Role of *dupA* in virulence of *Helicobacter pylori*

Amin Talebi Bezmin Abadi, Guillermo Perez-Perez

Amin Talebi Bezmin Abadi, Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran 14115-111, Iran

Guillermo Perez-Perez, Departments of Medicine and Microbiology, New York University School of Medicine and VA Medical Center, New York, NY 10010, United States

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Correspondence to: Amin Talebi Bezmin Abadi, PhD, Assistant Professor, Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran 14115-111, Iran. amin.talebi@modares.ac.ir
Telephone: +98-21-82884883
Fax: +98-21-82884883

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Abstract

*Helicobacter pylori* (*H. pylori*) is a gastric human pathogen associated with acute and chronic gastritis, 70% of all gastric ulcers, 85% of all duodenal ulcers, and both forms of stomach cancer, mucosal-associated lymphoid tissue (MALT) lymphoma and adenocarcinoma. Recently, attention has focused on possible relationship between presence of certain virulence factor and *H. pylori*-associated diseases. Some contradictory data between this bacterium and related disorders has been observed since not all the colonized individuals develop to severe disease. The reported diseases plausibility related to *H. pylori* specific virulence factors became an interesting story about this organism. Although a number of putative virulence factors have been identified including cytotoxin-associated gene a (*cagA*) and *vacA*, there are conflicting data about their actual participation as specific risk factor for *H. pylori*-related diseases. Duodenal ulcer promoting gene a (*dupA*) is a virulence factor of *H. pylori* that is highly associated with duodenal ulcer development and reduced risk of gastric cancer. The prevalence of *dupA* in *H. pylori* strains isolated from western countries is relatively higher than in *H. pylori* strains from Asian countries. Current confusing epidemiological reports will continue unless future sophisticated and molecular studies provide data on functional and complete *dupA* cluster in *H. pylori* infected individuals. This paper elucidates available knowledge concerning role of *dupA* in virulence of *H. pylori* after a decade of its discovery.

Key words: *Helicobacter pylori*; *dupA*; Bacterial virulence; Infection; Clinical outcome

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Core tip: *Helicobacter pylori* (*H. pylori*) is one of the most common bacterial infections worldwide. Ten years ago, *virB4* homologue was identified as a new virulence factor, *dupA* "duodenal ulcer promoting gene A" by Lu and her colleagues. Nowadays, new genetical analysis using available sequences can help scientists to draw a better conclusion about *dupA* and its actual role in pathogenesis of *H. pylori*-related diseases. In this paper, we aim to draw a new shaped overview regarding *H. pylori* and its virulence factors with emphasis of *dupA*.
INTRODUCTION

Due to the difficulty in diagnosis and fastidious condition of an optimal growth, Helicobacter pylori (H. pylori) was an unculturable and thus forgotten microorganism for many years[1]. Following the clinical and histological observations in gastritis and duodenal ulcer patients, Marshall and Warren were able to isolate and characterize this bacterium around thirty-three years ago[2]. New era had been started after this groundbreaking discovery and revealed as a publication in Lancet written by those Australian scientists[11]. As most of other human bacteria, H. pylori is mainly acquired during childhood and persists for the whole life of the colonized individual if not treated efficiently[3]. From bacteriologic point of view, H. pylori is a rod-shaped, microaerophilic Gram-negative organism which colonizing more than half of the world population[4]. Bacterial colonization induces acute inflammation in the gastric mucosa, a clinical manifestation which can be followed by diverse gastroduodenal disorders, but noted that only a minority of infected individuals develop severe diseases include duodenal ulcer and gastric cancer[4-8]. Many virulence-associated genes of H. pylori, including outer inflammatory protein a (OipA), vacuolating cytotoxin gene a (vacA), cytotoxin-associated gene a (cagA) and blood-group antigen-binding adhesion (babA) are believed to have a critical role in determining the final clinical manifestation of the infection[9,10]. Therefore, various studies have conducted to discover new insights into the role of these proposed virulence factors in pathogenesis of digestive diseases[11-14]. None of the mentioned virulence factors have distinguished as discriminating factor in the development of peptic ulcer disease and gastric cancer. The main rationale for different diseases outcome observed among colonized individuals is still under debate, though scientists proposed different array of virulence biomarkers in this bacterium as regular answer to this question. In this paper, we aim to open a new window for defining a bacterium as regular answer to this question. In this study, we will focus on the cagA gene which is in the group of subgroup A1[10,11].

VIRULENCE OF H. PYLORI

The definition of a virulence factor is referring to the ability of a bacterium to induce and develop a disease with a spectrum of severity[15]. Strains possessing these virulence factors are isolated more frequently from patients with the more serious clinical manifestations. It is logic to consider that for increase the chance of survival within harsh gastric condition H. pylori needs such smart strategies to keep the colonization. However, virulence factors can induce more cell damage with infiltrate immune cells to the location and thus inflammation will be the high priority event in epithelial cells[16]. Due to the chronic characteristic of H. pylori infection, scientists should expect to have particular definition of virulence factors for this bacterium.

Virulence factors of H. pylori play an inevitable role in the development of gastroduodenal diseases through mucosal inflammation[16]. Basically, the criteria for being a virulence factor are (1) biologic rationale; (2) epidemiologic consistency; and (3) enough evidences for being linked with certain disease[17,18]. In order to define a virulence factor for each bacterium, it should pass many in vivo and in vitro experiments[19-21]. However, it is worthwhile to emphasize that only a limited number of proposed virulence factors had been successfully confirmed for H. pylori[22-24]. It had been well-documented that all H. pylori strains have several virulence factors such as flagella and urease enzyme since they have a critical role in bacterial colonization[4]. Urease enzyme (as cytoplasmic protein) is necessary to establish primary bacterial colonization in the gastric mucosa. H. pylori flagella provide sufficient ability to quickly penetrate the gastric mucosa layer to avoid exposure with harsh acid condition in the stomach[4]. In addition, some adhesines such as babA2, iceA and Sialic acid-binding adhesin (sabA) are mostly present in H. pylori strains, and these factors help the bacterium to attach properly to the epithelial cells and serve as a unique virulence factor[25,26]. Clinically, gastric cancer and duodenal ulcer are standing in quite opposite sides of H. pylori-related disease spectrum. It brings a big query in the mind about disease plausibility which only can be explained with existence of diverse, but, specific virulence factors in this microorganism.

**cagA**

cagA is located at the end of the cag pathogenicity island (PAI), which is a 39-kb region transferred horizontally from an unknown bacterial source. The “pathogenicity islands” include cagA encode proteins contributing in signal transduction cascades that result in cytoskeletal rearrangement via actin polymerization and host cell protein phosphorylation[4]. Virulent strains of H. pylori possess the cagPAI. Many of H. pylori strains from patients with peptic ulcer or gastric cancer carry cagA, whereas many of those strains from asymptptomatically infected persons lack this gene[4]. Currently, we identify two major types of H. pylori isolates: cagA gene-negative and cagA gene-positive strains. Counting a virulence factor for cagA needs another classification which is based on polymorphism in Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs[4]. In cagA positive strains, there is a region contains the EPIYA motifs, which contains a tyrosine phosphorylation site[4]. Brief-
ly, two major types (Western and Eastern cagA) were determined according to this polymorphism. Though, we need more biologic rationale to be consistent with clinical evidences to present better information on how to interoperate this classic virulence factor in *H. pylori*.

### vacA

To now, vacA is the second most extensively investigated virulence factor of *H. pylori*. Virtually all *H. pylori* strains have a functional vacA gene which codes for the secreted pore-forming protein vacA\(^{[22]}\). The main difference in bacteria carrying vacA is expression levels and disease severity which are associated with sequence variation in different domains of secreted protein\(^{[49]}\). There is a big gap on our knowledge regarding biologic function of this protein since still many contradictory findings are exist\(^{[23-26]}\). So we need more investigation to determine how to count on vacA as useful *H. pylori* virulence factor.

### dupA

As first time, in 2005, it has been described that a new virulence factor which was located in the plasticity region of the *H. pylori* genome, PR or "plasticity region" where composed the dupA, has a relatively high rate of allelic diversity in *H. pylori* genomic DNA\(^{[27,28]}\). Whole genome analysis of 399 and 26695 revealed regions where G + C content was lower than rest of the *H. pylori* genome (34% against 40%)\(^{[29]}\). Later, since high variability was observed in this region, it termed as "plasticity region". Currently, we know that more than 60% of strain-specific genes of *H. pylori* are located in this area. In 399 and 26695 strains, two regions with lower G + C content and 45 kb and 69 kb long has been named as plasticity zones\(^{[40]}\). More than 50% of strain specific open reading frames (ORFs) are located in plasticity zone which are 46% and 48% unique genes from 26695 and 399, respectively. Interestingly, in comparison with 26695, the strain 399 has 33 more ORF in plasticity region (jhp914-jhp951)\(^{[30]}\). Lu *et al*\(^{[31]}\) investigated this region and reported a continuous gene covering jhp0917 and jhp0918 genes for first time which is a risk factor for duodenal ulcer diseases. Accordingly, they named the jhp0917-jhp0918 gene the dupA gene. To date, many of putative *H. pylori* genes have been suggested to be linked with increasing risk of digestive diseases, while none have been confirmed to be actually associated with unique and specific *H. pylori*-related disease such as gastric cancer or duodenal ulcer. Therefore, dupA can be named as first candidate to have achieved this distinction. Following the primary study by Lu *et al*\(^{[31]}\), a large number of controversial examinations has been published\(^{[32-42]}\). The global prevalence of dupA in patients with gastritis was reported around 45% which is highly differed among subjects with various nationality (31% in Asian and 64% in Western countries)\(^{[43,44]}\). Therefore, among most of Asian countries, a significant association between disease development and dupA status can be reported\(^{[38,45-54]}\). In two studies, first by Imagawa *et al*\(^{[37]}\) patients infected with dupA-positive strains showed higher risk to suffer from duodenal ulcer than dupA-negative patients. In second study, we have found that higher acid resistance of the dupA-positive strains can explain the adaptation of those strains to human stomach with high gastric acid output\(^{[35]}\). Indeed, Lu *et al*\(^{[31]}\) described that infections with *H. pylori* dupA-negative strains can increase the risk for duodenal ulcer; but it reduce the chance of occurrence for gastric\(^{[31]}\). Antral induction of IL-8 production is a main character of dupA pathogenesis causing predominant gastritis\(^{[36]}\). The mentioned mucosal inflammation and polymorphonuclear leukocytes (PMN) infiltration can lead to the occurrence of duodenal ulcer\(^{[31]}\). In a systematic review by Shiota *et al*\(^{[35]}\) with more than 2466 patients, they confirmed an association between certain clinical outcomes and the dupA status. Moreover, presence of an extra 600 bp in dupA ORF in *H. pylori* strains such as g27 showed that the length of the dupA is differ among various strain, mostly declared that dupA has two main genotypes accordingly, (long and short type)\(^{[35,38,55]}\). Unfortunately, most of studies in past did not consider this two types of dupA and thus the final results by them might be cautiously useful. Another interesting topics about dupA is existence of several mutations in gene length\(^{[38,56]}\). At different positions, these mutations can create a premature stop codon with considerable effects on its produced proteins function\(^{[56]}\). Strains isolated from patients with duodenal ulcer mostly carrying dupA without stop codon in comparison with other diseases types\(^{[27]}\). Notwithstanding, without frameshift mutation dupA which called intact long-type dupA rather short-type dupA is highly associated with gastric cancer\(^{[57]}\). It has been extensively reported that there is an association between increased expression levels of IL-8 and dupA in the gastric mucosa of *H. pylori*-colonized individuals. As expected, many reports are indicating on gastric mucosal inflammatory cell infiltration was significantly higher in patients with dupA-positive *H. pylori* than in patients with dupA-negative strain\(^{[56,57]}\). As such, current data suggesting that only intact long type dupA can produce DupA protein and also serve as real virulence factor for *H. pylori* strains. In brief, current knowledge about dupA positive strain and its subsequent diseases vulnerability insist on significant associations between the dupA gene and an increased risk for duodenal ulceration rather gastric cancer. As final remarks about dupA, we can mention to these sentences as follow: (1) Additional tests of the dupA DNA sequence are necessary to determine actual importance of intact dupA; also in level of proteins with immunoblotting techniques; (2) Similar to the caga, it has been asserted that dupA is forming a Type 4 secretion system (T4SS) as a full gene cluster. Noted that virB4 and dupA as homologous genes together.
are the major constituents of T4SS where located in plasticity region[52]; (3) Jung et al[58] recently examined South American population from Colombia to see association between dupA and virB gene homologs and clinical outcomes. In total, we concluded that intact dupA without shift mutation can serve as actual virulence factor with consistent results worldwide. It is no doubt that evaluation of various genes located in plasticity region are required and new data in close future can enrich our knowledge about this mysterious region of H. pylori genome; and (4) Broadly defined, virulence of H. pylori play an essential role in the development of severe gastroduodenal diseases such as duodenal ulcer through mucosal inflammation. With this regard, dupA as one of important risk factor was in focus of many researches in last years. The discrepancy observed among the epidemiologic studies can be explained by using various methods to determine existence of dupA, variation in ORF and different population’s bias. Thus, despite advances in our understanding of the development of H. pylori-related diseases, further work is required to clarify the roles of H. pylori virulence factors.

CONCLUSION

H. pylori plays a critical role in the development of severe digestive diseases; though, the main virulence determinant acting in this field are still not completely defined. Now the question is to find the determining item to represent this interesting disease pattern. For sure, we admitted that H. pylori is involved in pathogenesis of both gastric cancer and duodenal ulcer while they are in quite opposite side of digestive diseases, again, how we can still accept a crucial role for H. pylori in these gastroduodenal diseases? Many studies had been performed to elucidate actual biologic role of dupA in development of severe gastroduodenal diseases such as gastric cancer[48-49]. The observed discrepancy of dupA link with disease outcomes might be associate with the plasticity region of H. pylori or the limitation of PCR to detect the various forms of dupA gene; however, in order to draw a better conclusion further experiments are required[58,59]. Interestingly, the presence of dupA was significantly associated with H. pylori eradication failure with no biologic explanation[50-52]. In conclusion, it sounds that rather than promoting gastric cancer or duodenal ulceration in all populations, dupA is an effective factor for some of populations. Because of microarray analysis as new technology many new genes can be proposed as novel virulence biomarker for H. pylori.

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