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A report on allelic variation in *Helicobacter pylori dupA*: A viewpoint

Golzar Fatahi, Amin Talebi Bezmin Abadi *

Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

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Corresponding Author:
Amin Talebi Bezmin Abadi
Department of Bacteriology, Tarbiat Modares University, P.O. Box: 14115-111, Tehran, Iran.
Phone: +989120310214
E-mail: amin.talebi@modares.ac.ir

Abstract

Helicobacter pylori (H. pylori) is the pivotal cause of chronic gastritis, peptic ulcer diseases (PUD) and gastric cancer. Morphologically, the bacterium is spiral, Gram-negative and microaerophilic which survives lifespan in the human stomach in case of weak antibiotic therapy. There is a major difference in the pattern of global prevalence of *H. pylori* infection based on different levels of urbanization, hygiene, sanitation, access to clean water and other socioeconomic factors. To date, many studies have attempted to find significant associations between specific gastroduodenal diseases and *dupA*-positive strains, but no conclusive conclusion has been declared. The main reason for these inconsistent findings is the various methodologies applied in experiments which in turn have resulted in inaccurate observation. Our analysis showed that the existence of various alleles located in the *dupA* cluster would be a novel explanation for different associations found between this bacterial gene and diseases. In detailed experiments examining our proposed alleles using a large number of patients can be useful to disclose a significant clinical association between *H. pylori dupA*-positive strains and duodenal ulcer.

Keywords: *Helicobacter pylori*; *dupA*; duodenal ulcer; allelic variation

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Current dilemma: Viewpoints

Helicobacter pylori (*H. pylori*) is a spiral, Gram-negative and microaerophilic bacillus whose biologic territory is human gastric surface (1). There are great differences in prevalence patterns of *H. pylori* infection worldwide which depend on the level of urbanization, hygiene, sanitation, access to clean water and other socioeconomic factors (2-4). Bacterial virulence factors are affecting the final outcome of the diseases and currently, finding those factors are the main question mark for gastroenterologists and microbiologists (5). CagA was a classically recognized virulence factor, but newer studies showed that other factors are influencing the virulence pattern of this rouge bacterium. Lu *et al.* was the first research group to introduce ‘‘duodenal ulcer promoting’’ (*dupA*) gene as a novel virulence factor, which is located in the plasticity region of *H. pylori* genome (6, 7). They showed that *dupA*-positive strains were significantly associated with increased production of interleukin-8 (IL8) in the antral gastric mucosa (6). Interestingly, similar findings have been reported in both *in vivo* and *in vitro* experiments with sufficient clinical evidence (8-18). However, the accuracy of *dupA* as a practically useful biomarker, at least for duodenal ulcer (DU) patients, has attracted many types of research in recent years (15, 17, 19-23). Unfortunately, contradictory results obtained from different studies have prevented clinicians and microbiologists from having a straight conclusion about the association of that factor with digestive diseases (9, 11, 13, 19-21, 24-26). DupA is homologous to *virB4* and has been proposed as a component of a new cluster of *vir* homologue genes in the bacterial plasticity region (PR) that might form a type IV secretion system similar to *cag* pathogenicity island (PAI). Clinically, it is generally accepted that colonization with *dupA*-positive strains increases the risk of DU, but it is protective against GC both in Asian and Western populations (6). Following studies showed that two mutations in *dupA*, a deletion of an adenine at position 1311 and an insertion of an adenine at position 1426, were more frequently reported in *H. pylori* strains isolated from gastric cancer patients, considered as the negative-*dupA* strains that carried one or two mutations might lead to a defective DupA protein

(13). To the best of our knowledge, no investigation has been performed on different elements influencing inconsistent results obtained by these researches, although Talebi *et al*. have reported that various PCR designs might be effective, insufficient sample size was a limiting factor in their examination (27).

dupA alleles

The primer binding site of the *dupA* gene was highly variable in the designed PCR and application of different sets of primers had a great impact on reporting diverse percentages of *dupA*-positive strains from different populations (9, 10, 14, 17, 19, 21, 23, 24, 26-28). Many studies from different regions of the world have reported that the prevalence of *dupA* in DU patients was higher than gastric ulcer (GU) , but in studies on Swedish, Australian, Chinese, Indian and Malaysian populations, no association was observed between dupA and DU or GC development; this result is consistent with results from South America and East Asia (9, 11, 16-18, 20-23, 25, 29-34). In other studies from Belgium, South Africa, China and the United States, there were not only associations between *dupA* and duodenal ulcer disease, but also there were initiatives to the occurrence of gastric cancer (21, 26, 32, 35-37). Comparison of the complete genome sequences of two *H. pylori* strains (26695 and J99) revealed several regions where G+C content was lower than the rest of the *H. pylori* genome (35% compared with 39 %) suggesting that these genes may have been acquired horizontally from other bacterial species or transferred from other *H. pylori* strains (38, 39). Although ORFs of the major part of the plasticity region encode putative proteins with unknown functions, some have been found to share similarity with genes encoding functional proteins (40). A recent full-sequenced study of *H. pylori* revealed that the length of *dupA* open reading frame depended on the strains; Shi470 and G27 were approximately 600-bp longer ORF (approximately 2500-bp) than strain J99 as an additional 5′ region of *dupA*. This suggested that *dupA* had two genotypes according to the sequence of the putative 5′ region (presence, long-type and absence, short-type). Intact long-type *dupA* is a real virulence marker responsible for severe outcomes in Okinawa, Japan (41). Apparently, our molecular understanding of PR in *H. pylori* is poor. Since plasticity regions are transportable and have point mutations (frameshift, internal stop), insertions or deletions, they exhibit a wide variety of sequences in different strains. Given current *in silico* analysis, in this article, we will show that there might be a series of alleles in this region which can be greatly dominant in different strains (Figure 1). Of course, investigating these alleles in clinical isolates of *H. pylori* and comparing them among DU and GC patients can be helpful to elucidate the main determinant factors of *H. pylori* in individuals with severe implications, including duodenal ulcer.

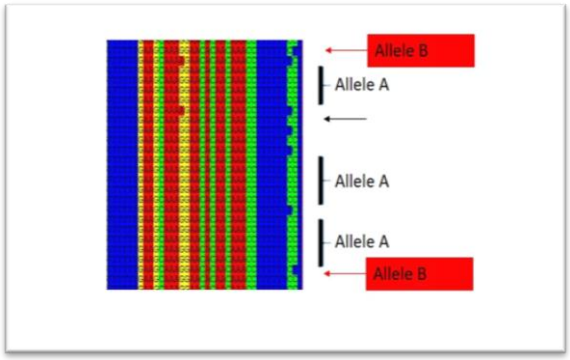

Figure 1. Demonstration of alleles A and B in *H. pylori dupA* genes in different clinical sequences.

In silico analysis

First, all *dupA* gene sequences were collected from all GeneBank strains using Laser gene software and then they were aligned to identify similarities in the selected sequences using Multiple Alignment software. During the sequence puzzling, we found the areas with significant dominance in different sequences.

Discussion

The alleles introduced in *H. pylori* plasticity regions can be inserted or removed in some bacterial isolates. In fact, this genetic change can increase the complexity of the present situation and make the discovery of true alleles affecting the consequences of pathogenicity more difficult. By investigating these alleles in different groups of *H. pylori*-related gastroduodenal disorders, it may be possible to address the ambiguity of *dupA* relationship with gastrointestinal diseases, especially duodenal ulcers. The logic behind these contradictory findings is different methods used (27). Totally, we assumed that *H. pylori* pathogenic strains that cause duodenal ulcer were associated with the presence of specific alleles rather than different sizes of *dupA* gene, both long and short (20, 21, 23, 33). The existence of different alleles (alleles A & B) located in *dupA* cluster (**Figure 1**) is a novel explanations for clarifying various disease associations reported between the gene and diseases. A prospective study using those alleles as determinants of specific diseases could be useful to disclose the 12-year-old investigations about *H. pylori* infection. Undoubtedly, clinical isolates are necessary to enrich the suggested hypothesis to solve this long-term investigation. Based on *in silico* analysis, we showed that virulent *H. pylori* strains

caused the duodenal ulcer in patients carrying specific alleles rather than *dupA* gene size. To be honest H. pylori virulence is hard to be predicted. The best evidence of the current claim is that we have no actual virulence determinant for this bacterium even 36 years after its discovery. Status of chronic infection and uninvestigated genetic vulnerabilities/eligibility bound to induce severe digestive diseases are the two major unanswered queries to fulfill our puzzle. Within 15 years of research, we are close to finding better biomarkers determining diseases status based on certain bacterial virutype. To now, it has been well documented that human colonization with *dupA*-positive *H. pylori* can result in various gastric disease outcome. In detailed experiments examining our proposed alleles using a large number of patients can be useful to disclose a significant clinical association between *H. pylori dupA*-positive strains and duodenal ulcer. Among available experiments, metagenomic analysis on clinical samples of individuals carrying *H. pylori* during the long-term assay can be an option. Personalized medicine for *H. pylori*-positive subjects to design better management of these patients will be another choice.

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Conflict of interests
The authors declare no conflict of interests.

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