

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.

Received: 15 Aug 2017

Revised : 8 Sep 2017

Accepted: 20 Sep 2017

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

Please cite this article as: Fatahi G, Talebi Bezmin Abadi A. A report on allelic variation in *Helicobacter pylori dupA*: A viewpoint. Res Mol Med, 2017; 5(3): 1-4

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 rouse 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.

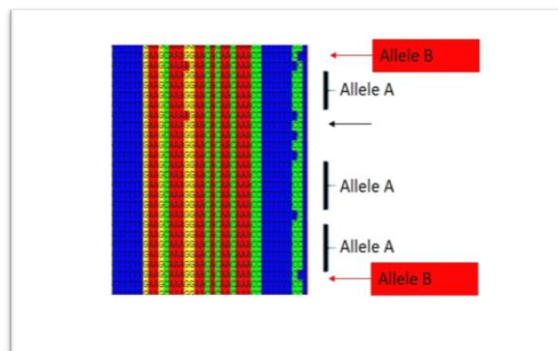


Figure 1. Demonstration of alleles A and B in *H. pylori dupA* genes in different clinical sequences.

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 explanation 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 virulence. 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.

Acknowledgments

The contents of the paper are the sole responsibility of the authors and do not necessarily represent the official views of any institute or organization.

Conflict of interests

The authors declare no conflict of interests.

References

- Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet*. 1983; 321(8336):1273-5. PMID: 6134060
- Hooi JK, Lai WY, Ng WK, Suen MM, Underwood FE, Tanyingoh D, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-analysis. *Gastroenterology*. 2017; 153(2):420-429. PMID: 28456631
- Leja M, Axon A, Brenner H. Epidemiology of *Helicobacter pylori* infection. *Helicobacter*. 2016; 21(S1):3-7. PMID: 27531531
- Abadi ATB, Kusters JG. Management of Helicobacter pylori infections. *BMC Gastroenterol*. 2016; 16(1):94. PMID: 27520775
- Abadi ATB. Strategies used by helicobacter pylori to establish persistent infection. *World J Gastroenterol*. 2017; 23(16):2870 - 2882. PMID: 28522905
- Lu H, Hsu P-I, Graham DY, Yamaoka Y. Duodenal ulcer promoting gene of Helicobacter pylori. *Gastroenterology*. 2005; 128(4):833-48. PMID: 15825067
- Lu H, Yamaoka Y, Graham DY. Helicobacter pylori virulence factors: facts and fantasies. *Curr Opin Gastroenterol*. 2005; 21(6):653-9. PMID: 16220040

- Arachchi H, Kalra V, Lal B, Bhatia V, Baba C, Chakravarthy S, et al. Prevalence of Duodenal Ulcer-Promoting Gene (*dupA*) of Helicobacter pylori in Patients with Duodenal Ulcer in North Indian Population. *Helicobacter*. 2007; 12(6):591-7. PMID: 18001398
- Paredes-Osses E, Saez K, Sanhueza E, Hebel S, Gonzalez C, Briceno C, et al. Association between *cagA*, *vacA*, and *dupA* genes of Helicobacter pylori and gastroduodenal pathologies in Chilean patients. *Folia Microbiol (Praha)*. 2017; 62(5):437-44. PMID: 28283946
- Wang MY, Chen C, Shao C, Wang SB, Wang AC, Yang YC, et al. Intact long-type DupA protein in Helicobacter pylori is an ATPase involved in multifunctional biological activities. *Microb Pathog*. 2015; 81:53-9. PMID: 25745877
- Nagashima H, Yamaoka Y. Helicobacter pylori *dupA* and smoking are associated with increased levels of interleukin-8 in gastric mucosa in Iraq-reply. *Hum Pathol*. 2015; 46(6):931. PMID: 25804905
- 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(3):290-3. PMID: 23793434
- Queiroz DM, Rocha GA, Rocha AM, Moura SB, Saraiva IE, Gomes LI, et al. *dupA* polymorphisms and risk of Helicobacter pylori-associated diseases. *Int J Med Microbiol*. 2011; 301(3):225-8. PMID: 21050811
- 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(7):1033-4. PMID: 21402555
- 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
- Gomes LI, Rocha GA, Rocha AM, Soares TF, Oliveira CA, Bittencourt PF, et al. Lack of association between Helicobacter pylori infection with *dupA*-positive strains and gastroduodenal diseases in Brazilian patients. *Int J Med Microbiol*. 2008; 298(3-4):223-30. PMID: 17897881
- Douraghi M, Mohammadi M, Oghalaie A, Abdirad A, Mohagheghi MA, Hosseini ME, et al. *dupA* as a risk determinant in Helicobacter pylori infection. *J Med Microbiol*. 2008; 57(Pt 5):554-62. PMID: 18436587
- Arachchi HS, Kalra V, Lal B, Bhatia V, Baba CS, Chakravarthy S, et al. Prevalence of duodenal ulcer-promoting gene (*dupA*) of Helicobacter pylori in patients with duodenal ulcer in North Indian population. *Helicobacter*. 2007; 12(6):591-7. PMID: 18001398
- Talebi Bezmin Abadi A, Perez-Perez G. Role of *dupA* in virulence of Helicobacter pylori. *World J Gastroenterol*. 2016; 22(46):10118-23. PMID: 28028359
- Jung SW, Sugimoto M, Shiota S, Graham DY, Yamaoka Y. The intact *dupA* cluster is a more reliable Helicobacter pylori virulence marker than *dupA* alone. *Infect Immun*. 2012; 80(1):381-7. PMID: 22038914
- Alam J, Maiti S, Ghosh P, De R, Chowdhury A, Das S, et al. Significant association of the *dupA* gene of Helicobacter pylori with duodenal ulcer development in a South-east Indian

- population. *J Med Microbiol.* 2012; 61(Pt 9):1295-302. PMID: 22653921
22. Matteo MJ, Armitano RI, Granados G, Wonaga AD, Sanches C, Olmos M, et al. *Helicobacter pylori* oipA, vacA and dupA genetic diversity in individual hosts. *J Med Microbiol.* 2010; 59(Pt 1):89-95. PMID: 19643933
23. Argent RH, Burette A, Miendje 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(9):1204-6. PMID: 17918084
24. Talebi Bezmin Abadi A. Therapy of *Helicobacter pylori*: present medley and future prospective. *Biomed Res Int.* 2014; 2014:124607. PMID: 24800203
25. Moura SB, Costa RF, Anacleto C, Rocha GA, Rocha AM, Queiroz DM. Single nucleotide polymorphisms of *Helicobacter pylori* dupA that lead to premature stop codons. *Helicobacter.* 2012; 17(3):176-80. PMID: 22515354
26. Abadi AT, 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(Pt 1):23-30. PMID: 21903829
27. Abadi AT, Loffeld RJ, Constancia AC, Wagenaar JA, Kusters JG. Detection of the *Helicobacter pylori* dupA gene is strongly affected by the PCR design. *J Microbiol Methods.* 2014; 106:55-6. PMID: 25128081
28. Osman HA, Hasan H, Suppian R, Hassan S, Andee DZ, Abdul Majid N, et al. 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(4):940-6. PMID: 26422871
29. 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(6):929-30. PMID: 25791584
30. Haddadi MH, Bazargani A, Khashei R, Fattahi MR, Bagheri Lankarani K, Moini M, et al. Different distribution of *Helicobacter pylori* EPIYA- cagA motifs and dupA genes in the upper gastrointestinal diseases and correlation with clinical outcomes in iranian patients. *Gastroenterol Hepatol Bed Bench.* 2015; 8(Suppl 1):S37-46. PMID: 26171136
31. Parzecka M, Szaflarska-Poplawska A, Gasiorowska J, Gorzkiewicz M, Grzybowski T. [The prevalence of dupA (duodenal ulcer-promoting gene) of *Helicobacter pylori* in children and adolescents--own observation]. *Pol Merkur Lekarski.* 2013; 34(203):277-80. PMID: 23894779
32. 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. *J Med Microbiol.* 2010; 59(Pt 12):1484-9. PMID: 20829397
33. 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 Dis.* 2010; 202(2):261-9. PMID: 20533870
34. Hussein NR. The association of dupA and *Helicobacter pylori*-related gastroduodenal diseases. *Eur J Clin Microbiol Infect Dis.* 2010; 29(7):817-21. PMID: 20419465
35. Souod N, Sarshar M, Dabiri H, Momtaz H, Kargar M, Mohammadzadeh A, et al. The study of the oipA and dupA genes in *Helicobacter pylori* strains and their relationship with different gastroduodenal diseases. *Gastroenterol Hepatol Bed Bench.* 2015; 8(Suppl 1):S47-53. PMID: 26171137
36. Roesler BM, Oliveira T, Costa SCB, Zeitune JMR. Is there any relationship between *Helicobacter pylori* dupA gene and the development of early and advanced gastric cancer in Brazilian patients. *Journal of Medical Research and Science.* 2012; 2(1):15-24.
37. Hussein NR, Tunjel I, Majed HS, Yousif ST, Aswad SI, Assafi MS. Duodenal ulcer promoting gene 1 (dupA1) is associated with A2147G clarithromycin-resistance mutation but not interleukin-8 secretion from gastric mucosa in Iraqi patients. *New Microbes New Infect.* 2015; 6:5-10. PMID: 26042186
38. Tomb J-F, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, et al. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature.* 1997; 388(6642):539-47. PMID: 9252185
39. Alm RA, Ling L-SL, Moir DT, King BL, Brown ED, Doig PC, et al. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature.* 1999; 397(6715):176-80. PMID: 9923682
40. Yamaoka Y. Roles of the plasticity regions of *Helicobacter pylori* in gastroduodenal pathogenesis. *J Med Microbiol.* 2008; 57(5):545-53. PMID: 18436586
41. Takahashi A, Shiota S, Matsunari O, Watada M, Suzuki R, Nakachi S, et al. Intact Long-Type dupA as a Marker for Gastroduodenal Diseases in Okinawan Subpopulation, Japan. *Helicobacter.* 2013; 18(1):66-72. PMID: 23067336