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Adaptive Evolution of BabA Virulence Factor of *Helicobacter pylori*

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ABSTRACT

Over half of the world's population suffers from Helicobacter pylori infection. However, the infection rate considerably varies across different countries and regions. Long-term Helicobacter pylori infection can pose serious health risks. Various outer membrane proteins synthesized by Helicobacter pylori participate in colonization, of which blood group antigen-binding adhesin is a key protein involved during the initial colonization process. Thus, this paper aimed to elucidate the adaptive evolution of BabA, a virulent factor of Helicobacter pylori, in human hosts from four aspects: the function of blood-group-anti-binding adhesin (BabA), the diversification of protein expression regulation, the effect of selection pressure on genes, and the relationship between gene evolution and diseases.

Keywords

Helicobacter pylori, Blood group antigen binding adhesion, Lewis b antigen, Evolve.

As is well documented, *Helicobacter pylori* (*H.pylori*) is a pathogenic bacterium that colonizes the stomach. Despite being mostly asymptomatic in humans, prolonged infection may lead to severe conditions, such as gastric ulcers, duodenal ulcers,gastric cancer, or gastric mucosa-associated lymphoid tissue lymphoma. It adheres to mucins secreted by the gastric mucosa via an outer membrane protein (OMP) and resists clearance even during intense gastric peristalsis. Adhesion is the initial interaction between *H. pylori* and gastric mucosal epithelial cells, serving as the foundation for gastric mucosal injury. Blood group antigen-binding adhesin (BabA) was the first isolated and is currently the most explored *Helicobacter pylori* outer membrane protein. In this paper, the biological function, expression regulation diversity, virulence evolution of *Helicobacter pylori* BabA, and its relationship with disease were explored in order to formulate effective preventive and therapeuticstrategies for *H. pylori* infection.

The biological function of BabA

The BabA protein, a member of the Hop family, has a molecular weight of 75-80 kDa and is encoded by the babA2 gene. It plays a pivotal role in the adhesion of *H.pylori*. A previous study reported that *H. pylori* can adhere to the gastric mucosa by binding to the Lewis b antigen expressed on the surface of gastric epithelial cells [1]. Falk et al. [2] corroborated this hypothesis in transgenic mice expressing human Lewis b antigen. Notably, researchers identified the adhesion factor BabA that specifically binds to the Lewis b antigen via affinity labeling [3]. To date, numerous antigens that can bind to BabA protein have been identified, encompassing blood group antigen H1,blood group antigen A, blood group antigen B, and globohexase ceramide [4,5]. However, Lewis b antigen remains the most extensively studied antigen, given that it is primarily expressed on the surface of mucins secreted by gastric mucosa and gastric epithelial cells. Backstrom et al. [6] analyzed the binding property of BabA to Lewis b antigen in clinical *H. pylori* isolates from Sweden and the United States but occasionally encountered *H. pylori* exhibiting a Lewis b antigen-binding phenotype that did not bind to Lewis b antigen, suggesting the presence of a metastable phenotype. In addition, they observed that the BabA protein derived from *H. pylori* strains isolated from different blood group populations displayed varying antigen recognition abilities. Interestingly, some *H. pylori* strains could only bind to H1 and Lewis b antigens of the O antigen but not to A antigen, B antigen,

A-Lewis b antigen, and B-Lewis b antigen [4]. Therefore, *H.pylori* strains that can recognize the O antigen were classified as "specific types". On the other hand, *H.pylori* strains that canrecognize both the O, A, and B blood group antigens were classified as "universal types". Naim Hage et al. [7] analyzed the crystal structure of BabA using X-ray diffraction technology and highlighted the critical role of diversity loop 1 (DL1) in its adhesion region for binding to the Lewis b antigen. Notably, the DL1 space of the "universal" strain BabA was large, whereas that of the "specific" strain BabA was small. In addition, the binding of BabA to Lewis b antigen has been established to enhance the translocation of cytotoxin-associated protein (CagA) in host cells [8] and induce independent double-strand breaks of vacuolating cytotoxin (VacA), γ-glutamyl transpeptidase and toxin-associated gene pathogenic island [9,10]. BabA-mediated adhesion in *H. pylori* enhances cag-T4SS system activity and inducesthe release of pro-inflammatory cytokines and precancerous-related factors [8,11].

The binding level of BabA and Lewis b antigen is highly variable

The frequency of sequence variation of outer membrane protein-encoding genes during *H. pylori* infection is higher than that of housekeeping genes, with one of the genes most affected by genomic variation being babA [12,13]. Alterations in the specificity and strength of binding of the BabA protein to the Lewis b antigen are the result of mutations in the truncated protein, frameshift due to changes in repeat length, or amino acid substitutions. The babA gene was initially cloned from *H. pylori* CCUG 17875 and contains a silenced babA1 gene and an expressed babA2 gene. The chief difference between the two genes was a deletion of 10 bp in the signal peptide sequence of babA1, which resulted in gene silencing. Colbeck et al. [14] reported the occurrence of the deletion of this translation initiation codon by *H. pylori*; nonetheless, this phenomenon seems to be relatively rare [15]. Slippery strand mispair (SSM) is a phenomenon wherein short and continuous repetitive DNA sequences are deleted or inserted during replication. The coding region of the babA gene at the 5' end has a CT dinucleotide repeat, and the number of repeat sequences determines protein expression [6]. So far, three genomic sites have been detected in bab, namely babA, babB, and babC, which have almost identical 5' end and 3' end domains but variable intermediate regions. The latter may bind to specific ligands, whereas the conservative domains facilitate intraspecific and intraspecific gene recombination to form chimeric genes [14,16,17], resulting in phenotypic changes in binding capacity to Lewis b antigens [18,19]. Pride [16] initially documented the formation of chimeras between babA and babB and observed that among the 42 (5%) clinical isolates, two babB 5' regions were substituted by the first 56 bp of babA (babA/B chimerism). Meanwhile, Matteo et al. [20] considered the formation of babA/B chimeras as a common mechanism for regulating the expression of babA protein. Indeed, these chimeras are capable of converting non-Lewis b antigen-binding strains into Lewis b antigen-binding strains or eliminating BabA-dependent adhesion [6,21]. Of note, the protein expression levels of BabA and Lewis b antigen-binding activity were lower in strains with mixed babA/B genotypes compared with those with non-mixed genotypes [22]. The formation of chimeric proteins appears to play a fundamental role in translation regulation. Ohno et al. [23] found the deletion of BabA expression post-infection in Mongolian gerbils, which was potentially caused by nucleotide changes in BabA. In addition, Aspholm-Hurtig et al. [4] analyzed the central variable region of the babA gene of *Helicobacter pylori* strains from different regions (Peru, Spain, Japan, and Alaska) using a phylogenetic tree and demonstrated that they could be divided into several different groups, suggesting that differences in binding strength and specificity of the babA protein might contribute to variations in amino acid sequence, thereby facilitating the differentiation of BabA and its adaptation to the host environment. Moonens et al. [24] concluded that a single amino acid substitution during the evolution of *H. pylori* affected the function of BabA protein and changedits blood group binding preference. Sweeney et al. [25] found that a mutation from leucine at position 198 on DL1 in the carbohydrate-binding region on the surface of BabA to serine converted *H. pylori* from the specific type to the universal type. Takentogether, these findings highlight that BabA generates a multi-level variation patternto adapt to the complex and volatile environment in the stomach, which plays a crucial role in the adaptation of the *H. pylori* population to the host.

Selective Pressure Drives the Evolution of the BabA Gene

H. pylori has been migrating with human ancestors for millennia, undergoing genetic mutations and diversification [26]. Genetic variation between populations may be driven by selection pressure, which assists bacteria in adapting to the physiological environment of the native host and eventually leads to geographic differentiation. BabA binding to host receptors was found to be acid-sensitive, decreasing at low pH and recovering at elevated pH. At the same time, this acid sensitivity varied among different gastric region isolates. For instance, *H. pylori* isolated from the gastric and gastric antrum displayed distinct acid adaptability, which was principally contingent upon the pH sensor in the BabA sequence and the pH-reversible remodeling that facilitated the formation of high-affinity BabA dimers. Importantly, the pH-sensitive regulatory sequence involved in BabA-mediated acid adaptation is located at amino acids 198-202. This sequence forms a critical coil in the spatial conformation betweenthe carbohydrate-binding region and the head region of the BabA surface, with position 199 of the critical coil being its key site. The diversity of BabA-mediated acid adaptation may be attributed to differences in this critical coil and critical site [27].This observation indicates that the different binding properties of BabA enable *H. pylori* to effectively adapt to changes in gastric mucosal pH, and this acid sensitivity is changed in different individuals and during chronic infection and disease progression (such as chronic inflammation and long-term administration of drugs inhibiting gastric acid secretion). In addition, the virulence factor of *Helicobacter pylori* BabA plays an instrumental role in the pathogenesis of the disease, not only mediating bacterial colonization but also promoting local immune response. Olfat et al. [28] performed an in vitro experiment and described that BabA-mediated bacterial adhesion to the human

gastric epithelium was associated with increased secretion of interleukin-8. Likewise, Sinem et al. [29] identified a positive correlation between babA and IL-17 expression in patients with gastric ulcers. Noteworthily, animal studies showed that BabA-expressing strains promoted inflammation, and the induction of epithelial cells in vitro was positively correlated with interleukin -8 secretion [30]. However, in response to a strong immune response from the host, *H. pylori* may inhibit the expression of certain adhesins to evade the defense mechanisms of the host. In a rhesus monkey model of *H. pylori* infection, although BabA was a prerequisite for initial infection, the expression of BabA was down-regulated within a few weeks following infection, implying that BabA may be lost due to selective pressure of the immune system [31]. Different selective pressures imposed on the BabAprotein necessitate rapid adaptation, showcasing the adaptive evolution of *H. pylori*.

Evolution of virulence gene of BabA is related to disease

While *H. pylori* asymptomatically colonized the stomach for decades, progression to serious clinical disease may be associated with certain virulent genes harbored by this bacterium. Gerhard and Yoshio [32,33] evinced that strains expressing BabA are more common in patients with peptic ulcers and gastric adenocarcinomas. Arecent study highlighted BabA-mediated adhesion in *H. pylori* strains associated with childhood ulcerative disease [34]. The evolutionary trajectory of virulence genes of *Helicobacter pylori* in different regions is unique, and infection in hosts with different genetic backgrounds leads to different infection outcomes. Araceli et al. [35] assessed the evolutionary ability of variable regions of the babA virulence gene and pointed outthat the positive selection predominantly influenced loci in East Asian strains, indicating that the babA gene was not randomly distributed in *H. pylori* strains of different human populations but exhibited geographical differences. Aspholm-Hurtig et al. [4] examined the binding ability of BabA to Lewis b antigen, A-Lewis b antigen, and B-Lewis b antigen and found that over 95% of the 265 *H. pylori* strains from different regions were of the "universal type". At the same time, a small proportion of these strains were "type specific" strains, mainly in the South American population and those with O-type blood. This binding property revealed that individuals with O-type blood are at higher risk of developing duodenal ulcers. The most prevalent disease in Peru is gastric gastritis, characterized by a high pH value of gastric juice and a high incidence of gastric cancer [36]. Contrastingly, gastric antral gastritis is dominant in India, featuring a low gastric juice pH and a low risk of gastric cancer [37]. Bugaytsova et al. [27] analyzed the pH-sensitive regulatory sequence of BabA in *H. pylori* strains from epidemic regions such as Peru and India and determined that the amino acid at the key site of BabA's pH-sensitive regulatory sequence was proline in the majority of Peruvian strains (adapted to high pH environments), whereas proline was missing at the key site in most Indian strains (adapted to low pH environments). Moreover, Moonens et al. [24] validated that sequence changes in DL1 and DL2 on the BabA surface carbohydrate-binding region of Alaska strain *H. pylori* A730 and Peru strain *H. pylori* P436 resulted in high structural heterogeneity in BabA's Lewis b binding pocket. These findings partly account for the regional differences in the incidence of *H. pylori*-associated malignancies.

In summary, under the action of multiple selection pressures, mutations that are beneficial to the survival and reproduction of bacteria are stably inherited in the population and promote the evolution of phenotypic traits. BabA protein is a critical adhesion molecule in *H. pylori* infection. Its adaptive virulence evolution partially reflects the long-term symbiotic evolution with humans. A comprehensive understanding of Baba protein is anticipated to provide insights into the prevention and treatment of *Helicobacter pylori* infection.

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