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Original Article

Helicobacter pylori sabA, *hopQ* and *hom* genotypes as potential genetic biomarkers for gastric mucosal inflammation

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Abstract

Helicobacter pylori infection drives heterogeneous gastric pathologies, yet genotype-phenotype correlations in diverse populations remain underexplored. The aim of this cross-sectional study was to investigate the associations between *H. pylori* virulence genotypes (*sabA*, *hopQ*, *hom* family) and histopathological severity in gastric mucosa among 113 Indonesian dyspepsia patients (mean age: 49.6 years; male predominance: 64.6%). Whole-genome sequencing characterized virulence genotypes, while histopathological grading system using the Updated Sydney System assessed inflammation, atrophy, and bacterial density in the antral and corporal gastric regions. Phylogenetic analysis elucidated strain relatedness. Key genotype frequencies included *sabA* "on" (40.6%, 43/106), *hopQ* type I (53.7%, 43/80), and *homC*^{L} (82.4%, 75/91). Statistical analysis revealed *sabA* "on" status significantly associated with elevated antral bacterial density (odds ratio (OR) 2.70 and 95% confidence interval (95%CI) 1.10–6.60, p=0.027). The *homC* variants (*homC*^{L}/*homC*^{S}) demonstrated robust associations with chronic inflammation severity (OR: 3.04; 95%CI: 0.99–9.36, p=0.046) and atrophy progression (OR: 4.78; 95%CI: 1.00–22.86, p=0.035), in contrast to the *hopQ* genotype, which showed no histopathological association. These findings indicated that *sabA* and *homC* as critical determinants of gastric microenvironment modulation, potentially through *sabA*-mediated colonization efficiency and *homC*^{L}-*babA* synergistic interactions. While histological profiles predominantly indicated mild atrophy, widespread severe chronic inflammation signals latent progression risks.

Keywords: *Helicobacter pylori*, *sabA*, *hopQ*, *hom*, gastric histopathology

Introduction

Despite significant advancements in healthcare, *Helicobacter pylori* (*H. pylori*) infection continues to be a major global health concern, disproportionately affecting developing nations like Indonesia. Although Indonesia's age-standardized incidence rate of gastric cancer is relatively low at 2.8 per 100,000 [1], recent data from GLOBOCAN 2022 indicated that gastric cancer caused 3,242 deaths, ranking as the 14th leading cause of cancer-related mortality in the country [2]. The substantial public health burden posed by *H. pylori* infection, which exceeds

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Received: December 13, 2024 - Accepted: March 15, 2025 - Published online: April 28, 2025

50% prevalence in many populations [2], arises from a multifactorial pathogenesis driven by the complex interplay of environmental exposures, host genetic susceptibility, and bacterial virulence determinants [3]. Notably, the heterogeneous clinical outcomes associated with H. pylori infection—ranging from asymptomatic carriage to severe gastroduodenal pathologies—show marked regional disparities, likely attributable to variations in these interacting factors across geographic and sociodemographic contexts. This is particularly evident in archipelagic nations such as Indonesia [1], where island-specific ecological conditions, ethnic diversity, and socioeconomic gradients may differentially influence infection dynamics, disease manifestations, and therapeutic responses. Understanding these region-specific drivers is critical for advancing evidence-based, context-customized strategies for prevention, diagnosis, and treatment, ensuring alignment with the unique epidemiological and demographic profiles of diverse populations.

Southeast Asian countries, including Thailand and the Philippines, have high *H. pylori* prevalence rates of 54.1–76.1% and 60%, respectively [4,5]. In contrast, Indonesia reports a lower overall prevalence, with an estimated 22.1% infection rate across its five largest islands [6-13]. However, this seemingly low prevalence obscures significant interethnic disparities. While the predominant Javanese population has an exceptionally low prevalence of 2.4%, higher rates are observed among other ethnic groups: 42.9% in Papua, 40.0% in Batak, 36.7% in Bugis, 13.0% in Tionghoa, and 7.5% in Dayak populations [14,15]. These variations emphasize the importance of examining the interplay between ethnic-specific host factors and bacterial genotypes in understanding *H. pylori*-associated diseases.

Although Indonesia's gastric cancer incidence is relatively low compared to other Asian countries, the combination of high antibiotic resistance rates and virulent *H. pylori* genotypes highlights the urgent need to address *H. pylori*-associated conditions promptly [13,16,17]. The wide spectrum of clinical outcomes observed in *H. pylori*-infected individuals suggests a complex relationship between bacterial virulence determinants and host susceptibility [18]. Among the virulence factors identified, genes such as sialic acid-binding adhesin (*sabA*), outer membrane protein (*hopQ*), and outer membrane family (*hom*) are key contributors to pathogenesis [19-21]. These factors facilitate bacterial adhesion, colonization, immune evasion, and modulation of host immune responses, ultimately influencing disease severity and progression [22,23].

The *H. pylori* genome encodes several virulence factors critical for persistent infection and gastric mucosal damage. *SabA* mediates bacterial adhesion to gastric epithelial cells by binding to sialylated Lewis antigens, thus enabling colonization and the establishment of a stable niche for survival [24,25]. *HopQ* plays a pivotal role in bacterial adhesion and immune modulation, interacting with host receptors to activate signaling pathways and induce pro-inflammatory cytokine production, particularly interleukin-8 (IL-8) [26,27]. This inflammatory response contributes to tissue damage and the progression of conditions such as chronic gastritis [28]. The *hom* family genes (*homA*, *homB*, *homC*) encode outer membrane proteins essential for bacterial survival, nutrient acquisition, and immune system evasion [29-32]. The *homC*, in particular, has been implicated in modulating host inflammatory responses and is associated with severe gastric pathologies [33].

While numerous studies have explored the associations between *H. pylori* virulence genotypes and gastric pathology, the majority of these investigations have focused on Western populations [34]. Data on these associations in Indonesian populations remain limited. Considering the genetic diversity of *H. pylori* strains and variations in host susceptibility across ethnic groups, it is essential to investigate the relationships between specific *H. pylori* genotypes and gastric mucosal histopathology in Indonesia. Therefore, the aim of this study was to explore the association between *H. pylori sabA*, *hopQ*, and *hom* family (*homA*, *homB*, *homC*) genotypes and gastric mucosal histopathology in Indonesians infected with *H. pylori*.

Methods

Study design and setting

This cross-sectional study was conducted to investigate the associations between *H. pylori* virulence genotypes (*sabA, hopQ, homA, homB, homC*) and histopathological features of

gastric mucosa. The study utilized 113 whole-genome sequenced *H. pylori* isolates obtained from a multicenter cohort of 1,172 dyspeptic patients across 12 Indonesian gastroenterology centers (August 2012–March 2017) [14,35-38]. Specimens were collected from adults (≥17 years) with chronic dyspepsia undergoing endoscopy, excluding individuals with gastrectomy or contraindications for the procedure. Genotypic profiles were characterized via Illumina-based next-generation sequencing, while histopathological outcomes (inflammation, atrophy, metaplasia, bacterial density) were assessed using the Updated Sydney System on antral and corpus biopsies [39]. The study design integrated molecular genotyping, histopathological grading, and geographic diversity to explore clinical implications of *H. pylori* genetic variability in an understudied Indonesian population.

Samples and DNA sequencing

A total of 1,172 gastric biopsy specimens were collected between August 2012 and March 2017 and some of the data have been published [14,35-38]. The specimens were obtained from dyspeptic patients who underwent endoscopy at 12 Gastroentero-Hepatology centers across Indonesia: Surabaya Bangli, Cimacan, Jakarta, Kolaka, Kupang, Makassar, Medan, Merauke, Palembang, Pontianak, and Samosir Island. The inclusion criteria for specimen collection were male or female outpatients aged ≥17 years with dyspeptic symptoms persisting for at least three months prior to the study. Patients with partial or total gastrectomy, non-fasted individuals, and those with contraindications for upper endoscopy were excluded. During endoscopy, biopsies were collected from both the antrum and corpus of the stomach.

DNA isolation was performed using antral gastric tissue samples, while histological evaluation was conducted on biopsies from both the antrum and corpus. Endoscopic findings, including diagnoses of gastric ulcer (GU), gastroesophageal reflux disease (GERD), gastric cancer (GC), duodenitis, and gastritis, were systematically recorded during the initial clinical assessment.

Of the 1,172 specimens, 113 samples were successfully cultured and subjected to next-generation sequencing (NGS). These 113 NGS results were utilized in the present study. *H. pylori* isolates were cultured from bacterial stocks preserved at -80°C in Brucella broth supplemented with 10% glycerol and 10% horse serum. The bacterial stocks were maintained at the Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Yufu City, Japan. From the 113 NGS samples, a subset was further selected based on DNA quality, ensuring suitability for genotype analysis, and the availability of complete histopathological data. Briefly, genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) in accordance with the manufacturer's protocol. Whole genome sequencing was conducted on high-throughput NGS platforms, the Illumina HiSeq 2000 and MiSeq, as previously described [36]. Briefly, high-quality genomic DNA was processed to create dual-indexed Nextera XT Illumina libraries, followed by cluster generation and paired-end sequencing. The MiSeq platform generated reads of 2×300 bp, while the HiSeq produced reads of 2×150 bp. Quality control measures included trimming reads and de novo genome assembly using SPAdes v3.6.2 [40] with default settings. The default settings included automatic selection of k-mer sizes, no strict minimum contig length cutoff, dynamic coverage cutoff, and error correction on reads. The assembled contigs were further validated through reference mapping to assess coverage. Initial analyses were conducted using commercial software, CLC Genomic Workbench v. 7.04, (Qiagen Inc., Redwood, California, USA). The coverage achieved ranged from 81× to 400× across the genomes, as detailed in our previous work [36]. For downstream analysis, we applied a quality threshold of Q30>80%, as recommended by Illumina, and required an average coverage of at least 80×, consistent with previously established criteria [36]. Genome annotation was performed using Prokka [41] yielding coverage depths of 81-400-fold per genome.

Genotyping of *Helicobacter pylori* virulence genes

This study analyzed 113 NGS samples of *H. pylori* obtained from our prior investigation [35,36], sequenced using the Illumina MiSeq platform and annotated via Prokka in .ffn format. The .ffn files were consolidated into a single FASTA file using command-line interface tools on the Ubuntu Linux operating system (Canonical Ltd., London, England). Raw sequence data were converted to FASTA format and subjected to BLASTN algorithm analysis against reference

genomes for virulence-associated loci: *sabA* (NC_000915.1:779008-780294), *hopQ* (NC_000915.1:1243583-1245508), *homA* (NC_000915.1:763982-765964), *homC* (NC_000915.1:380965-383067), from *H. pylori* strain 26695, and *homB* (NC_000921.1:962682-964688) from strain J99. These reference strains were selected due to their widespread use in research and their isolation from hosts exhibiting diverse clinical conditions. Default BLASTN parameters were applied: E-value threshold of 10, word size 11, gap costs of 5 (existence) and 2 (extension), scoring matrix values of +2 (match) and -3 (mismatch), with low-complexity filtering enabled to minimize alignment errors.

Each gene sequence was aligned to its corresponding reference sequence using Geneious Prime 2024.0.5 [42]. Sequences with ≥90% identity and ≥90% coverage were considered matches to specific genotypes, while ambiguous sequences failing these criteria were excluded to ensure data reliability. Alignments were performed with an E-value cutoff of 1e-5 to maintain reproducibility. Outer membrane protein (OMP)-encoding genes were subsequently segregated into individual FASTA files for downstream phylogenetic and structural analyses. To evaluate genetic diversity and evolutionary relationships among the strains, a phylogenetic tree was reconstructed using the Neighbor-Joining method in MEGA XI software [43] incorporating bootstrap analysis with 1,000 replicates to assess nodal support. The phylogenetic reconstruction was performed under the pairwise deletion model to account for missing data, ensuring robust inference of evolutionary patterns.

Genotyping was conducted by comparing sample-derived DNA or amino acid sequences with established reference genotypes. For *sabA*, samples were classified as "on" or "off" based on the presence or absence of an early stop codon. The "on" genotype indicated a full-length, potentially functional protein, while the "off" genotype denoted a truncated, likely non-functional protein due to an early stop codon, consistent with a previous study [44]. For *hopQ*, *homA*, and *homB*, genotyping was based on allele types as previously described [32,45]. For *homC*, variant types were classified according to established criteria [46].

Histopathological assessment

To determine the histopathological categories of gastric mucosa among *H. pylori* infection individuals, a histopathological assessment was conducted. The gastric biopsy specimens, previously obtained and processed for histological analysis [36], were utilized in this study. Tissues were fixed in 10% buffered formalin for 24 hours to preserve structural integrity, followed by paraffin embedding. Prior to embedding, fixed specimens were stored at room temperature for 1 to 6 months. Thin sections (3–4 μm) were obtained using a microtome and mounted on glass slides. Serial sections were stained with hematoxylin and eosin (H&E) for general histopathological assessment and May–Giemsa stain for *H. pylori* detection [36]. Histological features, including inflammation, neutrophil infiltration, atrophy, intestinal metaplasia, and bacterial density, were evaluated using the Updated Sydney System, which grades each parameter from 0 (normal), 1 (mild), 2 (moderate) and 3 (marked) [39].

To detect *H. pylori* in gastric tissue, immunohistochemical staining was conducted following the established method [47]. Briefly, tissue sections underwent antigen retrieval using citrate buffer (pH 6.0) and endogenous peroxidase blocking with 3% hydrogen peroxide. Sections were then incubated overnight at 4°C with α-*H. pylori* Antibody (DAKO, Glostrup, Denmark). After washing with phosphate-buffered saline (PBS), sections were treated with biotinylated goat anti-rabbit IgG (Nichirei, Tokyo, Japan), followed by avidin-conjugated horseradish peroxidase (HRP) complex Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA). Peroxidase activity was visualized using a hydrogen peroxide/diaminobenzidine (H_2O_2/DAB) substrate, resulting in a brown precipitate at antigen sites. Negative controls, in which the primary antibody was omitted, were included to confirm staining specificity.

Statistical analysis

The association between *H. pylori* genotypes (*sabA*, *hopQ*, *homA*, *homB*, and *homC*) and histopathological categories of gastric mucosa (acute inflammation, chronic inflammation, atrophy, metaplasia, and *H. pylori* density) were assessed using Chi-squared test or Fisher's exact test, as appropriate. Statistical significance was defined as a two-tailed $p<0.05$ and analyses were conducted using IBM SPSS Statistics version 26 (IBM Corporation, New York, USA).

Results

Prevalence of *sabA*, *hopQ*, *homA*, *homB* and *homC* genes in *H. pylori* isolates

A total of 113 *H. pylori* DNA samples were initially included in this study. However, variations in the fragment lengths of the target genes (*sabA*, *hopQ*, *homA*, *homB*, and *homC*) led to differential sample availability for genotype analysis. Some samples with shorter DNA fragments were excluded from specific assessments. Additionally, complete histopathological data were available for only 100 of the 113 samples, further contributing to variations in sample size across different genotypes. The distribution of *H. pylori* genotypes and their corresponding histopathological data is presented in **Table 1**.

The prevalence of the virulence gene variants was as follows: *sabA* "on" in 40.6% (43/106) and "off" in 59.4% (63/106); *hopQ* type I in 53.7% (43/80), type II in 31.3% (25/80), and unclassified in 15% (12/80); *homA* type II in 35.9% (23/64) and unclassified in 64.1% (41/64); *homB* type I in 18.9% (11/58), type II in 8.6% (5/58), type VI in 1.9% (1/58), and unclassified in 70.6% (41/58); and *homC* (*homC*^{S}) in 17.6% (16/91) and *homC*^{L} in 82.4% (75/91) (**Table 1**). The number of strains available for histopathological assessment is also presented in **Table 1**.

Table 1. Number of samples analyzed for *sabA, hopQ, homA, homB*, and *homC* genotypes after quality and histopathological data filtering

Gene	Genotype	Samples after quality filtering, n (%)	Samples with histopathology data, n (%)
sabA	*sabA* on	43 (40.6)	37 (39.9)
	sabA off	63 (59.4)	56 (60.2)
	Total	106 (100)	93 (100)
hopQ	*hopQ* allele type I	43 (53.8)	38 (53.5)
	hopQ allele type II	25 (31.2)	22 (31.0)
	Unclassified	12 (15.0)	11 (15.5)
	Total	80 (100)	71 (100)
homA	*homA* allele type II	23 (35.9)	17 (30.4)
	Unclassified	41 (64.1)	39 (69.6)
	Total	64 (100)	56 (100)
homB	*homB* allele type I	11 (19.0)	9 (17.6)
	homB allele type II	5 (8.6)	4 (7.8)
	homB allele type VI	1 (1.7)	1 (2.0)
	Unclassified	41 (70.7)	37 (72.5)
	Total	58 (100)	51 (100)
homC	*homC*^{S}	16 (17.6)	16 (20.0)
	homC^{L}	75 (82.4)	64 (80.0)
	Total	91 (100)	80 (100)

Phylogenetic tree analysis

The phylogenetic trees presenting the genetic relationships among *H. pylori* strains including 106 (*sabA*), 80 (*hopQ*), 64 (*homA*), 58 (*homB*), and 91 (*homC*) clinical isolates, alongside their respective reference strains (*H. pylori* 26695 for *sabA*, *hopQ*, *homA*, and *homC*; *H. pylori* J99 for *homB*) are presented in **Figures 1–3**. The trees reveal distinct clustering patterns correlated with geographical origins. Notably, several strains formed cohesive clades, suggesting shared evolutionary trajectories or potential epidemiological linkages in infection sources. In contrast, sporadic isolates exhibited phylogenetic divergence, indicative of independent evolutionary histories or alternative transmission pathways. These observations underscored the genetic diversity of *H. pylori* populations and their adaptation to localized host and environmental factors.

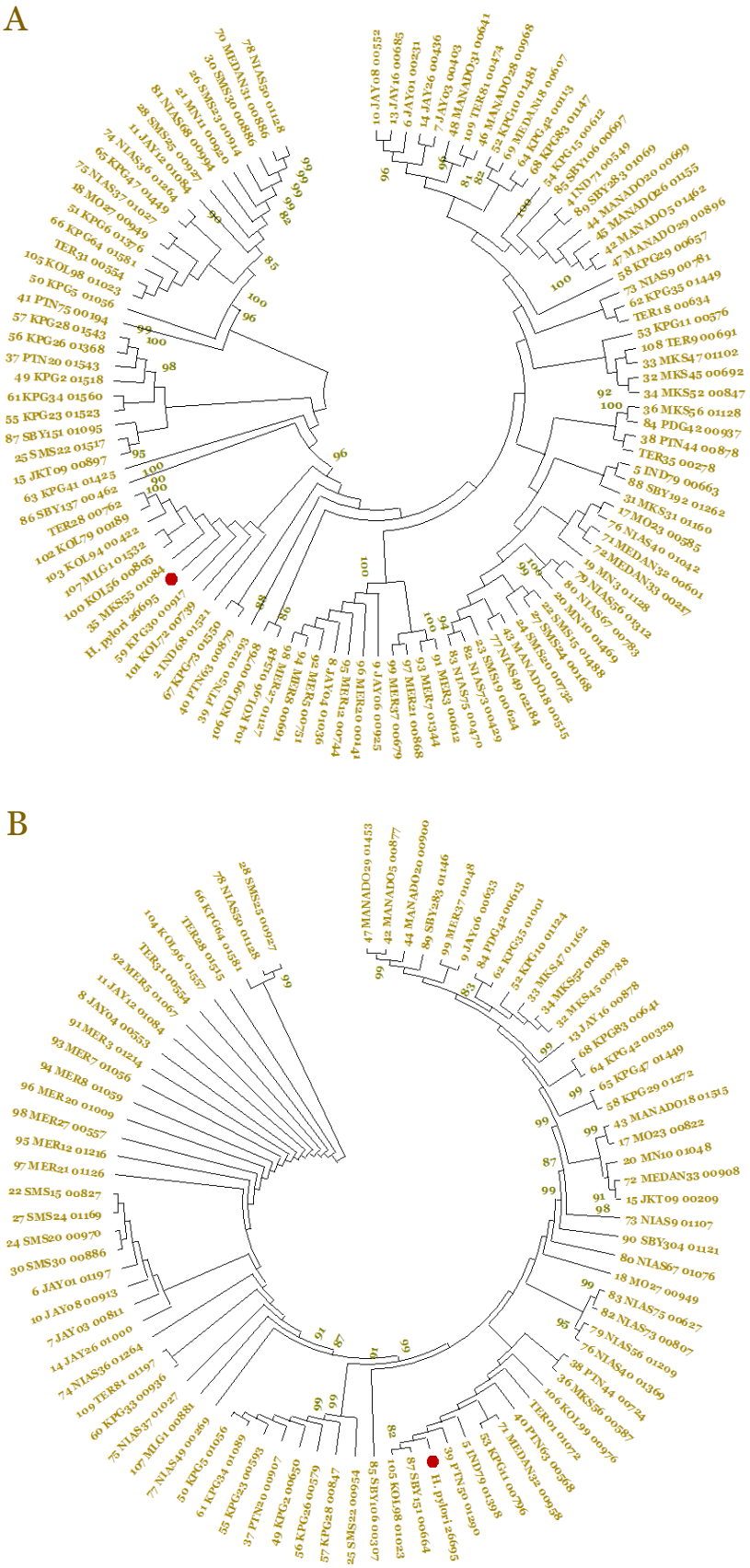

Figure 1. Phylogenetic tree analysis of the *sabA* gene (A) and *hopQ* gene (B) from isolated *Helicobacter pylori*. Phylogenetic tree analysis of the *sabA* gene and *hopQ* genes were created from 106 and 80 *H. pylori* strains, respectively. The reference strain used was *H. pylori* 26695, indicated by red circles in the figure.

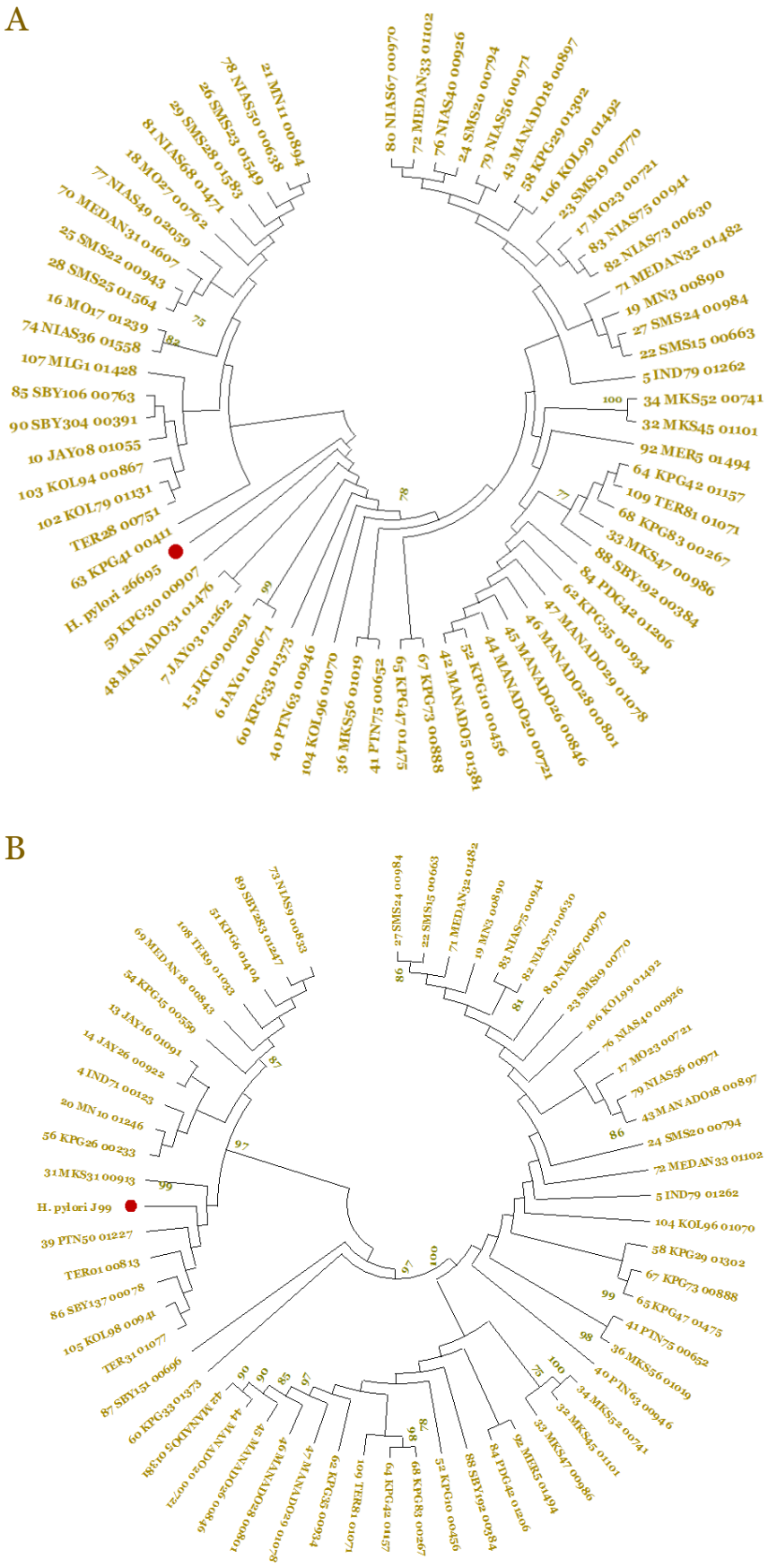

Figure 2. Phylogenetic tree analysis of the *homA* gene (A) and *homB* gene (B) from isolated *Helicobacter pylori*. Phylogenetic tree analysis of the *homA* gene and *homB* genes were created from 64 and 58 *H. pylori* strains, respectively. The reference strains are indicated by red circles in the figures; *H. pylori* 26695 for *homA* and *H. pylori* J99 for *homB*.

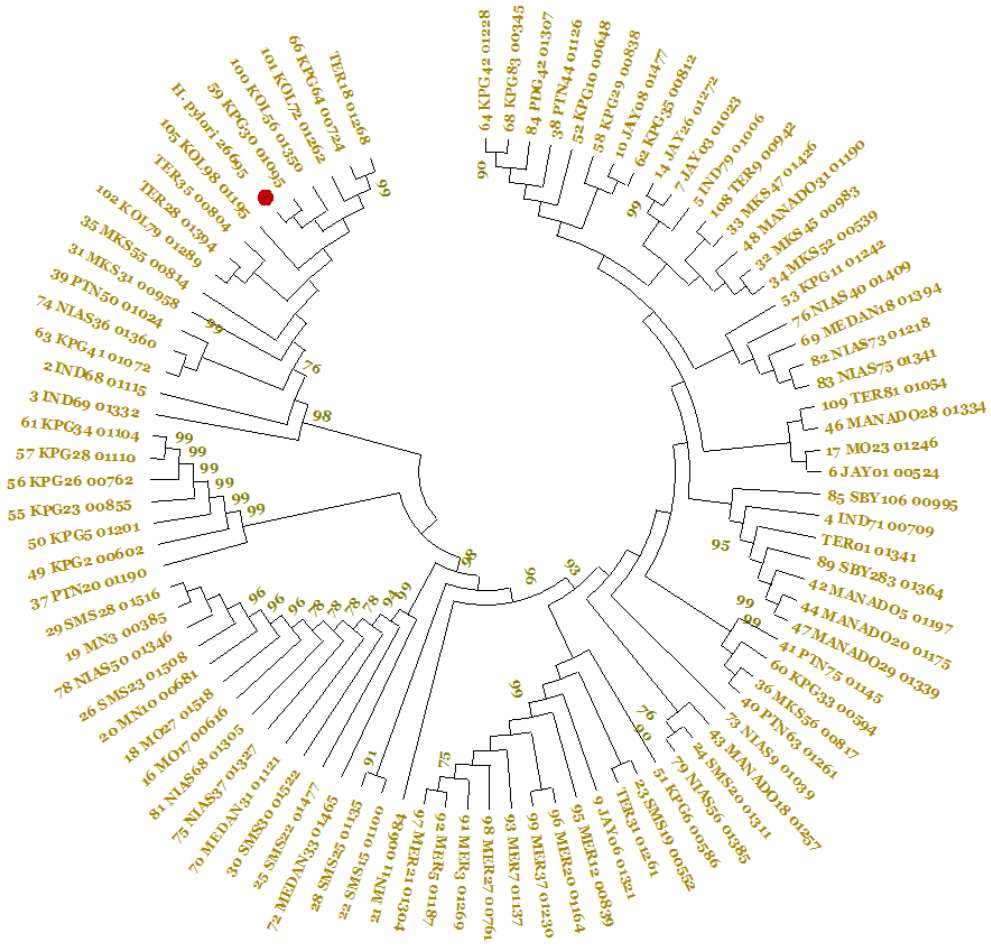

Figure 3. Phylogenetic tree analysis of the *homC* gene in 91 isolated *Helicobacter pylori* strains. The reference strain used was *H. pylori* 26695, indicated by red circles in the figure.

Association of *sabA* genotypes and gastric mucosal histology scores

In this study, 63 out of 106 *H. pylori* strains were identified as having the *sabA* "off" genotype, while 43 strains were identified as *sabA* "on" (**Table 2**). The *sabA* gene encodes the OMP, SabA, which is a key virulence factor involved in *H. pylori* pathogenesis. The "on" status, indicative of a functional SabA protein, was predominantly associated with the leucine (CL) (n=20) and leucine (RL) (n=10) amino acid sequences. In contrast, the "off" status, often resulting from a stop codon, was most commonly found in strains exhibiting the Y* sequence (n=58).

Table 2. Frequency of *sabA* gene in *Helicobacter pylori* strains

Nucleotide sequence	Amino acid sequence*	*sabA* status	Disease (based on endoscopy results)					Frequency (n)
			GU	GERD	GC	Duodenitis	Gastritis	
TACTGA	Y**	Off	6	2	1	1	48	58
TGCTTA	CL	On	4	1	0	0	15	20
CGCTTA	RL	On	1	1	0	0	8	10
TGCGTA	CV	On	0	0	0	0	6	6
TATTGA	Y**	Off	0	0	0	0	4	4
TTCTGA	F*	Off	0	0	0	0	1	1
TTGCGT	LR	On	0	0	0	0	1	1
CTTACT	LT	On	0	1	0	0	4	5
CCTACT	PT	On	0	0	0	0	1	1

GC: gastric ulcer; GERD: gastroesophageal reflux disease; GU: gastric ulcer
* Y: tyrosine; CL: leucine; RL: leucine; CV: valine; F: phenylalanine; LR: arginine; LT: threonine; PT: threonine
** Indicates a stop codon

The most prevalent nucleotide sequence identified was TACTGA, which results in a premature stop codon (Y*) and an "off" *sabA* status. This sequence was present in 58 *H. pylori* strains, with the majority (48 strains) associated with gastritis. The second most frequent sequence, TGCTTA (leucine, was observed in 20 strains, predominantly linked to gastritis (15 strains). Other nucleotide sequences were less frequent, with each identified in fewer than 10 strains. Endoscopic findings revealed that gastritis was the most common condition observed across all nucleotide sequences, followed by gastric ulcer and GERD, with gastric cancer and duodenitis being less frequent. Some sequences, including TGCGTA, TATTGA, and TTCTGA, were exclusively associated with gastritis (**Table 2**).

The relationship between the *H. pylori sabA* on/off genotype and histopathological features was further evaluated in both the antral and corporal gastric regions (**Table 3**), using samples with complete histopathological data (*sabA* on: n=37, *sabA* off: n=56). In the antrum, *sabA* "on" was significantly associated with higher *H. pylori* density (p=0.027). However, no significant associations were found between the *sabA* status and other antral histological parameters, such as acute and chronic inflammation, glandular atrophy, or metaplasia. Similarly, no significant associations were observed in the corpus between the *sabA* genotype and any histological parameters, including *H. pylori* density (**Table 3**).

Association of the *hopQ* genotypes and gastric mucosal histology scores

Among the 71 strains with complete histopathological data, 38 were categorized as *hopQ* allele type I and 22 as allele type II [45]. The evaluation of the association between *H. pylori hopQ* allele types (I/II) and histological parameters in both the antral and corporal regions of the stomach revealed no significant relationships. In the antrum, no significant associations were found between *hopQ* allele types and acute inflammation, chronic inflammation, glandular atrophy, metaplasia, or *H. pylori* density. Similarly, in the corpus, no significant associations were observed between *hopQ* allele types and acute inflammation, chronic inflammation, glandular atrophy, metaplasia, or *H. pylori* density (**Table 4**). These findings suggested that variation in *hopQ* alleles may not play a significant role in the induction of gastric inflammation, precancerous lesions, or *H. pylori* colonization density.

Association of the *homA* and *homB* genotypes and gastric mucosal histology scores

Genetic analysis of 64 *H. pylori* strains in the present study revealed limited diversity in *homA* gene. Only one allele variant was identified, with 23 strains had *homA* type II allele. The remaining 41 strains could not be classified due to deletions in the defining region of the gene. Analysis of the *homB* gene in 58 strains identified three allele types: type I (11 strains), type II (5 strains), and type VI (1 strain). The remaining 41 strains lacked classifiable *homB* alleles due to deletions in the defining region, resulting in the absence of the analysis.

Association of the *homC* genotypes and gastric mucosal histology scores

Of the 80 *H. pylori* strains with complete histopathological data, 64 were categorized as *homC* variant S, and 16 as variant L. The analysis of the association between *H. pylori homC* genotypes ($homC^S$/$homC^L$) and histological parameters in both the antrum and corpus revealed a region-specific effect (**Table 5**). In the antrum, the $homC^L$ genotype was significantly associated with an increased risk of chronic inflammation (p=0.046) and glandular atrophy (p=0.035) (**Table 5**). However, no significant associations were observed between *homC* genotypes and acute inflammation, metaplasia, or *H. pylori* density in the antrum. In contrast, no significant associations were found between *homC* genotypes and any histological parameters in the corpus, including acute inflammation, chronic inflammation, glandular atrophy, metaplasia, or *H. pylori* density (**Table 5**).

Table 3. Association of *Helicobacter pylori sabA* genotypes with antral and corporal histological parameters

Genotype profile (number of strains)	Acute inflammation grade (n)		OR (95%CI)	*p*-value	Chronic inflammation grade (n)		OR (95%CI)	*p*-value	Atrophy grade (n)		OR (95%CI)	*p*-value	Metaplasia grade (n)		OR (95%CI)	*p*-value	*H. pylori* density grade (n)		OR (95%CI)	*p*-value
Antral																				
	2/3 (38)	0/1 (55)	1.41 (0.61—3.29)	0.417[a]	2/3 (61)	0/1 (32)	0.77 (0.32—1.85)	0.572[a]	2/3 (35)	0/1 (58)	1.22 (0.52—2.88)	0.638[a]	2/3 (2)	0/1 (91)	1.52 (0.09—25.21)	1.000[b]	2/3 (38)	0/1 (55)	2.70 (1.10—6.60)	0.027[a] *
sabA on (37)	17	20			23	14			15	22			1	36			10	27		
sabA off (56)	21	35			48	18			20	36			1	55			28	28		
Corporal																				
	2/3 (8)	0/1 (85)	1.57 (0.36—6.73)	0.709[b]	2/3 (20)	0/1 (73)	1.01 (0.36—2.77)	0.982[a]	2/3 (5)	0/1 (88)	2.38 (0.37—15.00)	0.383[b]	2/3 (1)	0/1 (92)	0.00	0.398[b]	2/3 (23)	0/1 (70)	0.96 (0.36—2.52)	0.941[a]
sabA on (37)	4	33			8	29			3	34			1	36			9	28		
sabA off (56)	4	52			12	44			2	54			0	56			14	42		

[a] Analyzed using Chi-squared test
[b] Analyzed using Fisher's exact test
* Statistically significant at $p<0.05$

Table 4. Association of *Helicobacter pylori hopQ* genotypes with antral and corporal histological parameters

Genotype profile (number of strains)	Acute inflammation grade (n)		OR (95%CI)	*p*-value	Chronic inflammation grade (n)		OR (95%CI)	*p*-value	Atrophy grade (n)		OR (95%CI)	*p*-value	Metaplasia grade (n)		OR (95%CI)	*p*-value	*H. pylori* density grade (n)		OR (95%CI)	*p*-value
Antral																				
	2/3 (25)	0/1 (35)	1.05 (0.36—3.05)	0.928[a]	2/3 (35)	0/1 (25)	0.95 (0.32—2.76)	0.928[a]	2/3 (22)	0/1 (38)	1.39 (0.46—4.23)	0.553[a]	2/3 (1)	0/1 (59)	0.00	0.367[b]	2/3 (27)	0/1 (33)	0.97 (0.33—2.79)	0.957[a]
hopQ allele type I (38)	16	22			22	16			15	23			0	38			17	21		
hopQ allele type II (22)	9	13			13	9			7	15			1	21			10	12		
Corporal																				
	2/3 (5)	0/1 (55)	2.47 (0.25—23.62)	0.643[b]	2/3 (12)	0/1 (48)	0.50 (0.12—2.12)	0.507[b]	2/3 (3)	0/1 (57)	0.85 (0.07—10.03)	1.000[b]	2/3 (1)	0/1 (59)	0.00	1.000[b]	2/3 (15)	0/1 (45)	0.82 (0.24—2.82)	0.757[a]
hopQ allele type I (38)	4	34			9	29			2	36			1	37			10	28		
hopQ allele type II (22)	1	21			3	19			1	21			0	22			5	17		

[a] Analyzed using Chi-squared test
[b] Analyzed using Fisher's exact test
* Statistically significant at $p<0.05$

Table 5. Association of *Helicobacter pylori homC* genotypes with antral and corporal histological parameters

Genotype profile (number of strains)	Acute inflammation grade (n)		OR (95%CI)	*p*-value	Chronic inflammation grade (n)		OR (95%CI)	*p*-value	Atrophy grade (n)		OR (95%CI)	*p*-value	Metaplasia grade (n)		OR (95%CI)	*p*-value	*H. pylori* density grade (n)		OR (95%CI)	*p*-value
Antral																				
	2/3 (30)	0/1 (50)	2.05 (0.59—7.06)	0.248[a]	2/3 (52)	0/1 (28)	3.04 (0.99—9.36)	0.046[a*]	2/3 (28)	0/1 (52)	4.78 (1.00—22.86)	0.035[a*]	2/3 (1)	0/1 (79)	0.00	1.000[b]	2/3 (34)	0/1 (46)	1.82 (0.56—5.85)	0.309[a]
homC^{S} (16)	26	38			45	19			26	38			1	63			29	35		
homC^{L} (64)	4	12			7	9			2	14			0	16			5	11		
Corporal																				
	2/3 (7)	0/1 (73)	0.00	0.334[b]	2/3 (15)	0/1 (65)	4.20 (0.51—34.61)	0.281[b]	2/3 (3)	0/1 (77)	0.00	1.000[b]	2/3 (1)	0/1 (79)	0.00	1.000[b]	2/3 (22)	0/1 (58)	1.17 (0.33—4.12)	1.000[b]
homC^{S} (16)	7	57			14	50			3	61			1	63			18	46		
homC^{L} (64)	0	16			1	15			0	16			0	16			4	12		

[a] Analyzed using Chi-squared test
[b] Analyzed using Fisher's exact test
* Statistically significant at $p<0.05$

Discussion

This study presented novel insights into *Helicobacter pylori* virulence determinants, identifying *homC* variants—particularly *homC*^{L}—as critical mediators of gastric mucosal pathology in an Indonesian cohort. As the first investigation to systematically associate *homC* genotypes with histopathological outcomes using the Updated Sydney System, our analysis revealed *homC*^{L}'s significant association with antral-predominant chronic inflammation (OR: 3.04; p=0.046) and glandular atrophy (OR: 4.78; p=0.035). The spatial localization of tissue damage aligns with Kim et al.'s [46] structural classification distinguishing *homC*^{L} (VMAYDKDNAEFE motif) from *homC*^{S} (ANVNGS motif with 129–140 deletions) (**Figure S1**), suggesting *homC*^{L} may enhance bacterial adherence through synergism with adhesins like BabA or amplify inflammatory cascades that compromise mucosal integrity. The predominance of severe chronic inflammation in *homC*-positive biopsies supports the hypothesis that *homC* synergizes with babA to increase disease risk. Mechanistically, *homC* may enhance inflammatory responses, creating a microenvironment conducive to *H. pylori* colonization and persistence. Alternatively, *homC* could augment *babA*-mediated bacterial adherence to gastric epithelial cells, expediting colonization and tissue damage. Furthermore, *homC*^{S} association with gastric atrophy—a precursor to gastric cancer—indicates its potential involvement in carcinogenesis, suggesting its utility as a prognostic marker for high-risk individuals.

sabA genotypes were determined based on the presence or absence of a stop codon within the strain sequence (**Table 2**), consistent with prior methodologies [44,48]. Our analysis revealed a significant correlation between the *sabA* “on” state and elevated *H. pylori* density in the antrum (p<0.05), aligning with previous reports [49,50]. This supports the hypothesis that active *sabA* contributes to gastroduodenal disease progression by enhancing colonization and inflammation [51,52]. Despite the higher prevalence of the "off" *sabA* genotype in our cohort, no significant association was found between *sabA* status and any histopathological parameter. Considering the established link between the "on" state and increased virulence potential, this unexpected result underscores the multifaceted nature of *sabA* pathogenicity. It suggested that virulence is not solely dependent on its binary on/off status, but is also modulated by factors such as gene expression, receptor binding affinity, and the gastric microenvironment [22,44,50]. The association between *sabA* and *H. pylori* density may be more intricate than previously thought. *H. pylori*-induced inflammation can modify gastric epithelial cell glycosylation, potentially affecting *sabA* expression and binding affinity to sialylated Lewis x receptors [53,54]. Furthermore, genetic variations within the *sabA* gene can influence receptor binding affinity, impacting bacterial colonization and virulence [44]. While our results suggested that the *sabA* "on" state may be associated with increased bacterial density, this does not necessarily correlate with disease severity.

Other factors, such as host immune response and the presence of other virulence factors, likely modulate the effects of *sabA* on pathogenesis. Further studies are needed to elucidate the complex interplay between *sabA* status, *H. pylori* density, and disease progression. The diversity of *sabA* sequences observed in this study is consistent with previous research, highlighting the genetic variability of *H. pylori* strains. This diversity may influence adherence to the gastric mucosa, potentially affecting disease outcomes. The predominance of the *sabA* "off" status in our cohort suggests a possible role for *sabA* inactivation in pathogenesis. Further research is needed to determine the functional implications of this finding and the specific effects of *sabA* variants on clinical outcomes.

Analysis of the *hopQ* OMP amino acid sequences showed that *hopQ* allele type I is distinguished by the amino acid sequence QLSRL at positions 56-60, while *hopQ* allele type II, NLNKL in the same region (**Figure S2**). Meanwhile, 11 strains exhibited deletions in this region and were categorized as unclassified. Additionally, analysis of *hopQ* allele type II strains identified some strains with an amino acid change at position 56, wherein Q (Gln) code replaced with K (Lys). In contrast to *sabA*, the *hopQ* genotype was not significantly associated with any of the evaluated histopathological parameters, which is in stark contrast with previous studies that reported an association between *hopQ* allele type I and peptic ulcer disease and gastric cancer [55–58]. This may be attributed to the presence of unclassified *hopQ* genotypes in our study,

which may have disguised possible associations. Additional research with a larger and more comprehensively genotyped sample set is needed to clarify the role of *hopQ* in gastritis.

Of the 56 strains with complete histopathological data, 17 were categorized as *homA* allele type II, which was characterized by the amino acid sequence DDGKH at positions 338–342 (**Figure S3**). Meanwhile, no strains were identified as *homA* allele type I. The other 39 strains were unclassified due to variation and deletion in the defining regions. Regarding the *homB* gene, nine strains were identified as *homB* allele type I that was characterized by the unique motif SSTDCD at positions 261–266, four strains were categorized as *homB* allele type II, and characterized by the motif KGGGGE at the same positions, and one strain was categorized as *homB* allele type VI, which share the KGGGGE motif with type II at positions 261–266 but differed at positions 323–336 (**Figure S4**). Regarding *homB*, while we identified three allele types (I, II, and VI), their frequencies were extremely low (11, 5, and 1 isolate, respectively) (**Table 1**). Limited sample size and unclassified variants precluded robust associations. However, previous studies have suggested a potential interaction between *homA* and *homB* with other virulence factors that result in severe gastritis in children [59]. Furthermore, *homB* is associated with peptic ulcer disease and gastric cancer in specific populations [30,31,60]. Due to the geographic variability in the impact of *homB* alleles [61], additional research on diverse populations are warranted to fully grasp the contributions of these genes to gastric disease.

This study offers important insights into the function of specific *H. pylori* genes in gastric disease pathogenesis. However, this study has limitations. As we focused only on a select group of genes, results only provide an overview of the specific roles and impact of *H. pylori* genes on virulence gastric disease. Furthermore, the complex interacton between *H. pylori* and the host immune response, which is an essential factor in disease severity, warrants further research. Our study highlighted the complex role of *H. pylori* virulence factors in initiating histopathological changes in the gastric mucosa. The relationship of *sabA* to increased bacterial density, and the association of *homC* genotypes ($homC^{L}$ and $homC^{S}$) with chronic inflammation and atrophy reveal a complex and dynamic interaction among these factors.

Conclusion

Our study highlighted the complex role of *H. pylori* virulence factors in initiating histopathological changes in the gastric mucosa. The *sabA* genotype demonstrated a significant association with *H. pylori* density in the antrum, one of the parameters assessed using the Updated Sydney System. The relationship between *sabA* and *H. pylori* density observed in this study strengthens the evidence that *sabA* may contribute to the severity of *H. pylori* colonization, which in turn could influence the degree of inflammation and the progression of gastroduodenal diseases. Furthermore, this study revealed a significant association between the *homC* genotype, particularly the $homC^{L}$ and $homC^{S}$ variants, and parameters of chronic inflammation and atrophy degree in the antrum. These findings reinforced previous evidence linking $homC^{L}$ to the presence of the *babA* gene, which is known to contribute to the development of severe gastric diseases. Although histopathological findings showed a predominance of mild atrophy, the high prevalence of severe chronic inflammation in most samples indicates a risk of progression to more serious gastric conditions. Together, these results underscore the importance of *H. pylori* virulence factors, such as *sabA* and *homC*, in modulating gastric pathology and suggest potential targets for further research and therapeutic intervention.

Ethics approval

This research adhered to the ethical principles outlined in the Declaration of Helsinki, ensuring the protection and well-being of all participants. This study was approved by the the Ethical Committee of Dr. Soetomo Teaching Hospital (Surabaya, Indonesia, 221/Panke.KKE/IX/2012, 25 September 2012) and Airlangga University Faculty of Medicine (Surabaya, Indonesia; approval number: 122/EC/KEPK/FKUA/2024; date of approval: 22 July 2024).

Acknowledgments

None.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study was supported by the Directorate of Research, Technology and Community Service, Ministry of Education, Culture, Research and Technology 2024 (RH and MM). This work is also supported by the National Institutes of Health (DK62813) (YY), and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan (26640114, 221S0002, 16H06279, 15H02657 and 16H05191, 18KK0266, 19H03473, 21H00346, 22H02871, and 23K24133 (YY), the Japan Society for the Promotion of Science Institutional Program for Young Researcher Overseas Visits and the Strategic Funds for the Promotion of Science and Technology Agency (JST) for YY. RIA and KAF are Ph.D. students supported by the Japanese Government (MEXT) scholarship program for 2017, and 2019, respectively. This work is also supported by the Japan Agency for Medical Research and Development (AMED) [e-ASIA JRP] (YY).

Underlying data

As detailed in our previous study (https://www.nature.com/articles/s41586-024-07991-z#MOESM3), the whole genome sequencing data have been deposited in GenBank under accession number PRJDB17566. These data are also accessible via the Enterobase worksheet (https://enterobase.warwick.ac.uk/a/108555). All supplementary figures are available at https://figshare.com/s/99637276db9b9ca0a3ec.

Declaration of artificial intelligence use

This study employed artificial intelligence (AI) tools and methodologies for manuscript writing support, specifically using Gemini Advanced for language refinement. This included improving grammar, sentence structure, and overall readability. We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

How to cite

Hunowu R, Fauzia KA, Alfaray RI, *et al. Helicobacter pylori sabA, hopQ and hom* genotypes as potential genetic biomarkers for gastric mucosal inflammation. Narra J 2025; 5 (2): e1917 - http://doi.org/10.52225/narra.v5i2.1917.

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