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FREQUENCY OF HELICOBACTER PYLORI VIRULENCE GENES IN PATIENTS WITH RHEUMATOID ARTHRITIS AND NSAID-ASSOCIATED GASTROPATHY

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ABSTRACT
This study examined the frequency of Helicobacter pylori (Hp) virulence gene occurrence and their combinations in patients with rheumatoid arthritis (RA) receiving nonsteroidal anti-inflammatory drugs (NSAIDs). Sixty-nine patients with RA and gastrointestinal complaints were examined and divided into two groups: 45 patients (66%) with gastropathy and 24 patients (34%) without gastropathy. Hp genotypes were characterized in gastric biopsy material using polymerase chain reaction. The cagA, iceA2, and vacAs2 genotypes were significantly more frequent in patients with gastropathy than in those without (cagA: 42.2% vs 16.7%, OR=3.6, P=0.029; iceA2: 35.6% vs 20.8%, OR=2.1, P=0.2; vacAs2: 46.7% vs 16.7%, OR=4.4, P=0.011). The combination of cagA, iceA2, vacAm1, and vacAs2 genotypes occurred in 31.1% of patients with gastropathy versus 4.2% of patients without gastropathy, corresponding to a 10.4-fold increase in gastropathy risk (OR=10.4, 95% CI 1.273-84.75, P=0.011). These findings indicate that specific Hp genotype combinations, rather than individual virulence genes alone, are associated with a substantially elevated risk of NSAID-associated gastropathy in patients with RA, supporting the potential clinical value of combined genotyping for risk stratification prior to long-term NSAID therapy.

KEYWORDS: Helicobacter pylori; Genotype; Rheumatoid arthritis; Gastropathy; NSAID; cagA

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by symmetrical erosive arthritis (synovitis) and a range of extra-articular manifestations. Beyond its direct clinical burden, RA represents a substantial public health and economic problem: according to Global Burden of Disease estimates, RA prevalence has increased by approximately 14% since 1990, and the disease remains a major contributor to disability-adjusted life years and premature mortality worldwide, with a disproportionate burden among women (Safiri et al., 2023).

Helicobacter pylori (Hp) is a gram-negative, microaerophilic, spiral-flagellated bacterium with urease activity that colonizes the gastric mucosa and is causally linked to chronic gastritis, peptic ulcer disease, and gastric malignancy (Wotherspoon et al., 1991). Global prevalence of Hp infection varies markedly with the level of socioeconomic development, with pooled estimates indicating substantially lower prevalence in high-income countries compared with developing regions of Africa, Latin America, and Asia (Hooi et al., 2017).

Since its initial description in 1984, intensive research on Hp has revealed a highly diverse genome that serves as a valuable tool for studying bacterial evolution and disease pathogenesis, and for identifying virulence factors associated with more severe clinical outcomes. Hp pathogenicity is geographically heterogeneous: specific genotypes correlate with more severe clinical disease in some populations while appearing comparatively harmless in others, with well-documented contrasts between East Asian and Western strains attributable to differences in the cagA and vacA virulence loci, host genetic background, and environmental co-factors (Namikawa et al., 2025). The bacterial chromosome encodes a cluster of urease genes, several cytotoxins, and the cag pathogenicity island; key virulence determinants include the vacuolating cytotoxin (VacA), which induces host epithelial cell apoptosis, and the cytotoxin-associated antigen (CagA), which is translocated into host cells via a type IV secretion system encoded by the cag pathogenicity island and interferes with host cell signaling.

In patients with RA, the relationship between Hp infection and gastrointestinal injury is further complicated by concomitant NSAID use. Direct clinical studies in RA patients receiving long-term NSAID therapy have reported a significantly higher prevalence of endoscopically confirmed peptic ulceration compared with non-RA dyspeptic controls, implicating an interaction between NSAID-induced mucosal injury and Hp colonization in the pathogenesis of gastropathy in this population (Voutilainen et al., 1998; Stack et al., 2002). However, the specific contribution of individual Hp virulence genotypes, as opposed to Hp infection status alone, to this risk has not been well characterized in RA populations, motivating the molecular genetic analysis undertaken in the present study.

Objective
The purpose of this study was to characterize the genotypes of Hp in patients with RA using molecular genetic analysis, and to determine the association between individual Hp virulence genes and their combinations with the presence of NSAID-associated gastropathy.

MATERIAL AND METHODS
The study examined 69 patients with RA who presented with gastrointestinal complaints and were receiving complex treatment (glucocorticoids, anti-inflammatory agents, and disease-modifying antirheumatic drugs). Patients were divided into two groups: Group 1 comprised 45 patients (66%) with endoscopically confirmed gastropathy, and Group 2 comprised 24 patients (34%) without gastropathy. All patients underwent clinical, laboratory-instrumental, molecular-genetic, chromatographic, and statistical evaluation.
Molecular genetic analysis characterized the frequency of Hp gene occurrence (cagA, iceA1, iceA2, vacAm1, vacAs2) in gastric biopsy material by polymerase chain reaction in patients with and without gastropathy, together with the frequency of specific gastroduodenal pathology according to Hp genotype. Statistical analysis included calculation of odds ratios (OR) with 95% confidence intervals and chi-square ($\chi 2$) testing; $P<0.05$ was considered statistically significant.

RESULTS
Analysis of Hp gene frequency among RA patients with and without gastropathy showed that the vacAs2, cagA, and iceA2 genotypes were significantly or near-significantly more common in patients with gastropathy. The vacAs2 genotype was present in 21 of 45 patients with gastropathy (46.7%) versus 4 of 24 patients without gastropathy (16.7%) ($\chi 2=6.1$, P=0.011, OR=4.4, 95% CI 1.288-14.86). The cagA genotype was present in 19 of 45 patients with gastropathy (42.2%) versus 4 of 24 without gastropathy (16.7%) ($\chi 2=4.5$, P=0.029, OR=3.6, 95% CI 1.073-12.45). The iceA2 genotype was present in 16 of 45 patients with gastropathy (35.6%) versus 5 of 24 without gastropathy (20.8%) ($\chi 2=1.6$, P=0.2, OR=2.1, 95% CI 0.658-6.68). The iceA1 and vacAm1 genotypes did not differ significantly between groups (Table 1).

Table 1 Frequency of individual Hp genotypes in RA patients with and without gastropathy

Gene	Gastropathy (n=45), n (%)	No gastropathy (n=24), n (%)	$\chi 2$	P	OR (95% CI)
cagA	19 (42.2)	4 (16.7)	4.5	0.029	3.6 (1.073-12.45)
iceA1	9 (20.0)	3 (12.5)	0.6	0.4	1.7 (0.425-7.19)
iceA2	16 (35.6)	5 (20.8)	1.6	0.2	2.1 (0.658-6.68)
vacAm1	17 (37.8)	7 (29.2)	0.5	0.5	1.4 (0.50-4.28)
vacAs2	21 (46.7)	4 (16.7)	6.1	0.011	4.4 (1.288-14.86)

Analysis of genotype combinations showed that the vacAm1+vacAs2 combination occurred in 19 patients with gastropathy (42.2%) versus 4 without gastropathy (16.7%) ($\chi 2=4.6$, P=0.031, OR=3.6, 95% CI 1.073-12.45). The four-gene combination cagA+iceA2+vacAm1+vacAs2 occurred in 14 patients with gastropathy (31.1%) versus 1 patient without gastropathy (4.2%) ($\chi 2=6.7$, P=0.011, OR=10.4, 95% CI 1.273-84.75), representing the strongest association identified in this analysis. The combination iceA2+vacAm1+vacAs2 occurred in 11 patients with gastropathy (24.4%) versus 2 without gastropathy (8.3%) ($\chi 2=2.7$, P=0.1, OR=3.6, 95% CI 0.7191-17.61), and the combination iceA1+vacAm1+vacAs2 occurred in 8 patients with gastropathy (17.8%) versus 2 without gastropathy (8.3%) ($\chi 2=1.1$, P=0.3, OR=2.4, 95% CI 0.462-12.22). The combination cagA+iceA1 was not significantly different between groups (24.4% vs 20.8%), nor was the full five-gene combination cagA+iceA1+iceA2+vacAm1+vacAs2 ($\chi 2=0.2$, P=0.6) (Table 2).

Table 2 Frequency of Hp genotype combinations in RA patients with and without gastropathy

Genotype combination	Gastropathy (n=45), n (%)	No gastropathy (n=24), n (%)	$\chi 2$	P	OR (95% CI)
cagA+iceA2+vacAm1+vacAs2	14 (31.1)	1 (4.2)	6.7	0.011	10.4 (1.273-84.75)
vacAm1+vacAs2	19 (42.2)	4 (16.7)	4.6	0.031	3.6 (1.073-12.45)
iceA2+vacAm1+vacAs2	11 (24.4)	2 (8.3)	2.7	0.1	3.6 (0.7191-17.61)
iceA1+vacAm1+vacAs2	8 (17.8)	2 (8.3)	1.1	0.3	2.4 (0.462-12.22)
cagA+iceA1	11 (24.4)	5 (20.8)	0.1	0.7	1.2 (0.371-4.069)

cagA+iceA1+iceA2+vacAm1+ vacAs2	3 (6.7)	0 (0)	0.2	0.6	—

DISCUSSION

The principal finding of this study is that the risk of NSAID-associated gastropathy in patients with RA is most strongly predicted not by any single Hp virulence gene but by a specific four-gene combination, cagA+iceA2+vacAm1+vacAs2, which was associated with a more than tenfold increase in gastropathy risk, substantially exceeding the risk conferred by any individual genotype examined (maximum OR=4.4 for vacAs2 alone). This pattern is consistent with the broader understanding that Hp virulence is combinatorial and geographically heterogeneous, with specific genotype constellations, rather than isolated genes, driving clinically relevant differences in disease outcome across populations (Namikawa et al., 2025).

These findings extend earlier clinical work demonstrating that RA patients receiving long-term NSAID therapy face a significantly elevated risk of endoscopically confirmed gastric mucosal injury compared with non-RA dyspeptic controls (Voutilainen et al., 1998; Stack et al., 2002), by identifying a specific molecular genetic basis, namely concurrent carriage of cagA, iceA2, vacAm1, and vacAs2, that may help explain why this risk is not uniformly distributed across all Hp-infected RA patients receiving NSAIDs. From a mechanistic standpoint, this is biologically plausible: cagA-positive strains translocate the CagA oncoprotein into host epithelial cells via the type IV secretion system with downstream effects on cell signaling, while vacAs2 and iceA2 are established markers of more virulent Hp subtypes associated with more pronounced mucosal inflammation, and concurrent carriage of multiple virulence determinants may produce additive or synergistic epithelial injury that compounds the direct mucosal toxicity of NSAIDs themselves.

From a clinical standpoint, these findings suggest that molecular genotyping of Hp, rather than simple infection status, could in principle be used to stratify RA patients by gastropathy risk before initiating long-term NSAID therapy, allowing more selective use of prophylactic gastroprotection (for example, proton pump inhibitor co-therapy) in genotypically high-risk patients rather than uniform prophylaxis across the whole RA population. This approach would be consistent with international guidance recommending Hp testing before long-term NSAID use in at-risk patients, while adding a genotype-based refinement not currently incorporated into standard risk-stratification algorithms.

This study has several limitations. The sample size, while sufficient to detect associations for the strongest genotype combinations, resulted in wide confidence intervals for several estimates, particularly the four-gene combination (95% CI 1.273-84.75), reflecting the relatively small number of patients carrying this specific combination; larger, multicenter cohorts are needed to confirm these findings with greater precision. Additionally, this cross-sectional analysis cannot establish whether genotype-associated gastropathy risk is modified by specific NSAID agent, dose, or duration of use, an important direction for future prospective study.

CONCLUSION

In patients with RA receiving NSAID therapy, individual Hp genotypes vacAs2, cagA, and iceA2 were each associated with an increased risk of gastropathy, but the combination of cagA, iceA2, vacAm1, and vacAs2 conferred the greatest risk, increasing the odds of gastropathy more than tenfold. Thorough characterization of Hp virulence gene combinations may allow identification of RA patients at highest risk of NSAID-associated gastropathy, informing individualized gastroprotective strategies and monitoring protocols for this population.

ACKNOWLEDGMENTS

The authors received no specific funding for this study and declare no conflicts of interest.

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