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Genotypic and phenotypic variation of Lewis antigen expression in geographically diverse *Helicobacter pylori* isolates

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Abstract

Background—*Helicobacter pylori* is a persistent colonizer of the human gastric mucosa, which can lead to the development peptic ulcer disease and gastric adenocarcinomas. However, *H. pylori* can asymptomatically colonize a host for years. One factor that has been hypothesized to contribute to such persistence is the production of Lewis (Le) antigens in the lipopolysaccharide layer of the bacterial outer membrane as a form of molecular mimicry, since humans also express these antigens on their gastric mucosa. Humans and *H. pylori* both are polymorphic for Le expression, which is driven in *H. pylori* by variation at the Le synthesis loci. In this report we sought to characterize Le genotypic and phenotypic variation in geographically diverse *H. pylori* isolates.

Materials and Methods—From patients undergoing endoscopy in 29 countries, we determined Le phenotypes of 78 *H. pylori* strains, and performed genotyping of the *galT* and *β-(1,3)galT* loci in 113 *H. pylori* strains.

Results—Le antigen phenotyping revealed a significant (p <0.0001) association between type 1 (Le^a and Le^b) expression and strains of East-Asian origin. Genotyping revealed a significant correlation between strain origin and the size of the promoter region upstream of the Le synthesis gene, *galT* (p <0.0001).

Conclusion—These results indicate that the heterogeneity of human Le phenotypes are reflected in their *H. pylori* colonizing strains, and suggest new loci that can be studied to assess variation of Le expression.

Introduction

Helicobacter pylori are Gram negative, microaerophilic bacteria that colonize the human stomach. This persistent colonization has been linked to gastric ulcers, gastric adenocarcinoma (1), and mucosa-associated lymphoid tissue (MALT) lymphoma (2). As a result of these serious consequences, currently most treatment protocols for peptic ulcer disease include eradication of *H. pylori* as part of their regimens (3). However, colonization with *H. pylori* can go undetected for decades, and may have some early-in-life benefits (4, 5); how *H. pylori* is able to persist within the host for such long periods is not clearly understood.

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Lewis (Le) antigens are cell-surface fucosylated oligosaccharides that are expressed in both humans (6) and *H. pylori* (7–10). It has been hypothesized that *H. pylori* presents these antigens within its lipopolysaccharide (LPS) layer as a form of molecular mimicry, perhaps aiding in niche adaptation and evasion of host immune responses (11–17). Type 2 antigens (Le^x and Le^y) are most commonly expressed (~85 % of strains, (18, 19)), while type 1 antigens (Le^a and Le^b) are expressed in less than 5% of collections of *H. pylori* strains studied (18, 19). Both observational (16) and experimental (15, 20) studies have demonstrated a relationship between host and bacterial Le phenotype, suggesting that the host Le phenotype selects for bacterial Le phenotype. Furthermore, Le expression in *H. pylori* appears to correlate with the geographic origin of its human host; North American and European strains predominantly express type 2 Le antigens only, while type 1 Le antigen expression, along with simultaneous type 2 Le antigen expression, appears to be more prevalent in Asian and the limited numbers of South American strains studied (7–9, 18, 21–23).

Lewis antigens are synthesized from a common precursor, N–acetylglucosamine, which is galactosylated in the type 1 or 2 synthesis pathways by β-(1,3)galT or GalT, respectively (15, 24–26). These precursor disaccharides then can be mono-fucosylated to form the trisaccharides Le^x and Le^a (27–31), or difucosylated to form the tetrasaccharides Le^y or Le^b (28, 32).

Recently it has been reported that the *β-(1,3)galT* upstream homolog, *jhp0562*, is a potential marker for peptic ulcer disease (PUD) in children (33, 34), and its presence has been associated with the presence of *H. pylori* proteins (e.g. CagA) associated with high intensity host interactions (34). While only present in some strains of *H. pylori*, in those strains that possess a copy of *jhp0562*, mutagenesis has shown that this gene is essential for synthesis of all Le antigens (35). However, strains that naturally lack *jhp0562* also have the ability to produce type 2 antigens (34, 36, 37). In this study, we aimed to further elucidate the variation at the *β-(1,3)galT* and *galT* loci amongst *H. pylori* strains isolated from different human populations, especially in relation to Lewis antigen expression, and to examine the relationship between *H. pylori* Le antigen polymorphisms and the geographic origin of the host.

Methods

Patient population

The *H. pylori*-positive population consisted of patients undergoing upper gastrointestinal (UGI) endoscopy as part of routine treatment at the New York Harbor (Manhattan) VA Medical Center, Bellevue Hospital Center, and New York Downtown Hospital as well as patient samples isolated in other parts of the world, including Latin American countries and Europe, collected between 1984 and 2003 (Table S1). *H. pylori*-positive patients were defined on the basis of positive *H. pylori* cultures obtained from gastric biopsies. Patients were separated into two groups, " East Asian" and "Non-East Asian", with the former defined as having an East-Asian ethnicity, and which for the purposes of this study included patients originating in China, Taiwan, Japan, Hong Kong, Burma, Malaysia, and Thailand. The "Non-East Asian" group included all other patients (n = 116 subjects).

Bacterial strains and growth conditions

H. pylori strains were isolated from biopsy samples by plating on Skirrow's medium (BBL Microbiology Systems, Cockeysville, MD), and grown at 37°C under microaerobic conditions. Frozen stocks were maintained at −80°C in Brucella broth (BB) with 15% glycerol. Strains were routinely grown on 5% sheep's blood agar (BBL Microbiology

Systems, Cockeysville, MD) at 37°C and 5% CO_2, or in culture jars under microaerobic conditions.

Determination of Lewis antigen phenotypes

H. pylori Le antigen phenotypes were determined by ELISA using monoclonal antibodies to Le^a, Le^b, Le^x, or Le^y (Signet Laboratories, Inc., Dedham, MA) by methods described (19). Optical densities (OD) at 410 nm were determined on a microplate reader (MRX; Dynatech Laboratories, Inc., Chantilly, VA). Two previously defined strains, JP26, a wild-type Le^b-positive strain isolated in Japan, and 99-8, a Le^a-positive strain from our collection were included as controls (15). Corrected OD values were determined by obtaining the mean of the OD values from three wells per sample and subtracting the blank (*Escherichia coli* strain HB101). Lewis antigen expression was considered to be positive if the OD values were greater than 0.10. A subset (n=78) of the strains analyzed in this study were Le antigen phenotyped, based on our ability to recover a sufficient number of viable cells from frozen stock for ELISA.

PCR analysis of the *β-(1,3)galT* locus and *galT* promoter region

H. pylori strains were harvested from a single plate in 1.0 ml sterile phosphate buffered saline (PBS, pH 7.4), cells pelleted, and genomic DNA was extracted with the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Purified DNA was used as template to screen for the presence of *jhp0562* using primers Jhp0561(+419)F and Jhp0564(−10)R (15), flanking the *jhp0562-β-(1,3)galT* locus, and primers Jhp0562(+211)F (15) and jhp0562 (+577)R CATGCGTTGAGTAATAGCTTTTTTG, specific for *jhp0562*. To determine the relative size of the *galT* upstream region, primers 2(1,4)galT(−391)F and Gal(1,4)R(+50) were used (15).

Statistical analysis

Either Chi-squared analysis or Fisher's exact test was used as appropriate based on cell size, with $p < 0.05$ considered significant.

Results

Prevalence of *jhp0562* in *H. pylori* isolates of East Asian and Non-East Asian origin

To determine the prevalence of the presence of *jhp0562* at the *β-(1,3)galT* locus, PCR analysis of 111 *H. pylori* isolates was performed using primers flanking the region as well as with primers specific for *jhp0562* (Figure 1). Isolates harboring both genes yield a band of ~2.6kb, while isolates lacking *jhp0562* yield a band of ~1.5kb (Figure 1A). In some isolates producing the larger 2.6kb band, a faint band 1.5kb band is also observed (Figure 1A), which can be attributed to intragenomic recombination between the two homologous alleles (35). The results in Figure 1A were confirmed by a *jhp0562*-specific PCR (Figure 1B). The *jhp0562* allele was detected in 56/68 (82.4%) of Non-Asian strains, while 41/43 (95.3%) of East-Asian strains were *jhp0562* positive (Table 1). These results show that the majority of *H. pylori* strains tested harbor *jhp0562*, but *jhp0562* status trended toward significance between the two groups ($p = 0.075$, Table 1).

Prevalence of type 1 Le antigen between East Asian and Non-East Asian-derived *H. pylori* populations

Le antigen phenotyping by ELISA was performed on 78 strains: 39 non-Asian and 39 East Asian strains. No strains of non-East Asian origin were positive for type 1 Le antigens, while 13 East Asian strains expressed type 1 Le antigens, a difference that was significant ($p < 0.0001$, Table 1).

Correlation of type 1 Le antigen expression with *jhp0562* status

All 11 *H. pylori* strains that expressed type 1 Le antigens that were screened for the presence of *jhp0562* carried the gene. However, there was no correlation between the presence of *jhp0562* and type 1 expression (p=0.58).

Association between ethinic origin of *H. pylori* strains and *galT* promoter region

Although the experimentally defined transcripton start site for *galT* begins 31–33 nucleotides before the *galT* translational start site [(38), Figure 2], the promoter region of *galT* varies in size amongst *H. pylori* strains [e.g. 26695 and J99 (37, 39), Figure 2]. Using a forward primer 391nucleotides upstream of the *galT* start codon and a reverse primer annealing 50 nucleotides downstream of the start codon produces alleles of three different sizes, termed “small”, “medium” and “large” among isolates characterized in this study (Figure 3). A PCR screen with these primers was performed on 115 *H. pylori* strains. All strains produced a single band, with the exception of two strains, 02-363 and 03-151, which produced two bands, one small and one large, suggesting that these strains represent mixed populations (Figure 3). Chi-squared analysis revealed that strains of Non-East Asian origin most commonly possess a “large” *galT* promoter region, while Asian strains were significantly ($p < 0.0001$) associated with “small” *galT* promoter regions (Table 1).

Discussion

H. pylori strains are varied in their expression of Le antigens (7–10, 13, 15, 16, 19–22, 24, 28, 40–45), and multiple genetic mechanisms to create such variation have been described (15, 24, 32, 40, 41, 44, 46–50). The results of the genotypic and phenotypic screens in this study demonstrate the high levels of variation observed at the sites that are critical to Le antigen synthesis and identify new loci that are relevant to variation. Our results showed that *H. pylori* strains of East-Asian origin are more likely to express type 1 Le antigens on their LPS than strains of North American or European origin, confirming and extending prior studies (21, 22). In addition, it has been shown that *H. pylori* strains of South American origin express type 1 Le antigens more often than Western strains (23), suggesting that there are global distribution patterns of *H. pylori* Le antigen phenotypes. These patterns could reflect the prevalence of particular Le expression profiles on the gastric epithelia of different human populations (51), which would further support the hypothesis that *H. pylori* strains in which Le expression matches that of their host are selected (15, 16, 20). However, to our knowledge no studies comparing human and bacterial Le expression in geographically diverse isolates have been performed.

Our results showed no correlation between type 1 Le expression and the presence of *jhp0562*. However, previous work in our lab has shown that in strains in which it is present, *jhp0562* is essential for production of all Le antigens (35). However, from prior studies (34), and our current work, a substantial (37.6% and 9.7%, respectively) proportion of clinical isolates lack *jhp0562* but express type 2 Le antigens. Thus, there appears to be a fundamental difference between strains carrying *jhp0562* in which it is essential for Le antigen synthesis, and those without it, which can at least produce type 2 antigens. The mechanisms that underlie this difference are not known, although it is possible that another Le antigen synthesis gene is compensating for the lack of *jhp0562* in strains that do not have a copy of this gene, thus allowing for type 2 Le antigen production in these strains. The presence of *jhp0562* is correlated with PUD in children (33, 34), as well as other *H. pylori* virulence genes, including *vacA* s1, *babA*, *homB*, *oipA*, *hopQ* I, and those on the *cag* PAI, (34). Thus, although *jhp0562* is not an essential gene, its association with PUD and host-associated genes suggests a role in host interaction, perhaps by aiding in colonization and niche adaptation via its role in Le antigen synthesis.

PCR analysis of the *galT* promoter region revealed major alleles of three different sizes, “small”, “medium” and “large”, with East-Asian strains strongly associated with the small allele and Non-East Asian (Western) strains predominantly harboring the large allele. However, the predicted transcriptional start site (38) is conserved amongst the alleles (Figure 2). Thus, further studies are necessary to determine whether size variation of the promoter region affects transcriptional activity of *galT*. Additionally, two strains showed multiple bands, indicating a mixed population of cells within the isolate, suggesting that the patient was colonized with multiple *H. pylori* strains or a single strain with clonal variants, phenomena that have been observed previously (43, 52–55). Alternatively, these multiple bands could be the result of intragenomic or intergenomic recombination, commonly observed in *H. pylori* at various loci (35, 56–58), including between Le synthesis genes (44). Harboring alleles of different sizes within a particular population could be advantageous by allowing for variable expression of Le antigens, thus aiding in adaptation to different microniches within the host (43, 55, 56, 59).

Our results have shown that distinct *H. pylori* genotypes and phenotypes related to Le antigens are associated with geographic origins of the strain. Such extensive phenotypic variation perhaps reflects *H. pylori’s* ability to mimic the Le phenotype of its host as a means of niche adaptation and survival (15, 47). Divergence of other Le antigen synthesis genes between European and East-Asian strains further supports this hypothesis (36, 60). These results provide the framework for future studies to investigate the relationship between host and *H. pylori* Le antigen phenotypes, and how such phenotypic variation may contribute to *H. pylori’s* ability to persistently colonize the human gastric mucosa.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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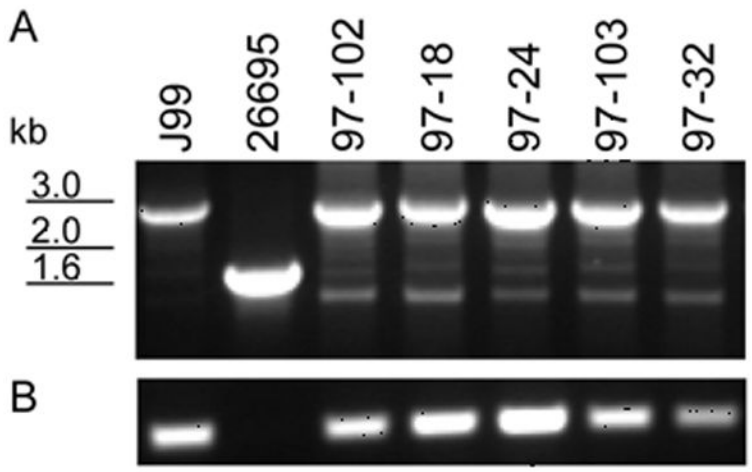

Figure 1. PCR amplification of the *jhp0562-β-(1,3)galT*(*jhp0563*) locus

Panel A. Representative results of PCR screens for the presence of *jhp0562* with primers flanking jhp0562- *β-(1,3)galT*. Strains harboring a copy of both *jhp0562* and *β-(1,3)galT* produce a band of ~2.6 kb, while strains lacking *jhp0562* amplify a band of ~1.5 kb. Whole genomic sequencing of strains J99 and 26695 indicate that strain J99 possesses both genes, whereas 26695 only possess *β-(1,3)galT* (37, 39)**. Panel B.** *jhp0562*-specific PCR. Only *H. pylori* strains that contain a copy of *jhp0562* yield a band in this assay.

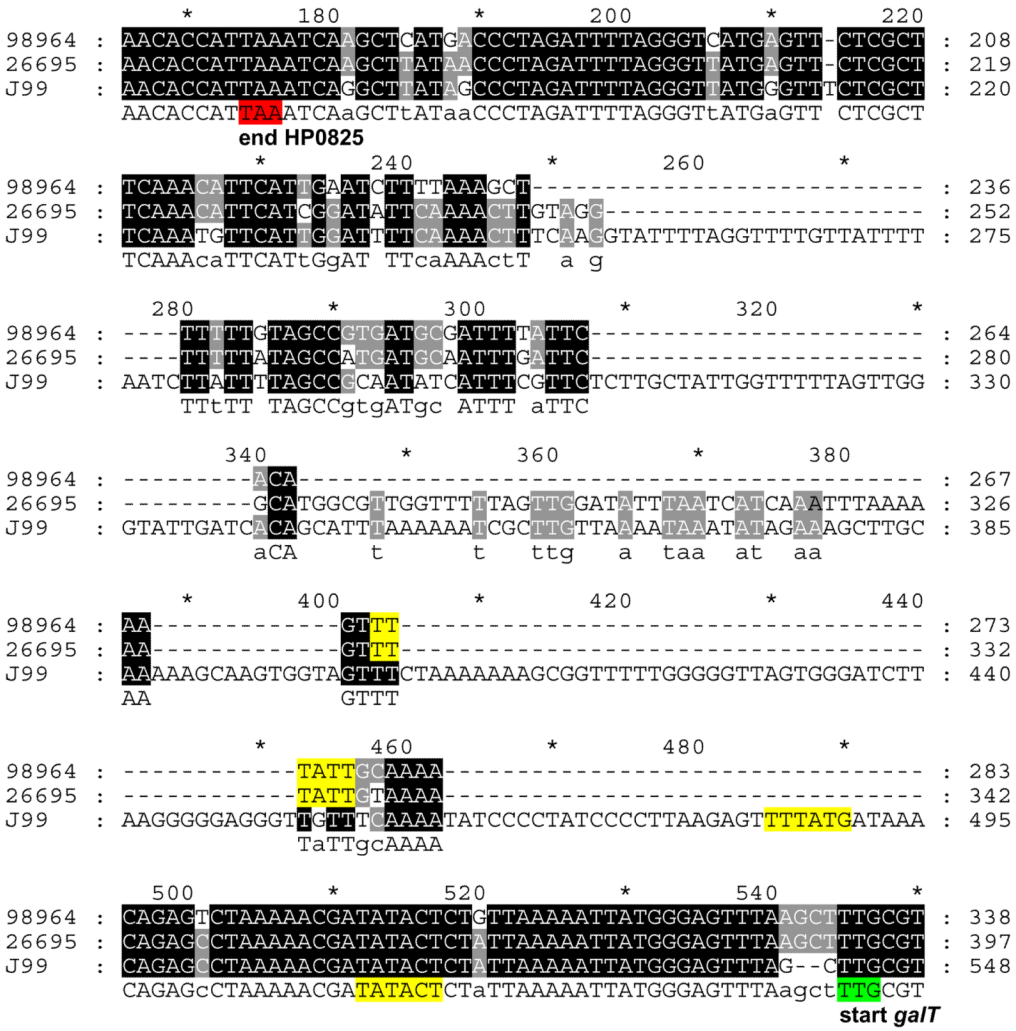

Figure 2. Nucleotide alignment of the region upstream of *galT* in three representative *H. pylori* strains

The strains are 98-964, 26695 and J99, with small, medium, and large *galT* upstream regions, respectively, as detected by PCR (Figure 3). The stop codon of the gene upstream of *galT*, HP0825 is highlighted in red, while the start codon of *galT* is shown in green. Predicted −35 and −10 sequences (38, 61), are highlighted in yellow.

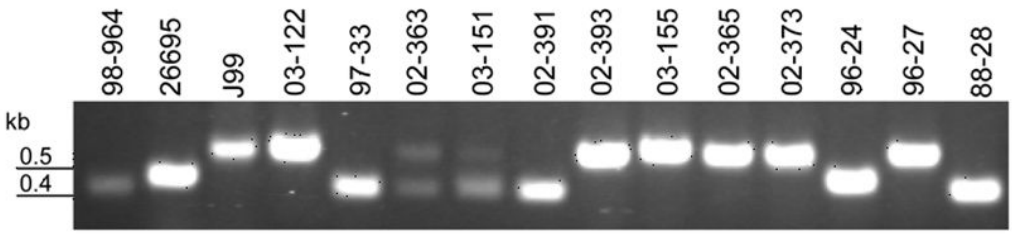

Figure 3. PCR screen of the *galT* promoter region in *H. pylori* strains

Results of representive PCR amplification of the *galT* promoter region. Three different promoter sizes were detected: small, as in control strain 98-964, medium, as in strain 26695; and large, as in control strain J99.

Table 1

Le antigen phenotypes and genotypes of *H. pylori* strains, according to their geographic origin.

Phenotypes (n=78)	East Asian	Non-East Asian	Total	*p*-value[a]
Le negative	3	8	11	
Le positive				
Type 1 only	0	0	0	-
Type 2 only	23	31	54	-
Type 1 & 2	13	0	13	<0.0001
Genotypes				
jhp0562 + (n =111)	41	56	97	
jhp0562 −	2	12	14	0.075
galT promoter (n=113)				
Small	33	3	36	-
Medium	7	7	14	0.0026[b]
Large	2	61	63	<0.0001[c]

[a] Fisher's exact test

[b] In relation to the number of small *galT* promoter regions.

[c] In relation to the number of strains that have smaller *galT* promoter sizes (small and medium), compared to the number of strains that have large *galT* promoter regions.