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Genotypic Profile of the Outer Membrane Proteins BabA and BabB in Clinical Isolates of *Helicobacter pylori*

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***Helicobacter pylori* BabA is the ABO blood group antigen binding adhesin, which has a closely related paralogue (BabB) whose function is unknown. PCR and DNA sequence analysis showed extensive genotypic diversity in *babA* and *babB* across different strains, as well as within a strain colonizing an individual patient. We hypothesize that diverse profiles of *babA* and *babB* reflect selective pressures for adhesion, which may differ across different hosts and within an individual over time.**

Approximately 4% of the *Helicobacter pylori* genome encodes a diverse repertoire of outer membrane proteins (OMPs), the largest of which is the 21-gene Hop family (18). Several of the *H. pylori* Hop proteins have been identified as adhesins, the best studied of which is the ABO blood group binding antigen, BabA (4, 6, 7, 9). The predicted *H. pylori* OMPs all have one region of similarity at the amino-terminal end and seven regions of similarity at the carboxy-terminal end (1, 18). This has led to the suggestion that recombination events might lead to a mosaic organization of OMPs that could be the basis for antigenic variation (18). This suggestion is supported by the observation that *H. pylori* J99 and 26695 have *babA* and *babB* in complementary loci (2, 18) and by evidence of RecA-dependent recombination in vitro (11). Many of the OMPs also have dinucleotide CT repeats in the 5′ coding region, which have been postulated to regulate their expression by phase variation through slipped-strand mispairing (10, 14, 20). This plasticity in the profile of *H. pylori* OMPs may provide a mechanism for adaptation to the different niches and microenvironments within the stomach, either by immune evasion, adhesion, or both, or perhaps by some other mechanism.

We recently observed an apparent gene conversion after experimental infection of rhesus macaques with *H. pylori* strain J166 (17). The inoculated J166 strain had *babA* and *babB* in the same chromosomal loci as J99, but most of the strains recovered after infection had replaced *babA* with a second copy of *babB*. The few recovered strains that retained *babA* did not express it, as a result of changes in the number of CT dinucleotide repeats preceding the 5′ signal peptide sequence. This result, combined with evidence that *babA* is absent in some human strains (13, 21), led us to hypothesize that the recombination events we observed in monkeys might reflect a response to selective pressure that may also be apparent in human clinical isolates of *H. pylori*. In this report we describe the composition of *babA* and *babB* at two loci in 44 human *H. pylori* isolates and provide evidence that the genotypes of these two OMPs are highly variable.

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Determination of the *babA and babB* genotypes. In *H. pylori* J99, *babA* (JHP0833) is downstream of *hypD* (JHP0835) and *babB* (JHP1164) is downstream of *s18* (JHP1165). In strain 26695, the locations of *babA* (HP1243) and *babB* (HP0317) are reversed (2). For simplicity, the chromosomal locations downstream of *hypD* and *s18* will be referred to as the *babA* and *babB* loci, respectively. We performed four anchored PCRs with primer pairs designed to query whether the gene at the *babA* locus was *babA* (primer pair HypDF1–BabAR1 [Table 1]) or *babB* (HypDF1–BabBR1) and whether the gene at the *babB* locus was *babA* (S18F1–BabAR1) or *babB* (S18F1–BabBR1). Each of the four PCRs (94°C for 5 min; 40 cycles of 94°C for 30 s, 56.5°C for 30 s, 72°C for 2 min; 72°C for 5 min) was performed on chromosomal DNA from low-passage-number, single-colony isolates ($n = 44$) by using standard concentrations of reagents. Primers (Table 1) were designed from *H. pylori* J99 (JHP0835 and JHP1165) or from multiple sequence alignments used to identify conserved regions that reliably discriminate *babA* and *babB* (12). PCR products were electrophoresed in 1% agarose (Gibco BRL) using J99 and 26695 as controls. All PCR products of the predicted size (*babA* locus, 2.1 to 2.6 kb; *babB* locus, 1.0 to 1.5 kb) from each of these four reactions were sequenced over a mean (standard deviation [SD]) of 676 (85) bp and compared to the GenBank nucleotide database using FASTA. PCR products for which it appeared that both *babA* and *babB* were present at the same locus (e.g., both S18F1–BabAR1 and S18F1–BabBR1 PCRs positive) were also sequenced. The presence of *babA* or *babB* at a given locus was defined in all cases as a positive PCR product of the predicted size whose DNA sequence was most closely related to *babA* or *babB*. To determine if a gene could be assigned correctly to *babA* or *babB* based on a mean of 676 bp, we performed the analysis on 10 complete sequences of *babA* and 10 of *babB* available in GenBank. In all 20 instances, the assignment was made correctly (data not shown). Mean (SD) percent similarities of the identified *babA* and *babB* genes to their orthologues in strain J99 were 90.4% (3.3%) and 89.2% (4.3%), respectively.

A representative agarose gel for the two sequenced strains, J99 and 26695, and a clinical isolate is shown in Fig. 1. In total, 27% (12/44) of isolates were J99-like, with *babA* in the *babA* locus and *babB* in the *babB* locus (designated AB), and 9%

TABLE 1. Primers used for amplification, cloning, and real-time PCR

Primer designation	Gene[a]	Position	Sequence (5′–3′)
S18F1	JHP1165	197–218	CTTTAATCCCCTACATTGTGGA
S18F2	JHP1165	137–157	GCAATAGCAAAAAGTGGC AAG
S18F3	JHP1165	190–211	CACATGGCTTTAATCCCCTACA
HypDF1	JHP0835	675–697	TTTTGAGCCGGTGGATATATTAG
HypDF2	JHP0835	732–754	CAAAGAAGCCAAGCTAGAAATCC
BabAR1	JHP0833	723–705	TTTGCCGTCTATGGTTTGG
BabAR2	JHP0833	444–424	ATACCCTGGCTCGTTGTTGAA
BabAR3	JHP0833	255–236	ATCGTTACGCACCCCATTGA
BabAR4	JHP0833	363–341	GCCTAAGACATTCCAAAACCCTA
BabBR1	JHP1164	772–750	TCGCTTGTTTTAAAAGCTCTTGA
BabBR2	JHP1164	481–459	CATGTCCTGGCTCATAATACGAA
BabBR3	JHP1164	389–370	TCATTGCTACCAGGACCACA
BabBR4	JHP1164	285–261	GGTTTTGACATCAAGCAAATTCCTA

[a] *H. pylori* strain J99 designation.

(4/44) were 26695-like, with *babA* in the *babB* locus and *babB* in the *babA* locus (designated BA) (Table 2; Fig. 2). In 23% (10/44) of the isolates, either *babA* or *babB* alone was present at both loci (AA or BB). In 30% (13/44) of the isolates, there was a mixed genotype, where the population of cells (derived from a single colony) contained both *babA* and *babB* at the same locus, a phenomenon previously observed for strain J166 (17). In all but 2 of these 15 isolates, the mixed genotype was at the *babB* locus (e.g., A AB). To determine the limit of detection for *babA* at the *babB* locus in a mixed-genotype strain, PCR was performed (primers S18F1 and BabAR1) using a mixture of DNA from 26695 (AB) and serial dilutions of strain H45 (BB) in ratios from 1 to 10^{-6}. The results showed that *babA* could be detected as a minority population in the *babB* locus when it was present at a fraction of 10^{-5} or greater (data not shown). Interestingly, 9% (4/44) of the PCR products yielded sequences that were most closely related (mean homology, 85%; SD, 1.9%) to HP0317 (*hopU*) in strain 26695. The function of HP0317 is unknown, but it is closely related to *babA* and *babB* and is here designated *babC*. One strain (D5131) contained *babB* at the *babB* locus but did not contain an identified OMP at the *babA* locus. These results suggest that the *babAB* genotype of *H. pylori* is complex and that some

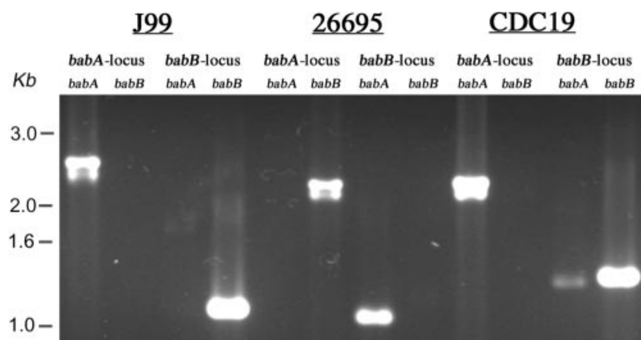

FIG. 1. PCR products stained with ethidium bromide and electrophoresed in a 1% agarose gel. Results are shown for each of the four PCRs (*babA* in the *babA* and *babB* loci; *babB* in the *babA* and *babB* loci [see Materials and Methods for details]) performed on control strains J99 (genotype AB) and 26695 (genotype BA) and on a sample clinical isolate (CDC19 [genotype A AB]). A kilobase ladder is shown on the left.

TABLE 2. *H. pylori* strain genotype and Leb attachment

Strain	Genotype[a]		Leb attachment[b]
	babA locus	*babB* locus	
CDC2	A	A	+
TR86-1	A	A	+
H135-1	A	A	+
CDC12	A	AB	−
CDC22	A	AB	−
D5135	A	AB	−
H106-1	A	AB	−
95-66	A	AB	+
CDC19	A	AB	+
CDC3	A	AB	+
H28-2	A	AB	+
CDC16	A	B	−
AR20	A	B	−
AR3-1	A	B	−
D5129	A	B	−
H116B	A	B	−
878787B6	A	B	+
CDC21	A	B	+
CDC24	A	B	+
D5127	A	B	+
J99	A	B	+
D5132	A	B	+
CDC1	A	B	−
CDC10	AB	B	+
J166	AB	B	+
26695	B	A	−
CDC11	B	A	−
D2371	B	A	+
TR106-3	B	A	+
AR19	B	AB	−
TR56-5	B	AB	+
H13-1	B	AB	+
88-23	B	B	−
CDC25	B	B	−
H34-JP	B	B	−
H11	B	B	ND
H40-2	B	B	ND
H45-1	B	B	−
H48-1	B	B	ND
AR18	B	C	−
CDC8	C	B	−
H12	C	B	−
H100	C	B	−
D5131		B	−

[a] A, *babA*; B, *babB*; AB, *babA* and *babB*; C, *babC*.
[b] +, positive; −, negative; ND, not determined.

strains may have undergone a gene conversion event much like that observed in experimentally infected rhesus monkeys (17), with deletion of *babA* and duplication of *babB*.

Intrastrain genotype variation. Since *H. pylori* shows marked genomic diversity, we next examined multiple single-colony isolates from each of three patients to determine if the diversity we found across patients could also be detected within a patient at a single point in time. Multiple (9 to 12) additional single colonies were isolated from the original cultures of a strain (H106) with genotype A AB (*babA* at the *babA* locus and both *babA* and *babB* at the *babB* locus), a J99-like strain (H116) with genotype AB, and a strain with genotype AA (H135). Each of the four PCRs and DNA sequencing of the products were performed as before on each isolate. For strain H106, the *babAB* genotype was A AB in all 12 isolates. However, although most isolates of strain H135 and H116 were AA and AB, respectively, like the original isolate (Table 2), 4 of 12 (33%) and 4 of 9 (44%), respectively, were A AB. Therefore, all patients from whom we examined multiple isolates were

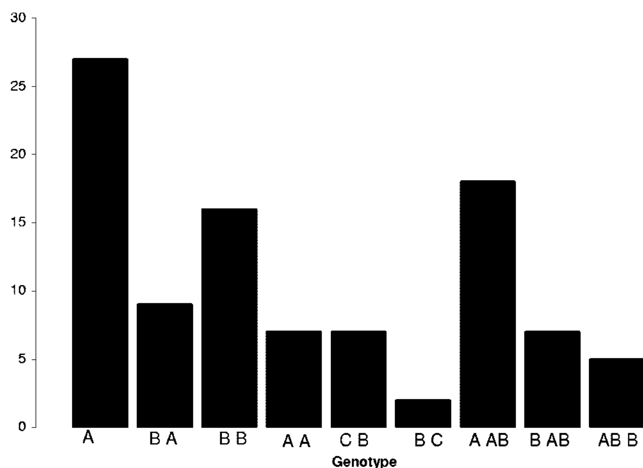

FIG. 2. Percentage of strains (n = 44) that showed each of the genotypes indicated. For example, strains with genotype AB have *babA* at the *babA* locus and *babB* at the *babB* locus. A AB, B AB, and AB B indicate the genotypes of strains that had more than one *bab* gene present at a given locus within the population derived from a single colony.

colonized with strains that were least partially AB at the *babB* locus. Repetitive extragenic palindromic PCR fingerprints (16) were identical for all isolates from each of the three patients (data not shown), indicating that patients are commonly colonized with a single strain but that there is considerable heterogeneity in the *babAB* genotype within the population, particularly at the *babB* locus.

Rates of recombination. Relative abundances of *babA* and *babB* at the *babB* locus were determined within the population for four strains (H106, D5135, CDC12, and CDC3) with genotype A AB. In each case *babB* appeared predominant by standard PCR (for example, strain CDC19 in Fig. 1). Copy numbers of *babA* and *babB* at the *babB* locus were determined using quantitative real-time DNA PCR as previously described (17). The results showed that for each strain, the frequency of *babA* at the *babB* locus was between 10^{-3} and 10^{-4}, a result similar to the finding we reported previously for strain J166 (17). The relative frequency of *babA* at the *babB* locus appeared to be stable for a given strain, since assays performed on 10 independent colonies of H106 were highly reproducible (mean, 2.2×10^{-4}; SD, 1.9×10^{-4}).

Site of recombination. DNA sequences from 10 strains (8 with genotype A AB, 1 with genotype B AB, and 1 with genotype AB B) were inspected to determine the upstream location at which the recombination event occurred for the second copy of *babA* or *babB*. The approximate site of recombination was identified as the position at which the second copy of *babA*, for example (in a strain with genotype A AB), showed homology to *babA* and not *babB*. In five strains (all genotype A AB), the recombination event was identified from approximately 50 to 200 bp downstream of the ATG, while in the other five strains, it was upstream of the ATG. Therefore, recombination of a second copy of *babA* or *babB* occurs before the unique region of the gene that most distinguishes *babA* from *babB*.

CT Repeats in *babA* and *babB*. *H. pylori* strains 26695 and J99 each contain CT repeats in the 5′ coding region of *babB*, which likely serve to make it phase variable, while in other strains, CT repeats are absent (12). BabA has not been reported to be phase variable (14) until recently (17). We therefore analyzed the *babA* and *babB* sequences from each of the 44 strains for the presence of 5′ CT repeats. The results show that CT repeats (minimum, 5; maximum, 11) are more common in *babB* (43/53 [81%]) than in *babA* (13/43 [30%]; χ^2 = 25.5; $P < 0.001$) and also more common at the *babB* locus (42/54 [78%]) than at the *babA* locus (14/42 [33%]; χ^2 = 19.2; $P < 0.001$). In strains that had duplicate copies of *babA* (n = 11) or *babB* (n = 12), both copies were in frame more often for *babB* (33%) than for *babA* (9%), but the results were not statistically significant.

Leb attachment. We used an enzyme-linked immunosorbent assay as described previously (17) to measure attachment to Leb, which serves as an assay for functional binding of *babA* to blood group antigens. Of 41 strains tested, 19 (46%) showed binding to Leb (Table 2). All strains that bound Leb had at least one copy of *babA*, though many strains had one or more copies of *babA* and did not bind Leb. In one case, a strain (AR19) with *babA* did not bind Leb due to a frameshift, which produced a stop codon; in other cases, one or both copies of *babA* were in frame but no binding was seen (e.g., AR20, CDC1, D5129). This suggests that there is heterogeneity in binding of BabA to Leb, which is consistent with recent work (8) as well as with the observation that *H. pylori* J99 binds Leb while 26695 does not (17), even though *babA* is transcribed and translated in both strains.

Perspective. The primary ecological niche for *H. pylori* is the mucus that overlies human gastric mucosa. Elegant studies in the gerbil model suggest that of the approximately 100 μm total thickness of this mucus layer, *H. pylori* is found only in the 25 μm closest to the epithelium, and only a small minority of bacteria are actually attached to host cells (15). The precise localization of bacteria within the mucus layer may represent a compromise between selective factors that promote adherence, such as nutrient acquisition (19) from disruption of epithelial-cell tight junctions (3), and those that oppose it, such as evasion of reactive oxygen species and other aspects of the host immune response. This hypothesis suggests that fine control and modulation of adhesion may be a critical feature that permits *H. pylori* to persistently colonize its host despite an active and multifaceted innate and adaptive immune response. Here we show that while most *H. pylori* strains have *babA* and *babB*, which are typically located at the same genomic loci as in strain J99, in 27% of strains (12/44) *babA* appeared to be absent. This result is in close agreement with results obtained using PCR with Western blotting (21) and a whole-genome DNA microarray (13). Most strains that did not contain *babA* had a second copy of *babB* in its place, like strains recovered from experimentally or naturally infected macaques (17). All strains without evidence of *babA* also tested negative for attachment to Leb (Table 2). The *babB* gene was identified in 95% of our isolates (41/44) and in 100% (15/15) of isolates studied by DNA microarray (13), suggesting that the presence of *babB* confers a stronger selective advantage than does the presence of *babA*. BabB may itself serve as a lectin to bind an unidentified receptor on the host epithelium, or perhaps to modulate binding of other adhesins.

Diversity at the *babAB* loci is not limited simply to selection

for a particular combination of *babA*, *babB*, or *babC*. Examination of multiple colonies from individual patients with *babA* at the *babA* locus showed that in all cases both *babA* and *babB* were expressed at the *babB* locus, though in different proportions. This may in fact be true for all such strains. We have, for example, occasionally found strain J99 to have genotype A AB, which may suggest that the mixed genotype of this strain at the *babB* locus is near the limit of our detection (10^{-5}). CT dinucleotide repeats that permit phase variation were common in the 5′ coding region, particularly in *babB*, but also in *babA* when it was found in the *babB* locus. This evidence for marked phase variation (CT repeats) and antigenic variation (presence of *babA* and *B* in different proportions) is consistent with the recent suggestion that *babB* constitutes an expression or contingency locus (5). We hypothesize that the diversity in *babABC* reflects both the glycophenotype of the host and selective pressures for adhesion, which may differ across different hosts, across gastric regions within a host, and also within an individual over time.

Nucleotide sequence accession numbers. All DNA sequences from this study were deposited in the NCBI GenBank database with accession numbers AY743975 to AY744069.

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