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Evaluation of the Association of Nine *Helicobacter pylori* Virulence Factors with Strains Involved in Low-Grade Gastric Mucosa-Associated Lymphoid Tissue Lymphoma

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Helicobacter pylori **has been associated with the development of two malignant diseases: gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Although the *cag* pathogenicity island, especially the *cagA* gene, has been linked with adenocarcinoma, few data concerning *H. pylori* pathogenic factors involved in low-grade gastric MALT lymphoma are available. The goal of this study was to analyze the prevalence of and correlation between genes coding for seven *H. pylori* virulence factors (*cagA*, *cagE*, *vacA*, *iceA*, *babA*, *hopQ*, and *oipA*) and two novel adhesins (*sabA* and *hopZ*) by comparing a collection of 43 *H. pylori* strains isolated from patients with low-grade gastric MALT lymphoma to 39 strains isolated from age-matched patients with gastritis only. Our results show that taken individually, none of the nine genes tested can be considered associated with MALT strains and allow us to conclude that MALT pathogenesis is not linked with more proinflammatory *H. pylori* strains. We demonstrated that in patients infected with strains harboring the *iceA1* allele, *sabA* functional status, and *hopZ* "off" status, the odds of developing a MALT lymphoma were 10 times higher. However, the low prevalence of such strains (10 of 43 MALT strains) renders this triple association a low-sensitivity marker for MALT strains. Our data confirmed that *H. pylori* virulence factors are correlated with one another. If the involvement of *H. pylori* in MALT lymphoma is well established, the pathomechanism by which gastric lymphoma occurs remains to be identified.**

Helicobacter pylori infection is the essential etiological factor of type B chronic gastritis and peptic ulcer disease (29, 35). It is also the first bacterium discovered to be involved in the development of malignant diseases: i.e., gastric carcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (14, 21, 40). Following *H. pylori* colonization, inflammation occurs. Untreated gastritis may persist for years with different grades of severity. In some individuals, immunological stimulation induces lymphoid follicles in the gastric mucosa and provides the background for MALT lymphoma development (11), the polyclonal lymphoid hyperplasia evolving further toward a monoclonal lymphoid population (36). The reasons for this evolution, which is accompanied by genetic abnormalities, remain to be elucidated (33, 37). MALT lymphoma induction and growth are probably antigen driven, and it has been shown that *H. pylori* could be the trigger (18, 23, 24, 26). Moreover, there is a demonstrated causal association between *H. pylori* infection and MALT-type lymphoma development, because it is possible to cure this cancer by eradicating *H. pylori*, suggesting that bacterial virulence factors exist and are associated with the disease development. *H. pylori* is the cause of approximately 80% of MALT lymphomas (8, 57).

Among the factors that may be linked to the outcome of infection, bacterial virulence factors such as cytotoxins and the *cag* pathogenicity island (PAI) have been extensively investigated but have not been clearly associated with MALT lymphoma strains (19, 39). The goal of this study was to analyze the prevalence of and correlation between genes coding for seven *H. pylori* virulence factors (*cagA*, *cagE*, *vacA*, *iceA*, *babA*, *hopQ*, and *oipA*) and two novel adhesins (*sabA* [sialic acid-binding adhesin] and *hopZ* [*H. pylori* outer membrane protein]) by comparing a collection of 43 *H. pylori* strains isolated from patients with low-grade gastric MALT lymphoma to strains isolated from age-matched patients with gastritis only (4, 10, 20, 22, 25, 44, 46, 55, 58, 60, 61). However, because MALT lymphoma is a rare disease, few studies have evaluated these new putative virulence markers in strains isolated from gastric MALT lymphoma.

Because disease-associated bacterial virulence factors may be correlated with one another, the aim of our study was to analyze the prevalence of and correlation between all of these genes by using a collection of 43 *H. pylori* strains isolated from patients with low-grade gastric MALT lymphoma and comparing them with 39 strains isolated from age-matched patients with gastritis only.

MATERIALS AND METHODS

Patients and *H. pylori* strains. We examined 82 *H. pylori* strains: 43 were isolated from patients with gastric low-grade MALT lymphoma (27 men and 16 women; mean age, 48.2 ± 13.4 years), and 39 were isolated from age-matched patients with histological gastritis only (29 men and 10 women; mean age, 48.3 ± 14.7 years) suffering from dyspepsia. Patients with gastric MALT lymphoma were enrolled from different areas of France in prospective multicenter studies carried out by the Groupe d'Etude Français des Lymphomes Digestifs of the Fédération

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TABLE 1. Comparison of genes from *H. pylori* strains isolated from patients suffering from gastric MALT lymphoma and from patients with gastritis only

Gene characteristic	Strain type				P^a
	MALT		Gastritis		
	n	%	*n*	%	
cagA					
Absent	21	48.8	19	48.7	1.000
Present	22	51.2	20	51.3	
cagE					
Absent	22	51.2	17	43.6	0.515
Present	21	48.8	22	56.4	
vacA					
s1m1	13	30.2	22	56.4	0.064
s1m2	12	27.9	6	15.4	
s2m2	18	41.9	11	28.2	
iceA					
A1	24	55.8	15	38.5	0.128
A2	19	44.2	24	61.5	
babA					
A1	24	55.8	18	46.1	0.507
A2	19	44.2	21	53.9	
hopQ					
I	21	48.8	24	61.5	0.274
II	22	51.2	15	38.5	
oipA					
Off	23	53.5	18	46.1	0.659
On	20	46.5	21	53.9	
sabA[b]					
Off	16	39.0	20	57.1	0.167
On	25	61.0	15	42.9	
hopZ[c]					
Off	23	56.1	14	40.0	0.176
On	18	43.9	21	60.0	

[a] *P* value by Fisher's exact test.
[b] *sabA* gene was not amplified for two MALT strains and four gastritis strains.
[c] *hopZ* gene was not amplified for two MALT strains and three gastritis strains. The *hopZ* gene was absent in one gastritis strain.

Française de Cancérologie Digestive (GELD) (48) and the Groupe d'Etude des Lymphomes de l'Adulte (GELA) (32).

Briefly, once a lesion suspected to be a MALT lymphoma was observed during endoscopy, the patient was invited to come back for a complete exploration, including *H. pylori* detection within 3 weeks.

Isolation of *H. pylori* was carried out from two gastric biopsy specimens (one from the lesion and one from macroscopically normal mucosa) by standard culture methods as previously described (31).

All strains were cloned, and genomic DNA was extracted from culture originating from simple colonies by using a commercial kit (Qiagen SA, Courtaboeuf, France).

In addition, the two *H. pylori* strains for which the genome has been sequenced, 26695 and J99, were used as reference strains (3, 52, 53).

Detection of genes encoding pathogenic factors. The genes explored (Table 1) were detected by PCR, except for the determination of the functional status of the *oipA*, *hopZ*, and *sabA* genes, for which sequencing was also performed. All of the primers used in this study were desalted and provided by Q-BIOgene (Strasbourg, France).

cag PAI status was evaluated by PCR for two loci: *cagA* (with two sets of primers) (54) and *cagE* (59). A *cagA*-positive status was defined when the *cagA* gene was detected by at least one of the two primer pairs. Specific primers for the *cag* empty site were also used to confirm the absence of the *cag* PAI (1, 27). The *vacA* (s [signal] and m [middle] regions), *babA*, and *iceA* alleles were detected by PCR as previously described [5, 6, 13, 22; R. M. Peek, S. A. Thompson, J. C. Atherton, M. J. Blaser, and G. G. Miller, abstract from the Ninth International Workshop on the Gastroduodenal Pathology of *Helicobacter pylori* 1996, Gut **39**(Suppl. 2)**:**A71, 1996]. The *hopQ* type I and II alleles were determined by allele-specific primers (Table 2). The functional status of the *oipA* (HP638/jhp581), *sabA* (HP725/jhp662), and *hopZ* genes is regulated by a slipped strand repair based on the number of CT dinucleotide repeats in the signal coding region. The signal sequences of the *oipA* and *sabA* genes including the CT repeats were amplified by PCR with the primers listed in Table 2 with sense and antisense primers designed on the flanking gene and behind the CT dinucleotide repeats, respectively. The primer sense was also used for sequencing. For the amplification of the *hopZ* gene, we used the previously described primers (41), and sequencing was done with the R2-hopZ primer (Table 2).

PCR amplifications for *cagA*, *cagE*, the *cag* PAI empty site, *vacA*, *iceA*, *babA2*, *hopQ*, *oipA*, and *sabA* were carried out in a 25-μl volume containing 2.5 μl of 10× PCR buffer (Eurobio, Les Ulis, France), 1.5 mM $MgCl_2$ (Eurobio), 200 μM (each) deoxynucleoside triphosphates (dNTPs) (Eurobio), 2 U of *Taq* DNA polymerase (Eurobio), 1 μM (each) primers, and 10 ng of *H. pylori* DNA. After 4 min of denaturation at 94°C, each reaction mixture was amplified for 35 cycles as follows: 30 s at 94°C, 30 s of annealing at 60 (for *cagA*, *iceA*, *hopQ*, and *oipA*), 58 (for *cagE* and *cag* PAI empty site), 55 (for *babA2* and *sabA*), or 53°C (for *vacA*); and 30 s at 72°C. After the last cycle, extension was continued for another 7 min (except for *vacA* [2 min]).

The *hopZ* gene was amplified in a 25-μl volume containing 2.5 μl of 10× PCR buffer (Invitrogen, Cergy Pontoise, France), 1.5 mM $MgCl_2$ (Invitrogen), 200 μM (each) dNTPs (Eurobio), 1 U of Platinum *Taq* polymerase (Invitrogen), 1.25 μM (each) primers, and 10 ng of *H. pylori* DNA. After 4 min of denaturation at 94°C, each reaction mixture was amplified for 40 cycles as follows: 30 s at 94°C then 30 s of annealing at 58°C and 2 min 30 s at 72°C. After the last cycle, extension was continued for another 7 min.

All PCR products were analyzed on a 1.5% agarose gel (except for *iceA* [3% agarose]) stained with ethidium bromide.

To determine the *oipA*, *hopZ*, and *sabA* status, amplified DNA fragments were

TABLE 2. Designation and sequences of the primers designed for this study

Region amplified	Primer designation	Sequences (5′ to 3′)
Type I *hopQ*	F2-hopQ-T1	GCGGTGGGAGCACAAATAG (sense)
	R3-hopQ-T1	GATGTGGTTACATGCGCTTC (antisense)
Type II *hopQ*	F1-hopQ-T2	CATATGCGGAGGCTATACGG (sense)
	R3-hopQ-T2	GCGTTGGTTGCTGTTTTAATG (antisense)
oipA flanking gene and *oipA*	F1-HP0637-jhp580	CCCCACAAGCGCTTAACAG (sense)
	F1-HP0638-jhp581	GAGAGTGCCTAAACCCTATAATCC (antisense)
sabA flanking gene and *sabA*	F1-HP726-jhp663	TTTTTGTCAGCTACGCGTTC (sense)
	R1-HP725-jhp662	ACCGAAGTGATAACGGCTTG (antisense)
hopZ flanking gene[a]	R2-hopZ	GCGTGTTBGCTTTTAAATTATCG (antisense)

[a] This primer was only used for sequencing the region containing the CT repeats of the *hopZ* gene.

purified with Microspin S-400 HR columns (Amersham Pharmacia Biotech, Inc., Uppsala, Sweden), and direct sequencing was performed with the ABI PRISM BigDye Terminators v3.0 cycle sequencing kit (PE Applied Biosystems, Foster City, Calif.) using an ABI 3700 analyzer DNA sequencer (PE Applied Biosystems).

Statistical analysis. Statistical analysis was performed with STATA 7.0 statistical software (Stata Corporation, Texas). A first comparison between MALT and gastritis strains was performed by univariate analysis and Fisher's exact test. Spearman rank coefficients (r) were also determined to study the association between the different characteristics of the strains.

In order to create a variable describing how the different strain characteristics were associated, a multiple correspondence analysis (MCA) was performed (47). To confirm the results, a cluster analysis was used to draw a dendrogram based on the same variables, by using the average aggregation method and Euclidian distance. Both analyses were performed with the Statbox 2.5 program (Grimmer Logiciels, Paris, France). These analyses were performed for all of the subjects included in this study for whom information on all genotypes considered was available as well as for the groups of patients with MALT lymphoma and with gastritis, separately.

The odds for association with MALT lymphoma and the 95% confidence interval (CI) were compared within groups of strains and between the different strata of the summary variables obtained by the MCA.

RESULTS

Relationship between each of the nine *H. pylori* virulence factors tested in strains of patients suffering from gastric MALT lymphoma and gastritis (Table 1). (i) *cag* PAI. Twenty-two MALT strains (51.2%) and 20 gastritis strains (51.3%) were *cagA* positive. Twenty-one MALT strains (48.8%) and 22 gastritis strains (56.4%) were *cagE* positive. Among the 43 MALT strains, 41 (95.3%) were either *cagA* positive, *cagE* positive, and *cag* PAI empty site-negative or *cagA* negative, *cagE* negative, and empty site positive, suggesting that the *cag* PAI is usually either complete or totally deleted. However, one MALT strain was *cagA* negative, *cagE* negative, and *cag* PAI empty site negative, and another one was *cagA* positive, *cagE* negative, and *cag* PAI empty site negative. Among the 39 gastritis strains, 34 (87.2%) were either *cagA* or *cagE* positive and *cag* empty site negative or *cagA* or *cagE* negative and empty site positive (data not shown). Two of these strains were *cagA* or *cagE* negative, but the *cag* PAI empty site PCR revealed an amplification fragment of 1,630 and 2,036 bp, respectively, suggesting that in these two strains, the *cag* PAI had partial deletions that allowed PCR amplification under our PCR conditions. Three other gastritis strains were *cagA* negative, *cagE* positive, and *cag* PAI empty site negative. At least one *cagA* or *cagE*-negative strain and one *cagA*-positive, *cagE*-negative strain were empty site negative. The distribution of *cagA* and *cagE* genes was not significantly different in *H. pylori* isolates from MALT patients compared to isolates from gastritis patients ($P = 1.0$ and 0.515, respectively).

(ii) *vacA* alleles. All 82 strains were classified according to the *vacA* gene signal region (either s1 or s2) or middle region (m1 or m2). The distributions of the different alleles s1m1, s1m2, and s2m2 among the MALT strains were 13 (30.2%), 12 (27.9%), and 18 (41.9%), respectively, and those for the gastritis strains were 22 (56.4%), 6 (15.4%), and 11 (28.2%), respectively. The type s2m1 was never detected in either group of strains. The distribution of *vacA* genotypes was not significantly different ($P = 0.064$). However, when considering the m genotype only, a slight association was found between m2 genotype and MALT strains ($P = 0.025$).

(iii) *iceA* status. The primers used allowed us to determine *iceA* genotypes in all strains. Twenty-four MALT strains (55.8%) were *iceA1*, and 19 (44.2%) were *iceA2* versus 15 (38.5%) *iceA1* and 24 (61.5%) *iceA2* among the gastritis strains. With *iceA2*-specific primers, PCR products for 10 gastritis and 6 MALT strains were longer than the expected size (344 versus 230 bp), suggesting the presence of *iceA2* variants in these strains. The prevalence of the two genotypes was not different in the two groups ($P = 0.128$).

(iv) *babA2* status. Nineteen MALT strains (44.2%) and 21 gastritis strains (53.9%) were *babA2* positive. The prevalence of *babA2* was not significantly different between the two groups ($P = 0.507$).

(v) *hopQ* status. The primers used for this new PCR allowed us to determine *hopQ* alleles in all strains. Type I *hopQ* alleles were detected in 21 MALT strains (48.8%) and 24 gastritis strains (61.5%), and type II *hopQ* alleles were detected in 22 MALT strains (51.2%) and 15 gastritis strains (38.5%). The distribution of type I and II *hopQ* alleles was not different in both groups of strains ($P = 0.274$).

(vi) *oipA* functional status. The primers used for this new PCR amplified the *oipA* gene in all strains. Based on the CT dinucleotide repeats present in the signal sequence coding region of the *oipA* gene, 20 MALT strains (46.5%) and 21 gastritis strains (53.9%) were considered to be *oipA* "on," and 23 (53.5%) and 18 (46.2%) strains, respectively, were considered *oipA* "off." The number of CT dinucleotide repeats in MALT strains ranged from 5 to 11, and the number in gastritis strains ranged from 5 to 12, in agreement with Yamaoka et al. (58). CT repeats began at position +23 in 72.1% of the MALT strains and 74.4% of the gastritis strains. There was no difference in the distributions of *oipA* functional status between these two groups of strains ($P = 0.659$).

(vii) *sabA* functional status. The primers used for this new PCR failed to obtain *sabA* gene amplification for two MALT strains and four gastritis strains. For the 76 remaining strains, 25 (61.0%) MALT strains and 15 (42.9%) gastritis strains contained a functional *sabA* gene. The number of CT dinucleotide repeats, which began at position +18, ranged from 3 to 12 for both MALT and gastritis strains (Table 3). When three CT repeats were observed, a thymidine-thymidine was always present in the middle of the repetition in frame with the rest of the gene. Interestingly, for strains harboring seven CT repeats, the *sabA* gene can be in or out of frame depending on the nucleotide sequence after the CT repeats (Table 3). There was no difference in *sabA* functional statuses between the two groups of strains ($P = 0.167$).

(viii) *hopZ* functional status. We failed to obtain PCR *hopZ* gene amplification for two MALT strains and three gastritis strains. In addition, for one gastritis strain, an 800-bp PCR product, was obtained (versus products of approximately 3,000 bp according to the 26695 and J99 reference strains), and direct sequencing revealed the lack of the *hopZ* gene. For the remaining strains, 18 (43.9%) MALT strains and 21 (60.0%) gastritis strains contained a functional *hopZ* gene. Analysis of the gene sequences revealed that CT repeats began at position +18, and the number of repeats ranged from 6 to 12 as previously described (41). Interestingly, two MALT strains contained four dinucleotide repeats in frame with the residual gene (GenBank accession no. AY299993 and AY299994).

Moreover, 11 (26.8%) MALT strains and 4 (11.4%) gastritis strains contained eight dinucleotide repeats out of frame (GenBank accession no. AY299995 to AY300009). There was no difference in the distributions of *hopZ* functional status between the two groups of strains ($P = 0.176$).

Relationship between the nine *H. pylori* virulence factors tested in strains of patients suffering from gastric MALT lymphoma and gastritis. In MALT strains, we found a positive correlation between *cagA* and *cagE* ($r = 0.9545$, $P < 10^{-4}$) and between *vacA* s1m1 genotype ($r = 0.6431$, $P < 10^{-4}$) and *babA2* ($r = 0.4009$, $P = 0.008$), and *oipA* on ($r = 0.7245$, $P < 10^{-4}$) and *hopQ* type I ($r = 0.7684$, $P < 10^{-4}$), but there was a negative correlation between *cagA* and *vacA* s2m2 ($r = 0.7742$, $P < 10^{-4}$). The *babA2* gene was correlated with *cagE* ($r = 0.4423$, $P = 0.003$) and *hopQ* type I ($r = 0.3486$, $P = 0.0022$). Among the gastritis strains, *cagA* was associated with the same markers as in MALT strains, with the exception of *babA2*, which was correlated with *vacA* s1m1 alleles ($r = 0.5346$, $P = 0.0005$) and with *hopQ* type I ($r = 0.4310$, $P = 0.0062$).

Results of the MCA for construction of the summary variable. First, the MCA was performed on 70 strains for which complete data were available for all variables, yielding the F1/F2 plane, which accounted for 82.4% of the total information (73.9% for the horizontal axis [F1] and 8.6% for the vertical axis [F2]). Two main clusters were identified on each side of the horizontal axis: the first included strains positive for *cagA*, *cagE*, *vacA* s1m1 type, *babA2*, *oipA* on, and *hopQ* type I (group A), and the second included strains negative for *cagA*, *cagE*, *vacA* s2m2 type, *babA1*, *oipA* off, and *hopQ* type II (group B). On the vertical axis, *iceA1* and *sabA* on strains were on one side, and *iceA2* and *sab*A off strains were on the other. The *hopZ* strains were found on the third axis (F3) (data not shown).

The dendrogram drawn from the results of the cluster analysis of both populations considered together confirmed the presence of four groups: the A and B groups, which were formally identified; and two others. Group C contained *iceA2*, *sabA* off, and *hopZ* on strains, and group D contained *iceA1* and *sabA* on strains (Fig. 1).

The same approach was then performed for each population: i.e., MALT patients (39 strains) or gastritis patients (31 strains) from whom complete data were available for each variable. The analysis of the MCAs revealed that groups A and B were similar and that the main difference between MALT and gastritis strains was based on the distribution of *iceA* genotype, *sabA* status, and *hopZ* status (Fig. 2A and B). In MALT strains, a cluster absent in gastritis strains and containing *iceA1*, *sabA* on, and *hopZ* off was identified (Fig. 2B).

Relationship between the genotype of *H. pylori* strains and MALT diagnosis. The possible association between the presence of MALT and the characteristics of strains cultured from these patients was tested. As shown in Table 4, the distribution of the dendrogram deduced A and B groups (based on *cagA*, *cagE*, and *vacA* alleles; *babA* and *oipA* status; and *hopQ* genotype) revealed no difference between MALT and gastritis strains ($P = 0.561$) (Table 4 and Fig. 3). The proportion of MALT patients was not different in those harboring strains with the *sabA* gene on, with *iceA1*, or with the *hopZ* gene on (62.5, 61.5, and 75.7%, respectively). The odds ratios (ORs)

TABLE 3. Signal sequence coding region of the *sabA* gene and deduced amino acid sequences of 18 different *Helicobacter pylori* strains[a]

Strain	Sequence of the signal peptide coding region	No. of CT repeats	Deduced amino acid sequence	Gene status
G1	ATGAAAAAACGATTTTTACTTTCTCTATCCC----------TTGCATCGTCATTACTTTATGCTGAAGACAACGGCTTTTTTGTGA	3	MKKRFLLSLSL----ASSLLYAEDNGFFVSAGYQIGEAVQMVKNTGEL	On
M30	ATGAAAAAACGATTTTTACTTTCTCTATCCC----------TTGCAGGTCATTACTTTATGCTGAAGACAACGGCTTTTTTGTGA	3	MKKRFLLSLSL----AASLLYAEDNGFFVSAGYQIGEAVQMVKNTGEL	On
M32	ATGAAAAAGACAATTCTGCTCTCTC----------GCTTCATCGCTCTTGCACGCTGAAGACAACGGCTTTTTTGTGA	4	MKKTILLSL------ASSLLHAEDNGFFVSAGYQIGEAVQMVKNTGEL	On
M45	ATGAAAAAACGATTTTTACTCTCTCTC----------GCTTGCGGTATCATCGCTCCATGCTGAAGACAACGGCTTTTTTGTGG	5	MKKRFLLSLSL----AVSSLHAEDNGFFVGVGYQIGEAVQMVKNTGEL	On
G15	ATGAAAAAACGATTTTTACTCTCTCTC----------GTTTGCGGTATCATCGCTCCACGCTGAAGACAACGGCTTTTTTATCG	6	MKKRFLLSLS----RLRYHRSTLKTTAFLSAWAIKSAKRCKWSKTPVN	Off
M59	ATGAAAAAGACAATTCTACTCTCTCTC----------GCTTCATCGCTCTTGCACGCTGAAGACAACGGCTTTTTTGTGG	6	MKKTILLSLS----RFIALAR*	Off
G5	ATGAAAAAACGATTTTTACTCTCTCTC----------GCTTGCGGTACCATCGCTCCACGCTGAAGACAACGGCTTTTTTGTGG	7	MKKRFLLSLSL-ACGTIAPR*	Off
M61	ATGAAAAAGACAATTTTACTCTCTCTC----------GCTTCATCGCTCTTGCATGCTGAAGACAACGGCTTTTTTGTAG	7	MKKTILLSLSL----ASSLHAEDNGFFVGVGYQIGEAVQMVKNTGEL	On
G21	ATGAAAAAACGATTTTTACTCTCTCTC----------GTTTGCGGTATCATCGCTCCACGCTGAAGACAACGGCTTTTTTGTGA	8	MKKRFLLSLSL----AVSSLHAEDNGFFVSAGYQIGEAVQMVKNTGEL	On
M23	ATGAAAAAACGATTTTTACTCTCTCTC----------GCTTGCGGTATCATCGCTCCACGCTGAAGACAACGGCTTTTTTGTGA	8	MKKRFLLSLSL--AVSSLHAEDNGFFVSAGYQIGEAVQMVKNTGEL	On
G16	ATGAAAAAACGATTTTTACTCTCTCTC------GCTTGCGGTATCATCGCTCCACGCTGAAGACAACGGCTTTTTTATCG	9	MKKRFLLSLSLS--RLRYHRSTLKTTAFLSVWAIKSAKRCKWSKTPVN	Off
M25	ATGAAAAAACGATTTTTACTCTCTCTC------GTTTGCGGTATCATCGCTCCACGCTGAAGACAACGGCTTTTTTATCG	9	MKKRFLLSLSLS--RLRYHRSTLKTTAFLSVWAIKSAKRCKWSKTPVN	Off
G13	ATGAAAAACGATTTTTACTCTCTCTCTC---GCTTGCGGTATCATCGCTCCACGCTGAAGACAACGGCTTTTTTGTGG	10	MKKRFLLSLSLSLACGIIAPR*	Off
M31	ATGAAAAAACGATTTTTACTCTCTCTCTC---GCTTGCGGCATCATCGCTCCACGCTGAAGACAACGGCTTTTTTGTGA	10	MKKRFLLSLSLSLACGIIAPR*	Off
G26	ATGAAAAAACGAATTTTACTCTCTCTCTC--GCTTGCGGTATCATCGCTCCACGCTGAAGACAACGGCTTTTTTGTAA	11	MKKRILLSLSLSLSLAVSSLHAEDNGFFVXVGYQIGEAVQMVKNTGEL	On
M65	ATGAAAAAACGATTTTTACTCTCTCTCTC--GCTTGCGGTATCATCGCTCCACGCTGAAGACAACGGCTTTTTTGTGA	11	MKKRFLLSLSLSLSLAVSSLHAEDNGFFVSAGYQIGEAVQMVKNTGEL	On
G6	ATGAAAAAACGATTTTTACTCTCTCTCTCTGCTTGCGGGCGCCATCGCTCCACGCTGAAGACAACGGCTTTTTTGTGG	12	MKKRFLLSLSLSLSLRRHRSTLKTTAFLWARAIKSVKRCKWSKTPAN	Off
M38	ATGAAAAAACGATTTTTACTCTCTCTCTCTCTCTGTTTGCGGTATCATCGCTCCACGCTGAAGACAACGGCTTTTTTGTAG	12	MKKRFLLSLSLSLSLRLRYHRSTLKTTAFL*	Off

[a] G, gastritis strain; M, MALT strain. The stop codon is boxed for strains having the off status (except for strains G15, G16, M25, and G6), and the end of the protein is indicated by an asterisk. Concerning these strains, the stop codon is at positions 133 and 144 for the G15 and G6 strains, respectively, and position 139 for the G16 and M25 strains. All 86 strains tested in this study having the on status presented the italicized conserved motif. CT repeats are underlined, and the thymidine-thymidine sequences in the middles of the repetitions are in boldface. The *sabA* gene sequences from G1 to M38 strains were submitted to GenBank and assigned accession no. AY299975 to AY299992, respectively.

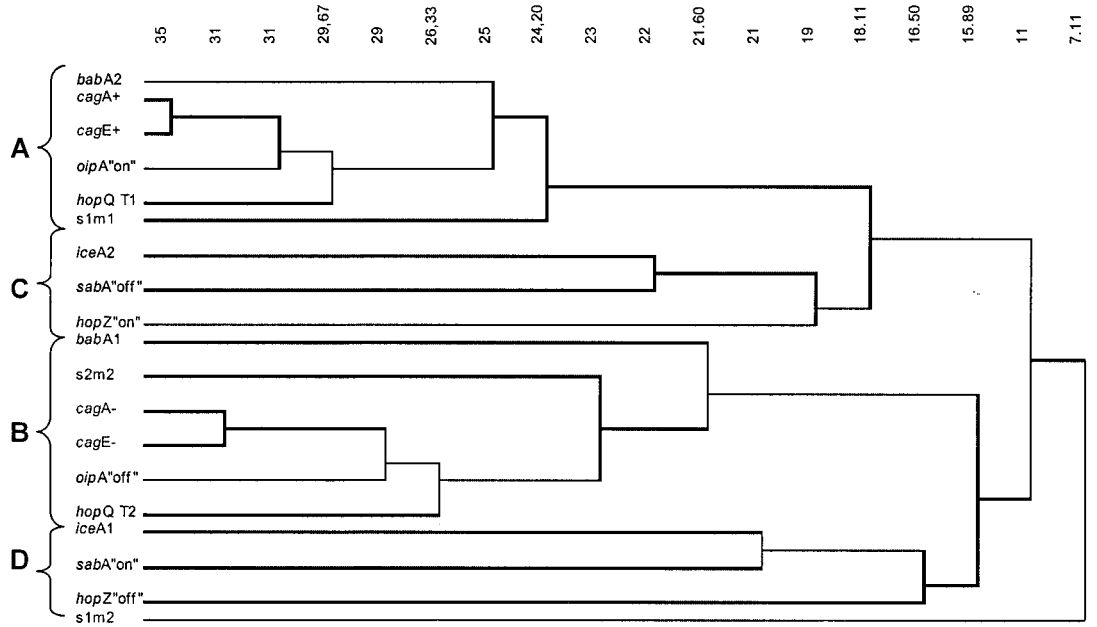

FIG. 1. Dendrogram of the distribution of 70 *H. pylori* strains from both strain populations for which complete data were available for all variables. Strains have been grouped according to *cagA*, *cagE*, *vacA*, *babA*, *iceA*, *hopQ*, *oipA*, *hopZ*, and *sabA* status.

were in line with these results. In contrast, when analyzing the summary variables according to the MCA results, it was remarkable that the odds of having MALT lymphoma among patients harboring *iceA1*, *sabA* on, and *hopZ* off strains (OR, 10.3; 95% CI, 1.2 to 86.0) were 10 times higher than for the other defined groups; indeed among a total of 11 strains having these three markers, 10 (90.9%) corresponded to MALT strains ($P = 0.018$) (Table 4 and Fig. 4).

DISCUSSION

The present study investigated the influence of the *cagA*, *cagE*, *vacA*, *babA*, *iceA*, *hopQ*, *oipA*, *hopZ*, and *sabA* genes on the outcome of *H. pylori*-associated disease by comparing two populations of *H. pylori* strains involved in gastric MALT lymphoma and in gastritis only. In comparison with previous studies concerning MALT lymphoma, the present study evaluated the prevalence and correlation of most *H. pylori* virulence factors and included the largest collection of strains.

We found that among the various putative virulence factors tested considered individually, none can be associated with MALT strains; however, the MCA allowed us to define two main clusters (groups A, *cagA* positive, *cagE* positive, *vacA* s1m1 *babA2 hopQ* T1 *oipA* on; and group B, *cagA* negative, *cagE* negative, *vacA* s2m2 *babA1 hopQ* T2 *oipA* off). The *cagA* gene, the first to be found differentially present in *H. pylori* strains, is considered a marker for the presence of the *cag* PAI (15). This region includes a number of other genes forming a type IV secretion system and is associated with increased virulence and severe clinical outcome, such as severe gastritis, duodenal ulceration, and gastric adenocarcinoma (2, 12). However, the region has not been clearly associated with gastric MALT lymphoma. In 1997, Witherell et al. (56) reviewed all of the publications that examined the role of *cagA*-positive strains in gastric MALT lymphoma and concluded that CagA-positive strains did not contribute to the disease outcome. Since then, several controversial results essentially based on serological data (detection of CagA antibodies) have been published (17, 30, 45, 50, 51). However, a consensus seems to have been found, indicating that *H. pylori* strains expressing CagA protein are not associated with gastric MALT lymphoma but are associated with diffuse large B-cell lymphoma. Our results based on a large collection of well-characterized MALT strains are consistent with this observation and allowed us to conclude that the CagA pathogenicity marker is not associated with gastric MALT lymphoma. We also tested the *cagE* gene of the *cag* PAI, which was found associated with the development of precancerous lesions in the Mongolian gerbil model (38). We observed a strong correlation between *cagA* and *cagE*, which is in favor of the accuracy of our results. Furthermore, it may happen that the *cag* PAI even when present is not functional (i.e., is not capable of transferring CagA into the cells), and this possibility has not been taken into account in most of the studies. Approximately 50% of the *H. pylori* strains produced the VacA cytotoxin (16). Specific *vacA* genotypes have been associated with different levels of cytotoxin production as well as with different clinical outcomes (5, 7). While the distributions of *vacA* alleles were not different between MALT and gastritis strains, we found as expected a good correlation between the *vacA* s1m1 genotype (corresponding to a high level of cytotoxin production) and *cagA* and *cagE*, suggesting that these virulence genes are closely associated: however, this does not explain the evolution toward MALT lymphoma.

Our results concerning *iceA* are in line with previous published data (20, 43) that showed that a heterogeneity of the *iceA2* gene did exist, due to the presence of a varying number of cassettes.

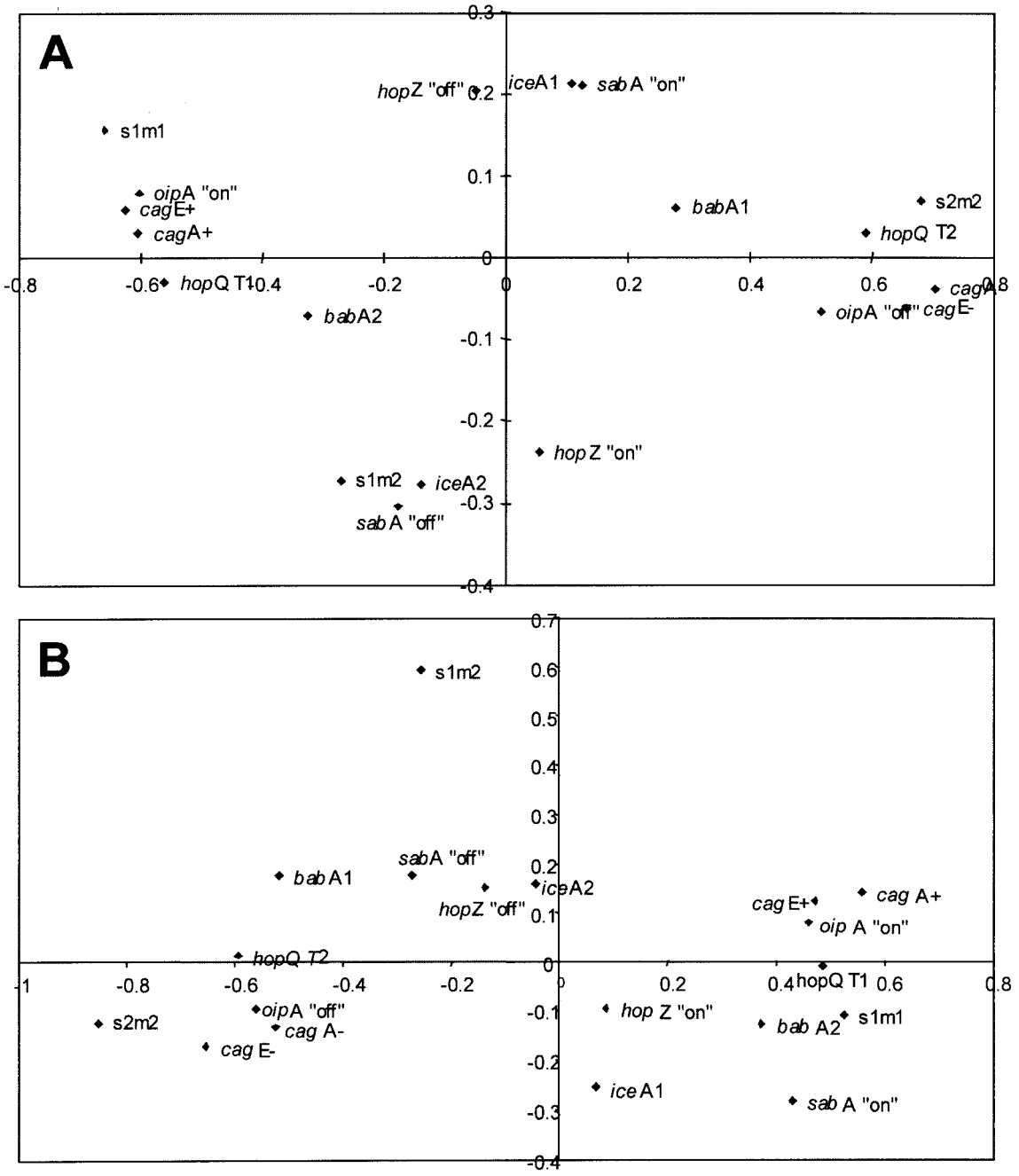

FIG. 2. MCA plot of 39 and 31 *H. pylori* strains isolated from patients suffering from gastric MALT lymphoma (A) and from gastritis (B), respectively, for which complete data were available according to *cagA*, *cagE*, *vacA*, *babA*, *iceA*, *hopQ*, *oipA*, *hopZ*, and *sabA* status.

Increased interleukin-8 (IL-8) production has been found to be linked to the presence of a functional *oipA* gene. This IL-8 production is enhanced when the *cag* PAI is present (61). The *oipA* gene has also been used to discriminate duodenal ulcer from gastritis (58). Our results are in agreement with those of the previous study, which showed a good correlation between *oipA* on status and *cagA*, *cagE*, and *vacA* s1m1 genotypes. The o*ipA* on status was significantly related to *H. pylori* density and neutrophil infiltration (58). Because we found no difference between MALT and gastritis strains in *oipA* functional status as well as for *cag* PAI, we can hypothesize that the MALT pathogenesis is not linked to proinflammatory properties of *H. pylori* strains.

The *babA2* and *hopQ* genes encoding outer membrane proteins were reported to be related to the *cag* PAI and might contribute to virulence (49). For example, *babA2*, which plays a central role in *H. pylori* adherence (9, 25), has been associated with duodenal ulcer and gastric cancer (22), and the type I *hopQ* allele has been associated with *cagA*-positive s1 *vacA*-type strains from patients with ulcer disease (10). Our results confirm this correlation for both groups of strains (MALT and gastritis). However, Gerhard et al. found an inverse correlation

TABLE 4. Relationship between the genotypes of *H. pylori* strains and MALT diagnosis[a]

Gene characteristic	Population with gene	Population with MALT	% MALT (95% CI)[b]	OR (95% CI)
sabA	76			
Off	36	16	44.4 (27.9–61.9)	Baseline
On	40	25	62.5 (45.8–77.3)	2.1 (0.8–5.2)
iceA	82			
iceA2	43	19	44.2 (29.1–60.1)	Baseline
iceA1	39	24	61.5 (44.6–76.6)	2.0 (0.8–4.9)
hopZ	76			
Off	39	18	46.2 (30.1–62.8)	Baseline
On	37	28	75.7 (58.8–88.2)	1.9 (0.8–4.8)
MCA summary variables	70			
ice and *sabA* genes				
Others	49	24	49.0 (34.4–63.7)	Baseline
iceA1 and *sabA* on	21	15	71.4 (47.8–88.7)	2.6 (0.9–7.8)
iceA, *sabA*, and *hopZ* genes				
Others	59	29	49.2 (35.9–62.5)	Baseline
iceA1, *sabA* on, and *hopZ* off	11	10	99.9 (58.7–99.8)	10.3 (1.2–86.0)
3rd MCA variable				
Group A[c]	12	5	41.7 (15.2–72.3)	Baseline
Group B[d]	16	10	62.5 (35.4–84.8)	2.3 (0.5–10.8)
Others	42	24	57.1 (41.0–72.3)	1.9 (0.5–6.9)

[a] ORs are presented as univariate results.
[b] CI, exact binomial 95% CI.
[c] Group A, isolates *cagA* positive, *cagE* positive, *vacA* s1m1 *babA2 hopQ* T1 *oipA* on.
[d] Group B, isolates *cagA* negative, *cagE* negative, *vacA* s2m2 *babA1 hopQ* T2 *oipA* off.

of triple-positive strains (*babA2*, *cagA* positive, and *vacA* s1m1 genotype) with the presence of MALT strains (22). But, in their study, 29 MALT strains isolated in Germany were compared to a group of strains isolated mainly from gastric adenocarcima and duodenal ulcer patients, which were essentially *cagA* positive (92.6 and 100%, respectively), and were not compared to gastritis strains only, in contrast to our study. This can explain the different results. The distribution of the A and B groups (based on *cagA*, *cagE*, *vacA* alleles, *babA*, *oipA* status, and *hopQ* genotypes) did not allow identification of any dif-

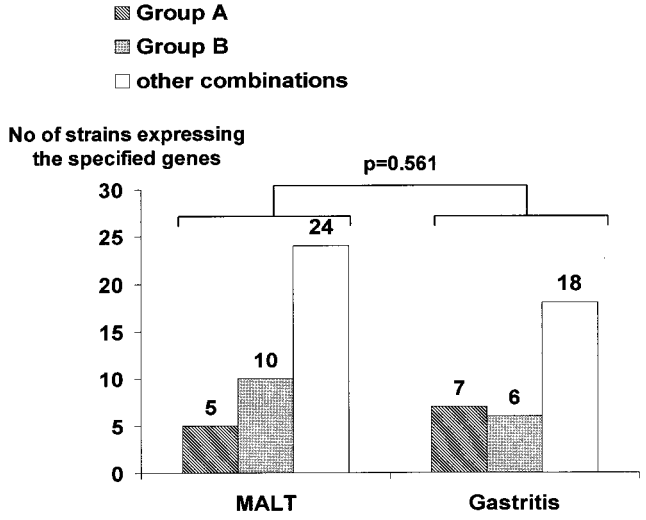

FIG. 3. Distribution of the strains harboring the group A and group B combinations among *Helicobacter pylori* strains isolated from patients with gastric MALT lymphoma or gastritis only.

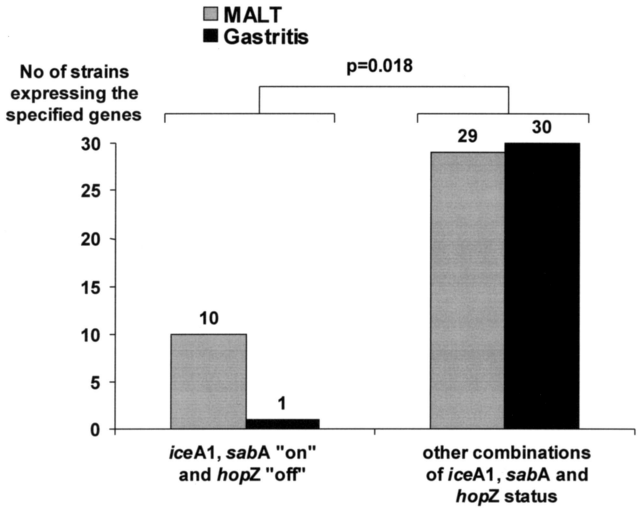

FIG. 4. Distribution of strains harboring the triple combination *iceA1*, *sab*A on, and *hop*Z off among *H. pylori* strains isolated from patients with gastric MALT lymphoma or gastritis only.

ference, but the association of these genes in a cluster probably indicates that such strains have a selective advantage.

Nevertheless, MCA results also show that for patients harboring *iceA1*, *sabA* on, and *hopZ* off strains, the odds of developing a gastric MALT lymphoma are 10 times higher. The *iceA* gene, which has a significant homology to a type II restriction endonuclease, has been described as a pathogenic factor. The *iceA1* allelic variant is upregulated upon contact of *H. pylori* with human gastric epithelial cells and has been associated with peptic ulcer disease and gastric adenocarcinoma (28, 42, 44, 55). The *sabA* and *hopZ* genes code for two adhesins that have been identified, but no association with a clinical outcome of *H. pylori* infection has yet been reported (34, 41). For the first time, we have demonstrated in this study an association between the presence of a *sabA* functional status with an *iceA1* allele and *hopZ* off status of *H. pylori* strains and MALT lymphoma. The association between a gene that is activated by contact with the gastric epithelium and an adhesin seems to be in line with their primary roles. The analysis of the *sabA* functional status confirms the heterogeneity of this gene among *H. pylori* strains and shows for the first time that this marker can be associated with an *iceA1* allele and, to a lesser extent, with a clinical outcome. However, the low prevalence of such strains (10 of 43 MALT strains) makes this triple association as a marker poorly sensitive while specific for MALT strains. It has been shown that *sabA* inactivation does not affect adherence mediated by the *babA* adhesin, implying that these two adhesins are organized and expressed as independent units (34). Our results support this observation, because we found no correlation between them, and moreover the *sabA* functional status and *babA2* are not present in the same clusters.

Since a very low proportion of *H. pylori*-infected patients will develop a gastric MALT lymphoma, our results show that current pathogenicity markers cannot identify the strains associated with gastric MALT lymphoma. Although the involvement of *H. pylori* in this disease is now well established, the pathomechanism by which a clonal expansion of B cells occurs in a background of chronic gastritis remains to be identified. However, our current data identified a polymorphism in the recently described *sabA* gene that supports the hypothesis that a small proportion of MALT strains may use the SabA adhesin to adhere to sialylated glycoconjugates expressed during chronic inflammation and thus may be able to contribute to the chronicity of *H. pylori* infection.

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