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Characterization of *Helicobacter pylori cagA* and *vacA* Genotypes among Alaskans and Their Correlation with Clinical Disease▽

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Helicobacter pylori* infection is common in Alaska. The development of severe *H. pylori* disease is partially determined by the virulence of the infecting strain. Here we present *vacA* and *cagA* genotype data for *H. pylori* strains isolated from Alaskans and their correlation with clinical disease. We enrolled patients scheduled for esophagogastroduodenoscopy and positive for *H. pylori* infection. Gastric biopsy specimens from the stomach antrum and fundus were cultured. We performed PCR analysis of the *H. pylori vacA* gene and for the presence of the *cagA* gene and *cagA* empty site. We genotyped 515 *H. pylori* samples from 220 Native and 66 non-Native Alaskans. We detected the *cagA* gene in 242/286 (85%) persons; of 222 strains that could be subtyped, 95% (212) were non-Asian *cagA* and 3% (6) were East Asian *cagA*. After removing mixed infections ($n = 17$), 83% of *H. pylori* strains had either the *vacA* s1m1 (120/269) or s2m2 (103/269) genotype. Sixty-six percent (68/103) of *H. pylori* strains with the *vacA* s2m2 genotype also contained the *cagA* gene. Infection with an *H. pylori* strain having the *cagA* gene or *vacA* s1m1 genotype (compared with s1m2 and s2m2) was associated with a decreased risk of esophagitis ($P = 0.003$ and 0.0003, respectively). Infection with an *H. pylori* strain having the *vacA* s1m1 genotype (compared with s1m2 and s2m2) was associated with an increased risk of peptic ulcer disease (PUD) ($P = 0.003$). The majority of *H. pylori* strains in this study carried the non-Asian *cagA* gene and either the *vacA* s1m1 or s2m2 genotype. A majority of *H. pylori* strains with the *vacA* s2m2 genotype also contained the *cagA* gene. There was an association of *H. pylori* genotype with esophagitis and PUD.

Helicobacter pylori is one of the most common infections of humans, with over 80% of persons infected in some developing countries (5). Persons infected with *H. pylori* have mild to severe gastric mucosal inflammation, but in some people infection leads to peptic ulcer disease (PUD) (28). Additionally, *H. pylori*-infected persons have at least a 2-fold increased risk of developing gastric cancer compared with uninfected persons, and *H. pylori* is characterized by the World Health Organization as a class 1 carcinogen and a risk factor for noncardia gastric adenocarcinoma (18, 45). It is estimated that persons infected with this organism have a 10 to 20% lifetime risk of developing PUD and a 1 to 2% lifetime risk of developing gastric cancer (20, 21). The risk of developing these diseases depends upon the inflammatory response to chronic colonization, which is thought to be determined by a combination of factors, including the virulence of the infecting strain, the host's response to infection, and environmental cofactors.

Two putative bacterial markers of virulence are the cytotoxin-associated gene pathogenicity island (*cag* PAI) and the vacuolating cytotoxin gene (*vacA*). The *cagA* gene, which codes for a 125- to 145-kDa protein, CagA, is a marker for the presence of the *cag* PAI; however, not all strains with an expressed CagA protein express the entire set of *cag* PAI proteins (6, 7, 12). In western populations, persons infected by *H. pylori* strains containing the *cag* PAI usually have an elevated inflammatory response and are at a higher risk of developing PUD or gastric cancer than persons infected by *H. pylori* strains without the *cag* PAI (34, 36). Because over 90% of *H. pylori* strains isolated from East Asia contain the *cag* PAI, the relationship between the *cag* PAI and clinical outcome is difficult to establish in Asian populations (39).

Active VacA is a toxin that induces massive vacuolization in epithelial cells *in vitro*. Although all strains of *H. pylori* have a *vacA* gene, there is variation in the amount of vacuolating activity due to sequence heterogeneity within the *vacA* gene at the 5′ end (signal [s] region) and the middle (m) region. Two allelic s region types have been identified, s1 and s2. The s1 type can be further subtyped as s1a, s1b, and s1c. Two allelic m region types have been identified, m1 and m2. The m1 type can be further subtyped as m1a and m1b. In HeLa cells, the level of vacuolating activity is high in *H. pylori* strains with the s1m1 genotype, intermediate in those with the s1m2 genotype, and low or absent in those with the s2m2 genotype (3). Many groups have found a correlation between toxin activity and the pathogenicity of *H. pylori*; the v*acA* s1m1 genotype is often associated with PUD and gastric cancer in western populations and some multiregional studies (3, 33, 40). The *vacA* s1m1 genotype is often linked with the presence of the *cag* PAI, and the *vacA* s2m2 genotype is usually linked with the absence of the *cag* PAI (3).

H. pylori infection is common in Alaska. In a statewide survey of over 2,000 samples of blood collected in the 1980s,

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75% of Alaska Native persons were positive for antibodies to *H. pylori*, indicating a current or past infection (35). The high prevalence of *H. pylori* in Alaska, along with high levels of antimicrobial resistance (8–10), makes it difficult to test and treat all infected persons. Therefore, it is important to identify those persons at a higher risk for severe disease to allow optimal clinical treatment and intensive follow-up. In addition, the determination of the genotype of *H. pylori* isolates from this population may allow us to further understand the relationship between putative virulence genes and clinical disease. We present here the first large investigation of the *vacA* and *cagA* genotypes from *H. pylori* strains isolated in different ethnic groups in Alaska and their correlation with clinical disease.

MATERIALS AND METHODS

Participants. Participants in this study were a subset of those recruited as part of a previously described study of *H. pylori* reinfection conducted from September 1998 through January 2005 (29). Briefly, patients ≥18 years of age undergoing esophagogastroduodenoscopy (EGD) were recruited at three urban medical facilities in Anchorage, AK, and three rural medical facilities in western Alaska. Patients were eligible for enrollment into the parent study if they had a positive [^{13}C]urea breath test (UBT; Meretek Diagnostics Inc., Lafayette, CO) at the time of their EGD. Persons were eligible for this study if their *H. pylori* infection was also confirmed by culture. Persons were excluded from study consideration if they were pregnant, had a prior gastric resection, had gastric cancer, or were undergoing active chemotherapy. There were two groups of patients: (i) Alaska Native persons (the indigenous peoples of Alaska) living in Anchorage or one of three regions in western Alaska and (ii) non-Native persons (nonindigenous people residing in Alaska) living in Anchorage. At enrollment, a research nurse interviewed participants by using a standardized form to obtain demographic data and information about symptoms of abdominal discomfort. A medical chart review was conducted to determine if there was a history of chronic stomach problems, specifically PUD, gastritis, or gastric surgery. Clinical diagnoses of PUD, gastritis, duodenitis, and esophagitis were determined by endoscopic evaluation using a scoring system described previously (29). All endoscopists went through extensive training to ensure that patients were evaluated in a standardized manner.

The study was approved by the Centers for Disease Control and Prevention, the Alaska Area, and the Western Institutional Review Boards as well as the Alaska Native Tribal Health Consortium and four tribal health boards: Southcentral Foundation, Yukon-Kuskokwim Health Corporation, Bristol Bay Native Health Corporation, and Norton Sound Health Corporation. Study participants provided written informed consent.

***H. pylori* isolates and DNA extraction.** During the EGD, gastric biopsy specimens were collected from the antrum and/or fundus of the stomach. The participant's endoscopist determined how many biopsy specimens were collected and from where they were collected based upon the clinical needs of the patient. Biopsy specimens were cultured as previously described (30), and all *H. pylori* organisms were stored at −80°C. At the time of DNA extraction, stored *H. pylori* organisms were cultured on Trypticase soy agar plates containing 5% sheep blood (Remel, Lenexa, KS) and incubated for 3 to 5 days at 37°C in 12% CO_2 and high humidity. *H. pylori* cells from each plate were collected together by sweeping a 1-μl inoculating loop across the medium. The *H. pylori* cells were placed into 100 μl of phosphate-buffered saline and vortexed to create a uniform turbidity. DNA was extracted with the MagNA Pure Compact using MagNA Pure Compact nucleic acid isolation kit I and the DNA Cultured Cells v3.1 protocol (Roche Applied Science, Indianapolis, IN). This protocol uses proteinase K and a chaotropic salt-containing lysis buffer to lyse the cells and magnetic glass particles to collect the nucleic acids.

PCR primers and probes. PCR analysis was performed on *H. pylori* DNA samples to genotype the *vacA* s and m regions and to detect the presence of the *cagA* gene and the *cagA* empty site using previously described primers (Table 1). Real-time PCR probes were designed from sequences obtained from the National Center for Biotechnology Information database by using Primer3 software (Table 1). Probes were tested for potential cross-reactivity with nonhomologous viral and bacterial sequences by using the Basic Local Alignment Search Tool software and were tested for specificity by using DNA collected from various *Helicobacter*, *Campylobacter*, and *Wolinella* species.

***cagA* genotyping.** All samples were initially tested with the Smartcycler real-time detection system (Cephid, Sunnyvale, CA), using Omnimix beads and fluorescent probes (Table 1) to amplify the 3′ end of the *cagA* gene and identify either the East Asian or non-Asian *cagA* gene (48). Samples negative by these two reactions were tested with a Stratagene MX3000 instrument using the Stratagene Brilliant reaction mixture (Stratagene, La Jolla, CA) to detect the 5′ end of the *cagA* gene (32). Products from this reaction were viewed on a 1% agarose gel. Samples positive for the *cagA* 5′ reaction were tested once more to delineate between the East Asian and non-Asian *cagA* genes; however, this time, a conventional detection system (1% agarose gel), rather than a real-time system, was used to view the products.

To detect the *cag* PAI empty site (indicating the absence of the *cag* PAI) (32), samples were tested with an Applied Biosystems 9700 thermal cycler using Applied Biosystems 10× PCR buffer (Applied Biosystems Inc., Foster City, CA). Products were viewed on a 1% agarose gel.

***vacA* genotyping.** All samples were tested with the Stratagene MX3000 real-time detection system using the Stratagene Brilliant quantitative PCR (QPCR) mixture (Stratagene, La Jolla, CA) and previously described primers (Table 1) (3, 32, 46). Products from the v*acA* s1b and s1c subtypes were viewed on a 3% agarose gel. All other *vacA* allelic types and subtypes were detected by using fluorescent probes (Table 1). To confirm the results generated by PCR, the *vacA* s and m regions from a subset of samples were sequenced on an Applied Biosystems 3130 genetic analyzer (Applied Biosystems Inc., Foster City, CA) according to previously described methods (3, 4).

Statistical analysis. For all statistical comparisons, persons infected by *H. pylori* strains with mixed *vacA* allelic types (s1/s2 or m1/m2) or incomplete *vacA* genotypes (the m region or s region could not be typed) were removed. Persons with mixed allelic subtypes (i.e., s1a/s1b or m1a/m1b) were considered to be s1 and m1, respectively, and were not excluded from the analysis. Mixed *vacA* infections occurred because (i) *H. pylori* strains cultured from the antrum and fundus of one participant had different genotypes or (ii) *H. pylori* strains cultured from a single site (antrum or fundus) contained more than one genotype. Unless stated otherwise, persons infected with both *cagA*-positive and *cagA*-negative colonies of *H. pylori* were considered to be infected with a *cagA*-positive strain. The likelihood ratio χ^2 test was used to test for differences in the two groups (Alaska Native and non-Native persons), and *P* values were exact when the sample size necessitated.

For the correlation of *H. pylori* genotypes with clinical data, we restricted the evaluation to three combinations of *vacA* s and *vacA* m regions with adequate sample sizes for evaluation: s1m1, s1m2, and s2m2. We compared clinical variables between these three genotypes with the likelihood ratio χ^2 test and analysis of variance (ANOVA) for categorical and continuous variables, respectively. All *P* values are two sided, and a *P* value of <0.05 was considered statistically significant.

RESULTS

Study participants. Genotyping was performed on 515 *H. pylori* samples collected from 220 Alaska Native and 66 non-Native persons (Table 2). Sixty-eight participants (24%) had one sample available (from either the antrum or fundus), 211 participants (74%) had two samples available (from both the antrum and fundus), and 7 participants (2%) had three or more samples available (more than one sample from the antrum and/or fundus). Endoscopic reports were available for 260 (91%) of the 286 participants. The most common clinical diagnoses were mild to moderate gastritis (198/260 [76%]), followed by esophagitis (63/260 [24%]). Fifty-eight percent (152/260) of participants had either a healthy stomach (33/260 [13%]) or gastritis only (119/260 [46%]). All Alaska Native persons and 68% (45/66) of non-Native persons were born in the United States.

***cagA* genotyping.** The *cagA* gene was detected in *H. pylori* isolates from 242/286 (85%) participants (Table 3). The *cagA* empty site (indicating the absence of the *cag* PAI) was detected in *H. pylori* strains from 99/286 (35%) participants. Among the 99 participants with the *cagA* empty site detected, 55 (56%) also had the *cagA* gene, and 44 (44%) were positive for the

TABLE 1. PCR primer and probe sequences for amplification of the *cagA* and *vacA* genes

Region	Primer or probe	Oligonucleotide sequence	Reference
cagA, East Asian	CAG1F	5′-TGG AAC CCT AGT CGG TAA TGG G	48
	CAG1R	5′-TGA TGC AAT TTT GTT AAT CCG GTC	48
	CAG1P	5′-TCT AAA ACA GAA GCC ACA ACG CTC ACC	
cagA, non-Asian	CAG2F	5′-AAT GCA AAA ATT GAC CRA CTC AAT C	48
	CAG2R	5′-AAA CCT GCT TTA GCT TCT GAY ACY GC	48
	CAG2P	5′-CTT CCC TTT GAA AAG GCA TGA TAA AGT TG	
cagA, 5′ end	CAGF	5′-AAT GGT GGT CCT GGA GCT AG	32
	CAGR	5′-GGA AAT CTT TAA TCT CAG TTC GGA A	32
cag PAI empty site	Luni1	5′-CA TTT TGG CTA AAT AAA CGC TG	32
	r5280 M	5′-TTG CAC GCA TTT TCC CTT AAT C	32
vacA s1a	SS1-F	5′-GTC AGC ATC ACA CCG CAA C	3
	VAI-R	5′-CTG CTT GAA TGC GCC AAA C	3
	S1aP	5′-AAA CAA GCC GAA GAA GCC AAT AAA ACC CC	
vacA s1b	SS3-F	5′-AGC GCC ATA CCG CAA GAG	3
	VAI-R	5′-CTG CTT GAA TGC GCC AAA C	3
vacA s1c	S1c-F	5′-CTY GCT TTA GTR GGG YTA	46
	VAI-R	5′-CTG CTT GAA TGC GCC AAA C	3
vacA s2	SS2-F	5′-GCT AAC ACG CCA AAT GAT CC	3
	VAI-R	5′-CTG CTT GAA TGC GCC AAA C	3
	S2P	5′-ACA ACY GTG ATY ATT CCA GCC ATT GTT GG	
vacA m1a	VA3-F	5′-GGT CAA AAT GCG GTC ATG G	3
	VA3-R	5′-CTA ATG CCA TTG GTA CCT GTA GAA AC	3
	M1aP	5′-YGT AGG CAA TGC AGC AGC TAT GAT GTT TA	
vacA m1b	VAM-F3	5′-CCC CAA TGC AGT CAT GGA T	32
	VAM-R3	5′-GCT GTT AGT GCC TAA AGA AGC AT	32
	M1bP	5′-CAC TAT CAA TTA TTT GGT TCG AGG CGG GAA	
vacA m2	VA4-F	5′-GGA GCC CCA GGA AAC ATT G	3
	VA4-R	5′-TGT CAT AAC TAG CGC CTT GCA C	3
	M2P	5′-CTT TTT GTC CAA GAT GGG CGT GTA GC	

cagA empty site only. Alaska Native persons were more likely than non-Native persons to be infected with *H. pylori* containing the *cagA* gene (198/220 [90%] versus 44/66 [67%]; $P < 0.0001$). The *cagA* genes from 222/242 (92%) participants were further characterized. A total of 212/222 (95%) participants had a non-Asian *cagA*-positive strain, 6/222 (3%) participants had an East Asian *cagA*-positive strain, and 4/222 (2%) participants had both non-Asian and East Asian *cagA*-positive strains.

***vacA* genotyping.** The *vacA* s1a subtype was identified in *H. pylori* strains from 130/286 (45%) participants, the *vacA* s1b subtype was identified from 31/286 (11%) participants, the *vacA* s1c subtype was identified from 6/286 (2%) participants, and the *vacA* s2 type was identified from 105/286 (37%) participants (Table 3). The remaining 14 participants had mixed *H. pylori vacA* s region types or subtypes (s1a/s2, $n = 8$; s1b/s2, $n = 2$; s1c/s2, $n = 1$; s1a/s1b, $n = 1$; s1a/s1c, $n = 1$; s1a/s1b/s2, $n = 1$).

DNA sequencing of the *vacA* s region was completed on *H. pylori* samples from 33% (94/286) of study participants. The samples represented a variety of *vacA* s region types or subtypes, as determined by PCR (s1a, $n = 35$; s1b, $n = 12$; s1c, $n = 2$; s2, $n = 45$). All 94 sequences corroborated the PCR results.

The *vacA* m1a subtype was identified in *H. pylori* isolates from 73/286 (26%) participants, the *vacA* m1b subtype was identified from 24/286 (8%) participants, and the *vacA* m2 type was identified from 157/286 (55%) participants (Table 3). The remaining 30 participants had mixed *H. pylori vacA* m region types or subtypes (m1a/m1b, $n = 24$; m1a/m2, $n = 4$; m1b/m2, $n = 1$; m1a/m1b/m2, $n = 1$).

DNA sequencing of the *vacA* m region was completed on *H. pylori* samples from 33% (94/286) of study participants. The samples represented a variety of *vacA* m region types or subtypes, as determined by PCR (m1a, $n = 27$; m1b, $n = 6$; m2, $n = 61$). All 94 sequences corroborated the PCR results.

Two hundred sixty-nine participants (94%) had complete *vacA* genotyping results with no mixed infections. The *vacA* s1m1 genotype was identified in 120 (45%) participants, the *vacA* s1m2 genotype was identified in 48 (18%) participants, the *vacA* s2m1 was identified in 1 (0.4%) participant, and the *vacA* s2m2 genotype was identified in 103 (38%) participants (Fig. 1). Compared with the other allelic combinations, Alaska Native persons were less likely than non-Native persons to be

TABLE 2. Study participant demographics and clinical diagnoses, Alaska, 1998 to 2005

Characteristic	Value
Demographics (n = 286)	
Mean age (yr) (min, max)	49 (18, 88)
No. (%) of patients	
Female	173 (60)
Alaska Native	220 (77)
Place of birth	
United States	265 (93)
Asia	9 (3)
Non-U.S. Americas	7 (2)
Europe	4 (1)
Middle East	1 (0.3)
No. (%) of patients taking medications (n = 286)	
NSAID[a]	34 (12)
PPI[b]	81 (28)
H_2 blocker[c]	113 (40)
Heavy alcohol[d]	14 (5)
Use of ≥1 of the above medications	196 (69)
No. (%) of patients with medical history (n = 286) of:	
Gastritis (5 yr)	110 (38)
Peptic ulcer disease	38 (13)
No. (%) of patients with endoscopic evaluation result[e] (n = 260) of:	
Duodenitis	46 (18)
Esophagitis	63 (24)
Ulcer	23 (9)
Gastric ulcer	18 (7)
Gastritis	
Mild	103 (40)
Moderate	95 (37)
Severe	20 (8)

[a] NSAID, nonsteroidal anti-inflammatory drugs (daily use).
[b] PPI, protein pump inhibitor (use within 30 days of enrollment).
[c] Use within 30 days of enrollment.
[d] At least 3 drinks daily.
[e] Thirty-three (13%) persons had healthy stomachs; 100 (38%) persons had more than one diagnosis.

TABLE 3. *cagA* and *vacA* genotypes of *Helicobacter pylori* strains isolated from Alaska Native and non-Native persons in Alaska, 1998 to 2005

Genotype	No. (%) of patients with genotype		
	Alaska Native (n = 220)	Non-Native (n = 66)	Total (n = 286)
cagA gene			
Absent[a]	22 (10)	22 (33)	44 (15)
Present[b]	198 (90)	44 (67)	242 (85)
vacA s region type			
s1a	108 (49)	22 (33)	130 (45)
s1b	14 (6)	17 (26)	31 (11)
s1c	0 (0)	6 (9)	6 (2)
s2	86 (39)	19 (29)	105 (37)
Mixed[c]	12 (5)	2 (3)	14 (5)
vacA m region type			
m1a	55 (25)	19 (29)	73 (26)
m1b	19 (9)	5 (8)	24 (8)
m2	117 (53)	40 (61)	157 (55)
Mixed[c]	29 (13)	3 (5)	32 (11)

[a] Only the *cagA* empty site is present.
[b] Includes isolates from persons where both the *cagA* gene and the *cagA* empty site are present.
[c] More than one allelic type/subtype is present in the same participant, suggesting infection with more than one *H. pylori* strain.

infected with *H. pylori* strains having the *vacA* s1m2 genotype (26/205 [13%] versus 19/64 [30%]; $P = 0.003$).

***vacA* genotyping and *cagA* status.** A total of 84 to 100% of *H. pylori* strains having the *vacA* s1m1 (119/120), s1m2 (38/45), and s2m1 (1/1) genotypes contained the *cagA* gene (Fig. 2). Sixty-six percent (68/103) of *H. pylori* strains with the *vacA* s2m2 genotype contained the *cagA* gene. Of *H. pylori* strains having the *vacA* s2m2 genotype, those that also contained the *cagA* gene were more likely to be isolated from Alaska Native persons than non-Native persons (67/84 [80%] versus 1/19 [5%]; $P < 0.0001$). When persons infected with both *cagA*-positive and *cagA*-negative colonies of *H. pylori* (n = 23 Alaska Native persons and 0 non-Native persons) were removed from the analysis, Alaska Native persons remained more likely than non-Native persons to be infected with *H. pylori* strains having the *vacA* s2m2 genotype and the *cagA* gene (44/61 [72%] versus 1/19 [5%]; $P < 0.0001$).

***H. pylori* genotypes and place of birth.** *vacA* and *cagA* genotyping results were stratified by the place of birth of the study participants (Table 4). All participants (6/6) infected with an East Asian *cagA*-positive strain of *H. pylori* were born in Asia, and 99% (209/212) of participants infected with a non-Asian *cagA*-positive strain were born in a non-Asian country. Participants born in Asia were more likely to be infected with *H. pylori* having the *vacA* m1b subtype than those born in a non-Asian country (5/8 [63%] versus 19/246 [8%]; $P < 0.001$). Additionally, all participants infected with a strain of *H. pylori* having the *vacA* s1c subtype (6/6) were born in Asia. The six strains of *H. pylori* with an East Asian *cagA* gene were more likely to have the *vacA* s1c (5/6 [83%]) and m1b (5/6 [83%]) subtypes than *H. pylori* strains with a non-Asian *cagA* gene

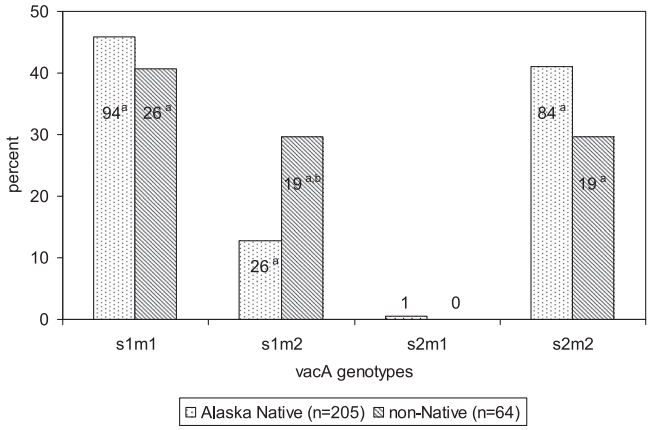

FIG. 1. Proportion of *Helicobacter pylori vacA* genotypes isolated from Alaska Native and non-Native persons in Alaska from 1998 to 2005. a, numbers within the bars represent the number of persons infected with *H. pylori* having that *vacA* genotype; b, $P = 0.003$ for Alaska Native people versus non-Native people.

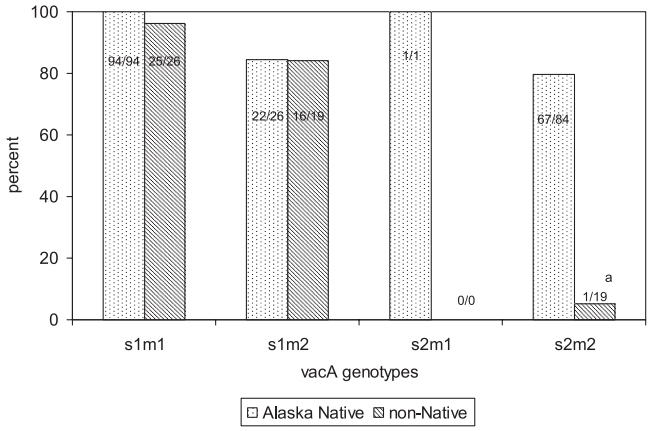

FIG. 2. Percentage of Alaska Native and non-Native persons infected with a *cagA*-positive strain of *Helicobacter pylori* stratified by the *vacA* genotype of their infecting organism in Alaska from 1998 to 2005. Persons with both *cagA*-positive and *cagA*-negative *H. pylori* colonies were considered to be infected with a *cagA*-positive strain. a, $p < 0.0001$ for Alaska Native people versus non-Native people.

(1/200 [0.5%] and 19/181 [10%], respectively; $P < 0.001$ for both).

***H. pylori* genotypes and clinical presentation.** Infection with *H. pylori* strains having the *cagA* gene or the *vacA* s1m1 genotype was associated with a decreased risk of esophagitis (Table 5). When restricting the analysis to participants with a *cagA*-positive strain of *H. pylori*, the *vacA* s1m1 genotype remained significantly associated with a decreased risk of esophagitis (s1m1, 15/107 [14%]; s1m2, 7/31 [23%]; s2m2, 22/67 [33%]; $P = 0.01$). Likewise, when controlling for the two *vacA* genotypes with both *cagA*-positive and *cagA*-negative strains (s1m2 and s2m2), the absence of the *cagA* gene remained associated with esophagitis; however, it was no longer statistically significant (*cagA* positive, 27/98 [28%]; *cagA* negative, 17/37 [46%]; $P = 0.07$). Infection with *H. pylori* having the *vacA* s1m1 genotype was also associated with an increased risk of having an ulcer at study enrollment or a history of PUD. This remained true after the analysis was restricted to participants with a *cagA*-positive strain of *H. pylori* (s1m1, 29/107 [27%]; s1m2, 4/31 [13%]; s2m2, 7/67 [10%]; $P = 0.01$). When the analysis was restricted to participants without possible confounding factors (use of nonsteroidal anti-inflammatory drugs, protein pump inhibitors, H_2 blockers, and/or heavy alcohol), all trends remained the same (data not shown). Participants infected with a strain of *H. pylori* having the *vacA* s2m2 genotype did not differ in their clinical presentations based upon the presence or absence of the *cagA* gene. No other associations were found between *H. pylori cagA* and *vacA* genotypes and clinical diagnoses in this study population, and all associations were similar in Alaska Native and non-Native persons.

DISCUSSION

This paper presents the largest characterization to date of *H. pylori* isolates collected from persons living in Alaska. *H. pylori* infection is common in Alaska (35, 38), and a high proportion of these strains demonstrate antimicrobial resistance (8–10), making it difficult to test and treat all infected persons. It is therefore important to identify persons at a higher risk for disease to allow optimal clinical treatment and intensive follow-up. Previous studies suggested that the *vacA* genotype and the prevalence of the *cagA* gene vary in *H. pylori* isolates collected from different parts of the world and that these genotypic variations affect the clinical presentation in persons infected with the organism (17, 44). It is important, therefore, to collect and report these data from different regions of the world so that we can better understand the relationship between putative virulence genes and clinical disease. The majority of *H. pylori* strains in this study contained the *cagA* gene, and persons infected with *cagA*-positive *H. pylori* strains were less likely to have an esophagitis diagnosis than persons infected with *cagA*-negative *H. pylori* strains. Persons infected with an *H. pylori* strain having the *vacA* s1m1 genotype had a decreased risk of esophagitis and an increased risk of PUD. We also identified a large number of organisms with the *vacA* s2m2 genotype that also contained the *cagA* gene; however, the presence or absence of the *cagA* gene in these organisms did not affect the clinical presentation of the patient.

We detected the *cagA* gene in 85% of *H. pylori* isolates collected from our study participants. Worldwide, the presence of the *cagA* gene varies, from a low of 50% in some Middle Eastern countries (2) to a high of 99% in many East Asian countries (11, 23). The percentage of *cagA*-positive *H. pylori* strains found in our study is most similar to data reported from Europe and regions of North America (74% to 88%) (31, 42, 46). Eighty-three percent of participants in our study were infected with organisms having either the *vacA* s1m1 (45%) or s2m2 (38%) genotype. The *H. pylori vacA* s1m1 genotype is

TABLE 4. *cagA* and *vacA* genotypes of *Helicobacter pylori* isolated from Alaska Native and non-Native persons stratified by the participants' place of birth, Alaska, 1998 to 2005

Place of birth	No. (%) of patients with isolates of genotype								
	cagA[a]		*vacA* s region[b]				*vacA* m region[b]		
	East Asian	Non-Asian	s1a	s1b	s1c	s2	m1a	m1b	m2
United States	0 (0)	201 (95)	124 (95)	27 (87)	0 (0)	101 (96)	68 (93)	19 (79)	147 (94)
Asia	6 (100)	3 (1)	2 (2)	1 (3)	6 (100)[c]	0 (0)	1 (1)	5 (21)[c]	2 (1)
Non-U.S. Americas	0 (0)	5 (2)	2 (2)	3 (10)	0 (0)	2 (2)	4 (5)	0 (0)	3 (2)
Europe	0 (0)	2 (1)	2 (2)	0 (0)	0 (0)	2 (2)	0 (0)	0 (0)	4 (3)
Middle East	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)

[a] Persons with both East Asian and non-Asian *cagA* colony types were removed.
[b] Persons with mixed *vacA* infections were removed.
[c] All isolates also had an East Asian *cagA* gene.

TABLE 5. Clinical presentation of Alaska Native and non-Native persons infected with *Helicobacter pylori* strains with and without the *cagA* gene and with *vacA* genotypes s1m1, s1m2, and s2m2 in Alaska from 1998 to 2005

Clinical presentation	*cagA* status[a]			*vacA* genotype[b]			
	No. (%) of patients		*P* value	No. (%) of patients with genotype			*P* value[c]
	Positive (n = 222)	Negative (n = 38)		s1m1 (n = 107)	s1m2 (n = 37)	s2m2 (n = 98)	
Endoscopic evaluation							
Duodenitis	40 (18)	6 (16)	0.74	22 (21)	9 (24)	15 (15)	0.42
Esophagitis	46 (21)	17 (45)	**0.003**	15 (14)	11 (30)	35 (36)	**0.001**[d]
Moderate or severe gastritis	96 (43)	19 (50)	0.44	48 (45)	19 (51)	44 (45)	0.77
All ulcers	20 (9)	3 (8)	0.82	12 (11)	3 (8)	7 (7)	0.58
Gastric ulcer	16 (7)	2 (5)	0.65	12 (11)	1 (3)	4 (4)	0.07
Medical chart review							
History of gastritis	93 (42)	13 (34)	0.37	46 (43)	11 (30)	43 (44)	0.28
History of PUD	32 (14)	4 (11)	0.51	21 (20)	4 (11)	9 (9)	0.08
Ulcer at enrollment or history of PUD	46 (21)	6 (16)	0.47	29 (27)	4 (11)	12 (12)	**0.01**[e]

[a] Persons with both *cagA*-positive and *cagA*-negative *H. pylori* colonies were considered to be infected with a *cagA*-positive strain.
[b] *vacA* genotype s2m1 was left out due to the small sample size (n = 1).
[c] The *P* value examines differences in clinical outcomes among the 3 *vacA* genotypes. Boldface type indicates significance.
[d] *P* value for s1m1 compared to the other 2 genotypes, 0.0003.
[e] *P* value for s1m1 compared to the other 2 genotypes, 0.003.

common worldwide, with reports of 42% to 84% of *H. pylori* strains from around the globe having this genotype (42). The *vacA* s2m2 genotype is less common, where the reported worldwide prevalences vary from 0% to 57% (19, 42). The *vacA* s2m2 percentage found in our study is among the highest reported, 44% in Alaska Native participants and 29% in non-Native participants. Such a high prevalence has been found in only two other small studies of organisms collected from Australia (8/24 [35%]) and North Africa (16/28 [57%]) (42).

Almost all of the *H. pylori* strains in our study with the *vacA* s1m1, s1m2, and s2m1 genotypes carried the *cagA* gene. Additionally, 66% of *H. pylori* strains with the *vacA* s2m2 genotype also contained the *cagA* gene. In contrast to our data, most studies have found an association between the low-toxin-producing *vacA* s2m2 genotype and less virulent *cagA*-negative *H. pylori* strains (13). With the exception of a single report of a small number of isolates from Portugal and Spain, where 57% (4/7) of the organisms with the *vacA* s2m2 genotype also contained the *cagA* gene (42), we know of no other study with such a high percentage of *vacA* s2m2 *H. pylori* strains that are also *cagA* positive. Almost all (66/67) of the *vacA* s2m2- and *cagA*-positive organisms in our study were isolated from Alaska Native persons. When the analysis was restricted to only Alaska Native persons, 80% of the *H. pylori* strains with the *vacA* s2m2 genotype also contained the *cagA* gene. We did not detect a difference in clinical presentation according to the presence or absence of the *cagA* gene in persons infected with *H. pylori* strains having the *vacA* s2m2 genotype, so it is unclear from our study if these strains are of clinical importance. It is possible that severe disease requires that the more virulent phenotype of both of these genes be present.

In addition to the differences noted above, we did find other significant genotypic differences between our two study groups (Alaska Native and non-Native persons). Specifically, Alaska Native persons were more likely than non-Native persons to be infected with *H. pylori* strains containing the *cagA* gene. We know of only one study investigating the prevalence of *H. pylori* infection in non-Native Alaskans. Those authors found an *H. pylori* seroprevalence of 24% in non-Native school teachers who were living in rural Alaska and were predominantly Caucasian persons born in the contiguous 48 U.S. states; testing for pathogenic genes was not performed in that study (27). In Alaska Native persons, the *H. pylori* seroprevalence is more than three times higher than what was found in that study (35). Documented high rates of gastric cancer in Alaska Native persons (24) may be due in part to the high prevalence of *cagA*-positive *H. pylori* strains in this population.

Many investigators have reported differences in the *cagA* genes in *H. pylori* isolates collected from persons living in East Asian versus non-Asian countries (31, 41, 48). It may be important to distinguish between these two types of *cagA* genes, as the CagA protein coded for by most East Asian strains appears to be more biologically active than most CagA proteins coded for by non-Asian *cagA* genes (16, 26), and this has been associated with an increased risk of PUD and gastric cancer (22). In our study population, 95% of persons infected with a *cagA*-positive strain of *H. pylori* that could be subtyped had a non-Asian *cagA*-positive strain. Only six persons had an East Asian *cagA*-positive strain. The six strains of *H. pylori* with East Asian *cagA* were also more likely to have the *vacA* s1c and m1b subtypes than *H. pylori* strains with non-Asian *cagA*. These two *vacA* subtypes have been linked to *H. pylori* isolates collected from persons living in East Asian countries and are often found in *H. pylori* strains having a non-Asian *cagA* gene (43, 46, 47). All persons infected with an East Asian *cagA* strain in this study were born in an East Asian country, and all but three of the persons infected with *H. pylori* strains having a non-Asian *cagA* gene were born in a non-Asian country.

Three previously published papers have reported data on the genotyping of *H. pylori* strains isolated from persons living in Alaska; however, the number of isolates included in those studies was small, ranging from 2 to 20 isolates per study (1, 15,

47). Two of those studies used multilocus sequence typing to group *H. pylori* isolates (1, 15), while the third study used a PCR methodology similar to that used in this study (47). Just as we have shown in our study, all three of those papers concluded that the majority of *H. pylori* strains that they tested had genes that grouped them with *H. pylori* strains of non-Asian origin.

We found that persons infected with a *cagA*-positive *H. pylori* strain were less likely than persons infected with a *cagA*-negative *H. pylori* strain to have a diagnosis of esophagitis upon endoscopic evaluation. Although it is not universal, many other researchers have seen this same relationship, suggesting a protective role for *cagA*-positive strains of *H. pylori* (25, 37). Persons infected with *H. pylori* isolates having the *vacA* s1m1 genotype were also less likely to have an esophagitis diagnosis than those infected with an isolate having either the *vacA* s1m2 or s2m2 genotype. We continued to see this association even when we restricted the analysis to only *cagA*-positive *H. pylori* strains, indicating that the *vacA* s1m1 genotype is independently negatively associated with esophagitis in our study population. It is biologically plausible that an *H. pylori* strain with the *vacA* s1m1 genotype would be protective against esophagitis because of its high toxin activity, and we hypothesize that *H. pylori* strains having the more virulent *vacA* s1m1 genotype could increase gastric atrophy and decrease acid production and the development of esophagitis.

Persons infected with *H. pylori* strains having the *vacA* s1m1 genotype were also more likely to have an ulcer at enrollment or a history of PUD than those infected with *H. pylori* strains having either the *vacA* s1m2 or s2m2 genotype. Many groups have found a correlation between vacuolating toxin activity and the pathogenicity of *H. pylori*, with the *vacA* s1m1 genotype having high toxin activity, s1m2 having intermediate activity, and s2m2 having low activity (3). Consistent with the findings in our study, in other western countries, infection with an *H. pylori* strain having the *vacA* s1m1 genotype is associated with more severe clinical disease (3, 33).

The original study from which these data were collected was a clinic-based, convenience sample of Alaskans with clinical symptoms requiring EGD evaluation. A limitation of our study, therefore, is that the comparison group for each clinical association was all persons without that diagnosis rather than strictly healthy persons. This could limit our ability to detect all clinical associations with the *H. pylori cagA* and *vacA* genotype. An additional limitation of our study is that over three-quarters of the participants were Alaska Native people, so the results presented here may not be generalizable outside Alaska. However, as rates of gastric cancer, PUD, and *H. pylori* infection are high in the Alaska Native people (14, 24, 35), it is important to specifically investigate organisms causing disease in this population. Finally, other *H. pylori* virulence factors, such as the *vacA* intermediate (i) region genotype and the blood group antigen binding adhesion (*babA*) genotype, may be equally, or more, important predictors of pathogenicity as the presence of the *cagA* gene and the *vacA* s and m region genotypes. It is also possible that the series of amino acids that make up the CagA protein, especially those of the EPIYA motifs, may be important predictors. It was beyond the scope of this project to test for these potential additional predictors of disease; however, we have plans to further analyze these samples as part of a future investigation of possible gastric cancer risk factors.

In conclusion, in this Alaskan population, where we have found high rates of *H. pylori* infection, gastric cancer, and PUD, we found that over 80% of *H. pylori* organisms carry the *cagA* gene, indicating the presence of the putative *cag* PAI virulence marker. In addition, almost half of the *H. pylori* strains contain the high-toxin-producing, and potentially more virulent, *vacA* s1m1 genotype. Infection with an *H. pylori* strain containing the *cagA* gene or the *vacA* s1m1 genotype was associated with a decreased incidence of esophagitis, supporting the hypothesis that infection with more virulent *H. pylori* organisms can protect persons from esophageal diseases. Similar to studies in other western parts of the world, infection with an *H. pylori* strain containing the *vacA* s1m1 genotype was also associated with more severe disease. Finally, two-thirds of the *H. pylori* organisms that had the less virulent *vacA* s2m2 genotype also contained the *cagA* gene, a novel finding for our population.

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