Original Article

Genetic diversity of the *Helicobacter pylori* sialic acid-binding adhesin (*sabA*) gene

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Summary **A putative virulence factor, SabA, a sialic acid-binding adhesin, has recently been characterized in *Helicobacter pylori* from European isolates. However, little genetic information is available for *sabA* genes in strains isolated from Japanese patients. Here, we investigated the presence of the *sabA* gene in 23 *H. pylori* clinical isolates using polymerase chain reaction detection. It was found that 91.3% of *H. pylori* isolates examined contain the *sabA* gene. Sequence comparison and phylogenetic analysis based on the deduced amino acid sequence of *sabA* in nine *H. pylori* isolates from Japanese patients and three *H. pylori* strains from Western individuals suggested that *sabA* is genetically diverse and the clustering of the strains based on SabA is related to their geographical origin. It needs to be further assessed whether the genetic diversity of *sabA* is associated with the clinical outcomes of *H. pylori* infection.**

Keywords: *Helicobacter pylori*, SabA, PCR, phylogenetic tree

1. Introduction

Helicobacter pylori (*H. pylori*) is a human gastroduodenal pathogen that affects at least half the world's population. Colonization of this bacterium in the stomach mucosa results in chronic mucosal inflammation without any clinical symptoms in the majority of infected people. In approximately 10% of infected individuals, however, the chronic inflammation causes a diverse spectrum of gastric diseases, ranging from peptic ulcer to gastric cancer and mucosa-associated lymphoid-tissue (MALT) lymphoma as well (*1*,*2*). Although it is yet unclear what exactly determines the clinical outcomes of *H. pylori* infection, a complex interaction of bacterial virulence factors, host immune response and environmental influences is thought to contribute to different clinical outcomes in *H. pylori* infection (*3*,*4*).

Among the bacterial virulence factors identified so far, *H. pylori* cytotoxin-associated gene A (*CagA*), its related pathogenicity island (*CagPAI*), and vacuolating cytotoxin A (*VacA*) are the best characterized toxins that are strongly linked to the pathogenicity of *H. pylori* (*5*). We currently know that CagA is delivered into gastric epithelial cells by a type IV bacterial secretion system (*6*,*7*). Within gastric epithelial cells, CagA is usually phosphorylated by host cell kinases, and it interacts with multiple host signaling molecules, which result in the morphological changes. Nonphosphorylated CagA can interact with certain host cell proteins and elicit numerous cellular effects (*8-10*), including disruption of tight cell-to-cell junctions, loss of cell polarity, and pro-inflammatory responses. Unlike CagA, VacA is secreted by *H. pylori* and it induces massive vacuolization in gastric epithelial cells (*5*). Recent studies demonstrated that VacA not only causes functional alterations such as increased membrane permeability and apoptosis in gastric epithelial cells but also has multiple effects on both T and B cells (*11*,*12*).

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In addition to these virulence factors, *H. pylori* adhesins such as the Lewis blood group antigen-binding adhesin (BabA) (*13*) and the sialic acid-binding adhesin (SabA) (*14*) are widely believed to play a critical role in initial colonization of *H. pylori* and subsequent persistence of infection. Over the past decade, extensive studies on adherence properties of *H. pylori* have demonstrated that BabA mediates strong binding of the bacteria to gastric epithelial cells and facilitates efficient delivery of virulence factors such as CagA and VacA into host cells (*5,15*). Another adhesin, SabA, which was identified and purified from the *babA*-mutant *H. pylori* strain by Mahdavi *et al*. (*14*), was shown to mediate a weaker binding of the bacteria to sialylated glycoconjugates expressed in inflamed gastric epithelium. Recently, it has also been found that SabA binds specifically to sialylated carbohydrates on the surface of neutrophils and thereby induces pro-inflammatory and immune responses (*5,16*), suggesting that SabA might play an important role in determining the clinical outcome of *H. pylori* infection.

Indeed, epidemiological studies from European and North American countries have revealed the close relationship between gastric cancer and SabA positive status (*14,17-19*). Two reports from Asia (*20,21*), however, indicated that SabA status has little influence on the clinical outcome of *H. pylori* infection. It is not yet known whether the inconsistent result is due to geographic or disease-associated allelic variation in *H. pylori* because there is little genetic information available for *sabA* genes from strains around the world. In the present study, we investigated the presence of the *sabA* gene in *H. pylori* isolates from Japanese patients. Moreover, the nucleotide sequences of the *sabA* gene in ten *H. pylori* isolates from our patients were determined and compared with those of the three *H. pylori* strains whose genome sequences were released previously in the GenBank database (*22-24*).

2. Methods

2.1. *H. pylori clinical isolates*

All *H. pylori* strains used in the study were deep-frozen primary pure *H. pylori* strains isolated from 23 outpatients with gastroduodenal ulcers who underwent upper endoscopy in our university hospital between 2001 and 2003.

2.2. *Culture of H. pylori*

An aliquot of the frozen primary pure *H. pylori* strains described above was thawed and inoculated onto the Pourmedia Vi HELICO AGAR plates (Eiken, Tokyo, Japan). Plates were incubated in a microaerophilic atmosphere with 5-8% CO_2 at 37°C for up to 5 days. The colony was picked up by a toothpick, and transported to a vial containing 20 μL of 1× phosphate-buffered saline (PBS, pH 7.0) and stored at –80°C until polymerase chain reaction (PCR) was performed.

2.3. *Detection of sabA gene by PCR*

The presence of *sabA* in *H. pylori* clinical isolates was analyzed by PCR using three primer pairs separately. Primer pairs 1, 3Fm (5'-CCGCTAGTGTCCAGGGTAAC-3')-5Rm (5'-CACCGCGTATTGCGTTGGGTA-3'), pair 2, 1Fm (5'-GGCTCTAGCAATGTGTGGCAG-3')-5Rm, and pair 3, 3Fm-1Rm (5'-CGCGCTGTAAGG GTTATTGAAC-3'), were used to amplified different fragments of *sabA* gene as reported by Mahdavi *et al*. with mild modification (*14*). Briefly, each amplification was performed in a reaction volume of 25 μL with GeneAmp 10× PCR Buffer (Applied Biosystems, Carlsbad, CA, USA), 1 unit of AmpliTaq Gold DNA Polymerase (Applied Biosystems), 0.2 μM of dNTPs, with 0.2 μM of the forward and reverse primers and with 5 μL *H. pylori* solution from each sample. The amplification consisted of an initial denaturation step at 95°C for 10 min, followed by 35 cycles, including a denaturation step at 94°C for 20 sec, annealing at 45°C for 20 sec, and extension at 72°C for 90 sec. The final extension was performed for 5 min at 72°C. The resulting PCR product was analyzed on 1% agarose gel stained with ethidium bromide.

2.4. *DNA sequence analysis*

The amplified DNA was purified with a QIA quick PCR purification kit (QIAGEN, Hilden, Germany), and directly sequenced using an ABI Prism Bigdye terminator v1.1 cycle sequencing kit (Applied Biosystems). Sequence analysis was performed utilizing programs of the GENETYX package (version 9.0; Genetyx Co., Tokyo, Japan). The evolutionary relationship among different *H. pylori* isolates was elucidated by the 6-parameter method. The phylogenetic tree was constructed with the neighbor-joining method in the ODEN program (version 1.1) (*25*).

3. Results

The presence of the *sabA* gene in 23 *H. pylori* clinical isolates was detected by PCR. We utilized the same primers as described by Mahdavi *et al*. with three different combinations for PCR detection (Figure 1) because there is very little sequence data available for the *sabA* gene in Japanese isolates. The PCR amplification with primer pairs 1, 2, and 3 yielded products of 364, 1,087, and 1,330 bp in length, respectively. As shown in Table 1, three PCR assays yielded different positive rates for the *sabA* gene in 23 *H. pylori* isolates, with the highest one (91.3%) using primer pair 1, suggesting that there is considerable sequence diversity of the *sabA* gene

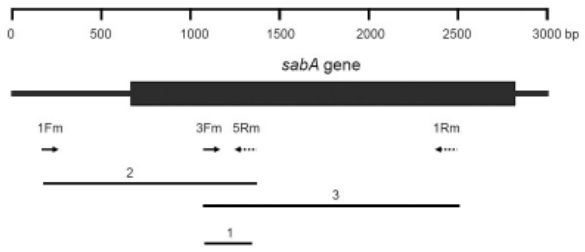

Figure 1. Detection of *sabA* gene by PCR. The line at the top indicates the gene length. The black rectangle under the ruler indicates the *sabA* gene. The numbered solid lines below represent the fragments that were amplified by different primer pairs. Solid and dashed arrows represent foreword and reverse primers used in this study.

Table 1. Detection of *sabA* gene by PCR in 23 *H. pylori* clinical isolates

Number of *H. pylori* strains	PCR results (n = 23)		
	Primer pair 1	Primer pair 2	Primer pair 3
Y03	+	+	+
Y04	+	+	–
Y06	+	–	+
Y07	+	+	–
Y09	–	+	–
Y11	+	–	–
Y13	+	+	–
Y14	+	+	+
Y15	+	+	+
Y16	–	–	–
Y17	+	+	–
Y19	+	+	+
Y21	+	–	–
Y22	+	+	+
Yno1	+	+	+
Y26	+	–	–
Yno3	+	+	+
Y28	+	–	–
Y29	+	+	–
Y31	+	+	–
Y37	+	+	+
Y38	+	+	+
Y40	+	+	+
Number of *sabA*-genopositive	21	17	11

in *H. pylori* clinical isolates.

In order to evaluate the genetic diversity of *sabA*, the fragments amplified by PCR with primer pairs 2 and 3 were subjected to sequence analysis in ten *H. pylori* isolates. Nucleotide point mutations, deletions, and insertions were commonly observed in the *sabA* gene. Tables 2 and 3 summarize the homology of *sabA* nucleotide and amino acid sequences among different *H. pylori* strains. The amino acid sequence diversity of SabA ranged from 5.1% to 13.3% in the *H. pylori* clinical isolates analyzed.

Figure 2 shows a phylogenetic tree constructed using the amino acid sequences of SabA in nine *H. pylori* isolates from Japanese patients and three *H. pylori* strains from European and American patients whose genomic sequences were released in the GenBank database. The pattern clearly indicated that the *H. pylori* isolates in Japan are more closely related to each other than to those from Western countries, suggesting that *sabA* genes may be divided into two groups, a Western group and a Japanese group.

4. Discussion

H. pylori virulence factors, such as urease, CagA, VacA, or BabA, might account for the development of different gastric diseases, including gastritis, peptic ulcer, and gastric cancer (*1,11*). Recent studies from Europe provided evidence that another adherence factor, SabA, may contribute further to the enhanced pathogenicity of *H. pylori* in human gastric epithelium (*14*). However, previous studies on *H. pylori* have revealed that clinical relevance of virulence factors observed in Western individuals vary apparently from those in Asian populations, due in large part to the remarkable genetic diversity of *H. pylori* (*26*). Therefore, the presence and characteristics of the *sabA* gene in *H. pylori* clinical isolates in Japan needed to be assessed.

In the present study, we used three primer sets whose sequences were derived from European strains to detect the *sabA* gene, using PCR, in the *H. pylori*

Table 2. Comparison of nucleotide sequence homology of *sabA* genes (%)

Strain No.	Y14	Y5	Y19	Y22	Y37	Y38	Y40	Yno1	Yno3	26695	J99	HPAG1
Y03	93.7	94.5	92.8	93.7	93.5	93.6	93.2	94.5	93.2	89.2	89.4	89.5
Y14		94.4	94.0	94.5	94.3	95.3	94.1	93.8	95.0	88.7	90.3	89.4
Y15			92.8	94.5	93.9	95.8	94.2	92.0	92.9	88.5	89.8	89.0
Y19				94.0	93.9	93.5	93.8	92.5	93.9	89.3	90.0	90.3
Y22					94.8	94.8	94.9	92.9	94.0	89.2	89.9	89.4
Y37						95.0	95.0	93.4	94.8	88.6	89.3	88.8
Y38							93.9	93.0	93.7	88.9	90.0	89.4
Y40								92.4	93.7	89.2	89.8	90.0
Yno1									93.3	89.6	89.4	89.7
Yno3										88.7	90.0	88.7
26695											91.9	94.3
J99												91.9

The *H. pylori sabA* gene sequences determined in this study have been submitted into GenBank with accession number AB244057-AB244066.

Table 3. Comparison of amino acid sequence homology of SabA (%)

Strain No.	Y14	Y15	Y19	Y22	Y37	Y40	Yno1	Yno3	26695	J99	HPAG1
Y03	91.6	91.7	90.9	90.9	91.1	90.9	92.5	91.3	87.2	88.3	87.3
Y14		92.5	93.2	93.2	92.6	92.9	92.0	93.2	87.6	88.6	87.8
Y15			92.0	94.3	91.7	92.8	90.1	90.9	87.3	89.0	88.7
Y19				93.2	93.3	93.6	90.7	92.4	88.2	88.4	88.3
Y22					93.2	93.9	90.5	91.8	88.6	88.9	89.0
Y37						93.3	90.8	92.7	87.4	88.0	87.5
Y40							90.2	92.3	87.7	88.6	88.7
Yno1								90.8	88.7	88.8	88.2
Yno3									86.7	89.0	86.9
26695										90.9	94.9
J99											90.6

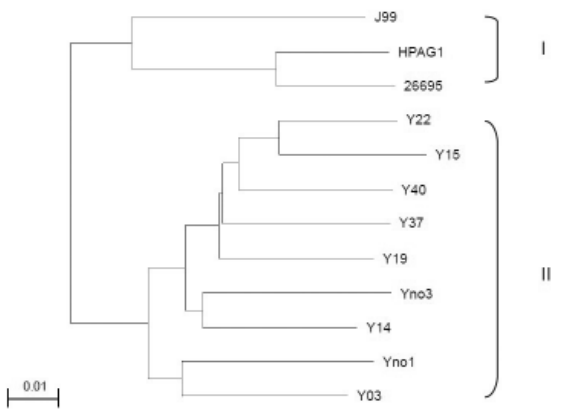

Figure 2. A phylogenetic tree constructed using the amino acid sequences of *H. pylori* SabA in 9 clinical isolates from Japanese patients and 3 strains from the GenBank database. The length of the horizontal bar indicates the number of amino acid substitutions per site.

strains isolated from Japanese patients. As shown in Table 1, the *sabA* gene was detected in 21 of 23 isolates using PCR with primer pair 1, while 17 and 11 out of 23 isolates with primer pair 2 and pair 3, respectively. These data suggested that 1) most, if not all, *H. pylori* isolates in the current study contain the *sabA* gene; and 2) the *sabA* gene is diverse, which causes ambiguous PCR results. Hence, it is necessary to design new primers based on the conserved sequences of *sabA* in Japanese *H. pylori* isolates for further epidemiological studies.

Sequence analysis showed considerable sequence diversity of the *sabA* gene among *H. pylori* clinical isolates, ranging from 5.0% to 11.5% at the nucleotide level and 5.1% to 13.3% at the amino acid level. Phylogenetic analysis demonstrated clustering of strains according to their geographical origin; cluster I contained all isolates from Japan whereas cluster II consisted of three isolates from Western countries. From these observations, two questions arose: 1) whether SabA is associated with pathogenicity of *H. pylori* given that *sabA*-genopositive strains were prevalent in Japanese patients studied here, and 2) what is the clinical relevance of the genetic diversity in *sabA*. Recently, Yanai *et al*. reported a high prevalence of functional *sabA* (81%) in 108 Japanese clinical *H. pylori* isolates based on sequence analysis of the CT dinucleotide repeats numbers at the 5' region of *sabA* gene, and indicated a close relationship between *sabA* status and atrophic gastritis. Nevertheless, there is increasing evidence that complicated regulatory mechanisms are involved in control of expression of the *sabA* gene. A report from Taiwan based on Western blotting analysis on 145 *H. pylori* clinical isolates indicated that only 31% of the isolates expressed SabA. Hence, to assess the clinical relevance of the *sabA* gene diversity, further genetic epidemiology studies on SabA and other virulence factors are needed.

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