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BRIEF ARTICLE

Clinical relevance of *Helicobacter pylori babA2* and *babA2/B* in Costa Rica and Japan

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Abstract

AIM: To evaluate the prevalence of *Helicobacter pylori* (*H. pylori*) *babA2*, *babB* and a recombinant gene between *babA2* and *babB* (*babA2/B*), and their role in the development of atrophic gastritis in Costa Rican and Japanese clinical isolates.

METHODS: A total of 95 continuous *H. pylori*-positive Costa Rican (41 males and 54 females; mean age, 50.65 years; SD, ± 13.04 years) and 95 continuous *H. pylori*-positive Japanese (50 males and 45 females; mean age, 63.43; SD, ± 13.21 years) patients underwent upper endoscopy from October 2005 to July 2006. They were enrolled for the polymerase chain reaction (PCR)-based genotyping of the *H. pylori babA2*, *babB* and *babA2/B* genes. Statistical analysis was performed using the χ^2 test and the Fisher's exact probability test and multivariate analysis was performed by logistic regression adjusting for gender and age. $P < 0.05$ was regarded as statistically significant.

RESULTS: The PCR-based genotyping of 95 Costa Rican and 95 Japanese isolates showed a higher prevalence of *babA2* in Japan (96.8%) than in Costa Rica (73.7%), while that of *babA2/B* was higher in Costa Rica (11.6%) than in Japan (1.1%). In Costa Rican isolates only, *babA2* was significantly associated with atrophic gastritis ($P = 0.01$).

CONCLUSION: These results suggest that the status of *babA2* and *babA2/B* shows geographic differences, and that *babA2* has clinical relevance in Costa Rica.

Key words: *babA2*; *babA2/B*; Costa Rica; *Helicobacter pylori*; Japan

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infects the human stomach causing chronic inflammation, which can lead to peptic ulcer and gastric cancer[1,2]. The diverse clinical outcome of

gastric disease may involve differences in the prevalence or expression of bacterial virulence factors. *H. pylori* BabA is a blood-group antigen-binding adhesin encoded by the *babA2* gene, which has been shown to mediate adherence of *H. pylori* to human Lewis b blood-group (Le^b) antigens[3,4]. Although three *bab* alleles have been identified (*babA1*, *babA2* and *babB*), only the *babA2* gene product is functional for Le^b binding activity[5,6]. Studies in Western countries have disclosed associations between the presence of *babA2* gene and digestive diseases such as duodenal ulcer and gastric cancer[4]. However, in Asia, most of the *H. pylori* strains are *babA2*-positive, irrespective of clinical outcome[7,8]. Thus, conclusions about the relationship between *H. pylori* genotypes and clinical outcome derived from one geographic region may not be true for other geographic regions. Evidence concerning BabA adhesin-associated genes is insufficient in Costa Rica, where the incidence of gastric cancer is very high, similar to Japan[9]. The *babA2* gene, which encodes BabA, may play a role in the development of gastric cancer in the Costa Rican population. In order to investigate this hypothesis we aimed to correlate the status of *babA2* in Costa Rican clinical isolates with atrophic gastritis, a gastric premalignant lesion. In addition, because *H. pylori* populations are highly diverse and are constantly changing their genome by point mutations, substitutions, insertions, and/or deletions of their genome[10-12], we decided to evaluate the prevalence of a recombinant gene between *babA2* and *babB* (*babA2/B*), already identified *in vitro*[13,14], in Costa Rican as well as in Japanese clinical isolates, which were used also in this study for comparative purposes.

MATERIALS AND METHODS

Study population

Half of the patients in this study attended a digestive center in San Jose, Costa Rica and the other half attended a National University in Kochi, Japan. A total of 95 continuous *H. pylori*-positive Costa Rican (41 males and 54 females; mean age, 50.65 years; SD, ± 13.04 years) and 95 continuous *H. pylori*-positive Japanese (50 males and 45 females; mean age, 63.43; SD, ± 13.21 years) patients underwent upper endoscopy from October 2005 to July 2006. They were enrolled for the polymerase chain reaction (PCR)-based genotyping of *H. pylori babA2*, *babB* and *babA2/B* genes. Informed consent was obtained from each patient and the study was approved by the Ethics Committee of the institutions. Information was collected on age, gender, symptoms and medication. None of the participating patients had undergone *H. pylori* eradication therapy or gastric surgery. In addition, none of the patients had recent intake of proton pump inhibitors, antibiotics, or non-steroidal anti-inflammatory drugs. The patients were histopathologically classified into two groups; atrophic gastritis (AG) group (29 Costa Rican and 48 Japanese) and non-atrophic gastritis (NAG) group (66 Costa Rican and 47 Japanese) according to the updated Sydney System for the classification of gastritis[15].

Endoscopical and histological evaluations

Endoscopy was performed with Olympus EVIS EXERA Ⅰ/Ⅱ systems (Olympus America Inc., San Jose, CA, USA). From each participating subject, at least four biopsies (from the antrum, corpus and cisura angularis) were collected for histological examination. In addition, one antral biopsy was also taken to obtain the clinical isolates following bacterial culture.

The biopsy samples were conventionally fixed in 100 mL/L formaldehyde anidre and embedded in paraffin. Serial 3- to 4-μm sections were stained with hematoxylin and eosin for histological observation. All biopsies were examined for the presence of glandular atrophy and were scored into four grades (0: none, 1: mild, 2: moderate and 3: marked) for both the antrum and the body of the stomach, according to the updated Sydney System of classification and grading of gastritis[15]. Gastric glandular atrophy was defined as the loss of gastric glands and its replacement with fibrosis or metaplastic epithelium.

Determination of H. pylori infection

H. pylori infection was determined by either the rapid urease test (RUT) or histological examination in biopsy specimens obtained from the antrum, cisura angularis and body of the stomach. Patients were considered *H. pylori*-positive if either the biopsy specimen was positive for RUT or the bacterium was observed in any of the hematoxylin and eosin-stained sections.

Isolation of H. pylori from biopsy specimens and DNA extraction

The homogenized biopsy specimens were placed on *H. pylori* selective agar plates (Helico VI agar, E-MS70, Eiken Chemical Co., Ltd., Japan) and cultured at 37℃ under microaerobic conditions (100 mL/L CO_2) for five to seven days. The presence of *H. pylori* colonies was confirmed by typical morphology, Gram staining and a positive urease test. Eventually, a total of 190 clinical isolates obtained from antrum specimens were subjected to genomic DNA (gDNA) extraction using a DNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions.

Detection of H. pylori babA2, babB and babA2/B genes by PCR

The genomic DNAs were subjected to PCR-based genotyping of *babA2*, *babB* and *babA2/B* using two primer pairs including primers previously described[4,16] and new primers (Table 1) designed based on sequences of referential *H. pylori* strains 26 695 and J99. We used PCR conditions exactly matching those described[4,16] and the conditions for the new primers used in this study are shown in Table 1. Whenever necessary, in particular, to determine *babA2/B*, sequence analysis of the putative products was performed using Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and these sequences were compared with *babA* and *babB* genes of strains 26 695 (HP1243 and HP896, respectively) and J99 (jhp833 and jhp1164, respectively)

Table 1 PCR primers and conditions for detection of *babA2*, *babB* and *babA2/B* genes

Region	Primer	Nucleotide sequence (5'- 3')	PCR conditions	Ref.
babA2	babA-F	AATCCAAAAAGGAGAAAAAGTATGAAA		[4]
	babA-R	TGTTAG TGATTTCGGTGTAGGACA		
	babA2-Fnc1	GAAAAAACATGAAAAAACACATCCTTTCAT		[16]
	babA2-Rmn2	TCTGGGTTAATGGCTTGCC		
babB	babB-Fnc1	CTCTCTCTCGTTTTTGCTCCA	96℃ for 2 min, 30 cycles (96℃ for 30 s, 48℃ for 30 s, 72℃ for 1 min)	This study
	babB-Rnc1	CTTCATAACACACCCCTAAAGAGTC		
	babB-Fnc3	ATGAAAAAAACCCTTTTACTC	96℃ for 2 min, 30 cycles (96℃ for 30 s, 46℃ for 30 s, 72℃ for 1 min)	This study
	babB-Rnc3	TGACCTGGATTGGTGCCCCTACG		
babA2/B	babA-F	AATCCAAAAAGGAGAAAAAGTATGAAA		[4]
	babB-Rnc1	CTTCATAACACACCCCTAAAGAGTC		
	babA2-Fnc1	GAAAAAACATGAAAAAACACATCCTTTCAT		[16]
	babB-Rnc1	CTTCATAACACACCCCTAAAGAGTC	96℃ for 2 min, 30 cycles (96℃ for 30 s, 62℃ for 30 s, 72℃ for 1 min)	This study
	babB-Rnc2[1]	CTACGCTCACCCCCTTGTACTTC	96℃ for 2 min, 30 cycles (96℃ for 30 s, 63℃ for 30 s, 72℃ for 1 min)	This study
	babB-Rnc3[1]	TGACCTGGATTGGTGCCCCTACG	96℃ for 2 min, 30 cycles (96℃ for 30 s, 62℃ for 30 s, 72℃ for 1 min)	This study

[1]Used with babA2-Fnc1.

Table 2 Characteristics of Costa Rican and Japanese dyspeptic patients

	Costa Rican			Japanese		
	AG	NAG	*P*-value	AG	NAG	*P*-value
Patients number	29	66		48	47	
Sex (M/F)	15/14	26/40	0.264	24/24	26/21	0.604
mean age ± SD (yr)	54.8 ± 12.9	48.9 ± 12.8	0.041	68.4 ± 10.2	58.4 ± 14.1	< 0.001

AG: Atrophic gastritis; NAG: Non-atrophic gastritis; SD: Standard deviation.

using the BLAST 2 SEQUENCES system (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi)[17]. When the putative recombinant gene was shown to be only homologous at the 5' and 3' positions of *babA2* gene open reading frame (ORF) and *babB* gene ORF, respectively, the gene was considered to be a recombinant *babA2/B* gene.

Statistical analysis

Statistical analysis was performed using the χ^2 test and the Fisher's exact probability test [STATA SE (version 8) statistical software]. $P < 0.05$ was regarded as statistically significant. Multivariate analysis was performed by logistic regression [SPSS 13.0 Japanese version (SPSS Japan Inc., 2005)] adjusting for gender and age. Odds ratios with 95% confidence intervals were used to study the influence of these genes on the development of gastric atrophy.

RESULTS

Comparison of gender and age of patients between AG and NAG

There was no significant difference in gender between the AG group and NAG group from either Costa Rica or Japan (Table 2). However, mean age was significantly higher in the AG group than in the NAG group of both Costa Rican and Japanese patients.

Prevalence of gastric atrophy in Costa Rican and Japanese patients

In Costa Rican patients, the prevalence of gastric atrophy was 30.5% (29/95) while that in Japanese patients was considerably higher (50.5%, 48/95).

H. pylori babA2, *babB* and *babA2/B* genes in clinical isolates

In Costa Rican patients, the prevalence of *babA2* was 73.7% (70/95) and after gender and age adjustment, this gene was found to be significantly associated with AG in this population ($P = 0.01$) (Table 3). The prevalence of *babB* and *babA2/B* was 81.1% (77/95) and 11.6% (11/95), respectively, and no significant differences were found between any of these genes and AG.

In Japanese patients, almost all patients were found to be *babA2*-positive (96.8%, 92/95), while only one patient had the *babA2/B* gene (98.9%). The prevalence of *babB* was 90.5% (86/95). After gender and age adjustment, no significant differences were found between any of these genes and AG in this population.

DISCUSSION

The prevalence of *babA2* in Costa Rican isolates was 73.7%, which was higher than that shown in Western studies (38%-43%)[18-20], but lower than that in Asian studies (80%-100%)[7,21-23], including that in our Japanese

Table 3 *H. pylori babA2*, *babB* and *babA2/B* genes according to atrophic gastritis in Costa Rican and Japanese patients

	Costa Rican patients				Japanese patients			
Gene	AG/NAG	*P*	OR	95% CI	AG/NAG	*P*	OR	95% CI
babA2								
Pos	27/43	0.01	7.80	1.63-37.40	46/46	0.88	0.82	0.06-10.70
Neg	2/23		1.00	(Reference)	2/1		1.00	(Ref.)
babB								
Pos	25/52	0.54	1.47	0.42-5.12	45/41	0.18	3.00	0.61-14.70
Neg	4/14		1.00	(Reference)	3/6		1.00	(Ref.)
babA2/B								
Pos	6/5	0.10	3.07	0.80-11.80	0/1	1.00	0.00	
Neg	23/61		1.00	(Reference)	48/46		1.00	(Ref.)

Pos: Positive; Neg: Negative; OR: Odds ratio; CI: Confidence intervals. Separate models were fitted to obtain an odds ratio for each gene with adjustment for gender and age in each country. $P < 0.05$ was considered significant.

isolates (96.8%) (Table 3). It seems that the prevalence of *babA2* does not parallel the incidence rate of gastric cancer in those countries, since Costa Rica, has an incidence rate comparable to that of Japan and China[9].

The *babA2* and *babB* genes exhibit extensive homologies at their 5' and 3' ends that should facilitate frequent recombination between them, suggesting that a recombination might interfere in the expression and functional activity of BabA. In this study, this recombination was found in 11 and 1 Costa Rican and Japanese (KMT28) isolates, respectively, irrespective of clinical outcome. However, in the Costa Rican strains, the *babA2* gene was found to be significantly associated with AG ($P = 0.01$ and OR = 7.8) (Table 3). This association has been reported previously in Western studies[9].

The PCR products of 12 *babA2/B* recombinant strains were employed for sequence analysis, revealing that several stop-codons in the amino acid sequence were found in all 11 Costa Rican strains, suggesting that these genes were non-functional. In contrast, since the Japanese strain KMT28 had complete in-frame sequence, the *babA2/B* gene was thought to be functional. In addition, reverse transcription-PCR (Toyobo Co., Ltd., Japan) using mRNA extracted from the KMT28 strain possessing the *babA2/B* gene with Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA), and sequence analysis were performed, demonstrating that the *babA2/B* transcript of KMT28 was definitely obtained and the sequence was identical to the *babA2/B* sequence (data not shown).

The relationship between *babA2*-positive *H. pylori* and an increased risk of developing clinical outcomes is controversial[7,18,20,24-26], because the presence of *babA2* is not always to reflect the BabA binding activity due to regulation by the number of transcriptional start adenine [poly (A)] residues in the promoter region[5] and the presence of chimeric *babA/B* or *babB/A* genes[14,27]. Moreover, it is relatively difficult to detect the *babA2* gene by PCR with a single primer pair due to high homology between the sequences of *babA1* and *babA2*. Thus, to determine BabA binding activity and/or the presence of its transcript it was critical to consider the functionality of BabA and its pathogenesis. Therefore we used at least two primer pairs to confirm the presence of *babA2* and recombinant *babA2/B* genes and investigated the relationship between the status of these genes and clinical outcomes.

Taken together, the status of *babA2* and *babA2/B* shows geographic differences, and *babA2* seems to have clinical relevance only in Costa Rica. A functional *babA2/B* was found in one Japanese isolate. However, we believe that a binding assay with Le^b antigen is necessary to confirm whether the BabA is functional and/or the adhesive strength is regulated individually depending on an adaptation of the microorganism in the stomach involved in clinical manifestation.

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COMMENTS

Background

The clinical outcome of gastric disease may involve differences in the prevalence or expression of bacterial virulence factors. Contrary to Asian studies, western studies have disclosed associations between the presence of *babA2* gene and gastric cancer. Evidence concerning BabA adhesin-associated genes is insufficient in Costa Rica, where the incidence of gastric cancer is very high, similar to Japan. The *babA2* gene, which encodes BabA, may play a role in the development of gastric cancer in the Costa Rican population.

Research frontiers

The research in this area is focused on the correlation between the status of *babA2* in Costa Rican clinical isolates and atrophic gastritis, a gastric premalignant lesion, and on the evaluation of the prevalence of a recombinant gene between *babA2* and *babB* (*babA2/B*), in Costa Rican and Japanese clinical isolates.

Innovations and breakthroughs

The PCR-based genotyping of 95 Costa Rican and 95 Japanese isolates showed a higher prevalence of *babA2* in Japan (96.8%) than in Costa Rica (73.7%), while that of *babA2/B* was higher in Costa Rica (11.6%) than in Japan (1.1%). In Costa Rican isolates only, *babA2* was significantly associated with atrophic gastritis ($P = 0.01$).

Applications

These results suggest that the status of *babA2* and *babA2/B* shows geographic differences, and that *babA2* has clinical relevance in Costa Rica.

Terminology

Helicobacter pylori (*H. pylori*) is a Gram-negative microaerobic bacterium that persistently colonizes the human gastric mucosa. *H. pylori* BabA is a blood-group antigen-binding adhesin encoded by the *babA2* gene, which has been shown to mediate adherence of *H. pylori* to human Lewis b blood-group antigens.

Peer review

This paper has a correct design and is presented adequately. Title, results and discussion are clear and properly expressed. This topic is controversial, in some way, and this investigation constitutes an interesting contribution.

REFERENCES

1 **Kuipers EJ**, Uyterlinde AM, Peña AS, Roosendaal R, Pals G, Nelis GF, Festen HP, Meuwissen SG. Long-term sequelae of Helicobacter pylori gastritis. *Lancet* 1995; **345**: 1525-1528

2 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789

3 **Borén T**, Falk P, Roth KA, Larson G, Normark S. Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. *Science* 1993; **262**: 1892-1895

4 **Gerhard M**, Lehn N, Neumayer N, Borén T, Rad R, Schepp W, Miehlke S, Classen M, Prinz C. Clinical relevance of the Helicobacter pylori gene for blood-group antigen-binding adhesin. *Proc Natl Acad Sci USA* 1999; **96**: 12778-12783

5 **Ilver D**, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Borén T. Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* 1998; **279**: 373-377

6 **Pride DT**, Meinersmann RJ, Blaser MJ. Allelic Variation within Helicobacter pylori babA and babB. *Infect Immun* 2001; **69**: 1160-1171

7 **Mizushima T**, Sugiyama T, Komatsu Y, Ishizuka J, Kato M, Asaka M. Clinical relevance of the babA2 genotype of Helicobacter pylori in Japanese clinical isolates. *J Clin Microbiol* 2001; **39**: 2463-2465

8 **Sheu BS**, Sheu SM, Yang HB, Huang AH, Wu JJ. Host gastric Lewis expression determines the bacterial density of Helicobacter pylori in babA2 genopositive infection. *Gut* 2003; **52**: 927-932

9 **Kuroishi T**, Hirose K, Takezaki T, Tominaga S, Aoki K, Tajima K. Cancer mortality statistics in 30 countries (1953-1997). In: Tajima K, Kuroishi T, Oshima A, editors. Cancer mortality and morbidity statistics. Tokyo: Japan Scientific Societies Press, 2004: 165-229

10 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186

11 **Peek RM Jr**, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2002; **2**: 28-37

12 **Blaser MJ**, Berg DE. Helicobacter pylori genetic diversity and risk of human disease. *J Clin Invest* 2001; **107**: 767-773

13 **Aspholm-Hurtig M**, Dailide G, Lahmann M, Kalia A, Ilver D, Roche N, Vikström S, Sjöström R, Lindén S, Bäckström A, Lundberg C, Arnqvist A, Mahdavi J, Nilsson UJ, Velapatiño B, Gilman RH, Gerhard M, Alarcon T, López-Brea M, Nakazawa T, Fox JG, Correa P, Dominguez-Bello MG, Perez-Perez GI, Blaser MJ, Normark S, Carlstedt I, Oscarson S, Teneberg S, Berg DE, Borén T. Functional adaptation of BabA, the H. pylori ABO blood group antigen binding adhesin. *Science* 2004; **305**: 519-522

14 **Bäckström A**, Lundberg C, Kersulyte D, Berg DE, Borén T, Arnqvist A. Metastability of Helicobacter pylori bab adhesin genes and dynamics in Lewis b antigen binding. *Proc Natl Acad Sci USA* 2004; **101**: 16923-16928

15 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181

16 **Con SA**, Takeuchi H, Con-Chin GR, Con-Chin VG, Yasuda N, Con-Wong R. Role of bacterial and genetic factors in gastric cancer in Costa Rica. *World J Gastroenterol* 2009; **15**: 211-218

17 **Tatusova TA**, Madden TL. BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. *FEMS Microbiol Lett* 1999; **174**: 247-250

18 **Oliveira AG**, Santos A, Guerra JB, Rocha GA, Rocha AM, Oliveira CA, Cabral MM, Nogueira AM, Queiroz DM. babA2- and cagA-positive Helicobacter pylori strains are associated with duodenal ulcer and gastric carcinoma in Brazil. *J Clin Microbiol* 2003; **41**: 3964-3966

19 **Zambon CF**, Navaglia F, Basso D, Rugge M, Plebani M. Helicobacter pylori babA2, cagA, and s1 vacA genes work synergistically in causing intestinal metaplasia. *J Clin Pathol* 2003; **56**: 287-291

20 **Oleastro M**, Gerhard M, Lopes AI, Ramalho P, Cabral J, Sousa Guerreiro A, Monteiro L. Helicobacter pylori virulence genotypes in Portuguese children and adults with gastroduodenal pathology. *Eur J Clin Microbiol Infect Dis* 2003; **22**: 85-91

21 **Yu J**, Leung WK, Go MY, Chan MC, To KF, Ng EK, Chan FK, Ling TK, Chung SC, Sung JJ. Relationship between Helicobacter pylori babA2 status with gastric epithelial cell turnover and premalignant gastric lesions. *Gut* 2002; **51**: 480-484

22 **Lai CH**, Kuo CH, Chen YC, Chao FY, Poon SK, Chang CS, Wang WC. High prevalence of cagA- and babA2-positive Helicobacter pylori clinical isolates in Taiwan. *J Clin Microbiol* 2002; **40**: 3860-3862

23 **Kim SY**, Woo CW, Lee YM, Son BR, Kim JW, Chae HB, Youn SJ, Park SM. Genotyping CagA, VacA subtype, IceA1, and BabA of Helicobacter pylori isolates from Korean patients, and their association with gastroduodenal diseases. *J Korean Med Sci* 2001; **16**: 579-584

24 **Gatti LL**, Fagundes e Souza EK, Leite KR, Bastos EL, Vicentini LR, Silva LC, Smith Mde A, Payão SL. cagA vacA alelles and babA2 genotypes of Helicobacter pylori associated with gastric disease in Brazilian adult patients. *Diagn Microbiol Infect Dis* 2005; **51**: 231-235

25 **Olfat FO**, Zheng Q, Oleastro M, Voland P, Borén T, Karttunen R, Engstrand L, Rad R, Prinz C, Gerhard M. Correlation of the Helicobacter pylori adherence factor BabA with duodenal ulcer disease in four European countries. *FEMS Immunol Med Microbiol* 2005; **44**: 151-156

26 **Han YH**, Liu WZ, Zhu HY, Xiao SD. Clinical relevance of iceA and babA2 genotypes of Helicobacter pylori in a Shanghai population. *Chin J Dig Dis* 2004; **5**: 181-185

27 **Solnick JV**, Hansen LM, Salama NR, Boonjakuakul JK, Syvanen M. Modification of Helicobacter pylori outer membrane protein expression during experimental infection of rhesus macaques. *Proc Natl Acad Sci USA* 2004; **101**: 2106-2111

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