## Section 4 Management of gastritis

### CQ17. Should all *H. pylori*-positive individuals receive eradication therapy?

**Statement 17**

*H. pylori* infected individuals should be offered eradication therapy, unless there are competing considerations.

Grade of recommendation strong

Evidence level: high

*Consensus level: 100%*

#### Comment

*H. pylori* is a major human pathogen that causes chronic and progressive gastric mucosal damage and is aetiologically related to peptic ulcer, gastric cancer and gastric atrophy. It is also closely associated with gastric MALT lymphoma, dyspepsia, hyperplastic gastric polyps and idiopathic thrombocytopenic purpura.[5 12 46 47 61 98–104] *H. pylori*-positive individuals are also the major reservoir for transmission of the infection.

The decision to eradicate a chronic infection in a society should be based on quantitative data regarding the outcome of untreated infections. *H. pylori* causes a chronic infection, similar, for example, to asymptomatic syphilis or tuberculosis, and the final outcome for any individual cannot be predicted.[105] *H. pylori* infection differs from many other chronic infectious diseases because it is always transmissible, thus putting others at risk. Because the gastric damage is progressive, the lack of an obvious clinical manifestation at diagnosis has no predictive value for life-time risk to an individual patient, their family or to the community. Benefits of *H. pylori* eradication for an individual depend in part on the degree and extent of damage that has already occurred and the reversibility of that damage. Potential benefits of eradication include stopping the progression of mucosal damage, stabilisation or reduction in risk of developing gastric cancer, resolution of mucosal inflammation, stabilisation or improvement of gastric mucosal function, return of the normal mechanisms governing acid secretion, cure of *H. pylori*-related PUD, reduction in risk of gastrointestinal complications of NSAID therapy and prevention of future development of *H. pylori*-related peptic ulcer.[2 5 11 28 46 47 106–115]

For society, the benefits include reduction of the reservoir of infected individuals capable of transmitting the infection to others, and avoidance of the costs associated with diagnosis, management and outcomes of *H. pylori*-related diseases that are prevented. Thus, *H. pylori*-infected patients should be offered eradication therapy unless there are competing considerations such as comorbidities, re-infection rates in their communities, competing health priorities of society and financial cost. It has to be remembered, however, that there are concerns about the negative impact of eradication therapies on human health, such as increase in allergy or obesity and perturbation of microbiota.[116 117]

### CQ18. What is the optimal timing for *H. pylori* eradication in asymptomatic subjects?

**Statement 18**

The maximum benefit of *H. pylori* eradication is obtained if it is done while the mucosal damage is still non-atrophic.

Grade of recommendation: strong

Evidence level: high

*Consensus level: 100%*

#### Comment

*H. pylori* eradication always confers a benefit by halting progression of gastric mucosal damage, reducing the reservoir of infected individuals and reducing or preventing *H. pylori*-associated diseases. The maximum benefit of eradication for an individual is obtained if eradication is done while the *H. pylori*-induced mucosal damage has not progressed beyond the non-atrophic stage. This population is found in countries where gastric cancer is still prevalent and is concentrated in the younger generation. *H. pylori* eradication of adolescents and young adults has an additional advantage of reducing or preventing transmission of the infection to their children.

As noted above (Section 3), the risk for development of gastric cancer correlates with the extent and severity of atrophic gastritis. It is impossible to define the risk for an individual based on age. Cancer risk in any population relates to the rate of progression of gastric mucosal damage, which is high in populations at high risk of cancer and low in *H. pylori*-infected populations with a low cancer risk. Thus, while it is possible to identify an average age at which the transition from non-atrophic to atrophic phenotype occurs for any population, one should expect that any age group will contain individuals with a wide range of damage, ranging from uninfected (normal) to advanced atrophy. This emphasises the need for risk stratification based on objective parameters including a validated histological staging system rather than on age, to identify whether one eradication treatment is needed or whether the patient might require surveillance.

The incidence of gastric cancer increases with age, which is a surrogate marker for the time required for progression of atrophic gastritis. When atrophic gastritis becomes extensive and severe, the risk increases exponentially. Cancer is the culmination of a multistep process of genetic instability, with cancer cells possessing mutations in coding regions, somatic gene rearrangements and epigenetic changes such as methylation. Current data are consistent with the notion that *H. pylori* eradication halts the progression of damage and reduces or eliminates the *H. pylori*-associated events that increase genetic instability in the gastric mucosa. These include infection-associated DNA double-strand breaks,[118] impaired DNA mismatch repair,[119] aberrant activation-induced cytidine deaminase expression, which induces nucleotide alterations involved in DNA mutations,[120] aberrant methylation in a number of gene promoters in the gastric mucosa, including cell growth-related genes, DNA-repair genes, tumour-suppressor genes, the cell adherence gene E-cadherin and CpG islands of microRNA genes[121–123] and aberrant microRNA expression.[124] *H. pylori* infection also causes an inflammatory response with mucosal infiltration of acute and chronic inflammatory cells. Cancer risk is increased in relation to the ability of the infecting strain to cause inflammation (eg, those possessing the Cag pathogenicity island). However, all strains cause inflammation, and gastric cancer is associated with infections lacking putative virulence factors. Thus all *H. pylori* infections should be considered pathogenic and should be eradicated.

Because of the damage and premalignant changes, *H. pylori* eradication cannot 'reset the clock' to zero (ie, no risk) but can stop the progression of risk and stabilise or decrease the subsequent risk.

### CQ19. Do we need to adopt eradication regimens according to the geographical area?

**Statement 19**

Eradication regimens should be based on the best locally effective regimen, ideally using individual susceptibility testing or community antibiotic susceptibility, or antibiotic consumption data and clinical outcome data. The agents available differ in

different regions and this, in part, dictates what regimens are possible.

Grade of recommendation: strong
Evidence level: high
*Consensus level: 100%*

### Comment

The success of a proven successful *H. pylori* eradication regimen depends on the pattern of resistance in the population and on the common host genotypes of drug metabolising enzymes in the population. The prevalence of *H. pylori* resistance to commonly used antimicrobial agents greatly varies geographically and is linked to consumption of antibiotics in the region,[125] so the preferred eradication regimen often differs between regions. Ideally, treatment regimens should be chosen based on susceptibility testing. Within any region, only regimens that reliably produce eradication rates of ≥90% in that population should be used for empirical treatment.[5 126–129]

### CQ20. Does eradication of *H. pylori* prevent gastric cancer?

**Statement 20**

Eradication of *H. pylori* reduces the risk of gastric cancer. The degree of risk reduction depends on the presence, severity and extent of atrophic damage at the time of eradication.

Grade of recommendation: strong
Evidence level: high
*Consensus level: 100%*

### Comment

*H. pylori* infection is the most important cause of gastric cancer as it is estimated that 89% of non-cardia gastric cancer, representing 78% of all cases of gastric cancer, can be attributed to chronic *H. pylori* infection.[130] Prevention of *H. pylori* infections removes the primary cause of gastric cancer and will thus reduce the incidence of gastric cancer in that population. The effectiveness of *H. pylori* eradication for prevention of gastric cancer depends on the severity and extent of atrophic damage at the time of eradication and ranges from essentially complete prevention for those with non-atrophic gastritis to stabilisation or reduction of risk in those with established atrophic changes.[94 95] As noted in Section 3, risk can be stratified using a variety of approaches, such as one of the validated histological stratification systems (eg, OLGA or OLGIM),[16–18] and *H. pylori* eradication can stabilise risk and halt the progression of risk.[28 94] Prevention of acquisition of *H. pylori* infections and eradication of the infection before the development of atrophic changes are forms of primary prevention. Secondary prevention involves identification and surveillance of those at risk in order to remove intraepithelial lesions and early gastric cancer(s) before they become invasive.[5 71 72 77 131] There may be also a role for cancer immunotherapy to treat premalignant lesions and halt their progression to more advanced lesions.[132]

### CQ21. Should the outcome of eradication therapy always be assessed (ie, test for cure)?

**Statement 21**

The outcome of eradication therapy should always be assessed, preferably non-invasively.

Grade of recommendation: strong
Evidence level: high
*Consensus level: 100%*

### Comment

Failure of eradication is common and allows the mucosal damage to progress, and so eradication should always be confirmed, preferably using a non-invasive test such as a urea breath test or a validated monoclonal-based stool antigen test.[5] For patients requiring endoscopic follow-up, such as after endoscopic removal of a gastric adenoma, histological assessment can be used. Confirmation of cure also provides an early warning system for the increasing antibiotic resistance in a population that will manifest as increasing rates of treatment failure.[125 128 129]

### CQ22. Which patients need long-term follow-up after eradication?

**Statement 22**

*H. pylori* eradication may not completely eliminate the risk of gastric cancer. Patients who remain at risk, as defined by the extent and severity of atrophy, should be offered endoscopic and histological surveillance.

Grade of recommendation: strong
Evidence level: high
*Consensus level: 97.3%*

### Comment

Long-term follow-up such as regular endoscopic surveillance should be based on estimating the risk of developing gastric cancer after *H. pylori* eradication (ie, risk stratification).[95 133] Cancer risk correlates with the extent and severity of atrophic gastritis and risk stratification should be confirmed using a validated histological risk scoring systems such as OLGA or OLGIM.[16–18] In areas with proven expertise in endoscopic scoring, a system such as that of Kimura and Takemoto can be used initially, although histological confirmation is still recommended.[134 135] Patients whose *H. pylori* infection was diagnosed non-invasively (eg, urea breath test or stool antigen) should be considered for histological assessment. These patients should include those within the age range in which atrophic changes are common in that population and those with a history of gastric ulcer as well as those with a pretreatment serum pepsinogen I of ≤70 ng/mL and a pepsinogen I:II ratio ≤3.[136–138] All those at especially high risk, including those at risk for intraepithelial neoplasia (dysplasia) or early gastric cancer, are candidates for regular endoscopic surveillance.

## DISCUSSION

The global consensus meeting on *H. pylori* gastritis has set a new landmark for gastritis, which has continued to be an ill-conceived clinical entity placed between a histological picture and upper abdominal symptoms.

In spite of the fact that gastritis had been long recognised as an important clinical entity, generations of gastroenterologists have neglected the importance of treatment of this nosological entity. Rudolf Schindler described chronic gastritis as a serious disease and a precursor of gastric cancer and considered their relationship as being of outstanding importance in the fight against gastric cancer.[139]

The discovery of *H. pylori* has revolutionised the pre-existing concepts of gastritis by assigning a specific aetiology to this entity underlying PUD and gastric cancer. The majority of these serious conditions are manifestations developed on the background of chronic gastritis caused by a unique infectious agent, *H. pylori*. For PUD, guidelines unanimously recommend eradication as the primary treatment for those with positive *H. pylori* tests. However, there has been no consensus on how and when to manage individuals with *H. pylori* gastritis itself, which is crucial to the efficiency of gastric cancer prevention because most patients with chronic gastritis may remain asymptomatic until the appearance of severe complications. Furthermore, both gastritis and duodenitis were recognised as important causes of upper gastrointestinal bleeding,[140] encouraging our attention to

these conditions now that anti-thrombotic therapies are increasingly being used.

To further compromise the concept of gastritis as a significant clinical entity, the term 'gastritis' has historically, but wrongly, been used as a substitute for a clinical diagnosis of FD. Historical studies, however, failed to demonstrate a significant association between histological findings of gastritis and the dyspeptic symptom complex.[141 142] Hence, a potential pathogenetic role for *H. pylori* in causing dyspeptic symptoms was initially considered doubtful and its eradication in FD controversial.[143 144] Meta-analysis of a large number of controlled trials with longer follow-up confirmed that eradication of *H. pylori* in patients with FD conveys a small but statistically significant benefit.[12] Consequently, dyspepsia attributable to *H. pylori* gastritis involves an underlying organic cause and should be excluded from the FD category. Additionally, 'dyspeptic' patients should not automatically be labelled as having 'gastritis' without any histological confirmation.

Diagnostic assessment of gastritis has been advanced by the recent introduction of high-resolution endoscopy with image-enhanced modalities, and magnification is now used routinely in major hospitals in Japan. This endoscopic technology allows the identification of mucosal changes (for targeted biopsies) more precisely, leading to more accurate evaluation of cancer risks such as preneoplastic changes. Wider use of this new endoscopic system outside Japan may be limited at present.

The Kyoto consensus meeting focused attention on gastritis in all its clinical expression and dealt with four main topics: classification of gastritis in relation to ongoing ICD revision, FD and *H. pylori* infection, diagnosis of gastritis and the management of gastritis. The methodology of the meeting adopted all modern means for reaching consensus and included an internet-based Delphi method with full access to published data in a completely 'neutral' environment.

In summary, The Kyoto meeting proposed an aetiology-based classification for gastritis and concluded that *H. pylori* gastritis is an infectious disease. As such, *H. pylori* gastritis requires treatment whether or not it is associated with symptoms because it represents a condition that may evolve towards serious complications, including peptic ulcer and gastric neoplasia.

Consensus was reached on the existence of a separate category of patients with dyspeptic symptoms that are due to *H. pylori* gastritis. In these patients, eradication therapy is the recommended first-line treatment. Because of the diagnostic problems related to 'gastritis', these patients should be labelled as having *H. pylori*-associated dyspepsia and are identified by sustained dyspeptic symptom relief after eradication.

For the diagnosis of gastritis, it was agreed that risk stratification systems such as OLGA and OLGIM are useful as are the serological markers. In view of recent technological advancements, image-enhanced endoscopy should be encouraged for identifying mucosal changes which carry a high risk of developing into gastric neoplasia. Finally, it was recommended that early eradication therapy, ideally before preneoplastic changes occur, should be undertaken. However, the feasibility of implementing this strategy should be regionally tailored. As eradication therapy does not guarantee elimination of the risk of gastric cancer, follow-up should be considered for patients who have preneoplastic conditions.

Although there are still many remaining areas to be discussed, we believe the outcome of the Kyoto consensus meeting presented in this report will improve patient care and will provide a cornerstone for further refinement and research in the area of gastritis.

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**Contributors** KS, NU, EME-O and PM planned the meeting and prepared the clinical questions. KS, JT, EJK, EME-O, DYG and PM wrote the manuscipt. SM, KH and MA helped to summarise the voting results of sections 1, 3 and 4, respectively. All other faculty members contributed to formulating one or two statements for each clinical question, participated in the meeting and voted. They also read and approved the manuscript.

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## REFERENCES


1 Strickland RG. Gastritis. *Front Gastrointest Res* 1975;1:12–48.
2 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984;323:1311–15.
3 Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. *Lancet* 2009;374:1449–61.
4 Fock KM, Graham DY, Malfertheiner P. *Helicobacter pylori* research: historical insights and future directions. *Nat Rev Gastroenterol Hepatol* 2013;10:495–500.
5 Malfertheiner P, Mégraud F, O'Morain CA, *et al*. Management of *Helicobacter pylori* infection—the Maastricht IV Florence Consensus Report. *Gut* 2012;61:646–64.
6 http://apps.who.int/classifications/icd10/browse/2015/en#/K29 (20 January 2015).
7 Sugano K. Should we still subcategorize *Helicobacter pylori*-associated dyspepsia as functional disease? *J Neurogastroenterol Motil* 2011;17:366–71.
8 Suzuki H, Nishizawa T, Hibi T. Can *Helicobacter pylori*-associated dyspepsia be categorized as functional dyspepsia? *J Gastroenterol Hepatol* 2011;26 (Suppl 3):42–5.
9 Tack J, Talley N, Camilleri M, *et al*. Functional gastrodudenal disorders. In Drossman DA, Corazziari F, Delvaux M, *et al*. eds. *Rome III: the functional gastrointestinal disorders*. 3rd edn. McLean, VA: Denon Associates, Inc, 2006:419–86.
10 Miwa H, Ghoshal UC, Fock KM, *et al*. Asian consensus report on functional dyspepsia. *J Gastroenterol Hepatol* 2012;27:626–41.
11 Asaka M, Kato M, Takahashi S, *et al*. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. *Helicobacter* 2010;15:1–20.
12 Moayyedi P, Soo S, Deeks JJ, *et al*. Eradication of *Helicobacter pylori* for non-ulcer dyspepsia. *Cochrane Database Syst Rev* 2011;16(2):CD002096.
13 Yagi K, Nakamura A, Sekine A. Characteristic endoscopic and magnified endoscopic findings in the normal stomach without *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 2002;17:39–45.
14 Yagi K, Nakamura A, Sekine A. Comparison between magnification endoscopy and histological, culture and urease test findings from the gastric mucosa of the corpus. *Endoscopy* 2002;24:376–81.
15 Okubo M, Tahara T, Shibata T, *et al*. Usefulness of magnifying narrow-band imaging endoscopy in the *Helicobacter pylori*-associated chronic gastritis. *Digestion* 2011;83:161–6.
16 Rugge M, Genta RM. Staging and grading of chronic gastritis. *Hum Pathol* 2005;36:228–33.
17 Rugge M, Correa P, Di Mario F, *et al*. OLGA staging for gastritis: a tutorial. *Dig Liv Dis* 2008;40:650–8.
18 Capelle LG, de Vries AC, Haringsma J, *et al*. The staging of gastritis with the OLGA system by using intestinal metaplasia as an accurate alternative for atrophic gastritis. *Gastrointest Endosc* 2010;71:1150–8.
19 Price AB. The Sydney system: histological division. *J Gastroenterol Hepatol* 1991;6:209–22.
20 Dixon MF, Genta RM, Yardley JH, *et al*. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20:1161–81.
21 Quaseem A, Snow V, Owens DK, *et al*. The development of Clinical Practice guidelines and guidance statement of the American College of Physicians: summary of methods. *Ann Intern Med* 2010;153:194–9.
22 Atkins D, Best D, Briss PA, *et al*. Grading quality of evidence and strength of recommendations. *BMJ* 2004;328:1490.
23 Jones DJ, Barkun AN, Lu Y, *et al*. Conflicts of interest ethics; silencing expertise in the development of international practice guidelines. *Ann Intern Med* 2012;56:809–16.
24 Marshall BJ, Armstrong JA, McGechie DB, *et al*. Attempt to fulfil Koch's postulates for pyloric *Campylobacter*. *Med J Aust* 1985;142:436–9.
25 Morris A, Nicholson G. Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. *Am J Gastroenterol* 1987;82:192–9.
26 Buckley M, O'Morain C. Prevalence of *Helicobacter pylori* in non-ulcer dyspepsia. *Aliment Pharmacol Ther* 1995;9(Suppl 2):53–8.
27 Rosenstock S, Kay L, Rosenstock C, *et al*. Relation between *Helicobacter pylori* infection and gastrointestinal symptoms and syndromes. *Gut* 1997;41:169–76.
28 Malfertheiner P, Sipponen P, Naumann M, *et al*. *Helicobacter pylori* eradication has the potential to prevent gastric cancer: a state-of-the-art critique. *Am J Gastroenterol* 2005;100:2100–15.
29 Nordenstedt H, Graham DY, Kramer JR, *et al*. *Helicobacter pylori*-negative gastritis: prevalence and risk factors. *Am J Gastroenterol* 2013;108:65–71.
30 Meining A, Stolte M, Hatz R, *et al*. Differing degree and distribution of gastritis in *Helicobacter pylori*-associated diseases. *Virchows Arch* 1997;431:11–15.
31 Uemura N, Okamoto S, Yamamoto S, *et al*. *H. pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;345:784–9.
32 Sipponen M, Kosunen TU, Valle J, *et al*. *H. pylori* infection and chronic gastritis in gastric cancer. *J Clin Pathol* 1992;45:319–23.
33 El-Omar EM, Penman ID, Ardill JE, *et al*. *Helicobacter pylori* infection and abnormalities of acid secretion in patients with duodenal ulcer disease. *Gastroenterology* 1995;109:681–91.
34 El-Omar EM, Oien K, El-Nujumi A, *et al*. *Helicobacter pylori* infection and chronic gastric acid hyposecretion. *Gastroenterology* 1997;113:15–24.
35 Malfertheiner P. The intriguing relationship of *Helicobacter pylori* infection and acid secretion in peptic ulcer disease and gastric cancer. *Dig Dis* 2011;29:459–64.
36 Dinis-Ribeiro M, Areia M, de Vries AC, *et al*. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP) and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). *Endoscopy* 2012;44:74–94.
37 Correa P. Gastric cancer: overview. *Gastroenterol Clin North Am* 2013;42:211–17.
38 Tanaka A, Kamada T, Inoue K, *et al*. Histological evaluation of patients with gastritis at high risk of developing gastric cancer using a conventional index. *Pathol Res Pract* 2011;207:354–8.
39 Meining A, Bayerdörffer E, Müller P, *et al*. Gastric carcinoma risk index in patients infected with *Helicobacter pylori*. *Virchows Arch* 1998;432:311–14.
40 Naylor GM, Gotoda T, Dixon M, *et al*. Why does Japan have a high incidence of gastric cancer? Comparison of gastritis between UK and Japanese patients. *Gut* 2006;55:1545–52.
41 Miehlke S, Hackelsberger A, Meining A, *et al*. Severe expression of corpus gastritis is characteristic in gastric cancer patients infected with *Helicobacter pylori*. *Br J Cancer* 1998;78:263–6.
42 Yeomans ND, Naesdal J. Systematic Review: ulcer definition in NSAID ulcer prevention. *Aliment Pharamcol Ther* 2008;27:465–72.
43 Toljamo K, Niemelä S, Karvonen AL, *et al*. Histopathology of gastric erosions. Association with etiological factors and chronicity. *Helicobacter* 2011;16:444–51.
44 Hart J, Hawkey CJ, Lanas A, *et al*. Predictors of gastroduodenal erosions in patients taking low-dose aspirin. *Aliment Pharmacol Ther* 2010;31:143–9.
45 Kato M, Terao S, Adachi K, *et al*. Changes in endoscopic findings of gastritis after cure of *H. pylori* infection: multicenter prospective trial. *Dig Endosc* 2013;25:264–73.
46 Annibale B, Sprile MR, D'ambra G, *et al*. Cure of *Helicobacter pylori* infection in atrophic body gastritis patients does not improve mucosal atrophy but reduces hypergastrinemia and its related effects on body ECL-cell hyperplasia. *Aliment Pharmacol Ther* 2000;14:625–34.
47 Iijima K, Ohara S, Sekine H, *et al*. Changes in gastric secretion assayed by endoscopic gastrin test before and after *Helicobacter pylori* eradication. *Gut* 2000;46:20–6.
48 Hawkey CJ, Wilson I, Naesdal J, *et al*. Influence of sex and *Helicobacter pylori* on development and healing of gastroduodenal lesions in non-steroidal anti-inflammatory drug users. *Gut* 2002;51:344–50.
49 Lanza FL, Evans DG, Graham DY. Effect of *Helicobacter pylori* infection on the severity of gastroduodenal mucosal injury after the acute administration of naproxen or aspirin to normal volunteers. *Am J Gastroenterol* 1991;86:735–7.
50 Sonnenberg A, Lash RH, Genta RM. A national study of *Helicobacter pylori* infection in gastric biopsy specimens. *Gastroenterology* 2010;139:1894–901.
51 Graham DY, Opekun AR, Osato MS, *et al*. Challenge model for *Helicobacter pylori* infection in human volunteers. *Gut* 2004;53:1235–43.
52 Bode G, Brenner H, Adler G, *et al*. Dyspeptic symptoms in middle-aged to old adults: the role of *Helicobacter pylori* infection and various demographic and lifestyle factors. *J Intern Med* 2002;252:41–7.
53 Moayyedi P, Forman D, Braunholtz D, *et al*. The proportion of upper gastrointestinal symptoms in the community associated with *Helicobacter pylori*, lifestyle factors and nonsteroidal anti-inflammatory drugs. Leeds HELP Study Group. *Am J Gastroenterol* 2000;95:1448–55.
54 Wildner-Christensen M, Hansen JM, De Muckadell OB. Risk factors for dyspepsia in a general population: non-steroidal anti-inflammatory drugs, cigarette smoking and unemployment are more important than *Helicobacter pylori* infection. *Scand J Gastroenterol* 2006;41:149–54.
55 Nandurkar S, Talley NJ, Xia H, *et al*. Dyspepsia in the community is linked to smoking and aspirin use but not to *Helicobacter pylori* infection. *Arch Intern Med* 1998;158:1427–33.
56 Makris N, Barkun A, Crott R, *et al*. Cost-effectiveness of alternative approaches in the management of dyspepsia. *Int J Technol Assess Health Care* 2003;19:446–64.
57 Ang T, Fock KM, Teo EK, *et al*. *Helicobacter pylori* eradication versus prokinetics in the treatment of functional dyspepsia: a randomized, double-blind study. *J Gastroenterol* 2006;41:647–53.

58 Delaney B, Ford AC, Forman D, *et al*. Initial management strategies for dyspepsia. *Cochrane Database Syst Rev* 2009;7:CD001961.
59 Suzuki H, Moayyedi P. *Helicobacter pylori* infection in functional dyspepsia. *Nat Rev Gastroenterol Hepatol* 2013;10:168–74.
60 Malfertheiner P, Mossner J, Fischbach W, *et al*. *Helicobacter pylori* eradication is beneficial in the treatment of functional dyspepsia. *Aliment Pharmacol Ther* 2003;18:615–25.
61 Mazzoleni LE, Sander GB, Francesconi CF, *et al*. *Helicobacter pylori* eradication in functional dyspepsia: HEROES trial. *Arch Intern Med* 2011;171:1929–36.
62 Tack J, Talley NJ, Camilleri M, *et al*. Functional gastroduodenal disorders. *Gastroenterology* 2006;130:1466–79.
63 Camilleri M, Stanghellini V. Current management strategies and emerging treatments for functional dyspepsia. *Nat Rev Gastroenterol Hepatol* 2013;10:187–94.
64 Tack J, Talley NJ. Gastroduodenal disorders. *Am J Gastroenterol* 2010;105:757–63.
65 Dinis-Ribeiro M, da Costa-Pereira A, Lopes C, *et al*. Magnification chromoendoscopy for the diagnosis of gastric intestinal metaplasia and dysplasia. *Gastrointest Endosc* 2003;57:498–504.
66 Anagnostopoulos GK, Yao K, Kaye P, *et al*. High-resolution magnification endoscopy can reliably identify normal gastric mucosa, *Helicobacter pylori*-associated gastritis and gastric atrophy. *Endoscopy* 2007;39:202–7.
67 Gonen C, Simsek I, Sarioglu S, *et al*. Comparison of high resolution magnifying endoscopy and standard videoendoscopy for the diagnosis of *Helicobacter pylori* gastritis in routine clinical practice: a prospective study. *Helicobacter* 2009;14:12–21.
68 Uedo N, Ishihara R, Iishi H, *et al*. A new method of diagnosing gastric intestinal metaplasia: narrow-band imaging with magnifying endoscopy. *Endoscopy* 2006;38:819–24.
69 Tahara T, Shibata T, Nakamura M, *et al*. Gastric mucosal pattern by using magnifying narrow-band imaging endoscopy clearly distinguishes histological and serological severity of chronic gastritis. *Gastrointest Endosc* 2009;70:246–53.
70 Kato M, Kaise M, Yonezawa J, *et al*. Magnifying endoscopy with narrow-band imaging achieves superior accuracy in the differential diagnosis of superficial gastric lesions identified with white-light endoscopy: a prospective study. *Gastrointest Endosc* 2010;72:523–9.
71 Capelle LG, Haringsma J, de Vries AC, *et al*. Narrow band imaging for the detection of gastric intestinal metaplasia and dysplasia during surveillance endoscopy. *Dig Dis Sci* 2010;55:3442–8.
72 Osawa H, Yamamoto H, Miura Y, *et al*. Blue laser imaging provides excellent endsocopic images of upper gastrointestinal lesions. *Video J Encyclopedia of GI endoscopy* 2014;1:607–10.
73 Lim JH, Kim N, Lee HS, *et al*. Correlation between endoscopic and histological diagnoses of gastric intestinal metaplasia. *Gut Liver* 2013;7:41–50.
74 de Vries AC, Haringsma J, de Vries RA, *et al*. Biopsy strategies for endoscopic surveillance of pre-malignant gastric lesions. *Helicobacter* 2010;15:259–64.
75 El-Zimaity HM, Graham DY. Evaluation of gastric mucosal biopsy site and number for identification of *Helicobacter pylori* or intestinal metaplasia: role of the Sydney System. *Hum Pathol* 1999;30:72–7.
76 Satoh K, Kimura K, Taniguchi Y, *et al*. Biopsy sites suitable for the diagnosis of *Helicobacter pylori* infection and the assessment of the extent of atrophic gastritis. *Am J Gastroenterol* 1998;93:569–73.
77 Pimentel-Nunes P, Dinis-Ribeiro M, Soares JB, *et al*. A multicenter validation of an endoscopic classification with narrow band imaging for gastric precancerous and cancerous lesions. *Endsocopy* 2012;44:236–46.
78 Kikuste I, Stirna D, Liepniece-Karele I, *et al*. The accuracy of flexible spectral imaging colour enhancement for the diagnosis of gastric intestinal metaplasia: do we still need histology to select individuals at risk of adenocarcinoma? *Eur J Gastroenterol Hepatol* 2014;26:704–9.
79 de Vries AC, van Grieken NC, Looman CW, *et al*. Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. *Gastroenterology* 2008;134:945–52.
80 Rugge M, Kim JG, Mahachai V, *et al*. OLGA gastritis staging in young adults and country-specific gastric cancer risk. *Int J Surg Pathol* 2008;16:150–4.
81 Rugge M, Meggio A, Pennelli G, *et al*. Gastritis staging in clinical practice: the OLGA staging system. *Gut* 2007;l56:631–6.
82 Rugge M, de Boni M, Pennelli G, *et al*. Gastritis OLGA-staging and gastric cancer risk: a twelve-year clinico-pathological follow-up study. *Aliment Pharmacol Ther* 2010;31:1104–11.
83 den Hoed CM, Holster IL, Capelle LG, *et al*. Follow-up of premalignant lesions in patients at risk for progression to gastric cancer. *Endoscopy* 2013;45:249–56.
84 Rugge M, Fassan M, Pizzi M, *et al*. Operative link for gastritis assessment vs operative link on intestinal metaplasia assessment. *World J Gastroenterol* 2011;17:4596–601.
85 Kuipers EJ. In through the out door: serology for atrophic gastritis. *Eur J Gastroenterol Hepatol* 2003;15:877–9.
86 Watabe H, Mitsushima T, Yamaji Y, *et al*. Predicting the development of gastric cancer from combining *Helicobacter pylori* antibodies and serum pepsinogen status: a prospective endoscopic cohort study. *Gut* 2005;54:764–8.
87 Miki K. Gastric cancer screening by combined assay for serum anti-*Helicobacter pylori* IgG antibody and serum pepsinogen levels-"ABC method". *Proc Jpn Acad SerB Phys Biol Sci* 2011;87:405–14.
88 Broutet N, Plebani M, Sakarovitch C, *et al*. Pepsinogen A, pepsinogen C and gastrin as markers of atrophic chronic gastritis in European dyspeptics. *Br J Cancer* 2003;88:1239–47.
89 Agreus L, Kuipers EJ, Kupcinskas L, *et al*. Rationale in diagnosis and screening of atrophic gastritis with stomach-specific plasma biomarkers. *Scand J Gastroenterol* 2012;47:136–47.
90 Okuda M, Nakazawa T, Booka M, *et al*. Evaluation of a urine antibody test for *Helicobacter pylori* in Japanese children. *J Pediatr* 2004;144:196–9.
91 Okuda M, Sugiyama T, Fukunaga K, *et al*. A strain-specific antigen in Japanese *Helicobacter pylori* recognized in sera of Japanese children. *Clin Diagn Lab Immunol* 2005;12:1280–4.
92 Mégraud F, European Paediatric Task Force on *Helicobacter pylori*. Comparison of non-invasive tests to detect *Helicobacter pylori* infection in children and adolescents: results of a multicenter European study. *J Pediatr* 2005;146:198–203.
93 Ford AC, Forman D, Hunt RH, *et al*. *Helicobacter pylori* eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. *BMJ* 2014;348:g3174.
94 Take S, Mizuno M, Ishiki K, *et al*. Baseline gastric mucosal atrophy is a risk factor associated with the development of gastric cancer after *Helicobacter pylori* eradication therapy in patients with peptic ulcer diseases. *J Gastroenterol* 2007;42 (Suppl 7):21–7.
95 Chen HN, Wang Z, Li X, *et al*. *Helicobacter pylori* eradication cannot reduce the risk of Gastric Cancer in patients with intestinal metaplasia and dysplasia: evidence from a meta-analysis. *Gastric Cancer* Published Online First: 22 Jan 2015. doi:10.1007/s10120-015-0462-7.
96 de Vries AC, Kuipers EJ, Rauws EA. *Helicobacter pylori* eradication and gastric cancer: when is the horse out of the barn? *Am J Gastroenterol* 2009;104: 1342–5.
97 Zhang YY, Xia HH, Zhuang ZH, *et al*. Review article: 'true' re-infection of *Helicobacter pylori* after successful eradication--worldwide annual rates, risk factors and clinical implications. *Aliment Pharmacol Ther* 2009;29:145–60.
98 Franchini M, Vescovi PP, Garofano M, *et al*. *Helicobacter pylori*-associated idiopathic thrombocytopenic purpura: a narrative review. *Semin in Thromb Hemost* 2012;38:463–8.
99 Franchini M, Cruciani M, Mengoli C, *et al*. Effect of *Helicobacter pylori* eradication on platelet count in idiopathic thrombocytopenic purpura: a systematic review and meta-analysis. *J Antimicrob Chemother* 2007;60:237–46.
100 Harvey RF, Lane JA, Nair P, *et al*. Clinical trial: prolonged beneficial effect of *Helicobacter pylori* eradication on dyspepsia consultations—the Bristol Helicobacter Project. *Aliment Pharmacol Ther* 2010;32:394–400.
101 Veldhuyzen van Zanten SJ, Flook N, Chiba N, *et al*. An evidence-based approach to the management of uninvestigated dyspepsia in the era of *Helicobacter pylori*. Canadian Dyspepsia Working Group. *CMAJ* 2000;162:S3–23.
102 Ohkusa T, Takashimizu I, Fujiki K, *et al*. Disappearance of hyperplastic polyps in the stomach after eradication of *Helicobacter pylori*. A randomized, clinical trial. *Ann Intern Med* 1998;129:712–15.
103 Suzuki H, Saito Y, Hibi T. *Helicobacter pylori* and Gastric Mucosa-associated Lymphoid Tissue (MALT) Lymphoma: updated review of clinical outcomes and the molecular pathogenesis. *Gut Liver* 2009;3:81–7.
104 Du MQ, Diss TC, Dogan A, *et al*. Clone-specific PCR reveals wide dissemination of gastric MALT lymphoma to the gastric mucosa. *J Pathol* 2000;192: 488–93.
105 Clark EG, Danbolt N. The Oslo study of the natural course of untreated syphilis. An epidemiologic investigation based on re-study of the Boeck-Burrsgaard material. *Med Clin North Am* 1964;48:613–23.
106 Gutierrez O, Melo M, Segura AM, *et al*. Cure of *Helicobacter pylori* infection improves gastric acid secretion in patients with corpus gastritis. *Scand J Gastroenterol* 1997;32:664–8.
107 Gisbert JP, Khorrami S, Carballo F, *et al*. *H. pylori* eradication therapy vs. antisecretory non-eradication therapy (with or without long-term maintenance antisecretory therapy) for the prevention of recurrent bleeding from peptic ulcer. *Cochrane Database Syst Rev* 2004;(2):CD004062.
108 Vergara M, Catalan M, Gisbert JP, *et al*. Meta-analysis: role of *Helicobacter pylori* eradication in the prevention of peptic ulcer in NSAID users. *Aliment Pharmacol Ther* 2005;21:1411–18.
109 Genta RM, Lew GM, Graham DY. Changes in gastric mucosa following eradication of *Helicobacter pylori*. *Mod Pathol* 1993;6:281–9.
110 Franceschi F, Genta RM, Sepulveda AR. Gastric mucosa: long-term outcome after cure of *Helicobacter pylori* infection. *J Gastroenterol* 2002;37(Suppl 13):17–23.
111 Graham DY, Shiotani A, El-Zimaity HM. Chromoendoscopy points the way to understanding recovery of gastric function after *Helicobacter pylori* eradication. *Gastrointest Endosc* 2006;64:686–9.
112 Graham DY. *Helicobacter pylori* and perturbations in acid secretion: the end of the beginning [editorial]. *Gastroenterology* 1996;110:1647–50.

113 Haruma K, Mihara M, Okamoto E, *et al*. Eradication of *Helicobacter pylori* increases gastric acidity in patients with atrophic gastritis of the corpus-evaluation of 24-h pH monitoring. *Aliment Pharmacol Ther* 1999;13:155–62.
114 Yasunaga Y, Shinomura Y, Kanayama S, *et al*. Improved fold width and increased acid secretion after eradication of the organism in *Helicobacter pylori* associated enlarged fold gastritis. *Gut* 1994;35:1571–4.
115 Lee YC, Chen TH, Chiu HM, *et al*. The benefit of mass eradication of *Helicobacter pylori* infection: a community-based study of gastric cancer prevention. *Gut* 2013;62:676–82.
116 Atherton JC, Blaser MJ. Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications. *J Clin Invest* 2009;119:2475–87.
117 Dorer MS, Talarico S, Salama NR. *Helicobacter pylori*'s unconventional role in health and disease. *PLoS Pathog* 2009;5:e1000544.
118 Toller IM, Neelsen KJ, Steger M, *et al*. Carcinogenic bacterial pathogen *Helicobacter pylori* triggers DNA double-strand breaks and a DNA damage response in its host cells. *Proc Natl Acad Sci* 2011;108:14944–9.
119 Kim JJ, Tao H, Carloni E, *et al*. *Helicobacter pylori* impairs DNA mismatch repair in gastric epithelial cells. *Gastroenterology* 2002;123:542–53.
120 Matsumoto Y, Marusawa H, Kinoshita K, *et al*. *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. *Nat Med* 2007;13:470–6.
121 Shin CM, Kim N, Jung Y, *et al*. Genome-wide DNA methylation profiles in noncancerous gastric mucosae with regard to *Helicobacter pylori* infection and the presence of gastric cancer. *Helicobacter* 2011;16:179–88.
122 Ushijima T, Hattori N. Molecular pathways: involvement of *Helicobacter pylori*-triggered inflammation in the formation of an epigenetic field defect and its usefulness as cancer risk and exposure markers. *Clin Cancer Res* 2012;18:923–9.
123 Zabaleta J. MicroRNA: a bridge from *H. pylori* infection to gastritis and gastric cancer development. *Front Genet* 2012;3:294.
124 Nishizawa T, Suzuki H. The role of microRNA in gastric malignancy. *Int J Mol Sci* 2013;14:9487–96.
125 Mégraud F, Coenen S, Versporten A, *et al*. *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* 2013;62:34–42.
126 Furuta T, Graham DY. Pharmacologic aspects of eradication therapy for *Helicobacter pylori* Infection. *Gastroenterol Clin North Am* 2010;39:465–80.
127 Graham DY, Shiotani A. Which therapy for *Helicobacter pylori* infection? *Gastroenterology* 2012;143:10–12.
128 Graham DY, Lee YC, Wu MS. Rational *Helicobacter pylori* therapy: evidence-based medicine rather than medicine-based evidence. *Clin Gastroenterol Hepatol* 2014;12:177–86.
129 Wu JY, Liou JM, Graham DY. Evidence-based recommendations for successful *Helicobacter pylori* treatment. *Expert Rev Gastroenterol Hepatol* 2014;8:21–8.
130 International Agency for Research on Cancer. *Helicobacter pylori* eradication as a strategy for preventing gastric cancer. IARC Working Group Reports. Vol 8, WHO Press, World Health Organization, Geneva, Switzerland, 2014.
131 Shiotani A, Cen P, Graham DY. Eradication of gastric cancer is now both possible and practical. *Semin Cancer Biol* 2013;23:492–501.
132 Matsueda S, Graham DY. Immunotherapy in gastric cancer. *World J Gastroenterol* 2014;20:1657–66.
133 Graham DY, Shiotani A. The time to eradicate gastric cancer is now. *Gut* 2005;54:735–8.
134 Kimura K, Satoh K, Ido K, *et al*. Gastritis in the Japanese stomach. *Scand J Gastroenterol* 1996;31(Suppl 214):17–20.
135 Kimura K, Takemoto T. An endoscopic recognition of the atrophic border and its significance in chronic gastritis. *Endoscopy* 1969;1:87–97.
136 Miki K. Gastric cancer screening using the serum pepsinogen test method. *Gastric Cancer* 2006;9:245–53.
137 Dinis-Ribeiro M, Yamaki G, Miki K, *et al*. Meta-analysis on the validity of pepsinogen test for gastric carcinoma, dysplasia or chronic atrophic gastritis screening. *J Med Screen* 2004;11:141–7.
138 Dinis-Ribeiro M, da Costa-Pereira A, Lopes C, *et al*. Validity of serum pepsinogen I/II ratio for the diagnosis of gastric epithelial dysplasia and intestinal metaplasia during the follow-up of patients at risk for intestinal-type gastric adenocarcinoma. *Neoplasia* 2004;6:449–56.
139 Schindler R. Chronic gastritis. *Bull NY Acad Med* 1939;15:322–37.
140 Lau JY, Barkun A, Fan DM, *et al*. Challenges in the management of acute peptic ulcer bleeding. *Lancet* 2013;381:2033–43.
141 Jönsson KA, Gotthard R, Bodemar G, *et al*. The clinical relevance of endoscopic and histologic inflammation of gastroduodenal mucosa in dyspepsia of unknown origin. *Scand J Gastroenterol* 1989;24:385–95.
142 Talley N, Phillips SF. Non-ulcer dyspepsia: potential causes and pathophysiology. *Ann Intern Med* 1988;108:865–79.
143 Lambert JR. The role of *Helicobacter pylori* in nonulcer dyspepsia. A debate—for. *Gastroenterol Clin North Am* 1993;22:141–51.
144 Talley NJ. The role of *Helicobacter pylori* in nonulcer dyspepsia. A debate—against. *Gastroenterol Clin North Am* 1993;22:153–67.

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ORIGINAL ARTICLE

# Analysis of *Helicobacter pylori* genotypes in clinical gastric wash samples

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**Abstract** *Helicobacter pylori* is a key factor in the development of gastric cancer; indeed, clearance of *H. pylori* helps prevent gastric cancer. However, the relationship between gastric cancer and the abundance and diversity of *H. pylori* genotypes in the stomach remains unknown. Here, we present, for the first time, a quantitative analysis of *H. pylori* genotypes in gastric washes. A method was first developed to assess diversity and abundance by pyrosequencing and analysis of single nucleotide polymorphisms in 23S ribosomal RNA (rRNA), a gene associated with clarithromycin resistance. This method was then validated using arbitrarily mixed plasmids carrying 23S rRNA with single nucleotide polymorphisms. Multiple strains were detected in many of 34 clinical samples, with frequency 24.3 ± 24.2 and 26.3 ± 33.8 % for the A2143G and A2144G strains, respectively. Importantly, results obtained from gastric washes were similar to those obtained from biopsy samples. The method provides opportunities to investigate drug resistance in *H. pylori* and assess potential biomarkers of gastric cancer risk, and should thus be validated in large-scale clinical trials.

**Keywords** *Helicobacter pylori* · Gastric wash · 23S rRNA · Pyrosequencing

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## Introduction

Gastric cancer is the third leading cause of cancer death in the world, with prognosis determined by tumor stage at diagnosis and treatment [1, 2]. Diagnostic tools such as gastrointestinal endoscopy followed by pathological analysis and/or fluoroscopy have proven useful; however, the mortality rate has remained high throughout the world [3]. Gastric cancer is believed to result in part from the accumulation of genetic alterations that stimulate oncogene overexpression and/or silence tumor suppressors [4–6]. Epigenetic silencing of tumor-related genes due to aberrant DNA methylation has also been recently reported [7–14]. Notably, aberrant DNA methylation occurs more frequently than mutations in pathologically differentiated adenocarcinoma [15]. *Helicobacter pylori*, which elicits chronic inflammation, is a key factor underlying such aberrant DNA methylation in the stomach mucosa [5].

*H. pylori*, identified in 1982 by Barry Marshall and Robin Warren [16], is a helix-shaped Gram-negative bacterium associated with gastric diseases such as gastritis [17, 18], gastric and duodenum ulcer [19, 20], gastric cancer [4, 6, 12, 13, 21, 22], mucosa-associated lymphoid tissue lymphoma [23], as well as ulcers due to non-steroidal anti-inflammatory drugs [24–26]. *H. pylori* is typically cleared by standard first-line therapies, including proton-pump inhibitors, potassium-competitive acid blockers, amoxicillin, and clarithromycin

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[27, 28]. However, these therapies are becoming less effective because of clarithromycin resistance [29], which, in turn, is reportedly due to A2143G, A2143C, and A2144G point mutations in 23S ribosomal RNA (rRNA) [29–32]. Unfortunately, clarithromycin-resistant strains cannot be identified based on currently available qualitative methods such as *H. pylori* IgG test [33, 34], stool antigen test [35, 36], urea breath test (UBT) [37, 38], and rapid urease test [37, 39].

In this manuscript, we describe a new approach to quantify multiple strains of *H. pylori* in gastric washes. In this approach, developed by co-author Watanabe and colleagues (patent WO2007/132844) [30, 40–43], stomach washes are performed with saline during endoscopy, a topical procedure in which only a small portion of abnormal tissue is excised. As cells are abundantly exfoliated from the gastric mucosa during washing, undamaged human and *H. pylori* DNA can be recovered from the wash and assayed by sensitive and quantitative techniques. Of these, Sanger sequencing is the most reliable for detection of microbial species, although the abundance of multiple genotypes is difficult to quantify by this method. In addition, the method is unsuitable for the clinic because of complex sample processing, long turn-around time, low throughput, and high cost. On the other hand, pyrosequencing methods, in which bases are called by sequential addition of dNTPs, enable genotyping, as well as quantitative analysis of single nucleotide polymorphisms, insertions, and deletions. Thus, we analyzed gastric washes by pyrosequencing to determine the abundance and diversity of *H. pylori* genotypes in each patient [30].

## Material and methods

### Patient characteristics

Both gastric wash and biopsy samples were collected from 17 patients (34 samples) who underwent endoscopy at Hokkaido University Hospital, Hokkaido, Japan, from April 2014 to December 2015. The study was conducted in accordance with all rules and regulations of the Hokkaido University Institutional Review Board (#014-0459), and informed consent was obtained from each patient. Patients were tested by UBT urea breath test (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), Helicocheck rapid urease test (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), and *H. pylori* IgG E-plate (Eiken Chemical Co., Ltd., Tokyo, Japan). A patient who tested positive on at least one test was deemed infected (Table 1).

### Sample collection of gastric washes

Approximately 10 min prior to endoscopy, patients were asked to swallow 100 mL water containing 80 mg dimethylpolysiloxane (Gascon, Kissei Pharmaceutical Co., Ltd., Matsumoto, Japan), 1 g sodium bicarbonate, and 20, 000 units pronase (Pronase MS, Kaken Pharmaceutical Co., Ltd., Tokyo, Japan). Gastric washes were collected in specimen containers (No. 111219, Fortegrow Medical, Tochigi, Japan), which were directly fitted to the endoscope modulator. Gastric washes were manually aspirated under vacuum

**Table 1** Patient characteristics

| No. | Age | Sex | RUT | UBT | HP-IgG | Histological assessment[a] | Clinical HP examination |
|---|---|---|---|---|---|---|---|
| 1 | 52 | M | − | 1.3 | <3 | Normal | − |
| 2 | 67 | F | − | 1.8 | <3 | Normal | − |
| 3 | 71 | F | − | 0 | <3 | Normal | − |
| 4 | 65 | F | + | 54.6 | 1.1 | Marked | + |
| 5 | 63 | M | + | 24.7 | 29 | Marked | + |
| 6 | 57 | F | + | 13.6 | 27 | Marked | + |
| 7 | 61 | M | − | 0.1 | <3 | Normal | − |
| 8 | 68 | F | + | 10.6 | 27 | Marked | + |
| 9 | 84 | M | + | 4.6 | 43 | Marked | + |
| 10 | 73 | F | + | 25.3 | 70 | Marked | + |
| 11 | 40 | F | − | 0 | <3 | Normal | − |
| 12 | 83 | F | + | 4.7 | 25 | Marked | + |
| 13 | 62 | M | + | 5.1 | 14 | Marked | + |
| 14 | 69 | M | + | 2.8 | 18 | Marked | + |
| 15 | 77 | M | + | 4.3 | 21 | Marked | + |
| 16 | 63 | M | + | 21 | 26 | Marked | + |
| 17 | 50 | M | + | 3.8 | 56 | Marked | + |

[a] Histological assessment was graded by updated Sydney system

through the suction channel of the endoscope, centrifuged immediately, and resulting pellets were frozen at −80 °C. Tissue samples with diameter approximately 5 mm were then collected using Radial Jaw (Boston Scientific Corp., Natick, MA), with guidance from a GIF-Q260 endoscope fitted to an EVIS LUCERA system (Olympus, Inc., Tokyo, Japan). Disposable sample collection tubes, connector tubes, and other endoscopic devices were used. The endoscope was washed after each procedure according to guidelines, using 0.55 % DISOPA (Johnson and Johnson, Langhorne, PA) and an automatic washing machine.

### DNA extraction

DNA was extracted from gastric wash and biopsy samples by phenol chloroform. Briefly, tissues or frozen pellets from gastric washes were resuspended in 10 % SDS and proteinase K, mixed vigorously, and incubated at 37 °C for 6 h. Samples were then mixed vigorously for approximately 30 s with one volume of 25:24:1 phenol:chloroform:isoamyl alcohol and centrifuged at room temperature for 5 min at 16,000×*g*. The aqueous layer was then transferred to a fresh tube and extracted in the same manner another two times. Final extracts were then mixed with 100 % ethanol, centrifuged at 4 °C for 5 min at 16,000×*g*, and stored at −20 °C overnight to precipitate DNA. Precipitates were collected by centrifugation, washed with 70 % ethanol, and centrifuged at 4 °C for 2 min at 16,000×*g*. As much of the supernatant was removed as possible, and pellets were resuspended in 500 μL Tris-EDTA buffer. Final DNA concentration and quality were measured on a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

### Construction of plasmids encoding variants of *H. pylori* 23S rRNA

Wild-type, A2143G, A2143C, and A2144G point mutants of *H. pylori* 23S rRNA (NCBI database accession no. U27270) were separately inserted into pTAKN-2 plasmids.

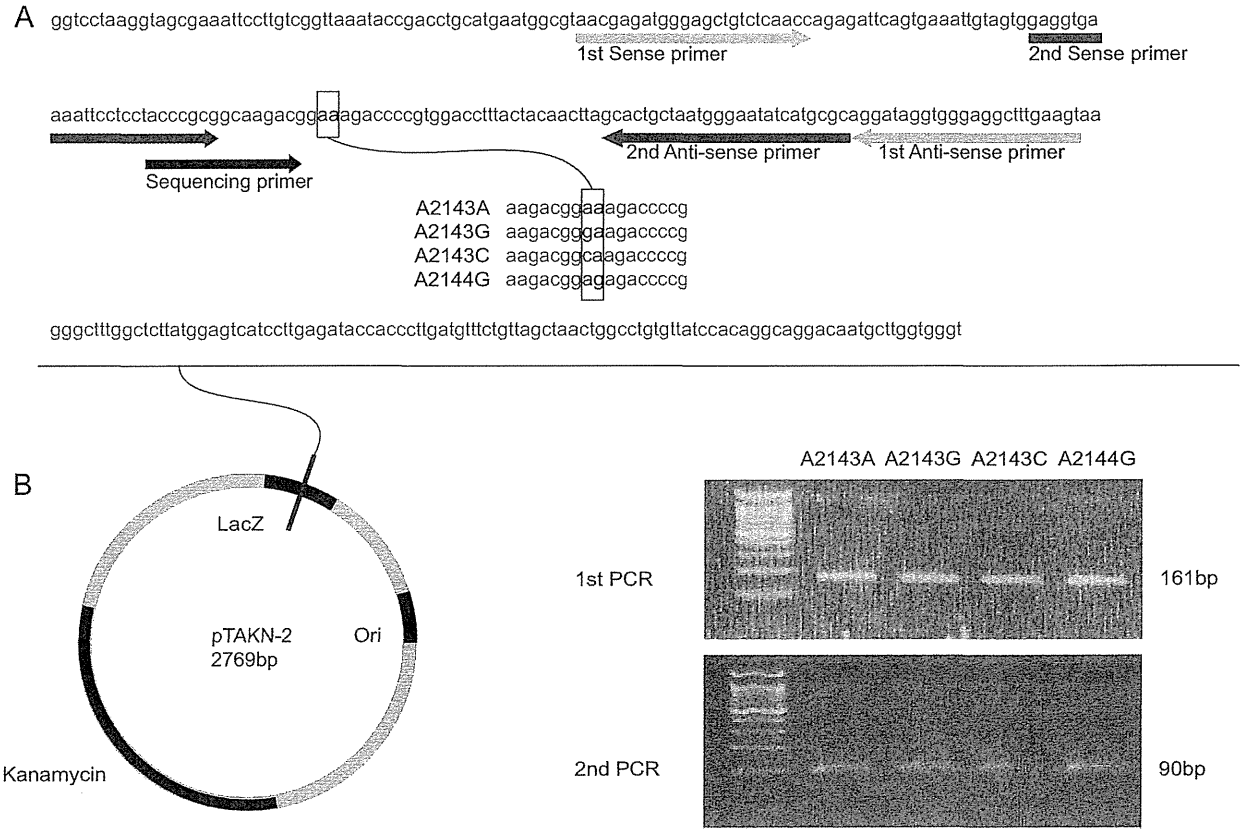



**Fig. 1** 23S rRNA sequence, primers, and plasmids. **a** Primers for nested PCR and pyrosequencing. *Helicobacter pylori* 23S rRNA was first amplified with the 1st sense/antisense primer set, and then by the 2nd sense/biotin antisense primer set. Biotin-tagged products from nested PCR were then analyzed by pyrosequencing to quantify A2143C, A2143G, and A2144G point mutants. **b** Fragments of wild-type or mutated 23S rRNA were separately inserted into pTAKN-2, *left*. *Right*, gel electrophoresis of products from the first PCR reaction, with expected size 161 bp, and of products from the second PCR reaction, which amplified a 90-bp region encompassing A2143 (wild type), A2143C, A2143G, and A2144G

### Quantitative pyrosequencing analysis of multiple *HPs*

Pyrosequencing enables quantitative analysis of the abundance of polymorphisms at a given site. To validate this approach, plasmids containing wild-type and mutated 23S rRNA in arbitrary ratios of 0, 30, 50, 70, and 100 % were analyzed. 23S rRNA was then amplified and biotinylated by nested PCR from gastric wash and biopsy samples. In

**Table 2** Ratio of *H. pylori* genotypes in plasmids, gastric wash, and biopsy samples

| | | | 161 bp | | | | |
|---|---|---|---|---|---|---|---|
| | | | 2143 | | | 2144 | |
| No. | Template DNA | | A | C | G | A | G |
| A | | | | | | | |
| 1 | Plasmid DNA A 100 % G 0 % | | 100 | 0 | 0 | 99 | 1 |
| 2 | Plasmid DNA A 70 % G 30 % | | 69 | 0 | 31 | 72 | 28 |
| 3 | Plasmid DNA A 50 % G 50 % | | 51 | 0 | 49 | 54 | 46 |
| 4 | Plasmid DNA A 30 % G 70 % | | 25 | 0 | 75 | 35 | 65 |
| 5 | Plasmid DNA A 0 % G 100 % | | 1 | 0 | 99 | 7 | 93 |
| | | | 161 bp | | | | |
| | | | 2143 | | | 2144 | |
| No. | Biopsy/gastric washes | Clinical HP exam | A | C | G | A | G |
| B | | | | | | | |
| 1 | Biopsy | – | 48 | 0 | 52 | 68 | 32 |
| | Gastric washes | | | N/A | | 88 | 12 |
| 2 | Biopsy | – | 56 | 0 | 44 | 81 | 19 |
| | Gastric washes | | | N/A | | N/A | |
| 3 | Biopsy | – | 56 | 0 | 44 | 62 | 38 |
| | Gastric washes | | | N/A | | N/A | |
| 4 | Biopsy | + | 30 | 0 | 70 | 10 | 90 |
| | Gastric washes | | 54 | 0 | 46 | 14 | 86 |
| 5 | Biopsy | + | 100 | 0 | 0 | 99 | 1 |
| | Gastric washes | | 100 | 0 | 0 | 99 | 1 |
| 6 | Biopsy | + | 41 | 0 | 59 | 14 | 86 |
| | Gastric washes | | 22 | 0 | 78 | 5 | 95 |
| 7 | Biopsy | – | 65 | 1 | 34 | 82 | 18 |
| | Gastric washes | | | N/A | | N/A | |
| 8 | Biopsy | + | 100 | 0 | 0 | 98 | 2 |
| | Gastric washes | | 100 | 0 | 0 | 99 | 1 |
| 9 | Biopsy | + | 100 | 0 | 0 | 99 | 1 |
| | Gastric washes | | 100 | 0 | 0 | 99 | 1 |
| 10 | Biopsy | + | 100 | 0 | 0 | 99 | 1 |
| | Gastric washes | | 100 | 0 | 0 | 99 | 1 |
| 11 | Biopsy | – | 75 | 1 | 25 | 85 | 15 |
| | Gastric washes | | | N/A | | N/A | |
| 12 | Biopsy | + | 100 | 0 | 0 | 97 | 3 |
| | Gastric washes | | 100 | 0 | 0 | 98 | 2 |
| 13 | Biopsy | + | 82 | 0 | 17 | 92 | 8 |
| | Gastric washes | | 100 | 0 | 0 | 98 | 2 |
| 14 | Biopsy | + | 71 | 0 | 28 | 84 | 16 |
| | Gastric washes | | 58 | 11 | 31 | 79 | 21 |
| 15 | Biopsy | + | 63 | 0 | 36 | 86 | 14 |
| | Gastric washes | | 57 | 5 | 38 | 79 | 21 |
| 16 | Biopsy | + | 76 | 0 | 24 | 51 | 49 |
| | Gastric washes | | 79 | 0 | 21 | 48 | 52 |
| 17 | Biopsy | + | 29 | 0 | 71 | 3 | 97 |
| | Gastric washes | | 25 | 0 | 75 | 1 | 99 |

the first reaction, a 255-bp fragment was amplified with sense primer "acgagatgggagctgtctcaacc" and antisense primer "agcattgtcctgcctgtggataac." Amplification products were then used as template in the second reaction to amplify a 90-bp fragment with sense primer "gaggtgaaaattcctcctacccgcg" and antisense primer "gcgcatgatattcccattagcagtgc." Reactions consisted of touchdown PCR with denaturation at 95 °C for 30 s, annealing at appropriate temperatures for 30 s, and extension at 72 °C for 30 s. Finally, amplification products were analyzed by pyrosequencing on a Pyromark Q24 (QIAGEN, Valencia, CA) using primer "acccgcggcaagacg." Sequence to analyze A2143V "GVAAGACCCC GTGGACCTTT ACTACAA" and A2144R "RAGACCCCGT GGACCTTT AC TACAAC."

### Abundance of *H. pylori* 23S rRNA in clinical samples

We analyzed 34 clinical samples (17 gastric washes and 17 biopsies) by SYBR Green real-time PCR, with *H. pylori* 23S rRNA as target and human *GAPDH* as reference. *H. pylori* 23S rRNA was amplified with sense primer "acgagatgggagctgtctcaacc" and antisense primer "agcattgtcctgcctgtggataac," while human *GAPDH* was amplified with sense primer "cgagatccctccaaaatcaa" and antisense primer "ctgcaaatgagcctacagca." Reactions were performed on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster, CA) according to the manufacturer's instructions. Data were analyzed in SDS2.1 by comparative cycle threshold (ΔCt).

### Statistical analysis

Mean and 95 % confidential interval were calculated for clinicopathologic features. Correlation between results from gastric wash and biopsy samples was analyzed by Mann–Whitney test for continuous variables, with $p < 0.05$ considered significant. All statistical analyses were performed in PRISM for Windows, Version 4 (GraphPad Prism 6.0.7, Inc., San Diego, CA).

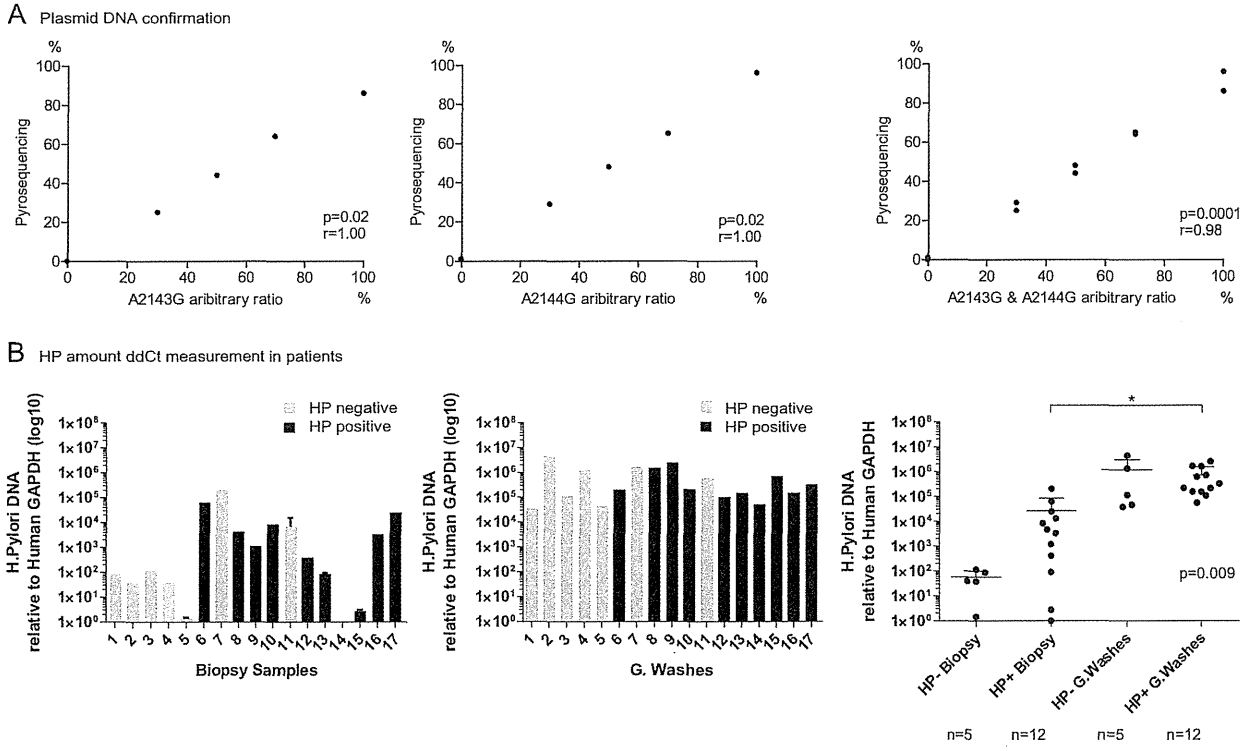



**Fig. 2** Validation of pyrosequencing, and abundance of *Helicobacter pylori* in clinical samples. **a** Plasmids containing wild-type, A2143G, and A2144G 23S rRNA were mixed in arbitrary ratios of 0, 30, 50, 70, and 100 % and analyzed by pyrosequencing. Significant correlation was observed between expected and experimentally determined ratios, with $r = 1.00$ for A2143G ($p = 0.02$), 1.00 for A2144G ($p = 0.02$), and 0.98 for both ($p = 0.0001$). **b** *H. pylori* 23S rRNA was quantified using real-time PCR, with human *GAPDH* as reference. Results are copy numbers obtained by ΔCt. Copy numbers were lower in biopsy samples (56.94 ± 45.1) that tested negative on qualitative tests than in samples that tested positive (27,013 ± 58,715), although the difference was not statistically significant ($p = 0.33$). However, copy numbers were comparable in all gastric wash samples. Further, copy numbers were significantly higher in gastric wash samples that tested positive on qualitative tests (707,706 ± 821,525) than in biopsy samples that tested positive (27,013 ± 58,715; $p < 0.009$)

## Results

### Pyrosequencing is suitable to genotype *H. pylori*

Pyrosequencing was validated as a tool to genotype *H. pylori* using pTAKN-2 plasmids containing wild-type 23S rRNA mixed in arbitrary ratios of 0, 30, 50, 70, and 100 % with A2143G, A2143C, and A2144G point mutations (Figs. 1a, b, and 5 and Table 2a), which are associated with resistance to clarithromycin. Ratios were strongly correlated between pyrosequencing and expected values, with $r=1.00$ for A2143G ($p=0.02$), 1.00 for A2144G ($p=0.02$), and 0.98 for both ($p=0.0001$), indicating that pyrosequencing is a robust method to genotype *H. pylori* (Fig. 2a).

### Amplification of *H. pylori* 23S rRNA from clinical samples

The abundance of *H. pylori* genotypes were assessed in gastric wash and biopsy samples (Table 1) obtained from 17 patients without critical primary illnesses, and without ulcers or tumors, as determined by endoscopy. The patient population consisted of nine males and eight females with mean age 65 ± 11.4 years, of whom five tested negative for *H. pylori* on urea breath test, rapid urease test, and *H. pylori* IgG test. The remaining 12 patients tested positive for *H. pylori* on at least one test. DNA was successfully extracted from all 34 samples, and *H. pylori* 23S rRNA was amplified by nested PCR from all samples, including those determined by qualitative tests to be uninfected (Fig. 2b).

### *H. pylori* abundance in clinical samples

To investigate the discrepancy between nested PCR and qualitative tests, we used real-time PCR to estimate the abundance of *H. pylori* in all clinical samples, as measured by 23S rRNA relative to human *GAPDH*. On average, the abundance of 23S rRNA was lower in biopsy samples that tested negative on qualitative tests (56.94 ± 45.1) than in gastric washes samples that tested negative (27,013 ± 58,715). However, the difference was not statistically significant ($p=0.33$, Fig. 2b). On the other hand, 23S rRNA was significantly more abundant ($p<0.009$) in gastric wash samples that qualitatively tested positive (707,706 ± 821,525) than in biopsy samples that tested positive (27,013 ± 58,715) (Fig. 2b).

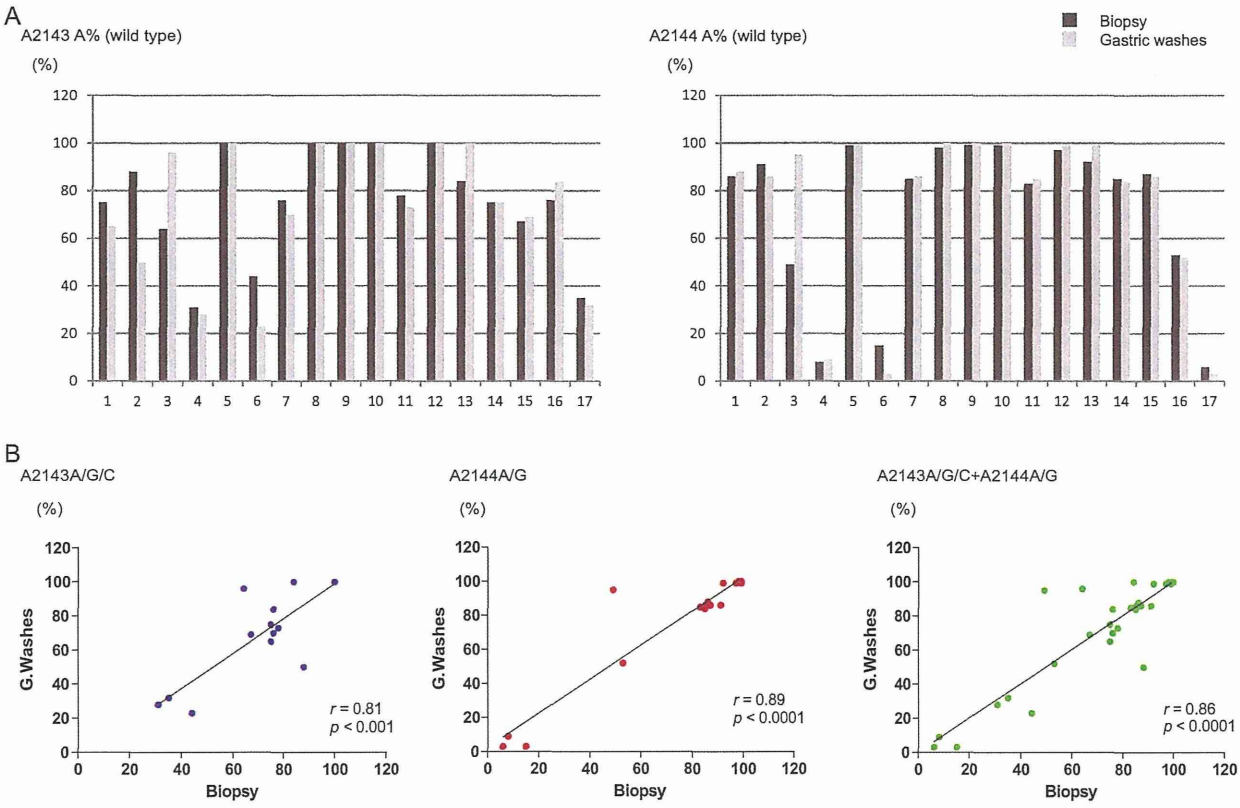



**Fig. 3** Analysis of *Helicobacter pylori* genotypes in clinical samples. The ratio of wild type to A2143G and A2144G strains of *H. pylori* was significantly correlated between biopsy and gastric wash samples, as shown in **a** bar graphs and **b** scatter plots. The correlation coefficient *r* was 0.81 for A2143 ($p=0.001$), 0.89 for A2144 ($p=0.0001$), and 0.86 for both ($p=0.0001$)

### Abundance of *H. pylori* genotypes in clinical samples

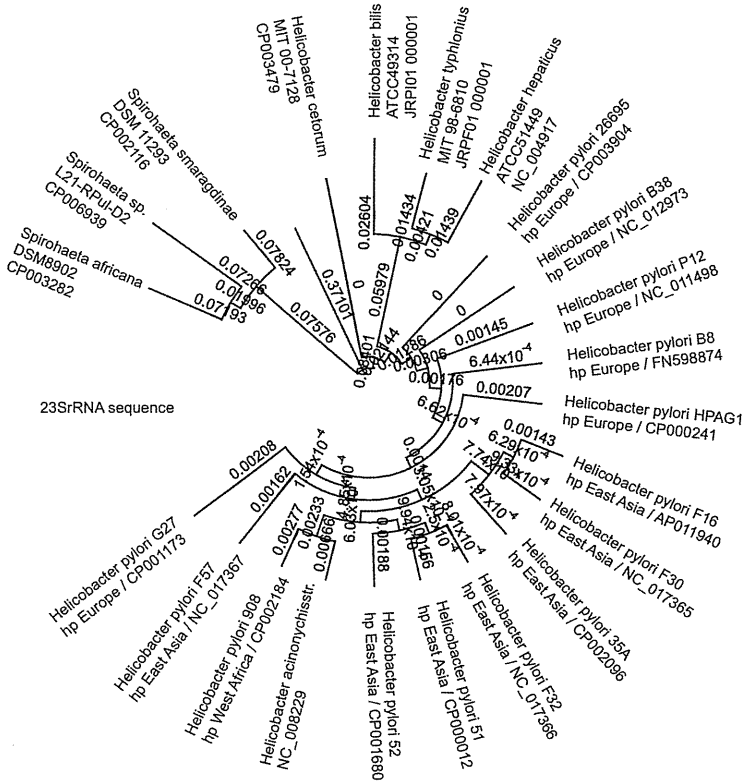
We examined the prevalence of A2143G, A2143C, and A2144G point mutants in gastric wash and biopsy samples. A2143C was not detected (Table 2b). However, the prevalence of A2143G and A2144G was significantly correlated between gastric wash and biopsy samples, with $r=0.81$ for A2143G ($p=0.001$), 0.89 for A2144G ($p=0.0001$), and 0.86 for both ($p=0.001$) (Fig. 3b). Wild-type alleles (A2143 and A2144) were similar in frequency in both samples. However, these alleles were, in most cases, slightly more abundant in biopsy samples than in gastric washes, except for samples 3 and 13, in which these alleles are notably more abundant in the gastric wash. It is possible that the difference in these cases is due to the nature of the data collected, which is localized (or "point") for biopsies, but more extensive (or "plane") for gastric washes. Thus, analyses using gastric washes are probably more informative, because "plane" information may better reflect the state of the whole stomach (Fig. 3a, b).

## Discussion

According to the World Health Organization, cancer is a major cause of mortality worldwide, with 8.2 million deaths reported in 2012. Of these, 1.59 million were attributed to lung cancer, 745,000 were due to liver cancer, 723,000 were due to gastric cancer, and 694,000 were due to colorectal cancer. Breast and esophageal cancer accounted for 521,000 and 400,000 deaths, respectively. Although morbidity from gastric cancer has gradually decreased in recent years, it remains a serious issue [44], especially in Japan, where gastric cancer is just behind lung cancer in terms mortality and where incidence is the second highest in the world after South Korea.

The development and progression of gastric cancer have been examined in many studies, and several risk factors have been identified. Of these, persistent infection with *H. pylori* is considered a key risk factor, along with lifestyle habits, smoking, and diet. *H. pylori* was first classified as a carcinogen in 1994 by the International Agency for Research on Cancer, a specialized arm of the World Health Organization. Indeed, two independent clinical studies by the Agency and by the Japan Gast Study Group indicated that *H. pylori* is involved in more than 80 % of gastric cancer cases and in 90 % of cases of cardia cancer, which is common in Japan. This finding suggested that clearance of *H. pylori* may reduce the incidence of gastric cancer [22].

*H. pylori* is believed to have originated in the human stomach in Africa approximately 60,000 years ago and spread to the Americas through Central Asia, Europe, and East Asia. This long period of interaction with humans and consequent co-evolution was accompanied by several mutations and has




| Accession number | Group | Bacterias |
|---|---|---|
| AP011940 | East Asia | Helicobacter pylori F16 |
| CP000012 | East Asia | Helicobacter pylori 51 |
| CP001680 | East Asia | Helicobacter pylori 52 |
| CP002096 | East Asia | Helicobacter pylori 35A |
| NC_017365 | East Asia | Helicobacter pylori F30 |
| NC_017366 | East Asia | Helicobacter pylori F32 |
| NC_017367 | East Asia | Helicobacter pylori F57 |
| CP000241 | Europe | Helicobacter pylori HPAG1 |
| CP001173 | Europe | Helicobacter pylori G27 |
| CP003904 | Europe | Helicobacter pylori 26695 |
| FN598874 | Europe | Helicobacter pylori B8 |
| NC_011498 | Europe | Helicobacter pylori P12 |
| NC_012973 | Europe | Helicobacter pylori B38 |
| CP002184 | West Africa | Helicobacter pylori 908 |
| NC_000921 | West Africa | Helicobacter pylori J99 |
| CP003479 | | Helicobacter cetorum |
| JRPF01000001 | | Helicobacter typhlonius |
| JRPI01000001 | | Helicobacter bilis |
| NC_004917 | | Helicobacter hepaticus |
| NC_008229 | | Helicobacter acinonychis |
| CP002116 | | Spirochaeta smaragdinae DSM11293 |
| CP003282 | | Spirochaeta africana DSM8902 |
| CP006939 | | Spirochaeta sp. L21-RPul-D2 |

**Fig. 4** 23S rRNA in *Helicobacter* sp. A fragment of 23S rRNA nearly specific to *H. pylori* was identified by bioinformatic analysis

resulted in several distinct lineages, including hpAfrica, hpSahul, hpAsia, hspEastAsia, hpEurope, hspMaori, and hpAmerica. The East Asian lineage harbors a *cag* pathogenicity island, a genetic element associated with atrophic gastritis or gastric cancer and is believed to have been exogenously acquired by horizontal gene transfer [45].

Clarithromycin-resistant *H. pylori* has recently emerged as a consequence of therapy, which typically consists of clarithromycin, amoxicillin, and proton-pump inhibitors. This resistance has been attributed to A2143C/G and A2144G polymorphisms in 23S rRNA, a component of the 50S subunit of the ribosome. However, we hypothesized that these variants do not accumulate by sudden mutation events, but by gradually displacing wild-type *H. pylori* from the stomach. We tested this hypothesis by evaluating 23S rRNA in clinical samples. This study represents the first comprehensive and quantitative analysis of *H. pylori* genotypes in a single stomach [46–48].

We first identified a specific target sequence in 23S rRNA by bioinformatic analysis in Geneious Pro 5.5.6 (Biomatters Ltd., Auckland, New Zealand) and developed a diagnostic procedure based on pyrosequencing, which enables quantification of multiple genotypes in a given sample using primers and probes that target the same site (Fig. 4). We first validated this approach using arbitrary mixtures of plasmids containing fragments of wild-type or polymorphic 23S rRNA (Figs. 2a and 5 and Table 2). Although an endoscopic biopsy is more commonly used for analysis, we next also used gastric wash samples after lavage of the whole stomach, because we believe it is important to obtain more general data on the abundance and diversity of *H. pylori* in the entire stomach (Figs. 2b and 3a, b). This approach is similar to pyrosequencing analysis of DNA methylation in the cancer-specific genes MINT25, RORA, GDNF, ADAM23, PRDM5, and MLF1 using biopsy and gastric wash samples from patients with primary gastric cancer [41, 42]. These protocols were described in *Gastroenterology* in 2009 and in *Tumor Biology* in 2011.

Overall, there was significant correlation between results obtained from gastric wash and biopsy samples, although moderate differences were observed in two cases. Indeed, these differences may be due to the "point" and "plane" nature of data collected from biopsy and gastric wash, respectively (Fig. 3a, b).

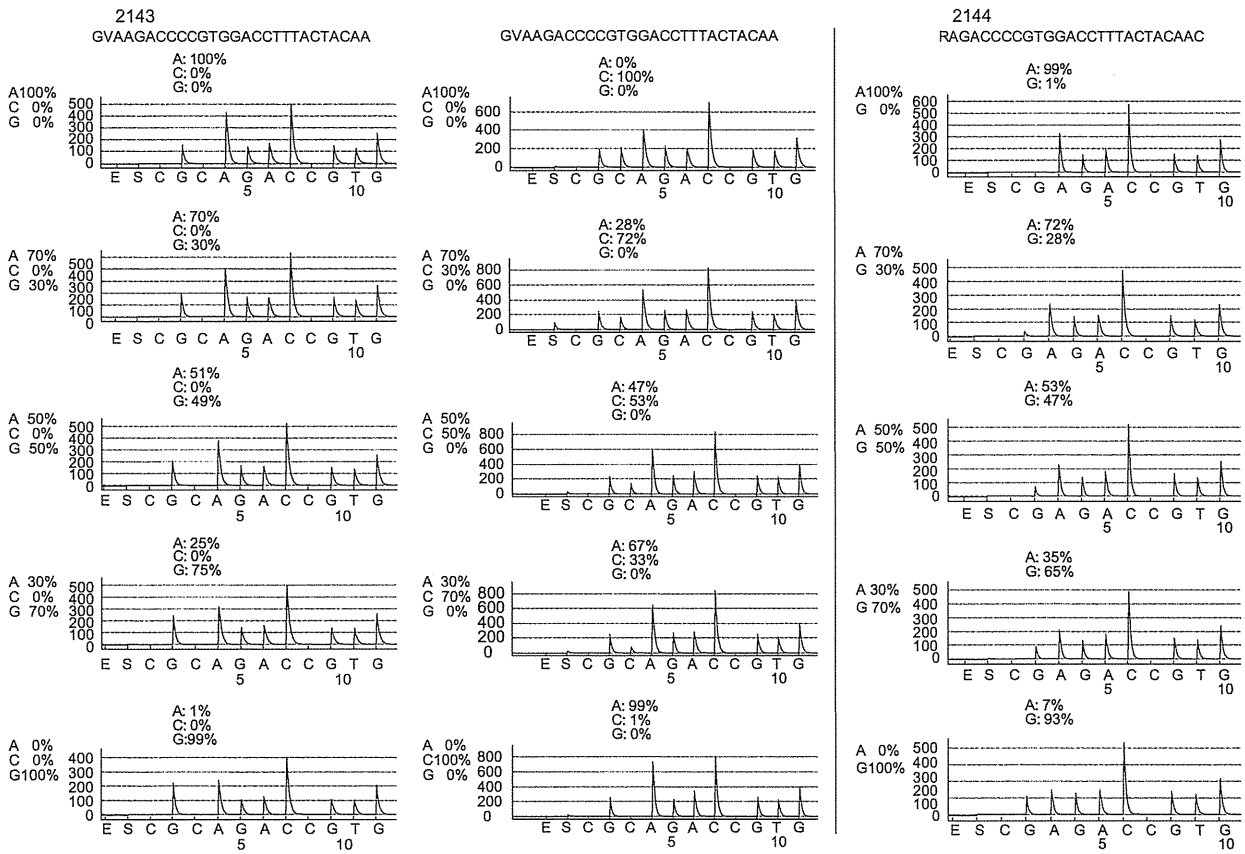



**Fig. 5** Pyrograms of polymorphisms in *Helicobacter pylori* 23S rRNA. Pyrosequencing was validated using wild-type 23S rRNA mixed at arbitrary ratios of 0, 30, 50, 70, and 100 % with **a** A2143G, **b** A2143C, and **c** A2144G

In any case, *H. pylori* was detected in all samples, including those that tested negative on multiple, currently available qualitative tests (Fig. 3a). We suggest that PCR is more sensitive than antibody, antigen and radioactive material-based tests. Indeed, we were able to amplify *H. pylori* 23S rRNA from all samples by nested PCR. We note that dense bands at 161 bp were not observed in the first round of PCR for gastric wash samples 1–3, 7, and 11. These samples are annotated "N/A" in Table 2b. However, 90-bp bands were observed for these samples in the second PCR reaction (Table 2b), suggesting that DNA in these samples may be damaged, fragmented, etc [49]. (Fig. 2b). Notably, we found that multiple *H. pylori* genotypes are simultaneously present at various ratios in a single stomach.

However, we note that many elderly patients receive anticoagulant drugs for cerebral infarction, atrial thrombus, and cardiac arrhythmia. Consequently, attending physicians sometimes hesitate to prescribe endoscopic biopsy for these patients because of the risk of GI bleeding. Thus, gastric washes may be more suitable instead. In addition, more general data on the entire stomach may be collected from such washes. Indeed, we have already reported in *Gastroenterology* in 2009 and *Tumor Biology* in 2011 that DNA methylation analysis of gastric washes is useful to assess markers of cancer risk. However, this is the first analysis of gastric washes for *H. pylori*. Nevertheless, a large-scale prospective clinical trial with a more heterogeneous patient population is needed in order to compare biopsies and gastric washes more fully.

In summary, our procedure enables detection of *H. pylori* at higher sensitivity than qualitative tests, as well as quantification of multiple genotypes. Hence, this method might be useful to diagnose *H. pylori* infection, devise personalized treatment strategies, and measure or monitor the impact of treatments on drug-resistant strains. However, the approach was tested on a limited number of samples from patients with a narrow range of clinical characteristics and should be further evaluated in a large-scale prospective study with a more heterogeneous patient population.

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**Authors' contributions** S.M., Y.W., and H.Y. conceived the study, designed and executed experiments, analyzed data, prepared figures and tables, and wrote the manuscript. S.O. analyzed data, and K.M. helped write the manuscript. R.O. and S.M. executed experiments and analyzed data. S.O. and K.M. obtained clinical samples. M.K. designed experiments, analyzed data, and helped write the manuscript. F.I. provided intellectual support. T.K., M.K., and N.S. supervised all aspects of the study.

**Compliance with ethical standards** The study was conducted in accordance with all rules and regulations of the Hokkaido University Institutional Review Board (#014-0459), and informed consent was obtained from each patient.

**Conflicts of interest** None

## References

1. Hohenberger P, Gretschel S. Gastric cancer. Lancet. 2003;362:305–15.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359–86.
3. Nardone G. Review article: molecular basis of gastric carcinogenesis. Aliment Pharmacol Ther. 2003;17 Suppl 2:75–81.
4. Nojima M, Suzuki H, Toyota M, Watanabe Y, Maruyama R, Sasaki S, et al. Frequent epigenetic inactivation of SFRP genes and constitutive activation of Wnt signaling in gastric cancer. Oncogene. 2007;26:4699–713.
5. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci U S A. 1999;96:8681–6.
6. Watanabe Y, Toyota M, Kondo Y, Suzuki H, Imai T, Ohe-Toyota M, et al. PRDM5 identified as a target of epigenetic silencing in colorectal and gastric cancer. Clin Cancer Res. 2007;13:4786–94.
7. Horii A, Nakatsuru S, Miyoshi Y, Ichii S, Nagase H, Kato Y, et al. The APC gene, responsible for familial adenomatous polyposis, is mutated in human gastric cancer. Cancer Res. 1992;52:3231–3.
8. Issa JP. CpG island methylator phenotype in cancer. Nat Rev Cancer. 2004;4:988–93.
9. Laird PW. The power and the promise of DNA methylation markers. Nat Rev Cancer. 2003;3:253–66.
10. Maesawa C, Tamura G, Suzuki Y, Ogasawara S, Sakata K, Kashiwaba M, et al. The sequential accumulation of genetic alterations characteristic of the colorectal adenoma-carcinoma sequence does not occur between gastric adenoma and adenocarcinoma. J Pathol. 1995;176:249–58.
11. Nanus DM, Kelsen DP, Mentle IR, Altorki N, Albino AP. Infrequent point mutations of ras oncogenes in gastric cancers. Gastroenterology. 1990;98:955–60.
12. Suzuki H, Yamamoto E, Nojima M, Kai M, Yamano HO, Yoshikawa K, et al. Methylation-associated silencing of microRNA-34b/c in gastric cancer and its involvement in an epigenetic field defect. Carcinogenesis. 2010;31:2066–73.
13. Ushijima T, Sasako M. Focus on gastric cancer. Cancer Cell. 2004;5:121–5.
14. Ushijima T. Detection and interpretation of altered methylation patterns in cancer cells. Nat Rev Cancer. 2005;5:223–31.
15. Kusano M, Toyota M, Suzuki H, Akino K, Aoki F, Fujita M, et al. Genetic, epigenetic, and clinicopathologic features of gastric carcinomas with the CpG island methylator phenotype and an association with Epstein-Barr virus. Cancer. 2006;106:1467–79.
16. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1:1311–5.
17. Asaka M, Sugiyama T, Nobuta A, Kato M, Takeda H, Graham DY. Atrophic gastritis and intestinal metaplasia in Japan: results of a large multicenter study. Helicobacter. 2001;6:294–9.
18. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut. 2015;64:1353–67.
19. Asaka M, Kato M, Kudo M, Meguro T, Kimura T, Miyazaki T, et al. The role of Helicobacter pylori in peptic ulcer disease. Gastroenterol Jpn. 1993;28 Suppl 5:163–7.
20. Mabe K, Takahashi M, Oizumi H, Tsukuma H, Shibata A, Fukase K, et al. Does Helicobacter pylori eradication therapy for peptic ulcer prevent gastric cancer? World J Gastroenterol. 2009;15:4290–7.
21. Asaka M, Kato M, Graham DY. Strategy for eliminating gastric cancer in Japan. Helicobacter. 2010;15:486–90.

22. Fukase K, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, et al. Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet. 2008;372:392–7.
23. Ono S, Kato M, Ono Y, Nishida U, Yamamoto K, Shimizu Y, et al. Target biopsy using magnifying endoscopy in clinical management of gastric mucosa-associated lymphoid tissue lymphoma. J Gastroenterol Hepatol. 2011;26:1133–8.
24. Kato M, Kamada G, Yamamoto K, Nishida U, Imai A, Yoshida T, et al. Lafutidine prevents low-dose aspirin and loxoprofen induced gastric injury: a randomized, double-blinded, placebo controlled study. J Gastroenterol Hepatol. 2010;25:1631–5.
25. Michikawa Y, Yasuda H, Watanabe Y, Oikawa R, Ohishi Y, Maehata T, et al. COX-2 gene promoter methylation in patients infected with Helicobacter pylori. Clin Med Insights Gastroenterol. 2013;6:13–9.
26. Lanza FL, Evans DG, Graham DY. Effect of Helicobacter pylori infection on the severity of gastroduodenal mucosal injury after the acute administration of naproxen or aspirin to normal volunteers. Am J Gastroenterol. 1991;86:735–7.
27. Asaka M, Sugiyama T, Kato M, Satoh K, Kuwayama H, Fukuda Y, et al. A multicenter, double-blind study on triple therapy with lansoprazole, amoxicillin and clarithromycin for eradication of Helicobacter pylori in Japanese peptic ulcer patients. Helicobacter. 2001;6:254–61.
28. Asaka M, Kato M, Sakamoto N. Roadmap to eliminate gastric cancer with Helicobacter pylori eradication and consecutive surveillance in Japan. J Gastroenterol. 2014;49:1–8.
29. Kawai T, Takahashi S, Suzuki H, Sasaki H, Nagahara A, Asaoka D, et al. Changes in the first line Helicobacter pylori eradication rates using the triple therapy—a multicenter study in the Tokyo metropolitan area (Tokyo Helicobacter pylori study group). J Gastroenterol Hepatol. 2014;29 Suppl 4:29–32.
30. Baba S, Oishi Y, Watanabe Y, Oikawa R, Morita R, Yoshida Y, et al. Gastric wash-based molecular testing for antibiotic resistance in Helicobacter pylori. Digestion. 2011;84:299–305.
31. Matsumura M, Hikiba Y, Ogura K, Togo G, Tsukuda I, Ushikawa K, et al. Rapid detection of mutations in the 23S rRNA gene of Helicobacter pylori that confers resistance to clarithromycin treatment to the bacterium. J Clin Microbiol. 2001;39:691–5.
32. Matsuoka M, Yoshida Y, Hayakawa K, Fukuchi S, Sugano K. Simultaneous colonisation of Helicobacter pylori with and without mutations in the 23S rRNA gene in patients with no history of clarithromycin exposure. Gut. 1999;45:503–7.
33. Asaka M, Kimura T, Kato M, Kudo M, Miki K, Ogoshi K, et al. Possible role of Helicobacter pylori infection in early gastric cancer development. Cancer. 1994;73:2691–4.
34. Evans Jr DJ, Evans DG, Smith KE, Graham DY. Serum antibody responses to the N-acetylneuraminyllactose-binding hemagglutinin of Campylobacter pylori. Infect Immun. 1989;57:664–7.
35. Thomas JE, Gibson GR, Darboe MK, Dale A, Weaver LT. Isolation of Helicobacter pylori from human faeces. Lancet. 1992;340:1194–5.
36. Vilaichone RK, Mahachai V, Graham DY. Helicobacter pylori diagnosis and management. Gastroenterol Clin North Am. 2006;35:229–47.
37. Graham DY, Klein PD, Evans Jr DJ, Evans DG, Alpert LC, Opekun AR, et al. Campylobacter pylori detected noninvasively by the 13C-urea breath test. Lancet. 1987;1:1174–7.
38. Marchildon PA, Sugiyama T, Fukuda Y, Peacock JS, Asaka M, Shimoyama T, et al. Evaluation of the effects of strain-specific antigen variation on the accuracy of serologic diagnosis of Helicobacter pylori infection. J Clin Microbiol. 2003;41:1480–5.
39. Kato M, Asaka M, Kudo T, Katagiri M, Komatsu Y, Sato F, et al. Ten minute 13C-urea breath test for the diagnosis of Helicobacter pylori infection. J Gastroenterol. 1998;33 Suppl 10:40–3.
40. Yamamoto H, Watanabe Y, Maehata T, Morita R, Yoshida Y, Oikawa R, et al. An updated review of gastric cancer in the next-generation sequencing era: insights from bench to bedside and vice versa. World J Gastroenterol. 2014;20:3927–37.
41. Watanabe Y, Kim HS, Castoro RJ, Chung W, Estecio MR, Kondo K, et al. Sensitive and specific detection of early gastric cancer with DNA methylation analysis of gastric washes. Gastroenterology. 2009;136:2149–58.
42. Oishi Y, Watanabe Y, Yoshida Y, Sato Y, Hiraishi T, Oikawa R, et al. Hypermethylation of Sox17 gene is useful as a molecular diagnostic application in early gastric cancer. Tumour Biol. 2012;33:383–93.
43. Hama R, Watanabe Y, Shinada K, Yamada Y, Ogata Y, Yoshida Y, et al. Characterization of DNA hypermethylation in two cases of peritoneal mesothelioma. Tumour Biol. 2012;33:2031–40.
44. Asaka M, Mabe K, Matsushima R, Tsuda M. Helicobacter pylori eradication to eliminate gastric cancer: the Japanese strategy. Gastroenterol Clin North Am. 2015;44:639–48.
45. Saadat I, Higashi H, Obuse C, Umeda M, Murata-Kamiya N, Saito Y, et al. Helicobacter pylori CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. Nature. 2007;447:330–3.
46. Maeda S, Yoshida H, Ogura K, Kanai F, Shiratori Y, Omata M. Helicobacter pylori specific nested PCR assay for the detection of 23S rRNA mutation associated with clarithromycin resistance. Gut. 1998;43:317–21.
47. Versalovic J, Shortridge D, Kibler K, Griffy MV, Beyer J, Flamm RK, et al. Mutations in 23S rRNA are associated with clarithromycin resistance in Helicobacter pylori. Antimicrob Agents Chemother. 1996;40:477–80.
48. Stone GG, Shortridge D, Flamm RK, Versalovic J, Beyer J, Idler K, et al. Identification of a 23S rRNA gene mutation in clarithromycin-resistant Helicobacter pylori. Helicobacter. 1996;1:227–8.
49. Kalach N, Gosset P, Dehecq E, Decoster A, Spyckerelle C, Papadopolos S, et al. Usefulness of gastric biopsy-based real-time polymerase chain reaction for the diagnosis of Helicobacter pylori infection in children. J Pediatr Gastroenterol Nutr. 2015;61:307–12.

各論

# どこまで行うHelicobacter pylori除菌療法

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KeyWords ◎*Helicobacter pylori* ◎胃癌予防 ◎除菌治療

## Headline

1. 胃がんは*Helicobacter pylori*感染に伴って起こる感染症である．*H. pylori*除菌による胃がん予防効果についての臨床研究が報告されている．
2. わが国のコホート試験では，対象者の胃がん発症率が高い場合に，*H. pylori*除菌の胃がん抑制効果が明らかになっている．また，わが国のランダム化試験では*H. pylori*除菌により早期胃がん内視鏡治療後の異時性がんは有意に抑制された．
3. 初発がんに対するランダム化試験では，胃がん発症率が低いので，多数例の登録と長期間の観察が必要となるが，メタ解析では有意が出ている．ただ，*H. pylori*除菌の成功後も胃がんリスクは長期に継続することも分かっており，除菌後の経過観察も重要である．

胃の分化型および未分化型腺がんは*H. pylori*感染に伴う慢性炎症を背景にして発症するので，胃がんは*H. pylori*感染症の一つである．胃がんの発生因子は*H. pylori*感染以外もあるが，*H. pylori*感染に他の因子が加わることで発がんしやすくなる．すなわち，*H. pylori*未感染者に高濃度塩分の摂取等の環境因子や加齢因子が存在しても発がんは起こりにくい．一方，*H. pylori*感染者の全員に胃がんが起こるわけではなく，発がんには*H. pylori*感染に伴う炎症状態が重要な役割を負っている[1]．*H. pylori*未感染者胃がんは胃がん全体の1%に満たない[2,3]（図1）．

*H. pylori*の除菌治療が胃がんの一次予防法として期待されている[4]．これまで，スナネズミを用いた動物実験では除菌治療が有意に胃がん発生を抑制し，さらには早い時期に除菌したほうがその効果は強いという成績がある[5,6]．

## 初発胃がんに対するH. pylori除菌効果

わが国から*H. pylori*除菌群と対照群における胃がん発症率を比較したコホート試験がいくつか報告されている[7~11]．胃がん発症率が低い企業健診者を対象にした試験では有意な結果を認めなかったが，胃・十二指腸潰瘍患者を対象に除菌成功群と非除菌群を3～5年の経過観察を行った四つの試験では，per protocol解析では除菌群での胃がん発生が有意に少ない結果であった．また，台湾のnational data base（NDB）を用いた大規模なコホート試験では，除菌まで平均1年を要した晩期除菌群よりも，すぐに除菌した早期除菌群での胃がん発生率は有意に低値であった[12]．除菌が1年遅くなることで，その後の胃がん発生率に差が生じるとの成績であった．

初発胃がんに対する*H. pylori*除菌の予防効果を検討したランダム化比較試験は，論文化されていないわが国の研究も含めて重複試験を除くと5試験ある[13~18]．平均7.3年の経過観察では有意差がなかったが，15年の経過観察報告で，*H. pylori*除菌群における胃がん発生は有意に抑制されたとの報告以外は，個々の試験では有意差を認めていない．初発がんを対象にした場合には，登録症例数に限りがあるために有意差が出るには15年以上の経過観察が必要である．最近のメタ解析ではリスク比が0.66（95%信頼区

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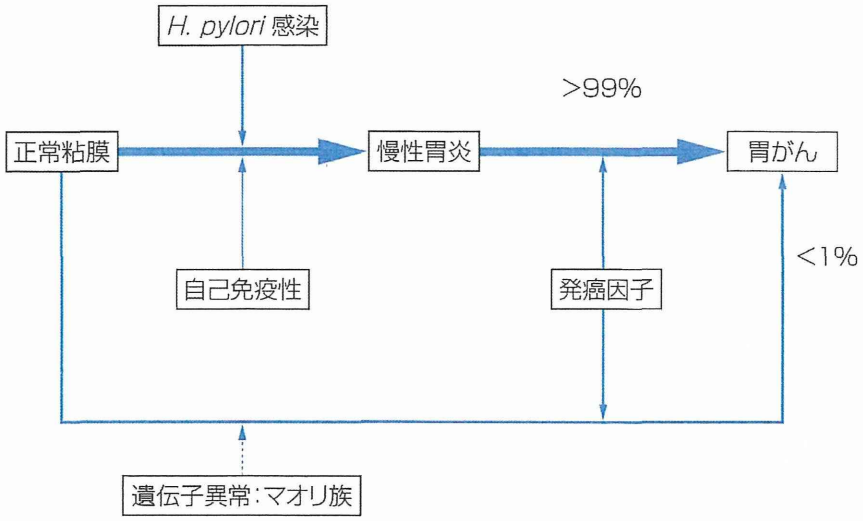


図 1　胃癌発生と *H. pylori* 感染

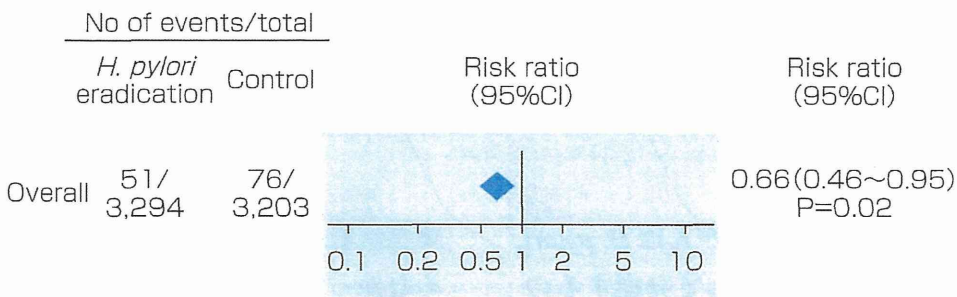


図 2　初発胃癌に対する *H. pylori* 除菌のメタ解析

間 0.46-0.95）と *H. pylori* 除菌の胃がん予防効果が確認された[19]（図 2）．

## 内視鏡治療後の異時がんに対する *H. pylori* 除菌効果

早期胃がんの内視鏡的治療後に，胃がん再発病変として遺残再発や異時性多発がんを認めることは少なくない．内視鏡的切除後の遺残再発については，経過観察中に切除瘢痕に接してがんを再び認めたものと定義でき，不十分な内視鏡的治療が原因とされる．一方，内視鏡的切除後の経過観察中に，切除した部位とは別の部位に異時がん（二次がん）を認めることがある．異時がんの発生は内視鏡治療後長期に渡って年率 1～5% と一定の割合で続く．

これまで早期胃がん内視鏡治療後の異時がんに対する *H. pylori* 除菌効果を検討した論文は，ランダム化比較試験，非ランダム化比較試験，後ろ向き試験が報告されている．Uemura らの非ランダム化比較試験では，早期胃がんの内視鏡的治療後に，*H. pylori* 除菌群と非除菌群に分け除菌群で有意に異時がんの発生が抑制された[20]．Japan Gast Study Group（JGSG）はランダム化試験として行い，異時がんの発生は有意差に除菌群で低く，不完全切除による遺残再発には有意差を認めなかった[21]．除菌後最長 10 年（平均 5 年）までの長期観察で，*H. pylori* 除菌による胃がん抑制作用は長期間に渡って継続されている．韓国でのランダム化試験の成績では有意差を認めなかったが，わが国の試験とは登録時期が内視鏡治療後直後である点に違いがある[22]．わが国の成績でも内視鏡治療直後に登録された群と内視鏡治療後の経過観察中に登録された群とでサブ解析すると，*H. pylori* 除菌に有意差を認めるのは経過観察群のみであった．内視鏡治療直後の群では，除菌時に存在していた見逃し

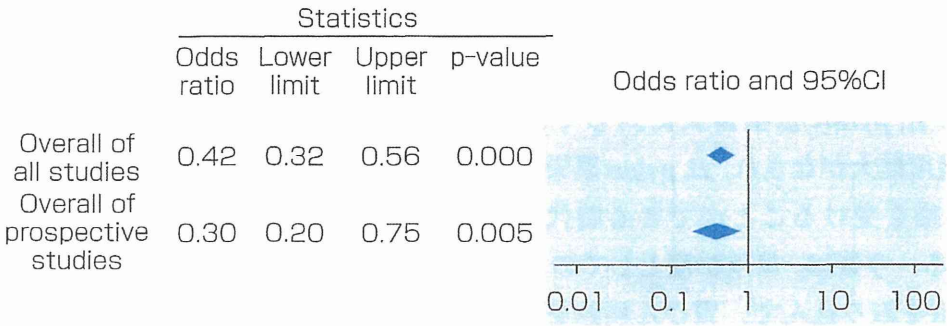


図 3　異時胃癌に対する *H. pylori* 除菌のメタ解析

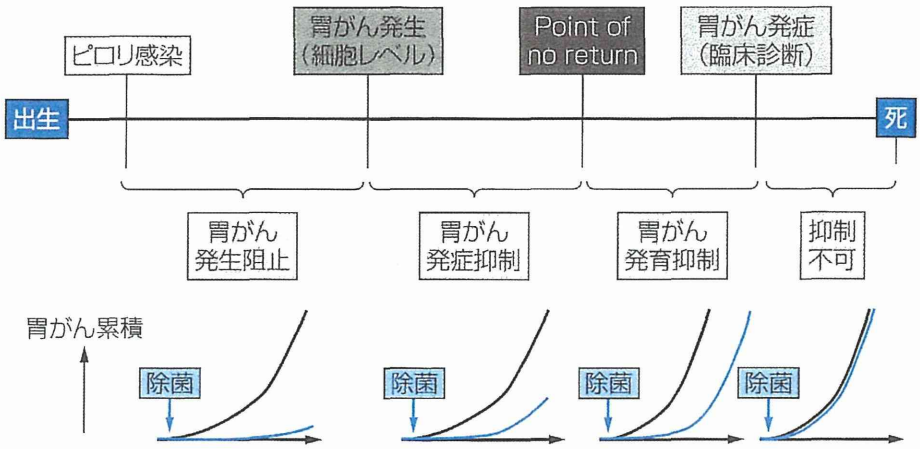


図 4　*H. pylori* 除菌時期と胃癌の発育進展に与える影響

がんが早期の経過観察中に発見される割合が多くなるために，除菌効果がマスクされる．最近，前向き試験や後ろ向き試験のメタ解析が報告され，*H. pylori* 除菌治療が有意に異時がんの発生を抑制することが確認された（オッズ：0.42，95% CI：0.32-0.56）[23]（図 3）．

## *H. pylori* 除菌による胃がん予防の限界

早期胃がんの成長速度は対数正規分布を示すことが報告されており，胃がんの発育速度は doubling time で評価できる[24]．胃がん細胞が発生してから，臨床的に胃がんと診断される 5 mm の胃がんになる期間はおよそ 5～20 年とされる．*H. pylori* 感染（A）から胃がん細胞の発生（B0）までは胃粘膜の炎症状態によるが通常は 40～60 年位はかかると考えられる．*H. pylori* 除菌が胃がんの発育進展に与える影響は除菌の時期によって異なり，A～B0 では細胞レベルでの胃がん発生の阻止，B0～B1 では胃がん発症の抑制，B1～B2 では胃がんの発育進展の抑制，B2～C は抑制不可である（図 4）．臨床試験では B1 の手前で除菌すると，胃がん発生は抑制できるが，B1 を過ぎた時点で除菌を行っても最終的には胃がんが発症する．

除菌後症例を長期観察を行うと，除菌後 10 年以上経過しても胃がんの発生が認められることが明らかになった[25]．すなわち，*H. pylori* 除菌によって胃がん予防が可能であっても，除菌時期によっては，除菌後にも胃がんリスクは長期に渡って継続する．したがって，*H. pylori* 除菌後も長期にわたって内視鏡検査を中心とした画像検査を続けることが非常に重要である．

## おわりに

胃がん，肝がん，子宮頸がんは感染症が原因で，感染のコントロールによって予防が可能ながんである．わが国の胃がん死亡を減らすには，*H. pylori* 除菌による一次予防，胃がんサー

ベイランスによる二次予防を組み合わせた胃がん予防策を軌道に乗せることが必要である[26]．2013年2月に*H. pylori*感染胃炎に対して*H. pylori*除菌の適用拡大がなされ，*H. pylori*感染者全員が除菌治療を受けることができる時代になったといえる．今後は，保険診療としての*H. pylori*除菌治療を取り込んで，胃がん検診を取り込んだ胃がん予防対策が重要である．除菌による胃がん予防効果は70歳以降でもある程度期待できので，重篤な合併症がなく本人が除菌治療を受ける意志があれば高齢者は除菌を施行しない理由にはならない．

## 文　献


1) Kato M, et al.: Recent knowledge of the relationship between Helicobacter pylori and gastric cancer and recent progress of gastroendoscopic diagnosis and treatment for gastric cancer. Jpn J Clin Oncol 40, 828-1837, 2010
2) Matsuo T, et al.: Low prevalence of Helicobacter pylori-negative gastric cancer among Japanese. Helicobacter 16: 415-419, 2011
3) Ono S, et al.: Frequency of Helicobacter pylori-Negative Gastric Cancer and Gastric Mucosal Atrophy in a Japanese Endoscopic Submucosal Dissection Series Including Histological, Endoscopic and Serological Atrophy. Digestion 86: 59-65, 2012
4) Kato M, et al.: Recent Development of Gastric Cancer Prevention. Jpn J Clin Oncol. 42: 987-994, 2012
5) Shimizu N, et al.: Eradication diminishes enhancing effects of *Helicobacter pylori* infection on glandular stomach carcinogenesis in Mongolian gerbils. Cancer Res 60: 1512-1514, 2000
6) Nozaki K, et al.: Effect of early eradication on *Helicobacter pylori*-related gastric carcinogenesis in Mongolian gerbils. Cancer Sci 94: 235-239, 2003
7) Take S, et al.: The effect of eradicating *Helicobacter pylori* on the development of gastric cancer in patients with peptic ulcer disease. Am J Gastroenterol 100: 1037-1042, 2005
8) Takenaka R, et al.: Helicobacter pylori eradication reduced the incidence of gastric cancer, especially of the intestinal type. Aliment Pharmacol Ther 25: 805-812, 2007
9) Ogura K, et al.: The effect of Helicobacter pylori eradication on reducing the incidence of gastric cancer. J Clin Gastroenterol 42: 279-283, 2008
10) Yanaoka K, et al.: Eradication of Helicobacter pylori prevents cancer development in subjects with mild gastric atrophy identified by serum pepsinogen levels. Int J Cancer 125: 2697-2703, 2009
11) Mabe K, et al.: Does Helicobacter pylori eradication therapy for peptic ulcer prevent gastric cancer? World J Gastroenterol 15: 4290-4297, 2009
12) Wu CY, et al.: Early Helicobacter pylori eradication decreases risk of gastric cancer in patients with peptic ulcer disease. Gastroenterology 137: 1641-1648, 2009
13) Wong BC, et al.: *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA 291: 187-194, 2004
14) Zhou L.: Ten-year follow-up study on the incidence of gastric cancer and the pathological changes of gastric mucosa after *H. pylori* eradication in China [Abstract]. Gastroenterology 134: A233, 2008
15) You WC, et al. Randomized double-blind factorial trial of three treatments to reduce the prevalence of precancerous gastric lesions. J Natl Cancer Inst 98: 974-983, 2006
16) Saito D, et al.: mpact of *H. pylori* eradication on gastric cancer prevention: endoscopic results of the Japanese Intervention Trial (JITHP-Study). A randomized multi-center trial. [Abstract]. Gastroenterology 28: A4, 2005
17) Correa P, et al.: hemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-*Helicobacter pylori* therapy. J Natl Cancer Inst 92: 1881-1888, 2000
18) Ma JL, et al.: Fifteen-year effects of Helicobacter pylori, garlic, and vitamin treatments on gastric cancer incidence and mortality. J Natl Cancer Inst 104: 488-492, 2012
19) Ford AC, et al.: Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. BMJ 348: g3174, 2014
20) Uemura N, et al.: Effect of Helicobacter pylori eradication on subsequent development of cancer after endoscopic resection of early gastric cancer in Japan. Gastroenterol Clin North Am 29: 819-827, 2000
21) Fukase K, et al.: Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet 372: 392-397, 2008
22) Choi J, et al.: Eradication of Helicobacter pylori after endoscopic resection of gastric tumors does not reduce incidence of metachronous gastric carcinoma. Clin Gastroenterol Hepatol 12: 793-800, 2014
23) Yoon SB, et al.: Effect of Helicobacter pylori eradication on metachronous gastric cancer after endoscopic resection of gastric tumors: a meta-analysis. Helicobacter. 19: 243-248, 2014
24) 中村恭一，他：胃がんの大きさと時間の関係―いわゆる胃がんの成長曲線．胃と腸 13: 89-93，1978
25) Take S, et al.: The long-term risk of gastric cancer after the successful eradication of Helicobacter pylori. J Gastroenterol 46: 318-324, 2011
26) Asaka M.: A new approach for elimination of gastric cancer deaths in Japan. Int J cancer 132: 1272-1276, 2013


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