

Rapid Automated Genotyping of CYP2C19 and *Helicobacter pylori* 23S rRNA Gene in Gastric Juice

Takahisa Furuta, Yoshihiro Soya, Mitsushige Sugimoto, Masafumi Nishino, Mihoko Yamade, Takahiro Uotani, Chise Kodaira, Shu Sahara, Hitomi Ichikawa, Takanori Yamada, Satoshi Osawa, Ken Sugimoto, Masato Maekawa, Hiroshi Watanabe, Kazuo Umemura

Takahisa Furuta, Center for Clinical Research, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, 431-3192, Japan

Yoshihiro Soya, Tsuruga Institute of Biotechnology, Toyobo Co., Ltd., Osaka, Japan

Mitsushige Sugimoto, Masafumi Nishino, Mihoko Yamade, Takahiro Uotani, Chise Kodaira, Shu Sahara, Hitomi Ichikawa, Takanori Yamada, Satoshi Osawa, Ken Sugimoto, First Department of Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, 431-3192, Japan

Masahito Maekawa, Department of Laboratory Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, 431-3192, Japan

Hiroshi Watanabe, Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Hamamatsu, Japan

Kazuo Umemura, Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Correspondence to: Takahisa Furuta, MD, PhD, Center for Clinical Research, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, 431-3192, Japan. furuta@hama-med.ac.jp

Telephone: +081-53-435-2950 Fax: +081-53-435-2951

Received: October 14, 2012 Revised: December 17, 2012

Accepted: December 20, 2012

Published online: April 21, 2013

ABSTRACT

AIM: Genotypes of *Helicobacter pylori* (*H. pylori*) 23S rRNA gene and human *CYP2C19* are predictive determinants of efficacy of triple therapy with proton pump inhibitor, clarithromycin, and amoxicillin for eradicating *H. pylori*. We intended to develop a rapid assay system to determine these genotypes.

METHODS: We designed primer sets and specific probes for determining SNPs from adenine to guanine at positions 2142 or 2143 of *H. pylori* 23S rRNA gene and *2 and *3 alleles of *CYP2C19*, using the novel full-automated rapid genetic analyzer (GENECUBE[®]). The results were compared with those acquired by direct nucleotide sequencing of 50 gastric tissue samples. We also determined the presence of SNPs in 132 additional samples of patients' gastric juices.

RESULTS: The GENECUBE[®] genotyping results of the 23S rRNA gene and *CYP2C19* from gastric tissue samples were in complete

agreement with those for direct sequencing. The sensitivity, specificity, and validity of GENECUBE[®] results were all 100% when compared with clinical data. GENECUBE[®] genotyping required approximately 30 min for a single test.

CONCLUSIONS: The GENECUBE[®]-based system can simultaneously measure genotypes of *H. pylori* 23S rRNA gene and human *CYP2C19* from gastric tissues and juices rapidly, which can greatly aid the tailored *H. pylori* eradication therapies.

© 2013 ACT. All rights reserved.

Key words: *Helicobacter pylori* (*H. pylori*); 23S rRNA; CYP2C19; PCR; SNP

Furuta T, Soya Y, Sugimoto M, Nishino M, Yamade M, Uotani T, Kodaira C, Sahara S, Ichikawa H, Yamada T, Osawa S, Sugimoto K, Maekawa M, Watanabe H, Umemura K. Rapid Automated Genotyping of CYP2C19 and the *Helicobacter Pylori* 23S rRNA Gene in Gastric Juice. *Journal of Gastroenterology and Hepatology Research* 2013; 2(4): 508-512 Available from: URL: <http://www.ghrnet.org/index./joghr/>

INTRODUCTION

Eradication of *H. pylori* infection is employed as a method of treating disorders of the upper gastrointestinal tract, such as peptic ulcer, gastric mucosa associated lymphoid tissue (MALT) lymphoma, and idiopathic thrombocytopenic purpura, as well as to prevent gastric cancer^[1,2]. Eradication therapy includes administration of a proton pump inhibitor (PPI) and two antibiotics. Triple therapy with a PPI (either omeprazole, lansoprazole, rabeprazole, or esomeprazole), clarithromycin, and amoxicillin is now the first-line regimen among modalities currently in use in Japan^[3]. The initial eradication rate of this therapy was approximately 90%. This feat attributed to the pathogen's susceptibility to clarithromycin^[4-7]. Thus, as the frequency of clarithromycin-resistant strains of *H. pylori* has increased^[8], the efficacy of triple therapy regimens has decreased^[9], falling to rates as low as 70%^[10].

Culture tests are typically employed to test the susceptibility of *H. pylori* strains to antimicrobial agents, such as clarithromycin; however, they require 10-14 days and are of little value in daily clinical practice. Susceptibility of most *H. pylori* strains is caused

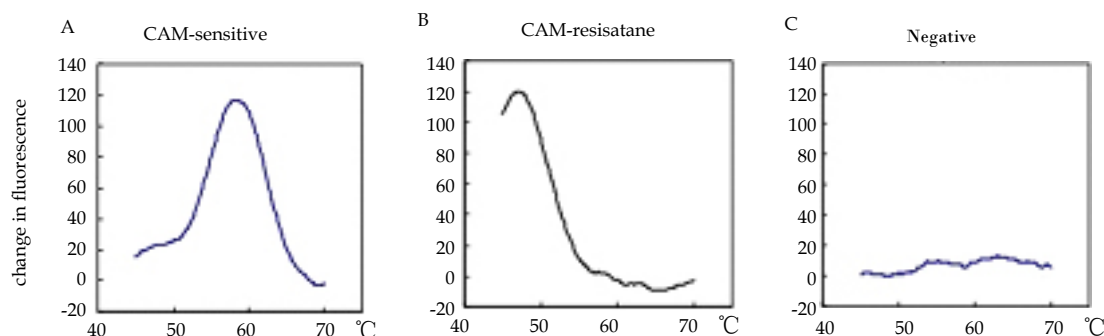


Figure 1 Analyses of 23S rRNA mutations by GENECUBE®. A: When there is no mutation at nucleotide position 2142 or 2143, the peak is observed around 55°C. The strain shown is shown to be sensitive to clarithromycin(CAM); B: When there is a mutation in the *H. pylori* 23S rRNA gene at nucleotide position 2142 or 2143, the peak is shifted to the left at around 46°C. The strain shown was diagnosed to be resistant to clarithromycin; C: When the sample is negative for *H. pylori*, there is no signal.

Table 1 Analyses of 23S rRNA gene mutations associated with susceptibility of *H. pylori* to clarithromycin.

| | 23S rRNA | | Direct Sequence | GENECUBE® |
|-----------|----------|--------|-----------------|-----------|
| | A2142G | A2143G | | |
| Sensitive | A | A | 16 | 16 |
| Resistant | G | A | 1 | 34 |
| | A | G | 33 | |
| Time | | | 8 h | 30 min |

Table 2 Comparison of results of CYP2C19 genotyping.

| | | PCR-Direct sequence | GENECUBE® | Allele frequency |
|---------------------|-----|---------------------|-----------|------------------|
| | | | | |
| CYP2C19*2 (681 G→A) | G/G | 25 | 25 | G: 0.72 |
| | G/A | 22 | 22 | A: 0.28 |
| | A/A | 3 | 3 | |
| CYP2C19*3 (636 G→A) | G/G | 38 | 38 | G: 0.87 |
| | G/A | 11 | 11 | A: 0.13 |
| | A/A | 1 | 1 | |
| Time | 8 h | | 30 min | |

by a point mutation from adenine to guanine at nucleotide positions 2142 or 2143 in the 23S rRNA gene and are recognized as single nucleotide polymorphisms (SNPs), designated A2142G or A2143G, respectively^[11-15]. In this manner, drug susceptibility can be determined by genetic testing.

Another factor associated with the ability to eradicate *H. pylori* by the standard triple therapy is the genotype of CYP2C19^[16]. We previously reported that patients with the rapid metabolizer genotype of CYP2C19 (designated *1/*1) have a lower chance of being successfully treated with PPI-based therapy at the standard dose^[5]. The CYP2C19 genotype is now well known to influence the eradication rates of *H. pylori* by PPI-based regimens^[17]. Based on the results of CYP2C19 and 23S rRNA genotyping studies, we planned a tailored regimen based on the genotypes of CYP2C19 in our patient population and *H. pylori* 23S rRNA gene, which resulted in higher eradication rates than those achieved with the standard regimen^[10].

Because human gastric epithelial cells are often present in gastric juice, DNA extracted from gastric juice samples will contain both the patient and *H. pylori* DNA. Therefore, using the DNA sample extracted from gastric juice samples, genotypes of CYP2C19 in patient population and *H. pylori* 23S rRNA gene can theoretically be measured.

Here, in order to make it easy to perform the individualized eradication therapy of *H. pylori* based on genotype of CYP2C19 and 23S rRNA genes of *H. pylori* in all patients, we developed a high-throughput system for reliably determining CYP2C19 and 23S rRNA gene genotypes using an automated DNA analyzer (GENECUBE®) with gastric juice and tissue samples.

MATERIALS AND METHODS

Study 1 Development of a system that rapidly determines the genotypes of CYP2C19 and the *H. pylori* 23S rRNA gene using the GENECUBE® automated DNA analyzer

This study enrolled 50 patients with gastric ulcers (GU, $n=22$), duodenal ulcers (DU, $n=12$), or gastritis ($n=19$). The subjects were 36 men and 14 women whose median age (range) was 53 (31-78) years. All had endoscopically and histologically proven ulcers or active chronic gastritis and were *H. pylori*-positive based on the rapid urease test (RUT) (see below). Written informed consent for participation was obtained from all patients before the study, and the Human Institutional Review Board of Hamamatsu University School of Medicine approved the protocol.

Biopsies for the RUT obtained from both the antrum and corpus of the greater curvature by gastroduodenoscopy were analyzed using a commercial kit (HelicoCheck; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). A color change from yellow to pink within 24 hours was judged as a positive result.

The GENECUBE® used in the present study was a novel, fully automated rapid genetic analyzer capable of extracting nucleic acids from biological material, preparing reaction mixtures, and amplifying the target gene all within 30 min. Analysis with this instrument is based on the hybridization of an allele-specific fluorescence-conjugated probe and PCR amplification within 20 min followed by melting point analysis. A total of 16 SNPs analyses can be handled simultaneously. In this study, 4 SNPs, including *2 and *3 mutations of CYP2C19 and A2142G and A2143G mutations of 23S rRNA of *H. pylori* were analyzed per one sample. Therefore, a total of 4 samples can be handled simultaneously.

We designed primers to amplify the 23S rRNA gene based on the *H. pylori* sequence deposited in the GenBank: U27270 as follows: forward primer: 5'-GTGGAGGTGAAAATTCCTCCTACCC-3', reverse primer 5'-GGCTCCATAAGAGCCAAAGCCCTTAC-3', probe for detecting the product (5'-CAAGACGGAAAGACCC-3'-BODIPYFL). The length of the expected polymerase chain reaction (PCR) product was 141 bp.

PCR was carried out using approximately 0.1 μ g of DNA, and TEST BASIC KIT contains KOD mix, PPDmix (TOYOBO, Ltd., Osaka, Japan), primers and probe. PCR conditions were as follows: denaturation at 95°C for 30 s, 50 cycles of denaturation at 97°C for 1 s, annealing at 58°C for 3 s, and extension at 63°C for 5 s. The PCR products were automatically subjected to melting point analysis, which consisted of denaturing at 98°C for 30 s, annealing at 40°C for 30 s, and then heating to 75°C in increments of 0.09°C/s. The display

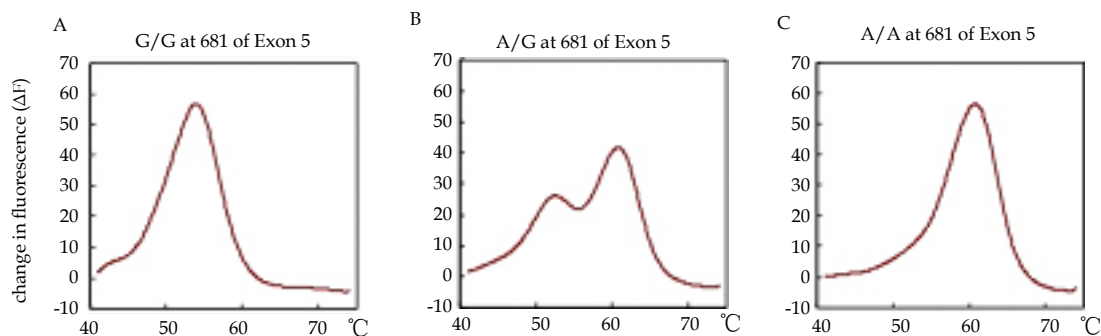


Figure 2 GENECUBE® detection of G to A polymorphism at nucleotide position 681 in exon 5 of CYP2C19 (CYP2C19*2) by GENECUBE®. A: When there is not a SNP at nucleotide 681 in exon 5 of CYP2C19, the peak is observed around 53°C; B: When the sample is heterozygous for G and A, two peaks result. The genotype of this sample was diagnosed as CYP2C19*1/*2; C: When the sample is homozygous for A, the peak is displayed at around 61°C. The genotype of this sample was diagnosed as CYP2C19*2/*2.

Table 3 GENECUBE®-based detection of *H. pylori* from gastric juice samples of subjects positive or negative for *H. pylori*.

| Sample type | GENECUBE® | | |
|------------------------------------|--------------|-----------|---------|
| | Not detected | Wild type | Mutated |
| <i>H. pylori</i> -negative (n=26) | 26 | 0 | 0 |
| <i>H. pylori</i> -positive (n=106) | 0 | 64 | 42 |

Table 4 GENECUBE®-based analyses of CYP2C19 genotypes with gastric juice samples.

| | | GENECUBE® | |
|------------------------|-----|-----------|------------------|
| | | | Allele frequency |
| CYP2C19*2 (681 G→A) | G/G | 77 | G: 0.75 |
| | G/A | 45 | A: 0.25 |
| | A/A | 10 | |
| CYP2C19*3 (636 G→A) | G/G | 108 | G: 0.91 |
| | G/A | 23 | A: 0.09 |
| | A/A | 1 | |

of the data on the GENECUBE® monitor is shown in Figure 1.

For the measurement of CYP2C19 genotypes, we designed the oligonucleotide primers (forward primer: 5'-ACCAGAGCTTGGCATATTGTATCTATAACC-3' and reverse primer 5'-CCAAAATATCACTTCCATAAAAGCAAG-3') and probe (5'-TTGATTATTTCCAGGAACC-3'-BODIPYFL) for CYP2C19*2 and the oligonucleotide primers (forward primer: 5'-GATGGAAA AATTGAATGAAAACATCAGGATTGTA-3' and reverse primer 5'-AAAATGTACTTCAGGGCTTGGTCAATA-3') and probe (5'-TAAGCACCCCCTGAATCC-3'-BODIPYFL) for CYP2C19*3 based on CYP2C19 sequence deposited in the GenBank (NG_008384.1). To enable simultaneous measurements of both genotypes, the PCR conditions were the same as described above for detecting 23S rRNA gene mutations. To confirm the GENECUBE® SNP analysis results, we determined the genotypes of CYP2C19 and 23S rRNA gene using a published direct sequencing method^[18,19], with minor modifications.

Study 2: Analysis of gastric juice sample

Gastric juice samples were collected by aspiration during gastroduodenoscopy in 132 patients not included in Study 1. These patients suffered from GU (n=31), DU (n=20), or gastritis (n=81). This group included 52 men and 80 women, whose median age (range) was 52 (21-80) years. *H. pylori* infections in 20 of these patients had been eradicated before the present study. Immediately after the endoscope reached the stomach, 1-10 mL of gastric juice was aspirated through the suction channel and collected in a trap placed in the suction line. When gastric juice was difficult to aspirate due to achlorhydria, 20 mL of water was sprinkled vigorously on the gastric mucosa using a syringe attached to the suction channel

and collected the water by aspiration. A 20-μL aliquot of gastric juice was then added to 180 μL of sample solution buffer (Toyobo) (the final volume was 200 μL) and heated at 95°C for 5 min to extract the DNA. 2-μL aliquot of 200 μL was subjected to GENECUBE® analysis to test whether GENECUBE® system could detect the presence or absence of *H. pylori* infection using juice samples. The presence of *H. pylori* infection was also assessed by RUT and ¹³C-urea breath test, and tests for serum anti-*H. pylori* IgG antibody. When any one of these tests (RUT, ¹³C-urea breath test and serum anti-*H. pylori* IgG antibody test) was positive, the patient was diagnosed with an *H. pylori* infection. The sensitivity and specificity of the GENECUBE® analysis was evaluated based on the three conventional test

RESULTS

Validation of GENECUBE® genotyping

Figure 1 shows that GENECUBE® analysis unambiguously demonstrated wild type or mutant sequences in the 23S rRNA gene at nucleotide positions 2142 or 2143. Of note is the fact that the analysis took only 30 min. In contrast, direct sequence analysis of these same 50 samples took approximately 8 hours.

Table 1 presents the results of the analysis of 23S rRNA gene mutations associated with susceptibility to clarithromycin by GENECUBE® and direct sequence analyses. Both methods detected the same mutant sequences in the same 34 samples. Further, sequencing of CYP2C19 by both techniques yielded identical results and were able to detect the CYP2C19*2 and CYP2C19*3 mutations (Table 2).

Analysis of gastric juice samples

In Study 2, 26 of the 132 samples were *H. pylori*-negative based on analysis of serum anti-*H. pylori*-IgG, urease and the ¹³C-urea breath test, and the remaining 106 were *H. pylori*-positive based on RUT, serum IgG, or ¹³C-urea breath test findings. The results of GENECUBE® analysis of 23S rRNA gene mutations in subjects based on *H. pylori* infection status are displayed in table 3. The GENECUBE® could detect *H. pylori* infection in all patients infected with *H. pylori* based on analysis of serum anti-*H. pylori*-IgG, urease and the ¹³C-urea breath test. Moreover, there were no false positive results. Thus, the sensitivity, specificity and validity of the GENECUBE® assay were all 100% in the present study results. Forty-two of the 106 *H. pylori*-positive patients harbored clarithromycin-resistant strains of *H. pylori* based on 23S rRNA gene sequences. CYP2C19 genotyping was successfully performed for each sample (Table 4), and we found that the CYP2C19 allele frequency was almost the same as those reported for samples analyzed in Japan^[20].

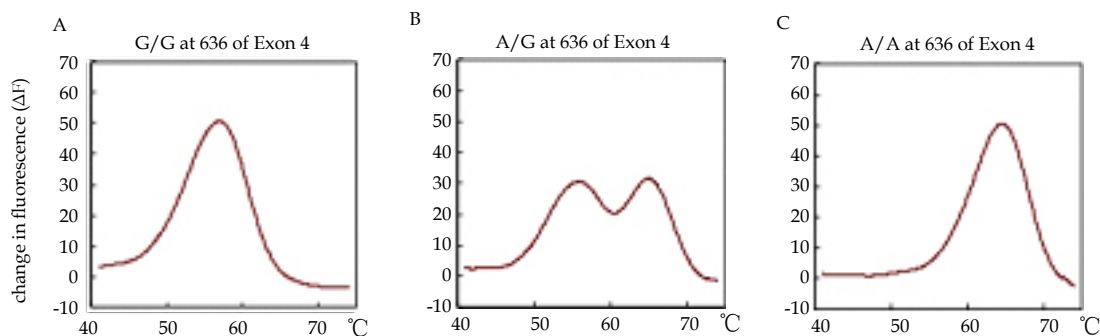


Figure 3 GENECUBE® analyses of G to A polymorphism at nucleotide position 636 in exon 4 of *CYP2C19* (*CYP2C19**3). A: When there is no SNP at 636 in exon 4 of *CYP2C19*, the peak is observed around 57°C; B: When the sample is heterozygous for G and A, two peaks are displayed. The genotype of this sample was diagnosed as *CYP2C19**1/*3; C: When the sample is homozygous for A, the peak is displayed at around 65°C. The genotype of this sample was diagnosed as *CYP2C19**3/*3.

DISCUSSION

We report here the development of a rapid (30 min per sample) detection system for determining *CYP2C19* genotypes and mutations in the *H. pylori* 23S rRNA gene associated with resistance to clarithromycin. We were able to successfully analyze gastric tissue and fluid samples and validated the assay system by comparing its results to those acquired by direct nucleotide sequencing. The sensitivity of detection of *H. pylori* was equal to that of standard immunological, biochemical, and biological tests. In addition, the time required to complete the assay makes it suitable for use in daily clinical practice.

The samples usually used for the determination of *H. pylori* infection are taken from gastric tissue collected by mucosal biopsy during endoscopy. These samples can be used to detect *H. pylori* by culture tests, RUT, histology, and PCR. Genotyping of human DNA can also be performed using these samples^[21]. Unfortunately, not all patients can tolerate this procedure, which is contraindicated in patients with bleeding tendency and those being treated with either or both anti-coagulants or anti-platelet agents, such as warfarin or clopidogrel. In contrast, collection of gastric juice samples during endoscopy can be easily and safely performed in all patients.

In the present study, we analyzed gastric juice samples acquired during endoscopy to determine the genotypes of *CYP2C19* and the *H. pylori* 23S rRNA gene, the latter of which is associated with susceptibility to clarithromycin^[13,15,22]. *H. pylori* and human DNA can be detected by PCR using gastric juice samples^[23], as gastric juice often contains gastric epithelial cells. Therefore, once DNA is extracted from the gastric juice, we can test for human and *H. pylori* DNA. This method of sample collection enabled us to perform simultaneous analyses of *CYP2C19* mutations and 23S rRNA mutation of *H. pylori*.

We previously reported that bacterial susceptibility to clarithromycin and the patient's *CYP2C19* genotype were each associated with the success or failure to eradicate *H. pylori* infection⁵ and that tailored regimens based on *CYP2C19* and 23S rRNA gene mutations yielded higher eradication rates than with the empirical standard therapy^[10]. However, standard genotyping methods, such as PCR-RFLP and direct nucleotide sequencing, require at least 5-10 hours, starting with DNA extraction sequence analysis, a length which has rendered such methods virtually useless in daily clinical practice. In the present study, we used the GENECUBE® system, which enabled us to complete simultaneous analysis of *CYP2C19* and 23S rRNA gene mutations within 30 min from start to finish. In our hospital, the genotyping of *CYP2C19* and 23S rRNA is now completed while patients take a rest for a while after endoscopy.

Therefore, the individualized eradication therapy can be given to patients on the same of the endoscopy.

Our goal here was to develop a GENECUBE®-based system for the simultaneous analyses of SNPs in *CYP2C19* and in the *H. pylori* 23S rRNA gene and to compare the results with those acquired by direct DNA sequencing to determine the validity of the new system. This was accomplished successfully, and we were able to show that the sensitivity, specificity and validity of the system correlated perfectly with non-genetic tests

Determining a patient's *CYP2C19* genotype and clarithromycin sensitivity is useful for devising optimal eradication therapy. In our hospital, we have used this system for advanced treatment in patients infected with *H. pylori*. We confess that performing a genotyping test in advance may seem costly (i.e., around 150 US dollars), but that this cost can be offset by achieving higher eradication rates after the first round of individualized therapy. Given that one of the limitations of the present study is its relatively small sample size, large-scale prospective studies must be performed to assess the full clinical potential of the GENECUBE® system and its economic feasibility.

CONCLUSION

The GENECUBE®-based system can simultaneously measure genotypes of *H. pylori* 23S rRNA gene and human *CYP2C19* from gastric tissues and juices rapidly, which can greatly aid the tailored *H. pylori* eradication therapies.

ACKNOWLEDGMENTS

This work was supported in part by a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (20590913). We thank the staff of the endoscopy unit (Tomoko Tsurumi, Kinuko Maruyama, Chieko Matsumoto, Akiko Ogi, Urara Kurosawa, Nobue Takahashi, Masayo Serizawa, Keiko Kikuchi, Satoko Takebayashi, Chikako Sasagase, and Akiko Tomita) for their help. The contributions of Etsuko Hamada, Ayano Fujiwara, and Katsumasa Yamanaka in the clinical laboratory unit are also greatly appreciated.

REFERENCES

- 1 Fock KM, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, Lam SK, Xiao SD, Tan HJ, Wu CY, Jung HC, Hoang BH, Kachintorn U, Goh KL, Chiba T, Rani AA. Second Asia-Pacific Consensus Guidelines for *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 2009; **24**: 1587-1600

- 2 Yuan Y, Padol IT, Hunt RH. Peptic ulcer disease today. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 80-89
 - 3 Asaka M, Sugiyama T, Kato M, Satoh K, Kuwayama H, Fukuda Y, Fujioka T, Takemoto T, Kimura K, Shimoyama T, Shimizu K, Kobayashi S. 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**: 254-261
 - 4 Murakami K, Sato R, Okimoto T, Nasu M, Fujioka T, Kodama M, Kagawa J, Sato S, Abe H, Arita T. Eradication rates of clarithromycin-resistant *Helicobacter pylori* using either rabeprazole or lansoprazole plus amoxicillin and clarithromycin. *Aliment Pharmacol Ther* 2002; **16**: 1933-1938
 - 5 Furuta T, Shirai N, Takashima M, Xiao F, Hanai H, Sugimura H, Ohashi K, Ishizaki T, Kaneko E. Effect of genotypic differences in CYP2C19 on cure rates for *Helicobacter pylori* infection by triple therapy with a proton pump inhibitor, amoxicillin, and clarithromycin. *Clin Pharmacol Ther* 2001; **69**: 158-168
 - 6 Lengyel G, Zsolt T. Failure in the eradication of *Helicobacter pylori*: the role of antibiotic resistance. *Orv Hetil* 2005; **146**: 839-842
 - 7 Tankovic J, Lamarque D, Lascols C, Soussy CJ, Delchier JC. Clarithromycin resistance of *Helicobacter pylori* has a major impact on the efficacy of the omeprazole-amoxicillin-clarithromycin therapy. *Pathol Biol (Paris)* 2001; **49**: 528-533
 - 8 Horiki N, Omata F, Uemura M, Suzuki S, Ishii N, Iizuka Y, Fukuda K, Fujita Y, Katsurahara M, Ito T, Cesar GE, Imoto I, Takei Y. Annual change of primary resistance to clarithromycin among *Helicobacter pylori* isolates from 1996 through 2008 in Japan. *Helicobacter* 2009; **14**: 86-90
 - 9 Sasaki M, Ogasawara N, Utsumi K, Kawamura N, Kamiya T, Kataoka H, Tanida S, Mizoshita T, Kasugai K, Joh T. Changes in 12-Year First-Line Eradication Rate of *Helicobacter pylori* Based on Triple Therapy with Proton Pump Inhibitor, Amoxicillin and Clarithromycin. *J Clin Biochem Nutr* 2010; **47**: 53-58
 - 10 Furuta T, Shirai N, Kodaira M, Sugimoto M, Nogaki A, Kuriyama S, Iwaizumi M, Yamade M, Terakawa I, Ohashi K, Ishizaki T, Hishida A. Pharmacogenomics-based tailored versus standard therapeutic regimen for eradication of *H. pylori*. *Clin Pharmacol Ther* 2007; **81**: 521-528
 - 11 Ménard A, Santos A, Mégraud F, Oleastro M. PCR-restriction fragment length polymorphism can also detect point mutation A2142C in the 23S rRNA gene, associated with *Helicobacter pylori* resistance to clarithromycin. *Antimicrob Agents Chemother* 2002; **46**: 1156-1157
 - 12 Maeda S, Yoshida H, Matsunaga H, et al. Detection of clarithromycin-resistant *helicobacter pylori* strains by a preferential homoduplex formation assay. *Journal of clinical microbiology*. 2000; **38**: 210-214
 - 13 Stone GG, Shortridge D, Versalovic J, Beyer J, Flamm RK, Graham DY, Ghoneim AT, Tanaka SK. A PCR-oligonucleotide ligation assay to determine the prevalence of 23S rRNA gene mutations in clarithromycin-resistant *Helicobacter pylori*. *Antimicrob Agents Chemother* 1997; **41**: 712-714
 - 14 Fontana C, Favaro M, Minelli S, Criscuolo AA, Pietroiusti A, Galante A, Favalli C. New site of modification of 23S rRNA associated with clarithromycin resistance of *Helicobacter pylori* clinical isolates. *Antimicrob Agents Chemother* 2002; **46**: 3765-3769
 - 15 Taylor DE, Ge Z, Purych D, Lo T, Hiratsuka K. Cloning and sequence analysis of two copies of a 23S rRNA gene from *Helicobacter pylori* and association of clarithromycin resistance with 23S rRNA mutations. *Antimicrob Agents Chemother* 1997; **41**: 2621-2628
 - 16 Furuta T, Shirai N, Ohashi K, Ishizaki T. Therapeutic impact of CYP2C19 pharmacogenetics on proton pump inhibitor-based eradication therapy for *Helicobacter pylori*. *Methods Find Exp Clin Pharmacol* 2003; **25**: 131-143
 - 17 Zhao F, Wang J, Yang Y, Wang X, Shi R, Xu Z, Huang Z, Zhang G. Effect of CYP2C19 genetic polymorphisms on the efficacy of proton pump inhibitor-based triple therapy for *Helicobacter pylori* eradication: a meta-analysis. *Helicobacter* 2008; **13**: 532-541
 - 18 Chen L, Qin S, Xie J, Tang J, Yang L, Shen W, Zhao X, Du J, He G, Feng G, He L, Xing Q. Genetic polymorphism analysis of CYP2C19 in Chinese Han populations from different geographic areas of mainland China. *Pharmacogenomics* 2008; **9**: 691-702
 - 19 Noguchi N, Rimbara E, Kato A, Tanaka A, Tokunaga K, Kawai T, Takahashi S, Sasatsu M. Detection of mixed clarithromycin-resistant and -susceptible *Helicobacter pylori* using nested PCR and direct sequencing of DNA extracted from faeces. *J Med Microbiol* 2007; **56**: 1174-1180
 - 20 Kubota T, Chiba K, Ishizaki T. Genotyping of S-mephenytoin 4'-hydroxylation in an extended Japanese population. *Clin Pharmacol Ther* 1996; **60**: 661-666
 - 21 Furuta T, Sagehashi Y, Shirai N, Sugimoto M, Nakamura A, Kodaira M, Kenmotsu K, Nagano M, Egashira T, Ueda K, Yoneyama M, Ohashi K, Ishizaki T, Hishida A. Influence of CYP2C19 polymorphism and *Helicobacter pylori* genotype determined from gastric tissue samples on response to triple therapy for H pylori infection. *Clin Gastroenterol Hepatol* 2005; **3**: 564-573
 - 22 Versalovic J, Osato MS, Spakovsky K, Dore MP, Reddy R, Stone GG, Shortridge D, Flamm RK, Tanaka SK, Graham DY. Point mutations in the 23S rRNA gene of *Helicobacter pylori* associated with different levels of clarithromycin resistance. *J Antimicrob Chemother* 1997; **40**: 283-286
 - 23 Furuta T, Kaneko E, Suzuki M, Arai H, Futami H. Quantitative study of *Helicobacter pylori* in gastric mucus by competitive PCR using synthetic DNA fragments. *J Clin Microbiol* 1996; **34**: 2421-2425
- Peer reviewer:** Aydin Seref Koksal, Associate Professor, Department of Gastroenterology, Türkiye Yüksek İhtisas Education and Research Hospital, 06010, Ankara, Turkey.