

## Influence of smoking and *CYP2C19* genotypes on *H. pylori* eradication success

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(Accepted 6 April 2006, first published online 2 June 2006)

### SUMMARY

*CYP2C19* polymorphisms and smoking influence the efficacy of *H. pylori* eradication therapy, but interaction between the two have hitherto not been examined. A total of 142 *H. pylori*-positive patients who received triple drug therapy with lansoprazole, amoxicillin and clarithromycin were categorized into three groups with regard to diplotypes of *CYP2C19*: homozygous extensive metabolizer (homEM), heterozygous EM (hetEM), and poor metabolizer (PM). The overall success rate was 61·3%. Smoking was an independent risk factor of eradication failure (OR 2·81, 95% CI 1·14–6·91), whereas *CYP2C19* polymorphisms were less influential. Among non-smokers, the homEM and hetEM groups showed worse eradication rates (58·5 and 67·3%) relative to PM (76·2%) as expected; however, an opposite trend was observed among smokers (homEM 50·0%, hetEM 46·7%, PM 20·0%), indicating possible interactions with *CYP2C19* polymorphisms. Smoking has a greater influence on *H. pylori* eradication than the *CYP2C19* genotype. Interaction between smoking and *CYP2C19* should be examined in the future.

### INTRODUCTION

Proton pump inhibitor (PPI)-based triple therapy involving two antibiotics for *Helicobacter pylori* eradication has been developed as a standard therapy. Eradication rates are more than 80%, but that leaves a considerable proportion of patients who encounter difficulty [1]. Several factors have been identified as predictive for successful *H. pylori* eradication [2–5].

Cytochrome P450 (CYP) 2C19 mediates the major metabolic transformations of PPIs and genetic polymorphisms of *CYP2C19* which lead to significant variation in activity and can thus exert an influence. More rapid PPI clearance in the extensive metabolizer (EM) is associated with a poorer *H. pylori* eradication outcome than with the poor metabolizer (PM) [6, 7]. Some researchers have reported that more extensive use of PPIs for EM compared with PM individuals might be a solution to overcome this phenotypic difference in *CYP2C19* activities [8, 9]. Smoking is another critical factor for *H. pylori* eradication. A recent meta-analysis demonstrated smokers to have

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an approximately twofold higher risk of eradication failure [10]. We therefore suggested smoking cessation as one solution for the issue [11]. Considering the significance of both *CYP2C19* polymorphisms and the smoking habit for *H. pylori* eradication, it is important to investigate interactions between these factors for eradication success; however, currently available evidence is limited [12, 13].

To clarify associations of *CYP2C19* genotypes in combination with smoking with regard to *H. pylori* eradication therapy, we here evaluated data from a prospective *H. pylori* eradication study conducted at Aichi Cancer Center (ACC) [14].

## METHODS

### Design and subjects

The investigation was conducted as a pilot study to assess the feasibility of *H. pylori* eradication intervention at ACC, for which background information and eradication results have been described elsewhere [11, 14]. In brief, during March to December 1999, 328 outpatients aged 40–69 years receiving no cancer treatment were recruited. Of the total, 283 (86.3%) agreed to participate in the study and finally 282 were examined by *H. pylori* antibody test (HM-CAP, Enteric Products Inc., Westbury, NY, USA) [15, 16]. With application of the set cut-off value (ELISA value  $\geq 2.3$ ), the seropositivity was 63.6% (180 patients). A total of 255 subjects underwent gastroscopy, and the *H. pylori* culture-positive (Dia Helico Pack for Jar, Dia-iatron, Tokyo, Japan) rate was 67.1% (171 patients) with biopsies from two gastric sites, the large curvature of the pylorus and the body. The subjects who were positive in at least one of the tests were defined as *H. pylori* infected (207 patients, 73.4%). Among them, 186 agreed to receive the LAC regimen (lansoprazole 30 mg/day, amoxicillin 500 mg t.i.d., clarithromycin 200 mg b.i.d.). Actually 173 participants took the drugs (compliance 93%, 173/186; participation rate 52.7%, 173/328). At entry and during follow-up, 31 patients proved to have a cancer (14 stomach, 5 breast, 4 colorectal and 8 miscellaneous cancers) and were excluded from this analysis. Finally, 142 subjects remained for analysis.

Subjects were followed up for 1 year after completing the eradication regimen. They were asked about symptomatic change and smoking status during the eradication therapy and the following 1 year. *H. pylori* IgG levels were examined serologically at

1 year after the medication. Gastroscopy at 1 year after the medication was optional. We defined success of eradication as a >25% decline in the *H. pylori* IgG value from the baseline, in line with the criteria of Marchildon *et al.* [17].

Written informed consent for genotyping was received from all subjects. This study was approved by the Institutional Review Board of ACC.

### Genotyping

Aliquots of 7 ml of peripheral blood were obtained with application of 2 Na-EDTA and the buffy coat of each sample was separated to extract genomic DNA using a QIAamp DNA Blood Mini kit (Qiagen Inc., Valencia, CA, USA). Definition of genotype for the *CYP2C19*\*1 (wild-type) allele and two mutated alleles, *CYP2C19*\*2, G681A in exon 5 (dbSNP ID: rs4244285; assay ID: C\_25986767\_70) and *CYP2C19*\*3, G636A in exon 4 G636A (dbSNP ID: rs4986893; assay ID: C\_27861809\_10) was executed with TaqMan assays by Applied Biosystems (Foster City, CA). The patients were classified into the following three genotype groups: homozygous EM (homEM): \*1/\*1; heterozygous EM (hetEM): \*1/\*2 or \*1/\*3; and PM: \*2/\*2, \*3/\*3, or \*2/\*3.

### Statistical analyses

Logistic regression models were employed to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) to assess the risk of eradication failure. Smoking habit was entered under the two categories of non-smoker (never) and smoker (former or current). The interactions between smoking habit and *CYP2C19* genotypes were evaluated under multiplicative assumption. Products of scores for genotype (0, homEM; 1, hetEM; and 2, PM) and smoking habit (0, non-smoker; and 1, smoker) were included as interaction terms. Accordance with Hardy–Weinberg equilibrium was checked using the  $\chi^2$  test, and the exact *P* value was used to assess any discrepancies between genotype and allele frequencies. A *P* value of <0.05 was considered as statistically significant. All analyses were performed using STATA version 8 (Stata Corp., College Station, TX, USA).

## RESULTS

A total of 142 patients consisted of 22 gastric ulcer (including scar stage), 18 duodenal ulcer (including

Table 1. ORs for the association of patients' characteristic with eradication failure

	Success (%)	Failure (%)	Adjusted OR* (95% CI)
Total	87 (61.3)	55 (38.7)	
Age (yr)			
< 60	41 (58.6)	29 (41.4)	1.00 (ref.)
≥ 60	46 (63.9)	26 (36.1)	0.82 (0.40–1.67)
Sex			
Male	47 (60.3)	31 (39.7)	1.00 (ref.)
Female	40 (62.5)	24 (37.5)	1.17 (0.56–2.45)
Smoking			
Non-smoker	75 (65.8)	39 (34.2)	1.00 (ref.)
Smoker	12 (42.9)	16 (57.1)	2.81 (1.14–6.91)
<i>CYP2C19</i> genotypes			
HomEM	28 (57.1)	21 (42.9)	1.00 (ref.)
HetEM	40 (62.5)	24 (37.5)	0.72 (0.33–1.58)
PM	17 (65.4)	9 (34.6)	0.68 (0.25–1.86)

OR, Odds ratio; CI, confidence interval; HomEM, homozygous extensive metabolizer; HetEM, heterozygous extensive metabolizer; PM, poor metabolizer.

\* ORs were adjusted for age, sex, smoking and *CYP2C19* genotypes.

scar stage), 9 gastroduodenal ulcer (including scar stage), 46 atrophic gastritis, 37 other abnormal finding such as erosion or polyps and 10 without specific abnormal finding in the gastroscopy. Table 1 shows the results of *H. pylori* eradication by patient characteristics. The median age was 60 years, ranging from 40–69 years. The overall eradication rate was 61.3% (95% CI 52.7–69.3) and age and sex were without influence. Eradication rates in smokers and non-smokers were 42.9% and 65.8%, respectively, the difference being significant (OR 2.81, 95% CI 1.14–6.91). Concerning the *CYP2C19* genotypes, of 142 patients, 139 were examined because no DNA samples were available for three individuals. Patients were classified into three genotype groups as follows: homEM group (\*1/\*1; n=49, 35%), hetEM group (\*1/\*2 or \*1/\*3; n=64, 46%), and PM group (\*2/\*2, \*3/\*3, or \*2/\*3; n=26, 19%). The observed distribution corresponded to the one predicted according to Hardy–Weinberg law (P=0.53). Eradication rates were 57.1% in homEM, 62.5% in hetEM and 65.4% in PM, respectively. The adjusted ORs of eradication failure for hetEM or PM, compared to homEM, were lower, but without significance.

To examine the impact of the combined effects of smoking and *CYP2C19* genotypes on eradication therapy, stratified analyses according to the *CYP2C19* genotype were conducted (Table 2, upper panel). The

eradication rates with homEM and hetEM were lower than that of PM among non-smokers (homEM 58.5%, hetEM 67.3% and PM 76.2%), as with the subjects overall. However, the opposite trend was observed among smokers (homEM 50.0%, hetEM 46.7%, and PM 20.0%). Consequently, the ORs for eradication failure in smokers, compared with non-smokers, were increased in hetEM (OR 3.97, 95% CI 1.01–15.68) and PM (OR 12.26, 95% CI 0.95–158.82) cases. Further, evaluating interactions between smoking and *CYP2C19* genotypes (Table 2, lower panel), we observed the same trends. These results implied interactions of these factors on efficacy of *H. pylori* treatment, although the P value was not significant.

## DISCUSSION

In this study, we found individual influences of smoking and *CYP2C19* gene polymorphisms, along with a tendency for a combined impact on *H. pylori* eradication with 1-week LAC therapy. Of the two factors, smoking exerted the greatest influence.

Patients without mutation of the *CYP2C19* gene metabolize PPI very extensively and, therefore, may not achieve enough acid suppression for amoxicillin, an acid-sensitive antibiotic, to be effective. Such a phenomenon has been suggested to be one of the

Table 2. Eradication rate and adjusted ORs of eradication failure according to smoking habits and CYP2C19 genotypes

	HomEM		HetEM		PM	
	% Eradication (no.)	OR* (95% CI)	% Eradication (no.)	OR* (95% CI)	% Eradication (no.)	OR* (95% CI)
<i>CYP2C19</i> stratified†						
Non-smoker	58.5 (24/41)	1.00 (ref.)	67.3 (33/49)	1.00 (ref.)	76.2 (16/21)	1.00 (ref.)
Smoker	50.0 (4/8)	1.21 (0.25–5.93)	46.7 (7/15)	3.97 (1.01–15.68)	20.0 (1/5)	12.26 (0.95–158.82)
Interaction with smoking and <i>CYP2C19</i> ‡						
Non-smoker		1.00 (ref.)		0.63 (0.27–1.50)		0.42 (0.13–1.35)
Smoker		1.35 (0.29–6.26)		1.56 (0.47–5.19)		5.51 (0.56–54.30)

HomEM, Homozygous extensive metabolizer; HetEM, heterogenous extensive metabolizer; PM, poor metabolizer; OR, odds ratio; CI, confidence interval.

\* ORs were adjusted for age and sex.

† References are non-smoker in each *CYP2C19* genotype.

‡ Reference is non-smoker and HomEM genotype.

causes of treatment failure with the PPI-based triple therapy, and the cure rate correlates with the *CYP2C19* genotype. Smoking has been also reported to influence the response to *H. pylori* eradication therapy in terms of mechanisms like decreased blood flow in the stomach [18, 19], stimulation of acid secretion [20] and poor compliance [2]. Several trials demonstrated cure rates of *H. pylori* infection to be significantly lower in extensive metabolizers of *CYP2C19* when compared to poor metabolizers [6, 7]; however, studies considering both smoking habits and *CYP2C19* genotypes are scarce and the evidence is inconsistent. Using a multivariate model, we here found that smoking is an independent factor that has a significant impact on treatment. Furthermore, the influence of the *CYP2C19* genotype on eradication differed between non-smokers and smokers. Our findings might suggest that smoking affects the metabolism of drugs used for eradication therapy via modification of *CYP2C19* activity, although induction of *CYP2C19* by smoking has not been reported.

The overall successful eradication rate (61.3%) in this study was relatively low. The result may be explained from the lower dose of PPI in this trial. The dosage of lansoprazole used in this study was 30 mg. In Japan, however, at present, 60 mg lansoprazole was used as the conventional LAC regimen [21, 22]. Another explanation may be to have evaluated eradication success using serum titre of *H. pylori* antibody. We examined *H. pylori* IgG antibody values at enrolment and at 1 year after the medication to judge the eradication success using a cut-off value of 25% decrease from the baseline. Sensitivity of this test is a little lower compared with urea breath test [17].

Unlike a previous report, our results did not show a statistical difference in eradication results among *CYP2C19* metabolizer types using 1-week LAC therapy in the usual clinical setting. Relatively lower sensitivity of evaluation of eradication success by *H. pylori* IgG testing might be one possible explanation. Alternatively, possible interactions between lansoprazole and clarithromycin that are metabolized by *CYP3A4* and *CYP2C19* might be of importance [23, 24]. Although all PPIs are metabolized by *CYP2C19* and *CYP3A4* to varying degrees, lansoprazole metabolism is more dependent on *CYP3A4* than that of other PPIs [25]. In regimens with lansoprazole, clarithromycin, which is metabolized by *CYP3A4* and competitively inhibits the metabolism

of PPI by the CYP3A4 enzyme, might have decreased the magnitude of CYP2C19-mediated metabolism of lansoprazole. A randomized trial showed that an eradication regimen with lansoprazole was more effective in patients with the homEM genotype of CYP2C19 than regimens with other PPIs [26].

The present study has several limitations. First, our sample size was not sufficiently large to detect interactions between the two factors. Second, we could not evaluate other important factors for eradication, such as antibiotic resistance and *H. pylori* strain types (e.g. CagA) [5]. Third, eradication success was evaluated using *H. pylori* antibody levels. Regarding confirmation of eradication, urea breath test or an endoscopy-based test have been recommended by a European consensus panel [27]. Therefore, the results of this study must be carefully interpreted.

In conclusion, we demonstrated smoking is a consistent factor for eradication failure and, further that effects of CYP2C19 genetic polymorphisms on eradication are modified by smoking. These findings indicate that we need to take into account the smoking status as well as CYP2C19 genotyping for eradication therapy.

## ACKNOWLEDGEMENTS

The study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, Culture and Technology of Japan and a Grant-in-Aid for the Third Term Comprehensive 10-Year-Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan.

## DECLARATION OF INTEREST

None.

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