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Smoking and polymorphisms of fucosyltransferase gene *Le* affect success of *H. pylori* eradication with lansoprazole, amoxicillin, and clarithromycin

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SUMMARY

Identification of factors influencing success of *Helicobacter pylori* (*HP*) eradication is important for clinical practice. We have prospectively conducted an *HP* eradication study in the Aichi Cancer Center with a total of 142 patients available for analysis. The overall success rate was 61·3% (95% confidence interval 52·7–69·3%). Smoking during the medication for eradication significantly decreased the success rate (42·9%), whereas smoking cessation during the treatment was associated with a similar rate as for non-smokers (66·7%). We also examined links between an eradication outcome and polymorphisms of *Le*, *Se*, *IL1A*, *IL1B*, *IL1RN* and *MPO* genes, but with one exception none showed any association. The non-functional *le* allele of *Le* polymorphisms, leading to decreased expression of Le^b antigen to which *HP* attaches with adhesin, showed a beneficial effect for success. Although further clarification is necessary, our study indicated that smoking cessation and *Le* gene polymorphisms may affect the success rate of *HP* eradication.

INTRODUCTION

Helicobacter pylori (*HP*) is recognized as the cause of gastric cancer [1] as well as gastric ulcers [2], and mucosa-associated-lymphoid tissue (MALT) lymphoma [3]. Accumulating evidence supports *HP* eradication as a promising strategy for gastric cancer prevention and regimens have been developed which allow a high success rate to be achieved. However, a certain percentage of cases fail to eradicate *HP* after treatment. Clearly, host factors may influence not only the infection process of *HP* but also eradication of *HP*, but these have yet to be clarified. In this paper, we focus on host genetic factors and the smoking habit as probable influential factors.

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A number of genetic variations have been reported to predispose to *HP* infection. For example, we previously reported an association between the *HP* infection rate, and polymorphisms of two fucosyltransferase genes, *secretor* (*Se*) and *Lewis* (*Le*) [4]. Those gene polymorphisms in Japanese affect the fucose transfer activity [5–10], thus leading to modified synthesis of type I Le antigen expressed on gastric mucosa [4] to which *HP* binds [9, 10]. Observed lower infection rate for harbouring functional alleles of *Le* gene (*Le*) and non-functional alleles of *Se* gene (*se*) indicated that decreased ligand expression due to these polymorphisms may lead to decreased adhesion of *HP* to gastric mucosa.

We have also reported the association between infection of *IL-1B* and *MPO* polymorphisms [11, 12]. A higher susceptibility was observed for *IL-1B–31 T*

allele carriers and *MPO–463* G allele carriers, and current smoking modifies the effect of these alleles. The *IL-1B–31 T* allele is associated with higher IL-1β production. Enhanced production of IL-1β inhibits acid production in the gastric mucosa [13]. With the *MPO–463* G/A polymorphism, the *A* allele suppresses the expression of myeloperoxidase [14], resulting in reduced tissue inflammation. Interactions with inflammation-related molecules are regarded as important not only for damage to the gastric mucosa but also for continuation of the infection, but the detailed mechanisms are still unknown [15].

Although the susceptibility to *HP* infection is thus influenced by genetic factors, whether this is also the case for the success rate of *HP* eradication still needs evaluation. Several investigators have reported effects of polymorphisms of the *CYP2C19* gene, encoding an enzyme metabolizing the proton pump inhibitor (PPI) [16]. The studies showed that a poor metabolizer produced higher eradication rates [17, 18]. However, there is only limited information available regarding the impact of other genetic polymorphisms on *HP* eradication.

In addition to genetic factors, current smoking may also be influential regarding the success rate of *HP* eradication [19–25]. Although controversy exists [26, 27], most of the reports have pointed to current smoking decreasing the success rate, but whether smoking cessation during eradication might be beneficial has not been examined to our knowledge.

Taken the available data into consideration, we hypothesized that genetic polymorphisms influencing the susceptibility to *HP*, in combination with smoking habit, influence the success rate of *HP* eradication. To evaluate the hypothesis, we evaluated associations with *Le*, *Se*, *IL-1A*, *IL-1B*, *IL-1RN* and *MPO* gene polymorphisms and patients' smoking status using the data set from an *HP* eradication study conducted at the Aichi Cancer Center (ACC) [28].

METHODS

Design and subjects

The investigation was conducted as a part of pilot studies to assess the feasibility of *HP* eradication intervention at the ACC, for which background information has been described elsewhere [28]. Briefly, subjects were outpatients aged 40–69 years not under cancer treatment, who had not undergone gastrectomy and were scheduled for a gastroscopy. Those examined by three out of eight gastroenterology physicians were invited to join the study by the staff of Epidemiology and Prevention to avoid psychological pressure on the subjects. After giving informed consent, patients provided 7 ml of venous blood and answered a questionnaire.

During March to December 1999, 328 eligible patients saw three doctors in charge. Of the 328, 283 (86·3%) agreed to participate in the study and finally 282 were examined by the *HP* antibody test (HM-CAP, Enteric Products Inc., Westbury, NY). With application of the set cut-off value (EV value $\geqslant$2·3), the seropositivity was 63·6% (180 patients). A total of 255 subjects underwent gastroscopy, and the *HP* culture (Dia Helico Pack for Jar, Dia-iatron, Tokyo, Japan) positive 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 *HP* infected (207 patients: 73·4%). Among them, 186 agreed to receiving the eradication regimen (amoxicillin 1500 mg t.i.d., clarithromycin 400 mg b.i.d., lansoprazole 40 mg q.d. for 1 week). Actually 173 participants with a median age of 60 years took the drugs (compliance 93% 173/186; participation rate 52·7% 173/328). At entry and during follow-up, 31 patients were proved to have a cancer (14 stomach, 5 breast, 4 colorectal and 8 miscellaneous cancers).

Subjects were followed up for 1 year after completing the eradication regimen. They were asked about symptomatic change and smoking status during the eradication and during the following 1 year. *HP* IgG levels were examined serologically. Gastroscopy 1 year after the medication was optional. We defined success of eradication as a greater than 25% decline in the *HP* IgG value, from the baseline, in line with the criteria of Marchildon et al. [29].

Written informed consent for genotyping was received from all subjects. The ethical committee in the ACC approved this study (Approval no. 12–23).

Genotyping

Aliquots of 7 ml of peripheral blood were obtained with 2Na-EDTA and the buffy coat of each sample was separated to extract genomic DNA using a QIAamp DNA Blood Mini Kit (Qiagen Inc., CA).

Detailed genotyping methods for *Se* and *Le* genes have been described elsewhere [8, 30]. For *Se* genotyping, only the A385T polymorphism in the *sej* and the *se5* alleles was assessed because the *se3* and *se4*

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Table 1. *Smoking status and Odds Ratios (ORs) for* HP *eradication*

	Eradication result			
	Success	Failure	OR	(95% CI)
Total	87 (61·3%)	55 (38·7%)		
Age				
<60 years	41 (58·6%)	29 (41·4%)	1·0	(reference)
⩾60 years	46 (63·9%)	26 (36·1%)	1·3	(0·6–2·5)
Sex				
Male	47 (60·3%)	31 (39·7%)	1·0	(reference)
Female	40 (62·5%)	24 (37·5%)	1·1	(0·6–2·2)
Non-smokers before Tx*	71 (65·7%)	37 (34·3%)	1·0	(reference)
Smokers				
Cessation during Tx*	4 (66·7%)	2 (33·3%)	1·04	(0·2–6·0)
Non-cessation during Tx*	12 (42·9%)	16 (57·1%)	0·4	(0·2–0·9)
				P for trend = 0·03

* Tx indicates the medication for *HP* eradication. None of non-smokers smoked during the medication.

alleles were not found in a previous analysis of more than 600 Japanese samples [30]. The *Le* allele was determined based on detection of three missense mutations, T59G, G508A and T1067A, in the *Le* gene [5]. The genotyping of *sej*, *le1*, *le2* and *le3* alleles was based on the polymerase chain reaction restriction fragment length polymorphism (PCR–RFLP) described earlier [8, 30]. Briefly, the full-length open reading frame of the *Se* or *Le* gene was amplified with a specific primer set and subjected to the second PCR reaction for the PCR–RFLP. The second PCR products were digested with *Alu*I to detect A385T mutations of the *sej* allele, with *Pvu*II for G508A of *le1*, with *Hin*dIII for T1067A of *le2*, and with *Msp*I for T59G of *le1*, *le2* and *le3*. The *se5* allele was detected by simple PCR, with a specific primer set as previously described [8, 30].

For *IL-1A* C-899T and *IL-1B* C-31T genotyping, we applied PCR with confronting two pair primer (PCR–CTPP) system which we have developed in our laboratory [31]. In brief, the confronting two-pair primers for the polymorphic nucleotides produce allele-specific PCR products as well as common PCR products. The specific primers for these polymorphisms have been described elsewhere [32]. The genotyping *IL-1RN* 86-bp variable number of tandem repeats (VNTR) at intron 2 [33] and *MPO* G-463A [34] was accomplished in line with previous reports.

Statistical analysis

In order to estimate the effect for the functional *Se* allele, we categorized the patients into three groups: *Se*/*Se*, *Se*/*se*, *se*/*se*, homozygous for the functional *Se* alleles, heterozygous for the functional and non-functional alleles (*Se*/*sej* and *Se*/*se5*), and homozygous for the non-functional alleles (*sej*/*sej*, *sej*/*se5* and *se5*/*se5*), respectively. Patients were also grouped depending on the *Le* alleles into three groups: *Le*/*Le*, consisting *Le*/*Le* and *Le*/*le3*; *Le*/*le*, consisting of *Le*/*le1* and *Le*/*le2*; and *le*/*le*, consisting of *le1*/*le1*, *le1*/*le2*, and *le2*/*le2*.

All statistical analyses were performed using STATA version 7 software (College Station, TX). Variation in genotype frequencies among eradicated and non-eradicated subjects was examined by a χ^2 test. Differences in eradication success rate were measured in terms of odds ratios (ORs) and 95% confidence intervals (CIs) estimated by logistic regression models.

RESULTS

Patients and their results of *HP* eradication

A total of 142 (78 males and 64 females) patients were analysed for *HP* eradication because 31 cancer cases and 1 individual who discontinued taking the drugs of their own accord were excluded. The median age was 60 years, range 40–69, and the age distribution did not differ between the sexes. During the medication, 37 patients showed gastrointestinal discomfort with diarrhoea as the main symptom, 6 suffered oral mucosal change, 4 demonstrated skin eruption, 2 showed dysuria. However, all of them completed the medication schedule. As shown in Table 1, the overall

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Table 2. *Genotype distributions of 142* HP *eradication results*

	No. = 142	Eradicated	Not-eradicated	OR (95% CI)	*P* for trend
Le					
Le/*Le*	70 (49·3%)	38 (54·3%)	32 (45·7%)	1·0 (reference)	
Le/*le*	63 (44·4%)	41 (65·1%)	22 (34·9%)	1·6 (0·8–3·2)	
le/*le*	9 (6·3%)	8 (88·9%)	1 (1·8%)	6·7 (0·8–56·8)	0·04
Se*					
Se/*Se*	42 (29·8%)	23 (54·8%)	19 (45·2%)	1·0 (reference)	
Se/*se*	78 (54·9%)	50 (64·1%)	28 (35·9%)	1·5 (0·7–3·2)	
se/*se*	21 (14·8%)	14 (66·7%)	7 (33·3%)	1·7 (0·6–4·9)	0·29
IL1A C-889T					
C/*C*	117 (82·4%)	71 (60·7%)	46 (39·3%)	1·0 (reference)	
C/*T*	24 (16·9%)	16 (66·7%)	8 (33·3%)	1·3 (0·5–3·3)	
T/*T*	1 (0·7%)	0 (0·0%)	1 (100%)	NA†	0·98
IL1B C-31T					
T/*T*	41 (28·9%)	25 (61·0%)	16 (39·0%)	1·0 (reference)	
T/*C*	78 (54·9%)	47 (60·3%)	31 (39·7%)	1·0 (0·5–2·1)	
C/*C*	23 (16·2%)	15 (65·2%)	8 (34·8%)	1·2 (0·4–3·5)	0·79
IL1RN 86-bp VNTR at intron 2					
4/*4*	127 (89·4%)	81 (63·8%)	46 (36·2%)	1·0 (reference)	
Other than *4*/*4*	15 (10·6%)	6 (40·0%)	9 (60·0%)	0·4 (0·1–1·1)	0·08
2/*2*	1 (0·7%)	1 (100%)	0 (0·0%)		
2/*4*	10 (7·0%)	3 (30·0%)	7 (70·0%)		
3/*4*	1 (0·7%)	0 (0·0%)	1 (100%)		
4/*5*	3 (2·1%)	2 (66·7%)	1 (33·3%)		
MPO G-463A					
G/*G*	117 (82·4%)	17 (68·0%)	8 (32·0%)	1·0 (reference)	
G/*A*	25 (17·6%)	70 (59·8%)	47 (40·2%)	1·4 (0·6–3·6)	
A/*A*	0 (0·0%)	—	—	NA†	0·45

* One subject could not be genotyped.
† NA indicates not analysed because of no subject.

eradication rate confirmed by serological testing was 61·3% (95% CI 52·7–69·3%). Although age and sex did not affect the outcome, smoking status exerted an influence. The eradication rate for non-smokers was 65·7%. When current smokers were divided into two groups: a cessation group who did not smoke during the period of medication and non-cessation group who continued smoking, success rates were 66·7 and 42·9%, respectively, pointing to significant benefit of cessation (OR 0·4, 95% CI 0·2–0·9, $P = 0·03$). Smoking after the medication did not change the success rate.

Genotype distributions of polymorphisms and their contribution to *HP* eradication

As shown in Table 2, the genotype distributions of each polymorphism were not different between eradicated and not-eradicated patients except for the *Le* genotype which showed a trend for increasing risk with harbouring non-functional *le* allele. The ORs for success of *HP* eradication were 1·6 (0·8–3·2) and 6·7 (0·8–56·8) for *Le*/*le* and *le*/*le* genotype, respectively. The ORs were not statistically significant, but the trend was significant ($P = 0·04$). For *IL1A*, *IL1RN* and *MPO* gene polymorphisms, evaluation of specific genotype was difficult because the allele frequencies for non-dominant allele were rare compared to *Le*, *Se* and *IL1B* polymorphisms.

We also analysed the effect of *Le* genotype in combination with smoking status (Table 3). Among the non-smokers, similarity to the overall analysis was apparent, and trend was also statistically significant. Since the numbers of patients in the cessation and non-cessation group were limited, the effect of *Le* genotype in the smoker category could not be evaluated.

DISCUSSION

In this study, we found that smoking during the medication period for *HP* eradication significantly decreased the success rate compared with non-smoking,

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Table 3. HP *eradication results according to* Le *genotype and smoking status*

	Non-smokers	Smokers	
		Cessation during Tx*	Non-cessation during Tx*
	No. of subjects Success/total (%) OR (95% CI)	No. of subjects Success/total (%) OR (95% CI)	No. of subjects Success/total (%) OR (95% CI)
Le†			
Le/*Le*	27/48 (56·3%) 1·0 (reference)	2/3 (66·7%) 1·0 (reference)	9/19 (47·4%) 1·0 (reference)
Le/*le*	38/53 (71·7%) 2·0 (0·9–4·5)	1/1 (100%) 0·5 (0·01–19·6)	2/8 (25·0%) 0·4 (0·1–2·3)
le/*le*	6/7 (85·7%) 4·7 (0·5–41·8)	1/1 (100%) NA‡	1/1 (100%) NA‡
P for trend	0·049	0·71	0·29

* Tx indicates the medication for *HP* eradication.
† One case could not be genotyped.
‡ NA indicates not analysed because of no subject.

and that smoking cessation during treatment was associated with a similar rate to non-smoking. Regarding genetic polymorphisms only *Le* polymorphisms showed any significant link with *HP* eradication results. Compared with the *Le*/*Le* genotype, *Le*/*le* and *le*/*le* showed higher ORs for eradication success. Patients homozygous for non-functional *le* allele had approximately six times higher success rate. In non-smokers, *Le* genotype showed a similar influence.

Smoking is reported to be associated with *HP* infection in both the general public [35, 36] and outpatients [37, 38]. Our results are in line with the large number of reports of smoking as a negative modifier of *HP* eradication [19–25]. Several cross-sectional studies revealed no association [26, 27]. However, none of those studies clearly defined smoking status during *HP* eradication. In this context, the finding in our study of a similar success rate in 108 non-smokers and in 6 patients who did not smoke during the eradication period is of obvious interest. Although further evaluations are required with larger sample sizes, our observations imply that smoking cessation during the medication is important for successful *HP* eradication.

The polymorphisms relevant to vulnerability of *HP* infection did not appear to be factors affecting outcome of *HP* eradication in this study, with the exception of the *Le* gene case. We reported previously that the non-functional *le* allele is a risk factor for *HP* infection, while the same allele demonstrated advantage for eradication in this study, especially in homozygous patients. Se and Le enzymes metabolize Type I precursor into H Type I blood group carbohydrate structure and Le^a, respectively. Le enzyme further converts H Type I structure into Le^b [39–42]. Lower Le enzyme activity in concert with higher Se enzyme activity may produce more H Type I on gastric foveolar cell [4] to which *HP* binds via blood group antigen-binding adhesin BabA [9, 10, 43]. In our previous study with the same subjects, the *le* allele and the *Se* allele independently increased the risk of persistent infection. Although biological mechanisms are not fully understood, our observation may give a clue for the eradication process.

The triple drug therapy applied in this study was a standard protocol for *HP* eradication, and its success rate (61·3%) was slightly lower than former studies. We examined *HP* IgG antibody values at enrolment and 1 year after the medication to judge the eradication using a cut-off value of 25% decrease from the baseline, as reported by Marchildon et al. [29]. Although their study demonstrated that this cut-off value at the 6 months after eradication has high sensitivity, Cutler et al. reported that *HP* IgG continues to be elevated above the normal value and that using a cut-off value of 20% decrease from the baseline at 12–21 months is highly sensitive compared with culture and urea breath testing [44]. Further studies are necessary to clarify the time dependence.

In conclusion, smoking during the medication for *HP* eradication may decrease the success rate, while cessation for the treatment period may be beneficial. The polymorphisms examined, except for the *Le* gene,

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appear not to be associated with success rates of eradication. Harbouring non-functional *le* allele, however, may be beneficial in this regard. Although the number of patients in this study is limited, our results suggest important insights into *HP* eradication.

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