Original Article

Role of Cytochrome P450 2C19 Genetic Polymorphisms in the Therapeutic Efficacy of Omeprazole in Iranian Patients with Erosive Reflux Esophagitis

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Abstract

Background: There are different clinical responses to omeprazole treatment in Iranian patients with gastroesophageal reflux disease. Omeprazole is metabolized in the liver by the cytochrome p450 2c19 (CYP2C19) enzyme. Two common polymorphisms of the CYP2C19 gene affect CYP2C19 enzyme activity. We investigated the effect of CYP2C19 gene polymorphisms on the clinical response to treatment with omeprazole in Iranian patients with erosive reflux esophagitis.

Methods: Eighty-two Iranian patients with reflux esophagitis were enrolled in the study and underwent treatment with omeprazole at 40 mg daily for 4 weeks. A 2 mL sample of venous blood was obtained from each subject. CYP2C19 genetic polymorphisms were detected using the PCR-RFLP method. The patients were grouped into homo-extensive metabolizers and hetero-extensive metabolizers based on their CYP2C19 polymorphism. The grade of esophagitis was determined via endoscopy. The symptoms score was assessed at the beginning of treatment.

Results: Our results showed that the rate of complete clinical response to treatment with omeprazole was 95% in the hetero-extensive metabolizers group, which was higher than in the homo-extensive metabolizers group ($P<0.001$).

Conclusion: CYP2C19 polymorphism influences the therapeutic efficacy of omeprazole in the treatment of Iranian patients with erosive reflux esophagitis. The clinical response and endoscopic healing of esophagitis are both affected by CYP2C19 genotype condition.

Keywords: clinical response, cytochrome P450 2C19, erosive reflux esophagitis, genetic polymorphism, omeprazole

Introduction

Gastroesophageal reflux disease (GERD) is a common disease across the world, including in Iran.[1,2] It is characterized by the reflux of acidic stomach contents into the esophagus, causing symptoms such as heartburn, acid regurgitation, and dysphagia, adversely affecting the patient's quality of life.[1,3–5] Without treatment, the lower esophageal mucosa is damaged by gastric acid, leading to reflux esophagitis.[6,7] Proton pump inhibitors (PPIs) such as omeprazole are the most effective agents available for the treatment of GERD. PPIs inhibit gastric H^+/K^+ATPase and this leads to less acid secretion.[8,9] Cytochrome p450 (CYPs) enzymes, located mostly in the liver, are responsible for the metabolism of drugs.[10] Cytochrome P450 2C19 (CYP2C19) is an isoform of the CYPs and is correlated with the metabolism of several therapeutically important drugs, including PPIs (omeprazole, lansoprazole, and pantoprazole).[11] The CYP2C19 gene has several polymorphisms, the most common of which are the CYP2C19*2 and CYP2C19*3 alleles.[12] In the CYP2C19*2 genotype, the G>A substitution in the position of 681 in exon 5 creates an aberrant splice site.[13] In the CYP2C19*3 genotype, the substitution of G>A in the position of 636 in exon 4 leads to a premature stop codon.[14] These two polymorphisms both decrease enzyme activity, and individuals can be grouped into two phenotypes, extensive metabo-

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lizers (EMs) and poor metabolizers (PMs; two mutated alleles). EMs can also be subdivided as hetero-EMs (one mutated allele) and homo-EMs (no mutated allele). In homo-EMs, PPIs are less effective at gastric acid secretion because of rapid metabolism due to higher CYP2C19 enzyme activity but they have intermediate effectiveness in hetero-EMs. PPIs have the most effectiveness on gastric acid secretion in PMs because of delayed metabolism due to lower enzyme activity. The frequency of the PM phenotype is greater in Asian populations (18 – 23%) than in Caucasians (2 – 5%).[13] The CYP2C19*2 allele is the most common variant among the normal Iranian population, while CYP2C19*3 is absent in the normal Iranian population.[15] The presence of the CYP2C19 (*2, *3) polymorphism can predict gastric acid suppression,[16] but there are various and sometimes conflicting data about the influence of CYP2C19 polymorphisms on the clinical response to treatment with PPIs among different populations.[8,17] We performed this study to evaluate the influence of two common CYP2C19 polymorphisms on the clinical and endoscopic responses to omeprazole in Iranian patients with erosive reflux esophagitis.

Materials and Methods

Patients

Eighty-two patients (37 men and 45 women, 38±14 years old) with at least one of three important symptoms of GERD (heart burn, acid regurgitation, or dysphagia) and erosive reflux esophagitis as diagnosed by endoscopy were enrolled in this study. This study was performed from April 2008 until October 2008 in the Endoscopy Ward of Fayazbakhsh Hospital (Tehran, Iran). Exclusion criteria were: presence of esophageal varices, concomitant active gastric or duodenal ulcers, esophageal abnormalities, and a history of gastric, duodenal, or esophageal surgery. None of the patients had taken PPIs in the 4 weeks prior to the beginning of the study. The study protocol was approved by the Ethics Committee of Tehran University of Medical Science (Tehran, Iran). Written informed consent was obtained from all participants. Subjects not consenting to the study were excluded.

Intervention

In all patients, endoscopy was performed before treatment, and the severity of erosive esophagitis was graded from A to D according to the Los Angeles classification. This classification grades esophagitis severity by the extent of mucosal abnormality, with complications recorded separately. In this classification: grade A includes one or more mucosal breaks each ≤5 mm in length; grade B includes at least one mucosal break >5 mm long, but not continuous between the tops of adjacent mucosal folds; grade C includes at least one mucosal break that is continuous between the tops of adjacent mucosal folds, which is not circumferential; and grade D includes a mucosal break that involves at least three-fourths of the luminal circumference.[18] The presence of hiatal hernia was also recorded. In addition, *H. pylori* infection was detected by the rapid urease test (RUT) in all patients. All patients underwent treatment with 40 mg of omeprazole daily for 4 weeks; one 20 mg capsule 30 min before breakfast and another one 30 min before dinner. Patients were given a form and were instructed by a trained general physician to record any new or changed symptoms during the period of treatment with omeprazole. Patients regular weekly consumption of omeprazole was carefully monitored and they were recommended not to take any other similar medications during the treatment period. They were also counseled to change their lifestyle, for example to avoid smoking, drinking coffee, and eating chocolate. Of the 82 subjects at the initial part of the study, 41 subjects were randomly allocated for endoscopy by simple random allocation. Of these, 5 subjects did not agree to undergo endoscopy. Therefore, a second endoscopy was performed in 36 subjects after they had completed treatment with omeprazole. In the second endoscopy, a full endoscopy was not performed instead only the esophagus was monitored.

Symptom evaluation

Gastroesophageal reflux questionnaires comprised of information such as age, sex, and body mass index (BMI) were completed for all patients. The symptom scores were derived from the sum of the frequency and severity of symptoms. The symptom frequency was measured on the following scale: 1) with specific foods, 2) less than once a week, 3) about 1 – 3 times during the week, and 4) daily. The scale of symptom severity was: 1) mild (awareness of heartburn, but easily tolerated), 2) moderate (discomfort from heartburn, but no interference with daily activities), or 3) severe (discomfort from heartburn that interferes with daily activities).

Table 1. Genotype and allele frequency of CYP2C19 in Fayazbakhsh Hospital patients diagnosed with reflux esophagtis (n=82)

Cyp2c19 genotype and allele	*n*	Frequency (%)	95%CI*
*1/*1	58	70.7	80.82–61.18
*1/*2	20	24.3	33.24–14.76
*1/*3	3	3.7	12.24–4.24
*2/*2	1	1.3	1.50– 6.25
*2/*3	0	—	—
*3/*3	0	—	—
Allele *1	139	84.75	91.49–78.51
Allele *2	22	13.41	19.11–6.89
Allele *3	3	1.8	7.31–2.11
*CI=confidence interval for variant allele frequency			

DNA extraction and genotyping

A 2 mL sample of venous blood was obtained from each subject immediately before starting the experiments. DNA was extracted from peripheral blood leucocytes by using the salting out method.[19] A 167 base pair (bp) fragment that contained the CYP2C19*2 mutation was amplified by the sequencing of primers that were: 5'-AATTACAACCAGAGCTTGGC-3' and 5'-ATCACTTTCCATAAAAGCAA G-3'. PCR amplification steps included an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, an annealing step at 55ºC for 30 s, and an extension step at 72°C for 30 s. Eventually, there was a final extension step at 72°C for 5 min. The PCR product was incubated at 25°C for 3 hr with the restriction enzyme SmaI. Electrophoresis was then performed on 3.5% agarose gel, and visualized after ethidium bromide staining. The CYP2C19*1 allele (wild-type) is typically spliced into 118 and 49 bp fragments, whereas the CYP2C19*2 allele is not digested which means that samples containing the CYP2C19*1 and CYP2C19*2 alleles (heterozygous) showed 167, 118, and 49 bp after digestion with SmaI, while samples containing only the CYP2C19*1 allele (homozygous) showed only a 167 bp fragment.

CYP2C19*3 allele was detected using the sense primer 5'-AAATTGTTTCCAATCAT TTAGCT-3' and the antisense primer 5'-ACTTCAGGGCTTG-GTCAATA-3'. The first PCR step was initial denaturation at 94°C for 5 min and was followed by 37 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 30 s, and extension at 72°C for 30 s. There was a final extension at 72°C for 5 min. PCR products were digested with the restriction enzyme BamHI during 4 hr of incubation at 37°C. The digested PCR products were separated by electrophoresis on a 2% agarose gel followed by staining with

Table 2. Descriptive characteristics of patients with reflux esophagitis according to CYP2C19 Phenotype groups

	Homo-EM* (*n*=58) *n* (%)	Hetero-EM† (*n*=23) *n* (%)	PM‡ (*n*=1) *n* (%)
Sex			
Male	28 (48.2%)	8 (34.7%)	1 (100%)
Female	30 (51.8%)	15 (65.3%)	0 (0%)
Age (years)			
Mean ± SD (range)	36±12 (18-66)	42±17 (18–78)	25.7
Los Angeles classification			
Grade A	49 (84.5%)	16 (69.5%)	1 (100%)
Grade B	8 (13.8%)	7 (30.5%)	0 (0%)
Grade C	1 (1.7%)	0 (0%)	0 (0%)
Grade D	0 (0%)	0 (0%)	0 (0%)
Helicobacter pylori			
Negative	18 (31%)	6 (26%)	0 (0%)
Positive	40 (69%)	17 (74%)	1 (100%)
Hiatal hernia			
Absent	31 (53.4%)	11 (47.8%)	1 (100%)
Present	27 (46.6%)	12 (52.2%)	0 (0%)
Symptom score			
Mean±SD	4 ±1.6	5±1.9	3
*Homo-EM=homozygous extensive metabolizer; †Hetero EM=heterozygous extensive metabolizer; ‡PM=poor metabolizer			

Table 3. Estimation of complete clinical response odds ratio in different groups of variables

Variables	Groups	Odds ratio			*P*-value¶
		Estimate	95%CI*		
			Lower	Upper	
Phenotype	†Hetero EM vs. ‡homo-EM	30.36	3.83	240	0.001
Sex	Female vs. male	1.072	0.443	2.593	0.767
Age	<40 vs. ≥40	1.310	0.528	3.246	0.560
Hiatal hernia	Absent vs. present	0.846	0.351	2.040	0.710
H. pylori	Positive vs. negative	1.288	0.493	3.363	0.605
L.A. classification	Grade B vs. grade A	3.838	1.000	14.735	0.040

*CI=confidence interval; †Hetero EM=heterozygous extensive metabolizer; ‡Homo-E.M=homozygous extensive metabolizer; ¶calculated with Chi-square test

ethidium bromide. The CYP2C19*1 (wild-type) allele produced 175 and 96 bp fragments after digestion with BamH1, while those containing the CYP2C19*3 allele were not digested. The samples containing the CYP2C19*1 and CYP2C19*3 alleles (heterozygote) produced 271, 175, and 96 bp fragments after digestion with BamH1.

Statistical analysis

Statistical analysis was performed with SPSS 16. We used the Chi-square test to compare all qualitative variables. The mean severity score and other quantitative variables were compared across two genotype groups with the *t*-test. Multivariate analysis was used when there were multiple confounding variables involved.

Results

CYP2C19 phenotype and genotype frequencies

Fifty-eight (70.8%) out of a total of 82 patients were homo-EMs, 23 (28%) were hetero-EMs and 1 (1.2%) was a PM. The genotype and allele frequencies for the CYP2C19 gene are shown in Table 1.

There were no significant differences in gender, age, presence of hiatal hernia, grade of esophagitis, *H. pylori* infection status, mean of symptoms score, and mean of BMI across the three phenotype groups (Table 2).

Clinical response and phenotype groups

In assessing the response to treatment, because of the low number of patients in the PM group (n=1), we considered only the hetero-EM group versus the homo-EM group. The rate of complete clinical response (symptom free) to treatment with omeprazole was 95% in the hetero-EM group, and was greater than 43% obtained with the homo-EM group. A statistically significant difference was seen using the Chi-square test (*P*=0.001) (Table 3).

Clinical response and grade of esophagitis

None of the patients had the grade D esophagitis and due to the low number of grade C esophagitis cases (n=1), we considered it within the grade B esophagitis group. The grade of esophagitis according to the Los Angeles classification was significantly associated with complete clinical response to treatment with omeprazole, and grade B esophagitis was associated with a higher rate of complete clinical response (*P*=0.040) (Table 3). However, this association was not significant after adjusting for all other factors, which as shown Table 3 (*P*=0.07).

Clinical response and other variables

There were no significant correlations between complete clinical response and the sex, age, *H. pylori* infection status, BMI, and hiatus hernia variables (Table 3).

Endoscopic response

A second endoscopy was performed on 38 randomized selected patients after 4 weeks of treatment with omeprazole. Twenty-two cases were homo-EM and 16 were hetero-EM. Endoscopic response was considered as healing of esophagitis with at least one score change in grading. The results of clinical and endoscopic responses of both groups of cases have been shown in Figure 1. The correlation between endoscopic and complete clinical responses was statistically significant in the hetero-EM group (*P*=0.04; Phi= 0.53).

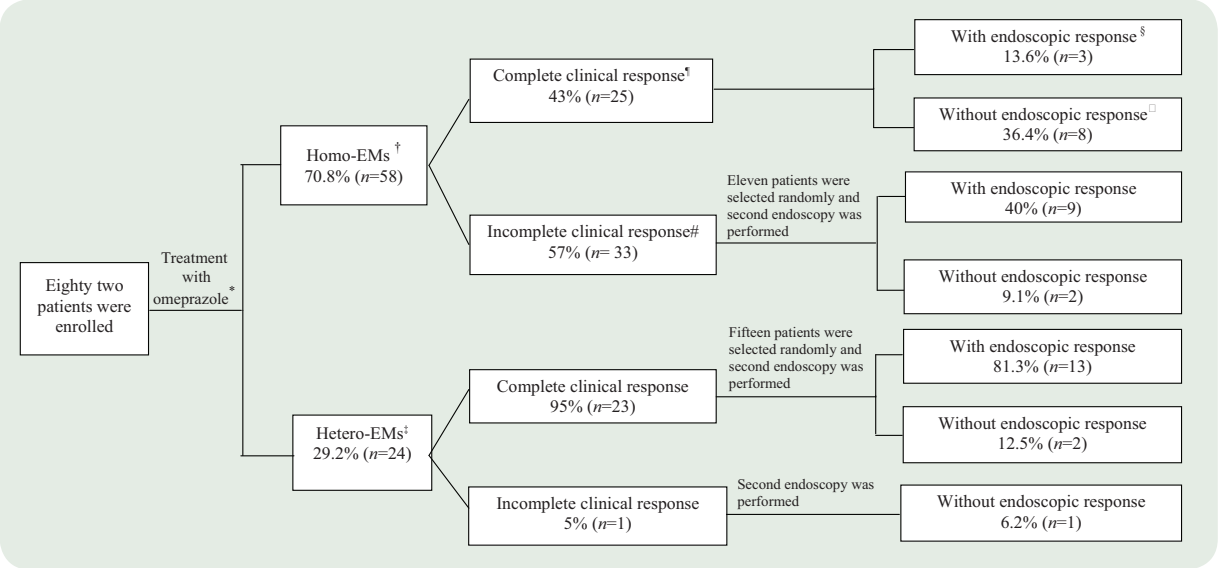

Figure 1. Flow chart of patient's enrollment and evaluation of clinical and endoscopical responses. *treatment with omeprazole 20 mg/bd for 4 weeks, †homo-EMs, ‡includes hetero-E.Ms [26.8% (n=22)] and PM [2.4% (n=2)], ¶complete clinical response regarded as symptom-free, #incomplete clinical response regarding as not symptom-free, §with endoscopic response regarded as healing of esophagitis with at least one score change in grading, □ without endoscopic response regarded as no change in esophagitis grading

Discussion

The omeprazole drug regimen for treatment of GERD is 20 mg daily or 20 mg twice/day. In most patients with erosive reflux esophagitis, once-daily dosing is sufficient to produce the desired level of acid inhibition, and a second dose, which is occasionally necessary, should be administered before the evening meal.[20] In this study, we showed that individual differences in CYP2C19 genotype status between groups influence the complete clinical response rates in patients with erosive reflux esophagitis treated with omeprazole at 40 mg daily for 4 weeks. In addition the complete clinical response rate in the homo-EM group was significantly less than in the hetero-EM group. In previous studies, it was shown that CYP2C19 genotype status can affect gastric acid secretion, but it does not affect the clinical symptomatic cure rate.[16] We showed that in the hetero-EM group, the majority of patients (95%) with erosive reflux esophagitis exhibited a complete clinical response to treatment, but in the homo-EM group only 50% of the patients exhibited a complete clinical response. Therefore, it seems that the dosage of omeprazole is insufficient in 50% of the homo-EM cases. This may also indicate that there are other unknown mutations in the CYP2C19 gene in Iranian patients that reduces CYP2C19 enzyme activity. It has been shown that CYP2C19*2 is the most common allele in the Caucasian population. In contrast, the CYP2C19*3 allele is very rare or totally absent in the Caucasian population, while it is common in Asians.[21] The frequency of the CYP2C19*2/*2 genotype is also low in the Caucasian population, but is more common in the Asian population.[22–25] Previous studies in the normal Iranian population have shown similarities to the Caucasian population in this regard.[15] We showed that the CYP2C19 allele distribution in Iranian patients with reflux esophagitis was similar to that of the normal Iranian population, except that the CYP2C19*3 allele frequency was not zero. In other countries, a mutant CYP2C19 allele (CYP2C19*17) has been detected with a mutation in the promoter region of the CYP2C19 gene. This allele increases CYP2C19 enzyme activity and leads to an incomplete clinical response in the homo-EM group.[26] As such, more studies should be performed for the assessment of other unknown mutations in the CYP2C19 gene.

Our study showed a difference between grade A and grade B esophagitis with regard to the achievement of a complete clinical response, but multivariate analysis showed that it was not statistically significant. The complete clinical response rate in grade B esophagitis was higher than that of grade A esophagitis. This re-

sult was similar to the complete clinical response rate of patients with grade B esophagitis who had severe symptoms at the beginning of treatment. These results are in accord with those of Aanen et al.,[27] who showed that patients with reflux symptoms and pathologic reflux had a better response to PPIs than patients with no evidence of reflux disease.

By comparing the rate of complete clinical and endoscopic responses separately in the homo-EM and hetero-EM groups, we found that in the hetero-EM group, complete clinical and endoscopic responses were univocal, and an endoscopic response was associated with a complete clinical response. Several studies have shown that CYP2C19*2,*3 polymorphisms can influence drug metabolism and increase therapeutic effects.[28] However, it seems that the optimal doses of omeprazole for the treatment of symptoms and healing of esophagitis may differ from one another, thus this leads to the incomplete healing of symptoms and lesions of esophagitis in the homo-EM group, though in the hetero-EM group the two responses occur together. However additional studies with larger sample sizes are needed to statistically enrich this result.

In conclusion, the results of this study showed that CYP2C19 polymorphism influences the therapeutic efficacy of omeprazole in the treatment of Iranian patients with erosive reflux esophagitis. It indicates that the clinical response and endoscopic healing of esophagitis are both affected by CYP2C19 genotype condition. Additional studies with a larger sample size must be performed in Iranian patients with erosive reflux esophagitis to determine the optimal dosage of omeprazole required for effective treatment, especially in the homo-EM group. In addition, by performing a clinical trial with two different doses of omeprazole with two groups of homo-EM patients affected by erosive reflux esophagitis, the optimal dosage of omeprazole required for this phenotype group can be determined.

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References

1. Malekzadeh R, Nasseri-Moghaddam S, Sotoudeh M. Gstroesophageal reflux disease: the new epidemic. *Arch Iran Med.* 2003; **6:** 127 – 140.
2. Nouraie M, Radmard AR, Zaer-Rezaii H, Razjouyan H, Nasseri-Moghaddam S, Malekzadeh R. Hygiene could affect GERD prevalence independently: a population-based study in Tehran. *Am J Gastroenterol.* 2007; **102:** 1353 – 1360.
3. Iwakiri K, Kawami N, Sano H, Tanaka Y, Umezawa M, Kotoyori M, et al. Mechanisms of excessive esophageal acid exposure in patients with reflux esophagitis. *Dig Dis Sci*. 2009; **54:** 1686 – 1692.
4. Kulig M, Leodolter A, Vieth M, Schulte E, Jaspersen D, Labenz J, et al. Quality of life in relation to symptoms in patients with gastro-oesophageal reflux disease--an analysis based on the ProGERD initiative. *Aliment Pharmacol Ther.* 2003; **18:** 767 – 776.
5. Mokrowiecka A, Jurek K, Pinkowski D, Malecka-Panas E. The comparison of Health-Related Quality of Life (HRQL) in patients with GERD, peptic ulcer disease, and ulcerative colitis. *Adv Med Sci.* 2006; **51:** 142 – 147.
6. Boeckxstaens GE. Review article: the pathophysiology of gastro-oesophageal reflux disease. *Aliment Pharmacol Ther.* 2007; **26:** 149 – 160.
7. Castell DO, Murray JA, Tutuian R, Orlando RC, Arnold R. Review article: the pathophysiology of gastro-oesophageal reflux disease-oesophageal manifestations. *Aliment Pharmacol Ther.* 2004; **20 (suppl 9):** 14 – 25.
8. Sachs G, Shin JM, Howden CW. Review article: the clinical pharmacology of proton pump inhibitors. *Aliment Pharmacol Ther.* 2006; **23 (suppl 2):** 2 – 8.
9. Shi S, Klotz U. Proton pump inhibitors: an update of their clinical use and pharmacokinetics. *Eur J Clin Pharmacol.* 2008; **64:** 935 – 951.
10. Chaudhry AS, Kochhar R, Kohli KK. Genetic polymorphism of CYP2C19 and therapeutic response to proton pump inhibitors. *Indian J Med Res.*2008 ; **127:** 521 – 530.
11. Zanger UM, Turpeinen M, Klein K, Schwab M. Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. *Anal Bioanal Chem.* 2008; **392:** 1093 – 1108.
12. Klotz U, SchwAB, M, Treiber G. CYP2C19 polymorphism and proton pump inhibitors. *Basic Clin Pharmacol Toxicol.* 2004; **95:** 2 – 8.
13. De Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of *S*-mephenytoin metabolism in humans. *J Biol Chem.* 1994b; **269:** 15419 – 15422.

14. De Morais SM, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, Goldstein JA. Identification of a new genetic defect responsible for the polymorphism of (*S*)-mephenytoin metabolism in Japanese. *Mol Pharmacol.* 1994a; **46:** 594 – 598.
15. Zand N, Tajik N, Moghaddam AS, Milanian I. Genetic polymorphisms of cytochrome P450 enzymes 2C9 and 2C19 in a healthy Iranian population. *Clin Exp Pharmacol Physiol.* 2007; **34:** 102 – 105.
16. Egan LJ, Myhre GM, Mays DC, Dierkhising RA, Kammer PP, Murray JA. CYP2C19 pharmacogenetics in the clinical use of proton-pump inhibitors for gastro-oesophageal reflux disease: variant alleles predict gastric acid suppression, but not oesophageal acid exposure or reflux symptoms. *Aliment Pharmacol Ther.* 2003; **17:** 1521 – 1528.
17. Ohkusa T, Maekawa, T, Arakawa T, Nakajima M, Fujimoto K, Hoshino E, et al. Effect of CYP2C19 polymorphism on the safety and efficacy of omeprazole in Japanese patients with recurrent reflux oesophagitis. *Aliment Pharmacol Ther.*2005; **21:** 1331 – 1339.
18. Kusano M, Ino K, Yamada T, Kawamura O, Toki M, Ohwada T, et al. Interobserver and intraobserver variation in endoscopic assessment of GERD using the "Los Angeles" classification. *Gastrointest Endosc*. 1999; **49:** 700 – 704.
19. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988; **16:** 1215.
20. Wolfe MM, Sachs G. Acid suppression: optimizing therapy for gastroduodenal ulcer healing, gastroesophageal reflux disease, and stress-related erosive syndrome. *Gastroenterology*. 2000; **118:** S9 – S31.
21. Brosen K, de Morais SMF, Meyer UA, Goldstein JA. A multifamily study on the relationship between CYP2C19 genotype and S-mephenytoin oxidation phenotype. *Pharmacogenetics*. 1995; **5:** 312 – 317.
22. Bathum L, Skjelbo E, Mutabingwa TK, Madsen H, Horder M, Brosen K. Phenotypes and genotypes for CYP2D6 and CYP2C19 in a black Tanzanian population. *Br J Clin Pharmacol.* 1999, **48:** 395 – 401.
23. Goldstein JA, Ishizaki T, Chiba K, De Morais SM, Bell D, Krahn PM, et al. Frequencies of the defective CYP2C19 alleles responsible for the mephenytoin poor metabolizer phenotype in various Oriental, Caucasian, Saudi Arabian and American black populations. *Pharmacogenetics.* 1997; **7:** 59 – 64.
24. Ozawa S, Soyama A, Saeki M, Fukushima-Uesaka H, Itoda M, Koyano S, et al. Ethnic differences in genetic polymorphisms of CYP2D6, CYP2C19, CYP3As, and MDR1/ABCB1. *Drug Metab Pharmacokinet.* 2004; **19:** 83 – 95.
25. Tassaneeyakul W, Tawalee A, Tassaneeyakul W, Kukongviriyapan V, Blaisdell J, Goldstein JA, et al. Analysis of the CYP2C19 polymorphism in a northeastern Thai population. *Pharmacogenetics.* 2002, **12:** 221 – 225.
26. Baldwin RM, Ohlsson S, Pedersen RS, Mwinyi J, Ingelman-Sundberg M, Eliasson E, et al. Increased omeprazole metabolism in carriers of the CYP2C19*17 allele; a pharmacokinetic study in healthy volunteers. *Br J Clin Pharmacol.* 2008; **65:** 767 – 774.
27. Aanen MC, Weusten BL, Numans ME, De Wit NJ, Samsom M, Smout A J. Effect of proton-pump inhibitor treatment on symptoms and quality of life in GERD patients depends on the symptom-reflux association. *J Clin Gastroenterol.* 2008; **42:** 441 – 447.
28. Uno T, Niioka T, Hayakari M, Yasui-Furukori N, Sugawara K, Tateishi T. Absolute bioavailability and metabolism of omeprazole in relation to CYP2C19 genotypes following single intravenous and oral administrations. *Eur J Clin Pharmacol.* 2007; **63:** 143 – 149.