

## Influence of *CYP2C19* on *Helicobacter pylori* eradication in Brazilian patients with functional dyspepsia

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**ABSTRACT.** The aim of this study was to examine the effect of polymorphisms in the cytochrome P450 (CYP) 2C19 gene (*CYP2C19*) on the *Helicobacter pylori* eradication rate in Brazilian patients with functional dyspepsia. Adults diagnosed with functional dyspepsia based on the ROME III criteria and infected with *H. pylori* were recruited to this study. The patients were subjected to gastrointestinal endoscopy and the *H. pylori* status was defined when both urease test and histopathology results were negative or positive. The patients were treated with proton pump inhibitor-based triple therapy (omeprazole,

amoxicillin, and clarithromycin). *CYP2C19\*2* and *CYP2C19\*3* were genotyped by polymerase chain reaction-restriction fragment length polymorphism. One hundred and forty-eight patients (81.8% women) with a mean ( $\pm$  SD) age of 46.1 (12.2) years were included in this study. Based on the *CYP2C19* genotypes, the patients were classified as homozygous extensive metabolizer (HomEM; 67.6%), heterozygous extensive metabolizer (HetEM; 26.3%), or poor metabolizer (PM; 6.1%). The *H. pylori* eradication rates in patients with HomEM, HetEM, and PM were 85.0, 89.7, and 100.0% ( $P=0.376$ ), respectively. The included study population comprised a high frequency of patients carrying the HomEM genotype. Although the genotypes of *CYP2C19* variants were not statistically significant, the results of this study suggest a possible effect of the PM genotype on the efficacy of *H. pylori* eradication.

**Key words:** Cytochrome P450 CYP2C19; *Helicobacter pylori* eradication; Functional dyspepsia; Poor metabolizer genotype

## INTRODUCTION

*Helicobacter pylori* infection is associated with chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma (Wotherspoon, 1998; Marshall and Windsor, 2005; McColl, 2010). The prevalence of *H. pylori* infection in patients with functional dyspepsia (FD) has been reported to be in the range of 40 to 70% (Talley, 1994; Egan et al., 2007). The recommended treatment method in such patients is the eradication of *H. pylori*, which could relieve the symptoms of FD and help prevent other gastric complications (Mazzoleni et al., 2011; Malfertheiner et al., 2012). Some studies have reported a lower *H. pylori* eradication rate in patients with FD than that in patients with other gastric disorders such as peptic ulcer disease (Jones and Lydeard, 1989; Jones et al., 1990).

Dyspepsia is a common complaint in gastroenterology practice, with a prevalence rate of ~30% (Tack et al., 2006). Most affected individuals do not have structural or biochemical abnormalities that can explain their symptoms and are, therefore, diagnosed with functional or non-ulcer dyspepsia (Fuccio et al., 2008). The symptoms have a major impact on the quality of life of FD patients, often prompting them to seek medical advice, which represents ~3-4% of all general practitioner consultations (Tack et al., 2006). Dyspepsia is also the most common source of referrals to gastroenterologists, imposing a huge economic burden on the individual and budgets of many countries through decreased work productivity and missed workdays (Sander et al., 2011; Matsuhisa and Tsukui, 2014).

The most common therapeutic regimens for the eradication of *H. pylori* include the concomitant use of proton pump inhibitors (PPIs) and antibiotic agents, resulting in eradication rates of 80 to 90% (Tang et al., 2013). PPIs are mostly metabolized by the cytochrome P450 (CYP) enzyme system, particularly the CYP2C19 enzyme (Ishizaki and Horai, 1999). The *CYP2C19* gene is highly polymorphic, with several functional polymorphisms or alleles that can influence the pharmacokinetics and pharmacodynamics of PPIs (Furuta et al., 2005). The wild-type allele, *CYP2C19\*1*, translates into an enzyme with high activity. *CYP2C19\*2* and *CYP2C19\*3*, however, are the most commonly reported alleles and may be involved in inter-individual variability of drug metabolism (Hunfeldt et al., 2010).

*CYP2C19*\*2 (rs4244285) is a defective allele that carries a single nucleotide polymorphism (681G→A) in exon 5, resulting in an aberrant splice site. *CYP2C19*\*3 (rs4986893) undergoes a G-to-A transition (636G→A) in exon 4, leading to a premature stop codon. These mutations encode a non-functional protein, dramatically decreasing drug metabolism (De Morais et al., 1994a,b; Hirota et al., 2013). The *CYP2C19* phenotype can be categorized as extensive metabolizer (EM) or poor metabolizer (PM) (Ishizaki and Horai, 1999). The homozygous EM (HomEM) has two wild-type alleles (\*1/\*1) that have a functional enzyme, thereby accelerating the PPI metabolism. The heterozygous EM (HetEM) genotype carries one wild-type allele and one loss-of-function variant allele (\*1/\*2 or \*1/\*3), translating into an enzyme with intermediate functional capacity. The PM genotype comprises two loss-of-function variant alleles (\*2/\*2, \*2/\*3, or \*3/\*3) (Padol et al., 2006); therefore, individuals with the PM genotypes display a much slower PPI metabolism rate. This leads to higher PPI bioavailability (approximately 13 higher than that seen in HomEM individuals) (Furuta et al., 2005).

The aim of this study was to examine the prevalence of the *CYP2C19* variant genotypes and their influence on the *H. pylori* eradication rate in patients with FD treated with clarithromycin, amoxicillin, and omeprazole for 10 days.

## MATERIAL AND METHODS

### Patients

Patients with FD were selected for this study from the sample population included in the *Helicobacter* Eradication Relief of Dyspeptic Symptoms Trial (HEROES Trial, ClinicalTrials.gov No. NCT00404534). The HEROES Trial is a randomized double-blind, placebo-controlled clinical trial carried out at Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil (Mazzoleni et al., 2011). Briefly, community and primary care patients of either gender who were 18 years of age or older diagnosed with FD according to the Roma III criteria (Fuccio et al., 2008), and who tested positive for *H. pylori* by histological and biochemical diagnostic tests were enrolled in the trial. Patients who had used antibiotics or bismuth 4 weeks before enrollment, PPIs 2 weeks before enrollment, or those treated with histamine-2 receptor blockers in the week before enrollment were excluded. The complete exclusion criteria have been described in detail elsewhere (Mazzoleni et al., 2011).

Upper gastrointestinal endoscopies were performed at screening and at least 12 months after screening. Three biopsy specimens were obtained from the body of the stomach, three from the antrum, and two from the incisura angularis. The *H. pylori* status was defined when the results of both the urease test and histopathological examination were negative or positive for infection (stains: Giemsa and hematoxylin-eosin). A third pathologist was consulted in case of disagreement between the primary pathologists.

Eligible patients enrolled in the HEROES Trial were randomly assigned to receive the omeprazole (20 mg twice daily), amoxicillin (1000 mg twice daily), and clarithromycin (500 mg twice daily) (Omepramix®; Aché Laboratórios Farmacêuticos SA, São Paulo, SP, Brazil) treatment for 10 days (antibiotics group) or omeprazole (20 mg twice daily) and placebo antibiotic (control group) treatment. Adherence to study medication was assessed by the pill count of returned medication. Patients were considered to be adherent if at least 80% of the prescribed medications for *H. pylori* eradication were consumed.

In this study, we examined the prevalence of *CYP2C19* variants and their role in *H. pylori* eradication rate in patients belonging to the antibiotics group. Patients who agreed to participate in genetic studies were included in this study. DNA was extracted from the stored blood samples for subsequent analysis of *CYP2C19* polymorphisms. The study protocol was approved by the local Institutional Review Board and informed consent was obtained from all patients prior to enrollment.

## Genetic analysis

Genomic DNA was extracted from blood samples using a salting-out method (Lahiri and Nurnberger, 1991). The variant genotypes of the *CYP2C19*\*2 (681G→A; rs4244285) and *CYP2C19*\*3 (636G→A; rs4986893) polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), using previously described primers and conditions (De Morais et al., 1994a,b; Kubota et al., 1996). The amplified 168-bp (for *CYP2C19*\*2) and 119-bp (for *CYP2C19*\*3) PCR products were digested with *MspI* and *BamHI* restriction endonucleases, respectively, and subsequently separated and analyzed in 10% polyacrylamide gels stained with silver nitrate.

Patients were classified according to their *CYP2C19* genotypes, as follows: HomEM, homozygous for the wild-type allele (*CYP2C19*\*1/*CYP2C19*\*1); HetEM, heterozygous for *CYP2C19*\*2 or *CYP2C19*\*3 (*CYP2C19*\*1/*CYP2C19*\*2 or *CYP2C19*\*1/*CYP2C19*\*3); and PM, homozygous for *CYP2C19*\*2 or *CYP2C19*\*3 or compound heterozygous (*CYP2C19*\*2/*CYP2C19*\*2 or *CYP2C19*\*3/*CYP2C19*\*3 or *CYP2C19*\*2/*CYP2C19*\*3).

## Statistical analysis

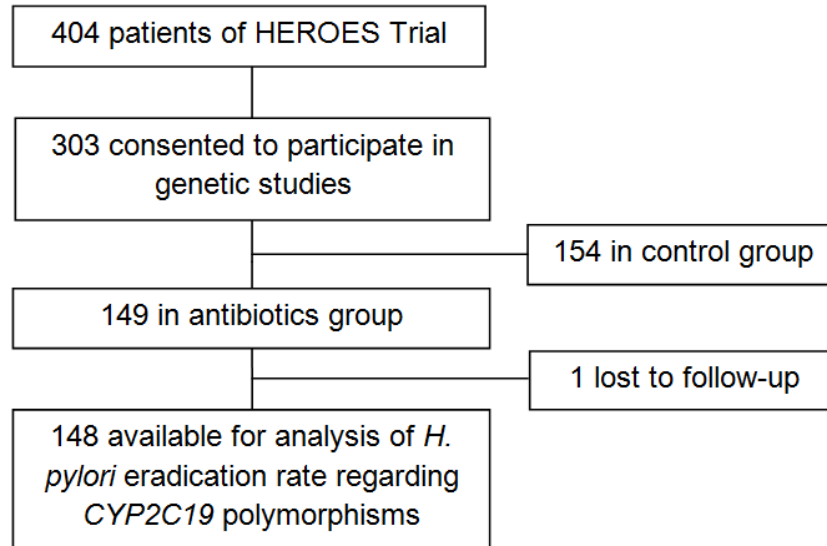
Allele frequencies were determined by direct count of alleles, and deviations from Hardy-Weinberg equilibrium were evaluated by the chi-square test. Associations between categorical variables were tested using the chi-square or Fisher exact tests. Continuous variables were analyzed by the Student *t*-test or Mann-Whitney *U* test. Data analysis was conducted using SPSS v.18.0 (SPSS Inc., Chicago, IL, USA). A two-tailed *P* value <0.05 was considered statistically significant.

## RESULTS

A total of 404 patients were recruited for the intention-to-treat analysis in the HEROES Trial. Of these, 303 patients consented to participate in genetic studies (154 in the control group and 149 in the antibiotics group). One patient in the antibiotics group was lost to follow-up (Figure 1) because of missing data.

In this study, we analyzed 148 *H. pylori*-positive patients with FD (121 women, 81.8%; 27 men, 19.2%) with a mean (SD) age of 46.1 (12.2) years. All patients completed the study; the adherence to study medication was 94.6%. The baseline characteristics of the included patients are shown in Table 1.

Of the 148 patients, 100 (67.6%) had the HomEM genotype, 39 (26.3%) had the HetEM genotype, and 9 (6.1%) had the PM genotype. The overall *H. pylori*-eradication rate was 129/148 (87.2%). The *H. pylori*-eradication rates in the HomEM, HetEM, and PM groups were 85.0, 89.7, and 100.0%, respectively (*P*=0.376) (Table 2).



**Figure 1.** Flow chart depicting study participants.

**Table 1.** Baseline characteristics of patients with functional dyspepsia (N = 148).

Variable	N (%)
Age (mean ± SD, years)	46.1 ± 12.2
Female	121 (81.8)
Caucasian ethnicity	117 (79.1)
Education	
≤10 years	89 (60.2)
>10 years	59 (39.8)
Smoking status	
Never smoked	85 (57.4)
Former	36 (24.3)
Current	27 (18.2)
Alcohol consumption	
No consumption	127 (85.8)
Former	4 (2.7)
Current	17 (11.5)
Coffee drinker	98 (66.2)

**Table 2.** Genotype frequencies of patients stratified by *Helicobacter pylori* status post-treatment.

Genotype <sup>1</sup>	Total (N = 148)	<i>H. pylori</i> -negative (N = 129)	<i>H. pylori</i> -positive (N = 19)	P
HomEM	100 (67.6)	85 (85.0)	15 (15.0)	0.376
HetEM	39 (26.3)	35 (89.7)	4 (10.3)	
PM	9 (6.1)	9 (100.0)	-	

<sup>1</sup>HomEM = homozygous extensive metabolizer; HetEM = heterozygous extensive metabolizer; PM = homozygous poor metabolizer.

## DISCUSSION

In this study, we analyzed patients with FD to investigate the influence of the *CYP2C19* genotypes on *H. pylori* eradication rate. We observed a high frequency of the HomEM genotype (followed by HetEM) and a low frequency of the PM genotype. *H. pylori* was eradicated in all PM patients, and the success rate of *H. pylori* treatment was slightly higher in patients with HetEM than that in patients with the HomEM genotype. However, the differences in eradication rates between *CYP2C19* genotypes were not statistically significant.

A major strength of this study is that the study population was a well-selected group of patients with FD (diagnosed according to the ROME III criteria) who were positive for *H. pylori* infection. All patients underwent upper gastrointestinal endoscopy with gastric and duodenal biopsies, and only those who tested positive for *H. pylori* were recruited to the trial. The infection status was rigorously assessed by two pathologists based on the results of the urease test and histopathological examinations; a third pathologist was consulted in case of disagreement. All patients received daily doses of clarithromycin, amoxicillin, and omeprazole (twice each) for 10 days. This treatment period, longer than the usual 7-day treatment period, was chosen because *H. pylori* eradication is less effective in patients with FD than that in patients with other gastric disorders. Additional strengths of this study were that only one patient was not followed-up and high proportion of adherence to study medication was achieved for all participants.

*CYP2C19* is an enzyme involved in the metabolism of several clinically used drugs, including some barbiturates, diazepam, proguanil, propranolol, and clopidogrel (Hirota et al., 2013; Liboredo and Pena, 2014). This enzyme is also responsible for the *in vivo* metabolism of most PPIs, such as omeprazole, lansoprazole, and pantoprazole (Ishizaki and Horai, 1999; Furuta et al., 2005). Genetic polymorphisms in *CYP2C19* are associated with increased clinical efficacy of PPI-based triple therapies for *H. pylori* eradication, and they could also be of clinical concern in the treatment of acid-related diseases with PPIs (Ishizaki and Horai, 1999; Zhao et al., 2008). It is known that the rate of PPI clearance (from the circulation) in individuals expressing the PM genotype is slower than that in individuals expressing the HomEM or HetEM genotypes (Zhao et al., 2008; Lee et al., 2014). Studies on the pharmacogenetics, pharmacodynamics, and pharmacokinetics of PPIs have shown that the plasma omeprazole concentrations in patients receiving a single dose of omeprazole differed between those carrying the three different genotype of *CYP2C19*. Plasma omeprazole levels in individuals with the PM genotype are sustained long after drug administration. Furthermore, the mean values of the areas under the plasma omeprazole concentration-time curves in the PM group are ~13 times higher than those in the HomEM group (Furuta et al., 2005).

The *CYP2C19* genotype frequencies observed in our study are consistent with those of previous studies in Brazilian populations (Suarez-Kurtz et al., 2012). A recent study conducted in Southern Brazil (at the same geographic location sampled in this study) reported HomEM, HetEM, and PM genotype frequencies of 71.5, 26.3, and 2.2%, respectively (Kohlrausch et al., 2014). Other studies conducted in western populations have also reported similar *CYP2C19* genotype distributions (Ishizaki and Horai, 1999; Kurose et al., 2012; Nastasi-Catanese et al., 2013). However, a significantly different distribution was reported in Asian populations, where the frequency of the PM genotype was ~23% higher than that in western populations (approximately 5%) (Ishizaki and Horai, 1999; Hirota et al., 2013).

Studies evaluating *CYP2C19* variants and *H. pylori* eradication in omeprazole-based

regimens have shown conflicting results. While some studies have reported a significant association between *H. pylori* cure rates and *CYP2C19* genotypes (Sheu et al., 2005; Sugimoto et al., 2006; Chaudhry et al., 2009), others have been unable to statistically confirm the association between the *CYP2C19* genotypes and successful *H. pylori* eradication; however, the latter studies have reported a high *H. pylori* eradication rate in individuals carrying the PM genotype, compared to those carrying the EM genotype (Inaba et al., 2002; Sapone et al., 2003; Zhang et al., 2010). These results are similar to the results seen in our sample population; that is, bacteria were eradicated in all (100%) individuals carrying the PM genotype, although statistical significance was not achieved. It is worth noting that several meta-analyses have reported a significant association between the *CYP2C19* genotypes and *H. pylori* eradication rates (Padol et al., 2006; Zhao et al., 2008; Tang et al., 2013).

PPI, clarithromycin, and amoxicillin or metronidazole triple therapy has been the universally recommended treatment method for *H. pylori*-infection, since its proposal at the First Maastricht conference (Malfertheiner et al., 1997). Recent studies have indicated the decreased efficacy of this combination over the years, resulting *H. pylori*-eradication rates of <70% (Malfertheiner et al., 2012). The reduced efficacy of standard triple therapy has been attributed to antibiotic resistance (Furuta et al., 2005; Lee et al., 2014). Recent years have seen the emergence of a few amoxicillin-resistant *H. pylori* strains; however, the major limiting factor of PPI-amoxicillin-clarithromycin triple therapy is the resistance of recent *H. pylori* strains to clarithromycin (Furuta et al., 2005). *H. pylori* shows varied antibiotic susceptibility patterns in various geographical locations; moreover, this bacterium has been substantially influenced by the prior use of these drugs (Han et al., 1999). Previous studies conducted in Brazil have reported varying rates of *H. pylori* resistance to clarithromycin, ranging from 11.1 to 17.3% (Prazeres Magalhães et al., 2002; Eisig et al., 2011; Picoli et al., 2014). In this study, we could not show an association between the *CYP2C19* genotype and efficacy of *H. pylori* eradication; however, *H. pylori* eradication was observed in a large proportion of patients (87.2%). This may be attributed to the low rates of clarithromycin resistance in *H. pylori* strains in Brazil, as well as the high patient compliance and prolonged treatment time of this study.

The results of this study are subject to certain limitations. The statistical power to detect differences was limited by the small sample size and low frequency of individuals carrying the PM genotype. Additionally, the *CYP2C19* gene shows great variability, with more than 30 alleles being identified, some of which have been classified as ultrarapid metabolizers (*CYP2C19\*17*). This increases the complexity of genotype-phenotype studies investigating the *CYP2C19* gene. In this study, the most commonly reported *CYP2C19* variants were analyzed.

In conclusion, no association was found between the *CYP2C19* genotype variants and *H. pylori* eradication rates. However, the results suggest a possible role of the PM genotype in determining the efficacy of *H. pylori* eradication. Therefore, we suggest that the dosage of PPI (in triple therapy) could be increased in populations with a high frequency of individuals carrying the EM genotype.

### Conflicts of interest

The authors declare no conflict of interest.

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