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# CYP2C19 Polymorphism in Patients with Gastroesophageal Reflux Disease – a Pilot Study

## Polimorfizm CYP2C19 u pacjentów z chorobą refluksową przełyku – badanie wstępne

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## Abstract

**Background.** Gastroesophageal reflux disease (GERD) is a disease of modern civilization whose symptoms occur in 5–10% of individuals living in Western countries. Proton pump inhibitors (PPIs) are the basis of the medical treatment of GERD. PPIs are metabolized with the system of enzymes of cytochrome P450. Polymorphism of the isoenzymes comprising this system determines the different speeds of the metabolism of the drugs.
**Objectives.** The aim of the study was the analysis of *CYP2C19* polymorphism in GERD patients in relation to the presence of GERD complications.
**Material and Methods.** The study group consisted of 40 patients hospitalized in the Department of Gastroenterology and Hepatology of Wroclaw Medical University with a diagnosis of GERD and 17 patients treated surgically in the Clinic of Gastrointestinal and General Surgery of Wroclaw Medical University due to GERD complications. Two SNP-type polymorphisms in gene *CYP2C19* were studied with the PCR-RFLP (polymerase chain reaction, restriction fragment length polymorphism) method.
**Results.** Most of the patients were found to belong to the phenotype of extensive metabolizers (EM). Genotypes and frequency of the alleles of polymorphism 681G→A in gene *CYP2C19* in GERD patient groups with and without complications are shown in table 2.
**Conclusions.** Most of the patients with uncomplicated GERD belong to the phenotype of extensive metabolizers (EM). Our results do not suggest that the polymorphism of gene *CYP2C19* plays a role in the development of severe GERD complications (**Adv Clin Exp Med 2011, 20, 1, 65–69**).

**Key words:** *CYP2C19*, gastroesophageal reflux disease, proton pump inhibitors.

## Streszczenie

**Wprowadzenie.** Choroba refluksowa przełyku jest chorobą cywilizacyjną, której objawy występują u 5–10% mieszkańców krajów zachodnich. Podstawą terapii zachowawczej GERD są inhibitory pompy protonowej (i.p.p.). W metabolizmie tych leków bierze udział system enzymów cytochromu P-450. Polimorfizm izoenzymów wchodzących w skład systemu warunkuje różną szybkość metabolizmu leków.
**Cel pracy.** Analiza polimorfizmu genu *CYP2C19* u pacjentów z GERD.
**Materiał i metody.** Grupę badaną tworzyło 40 pacjentów hospitalizowanych w Klinice Gastroenterologii i Hepatologii AM we Wrocławiu z rozpoznaniem GERD oraz 17 pacjentów leczonych chirurgicznie w Klinice Chirurgii Przewodu Pokarmowego i Chirurgii Ogólnej AM we Wrocławiu z powodu powikłań GERD. Do badania dwóch polimorfizmów typu SNP w genie *CYP2C19* wykorzystano metodę PCR-RFLP.
**Wyniki.** Większość pacjentów z niepowikłaną chorobą refluksową przełyku należy do grupy osób szybko metabolizujących. Rozkład genotypów i częstości alleli polimorfizmu 681G→A w genie *CYP2C19* w grupie pacjentów z GERD bez powikłań i u pacjentów leczonych chirurgicznie z powodu powikłań GERD przedstawiono w tabeli 2.
**Wnioski.** Większość pacjentów z niepowikłaną chorobą refluksową przełyku należy do osób szybko metabolizujących. Uzyskane wyniki nie przemawiają za udziałem polimorfizmu genu *CYP2C19* w rozwoju ciężkich powikłań choroby (**Adv Clin Exp Med 2011, 20, 1, 65–69**).

**Słowa kluczowe:** *CYP2C19*, choroba refluksowa przełyku, inhibitory pompy protonowej.

Gastroesophageal reflux disease (GERD) is a disease of modern civilization whose symptoms occur in 5–10% of individuals living in Western countries. Proton pump inhibitors ((PPIs) e.g. omeprazole, esomeprazole, lansoprazole, pantoprazole, rabeprazole) are the basis of the medical treatment of GERD and some of its complications (e.g. esophagitis, Barrett esophagus). It should be highlighted that no other medical GERD treatments that are safe and effective are currently available. Alternative treatment of GERD is surgical intervention. However, surgical treatment is limited to the small group of patients with uncomplicated GERD who fulfill special criteria and to patients with severe complications of the disease (e.g. stricture of the esophagus due to esophagitis). Thus, the failure of PPI treatment in GERD patients can be a significant clinical problem. Additionally, PPIs play a significant role in other acid-related diseases of the digestive tract.

PPIs are metabolized with the system of enzymes of cytochrome P-450. Cytochrome P450 enzymes play an important role in the metabolism and elimination of many xenobiotics and drugs. They are responsible for the first phase of the metabolism of about 80% of drugs used in the treatment of many different diseases. CYP enzymes participate in the change of lipophilic substances into polar metabolites, which can be eliminated with urine or with bile. The cytochrome P450 enzyme system in the liver consists of more than 30 isoenzymes [1]. Polymorphic isoenzymes CYP2C9, CYP2C19 and CYP2D6 take part in the metabolism of around 30–40% of drugs [2].

Polymorphism of the coding sequences of enzymes metabolizing drugs determines the synthesis of enzymes with different activity (high, low or total lack of activity). As a consequence, they can be four different phenotypes in population: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizers (UM).

Enzyme CYP2C19 takes part in the metabolism of e.g. proton pump inhibitors, but also some antidepressive, antimalaric and antiepileptic drugs [3–5].

The first defect described in gene CYP2C19, known as allele *2 (CYP2C19*2), is related to single nucleotide polymorphism (SNP) – a change of the single nucleotide G→A in exon 5 (rs4244285). As a result, a shortened protein is produced (with a length of 234 amino acids when normal protein has 246 amino acids), which loses the hem binding region and catalytic activity [6]. The second change, allele *3 (CYP2C19*3), is also determined with the change of the single nucleotide G→A in exon 4 (rs4986893). SNP is located in position 636 in the cDNA sequence and leads to the production of a shortened protein (211 amino acids) [7]. Lack of the hem binding regions and also other region-binding substrates causes a loss of enzymatic activity. Wild-type allele is described as *1. Variants *2 and *3 show big race and individual differentiation (Tab. 1).

Wild-type homozygotes (*1/*1) and heterozygotes (*1/*2) are extensive metabolizers (EM), and mutated homozygotes (*2/*2) are poor metabolizers (PM). The PM phenotype is inherited as an autosomal recessive feature.

Another mutated allele, described as CYP2C19*17 and present in extensive metabolizing individuals, was discovered in 2006. Its frequency in the Swedish population is estimated to be around 18% (in the Chinese population 4%). This allele is related to the increase of activity of enzyme CYP2C19 *in vivo* and has two SNPs (–806C→T and –3403C→T) in flanking region 5` [13]. Individuals with allele *17 have a 30–40% lower concentration of the medication comparing to individuals with normal allele.

Some studies have shown an influence of the genotype on the healing of peptic ulcers and effective treatment of GERD with a positive relation between the number of damaged alleles and treatment effectiveness [8]. Furuta et al. first described

**Table 1.** Distribution of the allelic variants *1, *2 and *3 in different ethnic groups [7]

**Tabela 1.** Rozkład wariantów allelicznych *1, *2 i *3 w różnych grupach etnicznych [7]

| Population (Populacja) | Frequency of allele (%) (Częstość alleli) | | |
|---|---|---|---|
| | allele CYP2C19 *1 | allele CYP2C19*2 | allele CYP2C19*3 |
| Caucasian (Kaukaska) | 87 | 13 | 0 |
| African (Afrykańska) | 75 | 25 | 0 |
| Iranian (Irańska) | 86 | 14 | 0 |
| Saudi Arabian (Arabia Saudyjska) | 85 | 15 | 0 |
| Japanese (Japońska) | 67 | 23 | 10 |
| Filipino (Filipiny) | 54 | 39 | 7 |
| Chinese-Taiwanese (Chiny-Tajwan) | 63 | 32 | 5 |
| Asian – summarized (Azjatycka – suma) | 62 | 32 | 6 |

the influence of genotype CYP2C19 in the healing of peptic ulcers with PPIs [9]. In the majority of cases, *Helicobacter pylori* eradication in EM failed due to the low level of PPIs. A relation between the genotype CYP2C19 and effective treatment of GERD with PPIs was demonstrated [10, 11]. The treatment was less effective in wild-type homozygotes for the following doses of PPIs: omeprazole 20 mg, rabeprazole 10 mg and lansoprazole 30 mg [12]. Adjusting the dose of PPIs in EM homozygotes among the genotype CYP2C19b is being tried, either with an increase of the single daily dose or the number of the doses administered during the day [13].

Our study was designed to analyze CYP2C19 polymorphism in patients with GERD in relation to the presence of GERD complications.

# Material and Methods

## DNA Isolation

Peripheral blood from the patients studied was taken in the presence of an anticoagulant (EDTA) and stored at a temperature of –20°C. DNA was isolated from 200 µl of frozen blood with the High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland).

## Analysis of CYP2C19 Polymorphism Using the PCR-RFLP Method

The PCR-RFLP (polymerase chain reaction, restriction fragment length polymorphism) method was used to study the two SNPs in gene CYP2C19. A QIAGEN® Multiplex PCR Kit (QIAGEN®, Düsseldorf, Germany) and the following starter pairs were used for the amplification of the fragments with polymorphic places:

allele CYP2C19*2 (change 681G→A)
forward: 5`-AATTACAACCAGAGCTTGGC-3`
reverse: 5`-TATCACTTTC-
CATAAAAGCAAG-3`
allele CYP2C19*3 (change 636G→A)
forward: 5`-TATTATTATCTGT-
TAACAAATATGA-3`
reverse: 5`-ACTTCAGGGCTTGGTCAATA-3`

## Statistics

Statistical analysis was performed with a $\chi^2$ test.

## Ethical Considerations

Our study was accepted by the Bioethical Commission of Wroclaw Medical University.

# Results

Two polymorphisms of gene CYP2C19 (681G→A (allele CYP2C19*2) and 636G→A (allele CYP2C19*3)) were studied in 40 patients with uncomplicated GERD and 17 patients with severe GERD complications.

The distribution of the frequency of genotypes in the studied population was in agreement with the Hardy-Weinberg equilibrium. The genotype distribution and allele frequency in the studied groups are presented in table 2. We did not find any polymorphism 681G→A (allele CYP2C19*3) in our study.

There was one person (2.5%) in the group of GERD patients with the homozygous genotype

CYP2C19*2/*2, which determines the phenotype of poor metabolizers (PM). 7 individuals (17.5%) with one mutant allele were heterozygotes CYP2C19*1/*2. The other 80% of the GERD patients studied were wild-type homozygotes CYP2C19*1/*1. Heterozygotes CYP2C19*1/*2 and homozygotes *1/*1 belong to the extensive metabolizers (EM).

**Table 2.** Genotype distribution and frequency of polymorphism of allele 681G→A in gene *CYP2C19* in the studied groups

**Tabela 2.** Rozkład genotypów i częstości alleli polimorfizmu 681G→A w genie *CYP2C19* w grupach badanych

| Genotype/ Allele (Genotyp/allele) | Patients with GERD (n = 40) (Pacjenci z GERD bez powikłań) | | Patients with complicated GERD treated surgically (n = 17) (Pacjenci leczeni chirurgicznie z powodu powikłań GERD) | |
|---|---|---|---|---|
| | number | frequency | number | frequency |
| **GG** (homozygote *1/*1) | 32 | 0.800 | 16 | 0.94 |
| **GA** (heterozygote *1/*2) | 7 | 0.175 | 1 | 0.06 |
| **AA** (homozygote *2/*2) | 1 | 0.025 | 0 | 0.00 |
| **G** (allele *1) | 71 | 0.887 | 33 | 0.97 |
| **A** (allele *1) | 9 | 0.113 | 1 | 0.03 |

Based on the frequency of genotypes, a frequency of alleles was estimated. In uncomplicated GERD patients, the frequencies of wild-type allele (CYP2C19*1) and mutated allele (CYP2C19*2) were 88.7% and 11.3% respectively and in complicated GERD patients, 97% and 3% respectively.

The frequency of mutated alleles differs significantly between both groups. The mutated allele was almost four times more frequent in GERD patients without complications.

## Discussion

The results of our pilot study have shown that, surprisingly, most of the patients with GERD without complications were extensive metabolizers (EM).

As proton pump inhibitors, metabolized with the CYP enzyme, are the key drugs in the medical treatment of GERD, we hypothesized that the failure of PPI treatment in GERD patients and the development of severe GERD complications requiring surgical intervention may be partly explained by CYP2C19 polymorphism. CYP2C19 analysis in PPI resistant GERD patients could be helpful in the elucidation of the background of the problem and could lead to a modification of the medical treatment (e.g. increased dosage) or to a change of the approach to the patients (e.g. consideration of antirefux surgical treatment). According to a review by Klotz, systemic drug exposure varies between phenotypes and genotype-adjusted treatment will improve effectiveness of PPIs [14].

Our results do not support this hypothesis and are contradictory to the results of some other studies addressing similar problems. For instance, Tseng et al., in a study conducted in the Chinese population (n = 178) with a higher prevalence of PM, found out that the diagnostic specificity of the PPJ test in the prediction of erosive esophagitis was higher in EM. Moreover, the possibility of false-positive results of the PPI test for GERD diagnosis was higher in PM [15]. In an earlier study of Lee et al., which was also conducted in the Chinese population, polymorphism of cytochrome CYP2C19 did not influence the results of PPI tests of GERD [16]. Saitoh et al. showed that CYP2C19 polymorphism influenced the recurrence of GERD symptoms during PPI maintenance treatment [17].

Our results can be elucidated in two ways.

First, our study group consisted of hospitalized patients with uncomplicated GERD, which means that they were subjects with atypical GERD symptoms, were resistant to the classic treatment or presented diagnostic difficulties. This could partly explain the high percentage of the EM individuals in this group.

Second, the other limitation of our study is the small number of studied individuals and the fact that we have not analyzed the presence of allele *17, which determines the very fast metabolism of the drug.

Further studies addressing the correlation between CYP polymorphism and its influence on PPI treatment of GERD are needed.

### References


[1] **Van der Wende J, Steijns LS:** Cytochrome P450 enzyme system: genetic polymorphisms and impact on clinical pharmacology. Ann Clin Biochem 1999, 36, 722–729.

[2] **Evans WE, Relling MV:** Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999, 286, 487–491.

[3] **Klotz U, Schwab M, Treiber G:** CYP2C19 polymorphism and proton pump inhibitors. Basic Clin Pharmacol Toxicol 2004, 95, 2–8.

[4] **Desta Z, Zhao X, Shin JG, Flockhart DA:** Clinical significance of the cytochrome P450 CYP2C19 genetic polymorphism. Clin Pharmacokinet 2002, 41, 913–958.

[5] **Kirchheiner J, Nickchen K, Bauer M, Wong ML, Licinio J, Roots I, Brockmoller J:** Pharmacogenetics of antidepressants and antipsychotic: the contribution of allelic variations to the phenotype of drug response. Mol Psychiatry 2004, 9, 442–473.

[6] **de Morais SMF, 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 1994, 269, 15419–15422.

[7] **Zand N, Tajik N, Hoormand M, Moghaddam AS, Milanian I:** Allele frequency of CYP2C19 gene polymorphisms in healthy Iranian population. IJPT 2005, 4, 124–127.

[8] **Furuta T, Shirai N, Sugimoto M, Ohashi K, Ishizaki T:** Pharmacogenomics of proton pump inhibitors. Pharmacogenomics 2004, 5, 181–202.

[9] **Furuta T, Ohashi K, Kamata T, Takashima M, Kosuge K, Kawasami T et al.:** Effect of genetic differences in omeprazole metabolism on cure rates for *Helicobacter pylori* infection and peptic ulcer. Ann Intern Med 1998, 129, 1027–1030.

[10] **Furtura T, Shari N, Watanabe F, Honda S, Takeuchi K, Iida T et al.:** Effect of cytochrome P450 2C19 genetic differences on cure rates for gastroesophageal reflux disease by lansoprazole. Clin Pharmacol Ther 2002, 72, 453–460.

[11] **Kawamura M, Ohara S, Koine T, Iijima K, Suzuki J, Kaaba S et al.:** The effects of lansoprazole on erosie reflux oesophagitis are influence by CYP2C19 polymorphisms. Aliment Pharmacol Ther 2003, 17, 965–973.
[12] **Saitoh T, Fukushima Y, Otsuka H, Hirakawa J, Mori H, Asano T, Ishikawa T, Katsube T, Ogawa K, Ohkawa S:** Effects of rabeprazole, lansoprazole and omeprazole on intragastric pH in CYP2C19 extensive metabolizers. Aliment Pharmacol Ther 2002, 16, 1811–1817.
[13] **Sim SS, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M:** A common novel VYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clin Pharmacol Ther 2006, 79, 103–113.
[14] **Klotz U:** Clinical impact of CYP2C19 polymorphism on the action of proton pump inhibitors: a review of a special problem. Int J Clin Pharmacol Ther 2006, 44, 297–302.
[15] **Tseng PH, Lee YC, Chiu HM, Wang HP, Lin JT, Wu MS:** A comparative study of proton-pump inhibitor tests for Chinese reflux patients in relation to the CYP2C19 genotypes. J Clin Gastroenterol 2009, 43, 920–925.
[16] **Lee YC, Lin JT, Wang HP, Chiu HM, Wu MS**: Influence of cytochrome P450 2C19 genetic polymorphism and dosage of rabeprazole on accuracy of proton-pump inhibitor testing in Chinese patients with gastroesophageal reflux disease. J Gastroenterol Hepatol 2007, 22, 1286–1292.
[17] **Saitoh T, Otsuka H, Kawasaki T, Endo H, Iga D, Tomimatsu M, Fukushima Y, Katsube T, Ogawa K, Otsuka K:** Influences of CYP2C19 polymorphism on recurrence of reflux esophagitis during proton pump inhibitor maintenance therapy. Hepatogastroenterology 2009, 56, 703–706.

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