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Original article

*CYP2C19*2* polymorphism in Polish peptic ulcer patients

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ABSTRACT

Background: CYP2C19 isoenzyme of cytochrome P450 in the liver catabolises proton pump inhibitors, one of the therapeutics utilized in *Helicobacter pylori* eradication therapy, and in this way could influence the eradication effectiveness. The isoensyme contributes also to metabolism of endogenous substances, which derivatives are involved in the pathogenesis of peptic ulceration. *CYP2C19**2 polymorphism (rs4244285) changing the CYP2C19 function could be relevant in the predisposition to peptic ulcer disease.

*Methods: CYP2C19**2 polymorphism in 197 peptic ulcer patients and 107 healthy subjects of Polish origin by PCR-RFLP method was investigated.

Results: There were no statistically significant differences in genotypes and alleles frequencies for investigated polymorphism between peptic ulcer patients and healthy individuals. No associations between frequencies of particular *CYP2C19* genotypes and alleles and the presence of *H. pylori* infection in peptic ulcer patients were stated. However, significant association between *CYP2C19**2 and gender in *H. pylori*-infected but not -uninfected peptic ulcer individuals was found.

Conclusions: Investigated polymorphism is not a risk factor for peptic ulcer in Polish population. Obtained results could suggested there is some interaction between gender, CYP2C19*2 polymorphism, and pathogenesis of *H. pylori* infection development. However, this hypothesis should be verified in the further studies.

Introduction

Helicobacter pylori infection is the main etiological factor of peptic ulcer disease (PUD), chronic gastritis and cancer development. It has been recognized as a class-1 gastric carcinogen. According to national [1] and European [2] recommendation a first-line treatment of the infection containing a proton pump inhibitor (PPI) plus two antibiotics (amoxicillin and either clarithromycin or metronidazole) should be administered to all *H. pylori*-positive symptomatic patients. The effectiveness of *H. pylori* eradication and also antisecretory therapy in patients with peptic ulcer without *H. pylori* infection is strongly connected with metabolism of PPIs.

CYP2C19, which is one of the isoenzymes of cytochrome P450 (CYP) in the liver, has important roles in the catabolism of these drugs [3]. There are strong inter-individual differences in CYP2C19 activity, mainly as a result of genetic polymorphism of *CYP2C19* gene. Two defective *CYP2C19* alleles, *CYP2C19**2 and *CYP2C19**3, cause the majority of poor metabolizer (PM) phenotypes and *CYP2C19**1 as an extensive metabolizers (EMs). Sapone et al. [4] have been reported that genetic polymorphism of *CYP2C19* and also *CYP3A4* influences the efficacy of *H. pylori* eradication therapy with a PPI (omeprazole, pantoprazole, and lansoprazole) and amoxicillin or clarithromycin. A large number of studies suggesting higher cure rates in *CYP2C19**2 and *CYP2C19**3 carriers [5–8]. There is also a *CYP2C19* gene variant (*CYP2C19**17), associated with increased gene transcription, and faster metabolism of CYP2C19 substrates but less clinical data is available on the *CYP2C19**17 allele's cure rate of *H. pylori* eradication [9,10]. Considering the role of CYP2C19 in the catabolism of PPIs it is reasonable to expect that genetic polymorphism impairing the CYP2C19 function could indirectly predispose to PUD.

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It was also stated that CYP2C19 could increase the risk of PUD regardless the drug metabolism [11]. *CYP2C19**17 was found to be connected with PUD independent of usage of PPIs or non-steroidal anti-inflammatory drugs (NSAIDs), another CYP2C19-metabolized medications. Besides various xenobiotics the isoenzyme catalizes some endogenous substances, *i.e.* arachodonic acid which metabolites could be involved in inflammation, proliferation or cellular migration [12]. As suggested Musumba et al. [11] in this way the CYP2C19 may influence peptic ulcer formation.

In the present study we investigated single nucleotide polymorphism *CYP2C19* NM_000769.1:c.681 G > A/C rs4244285 in group of Polish patients with peptic ulcer and in control group of healthy subjects. Comparison of alleles/genotypes distribution between these groups could give us an answer if CYP2C19 681 G > A/C polymorphism could influence the PUD risk. Additionally, we wanted to assess the interaction between the polymorphism and *H. pylori* infection in pathogenesis of peptic ulcers.

Materials and methods

Investigated and control groups

197 patients suffering from peptic ulcers (investigated group), 127 females: median age 52 yrs, min. 14 yrs, max. 85 yrs; 70 males: median age 55 yrs, min. 20 yrs, max. 84 yrs) were enrolled in the study. *Helicobacter* status was stated at the time of gastroduodenoscopy. To all patients with *H. pylori* infection and also to those with clinical symptoms of gastritis recommended treatment was administered. Patients treated with non-steroidal anti-inflammatory drugs were excluded. Control group was 107 healthy individuals, geographically and ethnically matched to the patients, who during interview declared having no symptoms of active gastroduodenal diseases. Data concerning exposure to carcinogens in patients and controls were not available. The investigation was in accordance with the principles of the Declaration of Helsinki and was approved by the Ethical Committee (No: RNN/195/13/KE). All subjects included in the study gave informed consent.

Rapid urease test

Diagnosis of *Helicobacter pylori* infection was performed by rapid urease test (Institute of Food and Nutrition, Poland) at the time of gastroscopy. Mucosa collected during gastroscopy from the antrum of the stomach was placed into medium containing urea and an indicator such as phenol. The test uses the ability of *H. pylori* to secrete urease enzyme which break down urea to ammonium and bicarbonate. Ammonium raises the pH of test`s medium and changes the color of the specimen from yellow (negative) to red (positive).

Genotyping of CYP2C19

Genotyping of *CYP2C19* was performed by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method. Genomic DNA from peripheral blood cells (control group) and biopsy specimens of gastric mucosa (investigation group) was isolated according to "Blood Mini DNA/Genomic Mini DNA" protocol (A&A Biotechnology, Poland). The purity and concentration of DNA samples were assessed spectrophotometrically. Then, until analysis, samples were stored at -20 °C. The mixture for PCR composed of 10 μl of REDTaq® ReadyMix™ PCR Reaction Mix (Sigma Aldrich, Germany), 0.2 μl (0.5 μM) of each primer (primer sequence published earlier [8]), 0.5 μl of DNA template and water to final volume of 20 μl. In each experiment negative control was included (reaction without DNA template). Products of the PCR reactions were electrophoresed in 2% agarose gel. Amplified DNA fragments were digested by SmaI(EURx,Poland) for 16 h at 25 °C. The genotypes were identified by electrophoresis of amplified DNA fragments (300 bp) after restriction enzyme digestion (two bands of 200 and 100 bp for *1/*1; one band of 300 bp for *2 /*2; three bands of 300, 200 and 100 bp for *1/*2).

Statistical analysis

Statistical analysis were performed using the STATISTICA version 10. (StatSoft, Inc., Tulsa, OK, USA) software package. The χ^2 test was applied to evaluate conformity between the observed and expected genotype frequencies according to the Hardy-Weinberg rule and to determine the significance of differences in allele and genotype frequencies between cases and controls. A p-value <0.05 was assumed as significant in all tests conducted.

Due to small number of *2/*2 homozygotes, *2/*2 and *1/*2 cases was analyzed as a one cohort.

Results

In all 197 gastric mucosa biopsy specimen (investigated group) and 107 blood samples (control group) *CYPC19* 681 G > A SNP were successfully genotyped. Obtained genotyping data are presented in Table 1.

Genotypes for investigated polymorphisms were distributed in accordance with Hardy-Weinberg equilibrium (HWE) within both investigated and control groups ($p = 0.2072$ and $p = 0.5238$, respectively) which confirmed the cohorts as representative. Genotype and allele frequencies occurred with similar frequencies in peptic ulcer and control groups. No statistically significant differences between investigated and control group were found (Table 1).

On the basis of the results of rapid urease tests peptic ulcer patients were divided into two subgroups: *Helicobacter pylori* infected and uninfected cases. Because of low frequency of mutated allele *2 in the investigated subgroups cases of genotype *1/*2 and *2/*2 were merged into one group for analysis. There was statistically significant association between neither genotype nor allele frequencies of investigated polymorphism and the presence of *H. pylori* infection ($p = 0.8369$ and $p = 0.8514$, respectively, Table 2). Connection between the presence of infection and the frequencies of genotypes or alleles of *CYP2C19**2 polymorphism was also analysed separately in peptic ulcer women and peptic ulcer men. As in whole peptic ulcer cohort, no statistically significant association was found (*p values* in Table 2). However, some differences in genotype and allele distribution between *H. pylori* infected and -uninfected women and between *H. pylori*-infected and -uninfected men was stated (Table 2).

In the next step, the frequencies of genotypes and allele in peptic ulcer woman and peptic ulcer man (Table 2) were compared. In the whole peptic ulcer cohort, there was a statistically significant association between the investigated polymorphisms and gender ($p = 0.0233$ for genotypes and

Table 1
Comparison of *CYP2C19* alleles and genotypes frequencies between peptic ulcers patients and healthy individuals.

CYP2C19 variants	Peptic ulcer n = 197	Controls n = 107	p (χ^2 test)
*1/*1	148 (75.2%)	74 (69.2%)	0.2520
*1/*2 or *2/*2	49 (24.8%)	33 (30.8%)	
*1/*2	45 (22.8%)	32 (29.9%)	
*2/*2	4 (2.0%)	1 (0.9%)	
*1	341 (86.5%)	180 (84.1%)	0.4126
*2	53 (13.5%)	34 (15.9%)	
HWE: p	0.2072	0.5238	

Table 2
Comparison of *CYP219* allele/ genotypes frequencies between peptic ulcers patients: infected *vs.* uninfected *H. pylori* and woman *vs.* men.

CYP2C19 variants	All cases			All cases		
	Infected, n = 98	Uninfected, n = 99	p (χ^2 test)	Women, n = 127	Men, n = 70	p (χ^2 test)
*1/*1	73 (74.5%)	75 (75.8%)	0.8369	102 (80.3%)	46 (65.7%)	**0.0233**
*1/*2 or *2/*2	25 (25.5%)	24 (24.2%)		25 (19.7%)	24 (34.3%)	
*1/*2	23 (23.5%)	22 (22.2%)		23 (18.1%)	22 (31.4%)	
*2/*2	2 (2.0%)	2 (2.0%)		2 (1.6%)	2 (2.9%)	
*1	169 (86.2%)	172 (86.9%)	0.8514	227 (89.4%)	114 (81.4%)	**0.0270**
*2	27 (13.8%)	26 (13.1%)		27 (10.6%)	26 (18.6%)	
	Women			Uninfected		
	Infected, n = 62	Uninfected, n = 65		Women, n = 65	Men, n = 34	
*1/*1	51 (82.3%)	51 (78.5%)	0.5907	51 (78.5%)	24 (70.6%)	0.3854
*1/*2 or *2/*2	11 (17.7%)	14 (21.5%)		14 (21.5%)	10 (29.4%)	
*1/*2	11 (17.7%)	12 (18.4%)		12 (18.4%)	10 (29.4%)	
*2/*2	0 (0.0%)	2 (3.1%)		2 (3.1%)	0 (0.0%)	
*1	113 (91.1%)	114 (87.7%)	0.3744	114 (87.7%)	58 (85.3%)	0.6352
*2	11 (8.9%)	16 (12.3%)		16 (12.3%)	10 (14.7%)	
	Men			Infected		
	Infected, n = 36	Uninfected, n = 34		Women, n = 62	Men, n = 36	
*1/*1	22 (61.1%)	24 (70.6%)	0.4038	51 (82.3%)	22 (61.1%)	**0.0206**
*1/*2 or *2/*2	14 (38.9%)	10 (29.4%)		11 (17.7%)	14 (38.9%)	
*1/*2	12 (33.3%)	10 (29.4%)		11 (17.7%)	12 (33.3%)	
*2/*2	2 (5.6%)	0 (0.0%)		0 (0.0%)	2 (5.6%)	
*1	56 (77.8%)	58 (85.3%)	0.2530	113 (91.1%)	56 (77.8%)	**0.0089**
*2	16 (22.2%)	10 (14.7%)		11 (8.9%)	16 (22.2%)	

$p = 0.0270$ for alleles, respectively). In the peptic ulcer men genotypes *1/*2 and *2/*2 were more frequent than in woman. The same association was found when a subgroup of peptic ulcer cases infected with *H. pylori* was analyzed ($p = 0.0207$ for genotypes and $p = 0.0089$ for alleles, respectively), but not in *H. pylori* uninfected peptic ulcer subgroup ($p = 0.3854$ for genotypes and $p = 0.6352$ for alleles, respectively).

Discussion

Peptic ulcer disease refers to a disruption of the mucosal integrity of the stomach and/or duodenum of complex and multifactorial pathogenesis. From many environmental factors contributing to ulcer formation the usage of NSAIDs and *H. pylori* infection are the most important. However, there is also genetic background of the host organism which acts concomitantly. In the presented study we research if there is the influence of host *CYP2C19**2 polymorphism in the occurrence of peptic ulcer disease, and it's connection with *H. pylori* infection in this disorder.

Frequencies of genotypes and alleles in the investigated cohort of peptic ulcer patients and healthy subjects did not differ substantially from these obtained earlier in Polish [13], UK Caucasians [11], Italian [14], Russians [15] population. Many earlier *in vivo* studies showed there are substantial differences among individuals having different *CYP2C19* genotypes in PPIs metabolism. For example, Gawrońska-Szklarz et al. [16] found that in Polish Caucasians treated with triple therapy (pantoprazole + amoxicillin + metronidazole) pantoprazole concentrations were the highest in patients with defective allele *CYP2C19**2 carriers and lowest in hyperactive genotype homozygotes *CYP2C19**17/*17, and median intragastric pH in *CYP2C19* PMs during lansoprazole dosing was higher than in homo-EMs and hetero-EMs in Japanese [6]. The eradication rate of *H. pylori* was found to be determined by *CYP2C19* genotypes in Polish individuals [5] and some other populations [7,8], at least for some particular treatment regimens. However, some contrary results were also published [17,18]. Assuming that wild type homozygous *1/*1 are EMs, three-quarters of investigated peptic ulcer subjects infected by *H. pylori* could potentially experience the successful eradication therapy. Only 2% of peptic ulcer individuals are predicted to be PMs with an excellent eradication rates. The *CYP2C19 2*/2** polymorphism is responsible for PMs phenotype. However, in this study due to the lack of full information about the treatment regimens used in patients forming the study group, the *CYP2C19* phenotypic analysis was not carried out.

It is well established that prostaglandins and leukotriens, elements of the arachidonic acid cascade have role in injury, protection, and healing of gastric mucosa [19], and any deregulation of the cascade may redound to gastrointesitinal disorders. As CYP2C19 is one of the enzymes metabolizing the arachidonic acid, it was proposed the *CYP2C19* polymorphisms influencing CYP2C19 activity could influence the individual susceptibility to ulceration [11]. We did not found any association between occurrence of peptic ulcer and the investigated *CYP2C19**2 polymorphism, so this hypothesis could not to be hold up. In the recently published research, Musumba et al. [11] found the among eight investigated *CYP2C* gene cluster functional polymorphisms (*i.e. CYP2C19**2) only the gain-of-function *CYP2C19**17 was significantly associated with the presence of PUD, which stays in agreement with our results.

Besides metabolizing drugs, CYP2C19 plays an important role in either the degradation of potential carcinogens or the bioactivation of some chemical procarcinogenes. Many studies showed the connection between *CYP2C19* variants and susceptibility to various cancers, *i.e.* biliary tract cancer [20], breast cancer [21], hepatocellular carcinoma [22], esophagus, lung and stomach cancer [23,24]. However, in the case of gastric cancer results obtained by Sugimoto et al. [24] indicate that *CYP2C19* variants can exert its effect only when severe inflammation or atrophic changes induced by *H. pylori* infection are present simultaneously. Considering this, it can be assumed that also in peptic ulcer pathogenesis the *CYP2C19* polymorphisms require simultaneous *H. pylori* infection. We did not found any differences in frequency of *CYP2C19**2 genotypes between the peptic ulcer subjects infected with *H. pylori* and these without *H. pylori* infection which does not support this hypothesis. Considering a significant over-representation of women in the investigated cohort of peptic ulcer patients we performed

analogous data analysis in separate subgroups of women and men. An association between investigated SNP and risk of *H. pylori* infection development neither in peptic ulcer women nor men was stated.

Some studies showed that men could be more susceptible than women to peptic ulcer disease [25–27], but the reason of this phenomenon is not clear. One of the possible explanations are female hormones which have a gastroprotective properties [25]. Additionally, CYP2C19 isoenzyme takes a part in metabolism of estrogens, so its polymorphism-derived variable activity could lead to individual differences in PUD activity. All things considered, some interaction between gender and *CYP2C19**2 would be expected in determining of PUD susceptibility. In the research a significant association between *CYP2C19**2 polymorphism and gender in PUD patients was found. Among PUD men frequency of *1/*2 and *2/*2 subjects was higher than in woman. When we repeated the analysis in the subgroup of *H. pylori*-infected PUD cases the difference in frequencies was hold up, but in PUD cases uninfected with *H. pylori* was virtually the same. It could suggested there is some interaction between gender, *CYP2C19*2* polymorphism, and *H. pylori* infection in the pathogenesis. However, this hypothesis should be verified in the further studies.

In conclusion, investigated polymorphism is not a risk factor for peptic ulcer in Polish population. Moreover, *CYP2C19**2 is not connected with *H. pylori* infection development in this group. However, a significant association between *CYP2C19**2 and gender in *H. pylori*-infected but not -uninfected peptic ulcer individuals was found.

Declarations of interest

None.

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