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Hydroxylation index of omeprazole in relation to CYP2C19 polymorphism and sex in a healthy Iranian population

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Abstract

Background: Polymorphism of *CYP2C19* gene is one of the important factors in pharmacokinetics of *CYP2C19* substrates. Omeprazole is a proton pump inhibitor which is mainly metabolized by cytochrome P450 2C19 (*CYP2C19*). The aim of present study was to assess omeprazole hydroxylation index as a measure of *CYP2C19* activity considering new variant allele (*CYP2C19*17*) in Iranian population and also to see if this activity is sex dependent.

Methods: One hundred and eighty healthy unrelated Iranian individuals attended in this study. Blood samples for genotyping and phenotyping were collected 3 hours after administration of 20 mg omeprazole orally. Genotyping of *2C19* variant alleles *2, *3 and *17 was performed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and semi-nested PCR methods. Plasma concentrations of omeprazole and hydroxyomeprazole were determined by high performance liquid chromatography (HPLC) technique and hydroxylation index (HI) (omeprazole/ hydroxyomeprazole) was calculated.

Results: The *CYP2C19*17* was the most common variant allele in the studied population (21.6%). Genotype frequencies of *CYP2C19*17*17*, *1*17, and *2*17 were 5.5%, 28.8% and 3.3% respectively. The lowest and the highest median omeprazole HI was observed in *17*17 and *2*2 genotypes respectively (0.36 vs. 13.09). The median HI of omeprazole in subjects homozygous for *CYP2C19*1* was 2.16-fold higher than individuals homozygous for *CYP2C19*17* ($P < 0.001$) and the median HI of *CYP2C19*1*17* genotype was 1.98-fold higher than *CYP2C19*17*17* subjects ($P < 0.001$). However, subjects with *CYP2C19*2*17* (median HI: 1.74) and *CYP2C19*1*2* (median HI: 1.98) genotypes and also *CYP2C19*1*17* (median HI: 0.71) and *CYP2C19*1*1* (mean HI: 0.78) did not show any significantly different enzyme activity. In addition, no statistically significant difference was found between women and men in distribution of *CYP2C19* genotypes. Furthermore, the hydroxylation index of Omeprazole was not different between women and men in the studied population.

Conclusion: Our data point out the importance of *CYP2C19*2* and *CYP2C19*17* variant alleles in metabolism of omeprazole and therefore *CYP2C19* activity. Regarding the high frequency of *CYP2C19*17* in Iranian population, the importance of this new variant allele in metabolism of *CYP2C19* substrates shall be considered.

Keywords: *CYP2C19*, Enzyme activity, Genotype, Omeprazole, Phenotype

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Introduction

Cytochrome P450 includes a wide variety of phase I metabolizing enzymes which are involved in metabolism of drugs and endogenous substances [1,2]. CYP2C19 is one of the members of cytochrome iso enzyme superfamily which contributes in metabolism of important drugs such as proton pump inhibitors (PPI) [3] psychotic drugs like venlafaxine [4] and citalopram [5,6], voriconazol [7], and clopidogrel [8,9].

CYP2C19 is represented by a gene located on chromosome 10 [10]. Genetic polymorphism of CYP2C19 is one of the major reasons of inter-individual variability in response to CYP2C19 substrate [11-13]. The main CYP2C19 polymorphisms that are associated with difference in therapeutic response are attributed to *CYP2C19*2*, *CYP2C19*3* and *CYP2C19*17* [14,15].

A point mutation in exon 5 (681 G > A, designated *2) causes a cryptic splice defect (*CYP2C19*2*) and a single nucleotide polymorphism (SNP) in exon 4 (636 G > A designated *3) creates a stop codon. Both mutations predominantly result in decreased CYP2C19 activity [9,16]. A recently discovered SNP in 5' -flanking region (-806 C > T and -3402 C > T) leads to increased CYP2C19 activity and therefore produces ultra rapid metabolizer phenotype [17,18].

The *CYP2C19*2*2* and *3*3 genotypes are more prevalent in oriental and Asian populations than in Caucasian (12-23% vs 3-5%). In contrast the *CYP2C19*17*17* is more frequent in Caucasian than in Asian populations (18-26% vs 0.4-1.4%) [19,20].

Omeprazole is a proton pump inhibitor that is administered in treatment of gastric acid related disease [21]. Polymorphism of CYP2C19 can affect pharmacokinetic and therefore efficacy of proton pump inhibitors [21,22]. Additionally non genetic factors like age, liver disease and combination therapy can result in resistance to *Helicobacter Pylori* eradication treatment [23,24].

Several studies have used hydroxylation index of omeprazole as an indicator of CYP2C19 activity however this enzyme activity, was mainly measured in relation to *2 and *3 variant alleles and not the new variant allele (*17) [16,25-28]. Although Sim *et al.* studied the effect of *CYP2C19*17* variant allele on enzyme activity, they only reported this activity in extensive metabolizers (*17*17, *1*17 and *1*1) and they did not determine CYP2C19 activity in *CYP2C19*2*17* carriers [17]. *CYP2C19*2* leads to decreased enzyme activity and *CYP2C19*17* causes increased enzyme activity [16,17] but the impact of combined alleles (*CYP2C19*2*17*) on CYP2C19 activity has not been reported comprehensively and it is unknown that the effect of which allele is more predominant in *CYP2C19*2*17* carriers.

Furthermore there are some controversies in publications about impact of sex on CYP2C19 activity [29,30].

To our best knowledge, currently there is no published data regarding CYP2C19 activity in relation to new variant allele in Iranian population. Thus, the objects of this study were to assess effect of *CYP2C19*17* on enzyme activity and also to see if there is any sex-dependent difference in CYP2C19 activity and finally to investigate genotype-phenotype relationship of CYP2C19 considering new variant allele (*CYP2C19*17*) in Iranian population.

Material and methods

Study subjects

The study protocol was approved by ethics committee of Tehran University of Medical Sciences (ethical no. 11208). Generally one hundred and eighty (60 women and 120 men) unrelated healthy Iranian volunteers with the mean age of between 20-55 years and average body weight of 45-89 kg took part in this study. All participants signed written informed consent of this project. The study was completed by contribution of faculties of pharmacy of Tehran, Yazd, Kermanshah and Kerman University of Medical Sciences. The participants were students or staffs of pharmacy schools, with no history of any illness or medicine consumption. No smoking and consumption of medicine that would affect CYP2C19 activity was permitted for one week before and during the study.

CYP2C19 phenotyping

After an overnight fast for at least 8 hours, volunteers took 20 mg omeprazole capsule (Abidi pharmaceuticals) with 250 milliliter tap water. Ten ml venous blood sample was collected from each subject 3 hours after administration of omeprazole and transferred into tubes containing 10 µl of 10% EDTA. Five ml of blood samples were centrifuged for 5 min at 4000 rpm and the plasma was separated and transferred to Eppendorf tube and stored at -80°C up to the day of analysis. The other 5 ml blood samples were stored directly in -80°C for genotyping analysis.

Analytical procedure

Omeprazole powder was purchased from TMAD (Iran). 5-hydroxyomeprazole was a kind donation by AstraZeneca (Sweden). The concentration of omeprazole and 5-hydroxyomeprazole was analyzed by HPLC method as described by Rezk *et al.* with a few modifications [31]. Briefly 500 µl plasma was extracted by liquid-liquid extraction using 1500 µl ethyl acetate. After orbital mixing for 10 min and centrifuging at 4000 × g for 10 min, the upper organic layer was separated and transferred to glass tube and then evaporated to dryness under gentle stream of air. Finally the residue was dissolved in 250 µl mobile phase and 100 µl of this sample was injected to HPLC system. The mobile phase was a combination of

dibasic sodium phosphate buffer (0.025 mol/lit, pH 6); acetonitrile: methanol (73: 18: 8 V/V/V). The HPLC apparatus consisted of a low pressure HPLC pump, UV detector ($\lambda = 302$ nm) all from Knauer (Berlin, Germany). The chromatographic separation was performed by using Chromolit™ Performance RP-18e 100 mm \times 4.6 mm, 5 μ m particle size. Flow rate was adjusted to 1 ml/min. The limits of quantification were about 15 μ g/ml for both compounds. Intraday and between day precisions were $< 5\%$ for both omeprazole and 5-hydroxyomeprazole.

CYP2C19 genotyping

The DNA was extracted from blood leucocytes by standard salting out method as explained by Miller *et al.* [32]. The extracted DNA was dissolved in sterile distilled water and stored at 4 °C until the day of analysis. Amplification of CYP2C19*2 and *3 allele was implemented using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described by De Morias [33]. The PCR product of each reaction was digested by specific endonuclease (all from New England Biolabs GmbH, Frankfurt, Germany); the 169 bp CYP2C19*2 product was digested by SmaI to 40 and 129 bp fragments. The 329 bp PCR product of CYP2C19*3 was digested by BamHI to 233 and 96 bp pieces. Genotyping of CYP2C19*17 -3402 C > T and -806 C > T polymorphisms was done by PCR-RFLP and nested-PCR assays as defined by Sim *et al.* [17]. For CYP2C19*17 -3402 C > T the PCR product (504 bp) was digested by MnlI and resulted in 224 and 280 bp fragments. But the PCR product of CYP2C19*17 -806 C > T (200 bp) was separated directly on 2.5% agarose gel without any digestion. In all PCR-RFLP assays mutation caused abolishment of restriction site and thus PCR product was not digested.

Statistical analysis

The allele frequencies differences between population were estimated using two-tailed Fisher's exact test. The 95% confidence intervals (CI) were calculated using Confidence Interval Analysis software. The relation of sex and genotype was assessed by two tailed Fisher's exact test. The observed and expected frequencies were calculated by using Hardy-Weinberg equation. The two-tailed Fisher's exact test was used to evaluate deviation of genotype frequencies in the studied population from Hardy-Weinberg equilibrium. The enzyme activity was compared by using omeprazole hydroxylation index. The hydroxylation index (HI) of omeprazole 3 hours after administration of omeprazole was calculated by dividing omeprazole to 5-hydroxyomeprazole plasma concentration. The mean HI in different genotypes were compared by Mann-Whitney two tailed test. The impact of sex on HI of omeprazole was also evaluated using Mann-Whitney two tailed test. The inter-individual variability in metabolism of

omeprazole was represented by probit plot. For drawing probit plot, the log of HI was calculated, the antimode value was determined using Microsoft office excel 2010. The normality of HI distribution was analyzed by frequency distribution histogram and also by Kolmogorov-Smirnov test. All statistical analyses were performed by Sigma Plot version 12.0 and Graph Pad Prism version 5 softwares and $P < 0.05$ was considered as statistically significant difference.

Results

The genotype and allele frequencies of CYP2C19 are reported in Table 1. According to the data presented in Table 1, CYP2C19 *17*17, *1*17 and *1*1 were detected in 10 (5.5%), 52 (28.9%) and 75 (41.7%) subjects respectively. The CYP2C19 *2*17 and *1*2 were identified in 6 (3.3%) and 33 (18.3%) individuals and finally the CYP2C19*2*2 was recognized in 4 (2.2%) of volunteers. CYP2C19*17 was the most common variant allele in Iranian population.

The hydroxylation index of omeprazole as mean \pm SD, median and 95% confidence interval is reported in Table 2. Subjects with CYP2C19 *17*17 genotype had a very high metabolic capacity with median hydroxylation index of 0.36 and were classified as Ultra-Rapid Metabolizers (URM). The median hydroxylation index of omeprazole in subjects homozygous for CYP2C19*1 was 2.17 fold higher than individual homozygous for CYP2C19*17 ($P < 0.001$) and the median hydroxylation index of CYP2C19*1*17 genotype was 1.97 fold higher than CYP2C19*17*17 subjects ($P < 0.001$). There was not a significant difference between HI of omeprazole in CYP2C19*1*17 and *1*1 carriers ($P > 0.05$) and these two groups were stratified as extensive metabolizers (EM).

The median HI of omeprazole was 1.74 in CYP2C19*2*17 and 1.98 in CYP2C19*1*2 carriers respectively. The difference in HI of omeprazole in CYP2C19*1*2 carriers were statistically significant with other CYP2C19 genotypes ($P < 0.05$) except for CYP2C19*2*17 genotype ($p > 0.05$). Individuals in these two groups had intermediate metabolic capacity and were designated as Intermediate-Metabolizers (IM). Homozygous carrier of CYP2C19*2 had a very low metabolic capacity with the median hydroxylation index of 13.03 and they were classified as poor metabolizers (PM). There was a significant difference between HI index of homozygous carriers of CYP2C19*2 with the other five genotypes ($p < 0.001$).

The plasma concentration of omeprazole and hydroxyomeprazole is illustrated in Figure 1. According to this figure there is a significant difference between omeprazole plasma concentration in individuals with CYP2C19*17*17 genotype with all other groups ($P < 0.01$), but the omeprazole plasma concentration was neither different between *1*17 and *1*1(EM) ($P > 0.5$) nor between *2*17 and *1*2 (IM)

Table 1 Genotype and allele frequencies of CYP2C19 in 180 healthy Iranian volunteers

CYP2C19 Genotype	Number of subjects		Frequency (%)	95% CI
	Men (120)	Women (60)		
*17*17	5	5	5.5	2.7 - 10.0
*1*17	37	15	28.8	22.4 - 36.1
*1*1	53	22	41.7	34.4 - 49.2
*1*2	20	13	18.3	13.0 - 24.7
*2*17	4	2	3.3	1.23 - 7.1
*2*2	1	3	2.2	0.6 - 5.5
CYP2C19 Alleles	No. of alleles		Frequency (%)	95% CI
CYP2C19*17	78		21.6	17.5 - 26.3
CYP2C19*1	235		65.3	60.1 - 70.2
CYP2C19*2	47		13.1	9.7 - 16.9
CYP2C19*3	0		0	0

*CI: Confidence Interval. (The 95% confidence intervals (CI) were calculated using Confidence Interval Analysis software).

genotypes. However, omeprazole plasma concentration was significantly different between EM (*I*17* and *I*1*) and IM (*2*17* and *I*2*). The plasma concentration of hydroxyomeprazole was significantly different between **2*2* and all other 5 genotypes while the plasma concentration of hydroxyomeprazole was not significantly different between other 5 genotypes (*17*17*, *I*17*, **1*1*, *2*17*, *I*2*).

Figure 2 indicates the hydroxylation index of omeprazole in 6 genotypes and also in predicted phenotype groups. As it is observed there is no significant difference between hydroxylation index of omeprazole in *I*17* and *I*1* groups or between *2*17* and *I*2* groups. While the difference between *17*17* or *2*2* with all other genotype groups were statistically significant.

The summary of omeprazole, hydroxyomeprazole plasma concentration and omeprazole HI in the total population, women and men is reported in Table 3. Omeprazole plasma concentration was significantly higher in *2*2* genotype than other genotype groups. Additionally there was a significant difference in omeprazole plasma concentration between different groups ($P < 0.001$) except for *CYP2C19*1*17* and **1*1* ($P > 0.05$), *CYP2C19*1*2* and *CYP2C19*2*17* ($P > 0.05$). Mean omeprazole plasma concentration was 19.0 fold higher in *CYP2C19*2*2* than *CYP2C19*17*17* and 11 fold higher than *CYP2C19*1*1* ($P < 0.001$), however hydroxyomeprazole concentration

was not statistically different among genotype groups ($P > 0.05$) except for *CYP2C19*2*2*. Moreover, omeprazole and hydroxyomeprazole plasma concentrations as well as omeprazole HI were not statistically different among women and men in the studied population ($P > 0.05$).

The effect of sex on hydroxylation index of omeprazole is illustrated in Figure 3. According to this figure there is not any significant difference between median hydroxylation index in women (0.84) and men (0.86) ($p > 0.05$).

The frequency distribution histogram of omeprazole hydroxylation index in 180 healthy Iranian volunteers is indicated in Figure 4. The graph shows a bimodal distribution with the antimode of around 0.8. Kolmogorov-Smirnov test showed that the omeprazole hydroxylation index was not normally distributed in the studied population (K-S Dist. = 0.296 $p < 0.001$). The bimodal distribution was also confirmed by probit plot.

The correlation of CYP2C19 genotype and phenotype was tested using Spearman rank correlation, and the results showed a well correlation between CYP2C19 genotype and phenotype ($r_s = 0.64$, $P < 0.0001$).

Discussion

Inter-individual variability in drug response always has been one of the main concerns in drug discovery and development. The important factors resulting in such

Table 2 Hydroxylation index of omeprazole (omeprazole/hydroxyomeprazole) in relation to CYP2C19 genotype in 180 healthy Iranian subjects

	<i>*17*17</i>	<i>*1*17</i>	<i>*1*1</i>	<i>*2*17</i>	<i>*1*2</i>	<i>*2*2</i>
Mean (SD)	0.35(0.06)	0.75(0.28)	0.85(0.30)	2.02(0.84)	2.27(1.04)	13.59(3.13)
Median	0.36 ^a	0.71	0.78	1.74 ^b	1.98 ^b	13.03 ^a
95% CI	0.31 - 0.39	0.68 - 0.83	0.79 - 0.92	1.33 - 2.72	1.92 - 2.63	10.51 - 16.67

^aRepresent statistically significant difference with other 5 genotypes.

^bRepresent statistically significant difference with **17*17*, **1*17*, **1*1* and **2*2*.

CI: Confidence Interval.

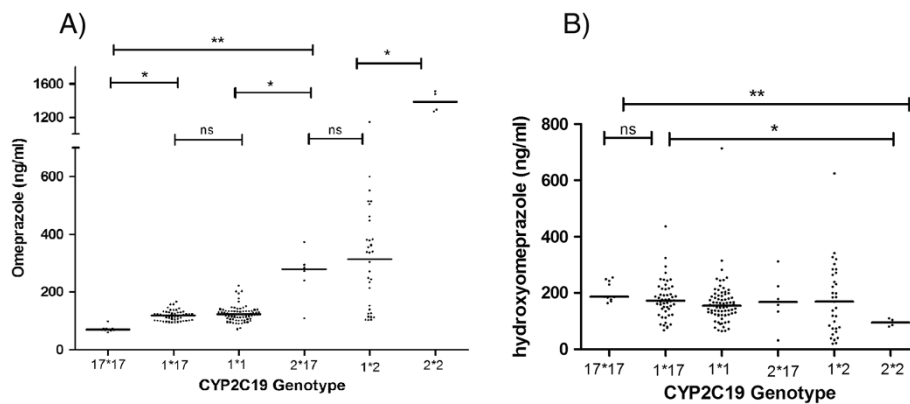


Figure 1 Plasma concentrations of Omeprazole (A) and hydroxyomeprazole (B) in different genotypes 3 hours after administration of Omeprazole orally. ns: not significant, * $p < 0.05$, ** $p < 0.001$.

variation include genetic, nongenetic and physiologic agents like change in protein structure, combination therapy, alcohol, smoking, sex, age and disease condition [34].

*CYP2C19*17* is a new variant allele which is associated with increased gene transcription and therefore higher enzyme activity [17]; which may lead to several clinical consequences including the lower susceptibility to breast cancer risk [35], higher risk of peptic ulcer disease [36], greater response to clopidogrel treatment and more risk of bleeding [37] in addition to a better treatment with tamoxifen [38].

In this study, omeprazole HI after 3 hours administration of omeprazole was used as indicator of *CYP2C19* activity. The HI in *CYP2C19*17*17* was significantly different with *CYP2C19*1*17* and *CYP2C19*1*1* genotypes and people in this group had very high metabolic activity, which is in agreement with what was reported by Sim *et al.* They found that median HI of omeprazole in homozygous carriers of *CYP2C19*1* is 2 fold higher than homozygous carriers of *CYP2C19*17* and 1.2 fold higher

than *CYP2C19*1*17* [17]. Ramsjö *et al.* has also reported that mean HI of omeprazole in *CYP2C19*1*1* was 3.2 fold higher than *CYP2C19*17*17* and 1.1 fold higher than *CYP2C19*1*17* [27].

*CYP2C19*2* allele is associated with decreased enzyme activity and *CYP2C19*17* variant allele is connected with increased enzyme activity. In Most of the genotype phenotype studies of *CYP2C19*17* variant allele, only HI of omeprazole in *CYP2C19*17*17*, *CYP2C19*1*17* and *CYP2C19*1*1* genotypes has been reported [17,27]. However, the capacity of *CYP2C19* enzyme activity in people carrying both defective mutant alleles of *2 and *17 (*CYP2C19*2*17*) was still unclear. Although Ragia *et al.* in the study for evaluation of distribution of *CYP2C19*17* genetic polymorphism in Greece people defined *CYP2C19*2*17* carriers as EM and people with *CYP2C19*1*2* as IM, they only predicted phenotype based on genotype and *CYP2C19* activity was not determined by using a probe drug [39]. Sugimoto *et al.* did not see any difference between metabolic capacities of *CYP2C19*1*1*, *1*17, 2*17 and 1*2 for metabolism of

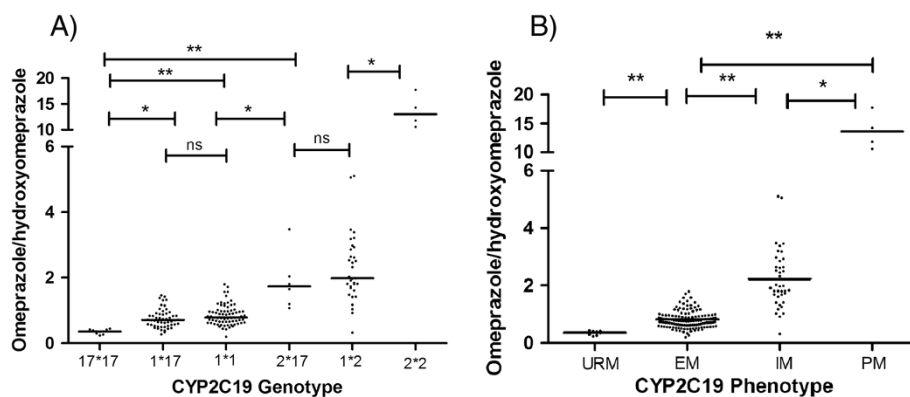


Figure 2 The hydroxylation index of Omeprazole in different genotypes (A) and in predicted phenotype groups (B) 3 hours after administration of Omeprazole orally. ns: not significant, * $p < 0.05$, ** $p < 0.001$.

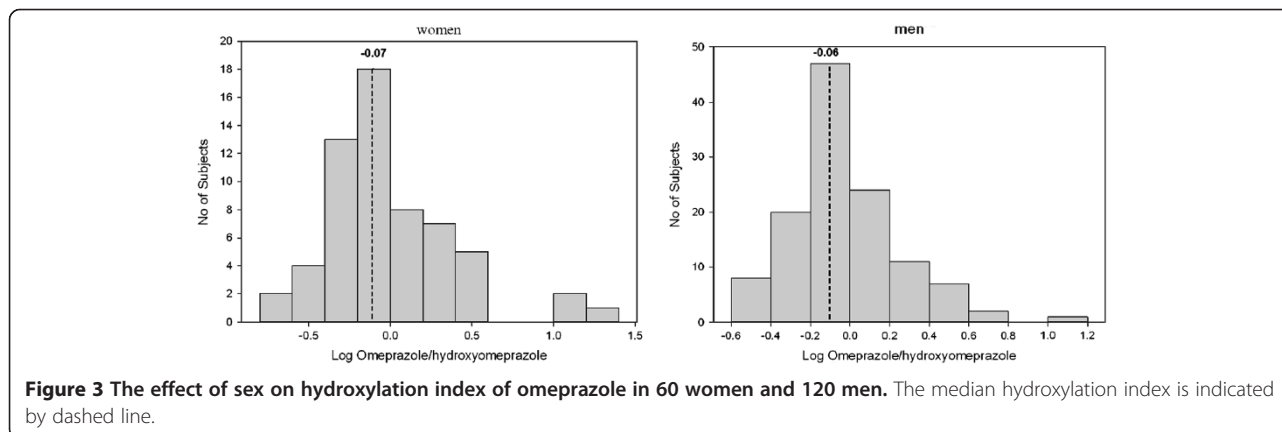
Table 3 Plasma concentration of omeprazole (OMP) and hydroxyomeprazole (OH-OMP) and hydroxylation index (HI) of omeprazole in relation to genotype in 60 women and 120 men 3 hour after administration of single oral dose of 20 mg omeprazole

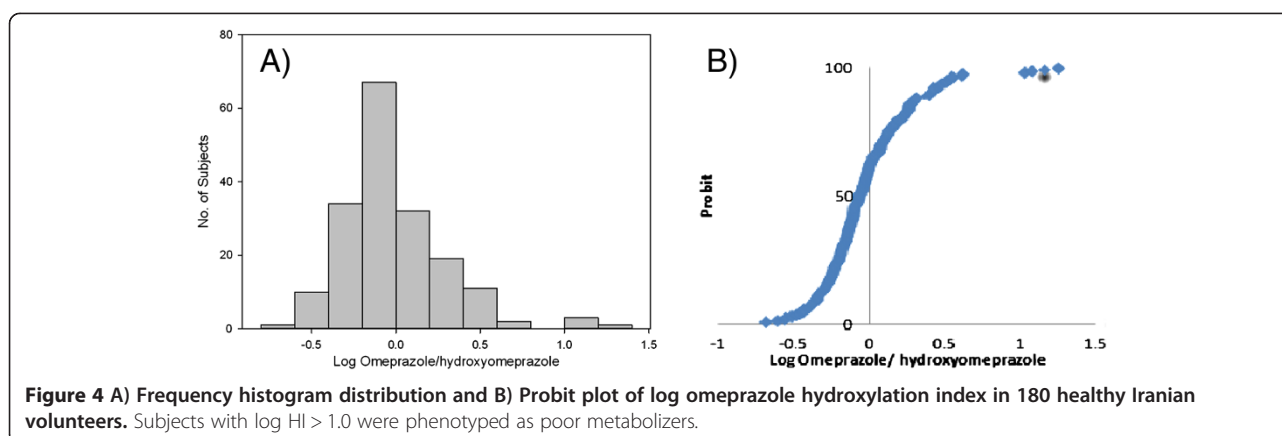
Genotype	No of subjects	Mean OMP concentration (ng/ml) ± SD	Mean OH-OMP concentration (ng/ml) ± SD	Mean HI ± SD
17*17	Total (10)	71.01 ± 10.28	203.31 ± 34.93	0.35 ± 0.06
	Women (5)	68.32 ± 5.73	204.92 ± 41.35	0.34 ± 0.08
	Men (5)	73.71 ± 13.67	201.69 ± 32.08	0.36 ± 0.04
1*17	Total (52)	118.28 ± 10.19	177.05 ± 37.53	0.75 ± 0.28
	Women (15)	120.22 ± 20.12	174.51 ± 28.01	0.72 ± 0.18
	Men (37)	117.49 ± 16.10	178.07 ± 44.57	0.77 ± 0.32
1*1	Total (75)	124.75 ± 27.18	163.74 ± 32.25	0.85 ± 0.30
	Women (22)	128.06 ± 39.90	194.40 ± 60.84	0.78 ± 0.27
	Men (53)	123.38 ± 20.83	151.01 ± 26.38	0.89 ± 0.30
2*17	Total (6)	289.01 ± 97.45	172.79 ± 63.58	2.02 ± 0.54
	Women (4)	324.25 ± 39.90	178.72 ± 33.59	1.97 ± 0.62
	Men (2)	271.39 ± 118.56	169.82 ± 84.95	2.05 ± 0.67
1*2	Total (33)	319.45 ± 150.34	177.79 ± 38.39	2.27 ± 1.04
	Women (13)	364.92 ± 265.16	194.66 ± 52.56	2.24 ± 0.79
	Men (20)	289.89 ± 167.55	165.53 ± 23.55	2.29 ± 1.19
2*2	Total (4)	1388.99 ± 123.41	104.67 ± 10.55	13.59 ± 3.13
	Women (3)	1359.84 ± 133.23	105.03 ± 13.80	13.37 ± 3.80
	Men (1)	1476.4	103.58	14.25

HI = (omeprazole concentration/hydroxyomeprazole concentration).

omeprazole and stratified these individuals as EM [20]. The omeprazole HI in subjects with *CYP2C19*2*17* genotype in this study was not significantly different from *CYP2C19*1*2* genotype ($P = 0.33$) so we designated them as IM. It seems that in heterozygous carriers of *CYP2C19*2* and **17* allele, the effect of **2* allele is more predominant than **17* allele and it can suppress induced enzyme activity by **17* allele. This observation is in agreement with classification of *CYP2C19*2*17* as IM by Gurbel *et al.* based on the study for genotype phenotype analysis of 2C19 in stented patient [40]. In contrast to these findings, in a study for evaluation of

CYP2C19 enzyme activity in Turkish children using lansoprazole as a probe drug, individuals with *CYP2C19*2*17* had similar enzyme activity to *CYP2C19*1*17* and *CYP2C19*1*1*; and this activity was significantly different from *CYP2C19*1*2* [41]. Involvement of individual with different age groups could possibly explain such different observations. Our study was conducted in adult individuals with the average age of 32 years but Gumus implemented the study in children with mean age of 10.2 years. The lower frequency of *CYP2C19*2*17* in our studied subjects in comparison to Turkish individuals (6 vs 16) can be considered as another justification.





In the present study effect of CYP2C19 genetic polymorphism and sex on metabolic activity of CYP2C19 was also assessed. Sex is an important factor in activity of some cytochrome P450 enzymes. CYP3A4 is an example of cytochrome enzymes which has higher activity in women than men [2]. There were some controversies in the previous published reports for impact of sex on CYP2C19 activity. Ramsjö *et al.* indicated a sex difference in CYP2C19 activity between Korean subjects and not in Swedish volunteers using Omeprazole as a probe drug [27]. Tamminga *et al.* observed a sex related decreased CYP2C19 activity in women when used mephenytoin as a probe for evaluation of CYP2C19 activity, however the author declared this reduction was more obvious in those who used oral contraceptive [42]. In contrast, Hägg *et al.* did not see any sex differences in CYP2C19 activity after administration of mephenytoin in Norwegian population [43]. By considering these reports one may conclude that sex dependency of CYP2C19 activity is influenced by environmental and epigenetic factors like diet and ethnic differences. The other possibility can be attributed to some new genetic mutation in some populations which has not been studied well. The result of this study represents no effect of sex on CYP2C19 activity which is in line with what is reported in Swedish and Norwegian population.

The frequency of PM and URM in Iranian population in this study was about 2.2% and 5.5% which is close to the study by Zand *et al.* [44] and other Caucasian population. In previous report by Akhlaghi *et al.* in Iranian patients with coronary artery disease the genotype frequency of CYP2C19*2*2 was reported 4.7% which is quite different from our results [45]. This can be due to difference in studied population (healthy volunteer's vs specific patients). The genotype frequency of CYP2C19*17*17 (URM) was 4% in Swedish and 3% in Ethiopian [17], 5.1% in Danish [19], 3.18% in Greece [39], 7% in Saudi Arabian [46] 1.2% in Indian [16] and 0% in Japanese, Korean and Thai population [20,27,47]. Accordingly the genotype frequency of

CYP2C19*2*2 (PM) is 2.2% in Danish [19], 2.1% in Greece [39], 0.4% in Saudi Arabian [46], 18.4% in Indian [16] and 18% in Japanese people [20].

To the best of authors' knowledge, this is the first study evaluating CYP2C19 genotype and phenotype in Iranian population in relation to new variant allele (CYP2C19*17). In the previous report, Zendehdel *et al.* investigated impact of CYP2C19 on therapeutic efficacy of omeprazole in Iranian patients with erosive reflux esophagitis; patients were genotyped only for CYP2C19*2 and CYP2C19*3, Individuals with HetEM genotype had better response to treatment with omeprazole than EM (95% vs 43% successful treatment response respectively) [48]. The high frequency of CYP2C19*17 allele (21.6%) detected in this study maybe one justification for 50% resistance rate in the EM group in Iranian patients in previous report. However impact of this variant allele (CYP2C19*17) on the efficacy of PPIs like omeprazole shall be evaluated in controlled clinical trials.

In this study the antimode of 0.8 was calculated for Iranian population. Different antimodes for HI of omeprazole have been reported in different ethnics groups: 14.4 in Indian [16], 7.0 in Koreans [25] and Thai population [47], 0.63 in Colombians [26], and 3.98 in West Mexicans [49]. The calculated antimode for Iranian population is similar to Colombian population, indicating comparable CYP2C19 activity in Iranians and Colombians and faster enzyme activity than Asian people.

A complete genotype phenotype correlation was observed in this study. However it should be noted that enzyme activity and therefore metabolic ratio may vary during some disease condition which may result in discrepancy in genotype-phenotype relationship of specific enzyme. Kimura *et al.* [50] indicated discordance of genotype-phenotype relationship of omeprazole in 14.5% of EM patients who had peptic ulcer disease. However this discrepancy was not observed in healthy individuals. Reduced hepatic enzyme activity as a result of old age or liver disease was reported as an explanation for such

finding. Long term treatment with omeprazole which has auto inhibition effect was the other possibility. Williams *et al.* [51] also did not see genotype-phenotype relationship of 2C19 using omeprazole in patients with advanced cancer. The authors concluded that increased level of some signaling molecules like interleukin (IL) and tumor necrosis factors (TNF α) may result in down-regulation of metabolizing enzymes [51,52]. So it should be considered that factors like age, disease state, and concomitant medication may have pronounced effect on enzyme activity. Although omeprazole hydroxylation index has been used as an indicator of CYP2C19 activity, it should be considered that hydroxyomeprazole which is formed by CYP2C19 is further metabolized by CYP3A4 to hydroxyomeprazole sulfone [53] which in turn may indirectly affect the hydroxylation index of omeprazole. Therefore the high concentration of CYP3A4 in liver microsomes of some human can explain the deviation from CYP2C19 genotype and also the sex dependent enzyme activity observed in some ethnic groups [54].

The prevalence of CYP2C19*2*17 in this study was only 6% which is a limitation of this study. Future studies to investigate impact of CYP2C19*2*17 genotype on CYP2C19 enzyme activity in larger groups specially by using drugs with narrow therapeutic window is suggested.

In conclusion, the result of this study shows that CYP2C19*2*17 has an intermediate metabolic activity which maybe important for drug dose adjustment regimens for treatment, specially in those having narrow therapeutic indices like clopidogrel. Additionally no effect of sex on CYP2C19 activity was observed in this study. Regarding the high frequency of CYP2C19*17 in Iranian population, the importance of this new variant allele in metabolism of CYP2C19 substrates shall be considered.

Abbreviations

CYP2C19: Cytochrome P450 2C19; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; HPLC: High performance liquid chromatography; HI: Hydroxylation index; SNP: Single nucleotide polymorphism; URM: Ultra-rapid metabolizers; EM: Extensive metabolizers; IM: Intermediate metabolizers; PM: Poor metabolizers; OMP: Omeprazole; OH-OMP: Hydroxyomeprazole.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

M-RR, MP, NT, M-H GH and RT conceived the study. MP performed the experimental work. All authors were involved in data analysis and interpretation. MP prepared the manuscript. All authors read and approved the final version.

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References

1. Sim SC, Nordin L, Andersson TM-L, Viriding S, Olsson M, Pedersen NL, Ingelman-Sundberg M: Association Between CYP2C19 Polymorphism and Depressive Symptoms. *Am J Med Genet* 2010, **153**(B):1160-1166.
2. Zanger UM, Schwab M: Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *J Pharmacol Ther* 2013, **138**:103-141.
3. Qiao H-L, Hu Y-R, Tian X, Jia L-J, Gao N, Zhang L-R, Guo Y-z: Pharmacokinetics of three proton pump inhibitors in Chinese subjects in relation to the CYP2C19 genotype. *Eur J Clin Pharmacol* 2006, **62**:107-112.
4. Weide JVD, Baalen-Benedek EHV, Kootstra-Ros JE: Metabolic ratios of psychotropics as indication of cytochrome P450 2D6/2C19 genotype. *Ther Drug Monit* 2005, **27**:478-483.
5. Yu B-N, Chen G-L, He N, Ouyang D-S, Chen X-P, Liu Z-Q, Zhou HH: Pharmacokinetic of citalopram in relation to genetic polymorphism of CYP2C19. *Drug Metabol Dispos* 2003, **31**(10):1255-1259.
6. Mrazek DA, Biernacka JM, O'Kane DJ, Black JL, Cunningham JM, Drews MS, Snyder KA, Stevens SR, Rush AJ, Weinshilboum RM: CYP2C19 variation and citalopram response. *Pharmacogenet Genomics* 2011, **21**:1-9.
7. Wang G, Lei HP, Li Z, Tan Z-R, Guo D, Fan L, Chen Y, Hu D-L, Wang D, Zhou H-H: The CYP2C19 ultra rapid metabolizer genotype influences the pharmacokinetics of voriconazol in healthy male volunteers. *Eur J Clin Pharmacol* 2009, **65**:281-285.
8. Sibbing S, Koch W, Gebhard D, Schuster T, Braun S, Stegherr J, Morath T, Schömig A, Beckerath NV, Kastrati A: Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation* 2010, **121**:512-518.
9. Hulot JS, Collet JP, Silvain J, Pena A, Bellemain-Appaix A, Barthélémy O, Cayla G, Beygui F, Montalescot G: Cardiovascular risk in clopidogrel-treated patients according to cytochrome P450 2C19*2 loss-of-function allele or proton pump inhibitor coadministration: a systematic meta-analysis. *J Am Coll Cardiol* 2010, **56**(2):134-143.
10. Mathijssen RHJ, Schaik RHN: Genotyping and phenotyping cytochrome P450: Perspectives for cancer treatment. *Eur J Cancer* 2006, **42**:141-148.
11. Gabriella Scordo M, Caputi AP, D'Arrigo C, Fava G, Spina E: Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. *Pharmacol Res* 2004, **50**:195-200.
12. Ingelman-Sundberg M, Sim SC, Ingelman-Sundberg A, Rodriguez-Antona C: Influence of cytochrome P450 polymorphism on drug therapies: Pharmacogenetic and clinical aspects. *Pharmacol Ther* 2007, **116**:496-526.
13. Chaudhry AS, Kochhar R, Kohli KK: Genetic polymorphism of CYP2C19 & therapeutic response to proton pump inhibitors. *Indian J Med Res* 2008, **127**(6):521-530.
14. Lee S-J, Kim W-Y, Kim H, Shon J-H, Lee SS, Shin J-G: Identification of new CYP2C19 variants exhibiting decreased enzyme activity in the metabolism of S-Mephenytoin and Omeprazole. *Drug Metab Dispos* 2009, **37**(11):2262-2269.
15. Li-Wan-Po A, Girard T, Farndon P, Cooley C, Lithgow J: Pharmacogenetics of CYP2C19: functional and clinical implications of a new variant CYP2C19*17. *Br J Clin Pharmacol* 2010, **69**(3):222-230.
16. Rosemary J, Adithan C, Padmaja N, Shashindran CH, Gerard N, Krishnamoorthy R: The effect of the CYP2C19 genotype on the hydroxylation index of Omeprazole in south Indians. *Eur J Clin Pharmacol* 2005, **61**:19-23.
17. Sim SC, Risinger C, Dahl ML, Akillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M: A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 2006, **79**:103-113.

18. Anichavezhi D, Roa C, Shewade DG, Krishnamoorthy R, Adithan C: **Distribution of CYP2C19*17 allele and genotype in an Indian population.** *J Clin Pharmacy Ther* 2012, **37**:313–318.
19. Pedersen RS, Brasch-Andersen C, Sim SC, Bergmann TK, Halling J, Petersen MS, Weihe P, Edvardsen H, Kristensen VN, Brøsen K, Ingelman-Sundberg M: **Linkage disequilibrium between the CYP2C19*17 allele and wide type CYP2C8 and CYP2C9 alleles: identification of CYP2C haplotypes in healthy Nordic population.** *Eur J Clin Pharmacol* 2010, **66**:1199–1205.
20. Sugimoto K, Uno T, Yamazaki H, Tateishi T: **Limited frequency of the CYP2C19*17 allele and its minor role in a Japanese population.** *Br J Clin Pharmacol* 2008, **65**(3):437–439.
21. Sakai T, Aoyama N, Kita T, Sakaeda T, Nishiguchi K, Nishitara Y, Hohda T, Sirasaka D, Tamura T, Tanigawara Y, Kasuga M, Okumura K: **CYP2C19 genotype and pharmacokinetics of three proton pump inhibitors in healthy subjects.** *Pharm Res* 2001, **18**(6):72–77.
22. Cho H, Choi MK, Cho DY, Yeo CW, Jeong HE, Shon JH, Lee JY: **Effect of CYP2C19 genetic polymorphism on pharmacokinetics and pharmacodynamics of a new proton pump inhibitor, ilaprazole.** *J Clin Pharmacol* 2012, **52**(7):976–984.
23. Ammon S, Treiber G, Kees F, Klotz U: **Influence of age on the steady state disposition of drugs commonly used for the eradication of *Helicobacter pylori*.** *Aliment Pharmacol Ther* 2000, **14**(6):759–766.
24. Ishizawa Y, Yasui-Furukori N, Takahata T, Sasaki M, Tateishi T: **The effect of aging on the relationship between the cytochrome P450 2C19 genotype and Omeprazole pharmacokinetics.** *Clin pharmacokinetics* 2005, **44**:1179–1189.
25. Chong E, Ensom M: **Pharmacogenetics of the Proton Pump Inhibitors: A Systematic Review.** *Pharmacotherapy* 2003, **23**(4):460–471.
26. Isaza C, Henao J: **Isaza Martínez J H, Sepúlveda Arias J, Beltrán L: Phenotype-genotype analysis of CYP2C19 in Colombian mestizo individuals.** *BMC Clin Pharmacol* 2007, **7**(6):1–5.
27. Ramsjö M, Aklilu E, Bohman L, Ingelman-Sundberg M, Roh HK: **CYP2C19 activity comparison between Swedish and Koreans: effect of genotype, sex, oral contraceptive use and smoking.** *Eur J Clin Pharmacol* 2010, **66**:871–877.
28. Niioka T, Uno T, Sugimoto K, Sugawara K, Hayakari M, Tateishi T: **Estimation of CYP2C19 activity by the Omeprazole hydroxylation index at a single point in time after intravenous and oral administration.** *Eur J Clin Pharmacol* 2007, **63**(11):1031–1038.
29. Xie HG, Huang SL, Xu ZH, Xiao ZS, He N, Zhou HH: **Evidence for the effect of gender on activity of (S)-mephenytoin 4'-hydroxylase (CYP2C19) in a Chinese population.** *Pharmacogenetics* 1997, **7**(2):115–119.
30. Laine K, Tybring G, Bertilsson L: **No sex-related differences but significant inhibition by oral contraceptives of CYP2C19 activity as measured by the probe drugs mephenytoin and Omeprazole in healthy Swedish white subjects.** *Clin Pharmacol Ther* 2000, **68**(2):151–159.
31. Rezk NL, Brown KC, Kashuba ADM: **A simple and sensitive bioanalytical assay for simultaneous determination of Omeprazole and its three major metabolites in human blood plasma using RP-HPLC after a simple liquid-liquid extraction procedure.** *J Chromatogr B* 2006, **844**:314–321.
32. Miller SA, Dykes DD, Polesky HF: **A simple salting out procedure for extracting DNA from human nucleated cells.** *Nucleic Acids Res* 1988, **16**:1215.
33. 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 Bio Chem* 1994, **269**(22):15419–15422.
34. Qiang M, Anthony YHL: **Pharmacogenetics, Pharmacogenomics, and Individualized Medicine.** *Pharmacol Rev* 2011, **63**:2437–2459.
35. Justenhoven C, Hamann U, Pierl CB, Baisch C, Harth V, Rabstein S, Spickenheuer A, Pesch B, Brüning T, Winter S, Ko YD, Brauch H: **CYP2C19*17 is associated with decreased breast cancer risk.** *Breast Cancer Res Treat* 2009, **115**(2):391–396.
36. Musumba CO, Jorgensen A, Sutton L, Eker DV, Zhang E, Hara NO, Carr DF, Pritchard DM, Pirmohamed M, Eker DV, Zhang E, Hara NO, Carr DF, Pritchard DM, Pirmohamed M: **CYP2C19*17 Gain-of-function polymorphism is associated with peptic ulcer disease.** *Clin Pharmacol Ther* 2013, **93**(2):195–203.
37. Li Y, Tang HL, Hu YF, Xie HG: **The gain-of-function variant CYP2C19*17: a double-edged sword between thrombosis and bleeding in clopidogrel-treated patients.** *J Thromb Haemost* 2012, **10**(2):199–206.
38. Schroth W, Antoniadou L, Fritz P, Schwab M, Muerdter T, Zanger UM, Simon W, Eichelbaum M, Brauch H: **Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes.** *J Clin Oncol* 2007, **25**(33):5187–5193.
39. Ragia G, Arvanitidis KI, Tavridou A, Manolopoulos VG: **Need for reassessment of reported CYP2C19 allele frequencies in various populations in view of CYP2C19*17 discovery: the case of Greece.** *Pharmacogenomics* 2009, **10**(1):43–49.
40. Gurbel PA, Shuldiner AR, Bliden KP, Ryan K, Pakyz RE, Tantry US: **The relation between CYP2C19 genotype and phenotype in stented patients on maintenance dual antiplatelet therapy.** *Am Heart J* 2011, **161**:598–604.
41. Gumus E, Karaca O, Babaoglu MO, Baysoy G, Balamtekin N, Demir H, Uslu N, Bozkurt A, Yuce A, Yasar U: **Evaluation of lansoprazole as a probe for assessing cytochrome P450 2C19 activity and genotype-phenotype correlation in childhood.** *Eur J Clin Pharmacol* 2012, **68**(5):629–636.
42. Tamminga WJ, Wemer J, Oosterhuis B, Weiling J, Wilffert B, de Leij LF, de Zeeuw RA, Jonkman JH: **CYP2D6 and CYP2C19 activity in a large population of Dutch healthy volunteers: indications for oral contraceptive-related gender differences.** *Eur J Clin Pharmacol* 1999, **55**(3):177–184.
43. Hägg S, Spigset O, Dahlqvist R: **Influence of gender and oral contraceptives on CYP2D6 and CYP2C19 activity in healthy volunteers.** *Br J Clin Pharmacol* 2001, **51**(2):169–173.
44. 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**(1–2):102–105.
45. Akhlaghi A, Shirani S, Ziaie N, Pirhaji O, Yaran M, Shahverdi G, Sarrafzadegan N, Khosravi A, Khosravi E: **Cytochrome P450 2C19 Polymorphism in Iranian Patients with Coronary Artery Disease.** *ARYA Atheroscler* 2011, **7**(3):106–110.
46. Saeed LH, Mayet AY: **Genotype-Phenotype analysis of CYP2C19 in healthy Saudi individuals and its potential clinical implication in drug therapy.** *Int J Med Sci* 2013, **10**:1497–1502.
47. Tassaneeyakul W, Tawalee A, Tassaneeyakul W, Kukongviriyapan V, Blaisdell J, Goldstein JA, Gaysomsiri D: **Analysis of the CYP2C19 polymorphism in a North-eastern Thai population.** *Pharmacogenetics* 2002, **12**:221–225.
48. Zendedel N, Biramijamal F, Hossein-Nezad A, Zendedel N, Sarie H, Doughaiemoghaddam M, Pourshams A: **Role of Cytochrome P450 2C19 genetic polymorphism in the therapeutic efficacy of omeprazole in Iranian patients with erosive reflux esophagitis.** *Arch Iran Med* 2010, **13**(5):406–412.
49. Gonzalez HM, Romero EM, Peregrina AA, de J Chavez T T, Escobar-Islas E, Lozano F, Hoyo-Vadillo C: **CYP2C19- and CYP3A4-dependent Omeprazole metabolism in West Mexicans.** *J Clin Pharmacol* 2003, **43**:1211–1215.
50. Kimura M, Ieiri I, Wada Y, Mamiya K, Urae A, Iimori E, Sakai T, Otsubo K, Higuchi S: **Reliability of the omeprazole hydroxylation index for CYP2C19 phenotyping: possible effect of age, liver disease and length of therapy.** *Br J Clin Pharmacol* 1999, **47**(1):115–119.
51. Williams ML, Bhargava P, Cherrouk I, Marshall JL, Flockhart DA, Wainer IW: **A discordance of the cytochrome P450 2C19 genotype and phenotype in patients with advanced cancer.** *Br J Clin Pharmacol* 2000, **49**(5):485–488.
52. Helsby NA, Lo WY, Sharples K, Riley G, Murray M, Spells K, Dzhelal M, Simpson A, Findlay M: **CYP2C19 pharmacogenetics in advanced cancer: compromised function independent of genotype.** *Br J Cancer* 2008, **99**(8):1251–1255.
53. Hagymási K, Müllner K, Herszényi L, Tulassay Z: **Update on the pharmacogenomics of proton pump inhibitors.** *Pharmacogenomics* 2011, **12**(6):873–888.
54. Yamazaki H, Inoue K, Shaw PM, Checovich WJ, Guengerich FP, Shimada T: **Different contributions of cytochrome P450 2C19 and 3A4 in the oxidation of omeprazole by human liver microsomes: effects of contents of these two forms in individual human samples.** *J Pharmacol Exp Ther* 1997, **283**(2):434–442.

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