



HAL
open science

CYP2C19 activity comparison between Swedes and Koreans: effect of genotype, sex, oral contraceptive use, and smoking

Margareta Ramsjö, Eleni Aklillu, Lilleba Bohman, Magnus Ingelman-Sundberg, Hyung-Keun Roh, Leif Bertilsson

► **To cite this version:**

Margareta Ramsjö, Eleni Aklillu, Lilleba Bohman, Magnus Ingelman-Sundberg, Hyung-Keun Roh, et al.. CYP2C19 activity comparison between Swedes and Koreans: effect of genotype, sex, oral contraceptive use, and smoking. *European Journal of Clinical Pharmacology*, 2010, 66 (9), pp.871-877. 10.1007/s00228-010-0835-0 . hal-00595508

HAL Id: hal-00595508

<https://hal.science/hal-00595508>

Submitted on 25 May 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**CYP2C19 activity comparison between Swedes and Koreans:
Effect of genotype, sex, oral contraceptives use and
smoking**

Journal:	<i>European Journal of Clinical Pharmacology</i>
Manuscript ID:	EJCP-2010-0050.R1
Type of submission:	Original
Date Submitted by the Author:	29-Apr-2010
Complete List of Authors:	Ramsjö, Margareta; Karolinska Institutet, Division of Clinical Pharmacology, Department of Laboratory Medicine Aklillu, Eleni; Karolinska Institutet, Div Clinical Pharmacolgy, Department of Laboratory Medicine Bohman, Lilleba; Karolinska Institutet, Division of Clinical Pharmacology, Department of Laboratory Medicine Ingelman-Sundberg, Magnus; Karolinska Institutet, Department of Pharmacology and Physiology, Section of Pharmacogenetics Roh, Hyung-Keun; Gachon University Hospital, Department of Internal Medicine, Division of Clinical Pharmacology Bertilsson, Leif; Karolinska Institutet, Division of Clinical Pharmacology, Department of Laboratory Medicine

CYP2C19 activity comparison between Swedes and Koreans: Effect of genotype, sex, oral contraceptives use and smoking

Margareta Ramsjö¹, Eleni Aklillu¹, Lilleba Bohman¹, Magnus Ingelman-Sundberg², Hyung-Keun Roh³ and Leif Bertilsson¹.

¹Department of Laboratory Medicine at Karolinska Institutet, Division of Clinical Pharmacology, Karolinska University Hospital-Huddinge C1:68, Stockholm, Sweden

²Department of Pharmacology and Physiology, Section of Pharmacogenetics, Karolinska Institutet, Stockholm, Sweden

³Department of Internal Medicine, Division of Clinical Pharmacology, Gachon University Hospital, Incheon, Korea

Corresponding Author

Eleni Aklillu, B.Pharm, M.Sc, PhD

Division of Clinical Pharmacology, Department of Laboratory Medicine,

Karolinska University Hospital Huddinge, Karolinska Institutet,

SE-14186 Stockholm, Sweden;

Phone: + 46 8 58 58 78 82; Fax: + 46 8 58 58 10 70,

E-mail: Eleni.Aklillu@ki.se

Abstract

Objectives To compare CYP2C19 enzyme activity between Swedes and Koreans controlling for the effect of CYP2C19 genotype, sex, oral contraceptive use and smoking habit.

Methods CYP2C19 activity was determined in 185 healthy Swedish and 150 Korean subjects as omeprazole/5-hydroxyomeprazole ratio (metabolic ratio; MR) using high-performance liquid chromatography. Genotyping was performed by PCR using Taqman assay.

Results As expected, a higher incidence of poor metabolizers (PM) was found in Koreans (14%) compared to Swedes (3.8%) and the frequency of the *CYP2C19*17* allele was very low in Koreans 0.3%. Among subjects homozygous for *CYP2C19*1*, Koreans displayed significantly lower CYP2C19 enzyme activity than Swedes ($p < 0.000001$). Interestingly in Koreans a pronounced gender difference was apparent: females ($n=24$) had significantly lower MR than males ($N=30$) ($p < 0.0001$) but such a gender difference was not seen among Swedes. Swedish OC users had a higher MR than non-users ($p < 0.00001$), whereas OC was only used by one Korean. No effect of smoking was observed.

Conclusions We find specific gender dependent effects of CYP2C19 activity in Koreans but not in Swedes. Controlling for the effect of genotype and sex, Koreans display lower CYP2C19 activity than Swedes. The genetic, epigenetic or environmental basis for this difference remains to be identified.

Keywords *CYP2C19*, omeprazole, gender, Korean, Swedes

Introduction

The enzyme CYP2C19 catalyses the metabolism of a wide variety of drugs such as antidepressants [1], benzodiazepines [2], proguanil [3] and proton pump inhibitors (PPIs) [4]. The major metabolites of omeprazole detected *in vivo* in human plasma are 5-hydroxyomeprazole formed by CYP2C19 and omeprazole sulphone formed by CYP3A4 [5, 6]. The metabolic disposition of omeprazole correlates well with the rate of S-mephenytoin hydroxylation [7] and those two drugs are the most commonly used probes of CYP2C19. About 3% of Caucasians are PM of S-mephenytoin [8, 9] and a higher incidence of PMs has been reported in Japanese (18-23%) [10, 11], Chinese (15-17%) [12, 13] and in Korean subjects (13-16%) [14]. The PM phenotype is caused by the *CYP2C19*2* [15] and *CYP2C19*3* alleles although the *CYP2C19*3* is very uncommon in Caucasian PMs [16]. Sim et al [17] identified a novel allele (*CYP2C19*17*) that was associated with increased CYP2C19 activity *in vivo* in two different ethnic populations. This allele is associated with higher levels of gene transcription and increased rates of omeprazole and mephenytoin metabolism. The frequency of this allele was 18% in both Swedes and Ethiopians but only 4% in Chinese subjects [17].

In general previous studies reported lower CYP2C19 enzyme activity among Asians compared to White population. Several factors may contribute for the observed inter ethnic differences in enzyme activity such as differences in functional variant allele frequencies between populations and other environmental factors like dietary, oral contraceptive (OC) use or smoking habit. Indeed *CYP2C19* defective variant alleles occur at a higher frequency in Asians as compared to whites and this may account partly for the observed lower CYP2C19 activity in this population. Furthermore previously it is reported that OC use inhibits CYP2C19 enzyme activity [18] and hence variation in OC use habit may also contribute to some extent. As to our knowledge no study has compared the enzyme activity between the

two populations controlling for the effect of confounding factors such as oral contraceptive use and *CYP2C19* genotype. The aim of the present study was to investigate the *CYP2C19* genotype phenotype relationship differences using the same methodology between healthy Swedish and Korean subjects having the same *CYP2C19* genotype, OC use and smoking habit. We find an important gender difference in the *CYP2C19* activity in Koreans but not in Swedes and a generally lower *CYP2C19* activity among Koreans in subjects homozygous for *CYP2C19*1*.

Materials and Methods

Study Subjects

We studied 185 healthy Swedish subjects at the Karolinska University Hospital, Huddinge, Sweden and 150 healthy Korean subjects at the Inha University Hospital, Incheon, Korea. The subjects were mainly students and staff at the two hospitals. In the Swedish subjects the mean age was $29 \pm \text{SD } 8.3$ years (range 18-60) with the mean weight $69 \pm \text{SD } 12$ kg (range 47-109) (no data on weight in two subjects). The Swedish subjects consisted of 112 women and 73 men and 39 (11 men and 28 women) were classified as smokers (definition of smokers > 2 cigarettes/ day) as described previously [19, 20]. There were 44 women using OC. Among the 28 women smokers, 6 were OC user. In four women no data on OC use was available. In the Korean subjects the mean age was 25 ± 3.9 years (range 20-46) with the mean weight 62 ± 12 kg (range 38-94). The Korean subjects consisted of 74 women and 76 men and 29 of the subjects were classified as smokers (26 men and 3 women of whom none were OC user). In one subject information on smoking habit was unavailable. Only one Korean woman was a user of OC. The participants were asked if they used OC but not which kind of contraceptives they used.

The local Ethics committees at Karolinska Institutet, Stockholm, Sweden and Inha University, Incheon, Korea approved the study and it was performed in accordance with the Helsinki Declaration. After written and oral information about the study, the participants were screened by clinical laboratory tests and interviewed for their medical histories. The subjects were not allowed to use other medications (except for OC) including herbal medicines one week before the study started. They refrained from alcohol, grapefruit juice during 2 days and caffeine products during 1, 5 days before the study started. The Karolinska cocktail included omeprazole 20 mg (enterotablet of Losec Mups 20 mg; Hässle, Stockholm, Sweden), losartan 25 mg, caffeine 100 mg, quinine 250 mg and debrisoquine 10 mg [21].

Phenotyping

After an 8-hour overnight fast, the subjects took the drug omeprazole at 8 a.m. Three hours after the intake of omeprazole, blood samples were collected and centrifuged, plasma was separated and stored frozen at -20° until analysis by reversed phase HPLC with UV detection. The Korean plasma and DNA samples were packed on dry ice and sent to Sweden for analysis. The method for measuring omeprazole and its metabolite 5-hydroxyomeprazole was described by Tybring et al [22] and modified by Christensen et al [21].

Genotyping

Blood samples were collected using tubes containing EDTA. Genomic DNA was extracted from leukocytes by using QIAamp®DNA Mini Blood Kit (distributed by AB Applied Biosystems, California, USA and Chestrite, United Kingdom). Genotyping methods are based on the PCR (Polymerase-chain-reaction) technique and specific primers to different parts of the

gene. Allelic discrimination was determined by using a Taqman assay. Genotyping of *CYP2C19*17* was carried out by PCR with specific primers as described by Sim et al [17].

Statistical analysis

The individual metabolic ratio MR of omeprazole was determined by dividing the molar concentrations of omeprazole and 5-hydroxyomeprazole in plasma.

The MRs was log transformed before application of statistical analysis. For comparisons of Swedes and Koreans within each genotype group and between groups based on smoking and OC use the independent t-test was used. P-Values less than 0.05 were considered statistically significant. Statistical analyses were performed by Statistical version 7 (Stat Soft® Scandinavia AB).

Results

CYP2C19 genotype: The study encompassed 185 Swedes and 150 Koreans. We evaluated results after administering the Karolinska cocktail with the use omeprazole as a probe drug for phenotyping of CYP2C19. The genotype analyses revealed allele frequencies of *CYP2C19*2*, and **17* in the Swedes of 16 and 20 %, respectively and 28, 11 and 0.3 % of **2*, **3* and **17* in the Koreans, respectively (Table 1). In both Koreans and Swedes, there was no significant difference between the observed and expected genotype frequency according to Hardy–Weinberg law (χ^2 test, $p>0.05$).

CYP2C19 phenotype: The subjects were phenotyped for omerazole and Figure 1 shows histograms of the MR (omeprazole/5-hydroxyomeprazole). A bimodal distribution especially in Koreans with an antimode of about 10 was observed. The median MR in Koreans was substantially higher (2.57) as compared to the Swedes (0.71) (p -value <0.00001). The mean

MR among Koreans was 3.04 while in Swedes 0.86.

CYP2C19 phenotype comparison between Swedes and Koreans: For statistical analysis and comparison of CYP2C19 enzyme activity, subjects were first stratified based on CYP2C19 genotype. The subjects homozygous for CYP2C19*1 were further stratified for gender and intake of OC (Figure 2). Interestingly the Koreans homozygous for CYP2C19*1 had significantly higher MR for omeprazole; the MR in Korean men was 1.91 vs. 0.56 in Swedish men ($p < 0.000001$) and an MR of 0.91 was seen in Korean females as compared to 0.58 in Swedish women who were none OC users ($p = 0.022$). In Koreans a gender difference was seen where men ($n = 30$) had higher MR (1.91) as compared to females ($n = 24$) (0.91) ($p < 0.0001$). By contrast, no gender differences were seen among the Swedes. Among the Swedish women, OC had a pronounced inhibitory effect ($p < 0.00001$), whereas no Korean female on OC was included in the study since OC usage was only reported by one Korean woman.

The results of the phenotyping of the two populations are also shown stratified according to the number of active genes (with CYP2C19*17 excluded) with the two genders together (Figure 3). Korean subjects homozygous for CYP2C19*1 had much higher MR (1.48) than the Swedish individuals (0.58) ($p < 0.000001$). A similar difference was also noted when comparing those with one functional allele (2.88 vs 1.10, $p < 0.0012$) and also a small difference (28.8 vs 20.9, $p < 0.028$) was noted among those homozygous for null alleles although the number of subjects in the Swedish *0/*0 group is very low.

The CYP2C19*17 allele was only present in one Korean subject. Analysis of the MR for omeprazole among Swedes revealed that the MR in *17/*17 was significantly lower (0.18,

n=4) than in *1/*1 (0.58, n=56, p=0.016) and *1/*17 (0.51, n=35; p=0.048), in line with our previous results [17].

There was no effect of smoking on MR neither in Swedes (p=0.75) nor in Koreans (p=0.50) in the genotype *CYP2C19* *1/*1 by using the independent t-test. Considering smokers and non-smokers separately, effect of *CYP2C19* genotype on enzyme activity remained significant in both Koreans (p<0.0001) and Swedes (P<0.0003). We further investigated for interaction between genotype, oral contraceptive use and smoking using factorial ANOVA in Swedes. Since there was only one OC user in Koreans we did not perform this analysis in Koreans. The result indicated only oral contraceptive use (p<0.001) and *CYP2C19* genotype (p<0.001) but not smoking affects *CYP2C19* activity. Regardless of smoking habit, oral contraceptive use inhibited and *CYP2C19* genotype influenced *CYP2C19* enzyme activity.

Discussion

In the present study we compared *CYP2C19* enzyme activity between Koreans and Swedes using the same methodology and taking into consideration effect of genotype, sex, OC use. Our major finding include quite unexpected and very interesting differences in the *CYP2C19* phenotype between Koreans and Swedes belonging to the same genotype group, namely for those homozygous for *CYP2C19**1. Phenotyping data based on the probe drug omeprazole revealed that the Korean men had a much higher MR for omeprazole than the Swedish men. Interestingly Korean women were significantly more rapid for omeprazole metabolism than the Korean men. Still the Korean women were slower than Swedish women without intake of oral contraceptives, but the difference was much less than observed between Swedish and Korean men. Oral contraceptive intake did decrease the rate of omeprazole metabolism in Swedish women to approach a similar MR for omeprazole as Korean men. Korean subjects

carrying one null allele were also slower as compared to Swedes and a slight difference was seen between men and women homozygous for null alleles (Figure 3), but this difference was very small and the number of Swedish subjects in this group was also very low. Stratifying the phenotype data for smokers or nonsmokers revealed no influence of smoking habit on CYP2C19 enzyme activity.

Previously a small hardly significant gender difference in CYP2C19 activity was observed in Chinese using S-mephenytoin as a probe drug [23]. Here the S/R-ratio for mephenytoin was lower in homozygous extensive metabolizers (EM) females than in homozygous EM males (0.22 +/- 0.14 vs. 0.33 +/- 0.09; $p = 0.046$). By using omeprazole as a probe drug in the current study in Koreans, much higher significance values were obtained for a gender difference indicating that such a gender difference is true in some Asian populations.

The mechanism behind the gender difference in Koreans in CYP2C19 activity is unknown. One could imagine environmental, epigenetic or genetic reasons. Regarding environmental influence differences in diets or drug habits between Korean men and women are unlikely, but could not be excluded. Also one could hypothesize that hormonal or environmental influence should result in gender specific epigenetic control of the *CYP2C19* gene expression which would influence the activity. This cannot be excluded and a true evaluation would require liver samples from both sexes where the pattern of gene methylation as well as histone acetylation and methylation could be investigated [24]. Perhaps the most likely explanation is of true genetic origin. The differences in sequence between the Caucasian and Korean *CYP2C* locus as revealed from whole genome sequencing in the *CYP2C* locus is extensive and encompasses several hundreds of mutations [25]. One could therefore suggest that the Korean variant of the *CYP2C19*1* allele carries mutations that makes it less expressed as compared to

the Swedish variant, and at the same time there are mutations in this locus that makes the Korean *CYP2C19*1* gene more sensitive for the estrogen receptor or other nuclear factors controlled by the action of estrogens, thereby causing an increased rate of CYP2C19 activity in the Korean females as compared to males. Further studies have to be designed that investigate these alternatives before the basis for the gender difference is understood.

The Swedish females taking oral contraceptives had lower CYP2C19 activity as compared to women without. Tamminga et al. have also describes an effect of OCs using S-mephynotoin as a probe drug [26]. The oral contraceptives may contain progestin with or without ethinyl oestradiol and it is not known whether it is the oestrogen or the progestin component that inhibits drug metabolism [10]. The inhibition of CYP2C19 activity by OC seems to be due to the ethinyloestradiol component of OCs [27, 28]. The mechanism behind this effect is unknown but has been suggested to be interaction at the active site between the hormones and the CYP2C19 substrates. A mechanism where the oral contraceptives directly inhibit the rate of CYP2C19 expression can however not be excluded.

Our result indicated no significant effect of cigarette smoking on CYP2C19 enzyme activity in both populations. Even controlling for the effect of genotype and OC use there was no significant difference between smokers and non-smokers on enzyme activity. Among cytochrome P450 drug metabolizing enzymes that expressed in the liver, CYP1A2 is the only one whose activity is affected by smoking habit [20]. Previous studies reported interaction between smoking and CYP2C19 genotype in influencing H.Pylori eradication success and suggested interaction between smoking and CYP2C19 should be examined in the future [29]. Apparently effect of smoking on H.Pylori eradication might be not by directly altering CYP2C19 enzyme activity and hence omeprazole metabolism.

The finding of significantly lower CYP2C19 enzyme activity in Koreans compared to Swedes that is not accountable or explained by the common CYP2C19 genotype or OC use may have some clinical relevance. In clinical practice the common CYP2C19 SNPs that are recommended or practiced to genotype for include *CYP2C19**2 and *3 in Asians. Our Study indicates genotyping for these SNPs only may not provide full information about individual's metabolic capacity or phenotype. Alternatively, phenotyping with a safe probe drug to reflect an individual's metabolic capacity or therapeutic drug monitoring may need to be considered. Our study also can be a base for further investigation to identify the underlying genetic, epigenetic or environmental factors responsible for the observed wide differences in CYP2C19 enzyme activity between Koreans and Swedes.

Limitation of present study includes that subjects were genotyped for the most common variant alleles in the respective population and other variants of the *CYP2C19* alleles were not analyzed because of their low frequencies. However contribution of these rare variant alleles to the observed difference in enzyme activity between the two populations cannot be ruled out. In summary, the present study describes an interesting gender difference in the CYP2C19 enzyme activity in Koreans which cannot be seen in Swedes. Further studies are needed to evaluate the mechanisms behind this difference, which however, would drastically influence the pharmacokinetics of CYP2C19 drugs as to motivate gender specific dosing regimens in Koreans.

Acknowledgment

The study was financially supported by the Swedish Research Council, Medicine, 3902 and the Swedish Capio Forskningsstiftelse.

References

1. Brosen K (2004) Some aspects of genetic polymorphism in the biotransformation of antidepressants. *Therapie* 59 (1): 5-12
2. Bertilsson L, Henthorn TK, Sanz E, Tybring G, Sawe J, Villen T (1989) Importance of genetic factors in the regulation of diazepam metabolism: relationship to S-mephenytoin, but not debrisoquin, hydroxylation phenotype. *Clin Pharmacol Ther* 45 (4): 348-355
3. Ward SA, Helsby NA, Skjelbo E, Brosen K, Gram LF, Breckenridge AM (1991) The activation of the biguanide antimalarial proguanil co-segregates with the mephenytoin oxidation polymorphism--a panel study. *Br J Clin Pharmacol* 31 (6): 689-692
4. Li XQ, Andersson TB, Ahlstrom M, Weidolf L (2004) Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos* 32 (8): 821-827
5. Andersson T, Miners JO, Veronese ME, Tassaneeyakul W, Meyer UA, Birkett DJ (1993) Identification of human liver cytochrome P450 isoforms mediating omeprazole metabolism. *Br J Clin Pharmacol* 36 (6): 521-530
6. Laine K, Tybring G, Bertilsson L (2000) 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* 68 (2): 151-159
7. Chang M, Dahl ML, Tybring G, Gotharson E, Bertilsson L (1995) Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. *Pharmacogenetics* 5 (6): 358-363.

8. Alvan G, Bechtel P, Iselius L, Gundert-Remy U (1990) Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. *Eur J Clin Pharmacol* 39 (6): 533-537
9. Jurima M, Inaba T, Kadar D, Kalow W (1985) Genetic polymorphism of mephenytoin p(4')-hydroxylation: difference between Orientals and Caucasians. *Br J Clin Pharmacol* 19 (4): 483-487
10. Nakamura K, Goto F, Ray WA, McAllister CB, Jacqz E, Wilkinson GR, Branch RA (1985) Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Caucasian populations. *Clin Pharmacol Ther* 38 (4): 402-408
11. Horai Y, Nakano M, Ishizaki T, Ishikawa K, Zhou HH, Zhou BI, Liao CL, Zhang LM (1989) Metoprolol and mephenytoin oxidation polymorphisms in Far Eastern Oriental subjects: Japanese versus mainland Chinese. *Clin Pharmacol Ther* 46 (2): 198-207
12. Bertilsson L, Lou YQ, Du YL, Liu Y, Kuang TY, Liao XM, Wang KY, Reviriego J, Iselius L, Sjoqvist F (1992) Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquin and S-mephenytoin. *Clin Pharmacol Ther* 51 (4): 388-397.
13. Ishizaki T, Sohn DR, Kobayashi K, Chiba K, Lee KH, Shin SG, Andersson T, Regardh CG, Lou YC, Zhang Y, et al. (1994) Interethnic differences in omeprazole metabolism in the two S-mephenytoin hydroxylation phenotypes studied in Caucasians and Orientals. *Ther Drug Monit* 16 (2): 214-215
14. Roh HK, Dahl ML, Tybring G, Yamada H, Cha YN, Bertilsson L (1996) CYP2C19 genotype and phenotype determined by omeprazole in a Korean population. *Pharmacogenetics* 6 (6): 547-551

15. de Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA (1994) The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J Biol Chem* 269 (22): 15419-15422
16. De Morais SM, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, Goldstein JA (1994) Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese. *Mol Pharmacol* 46 (4): 594-598.
17. Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 79 (1): 103-113
18. Sandberg M, Johansson I, Christensen M, Rane A, Eliasson E (2004) The impact of CYP2C9 genetics and oral contraceptives on cytochrome P450 2C9 phenotype. *Drug Metab Dispos* 32 (5): 484-489
19. Djordjevic N, Ghotbi R, Bertilsson L, Jankovic S, Aklillu E (2008) Induction of CYP1A2 by heavy coffee consumption in Serbs and Swedes. *Eur J Clin Pharmacol* 64 (4): 381-385
20. Ghotbi R, Christensen M, Roh HK, Ingelman-Sundberg M, Aklillu E, Bertilsson L (2007) Comparisons of CYP1A2 genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans. *Eur J Clin Pharmacol* 63 (6): 537-546
21. Christensen M, Andersson K, Dalen P, Mirghani RA, Muirhead GJ, Nordmark A, Tybring G, Wahlberg A, Yasar U, Bertilsson L (2003) The Karolinska cocktail for phenotyping of five human cytochrome P450 enzymes. *Clin Pharmacol Ther* 73 (6): 517-528

22. Tybring G, Bottiger Y, Widen J, Bertilsson L (1997) Enantioselective hydroxylation of omeprazole catalyzed by CYP2C19 in Swedish white subjects. *Clin Pharmacol Ther* 62 (2): 129-137
23. Xie HG, Huang SL, Xu ZH, Xiao ZS, He N, Zhou HH (1997) Evidence for the effect of gender on activity of (S)-mephenytoin 4'-hydroxylase (CYP2C19) in a Chinese population. *Pharmacogenetics* 7 (2): 115-119
24. Gomez A, Ingelman-Sundberg M (2009) Pharmacoeugenetics: its role in interindividual differences in drug response. *Clin Pharmacol Ther* 85 (4): 426-430
25. Kim JI, Ju YS, Park H, Kim S, Lee S, Yi JH, Mudge J, Miller NA, Hong D, Bell CJ, Kim HS, Chung IS, Lee WC, Lee JS, Seo SH, Yun JY, Woo HN, Lee H, Suh D, Kim HJ, Yavartanoo M, Kwak M, Zheng Y, Lee MK, Kim JY, Gokcumen O, Mills RE, Zaranek AW, Thakuria J, Wu X, Kim RW, Huntley JJ, Luo S, Schroth GP, Wu TD, Kim H, Yang KS, Park WY, Church GM, Lee C, Kingsmore SF, Seo JS (2009) A highly annotated whole-genome sequence of a Korean individual. *Nature* 460 (7258): 1011-1015
26. Tamminga WJ, Wemer J, Oosterhuis B, Weiling J, Wilffert B, de Leij LF, de Zeeuw RA, Jonkman JH (1999) CYP2D6 and CYP2C19 activity in a large population of Dutch healthy volunteers: indications for oral contraceptive-related gender differences. *Eur J Clin Pharmacol* 55 (3): 177-184
27. Hagg S, Spigset O, Dahlqvist R (2001) Influence of gender and oral contraceptives on CYP2D6 and CYP2C19 activity in healthy volunteers. *Br J Clin Pharmacol* 51 (2): 169-173
28. Palovaara S, Tybring G, Laine K (2003) The effect of ethinyloestradiol and levonorgestrel on the CYP2C19-mediated metabolism of omeprazole in healthy female subjects. *Br J Clin Pharmacol* 56 (2): 232-237

29. Suzuki T, Matsuo K, Sawaki A, Wakai K, Hirose K, Ito H, Saito T, Nakamura T, Yamao K, Hamajima N, Tajima K (2007) Influence of smoking and CYP2C19 genotypes on *H. pylori* eradication success. *Epidemiol Infect* 135 (1): 171-176

For Peer Review

Table 1 Distribution of CYP2C19 allele frequencies with the respective 95% CI in the Swedish Korean populations. *CYP2C19*1* denotes alleles not identified as *2, *3 or *17. The *3 allele was not analysed in the Swedish cohort due to its very rare occurrence.

<i>CYP2C19</i> allele	Koreans (n=150)	Swedes (n=185)
<i>CYP2C19*1</i>	0.61 (0.55 – 0.66)	0.64 (0.59 – 0.69)
<i>CYP2C19*2</i>	0.28 (0.23 – 0.33)	0.16 (0.12 – 0.20)
<i>CYP2C19*3</i>	0.11 (0.08 – 0.15)	n.d
<i>CYP2C19*17</i>	0.003 (-0.003 – 0.01)	0.20 (0.16 – 0.24)

n= number of subjects, n.d= not determined

Legends to figures.

Fig. 1

The distribution of the log metabolic ratio (MR) of omeprazole/5- hydroxyomeprazole in all Swedes (n= 185) and Koreans (n= 150) participating in this study. The arbitral vertical line indicates the position of omeprazole/5-hydroxyomeprazole ratio = 1. The respective median MR is indicated by arrow.

Fig.2

Effect of oral contraceptives (OC) and gender on omeprazole/5-hydroxyomeprazole ratio in Swedes and in Koreans with the genotype *CYP2C19* *1 / *1. Only one Korean female was user of OC and she has been excluded here. *CYP2C19**1 was assigned in the absence of *2,*3 or *17. The arbitral vertical line indicates MR (omeprazole/5-hydroxyomeprazole ratio) = 1. The respective median MR is indicated by arrow.

Fig.3

The distribution of omeprazole/5-hydroxyomeprazole ratio in genotypes * 1/* 1, * 1/* 0 and *0/ *0 in Swedish and Koreans without oral contraceptives (NOC) and without the * 17 allele. The respective median MR is indicated by arrow. In the Swedish samples *0= *2 allele and in Korean samples *0 = *2 or * 3 alleles. The arbitral vertical line indicates the position of omeprazole/5-hydroxyomeprazole ratio = 1.

Fig.1

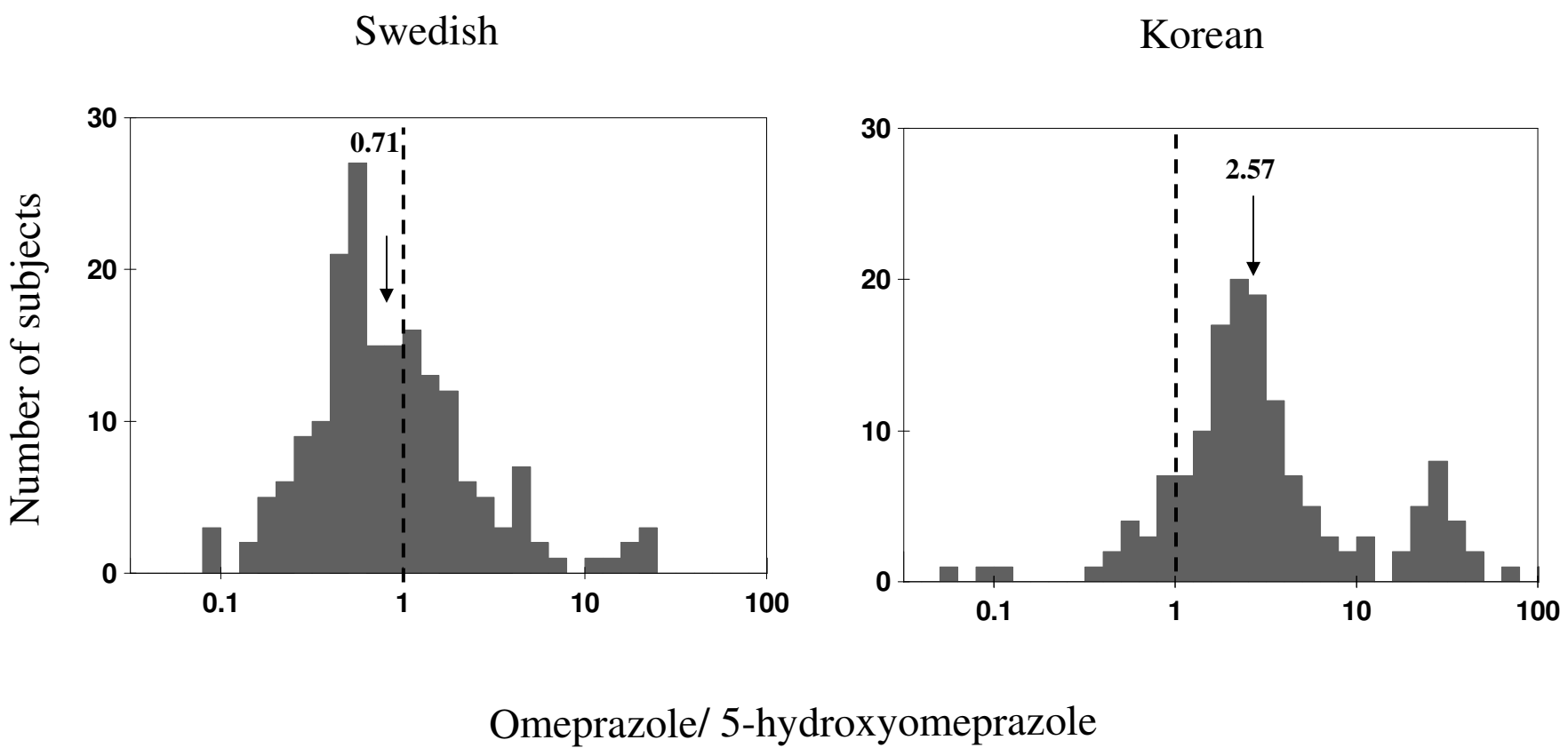


Fig.2

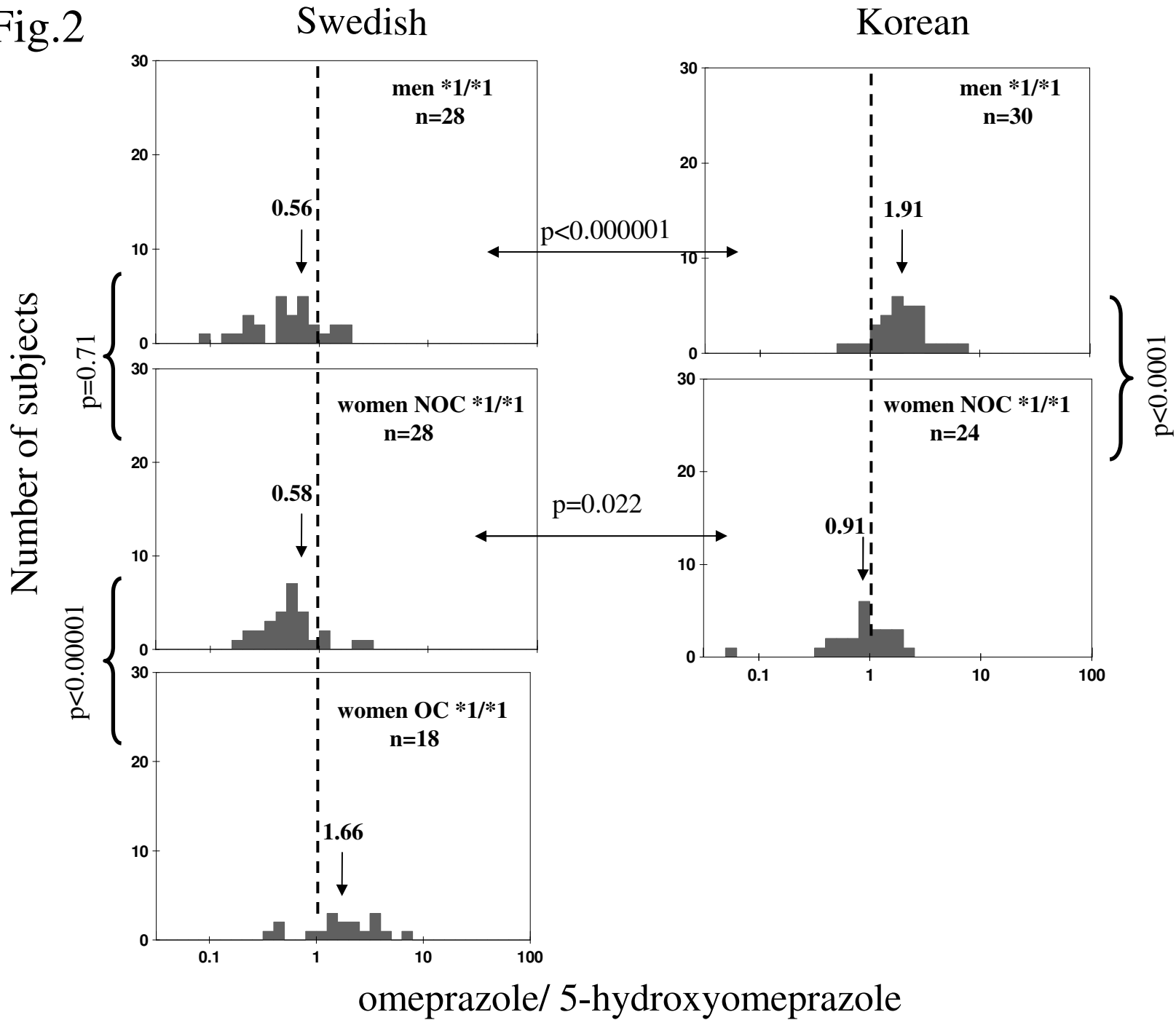
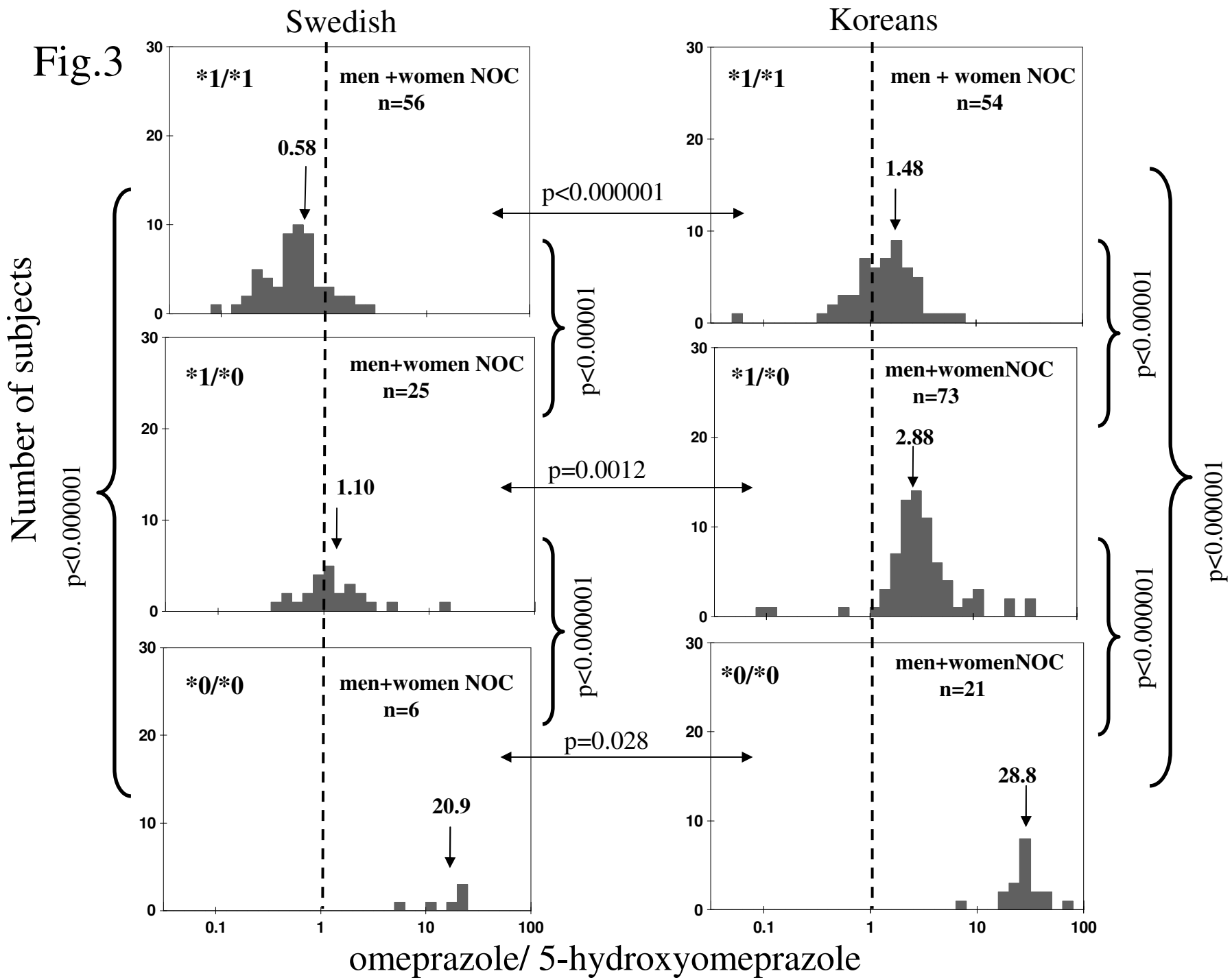


Fig.3



29 April 2010

SUMMARY OF REVISIONS AND RESPONSE TO REFEREE COMMENTS

(EJCP-2010-0050)

RE: entitled "Interethnic and gender dependent differences in CYP2C19 activity. A comparison between Swedes and Koreans"

We thank the referees for review of our manuscript and helpful feedback. We considered the peer-review feedback in great detail and made every attempt to incorporate the suggested amendments.

We present below our itemized responses to specific comments by the referees. The revised sections and points of major emphasis are highlighted in color throughout the manuscript as they relate to the referee comments. A separate clean copy of the manuscript is also provided as part of the revised manuscript dossier.

Changes in the revised manuscript are indicated in blue color.

Reviewer: 1

COMMENTS TO THE AUTHOR: Use the space below for comments to be transmitted to the author.

The authors investigated phenotype-genotype association for CYP2C19 in two different ethnic groups from Sweden and Korea. Interethnic and gender differences in omeprazole metabolic ratio and effect of oral contraceptives are original findings in the present study.

Manuscript is well written. The following points need to be considered/answered.

1. Distribution of sex and oral contraceptive use should be stated among the smokers/nonsmokers. It should be statistically tested if these factors are confounding factors for the comparison of parameters between smokers and nonsmokers.

Authors reply:

- Distribution of sex and oral contraceptive among smokers and non smokers for both populations is now indicated under **“Materials and Methods” section, 1st paragraph on page 4.**
- Interaction between smoking habit, oral contraceptive use and CYP2C19 genotype were performed using factorial ANOVA. Regardless of smoking habit, oral contraceptive use and genotype affected CYP2C19 enzyme activity. Effect of oral contraceptive on enzyme activity was tested only in Swedes since there was only one oral contraceptive user in Koreans. The result is now described under **“Result” Section, last paragraph on page 7**

2. Were quality controls included during the transport of the samples from Korea to Sweden to ensure that the samples were not affected by transport conditions?

Authors reply:

- We did not use any specific controls on the Korean samples as they were sent by courier packed with dry ice to Sweden. Upon arrival to Stockholm, the dry ice was still intact. Furthermore the samples were analyzed by the same methods and during the same conditions as the Swedish samples in the same laboratory with the same laboratory personnel.

3. Which oral contraceptives were used by Swedish subjects? This information should be added to the text.

Authors reply:

- The participants were asked if they used contraceptives, not which kind of contraceptives they used.
- This information is now included under **“Materials and Methods” section on the 1st paragraph, last sentence on page 4.**

4. Table 1: 95% confidence intervals should be added for the allele frequencies. Are the allele frequencies distributed in accordance with the Hardy Weinberg equation? This information should be added.

Authors reply:

- We have now inserted the respective 95% CI for allele frequency in **Table 1**.
- Statement about compliance to the Hardy Weinberg equation is now described under **"Result" Section, 1st paragraph on page 6** as follows

Table 1 Distribution of CYP2C19 allele frequencies with the respective 95% CI in the Swedish and Korean populations analysed. *CYP2C19*1* denotes alleles not identified as *2, *3 or *17. The *3 allele was not analysed in the Swedish cohort due to its very rare occurrence

<u>CYP2C19 allele</u>	<u>Koreans (n=150)</u>	<u>Swedes (n=185)</u>
<u>CYP2C19*1</u>	<u>0.61 (0.55 – 0.66)</u>	<u>0.64 (0.59 – 0.69)</u>
<u>CYP2C19*2</u>	<u>0.28 (0.23 – 0.33)</u>	<u>0.16 (0.12 – 0.20)</u>
<u>CYP2C19*3</u>	<u>0.11 (0.08 – 0.15)</u>	<u>n.d</u>
<u>CYP2C19*17</u>	<u>0.003 (-0.003 – 0.01)</u>	<u>0.20 (0.16 – 0.24)</u>

5. There are 26 different variants of CYP2C19 listed in the official website of allele nomenclature committee (www.cypalleles.ki.se). The *2, *3 and *17 alleles were analyzed in the present study. The contribution of other variants that were not analyzed cannot be ruled out. A statement about this matter should be added to the discussion.

Authors reply:

- The following sentence is now inserted Under **“Discussion” section Last paragraph on page 11.**

Limitation of present study includes that subjects were genotyped for the most common variant alleles in the respective population and other variants of the CYP2C19 alleles were not analyzed because of their low frequencies. However contribution of these rare variant alleles to the observed difference in enzyme activity between the two populations cannot be ruled out.

6. Definition of smoking was defined as “>2 cigarettes/day”. A reference or logic for this assumption could be provided.

Authors reply:

- This definition is a subjective definition of smoking and this factor as smoking has been studied before (*Eur J Clin Pharmacol* 2007; **63**:537-546, *Eur J Clin Pharmacol* 2008; **64**: 381-385) with the definition as a smoker; “smoking is to smoke between two or forty cigarettes per day”.
- We have now cited these papers as a reference under **“Materials and Methods” section on the 1st paragraph, last sentence on page 4.**

7. OC should be stated as “oral contraceptive” at the first place in the text.

Authors reply:

- The abbreviation for oral contraceptives (OC) is stated in the Abstract under Objectives
- It is now stated also at the first place in the text under **“Introduction” section on the last paragraph on page 3.**

8. Page 6 (Results, lines 18-19): The mean values should also be provided and a p value should be given.

Authors reply:

- The p value and mean value for Swedes and Koreans is now described under **"Result" Section, 1st paragraph on page 6** as follows

[\(p-value <0.00001\). The mean MR among Koreans was 3.04 while in Swedes 0.86.](#)

9. Page 6 (Results, lines: 30-32): It should be stated that subjects are non oral contraceptive.

Authors reply:

the following sentence is now inserted **"Result" Section, on page 7, 1st paragraph**

- "an MR of 0.91 was seen in Korean females as compared to 0.58 in Swedish women [who were none OC users](#) (p=0.022)

Reviewer: 2

General comments:

The article entitled "Interethnic and gender dependent differences in CYP2C19 activity. A comparison between Swedes and Koreans" by Margareta Ramsjö et al. studied the interethnic difference in CYP2C19 activity and investigated the effect of gender, smoking and oral contraceptives (OC). The authors found that in the genotype CYP2C19 *1/ *1 group, gender difference of CYP 2C19 activity was seen in Koreans where men had higher MR as compared to females (1.91 vs 0.91, $p < 0.0001$). No gender difference was seen among the Swedes. OC had a pronounced inhibitory effect among the Swedish women ($p < 0.00001$). No effect of smoking was found in Swedes and Koreans.

In my opinion, this study is well designed and the results are well presented with tables and figures. However the writing needs considerable improvement.

Specific comments:

Major

1. The title "Interethnic and gender dependent differences in CYP2C19 activity. A comparison between Swedes and Koreans" does not reflect the objectives of the study. I recommend the author revise the title and try to make it in accord with the objectives, main results and final conclusion. Providing there is no major change of the content, I would suggest the title be revised as "CYP2C19 activity comparison between Swedes and Koreans. Effect of gender, oral contraceptives (OC) and smoking".

Authors reply:

We agree and now have changed the title to "[CYP2C19 activity comparison between Swedes and Koreans: Effect of genotype, sex, oral contraceptives use and smoking](#)".

2. Present layout of the article is somewhat confusing and need improving:

Abstract:

Objectives, conclusions and the article title are not in accordance with one another.

Authors reply:

Introduction:

In this part, the authors should state the grounds for conducting the study and refer to what new information this article would provide. There needs a strong scientific rationale to conduct the study.

Authors reply:

- The rationale to conduct the study and aims of the study is now clearly stated in the introduction part "**Introduction**" section on the last paragraph on page 4 as follows.

["In general previous studies reported lower CYP2C19 activity among Asians compared to White population. Several factors may contribute for the observed inter ethnic differences in enzyme activity such as differences in functional](#)

variant allele frequencies between populations and other environmental factors such as dietary, oral contraceptive (OC) use or smoking habit. Indeed CYP2C19 defective variant alleles occur at a higher frequency in Asians as compared to whites and this may account partly for the observed lower CYP219 activity in this population. Furthermore previously it is reported that OC use inhibits CYP2C19 enzyme activity [18] and hence variation in OC use habit may also contribute to some extent. As to our knowledge no study has compared the enzyme activity between the two populations controlling for the effect of confounding factors such as oral contraceptive use and CYP2C19 genotype. The aim of the present study was to investigate the *CYP2C19* genotype phenotype relationship differences using the same methodology between healthy Swedish and Korean subjects having the same *CYP2C19* genotype, OC use and smoking habit by. ~~and to assess effect of sex, cigarette smoking and OC use on enzyme activity We evaluated results after administering the Karolinska cocktail with the use omeprazole as a probe drug for phenotyping of CYP2C19.~~ We find an important gender difference in the CYP2C19 activity in Koreans but not in Swedes and a generally lower CYP2C19 activity among Koreans in subjects homozygous for *CYP2C19*1*.

- L52-60 on P3 should sit in the part of results

Authors reply: Agreed. We have moved this sentence to the **Result section, 1st paragraph on page 6** as follows

The study encompassed 185 Swedes and 150 Koreans. We evaluated results after administering the Karolinska cocktail with the use omeprazole as a probe drug for phenotyping of CYP2C19.

- **Results:**

In order to avoid unnecessary confusion, recommend the author present the results in two parts, first the results of genotyping and then that of phenotyping.

Authors reply: Agreed: Now we have structured the result section under subheadings

- *CYP2C19 Genotype*
- *CYP2C19 Phenotype*
- *CYP2C19 phenotype comparison between Swedes and Koreans*

Discussion:

- The first paragraph states nothing new other than repeating the results. I recommend the author condense this paragraph.

Authors reply: We have now modified the first paragraph of the discussion as suggested (page 8)

- As to the reasons of gender difference, is it possible that the difference is related to the different probe drugs used?

Authors reply: We used the same probe drug omeprazole

- No effect of smoking was mentioned in this part.

Authors reply: Effect of smoking is now presented in the "**Result**" on page 8 and discussed in the "**Discussion# part on page 10-11**" as follows

Result:

There was no effect of smoking on MR neither in Swedes ($p=0.75$) nor in Koreans ($p=0.50$) in the genotype *CYP2C19* *1/ *1 by using the independent t-test. Considering smokers and non-smokers separately, effect of CYP2C19 genotype on enzyme activity remained significant in both Koreans ($p<0.0001$) and Swedes ($P<0.0003$). We further investigated for interaction between genotype, oral contraceptive use and smoking using factorial ANOVA in Swedes. Since there was only one OC user in Koreans we did not perform this analysis in Koreans. The result indicated only oral contraceptive use ($p<0.001$) and CYP2C19 genotype ($p<0.001$) but not smoking affects CYP2C19 activity. Regardless of smoking habit, oral contraceptive use inhibited and CYP2C19 genotype influenced CYP2C19 enzyme activity.

Discussion:

Our result indicated no significant effect of cigarette smoking on CYP2C19 enzyme activity in both populations. Even controlling for the effect of genotype and OC use there was no significant difference between smokers and non-smokers on enzyme activity.

Among cytochrome P450 drug metabolizing enzymes that expressed in the liver, CYP1A2 is the only one whose activity is affected by smoking habit [20]. Previous studies reported interaction between smoking and CYP2C19 genotype in influencing H.Pylori eradication success and suggested interaction between smoking and CYP2C19 should be examined in the future [29]. Apparently effect of smoking on H.Pylori eradication might be not by directly altering CYP2C19 enzyme activity and hence omeprazole metabolism.

- Clinical implications of the present study should be discussed. The author discussed very little about this issue.

Authors reply: The clinical implications of our study is now discussed in the "Discussion# part on page 11 as follows

The finding of significantly lower CYP2C19 enzyme activity in Koreans compared to Swedes that is not accountable or explained by the common CYP2C19 genotype or OC use may have clinical relevance. In clinical practice the common CYP2C19 SNPs that are recommended or practiced to genotype for include CYP2C19*2 and *3 in Asians. Our Study indicates genotyping for these SNPs only may not provide full information about individual's metabolic capacity or phenotype. Alternatively, phenotyping with a safe probe drug to reflect an individual's metabolic capacity or therapeutic drug monitoring may need to be considered. Our study can be a base for further investigation to identify the underlying genetic, epigenetic or environmental factors responsible for the observed wide differences in CYP2C19 enzyme activity between Koreans and Swedes.

- Limitation of present study should be discussed briefly

Authors reply: Limitation of present study now described under "Discussion" section Last paragraph on page 11 as follows.

Limitation of present study includes that subjects were genotyped for the most common variant alleles in the respective population and other variants of the CYP2C19 alleles were not analyzed because of their low frequencies. However contribution of these rare

[variant alleles to the observed difference in enzyme activity between the two populations cannot be ruled out.](#)

3. There are some spelling errors and the linguistics need improving. The authors should seek the services of those fluent in English before submitting the next version.

Authors reply: In the revised manuscript we have done our best to correct spelling errors and improve the linguistics.

Minor:

- ? "indeed was the case, i.e. that the reporting was true." (Line 51, page 7)

Authors reply: This sentence is now removed from the **Discussion part 1st paragraph on page 9**

- "EM female", EM here should be written in full for the first appearance in the article (Line 10, page 8)

Authors reply: This abbreviation EM is now fully stated where it first appears in the text **Discussion part 1st paragraph on page 9**

- "...could be investigated cf. [21].", cf should be deleted. (Line 39 and 46, page 8)

"indeed" is superfluous (Line 15, page 9)

"effect)" should be "effect" (Line 22, page 9)

? "would not drastically", here would or would not? (Line 39, page 9)

Authors reply: We agree and now made the correction in the revised manuscript as indicated in the **Discussion part on page 9 and 10.**

CYP2C19 activity comparison between Swedes and Koreans: Effect of genotype, gender, oral contraceptives use and smoking ~~Interethnic and gender dependent differences in CYP2C19 activity. A comparison between Swedes and Koreans~~

Margareta Ramsjö¹, Eleni Aklillu¹, Lilleba Bohman¹, Magnus Ingelman-Sundberg², Hyung-Keun Roh³ and Leif Bertilsson¹.

¹Department of Laboratory Medicine at Karolinska Institutet, Division of Clinical Pharmacology, Karolinska University Hospital-Huddinge C1:68, Stockholm, Sweden

²Department of Pharmacology and Physiology, Section of Pharmacogenetics, Karolinska Institutet, Stockholm, Sweden

³Department of Internal Medicine, Division of Clinical Pharmacology, Gachon University Hospital, Incheon, Korea

Corresponding Author

Eleni Aklillu, B.Pharm, M.Sc, PhD

Division of Clinical Pharmacology, Department of Laboratory Medicine,

Karolinska University Hospital Huddinge, Karolinska Institutet,

SE-14186 Stockholm, Sweden;

Phone: + 46 8 58 58 78 82; Fax: + 46 8 58 58 10 70,

E-mail: Eleni.Aklillu@ki.se

Abstract

Objectives To compare CYP2C19 enzyme activity between Swedes and Koreans controlling for the effect of CYP2C19 genotype, oral contraceptive use and smoking habit. ~~investigate the CYP2C19 geno phenotype relationship and the effect of smoking and oral contraceptives (OC) on enzyme activity in Swedes and Koreans.~~

Methods CYP2C19 activity was determined in 185 healthy Swedish and 150 Korean subjects as omeprazole/5-hydroxyomeprazole ratio (metabolic ratio; MR) ~~determined~~ using high-performance liquid chromatography. Genotyping was performed by PCR using Taqman assay.

Results As expected, a higher incidence of poor metabolizers (PM) was found in Koreans (14%) compared to Swedes (3.8%) and the frequency of the *CYP2C19*17* allele was very low in Koreans 0.3%. Among subjects homozygous for *CYP2C19*1* Koreans displayed significantly lower CYP2C19 enzyme activity than Swedes ($p < 0.000001$). ~~and interestingly~~ Interestingly in Koreans a pronounced gender difference was apparent: females ($n=24$) had significantly lower MR than males ($N=30$) ($p < 0.0001$) but such a gender difference was not seen among Swedes. Swedish OC users had a higher MR than non-users ($p < 0.00001$), whereas OC was only used by one Korean. No effect of smoking was observed.

Conclusions -We find specific gender dependent effects of CYP2C19 activity in Koreans but not in Swedes. Controlling for the effect of genotype and sex, Koreans display lower CYP2C19 activity than Swedes. The genetic, epigenetic or environmental basis for this difference ~~has~~ remains to be identified.

Keywords *CYP2C19*, omeprazole, gender, Korean, Swedes

Introduction

The enzyme CYP2C19 catalyses the metabolism of a wide variety of drugs such as antidepressants [1], benzodiazepines [2], proguanil [3] and proton pump inhibitors (PPIs) [4]. The major metabolites of omeprazole detected *in vivo* in human plasma are 5-hydroxyomeprazole formed by CYP2C19 and omeprazole sulphone formed by CYP3A4 [5, 6]. The metabolic disposition of omeprazole correlates well with the rate of S-mephenytoin hydroxylation [7] and those two drugs are the most commonly used probes of CYP2C19. About 3% of Caucasians are PM of S-mephenytoin [8, 9] and a higher incidence of PMs has been reported in Japanese (18-23%) [10, 11], Chinese (15-17%) [12, 13] and in Korean subjects (13-16%) [14]. The PM phenotype is caused by the *CYP2C19*2* [15] and *CYP2C19*3* alleles although the *CYP2C19*3* is very uncommon in Caucasian PMs [16]. Sim et al [17] identified a novel allele (*CYP2C19*17*) that was associated with increased CYP2C19 activity *in vivo* in two different ethnic populations. This allele is associated with higher levels of gene transcription and increased rates of omeprazole and mephenytoin metabolism. The frequency of this allele was 18% in both Swedes and Ethiopians but only 4% in Chinese subjects [17].

~~Christensen et al [18] developed the Karolinska cocktail based on five probe drugs to phenotype the enzymes CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP2C19. In general previous studies reported lower CYP2C19 enzyme activity among Asians compared to White population. Several factors may contribute for the observed inter ethnic differences in enzyme activity such as differences in functional variant allele frequencies between populations and other environmental factors like dietary, oral contraceptive (OC) use or smoking habit. Indeed *CYP2C19* defective variant alleles occur at a higher frequency in Asians as compared to whites and this may account partly for the observed lower CYP219 activity in this population. Furthermore previously it is reported that OC use inhibits CYP2C19 enzyme activity [18] and~~

hence variation in OC use habit may also contribute to some extent. As to our knowledge no study has compared the enzyme activity between the two populations controlling for the effect of confounding factors such as oral contraceptive use and CYP2C19 genotype. The aim of the present study was to investigate the CYP2C19 genotype phenotype relationship differences using the same methodology between healthy Swedish and Korean subjects having the same CYP2C19 genotype, OC use and smoking habit. ~~and to assess effect of sex, cigarette smoking and OC use on enzyme activity. We evaluated results after administering the Karolinska cocktail with the use omeprazole as a probe drug for phenotyping of CYP2C19.~~ We find an important gender difference in the CYP2C19 activity in Koreans but not in Swedes and a generally lower CYP2C19 activity among Koreans in subjects homozygous for CYP2C19*1.

Materials and Methods

Study Subjects

We studied 185 healthy Swedish subjects at the Karolinska University Hospital, Huddinge, Sweden and 150 healthy Korean subjects at the Inha University Hospital, Incheon, Korea. The subjects were mainly students and staff at the two hospitals. In the Swedish subjects the mean age was $29 \pm \text{SD } 8.3$ years (range 18-60) with the mean weight $69 \pm \text{SD } 12$ kg (range 47-109) (no data on weight in two subjects). The Swedish subjects consisted of 112 women and 73 men and 39 (11 men and 28 women) were classified as smokers (definition of smokers > 2 cigarettes/ day) as described previously [19, 20]. There were 44 women using OC. Among the 28 women smokers, 6 were OC user. In four women no data on OC use was available. In the Korean subjects the mean age was 25 ± 3.9 years (range 20-46) with the mean weight 62 ± 12 kg (range 38-94). The Korean subjects consisted of 74 women and 76 men and 29 of the subjects were classified as smokers (26 men and 3 women of whom none were OC user). In one subject information on smoking habit was unavailable. Only one Korean woman was a

user of OC. [The participants were asked if they used OC but not which kind of contraceptives they used.](#)

The local Ethics committees at Karolinska Institutet, Stockholm, Sweden and Inha University, Incheon, Korea approved the study and it was performed in accordance with the Helsinki Declaration. After written and oral information about the study, the participants were screened by clinical laboratory tests and interviewed for their medical histories. The subjects were not allowed to use other medications (except for OC) including herbal medicines one week before the study started. They refrained from alcohol, grapefruit juice during 2 days and caffeine products during 1, 5 days before the study started. The Karolinska cocktail included omeprazole 20 mg (enterotablet of Losec Mups 20 mg; Hässle, Stockholm, Sweden), losartan 25 mg, caffeine 100 mg, quinine 250 mg and debrisoquine 10 mg [21].

Phenotyping

After an 8-hour overnight fast, the subjects took the drug omeprazole at 8 a.m. Three hours after the intake of omeprazole, blood samples were collected and centrifuged, plasma was separated and stored frozen at -20° until analysis by reversed phase HPLC with UV detection. The Korean plasma and DNA samples were packed on dry ice and sent to Sweden for analysis. The method for measuring omeprazole and its metabolite 5-hydroxyomeprazole was described by Tybring et al [22] and modified by Christensen et al [21].

Genotyping

Blood samples were collected using tubes containing EDTA. Genomic DNA was extracted from leukocytes by using QIAamp®DNA Mini Blood Kit (distributed by AB Applied Bio

systems, California, USA and Chestrate, United Kingdom). Genotyping methods are based on the PCR (Polymerase-chain-reaction) technique and specific primers to different parts of the gene. Allelic discrimination was determined by using a Taqman assay. Genotyping of *CYP2C19*17* was carried out by PCR with specific primers as described by Sim et al [17].

Statistical analysis

The individual metabolic ratio MR of omeprazole was determined by dividing the molar concentrations of omeprazole and 5-hydroxyomeprazole in plasma.

The MRs was log transformed before application of statistical analysis. For comparisons of Swedes and Koreans within each genotype group and between groups based on smoking and OC use the independent t-test was used. P-Values less than 0.05 were considered statistically significant. Statistical analyses were performed by Statistical version 7 (Stat Soft® Scandinavia AB).

Results

CYP2C19 genotype: The study encompassed 185 Swedes and 150 Koreans. We evaluated results after administering the Karolinska cocktail with the use omeprazole as a probe drug for phenotyping of CYP2C19. The genotype analyses revealed allele frequencies of *CYP2C19*2*, and **17* in the Swedes of 16 and 20 %, respectively and 28, 11 and 0.3 % of **2*, **3* and **17* in the Koreans, respectively (Table 1). In both Koreans and Swedes, there was no significant difference between the observed and expected genotype frequency according to Hardy–Weinberg law (χ^2 test, $p>0.05$).

CYP2C19 phenotype: The subjects were phenotyped for omerazole and Figure 1 shows histograms of the MR (omeprazole/5-hydroxyomeprazole). A bimodal distribution especially

in Koreans with an antimode of about 10 was observed. The median MR in Koreans was substantially higher (2.57) as compared to the Swedes (0.71) (p-value <0.00001). The mean MR among Koreans was 3.04 while in Swedes 0.86.

CYP2C19 phenotype comparison between Swedes and Koreans: For statistical analysis and comparison of CYP2C19 enzyme activity, subjects were first stratified based on CYP2C19 genotype. The subjects homozygous for *CYP2C19*1* were further stratified for gender and intake of OC (Figure 2). Interestingly the Koreans homozygous for *CYP2C19*1* had significantly higher MR for omeprazole; the MR in Korean men was 1.91 vs. 0.56 in Swedish men ($p < 0.000001$) and an MR of 0.91 was seen in Korean females as compared to 0.58 in Swedish women who were none OC users ($p = 0.022$). In Koreans a gender difference was seen where men ($n = 30$) had higher MR (1.91) as compared to females ($n = 24$) (0.91) ($p < 0.0001$). By contrast, no gender differences were seen among the Swedes. Among the Swedish women, OC had a pronounced inhibitory effect ($p < 0.00001$), whereas no Korean female on OC was included in the study since OC usage was only reported by one Korean woman.

The results of the phenotyping of the two populations are also shown stratified according to the number of active genes (with *CYP2C19*17* excluded) with the two genders together (Figure 3). Korean subjects homozygous for *CYP2C19*1* had much higher MR (1.48) than the Swedish individuals (0.58) ($p < 0.000001$). A similar difference was also noted when comparing those with one functional allele (2.88 vs 1.10, $p < 0.0012$) and also a small difference (28.8 vs 20.9, $p < 0.028$) was noted among those homozygous for null alleles although the number of subjects in the Swedish **0/*0* group is very low.

The *CYP2C19**17 allele was only present in one Korean subject. Analysis of the MR for omeprazole among Swedes revealed that the MR in *17/ *17 was significantly lower (0.18, n=4) than in *1/ *1 (0.58, n=56, p=0.016) and *1/ *17 (0.51, n=35; p=0.048), in line with our previous results [17].

There was no effect of smoking on MR neither in Swedes (p=0.75) nor in Koreans (p=0.50) in the genotype *CYP2C19* *1/ *1 by using the independent t-test. Considering smokers and non-smokers separately, effect of *CYP2C19* genotype on enzyme activity remained significant in both Koreans (p<0.0001) and Swedes (P<0.0003). We further investigated for interaction between genotype, oral contraceptive use and smoking using factorial ANOVA in Swedes. Since there was only one OC user in Koreans we did not perform this analysis in Koreans. The result indicated only oral contraceptive use (p<0.001) and *CYP2C19* genotype (p<0.001) but not smoking affects *CYP2C19* activity. Regardless of smoking habit, oral contraceptive use inhibited and *CYP2C19* genotype influenced *CYP2C19* enzyme activity.

Discussion

In the ~~current investigation~~ present study we compared *CYP2C19* enzyme activity between Koreans and Swedes using the same methodology and taking into consideration effect of genotype, sex, OC use. Our major finding include ~~we found~~ quite unexpected and very interesting differences in the *CYP2C19* phenotype between Koreans and Swedes belonging to the same genotype group, namely for those homozygous for *CYP2C19**1. Phenotyping data based on the probe drug omeprazole revealed that the Korean men had a much higher MR for omeprazole than the Swedish men. (~~p<0.000001, Figure 2).~~ Interestingly Korean women were significantly more rapid for omeprazole metabolism than the Korean men (~~p<0.0001~~). Still the Korean women were slower than Swedish women without intake of oral

contraceptives, but the difference was much less than observed between Swedish and Korean men. Oral contraceptive intake did decrease the rate of omeprazole metabolism in Swedish women to approach a similar MR for omeprazole as Korean men. ~~Only one Korean woman was reported to take oral contraceptives and the relatively low MRs seen in among female Koreans indicate that this indeed was the case, i.e. that the reporting was true.~~ Korean subjects carrying one null allele were also slower as compared to Swedes and a slight difference was seen between men and women homozygous for null alleles (Figure 3), but this difference was very small and the number of Swedish subjects in this group was also very low. Stratifying the phenotype data for smokers or nonsmokers revealed no influence by of smoking habit on CYP2C19 enzyme activity.

Previously a small hardly significant gender difference in CYP2C19 activity was observed in Chinese using S-mephenytoin as a probe drug [23]. Here the S/R-ratio for mephenytoin was lower in homozygous ~~EM~~ extensive metabolizers (EM) females than in homozygous EM males (0.22 +/- 0.14 vs. 0.33 +/- 0.09; $p = 0.046$). By using omeprazole as a probe drug in the current study in Koreans, ~~interestingly~~ much higher significance values were obtained for a gender difference ~~indeed~~ indicating that such a gender difference ~~indeed~~ is true in some Asian populations.

The mechanism behind the gender difference in Koreans in CYP2C19 activity is unknown. One could imagine environmental, epigenetic or genetic reasons. Regarding environmental influence differences in diets or drug habits between Korean men and women are unlikely, but could not be excluded. Also one could hypothesize that hormonal or environmental influence should result in gender specific epigenetic control of the *CYP2C19* gene expression which would influence the activity. This cannot be excluded and a true evaluation would require

liver samples from both sexes where the pattern of gene methylation as well as histone acetylation and methylation could be investigated ~~ref.~~ [24]. Perhaps the most likely explanation is of true genetic origin. The differences in sequence between the Caucasian and Korean *CYP2C* locus as revealed from whole genome sequencing in the *CYP2C* locus is extensive and encompasses several hundreds of mutations ~~ref.~~[25]. One could therefore suggest that the Korean variant of the *CYP2C19*1* allele carries mutations that makes it less expressed as compared to the Swedish variant, and at the same time there are mutations in this locus that makes the Korean *CYP2C19*1* gene more sensitive for the estrogen receptor or other nuclear factors controlled by the action of estrogens, thereby causing an increased rate of CYP2C19 activity in the Korean females as compared to males. Further studies have to be designed that investigate these alternatives before the basis for the gender difference is understood.

The Swedish females taking oral contraceptives had lower CYP2C19 activity as compared to women without. Tamminga et al. have also describes an effect of OCs using S-mephynotoin as a probe drug [26]. The oral contraceptives may contain progestin with or without ethinyl oestradiol and it is not known whether it is the oestrogen or the progestin component that inhibits drug metabolism [10]. The inhibition of CYP2C19 activity by OC seems to be due to the ethinyloestradiol component of OCs [27, 28]. The mechanism behind this effect is unknown but has been suggested to be interaction at the active site between the hormones and the CYP2C19 substrates. A mechanism where the oral contraceptives directly inhibit the rate of CYP2C19 expression can however not be excluded.

Our result indicated no significant effect of cigarette smoking on CYP2C19 enzyme activity in both populations. Even controlling for the effect of genotype and OC use there was no

significant difference between smokers and non-smokers on enzyme activity. Among cytochrome P450 drug metabolizing enzymes that expressed in the liver, CYP1A2 is the only one whose activity is affected by smoking habit [20]. Previous studies reported interaction between smoking and CYP2C19 genotype in influencing H.Pylori eradication success and suggested interaction between smoking and CYP2C19 should be examined in the future [29]. Apparently effect of smoking on H.Pylori eradication might be not by directly altering CYP2C19 enzyme activity and hence omeprazole metabolism.

The finding of significantly lower CYP2C19 enzyme activity in Koreans compared to Swedes that is not accountable or explained by the common CYP2C19 genotype or OC use may have some clinical relevance. In clinical practice the common CYP2C19 SNPs that are recommended or practiced to genotype for include *CYP2C19**2 and *3 in Asians. Our Study indicates genotyping for these SNPs only may not provide full information about individual's metabolic capacity or phenotype. Alternatively, phenotyping with a safe probe drug to reflect an individual's metabolic capacity or therapeutic drug monitoring may need to be considered. Our study also can be a base for further investigation to identify the underlying genetic, epigenetic or environmental factors responsible for the observed wide differences in CYP2C19 enzyme activity between Koreans and Swedes.

Limitation of present study includes that subjects were genotyped for the most common variant alleles in the respective population and other variants of the *CYP2C19* alleles were not analyzed because of their low frequencies. However contribution of these rare variant alleles to the observed difference in enzyme activity between the two populations cannot be ruled out. In summary, the present study describes an interesting gender difference in the CYP2C19 enzyme activity in Koreans which cannot be seen in Swedes. Further studies are needed to

evaluate the mechanisms behind this difference, which however, would ~~not~~ drastically influence the pharmacokinetics of CYP2C19 drugs as to motivate gender specific dosing regimens in Koreans.

Acknowledgment

The study was financially supported by the Swedish Research Council, Medicine, 3902 and the Swedish Capio Forskningsstiftelse.

For Peer Review

References

1. Brosen K (2004) Some aspects of genetic polymorphism in the biotransformation of antidepressants. *Therapie* 59 (1): 5-12
2. Bertilsson L, Henthorn TK, Sanz E, Tybring G, Sawe J, Villen T (1989) Importance of genetic factors in the regulation of diazepam metabolism: relationship to S-mephenytoin, but not debrisoquin, hydroxylation phenotype. *Clin Pharmacol Ther* 45 (4): 348-355
3. Ward SA, Helsby NA, Skjelbo E, Brosen K, Gram LF, Breckenridge AM (1991) The activation of the biguanide antimalarial proguanil co-segregates with the mephenytoin oxidation polymorphism--a panel study. *Br J Clin Pharmacol* 31 (6): 689-692
4. Li XQ, Andersson TB, Ahlstrom M, Weidolf L (2004) Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos* 32 (8): 821-827
5. Andersson T, Miners JO, Veronese ME, Tassaneeyakul W, Meyer UA, Birkett DJ (1993) Identification of human liver cytochrome P450 isoforms mediating omeprazole metabolism. *Br J Clin Pharmacol* 36 (6): 521-530
6. Laine K, Tybring G, Bertilsson L (2000) 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* 68 (2): 151-159
7. Chang M, Dahl ML, Tybring G, Gotharson E, Bertilsson L (1995) Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. *Pharmacogenetics* 5 (6): 358-363.

8. Alvan G, Bechtel P, Iselius L, Gundert-Remy U (1990) Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. *Eur J Clin Pharmacol* 39 (6): 533-537
9. Jurima M, Inaba T, Kadar D, Kalow W (1985) Genetic polymorphism of mephenytoin p(4')-hydroxylation: difference between Orientals and Caucasians. *Br J Clin Pharmacol* 19 (4): 483-487
10. Nakamura K, Goto F, Ray WA, McAllister CB, Jacqz E, Wilkinson GR, Branch RA (1985) Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Caucasian populations. *Clin Pharmacol Ther* 38 (4): 402-408
11. Horai Y, Nakano M, Ishizaki T, Ishikawa K, Zhou HH, Zhou BI, Liao CL, Zhang LM (1989) Metoprolol and mephenytoin oxidation polymorphisms in Far Eastern Oriental subjects: Japanese versus mainland Chinese. *Clin Pharmacol Ther* 46 (2): 198-207
12. Bertilsson L, Lou YQ, Du YL, Liu Y, Kuang TY, Liao XM, Wang KY, Reviriego J, Iselius L, Sjoqvist F (1992) Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquin and S-mephenytoin. *Clin Pharmacol Ther* 51 (4): 388-397.
13. Ishizaki T, Sohn DR, Kobayashi K, Chiba K, Lee KH, Shin SG, Andersson T, Regardh CG, Lou YC, Zhang Y, et al. (1994) Interethnic differences in omeprazole metabolism in the two S-mephenytoin hydroxylation phenotypes studied in Caucasians and Orientals. *Ther Drug Monit* 16 (2): 214-215
14. Roh HK, Dahl ML, Tybring G, Yamada H, Cha YN, Bertilsson L (1996) CYP2C19 genotype and phenotype determined by omeprazole in a Korean population. *Pharmacogenetics* 6 (6): 547-551

15. de Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA (1994) The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J Biol Chem* 269 (22): 15419-15422
16. De Morais SM, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, Goldstein JA (1994) Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese. *Mol Pharmacol* 46 (4): 594-598.
17. Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 79 (1): 103-113
18. Sandberg M, Johansson I, Christensen M, Rane A, Eliasson E (2004) The impact of CYP2C9 genetics and oral contraceptives on cytochrome P450 2C9 phenotype. *Drug Metab Dispos* 32 (5): 484-489
19. Djordjevic N, Ghotbi R, Bertilsson L, Jankovic S, Aklillu E (2008) Induction of CYP1A2 by heavy coffee consumption in Serbs and Swedes. *Eur J Clin Pharmacol* 64 (4): 381-385
20. Ghotbi R, Christensen M, Roh HK, Ingelman-Sundberg M, Aklillu E, Bertilsson L (2007) Comparisons of CYP1A2 genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans. *Eur J Clin Pharmacol* 63 (6): 537-546
21. Christensen M, Andersson K, Dalen P, Mirghani RA, Muirhead GJ, Nordmark A, Tybring G, Wahlberg A, Yasar U, Bertilsson L (2003) The Karolinska cocktail for phenotyping of five human cytochrome P450 enzymes. *Clin Pharmacol Ther* 73 (6): 517-528

22. Tybring G, Bottiger Y, Widen J, Bertilsson L (1997) Enantioselective hydroxylation of omeprazole catalyzed by CYP2C19 in Swedish white subjects. *Clin Pharmacol Ther* 62 (2): 129-137
23. Xie HG, Huang SL, Xu ZH, Xiao ZS, He N, Zhou HH (1997) Evidence for the effect of gender on activity of (S)-mephenytoin 4'-hydroxylase (CYP2C19) in a Chinese population. *Pharmacogenetics* 7 (2): 115-119
24. Gomez A, Ingelman-Sundberg M (2009) Pharmacoeugenetics: its role in interindividual differences in drug response. *Clin Pharmacol Ther* 85 (4): 426-430
25. Kim JI, Ju YS, Park H, Kim S, Lee S, Yi JH, Mudge J, Miller NA, Hong D, Bell CJ, Kim HS, Chung IS, Lee WC, Lee JS, Seo SH, Yun JY, Woo HN, Lee H, Suh D, Kim HJ, Yavartanoo M, Kwak M, Zheng Y, Lee MK, Kim JY, Gokcumen O, Mills RE, Zaranek AW, Thakuria J, Wu X, Kim RW, Huntley JJ, Luo S, Schroth GP, Wu TD, Kim H, Yang KS, Park WY, Church GM, Lee C, Kingsmore SF, Seo JS (2009) A highly annotated whole-genome sequence of a Korean individual. *Nature* 460 (7258): 1011-1015
26. Tamminga WJ, Wemer J, Oosterhuis B, Weiling J, Wilffert B, de Leij LF, de Zeeuw RA, Jonkman JH (1999) CYP2D6 and CYP2C19 activity in a large population of Dutch healthy volunteers: indications for oral contraceptive-related gender differences. *Eur J Clin Pharmacol* 55 (3): 177-184
27. Hagg S, Spigset O, Dahlqvist R (2001) Influence of gender and oral contraceptives on CYP2D6 and CYP2C19 activity in healthy volunteers. *Br J Clin Pharmacol* 51 (2): 169-173
28. Palovaara S, Tybring G, Laine K (2003) The effect of ethinylloestradiol and levonorgestrel on the CYP2C19-mediated metabolism of omeprazole in healthy female subjects. *Br J Clin Pharmacol* 56 (2): 232-237

29. Suzuki T, Matsuo K, Sawaki A, Wakai K, Hirose K, Ito H, Saito T, Nakamura T, Yamao K, Hamajima N, Tajima K (2007) Influence of smoking and CYP2C19 genotypes on *H. pylori* eradication success. *Epidemiol Infect* 135 (1): 171-176

For Peer Review

Table 1 [Distribution of CYP2C19 allele frequencies with the respective 95% CI](#) in the Swedish and Korean populations—~~analysed~~. *CYP2C19*1* denotes alleles not identified as *2, *3 or *17. The *3 allele was not analysed in the Swedish cohort due to its very rare occurrence.

population	n	Allele frequencies (%)			
		*1	*2	*3	*17
Swedish	185	63.8	16.2	n.d	20.0
Korean	150	60.7	27.7	11.3	0.3

n= number of subjects, n.d= not determined

CYP2C19 allele	Koreans (n=150)	Swedes (n=185)
CYP2C19*1	0.61 (0.55 – 0.66)	0.64 (0.59 – 0.69)
CYP2C19*2	0.28 (0.23 – 0.33)	0.16 (0.12 – 0.20)
CYP2C19*3	0.11 (0.08 – 0.15)	n.d
CYP2C19*17	0.003 (-0.003 – 0.01)	0.20 (0.16 – 0.24)

[n= number of subjects, n.d= not determined](#)

Legends to figures.

Fig. 1

The distribution of the log metabolic ratio (MR) of omeprazole/5- hydroxyomeprazole in all Swedes (n= 185) and Koreans (n= 150) participating in this study. The arbitral vertical line indicates the position of omeprazole/5-hydroxyomeprazole ratio = 1. The respective median MR is indicated by arrow.

Fig.2

Effect of oral contraceptives (OC) and gender on omeprazole/5-hydroxyomeprazole ratio in Swedes and in Koreans with the genotype *CYP2C19* *1 / *1. Only one Korean female was user of OC and she has been excluded here. *CYP2C19**1 was assigned in the absence of *2,*3 or *17. The arbitral vertical line indicates MR (omeprazole/5-hydroxyomeprazole ratio) = 1. The respective median MR is indicated by arrow.

Fig.3

The distribution of omeprazole/5-hydroxyomeprazole ratio in genotypes * 1/* 1, * 1/* 0 and *0/ *0 in Swedish and Koreans without oral contraceptives (NOC) and without the * 17 allele. The respective median MR is indicated by arrow. In the Swedish samples *0= *2 allele and in Korean samples *0 = *2 or * 3 alleles. The arbitral vertical line indicates the position of omeprazole/5-hydroxyomeprazole ratio = 1.