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# **CYP2C19 AND CYP2C9 GENO-AND PHENOTYPES IN HEALTHY SWEDISH AND KOREAN SUBJECTS**

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“True knowledge exists in knowing that you know nothing”.

**Sokrates** (470 f.Kr-399 f.Kr)



## ABSTRACT

Cytochrome P450s (CYPs) are responsible for approximately 75% of the phase I-dependent drug metabolism. Several important polymorphisms in these enzymes are known to affect the individual drug response. CYP2C19 and CYP2C9 are both polymorphic enzymes and are responsible for the metabolism of many therapeutically used drugs, e.g. anticoagulants, antidepressants and antiulcer drugs. This thesis focuses on comparing genotypes and phenotypes in healthy Swedish and Korean subjects. A higher incidence of poor metabolizers (PMs) was found in the enzyme CYP2C19 in Koreans (14%) compared to Swedes (4%). The frequency of the CYP2C19\*2 allele was 16% and 28% in Swedes and Koreans, respectively. The Asian specific \*3 allele was present in 11% of Korean alleles. The frequency of the CYP2C19\*17 allele was very low in Koreans (0.3%) compared to Swedes (20%) and this allele caused an increased enzyme activity in the Swedish subjects. Among subjects homozygous for CYP2C19\*1, Koreans displayed significantly lower CYP2C19 enzyme activity than Swedes ( $p < 0.000001$ ). In Koreans a pronounced gender difference was apparent and females ( $n=24$ ) had significantly lower metabolic ratio (MR) of omeprazole and its metabolite hydroxyomeprazole than males ( $n=30$ ;  $p < 0.0001$ ). Such a gender difference was not seen among Swedes. Controlling for the effect of genotype and sex, Koreans display lower CYP2C19 activity than Swedes. Swedish females who used oral contraceptives (OC) had higher MR (lower activity) than non users (NOC) ( $p < 0.00001$ ). No effects of smoking were observed. A higher MR of losartan was found in Swedes compared to Koreans. The allele frequency of CYP2C9 \*2 was 10% in the Swedes and due to its infrequent occurrence not determined in Koreans. The frequency of the allele CYP2C9\*3 was higher in Swedes (10%) compared to Koreans (6%). Swedish females user of OC had a higher MR than NOC in the genotype group \*1/\*1 ( $p=0.001$ ). Only one Korean woman was user of OC. The woman in the case report in this thesis required treatment with high doses of phenytoin. When fluconazole, a potent inhibitor of CYP2C9 was added to the treatment regimen, the patient developed adverse drug reactions. The losartan MR was  $< 0.13$  for this patient which is lower than any of the 190 healthy Swedish subjects used for comparison. The patient is thus an UM (ultrarapid metabolizer) of the CYP2C9 substrates phenytoin and losartan. Ultrarapid metabolism of drugs may have a genetic, epigenetic or environmental basis that can explain the clinically observed differences in the metabolism of drugs.

## LIST OF PUBLICATIONS

The thesis is based on the following papers:

- I                                    **Ramsjö M**, Aklillu E, Bohman L, Ingelman-Sundberg,  
Roh H-K and Bertilsson L (2010). Interethnic and gender dependent  
differences in CYP2C19 activity. A comparison between Swedes and  
Koreans. *Eur J Clin Pharmacol*.66:871-7.
  
- II                                    **Ramsjö M**, \*, Sandberg Lundblad M\*, Bohman L, Kang H-J  
Roh H-K, Eliasson E, Aklillu E and Bertilsson L (2010). CYP2C9 geno- and  
phenotypes- in Swedish and Korean healthy subjects with focus on genetics  
and environmental factors (unpublished).  
\*M.R. and M- S-L contributed equally and share first authorship.
  
- III                                    Helldén A, Bergman U, Engström Hellgren K, Masquelier M,  
Nilsson Remahl I, Odar-Cederlöf I, **Ramsjö M** and Bertilsson L(2010).  
Fluconazole-induced intoxication with phenytoin in a patient with ultra-high  
activity of CYP2C9. *Eur J Clin Pharmacol* 66:791-5.

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## GLOSSARY

Acetylcholin (ACh)	a neurotransmitter in the peripheral nervous system (PNS) and the central nervous system (CNS)
Allele	one of several forms of a gene or DNA sequence at a specific chromosomal location
Chromosome	the genetic structures of cells containing the cellular DNA (contains many genes) regulatory elements and other nucleotide sequences that bears the genetic codes in an organism.
Exon	nucleic acid sequence that codes for the mature messenger RNA product
Gene	a gene contains genomic sequences corresponding to a unit of inheritance in a living organism.
Genetic polymorphism	genetic variation that results in different forms of individuals among the same population living in varied environments.
Genotype	genetic constitution of an individual, either overall or at a specific locus.
Heterozygous	containing two different alleles of the same gene
Homozygous	containing two copies of the same allele
Metabolism	conversion to another chemical species
Mutation	a permanent transmissible change in the genetic material
Pharmacodynamics	the effect(s) produced by a drug
Pharmacogenetics	the study or clinical testing of genetic variation in humans or laboratory organisms that gives variations in drug responses
Pharmacokinetics	the time course of the concentration of a drug and its metabolites in the body including processes as absorption, distribution, metabolism and excretion
Phenotype	the appearance or observable nature of an individual
Polymorphism	occurrence of more than one form; existence in the same species or other natural group of more than one morphologic type
Probe drug	a marker drug for a specific metabolizing enzyme
Ultra rapid metabolizer(UM)	high activity of the liver enzyme



# 1 INTRODUCTION

Patients show large interindividual variability in drug response. Inter/intra-individual variation in drug response can be of genetic, physiological (age, sexes) pathological (diseases) and/or environmental origin. Drug concentrations in plasma can vary between two individuals of the same weight on the same drug dosage. Genetic factors can account for 15-30% of interindividual differences in drug metabolism and response, but for certain classes of drugs, genetic factors are of utmost importance and can account for up to 95% of interindividual variability in drug response [1].

The metabolic conversion of drugs is mainly enzymatic and cytochrome P450 enzymes (CYPs) belong to a large protein family and are the most important group of xenobiotic metabolizing enzymes in humans. CYPs are characterized by a wide interindividual and intraindividual variability in enzyme activity. Major sources of these differences in enzyme activity are environmental factors and this thesis is focused on the CYP2C19 and CYP2C9 genes and factors that may affect variation in the enzyme activities.

## 1.1 DRUG METABOLISM

The concentration of a drug at its site of action or the plasma concentration achieved after administration of a drug and is determined by the following processes; absorption, distribution, metabolism and elimination. Drug metabolism occurs mainly in the liver, and usually converts lipophilic drugs to more polar metabolites prior to elimination. The drug metabolism is normally divided into phase I and phase II reactions. In phase I, which represents many of cytochrome P450 enzymes (CYP) a polar group is introduced to the parent drug through oxidation, reduction or hydrolysis. The more polar intermediates from phase I reactions may be conjugated with water-soluble groups in phase II reactions through the actions of uridine diphosphate (UDP), glucuronosyltransferase (UGTs), sulfotransferase to further facilitate excretion. Cytochrome P450s (CYP) are the most important phase I drug metabolizing enzymes and the enzyme systems are primarily located in the smooth endoplasmic reticulum of the liver which is the principal organ of drug metabolism, although every biological tissue has some ability to metabolize drugs.

## 1.2 CYTOCHROME P450 SYSTEM

The cytochrome P450s (CYP) are heme containing proteins found in most tissues with the greatest portion found in the liver. Cytochrome P450 was first named in 1961 because of an absorbance maximum at 450 nm spectral peaks when reduced and bound to carbonmonoxide [2]. The enzymes of the cytochrome P450 superfamily are classified to amino acid sequence homology [3]. The P450 nomenclature follows that of dividing isoenzymes into families (e.g.CYP2), subfamilies (CYP2C) and individual enzymes (CYP2C19). Separate alleles of the same gene are designed by an asterisk and a number (CYP2C19\*2). The major human drug metabolizing enzymes belong to the families *CYP1*, *CYP2*, and *CYP3* specifically CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1 and CYP3A4/5/7 [4]. The human CYP2C subfamily consists of four members clustering at the chromosomal location 10q24 and they comprise approximately 20% of the P450 enzymes in the human liver. The CYP2C subfamily consists of 2C8, 2C9, 2C18 and 2C19.

## 1.3 VARIATION IN PHARMACOKINETICS

Patients are commonly given the same doses of a drug and may not exhibit the same effect, and not the same concentrations in drug therapies. This can be due to interindividual and/or intraindividual variation. Genetic polymorphisms within genes involved in drug metabolism seem to be one of the major causes to the variable outcomes [5]. Other factors that can have influence on the pharmacokinetics of drugs are age [6], infections [7] and environmental factors [8, 9]. Compliance with, or adherence to a medication regimen is an important factor that can alter the drug responses and drug concentrations and can results in toxic reactions or subtherapeutic concentrations of a drug. Lack of compliance is a great problem, but this is very difficult to control [10].

- **Genetics**  $\Longrightarrow$  different populations, polymorphisms
- **Physiological**  $\Longrightarrow$  age, diseases, gender
- **Environmental factors**  $\Longrightarrow$  habitants (cultural, dietary, smoke, concomitant drugs, compliance)

## 1.4 PHARMACOGENETICS

Pharmacogenetics has for the last 40 years expanded to be a major part of clinical pharmacology. Pharmacogenetics deals with inherited differences in the response to drugs and has influenced the medical practices, preclinical and clinical drug research. Different populations may carry different alleles of CYP genes and genetic polymorphism seems to be one of the major causes to the variable outcomes [5]. Two single nucleotide polymorphisms constituting the CYP2C19\*17 allele cause an increased activity of the enzyme CYP2C19 [11]. The enzyme CYP2C9 is also a polymorphic enzyme and the alleles CYP2C9\*2 and CYP2C9\*3 encode enzymes with decreased activity compared with the most common allele CYP2C9\*1 [12, 13]. Gene duplication of cytochrome P4502D6 (CYP2D6) which metabolizes many antidepressants has been identified as a mechanism of poor response in the treatment of depression [14]. The patient's genetic information of drug transporters, drug metabolizing enzymes and drug receptors account to allow for an individual drug therapy and leading to optimal choice and dose of the drugs and reducing risks of intoxication and /or lack of efficacy to the drug therapy.

## 1.5 CYP2C19

The human CYP2C subfamily contains four homologous genes (-2C8, -2C9, -2C18 and -2C19), located in a 500-kbp cluster on chromosome 10q24 and the CYP2C19 gene is highly polymorphic [14]. CYP2C19 affects a number of clinically important drugs. The majority of antidepressants are metabolized by CYP2C19 [15, 16], benzodiazepines [17], proguanil [18] and proton pump inhibitors (PPIs) [19]. Individuals can be classified according to their CYP2C19 genotype and the associated CYP2C19 activity. The subjects can be divided into extensive metabolizers, intermediate metabolizers, poor metabolizers or ultrarapid metabolizers [11]. The poor metabolizer (PM) phenotype is caused by the CYP2C19\*2 and CYP2C19\*3 alleles although the CYP2C19\*3 is very uncommon in Caucasian PMs [20]. A novel allele CYP2C19\*17 has been identified and this allele is associated with an increased CYP2C19 activity *in vivo* in two different ethnic populations [11]. The 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 [11]. About 3 % of Caucasians are PM

of S-mephenytoin [21] and a higher incidence of PMs has been reported in Japanese (18-23 %) [22, 23] Chinese (15-17%) [24, 25] and in Korean subjects (13-16%) [26].

## **1.6 CYP2C9**

There are different allelic variants of CYP2C9 defined in different ethnic populations and the allelic variants are described in the official human nomenclature web site ([www.imm.ki.se/cypalleles](http://www.imm.ki.se/cypalleles)). CYP2C9 gene is 55 kb long, including 9 exons and located at chromosome 10. The CYP2C9 gene encodes a protein of 492 amino acids [15]. This enzyme is important for the metabolism of many therapeutically used drugs as tolbutamide, losartan, and nonsteroidal anti-inflammatory drugs (NSAID). Other prescribed drugs are drugs with a narrow therapeutic index such as warfarin and phenytoin. Two common coding variants, termed CYP2C9 \*2 and CYP2C9\*3 have functional consequences for enzyme activity and lead to reduced enzyme activity [12, 13] and thereby to a less effective metabolism towards CYP2C9 substrates, such as warfarin or S-naproxen. CYP2C9\*2 is more frequent in Caucasians while it is absent in Asians and very rare among African-Americans [27, 28-30]. The CYP2C9\*5 variant is specific for Black population and codes also for an enzyme with a reduced activity [31].

## **1.7 DRUG-DRUG INTERACTIONS**

Drug-drug interactions may cause interindividual differences in drug response [32]. In patients on multi-drug therapy, reduced or enhanced concentrations may be obtained and this can cause severe side effects [33]. Known CYP2C9 inhibitors are amiodarone, fluconazole, metronidazole, ketoconazole, cimetidine, and valproate [34, 35]. Inhibitors of CYP2C9 are felbamate, omeprazole, cimetidine, fluoxetine, diazepam, and ticlopidine [35]. Phenytoin is an anticonvulsant drug and is predominately eliminated by CYP2C9 and CYP2C19 dependent hepatic metabolism. This drug is associated with a wide range of drug interactions [34]. Phenytoin increases the metabolism of drugs metabolized by the CYP2C and CYP3A subfamilies and UGT enzymes. Known inhibitors of CYP2C9 and CYP2C19 will decrease the metabolism of phenytoin and the result may be increased plasma concentrations.

## **1.8 ORAL CONTRACEPTIVES**

Estradiol derivatives are ethinylestradiol, mestranol and estradiol which are mainly used in oral contraceptives. Estradiol can be used for hormone replacement therapy and in OC the estradiol derivatives are commonly combined with progesterone derivatives to obtain a better cycle control in women with intact uterus [36].

Several publications have shown that OC that contains ethinylestradiol inhibit the activity of the enzyme CYP2C19 [36, 37, 38]. Propanolol, proguanil and selegiline can also have an effect of OC but this can be due to other CYP enzymes that are involved in the metabolism of these drugs. The enzyme CYP2C9 has been found to be inhibited by ethinylestradiol in vitro [36]. A study with CYP2C9 and the substrate losartan showed that intake of OC led to a slower losartan metabolism [39].

## 2. THE PRESENT STUDY

### 2.1 AIMS OF THE STUDY

- To compare healthy Swedish and Korean populations with focus on genotypes and phenotypes in specific allelic variants of CYP2C19 and CYP2C9.
- To study the activity of CYP2C9 in a patient with fluconazole induced intoxication during treatment with high phenytoin doses.

### 2.2 MATERIALS AND METHODS

#### 2.2.1 Study populations

Healthy Swedish and Korean subjects were recruited in Study I and II for participating in a cocktail study. The subjects were given five different probe drugs of the cytochrome P450 enzymes including omeprazole (20 mg) and losartan (25 mg), but also caffeine, quinine and debrisoquine. The probe drugs were given at different times to overcome interaction risks.

Other drugs except OC were not allowed one week before the study started and the subjects refrained from alcohol, caffeine products and grapefruit juice at least two days before the study started [40]. Only one Korean woman was user of oral contraceptive and she was not included when studying the effect of oral contraceptives on phenotypes.

**In study I** 185 healthy Swedish and 150 Korean subjects were included. The metabolic ratio of omeprazole and its metabolite 5-OH-omeprazole and genotypes for CYP2C19 were determined.

**In study II** 190 healthy Swedish and 147 healthy Korean subjects were included. The metabolic ratio of losartan and its metabolite E-3174, and genotypes for CYP2C9, were determined.

**In study III** we present a patient with Bechet's disease (BD), who required treatment with high doses of phenytoin. When fluconazole, a potent inhibitor of CYP2C9 was added, the patient developed symptoms of intoxication of phenytoin. Phenytoin concentrations were determined and the losartan test was performed after an overnight fast and after voiding the night urine. Losartan (25 mg) was administered as a single

dose in the morning and urine was collected 8 hours thereafter. Genotyping for CYP2C9 was performed.

### **2.2.2 Genotyping**

Genotyping of study participants were performed by extracting DNA from leukocytes. Genotyping methods were based on the polymerase-chain-reaction (PCR) technique. An allelic discrimination was determined by using a Taqman assay with specific primers to identify known polymorphisms. For the allele specific reactions we designed our own specific primers and for the TaqMan assay we used either pre-developed reaction kits, or designed our own specific primers and probes. The samples from Korea were packed on dry ice and sent to Sweden for analyses by the same genotyping and phenotyping methods as the Swedish samples.

### **2.2.3 Phenotyping**

After an 8-overnight fast, the subjects were administered the drug omeprazole. Three hours after the intake of omeprazole, blood samples were collected and centrifuged. Plasma was separated and stored frozen at  $-20\text{ C}$  until analysis omeprazole and its metabolite 5-hydroxyomeprazole were quantified by using a reversed-phase high-performance liquid chromatography (HPLC) method. Paper I: The individual of omeprazole and its metabolite was determined by dividing the molar concentrations of omeprazole and 5-hydroxyomeprazole in plasma.

In papers II and III losartan was administered as a single oral dose in the morning. Thereafter, urine was collected during eight hours and the urine sample was stored at  $-20\text{ C}$  until HPLC-analysis of losartan and its metabolite E-3174 were performed according to Yasar et al [13]. The metabolic ratio of losartan was determined by dividing the molar concentrations of losartan by that of its metabolite E-3174.

In paper III phenytoin concentrations were determined by routine methods. The HPLC analysis of phenytoin was performed after plasma protein precipitation.

### **2.2.4 Statistical analyses**

For all statistical analysis P-Values less than 0.05 were considered statistically significant. MRs were log-transformed before statistical analysis and the logarithmic values were presented in histograms. Ethnic groups of the same genotypes, gender and

smoker-non smoker were compared and in the Swedish women were the group of women user of OC (OC) and non user of OC (NOC) compared. The independent t-test was used in Statistical version 7 (Stat Soft® Scandinavia AB).



### 3 RESULTS AND DISCUSSION

#### 3.1 Paper I

As expected, a higher incidence of CYP2C19 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% (table 1). Among subjects homozygous for *CYP2C19\*1*, Koreans displayed significantly higher MR (lower CYP2C19 enzyme activity) than Swedes ( $p < 0.000001$ ). In Koreans a pronounced gender difference was detected: 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 ( $p < 0.00001$ ) (fig. 1). 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.

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

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

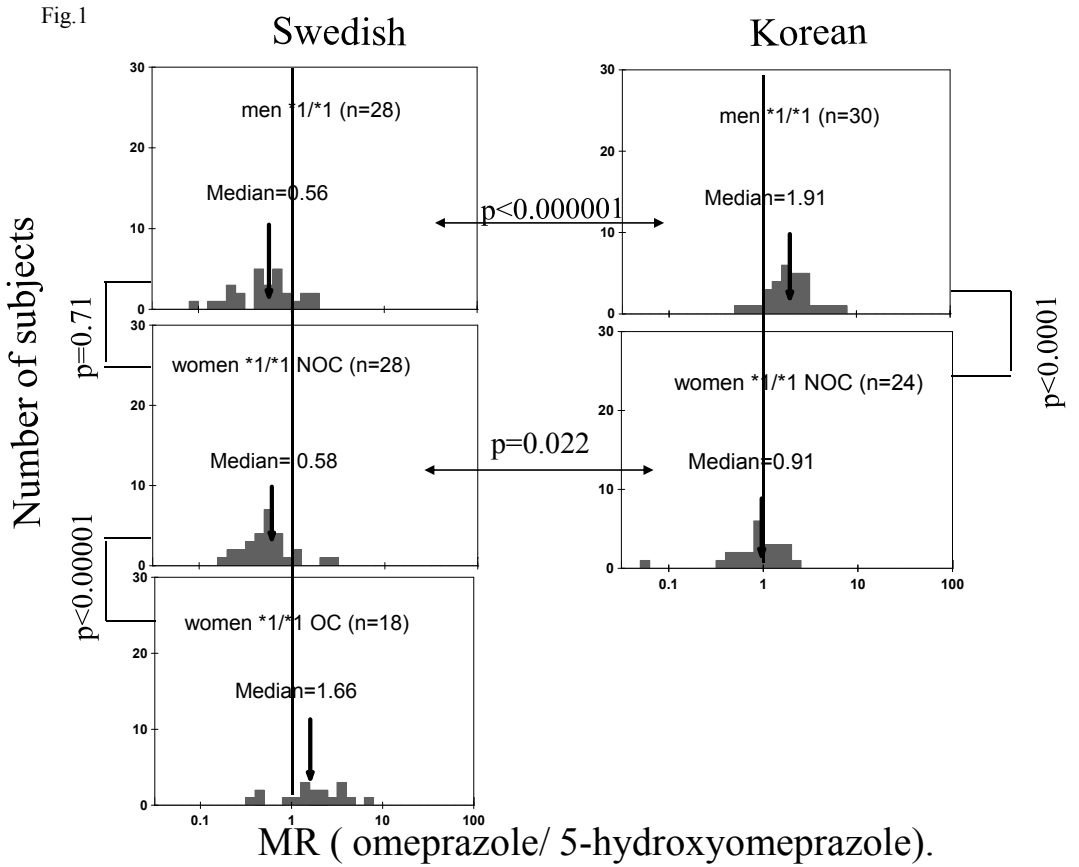


Figure 1.

CYP2C19 \*1 / \*1 are shown in this figure.

Effect of gender on MR in Swedes and in Koreans and effect of oral contraceptives

(OC) in Swedish OC users. Only one Korean female was user of OC and she has been excluded here.

OC= user of oral contraceptive; NOC= non user of oral contraceptive.

### 3.2 Paper II

The study encompassed 190 Swedes and 147 Koreans. The genotype analyses revealed allele frequencies of CYP2C9\*1, \*2 and \*3 in the Swedish subjects of 80.0, 9.7 and 10.3%, respectively. In Koreans the frequencies of the alleles \*1 and \*3 were 94.2% and 5.8% and the allele \*2 was not determined in Koreans because of its low occurrence in Asians (table 2).

The subjects were phenotyped with losartan and histogram of the MR (losartan/E-3174) is shown in figure 2. The median MR was higher in Swedes as compared to Koreans (1.07 vs. 0.58,  $p=0.00001$ ).

As shown in figure 3 Swedish women being users of OC had higher MR than NOC genotyped as CYP2C9 \*1/\*1 ( $p=0.0001$ ) whereas OC usage was only reported by one Korean woman. MR was higher in Swedes compared to Koreans both in men and women NOC genotyped as CYP2C9\*1/\*1 (0.87 vs. 0.52, 0.69 vs. 0.60) and statistically significant difference was detected ( $p=0.00001$ ,  $p=0.0061$ ).

Table 2.

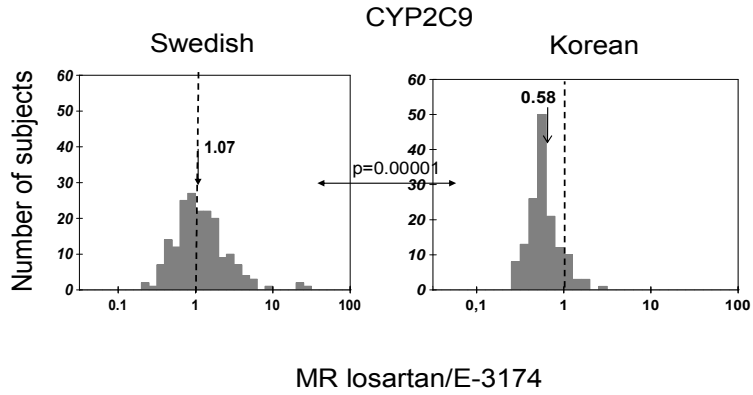
Allele frequencies of CYP2C9 \*1, \*2, \*3 in Swedish and Korean populations. Allele \*2 was not determined in Koreans as the allele frequency in Asians is very low.

Populations (n)	Allele frequencies %		
	*1	*2	*3
Swedish 190	80.0	9.7	10.3
Korean 147	94.2	n.d.	5.8

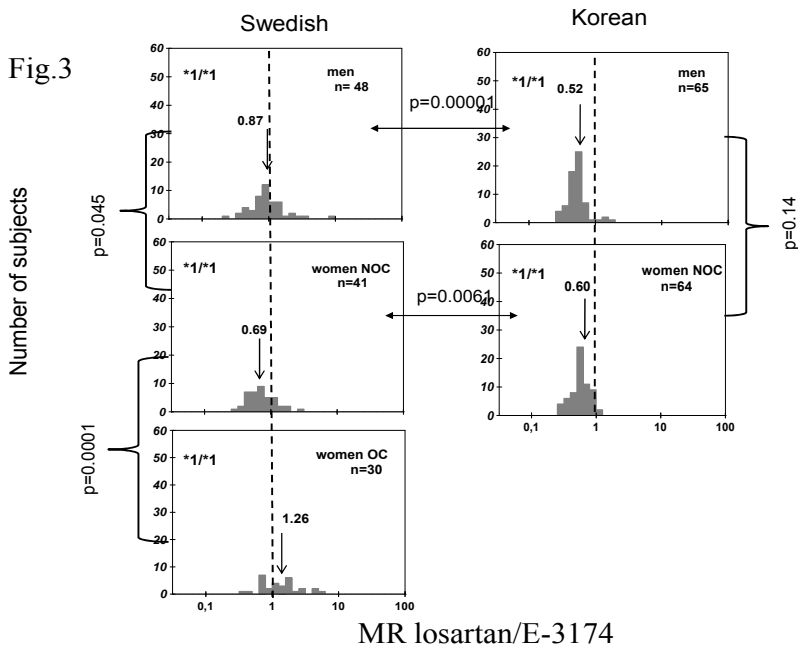
n = number of subjects

n.d.= not determined

Fig.2



(Figure 2) The distribution of the log MR of losartan/E-3174 in healthy 190 Swedish and 147 Korean subjects participating in this study.



(Figure3) Effect of gender in Swedes and Koreans on losartan/E-3174 MR within the genotype group CYP2C9\*1/\*1 (i.e.without \*2 and \*3) and comparing the populations in men and women NOC. Effect of OC was shown in the Swedish females.

### 3.3 Paper III

In paper III we present a patient with Bechet's disease who required treatment with high doses of phenytoin to obtain optimal plasma concentrations. When fluconazole, an inhibitor of CYP2C9 was added, she developed ataxia, tremor, fatigue, slurred speech and somnolence. This indicates phenytoin intoxication.

A phenotyping test for CYP2C9 with losartan was performed and showed that the patient had lower MR than the healthy Swedish subjects used as control material. This confirms that this patient is an outlier with ultra-high activity of CYP2C9 (fig 4).

None of the CYP2C9\*2 and CYP2C9\*3 or CYP2C19\*2 alleles were present in this patient and she thus had the genotypes CYP2C9\*1/\*1 and CYP2C19\*1/\*1. Among the drugs the patient used in close relation to the two intoxication episodes phenytoin was the only known substrate and fluconazole the only known inhibitor for CYP2C9. The MR of losartan was determined and potential and relevant drug interactions were investigated by using the drug interaction database SFINX [41].

Fig. 4

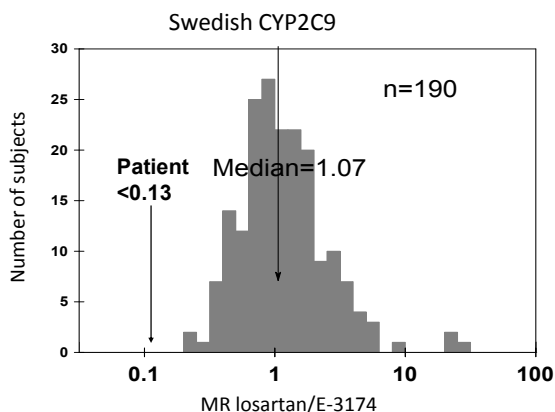


Figure 4 shows that the patient had a MR of  $< 0.13$  which is less than the MR in any of the 190 healthy Swedish subjects used for comparison. The patient is thus an ultra-rapid metabolizer of the CYP2C9 substrate losartan.

There are several polymorphisms and allelic variants reported for the CYP2C19 and CYP2C9 genes. Our results generally agree with previous reports. The Swedish and the Korean subjects were genotyped for the selected variants of CYP2C19 i.e. \*2,\*3 and\*17 and in the CYP2C9 gene selected for \*2 and \*3 alleles, based on previous reports. The thesis tries to find explanations to the differences in CYP2C19 and CYP2C9 between ethnic populations as well as between individuals of the same genotype. Environmental factors such as diet, physiological status and unidentified mutations in the gene might have influenced our results and remain to be further studied. The subjects were received the Karolinska cocktail as a tool for phenotyping the most important human drug metabolizing enzymes in P450s; CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. As reported by Christensen et al. we administered caffeine, losartan, omeprazole, debrisoquine, and quinine. Quinine inhibits CYP2D6 and it is a P-gp substrate and therefore the administration of quinine was separated from the other drugs [40]. Metabolic ratios (MRs) for omeprazole/5-OH omeprazole (metabolite of omeprazole) in three hour plasma sample and losartan/E-3174 (metabolite of losartan) in 0 to 8 hours urine were used as phenotypic indices for

CYP2C19 and CYP2C9 activities. An important aspect of the present study is that both genotyping and phenotyping were performed in exactly the same way in Swedes and in Koreans. Therefore a direct comparison may be performed.

**In study I** a gender difference was demonstrated in Koreans for CYP2C19 and this difference has not been demonstrated before. This gender difference was not found in the Swedish subjects. Factors can be due to gene mutations and/or other factors that have influence on the CYP2C19 activity in different populations.

In a study by Mwinyi et al [42] it was shown that the spectrum of genes regulated by GATA proteins play a role in the regulation of genes involved in the metabolism of endogenous and exogenous compounds. A novel mechanism of CYP2C9 and CYP2C19 transcriptional regulation were found and involves transcription factors from the GATA family and estrogen receptor. Further understanding of variation in the expression of special regulators of CYP2C19 and CYP2C9, such as transcription factors, may explain why the enzymes CYP2C19 and CYP2C9 show a wide interindividual range in gene expression. The enzyme CYP2C19 activity was inhibited by OC in Swedish females and an earlier study showed that estradiol and its derivatives are able to modulate CYP2C19 promoter activity. This occurs via the classic ERE (estrogen response element) dependent pathway. CYP2C19 is not the only enzyme of the cytochrome P450 family that is known to be influenced by female sex steroids [43].

**In study II** it was shown that OC inhibited the enzyme CYP2C9 activity in Swedish females and this finding confirms by an earlier study [39]. Study II showed also that the enzyme CYP2C9 activity was higher in Swedes than Koreans in specific genotypes, and this difference is unclear. Factors can play an important role in the endogenous constitutive expression of both CYP2C19 and CYP2C9 and these remains to be further investigated.

**Study III** demonstrates an ultra high activity of CYP2C9 and low phenytoin concentrations at normal doses. The result of the losartan test in the patient shows low urinary excretion of parent drug and high concentrations of the metabolite. This indicates that the patient does not have poor absorption, but has a high CYP2C9 activity. The MR was  $<0.13$  which shows that the patient had a lower MR than any of the 190 healthy Swedish Caucasians used as comparison material [44]. This confirms that the patient is an outlier with ultrahigh activity of CYP2C9. In clinical practice the patient needed high doses of the CYP2C9 substrate phenytoin to reach drug concentrations. Another important finding is that this patient demonstrates risk of severe intoxications in UM patients, when treated with strong CYP 2C9 inhibitors, such

as fluconazole. The molecular basis of CYP2C9 catalysed ultrarapid metabolism remains to be demonstrated.



## 4 CONCLUSIONS

### 4.1 PAPER I

A higher incidence of PM was found in Koreans compared to Swedes and these findings confirm by previous studies. The frequency of the *CYP2C19\*17* allele was very low in Koreans (0.3%) compared to Swedes (20.0%) which is in accordance with Sim et al [11]. Among subjects genotyped as *CYP2C19\*1/\*1* Koreans had lower CYP2C19 activity than Swedes. A gender difference was detected in Koreans where females had significantly lower MR than males but this difference was not seen among Swedes. Swedish user of OC had a higher MR of omeprazole than non users and confirms that OC inhibits CYP2C19. No effect of smoking was observed in the two populations.

### 4.2 PAPER II

The MR of losartan was higher in Swedes compared to Koreans. Swedish users of OC had higher MR than non users genotyped as *CYP2C9 \*1/\*1* and this finding confirms that OC inhibits CYP2C9 [39]. A higher MR was detected in subjects heterozygous for \*3 compared to subjects genotyped for *CYP2C9\*1/\*1* in both populations when comparing the two genotype groups (including men and women NOC in the genotype groups). In the Swedish subjects a higher MR was observed in the genotype group *CYP2C9\*1/\*3* compared to *CYP2C9\*1/\*2* but no significant difference was detected when comparing the Swedish subjects genotyped for *CYP2C9 \*1/\*1* and for *CYP2C9\*1/\*2*. This shows that \*3 has a more potent effect than \*2 to decrease the CYP2C9 activity. Effect of smoking was observed neither in Swedes nor in Koreans.

### 4.3 PAPER III

In one patient, we found an ultra-high rate of metabolism of phenytoin and this may apply to other CYP2C9 substrates, where inhibition of CYP2C9 might cause severe adverse drug reactions. The losartan MR was <0.13 in this patient and this was lower than in the control group of 190 Swedish subjects used for comparison.

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