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Differences in *CYP2C9* Genotype and Enzyme Activity Between Swedes and Koreans of Relevance for Personalized Medicine: Role of Ethnicity, Genotype, Smoking, Age, and Sex

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Abstract

Global personalized medicine demands the characterization of person-to-person and between-population differences in drug pharmacokinetics and pharmacodynamics. CYP2C9 pharmacokinetic pathway is subject to modulation by both genetic and environmental factors. *CYP2C9* genotype-based dose recommendations (e.g., for warfarin) is advocated. However, the overall contribution of genotype for variation in enzyme activity may differ between populations. We evaluated the importance of ethnicity, genotype, smoking, body weight, age, and sex for CYP2C9 enzyme activity. *CYP2C9* genotype and phenotype was determined in 148 Swedes and 146 Koreans using losartan as a probe. CYP2C9 enzyme activity was assed using urinary losartan/metabolite E-3174 ratio. The frequency of *CYP2C9* defective variant alleles (*2 and *3) was significantly higher in Swedes (10.8% and 12.5%) than in Koreans (0% and 5.8%). In matched genotypes, CYP2C9 enzyme activity was significantly lower in Swedes compared to Koreans ($p < 0.0001$). In a univariate analysis, age, weight, ethnicity, genotype, and smoking were significant predictors of CYP2C9 phenotype. A stepwise multivariate analysis indicated ethnicity, genotype, and smoking remained as significant predictors of CYP2C9 enzyme activity, accounting for 50% of the total variance. In both study populations, *CYP2C9* genotype was a significant predictor of CYP2C9 enzyme activity, but its contribution in explaining the total variance was lower in Koreans (26.6%) than Swedes (40%). In conclusion, we report significantly lower CYP2C9 enzyme activity in Swedes compared to Koreans, partly but not exclusively due to *CYP2C9* pharmacogenetic variations. Ethnicity and environment factors need to be considered together with genotype for population-specific dose optimization and global personalized medicine.

Introduction

The cytochrome P450 isoenzyme CYP2C9, one of the most abundant CYP enzymes in the liver, plays important roles in the metabolism of many therapeutically important drugs, including nonsteroidal anti-inflammatories, oral anticoagulants, and oral hypoglycemics (He et al., 2011; Rettie and Jones, 2005). *CYP2C9* is genetically polymorphic and displays wide between-patient differences in its metabolic activity, which can result in difficulties for rational dosing, inadequate therapeutic effect, or toxicity (Ninomiya et al., 2000; Wadelius et al., 2004). Induction and/or inhibition of CYP enzymes are the usual mechanisms by which concomitant medications can cause variations between patients in drug elimination capacity. Interactions between the genetic, environmental (e.g., dietary, lifestyle, socio-culture background) and biological (e.g., gender, age) factors add up to the complexity of this variation and may explain why a group of people from a certain population or ethnic background have different metabolic or adverse event profiles compared to others.

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Pharmacogenomics is the study of the role of genomics differences for drug safety and efficacy. Recent efforts for global characterization of human genetic variation and pharmacogenomics variability worldwide attest to the growing importance placed on drug-by-gene interactions and capacity building for rational therapeutics in both developed and developing countries (Dandara et al., 2014). Indeed, population and ethnic variation is associated with different distribution of functional variant alleles between populations, and hence variability in drug plasma exposure. The distribution of *CYP2C9* defective variant alleles displays wide inter-ethnic variation. *CYP2C9*2* (*Arg*144*Cys*) and *CYP2C9**3 (Ile359-Leu) alleles are most commonly present in Caucasians and Africans, while *CYP2C9**2 appears to be absent in Asians (Dickmann et al., 2001; Lee et al., 2003; Scordo et al., 2001; Wang et al., 2014; Yasar et al., 2002a). The *CYP2C9*4* (*Ile*359*Thr*) allele occurs exclusively in Japanese individuals (Imai et al., 2000), but is absent in Koreans (Bae et al., 2005), whereas *CYP2C9*5* and *CYP2C9*6* alleles occur specifically in black populations (Dickmann et al., 2001; Kidd et al., 2001; Yasar et al., 2002a).

To avoid toxicity or treatment failure, population-based dose recommendations for drugs metabolized by CYP2C9, particularly warfarin is suggested (Giri et al., 2014; Wadelius, 2014). Most of these recommendations and dose algorithms are based on genotype. However, the extent of correlation between *CYP2C9* genotype and phenotype varies between populations mainly due to the contribution of sociodemographic, epigenetic, or environmental factors, such as diet. Numerous factors affecting CYP2C9 enzyme activity have been described, including genotype, gender, age, cigarette smoking, oral contraceptives, and food constituents (Bazan et al., 2014; Giri et al., 2014; Hidaka et al., 2008; Sandberg et al., 2004). These factors might be important to consider for dose modifications (Bazan et al., 2014; Lee et al., 2005). But since these findings have not always been consistent, further investigations are warranted.

Effects of herbal medicine and food–drug interactions involving CYP2C9 enzyme inhibition and/or induction is increasingly recognized (Hidaka et al., 2008; Kimura et al., 2010; Srinivas, 2013). The likelihood of such interactions is theoretically higher than drug–drug interactions, as drugs usually contain single chemical entities whereas the herbs and food contains mixtures (Izzo, 2005). Hence, geographic and dietary differences between ethnic groups may contribute for variation in CYP2C9 metabolic profile. Although allele frequency distribution between populations provide indication about metabolic capacity of a give population, more comprehensive information relevant for population-specific dosage recommendation would be obtained by considering also the phenotypic profile, which reflects the influence of both genetic and environmental factors.

Previously we evaluated the importance of ethnicity, genetic, and environmental factors for between population variability in CYP3A (Diczfalusy et al., 2008), CYP2D6 (Aklillu et al., 2002), CYP2C19 (Ramsjo et al., 2010), CYP1A2 (Ghotbi et al., 2007), CYP2A6 (Djordjevic et al., 2013), NAT2 (Djordjevic et al., 2012), and CYP2B6 (Ngaimisi et al., 2013) enzyme activity among Caucasians, Asians, and black populations. In the present study, we investigated the relative importance of ethnicity, genotype, age, weight, sex differences, and smoking on CYP2C9 enzyme activity in Swedes and Koreans. Our results show lower CYP2C9 enzyme activity in Swedes compared to Koreans, regardless of genotype, indicating the importance of ethnicity, environmental, or epigenetic factors.

Materials and Methods

Subjects

The study population consisted of 148 healthy Swedish volunteers from Karolinska University Hospital, Huddinge, Sweden, and 146 healthy Korean volunteers from Inha University Hospital, Incheon, Korea. In the present study, oral contraceptive (OC) users were excluded because it inhibits CYP2C9 enzyme activity (Sandberg et al., 2004). The study was approved by the local ethics committee at Karolinska Institutet, Stockholm, Sweden, and Inha University Hospital, Incheon, Korea, and was performed in accordance with the Helsinki Declaration and The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (*ICH*) Guideline for Good Clinical Practice. After receiving written and oral information about the study, subjects were interviewed concerning their medical history and screened with general clinical laboratory tests. Subjects were requested to refrain from taking other medications for one week and from consuming alcohol, caffeinated products, and grapefruit juice for 2 days before the study started.

CYP2C9 *phenotyping*

The subjects received the Karolinska cocktail that included five probe drugs (omeprazole, caffeine, quinine, debrisoquine, and losartan) and the drugs were given at different time-points to overcome the interaction risks (Christensen et al., 2003). The active metabolite of losartan, E-3174, is formed mainly by CYP2C9 and to a minor extent by CYP3A4. After an overnight fast, losartan 50 mg (Cozaar; Merck, Darmstadt Germany) was given in the morning as a single oral dose. Urine was collected during 8 hours after drug intake and 10 mL aliquots was stored at −20°C until HPLC analyses of losartan and E-3174 were performed as described previously (Yasar et al., 2002b).

CYP2C9 *genotyping*

Blood samples were collected in EDTA tubes and stored at −20°C. The samples from Korea were packed in dry ice and sent to Sweden for analyses. Genomic DNA was extracted from the leukocytes using QiAmp DNA Blood Kit (Qiagen, Hilden, Germany). Genotyping for *CYP2C9*2* and *CYP2C9*3* was done using Taqman assays (Sandberg et al., 2004). In addition, genotyping for *CYP2C9*13* was done in Koreans by a PCR-restriction fragment length polymorphism (RFLP) method using the restriction enzyme *PspGI* (Bae et al., 2005).

Statistical analysis

Chi square test was used to compare the observed and expected allele frequencies according to the Hardy–Weinberg equilibrium. The metabolic ratio (MR) was determined by dividing the molar concentration of losartan/metabolite E-3174. CYP2C9 MR data were log-transformed before

application of statistical analysis. Distribution of log CYP2C9 MR between Swedes and Koreans is presented in histograms. Independent tests were done to compare log CYP2C9 MR between the two study populations and stratified by *CYP2C9* genotype. In cases where Levene's test for variance was greater than 0.05, equal variance was assumed, otherwise unequal variance was assumed. Analysis of variance was used to evaluate the effect of genotype on MR. Univariate linear regression analyses were used to identify the individual effect of age, gender, weight, genotype, smoking habit and ethnicity on CYP2C9 MR. Predictor variables that resulted in p-value < 0.2 were entered into a stepwise multivariate regression analysis to identify significant predictors in the final model. Graphical representation and statistical analyses were performed using Statistica, version 12 (StatSoft Inc, Tulsa, OK, USA) and SPSS Statistics (IBM Corporation, Somers, NY) software, version 22.0 respectively. P values < 0.05 were considered to be statistically significant.

Results

Among the Swedish subjects there were 85 males and 63 females. The mean age for Swedes was 31 ± SD 9 years. The mean weight was 72 ± SD 12 kg. Twenty-nine subjects were classified as daily smokers. Among the Korean subjects, the mean age was 25 ± SD 4 years and the mean weight was 62 ± SD 12 kg. There were 74 male and 72 female participants in the study, with 27 subjects classified as daily smokers. In one subject, no data on smoking habit was available.

Comparison of CYP2C9 *genotypes between Swedes and Koreans*

The observed CYP2C9 genotype and allele frequency between Swedes and Koreans is presented in Table 1. The frequency of CYP2C9 defective variant alleles (*2 and *3) was significantly higher in Swedes than Koreans. One Korean subject was genotyped as *CYP2C9*1/*13*. The Swedes were not screened for *CYP2C9*13* as it is mainly reported in Asian populations. Previously we found a significant relationship between an intronic SNP IVS8-109A > T with a lower CYP2C9 activity in Swedes (Hatta et al., 2012). In the present study, we extended the investigation by screening for this mutation in individuals with *CYP2C9* alleles *2 and *3. Our result showed that IVS8-109A > T was not linked to *CYP2C9* alleles *2 or *3 in the Swedish subjects.

The frequency distribution of genotype-deduced CYP2C9 phenotype was significantly different between the two populations (Chi square $p < 0.001$). The frequency of CYP2C9 extensive *(*1/*1)*, intermediate (heterozygous *2 *or*3), and poor metabolizers (homozygous for defective variant alleles) in Swedes was 59%, 36%, and 5%, respectively; whereas the corresponding frequency in Koreans was 88%, 12%, and 0%, respectively.

Comparison of CYP2C9 *phenotype between Swedes and Koreans*

Frequency distribution of log CYP2C9 MR (losartan/E-3174) between Swedes and Koreans is presented in Figure 1. The median CYP2C9 MR was significantly higher in Swedes as compared to the Koreans (1.02 vs. 0.57, Mann-Whitney; $p < 0.0001$). The mean log CYP2C9 MR was significantly higher in Swedes than Koreans (independent t-test, $p < 0.0001$; mean difference = −0.28, SE for differences = 0.03, 95% Confidence Interval of the mean difference = −0.34 to −0.21, not assuming equal variances, Levene's test for equality of variances $p = < 0.05$). Having the same *CYP2C9* genotype, Swedes displayed significantly higher CYP2C9 MR than Koreans (Fig. 2). Considering *CYP2C9*1/*1* genotypes only, the mean log CYP2C9 MR remained significantly higher in Swedes than Koreans (independent t-test $p < 0.0001$; mean difference = −0.18, SD for differences = 0.03, 95% Confidence Interval of the mean difference = −0.24 to −0.12).

Effect of CYP3A4 on the production of losartan metabolite E-3174

Both the Koreans and Swedish study participants were previously phenotyped for CYP3A4 enzyme activity using quinine as probe drug and 4 beta-hydroxycholesterol as endogenous marker for CYP3A4 activity (Diczfalusy et al.,

Table 1. Comparisons of CYP2C9 Genotype and Allele Frequencies (%) Between Swedes and Korean Populations

	Swedish (n = *148)*		*Koreans* (n = *146)*	
CYP2C9 Genotype	*Observed frequency*	*95% Confidence Interval*	*Observed frequency*	*95% Confidence Interval*
**1/*1*	0.588 (87/148)	0.509, 0.666	0.877 (128/146)	0.823, 0.930
**1/*2*	0.162 (24/148)	0.103, 0.222	0	–
**1/*3*	0.196 (29/148)	0.132, 0.260	0.116 (17/146)	0.064, 0.168
**1/*13*	n.d	–	0.007 (1/146)	0, 0.169
**2/*2*	0.012 (2/148)	0.005, 0.032	0	–
**2/*3*	0.027 (4/148)	0.001, 0.053	0	–
**3/*3*	0.014 (2/148)	0.005, 0.032	0	–
CYP2C9 allele				
*CYP2C9*1*	0.767	0.719, 0.815	0.938	0.911, 0.966
*CYP2C9*2*	0.108	0.073, 0.244	0	n.d
*CYP2C9*3*	0.125	0.087, 0.163	0.582	0.031, 0.083
*CYP2C9*13*	n.d		0.34	0, 0.101

*n.d, not determined.

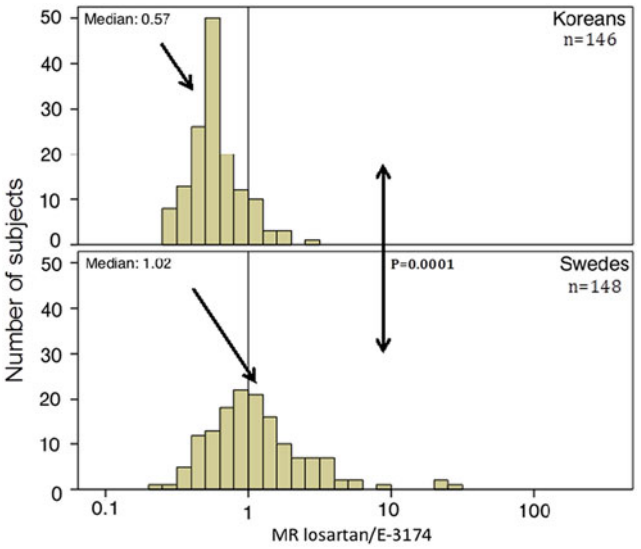

FIG. 1. Comparisons of frequency distribution of log losartan/E-3174 between 148 Swedish and 146 Korean subjects participating in this study. MR = metabolic ratio; MR = 1 is indicated as an *arbitral vertical line*.

2008). A previous study in human liver microsomes indicated contribution of CYP3A4, in addition to CYP2C9, for the formation of E-3174 from losartan (Stearns et al., 1995). However an *in vivo* study showed that ketoconazole, an inhibitor of CYP3A4, has no effect on the metabolism of losartan to E-3174 (McCrea et al., 1996). Likewise we found no significant correlations between log plasma losartan/E-3174 ratio and log plasma quinine/3-hydroxyquinine ratio in both Swedes (Spearman's correlations two-tailed, $p = 0.61$) and Koreans ($p = 0.87$). Similarly no correlation between losartan/E-3174 ratio and 4 beta-hydroxycholesterol was found in Swedes ($p = 0.56$) and Koreans ($p = 0.93$).

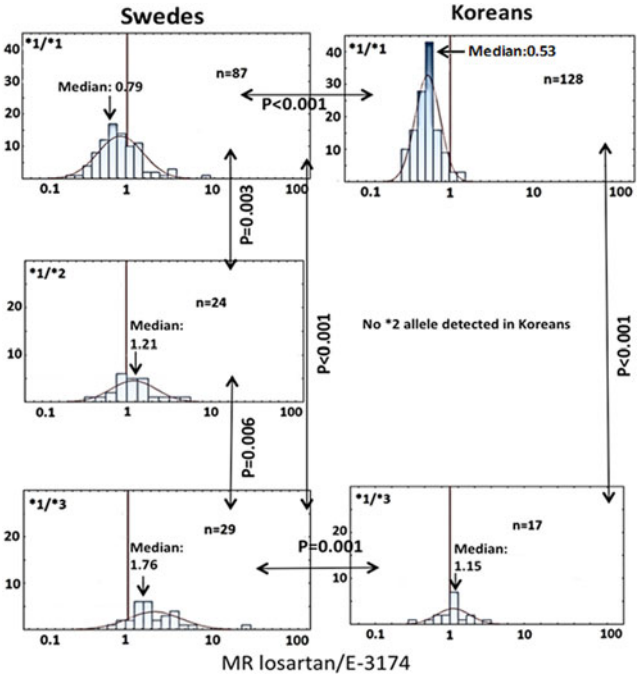

FIG. 2. Comparisons of frequency distribution of log losartan/E-3174 ratio between Swedes and Koreans stratified by CYP2C9 genotype. CYP2C9*2 was not detected in Koreans. MR = 1 is indicated as an *arbitral vertical line*.

Factors predicting variation in CYP2C9 enzyme activity

Data from the two study populations were merged for statistical analysis and linear regression analysis was applied to identify significant predictors of CYP2C9 phenotype (Table 2). Univariate regression analysis was first performed to identify individual effect of the predicting variables on CYP2C9 MR. Ethnicity was a significant predictor of variation in CYP2C9 MR ($p < 0.0001$). Similarly, *CYP2C9* genotype was a strong significant predictor of CYP2C9 enzyme activity in overall study population ($p < 0.0001$). Smoking was a significant predictor for log CYP2C9 MR ($p = 0.009$). The enzyme activity was higher in smokers than non-smokers both in Koreans ($p = 0.003$) and Swedes ($p = 0.057$) (Fig. 3). Significant positive correlation of weight and age with log CYP2C9 MR was also observed in the overall study population.

A multivariate stepwise discriminant regression analysis was performed to identify independent predicting factors while controlling for the effect of all variables. All predicting variables with $p < 0.2$ in the univariate analysis were included in the multivariate analysis. Stepwise multivariate regression analysis indicated that *CYP2C9* genotype, ethnicity, and smoking as significant predictors of CYP2C9 MR, accounted together for 50% of the variance ($p = 0.004$). *CYP2C9* genotype alone explained 40% (step 1) and *CYP2C9* genotype together with ethnicity explained 48% (step 2) of between subject variability in CYP2C9 enzyme activity. The effect of smoking was minor but significant to be retained in the model. Both age and weight were not significant predictors of CYP2C9 enzyme activity in the final multivariate model.

We also performed univariate and multivariate analysis in each study population separately. In Koreans, *CYP2C9* genotype ($p < 0.0001$) and smoking ($p = 0.003$) were significant predictors in a univariate analysis, whereas sex ($p = 0.08$) and weight ($p = 0.14$) had marginal effect. In a stepwise multivariate analysis only *CYP2C9* genotype and smoking were significant predictors of CYP2C9 MR explaining 31.0% of all the variance. *CYP2C9* genotype alone accounted for 26.6% of the variance in CYP2C9 MR among Koreans.

In Swedes, *CYP2C9* genotype ($p < 0.0001$) was the only significant predictor of CYP2C9 MR, whereas sex ($p = 0.08$) and smoking ($p = 0.057$) had marginal effect in a univariate analysis. But in a stepwise multivariate analysis, *CYP2C9* genotype was found to be the only significant predictor of CYP2C9 MR explaining 39.6% of the total variance.

Discussion

We investigated the relative importance of ethnicity for CYP2C9 enzyme activity, considering the effect of other confounding factors such as genotype, age, gender and cigarette smoking. Our result indicates that overall Swedes display lower CYP2C9 enzyme activity compared to Koreans, which remained significant even after controlling for the effect of genotype. In both populations, *CYP2C9* genotype is the most significant predictor of CYP2C9 enzyme activity but

Table 2. Univariate and Stepwise Multivariate Linear Regression Analysis to Identify Factors That Predict Log CYP2C9 Metabolic Ratio in Swedes and Koreans

	Univariate analysis		*Multivariate analysis*	
Predictor variable	*Beta(SE of beta)*	*p*-value	*Beta(SE of beta)*	*p*-value
Age	0.007 (0.002)	0.005	0.007 (0.002)	
Weight	0,003 (0.001)	0.019	0,003 (0.001)	
Ethnicity	0.28 (0.032)	<0.0001	0.18 (0.027)	<0.0001
Sex	0.044 (0.036)	0.23	0.044 (0.036)	
Smoking	−0.12 (0.046)	0.009	−0.096 (0.033)	0.004
CYP2C9 genotype	0.21 (0.015)	<0.0001	0.18 (0.014)	<0.0001

Data from the two study population were merged for the analysis and variables with p value <0.2 in the univariate analysis were added in the multivariate analysis.

its contribution in explaining the total variance in enzyme activity is much lower in Koreans (27%) than Swedes, where it accounts for ~40% of the variance. Our result indicates that apart from genotype, ethnicity and environmental factors such as smoking are relevant for CYP2C9 enzyme activity. The frequency of *CYP2C9* defective variant alleles is significantly higher in Swedes than Koreans. Hence, it is anticipated that CYP2C9 enzyme activity would be much lower in Swedes than Koreans and the observed significant variation in CYP2C9 enzyme activity between the two populations could be due to pharmacogenetic variation between the two populations. However, in both *CYP2C9* *1/*1 and *1/*3 genotype groups, Swedes still displayed significantly lower enzyme activity than Koreans (Fig. 2). This indicates that *CYP2C9* genetic polymorphisms significantly contribute to, but not fully explain, the observed inter-ethnic variability in CYP2C9 enzyme activity.

The finding of higher CYP2C9 enzyme activity in Koreans compared to Swedes, regardless of *CYP2C9* genotype, might be due to un-identified genetic, epigenetic, or environmental factors such as differences in dietary habits between the two populations. Inhibition of CYP3A4 or CYP2C9 enzymes activity by spices, beverages, including grapefruit, pomegranate, cranberry and pineapple juice, and flavonoids is documented (Hidaka et al., 2008; Kimura et al., 2010; Si et al., 2009; Srinivas, 2013). However based on our result, it is plausible to assume that CYP2C9 enzyme in Koreans is rather induced by regular dietary constituents present in Korean food that are not commonly consumed in Sweden. Actually a recent study from Korea reported that capsaicin, the principal pungent ingredient in hot red and chili peppers, induces CYP3A4 expression both *in vitro* and *in vivo* via human pregnane X receptor (hPXR) and C/EBPβ activation (Han et al., 2012). Another study from Japan also reported the induction of p-glycoprotein (P-gp) by capsaicin (Okura et al., 2010). The authors concluded that regular exposure to dietary ingredient containing capsaicin increases the metabolism of CYP3A4 substrate and P-gp potentially to cause food–drug interactions (Han et al., 2012; Okura et al., 2010). Hot red and chili peppers are common spices in regular Korean, Indian, Thai, and Ethiopian food.

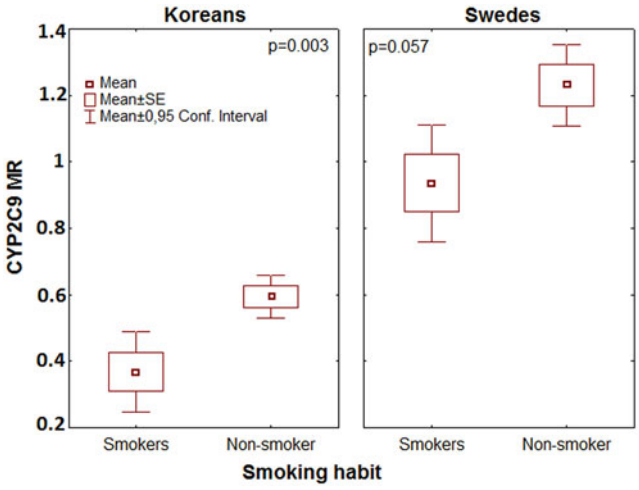

FIG. 3. Comparisons log losartan/E-3174 ratio between smokers and non-smokers in Koreans and Swedes using independent *t*-test.

Interestingly, we previously reported high CYP3A4 enzyme activity in Koreans (Diczfalusy et al., 2008) and Ethiopians (Gebeyehu et al., 2011) as compared to Swedes. We found that the contribution of CYP3A4 compared to CYP2C9 to the formation of E-3174 is negligible. This finding is also supported by a previous study reporting no significant effect of CYP3A4 for the systemic conversion of losartan to E-3174 (McCrea et al., 1996). Lack of correlation between the losartan/E-3174 and quinine MR or 4 beta-hydroxycholesterol confirms that plasma losartan/E-3174 ratio is a specific marker for CYP2C9 enzyme activity and it does not reflect CYP3A4 enzyme activity in human. Therefore the differences in plasma losartan/E-3174 ratio between the two study populations reflects differences in CYP2C9 enzyme activity but not differences in CYP3A enzyme activity.

Since induction of both CYP2C9 and CYP3A enzymes by drugs such as rifampicin, hyperforin (found in St. John's wort), and phenobarbital is mediated via activation of hPXR (Chen et al., 2004), it is highly likely that regular dietary components such as capsaicin, which also is a ligand for hPXR (Han et al., 2012), induce CYP2C9 enzyme (in Koreans), but this needs further investigation. One limitation of this study is the lack of further information on the daily basic diet consumed by the Koreans and Swedes. Presumably the Swedish and Korean subjects are accustomed to locally available foods in their respective countries.

Compared to ethnicity and genotype, the contribution of smoking on CYP2C9 enzyme activity is minor but significant.

The impact of smoking on CYP2C9 MR was significant, particularly in Koreans, but its effect in Swedes was marginal. Polycyclic aromatic hydrocarbon (PAH) from cigarette smoke activates CYP3A4 trough PXR (Luckert et al., 2013), which is also a regulator for CYP2C9 (Chen et al., 2004). A previous study demonstrated the effect of PAHs on the activation of CYP2C enzymes (Degawa et al., 1994). In bronchial biopsies of smokers, *CYP2C9* is significantly induced compared to non-smokers (Thum et al., 2006). However, the effect of smoking on CYP2C9 enzyme activity is inconsistent (Kim et al., 2006; Llerena et al., 2014). Although age and body weight were significant predictors of CYP2C9 enzyme activity in a univariate analysis, the contribution of these variables did not reach significance in the multivariate analysis. The effect of sex on CYP2C9 enzyme activity was also not significant. It is well known that oral contraceptives inhibit CYP2C9 enzyme activity (Sandberg et al., 2004). Thus in the present study women on OC use were excluded. Similar to our finding, no significant effect of sex on CYP2C9 enzyme activity and losartan pharmacokinetics is reported (Cabaleiro et al., 2013; Sica et al., 2005). A recent study highlighted the role of sex for the activity of CYP2C9, but the authors described the lack of information about the females' consumption of oral contraceptives as limitation of their findings (Llerena et al., 2014). The existing inconsistent findings about the relevance of age, smoking, and sex on CYP2C9 enzyme activity warrants further well-controlled studies.

Our study may have clinical relevance since the observed ethnic differences in CYP2C9 enzyme activity may possibly result in between population variability in clinical outcome and treatment safety profile. The optimal dose of warfarin, the most widely used oral anticoagulant metabolized by CYP2C9, varies among individuals, and the prediction of a maintenance dose is difficult (Dang et al., 2005). There have been extensive studies on the difference of prescribing dosage of warfarin among Asians and Caucasians. Contrary to what is expected from our findings, studies on warfarin show that maintenance doses in Asians are 30%–50% lower than in Caucasians (Takahashi et al., 2006; Tatsuno & Tatsuno, 2014). However, it is well known that the warfarin maintenance dose is also dependent on the activity of another genetically polymorphic gene that encodes for vitamin K epoxide reductase complex subunit-1, *VKORC1* and other factors such as age, co-morbidity, concurrent medication, and diet (Takahashi et al., 2006). A study by Teh et al., showed that age and genetic variants of *CYP2C9* and *VKORC1* account for nearly 37% of the variability in warfarin dose in Malaysian subjects (Teh et al., 2012).

A *CYP2C9* and *VKORC1* pharmacogenetic-based warfarin dosage recommendation is developed and genotyping before using warfarin is thought to be promising to optimize warfarin dosing. However warfarin dose requirements vary across ethnic groups even after being adjusted for confounding genetic factors, indicating the relevance of environmental factors (Kimmel et al., 2013; Stergiopoulos and Brown, 2014; Tatsuno and Tatsuno, 2014). Pharmacogenetic dosing algorithms incorporating the *CYP2C9* and *VKORC1* genotype account for at least $\sim 40\%$ of the variability in warfarin dose in Caucasians, whereas they only explain $\sim 20\%$ in people of African descent, largely due to the lower frequencies of these alleles in the latter population (Wadelius, 2014). Similarly we found that the relative importance of *CYP2C9* genotype in explaining variations in enzyme activity is much lower in Koreans than Swedes. Thus, the relevance of environmental factors for enzyme activity and dose recommendations may be higher in populations where the defective variant allele frequency is lower. In these populations, environmental factors such as dietary habits are equally important to consider and a universal genotype-based dosing algorithm may not be applicable to all populations. Indeed, a possible role of dietary factors and herbal medicines for instability of anticoagulation in warfarin-treated patients is well described (Greenblatt and von Moltke, 2005; Srinivas, 2013; Wells et al, 1994).

Ethnic differences in drug metabolizing enzyme activity may result in variation in treatment outcome, type, and severity of adverse event between populations receiving the same treatment (Ngaimisi et al., 2013). Importance of ethnicity for the activity of major drug metabolizing enzymes is well documented. Previously we reported higher CYP2A6 (Djordjevic et al., 2013), CYP1A2 (Ghotbi et al., 2007), and CYP2C19 (Ghotbi et al., 2007) enzyme activity in Swedes compared to Koreans. In contrast, Koreans display higher CYP3A (Diczfalusy et al., 2008) and NAT2 enzyme activity than Swedes (Djordjevic et al., 2012). By comparing the same population living in different geographical locations, it was reported that CYP2D6 (Aklillu et al., 2002) and xanthine oxidase (Aklillu et al., 2003) activities are significantly influenced by environmental factors, but no such effect was found for CYP2C19 or CYP2A6 (Aklillu et al., 2014).

To our knowledge there is only one study that compared CYP2C9 phenotype between populations using the same study design, in Hispanics, where the Cuban Mestizos showed a lower metabolic activity compared to other Latin American populations (Llerena et al., 2014). Apart from warfarin, yet little information is available regarding clinical importance of ethnicity for other CYP2C9 substrate drugs. One previous study found no significant difference in tolbutamide dosage requirement between Japanese and Caucasian Americans (Gross et al., 1999). However, future comparative studies are required to elucidate the impact of ethnicity on between population differences in dosage requirement for other CYP2C9 substrate drugs such as phenytoin.

Conclusion

In summary, we report significant ethnic differences in CYP2C9 enzyme activity between Swedes and Koreans, mainly but not entirely due *CYP2C9* pharmacogenetic variations between the two populations. The genetic, epigenetic, or environmental basis for this difference remains to be identified. *CYP2C9* genotype, ethnicity, and smoking are major determinants of CYP2C9 enzyme activity. We emphasize the importance of ethnicity, genotype, and smoking to be considered for population-specific dosage recommendations of narrow therapeutic index drugs metabolized by CYP2C9, such as warfarin.

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Author Disclosure Statement

The authors declare no conflict of interest.

References

Aklillu E, Carrillo J, Makonnen E, Bertilsson L, and Ingelman-Sundberg M. (2003). Xanthine oxidase activity is influenced by environmental factors in Ethiopians. Eur J Clin Pharmacol 59, 533–536.

Aklillu E, Djordjevic N, Carrillo JA, Makonnen E, Bertilsson L, and Ingelman-Sundberg M. (2014). High CYP2A6 enzyme activity as measured by a caffeine test and unique distribution of CYP2A6 variant alleles in Ethiopian population. OMICS 18, 446–453.

Aklillu E, Herrlin K, Gustafsson LL, Bertilsson L, and Ingelman-Sundberg M. (2002). Evidence for environmental influence on CYP2D6-catalysed debrisoquine hydroxylation as demonstrated by phenotyping and genotyping of Ethiopians living in Ethiopia or in Sweden. Pharmacogenetics 12, 375–383.

Bae JW, Kim HK, Kim JH, et al. (2005). Allele and genotype frequencies of CYP2C9 in a Korean population. Br J Clin Pharmacol 60, 418–422.

Bazan NS, Sabry NA, Rizk A, Mokhtar S, and Badary OA. (2014). Factors affecting warfarin dose requirements and quality of anticoagulation in adult Egyptian patients: Role of gene polymorphism. Ir J Med Sci 183, 161–172.

Cabaleiro T, Roman M, Ochoa D, et al. (2013). Evaluation of the relationship between sex, polymorphisms in CYP2C8 and CYP2C9, and pharmacokinetics of angiotensin receptor blockers. Drug Metab Dispos 41, 224–229.

Chen Y, Ferguson SS, Negishi M, and Goldstein JA. (2004). Induction of human CYP2C9 by rifampicin, hyperforin, and phenobarbital is mediated by the pregnane X receptor. J Pharmacol Exp Ther 308, 495–501.

Christensen M, Andersson K, Dalen P, et al. (2003). The Karolinska cocktail for phenotyping of five human cytochrome P450 enzymes. Clin Pharmacol Ther 73, 517–528.

Dandara C, Huzair F, Borda-Rodriguez A, et al. (2014). H3Africa and the African life sciences ecosystem: Building sustainable innovation. OMICS 18, 733–739.

Dang MT, Hambleton J, and Kayser SR. (2005). The influence of ethnicity on warfarin dosage requirement. Ann Pharmacother 39, 1008–1012.

Degawa M, Stern SJ, Martin MV, et al. (1994). Metabolic activation and carcinogen-DNA adduct detection in human larynx. Cancer Res 54, 4915–4919.

Dickmann LJ, Rettie AE, Kneller MB, et al. (2001). Identification and functional characterization of a new CYP2C9 variant (CYP2C9*5) expressed among African Americans. Mol Pharmacol 60, 382–387.

Diczfalusy U, Miura J, Roh HK, et al. (2008). 4 beta-Hydroxycholesterol is a new endogenous CYP3A marker: Relationship to CYP3A5 genotype, quinine 3-hydroxylation and sex in Koreans, Swedes and Tanzanians. Pharmacogenet Genomics 18, 201–208.

Djordjevic N, Carrillo J, Roh H, et al. (2012). Comparison of N-acetyltransferase-2 enzyme genotype-phenotype and xanthine oxidase enzyme activity between Swedes and Koreans. J Clin Pharmacol 52, 1527–1534.

Djordjevic N, Carrillo J, van den Broek M, et al. (2013). Comparisons of CYP2A6 genotype and enzyme activity between Swedes and Koreans. Drug Metab Pharmacokinet 28, 93–97.

Gebeyehu E, Engidawork E, Bijnsdorp A, Aminy A, Diczfalusy U, and Aklillu E. (2011). Sex and CYP3A5 genotype influence total CYP3A activity: High CYP3A activity and a unique distribution of CYP3A5 variant alleles in Ethiopians. Pharmacogenomics J 11, 130–137.

Ghotbi R, Christensen M, Roh H, Ingelman-Sundberg M, Aklillu E, and Bertilsson L. (2007). Comparisons of CYP1A2 genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans. Eur J Clin Pharmacol 63, 537–546.

Giri AK, Khan NM, Grover S, et al. (2014). Genetic epidemiology of pharmacogenetic variations in CYP2C9, CYP4F2 and VKORC1 genes associated with warfarin dosage in the Indian population. Pharmacogenomics 15, 1337–1354.

Greenblatt DJ, and von Moltke LL. (2005). Interaction of warfarin with drugs, natural substances, and foods. J Clin Pharmacol 45, 127–132.

Gross AS, Bridge S, and Shenfield GM. (1999). Pharmacokinetics of tolbutamide in ethnic Chinese. Br J Clin Pharmacol 47, 151–156.

Han EH, Kim HG, Choi JH, et al. (2012). Capsaicin induces CYP3A4 expression via pregnane X receptor and CCAAT/enhancer-binding protein beta activation. Mol Nutr Food Res 56, 797–809.

Hatta FH, Teh LK, Hellden A, et al. (2012). Search for the molecular basis of ultra-rapid CYP2C9-catalysed metabolism: relationship between SNP IVS8-109A>T and the losartan metabolism phenotype in Swedes. Eur J Clin Pharmacol 68, 1033–1042.

He SM, Zhou ZW, Li XT, and Zhou SF. (2011). Clinical drugs undergoing polymorphic metabolism by human cytochrome P450 2C9 and the implication in drug development. Curr Med Chem 18, 667–713.

Hidaka M, Nagata M, Kawano Y, et al. (2008). Inhibitory effects of fruit juices on cytochrome P450 2C9 activity in vitro. Biosci Biotechnol Biochem 72, 406–411.

Imai J, Ieiri I, Mamiya K, et al. (2000). Polymorphism of the cytochrome P450 (CYP) 2C9 gene in Japanese epileptic patients: Genetic analysis of the CYP2C9 locus. Pharmacogenetics 10, 85–89.

Izzo AA. (2005). Herb-drug interactions: An overview of the clinical evidence. Fundam Clin Pharmacol 19, 1–16.

Kidd RS, Curry TB, Gallagher S, Edeki T, Blaisdell J, and Goldstein JA. (2001). Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. Pharmacogenetics 11, 803–808.

Kim MJ, Nafziger AN, Kashuba AD, et al. (2006). Effects of fluvastatin and cigarette smoking on CYP2C9 activity measured using the probe S-warfarin. Eur J Clin Pharmacol 62, 431–436.

Kimmel SE, French B, Kasner SE, et al. (2013). A pharmacogenetic versus a clinical algorithm for warfarin dosing. N Engl J Med 369, 2283–2293.

Kimura Y, Ito H, and Hatano T. (2010). Effects of mace and nutmeg on human cytochrome P450 3A4 and 2C9 activity. Biol Pharm Bull 33, 1977–1982.

Lee CR, Pieper JA, Hinderliter AL, Blaisdell JA, and Goldstein JA. (2003). Losartan and E3174 pharmacokinetics in cytochrome P450 2C9*1/*1, *1/*2, and *1/*3 individuals. Pharmacotherapy 23, 720–725.

Lee VW, You JH, Lee KK, Chau TS, Waye MM, and Cheng G. (2005). Factors affecting the maintenance stable warfarin dosage in Hong Kong Chinese patients. J Thromb Thrombolysis 20, 33–38.

Llerena A, Alvarez M, Dorado P, et al. (2014). Interethnic differences in the relevance of CYP2C9 genotype and environmental factors for diclofenac metabolism in Hispanics from Cuba and Spain. Pharmacogenomics J 14, 229–234.

Luckert C, Ehlers A, Buhrke T, Seidel A, Lampen A, and Hessel S. (2013). Polycyclic aromatic hydrocarbons stimulate human CYP3A4 promoter activity via PXR. Toxicol Lett 222, 180–188.

McCrea JB, Lo MW, Furtek CI, et al. (1996). Ketoconazole does not effect the systemic conversion of losartan to e-3174. Clin Pharmacol Ther 59, 44–44.

Ngaimisi E, Habtewold A, Minzi O, et al. (2013). Importance of ethnicity, CYP2B6 and ABCB1 genotype for efavirenz pharmacokinetics and treatment outcomes: A parallel-group prospective cohort study in two sub-Saharan Africa populations. Plos One 8, e67946.

Ninomiya H, Mamiya K, Matsuo S, Ieiri I, Higuchi S, and Tashiro N. (2000). Genetic polymorphism of the CYP2C subfamily and excessive serum phenytoin concentration with central nervous system intoxication. Ther Drug Monit 22, 230–232.

Okura T, Ibe M, Umegaki K, Shinozuka K, and Yamada S. (2010). Effects of dietary ingredients on function and expression of P-glycoprotein in human intestinal epithelial cells. Biol Pharm Bull 33, 255–259.

Ramsjo M, Aklillu E, Bohman L, Ingelman-Sundberg M, Roh HK, and Bertilsson L. (2010). CYP2C19 activity comparison between Swedes and Koreans: Effect of genotype, sex, oral contraceptive use, and smoking. Eur J Clin Pharmacol 66, 871–877.

Rettie AE, and Jones JP. (2005). Clinical and toxicological relevance of CYP2C9: Drug–drug interactions and pharmacogenetics. Annu Rev Pharmacol Toxicol 45, 477–494.

Sandberg M, Johansson I, Christensen M, Rane A, and Eliasson E. (2004). The impact of CYP2C9 genetics and oral contraceptives on cytochrome P450 2C9 phenotype. Drug Metab Dispos 32, 484–489.

Scordo M, Aklillu E, Yasar U, Dahl M, Spina E, and Ingelman-Sundberg M. (2001). Genetic polymorphism of cytochrome P4502C9 in a Caucasian and a black African population. Br J Clin Pharmacol 52, 447–450.

Si D, Wang Y, Zhou YH, et al. (2009). Mechanism of CYP2C9 inhibition by flavones and flavonols. Drug Metab Dispos 37, 629–634.

Sica DA, Gehr TW, and Ghosh S. (2005). Clinical pharmacokinetics of losartan. Clin Pharmacokinet 44, 797–814.

Srinivas NR. (2013). Cranberry juice ingestion and clinical drug–drug interaction potentials; Review of case studies and perspectives. J Pharm Pharm Sci 16, 289–303.

Stearns RA, Chakravarty PK, Chen R, and Chiu SH. (1995). Biotransformation of losartan to its active carboxylic acid metabolite in human liver microsomes. Role of cytochrome P4502C and 3A subfamily members. Drug Metab Dispos 23, 207–215.

Stergiopoulos K, and Brown DL. (2014). Genotype-guided vs clinical dosing of warfarin and its analogues: Meta-analysis of randomized clinical trials. JAMA Intern Med 174, 1330–1338.

Takahashi H, Wilkinson GR, Nutescu EA, et al. (2006). Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans. Pharmacogenet Genomics 16, 101–110.

Tatsuno SY, and Tatsuno EM. (2014). Does ethnicity play a role in the dosing of warfarin in Hawai'i? Hawaii J Med Public Health 73, 76–79.

Teh LK, Langmia IM, Fazleen Haslinda MH, et al. (2012). Clinical relevance of VKORC1 (G-1639A and C1173T) and CYP2C9*3 among patients on warfarin. J Clin Pharm Ther 37, 232–236.

Thum T, Erpenbeck VJ, Moeller J, Hohlfeld JM, Krug N, and Borlak J. (2006). Expression of xenobiotic metabolizing enzymes in different lung compartments of smokers and nonsmokers. Environ Health Perspect 114, 1655–1661.

Wadelius M. (2014). Warfarin pharmacogenetics: It matters if you're black or white. Blood 124, 2171.

Wadelius M, Sorlin K, Wallerman O, et al. (2004). Warfarin sensitivity related to CYP2C9, CYP3A5, ABCB1 (MDR1) and other factors. Pharmacogenomics J 4, 40–48.

Wang YH, Pan PP, Dai DP, et al. (2014). Effect of 36 CYP2C9 variants found in the Chinese population on losartan metabolism in vitro. Xenobiotica 44, 270–275.

Wells PS, Holbrook AM, Crowther NR, and Hirsh J. (1994). Interactions of warfarin with drugs and food. Ann Intern Med 121, 676–683.

Yasar U, Aklillu E, Canaparo R, et al. (2002a). Analysis of CYP2C9*5 in Caucasian, Oriental and black-African populations. Eur J Clin Pharmacol 58, 555–558.

Yasar U, Forslund-Bergengren C, Tybring G, et al. (2002b). Pharmacokinetics of losartan and its metabolite E-3174 in relation to the CYP2C9 genotype. Clin Pharmacol Ther 71, 89–98.

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