Eur J Clin Pharmacol (2007) 63:537–546
DOI 10.1007/s00228-007-0288-2

PHARMACOGENETICS

Comparisons of *CYP1A2* genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans

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Received: 19 December 2006 / Accepted: 20 February 2007 / Published online: 17 March 2007

Abstract
Objectives To investigate the CYP1A2 genotype-phenotype relationship and to compare *CYP1A2* genetic polymorphisms and enzyme activity in terms of the effect of smoking and oral contraceptive (OC) use in Swedes and Koreans.
Methods CYP1A2 enzyme activity was determined in 194 and 150 healthy Swedish and Korean subjects, respectively, on the basis of the 4-h plasma paraxanthine/caffeine (17X/137X) ratio determined using high-performance liquid chromatography. Genotyping for the −3860G>A, −2467delT, −739 T>G, −729 C>T, −163C>A and −3113A>G polymorphisms was performed by PCR-restriction fragment length polymorphism analysis.
Results The mean 17X/137X ratio was 1.54-fold higher in Swedes than in Koreans (mean difference: 0.16; 95% CI of the mean difference: 0.12, 0.20; $p<0.0001$). Smokers had a significantly higher 17X/137X ratio (higher CYP1A2 activity) than non-smokers, while Swedish OC users had a significantly lower 17X/137X ratio than non-users (mean difference: 0.31, 95% CI of the mean difference: 0.23, 0.39; $p<0.0001$). No effect of gender differences on enzyme activity was observed. Four known (*CYP1A2*1A*, **1D*, **1F*, and **1L*) and two novel haplotypes (*CYP1A2*1V* and *CYP1A2*1W*) were found. *CYP1A2*1K* was rare in Swedes and absent in Koreans. No significant genotype-phenotype relationship was observed, with the exception of *CYP1A2*1F* in Swedish smokers, where it was associated with higher enzyme inducibility ($p=0.02$). Koreans displayed a significantly lower mean 17X/137X ratio than Swedes having the same *CYP1A2* genotype, smoking habit and OC use.
Conclusions We found significant differences in CYP1A2 enzyme activity between Swedes and Koreans that could not be explained by environmental factors or the *CYP1A2* haplotypes examined, despite differences in allele frequencies. None of the investigated *CYP1A2* haplotypes are critical in inducing variations in enzyme activity, with the exception of *CYP1A2*1F*.

Keywords Caffeine · CYP1A2 · Haplotype · Phenotype · Polymorphism

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Introduction

Cytochrome P450 1A2 (CYP1A2) accounts for about 15% of the total P450 content in the liver [1] and metabolises several important clinical drugs, such as clozapine [2], verapamil [3] and endogenous substrates such as melatonin [4] and estradiol [5]. Caffeine, which is predominantly metabolised by CYP1A2, is considered to be a "gold standard" probe for measuring CYP1A2 activity [6, 7].

Several studies have reported the presence of wide inter-individual and ethnic differences in CYP1A2 activity that might be of clinical relevance when prescribing drugs with a narrow therapeutic index, such as clozapine. Consequently, it is important to be able to identify an individual's metabolic capacity in order to avoid therapeutic failure or toxicity. A study on monozygotic and dizygotic twins revealed that variations in CYP1A2 activity are mainly governed by genetic factors although environmental factors also play a role [8]. In addition to the genetic factors causing altered drug metabolism, tobacco smoking and dietary constituents such as charcoal-broiled meat induce CYP1A2 activity whereas oral contraceptives (OCs) and fluoroquinolones [7, 9, 10] inhibit enzyme activity (http://medicine.iupui.edu/flockhart/table.htm).

To date, more than 20 variants of the *CYP1A2* gene have been identified (http://www.cypalleles.ki.se), and several single nucleotide polymorphisms (SNPs) have been found in the *CYP1A2* upstream sequence and intron 1 region. It has been suggested that some of these SNPs affect CYP1A2 expression/enzyme activity [11–13]. Of the polymorphic *CYP1A2* alleles showing variability in the promoter region, *CYP1A2*1C, CYP1A2*1D, CYP1A2*1F* and *CYP1A2*1K* have been associated with altered enzyme activity. C*YP1A2*1C*, located in the enhancer region, and the 3534G>A polymorphism, located in intron 6 of the *CYP1A2* gene, are reported to be associated with decreased enzymatic activity [12, 14]. The −163C>A polymorphism has been associated with higher enzyme inducibility by smoking [13], although this association is controversial [11, 15–18]. *CYP1A2*1D* and *CYP1A2*1F* exist at higher frequencies in Asians and Caucasians, respectively [13, 15, 16, 18]. In addition, *CYP1A2*1K* causes lower CYP1A2 activity in Ethiopians, whereas *CYP1A2*1J* does not have any effect on enzyme activity [11]. More recently, the −3113A>G polymorphism, with a frequency of 10% in a Chinese population, has been reported to be associated with decreased CYP1A2 activity [19]. Non-synonymous SNPs in the CYP1A2 coding region have also been reported, however their frequency in the population is very rare (<1%) [20, 21]. In the present study, common functional *CYP1A2* alleles were selected for investigation.

Inter-ethnic variations in genetic polymorphism and enzyme activity have been extensively investigated for the different CYP P450 enzymes. For example, the differences in CYP2D6 and CYP2C19 enzyme activity due to genetic variations between Asian and Caucasian populations are well documented. To our knowledge, no systematic comparisons of CYP1A2 have yet been carried out. The aims of our study were, therefore, to investigate the CYP1A2 genotype-phenotype relationship and compare CYP1A2 genetic polymorphisms as well as enzyme activity between Swedes and Koreans using caffeine as a probe.

Materials and methods

Study subjects

The study cohort consisted of 194 healthy unrelated Swedes from Karolinska University Hospital, Huddinge, Sweden and 150 unrelated Koreans from the Inha University Hospital, Incheon, Korea. The Swedish population comprised 80 men, of whom 12 were smokers (definition of smokers: >2 cigarettes/day), and 114 women, of whom 29 were smokers, with a median age of 26 years (range: 18–60 years) and a median weight of 68 kg (range: 47–109 kg). The Korean population comprised 76 men, of whom 26 were smokers, and 74 women, of whom two were smokers, with a median age of 24 years (range: 20–46 years) and a median weight of 60 kg (range: 38–94 kg). Permission was obtained from the Ethics Committees of Karolinska Institutet, Karolinska University Hospital, Huddinge, Sweden and Inha University Hospital, Korea. After oral and written explanations of the study, the subjects gave written informed consent for both phenotyping and genotyping analysis.

All subjects refrained from taking coffee, tea, Coca-Cola, chocolate or any caffeine-containing beverage for at least 36 h prior to and throughout the study. As a part of the Karolinska Cocktail [22], the subjects received a 100 mg oral dose of caffeine (Koffein, ACO AB Helsingborg, Sweden) at 7.00 a.m.; 4 h later, a 10-ml blood sample was collected, and plasma was prepared for phenotype analysis. A 10-ml blood sample was also drawn to prepare genomic DNA using QIAamp DNA Extraction kit (Qiagen, Hilden, Germany), and the DNA concentration was determined on a UV spectrophotometer (DU 530 Life Science UV/Vis spectrophotometer; Beckman Coulter, Fullerton, Calif.). Both the plasma and DNA samples from Korea were packed on dry ice and sent to Sweden for phenotype and genotype analysis.

Caffeine phenotyping

High-performance liquid chromatography (HPLC) was used to determine the plasma concentrations of paraxanthine (17X) and caffeine (137X) according to Christensen et al. [22] with some modifications. In brief, standards of 17X (Fluka, Buchs, Switzerland) in the concentration range of 0.67–26.6 μ*M* and 137X (Sigma-Aldrich, Buchs, Switzerland) in concentration range of 0.92–36.7 μ*M* were included in each run. Quality controls of low, medium and high concentrations of 137X and 17X were included in each run at concentrations of 1.7, 6.7 and 16.7 μ*M* and 2.5, 10.0 and 25.0 μ*M*, respectively. All samples, standards and quality controls were measured in one run and in duplicate. The CYP1A2 activity was calculated on the basis of the

17X/137X ratio index. The within-day coefficients of variation (CVs) were less than 9% at 6.7 μ*M* for caffeine (n=10) and less than 6% at 10.0 μ*M* for paraxanthine (n=10). The between-day CVs were less than 5% at 6.7 μ*M* for caffeine (n=10) and less than 3% at 10.0 μ*M* for paraxanthine (n=10).

SNP genotyping

Genotyping for five CYP1A2 polymorphisms, −3860G>A, −2467delT, −739 T>G, −729 C>T and −3113A>G, was carried out by PCR-restriction fragment length polymorphism (RFLP) analyses according to the references listed in Table 1, with some modifications. In brief, using the Perkin-Elmer GeneAmp PCR System 9700 (Applied Biosystems, Foster City, Calif.), we first PCR-amplified part of the *CYP1A2* gene spanning a polymorphic site using different forward and reverse primers (Invitrogen, Carlsbad, Calif.) designed for different parts of the gene. The PCR products were digested overnight with the respective restriction enzymes (New England Biolabs, Ipswich, Mass.), as indicated in Table 1, and the fragment patterns were analysed on a 2% agarose gel. Genotyping for the −163C>A polymorphism was carried out according to Nordmark et al. [16] using allele-specific primers and florescent-labelled reporter probes. PCR was performed using the Taqman Universal PCR Master Mix, and amplification and detection were performed in the ABI 7700 sequence detection system (Taqman; Applied Biosystems). Primers were from CyberGene (Novum, Stockholm, Sweden), and the fluorescence-labelled probes and Taqman Universal Master Mix were purchased from Applied Biosystems.

Statistical analysis

The chi-square and Fisher exact tests were used to assess differences in the allele and haplotype frequency distribution between Swedes and Koreans. The 17X/137X ratio was log transformed before the application of statistical analysis. The independent *t*-test was used to compare the 17X/137X ratio between Koreans and Swedes based on gender differences, smoking habit and OC use. ANOVA was used for comparing the 17X/137X ratios within each genotype/haplotype group based on smoking habit. Haplotype analysis and the estimation of expected haplotype frequency from the raw genotype data was carried out using the population genetic software programme, ARLEQUIN ver. 2.000 [23].

Results

Caffeine phenotyping

CYP1A2 activity was determined by the plasma 17X/137X ratio in 194 Swedes and 150 Koreans; the log-transformed 17X/137X ratio was found to be normally distributed for both Koreans (Fig. 1a, left) and Swedes (Fig. 1a, right). A 15-fold and six-fold variation in the 17X/137X ratio was observed in Swedes (range: 0.09–1.40) and Koreans (range: 0.11–0.72), respectively. A comparison of the mean 17X/137X ratio showed that the Swedish participants had a 1.54-fold higher ratio (mean difference: 0.160; 95% CI of the mean difference: 0.117, 0.197; $p<0.0001$) – i.e. higher CYP1A2 activity – than the Koreans (Fig. 1a). The effect of a smoking habit on enzyme activity was analysed within and between the Korean and Swedish groups using independent *t*-test. Smokers had significantly higher 17X/137X ratio than non-smokers in both Koreans (mean difference: 0.200; 95% CI of the mean difference: 0.143, 0.257; $p<0.0001$) and Swedes (mean difference: 0.103; 95% CI of the mean difference: 0.046, 0.160; $p<0.0007$). Korean smokers and non-smokers had a significantly lower mean 17X/137X

Table 1 Primers, PCR conditions and digestion enzymes used in the PCR-restriction fragment length polymorphism (RFLP) analyses for *CYP1A2* genotyping

Single nucleotide polymorphism	Primer name	Sequence (5′→3′)	Fragment length (bp)	$MgCl_2$ (m*M*)	Restriction enzyme	Reference
−3860G>A	1A2*1C-F	GCTACACATGATCGAGCTATAC	596	1.7	*Dde*I	[15]
	1A2*1C-R	CAGGTCTCTTCACTGTAAAGTTA				
−2467delT	1A2-delT	TGAGCCATGATTGTGGCATA	167	1.7	*Nde*I	[15]
	1A2-del-R	AGGAGTCTTTAATATGGACCCAG				
−739T>G	1A2-prom-F	CACTCACCTAGAGCCAGAAGCTC	167	1.4	*Ava*II	[11]
	1A2-prom-R	AGAGCTGGGTAGCAAAGCCTGGA				
−729C>T	1A2-prom-F	TGGAAGCTAGTGGGGACA	167	1.4	*Nci*I	[11]
	1A2-prom-R	TTGTGCTAAGGGGGAAGC				
−3113A>G	3113-F	AAGGAGAAGGAGCGTAATCC	441	1.4	*Hpy*CHIV	[19]
	3113-R	GTTCCAGGACCCATTGGA				

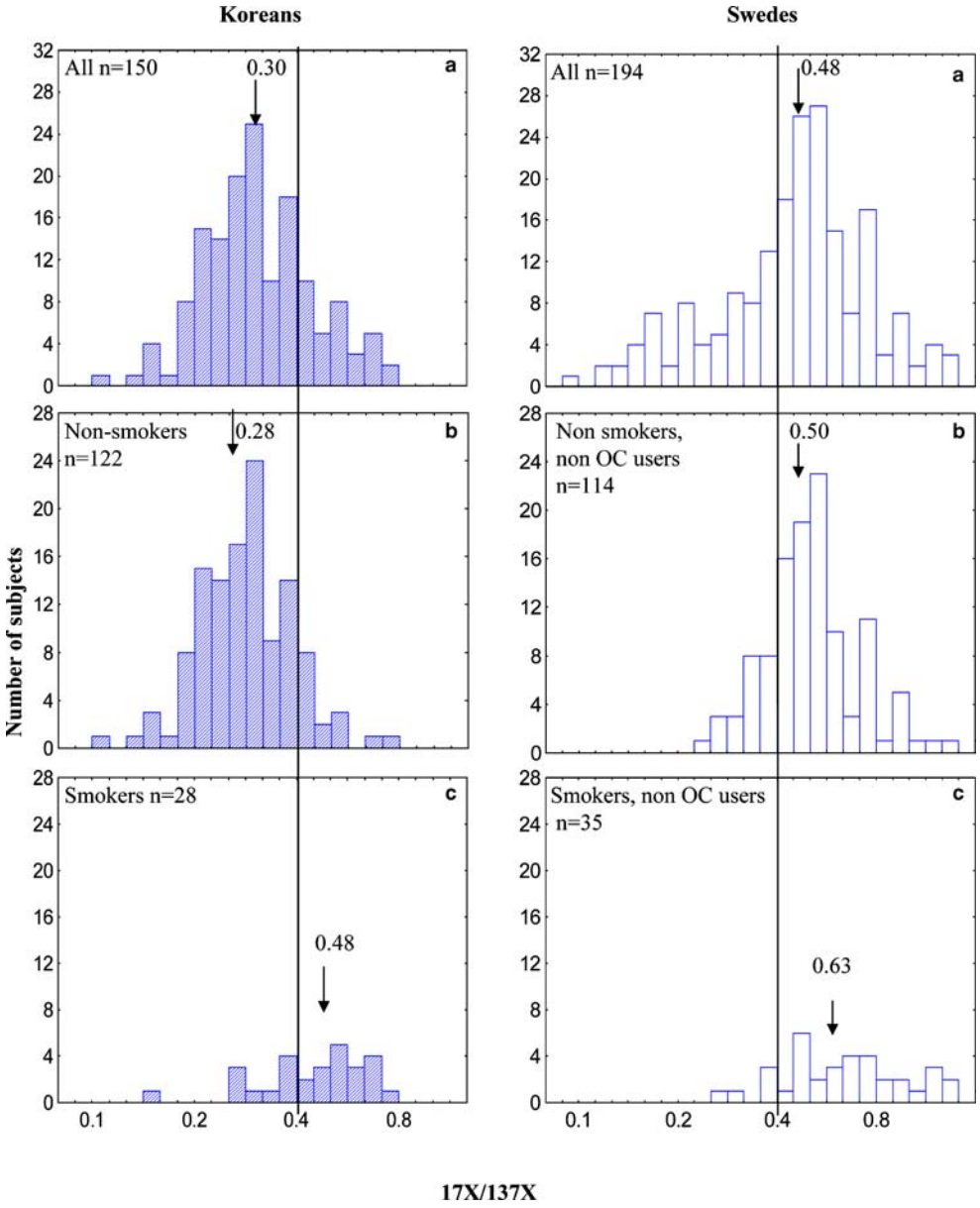

Fig. 1 The frequency distributions of the log-transformed 17X/137X ratio in 150 Koreans (*left*) and 194 Swedes (*right*). The *arrows* indicate the medians and the *numbers* along the *x*-axis are antilog values. The *vertical line* is shown at an arbitrary antilog value of 0.4 as reference

ratio than Swedish smokers ($p<0.0001$) and non-smokers ($p<0.0001$), respectively (Fig. 1b,c).

The effects of gender differences and OC use on enzyme activity were investigated (Fig. 2). There was no significant difference in the mean 17X/137X ratio between men and women (non-OC users) in both Swedes (mean difference: 0.051; 95% CI of the mean difference: −0.002, 0.104; $p=0.06$) and Koreans (mean difference: 0.036; 95% CI of the mean difference: −0.014, 0.086; $p=0.14$). Swedish OC users had a significantly lower 17X/137X ratio than non-OC users (mean difference: 0.309; 95% CI of the mean difference: 0.230, 0.388; $p<0.0001$; Fig. 2c). Korean men and women had a significantly lower mean 17X/137X ratio than Swedish men ($p<0.0001$) and women ($p<0.0001$), respectively (Fig. 2a and b). No comparison of OC users versus non-OC users was made in Koreans since only one woman used OC.

CYP1A2 genotypes and haplotypes

The SNP and haplotype frequencies found in our Swedish and Korean study cohort are listed in Tables 2 and 3, respectively. The frequency of the −3860G>A and −2467delT polymorphisms were significantly higher in Koreans than Swedes. The −163C>A was the most frequent SNP in Swedes, with a frequency of 71.4%, while in Koreans, the −2467delT was the most frequent SNP, with a frequency of 70.7%, followed by −163C>A, with a frequency of 62.7%. CYP1A2 haplotypes were constructed for the Korean and Swedish populations separately using the raw genotype data and the population genetic software, ARLEQUIN ver. 2.000 [23]. Six different haplotypes were found in both populations, including two novel ones that were assigned as *CYP1A2*1V* (−2467delT, −163C>A) and

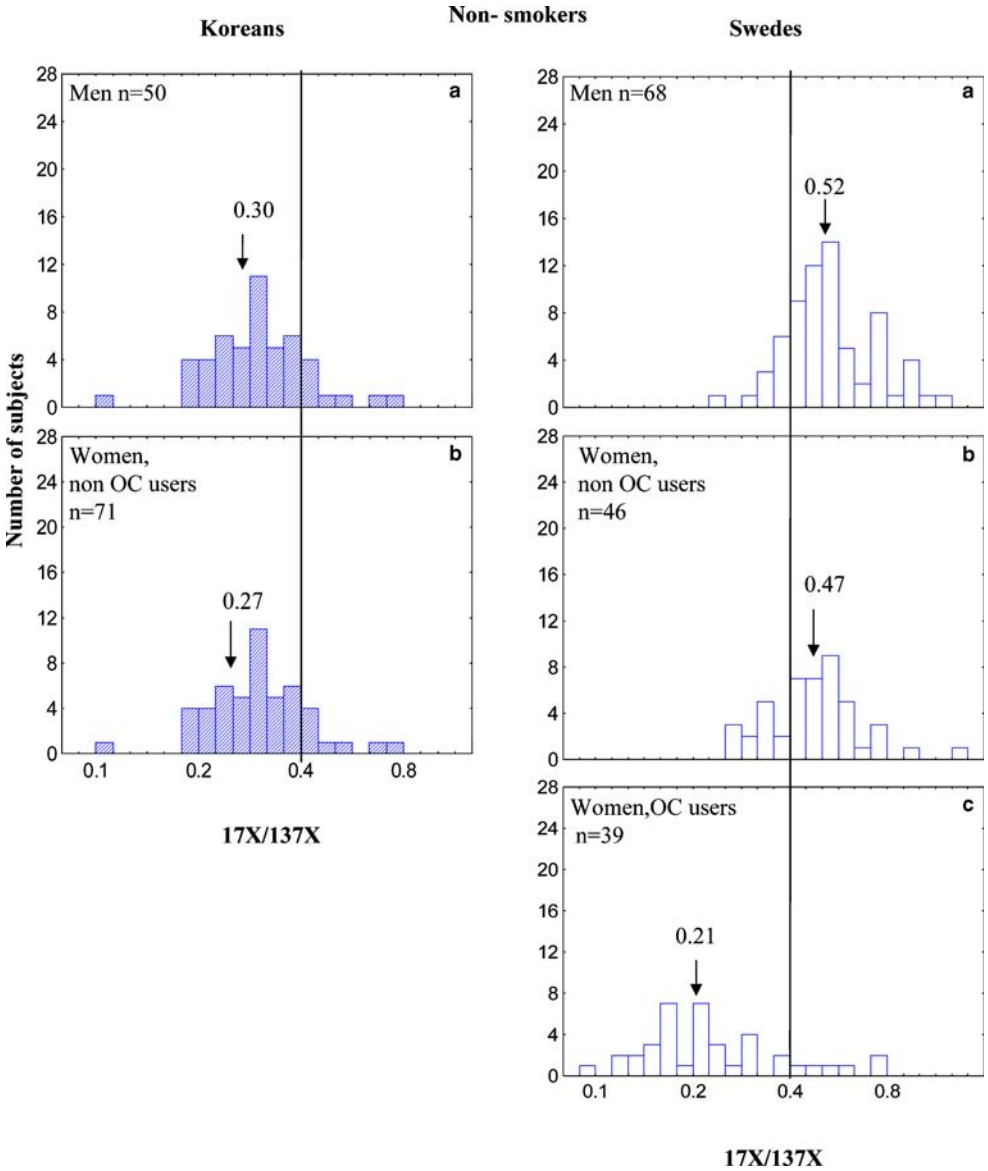

Fig. 2 The frequency distributions of the log-transformed 17X/137X ratio in 121 Korean (*left*) and 153 Swedish (*right*) non-smokers. The *arrows* indicate the medians, and the *numbers* along the *x*-axis are antilog values. The *vertical line* is shown at an arbitrary antilog value of 0.4 as reference

*CYP1A2*1W* (−2467delT, −163C>A, −739T>G, −3113A>G) according to *CYP1A2* allele nomenclature (http://www.cypalleles.ki.se). Similarly, there were significant differences in the haplotype frequency of *CYP1A2*1D*, **1F*, **1L* and **1V* between the Koreans and Swedes (Table 3). The most common haplotype in the Swedish subjects was *CYP1A2*1F*, containing only the −163C>A SNP, with a frequency of 56.7%. The most

Table 2 List of *CYP1A2* single nucleotide polymorphisms (SNPs) identified and their observed frequencies in the Swedish (n=194) and Korean subjects (n=150)

SNP	Swedes: observed frequency (95% CI)[a]	Koreans: observed frequency (95% CI)[a]	95% CI on the difference in allele frequency[a]	p value[b]
−3860G>A	0.008 (−0.001–0.017)	0.267 (0.217–0.317)	(−0.310–(−0.208))	<0.0001
−2467delT	0.193 (0.154–0.232)	0.707 (0.655–0.759)	(−0.579–(−0.449))	<0.0001
−163C>A	0.714 (0.669–0.760)	0.627 (0.572–0.682)	(0.016–0.157)	NS
−729T>C	0.003 (−0.002–0.008)	0.000	(−0.002–0.008)	NS
−739T>G	0.023 (0.008–0.038)	0.027 (0.009–0.045)	(−0.028–0.020)	NS
−3113A>G	0.023 (0.008–0.038)	0.027 (0.009–0.045)	(−0.028–0.020)	NS

[a] Chi-square and Fisher exact tests were used to calculate the confidence intervals (CI).
[b] NS, No significant differences

Table 3 List of CYP1A2 haplotypes identified and their observed frequencies in the Swedish (n=193) and Koreans subjects (n =50)

Haplotype	Nucleotide changes and SNP combinations	Swedes: observed frequency (95% CI)[a]	Koreans: observed frequency (95% CI)[a]	95% CI on the difference in frequency[a]	p value[b]
*CYP1A2*1A*	None	0.244 (0.201–0.287)	0.217 (0.170–0.264)	(−0.036–0.090)	NS
*CYP1A2*1D*	−2467delT	0.034 (0.016–0.052)	0.153 (0.112–0.194)	(−0.164–(−0.074))	0.0052
*CYP1A2*1F*	−163C>A	0.567 (0.518–0.616)	0.077 (0.047–0.107)	(0.431–0.548)	0.0001
*CYP1A2*1L*	−2467delT, −163C>A, −3860G>A	0.008 (−0.001–0.017)	0.267 (0.217–0.317)	(−0.310–(−0.208))	0.0001
*CYP1A2*1V*	−2467delT, −163C>A	0.123 (0.090–0.156)	0.260 (0.210–0.310)	(−0.196–(−0.078))	0.012
*CYP1A2*1W*	−2467delT, −163C>A, −739T>G, −3113A>G	0.021 (0.007–0.035)	0.027 (0.009–0.045)	(−0.029–0.017)	NS

[a] Chi-square and Fisher exact tests were used to calculate the CI.
[b] NS, No significant difference

frequent haplotypes in the Korean subjects were *CYP1A2*L* and *CYP1A2*1V*, with frequencies of 26.7 and 26.0%, respectively.

Association of individual SNPs and haplotype pairs with caffeine metabolic rate

The genotype and phenotype relationship was investigated using ANOVA by comparing the mean 17X/137X ratios within the genotype groups based on the smoking habit within and between the Swedish and Korean subjects. There were no significant differences in mean 17X/137X ratios between the different SNP genotype groups in Koreans and Swedes, with the exception of −163C>A, for which Swedish smokers homozygous for the −163 A/A SNP had a significantly higher mean 17X/137X ratio than those with the A/C or C/C genotype (p=0.04). However, no association was observed in the Koreans. In both the Koreans and Swedes, the mean 17X/137X ratios in smokers were significantly higher than those in non-smokers irregardless of the genotype groups (Table 4).

Table 4 Comparisons of the mean log 17X/137X ratios between Swedes and Koreans having the same genotype group, in smokers and non-smokers

CYP1A2 genotype	Non-smokers					Smokers				
	Swedes (n=114)		Koreans (n=121)		p (95% CI for mean difference)[a]	Swedes (n=35)		Koreans (n=28)		p (95% CI for mean difference)[a]
	n	Mean±SD	n	Mean±SD		n	Mean±SD	n	Mean±SD	
−3860G>A										
G/G	114	0.51±0.14	63	0.29±0.14	0.0001 (0.207, 0.292)	33	0.65±0.19	19	0.45±0.17	0.004 (0.053, 0.265)
G/A			48	0.28±0.12		2	0.51±0.04	6	0.39±0.09	0.16 (−0.054, 0.280)
A/A			10	0.27±0.17				3	0.54±0.15	
−2467delT										
T/T	90	0.55±0.15	3	0.28±0.10	0.002 (0.104, 0.440)	27	0.72±0.21	1	0.63	
T/delT	10	0.49±0.07	62	0.28±0.14	0.0001 (0.150, 0.321)	3	0.72±0.05	17	0.43±0.18	0.04 (0.005, 0.446)
delT/delT	14	0.50±0.16	56	0.8±0.13	0.0001 (0.135, 0.307)	5	0.58±0.09	10	0.46±0.12	0.18 (−0.046, 0.222)
−739T>G										
T/T	110	0.54±0.14	116	0.28±0.16	0.0001 (0.217, 0.293)	33	0.70±0.19	25	0.44±0.16	0.001 (0.064, 0.256)
T/G	4	0.52±0.11	5	0.28±0.15	0.03 (0.046, 0.470)	2	0.70±0.07	3	0.49±0.06	
−163C>A										
C/C	12	0.50±0.14	17	0.28±0.11	0.0001 (0.164, 0.348)	2	0.39±0.01	4	0.42±0.13	
C/A	42	0.49±0.16	55	0.28±0.13	0.0001 (0.180, 0.302)	17	0.58±0.19	14	0.44±0.19	0.10 (−0.024, 0.254)
A/A	60	0.52±0.13	49	0.28±0.14	0.0001 (0.210, 0.316)	16	0.76±0.16	10	0.46±0.12	0.001 (0.093, 0.341)
−3113A>G										
A/A	110	0.54±0.14	116	0.28±0.13	0.0001 (0.217, 0.293)	33	0.70±0.19	25	0.44±0.16	0.001 (0.064, 0.256)
A/G	4	0.52±0.11	5	0.28±0.15	0.03 (0.042, 0.466)	2	0.70±0.07	3	0.49±0.06	

For statistical comparison, the t- test or ANOVA was applied when the group consisted of two members or more than two members, respectively. Women using OC were excluded in the comparison.
[a] Confidence intervals (CIs) were calculated for the mean difference in 17X/137X ratios.

The haplotype and phenotype relationship was also investigated based on smoking habit (Table 5). No haplotype-phenotype relationships was found in both populations, with the exception of haplotype *CYP1A2*1F* in Swedish smokers, where smokers homozygous for **1F* had a significantly higher mean 17X/137X ratio than heterozygous or homozygous non-*1F (p=0.02). No effect of **1F* on the mean 17X/137X ratio was found in non-smokers. No individual was homozygous for **1F* among the Korean subjects, and only two individuals were heterozygous for **1F* among Korean smokers. Having the same haplotype group and smoking habit, Koreans had a significantly lower mean 17X/137X ratio than the Swedes. None of the Koreans and only one Swedish carried the −729C>T SNP and the *CYP1A2*1K* allele. Thus, no genotype-phenotype relationship analysis were carried out for the −729C>T SNP and *CYP1A2*1K*.

Discussion

CYP1A2 activity was measured in Swedish and Korean subjects using caffeine as a phenotyping probe. The induction of CYP1A2 by smoking and its inhibition by OCs was confirmed in this study. Previous studies have shown that smoking induces CYP1A2 [7] via the aryl hydrocarbon receptor and that OCs inhibit CYP1A2 [9]. Controlling for the effect of smoking and OCs, Swedes had a significantly higher 17X/137X ratio and, therefore, a higher CYP1A2 enzyme activity than the Koreans. Genetic factors, such as polymorphisms in the CYP1A2 gene causing altered enzyme activity, or environmental factors, such as dietary habits, could be an underlying cause for the observed differences in CYP1A2 enzyme activity between the Koreans and Swedes. Differences in dietary habits between these two populations could also in part explain the difference in CYP1A2 activity since dietary substances such as cruciferous vegetables and charcoal-broiled meat are known *CYP1A2* inducers [24].

Table 5 Comparisons of mean log 17X/137X ratios between Swedes and Koreans having the same haplotype group, in smokers and non-smokers

CYP1A2 haplotype	Non-smokers					Smokers				
	Swedes (n=114)		Koreans (n=121)		p (95% CI for mean difference)[a]	Swedes (n=35)		Koreans (n=28)		p (95% CI for mean difference)[a]
	n	Mean±SD	n	Mean±SD		n	Mean±SD	n	Mean±SD	
*CYP1A2*1A*										
**1A/*1A*	11	0.53±0.51	1	0.26		2	0.39±0.01			
*1A/non*1A	36	0.52±0.15	47	0.29±0.12	0.0001 (0.169, 0.291)	16	0.63±0.20	16	0.43±0.18	0.06 (−0.004, 0.270)
non**1A* /non**1A*	67	0.55±0.14	73	0.28±0.14	0.0001 (0.221, 0.320)	17	0.80±0.16	12	0.47±0.11	0.001 (0.086, 0.312)
*CYP1A2*1D*										
**1D/*1D*	1	0.52	8	0.27±0.11				1	0.52	
1D*/non1D*	6	0.51±0.21	24	0.28±0.14	0.001 (0.114, 0.422)	1	0.57	4	0.43±0.14	
non**1D*/non**1D*	107	0.51±0.14	89	0.28±0.14	0.0001 (0.210, 0.290)	34	0.64±0.19	23	0.45±0.16	0.002 (0.062, 0.254)
*CYP1A2*1F*										
**1F/*1F*	46	0.56±0.14				11	0.92±0.16			
1F*/non1F*	40	0.52±0.15	19	0.28±0.16	<0.0001 (0.170, 0.334)	16	0.61±0.19	2	0.56±0.07	1.00 (−0.290, 0.296)
non**1F*/non**1F*	28	0.50±0.15	102	0.28±0.13	<0.0001 (0.167, 0.281)	8	0.58±0.13	26	0.44±0.16	0.10 (−0.018, 0.230)
*CYP1A2*1L*										
**1L/*1L*			10	0.27±0.17				3	0.54±0.15	
1L*/non1L*			48	0.28±0.12		2	0.51±0.04	6	0.39±0.09	0.16 (−0.054, 0.280)
non**1L*/non**1L*	114	0.51±0.14	63	0.29±0.14	<0.0001 (0.205, 0.293)	33	0.65±0.19	19	0.45±0.17	0.004 (0.053, 0.265)
*CYP1A2*1V*										
**1V/*1V*	7	0.44±0.12	3	0.37±0.15	0.56 (−0.093, 0.271)	2	0.65±0.16			
1V*/non1V*	12	0.52±0.14	50	0.29±0.13	<0.0001 (0.144, 0.316)	4	0.59±0.09	15	0.42±0.18	0.15 (−0.061, 0.349)
non**1V*/non**1V*	95	0.54±0.14	68	0.27±0.13	<0.0001 (0.234, 0.322)	29	0.72±0.20	13	0.48±0.11	0.04 (0.009, 0.251)
*CYP1A2*1W*										
**1W/*1W*										
1W/non**1W*	7	0.47±0.11	5	0.28±0.15	0.01 (0.063, 0.397)	2	0.70±0.07	3	0.49±0.06	
non**1W*/non**1W*	107	0.51±0.15	116	0.28±0.13	<0.0001 (0.219, 0.295)	33	0.64±0.19	25	0.44±0.16	0.001 (0.064, 0.256)

For statistical comparison, t- test or ANOVA was applied when the group consisted of two members or more than two members, respectively. Women using OC were excluded in the comparison.

[a] Confidence intervals (CIs) were calculated for the mean difference in 17X/137X ratios.

We further investigated whether the observed inter-ethnic differences in enzyme activity was due to differences in CYP1A2 genetic polymorphisms and allele frequencies between the two populations. The frequencies of the *CYP1A2* SNPs in our Swedish and Korean populations are comparable with previous published data for Caucasians and Asians [12, 13, 15, 16, 19, 25]. The two SNPs that differed the most in frequency between the two populations were −3860G>A and the −2467delT, both of which existed more frequently in the Koreans than in the Swedes. Both SNPs have been identified in an Asian population in earlier studies, and −3860G>A has been reported to be associated with lower enzyme activity [12, 15]. One could speculate whether these SNPs could explain the observed differences in enzyme activity between the two populations. However, a recent study in a Japanese population reported that −3860G>A (*CYP1A2*1C*) has no influence on enzyme activity [26]. Accordingly, we did not find any significant differences in the 17X/137X ratio between the different genotype groups for the −3860G>A and −2467delT SNPs in both Swedes and Koreans. Therefore, variations in −3860G>A and −2467delT frequencies between our Korean and Swedish subjects could not be the underlying cause for the observed variation in enzyme activity between the two populations. Chen et al. described the −3113A>G polymorphism, which was present at a frequency of 10% in the Chinese population, as being associated with decreased CYP1A2 activity [19]. However, we found a lower frequency of the −3113A>G among both the Swedes (2.3%) and Koreans (2.7%) and there was no significant difference in CYP1A2 activity among the genotype groups with the −3113A>G polymorphism, neither in smokers nor in non-smokers.

Sachse et al. [13] reported that the −163A/A genotype in Caucasians is associated with significantly higher caffeine metabolic ratios compared with the A/C and C/C genotypes among smokers. The influence of the −163 C>A SNP on enzyme inducibility was replicated in the present study in the Swedish smokers (p=0.04). Haplotype analysis indicated that Swedish smokers carrying the haplotype *CYP1A2*1F* (only the −163 A/A) had significantly higher enzyme activity. *CYP1A2*1F* was the most frequent haplotype in Swedes (55.9%). However, the effect of the −163 A/A SNP on enzyme inducibility was observed only when it occurred alone: a comparison of the mean 17X/137X ratios in other haplotype groups where the −163C>A was present with the −2467delT and the −3860G>A indicated no significant difference on CYP1A2 enzyme inducibility. Thus, *CYP1A2*1F* conferred a higher enzyme inducibility of CYP1A2 by smoking in our Swedish subjects. However, a previous study in pregnant Swedish women indicated that the *−163C>A* SNP had not influence on enzyme activity; however, these researchers did not investigate other SNPs or carry out haplotype analysis [16].

In contrast to our results with the Swedish subjects, we found no influence of the −163C>A SNP on enzyme activity or inducibility in the Koreans. One possible explanation is that in Koreans, the −163 C>A SNP exists in linkage disequilibrium with other SNPs, such as the −2467delT and the −3860G>A SNPs, thereby generating other haplotypes, such as *CYP1A2*1L*, **1V* and **1W* at frequencies of 27, 26 and 3%, respectively. These haplotypes are rare in Swedes and exist at a frequency of 1, 12 and 2%, respectively. Although frequency of the −163C>A SNP between Koreans (63%) and Swedes (71%) was not significantly different, the frequency of the *CYP1A2*1F* haplotype in Koreans was significantly lower (7.7%) than in Swedes (57%). This result indicates that the −163C>A SNP confers a higher enzyme inducibility when it exists independent of the other SNPs. The present study illustrates the importance of studying haplotypes rather than focusing on individual SNPs in genetic association studies investigating genotype-phenotype relationships. The results of a recent study in a Japanese population also indicated that the −163C>A did not have an effect on enzyme activity [26]. Because of the low *CYP1A2*1F* allele frequency and hence low number of subjects carrying the **1F* haplotype, we were unable to investigate the effect of the *CYP1A2*1F* on enzyme inducibility in Koreans.

Despite the fact that *CYP1A2*1F* is argued to have an effect on enzyme inducibility, a recent study reported an association between coffee intake and an increased risk of nonfatal myocardial infarction among individuals carrying the *CYP1A2*1F* allele, who are defined as slow metabolisers, in both smokers and non-smokers [27]. However, these authors are actually describing the consensus allele −163C which is *CYP1A2*1A* but not *CYP1A2*1F*. Hitherto, it has not been shown that carriers of *CYP1A2*1F* differ from carriers of the *CYP1A2*1A* allele for non-inducible caffeine metabolism and, therefore, carriers of the *CYP1A2*1F* allele may not be classified as a "slow" or "rapid" caffeine metabolisers [28]. If any effect of the *CYP1A2*1F* polymorphism were to be seen, this would be restricted to smokers in whom an effect on inducibility may be evident.

Aklillu et al. described the −729C>T SNP and *CYP1A2*1K* allele found in an Ethiopian population to be associated with lower *CYP1A2* expression and enzyme activity [11]. *CYP1A2*1K* was found to be rare in Swedes (0.3%) and absent in Koreans. Thus, the effect of *CYP1A2*1K* could not be shown in these populations. Most of the *CYP1A2* polymorphisms known today do not seem to entirely explain the existing wide inter-individual and inter-ethnic variations in enzyme activity. A recent study aimed at investigating a genotype-phenotype relationship by sequencing the whole *CYP1A1_CYP1A2* locus from individuals having low or high enzyme activity/

expression revealed that no SNP or haplotype in the *CYP1A2* gene that has been identified to date could clearly be used to predict the metabolic phenotype [29]. Thus, yet unidentified polymorphisms that affect *CYP1A2* gene regulation could be one of the underlying causes for the observed differences in CYP1A2 enzyme activity [24].

In conclusion, we found significantly higher CYP1A2 enzyme activity in Swedes than in Koreans, when all of the subjects had the same smoking habit, OC use and genotype. Despite the presence of significant variations in CYP1A2 haplotype distribution, none of the genotypes or haplotypes investigated could explain the observed wide variation and differences in enzyme activity between the two populations. The observed difference in enzyme activity between Asians and Caucasians may be clinically relevant in terms of improving the therapeutic outcome when CYP1A2 substrate drugs with a narrow therapeutic index are prescribed. Further studies are required to explain inter-individual and inter-ethnic variations in CYP1A2 enzyme activity.

Acknowledgment We thank Birgitta Ask and Jolanta Widen for their excellent technical assistance. The study was supported by the Swedish Research Council, Medicine, 3902, and by NIH (R01 GM60548).

Conflict of interest statement The authors have identified no conflicts of interest in relation to this manuscript. This study complies with the current laws of Sweden and Korea, where it was performed, inclusive of ethics approvals.

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