



HAL
open science

Induction of CYP1A2 by heavy coffee consumption is associated with the -163C>A polymorphism

Natasa Djordjevic, Roza Ghotbi, Slobodan Jankovic, Eleni Aklillu

► **To cite this version:**

Natasa Djordjevic, Roza Ghotbi, Slobodan Jankovic, Eleni Aklillu. Induction of CYP1A2 by heavy coffee consumption is associated with the -163C>A polymorphism. *European Journal of Clinical Pharmacology*, 2010, 66 (7), pp.697-703. 10.1007/s00228-010-0823-4 . hal-00585807

HAL Id: hal-00585807

<https://hal.science/hal-00585807>

Submitted on 14 Apr 2011

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

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

Induction of CYP1A2 by heavy coffee consumption is associated with the *CYP1A2* –163C>A polymorphism

Natasa Djordjevic · Roza Ghotbi · Slobodan Jankovic · Eleni Aklillu

Received: 9 February 2010 / Accepted: 29 March 2010 / Published online: 14 April 2010
© Springer-Verlag 2010

Abstract

Objectives To investigate the association of *CYP1A2* genetic polymorphisms with the inducing effect of heavy coffee consumption on CYP1A2 activity in Serbian and Swedish populations, and to determine the frequency of the *CYP1A2* genetic polymorphisms in Serbs.

Methods Using PCR-RFLP and the tag-array minisequencing method, 126 Serbian healthy volunteers were genotyped for –3860G>A, –2467delT, –739T>G, –729C>T, –163C>A, 2159G>A, and 4795G>A. For 64 nonsmoking participants, the data on CYP1A2 activity (plasma paraxanthine/caffeine ratio) and coffee consumption habit were available from our previous study. The data on *CYP1A2* genotype, enzyme activity, and coffee consumption from 114 Swedish healthy nonsmoking subjects were included in the analyses.

Results In Serbs, *CYP1A2* polymorphisms –3860G>A, –2467delT, –739T>G, –729C>T, –163C>A, and 2159G>A were found at the frequencies of 0.4, 5.0, 3.4, 0.7, 61.1, and 56.0%, respectively, while 4795G>A was not detected. Significant association of heavy coffee consumption with high CYP1A2 enzyme activity was observed only in carriers of –163 A/A. Increasing effect of –163C>A on CYP1A2 inducibility was found in both Serbian ($P=0.022$)

and Swedish ($P=0.016$) nonsmoking heavy coffee consumers. There was no significant difference in CYP1A2 enzyme activity among genotypes in non-heavy coffee consumers. The results indicate that 22 and 14% of the phenotypic variability among Serbian and Swedish heavy coffee consumers, respectively, might be explained by –163C>A polymorphism.

Conclusions *CYP1A2* polymorphism –163C>A has an important increasing effect on CYP1A2 inducibility by heavy coffee consumption and may possibly be a contributing factor for interindividual variations in CYP1A2 enzyme activity.

Keywords CYP1A2 · Polymorphisms · Induction · Coffee

Introduction

Cytochrome P450 1A2 (CYP1A2) enzyme participates in the metabolism of numerous endogenous and foreign compounds and activates many procarcinogens [1, 2]. Therefore, any alteration in CYP1A2 activity affects drug metabolism and modifies the risk of developing cancers and other diseases [1–4]. CYP1A2 activity displays extensive inter- and intraindividual variability, and a number of environmental and genetic factors have been reported to contribute to these variations [5–10].

Polymorphism of the *CYP1A2* gene has been well described in many different populations, but only a few SNPs were associated with altered CYP1A2 activity [5, 10, 11]. On the other hand, numerous drugs, including oral contraceptives, have been reported to induce or inhibit CYP1A2 [2, 10, 12–14]. In addition, certain habits such as cigarette smoking also affect enzyme activity [9, 10], and this effect is observed to be associated with *CYP1A2*

N. Djordjevic · R. Ghotbi · E. Aklillu (✉)
Division of Clinical Pharmacology, Department of Laboratory
Medicine, Karolinska Institutet,
Karolinska University Hospital, Huddinge,
C1: 68 SE-141 86 Stockholm, Sweden
e-mail: Eleni.Aklillu@ki.se

N. Djordjevic · S. Jankovic
Department of Pharmacology and Toxicology, Medical Faculty,
University of Kragujevac,
Svetozara Markovica 69,
34 000 Kragujevac, Serbia

polymorphism. Namely, substitution C>A at position –163 (rs762551) leads to increased enzyme inducibility in smokers [7, 10, 15], while carriers of –3860G>A (rs2069514) who smoke cigarettes display decreased CYP1A2 activity [8].

Recently, we reported that heavy coffee consumption (daily consumption of at least three cups of coffee) significantly increases CYP1A2 activity [9]. This inducing effect has been observed in two separate populations, Serbs and Swedes, while controlling for the effect of drug intake and cigarette smoking. Yet, the association of the described effect with *CYP1A2* polymorphism has not been explored at this time.

CYP1A2 genetic polymorphisms in the Swedish population have been previously reported [10]. However, to the best of our knowledge, *CYP1A2* genotype in the Serbian population has not been investigated so far. The aim of the present study was to investigate the association of *CYP1A2* genetic polymorphisms with the inducing effect of heavy coffee consumption on CYP1A2 activity in Serbian and Swedish populations, and to determine the frequency of the *CYP1A2* genetic polymorphisms in Serbs.

Materials and methods

Study subjects One hundred twenty-six unrelated healthy Serbian volunteers, 60 men and 66 women, were enrolled in the genotype analyses. As a part of the previous study [16], a 20-ml venous blood sample was collected into EDTA-containing Vacutainer tubes (Sarstedt, Nümbrecht, Germany) and frozen at –80°C. All samples were packed on dry ice and sent to Karolinska Institutet, Stockholm, Sweden, for genotyping analyses. For 64 nonsmoking participants, the data on CYP1A2 activity [plasma paraxanthine (17X)/caffeine (137X) ratio] and coffee consumption were available from our previous study [9]. Additionally, data on *CYP1A2* genotype and activity of 114 Swedish nonsmoking subjects [10] were included in the analyses. There were no oral contraceptive users among the study subjects. All subjects participated voluntarily after written informed consent had been obtained. The study was approved by the ethics committees at the Medical Faculty, University of Kragujevac, Serbia, and at Karolinska Institutet, Sweden. The study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

***CYP1A2* genotyping** DNA was extracted from the whole blood samples using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Genotyping for six *CYP1A2* polymorphisms (–3860G>A, –163C>A, –739T>G, –729C>T, 2159G>A, and 4795G>A) was carried out using the tag-

array minisequencing method, described by Lindroos et al. [17] with some modifications. The multiplex PCR and minisequencing primers (Table 1) were designed using OligoPerfect Designer (www.invitrogen.com) and Auto-Primer software (<http://www.autoprimer.com/>) (Beckman Coulter, Fullerton, CA, USA), and purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA). Genotyping for –2467delT was carried out using the PCR-RFLP method described by Chida et al. [11].

Statistical analysis Haplotype analysis and haplotype frequency calculations were carried out using the population genetic software program Arlequin, version 3.11 (<http://cmpg.unibe.ch/software/arlequin3>). Chi-squared test was used to compare observed with expected allele frequencies (Hardy-Weinberg equilibrium). The 17X/137X ratio was log-transformed before statistical analyses. One-way ANOVA followed by the post-hoc analyses (Fisher test) was used to assess the effect of genotype and coffee consumption on CYP1A2 enzyme activity. Statistical analyses were performed with Statistica, version 7.1 (StatSoft, Tulsa, OK, USA), and $P < 0.05$ was considered as significant.

Results

The frequencies of the *CYP1A2* SNPs, haplotypes, and genotypes in the Serbian population are summarized in Table 2. The most frequent SNPs in Serbs were –163C>A and 2159G>A. Seven different haplotypes were found, including two novel ones that were assigned as *CYP1A2*IX* (–739T>G, –163C>A, 2159G>A) and *CYP1A2*IY* (–2467delT, –739T>G, –729G>A, –163C>A). All haplotypes were in Hardy-Weinberg equilibrium ($\chi^2 < 0.10$, $P = 0.05$).

To investigate the influence of genotype on CYP1A2 inducibility by coffee consumption, we analyzed both Serbian and Swedish heavy and non-heavy coffee consumers. Cigarette smokers and oral contraceptive users were not included in the comparison. In heavy coffee consumers, the only genotype-phenotype association revealed by comparison of log-transformed 17X/137X ratios included –163C>A polymorphism. The observed difference in CYP1A2 activity was significant in both Serbian (Fig. 1, $P = 0.022$) and Swedish (Fig. 2, $P = 0.016$) heavy coffee consumers, with the highest mean 17X/137X ratio in the –163 A/A genotype group (Table 3). The coefficients of determination indicate that 22% ($r^2 = 0.22$) and 14% ($r^2 = 0.14$) of the phenotypic variability among Serbian and Swedish heavy coffee consumers, respectively, might be explained by the –163C>A polymorphism. On the other

Table 1 SNPs and primers used in genotyping of *CYP1A2* gene by the tag-array minisequencing method

SNP	rs#	Primer name	Sequence 5' to 3'	PCR product (bp)
-3860G>A	rs2069514	CYP1A2*1C-F	TGGAGTGCAGTGGTGCGA	90
		CYP1A2*1C-R	TTCCAGCTACTCGGGAAG	
		CYP1A2*1C-MS-fo	GAGTAGCCTTCCCAGCATTGGGCTCACCGCAACCTCCGCCTCTC	
-739T>G	rs2069526	CYP1A2*1J*1K-F	AAGCTAGTGGGGACAGAAAGA	130
		CYP1A2*1J*1K-R	TTAAAAATGGCTTAGTCCAAACTG	
		CYP1A2*1J-MS-fo	AAACCATCGACTCACGGGATGGGGAGCCTGGGCTAGGTGTAGGGG	
-729C>T	rs12720461	CYP1A2*1J*1K-F	AAGCTAGTGGGGACAGAAAGA	130
		CYP1A2*1J*1K-R	TTAAAAATGGCTTAGTCCAAACTG	
		CYP1A2*1K-MS-re	ATTGACCAAACCTGCGGTGCGAGTCAAGAGCTGGGTAGCAAAGCCC	
-163C>A	rs762551	CYP1A2*1F-F	AATCTTGAGGCTCCTTTCCA	107
		CYP1A2*1F-R	AGCTGGATACCAGAAAGACTAAGC	
		CYP1A2*1F-MS-fo	ATTAACCTCGACTGCCGCGTGTGCTCAAAGGGTGAGCTCTGTGGGC	
2159G>A	rs2472304	CYP1A2rs2472304-F	AGAAGGAGCTGGGTACATGG	101
		CYP1A2rs2472304-R	AGCAGGCACATAACAGGTGT	
		CYP1A2rs2472304-MS-fo	AACAACGATGAGACCGGGCTACCCTATAGCCAGGAGAAGCCTTGA	
4795G>A	rs12904742	CYP1A2rs12904742-F	TACACGGGAGGCTCAGGT	97
		CYP1A2rs12904742-R	TATCACCCAGGCTGGAGTAC	
		CYP1A2rs12904742-MS-fo	AAGGCACGTATCATATCCCTGGTTGGCTTGAGCTGGGGAGGCAGA	

hand, in non-heavy coffee consumers there was no significant difference in *CYP1A2* enzyme activity among the different genotype groups, in Serbs ($P \geq 0.15$) or in Swedes ($P \geq 0.31$).

To further evaluate the effect of heavy coffee consumption on *CYP1A2* activity, we compared log-transformed 17X/137X ratios between heavy and non-heavy coffee consumers within each *CYP1A2* -163 genotype group. As expected, in both Serbs and Swedes the inducing effect of heavy coffee consumption on *CYP1A2* was observed only in carriers of -163 A/A (Table 3).

Discussion

In the present study, we investigated the association of *CYP1A2* genetic polymorphisms and the induction of *CYP1A2* by heavy coffee consumption in Serbs and Swedes, using caffeine as a probe drug and plasma 17X/137X ratio as an index of *CYP1A2* enzyme activity. To the best of our knowledge, this is the first study to report increased *CYP1A2* inducibility by heavy coffee consumption in carriers of -163 A/A genotype. In addition, we described the frequencies of the seven most important SNPs of *CYP1A2* gene, as well as haplotypes and genotypes, in the Serbian population.

The frequencies of investigated *CYP1A2* genetic variations, as well as of corresponding alleles and genotypes in Serbs, were comparable with the results previously pub-

lished for other Caucasians [5, 10, 18–20]. So far, only a few variations of the *CYP1A2* gene were associated with altered enzyme inducibility [2]. Nakajima et al. [8] described -3860G>A as a causal factor of decreased *CYP1A2* induction in Japanese smokers. On the contrary, in Korean smokers *CYP1A2* activity was not found to be affected by this polymorphism [10]. Substitution of G>A at the position -3860 of the *CYP1A2* gene is known as a feature of Asian populations [10, 12], and it is very rare in Caucasians [19]. As expected, in both Serbs and Swedes -3860G>A occurred at a very low frequency (less than 1%) [10]. Therefore, in the present study the proposed decreasing effect of this polymorphism on *CYP1A2* induction could not be tested. Association of *CYP1A2*1K* (-739T>G, -729C>T, -163C>A) with low enzyme activity in Ethiopians [5] was reported previously, but this allele is also rare in both Asians and Caucasians.

On the other hand, Sachse et al. [7] observed higher enzyme inducibility in the presence of -163 A/A genotype, and the effect was also restricted to smokers. The importance of -163C>A polymorphism for *CYP1A2* enzyme induction was confirmed by a number of studies, reporting higher *CYP1A2* enzyme activity in the presence of the *CYP1A2* inducers, such as cigarette smoking or omeprazol [10, 12, 15, 21]. Substitution C>A at position -163 is one of the most common *CYP1A2* polymorphisms in Caucasians, with frequencies that range from 33.8% in British [20] to 71.4% in Swedish population [10]. In Serbs, -163C>A was the most frequently observed SNP,

Table 2 SNP and haplotype frequencies of *CYP1A2* gene in Serbs

		Observed frequency	95% CI
SNP	-3860G>A	0.004 (1/274)	0.000, 0.023
	-2467delT	0.050 (14/280)	0.030, 0.083
	-739T>G	0.034 (9/264)	0.017, 0.065
	-729G>A	0.007 (2/274)	0.000, 0.028
	-163C>A	0.611 (160/262)	0.550, 0.668
	2159G>A	0.560 (149/266)	0.500, 0.618
	4795G>A	0.000 (0/274)	0.000, 0.017
Haplotype ^a	<i>CYP1A2*1A</i>	0.385 (97/252)	0.327, 0.446
	<i>CYP1A2*1C</i>	0.004 (1/252)	0.000, 0.025
	<i>CYP1A2*1M</i>	0.548 (138/252)	0.486, 0.608
	<i>CYP1A2*1V</i>	0.028 (7/252)	0.013, 0.058
	<i>CYP1A2*1W</i>	0.012 (3/252)	0.003, 0.036
	<i>CYP1A2*1X</i>	0.016 (4/252)	0.005, 0.042
	<i>CYP1A2*1Y</i>	0.008 (2/252)	0.000, 0.031
Genotype	<i>CYP1A2*1A</i> / <i>*1A</i>	0.143 (18/126)	0.092, 0.216
	<i>CYP1A2*1A</i> / <i>*1M</i>	0.429 (54/126)	0.346, 0.516
	<i>CYP1A2*1A</i> / <i>*1V</i>	0.024 (3/126)	0.005, 0.072
	<i>CYP1A2*1A</i> / <i>*1W</i>	0.016 (2/126)	0.001, 0.060
	<i>CYP1A2*1A</i> / <i>*1X</i>	0.008 (1/126)	0.000, 0.049
	<i>CYP1A2*1A</i> / <i>*1Y</i>	0.008 (1/126)	0.000, 0.049
	<i>CYP1A2*1C</i> / <i>*1M</i>	0.008 (1/126)	0.000, 0.049
	<i>CYP1A2*1M</i> / <i>*1M</i>	0.294 (37/126)	0.221, 0.379
	<i>CYP1A2*1M</i> / <i>*1V</i>	0.032 (4/126)	0.010, 0.082
	<i>CYP1A2*1M</i> / <i>*1W</i>	0.008 (1/126)	0.000, 0.049
	<i>CYP1A2*1M</i> / <i>*1X</i>	0.024 (3/126)	0.005, 0.072
	<i>CYP1A2*1M</i> / <i>*1Y</i>	0.008 (1/126)	0.000, 0.049

^a *CYP1A2*1A* (wild type), *CYP1A2*1C* (-3860G>A), *CYP1A2*1M* (-163C>A, 2159G>A), *CYP1A2*1V* (-2467delT, -163C>A), *CYP1A2*1W* (-2467delT, -163C>A, -739T>G), *CYP1A2*1X* (-739T>G, -163C>A, 2159G>A), *CYP1A2*1Y* (-2467delT, -739T>G, -729G>A, -163C>A)

with a frequency that fitted well with the expected range. To investigate the effect of -163C>A on induction of *CYP1A2* by heavy coffee consumption, we compared enzyme activity among Serbian and Swedish heavy coffee consumers, controlling for the effect of cigarette smoking and oral contraceptive use. Our results demonstrate significant differences in 17X/137X ratios among -163C>A genotypes in heavy coffee consumers, with the highest *CYP1A2* enzyme activity detected in carriers of -163 A/A genotype. The inducing effect of heavy coffee consumption was observed only in subjects homozygous for the variant type allele, which designates the -163A allele as a recessive factor necessary for the *CYP1A2* induction. As expected, the influence of -163C>A was not observed either in Serbian or in Swedish non-heavy coffee consumers.

Several previous studies using plasma levels of haloperidol [22]; caffeine as a probe; urinary (AFMU+1U+1X)/17U [23], (AAMU+AFMU+1U+1X)/17U [20], or (AFMU+1U+1X+17U+17X)/137X ratio [5] or theophylline as a probe; and plasma (1U+3X)/TP ratio [24] as index of *CYP1A2* activity failed to find any correlation of -163C>A polymorphism with *CYP1A2* induction. Nevertheless, some of the studies [20, 24] had too low sample power, which might be the reason for the lack of significance in genotype-phenotype association. In addition, incomplete determination of the different haplotypes existing in the populations was suggested as one of the possible explanations for the discrepant results [5, 10], suggesting that functional impact of -163C>A probably depends on whether this polymorphism occurs alone or in linkage disequilibrium with other SNPs. Furthermore, none of the previous studies took into account heavy

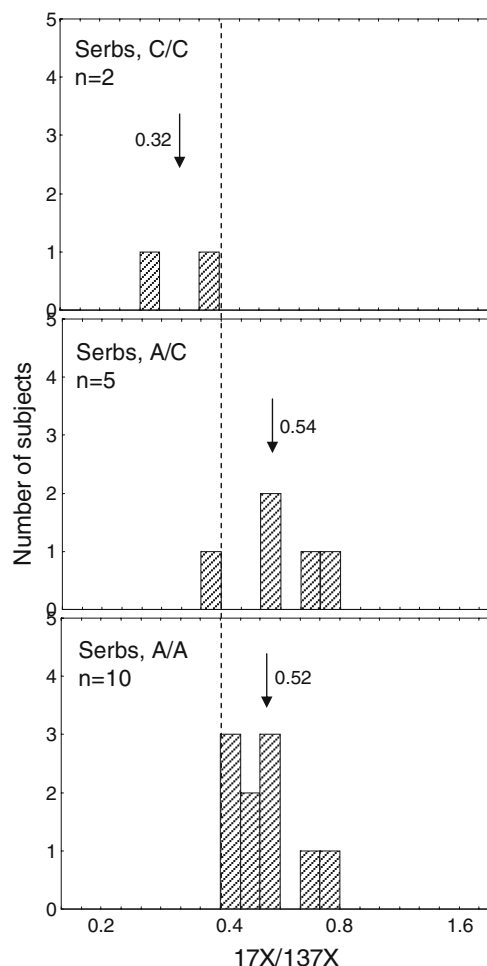


Fig. 1 The frequency distributions of the log-transformed 17X/137X ratios in Serbian heavy coffee consumers, with respect to -163C>A variation of *CYP1A2* gene. The arrows indicate the medians and the numbers along them are antilog values. The vertical line shows the reference antilog value of 0.4

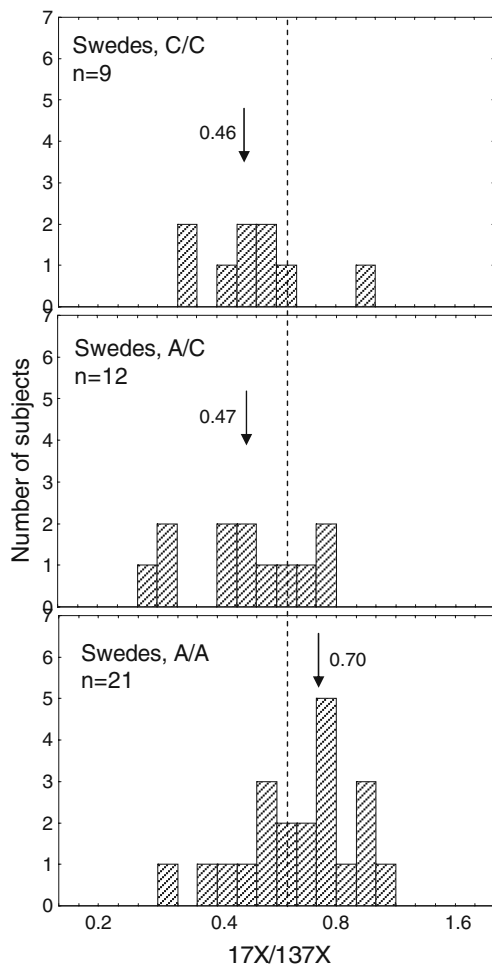


Fig. 2 The frequency distributions of the log-transformed 17X/137X ratios in Swedish heavy coffee consumers, with respect to -163C>A variation of *CYP1A2* gene. The arrows indicate the medians and the numbers along them are antilog values. The vertical line shows the reference antilog value of 0.6

coffee consumption as a possible confounding factor [9]. Our study demonstrates that heavy coffee consumption habit might be a possible confounding factor for the discrepant findings from studies that investigated the

association of the -163C>A polymorphism with *CYP1A2* enzyme induction.

Caffeine as a metabolic probe has several important advantages: rapid and complete absorption, wide distribution, low plasma protein binding, complete biotransformation, short half-life, and negligible renal excretion [25]. After ingestion, caffeine (137X) is predominantly metabolized by *CYP1A2* to paraxanthine (17X), and the 17X/137X ratio in plasma has been suggested as the most robust and valid measurement of *CYP1A2* activity [26, 27]. In the present study, *CYP1A2* activity was estimated by plasma 17X/137X ratio, after administration of 100 mg oral dose of caffeine [9, 10]. Possible competitive inhibition with caffeine from other dietary sources was avoided by abstaining from intake of any caffeine-containing food, beverage, and medication for at least 24 h prior to and during the study [9, 10]. On the other hand, it is known that cigarette smoking induces and oral contraceptive use inhibits *CYP1A2* [28, 29]. To avoid the effect of these confounders, in the present study smokers and oral contraceptive users were excluded from the comparison.

Recently, we reported significantly lower *CYP1A2* activity in Serbs compared to Swedes [9]. In addition, while in Swedes -163C>A polymorphism most frequently occurred alone (*CYP1A2**F) [10], in Serbs it was always in linkage disequilibrium with other SNPs, mostly with 2159G>A (*CYP1A2**M). To avoid the described confounding effect of ethnicity, Serbian and Swedish heavy coffee consumers were analyzed separately. The influence of -163C>A on *CYP1A2* activity was significant in both Serbian and Swedish heavy coffee consumers, while other *CYP1A2* polymorphisms, including 2159G>A, did not affect enzyme activity. Additionally, in both Serbs and Swedes, the inducing effect of heavy coffee consumption on *CYP1A2* was observed only in carriers of -163A/A. The results strongly suggest that -163C>A polymorphism has an important increasing effect on *CYP1A2* inducibility by heavy coffee consumption, regardless of possible linkage disequilibrium with other *CYP1A2* SNPs.

Table 3 Comparisons of mean 17X/137X ratios between non-heavy and heavy coffee consumers in Serbs and Swedes based on the *CYP1A2* -163C>A genotype

	Non-heavy coffee consumers		Heavy coffee consumers		P value
	n	Mean ± SD	n	Mean ± SD	
Serbs					
C/C	10	0.38±0.13	2	0.32±0.09	0.63
C/A	25	0.44±0.13	5	0.56±0.13	0.07
A/A	12	0.38±0.13	10	0.54±0.11	0.005
Swedes					
C/C	19	0.50±0.22	9	0.51±0.19	0.73
C/A	28	0.54±0.20	12	0.49±0.17	0.45
A/A	25	0.47±0.10	21	0.68±0.20	<0.0001

In conclusion, the results of the study indicate that the $-163C>A$ *CYP1A2* polymorphism has an important increasing effect on *CYP1A2* inducibility by heavy coffee consumption. In heavy coffee consumers, genotyping for $-163C>A$ should be considered as a factor affecting interindividual variations in drug metabolism, as well as susceptibility to diseases associated with *CYP1A2* activity.

Acknowledgments Authors would like to thank all volunteers who participated in the study. We are very grateful to Lilleba Bohman, Takashi Fukasawa, and Lili Milani for both technical support in the laboratory and scientific collaboration. The study was financially supported by the Swedish Research Council, Medicine, 3902; the Medical Faculty, University of Kragujevac, Republic of Serbia, JP 1/05; the Swedish Institute; Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I), Instituto de Salud Carlos III, Subdirección General de Evaluación y Fomento de la Investigación, PI071152; and Ayudas para la consolidación y apoyo a grupos de investigación de Extremadura, GRU09015 (Orden de 17 de diciembre de 2008, DOE 5 de enero de 2009).

Conflict of interest statement The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- Nakajima M, Yokoi T, Mizutani M, Shin S, Kadlubar FF, Kamataki T (1994) Phenotyping of *CYP1A2* in Japanese population by analysis of caffeine urinary metabolites: absence of mutation prescribing the phenotype in the *CYP1A2* gene. *Cancer Epidemiol Biomarkers Prev* 3:413–421
- Zhou SF, Wang B, Yang LP, Liu JP (2010) Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. *Drug Metab Rev* (in press)
- Bageman E, Ingvar C, Rose C, Jernstrom H (2008) Coffee consumption and *CYP1A2**1F genotype modify age at breast cancer diagnosis and estrogen receptor status. *Cancer Epidemiol Biomarkers Prev* 17:895–901
- Palatini P, Ceolotto G, Ragazzo F, Dorigatti F, Saladini F, Papparella I, Mos L, Zanata G, Santonastaso M (2009) *CYP1A2* genotype modifies the association between coffee intake and the risk of hypertension. *J Hypertens* 27:1594–1601
- Aklillu E, Carrillo JA, Makonnen E, Hellman K, Pitarque M, Bertilsson L, Ingelman-Sundberg M (2003) Genetic polymorphism of *CYP1A2* in Ethiopians affecting induction and expression: characterization of novel haplotypes with single-nucleotide polymorphisms in intron 1. *Mol Pharmacol* 64:659–669
- Kashuba AD, Bertino JS Jr, Kearns GL, Leeder JS, James AW, Gotschall R, Nafziger AN (1998) Quantitation of three-month intraindividual variability and influence of sex and menstrual cycle phase on *CYP1A2*, *N*-acetyltransferase-2, and xanthine oxidase activity determined with caffeine phenotyping. *Clin Pharmacol Ther* 63:540–551
- Sachse C, Brockmoller J, Bauer S, Roots I (1999) Functional significance of a $C\rightarrow A$ polymorphism in intron 1 of the cytochrome P450 *CYP1A2* gene tested with caffeine. *Br J Clin Pharmacol* 47:445–449
- Nakajima M, Yokoi T, Mizutani M, Kinoshita M, Funayama M, Kamataki T (1999) Genetic polymorphism in the 5'-flanking region of human *CYP1A2* gene: effect on the *CYP1A2* inducibility in humans. *J Biochem* 125:803–808
- Djordjevic N, Ghotbi R, Bertilsson L, Jankovic S, Aklillu E (2008) Induction of *CYP1A2* by heavy coffee consumption in Serbs and Swedes. *Eur J Clin Pharmacol* 64:381–385
- Ghotbi R, Christensen M, Roh HK, Ingelman-Sundberg M, Aklillu E, Bertilsson L (2007) Comparisons of *CYP1A2* genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans. *Eur J Clin Pharmacol* 63:537–546
- Chida M, Yokoi T, Fukui T, Kinoshita M, Yokota J, Kamataki T (1999) Detection of three genetic polymorphisms in the 5'-flanking region and intron 1 of human *CYP1A2* in the Japanese population. *Jpn J Cancer Res* 90:899–902
- Han XM, Ouyang DS, Chen XP, Shu Y, Jiang CH, Tan ZR, Zhou HH (2002) Inducibility of *CYP1A2* by omeprazole in vivo related to the genetic polymorphism of *CYP1A2*. *Br J Clin Pharmacol* 54:540–543
- Parker AC, Pritchard P, Preston T, Choonara I (1998) Induction of *CYP1A2* activity by carbamazepine in children using the caffeine breath test. *Br J Clin Pharmacol* 45:176–178
- Fuhr U, Anders EM, Mahr G, Sorgel F, Staib AH (1992) Inhibitory potency of quinolone antibacterial agents against cytochrome P450IA2 activity in vivo and in vitro. *Antimicrob Agents Chemother* 36:942–948
- Gunes A, Ozbey G, Vural EH, Uluoglu C, Scordo MG, Zengil H, Dahl ML (2009) Influence of genetic polymorphisms, smoking, gender and age on *CYP1A2* activity in a Turkish population. *Pharmacogenomics* 10:769–778
- Djordjevic N, Carrillo JA, Gervasini G, Jankovic S, Aklillu E (2010) In vivo evaluation of *CYP2A6* and xanthine oxidase enzyme activities in the Serbian population. *Eur J Clin Pharmacol* (in press)
- Lindroos K, Sigurdsson S, Johansson K, Ronnblom L, Syvanen AC (2002) Multiplex SNP genotyping in pooled DNA samples by a four-colour microarray system. *Nucleic Acids Res* 30:e70
- Chevalier D, Cauffiez C, Allorge D, Lo-Guidice JM, Lhermitte M, Lafitte JJ, Broly F (2001) Five novel natural allelic variants—951A>C, 1042G>A (D348N), 1156A>T (I386F), 1217G>A (C406Y) and 1291C>T (C431Y)—of the human *CYP1A2* gene in a French Caucasian population. *Hum Mutat* 17:355–356
- Kootstra-Ros JE, Smallegoor W, van der Weide J (2005) The cytochrome P450 *CYP1A2* genetic polymorphisms *1F and *1D do not affect clozapine clearance in a group of schizophrenic patients. *Ann Clin Biochem* 42:216–219
- Sachse C, Bhambra U, Smith G, Lightfoot TJ, Barrett JH, Scollay J, Garner RC, Boobis AR, Wolf CR, Gooderham NJ (2003) Polymorphisms in the cytochrome P450 *CYP1A2* gene (*CYP1A2*) in colorectal cancer patients and controls: allele frequencies, linkage disequilibrium and influence on caffeine metabolism. *Br J Clin Pharmacol* 55:68–76
- Pavanello S, Pulliero A, Lupi S, Gregorio P, Clonfero E (2005) Influence of the genetic polymorphism in the 5'-noncoding region of the *CYP1A2* gene on *CYP1A2* phenotype and urinary mutagenicity in smokers. *Mutat Res* 587:59–66
- Shimoda K, Someya T, Morita S, Hirokane G, Yokono A, Takahashi S, Okawa M (2002) Lack of impact of *CYP1A2* genetic polymorphism (C/A polymorphism at position 734 in intron 1 and G/A polymorphism at position -2964 in the 5'-flanking region of *CYP1A2*) on the plasma concentration of haloperidol in smoking male Japanese with schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 26:261–265
- Nordmark A, Lundgren S, Ask B, Granath F, Rane A (2002) The effect of the *CYP1A2* *1F mutation on *CYP1A2* inducibility in pregnant women. *Br J Clin Pharmacol* 54:504–510

24. Takata K, Saruwatari J, Nakada N, Nakagawa M, Fukuda K, Tanaka F, Takenaka S, Mihara S, Marubayashi T, Nakagawa K (2006) Phenotype-genotype analysis of CYP1A2 in Japanese patients receiving oral theophylline therapy. *Eur J Clin Pharmacol* 62:23–28
25. Krul C, Hageman G (1998) Analysis of urinary caffeine metabolites to assess biotransformation enzyme activities by reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 709:27–34
26. Fuhr U, Jetter A, Kirchheiner J (2007) Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the “cocktail” approach. *Clin Pharmacol Ther* 81:270–283
27. Fuhr U, Rost KL (1994) Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. *Pharmacogenetics* 4:109–116
28. Kalow W, Tang BK (1991) Caffeine as a metabolic probe: exploration of the enzyme-inducing effect of cigarette smoking. *Clin Pharmacol Ther* 49:44–48
29. Abernethy DR, Todd EL (1985) Impairment of caffeine clearance by chronic use of low-dose oestrogen-containing oral contraceptives. *Eur J Clin Pharmacol* 28:425–428