Influence of genetic polymorphisms, smoking, gender and age on CYP1A2 activity in a Turkish population

Aims: To study the variation in CYP1A2 activity in relation to smoking, gender, age and *CYP1A2* polymorphisms. **Materials & methods:** CYP1A2 activity was determined by plasma paraxanthine:caffeine ratio (17X:137X) 4 h after the intake of a standardized cup of coffee in 146 Turkish healthy volunteers. Seven *CYP1A2* polymorphisms (-3860G>A, -3113G>A, -2467del/T, -739T>G, -729C>T, -163C>A and 5347T>C) were analyzed. **Results:** The 17X:137X ratios were increased in smokers ($p < 0.0001$) and tended to be higher in men both among nonsmokers ($p = 0.051$) and smokers ($p = 0.064$). Age-related differences were observed only among nonsmoking women ($p = 0.024$). The -163C>A polymorphism correlated with 17X:137X ratios only in smokers ($p = 0.006$). Furthermore, increased 17X:137X ratios were observed in *CYP1A2* haplotype H4 (-3860G, -3113G, -2467del, -739T, -729C, -163A and 5347T) carriers in the overall study population ($p = 0.026$). Multiple regression analyses including smoking, gender, -163C>A genotype and age revealed a significant influence of smoking ($p < 0.0001$) and gender ($p = 0.002$) in the overall study population. However, in nonsmokers only the influence of gender remained significant ($p = 0.021$), while in smokers the influence of the -163C>A genotype held the statistical significance ($p = 0.019$). The influence of haplotype H4 remained significant ($p = 0.028$) in the overall study population in similar analyses. **Conclusion:** Smoking has the strongest impact on CYP1A2 activity, while gender and haplotype H4 showed marginal effects. The influence of the -163C>A polymorphism on CYP1A2 activity in smokers suggests an effect on the inducibility of the enzyme.

KEYWORDS: age · caffeine · CYP1A2 · gender · pharmacogenetics · polymorphisms · smoking

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CYP1A2 accounts for approximately 13% of the total liver cytochromes P450 (CYPs) and is involved in the metabolism of many commonly used drugs and endogenous compounds as well as in the bioactivation of procarcinogens [1,2]. The activity of this enzyme shows large interindividual variation. Caffeine, a substrate for CYP1A2, is considered as the gold standard probe for phenotyping CYP1A2 activity *in vivo* [3–5]. The paraxanthine (17X):caffeine (137X) ratio in plasma 4 h after caffeine intake can be used to estimate CYP1A2 activity as it correlates well with oral caffeine clearance [6].

Smoking is a well-known inducer of CYP1A2 [7–9]. Several drugs (e.g., omeprazole, oral contraceptives, fluvoxamine and furafylline), dietary factors, gender, age and pregnancy have also been shown to influence the activity of this enzyme [1,10]. Moreover, interethnic differences have been reported, possibly owing to diversity in lifestyles, dietary habits and genetic constitution [11–13].

A large number of SNPs have been identified in the *CYP1A2* gene [101] and a few have been suggested to alter the inducibility of the enzyme, -3860G>A (rs2069514) in the 5′-flanking region and -163C>A (rs762551) in intron 1 [7,9,14]. However, a number of studies failed to show any influence of these polymorphisms on enzyme activity and their functional importance is therefore controversial [15–17]. Another polymorphism, -3113G>A (rs2069521) in the 5′-flanking region of the gene, was reported to lead to decreased enzyme activity in a nonsmoker Chinese population [17]. However, this finding could not be replicated in two later studies [12,18]. The 5347T>C (rs2470890) polymorphism, a synonymous mutation in exon 7 of the gene, appears to have no significant impact on enzyme activity [19], even though a tendency for higher CYP1A2 activity in 5347T/T homozygotes compared with 5347C/C subjects ($p = 0.052$) was reported by Chen *et al.* [17].

Among *CYP1A2* haplotypes identified so far, *CYP1A2*1K* (-163A, -739G and -729T) has been reported to lead to a 40% lower inducibility of the enzyme *in vitro* and lower CYP1A2 activity, measured by urinary caffeine metabolic ratio, compared with *CYP1A2*1A* (wild-type, no variant alleles), *CYP1A2*1F* (-163A) or *CYP1A2*1J* (-163A and -739G) haplotypes in nonsmoking Ethiopians [16]. Due to the

low frequency of *CYP1A2*1K*, its influence on enzyme activity could not be evaluated in other populations [12,17]. Additionally, Swedish smokers homozygous for the *CYP1A2*1F* haplotype were shown to have higher plasma 17X:137X ratios compared with heterozygous or non-*CYP1A2*1F* carriers [12]. The possible influence of other rare variants (*CYP1A2*2–*16*) on enzyme activity is unclear as very few subjects carry these alleles [20–25]. Therefore, the impact of genetic polymorphisms on CYP1A2 activity is so far unclear and may also depend on ethnicity.

In the present study, we evaluated the effect of smoking, gender, age and *CYP1A2* polymorphisms on CYP1A2 enzyme activity, assessed using plasma 17X:137X ratio, in Turkish healthy volunteers.

Materials & methods

Subjects & study protocol

A total of 146 unrelated Turkish volunteers were recruited among students, faculty and hospital personnel at Faculty of Medicine, Gazi University, Ankara, Turkey. There were 53 smokers (22 women and 31 men), and 93 nonsmokers (59 women and 34 men), aged 19–57 years. All were healthy as assessed by medical history. No drugs that might interact with CYP1A2 (known substrates, inducers or inhibitors) were allowed 1 week before or during the study. Subjects using oral contraceptives were excluded. Smoking status was self-reported and subjects smoking less than three cigarettes a day were defined as nonsmokers.

The subjects were asked to refrain from caffeine-containing drinks (coffee, tea and soft drinks) for a minimum of 24 h before the study. A standardized cup of coffee (containing 100 mg caffeine) was given to the subjects and blood samples (10 ml) were collected 4 h later. Plasma and whole-blood samples were kept at -20°C until analysis. The study was approved by the local ethics committee at Gazi University Hospital, Ankara, Turkey, and informed consent was obtained from all subjects before inclusion to the study.

Genotyping

Genomic DNA was isolated from peripheral leukocytes with Qiagen Blood and Cell Culture kit (Qiagen, CA, USA) according to the guidelines of the manufacturer. The *CYP1A2* polymorphisms -3860G>A [26], -3113G>A [17], -2467del/T [26], -739T>G, -729C>T [16], -163C>A [7] and 5347T>C [17] were analyzed by previously described PCR-restriction fragment length polymorphism methods with minor modifications. Positive and negative controls were included in each run.

Analysis of plasma caffeine & paraxanthine levels

CYP1A2 activity was assessed by the 17X:137X ratio in 4 h plasma samples. Caffeine and paraxanthine concentrations were measured by HPLC according to the method described by Christensen *et al.* [5]. The limit of quantification was 0.5 µmol/l and the interday coefficient of variation less than 13% at 7.5 µmol/l for both caffeine and paraxanthine.

Statistics

The Kruskal–Wallis and Mann–Whitney tests, used for group comparisons after log-transformation of plasma 17X:137X ratios, and Spearman rank test for correlation analysis were performed using GraphPad Prism 4 (GraphPad Software, Inc., CA, USA). Regression analyses were performed using Statview 5.0.1 (SAS Institute, Inc., NC, USA). P-values below 0.05 were accepted as statistically significant. Haplotypes were constructed using Phase 2.0 program [27].

Results

The plasma 17X:137X ratio showed 13-fold variation (from 0.13 to 1.66) in the study population, with a median of 0.64 (Figure 1). Smokers (n = 53) had significantly higher 17X:137X ratios than nonsmokers (n = 93) (0.87 [0.32–1.66] vs 0.55 [0.13–1.26], $p < 0.0001$) (Figure 1). Men tended to have higher 17X:137X ratios compared with women both among smokers (median [range] 0.97 [0.46–1.66] vs 0.84 [0.32–1.44], $p = 0.064$) and nonsmokers (0.59 [0.38–1.13] vs 0.50 [0.13–1.26], $p = 0.051$), but the differences did not reach statistical significance. No correlation was observed between the age of subjects and their 17X:137X ratios (rs = -0.04 in nonsmokers and rs = -0.063 in smokers). However, a significant influence of age on 17X:137X was observed among nonsmoking women ($p = 0.024$), subjects in their twenties having higher median 17X:137X ratios (n = 37, 0.58 [0.30–1.30]), compared with those in their thirties (n = 19, 0.47 [0.13–1.26]) ($p = 0.049$) and forties (n = 3, 0.39 [0.36–0.46]) ($p = 0.033$), while the difference between subjects in their thirties and forties was not significant. No effect of age was observed in smoking women or in men either nonsmoking or smoking.

The distribution of *CYP1A2* genotypes and alleles did not deviate from Hardy–Weinberg equilibrium. The minor allele frequencies (95% CIs) were 0.065 (0.037–0.090) for -3860A, 0.062 (0.034–0.090) for -3113A, 0.202 (0.156–0.248) for -2467del, 0.048 (0.023–0.073) for -739G, 0.007 (0.003–0.017) for -729T, 0.332 (0.278–0.386) for -163C and 0.497 (0.440–0.554) for 5347T.

The 17X:137X ratios in relation to *CYP1A2* genotypes and smoking behavior are summarized in Table 1. Among the polymorphisms evaluated in this study, only -163C>A showed a significant association with the 17X:137X ratio ($p = 0.006$) (Table 1). Smokers homozygous or heterozygous for the -163A allele had higher 17X:137X ratios compared with -163C/C carriers ($p = 0.004$ and $p = 0.038$, respectively), while no difference was observed among nonsmokers. Smokers with the -163A/A genotype had 1.9-fold higher median 17X:137X ratio than nonsmokers with the same genotype ($p < 0.0001$, Figure 2). Similarly, among subjects carrying the -163C/A genotype, smokers had a significantly higher median 17X:137X ratio compared with nonsmokers ($p = 0.003$) while no difference was found among subjects with the -163C/C genotype.

In multiple regression analyses of the overall study population including smoking, gender, age and -163C>A genotype as co-variants, only smoking ($p < 0.0001$) and gender ($p = 0.002$) had significant association with the 17X:137X ratio. Accordingly, smoking status explained 24% and gender explained 10% of the variation in 17X:137X in our study population, while the contribution of -163C>A genotype and age were minimal (less than 1%) and not significant.

In nonsmokers, multiple regression analyses including gender, age and -163C>A genotype revealed a significant influence for gender only ($p = 0.021$) explaining 4.8% of the variation in 17X:137X. However, a similar analysis in smokers including -163C>A, gender and age as co-variants showed a significant influence of the -163C>A genotype ($p = 0.019$), while for gender only a tendency was observed ($p = 0.087$). The -163C>A genotype explained 19.4% of the variation in 17X:137X, gender contributing with 8% and age with less than 1%.

The data were further analyzed with respect to haplotypes. The pairwise linkage disequilibrium analyses (r^2) of the seven SNPs studied are shown in Figure 3 and the haplotype frequencies in Table 2. The plasma 17X:137X ratios (median and range) in nonsmokers and smokers carrying different haplotype pairs

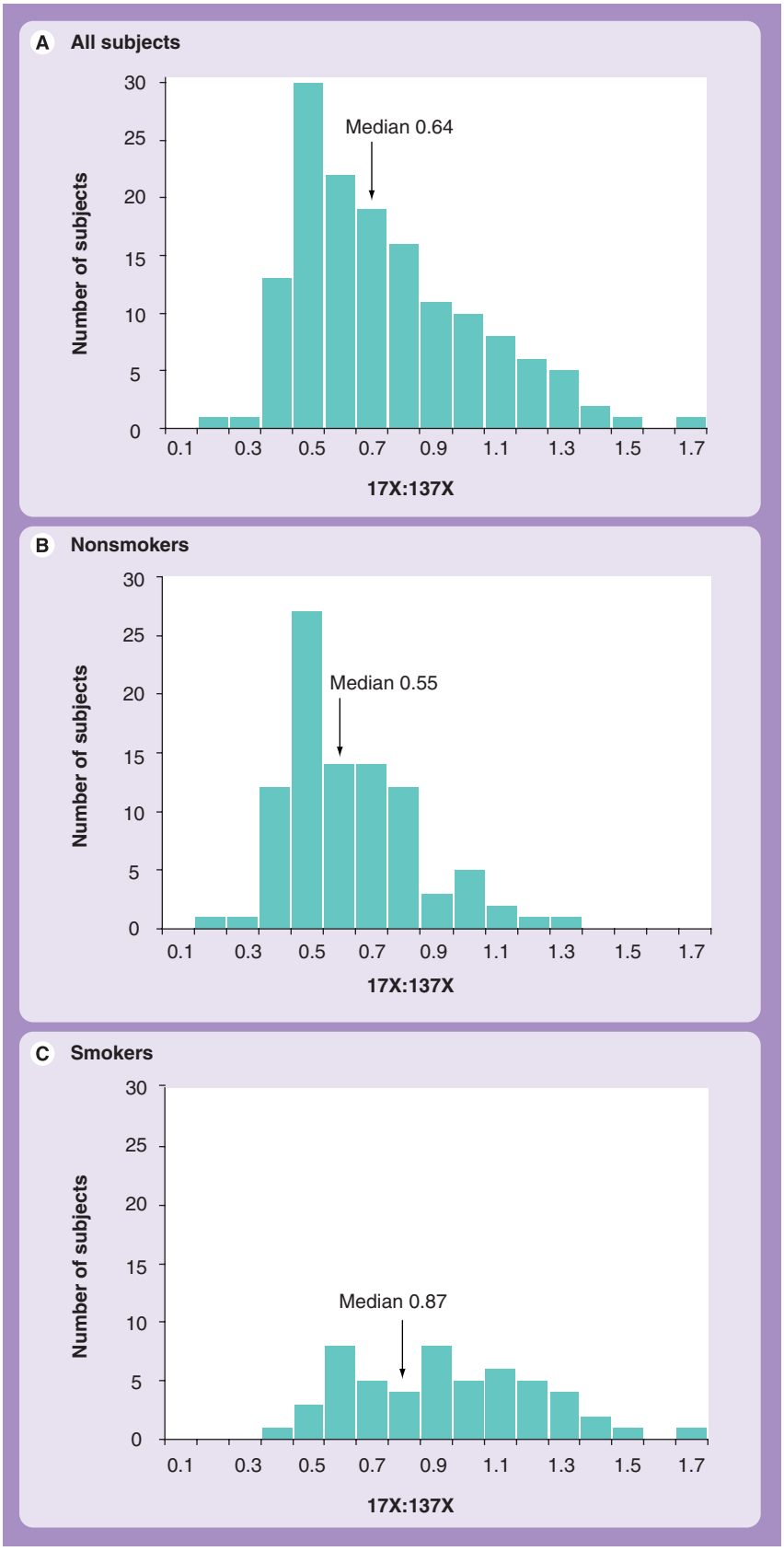

Figure 1. Distribution of log-transformed plasma 17X:137X ratios in the overall study population (A) and separately in nonsmokers (B) and in smokers (C). The 17X:137X ratios on the x-axis are the antilog values.

Table 1. *CYP1A2* genotype distribution and 17X:137X ratios (median, range) in smokers and nonsmokers.

Polymorphism	Genotype	Nonsmokers			Smokers		
		n	*17X:137X*	*p-value**	*n*	*17X:137X*	*p-value**
-3860G>A	G/G	86	0.56 (0.27–1.26)	0.551	43	0.84 (0.32–1.66)	0.782
	G/A	7	0.50 (0.13–0.79)		8	0.98 (0.58–1.15)	
	A/A	0	–		2	0.91 (0.84–0.97)	
-3113G>A	G/G	81	0.55 (0.13–1.26)	0.225	48	0.86 (0.32–1.66)	0.484
	G/A	11	0.60 (0.30–0.70)		5	1.00 (0.66–1.22)	
	A/A	1	0.27		0	–	
-2467del/T	T/T	58	0.55 (0.27–1.26)	0.391	36	0.82 (0.32–1.32)	0.263
	T/del	31	0.51 (0.13–1.06)		14	1.01 (0.58–1.66)	
	del/del	4	0.65 (0.50–0.89)		3	0.90 (0.84–1.00)	
-739T>G	T/T	84	0.56 (0.13–1.26)	0.447	48	0.86 (0.32–1.66)	0.484
	T/G	9	0.48 (0.38–0.70)		5	1.00 (0.66–1.22)	
	G/G	0	–		0	–	
-729C>T	C/C	91	0.56 (0.13–1.26)	–	53	0.87 (0.32–1.66)	–
	C/T	2	0.46 (0.45–0.47)		0	–	
	T/T	0	–		0	–	
-163C>A	C/C	50	0.53 (0.31–1.13)	0.353	18	0.58 (0.44–0.92)	0.006
	C/A	30	0.60 (0.30–1.26)		29	0.83 (0.32–1.44)	
	A/A	13	0.52 (0.13–0.97)		6	0.99 (0.57–1.66)	
5347T>C	T/T	30	0.55 (0.36–0.94)	0.360	10	0.98 (0.57–1.66)	0.230
	T/C	36	0.49 (0.13–1.26)		29	0.87 (0.32–1.44)	
	C/C	27	0.60 (0.27–1.13)		14	0.75 (0.44–1.15)	

**p-values are based on comparison of log transformed 17X:137X ratios between the genotype groups. The 17X:137X ratios are antilog values.*

are shown in Table 3. Smokers homozygous for H1 had significantly higher plasma 17X:137X ratios than nonsmokers with the same genotype (p = 0.005). However, there was no difference in 17X:137X ratios between carriers and noncarriers of this haplotype either among smokers or nonsmokers. Subjects carrying H4 (including the -3860G, -3113G, -2467del, -739T, -729C, -163A and 5347T alleles) had higher 17X:137X ratios compared with subjects who did not carry this haplotype (p = 0.026) in the overall study population (Figure 4A). However, comparisons of carriers and noncarriers of this haplotype separately among nonsmokers and smokers did not reveal any significant differences (p = 0.568 and p = 0.054, respectively) (Figure 4B). None of the other haplotypes showed any significant association with the 17X:137X ratio.

The influence of H4 haplotype on 17X:137X ratio remained significant (p = 0.028) in a multiple regression analysis with smoking, gender age and H4 genotype as co-variants and explained 4.1% of the variation of 17X:137X in the overall study population. This effect was not statistically significant when only nonsmokers were studied (1%, p > 0.05), while in smokers, a significant effect of H4 haplotype (p = 0.025) was observed in the multiple model analysis explaining 7.9% of the variation.

Discussion

In the present study, smoking and gender explained up to 34% of the variation, whilst a smaller contribution by haplotype H4 (4%) was observed in the present overall study population. However, when the analysis was restricted to smokers, the -163C>A polymorphism explained 19% of the variation only in smokers. These results strongly support the role of the -163C>A polymorphism in the inducibility of CYP1A2 by smoking. The influence of age was only observed among nonsmoking women. However, this effect did not hold statistical significance when evaluated together with other confounders. Moreover, the age span in the study population was narrow, with no subject being older than 57 years.

The inducer effect of smoking on CYP1A2 activity has been reported to be related to the enhanced transcription of the *CYP1A2* gene due to the interaction between polycyclic aromatic hydrocarbons present in tobacco smoke and aryl hydrocarbon receptor, a transcription factor that binds to the aryl hydrocarbon response element located in the 5′-flanking region of the gene [8]. The inducing effect of smoking on CYP1A2 activity has been reported to be dose dependent [28]. However, the classification of nonsmokers as subjects smoking less than three cigarettes per day has been used

previously in the literature and in our study concerning CYP1A2 activity [12]. We had separately evaluated our data in the subjects smoking zero, one, or two cigarettes per day and observed no differences in 17X:137X ratios (data not shown). Thus, this classification does not seem to influence our findings.

CYP1A2 activity shows large interindividual variation that has been shown to be determined to a great extent by heritability in twin studies [29,30]. So far, several environmental and constitutional factors have been shown to influence CYP1A2 activity [1]. Nevertheless, the genetic determinants of this variability have not been clear to date.

The SNPs evaluated in the present study were chosen based on literature data suggesting a functional impact on CYP1A2 activity [7,9,16,17] and presence in Caucasian populations [102]. In comparison to the available data from Hapmap database, allele frequencies for -3113A (0.062), -739G (0.048) and -729T (0.007) alleles in the Turkish population evaluated in the present study were lower than Chinese (CHB; 0.091, 0.083 and 0.023, respectively) and Yoruban (YRI; 0.100, 0.125 and 0) populations and were higher than in Japanese (JPT; 0.033, 0.023 and 0) and Caucasians (CEU; 0.017, 0 and 0) evaluated in Hapmap [102]. The frequency of the -163C allele was similar in Turkish (0.332), CEU (0.318), CHB (0.333) and in JPT (0.386) but slightly lower than in YRI (0.433). The 5347T allele was observed with the highest frequency in CEU (0.662) followed by Turkish (0.497), JPT (0.182) and CHB (0.122) but not observed in the YRI population. Thus, the differences in *CYP1A2* allele frequencies are in line with the geographic origin of the populations compared.

While our results were not able to confirm previously reported associations between -3860G>A, -3113G>A or *CYP1A2*1K* polymorphisms and CYP1A2 activity, a strong smoking-associated impact of -163C>A was observed. The inconsistent findings might be due to differences in allele frequencies, especially for the -3860A allele, which is much more common among Orientals (allele frequency 0.23 in Japanese [9]) than in Caucasians (0.065 in this study) or differences in environmental factors between study populations.

Among the 15 haplotypes observed in our study, H1 (*CYP1A2*1F*) had the highest frequency (0.408), similar to the frequencies previously reported in Swedes (0.567) [12] and Ethiopians (0.496) [16] and higher than in Koreans (0.077) [12] and Japanese (0.004) [31]. Smokers with H1/H1 genotype had significantly

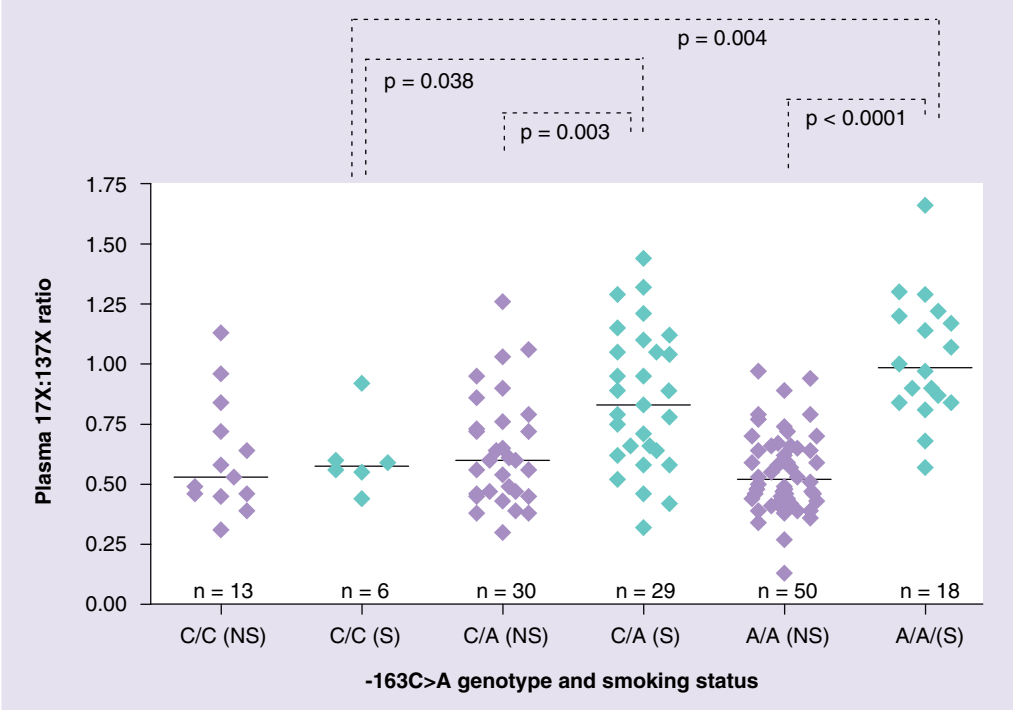

Figure 2. Plasma 17X:137X ratios in nonsmokers and smokers in relation to -163C>A genotype. The horizontal lines indicate median 17X:137X ratios.
NS: Nonsmokers; S: Smokers.

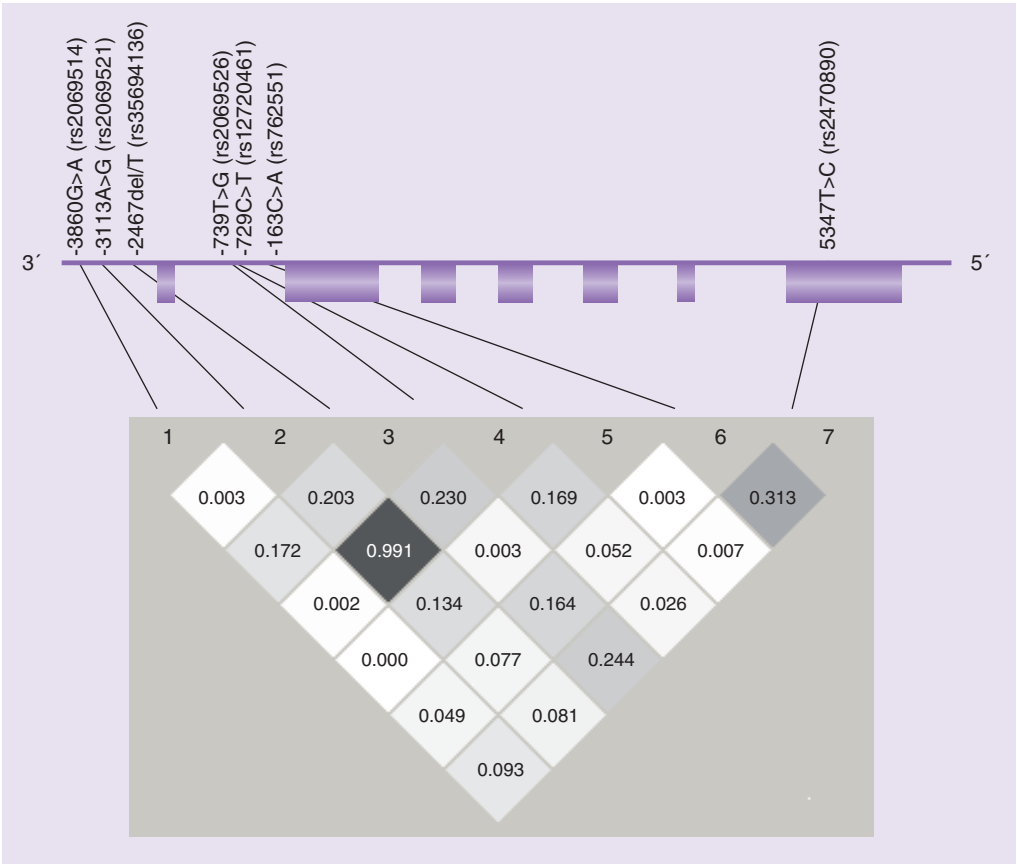

Figure 3. Pairwise linkage disequilibrium (r^2) between *CYP1A2* polymorphisms evaluated in this study.

higher 17X:137X ratios than nonsmokers with the same genotype (p = 0.005) in our study population. However, there was no significant difference in 17X:137X ratio between H1/H1, H1/non-H1 and non-H1/non-H1 carriers among smokers or nonsmokers. Therefore, it is difficult to interpret the influence of this haplotype on the inducibility of the enzyme. *CYP1A2*1F* haplotype carriers were previously shown to have significantly higher plasma 17X:137X ratios compared with subject without this haplotype in Swedish smokers but not in Koreans or Ethiopians [12,16].

The association of H4 (*CYP1A2*1V*) with CYP1A2 activity has not been shown previously. The frequency of this haplotype is higher in Koreans (0.260) than in Swedes (0.123) [12] and our Turkish population (0.051). As we included the 5347T>C polymorphism in our haplotype construction, it is probable that H3 (frequency 0.055) and H4 (0.051) in our population might have been classified as *CYP1A2*1V* in the study of Ghotbi *et al.* [12]. Moreover, as the -163A allele has been reported to associate with higher inducibility of the enzyme by smoking or omeprazole [7,14], it could be argued that this allele included in H4 might have led to the higher 17X:137X ratios in our study. In fact, the multiple regression analyses among smokers indicated a larger contribution by the -163C>A polymorphism alone on CYP1A2 activity compared with the H4 haplotype. Thus, among the seven SNPs evaluated in our study, testing for -163C>A might provide sufficient information for the impact of *CYP1A2* genotype on enzyme activity especially in smokers.

Higher CYP1A2 activity in men compared with women has been reported in several studies [11,29,32–34]. Oral contraceptives are known to inhibit CYP1A2 activity and therefore assumed to contribute to gender-related differences [1]. However, none of the subjects participating in the present study used oral contraceptives. Still, the 17X:137X ratio showed a trend for being higher in men compared with women both in smokers and nonsmokers, and the effect of gender remained significant in multiple regression analyses. It is thus possible that the expression and/or activity of this enzyme are hormonally regulated. Furthermore, we observed a small impact of age only in nonsmoking women, the

Table 2. *CYP1A2* haplotypes and their frequencies among 146 Turkish healthy volunteers.

Haplotypes	Frequency	-3860G>A	-3113G>A	-2467del/T	-739T>G	-729C>T	-163C>A	5347T>C
H1 (*CYP1A2*1F*)	0.408	G	G	T	T	C	A	T
H2 (*CYP1A2*1B*)	0.301	G	G	T	T	C	C	C
H3	0.055	G	G	Del	T	C	A	C
H4 (*CYP1A2*1V*)	0.051	G	G	Del	T	C	A	T
H5 (*CYP1A2*1L*)	0.045	A	G	Del	T	C	A	C
H6 (*CYP1A2*1A*)	0.031	G	G	T	T	C	C	T
H7	0.027	G	A	Del	G	C	A	C
H8	0.024	G	G	T	T	C	A	C
H9	0.021	A	G	T	T	C	A	C
H10	0.010	G	A	T	T	C	A	C
H11 (*CYP1A2*1W*)	0.007	G	A	Del	G	C	A	T
H12	0.007	G	A	Del	G	T	A	C
H13	0.007	G	A	del	T	C	A	C
H14	0.003	G	A	T	G	C	A	C
H15	0.003	G	G	Del	G	C	A	C

17X:137X ratio being higher in subjects in their twenties compared with those in their thirties and fourties. This finding is partly in line with previous reports, showing the effect of age on CYP1A2 activity among nonsmoking men and women but not among smokers [10,35]. Hence, age, especially in nonsmoker women, should be considered for its potential contribution to variability in CYP1A2 activity.

CYP1A2 activity also shows interethnic differences, being lower in Asian and African populations compared with Caucasians [11–13]. Swedish healthy volunteers were reported to have higher median CYP1A2 activity, measured by plasma 17X:137X ratios, compared with Koreans [12]. The median plasma 17X:137X ratio in Turkish nonsmokers evaluated in the present study (0.55) was similar to that in Swedish (0.50) and higher than in Korean nonsmokers (0.28). Likewise, it was 0.87 in smokers in our study population, while 0.63 in Swedish and 0.48 in Korean smokers. These differences might be related to environmental conditions, lifestyle, dietary

Table 3. Haplotype pairs observed in more than one subject and the corresponding 17X:137X ratios (median [range]) in nonsmokers and smokers.

Haplotype pairs	Nonsmokers		Smokers	
	n	*17X:137X ratio*	*n*	*17X:137X ratio*
H1/H1	23	0.53 (0.36–0.94)	6	0.86 (0.57–1.29)*
H1/H2	13	0.61 (0.39–1.26)	16	0.78 (0.32–1.32)
H1/H3	6	0.45 (0.34–0.59)	–	–
H1/H4	3	0.59 (0.46–0.72)	2	1.42 (1.17–1.66)
H1/H5	4	0.45 (0.13–0.79)	–	–
H1/H6	2	0.55 (0.54–0.56)	2	0.90 (0.75–1.05)
H1/H7	3	0.65 (0.48–0.70)	–	–
H1/H8	2	0.69 (0.41–0.98)	2	1.09 (0.87–1.30)
H1/H9	1	0.48	2	1.11 (1.07–1.14)
H2/H2	11	0.53 (0.39–1.13)	5	0.59 (0.44–0.92)
H2/H3	4	0.81 (0.47–1.06)	1	0.95
H2/H4	2	0.53 (0.46–0.60)	4	1.11 (0.58–1.44)
H2/H5	1	0.73	4	0.79 (0.58–1.15)
H2/H6	2	0.64 (0.30–0.96)	1	0.55
H2/H7	2	0.63(0.60–0.65)	2	0.86 (0.66–1.05)
H2/H8	2	0.87 (0.79–0.95)	–	–

**p = 0.005 for comparison of log transformed 17X:137X ratios in H1/H1 carrying nonsmokers and smokers.*

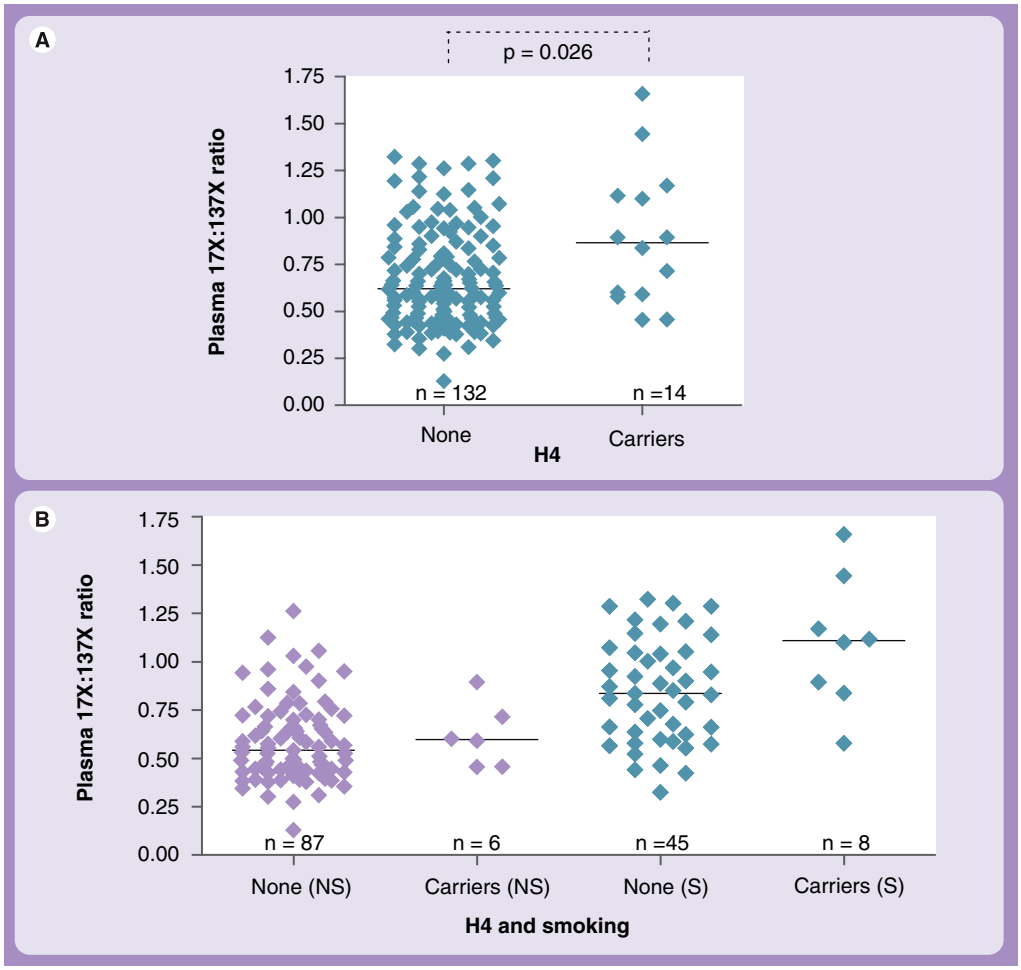

Figure 4. Plasma 17X:137X ratios in H4 carriers versus noncarriers. (A) In the total study population. **(B)** In NS and S.
NS: Nonsmokers; S: Smokers.

and smoking habits or the frequency of polymorphisms that influence the activity or inducibility of the enzyme.

The variation in CYP1A2 activity has been suggested to be of potential importance for the susceptibility for a few diseases (several cancer types, porphyria cutanea tarda) [1]. Both treatment resistance and adverse drug reactions during therapy with antipsychotics metabolized by CYP1A2 have also been reported to be related to smoking status and/or -163C/A genotype [36–42]. Our results provide further support for the impact of this polymorphism on the inducibility of CYP1A2 by smoking. However, the other *CYP1A2* polymorphisms evaluated in this study do not seem to influence the activity and/or inducibility of the enzyme significantly.

Conclusion

Smoking status, gender, haplotype H4 and the -163C>A polymorphism in *CYP1A2* contribute to the interindividual variation in CYP1A2 activity. Further studies evaluating the effect of *CYP1A2* haplotypes and age on enzyme activity are needed.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Introduction

- CYP1A2 activity shows a large interindividual variability influenced by environmental, constitutional and genetic factors.
- Up until now, the genetic basis of CYP1A2 variability has not been clarified.

Methods

- CYP1A2 activity was assessed by plasma paraxanthine:caffeine ratio, in Turkish healthy volunteers.
- Smoking status, gender, age and seven *CYP1A2* polymorphisms (-3860G>A, -3113G>A, -2467del/T, -739T>G, -729C>T, -163C>A and 5347T>C), as well as *CYP1A2* haplotypes were evaluated in relation to CYP1A2 activity.

Results

- CYP1A2 activity showed 13-fold variation in Turkish healthy volunteers.
- Smoking had the strongest influence on CYP1A2 activity among factors analyzed in the present study.
- A tendency for men to have higher CYP1A2 activity was observed both in smokers and nonsmokers.
- Nonsmoking women at their twenties had higher CYP1A2 activity than those at their thirties and forties.
- Among seven SNPs analyzed in the present study, the -163C>A polymorphism showed a significant influence on CYP1A2 activity only in smokers.
- Subjects carrying the *CYP1A2* haplotype H4, including -3860G, -3113G, -2467del, -739T, -729C, -163A and 5347T alleles, had increased CYP1A2 activity in the overall study population.

Discussion

- The smoking-related induction has a substantial impact on the variation in CYP1A2 activity while gender contributes to a smaller extent.
- In nonsmokers, only gender seems to contribute to the interindividual variability in CYP1A2 activity among the factors evaluated in this study, while in smokers -163C>A genotype has the highest impact, together with a smaller effect of gender.
- Except for the finding concerning -163C>A in smokers, none of the previously shown associations between *CYP1A2* polymorphisms and enzyme activity was confirmed in the present study.
- Increased CYP1A2 activity related to *CYP1A2* haplotype H4 (-3860G, -3113G, -2467del, -739T, -729C, -163A, 5347T) is a novel finding, which needs to be evaluated in further studies.

Conclusions

- Smoking status, gender, haplotype H4 and the -163C>A polymorphism in *CYP1A2* contribute to the interindividual variation in CYP1A2 activity.

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