Impact of Smoking, Smoking Cessation, and Genetic Polymorphisms on CYP1A2 Activity and Inducibility

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Cytochrome P4501A2 (CYP1A2) is involved in the metabolism of several drugs and is induced by smoking. We aimed to determine the interindividual change in CYP1A2 activity after smoking cessation and to relate it to *CYP1A2* genetic polymorphisms. CYP1A2 activity was determined from the paraxanthine:caffeine ratio in 194 smokers and in 118 of them who had abstained from smoking during a 4-week period. The participants were genotyped for *CYP1A2*1F*, **1D*, and **1C* polymorphisms. Smokers had 1.55-fold higher CYP1A2 activity than nonsmokers ($P < 0.0001$). The individual change in CYP1A2 activity after smoking cessation ranged from 1.0-fold (no change) to a 7.3-fold decrease in activity. In five participants with low initial CYP1A2 activity, an increase was observed after smoking cessation. Before smoking cessation, the following factors were found to influence CYP1A2 activity: *CYP1A2*1F* ($P = 0.005$), *CYP1A2*1D* ($P = 0.014$), the number of cigarettes/day ($P = 0.012$), the use of contraceptives ($P < 0.001$), and *−163A/−2467T/−3860G* haplotype ($P = 0.002$). After quitting smoking, only *CYP1A2*1F* ($P = 0.017$) and the use of contraceptives ($P = 0.05$) had an influence. No influence of *CYP1A2* polymorphisms on the inducibility of CYP1A2 was observed.

Cigarette smoking can interact with medication through pharmacokinetic or pharmacodynamic mechanisms. Among the complex mixture of chemicals found in tobacco smoke, the polycyclic aromatic hydrocarbons exert a potent induction effect on cytochrome P450 (CYP) isoenzymes 1A1, 1A2, and possibly 2E1.[1] CYP1A2 is one of the major cytochromes in the human liver (~13% of the total liver CYPs), and is involved in the metabolism of several commonly used drugs (e.g., caffeine, clozapine, flutamide, lidocaine, melatonin, mexiletine, olanzapine, ropivacaine, theophylline, tizanidine, triamterene, zolmitriptan) and endogenous compounds as well as in the bioactivation of procarcinogens.[2]

Several reports in the literature have shown that induction of CYP1A2 by smoking can result in decreased plasma levels of drugs that are mainly metabolized by this isoenzyme, thereby causing a decrease in therapeutic response. For instance, lower concentrations of clozapine, an antipsychotic drug, were observed in the plasma of smokers as compared to nonsmokers,[3] resulting in the requirement for higher maintenance doses in smokers.[4] Decreased plasma drug levels in smokers as compared to nonsmokers have also been reported for olanzapine,[5] fluvoxamine,[6] tacrine,[7] theopylline,[7] and haloperidol.[8]

The effect that discontinuation of smoking has on CYP1A2 activity has also been studied. A 40% decrease in CYP1A2 activity after smoking cessation has been described in a study involving 22 participants.[9] Another study showed a mean increase of 72% in plasma clozapine levels in 11 patients with schizophrenia after smoking cessation, but with a large variability (66%, range 14–261%).[10] However, neither study presented data on the interindividual variations in CYP1A2 activity after smoking cessation, and, to our knowledge, few data have been published on this topic.

Increased plasma drug levels after quitting smoking can lead to adverse effects. Adverse effects such as confusion,[11] antimuscarinic and sexual problems,[12] tonic-clonic seizures, stupor and coma,[13,14] and aspiration pneumonia[10] have been described for clozapine, and extrapyramidal motor symptoms have been observed for olanzapine.[11] Given the current trend toward implementation of nonsmoking policies in hospitals, it is of major interest to reconsider the dosage of drug substrates

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of CYP1A2 in patients depending on their smoking status (i.e., whether they are current smokers or have recently quit smoking).[15] For instance, after smoking cessation, an immediate dose reduction of 10% daily until the fourth day after the quit day was recommended.[16] This is in agreement with previously reported differences in CYP1A2 activity between smokers and nonsmokers.[17,18] However, such general dose-change recommendations may not cover the large variability in CYP1A2 activity in the same individual, depending on smoking status. Therefore, other measures, such as therapeutic drug monitoring, are required.[16]

Various factors such as gender, race, environmental exposure to inducers or inhibitors, and genetic factors are responsible for interindividual differences in the CYP1A2 phenotype.[19] With respect to genetic factors, several polymorphisms have been identified in the *CYP1A2* gene, in particular in the 5′-flanking region and in intron 1 (refs. 20–22) (see http://www.imm.ki.se/CYPalleles/cyp1a2.htm). Some of these polymorphisms may be associated with altered inducibility of gene expression in smokers.[20,23,24] In smokers, the presence of the *CYP1A2*1C* polymorphism, identified in the 5′-flanking region of the gene, could lead directly to a decrease in CYP1A2 activity, or indirectly to reduced inducibility.[20]

Of special interest is the *CYP1A2*1F* polymorphism in intron 1, which has been suggested to confer a higher inducibility of CYP1A2 through smoking, with a 1.6-fold higher metabolic activity in smokers homozygous for the A allele as compared to smokers with other genotypes, whereas no such differences were found between genotypes in nonsmokers.[23] The effect of *CYP1A2*1F* polymorphism on CYP1A2 activity was observed in a Turkish population[25] as well as in a group of Swedish smokers,[26] but not in Koreans.[26] Another polymorphism in the 5′-flanking region, *CYP1A2*1D,* was shown to have a main effect on CYP1A2 phenotype, leading to increased CYP1A2 activity in smokers.[27]

Because few data are yet available on the interindividual variations in CYP1A2 activity after smoking cessation, the present study aimed to examine the variation of CYP1A2 activity in a large group of smokers before and after smoking cessation. A second objective of the study was to determine whether genetic polymorphisms of the *CYP1A2* gene (including haplotypes) could influence the inducibility of CYP1A2.

RESULTS

Study population

A total of 211 volunteers gave their written informed consent to participate in the study; of these, 194 fulfilled the inclusion criteria and were enrolled in the study. The sample consisted of 93 (48%) men and 101 (52%) women; most of the participants were Caucasians (95%) and unrelated to one another (96%). The mean (±SD) age of the participants was 40 ± 11 years (range 19–64 years), and the mean body mass index was 25 ± 4.3 kg/m^2 (range 18–41 kg/m^2). On average, the participants had been smoking 22 ± 10 cigarettes/day (range 10–70 cigarettes) with a mean (±SD) smoking duration of 22 ± 10 years (range 3–45 years).

Of the total sample, 99 (51%) and 95 (49%) smokers were receiving nicotine replacement therapy (NRT) and varenicline, respectively. Forty-seven participants (24%) decreased their cigarette consumption from 24 ± 9 cigarettes/day (range 10–50 cigarettes) to 16 ± 8 cigarettes/day (range 3–45 cigarettes) during the week prior to the quit date and before the first CYP1A2 phenotyping test. Of the female participants, 53% (27% of the overall study population) were using contraceptives during the study and 10 participants (5%) were taking comedications that could potentially inhibit (paroxetine, sertraline, or fluoxetine)[28,29] or induce (proton pump inhibitors)[30] CYP1A2 activity. During the study, no participant started or stopped a pharmacologic treatment influencing CYP1A2 activity, and no change in treatment was recorded in participants who were already receiving such treatment at the time of entering the study.

CYP1A2 phenotyping

Phenotyping was performed in 194 participants while smoking, and in 137 after 4 weeks of smoking cessation. Among the 137 participants who completed 1 month of follow-up, 118 were completely abstinent. There was a complete concordance between the self-reported abstinence and the carbon monoxide (CO) concentrations (below 4 ppm) in these participants. Moreover, in 63 subjects who were either taking varenicline or had stopped NRT, there was a complete concordance between self-reported abstinence and cotinine plasma levels (<7 ng/ml).

To determine whether a 4-week smoking-free interval was sufficient for CYP1A2 to return to baseline activity, a supplementary phenotyping test was performed in a subgroup of 21 participants (12 with large variation and 9 with little variation in CYP1A2 activity) who were still abstinent at 6 months. No significant difference was found between the plasma paraxanthine:caffeine ratio at 4 weeks and the corresponding ratio at 6 months after smoking cessation (median 0.54 vs. 0.41, $P = 0.9$), even when the two groups were considered separately (data not shown). Moreover, with regard to the directional change in plasma paraxanthine:caffeine ratios, there was no observed tendency toward lower ratios at 6 months (data not shown). This was in agreement with the results from other studies in which the plasma paraxanthine:caffeine ratio did not vary over a period of 3 months regardless of age and smoking status,[31] and urinary caffeine metabolite ratios did not vary with gender or with the phase of the menstrual cycle.[32] The phenotyping test performed at 4 weeks after smoking cessation can therefore be considered indicative of a baseline CYP1A2 activity, the effect of induction by smoking being absent.

A 17-fold variation in the plasma paraxanthine:caffeine ratio was measured in the group of 194 smokers, with a median value of 1.00 (interquartile range (IQR): 0.75–1.24, **Figure 1a**). We compared median paraxanthine:caffeine ratios before and after quitting smoking in 118 participants who remained abstinent for 4 weeks. While they were smoking, these 118 participants had a median ratio of 0.98 (IQR: 0.74–1.19). After quitting smoking, they showed a 37-fold variation in the plasma ratio, with a median value of 0.63 (IQR: 0.43–0.79, **Figure 1b**). That is, a 1.55-fold higher CYP1A2 activity was measured in smokers as compared to nonsmokers ($P < 0.0001$).

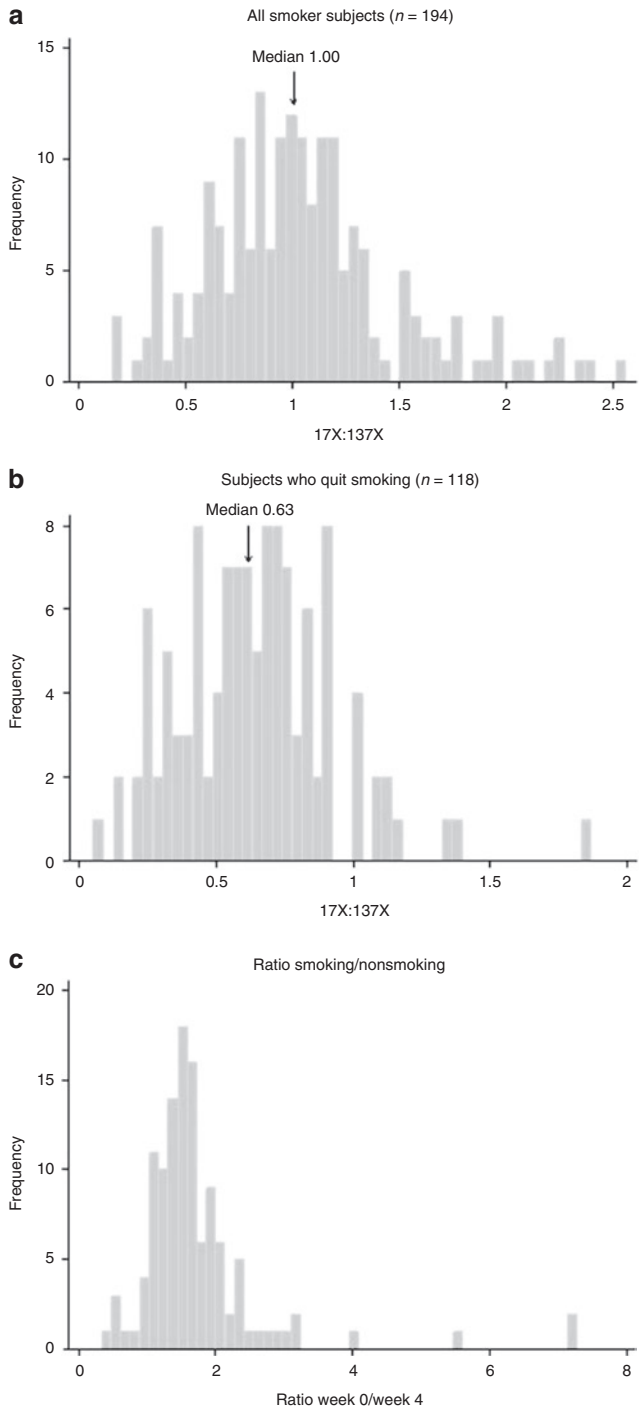

Figure 1 Distribution of plasma 17X:137X ratios in the study population (**a**) before quitting smoking ($n = 194$) and (**b**) after quitting smoking ($n = 118$). (**c**) Plasma 17X:137X ratios before and after quitting smoking ($n = 118$).

The interindividual variability in the change in CYP1A2 activity after smoking cessation (CYP1A2 inducibility) was calculated as the paraxanthine:caffeine ratio before and after quitting smoking (w0:w4 ratio). In five participants, the ratios were <0.8 (median 0.50; IQR: 0.47–0.55), suggesting an increase in CYP1A2 activity after smoking cessation, which was unexpected. It was confirmed that these participants had been active smokers at the time of the first phenotyping test (by CO and cotinine determinations) and that they were abstinent during the first month (by CO determination and self-declaration) and also at the second phenotyping test (by CO, cotinine determinations, and self-declaration). Moreover, these five subjects had low CYP1A2 activity even while they were smoking (median paraxanthine:caffeine value: 0.37; IQR: 0.30–0.46). After excluding the data for these five individuals, a median 1.6-fold decrease in CYP1A2 activity was measured following smoking cessation (IQR: 1.35–1.94), with a large interindividual variation: the w0:w4 ratios ranged from 1.0 (i.e., no change of CYP1A2 activity) to 7.3 (i.e., a 7.3-fold decrease in CYP1A2 activity after smoking cessation) (**Figure 1c**).

Influence of nongenetic factors on CYP1A2 activity and inducibility

The effects of factors that could potentially influence CYP1A2 activity (i.e., gender, age, number of cigarettes smoked per day, baseline plasma cotinine levels, smoking cessation treatment, and comedications) were examined in the subset of unrelated participants, in smokers ($n = 186$) and in those who had abstained for 4 weeks ($n = 114$) (**Table 1**). In the group of smokers, gender did not have a significant influence on plasma paraxanthine:caffeine ratios ($P = 0.22$), nor was there a significant difference in the results when contraceptive users and nonusers were considered separately ($P > 0.2$). Age was observed to have an effect on plasma paraxanthine:caffeine ratios ($P = 0.031$), but only a borderline significant difference was found between pairs of age categories when we used a Bonferroni adjusted multiple comparison ($P > 0.054$). Plasma paraxanthine:caffeine ratios were found to increase with the number of cigarettes smoked per day ($P = 0.007$), and the major differences were observed between the 10–19 and 30–39 or 40–70 cigarettes/day categories (Bonferroni adjusted $P = 0.042$ and 0.039, respectively). Similar results were obtained when the number of cigarettes smoked per day during the week before the first phenotyping test was considered (data not shown). Another measure of smoke exposure is the cotinine plasma level in smokers. We observed a significant association between increase in plasma paraxanthine:caffeine ratio and increase in cotinine plasma levels ($P < 0.001$), with the largest variation occuring between 1–150 and 151–250 ng/ml cotinine (Bonferroni adjusted $P = 0.007$). No significant difference in the plasma paraxanthine:caffeine ratios was observed between subjects taking varenicline and subjects taking NRT ($P = 0.39$). Women using contraceptives showed significantly lower plasma paraxanthine:caffeine ratios than the rest of the study population ($P < 0.001$). The other comedications did not show any statistically significant influence on CYP1A2 activity ($P = 0.93$), even when participants taking CYP1A2 inducers ($n = 6$) and those taking inhibitors ($n = 4$) were considered separately (data not shown).

After smoking cessation, gender, age, and comedications did not have a significant influence on plasma paraxanthine:caffeine ratios ($P = 0.15$, 0.067, and 0.18, respectively). No significant change in the ratios was observed between participants taking varenicline and those on NRT ($P = 0.58$). Contraceptives users showed lower paraxanthine:caffeine ratios than nonusers ($P = 0.017$).

Table 1 Influence of nongenetic factors on CYP1A2 activity while smoking and after smoking cessation and on CYP1A2 inducibility (univariate analysis)

		While smoking			After smoking cessation			Inducibility		
Nongenetic factor	Category	*n*	17X:137X ratio[a]	*P* value[b]	*n*	17X:137X ratio[a]	*P* value[b]	*n*	w0:w4 ratio[a]	*P* value[b]
Gender	Male	89	1.03 (0.83–1.19)		59	0.68 (0.53–0.77)				
	Female	97	0.90 (0.67–1.27)	0.22	55	0.59 (0.36–0.85)	0.15			0.61
Age (years)	19–30	41	0.75 (0.61–1.14)		21	0.53 (0.32–0.70)		21	1.44 (1.15–1.72)	
	31–40	62	1.00 (0.82–1.25)		43	0.67 (0.52–0.85)		43	1.54 (1.19–1.81)	
	41–50	47	1.04 (0.87–1.35)		26	0.67 (0.54–0.83)		26	1.58 (1.34–1.93)	
	51–64	36	1.03 (0.79–1.20)	0.031	24	0.60 (0.39–0.73)	0.067	24	1.67 (1.44–2.43)	0.19
Number of cigarettes/day	10–19	62	0.86 (0.61–1.15)					42	1.46 (1.15–1.65)	
	20–29	82	1.02 (0.77–1.25)					50	1.67 (1.35–1.98)	
	30–39	26	1.08 (0.87–1.53)					15	1.54 (1.40–1.92)	
	40–70	16	1.04 (1.00–1.21)	0.007			—	7	2.12 (1.57–2.32)	0.027
Cotinine plasma levels (ng/ml)	1–150	33	0.65 (0.46–0.89)					23	1.35 (1.13–1.72)	
	151–250	62	0.97 (0.77–1.15)					40	1.53 (1.40–1.91)	
	251–350	48	1.12 (0.85–1.31)					27	1.65 (1.39–1.95)	
	351–818	43	1.12 (0.93–1.54)	<0.001			—	24	1.65 (1.34–2.05)	0.21
Smoking cessation treatment	NRT	95	0.99 (0.76–1.29)		61	0.60 (0.46–0.75)		61	1.50 (1.20–1.81)	
	Varenicline	91	0.99 (0.74–1.16)	0.39	53	0.65 (0.43–0.83)	0.58	53	1.66 (1.39–1.94)	0.14
Contraceptives use	Yes	50	0.75 (0.46–1.15)		26	0.48 (0.32–0.74)		26	1.55 (1.19–1.98)	
	No	136	1.02 (0.84–1.25)	<0.001	88	0.67 (0.50–0.82)	0.017	88	1.57 (1.34–1.90)	0.60
Comedications	Yes	10	1.06 (0.76–1.21)		4	0.78 (0.64–0.93)		4	1.52 (1.29–1.66)	
	No	176	0.99 (0.75–1.24)	0.93	110	0.63 (0.43–0.78)	0.18	110	1.57 (1.28–1.9)	0.63

[a]Ratios are expressed as median (interquartile range). [b]*P* value determined by Kruskal–Wallis and Wilcoxon tests. NRT, nicotine replacement therapy.

Table 2 Influence of genetic factors on CYP1A2 activity while smoking and after smoking cessation and on CYP1A2 inducibility (univariate analysis)

		While smoking			After smoking cessation			Inducibility		
Genetic factor	Genotype	*n*	17X:137X ratio[a]	*P* value[b]	*n*	17X:137X ratio[a]	*P* value[b]	*n*	w0:w4 ratio[a]	*P* value[b]
*CYP1A2*1F* (−163 C>A)	*CC*	19	0.86 (0.46–1.15)		11	0.54 (0.32–0.69)		11	1.44 (1.19–2.13)	
	CA	81	1.00 (0.76–1.27)		46	0.61 (0.43–0.83)		46	1.61 (1.37–2.00)	
	AA	86	1.00 (0.75–1.19)	0.12	57	0.67 (0.50–0.77)	0.084	57	1.57 (1.23–1.85)	0.64
*CYP1A2*1D* (−2467T/delT)	*TT*	164	1.01 (0.76–1.25)		102	0.64 (0.44–0.83)		102	1.57 (1.35–1.95)	
	TdelT	21	0.77 (0.39–0.99)		11	0.61 (0.26–0.78)		11	1.28 (1.01–1.68)	
	delTdelT	1	0.99	0.011	1	0.60	0.45	1	1.65	0.17
*CYP1A2*1C* (−3860G>A)	*GG*	181	1.00 (0.76–1.24)		109	0.64 (0.44–0.79)		109	1.57 (1.34–1.93)	
	GA	5	0.69 (0.38–0.77)		5	0.61 (0.26–0.78)		5	1.28 (0.85–1.58)	
	AA	0	—	0.011	0	—	0.54	0	—	0.25
−163A/−2467delT/−3860A	Noncarriers	181	1.00 (0.76–1.24)		109	0.64 (0.44–0.79)		109	1.57 (1.34–1.93)	
	Carriers	5	0.69 (0.38–0.77)	0.011	5	0.61 (0.26–0.78)	0.54	5	1.28 (0.85–1.58)	0.25
−163A/−2467T/−3860G	Noncarriers	31	0.83 (0.46–1.05)		16	0.57 (0.32–0.70)		16	1.41 (1.14–1.67)	
	Carriers	155	1.02 (0.77–1.25)	0.009	98	0.66 (0.44–0.83)	0.14	98	1.58 (1.34–1.94)	0.15

[a]Ratios are expressed as median (interquartile range). [b]*P* value determined by Wilcoxon test; dominant model grouped by the presence of at least one variant allele vs. wild type.

The effects of these factors on the w0:w4 ratio were also tested. Gender, age, plasma cotinine levels, smoking cessation treatment, and comedications did not show any significant effect on w0:w4 ratios. The ratios increased with the number of cigarettes smoked per day ($P = 0.027$), but no significant difference was observed between categories of cigarettes/day when we used a Bonferroni adjusted multiple comparison ($P > 0.09$). However, subjects with little or no change (±20%) in CYP1A2 activity after smoking cessation

($n = 18$) smoked fewer cigarettes per day as compared to participants with high (paraxanthine:caffeine ratio >2, $n = 24$) CYP1A2 activity change (mean 17 vs. 24 cigarettes/day, $P = 0.04$).

CYP1A2 genotyping and influence on CYP1A2 activity and inducibility

The three genetic polymorphisms analyzed were in Hardy–Weinberg equilibrium ($P > 0.7$). Their allele frequencies are presented in **Supplementary Table S1b** online. The analysis of the three polymorphisms in the subset of unrelated participants when they were smoking ($n = 186$) revealed a significant association with plasma paraxanthine:caffeine ratios for *CYP1A2*1D* ($P = 0.011$) and *CYP1A2*1C* ($P = 0.011$). Homozygous and heterozygous carriers of the *−2467T/delT* polymorphism ($n = 22$) showed lower plasma paraxanthine:caffeine ratios than wild-type subjects ($n = 164$) (median 0.77 vs. 1.01, $P = 0.011$); also, carriers of the *−3860 GA* genotype ($n = 5$) had lower ratios than those with the *−3860 GG* genotype ($n = 181$) (median 0.69 vs. 1.00, $P = 0.011$). On the other hand, caffeine metabolite ratios were not significantly different between carriers of the *CYP1A2*1F CA* and *AA* genotypes ($n = 167$) relative to those with *CC* genotypes ($n = 19$) (median 1.00 vs. 0.86; $P = 0.12$) (**Table 2** and **Figure 2**).

Data were also analyzed with respect to haplotypes: linkage disequilibrium analyses and haplotype frequencies are shown in **Supplementary Table S2** online. The haplotype *−163A/−2467delT/−3860A* showed a significant influence on paraxanthine:caffeine ratios, with carriers ($n = 5$) having lower ratio values than noncarriers ($n = 181$) (median 0.69 vs. 1.00, $P = 0.011$). An opposite effect was observed for haplotype *−163A/−2467T/−3860G*, with carriers ($n = 155$) having significantly higher ratios than noncarriers ($n = 31$) (median 1.02 vs. 0.83, $P = 0.009$) (**Table 2** and **Figure 3**).

In subjects who had quit smoking, the influence of the genetic polymorphisms and haplotypes was not significant, although a trend was observed for genotypes of *CYP1A2*1F* polymorphism ($P = 0.084$) (**Table 2** and **Supplementary Figures S1 and S2** online).

Genotypes and haplotypes were not observed to have any significant influence on CYP1A2 inducibility (w0:w4 ratios, data not shown).

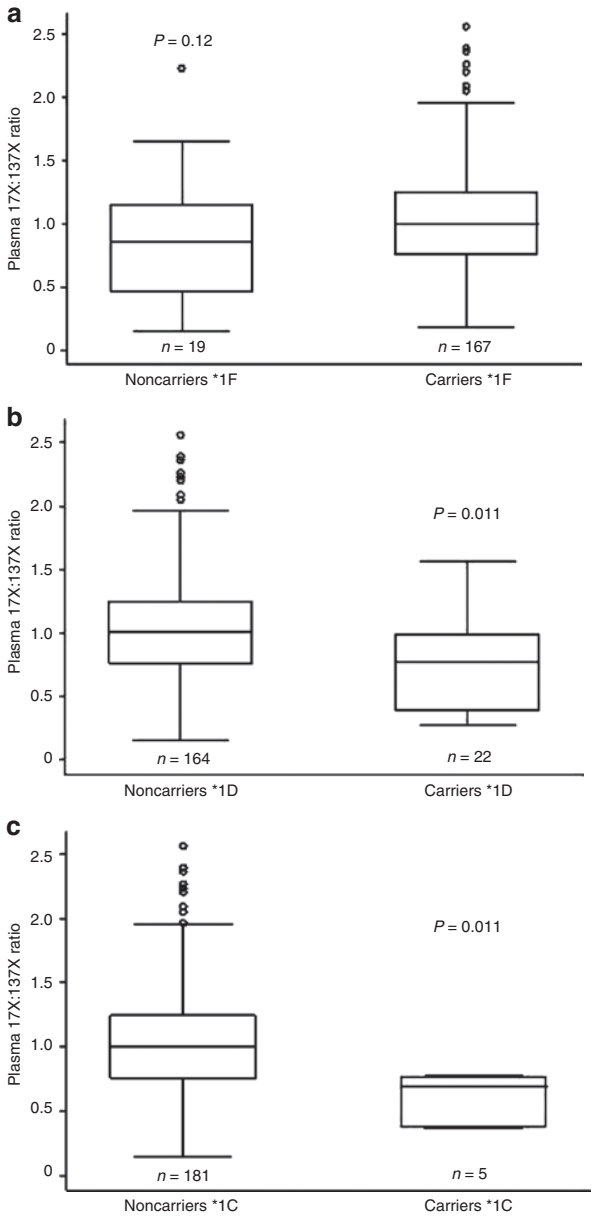

Figure 2 Plasma 17X:137X ratios in smokers in relation to (**a**) *CYP1A2 *1F*, (**b**) **1D*, and (**c**) **1C* genotypes.

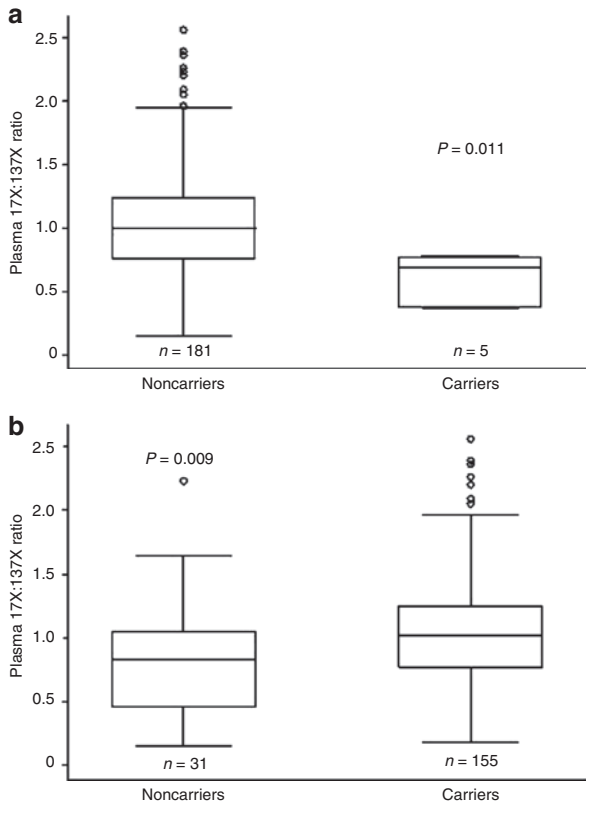

Figure 3 Plasma 17X:137X ratios in smokers in relation to (**a**) *−163A/−2467delT/−3860A* and (**b**) *−163A/−2467T/−3860G* haplotypes.

Table 3 Multivariate analysis on CYP1A2 activity considering genotypes and haplotypes

	While smoking		After smoking cessation		Inducibility (w0:w4)	
Variable	Coefficient (SE)	*P* value	Coefficient (SE)	*P* value	Coefficient (SE)	*P* value
Genotype						
*CYP1A2*1F (−163C>A)*	0.293 (0.103)	0.005	0.375 (0.157)	0.017	−0.028 (0.135)	0.833
*CYP1A2*1D (−2467delT)*	−0.264 (0.108)	0.014	−0.106 (0.197)	0.590	−0.106 (0.166)	0.523
*CYP1A2*1C (−3860G>A)*	−0.139 (0.205)	0.496	−0.114 (0.302)	0.705	−0.115 (0.255)	0.653
Gender	0.107 (0.076)	0.160	−0.036 (0.112)	0.744	0.112 (0.096)	0.245
Age (years)	0.010 (0.032)	0.742	−0.023 (0.048)	0.621	0.062 (0.041)	0.132
Number of cigarettes/day	0.081 (0.032)	0.012	—	—	0.033 (0.044)	0.454
Smoking cessation treatment	−0.045 (0.062)	0.474	0.012 (0.096)	0.899	0.079 (0.083)	0.341
Contraceptives use	−0.393 (0.085)	<0.001	−0.250 (0.128)	0.051	−0.158 (0.111)	0.154
Intercept	−0.216 (0.108)	0.046	−0.805 (0.157)	<0.001	0.447 (0.135)	0.001
Haplotype						
−163A/−2467delT/−3860A	−0.258 (0.183)	0.159	−0.057 (0.247)	0.817	−0.192 (0.209)	0.359
−163A/−2467T/−3860G	0.263 (0.085)	0.002	0.249 (0.138)	0.070	0.053 (0.118)	0.652
Gender	0.124 (0.076)	0.102	−0.037 (0.112)	0.741	0.126 (0.095)	0.184
Age (years)	0.008 (0.032)	0.803	−0.025 (0.048)	0.602	0.060 (0.041)	0.143
Number of cigarettes/day	0.085 (0.032)	0.009	—	—	0.034 (0.043)	0.427
Smoking cessation treatment	−0.035 (0.063)	0.580	0.031 (0.096)	0.741	0.077 (0.082)	0.348
Contraceptives use	−0.402 (0.086)	<0.001	−0.255 (0.128)	0.047	−0.167 (0.110)	0.132
Intercept	−0.214 (0.092)	0.021	−0.704 (0.141)	<0.001	0.363 (0.122)	0.003

Coefficients and *P* values were obtained from mixed-effects multiple-regression models using the log-transformed paraxanthine:caffeine and w0:w4 ratios as dependent variable and the genetic and nongenetic factors as independent variables (age and number of cigarettes/day were standardized).

Multivariate analysis of CYP1A2 activity and inducibility

Multiple-regression analyses were used to assess the influence of covariables on CYP1A2 activity, using a mixed-effects model with the entire study population ($n = 194$), including participants who were related to one another (Table 3). When participants were smoking, significant associations were found between the paraxanthine:caffeine ratio and *CYP1A2*1F* ($P = 0.005$), *CYP1A2*1D* ($P = 0.014$), contraceptives use ($P < 0.001$), the number of cigarettes smoked per day ($P = 0.012$), or the cotinine plasma levels ($P < 0.001$), with an explained variability of 19.6% (adjusted $R^2 = 0.196$). When haplotypes were considered, the influence of the *−163A/−2467T/−3860G* haplotype was significant ($P = 0.002$), together with the number of cigarettes per day ($P = 0.009$) and contraceptives use ($P < 0.001$) (adjusted $R^2 = 0.187$).

In subjects who had quit smoking, the influence of *CYP1A2*1F* ($P = 0.017$) and contraceptives use (0.05) remained significant, with an explained variability of 5% (adjusted $R^2 = 0.05$), whereas the haplotype *−163A/−2467T/−3860G* was at the limit of significance ($P = 0.07$, adjusted $R^2 = 0.036$). No influence was observed on CYP1A2 inducibility after fitting the same multiple-regression models, with both genotypes and haplotypes being considered, on w0:w4 ratios.

DISCUSSION

Smoking potently induces CYP1A2 through the binding of polycyclic aromatic hydrocarbons to the aryl hydrocarbon receptor.[33] Through this mechanism, smoking can influence the metabolism of drugs that are substrates of this enzyme, leading to a decrease in therapeutic response in smokers[3] and a higher risk of adverse effects after smoking cessation.[11,34] Wide interindividual variability in CYP1A2 activity has previously been reported;[17,35,36] however, to date, few data have been available on the interindividual variability in induction of CYP1A2 by smoking. The first aim of our study was to assess this variability in a large cohort of smokers by measuring CYP1A2 activity before and after smoking cessation.

We observed a higher CYP1A2 activity in smokers as compared to abstainers, a finding that confirms previous reports.[25,26] As expected, a significant decrease (1.55-fold) in CYP1A2 activity was shown after smoking cessation. High interindividual variabilities in CYP1A2 activity were measured, both in the group of smokers ($n = 194$, 17-fold variation) and in the group that had abstained from smoking for 4 weeks ($n = 118$, 37-fold). These findings are in agreement with those from previously published studies.[9,18]

Of particular interest is the high variability in the change in CYP1A2 activity after smoking cessation. Three groups of subjects merit discussion in this regard. The first group of five participants showed an increase in CYP1A2 activity after smoking cessation. An initial low level of CYP1A2 activity, as well as other possible confounding factors (e.g., change in diet during the study), might have contributed to this result. Most remarkably, little or no variation (±20%) in CYP1A2 activity

was measured in a second group consisting of 20 subjects after smoking cessation, suggesting that smoking has little or no CYP1A2-inducing activity in such individuals. Finally, in a group of 25 participants, a decrease ranging from 2- to 7.3-fold in CYP1A2 activity was measured, suggesting a strong inducing effect of smoking on CYP1A2 activity. The other 68 participants in the study population showed a decrease in CYP1A2 activity between 1.2- and 2-fold, in line with previously published differences between smokers and nonsmokers.[25,26] Overall, these results are in agreement with those of another study that measured clozapine serum levels in 11 patients before and after smoking cessation. A large interindividual variability was observed in that study, with a median increase of 72% in clozapine levels, but with a large standard deviation (66%) and a range from 14 up to 261%.[10]

From a clinical point of view, it has previously been proposed that, in the case of drugs that are substrates of CYP1A2, therapeutic drug monitoring accompanied by dose reduction as required should be carried out in patients who have quit smoking.[16] This study supports these recommendations because a determination of plasma drug levels would enable allowances to be made for the large interindividual variability in the induction of CYP1A2 by smoking.

The second aim of our study was to determine some of the factors that could possibly contribute to the interindividual variability in induction of CYP1A2 by smoking. For this purpose, we considered gender, age, number of cigarettes smoked per day, pharmacological treatment for smoking cessation, comedications, genetic polymorphisms, and haplotypes. Smoking was found to have a dose-dependent effect on CYP1A2 activity, with plasma paraxanthine:caffeine ratios increasing with an increase in the number of cigarettes smoked per day, and with plasma cotinine levels. This is in line with previously published data.[37] In addition, participants with little or no change in CYP1A2 activity after smoking cessation had smoked fewer cigarettes per day as compared with those who showed a large change in CYP1A2 activity. This could contribute to the finding of little or no change in CYP1A2 activity after smoking cessation in the latter group. However, the difference in the number of cigarettes/day was small between the groups (mean 17 vs. 24 cigarettes/day). Moreover, no significant difference was observed in the number of cigarettes/day between the group with large change in CYP1A2 activity and the group with average change. These results suggest that this variable (number of cigarettes smoked per day) contributes only partially to the variability in CYP1A2 induction by smoking.

An increase in CYP1A2 activity was found with increase in age among those who smoked, but this effect was not significant in the multiple-regression models. Gender had no effect on enzyme activity, a finding that is in agreement with the data from another study.[26] Although higher CYP1A2 activity has been reported in men as compared to women, these differences might be explained by the fact that some of the women were using contraceptives.[38] In the present study, 53% of the women were using contraceptives, but this was not reflected in a difference in CYP1A2 activity as compared to men.

In univariate analysis, the *CYP1A2 *1D* and*1C* genetic polymorphisms were associated with a decrease in CYP1A2 activity in smokers but had no effect after smoking cessation. In multivariate analysis, the association remained significant for *CYP1A2*1D* in smokers, whereas significant associations were observed for *CYP1A2*1F* both in smokers and in those who had quit smoking. In a previous study, *CYP1A2*1F* was shown to be associated with an increase in CYP1A2 activity in smokers but not in nonsmokers, suggesting higher inducibility in carriers of *CYP1A2*1F*.[23] However, that study was performed with a relatively low number of smokers ($n = 51$) and used different caffeine ratios to determine the phenotype of smokers and nonsmokers.[23] The significant association found in our study between the C*YP1A2*1F* polymorphism and CYP1A2 activity, both in smokers and in those who had quit smoking, could suggest a direct functional role for this polymorphism in transcription activation, rather than as specifically influencing the induction of CYP1A2.

A decrease in the inducibility of CYP1A2 by tobacco smoke in carriers of *CYP1A2*1C* allele has previously been reported.[20] The authors of that paper proposed an effect of the *−3860EG>A (*1C)* polymorphism on the binding of liver-specific factors to the 3-methylcholanthrene-responsive region that regulates the expression of CYP1A2.[20] We measured a statistically significant decrease in CYP1A2 activity in *−3860A* carriers in univariate analysis but not in multivariate analysis.

It is difficult to explain the discrepancy between our results, which showed the *CYP1A2 *1D* polymorphism as being associated with a decrease in CYP1A2 activity in smokers, and those from another study ($n = 95$) in which the *CYP1A2*1D* allele was linked to an increase in CYP1A2 activity.[27] One explanation for this discrepancy might be that in the latter study no haplotype analyses of the polymorphisms studied (*CYP1A2 *1F*, **1D*, and **1C*) were carried out.

The three polymorphisms studied were found to be in high linkage disequilibrium, and the haplotype analysis revealed the influence of haplotypes *-163A/-2467delT/-3860A* and *-163A/-2467T/-3860G* on CYP1A2 activity in smokers (decrease and increase in activity, respectively). Several haplotypes containing the three polymorphisms, alone or in combination, have previously been reported, such as *CYP1A2*1L*, *CYP1A2*1V*, and *CYP1A2*1W*.[39,26] However, to our knowledge, no association with CYP1A2 activity was found when the genotype–phenotype relationships were studied taking into account these haplotypes.[26]

It must also be mentioned that, in most of the studies on smoking and CYP1A2 genotype–phenotype interactions,[20,24–26] CYP1A2 activity was measured in various participants genotyped for the polymorphism of interest. Therefore, any difference in activity levels among the subjects could be due to the polymorphism of interest but could also be due to other genetic and environmental factors to which the various participants were exposed. We have overcome this limitation by assessing the influence of genetic polymorphisms on CYP1A2 activity before and after smoking cessation. However, in the multivariate analysis, no effect of either genetic or nongenetic factors was found on the ratio for CYP1A2 activity before and after smoking cessation

(w0:w4 ratio). In summary, the present study shows a large interindividual variability in the inducibility of CYP1A2 by smoking. This highlights the need for careful clinical management of patients receiving drugs that are metabolized by this enzyme and who smoke, start smoking, or wish to stop smoking. The number of cigarettes smoked per day, contraceptives use, as well as genetic polymorphisms of the *CYP1A2* gene were found to significantly influence CYP1A2 activity, but more investigation is needed to determine other clinical variables and/or genetic polymorphisms that could potentially influence CYP1A2 activity and/or inducibility.

METHODS

Study design and participants. This longitudinal study was conducted at the Centre for Psychiatric Neurosciences of the Department of Psychiatry and at the University Outpatient Clinic of Lausanne, Switzerland. A total of 194 regular smokers who wished to quit smoking and to participate in a smoking-cessation program were recruited from the general population. The program offered a 3-month follow-up (10 visits) composed of smoking-cessation counseling and pharmacologic treatment (NRT or varenicline) and a 6-month concluding visit. Body weight, waist circumference, and blood pressure were recorded during the study, and blood sampling was performed before the quit date, at 4 weeks after smoking cessation, and at the 6-month follow-up. Abstinence was assessed during the follow-up by self-declaration and by measuring expired CO levels (Micro Smokerlyzer; Bedfont Scientific, Rochester, England). Details of the inclusion criteria and the criteria of abstinence assessment are presented in **Supplementary Methods** online. The study was approved by the ethics committee of the Lausanne University Medical School. Written informed consent, including consent for genetic analysis, was obtained from all participants.

CYP1A2 phenotyping. CYP1A2 activity was determined before the quit day (W0) and at 4 weeks after smoking cessation (W4), using the plasma caffeine phenotyping test. The participants were asked to refrain from caffeine-containing beverages and foods beginning the night before the day of the scheduled test. They received a 200-mg caffeine capsule, and blood was collected 6 h after caffeine intake. Before caffeine intake (hour 0) and again before blood sampling (at 6 h), compliance regarding caffeine restriction was assessed by self-declaration, and a new test was programmed and performed if compliance was not assured ($n = 11$). The plasma levels of paraxanthine (17X) and caffeine (137X) were measured by gas chromatography/mass spectrometry, using a previously described method.[3] The 17X/137X ratio, which is a valid marker of CYP1A2 activity,[37] was calculated for all subjects. All comedications were recorded and participants were asked to report any change in their medications or lifestyles during the study. Although contraceptives are known inhibitors of CYP1A2,[37] they were allowed in the study, considering the fact that varenicline is contraindicated in pregnant women. Nevertheless, the use of smokers as their own controls takes into account possible factors (environmental factors, food, drugs, etc.) that might influence CYP1A2 activity.

Genotyping. The genotyping procedure involved the use of 5′-nuclease allele discrimination assays (TaqMan; Applied Biosystems, Rotkreuz, Switzerland). It is described in **Supplementary Methods** online.

Statistical analysis. The Kruskal–Wallis and Wilcoxon/Mann–Whitney U tests were used to determine the effects of genetic and nongenetic factors on plasma paraxanthine:caffeine ratios. Each Kruskal–Wallis analysis was accompanied by pairwise rank-sum tests between all pairs of categories, with Bonferroni adjustment. Multiple-regression analyses were carried out using a mixed-effects model to assess the influence of covariables on CYP1A2 activity in the entire population ($n = 194$), including related participants. For this purpose, a random effect was introduced in the family level. The log-transformed value of the paraxanthine:caffeine ratio or of the w0:w4 ratio was considered the dependent variable, whereas gender, age, number of cigarettes smoked per day, treatment, contraceptives use, and genetic polymorphisms or haplotypes were considered independent variables. The validity of fitted models was assessed graphically. These models were used to assess the effects of predictors on CYP1A2 activity before and after smoking cessation and on CYP1A2 inducibility (w0:w4 ratios). The variances of random effects in the mixed models were not significantly different from zero. However, from a conceptual point of view, we prefer to present the mixed models as final models. As the results are very similar to those of multiple linear regression without random effects, the adjusted values of R^2 obtained from the linear models are reported as a measure of the explained variability. Haplotypes were inferred using the R software (version 2.11.1, http://www.r-project.org). All tests were two-sided, and a P value ≤ 0.05 was considered statistically significant. All analyses were performed using STATA software (version 11.0; StataCorp, College Station, TX).

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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