Pharmacogenetics of CYP1A2 activity and inducibility in smokers and exsmokers

Maria Dobrinas[a], Jacques Cornuz[b] and Chin B. Eap[a,c]

Background **There is a high interindividual variability in cytochrome P4501A2 (*CYP1A2*) activity and in its inducibility by smoking, only poorly explained by known *CYP1A2* polymorphisms. We aimed to study the contribution of other regulatory pathways, including transcription factors and nuclear receptors, toward this variability.**

Methods **CYP1A2 activity was determined by the paraxanthine/caffeine ratio in 184 smokers and in 113 of them who were abstinent for 4 weeks. Participants were genotyped for 22 polymorphisms in 12 genes.**

Results **A significant influence on CYP1A2 inducibility was observed for the *NR1I3* rs2502815 ($P=0.0026$), rs4073054 ($P=0.029$), *NR2B1* rs3818740 ($P=0.0045$), rs3132297 ($P=0.036$), *AhR* rs2282885 ($P=0.040$), rs2066853 ($P=0.019$), *NR1I1* rs2228570 ($P=0.037$), and *NR1I2* rs1523130 ($P=0.044$) polymorphisms. Among these, the *NR1I3* rs2502815 ($P=0.0045$), rs4073054 ($P=0.048$), and *NR2B1* rs3818740 ($P=0.031$) also influenced CYP1A2 basal activity.**

Conclusion **This is the first *in-vivo* demonstration of the influence of genes involved in CYP1A2 regulatory pathways on its basal activity and inducibility by smoking. These results need to be confirmed by other studies. *Pharmacogenetics and Genomics* 23:286–292 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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[a]Unit of Pharmacogenetics and Clinical Psychopharmacology, Department of Psychiatry, Centre for Psychiatric Neurosciences, Lausanne University Hospital, Prilly, [b]Department of Ambulatory Care and Community Medicine, University of Lausanne, Lausanne and [c]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland

Correspondence to Prof. Chin B. Eap, PhD, Unit of Pharmacogenetics and Clinical Psychopharmacology, Department of Psychiatry, Centre for Psychiatric Neurosciences, Hospital of Cery, 1008 Prilly, Lausanne, Switzerland
Tel: +41 21 643 64 38; fax: +41 21 643 64 44; e-mail: chin.eap@chuv.ch

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Introduction

Cytochrome P4501A2 (CYP1A2) is one of the major cytochromes in the human liver and is involved in the metabolism of endogenous compounds, of several commonly used drugs (e.g. caffeine, clozapine, flutamide, lidocaine, olanzapine, zolmitriptan, etc.) and in the bioactivation of procarcinogens [1]. Smoking induces CYP1A2 activity, mainly through the polycyclic aromatic hydrocarbons produced by combustion and found in tobacco smoke [2]. Several studies have reported a high interindividual variability of CYP1A2 activity [3,4], but little was known on the interindividual variability of CYP1A2 induction by smoking, which can influence therapeutic and side effects of drug substrates of CYP1A2 in smokers and exsmokers [5–7]. This was studied in a large cohort of smokers by measuring CYP1A2 activity before and after smoking cessation [8]. We found higher CYP1A2 activity in smokers compared with nonsmokers (average 1.55-fold), thus confirming previous results [9,10], but more importantly, the individual change in CYP1A2 activity after smoking cessation ranged from 1.0-fold (no change) to 7.3-fold decreased activity [8]. We found several genetic and nongenetic factors influencing both basal and induced CYP1A2 activity, but explaining a limited percentage of its variability, and we observed no influence on CYP1A2 inducibility [8,11]. Therefore, other genetic and environmental factors that participate in the regulation of constitutive and/or inducible CYP1A2 expression must contribute toward the large interindividual variability of this enzyme.

First, as human *CYP1A1* and *CYP1A2* genes are orientated head to head on chromosome 15, sharing a common 5′-flanking region, regulatory elements for the two genes might either be distinct or may overlap one another [12]. Therefore, polymorphisms in the *CYP1A1* gene could influence CYP1A2 activity, as it was shown for the rs1048943 (Ile462Val; *CYP1A1*2C*) polymorphisms *in vivo* [13]. However, a previous study aimed at investigating the CYP1A2 genotype–phenotype relationship by sequencing the whole *CYP1A1_CYP1A2* locus [12] and testing selected single nucleotide polymorphisms (SNPs) in individuals with low or high enzyme activity/expression [14]. The study showed that no SNP or haplotype in the *CYP1A1_CYP1A2* locus could clearly be used to predict the metabolic phenotype. The authors concluded that unidentified genetic variations located either *cis* (in nearby sequences) or *trans* (not linked to the gene) could be responsible for the observed large differences in CYP1A2 enzyme activity [14].

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Interestingly, the aryl hydrocarbon receptor (AhR) pathway regulates the transcription of CYP1 family genes. It was thus shown to be responsible of CYP1A2 induction by tobacco smoke, through AhR-mediated transactivation, involving the *AhR* nuclear translocator (ARNT) and the AhR regulator (AhRR) [15]. Several genetic polymorphisms were described in AhR, among which the rs2066853 (Lys554Arg) SNP was found to be associated with an increase in CYP1A2 activity, measured by the urinary caffeine metabolite ratio in a group of healthy female smokers [16].

Moreover, several nuclear factors are involved in the regulation of CYPs gene expression, among which is CYP1A2 [17]. Thus, nuclear factors such as the pregnane X receptor (PXR; encoded by the *NR1I2* gene) [18,19], the constitutive androstane receptor (CAR; encoded by the *NR1I3* gene) [20], and their heterodimerization partner retinoid X receptor-α (RXRα; encoded by the *NR2B1* gene) were shown *in vitro* to regulate gene expression of CYP1A enzymes, and are then expected to have an influence on CYP1A2 activity. Liver-enriched transcription factors, such as the hepatocyte nuclear factors (HNF1α, 4α; encoded by the *TCF* genes) and the coactivators nuclear receptor coactivator 1 (*NCOA1*; SRC1) and peroxisome proliferator-activated receptor gamma (PPARG) coactivator 1-α (PGC1α), were also found to be involved in CYP1A2 constitutive expression *in vitro* [21,22]. Finally, under pathophysiological conditions of inflammation, the expression of CYP1A genes was downregulated through inflammation mediators such as interleukin 1β (IL1β) or tumor necrosis factor α [23,24].

A recent study focusing on a pathway-based approach to evaluate CYP1A2 pharmacogenetics *in vitro* found a significant influence of SNPs in genes of the AhR pathway, HNF1α, IL1β, SRC1, RXRα, and vitamin D receptor (VDR; encoded by the *NR1I1* gene), on CYP1A2 phenotypes [25].

In the present study, a thorough analysis of genetic factors influencing CYP1A2 activity and inducibility by smoking was carried out. For this purpose, multiple genes involved in CYP1A2 regulation pathways described in the above-mentioned *in-vitro* and *in-vivo* reports were analyzed in a large cohort of smokers and exsmokers.

Methods

Study design and participants

This cohort is based on a clinical and pharmacogenetic study on smoking cessation that has been described previously [8]. Briefly, smokers from the general population who wished to quit smoking and to participate in a smoking-cessation program were recruited. Details of the inclusion and exclusion criteria, clinical measures, blood sampling, and the criteria of abstinence assessment have been described previously [8]. The study was approved by the ethics committee of the Lausanne University Medical School and by the Swiss Agency for Therapeutic Products (Swissmedic). Written informed consent, including consent for genetic analysis, was obtained from all the participants.

CYP1A2 phenotyping

CYP1A2 activity was determined before the quit day (w0) and 4 weeks after smoking cessation (w4), according to the previously described caffeine test [8]. The plasma levels of paraxanthine (17X) and caffeine (137X) were measured by gas-chromatography/mass-spectrometry [5]. The 17X/137X ratio, which is a valid marker of CYP1A2 activity [4,26], was calculated for all participants. Moreover, in all cases, the ratio between CYP1A2 activity while smoking and after smoking cessation (w0/w4) was calculated and was used as a measure of CYP1A2 inducibility by smoking.

Genotyping

Genomic DNA was extracted from EDTA blood samples using the FlexiGene DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. We selected genetic polymorphisms in genes supposed to be involved in the regulation of CYP1A2 expression, which included *CYP1A1*, *AhR*, *AhRR*, *ARNT*, *TCF1* (HNF1α), *PPARGC1α* (PGC1α), *NCOA1* (SRC1), *IL1β*, *NR1I2* (PXR), *NR1I3* (CAR), *NR2B1* (RXRα), and *NR1I1* (VDR). SNPs were chosen according to the previous literature considering them as candidate polymorphisms, functional effects *in vivo* and/or *in vitro*, minor allele frequencies in Caucasian populations, and linkage disequilibrium patterns. Thus, 22 SNPs were selected for analyses and are listed in Table 1. All SNPs were genotyped by a real-time PCR using 5′-nuclease allele discrimination assays (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland). Genotyping was performed using commercially available assays (assay IDs are listed in Table 1). All reagents were purchased from Applied Biosystems and genotyping was performed according to the manufacturer's protocol.

Statistical analysis

Wilcoxon/Mann–Whitney tests were used to determine the effects of genetic polymorphisms on plasma paraxanthine/caffeine ratios. SNPs were considered in both dominant and recessive modes, that is, heterozygous and homozygous mutated versus wild-type participants in the first case, and homozygous mutated versus the two other groups in the second case.

Multiple regression analyses were carried out using a mixed-effects model to adjust for the influence of covariables on CYP1A2 activity in the entire population ($n = 184$), including related participants. For this purpose, a random effect was introduced at the family level. The log-transformed values of the paraxanthine/caffeine

Table 1 Selected SNPs, relative position, assay ID, and minor allelic frequency

Genes	dbSNP ID	Alleles	TaqMan SNP genotyping assay	MAF (NCBI)	MAF study
CYP1A1	rs1048943	1384A>G; Ile462Val; CYP1A1*2C	C_25624888_50	0.03	0.03
	rs4646421	−26−728C>T	C_1840674_10	0.10	0.11
AhR	rs2282885	66−3946A>G	C_2541460_1_	0.34	0.39
	rs7811989	705+853G>A	C_29150577_20	0.28	0.27
	rs2066853	1661G>A; Arg554Lys	C_11170747_20	0.10	0.09
AhRR	rs2241598	g.438564C>T	C_16056261_10	0.18	0.26
ARNT	rs2134688	227+1853A>G	C_26672991_10	0.10	0.10
TCF1 (HNF1α)	rs2464196	1460G>A; Ser487Asn	C_1263617_10	0.30	0.32
	rs1169306	1769−557C>T	C_1263610_10	0.40	0.39
PPARGC1A (PGC1α)	rs8192678	1444G>A; Gly482Ser	C_1643192_20	0.35	0.32
NCOA1 (SRC1)	rs2119115	90−1837A>C	C_15822627_20	0.28	0.25
IL1β	rs1143634	315C>T; Phe105Phe	C_9546517_10	0.26	0.21
NR1I2 (PXR)	rs7643645	22−579A>G	C_1834250_10	0.30	0.40
	rs2472677	−22−7659T>C	C_26079845_10	0.38	0.42
	rs1523130	−1663C>T	C_9152783_20	0.37	0.35
NR1I3 (CAR)	rs2502815	239−99C>T	C_16248625_10	0.23	0.27
	rs4073054	917+116T>G	C_25741543_10	0.41	0.37
	rs2307424	540C>T; Pro180Pro	C_25746794_20	0.34	0.34
NR2B1 (RXRα)	rs3818740	28+7589T>C	C_27521184_10	0.25	0.33
	rs3132297	610+901C>T	C_9199894_10	0.17	0.20
NR1I1 (VDR)	rs1540339	277+1504G>A	C_8716064_1_	0.36	0.35
	rs2228570	2T>A; Met1Thr	C_12060045_20	0.41	0.37

AhR, aryl hydrocarbon receptor; AhRR, AhR regulator; ARNT, AhR nuclear translocator; CAR: constitutive androstane receptor; CYP1A1, cytochrome P450 1A1; db, NCBI reference ID; HNF1α, hepatocyte nuclear factor 1α; IL1β, interleukin 1β; MAF, minor allele frequency; NCBI, National Center for Biotechnology Information; NCOA1, nuclear receptor coactivator 1; NR, nuclear receptor family; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1α; PPARG, peroxisome proliferator-activated receptor gamma; PXR, pregnane X receptor; RXRα, retinoid X receptor-α; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

ratio or of the w0/w4 ratio were considered the dependent variable, whereas sex, age, number of cigarettes smoked per day, smoking cessation treatment, contraceptive use, and genetic polymorphisms were considered independent variables. The validity of fitted models was assessed graphically. These models were used to assess the effect of predictors on CYP1A2 activity before and after smoking cessation and on CYP1A2 inducibility (w0/w4 ratios).

Multivariate analyses were also carried out considering all the genetic and nongenetic factors together, by fitting multiple linear regressions through step-wise model selection on the basis of Akaike's information criterion (AIC) (stepAIC command from the MASS package, R software version 2.11.1 [27]). In these analyses, the genetic polymorphisms were included as three-category variables, with wild type being the reference category. The results of the step-wise models are presented as final results.

All tests were two sided, and a *P*-value of up to 0.05 was considered statistically significant. All analyses were carried out using STATA software (version 11.0; StataCorp., College Station, Texas, USA).

Results

Study population

A total of 211 volunteers provided their written informed consent to participate in the study; of these, 194 fulfilled the inclusion criteria and were enrolled in the study. In the present analysis, we considered 184 Caucasian participants, among whom 89 (48%) were men and 95 (52%) were women, and most of them were unrelated to one another (96%). The characteristics of the study population have been described previously [11]. Phenotyping was performed in 184 participants when they were smoking and in 113 of them who were abstinent after 4 weeks of smoking cessation. The phenotyping test performed at 4 weeks following smoking cessation was considered as indicative of a baseline CYP1A2 activity, no longer induced by smoking [8].

Genotyping and influence on CYP1A2 activity and inducibility

In total, we genotyped 22 SNPs in 12 candidate genes and their minor allele frequencies in the study population were similar to the values reported in Caucasian populations in the SNP database of the National Center for Biotechnology Information (NCBI) [28] (Table 1). All SNPs were in Hardy–Weinberg equilibrium ($P > 0.14$).

The univariate analysis in the subset of unrelated individuals ($n = 177$) when they were smoking showed no significant influence of the tested polymorphisms on the induced CYP1A2 activity. However, lower plasma paraxanthine/caffeine ratios of borderline significance were observed in heterozygous and homozygous carriers of the *AhR* rs7811989 polymorphism ($n = 83$) compared with wild-type individuals ($n = 94$), when a dominant model of inheritance was considered (median 0.96 vs. 1.07, $P = 0.052$) (Supplementary Table S1, *http://links.lww.com/FPC/A584*).

After quitting smoking, several associations between the tested SNPs and plasma paraxanthine/caffeine ratios were observed. Thus, homozygous carriers of the *AhR* rs2282885 ($n = 11$), the *NR1I2* rs1523130 ($n = 10$), and

the *NR1I3* rs2502815 ($n = 10$) polymorphisms had significantly higher plasma paraxanthine/caffeine ratios than heterozygous and wild-type carriers ($n = 99$, 100, and 100, respectively) (median 0.77 vs. 0.63, $P = 0.040$; 0.85 vs. 0.62, $P = 0.036$; and 0.75 vs. 0.60, $P = 0.004$, respectively). In contrast, significantly lower ratios were found in heterozygous and homozygous carriers of the *NR1I1* rs2228570 polymorphism ($n = 71$) compared with wild-type individuals ($n = 39$) (median 0.59 vs. 0.69, $P = 0.041$), whereas borderline significant lower ratios were observed in carriers of the *NR1I3* rs4073054 polymorphism ($n = 70$) compared with wild-type individuals ($n = 40$) (median 0.58 vs. 0.71, $P = 0.053$) (Supplementary Table S1, *http://links.lww.com/FPC/A584*).

Moreover, several SNPs were found to influence CYP1A2 inducibility, defined as the w0/w4 ratios. As such, higher w0/w4 ratios were found in homozygous carriers of the *AhRR* rs2241598 polymorphism ($n = 3$) compared with heterozygous and wild-type individuals ($n = 107$) (median 2.38 vs. 1.57, $P = 0.026$) and in carriers of the *NR1I1* rs2228570 polymorphism ($n = 71$) compared with noncarriers ($n = 39$) (median 1.64 vs. 1.51, $P = 0.038$), whereas lower w0/w4 ratios were observed in homozygous carriers of the *NR1I3* rs2502815 polymorphism ($n = 10$) compared with heterozygous and wild-type individuals ($n = 110$) (median 1.46 vs. 1.58, $P = 0.051$) (Supplementary Table S1, *http://links.lww.com/FPC/A584*). Further, we tested the influence of the studied SNPs on the considered phenotypes when adjusting for the nongenetic factors (sex, age, number of cigarettes smoked per day, smoking-cessation treatment, contraceptive use) in the entire study population ($n = 184$). In the induced state, no significant influence was observed on the plasma paraxanthine/caffeine ratios and the *AhR* rs7811989 polymorphism (observed in the univariate analysis) was not significant after adjustment for covariables ($P = 0.50$) (Supplementary Table S1, *http://links.lww.com/FPC/A584*).

After smoking cessation, the influence of the *NR1I3* rs2502815 and the *NR1I1* rs2228570 polymorphisms on plasma paraxanthine/caffeine ratios was still significant after adjustment for covariables ($P = 0.007$ and 0.027, respectively); it was no longer present for the *AhR* rs2282885 and the *NR1I2* rs1523130 polymorphisms ($P = 0.082$ and 0.085, respectively) and it became more marked for the *NR1I3* rs4073054 polymorphism ($P = 0.012$) (Supplementary Table S1, *http://links.lww.com/FPC/A584*).

With respect to inducibility, the effects observed in the univariate analyses became more marked after adjustment for covariables for *NR1I3* rs2502815 ($P = 0.016$), *NR1I1* rs2228570 ($P = 0.017$), and *NR1I3* rs4073054 ($P = 0.037$) polymorphisms, except for the *AhRR* rs2241598 SNP, which reached borderline significance ($P = 0.053$) (Supplementary Table S1, *http://links.lww.com/FPC/A584*).

Multivariate analysis on CYP1A2 activity and inducibility

Genetic and nongenetic factors were considered together in an attempt to identify the most important factors influencing CYP1A2 activity and inducibility. For this purpose, a step-wise multiple regression using AIC was used. Significant effects were observed either for the heterozygous or the homozygous mutated versus wild-type individuals, but in all cases, both categories were present in the final model (Table 2).

In terms of induced CYP1A2 activity, there was a significant influence of the number of cigarettes smoked per day ($P = 0.012$), of contraceptive use ($P < 0.001$), as described previously [8], and a borderline significance for the *NR1I2* rs1523130 polymorphism ($P = 0.092$), with an explained variability of 15.3% (Table 2).

After smoking cessation, the influences of contraceptives reported previously [8] and of the *NR1I3* rs2502815 and *NR1I3* rs4073054 polymorphisms observed in the univariate analyses were confirmed ($P = 0.0063$, 0.048, and 0.0045, respectively). In addition, the model showed a significant influence of the *NR2B1* rs3818740 polymorphism ($P = 0.031$), and nonsignificant contributions of the *AhR* rs2282885, *AhR* rs2066853, and *NR1I2* rs1523130 polymorphisms, with a percentage of explained variability of 18.8% (Table 2).

Moreover, several effects were observed on CYP1A2 inducibility, confirming univariate analyses for *NR1I3* rs2502815, *NR1I3* rs4073054, and *NR1I1* rs2228570 polymorphisms ($P = 0.0026$, 0.029, and 0.037, respectively) and shown by the step-wise selection model for *NR2B1* rs3818740, *NR2B1* rs3132297, *AhR* rs2282885, *AhR* rs2066853, *NR1I2* rs1523130, and *IL1β* rs1143634 SNP ($P = 0.0045$, 0.036, 0.040, 0.019, 0.044, and 0.052, respectively) (adjusted $R^2 = 0.224$, Table 2).

As mentioned previously [8,11], other comedications (except for contraceptives) potentially inducing/inhibiting CYP1A2 activity were present in only 5% of the cohort and did not influence CYP1A2 phenotypes. Therefore, they were not adjusted for in the final models.

Finally, the step-wise multiple regression models were fitted to assess the effect of all the genetic and nongenetic factors investigated in this study, including previously reported *CYP1A2* and P450 oxydoreductase (*POR*) polymorphisms [8,11]. The results were in agreement with the previous findings, confirming the influence on CYP1A2-induced activity of the number of cigarettes per day ($P < 0.001$), contraceptive use ($P < 0.001$), *CYP1A2*1F* ($P < 0.001$), and *NR1I2* rs1523130 ($P = 0.027$) polymorphisms (adjusted $R^2 = 0.208$); on CYP1A2 basal activity of contraceptive use ($P = 0.006$), *CYP1A2*1F* ($P = 0.004$), *POR* rs2302429 ($P = 0.003$), *NR1I3* rs2502815 ($P < 0.001$),

Table 2 Multiple regression models for CYP1A2 induced and basal activity and for CYP1A2 inducibility

Nongenetic/genetic factors	Coefficient (SE)[a]	*P*-value[b]
While smoking		
Number of cigarettes/day	0.082 (0.033)	0.012
Contraceptive use (yes)	−0.309 (0.073)	<0.001
NR1I2 rs1523130 (CT vs. CC)	−0.117 (0.069)	0.092
NR1I2 rs1523130 (TT vs. CC)	0.058 (0.113)	0.610
Intercept	0.083 (0.054)	0.125
Adjusted R^2	0.153	
After smoking cessation		
Contraceptive use (yes)	−0.306 (0.110)	0.0063
NR1I3 rs4073054 (TG vs. TT)	0.247 (0.124)	0.048
NR1I3 rs4073054 (GG vs. TT)	−0.179 (0.171)	0.298
NR1I3 rs2502815 (CT vs. CC)	0.009 (0.108)	0.931
NR1I3 rs2502815 (TT vs. CC)	0.562 (0.193)	0.0045
NR2B1 rs3818740 (TC vs. TT)	−0.224 (0.102)	0.031
NR2B1 rs3818740 (CC vs. TT)	−0.048 (0.134)	0.721
AhR rs2282885 (AG vs. AA)	−0.042 (0.098)	0.672
AhR rs2282885 (GG vs. AA)	0.292 (0.165)	0.081
AhR rs2066853 (GA vs. GG)	−0.180 (0.132)	0.175
NR1I2 rs1523130 (CT vs. CC)	−0.161 (0.098)	0.105
NR1I2 rs1523130 (TT vs. CC)	0.114 (0.171)	0.507
Intercept	−0.430 (0.159)	0.0078
Adjusted R^2	0.188	
Inducibility (w0 : w4 ratio)		
Age (years)	0.081 (0.039)	0.039
NR1I3 rs4073054 (TG vs. TT)	−0.221 (0.099)	0.029
NR1I3 rs4073054 (GG vs. TT)	0.082 (0.144)	0.573
NR1I3 rs2502815 (CT vs. CC)	0.002 (0.089)	0.984
NR1I3 rs2502815 (TT vs. CC)	−0.499 (0.161)	0.0026
NR2B1 rs3818740 (TC vs. TT)	0.245 (0.084)	0.0045
NR2B1 rs3818740 (CC vs. TT)	0.061 (0.113)	0.593
NR2B1 rs3132297 (CT vs. CC)	−0.174 (0.082)	0.036
NR2B1 rs3132297 (TT vs. CC)	−0.214 (0.209)	0.308
AhR rs2282885 (AG vs. AA)	0.165 (0.0790)	0.040
AhR rs2282885 (GG vs. AA)	0.021 (0.134)	0.874
AhR rs2066853 (GA vs. GG)	0.250 (0.105)	0.019
NR1I2 rs1523130 (CT vs. CC)	0.167 (0.082)	0.044
NR1I2 rs1523130 (TT vs. CC)	0.009 (0.137)	0.946
NR1I1 rs2228570 (TA vs. TT)	0.175 (0.082)	0.037
NR1I1 rs2228570 (AA vs. TT)	0.086 (0.119)	0.471
IL1β rs1143634 (CT vs. CC)	−0.166 (0.084)	0.052
IL1β rs1143634 (TT vs. CC)	−0.075 (0.173)	0.664
Intercept	0.305 (0.145)	0.039
Adjusted R^2	0.224	

AhR, aryl hydrocarbon receptor; AIC, Akaike's information criterion; CYP1A1, cytochrome P450 1A1; IL1β, interleukin 1β; NR, nuclear receptor family.

[a, b]Coefficients and *P*-values obtained from multiple regression analyses by stepwise model selection based on AIC, using the log-transformed paraxanthine : caffeine and w0 : w4 ratios as a dependent variable and the genetic and nongenetic factors as independent variables (age and number of cigarettes/day were standardized).

NR1I3 rs4073054 ($P = 0.054$), and *NR2B1* rs3818740 ($P = 0.011$) polymorphisms (adjusted $R^2 = 0.314$); and finally, the same results on CYP1A2 inducibility as in the present report, without any influence of *CYP1A2* and *POR* polymorphisms.

Discussion

In the previous reports, our group has described a large interindividual variability in the induction of CYP1A2 by smoking and the influence of some genetic and nongenetic factors [8,11]. The aim of the present analyses was to assess the influence of other genetic factors, involved in the regulation of CYP1A2 constitutive and inducible expression, on CYP1A2 activity and inducibility.

Therefore, we included 22 polymorphisms in 12 genes that were previously shown to be involved in CYP1A2 expression *in vitro*: *NR1I2* (PXR) [19], *NR1I3* (CAR) [20], *NR2B1* (RXRα) that heterodimerizes with CAR [20], *NR1I1* (VDR) [25], *TCF1* (HNF1α), *PPARGC1A* (PGC1α), *NCOA1* (SRC1) [21,22] or *in vivo*: *CYP1A1* [29]; in the inflammation pathways: *IL1β* [24], or in the induction mechanisms: *AhR*, *AhRR*, *ARNT in vitro* [15] and *in vivo* [16,30].

There was no significant influence of the genetic factors on CYP1A2-induced activity, only a confirmation of the previously observed and discussed influence of the number of cigarettes smoked per day and the use of contraceptives [8], and a nonsignificant influence of the *NR1I2* rs1523130 SNP.

After smoking cessation, the strongest observed effect was of the *NR1I3* rs2502815 and to a lower extent of NR1I3 rs4073054 polymorphisms. This is consistent with a recent *in-vitro* study showing that CAR transactivates human CYP1A1 and CYP1A2 in human hepatocytes through a common cis-element (ER8) [20]. Also, the *NR1I3* rs2502815 polymorphism was associated with bone mineral density in healthy Japanese postmenopausal women [31] and both *NR1I3* rs2502815 and *NR1I3* rs4073054 SNPs were selected as tagging SNPs in previous pharmacogenetic studies [32,33]. Of note, other genetic variations have been identified in the *NR1I3* gene, among which three novel SNPs, for which a functional activity was described in a Japanese population [34], or several insertion/deletions generating different splice variants [35]. However, these variants occur at very low frequencies; thus, their effects should be tested in very large study populations.

Moreover, lower CYP1A2 basal activity was observed for *NR2B1* rs3818740 carriers, which confirms the findings of the *in-vitro* study showing a decrease in CYP1A2 mRNA levels for carriers of this polymorphism [25]. Interestingly, the contribution of CAR in the regulation of target genes is achieved through heterodimerization with RXRα; thus, an effect of SNPs in both genes would have been expected. With respect to the other genes, an additional contribution to the CYP1A2 basal activity, which did not reach statistical significance, was observed in the final model for *AhR* and *NR1I2* SNPs.

Most remarkably, several effects were found on CYP1A2 inducibility, most genes being common to the ones influencing the basal activity. Thus, a significant effect on the inducibility was observed for *NR1I3* rs2502815 and rs4073054 polymorphisms, as well as for *NR2B1* rs3818740 and rs3132297 SNPs. This is again in agreement with the previously mentioned *in-vitro* study, which suggested a fundamental role of CAR (as a heterodimer with RXRα) in the xenobiotic-induced expression of CYP1A1 and CYP1A2, independent of

AhR [20]. Of note, *NR1I3* and *NR2B1* polymorphisms showed different effects on basal activity and on inducibility. Thus, whereas *NR1I3* rs2502815 carriers presented a higher CYP1A2 basal activity, they had decreased inducibility, and the opposite was observed for *NR1I3* rs4073054 and *NR2B1* rs3818740 SNPs (lower CYP1A2 basal activity and increased inducibility). This can suggest that the CAR/RXRα heterodimer has an influence on both constitutive and inducible CYP1A2 expression, but the mechanisms underlying it might be different.

Furthermore, a significant influence of *AhR* rs2282885 and rs2066853 SNPs on CYP1A2 inducibility was observed, confirming the AhR-mediated pathway involved in the induction of the enzyme [15]. Also, this seems to confirm the effect observed for *AhR* rs2066853 on the increased CYP1A2 activity in a group of healthy female smokers [16] (even if in our study the medians between carriers and noncarriers were similar, the interquartile ranges suggest increased ratios in carriers, confirmed by the multivariate model). Another study showed a significant interaction between the *AhR* rs2066853 SNP and cumulative cigarette smoking, as well as an increased risk of lung cancer in heavy smokers carrying this polymorphism [30]. Interestingly for *AhR* polymorphisms, even if present in the model of CYP1A2 basal activity, they were not significant. However, they had a significant influence on the inducibility, suggesting a specific effect of the AhR pathway on CYP1A2 induction.

A marginal effect on CYP1A2 inducibility was also observed for *NR1I2* rs1523130, confirming the involvement of PXR in the regulation of CYP1A2 [19] and suggesting a stronger influence on the inducibility than on the constitutive expression (the SNP was also present in the final models for CYP1A2-induced and basal activity, but its influence was not significant). This SNP was associated *in vitro* with decreased hepatic CYP3A expression [36]. Again, it seems that the underlying mechanisms by which PXR regulates the constitutive or induced CYP1A2 expression are different, given that the directional change shows an increase in CYP1A2 constitutive expression and a decrease in inducibility.

A similar conclusion could be reached for the *NR1I1* rs2228570 polymorphism, which showed an increased CYP1A2 inducibility that was significant in the final model, but a decreased basal activity, observed in the univariate analysis. This SNP, in the gene coding for the VDR nuclear factor, was associated with an increased CYP1A2 activity and protein level in the *in-vitro* study [25]. The SNP seems to affect the N-terminal chain of the VDR protein, which can result in a shorter variant with higher transcriptional activity [37]. The authors hypothesized a direct action of VDR in CYP1A2 regulation at unidentified hormone response elements [25], and our finding seems to confirm this hypothesis and to suggest different regulation mechanisms for constitutive and induced expressions. Finally, a borderline significant effect on CYP1A2 inducibility was also observed for the *IL1β* SNP, confirming the result found *in vitro* [25] and suggesting an interaction between the induction and inflammation pathways.

In conclusion, this is, to our knowledge, the first study to confirm *in vivo* the involvement of nuclear factors such as CAR, RXRα, PXR, and VDR in the regulation of CYP1A2 constitutive and/or inducible activity. More interestingly, polymorphisms in these genes seem to have different effects on the constitutive and induced expressions. A similar observation was made in one of the few studies focusing specifically on CYP1A2 induction, which showed an opposite effect of the *CYP1A1*2C* polymorphism on constitutive and induced CYP1A2 activity [13]. However, the underlying mechanisms of CYP1A2 regulation are not yet understood, given the complexity of the nuclear receptors family, their overlapping activity patterns, and crosstalk during the transactivation of their target genes [17].

This also represents the first *in-vivo* study to show the influence of *AhR* specifically on CYP1A2 inducibility, compared with previous researches that were carried out either in smokers or in nonsmokers. This study presents the advantage of measuring the individual change in CYP1A2 activity in the same participants before and after smoking cessation, allowing therefore to have a measure of inducibility, as suggested by other authors [16]. Thus, the use of smokers as their own controls probably takes into account possible factors (environmental factors, food, drugs, etc.) that might influence CYP1A2 activity. Finally, when all the genetic and nongenetic factors studied until now were considered (including *CYP1A2* and *POR* genotypes), we managed to explain 21 and 31% of the variability in CYP1A2 induced and basal activity, respectively, and 22% in CYP1A2 inducibility.

In summary, this study improves our understanding on the factors influencing CYP1A2 activity and suggests genetic influences on CYP1A2 inducibility. However, no corrections for multiple testing were performed and, despite the fact that step-wise selection models based on AIC can be used without correction, these results are exploratory.

The limitations of our study also include a rather small sample size and a lack of functional analysis for the confirmation of the impact of studied polymorphisms on CYP1A2 phenotypes. Therefore, the specific mechanisms through which these genes regulate the induction of CYP1A2 should be further investigated thoroughly.

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Conflicts of interest

There are no conflicts of interest.

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