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A population and family study of CYP1A2 using caffeine urinary metabolites

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Abstract CYP1A2 is a cytochrome P450 which is inducible by polycyclic aromatic hydrocarbons. This induction could be mediated via the Ah locus, which encodes a cytosolic receptor responsible for the regulation of the CYP1A1 gene. Enzyme activity in vivo can be measured by the urinary caffeine metabolite ratio (AFMU + 1X + 1U)/17U. Our goal was to determine, using this ratio, the possible existence of a genetic polymorphism in CYP1A2 induction. For this purpose, a population and family study, including smokers, were undertaken. In a first step, we investigated factors influencing enzyme activity in a population of 245 unrelated individuals.

The induction effect of smoking and inhibiting effect of oral contraceptive use were confirmed. None of the other factors examined (age, sex, level of cigarette consumption, nicotine or tar amounts, filter, inhalation) accounted for the interindividual variability in the metabolic ratio. Using the statistical SKUMIX method, a unimodal (one peak) distribution of the ratio was concluded in 164 unrelated smokers, since a second distribution did not significantly improve the fit to the data ($\chi^2 1 = 1.39$, $P > 0.2$). Segregation analysis was performed on 68 nuclear families and no major gene effect could be shown. Furthermore, the polygenic model did not provide a higher likelihood than the sporadic one, which argues against the existence of any familial resemblance. Although we cannot rule out the possibility that some environmental factors could obscure the phenotypes and occult a genetic determinism, we conclude that genetic factors are probably negligible in the determination of CYP1A2 activity measured by this method.

These results suggest that CYP1A2 induction via the Ah locus would not be similar to that of CYP1A1.

Key words CYP1A2; caffeine, population study, genetic polymorphism, family study

Two forms of cytochrome P450, CYP1A1 and CYP1A2[1], are inducible by certain xenobiotics, in particular polycyclic aromatic hydrocarbons (PAH), procarcinogens present in cigarette smoke. Both cytochromes convert procarcinogens into potent carcinogenic metabolites [1–3]. The central role of these PAH-inducible cytochromes in the activation and disposition of drugs and chemical carcinogens implies that their polymorphic expression could be a crucial factor in cancer susceptibility [4]. CYP1A1 catalyses the oxidation of polycyclic hydrocarbons such as benzo(a)pyrene. CYP1A2 catalyses the metabolic activation of several primary arylamines and heterocyclic amines through N-oxidation [5]. Because of the type of inducers, the great majority of experimental studies have been performed in animals. In mice the induction of these activities by up-regulation of both corresponding genes is mediated via the Ah locus, which encodes the cytosolic aryl hydrocarbon (Ah) receptor [6]. The molecular mechanism of induction of the CYP1A1 gene has been determined. Induction involves firstly the binding of the agonist to the Ah receptor, leading to Hsp90 (90 kDa heat shock protein) dissociation from the Ah receptor. This is followed by the binding of the ligand Ah receptor complex with the Ah receptor nuclear translocator (ARNT) [7, 8] and the binding to DNA sequences

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[1] The nomenclature for cytochromes P450 used in this paper is that suggested in: Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsamus IC, Gotoh O, Okuda K, Nebert DW (1993) The P450 superfamily: update of new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. DNA Cell Biol 12: 1–51

known as Ah- or xenobiotic-responsive elements (AhRE, XRE) in the 5′ flanking region of the gene [9–11]. This leads to the transcriptional activation of CYP1A1, which is followed by increased expression of the enzyme.

It has been suggested that in humans most of the variability in CYP1A1 and CYP1A2 induction level might be dependent on a regulatory gene such as the Ah receptor gene and that Ah polymorphism in the human population would be similar to Ah polymorphism among inbred mouse strains [12]. But, despite many studies, it has not yet been established whether there is a phenotypic polymorphism due to differences in the Ah receptor gene in humans. The mechanism of induction has been explored in vitro, using cultured peripheral blood lymphocytes, exclusively for the CYP1A1 gene. Lymphocytes do not express CYP1A2. Using this system, which reflects the induced aryl hydrocarbon hydroxylase (AHH) activity associated with CYP1A1, the investigations (population and family studies) by Kellermann et al. [13, 14] have suggested an important effect of a single gene on AHH activity. The distribution was described as a trimodal one with heterozygotes being distinguishable by intermediate AHH activity. The population study of Trell et al. [15] displayed the same distribution, and Gahmberg et al. [16] reported a bimodal distribution with high inducibility being dominant.

In contrast to the CYP1A1 gene, there is a relative lack of CYP1A2 induction in most cultured cell lines. Moreover, only CYP1A2 is expressed in human liver. Recently, a urine test based on metabolite ratios derived from unlabelled caffeine to assess PAH-inducible P450 activity in vivo was developed [17–19]. It was demonstrated that the hepatic 3N-demethylation of caffeine to paraxanthine is mainly catalysed by CYP1A2 [20]. This activity is induced in human liver by smoking and can be measured by the caffeine metabolite ratio of AFMU (5-acetylamino-6-formylamino-3-methyluracil) + 1U (1-methylurate) + 1X (1-methylxanthine) to 17U (1,7-dimethylurate), all measured in urine. We refer to this ratio as the CYP1A2 index in this paper. Several population studies have indicated a large inter-individual variability in CYP1A2 activity. However, there is controversy as to the exact distribution of this phenotype: the distributions were described from population studies as being unimodal (one peak only) [21–24] as well as bimodal [25], and a possible trimodality was also reported [26].

The purpose of this work was, firstly, to investigate a possible polymorphism in inducible CYP1A2 activity by studying the distribution of the CYP1A2 index [using the urinary molar ratio (AFMU + 1X + 1U)/17U] among 164 unrelated smokers, and look for a genetic determinism of the phenotype by the study of 68 smoker nuclear families, and secondly, to assess the effects of potential modulating agents on the induction of CYP1A2 activity among 245 unrelated individuals.

Subjects and methods

The subjects gave their informed consent to the study, which received approval from the local ethics committee of the University Hospital (Besançon, France). They were requested to abstain from food and beverages containing methylxanthines for 12 h before the test and during the 24 h of the test. After interview, they were allowed to maintain prescribed medicine if it was known not to interact with CYP1A2, particularly fluvoxamine and omeprazole, and/or could not be discontinued (cardiovascular and psychotropic drugs, antibiotics). Apart from these exceptions, they had to stop taking medicine or be excluded from the study. The subjects were given 200 mg caffeine with a glass of water at 08.00 hours. The bladder having been voided at least once in the interim, urine was collected in a plastic jar containing 1 ml of HCl 6N from 20.00 to 08.00 hours next morning. Hashiguchi et al. [27] have recently shown good correlation between data obtained from urine samples collected during a 12-h period of time, either diurnal or nocturnal. The containers were collected, the volume of urine measured and two aliquots were transferred to a glass tube containing 20 mg of ascorbic acid per ml of urine. The tubes were frozen and kept at −20 °C until analysis, which was performed less than 2 days after the end of the test. Under these conditions, AFMU is stable enough to allow quantitative determination by high-performance liquid chromatography (HPLC) [28].

The families, of Caucasian origin and all from the region of Ile de France, were selected from: (1) students at the University of Paris V, Paris VI and Paris VII, (2) healthy people attending anti-tobacco consultations, (3) general practitioners with knowledge of the physical state and psychological behaviour of their families; this procedure permitted the selection of compliant subjects. Data were obtained from 70 smoker nuclear families (i.e. smoking father and/or mother and smoking children) whose members could be regarded as healthy. The study focused on subjects who reported, on a questionnaire, current (or until 1 month preceding the study), regular smoking of at least one cigarette per day. There were 17, 45, 3, 2 and 1 family with one to five sibs respectively. The parents ranged in age from 36 to 75 years [average age 54.2 (8.85) years] and the children from 15 to 52 years [average age 27.64 (7.85) years].

We assembled a population of 245 unrelated individuals by adding members of staff and students from our laboratories to the parents of the families, the sample included 164 smokers. Ten individuals had stopped smoking for 1 day to 1 month before the test. The consumption (or ex-consumption) averaged 20 (12) cigarettes per day, with a range from 1 to 60. Smokers were queried regarding the frequency of inhalation and brand choice. Information was also collected on the amount of tar and nicotine in the cigarette brands, filter, as well as flavour. The age ranged from 15 to 80 years (average age 43.5 years). In our study, a majority of women ($n = 53$) used oestroprogestative oral contraceptives (OC). The inhibitory action of the oestroprogenic part of OC on CYP1A2 reported in the literature [19, 22, 23, 29] was checked in this study and taken into account as a possible confounding factor. Indeed, it is crucial to be able to check the OC inhibitory effect, since a substantial proportion of women are OC users. Ignoring this effect would potentially lead to biases in the results. On the other hand, this effect could be cancelled by excluding OC users, which would drastically reduce the sample size and thus the power of the study.

Analytic procedure

AFMU, 1X, 1U and 17U were measured in urine by HPLC according to the method described by Grant et al. [17] with a Waters liquid chromatograph (Millipore, Milford, Mass., USA) incorporating a 6000 A pump, a Wisp/10B automatic injector and a Beckman ultrasphere ODS column 4.6 × 150 mm in internal diameter

(Beckman, Fullerton, Calif., USA) with a guard column. The mobile phase consisted of a mixture of 0.05 % acetic acid/methanol (920:80 v/v). In our hands, the coefficients of variation were 6.1 % (0.5 $\mu g \cdot ml^{-1}$) 2.7 % (1.5 $\mu g \cdot ml^{-1}$), 5.6 % (1.2 $\mu g \cdot ml^{-1}$) 4.1 % (3.16 $\mu g \cdot ml^{-1}$), 7.3 % (0.8 $\mu g \cdot ml^{-1}$) 5.2 % (3.3 $\mu g \cdot ml^{-1}$) and 7.5 % (0.8 $mg \cdot ml^{-1}$) 5.7 % (3.3 $\mu g \cdot ml^{-1}$) for AFMU, 1X, 1U and 17U respectively.

Data analysis

Study of the effects of factors on the induction of CYP1A2 activity among the 245 individuals

To assess their possible effects on the CYP1A2 index, the factors age, sex, smoking, the amount of tar and nicotine per cigarette, filter, flavour, and use of OC, were tested independently in the 245 unrelated individuals. Median values were compared using the *t*-test and/or the Mann-Whitney non-parametric *U*-test. In addition, interaction between cigarette and OC use was studied with analysis of variance (ANOVA). The correlation coefficients, determined by least-squares linear regression analysis, were calculated for age and other variables. All these factors were analysed in order to adjust the data with respect to the statistically significant variables. Since OC use reduces the CYP1A2 index, we adjusted the CYP1A2 index for OC use before analysing its distribution: the CYP1A2 index values within each subgroup (with or without OC use) were standardised to mean 0 and variance 1. The standardised CYP1A2 index values were $Z = (X - \mu)/\sigma$, where X were the original values and μ and σ were the mean and standard deviation, respectively, of the values in the given OC group. This procedure has the advantage of correcting for the OC effect without reducing the sample size.

The data were compared with the normal distribution and, if needed, a transformation of the data to approach normality in order to satisfy their suitability for segregation analysis was made.

Distribution among unrelated individuals

Normality tests were performed on the distribution of the CYP1A2 index among 164 smokers using the computer program SKUMIX [30]. This method estimates the relevant parameters and performs likelihood ratio tests of hypotheses that specify alternative numbers of commingled distributions, allowing for skewness through a transform parameter p. The following equation enables the variable y to be transformed into a normally distributed variable y′:

$$y' = 6/p[(y/6 + 1)^p - 1]$$

The parameters involved are v, u (variance and mean of the distribution); d, t, q (parameters which specify the number and characteristics of the commingled distributions when explained by a two-allele, single-locus model: d = degree of dominance, t = displacement between means of homozygotes, q = frequency of A allele giving highest values); and p (parameter that eliminates skewness).

The maximum log-likelihood and the parameter estimates are tested for six different hypotheses: (1) a single normal distribution (t, q and d are set to 0, which means that the whole population has only one mean, so that the distributions are confounded, and p is fixed to 1, which means no transformation of variable); (2) a single skewed distribution (t, q and d are fixed to 0 and p is allowed to vary); (3) two normal distributions (d is fixed to 0, which means complete dominance, t and q are allowed to vary, and p is fixed to 1); (4) two skewed distributions (d is fixed to 0 and t, q, and p are allowed to vary); (5) three normal distributions (p is fixed to 1 and d, t and q are allowed to vary); (6) three skewed distributions (all parameters are allowed to vary).

The likelihoods of the hypotheses on the number and the skewness of distributions are computed and tested using the maximum likelihood ratio test: if R is a restricted model compared with a more general one G, with respective maximum log-likelihood $\ln L_R$ and $\ln L_G$, then $-2(\ln L_R - \ln L_G)$ follows a χ^2 distribution with *n* degrees of freedom, *n* being the difference between the numbers of independent parameters of G and R.

Genetic analysis of family data

Complex segregation analysis was performed using the 'unified' model proposed by Lalouel et al. [31] and implemented in the computer program POINTER. Parameters involved are: v, u (variance and mean of the CYP1A2 index); d, t, q (parameters of the major locus; h (polygenic heritability); and τ_1, τ_2, τ_3 (transmission probabilities, i.e. probabilities that a parent transmits the a allele, when his/her genotype is aa, Aa, and AA, respectively).

The segregation analysis was carried out for the following models: (1) a sporadic model, (2) a polygenic effect expressed by h, (3) a major gene effect expressed in terms of t, d, q, and the transmission probabilities (τs) set to 1, 1/2 and 0, (4) the mixed model with both types of effect, (5) a transmission probability model with free transmission probabilities, (6) absence of transmission (environmental hypothesis) from parent to offspring with all τs equal to 1-q, the frequency of the a allele.

The following hypotheses were then tested: absence of a major gene by comparing models 2 and 4; absence of a polygenic component by comparing models 3 and 4; Mendelian transmission by comparing models 3 and 5; absence of transmission by comparing models 5 and 6. These hypotheses were tested using the maximum likelihood ratio test as explained above. After performing these tests, the model retained by segregation analysis was the one with the least number of parameters that was not rejected against a more general one. The parameter estimates are those which maximised the likelihood of this model.

Results

Factors affecting the CYP1A2 index

The opposite effects of smoking and OC on CYP1A2 activity [19, 22, 23] were confirmed by this study. Induction of CYP1A2 by cigarette smoking is reflected by an increased CYP1A2 index in smokers; on the other hand, inhibition of CYP1A2 by OC use is reflected by a reduced CYP1A2 index in women using OC (Table 1). We further investigated these effects in subjects stratified according to both smoking status and OC use (Table 2): induction of CYP1A2 by cigarette smoking was noted in OC users (4.86 vs 3.02; $P = 0.0092$) as well as in non-OC users (7.61 vs 4.22; $P = 0.0001$). On the other hand, women using OC exhibited a lower CYP1A2 index than women who did not, whether they were smokers or non-smokers. Moreover, interaction between smoking habits and OC use was investigated. As shown in Table 2, inhibition of CYP1A2 by OC seems to be more important in smokers (4.86 vs. 7.61; $P = 0.0001$) than in non-smokers (3.02 vs 4.22; $P = 0.0081$), but the interaction is not significant ($F = 2.51$; $P = 0.11$).

Before analysing the distribution of CYP1A2, we made an adjustment of the CYP1A2 index for OC use

Table 1 Comparison of CYP1A2 indexes [(AFMU + 1X + 1U)/17U] (raw data) in different populations and relation with host factors in 245 healthy subjects (*OC* oestro-progestative oral contraceptives)

	Population 1 Mean (SD)	Population 2 Mean (SD)	P^b
Smoking status	Smokers (n = 164) 6.96 (3.41)	Non-smokers (n = 81) 4.07 (1.79)	0.0001*
OC use in women	Not using OC (n = 130) 6.15 (3.38)	OC users (n = 53) 4.34 (2.36)	0.0005*
Sex	Males (n = 62) 7.23 (2.9)	Women (n = 183) 5.63 (3.23)	0.0006*
Cigarette flavour[a]	Blonde tobacco (n = 87) 6.54 (2.86)	Black tobacco (n = 48) 8.15 (3.59)	0.03*
Filter use	Filter (n = 133) 6.58 (3.14)	No filter (n = 31) 8.55 (4.08)	0.01*
Smoking behaviour[a]	Inhalation (n = 127) 7.12 (3.68)	No inhalation (n = 36) 6.38 (2.23)	0.6
	Mean range (SD)		r^c
Age: non-smokers (years)	39 (18)	(n = 81)	0.03
Age: smokers (years)	43 (15)	(n = 164)	0.20*
Age: smoker males (years)	58 (10)	(n = 52)	0.01
Age: smoker females (years)	36 (13)	(n = 112)	0.22*
Cigarettes per day[a]	20 (12)	(n = 162)	0.04
Nicotine content of cigarette (mg)[a]	0.86 (0.43)	(n = 147)	0.04
Tar content of cigarette (mg)[a]	10.8 (6.30)	(n = 147)	0.04

[a] Some missing values for these variables; [b] Tests of significance based on Mann-Whitney non-parametric test; * significant; [c] correlation coefficient; * significant at 5 % level (F-test)

Table 2 Comparison of mean CYP1A2 index [(AFMU + 1X + 1U)/17U] based on smoking status and OC use in women

	Women using OC		Women without OC		
	n	Mean (SD)	n	Mean (SD)	P^a
Smokers	38	4.86 (2.48)	74	7.61 (3.66)	0.0001*
Non-smokers	15	3.02 (1.35)	56	4.22 (1.56)	0.0081*
P^a		0.0092*		0.0001*	

[a] Tests of significance based on Mann-Whitney non-parametric test; * significant

in order to remove the reducing effect of OC as explained in the subjects and methods section. Thus, $Z = (X - 7.59)/3.43$[2] for the group without OC, and $Z = (X - 4.86)/2.48$ for the group with OC.

After adjustment of the CYP1A2 index values for OC (not shown), all the differences noted between various groups (Table 1) disappeared: men vs women ($P = 0.75$); smoking habits: blonde tobacco vs black tobacco ($P = 0.1$), filter vs no filter ($P > 0.05$). With regard to the age factor, there was no significant correlation with the CYP1A2 index in the group of women after adjustment for OC ($r = 0.07$, $P > 0.25$; before adjustment r = 0.22, $P < 0.05$; Table 1). The CYP1A2 index was not correlated with either the number of cigarettes smoked per day, or the nicotine or tar content (Table 1).

[2] These values are the mean and standard deviation of the whole group (men and women) and thus differ slightly from the values given in Table 2 for women alone

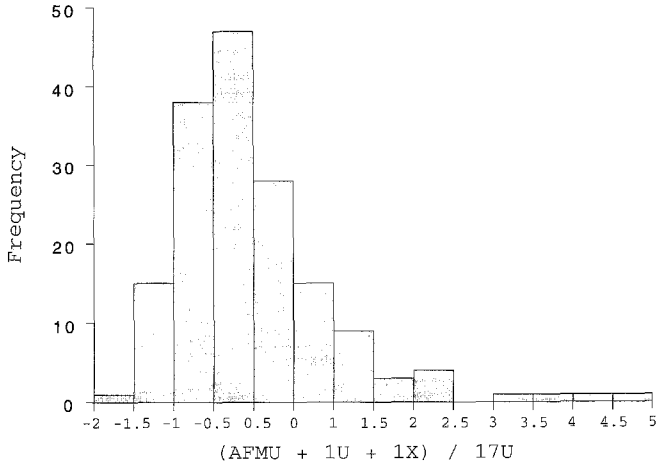

Fig. 1 Frequency distribution of the CYP1A2 index (standardised for each OC use) in 164 healthy unrelated smokers

Distribution of the CYP1A2 index in 164 unrelated smokers

The frequency distribution of the CYP1A2 index (after adjusting for OC use) among the 164 unrelated smokers is given in Fig. 1. The distribution appears unimodal and skewed. The unimodality is confirmed by the statistical analysis using SKUMIX. The results of this analysis are given in Table 3. Deviation from normality is obvious: under the hypothesis of one distribution, a p transform of –2.1 provided a significantly better fit to the data ($\chi^2_1 = 464.4 - 400.8 = 63.6$; $P < 0.001$) and could approximately normalise the skewed distribution. We found no evidence for a second component in the presence of skewness ($\chi^2_1 = 400.78 - 399.39 = 1.39$; $0.3 < P <$

Table 3 Analysis of the distribution of the CYP1A2 index among 164 unrelated smokers: estimate of parameters with standard errors (*v, u* variance and mean of CYP1A2 index; *d, t, q* parameters which specify number and characteristics of commingled distributions when explained by a two-allele single-locus model; *d* degree of dominance, *t* displacement between means of homozygotes, *q* frequency of *a* allele giving highest values; *p* parameter that eliminates skewness; *lnL* maximum log-likelihood of model; *ln* natural log; *italic numbers* fixed parameters)

Hypothesis	v	u	d	t	q	p	− 2 lnL
1. Single normal distribution	1.00 (0.08)	0.00 (0.05)	*0*	*0*	*0*	*1*	464.40
2. Single skewed distribution	0.73 (0.06)	− 0.20 (0.05)	*0*	*0*	*0*	− 2.10 (0.30)	400.78
3. Two normal distributions	0.54 (0.05)	0.00 (0.05)	*0*	3.38 (0.30)	0.20 (0.03)	*1*	413.84
4. Two skewed distributions	0.53 (0.06)	− 0.14 (0.06)	*0*	1.83 (0.47)	0.27 (0.07)	− 1.01 (0.48)	399.39
5. Three normal distributions	0.53 (0.05)	− 0.00 (0.05)	− 0.00 (0.13)	3.38 (0.34)	0.20 (0.03)	*1*	414.19
6. Three skewed distributions	0.29 (0.04)	− 0.07 (0.05)	0.35 (0.03)	3.59 (0.43)	0.14 (0.03)	0.18 (0.42)	397.02

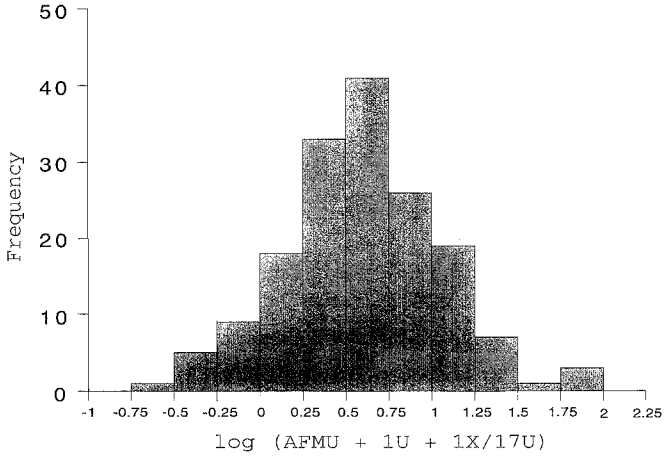

Fig. 2 Frequency distribution of the log CYP1A2 index (standardised for each OC use) in 164 healthy unrelated smokers

0.2). The distribution could also be normalised by logarithmic transformation of the data (e.g. the log normal distribution of the CYP1A2 index in Fig. 2).

Segregation analysis

Complex segregation analysis was then performed on the smoker-family data transformed with the *P* value assessed from SKUMIX. Two of the 70 nuclear families had to be excluded because of paternity exclusion [32]. A generation effect was noted in the families: children showed a greater mean value of CYP1A2 index than parents ($P = 0.027$). The data were then standardised within each group (parents and children) to mean 0 and variance 1 preliminary to the segregation analysis.

The results of the analysis of this sample of 68 families (207 smokers) are shown in Table 4. Comparison of the log-likelihoods between models 1 and 2 indicated no significant familial resemblance ($\chi^2_1 = 590.23 - 586.75 = 3.48$; $P > 0.05$). Comparison between models 2 and 4 provided no significant evidence for a major effect ($\chi^2_3 = 586.75 - 582.17 = 4.58$; $P > 0.05$). Since no major gene was detected, there was no need to test other models.

The analysis with logarithmic transformation of the data confirmed the conclusions (data not shown).

Discussion

As stated by Kalow and coworkers at the beginning of their research on caffeine metabolism [17–19] and recently confirmed by Gu et al. [33] using cDNA-expressed human P450, caffeine 3N- and 7N-demethylation pathways are exclusively CYP1A2 dependent. The 8-hydroxylation of paraxanthine to 17U depends mainly on CYP2A6 and CYP3A and to a minor extent on CYP1A2. Various urinary metabolite ratios have been proposed as an index of CYP1A2 activity [26]: (17X + 17U)/137X, 17X/137X and (AFMU + 1X + 1U)/17U. The first and second of these ratios are open to criticism [29]. Urinary elimination of 17X is strongly dependent on renal flow, and the excreted quantities of 137X are so small that the risk of analytical error is great. Because Kalow et al. [34] proposed the ratio (AFMU + 1U + 1X)/17U as the best empirical index of CYP1A2 and Tucker [35] endorsed this statement by computer simulation, we chose this ratio. In the present study, the expected induction effect of smoking and inhibition effect of OC use on CYP1A2 activity [19, 22, 23, 29] were confirmed. This implies that it is necessary to consider these factors in studies using caffeine as a probe for a drug or xenobiotic whose metabolism is CYP1A2 dependent. It should be emphasised that a number of variables which might seem to be responsible for a variation in the CYP1A2 index (sex, smoking habits) in population studies are no longer significant after adjusting for OC use. An age-related effect on the

Table 4 Segregation analysis of the 68 smoker nuclear families on the transformed data (p −2.1) (*h* polygenic heritability; τ_1, τ_2, τ_3 probability of a parent transmitting *a* allele when his/her genotype is *aa*, *Aa* and *AA* respectively; for other conventions see Table 3)

Hypothesis	d	t	q	h	τ_1	τ_2	τ_3	−lnL
1. Sporadic model	*0*	*0*	*0*	*0*	–	–	–	590.23
2. Polygenic model	*0*	*0*	*0*	0.23 (0.09)	–	–	–	586.75
3. Monogenic model	1[a]	1.33 (0.12)	0.33 (0.05)	*0*	*1*	*1/2*	*0*	581.42
4. Mixed model	1[a]	1.32 (0.13)	0.36 (0.06)	*0.05*	*1*	*1/2*	*0*	582.17
6. No transmission	1[a]	1.32 (0.12)	0.43 (0.05)	*0*	*0.90*	*0.90*	*0.90*	582.64

[a] Parameters that reach abound

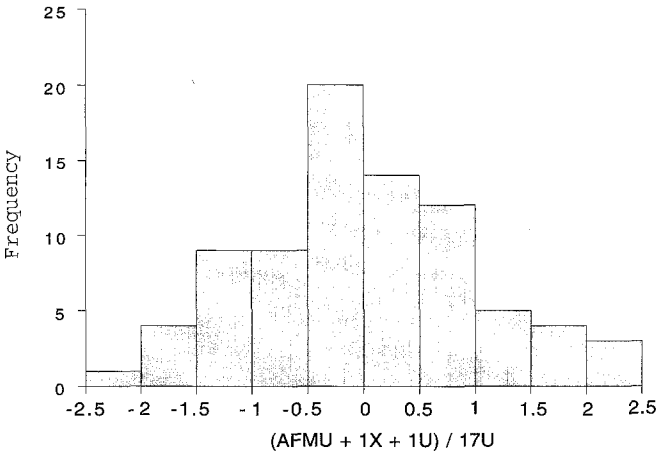

Fig. 3 Frequency distribution of the CYP1A2 index (standardised for each OC use) in 81 healthy non-smokers

CYP1A2 index cannot be totally excluded. Data from the family study showed a higher mean value of the CYP1A2 index in the children than in the parent group. We did not find any correlation of the CYP1A2 index corrected for OC use with age in the group of 164 unrelated individuals. Campbell et al. [19] found a higher value of the CYP1A2 index in children under 11 years of age. Because the youngest individuals in our study were older than 15 years and not numerous compared with the adults, we failed to detect any correlation which could confirm this finding.

Kalow et al. [24] reported a dose-response relationship of CYP1A2 induction with smoke exposure ascertained through cotinine excretion in urine. This was confirmed by Wagenknecht et al. [36], who found a strong correlation between cotinine urinary elimination rate and cigarette consumption per day. Cotinine may thus be a good indicator of smoke exposure [37]. We did not measure urinary excretion of cotinine and we did not find any correlation between the CYP1A2 index and cigarette consumption. This may be surprising. The CYP1A2 index mean values found in the smoker, the non-smoker and the OC-user groups were in complete agreement with the values recently published by Vistisen et al. [22] in a comparable study. That smoking affects CYP1A2 seems well established, but it should be noticed that this effect may be variable, heavy smokers having a CYP1A2 index well within the range of non-smokers and vice versa. Moreover, it is well known that under-reporting of tobacco consumption is usual [38]. Thus, interindividual variability in the effect of smoking on the CYP1A2 index, possible modification of CYP1A2 activity by other environmental or host factors and the understatement of cigarette consumption might explain the lack of correlation.

The distribution of the CYP1A2 index in the 164 unrelated smokers adjusted for OC use and reflecting CYP1A2 induced activity appears to be unimodal and skewed. The distribution can be normalised either with the p value assessed from SKUMIX or by logarithmic transformation of the data. Log normal distributions were also claimed in previous studies [21–24]. An apparent bimodality was reported by Kadlubar et al. [25] in 30 individuals and was attributed to the existence of slow and rapid metabolisers. Since smoking status was ignored in this investigation, it is possible that the bimodality is in fact due to the difference in CYP1A2 activity between smokers and non-smokers. Different results were recently found by Butler et al. [26] using the urinary metabolite ratio 17X/17U: the distributions were described as being normal or trimodal (with the suggested existence of slow, intermediary and rapid CYP1A2 phenotypes) depending on the ethnic origin and smoking status of the individuals. CYP1A2 activity among the three non-smoker populations studied [Arkansas ($n = 101$), Italy ($n = 95$) and China ($n = 78$)] appeared trimodal. This trimodality was conserved in the smoker population in only the Chinese group. In order to investigate a possible trimodality in the non-smoker population, we conducted a similar distribution analysis of 81 non-smokers. The SKUMIX analysis yielded a unimodal skewed distribution (data not shown). The frequency distribution of the CYP1A2 index (adjusted for OC use) among the 81 non-smokers is shown in Fig. 3.

Discrepancy between all these studies may be due to the use of a different CYP1A2 index. We re-analysed the data with the (AFMU + 1X + 1U)/17X ratio in order to compare the results. As expected, we found a urine volume effect, which is consistent with a strong dependence of 17X elimination on urine flow [17, 19, 29]. Adjusting for this effect (in addition to the OC use effect), we obtained very similar results (data not shown)

to these obtained with the CYP1A2 index used in this paper. Thus, different ratios could be affected by different environmental factors, which might explain the variability in the results mentioned above. It may also be due to differences in data management. We used hypothesis-testing methods which, even if they are highly dependent on their assumption, are less prone to errors in detecting multimodality than, for instance, probit plots [35].

A multifactorial variation is suggested by the log-normal population distribution observed with our data. To test whether the interindividual variability in CYP1A2 induced activity is partly accounted for genetic factors, we performed a complex segregation analysis. The fact that no major gene could be detected argues against the hypothesis that the Ah receptor gene is a major genetic determinant of the induction of CYP1A2 activity. It suggests that the genetic determinism described in the murine system for both cyp1-a1 and cyp1-a2 induction with resulting 'responsive' and 'nonresponsive' phenotype [39] is not similar in humans, at least for the *CYP1A2* gene.

In addition, no familial resemblance was shown by the analysis of the data. No genetic model underlying the variability of the phenotype could be postulated that had a better than sporadic fit. These results indicate that multiple environmental mechanisms are involved in the resulting induced CYP1A2 activity. Despite the care taken in the present study in considering possible factors influencing CYP1A2 induction, other kinds of factors not envisaged here could obscure the phenotype. Support for a substantial role of such other factors has come from recent reports. Of relevance are the observations of the inducing effect of exercise or a diet rich in cruciferous vegetables on the CYP1A2 ratio [21, 22]. Therefore, it is possible that other factors prevent different phenotypes from being distinguished and hence the existence of a subjacent genetic determinism from being evidenced.

In conclusion, we did not find a genetic polymorphism in CYP1A2 phenotypes in vivo using caffeine. We only observed the usual interindividual variability in CYP1A2 activity. As recently stated by Kalow and Tang [34], use of the (AFMU + 1X + 1U)/17U urinary metabolite ratio leads to an underestimation of variability, since CYP1A2 is partly responsible for the 8-hydroxylation of 17X to 17U. However, we used a standard dose of caffeine (200 mg) ingested by healthy subjects after a 12-h period devoid of any methylxanthine-containing food or beverage, and the urine collection was performed far from the time of caffeine intake. Under these conditions the caffeine metabolite ratio allows a population study to be performed [34]. The lack of observed polymorphism might imply that a polymorphism in human aromatic hydrocarbon responsiveness, suggested to be a major factor in differences between *CYP1A1* and *CYP1A2* gene induction as the result of genetic differences characterised first in the mouse, would not mirror the murine system. However, two points must be kept in mind. Firstly, the fact that a genetic component apparently plays no role in CYP1A induced activity must be considered with great caution, since numerous, as yet unknown inducers or inhibitors in the diet or from environmental exposure might probably be far the most part responsible for the observed variability; they should result in a 'noise' preventing the detection of a genetic polymorphism. In this respect, genotyping may be preferable for epidemiologic studies. No polymorphism associated with enzyme activity has yet been found for the *CYP1A2* gene and flanking regions. The human Ah receptor cDNA has recently been cloned [40] and will now permit screening for polymorphism. Secondly, the PAH receptor, which should be a major determinant in *CYP1A1* gene regulation through transcriptional activation, should be a relatively minor determinant in *CYP1A2* gene regulation. CYP1A1 and CYP1A2 activities are known to be tissue specific, and different cellular processes underlie the induction of these two genes. It has been shown in molecular transcriptional activation experiments [41] that the PAH-responsive DNA fragment found in the 5′ flanking sequences of the *CYP1A2* gene is not as efficient as the DNA fragment that contains the *CYP1A1* enhancer sequences.

Further studies on CYP1A2 induction are necessary to elucidate this problem and permit better clinical application. At the present time, because more and more drugs seem to be metabolised partly or totally by CYP1A2, important interindividual variability without polymorphism must be taken into account. Tobacco consumption or OC use, with their opposing effects on CYP1A2 activity, must also be considered. Caffeine appears to be a suitable, even if empirical probe drug to be used to check the level of CYP1A2 activity in cases where specific drug monitoring cannot be developed for a drug with a low therapeutic index.

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