The interindividual differences in the 3-demthylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors

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This study investigated the role of genetic factors (CYP1A2) in caffeine metabolism. The CYP1A2 activity was determined in 378 Danish twins following oral intake of a single dose of 200 mg caffeine and subsequent determination of the caffeine ratio (AFMU+1MU+1MX)/17DMU in a 6-h urine sample. The mean (± SD) caffeine ratio was 5.9 ± 3.4. The caffeine ratio was statistically significantly higher in men compared to women, in smoking men and women compared to non-smoking persons of the same gender and in women not taking oral contraceptives compared with women on oral contraceptives. Thus, we confirmed that CYP1A2 is more active in men than in women, that it is induced by smoking and inhibited by oral contraceptives. In the subsequent analysis of heritability, we included 49 monozygotic twin pairs and 34 same gender dizygotic twin pairs concordant for non-smoking and non-use of oral contraceptives. The intraclass correlation coefficient was 0.798 (95% confidence interval, 0.696–0.900) and 0.394 (95% confidence interval, 0.109–0.680) in the monozygotic and dizygotic twins, respectively. The correlation was statistically significantly higher ($P = 0.0015$) in the former compared with the latter. A biometrical model for the caffeine ratio including only additive genetic factors and unique environmental factors was the overall best fitting model. Estimates based on this model gave a heritability estimate of 0.725 (95% confidence interval 0.577–0.822). Unique environmental effects seem to account for the remainder 0.275 (95% confidence interval, 0.178–0.423). Our study shows that the CYP1A2 activity is mainly governed by genetic factors. *Pharmacogenetics* 12: 473–478

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Introduction

CYP1A2 is one of the most important drug-metabolizing enzymes accounting for nearly 15% of the cytochrome P450 in the human liver [1]. Accordingly, more than 20 clinically used drugs are partly or predominantly metabolized by CYP1A2 including caffeine, theophylline, imipramine, clozapine and propranolol [2]. CYP1A2 also activates certain procarcinogens and hence may play an etiological role in the development of cancer [3].

CYP1A2 shows a considerable inter- and intraindividual variability, and the content of CYP1A2 mRNA in human liver displays a more than 40-fold interindividual difference [4]. Using clozapine as an example, the clinical equivalent is an almost 40-fold variability in the steady-state plasma concentration in patients given the same dose [5, reviewed]. CYP1A2 displays higher activity in men than in women [6,7]. The enzyme is inhibited by oral contraceptives [6–8] and in particular by fluvoxamine [9]. Tobacco smoking induces CYP1A2, and this was first shown by lower plasma concentrations of phenacetin or accelerated metabolism of caffeine in smokers compared to non-smokers [10,11]. Other inducers of CYP1A2 include omeprazole [12], rifampicin [13] and cruciferous vegetables [6]. Recently, it has been suggested that the inducibility of CYP1A2 in itself is subject to interindividual differences as several single nucleotide polymorphisms in the *CYP1A2* gene causing high inducibility [14,15] have been reported.

Familial occurrence of defective *O*-de-ethylation of phenacetin, a marker reaction for CYP1A2 [16], was reported 35 years ago [17], and this was the first hint that genetic factors play an important role in variability in CYP1A2. This notion was supported by subsequent studies in twins [18,19] using theophylline to probe for CYP1A2 [20,21].

Assessment of caffeine metabolism still is the gold standard for studying CYP1A2 *in vivo*. The 3-demethylation of caffeine is catalysed by CYP1A2 [22], and the urinary ratios between various caffeine metabolites

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following the ingestion of a single oral dose of the test drug provide an estimate of the enzyme [23]. The urinary caffeine metabolic ratios displayed a unimodal distribution in some studies [6,7,24–26] and a bimodal [27,28] or even a trimodal distribution in others [29]. Accordingly, a definitive role of a genetic polymorphism in caffeine metabolism alias CYP1A2 has not been established.

This could be due to a strong influence from environmental and constitutional factors hiding a polymodal distribution. The study of twins provides one of the most efficient methods of dissecting the aetiology of complex traits [30]. Monozygotic twins share all their genes, while dizygotic twins, like ordinary sibs share on average half of their genes. Based on the assumption that monozygotic and dizygotic twin pairs share environmental influence to the same degree, a comparison of similarity allows one to estimate the relative importance of genetic and environmental effects for the variation in a trait.

In order to further explore the role of genetic factors for CYP1A2 activity, we have therefore determined the urinary caffeine metabolic ratio in a large sample of mono- and dizygotic twins, and we report our findings here.

Materials and methods

This study was a part of a twin project on the metabolic syndrome. Healthy twin pairs were recruited through the Danish Twin Registry and took part in a clinical assessment of glucose tolerance and cardiovascular risk factors, including health-related behaviour such as smoking habits and use of any medication. Correct zygosity was established by analysis of nine highly polymorphic restriction fragment length polymorphisms and micro satellite markers widely scattered at the genome. At the end of the clinical examination, the twin pairs were asked to participate in the present study. The Ethics Committee of Vejle and Funen Counties (j.no. 1997–0271) approved the study.

Three hundred and seventy-eight twins, 187 men and 191 women, were included in the study after informed written and verbal consent. The median age was 37 years (range 17–64 years). Eighty subjects stated that they were smokers (range 1–37 cigarettes per day), and 31 female subjects used oral contraceptives. Some of the subjects also took other drugs, none of which are known to influence the activity of CYP1A2. The study was performed in the subject's own home. The subjects abstained from ingesting methylxanthine-containing foods, beverages and medication for at least 40 h before the test and until delivery of the urine sample. Each subject ingested 200 mg caffeine (Central Pharmacy, Odense University Hospital, Denmark). Six hours later, a urine sample was given in a conical tube containing 300 µl 1 N HCl. The subjects were instructed to store the urine sample at 4 °C until the tube was posted to the research unit of Clinical Pharmacology by regular mail. Here, the urine was stored at −20 °C until analysis. The test was performed only on Mondays to Thursdays in order to avoid a long mailing time.

Urine samples were analysed for the caffeine metabolites 5-acetylamino-6-formylamino-3-methyluracil (AFMU), 1-methyluric acid (1MU), 1-methylxanthine (1MX) and 1,7-dimethyluric acid (17DMU) according to a previously published high-pressure liquid chromatography method [7]. The CYP1A2 activity was estimated from the urinary caffeine metabolic ratio (AFMU+1MU+1MX)/17DMU [23]. In the following, this ratio is referred to as the *caffeine ratio* and is used synonymously with the CYP1A2 activity. Pilot studies in our laboratory have shown that the caffeine ratio is not affected by the urine being stored at room temperature of up to 24 h after delivery and until storage at −20 °C (data not shown). Regardless of zygosity, the subjects were divided into groups according to gender, smoking habits and use of oral contraceptives. The caffeine ratio was compared between the groups by means of a student's *t*-test. *P*-values of less than 0.05 were considered statistically significant.

Twin resemblance for a variable trait, in this case the caffeine ratio, was assessed for monozygotic and same gender dizygotic pairs separately by the intraclass correlation coefficient. The test for significant difference between the coefficients of monozygotic and dizygotic twin pairs was based on the modified Fisher's *z*-transformation procedure [31]. In order to estimate the heritability of CYP1A2 activity (proportion of variance of the caffeine ratio attributable to genetic factors), we analysed the data according to the classic twin model by means of structural-equation biometric models. The details of the classic twin study and the model fitting procedure have been described elsewhere [30, 32,33]. Briefly, structural-equation modelling is based on comparison of the covariance of the caffeine ratio between monozygotic and dizygotic twins. It allows separation of the observed phenotypic variance into additive (A) and dominant (D) genetic components and into common (C) and unique (E) environmental components. Additive genetic factors (A) are the effects of genes taken singly and added over multiple loci, whereas dominant genetic factors (D) represent genetic interaction within loci. The common environmental component (C) reflects the effect of the shared family environment, whereas the unique component (E) applies only to the individual person. The latter component (E) also includes measurement error, which in this case is the analytical and intraindividual variation in the caffeine ratio.

The goal in model fitting is to explain the observed data as well as possible with as few parameters as possible (parsimony). The following models were fitted: ACE, ADE, DCE, AE, DE, CE and E. The fit of each model was assessed by a goodness of fit χ^2 test. The Akaike Information Criterion (AIC) [34], which equals the χ^2 value minus twice the degrees of freedom, was used to identify the model with the best balance between goodness of fit and parsimony.

The final step in our twin analysis was to test whether there was a gender difference in the genetic and environmental factors. This was done by comparing models in which parameter estimates were constrained to be equal across gender groups with models in which estimates were allowed to differ among gender.

The calculation of the intraclass correlation coefficients was carried out with Stata software [35]. All structural equation modelling was carried out with Mx software [36].

Results

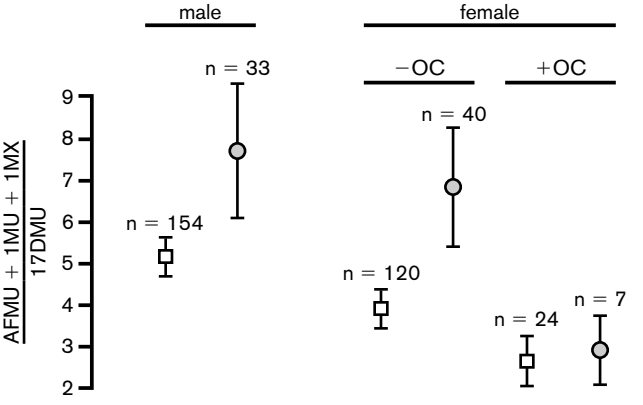
The caffeine ratio showed a unimodal distribution (data not shown) with a mean value of 5.9 ± 3.4 (mean $\pm$ SD). The ratio was statistically significantly lower in non-smoking women (not including oral contraceptive users) compared with non-smoking men, ($P < 0.001$) (Table 1 and Fig. 1). However, no gender-related difference was seen among smoking individuals ($P > 0.2$) (oral contraceptive users excluded).

The caffeine ratio was markedly increased in smoking men ($P < 0.001$) and in smoking women not taking oral contraceptives ($P < 0.001$), as compared to non-smoking individuals of the same gender. In line with this, the intraclass correlation dropped from 0.798 in monozygotic pairs concordant for non-smoking to 0.00 for monozygotic pairs discordant for smoking (Table 2). In pairs where one or both twins were smokers, we did not find any difference in the intraclass correlation between monozygotic and dizygotic pairs. Both observations indicate a gene-environment interaction.

No statistically significant increase in the caffeine ratio was seen among smoking women as compared to non-smoking women when they used oral contraceptives ($P > 0.5$). In women using oral contraceptives, the caffeine ratio was significantly lower than in women not using oral contraceptives ($P < 0.05$ for both non-smoking women and smoking women; Table 1 and Fig. 1).

The heritability analysis was restricted to same gender twin pairs concordant for non-smoking and no intake of oral contraceptives due to the fact that the caffeine

Fig. 1



The caffeine metabolic ratio AFMU+1MU+1MX/17DMU reflecting CYP1A2 activity in relation to smoking, oral contraceptive use and gender. Open squares are non-smokers and shaded circles are smokers. The points represent means and the error bars represent 95% confidence intervals. Non-overlapping confidence intervals indicate statistical significance ($P < 0.05$). OC, oral contraceptives; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; 1MU, 1-methyluric acid; 1MX, 1-methylxanthine; 17DMU, 1,7-dimethyluric acid.

Table 2 Intraclass correlation coefficients in 62 monozygotic twin pairs according to smoking habits

Smoking habits	Number	Intraclass correlation coefficient
Concordant for non-smoking	49	0.79 (0.70–0.90)[a,b]
Concordant for smoking	5	0.34 (0.01–0.97)[a]
Discordant for smoking	8	0.00 (0.00–0.72)[b]

Values in parenthesis represent 95% confidence intervals.
[a]Concordant for non-smoking vs. concordant for smoking, $P = 0.038$.
[b]Concordant for non-smoking vs. discordant for smoking, $P = 0.004$.

Table 1 The urinary caffeine metabolic ratio[a] in 378 mono- and dizygotic twins

	Men		Women −OC		Women +OC	
	Non-smokers	Smokers	Non-smokers	Smokers	Non-smokers	Smokers
Number	154	33	120	40	24	7
Mean ($\pm$ SD)	6.1 ± 3.0	8.7 ± 4.6	4.9 ± 2.5	7.8 ± 4.5	3.6 ± 1.4	3.9 ± 0.90

[a]Urinary caffeine metabolic ratio = AFMU+1MU+1MX/17DMU.
OC, oral contraceptives; AFMU, 5-acetylamino-6-formylamino-3-methyluracil; 1MU, 1-methyluric acid; 1MX: 1-methylxanthine; 17DMU, 1,7-dimethyluric acid.

ratio was heavily influenced by gender, smoking and oral contraceptives.

The correlations between the caffeine metabolic ratio among 49 monozygotic twin pairs (28 male pairs and 21 female pairs) and 34 dizygotic twin pairs (17 male pairs and 17 female pairs) are shown in Fig. 2. The intraclass correlation coefficient was 0.798 (95% confidence interval, 0.696–0.900) and 0.394 (95% confidence interval, 0.109–0.680) in monozygotic and dizygotic twins, respectively ($P = 0.0015$). The significantly higher correlation for CYP1A2 activity in monozygotic than in dizygotic twins indicates that genetic factors contribute to the variation.

A biometrical model including only additive (A) genetic factors and unique (E) environmental factors gave the overall best fit for CYP1A2 activity (Table 3). The fit of models allowing for different parameters in the two genders was then compared with the fit of a constrained model specifying equality of the genetic and environmental parameters across gender. These analyses indicated that the parameters and the heritability is the same in the two genders (data not shown). Estimates based on the best fitting model revealed a heritability estimate of 0.725 (95% confidence interval 0.577–0.822). Unique environmental effects seem to account for the remainder (0.275, 95% confidence interval 0.178–0.423).

Table 3 **A biometrical model of CYP1A2 activity**

	Goodness of fit tests		
Model	χ^2 (df)	*P*-value	AIC
A-C-E	7.31 (6)	0.29	−4.69
A-D-E	7.35 (6)	0.29	−4.65
D-C-E	7.29 (6)	0.30	−4.71
A-E	7.37 (8)	0.50	−8.63
D-E	8.35 (8)	0.40	−7.65
C-E	13.52 (8)	0.10	−2.48
E	51.43 (10)	0.000	31.43

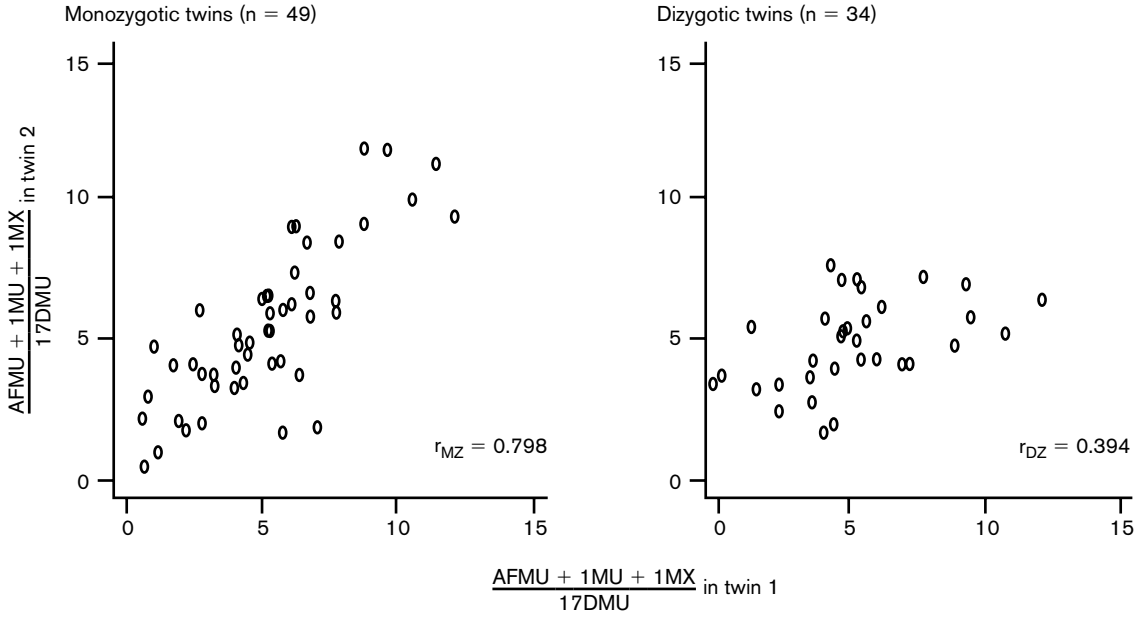
Note, a high *P*-value indicates a good fit or agreement between the observed data and the model. AIC, Akaike's Information Criterion; A, additive genetic components; D, dominant genetic components; C, common environmental components; E, unique environmental components.

Discussion

Twin studies played a key role in the early history of pharmacogenetics in drug oxidation. Thus, a role of genetic factors in the oxidation of a number of drugs was established through this approach: amylobarbitone [37], antipyrine [38], nortriptyline [39], phenytoin [40], tolbutamide [41], phenylbutazone [42], dicoumarol [43] and as mentioned previously, theophylline [18,19].

However, with the discovery of a genetic polymorphism in debrisoquine and sparteine oxidation [44,45], the focus in the pharmacogenetics of drug oxidation turned towards drugs whose oxidation show a bimodal distribu-

Fig. 2



Scatter plot of the caffeine ratio AFMU+1MU+1MX/17DMU reflecting CYP1A2 in 49 monozygotic and 34 dizygotic twins. The caffeine ratio for one twin is plotted against the caffeine ratio in the corresponding twin. The r_{MZ} and the r_{DZ} equal the intraclass correlation coefficient for monozygotic and dizygotic twin pairs, respectively. AFMU, 5-acetylamino-6-formylamino-3-methyluracil; 1MU, 1-methyluric acid; 1MX, 1-methylxanthine; 17DMU, 1,7-dimethyluric acid.

tion pattern. In addition to the sparteine/debrisoquine oxidation polymorphism, the source of which is CYP2D6, the focus in this research area has been on the *S*-mephenytoin oxidation or CYP2C19 polymorphism [46] and more recently also on the CYP2C9 oxidation polymorphism [47]. Studies on a bimodal distribution pattern in the caffeine metabolism capacity reflecting CYP1A2 have yielded conflicting results, and a genetic polymorphism in CYP1A2 has therefore not been established. In order to study the pharmacogenetics of CYP1A2, we have therefore carried out caffeine testing in 378 mono- and dizygotic twins. A mean caffeine ratio of 5.9 reported in the present study is very similar to the value obtained in our previous study of 277 unrelated healthy subjects [7]. The same applies to the influence of gender, smoking and intake of oral contraceptives. (Table 1 and Fig. 1). We therefore decided to exclude smokers, oral contraceptive users and dizygotic twin pairs discordant for gender for the subsequent statistical analysis.

The role of genetic factors for the caffeine ratio thus was estimated in 49 monozygotic and 34 dizygotic twin pairs. To our knowledge, this is the largest sample of twins included in a study of drug metabolism. We show that the caffeine ratio alias CYP1A2 mainly is under genetic control, i.e. the heritability is 0.725.

The heritability of a trait is one of its most important properties as it determines the degree of resemblance between relatives. The heritability is the proportion of the total phenotypic variance that is due to additive genetic variance. Since the environmental variance is also a part of the total phenotypic variance, the heritability is a property of a population and that population's culture and management. In the present study, the estimate of the heritability in CYP1A2 was obtained from Caucasians living in Denmark, among whom the cultural background and living conditions are generally very similar. The heritability will thus not necessarily be the same in another population. Nevertheless, within the range of sampling error, the estimates tend to be similar across populations [48], and a heritability estimate of CYP1A2 in other populations most likely will be within the 95% confidence intervals reported here.

A very large twin study [49] addressed caffeine use, intoxication, tolerance and withdrawal in 486 monozygotic and 335 dizygotic twin pairs, and the conclusion was that individual differences in these four parameters are heavily influenced by genetic factors. The relationship, if any, between these findings and the genetic control in caffeine metabolism reported in the present study remains to be established.

The clinical response to the two antipsychotics, clozapine and olanzapine, both metabolized by CYP1A2 show high concordance in monozygotic twins [50,51]. Likewise, a relationship, if any, to the genetic control in CYP1A2 remains to be established.

A recent literature search study [52] reported 27 drugs that were particularly frequently mentioned in adverse drug reaction studies, and of these there were 12 drugs metabolized by CYP1A2. Although CYP1A2 only is a minor pathway for most of these drugs except two (imipramine and theophylline), it suggests that CYP1A2 may play a more important role in adverse drug reactions than hitherto anticipated.

In conclusion, our study shows that CYP1A2 activity is mainly governed by genetic factors and it also shows that induction by smoking is a powerful environmental factor, which is able to completely obscure the effects of the genetic factors. Future studies are warranted on how this information is used best in avoiding adverse drug reactions and improving efficacy of drugs metabolized by CYP1A2.

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