Pharmacogenetics 1999, **9**:367–375

Original article

Detailed modelling of caffeine metabolism and examination of the *CYP1A2* gene: lack of a polymorphism in *CYP1A2* in Caucasians

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Received 4 June 1998; accepted 14 December 1998

The cytochrome P450 CYP1A2 is important in the metabolism of both drugs and procarcinogens such as heterocyclic amines. We aimed to clarify the existence of a phenotypic polymorphism and explore the molecular basis of such a polymorphism. Ninety-two non-smoking individuals underwent caffeine phenotyping. The distribution of the 1,7-dimethylxanthine + 1,7-dimethyluracil/caffeine (17U + 17X/137X) ratio and log-transformed data were determined. Probit plots were constructed and the distribution fitted using maximum likelihood method. The *CYP1A2* gene, including upstream regulatory regions, was examined for sequence polymorphisms using the single-strand conformation polymorphism technique in 19 individuals and by complete DNA sequencing in two individuals from the extremes of the distribution. We found a similar range (1.45–18.65) and median (6.7) for the 17U + 17X/137X ratio to that found in previous studies of non-smoking Caucasians and no effect of sex. The 17U + 17X/137X ratio gave a normal distribution when log-transformed. Maximum likelihood analysis showed that the log-normal and bimodal distributions had similar deviances but the log-normal distribution was favoured because it has fewer parameters. There was no evidence for significant DNA sequence differences between fast and slow metabolizers, although some differences from published sequences including a silent polymorhpism in exon 7 which were unlikely to be of functional significance were found. We therefore conclude that *CYP1A2* does not show functionally significant polymorphism but that the wide interindividual variation in activity may be due to environmental factors.

Pharmacogenetics 9:367–375 © 1999 Lippincott Williams & Wilkins

Keywords: CYP1A2, caffeine, genetic polymorphism

Introduction

The hepatic cytochrome P450 CYP1A2 plays a central role in the metabolism of drugs such as theophylline, imipramine and clozapine (Fuhr *et al.*, 1992; Lemoine *et al.*, 1993; Bertilsson *et al.*, 1994) but its role in the activation of procarcinogens such as heterocyclic amines and 4-aminobiphenyl has excited more interest. Heterocyclic amines are found in well-cooked meat and have been implicated in the aetiology of colorectal cancer (Sugimura & Sato, 1983; Ito *et al.*, 1991; Skog, 1993). CYP1A2 activity varies widely between individuals, partly due to the influence of environmental inducers such as cigarette smoking (Lang *et al.*, 1994; Nakajima *et al.*, 1994). It has also been suggested that there is a polymorphism in the activity of CYP1A2 and that individuals can be classified as fast, intermediate or slow metabolizers (Butler *et al.*, 1992; Nakajima *et al.*, 1994). One study has suggested that humans with a putative fast phenotype for CYP1A2 activity are predisposed to colorectal cancer (Lang *et al.*, 1994). However, the existence of a true phenotypic polymorphism in CYP1A2 has not been confirmed in all studies and if one does exist the molecular basis has not been demonstrated. The present study further examines the

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0960-314X

existence of a polymorphism in CYP1A2 activity and in the *CYP1A2* gene.

Caffeine (1,3,7-trimethylxanthine) is metabolized by CYP1A2 and this has proved useful in the evaluation of CYP1A2 activity *in vivo*. The metabolic pathways of caffeine are shown in Fig. 1. Some 94% of a dose of caffeine is initially metabolized by the combination of 1-, 3-, and 7-demethylation and less than 3% appears in the urine unchanged (Arnaud & Welsch, 1982; Bonati *et al.*, 1982; Lelo *et al.*, 1986). The 3-demethylation of caffeine to 1,7-dimethylxanthine (1,7X) has been confirmed to be the main pathway for caffeine metabolism, responsible for > 80% of demethylation at low or moderate dosage (Butler *et al.*, 1989; Berthou *et al.*, 1990; Gu *et al.*, 1992). The 3-demethylation of caffeine is catalysed principally by CYP1A2 (Butler *et al.*, 1989; Fuhr *et al.*, 1992; Tassaneeyakul *et al.*, 1994; Fuhr *et al.*, 1996). The metabolic steps following the 3-demethylation of caffeine are complex and are shown in detail in Fig. 1. It can be seen that the production of some metabolites is dependent on the activity of several enzymes and that 1,7-dimethylxanthine (17X) is both a metabolite and a substrate for CYP1A2.

Several methods for determining an individual's CYP1A2 activity using caffeine as a metabolic probe have been developed, including blood and salivary clearances and various urinary metabolite ratios. The systemic caffeine clearance is the most reliable and robust in-vivo surrogate for the activity of CYP1A2 in caffeine 3-demethylation (Fuhr *et al.*, 1993, 1996; Fuhr & Rost, 1994). The correlation between various other methods and systemic caffeine clearance is summarized in Table 1. It can be seen that the most accurate methods are those that rely on blood or salivary caffeine and metabolite measurements. Two urinary metabolite ratios have been widely studied, the 17X + 1,7-dimethyluric acid (17U):3,7-dimethylxanthine (137X) ratio described by Butler *et al.* (1989) and the 5-acetylamino-6-formylamino-3-methyluracil (AFMU) + 1-methylxanthine (1X) + 1-methyluric acid (1U)/17U

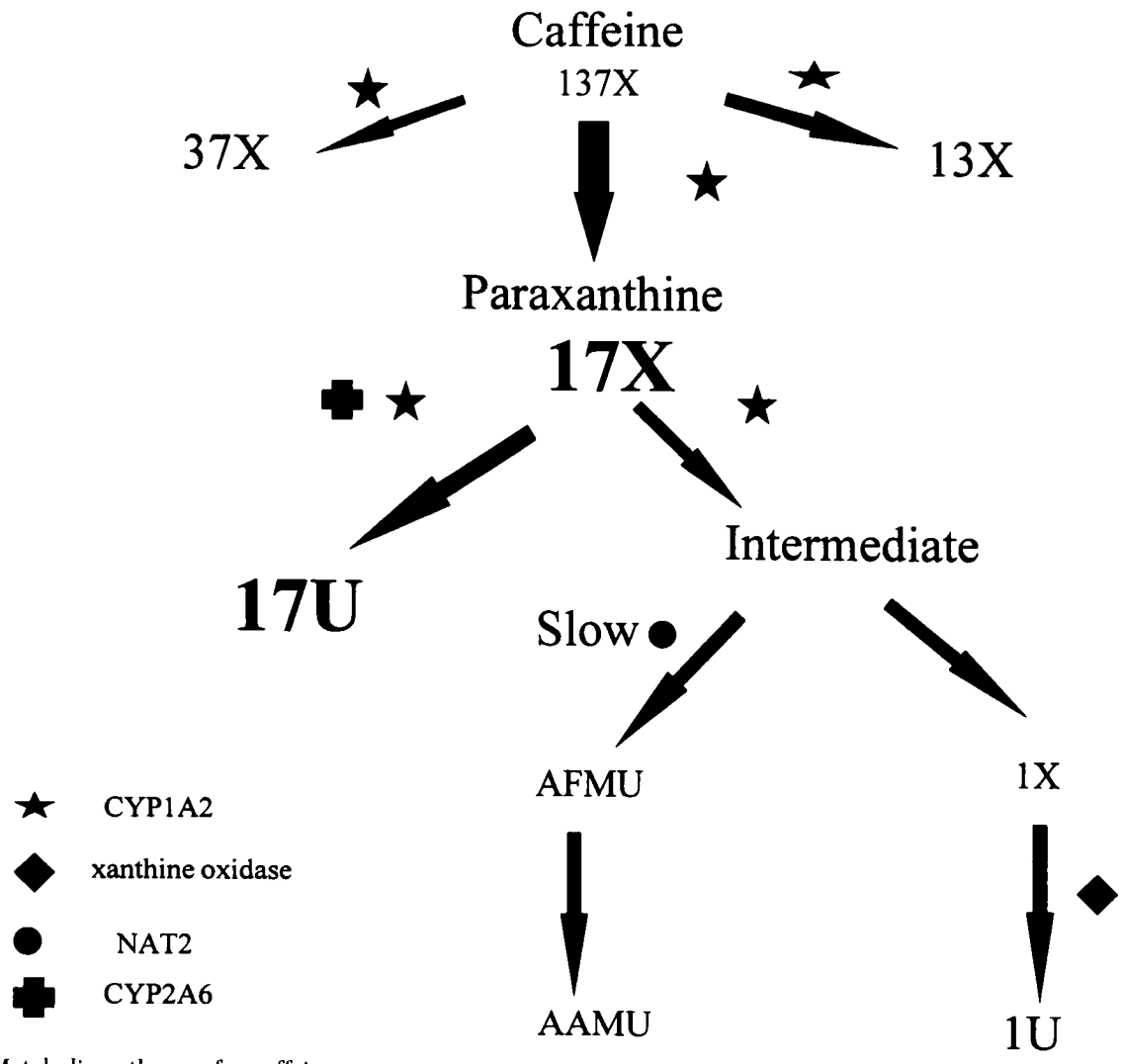

Fig. 1. Metabolic pathways for caffeine.

Table 1. Primers and annealing conditions used for polymerase chain reaction amplification of *CYP1A2* gene

Location of primers and sequence			*Annealing conditions*
5′ Upstream region			
T	-2522 → -2541	5′-TGG AAA GGT GGG AAA GGA GT-3′	58 °C, 1 min
U	-2241 → -2222	5′-TTG GAT TCC TCT TGG CTG TG-3′	
W	-2263 → -2244	5′-CAA GGA TAT CCT GAG AAA CT-3′	58 °C, 1 min
X	-2011 → -1992	5′-TTG TTT CCT GTC TAT ATC TCC-3′	
Exon 1 and 100 bp upstream			
X1A	-125 → -104	5′-ARG GCC CAA GGC CAA GAG TTG-3′	60 °C, 1 min
X1B	+94 → +113	5′-GCT TCC AGG TTC TAT AGT TG-3′	
Exon 2			
A	861 → 880	5′-TCA GCC TGG TCC CTC CTT TT-3′	59 °C, 1 min
B	1108 → 1127	5′-CTG CAG GAC GTC CCC GTA GC-3′	
C	1037 → 1056	5′-CTG GCC CTT GCT CGG GCA TG-3′	60 °C, 1 min
C2	1314 → 1333	5′-GAA GGT GTT GAG GGC ATT CT-3′	
D2	1246 → 1265	5′-ACT GAT GGC CAG AGC TTG AC-3′	60 °C, 1 min
D	1450 → 1469	5′-CCA CCT GAT TGT AAG GGT CG-3′	
E	1397 → 1416	5′-GCT AAG GCC CTG ATC AGC AG-3′	55 °C, 1 min
E1	1801 → 1820	5′-GGC AAC ACA GCA GCT GTG TG-3′	
Exon 3			
F	2303 → 2322	5′-CCT TGG AAG TGC CAG AGT GC-3′	55 °C, 1 min
G	2543 → 2562	5′-TGT AGG GTT GGT GGG TTA TT-3′	
Exon 4			
H	3414 → 3433	5′-AAT GCT GGA TAC ATA CAT AG-3′	59 °C, 1 min
I	3624 → 3643	5′-GGT TTC AAG GCT TCT CCT GG-3′	
Exon 5			
J	3797 → 3816	5′-AGG GGT ATT CAT GGG GCA GT-3′	58 °C, 1 min
K	4035 → 4054	5′-AGG GCT GAG AAG CCA GGA AG-3′	
Exon 6			
L	4622 → 4641	5′-GGG TGG AGG TAG GAG CAA CA-3′	57 °C, 1 min
M	4807 → 4826	5′-GAC TGC TGA ACC TGC ACA CA-3′	
Exon 7			
P	5896 → 5917	5′-TGT TCT CAA CAG AAG TCT CCC T-3′	58 °C, 1 min
Q	6152 → 6171	5′-AAC TCC AGT TGC TGT AGC AG-3′	
R	6113 → 6134	5′-AAG TCC TGG CCA AGT GGG AGA T-3′	56 °C, 1 min
S	6331 → 6352	5′-AAG AGA AAC AAG GGC TGA GTC C-3′	

ratio described by Grant *et al.* (1983). The urinary ratios appear to correlate less closely with caffeine clearance than techniques that rely on salivary or blood measurements (Tang *et al.*, 1994).

Many methods have shown that activity of CYP1A2 varies very widely between individuals and even in non-smokers there is up to 30-fold variation in activity (Lang *et al.*, 1994; Nakajima *et al.*, 1994). Two studies which utilized the urinary 17U + 17X/137X ratio as a measure of activity have suggested that the distribution is bimodal or trimodal (Butler *et al.*, 1992; Nakajima *et al.*, 1994). However, in four studies, with more than 750 individuals, the population distribution of the urinary AFMU + 1X + 1U/17U ratio was log-normally

distributed and the largest family study failed to show any evidence of a genetic effect on CYP1A2 activity (Kalow & Tang, 1991; Vistisen *et al.*, 1992; Carrillo & Benitez, 1994; Catteau *et al.*, 1995). Butler *et al.* (1992) suggest that individuals may be described as having a fast, an intermediate or a slow phenotype for CYP1A2 activity based on the results of their urinary 17X + 17U/137X ratio.

The human *CYP1A2* gene consists of an upstream region of approximately 2 kb, 7 exons and 6 introns encoding a protein with 515 amino-acid residues (Quattrochi *et al.*, 1986; Ikeya *et al.*, 1989). There are some differences between the four published gene/cDNA sequences, principally in exon 7, but no studies have evaluated the effect of the apparent differences on CYP1A2 activity (Jaiswal *et al.*, 1986; Quattrochi *et al.*, 1986; Ikeya *et al.*, 1989; Nakajima *et al.*, 1994). In one study of Japanese individuals, no sequence differences in the *CYP1A2* gene were seen between fast and slow metabolizers of caffeine (Nakajima *et al.*, 1994).

It is important to determine whether there is a functionally significant polymorphism in the *CYP1A2* gene to increase understanding of interindividual variation in drug metabolism and susceptibility to certain cancers. This study examines the population distribution of the 17U + 17X/137X ratio using more detailed statistical analysis than previous studies and is the first study to examine the *CYP1A2* gene for sequence polymorphisms in Caucasians.

Materials and methods

Participants

Ninety-two healthy non-smoking individuals aged 18–50 years who were employees at Newcastle University or local hospitals performed caffeine phenotyping as described by Butler *et al.* (1992) and had blood taken for DNA extraction. Forty-nine (53%) were women. Individuals were excluded if they were taking any medication at all, including the oral contraceptive pill, if they had recently smoked tobacco or if they were known to have any significant impairment of hepatic or renal function. The study was approved by the Newcastle and North Tyneside ethics committee and all individuals gave informed written consent.

Caffeine phenotyping

Foods containing caffeine, such as chocolate, or beverages including Coca-Cola, tea and coffee were stopped for 12 h before the study and until the end of the study. Individuals refrained from consuming grapefruit and orange juice as naringenin found in grapefruit juice can inhibit the expression of CYP1A2. After an overnight fast, individuals consumed two strong cups of their usual brand of instant coffee at 08.00 h, approximately equivalent to 200 mg of caffeine and similar to the 3 mg/kg given by Butler *et al.* (1992). Individuals emptied their bladder at 12.00 h and then collected a urine sample at 13.00 h for analysis. The urine samples were titrated to pH 3.5 with 1 M hydrochloric acid on the day of the phenotyping procedure and were stored at −80 °C. A venous blood sample was collected from each subject into plastic ethylenediaminetetraacetic acid (EDTA) tubes (Sarsted, Leicester, UK) for later DNA preparation and stored at −20 °C.

On the day of analysis the urine samples were rapidly thawed under flowing tepid water. Duplicate samples were prepared for analysis from 400 µl aliquots using the extraction method described by Butler *et al.* (1992). A five-point calibration curve was prepared for each of three standards (17U, 17X and 137X). High-pressure liquid chromatography (HPLC) analysis was performed on a Hewlett Packard Series (Cheshire, UK) 1050 instrument utilizing an ultra-violet detector at 280 nm using a Spherisorb ODS column. The solvents used for elution and the gradients applied were as described by Butler *et al.* (1992). The peaks of the metabolites of interest were identified by comparison with the retention times of the standards.

Statistical analysis

The reproducibility of the analytical technique used was tested by comparing the results from duplicate samples using the *t*-test for paired samples. The distribution of the urinary ratios in the 90 individuals (two had incomplete data) was examined using probit plots of the empirical distribution and by fitting several theoretical distributions by maximum likelihood (Aitkin *et al.*, 1989).

Single-strand conformation polymorphism analysis

In the 10 fastest and nine slowest metabolizers of caffeine in the phenotyping study, the *CYP1A2* gene was examined for sequence polymorphisms by single strand conformation polymorphism analysis (SSCP) using a modification of the method described by Iwahana *et al.* (1994). The primers were designed to amplify the *CYP1A2* gene in sections of approximately 300 bp. Primer sequences are detailed in Table 2. The annealing conditions used for amplifying each polymerase chain reaction (PCR) product are given in Table 2. The annealing temperature was adjusted to ensure no other genes were amplified. For SSCP, denaturation of the PCR product was achieved by adding 2.5–15 µl of the PCR product to 22.5 µl of 50:1 formamide:50 mM EDTA and heating to 90 °C for 4 min. Five µl of a STOP solution (95% formamide with 20 mM EDTA and 0.05% bromophenol blue and 0.05% xylene cyanol) were added and the sample placed on ice and then the entire

Table 2. Comparison of terminal section of exon 7 in the present study and previous studies

Base	6251	6250–6265	6277	6280
Present study	CAG GCG CGG	CTG CGC TTC TCC ATC AAT/C		**TGA**
Jaiswal *et al.* (1986)	CAG GCG CGG	- - - CGC TTC TCC ATC AAT		**TGA**
Nakajima *et al.* (1989)	CAG GCG CGG	CTG CGC TTC TCC ATC AAC		**TGA**
Quattrocci *et al.* (1986)	CAG GCG CGG	CTG CCG TTC TCC ATC AAT		**TGA**

The sequence of bases 6251–6280 (codons 507–516) of the *CYP1A2* gene from the present study and three previous publications. At base 6277 there is a silent C to T sequence polymorphism.

volume applied to a 10% polyacrylamide gel (147 mm × 171 mm × 15 mm) prepared using 0.5 × TBE buffer. These conditions produced nearly 100% denaturation. Electrophoresis in 0.5 × TBE buffer was carried out at a constant voltage of 250 V for between 08.00 h and 16.00 h, depending on speed of migration for each product. For each PCR product, electrophoresis was performed at three different electrophoretic/gel conditions; 10% polyacrylamide gels with a ratio of 37.5:1 acrylamide:bisacrylamide and with 10% glycerol run at room temperature; 10% polyacrylamide gels with a ratio of 37.5:1 acrylamide:bisacrylamide with no glycerol run at room temperature; 10% polyacrylamide gel with a ratio of 49:1 acrylamide:bisacrylamide with no glycerol run at 4 °C. This is similar to the conditions shown by Ravnik-Glavac *et al.* (1994) to have 98% sensitivity for single base mutations. After electrophoresis DNA bands were visualized by staining with either ethidium bromide followed by photography under ultraviolet transillumination, as described by Daly *et al.* (1996), or by silver staining with the Silver Staining Kit (BioRad cat. 161–0449) according to the manufacturer's protocol.

Sequencing of the CYP1A2 *gene*

The entire sequence of interest of the CYP1A2 gene was examined in two individuals, one a fast and one a slow metabolizer of caffeine. Long PCR was used to amplify exons 2–6 with primers A and M using the Expand Long Template PCR system (Boehringer Mannheim, Germany) according to the manufacturer's instructions. Thirty cycles of 94 °C for 1 min, 65 °C for 2 min and 68 °C for 10 min were used for the PCR. Sequencing of the long PCR product was performed directly using the primers B to L as internal primers. Exon 7 was amplified as a 580 bp section with primers P and S and the upstream region with primers T and X. Exon 1 and the two regions of the 5′ upstream sequence already analysed by SSCP were sequenced separately.

Direct sequencing of the PCR products was performed using a modification of the dideoxy chain termination method of Sanger *et al.* (1977) using a commercially available sequencing kit (Sequenase Version 2.0 USB; supplied by Amersham, Bucks, UK). For sequences that showed strong compression artefacts dITP was used for sequencing as recommended by the manufacturer. The terminal section of exon 7 was sequenced in six individuals with varying SSCP banding patterns after cloning using the pMOS Blue T-vector kit (Amersham).

Results

Caffeine phenotyping

Calibration curves of the integrated area under the curve against the known amount of compound injected were made for each of the substrates of interest. The correlations between the concentration of the substrate and the integrated area under the curve for the corresponding peaks were between 0.97 and 1.0. The 17U + 17X/137X ratio of duplicate samples from the same subject showed a high degree of between-test reproducibility with a correlation of 0.945 ($P < 0.0001$) and a paired t-test showed no difference between the two samples ($P = 0.8$).

The range for 17U + 17X/137X in the 92 individuals was from 1.45–18.65 with a median ratio of 6.7 and a mean of 7.78. The mean ratio in men was 7.56 and in women was 7.99; no difference between the two was detected by Mann–Whitney test (2-tailed $P = 0.74$).

The distribution of the untransformed ratios was positively skewed and the Lilliefors test showed that they were not normally distributed (Kolmogorov–Smirnov statistic 0.1334, $P = 0.0004$). The probit plot of the distribution of the 17U + 17X/137X ratio showed a non-linear pattern that could be interpreted as evidence for a polymorphism, implying a mixture of distributions. However, a log-normal distribution is also a possible model for positively skewed data and the distribution of the data after log-transformation is shown in Fig. 2.

To further assess these distributions, mixtures of normal and log-normal distributions with different means and variances were fitted by maximum likelihood and compared by their 'deviances' (values of −2 log maximized likelihood, see Aitkin *et al.*, 1989). The

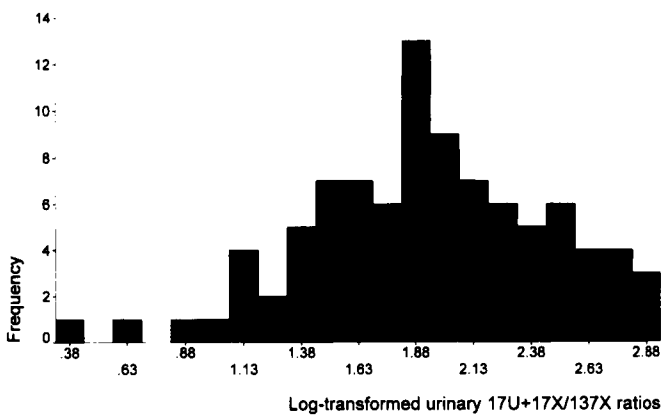

Fig. 2. The distribution of the log-transformed 17U + 17X/137X ratio.

smaller the deviance the better the fit of the model to the data. The distributions were also compared on the probit plot, with the addition of a confidence band for the true distribution based on the 95% confidence limits (± 2 SE) around the observed proportion at each data point.

The single normal distribution had a deviance of 501.91, and the two-component normal a deviance of 479.05, a substantial improvement for the three extra parameters. The single log-normal distribution had a deviance of 484.36, and a mixture of two log-normals had the same deviance: the two components could not be distinguished. Thus, the skewness in the sample distribution is satisfactorily explained by either a mixture of two normals or a single log-normal. Adding further components did not improve the fit further.

A two-parameter gamma distribution was also fitted (Aitkin *et al.*, 1989); this has a similar skew to the log-normal, and its deviance was 483.51. The log-normal and gamma fits are equivalent. The deviance difference of 5.31 between the log-normal and the two-component normal mixture is not large compared with the three extra parameters, and so there is no compelling evidence of polymorphism in the observed distribution.

Figure 3 shows the observed data and the fitted two-component normal (dotted line) and the single log-normal (solid line) distributions. Figure 4 shows the fitted distributions with the confidence band for the true distribution. Both the normal mixture and the log-normal fall completely within the confidence band, showing good fits to the true distribution. The band is quite wide as the sample size of 90 is relatively small.

Examination of the CYP1A2 *gene by single-strand conformation polymorphism*

The sequence examined included the 5′ upstream regions at −2011 to −2521 and −50 to −70 that influ-

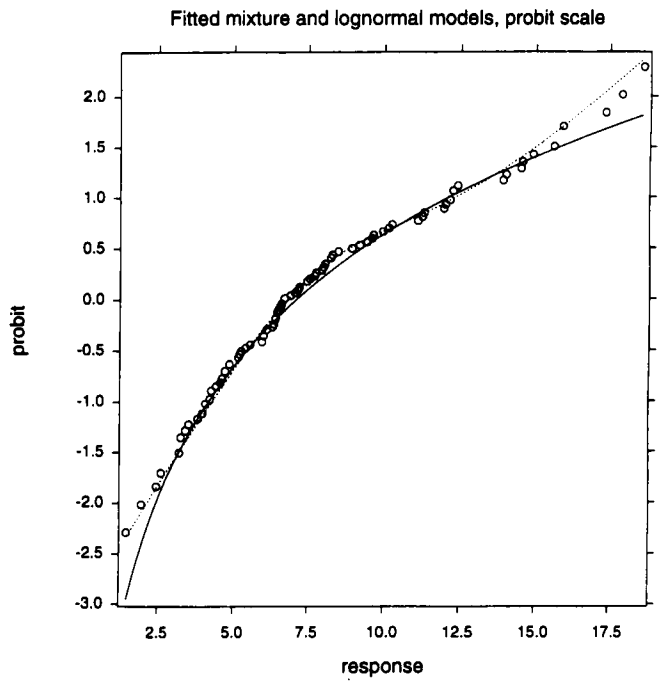

Fig. 3. Probit plot of the distribution of the 17U + 17X/137X ratio against the fitted two-component normal and the log-transformed distributions. The actual data points are shown as circles, the fitted two-component normal distribution as a dotted curve and the fitted log-normal distribution as a continuous curve.

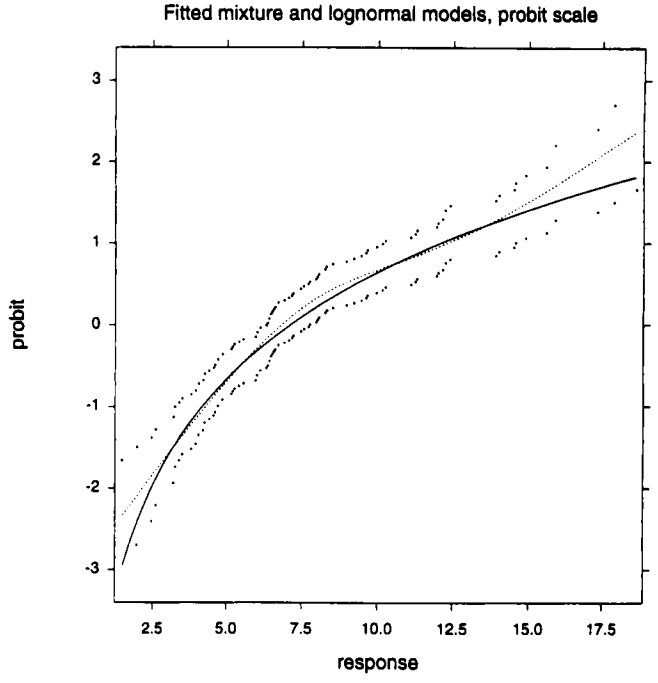

Fig. 4. Comparison of the confidence band of the distribution of the 17U + 17X/137X ratio with the fitted two-component normal and log-normal distributions. The 95% confidence bands for the 17U + 17X/137X distribution are shown as large dots, the fitted two-component normal distribution as a dotted curve and the fitted log-normal distribution as a continuous curve.

ence transcription of the *CYP1A2* gene (Quattrochi *et al.*, 1994) and the 7 exons and exon/intron boundaries. SSCP produced from 2 to 4 bands for each sample. No conformational polymorphisms were detected using the 10% polyacrylamide gels with a ratio of 37.5:1 acrylamide to bisacrylamide with or without the addition of 10% glycerol. With the 10% 49:1 polyacrylamide gel with no glycerol and electrophoresis at 4 °C the only conformational polymorphism detected was for the terminal segment of exon 7. The banding pattern was consistent with two alleles with frequencies of 0.74 and 0.26 respectively. Sequencing of the terminal section of exon 7 showed that the polymorphism detected by SSCP was a T:C transition at base 6279. This polymorphism has been described previously (Nakajima *et al.*, 1994). As both sequences encode asparagine this polymorphism is not predicted to affect CYP1A2 activity.

Sequencing of CYP1A2

No significant differences were seen in the sequence of the *CYP1A2* gene between the one fast and one slow metabolizer chosen for detailed analysis. Some differences were seen from the published sequences. In exon 2, codon 79 has been reported to be AGC (Ser) (Quattrochi *et al.*, 1986) or CGC (Arg) (Nakajima *et al.*, 1994; Jaiswal *et al.*, 1986; Ikeya *et al.*, 1989). The two individuals sequenced here have CGC at this position and SSCP of exon 2 detected no differences in the other 17 individuals. Other differences from published sequences were seen in introns 2, 3 and 5. However, these differences either were seen in both the fast and slow metabolizers of caffeine or were > 20 bases from the splice site and are therefore unlikely to influence RNA-splicing. It is unlikely that these differences from published sequences will affect enzyme activity.

Several sequences for the terminal part of exon 7 have been described (Jaiswal *et al.*, 1986; Quattrochi *et al.*, 1986; Nakajima *et al.*, 1994). It was found that the region of disagreement between the published sequences was the site of a sequencing compression artefact. It was not possible to characterize the sequence in this region using direct sequencing of the PCR product. Sequencing using dITP was then performed on a cloned PCR product. The sequence for exon 7 in all individuals studied in these experiments corresponds to the sequence described by Nakajima *et al.* (1994) for the Japanese population and is compared to the three published sequences in Fig. 5.

Base	6253	6262–6267	6279	6282
Present study	CAG GCG CGG	CTG CGC TTC TCC ATC AAT/C		TGA
Jaiswal *et al.* (1986)	CAG GCG CGG	--- CGC TTC TCC ATC AAT		TGA
Nakajima *et al.* (1989)	CAG GCG CGG	CTG CGC TTC TCC ATC AAC		TGA
Quattrocci *et al.* (1986)	CAG GCG CGG	CTG CCG TTC TCC ATC AAT		TGA

Fig. 5. Comparison of terminal section of exon 7 in the present study and previous studies. The sequence of bases 6253–6282 of the *CYP1A2* gene from the present study and three previous publications. At base 6279 there is a silent C-T sequence polymorphism. Sequence numbering is based on Ikeya *et al.* (1989).

Discussion

The range and mean of the 5-h 17U + 17X/137X ratio in the present study are similar to that found in previous studies; for example, the range was from 2 to 25 in non-smoking Caucasians in Arkansas with a median of 9.3 (Butler *et al.*, 1992). No difference was seen in the CYP1A2 activity between men and women. This is in contradiction to some previous studies that included women who were taking the oral contraceptive pill and that mixed smokers and non-smokers (Vistisen *et al.*, 1992; Catteau *et al.*, 1995). In the present study the distribution of the urinary 17U + 17X/137X ratio was log-normal and no break-point was found in the probit plot of the log-transformed ratios. Maximum log-likelihood analysis confirmed that a log-normal distribution was statistically more likely to represent the true distribution. A log-normal distribution of the 17U + 17X/137X ratio does not rule out a genetic polymorphism in the *CYP1A2* gene. Only one study, of five Japanese individuals, has been published that has looked systematically for genetic polymorphisms in *CYP1A2* and no differences in sequence were found between the fast and slow metabolizers. In the present study, a larger number of individuals were examined by SSCP and the *CYP1A2* gene was also completely sequenced in two individuals but no significant genetic polymorphisms were found. We believe that it is unlikely that we failed to detect any relatively common functionally significant polymorphisms for several reasons. The combination of gel and electrophoretic conditions that we used for SSCP has been shown to detect approximately 98% of single-base polymorphisms in the cystic fibrosis gene (CFTR) (Ravnic-Glavac *et al.*, 1994). Moreover, screening 19 individuals by SSCP analysis should enable detection of polymorphisms that occur at frequencies as low as 0.026 and we chose to screen individuals at the extremes of activity to increase the chances of detecting functionally significant polymorphisms. However, the fact that complete sequencing of coding regions and upstream regulatory sequences was only carried out on two samples means that there is a small chance that a functionally significant polymorphism could have been missed.

Log-normal distributions are usually seen in population parameters which are influenced by the multiplication of a number of other variables whereas normal distributions represent additive relations between variables (Bland, 1987). Metabolic measurements are frequently log-normal because the rate of a reaction depends in part on the concentration of the other compounds. Surprisingly, neither of the previous studies of the 17U + 17X/137X ratio have examined log-transformed data (Butler *et al.*, 1992; Nakajima *et al.*, 1994). In those studies the distribution of the raw urinary 17U + 17X/137X ratio was not normal and probit plots suggested that urinary ratios were trimodally distributed in a mixed racial group of non-smokers in Arkansas and bimodally distributed in Japanese smokers. The present study differs from other studies that have utilized the 17U + 17X/137X ratio in that it includes a more homogenous group of individuals and in that known exogenous influences on CYP1A2 activity such as smoking and the oral contraceptive pill were rigorously controlled for in the design of the study. The results presented here coincide with the findings of many studies which have examined the distribution of CYP1A2 activity using other methods such as the AFMU + 1X + 1U/17U ratio (e.g. Kalow & Tang, 1991; Vistisen *et al.*, 1992; Catteau *et al.*, 1995).

The presence or absence of a polymorphism in CYP1A2 activity is essential in determining the effect of CYP1A2 on the carcinogenicity of heterocyclic amines. There is substantial evidence to demonstrate that oxidation of heterocyclic amines by CYP1A2 is central to their mutagenic effect but there are only limited human data to implicate variation in CYP1A2 activity as a susceptibility factor for colorectal cancer. In one case–control study, individuals with the putative fast phenotype for CYP1A2 and the fast phenotype for NAT2 were at increased risk of colorectal cancer if they preferred well-done red meat (Lang *et al.*, 1994). However, the results were very dependent on the assignment of individuals to particular phenotypes because there was no difference between the mean urinary 17U + 17X/137X ratios of the cases and control individuals. The present study does not support the presence of distinct phenotypes for CYP1A2 activity.

More work is required on the role of CYP1A2 activity as a susceptibility factor for colorectal and other cancers. Because of the central role of CYP1A2 in activation of heterocyclic amines it is likely that variation induced by environmental factors, both recognized and unrecognized, is important in determining individual cancer susceptibility.

Acknowledgements

This research was sponsored by the North of England Cancer Research Campaign and the Northern Regional Health Authority. We wish to thank Annette Steward and A. Monesola Adeokun who contributed to the sequencing of *CYP1A2*.

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