CYP2D6 polymorphism in a Gabonese population: contribution of the *CYP2D6*2* and *CYP2D6*17* alleles to the high prevalence of the intermediate metabolic phenotype

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Aims To determine the molecular basis of the intermediate extensive metaboliser (EM) CYP2D6 phenotype in healthy Gabonese subjects.

Methods The CYP2D6 phenotype of 154 healthy Gabonese subjects was assessed by giving the subject a single dose of 30 mg dextromethorphan, and collecting their urine for the next 8 h. The *CYP2D6* genotype was determined for 50 individuals of the EM phenotypic group by Southern blotting and various PCR-based procedures aimed at identifying different *CYP2D6* alleles.

Results We found that in the studied Gabonese population, as compared with a French population, there is significantly higher frequency of intermediate EM phenotype having lower frequency of *CYP2D6* PM alleles. To clarify this discrepancy phenotype-genotype relationship was studied. We found that the *CYP2D6*17* and *CYP2D6*2* alleles, prevalent in this black population, are characterised by their low capacity for dextromethorphan demethylation. Our data also show that the *CYP2D6*1* allele is associated with the highest *in vivo* activity followed by the *CYP2D6*2* allele and then the *CYP2D6*17* allele.

Conclusions The higher frequencies of the *CYP2D6*2* and *CYP2D6*17* alleles than the *CYP2D6*1* allele account for the high frequency of the intermediate EM phenotype in this black population. The polymorphism of the CYP2D6 enzyme activity in African populations could have important implications for use of drugs that are substrates for CYP2D6 and have a narrow therapeutic window.

Keywords: CYP2D6 polymorphism, Bantu population, genotype, phenotype

Introduction

The debrisoquine/sparteine type polymorphism is a well-characterised genetic variation in drug metabolism associated with cytochrome P450 CYP2D6 [1]. People with active CYP2D6 are termed extensive metaboliser (EM). The enzyme activity in this group shows wide variation and individuals can be classified as ultrarapid, intermediate and slow EMs. Between 1 and 7% of Caucasians and 29% of Ethiopians are ultrarapid metabolisers with multiple copies of functional *CYP2D6* genes [2–4]. The genetic basis of the poor metaboliser (PM) group is now well

understood, but much less is known about the molecular basis of the heterogeneity within the EM group. EMs with intermediate activity are either heterozygotes for one of the poor metaboliser alleles or carry at least one of the functionally active but less efficient *CYP2D6* alleles (*CYP2D6*2* for Caucasians, *CYP2D6*10* for Chinese, *CYP2D6*17* for Zimbabweans [5, 6, 7, 8, 9, 10]).

We investigated the relationship between metabolic phenotype and *CYP2D6* genotype in a Bantu-speaking black population in Gabon. We also explored the molecular basis of interethnic differences in CYP2D6 activity by comparing data obtained from the Gabonese population with those previously obtained from a French population [11].

Methods

One hundred and fifty-four unrelated, healthy Gabonese volunteers (102 men and 52 women) gave their informed

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Received 17 March 1998, accepted 16 August 1998.

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consent to participate in the phenotype studies performed at the 'Centre International de Recherches Médicales de Franceville' (Gabon). CYP2D6 phenotype was assessed by giving the subject a single dose of 30 mg dextromethorphan, and collecting their urine for the next 8 h. The amount of dextromethorphan (DEM) and dextrorphan (DOR) in the urine was determined by high performance liquid chromatography (h.p.l.c.) [11] and the urinary metabolic ratio (MR) DEM/DOR was calculated. Subjects with a log DEM/DOR ratio higher than -0.5 were assigned to the PM phenotype.

Blood samples for genotyping were obtained from 50 individuals of the EM group. These DNA samples were analysed by Southern blotting (*Xba*I polymorphism) and using various published PCR-based procedures aimed at identifying the following CYP2D6 alleles: CYP2D6*1, *2, *3, *4, *10 and *17 [7, 9, 12, 13] except for the CYP2D6*2 allele. The CYP2D6*2 allele frequency was obtained by subtracting the frequency of the CYP2D6*17 allele (exon 2 variation [9]) from the overall frequency of the exon 6 mutation [7].

The analysis of variance test was used for statistical evaluation of the differences between mean MR in individual genotypes. Where statistically significant differences were found groups were compared using the Student's *t*-test with a Bonferroni correction. Chi-square analysis was used to test the differences in genotype and allelic variations between different ethnic groups.

Results

The distribution profile of the log MR was bimodal (Figure 1a) with a PM phenotype frequency of 0.02 ($n = 3$ subjects). The distribution of the data for this black population was right-skewed as compared with that obtained for a French Caucasian population (Figure 1b) [11], suggesting a higher frequency of intermediate EM phenotype.

The CYP2D6 genotype and phenotype observed in the Gabonese subjects are shown in Table 1a. Analysis of allele segregation in family members indicated that the 16+9 kb-*Xba*I allele was associated with the CYP2D6*4 poor metaboliser (PM) allele whereas the 42 kb-*Xba*I fragment was associated with the CYP2D6*2X2 extensive metaboliser (EM) allele. As with other black populations [14–16], the frequency of the CYP2D6 PM alleles in the Gabonese EM group was significantly lower (0.09) than that in Caucasians (0.21) ($P < 0.02$). The most prevalent mutant allele in the black population was the CYP2D6*5 allele (frequency 0.07). The heterozygotes (EM/PM) had a lower mean CYP2D6 activity, as shown by their low MR values (-1.4 ± 0.9 ($n = 9$) vs -2.1 ± 0.9 for the homozygotes *Xba*I 29 kb/29 kb EM/EM ($n = 39$)): ($P < 0.05$). Within

Figure 1 a) Frequency distribution of the log dextromethorphan (DEM) and dextrorphan (DOR) urinary metabolic ratio (MR) in 154 healthy Gabonese individuals. EM and PM individuals were defined as those with a log DEM/DOR MR lower (EM) and higher (PM) than -0.5 . b) Frequency distribution of the log DEM/DOR MR in 422 healthy French Caucasians. The data are from [11].

the homozygous EM *Xba*I 29/29 kb group ($n = 39$ individuals), the observed frequencies were 0.32 for CYP2D6*1, 0.44 for CYP2D6*2, and 0.24 for CYP2D6*17.

The relationship between MR values and six genotypes defined by the CYP2D6*1, CYP2D6*2, CYP2D6*17 alleles was analysed in the Gabonese EM group (Table 1b) (only subjects homozygous for 29 kb fragments were analysed to avoid a bias due to differences in CYP2D gene copy numbers). These results show a non random distribution of the CYP2D genotypes within the EM phenotypes. Finally, there was no significant difference in mean MR values between the French Caucasian [11] and Gabonese individuals with CYP2D6*1/CYP2D6*1, CYP2D6*2/CYP2D6*1 and CYP2D6*2/CYP2D6*2 genotypes (data not shown).

Table 1a CYP2D genotype and phenotype in the Gabonese.

CYP2D genotype EM/EM	n	Mean MR values $\pm$ s.d.	CYP2D genotype EM/PM	n	Mean MR values $\pm$ s.d.
D6*1/D6*1 (Xbal 29/29)	5	-2.9 ± 0.3	D6*2X2/d6*4 (Xbal 42/29)	1	-2.12
D6*1/D6*2 (Xbal 29/29)	11	-2.5 ± 0.2	D6*2/D6*4 (Xbal 29/16+9)	1	-1.5
D6*2/D6*2 (Xbal 29/29)	9	-2.4 ± 0.3	D6*2X2/D6*5 (Xbal 42/29)	1	-2.6
D6*1/D6*17 (Xbal 29/29)	4	-2.3 ± 0.5	D6*2/D6*5 (Xbal 29/11.5)	4	-1.3 ± 0.3
D6*2/D6*17 (Xbal 29/29)	5	-2 ± 0.3	D6*1/D6*5 (Xbal 29/11.5)	2	-1.5 ± 0.2
D6*17/D6*17 (Xbal 29/29)	5	-1.1 ± 0.6			
D6*2X2/D6*1 (Xbal 42/29)	1	-2.2			
D6*2X2/D6*1 (Xbal 42/29)	1	-1.9			

Genotypes are defined by various combinations of the D6*1, D6*2, D6*4, D6*5 alleles and Xbal RFLP (kb). The phenotype is defined by the log DEM/DOR metabolic ratio (MR). *n*: number of individuals.

Table 1b Genotype-phenotype relationships in the Gabonese EM group homozygous for Xbal-29kb fragments.

CYP2D6 genotype	Mean log MR values $\pm$ s.d.	CYP2D6 genotype	Mean log MR values $\pm$ s.d.	t-test P-value	Bonferroni correction $k \times P$ -value ($k = 15$)
D6*1/D6*1	-2.9 ± 0.3	D6*1/D6*2	-2.5 ± 0.2	< 0.02	< 0.3
		D6*1/D6*17	-2.3 ± 0.5	< 0.05	< 0.75
		D6*2/D6*2	-2.4 ± 0.3	< 0.02	< 0.3
		D6*2/D6*17	-2 ± 0.3	< 0.002	< 0.03
		D6*17/D6*17	-1.1 ± 0.6	< 0.0001	< 0.0015
D6*1/D6*2	-2.5 ± 0.2	D6*2/D6*17	-2 ± 0.3	< 0.005	< 0.075
		D6*17/D6*17	-1.1 ± 0.6	< 0.0001	< 0.0015
D6*1/D6*17	-2.3 ± 0.5	D6*17/D6*17	-1.1 ± 0.6	< 0.005	< 0.075
D6*2/D6*2	-2.4 ± 0.3	D6*17/D6*17	-1.1 ± 0.6	< 0.0001	< 0.0015
D6*2/D6*17	-2 ± 0.3	D6*17/D6*17	-1.1 ± 0.6	< 0.002	< 0.03

Genotypes are defined by various combinations of the CYP2D6*1, CYP2D*2 and CYP2D6*17 alleles and the phenotype is defined by the log DEM/DOR metabolic ratio (MR). Individual *t*-test (corrected by the Bonferroni method) was used for statistical evaluation of the differences in mean MR values (columns 2 *vs* 4) between two genotypic groups. Only differences which are statistically significant before correction are indicated. *k* = number of tests.

Discussion

Our results show that the CYP2D6*17 allele was associated with the lowest capacity for dextromethorphan demethylation in the Gabonese population (Table 1b). Although the CYP2D6*2 allele has been reported to have similar catalytic activity (for debrisoquine) to that of CYP2D6*1 [2, 9], we found that the CYP2D6*2 allele was associated with lower levels of CYP2D6 activity, consistent with previous *in vivo* observations in Caucasians [7, 10]. We do not know the reason for this difference but the association of the CYP2D6*2 allele with lower CYP2D6 activity might apply only to dextromethorphan and not to debrisoquine, as these substrates belong to two different chemical classes [17]. Recently, Oscarson *et al.* [18] reported *in vitro* studies showing that the CYP2D6*2 protein exhibits lower activity

with bufuralol than CYP2D6*1, which is in agreement with our data. Further studies using different substrates are required to determine the molecular basis for the lower CYP2D6 activity of the CYP2D6*2 enzyme. Nevertheless, the CYP2D6*2 allele *in vivo* exhibited higher CYP2D6 activity than the CYP2D6*17 allele (Table 1b) (also in agreement with *in vitro* studies [18]). The CYP2D6*17 allele was relatively frequent in the Gabonese population studied consistent with results for another black population [9]. However, the frequency of the CYP2D6*2 allele was lower in Zimbabwean [9] than in Gabonese (0.13 *vs* 0.40). This difference may be due to i) genuine ethnic differences between these two population groups, or ii) a difference in the CYP2D6*2 allele detection procedure. Concerning the latter possibility, the CYP2D6*2 allele was detected by analysis of sequence variation in intron 1 [9] rather than by

detection of *CYP2D6* coding sequence variations (in exons 3, 6 and 9) (see Method section).

This analysis in a Bantu population from Gabon shows that the *CYP2D6**1 allele is associated with the highest activity *in vivo* followed by the *CYP2D6**2 allele and then the *CYP2D6**17 allele. In conclusion, the higher frequencies of the *CYP2D6**2 and *CYP2D6**17 alleles than the *CYP2D6**1 allele may account for the high incidence of the intermediate EM phenotype in this black population. The polymorphism of the *CYP2D6* enzyme activity in African populations could have important implications for use of drugs that are substrates for *CYP2D6* and have a narrow therapeutic window [19, 20].

This work was supported by grants from 'Association Française contre les Myopathies', 'La Ligue Nationale Française contre le Cancer' and 'La Caisse Nationale d'Assurance Maladie des Travailleurs Salariés'. The 'Association pour la Recherche sur le Cancer' provided a fellowship to SP.

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