Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and a black African population

Maria Gabriella Scordo,^{1,2} Eleni Aklillu,³ Umit Yasar,^{1,3} Marja-Liisa Dahl,¹ Edoardo Spina² & Magnus Ingelman-Sundberg³

¹Department of Medical Laboratory Sciences and Technology, Division of Clinical Pharmacology at Karolinska Institutet, Huddinge University Hospital, Stockholm, Sweden, ²Institute of Pharmacology, University of Messina, Italy and ³Institute of Environmental Medicine, Division of Molecular Toxicology, Karolinska Institutet, Stockholm, Sweden

Aims CYP2C9 is a major enzyme in human drug metabolism and the polymorphism observed in the corresponding gene may affect the therapeutic outcome during treatment with several drugs. The distribution of variant *CYP2C9* alleles was therefore investigated in an Italian and an Ethiopian population.

Methods Allele-specific PCR analysis was carried out in order to determine the frequencies of the two most common variant alleles, *CYP2C9**2 and *CYP2C9**3 in genomic DNA isolated from 157 Italians and 150 Ethiopians.

Results The frequencies of *CYP2C9**1 (80%), *CYP2C9**2 (11%) and *CYP2C9**3 (9%) found in the Italian population were similar to other Caucasian groups. However in the Ethiopian population *CYP2C9**1, *CYP2C9**2 and *CYP2C9**3 were present at a frequency of 94, 4 and 2% respectively. The 95% confidence intervals in *CYP2C9**1, *CYP2C9**2 and *CYP2C9**3 between Italians and Ethiopians were 0.098, 0.176, 0.040, 0.098 and 0.040, 0.098, respectively.

Conclusions Our results indicate that the Ethiopian population has a unique relative distribution of the *CYP2C9* alleles, which is not similar to any other ethnic group hitherto described.

Keywords: interethnic differences, losartan, poor metabolisers, warfarin

Introduction

Cytochrome P450 2C9 (*CYP2C9*) catalyses the metabolism of many important drugs such as phenytoin, S-warfarin, tolbutamide, losartan, torasemide and as well as nonsteroidal anti-inflammatory drugs (NSAIDs) [1]. Four variants of the *CYP2C9* allele have been found in Caucasian and African-American populations [2–4] (see <http://www.imm.ki.se/CYPalleles/> for an update). These alleles encode *CYP2C9* enzymes with potentially different catalytic activity and specificity [1]. Several studies [2, 3] indicate that the allelic variants Arg₁₄₄Cys (*CYP2C9**2) and Ile₃₅₈Leu (*CYP2C9**3) encode enzymes with decreased substrate turnover. Thus, several investigators

have shown that the amino acid substitutions Arg₁₄₄Cys and Ile₃₅₈Leu decrease the rate of phenytoin hydroxylation [5], whereas the former substitution impairs the 6- and 7-hydroxylation of S-warfarin [6] and the latter decreases the metabolic rate of tolbutamide [7]. Apparently, these amino acid variants may not affect the *CYP2C9*-mediated metabolism of some NSAIDs like diclofenac to the same extent [1]. Since *CYP2C9* plays a role in the metabolism of benzo[a]pyrene, a major lung carcinogen [8, 9], it has been postulated that the *CYP2C9* genetic polymorphism could influence individual susceptibility to lung cancer.

Interethnic differences in *CYP2C9* allele distribution have been described between Caucasians and Orientals. However, no data are available from black African populations [3, 10]. Therefore, we studied samples of Italian and Ethiopian populations to examine the frequencies of *CYP2C9**1, *CYP2C9**2 and *CYP2C9**3 alleles and to compare them with previous findings in other populations.

Correspondence: Dr Magnus Ingelman-Sundberg, Division of Molecular Toxicology, IMM, Karolinska Institutet, SE-171 77 Stockholm, Sweden. Tel.: +728-7735; Fax: +468-337-327; E-mail: maging@ki.se

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Methods

Genotyping

One hundred and fifty-seven unrelated healthy Italian volunteers (95 males and 62 females aged 21–64 years, mean $\pm$ s.d.: 32 ± 9 years) of Caucasian origin and from South-Italy, were enrolled in the study. The protocol was approved by the Ethics Committee at the University of Messina, Italy, and written informed consent to participate in the study was obtained from the volunteers. The Ethiopian genomic DNA samples were from our previous CYP2D6 genotype and phenotype study [11]. Approval for sample collection and use of the samples in pharmacogenetic studies was obtained in 1995 from the Medical Faculty at the University of Addis Ababa. Approval for analysis of the CYP2C9 genotype was obtained from the ethics committee at the Karolinska Institute. Venous blood (5 ml) was obtained from each Italian volunteer, and DNA was isolated from peripheral leucocytes by Qiagen Blood and Cell Culture DNA kit[®] (Qiagen, Hilden, Germany), according to the guidelines of the manufacturer. Genotyping of variants, Arg₁₄₄Cys (CYP2C9*2) and Ile₃₅₉Leu (CYP2C9*3), was performed by PCR followed by restriction enzyme analysis, as previously validated by Yasar *et al.* [12].

Statistical analysis

The Chi-square test was used to compare allele frequencies in the two populations. A *P* value of 0.05 or less was regarded as significant.

Results

The frequencies of the CYP2C9*1, CYP2C9*2 and CYP2C9*3 alleles as well the genotype frequencies for both populations are summarized in Table 1. The CYP2C9*2 and CYP2C9*3 alleles were statistically more common among the 157 Italian subjects compared with the 150 Ethiopian volunteers. Among the Italian volunteers, nine individuals (5.7%) were found to carry two mutated alleles, four subjects were homozygous for CYP2C9*2, two homozygous for CYP2C9*3, and three subjects were heterozygous for both the Arg144Cys and Ile359Leu mutations. The latter genotype was classified as CYP2C9*2/*3, since there is no evidence for a linkage of the Cys144 variant with the Leu359 variant on the same chromosome [2, 13]. In contrast, no subject carrying two mutated alleles was detected in the Ethiopian population studied.

Discussion

The CYP2C9 genetic polymorphism has been well studied in Caucasians and Orientals, but not in African populations. The frequencies of the CYP2C9*2 and CYP2C9*3 alleles found in our Italian population were very close to those found in other Caucasian populations (Figure 1). By contrast, the CYP2C9*2 allele frequency is zero in different Oriental populations and very low in African-Americans (0.01). These findings suggest that the CYP2C9*2 allele evolved quite recently, after the separation of the Caucasian and Oriental racial groups.

Table 1 Allele (a) and genotype (b) frequencies for CYP2C9 in the Italian and Ethiopian populations.

a					
Variant allele	Frequency in Italian volunteers (n = 157)	Frequency of Ethiopian volunteers (n = 150)	Absolute difference	95% CI on the difference	P**
CYP2C9*1	0.796	0.751–0.841	0.933	0.905, 0.961	NS
CYP2C9*2	0.112	0.077–0.147	0.043	0.020, 0.066	<0.01
CYP2C9*3	0.092	0.060–0.124	0.023	0.006, 0.040	<0.001
b					
Genotype	Number of subjects	Observed (Predicted)* frequency (%)	Number of subjects	Observed (Predicted)* frequency (%)	
CYP2C9*1/*1	102	65.0 (65.0)	130	86.7 (87.0)	
CYP2C9*1/*2	24	15.3 (16.8)	13	8.7 (8.1)	
CYP2C9*1/*3	22	14.0 (14.5)	7	4.6 (4.3)	
CYP2C9*2/*2	4	2.5 (1.1)	0	0 (0.2)	
CYP2C9*3/*3	2	1.3 (0.8)	0	0 (0.05)	
CYP2C9*2/*3	3	1.9 (2.0)	0	0 (0.2)	

* Predicted frequencies calculated according to the Hardy–Weinberg equation. ** *P* difference between Italians and Ethiopians. CI confidence interval.

Figure 1 Global distribution of the variant *CYP2C9**2 (a) and *CYP2C9**3 (b) alleles (allele frequency in percentage). Data are from (a [10].; b [3].; c [2].; d [14].; e [17].; f [18].; g [12].; h [13].; i [19].; j [20].; k [21].; l. [this study]. * African-Americans.

The *CYP2C9**2 allele frequency of 0.043 here found among Ethiopians, was lower than in Caucasians, but higher than in African-Americans. Similarly the allele frequency of *CYP2C9**3 among Ethiopians was comparable with that found in Japanese (0.021) [14] and Chinese (0.026) [10] populations, but was much higher than in African Americans (0.005) [3].

There is pronounced genetic heterogeneity among different African populations. For instance *CYP2D6**17, which is prevalent among Zimbabweans and other black African populations, is very rare among Ethiopians [11]. In contrast, about 29% of Ethiopians, but less than 2% of other black Africans, for example, Zimbabweans, carry duplicated *CYP2D6* genes [15]. *CYP2D6**10, which encodes a partially active *CYP2D6* enzyme, is more prevalent among Orientals, and is present among Ethiopians at a relatively higher frequency compared with other black Africans [16]. Thus, our results concerning the variant *CYP2C9* alleles support the earlier data indicating that different African populations are heterogeneous with respect to *CYP* genotype.

In conclusion, our findings indicate a unique distribution of the *CYP2C9* alleles in the Ethiopian population. The characterisation of *CYP2C9* genetic polymorphism among different races might contribute to the optimization of therapy with a range of clinically important drugs.

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