Genetic polymorphism of (S)-mephenytoin 4′-hydroxylation in populations of African descent

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Aims The frequency of CYP2C19 poor metabolizers (PMs) in populations of African descent has been reported to range from 1.0% to 35.4%. In order to determine with greater certainty the frequency of CYP2C19 PMs in such black populations we have performed a meta-analysis of the studies.

Methods Relevant data on the frequency of both the PM phenotype of probe drugs (mephenytoin, omeprazole, and proguanil), and the distribution frequencies of *CYP2C19* alleles and genotypes in black populations were summarized and reanalysed using a meta-analytical approach.

Results Of nine reported studies two were excluded because of significant heterogeneity ($\chi^2 = 115$, $P < 0.0001$). The combined data from the remaining seven studies showed that the frequency of the PM phenotype in 922 healthy unrelated black Africans and black Americans ranged from 1.0% to 7.5% ($n = 7$ for combined data) with an overall frequency being 3.9% (36 of 922; 95%CI: 2.7%–5.2%). The frequency of the PM genotypes in blacks was 3.7% (36 of 966; 95%CI: 2.5%–4.9%), in agreement with the frequency of the PM phenotype. In the extensive metabolizers (EMs) 29% (271 of 930) were heterozygotes (*wt/m*). The observed frequencies of the three Mendelian genotypes were 0.68 for *wt/wt*, 0.28 for *wt/m*, and 0.04 for *m/m*. The allelic distribution was appropriate at 82.3% (95%CI: 80.5%-83.9%) for *CYP2C19*1*, 17.3% (95%CI:15.7%–19.0%) for *CYP2C19*2* (*m1*), *and* 0.4% (95%CI: 0.1%–0.7%) for *CYP2C19*3* (*m2*) in these populations.

Conclusions We conclude that subjects of African ancestry have a low frequency of the CYP2C19 PM phenotype and genotype; that the defective *CYP2C19* alleles are uncommon, and that a small proportion of heterozygotes exists in the EM subpopulation.

Keywords: (S)-mephenytoin hydroxylation, black subjects, CYP2C19, database, genetic polymorphism, meta-analysis

Introduction

Genetic polymorphism of (S)-mephenytoin 4′-hydroxylation is a well-documented polymorphically distributed pharmacogenetic trait in humans [1, 2]. Recently, human (S)-mephenytoin 4′-hydroxylase has been identified as cytochrome P450 (CYP) 2C19 [3, 4] and shown to be genetically polymorphic [5–8], with individuals being phenotypically characterized as either extensive (EMs) or poor metabolizers (PMs) according to their ability to oxidize CYP2C19 substrates. This polymorphism is relevant to the oxidative metabolism of a number of clinically important drugs [1, 2], such as diazepam, certain barbiturates, tricyclic antidepressants, omeprazole and proguanil. As a result, pronounced genetically determined differences in the disposition of these drugs may affect their efficacy and toxicity. Also, CYP2C19 polymorphism may contribute to a metabolic predisposition to certain diseases including non-aggressive bladder cancer [9], lung cancer (squamous cell carcinoma) [10], scleroderma or systemic sclerosis [11] and the eosinophilia–myalgia syndrome [12]. Thus defining the frequency of this polymorphism in different populations has considerable epidemiologic importance. Several polymorphisms in the *CYP2C19* gene have been identified and shown to cause phenotypic variability. To date, nine alleles have been described which include the active *CYP2C19*1A* (*wt1*) and *2C19*1B* (*wt2*) alleles and seven defective alleles *CYP2C19*2A* (*m1A*), *2C19*2B* (*m1B*), *2C19*3* (*m2*),

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*2C19*4* (*m3*), *2C19*5A* (*m4* or *TRP433*), *2C19*5B, and 2C19*6* (*m5*) (nomenclature from Ibeanu *et al.* 1998) [8]. There is an excellent correlation between genotype and phenotype, with defective *CYP2C19* forms resulting in the PM phenotype as determined using a range of substrates [1, 2].

The frequency of the CYP2C19 PM phenotype is 4–5 times more common in Asian than in Caucasian populations, for example, 14.3% (95% CI:12.3%–16.4%) of 1117 Chinese subjects were CYP2C19 PMs [13] as compared with 3.2%–3.4% in Caucasian populations [1, 14, 15]. However, a wide variation has been reported in the frequency of the PM phenotype in populations of African ancestry (1.0%–35.4%), depending on the populations tested and the probe drugs used for phenotyping [16–24]. To synthesize these data and determine an overall population frequency of the PM phenotype and/or genotype in black subjects of African ancestry, all relevant eligible data were reanalysed.

Methods

All available information was retrieved by electronic searching of the English-language publications which appeared in the MEDLINE database (1980– October 1998), hand-searching abstracts of major related conferences since 1980 and checking relevant references cited in other publications. Any publications describing nonrelated and randomly selected healthy black subjects phenotyped as EMs or PMs, or genotyped as carriers of defective and/or active *CYP2C19* alleles were included. As described elsewhere [13, 15, 25, 26], the text of each report was carefully reviewed using a meta-analytical approach, and the total sample size and numbers of phenotypes and/or genotypes of CYP2C19 were extracted. Because only the final data are eligible for meta-analysis, some reports in abstract form were not included in this meta-analysis.

Allocation of an individual to the PM phenotype is based on the subject's ability to oxidize CYP2C19 substrates, such as (S)-mephenytoin [1, 5, 6, 14, 16, 18, 24], omeprazole [19, 24], and proguanil [17, 22]. All genotyping analysis was based on the recently developed method by de Morais *et al.* [5, 27, 28]. The principal genetic defect found in PMs of (S)-mephenytoin is a single G→A mutation in exon 5 of *CYP2C19* gene (*CYP2C19*2*), creating an aberrant splice site [27]. A second defective allele (*CYP2C19*3*) is a single G→A mutation at position 636 of exon 4, creating a premature stop codon [28]. These single-point mutations remove, respectively, restriction endonucleases *BamH*I and *Sma*I sites [5, 6, 10, 18–21, 27–31].

The geographical distributions of the CYP2C19 phenotypes and genotypes reported in black populations are summarized in Table 1 and 2, respectively. To determine whether available data from each individual study could legitimately be pooled with the other studies, the homogeneity among the independent reports was first estimated on the basis of the overlap of the 95% confidence interval (CI) of the PM incidence in each study, since the intersection of plots of 95%CI of the actual incidence of PMs in each study may be used to visually judge the homogeneity of the studies [31]. Homogeneity across the studies was finally assessed with a standard χ^2 test on $2 \times n$– contingency tables as described elsewhere [13, 15, 25, 26]. Lack of a significant χ^2 test indicated the homogeneity of eligible data for pooled analysis [31].

It has been shown that the PM phenotype of CYP2C19 is inherited as an autosomal recessive trait [32–34]. According to the Hardy–Weinberg theory, the square root of the observed incidence of the PMs in a certain population tested should equal the total gene frequency (q) of the recessive alleles controlling the PM phenotype; the total gene frequency (p) of the dominant alleles controlling the expression of the EM phenotypes is equal to 1-q; the expected frequencies for the homozygous (*wt/wt*) and heterozygous (*wt/m*) high activity phenotypes are estimated to be p^2 and 2pq, respectively [35]. Thus, the actual and predicted frequencies of the PM phenotype and/or genotype were compared.

Results

CYP2C19 phenotype

A statistically significant inconsistency existed across the three studies in African-Americans ($\chi^2 = 26.62$; d.f. = 2, $P < 0.0001$), and across the six studies in black Africans ($\chi^2 = 75.63$; d.f. = 5, $P < 0.0001$). When the two small studies, showing a PM frequency of 18.5% [16] and 35.4% [17] in an African-American and Kenyan population, respectively, were excluded from the meta-analysis, an excellent consistency was found in African-Americans ($\chi^2 = 0.44$; d.f. = 1, $P = 0.51$), and in black Africans ($\chi^2 = 1.85$; d.f. = 4, $P = 0.76$). This was due to significant heterogeneity between these and the other studies ($\chi^2 = 114.96$; d.f. = 8, $P < 0.0001$). A significant overlap of the 95% CIs of the PM phenotype frequencies was found between the separate observations ($\chi^2 = 9.78$; d.f. = 6, $P = 0.13$) in the remaining seven studies (Table 1). Because all the data with homogeneity may be used for pooled analysis, the overall estimate of the PM phenotype was 3.9% (36 of 922; 95%CI: 2.7%-5.2%) of all the black populations tested, with a mean value of $4.1 \pm 2.1\%$. To further assess the potential population-based differences in this polymorphism, we compared the frequencies of CYP2C19 PM phenotype and found

Table 1 Distribution of S-mephenytoin 4′-hydroxylation phenotypes in various populations of African ancestry.

	n	*Probe*[a]	*EMs*	*PMs*	*PMs %*	*95% CI*	*Reference*
African-American	27[b]	M	22	5	18.5	3.8–33.2	16
	191	M	189	2	1.0	−0.4–2.4	18
	100	O	98	2	2.0	−0.7–4.7	19
	sum		sum	sum	mean ± s.d.		
	291		287	4	1.5 ± 0.7	0.0–2.7	
				$\chi^2 = 0.44$; $df = 1$, $P > 0.05$			
Ethiopian	114	M	108	6	5.3	1.2–9.4	20
Kenyan	65[b]	P	42	23	35.4	23.8–47.0	17
Nigerian	92	M	88	4	4.3	0.2–8.4	23
Tanzanian	216	M	206	10	4.6	1.8–7.4	22
	106	M	98	8	7.5	2.5–12.6	24
Zimbabwean (Shona)	103	M	99	4	3.9	0.2–7.6	21
	sum		sum	sum	mean ± s.d.		
	631		599	32[c]	5.1 ± 1.4	3.4–6.8	
				$\chi^2 = 1.85$; $df = 4$, $P > 0.05$			
	total		total	total	mean ± s.d.		
	922		886	36	4.1 ± 2.1	2.7–5.2	
				$\chi^2 = 9.78$; $df = 6$, $P > 0.05$			

[a]Probe drug, M: mephenytoin; O: omeprazole; P: proguanil.
[b]Excluded from this meta-analysis because of a significant heterogeneity between it and the other studies ($\chi^2 = 115$; $df = 8$; $P < 0.0001$).
[c]Significantly different from African-Americans, $P < 0.01$.

Table 2 Distribution of the *CYP2C19* genotypes in various populations of African ancestry.

		EMs					*PMs*			
	n	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	*% PMs*	*95% CI*	*Reference*
African-American	76	48	27	0	1	0	0	1.3	−1.2–3.8	18
	100	70	28	0	2	0	0	2.0	−0.7–4.7	19
	108	61	40	0	7	0	0	6.5	1.8–11.2	29
	233	164	60	0	8	1	0	3.9	1.4–6.4	30
Sum	517	343	155	0	18	1	0	3.7	2.1–5.3	
Frequency		(0.66)	(0.30)	(0.0)	(0.03)	(0.0)	(0.0)			
Ethiopian	114	85	22	1	3	3	0	5.3	1.2–9.4	20
Tanzanian	251	166	75	2	7	1	0	3.2	1.0–5.4	24
Zimbabwean	84	65	16	0	3	0	0	3.6	−0.4–7.6	21
Sum	449	316	113	3	13	4	0	3.8	2.0–5.6	
Frequency		(0.70)	(0.25)	(0.0)	(0.03)	(0.0)	(0.0)			
P value[1]		0.19	0.10	0.10	0.72	0.19	—	0.93		
	Sum	Sum	Sum	Sum	Sum	Sum	Sum	Mean ± s.d.		
Total	966	659	268	3	31	5	0	3.7 ± 1.8	2.5–4.9	
Frequency		(0.68)	(0.28)	(0.0)	(0.03)	(0.0)	(0.0)			
						$\chi^2 = 5.32$; $df = 6$, $P > 0.05$				

[1]African-Americans *vs* black Africans; Fisher's exact test.

that there was no intersection of 95%CIs of both subtotal PM frequencies between African-Americans (0%-2.7%) and black Africans (3.4%-6.8%), and that black Africans have a higher frequency of CYP2C19 PM phenotype than African-Americans (5.1% *vs* 1.4%; $P = 0.0056$) as shown in Table 1.

CYP2C19 genotype

In the African-Americans studied, a good consistency existed in the distributions of various genotypes ($\chi^2 = 12.24$; d.f. = 9, $P = 0.20$), and in the frequencies of EMs and PMs genotypically identified ($\chi^2 = 4.41$; d.f. = 3, $P = 0.22$) as presented in Table 2. Similarly, a significant homogeneity occurred in the black African populations for the distributions of the genotypes ($\chi^2 = 12.71$; d.f. = 8, $P = 0.12$), and for the frequencies of genotyped EMs and PMs ($\chi^2 = 0.94$; d.f. = 2, $P = 0.62$). By comparison, there were no population-based differences in the prevalence of genotyped PMs (African-Americans *vs* Africans; 19 of 517 *vs* 17 of 449; $\chi^2 = 0.0083$; d.f. = 1, $P = 0.93$), or in the distributions of both major genotypes *2C19*1/*1* (343 of 517 *vs* 316 of 449; $P = 0.19$) and *2C19*1/*2* (155 of 517 *vs* 113 of 449; $P = 0.10$) between African-Americans and black Africans. Furthermore, systematic analysis of the genotypic data on PMs in all 7 studies indicated that there was no significant heterogeneity between the different studies (Table 2) ($\chi^2 = 5.34$; d.f. = 6, $P = 0.50$). The frequency of the PM genotype was 3.7% (36 of 966; 95%CI:2.5%–4.9%), with a mean value of 3.7 ± 1.8%. Based on the estimate of the PM phenotype of 3.9%, the expected frequencies of the three Mendelian genotypes were calculated to be 0.64 for *wt/wt*, 0.32 for *wt/m*, and 0.04 for *m/m* in black populations (Table 3). In contrast, the observed frequencies of the three Mendelian genotypes were 0.68 (659 of 966) for *wt/wt*, 0.28 (271 of 966) for *wt/m*, and 0.04 (36 of 966) for *m/m* (Table 2 and 3), very close to expected.

As shown in Table 4, an excellent consistency was found in the distributions of various alleles in the African-Americans ($\chi^2 = 9.27$; d.f. = 6, $P = 0.16$) or black Africans ($\chi^2 = 7.70$; d.f. = 4, $P = 0.10$), and also found in the distributions of active (*wt*) and inactive (*m1* plus *m2*) alleles across the seven studies ($\chi^2 = 12.30$; d.f. = 6, $P = 0.06$) but not in the distributions of three major alleles (*wt*, *m1*, and *m2*) ($\chi^2 = 27.63$; d.f. = 12, $P = 0.006$), showing that black Africans have a relatively higher prevalence of the allele *m2* than African-Americans (0.8% *vs* 0.1%; $P = 0.03$). In both populations, the observed allelic frequencies were as follows: *CYP2C19*1* (82.3%; 95%CI: 80.5%-83.9%), *2C19*2* (17.3%; 95%CI: 15.7%-19.0%), and *2C19*3* (0.4%; 95%CI: 0.1%-0.7%).

Table 3 Comparison of both observed and expected frequencies of the three Mendelian genotypes of *CYP2C19* in various populations of African ancestry.

	Observed	*Expected*
wt/wt (p^2)	0.68	0.64
wt/m (2pq)	0.28	0.32
m/m (q^2)	0.04	0.04

Discussion

The interethnic differences in the frequency of defective *CYP2C19* alleles and/or diminished CYP2C19 catalytic activity are a subject of active research [1, 2]. This study has provided a systematic overview of the population distribution of the CYP2C19 PM phenotype and *CYP2C19* genotypes and alleles in black subjects in different geographical areas. From Table 1, a precise estimate of the PM phenotype frequency based on the overview of the data (Table 1) was 3.9% (95%CI: 2.7%-5.2%) in 922 healthy black subjects, in accordance with actual PM incidence (range:1.0%–7.5%; mean: 4.1%) in each study. As shown in Table 2, the frequency of PM genotypes was 3.7% (95%CI: 2.5%-4.9%) in 966 healthy black individuals. The PM frequencies by both phenotyping and genotyping are in complete agreement. The observed frequencies of the three Mendelian genotypes are in good agreement with the expected (Table 3), indicating that in this study the distribution of the defective or inactive alleles is in Hardy–Weinberg genetic equilibrium and the total sample size is adequate. Thus the combination of genotyping and phenotyping tests gives a 'true value' of the deficient PM frequency of CYP2C19 in black populations ($\sim$4%).

Greater variability in the frequency of CYP2C19 PM phenotype (1.0%-35.4%) was reported among different black populations (Table 1). The apparent discrepancy between the two excluded studies and the remaining seven studies may be due to their inadequate sample size, differences in ethnic origin or genetic background, or the different test drugs used for the phenotyping studies [16–24]. In the first study, a small study of elderly African-Americans ($n = 27$), 18.5% were found to be PMs [16], a finding perhaps influenced by the small sample size. In the second study, 35.4% of healthy Kenyan adults ($n = 65$) were identified as PMs using proguanil as the test drug [17]. Recent evidence has shown that the oxidative cyclization of proguanil (PG) to cycloguanil (CG) is catalysed by both CYP2C19 and CYP3A4 [36, 37] although the latter makes only a minor contribution *in vivo* [37]. The larger interindividual variability in CYP3A *in vivo* [38] and hence variability in its contribution to CG formation may mask a clear distinction between the ratios of PG to CG (PG/CG ratio) of the EMs and PMs, resulting in the frequency distribution pattern of the PG/CG ratio being highly skewed and continuous rather than bimodal in Caucasian [39] or black African [17] populations, and further making it difficult to assign an antimode. Also, the secondary parallel metabolic pathway of PG produces 4-chloro-

Table 4 The distribution frequency of the *CYP2C19* alleles in various populations of African ancestry.

	n	wt (*1)	m1 (*2)	m2 (*3)	*Reference*
African-American	152	123 (80.9)	29 (19.1)	0 (0.0)	18
	200	168 (84.0)	32 (16.0)	0 (0.0)	19
	216	162 (75.0)	54 (25.0)	0 (0.0)	29
	466	388 (83.3)	77 (16.5)	1 (0.2)	30
Sum (frequency)	1034	841 (81.3)	192 (18.6)	1 (0.1)	
Ethiopian	228	193 (84.6)	31 (13.6)	4 (1.8)	20
Tanzanian	502	409 (81.5)	90 (17.9)	3 (0.0)	24
Zimbabwean	168	146 (86.9)	22 (13.1)	0 (0.0)	21
Sum (frequency)	898	748 (83.3)[a]	143 (15.9)[b]	7 (0.8)[c]	
	Sum	Sum (%)	Sum (%)	Sum (%)	
	1932	1589 (82.3)	335 (17.3)	8 (0.4)	

Data are *n* (%). [a]$P=0.28$; [b]$P=0.13$; [c]$P=0.03$, black Africans *vs* African-Americans, Fisher's exact test.

phenylbiguanide (4-CPBG) under of the control of CYP2C19 [40]. In theory, the ratio of PG to both metabolites (CG + 4-CPBG), but not the PG/CG ratio, should reflect the true status of CYP2C19 activity in any individual. However, the two CYP2C19-mediated metabolites (CG and 4-CPBG) may not be the terminal metabolites, and hence variability in their subsequent metabolism will also affect the ratios, PG/CG, or PG/(CG + 4-CPBG).

Potential widespread use of antimalarial drug(s) (e.g. chloroquine, and/or proguanil) may have occurred in the black African subjects who reside in a malaria-endemic area. Although chloroquine is not likely to interact with drug metabolism catalysed by CYP2C19 [41], the use of proguanil as a malaria chemoprophylactic drug might contribute to the relatively higher frequency of the CYP2C19 PM phenotype in black Africans in contrast to the African-Americans who are not exposed to antimalarials (5.1% *vs* 1.4%; $P=0.0056$) (Table 1). The genotyping studies provide a direct marker for CYP2C19 status, and showed that the overall frequency of the CYP2C19 PM phenotype in black Africans, while not different from their genotypic frequency (phenotype *vs* genotype; 5.1% *vs* 3.8%, $P=0.21$), was much more similar to that in African-Americans (*CYP2C19* PM genotype: 3.8% *vs* 3.7%; $P=0.93$) as were the three major genotypes, *wt/wt*, *wt/m1*, and *m1/m1* (all $P>0.10$) (Table 2).

Black populations are evolutionarily distinct from Asian and Caucasian populations [2]. The ancestors of African-Americans originated in Africa but significant genetic admixture has subsequently occurred. Furthermore, within Africa genetic and cultural diversity is substantial, and may thus affect the distribution of CYP2C19 polymorphic drug oxidation in these populations. As shown in Table 1–3, there is some racial microheterogeneity in CYP2C19 activity between African-American and black African populations. Historically, it was from west Africa, particularly the coastal regions, that Africans were transported to North and South America as slaves. Thus the origin of many African-Americans is thought to have been west Africa. The African populations studied in Africa have been from east and central Africa. In fact, there were some distinct differences among the African populations living in ethnically diverse regions because of evolution in isolation and separation by race. The interaction with different genetic and environmental factors may result in the microheterogeneity of phenotypes and genotypes of CYP2C19 among the populations of African ancestry. In addition, it is possible that the estimated 20%–25% admixture of Caucasian alleles in the African-Americans' gene pool [42] has diluted some alleles (e.g. *m2*) in black Americans.

The antimalarial agent proguanil is widely used in Africa because of endemic malaria. This has potential clinical implications for black PMs in endemic malarial areas. Proguanil is an inactive pro-drug that requires biotransformation to its therapeutically active metabolite cycloguanil, which is predominantly catalysed by CYP2C19 and to a minor extent by CYP3A4 [36, 37]. Therefore, decreased activation of proguanil to cycloguanil in CYP2C19 PMs may result in failure of malaria chemoprophylaxis. However, the low prevalence of the PM trait (~4%) in black Africans suggests that this will not be a major problem. The results from this study show that the relative proportions of the heterozygotes and homozygotes in the EM group in blacks was 29% (271 of 930) and 71% (659 of 930), respectively, which are very close to those reported in white EMs [26] but different from those in Chinese subjects [26]. Based on the relevant evidence from omeprazole clinical trials [43, 44], it is expected that gene dosage of *CYP2C19* will affect the antimalarial action of proguanil when it is used in patients with malaria.

In summary, the present study has defined the prevalence of the genetic deficiency of CYP2C19 at 4%

in the black populations of African ancestry. This estimate has utility in designing large scale epidemiological investigations and clinical trials in the population.

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