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Cytochrome 1A1 and *1B1* gene diversity in the Zanzibar islands

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Abstract Amodiaquine (AQ) is a 4-aminoquinoline widely used in the treatment of malaria as part of the artemisinin combination therapy (ACT). AQ is metabolised towards its main metabolite desethylamodiaquine mainly by cytochrome P450 2C8 (CYP2C8). CYP1A1 and CYP1B1 play a minor role in the metabolism but they seem to be significantly involved in the formation of the short-lived quinine-imine. To complete the genetic variation picture of the main genes involved in AQ metabolism in the Zanzibar population, previously characterised for CYP2C8, we analysed in this study CYP1A1 and CYP1B1 main genetic polymorphisms. The results obtained show a low frequency of the *CYP1A1*2B/C* allele (2.4%) and a high frequency of *CYP1B1*6* (approximately 42%) followed by *CYP1B1*2* (approximately 27%) in Zanzibar islands. Genotype data for CYP1A1 and CYP1B1 show a low incidence of fast metabolisers, revealing a relatively safe genetic background in Zanzibar's population regarding the appearance of adverse effects.

keywords amodiaquine, CYP1A1, CYP1B1, African population, pharmacogenetic

Introduction

Amodiaquine (AQ), a 4-aminoquinoline, is widely used in African national malaria control programmes mainly by means of its combination with artesunate (AS-AQ, 4 + 10 mg/kg/day, respectively). AS-AQ is a pivotal artemsinin combination therapy (ACT) in the management of uncomplicated malaria, available as a fixed combination (Cuarsucam®, Sanofi-Aventis, Paris, France), with more than 20 million treatments formally documented in the continent in the last 5 years (WHO 2010).

Amodiaquine is mainly metabolised by the cytochrome P450 isoform 2C8 (CYP2C8) towards its active metabolite desethylamodiaquine (DEAQ) (Li *et al.* 2002), with a secondary participation of the CYP1A1 and CYP1B1 isoforms (Johansson *et al.* 2009). AQ-linked severe adverse side events under prophylaxis regimens are relatively rare (approximately 1:2000), but serious. These are mainly associated with netropenia effects, although severe liver failure has also been described (Jewell *et al.* 1995; Tingle *et al.* 1995; Naisbitt *et al.* 1997). These events are believed to appear from the generation of highly reactive, short-lived quinine-imine (QI) species from the metabolism of both AQ and DEAQ, albeit to a significantly lesser extent in the latter. Although these events are relatively rare – and involving generally higher doses – mild effects are frequently seen in the context of the management of uncomplicated forms of the disease, particularly gastrointestinal effects (reviewed in Gil 2012). Such events (abdominal pain) have been significantly associated with the presence of at least one low metaboliser *CYP2C8*2* allele in an AQ monotherapy trial performed in Burkina Faso (Parikh *et al.* 2007).

Recently, Johansson *et al.* (2009) provided strong evidence for a critical role of CYP1A1 and CYP1B1 in the formation of the toxic QI. The extra-hepatic localisation of the two enzymes could hence explain the occurrence of neutropenia because of the transformation of AQ and DEAQ to QI in, for example the blood, the location of the majority of serious side effects. Also, asymptomatic neutropenia is a frequent occurrence in AQ-based therapies, with unknown long-term consequences in the patient. This raises the possibility that CYP1A1 and CYP1B1 fast metabolisers are more prone to AQ therapy side effects.

AS-AQ was introduced in the islands of Zanzibar in 2005 (Bhattarai *et al.* 2007). In this context, we have previously analysed the main CYP2C8 polymorphism in

a cohort of malaria patients in these islands (Cavaco *et al.* 2005), having identified a non-negligible frequency of *CYP2C8* low activity alleles (3.6%) potentially associated with an increased risk of occurrence of side effects. Owing to the potential importance of CYP1A1 and CYP1B1 in the formation of the QI, we decided to re-visit the previous study and extend it with a retrospective analysis of the main mutations in these genes.

Methods

In *CYP1A1,* we analysed the pivotal I462V SNP (rs1049843, *CYP1A1*2B* and **2C*) associated with more active enzymes (increased V_{max}) (Cosma *et al.* 1993). In *CYP1B1*, we analysed the variability for eight SNPs [142 C>G (rs10012), 182 G>A (rs28936700), 203 A>G (rs9282670), 241 T>A (rs9282671), 355 G>T (rs1056827), 4326 C>G (rs1056836), 4360 C>G (rs4986888) and 4390 A>G (rs1800440)] defining the alleles *CYP1B1*1*, **2*, **3*, **4*, **5*, **6*, **7* and **12* (http://www.cypalleles.ki.se/cyp1b1.htm). *In vitro* data showed an altered enzyme activity for the *CYP1B1*3*, **5*, **6* and **7* alleles compared to *CYP1B1*1* (Aklillu *et al.* 2002; Helmig *et al.* 2009).

The analysed group of malaria patients was a random selection from the previously analysed sample for the *CYP2C8* polymorphism (Cavaco *et al.* 2005), comprising 103 unrelated patients (average age: 2.1 years, SD = 1.2 years, 60 female and 43 male) from the Zanzibar Islands (Unguja = 44; Pemba = 59). These studies were ethically cleared by the Zanzibar Medical Research Ethical Committee (ZAMREC) and the ethics committee of the Karolinska Institutet.

Peripheral blood samples were collected upon written informed consent in the local language (Swahili) from the children's responsible guardian. DNA was extracted from the preserved blood samples using the QIAmp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). The analysis of the *CYP1A1* A2455G (I462V) and the *CYP1B1* C4326G (L432V), C4360G (A443G) and A4390G (N453S) were performed through PCR-RFLP methods. For the determination of the *CYP1B1* 142 C>G, 182 G>A, 203 A>G, 241 T>A and 355 G>T SNPs, direct PCR amplicon sequencing was used (Macrogen, Seoul, Korea). DNA sequence analysis was performed with the Chromas© software (McCarthy, Qld, Australia). Details of primers and methods can be obtained with the authors.

Results

The *CYP1A1*2B/C* alleles are present in the Zanzibar population at a low combined allelic frequency of 2.4% (5/206), having been only found in heterozygote form (five subjects). This confirms the low frequency of this variant in African native populations previously described Tanzania with 1.3% and the non-detection in South African Venda and Zimbabweans (Dandara *et al.* 2002). In Nigeria, West Africa, this SNP was as well described as rare (Okobia *et al.* 2005). The presence of this SNP in mainland Tanzanians and in the Zanzibari population, but not in the other countries, might be related to the fact that in this Eastern African coastal regions gene mixing may have existed between the local Black population and the Orientals, where 2455 A>G was described to be more frequent (approximately 20%) (Dandara *et al.* 2002; Chowbay *et al.* 2005).

CYP1B1 was analysed for eight SNPs. Three were determined by PCR-RFLP (4326 C>G, 4360 C>G and 4390 A>G) all located in the region coding the catalytically important haem-binding domain of the enzyme (Bailey *et al.* 1998). From the five SNPs analysed through sequencing, 142 C>G and 355 G>T were described as not altering the catalytic properties and protein stability of CYP1B1 (McLellan *et al.* 2000); the 182 G>A, 203 A>G and 241 T>A SNPs have not been yet characterised. Owing to results in the sequencing that did not allow the unequivocal identification of the genotype (multiple SNP heterozygoty in three of the SNPs), some subjects had to be excluded, and the group shrank to 88 individuals.

The above-mentioned SNPs define the alleles *CYP1B1*1*, **2*, **3*, **4*, **5*, **6*, **7* and **12*, all present in the studied population (Table 1), except for the *CYP1B1*5* and *CYP1B1*12*. The 203 A>G and 241 T>A SNPs, not part of a haplotype (http://www.cypalleles.ki.se/cyp1b1.htm), were not found in our study. From the different possible genotypes, *CYP1B1*2/*6* and *CYP1B1*6/*6* are the most frequent (Table 1), resulting in a high frequency of *CYP1B1*2* and **6*, which together comprise about two-thirds of all the detected alleles.

There is very limited information on the *CYP1B1* in African native populations, a study in an Ethiopian population being until now the only available report (Aklillu *et al.* 2002). Although both being of East African origin, we observed significant allele prevalence differences between the two populations. The most frequent alleles in the Zanzibari islands are *CYP1B1*6* (41.5%) and *CYP1B1*2* (27.3%), while the highest frequency allele observed among Ethiopians as *CYP1B1*3* (39.0%), followed by *CYP1B1*2* with 36.7%. This study adds to previous reports on the description of the Zanzibar population (Cavaco *et al.* 2005; Ferreira *et al.* 2008), making this the pharmacogenetically most well-studied native African population.

Table 1 Genotype and allelic frequencies of *CYP1B1*

CYP1B1 genotypes (n = 88)			Allele frequencies (n = 176)		
Genotypes	*N*	Frequency (95% CI)	Allele (SNPs)	*N*	Frequency (95% CI)
**1/*1*	5	0.057 (0.019–0.128)	*CYP1B1*1* (*none*)	18	0.102 (0.058–0.147)
**1/*2*	4	0.046 (0.13–0.112)	*CYP1B1*2* (*142C>G; 355G>T*)	48	0.273 (0.207–0.339)
**1/*3*	4	0.046 (0.13–0.112)	*CYP1B1*3* (*4326C>G*)	28	0.159 (0.105–0.213)
**2/*2*	10	0.114 (0.056–0.199)	*CYP1B1*4* (*4390A>G*)	2	0.011 (0.001–0.041)
**2/*4*	2	0.023 (0.003–0.080)	*CYP1B1*6* (*142C>G*; *355G>T*; *4326C>G*)	73	0.415 (0.342–0.488)
**2/*6*	21	0.239 (0.154–0.341)	*CYP1B1*7* (*142C>G*; *355G>T*; *4326C>G*; *4360C>G*)	7	0.040 (0.016–0.080)
**2/*7*	1	0.011 (0.0003–0.062)			
**3/*3*	9	0.102 (0.048–0.185)			
**3/*6*	5	0.057 (0.019–0.128)			
**3/*7*	1	0.011 (0.0003–0.062)			
**6/*6*	23	0.261 (0.173–0.366)			
**6/*7*	1	0.011 (0.0003–0.062)			
**7/*7*	2	0.023 (0.003–0.080)			

Discussion

The data obtained in the Zanzibari population show that *CYP1A1*2B/C* allele, associated with a higher enzyme activity, is present in such low frequencies that will not play an important role in the appearance of adverse events. For *CYP1B1*, we found high frequencies for some of the alleles studied (**6*, **2* and **3*). From these, *CYP1B1*6* has been associated with lower enzyme activity (lower V_{max}) and for **2* and **3*, enzyme activity was comparable to **1* (Aklillu *et al.* 2002). The genotypes observed for *CYP1B1* may lead to the formation of less QI metabolite and then to lower risk of adverse events.

Although serious adverse events are rare in the treatment for uncomplicated malaria, mild events are common. In the particular case of AQ-based treatments, these have been consistently reported (Parikh *et al.* 2007; Ndiaye *et al.* 2011). From the present knowledge, the most likely genetic candidates modulating the risk for these events are the polymorphisms at *CYP1A1* and *CYP1B1*, particularly when associated with CYP2C8 variability. It is expected that a CYP2C8 low metaboliser will allow a higher exposure to AQ, permitting more chances for CYP1A1/CYP1B1 to metabolise a fraction of the drug towards the locally reactive QI.

Considering the characteristics of the Zanzibari population, with notable – for African standards – frequencies of the very low active *CYP2C8*3* and **4* alleles, and a massive exposure to AS-AQ, one would expect the emergence of reports concerning serious AQ-related adverse events. We speculate that the continuous success and safety of this drug in the islands might be partially related with the fact that the frequency of CYP1A1/CYP1B1 fast metabolisers is low, hence lowering the risk of adverse events. African populations are genetically very diverse (Lambert & Tishkoff 2009). Our present data should not be considered as referential for other regions in the continent, a view supported by documented differences between Africa countries regarding the frequency of *CYP1A1* alleles (reviewed in Gil 2012). It is likely that there are presently populations carrying significant frequencies of *CYP1A1* and *1B1* fast alleles metaboliser alleles being targeted with AS-AQ treatments, as this is one of the most adopted ACTs in Africa. Pharmacogenetic studies in such regions, focusing on *CYP2C8*, *CYP1A1* and *CYP1B1* polymorphisms, seem warranted.

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