Clin Chem Lab Med 2003; 41(4):606–609 © 2003 by Walter de Gruyter · Berlin · New York

*CYP3A4*1B* and *NAT2*14* Alleles in a Native African Population

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Single nucleotide polymorphisms were examined in the cytochrome 450 3A4 (*CYP3A4*) and *N*-acetyltransferase 2 (*NAT2*) genes, which code for major mediators of the metabolism of a wide variety of therapeutic drugs, as well as xenobiotics. We determined, in a population from Guinea-Bissau, the frequencies of *CYP3A4* and *NAT2* variants expected to be prevalent among Africans, due to the high frequency previously observed in African Americans. The observed frequencies were 72% for *CYP3A4*1B* and 19.2% for the *NAT2* 191 G>A variant. The high frequency found for these potentially function-altering polymorphisms suggests the possibility of impaired metabolism through CYP3A4 and NAT2 in this population. Strikingly, the frequency observed for the *NAT2* 191 G>A single nucleotide polymorphism (SNP), associated with the slow acetylator phenotype, was significantly higher than found in other African populations, suggesting the existence of a west to east gradient across Sub-Saharan Africa. The prevalence of these variants may be relevant with regard to therapeutic efficacy in African populations for it may potentially affect drug clearance and consequently, increase the incidence of side effects and drug-drug interactions. Clin Chem Lab Med 2003; 41(4):606–609

Key words: Polymorphism; CYP3A4; N-acetylation; NAT2; Africans.

Abbreviations: CYP, cytochrome P450; NAT2, *N*-acetyltransferase 2; SNP, single nucleotide polymorphism.

Introduction

Pharmacogenetic variation in drug metabolizing enzymes is responsible for differences in individual therapeutic response, susceptibility for disease, and incidence of side effects or drug interactions. Cytochrome P450 3A4 (CYP3A4) is the main phase I enzyme in the human liver, and is responsible for the biotransformation of at least 50% of the currently available therapeutic drugs, such as antiarrhythmics, antiretroviral, and antimalarial agents. Although a high degree of inter-individual variation in CYP3A4 activity has been known for some time, the underlying genetic variability has been unveiled in the last 2 years, with approximately 30 single nucleotide polymorphisms (SNPs) known presently (1).

*CYP3A4*1B* was the first variant described, consisting of an A→G transition in the 5′-flanking region (–392 bp) (2). This change was found in both black and white subjects, with a higher frequency in African Americans (3). Although the functional significance of this SNP is still controversial, it has been proposed to affect the enzyme concentration, to be associated with advanced stage prostate cancer (2), and to be protective for secondary cancer caused by leukemia chemotherapy (4).

The hepatic *N*-acetyltransferase enzyme encoded by the *N*-acetyltransferase 2 (*NAT2*) gene locus is a phase II enzyme, which plays a primary role in the activation and/or inactivation of a diverse number of aromatic amine (*e.g.*, procainamide) and hydrazine drugs (*e.g.*, isoniazid) used in therapeutics. The reference *NAT2*4* and 28 *NAT2* allelic variants have been identified in human populations (5) containing a combination of 12 different SNPs present throughout the *NAT2* coding region. The mutation 191 G>A, previously reported as "African-specific", defines the *NAT2*14* alleles and is known to have a drastic effect on NAT2 enzymatic activity, being associated with the slow acetylator phenotype (6).

The genetic basis underlying the human drug metabolizing polymorphisms has been extensively studied in several populations but native Africans are poorly documented. The knowledge of the pharmacogenetic status is of particular relevance in these populations with a heavy burden of infectious diseases, since it may affect therapeutic outcome and incidence of side effects. We describe here the characterization of a native population from Guinea-Bissau, regarding *CYP3A4*1B* and *NAT2*14* polymorphisms.

Materials and Methods

Blood samples were obtained from individuals of the Balanta ethnic group from Guinea-Bissau (linguistic lineage Niger-Congo, Atlantic Congo, Atlantic, Northern, Bak, Balant-Ganja, Balanta-Kentohe), upon informed consent. This study was given ethical clearance by the Ministry of Health, Guinea-Bissau.

Genomic DNA was extracted from peripheral blood, blotted and dried on filter paper (MN 818-filter paper, Macherey-Nagel, Düren, Germany) using the Chelex (Bio-Rad, Hercules, CA, USA) method (7), and amplified (200–700 ng) by the polymerase chain reaction (PCR) in a Mastercycler personal 5332 thermocycler (Eppendorf, Hamburg, Germany).

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A sample (n = 50) consisting of 23 men and 27 women was analyzed for the presence of *CYP3A4*1B* using as primers: forward 5′ -AAT GAG GAC AGC CAT AGA GAC AAG GcC A-3′, reverse 5′ -CAA TCA ATG TTA CTG GGG AGT CCA AGG G- 3′, where lower case indicates a mismatched nucleotide introduced to create a *Bst*NI restriction site. Thirty cycles of amplification were performed including 45 s denaturation at 94 °C, 45 s annealing at 59 °C, and 45 s extension at 72 °C. The PCR product was digested with *Bst*NI (Stratagene, La Jolla, USA) according to the manufacturer's recommendations.

For *NAT2* 191 G>A we analyzed a sample (n = 125) consisting of 66 men and 59 women, through a semi-nested PCR, with a first reaction using primers: forward 5′-GAT CAC ATT GTA AGA AGA AAC CG-3′ and reverse 5′-GGA TGA AAG TAT TTG ATG TTT AGG-3′, and the second reaction with primers: forward 5′-GAT CAC ATT GTA AGA AGA AAC CG-3′ and reverse 5′-TTG GGT GAT ACA TAC ACA AGG G-3′. Thirty-five cycles of amplification were performed including 30 s denaturation at 92 °C, 45 s annealing at 48 °C, and 1 min extension at 72 °C. The PCR product was digested with *Msp* I according to the manufacturer's recommendations (Promega Corp., Madison, WI, USA).

Restriction fragments were size-separated by 8% polyacrylamide gel electrophoresis/Tris-borate-EDTA buffer (TBE), stained with ethidium bromide and visualized by UV transillumination.

Allelic frequencies and confidence intervals were assessed using the CIA and Microstat software (8).

Results and Discussion

We have identified, in a rural population from Guinea-Bissau, 18 heterozygotes and 27 homozygotes for the *CYP3A4*1B* allele, corresponding to an allelic frequency of 72% (Table 1). This allele was first reported to be highly prevalent in African Americans, with a frequency ranging from 48% to 55% (3, 9, 10), significantly higher than the frequency found in Caucasians, which is up to 10% (2, 11).

A recent report suggests that population stratification is a potential problem for disease association studies in African American populations, with results that are strikingly different from native populations (12). However, few studies have addressed the pharmacogenetic status of *CYP3A4* in native Africans. While this work was in progress, reports were published showing *CYP3A4*1B* allelic frequencies of 87% in Nigerians (12) and 78% in Senegalese (13), while two independent studies have reported frequencies of 69% and 81% in Ghanaians (14, 13). Surprisingly, this allele was described as absent in Africans from North Sahara (11). The frequency of the *CYP3A4*1B* allele in the Balanta from Guinea Bissau, observed in the present study, does not differ significantly from those determined in other African populations, with the exception of Nigerians ($p = 0.003$).

With regard to the N-acetylation polymorphism, we determined the frequencies of the *NAT2* gene variant corresponding to the 191 G>A SNP, reported to be overrepresented in African-Americans (15). In the present study, we observed a frequency of 19.2% (Table 1), which is distinct from that found in previous studies performed on native African populations. Strikingly, the 191 G>A frequency found in the Balanta from Guinea Bissau is significantly higher than in Gabonese (8.6%, $p = 0.017$), non-caste Dogons (5%, $p = 0.0002$), black South Africans (8.4%, $p = 0.0011$) and Sudanese (2.9%, $p < 0.0001$) (16–18). Moreover, 191 G>A has been reported as absent in individuals from Somalia (18).

In the present study, we identified *CYP3A4* and *NAT2* variant alleles in a rural population from Guinea-Bissau. The high frequency observed for the *CYP3A4*1B* SNP is in keeping with recent reports on Nigerians, Ghanaians, and Senegalese (12–14). Being the main *CYP3A4* gene variant in native Africans, it might account for the major interethnic differences in the pharmacokinetics of some CYP3A4 substrates (19) and in the incidence of prostate cancer (20). Nevertheless, its functional relevance remains controversial. Although some data do support altered function, there is no consensus (9, 21–23). Initially, the effect of this mutation on transcription was believed to be a decrease, based on clinical presentation of prostate cancer (2), risk of drug-induced leukemia (4), and a lower clearance of midazolam (24). Later experiments showed a modest

Table 1 *CYP3A4*1B* and *NAT2*14* allelic frequencies in African populations.

	Population	Number of alleles	Allelic frequency*	Reference
	Guinea-Bissau	100	0.72 (0.62–0.81)	This study
	Ghana	200	0.69 (0.62–0.75)	14
*CYP3A4*1B*	Nigeria	164	0.87 (0.80–0.91)	12
–392 A>G	Ghana	236	0.81 (0.75–0.85)	13
	Senegal	346	0.78 (0.74–0.82)	13
	North Sahara	14	0	10
	Guinea-Bissau	250	0.192 (0.143–0.241)	This study
	Gabon	104	0.086 (0.044–0.158)	16
NAT2	Mali	130	0.05 (0.024–0.109)	16
191 G>A	Sudan	272	0.029 (0.013–0.058)	18
	Somalia	100	0	18
	South Africa	202	0.084 (0.053–0.131)	17

* Allelic frequencies and confidence intervals were assessed using the program CIA (Gardner and Altman, 1989).

increase in luciferase activity from the *CYP3A4* variant promoter when compared to the wild-type promoter (22). Recently the influence of *CYP3A4*1B* on prostate cancer risk was confirmed in a study that showed that the frequency of this SNP is higher among patients with benign prostate hyperplasia who subsequently develop prostate cancer than among benign prostate hyperplasia controls (25). This association was recently proposed to reflect linkage with a *CYP3A5* allele potentially leading to increased expression (26).

Further studies are needed to establish the correlation between the CYP3A4 phenotype and *CYP3A4*1B* allele, in view of its high frequency among Africans and its potential role in cancer susceptibility and therapeutic efficacy of CYP3A4 substrates.

The *NAT2* 191 G>A mutation, associated with slow acetylation, was suggested to be of African origin by Bell and co-workers, who found this mutation in African Americans (15). In 1996, a study among the Gabonese and the Dogons of Mali confirmed the African origin of 191 G>A (16). The fact that this SNP is absent in Somalis and present in Sudanese at a low frequency led to the suggestion that the incidence of this mutation would decrease from west to east across Sub-Saharan Africa (18). The Balanta population belongs to the Niger-Congo linguistic cluster, which also includes most of the native populations previously characterized – the Mali Dogons (Volta-Congo), Sudanese, Gabonese, and South African Venda (narrow Bantu) (27). The absence of the 191 G>A SNP in Somalis probably reflects their Hamitic origin (18). The higher incidence observed in our study is compatible with the fact that Guinea-Bissau occupies the most western location of the African countries studied so far, supporting the idea of a western African origin of the 191 G>A mutation.

The 191 G>A substitution has been described as a major function-altering change, giving rise to a NAT2 enzyme showing reduced catalytic activity (6). This may lead to interethnic differences in therapeutic efficiency or incidence of side effects, particularly in the treatment of infectious diseases.

Importantly, the studied population was shown to have a high prevalence of tuberculosis, in strong association with HIV-2 infection (28). Since anti-tuberculosis drugs, such as isoniazid and rifampicin, are NAT2 substrates (29), and most anti-retroviral drugs are CYP3A4 substrates, pharmacogenetic variations in the metabolism of these drugs may lead to important drug-drug interactions in situations of combined chemotherapy. Isoniazid was recently shown to inhibit major drug detoxification enzymes, namely CYP3A4 (30). As such, slow acetylators would be more prone to suffer side effects from HIV therapeutic agents. On the other hand, since rifampicin is a known CYP3A4 inducer, a decrease in rifampicin clearance in slow acetylators may lead to reduced levels of co-administered anti-HIV drugs, and therefore, to therapeutic failure (31).

Although phenotyping studies are necessary to assess the role for ethnicity and geographical variation in drug disposition in single or combined chemotherapy, genotyping of *CYP3A4* and *NAT2* variant alleles is a promising tool in the tailoring of therapeutic strategies for each population in African regions with a heavy burden of infectious diseases.

Acknowledgements

We are indebted to Abílio Antunes (Clinical and Tropical Diseases Unit, IHMT/UNL, Portugal) and Suzana David (Mycobacteria Unit, IHMT/UNL, Portugal) for sharing the Balanta samples. This work was partially supported by Fundação para a Ciência e Tecnologia, Portugal (Praxis Grant SFRH/BD/8887/2002 to IC, Praxis Grant SFRH/BPD/5590/2001 to JPG and Project POPCTI/ESP/39580/01).

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Received 3 December 2002, revised 12 February 2003, accepted 21 February 2003

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