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 therapeu-
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 AGC C-3', reverse 5'-CA A TCA ATG TTA CGG AGT CCA AGG G-3', where lower case indicates a mismatched nucleotide introduced to create a BstM 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 BstM (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'-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>
<thead>
<tr>
<th>Population</th>
<th>Number of alleles</th>
<th>Allelic frequency*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea-Bissau</td>
<td>100</td>
<td>0.72 (0.62–0.81)</td>
<td>This study</td>
</tr>
<tr>
<td>Ghana</td>
<td>200</td>
<td>0.69 (0.62–0.75)</td>
<td>14</td>
</tr>
<tr>
<td>Nigeria</td>
<td>164</td>
<td>0.87 (0.80–0.91)</td>
<td>12</td>
</tr>
<tr>
<td>Ghana</td>
<td>236</td>
<td>0.81 (0.75–0.85)</td>
<td>13</td>
</tr>
<tr>
<td>Senegal</td>
<td>346</td>
<td>0.78 (0.74–0.82)</td>
<td>13</td>
</tr>
<tr>
<td>North Sahara</td>
<td>14</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Guinea-Bissau</td>
<td>250</td>
<td>0.192 (0.143–0.241)</td>
<td>This study</td>
</tr>
<tr>
<td>Gabon</td>
<td>104</td>
<td>0.086 (0.044–0.158)</td>
<td>16</td>
</tr>
<tr>
<td>NAT2 191 G&gt;A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mali</td>
<td>130</td>
<td>0.05 (0.024–0.109)</td>
<td>16</td>
</tr>
<tr>
<td>Sudan</td>
<td>272</td>
<td>0.029 (0.013–0.058)</td>
<td>18</td>
</tr>
<tr>
<td>Somalia</td>
<td>100</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>South Africa</td>
<td>202</td>
<td>0.084 (0.053–0.131)</td>
<td>17</td>
</tr>
</tbody>
</table>

* 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 acetyulators 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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