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Short Communication

(TTTA)n polymorphism of *CYP19* (aromatase gene) in Euro- and Afro-Brazilians

Kátia Kvitko, Janice Corrêa de Barros Nunes and Mara Helena Hutz

Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Departamento de Genética, Porto Alegre, RS, Brazil.

Abstract

We investigated the polymorphic tetranucleotide repeat $(TTTA)_n$ located in the fourth intron of the *CYP19* gene in two Brazilian populations. The frequencies of the five common alleles (A) in Euro- and Afro-Brazilians were, respectively: seven repeats (*A5*), 0.586 and 0.80; eight repeats (*A4*), 0.092 and 0.06; nine repeats (*A3*), 0.014 and 0.01; eleven repeats (*A2*), 0.284 and 0.09; twelve repeats (*A1*), 0.021 and 0.04. In addition, one Euro-Brazilian individual had a rare allele with 13 repeats. The allelic frequencies in Euro- and Afro-Brazilians differed statistically ($p < 10^{-3}$). The two samples were found to be in Hardy-Weinberg equilibrium (p = 0,828 and p = 0,995).

Key words: *CYP19*, gene polymorphism.

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The *CYP19* gene encodes an aromatase protein, which has a role in the conversion of C19 androgenic steroids into estrogens. A polymorphic tetranucleotide repeat $(TTTA)_n$ is located in the fourth intron of *CYP19*, some alleles apparently determining a higher susceptibility to breast cancer (Kristensen *et al.*, 1998, 2000). A few studies have demonstrated ethnic differences related to this polymorphism (Probst-Hensch *et al.*, 1999; Gu *et al.*, 2001).

The aim of the present study was to investigate if this polymorphism differed between Euro-derived and Afro-derived Brazilian populations.

The European-derived sample consisted of 146 unrelated healthy individuals referred for paternity testing. The 124 Afro-Brazilians were ascertained at the central laboratory of a general public hospital to which they went for routine blood examination. The University Ethical Committee approved the investigation.

Genomic DNA was extracted from total blood by a salting out method (Miller *et al.*, 1988). The region encompassing the fourth intron of *CYP19* was amplified by the polymerase chain reaction (PCR), primers and the temperature profile of each cycle being as previously described (Kristensen *et al.*, 1998). Genotype patterns were determined after 10.5% polyacrilamide gel electrophoresis and ethidium bromide staining. Allelic frequencies were estimated by gene counting. Agreement of genotypic frequencies with Hardy-Weinberg expectations was evaluated by the χ^2 test (Roff and Bentzen, 1989) and Fisher's exact test. Comparisons of allelic frequencies were performed using the PEPI software program.

Allele and genotype frequencies are shown in Tables 1 and 2. The two samples were in Hardy-Weinberg equilibrium (p = 0,828, Euro-Brazilians; p = 0,995, Afro-Brazilians). The Euro-Brazilian allele prevalence was quite similar to those previously detected in a healthy Norwegian population (Kristensen *et al.*, 1998), in a study of Belgians (Pottelberg *et al.,* 2003), and in a British population (Baxter *et al*., 2001). However, it differed from those found in an Euro-American sample ($p < 10^{-3}$) (Siegelman-Danieli and Buetow, 1999), in Japanese women ($p < 10^{-3}$) (Miyoshi *et al*., 2000), in Russians ($p < 10^{-4}$) (Suspitsen *et al.,* 2002), in a British population (p = 0,006) (Healey *et al*., (2000) and in a Latin-American sample (p = 0,0029) (Probst-Hensch *et al.,* 1999). In addition to the five common alleles with seven (*A5*), eight (*A4*), nine (*A3*), eleven (*A2*) and twelve (*A1*) TTTA-repeats, we observed in one European-derived individual a rare allele with 13 repeats classified as *A1V*. The allele frequencies of the TTTA repeat differed statistically between Euro- and Afro-Brazilians, due to *A2* and *A5* allele distributions in these populations ($p < 10^{-3}$ for both alleles). Two previously studied Afro-derived populations also showed allelic differences when compared to European-derived populations (Probst-Hensch *et al.*, 1999; Gu *et al.*, 2001). The allelic frequencies reported by Probst-Hensch *et al.* (1999) in an Afro-American sample from California (USA) were similar to the frequencies detected in the present study.

Send correspondence to Kátia Kvitko. UFRGS, Instituto de Biociências, Departamento de Genética, Caixa Postal 15053, 91501-970 Porto Alegre, RS, Brazil. E-mail: katia.kvitko@ufrgs.br.

Table 1 - *CYP19* allele distribution in Afro- and Euro-Brazilians[1].

Alleles	Afro-Brazilians	Euro-Brazilians
A1V	-	0.003
A1	0.04	0.021
A2	0.09	0.284
A3	0.01	0.014
A4	0.06	0.092
A5	0.80	0.586

[1]$p < 10^{-4}$.

Table 2 - *CYP19* genotype distribution in Afro- and Euro-Brazilians[1].

Genotype	Afro-Brazilians	Euro-Brazilians
A1V-2	0	1
A1-A1	0	0
A1-A2	0	0
A1-A3	0	0
A1-A4	1	1
A1-A5	9	5
A2-A2	1	17
A2-A3	0	1
A2-A4	0	11
A2-A5	21	36
A3-A3	0	0
A3-A4	1	1
A3-A5	1	2
A4-A4	0	1
A4-A5	13	12
A5-A5	77	58
Total	124	146

[1]$p < 10^{-3}$.

Previous studies had shown that *CYP19* might be involved in breast cancer susceptibility (Kristensen *et al.*, 1998; 2000; Siegelmann-Danieli and Buetow, 1999; Miyoshi *et al.*, 2000), probably due to its role in the conversion of C19 steroids into estrogens. Population differences in the frequency of the *CYP19* polymorphism as we disclosed here between Afro- and Euro-derived Brazilian populations are crucial in the interpretation of these association studies.

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