



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

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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 ($p < 10^{-4}$) (Suspsitsen *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 A_{1V}. 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.

Table 1 - *CYP19* allele distribution in Afro- and Euro-Brazilians¹.

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

¹p < 10⁻⁴.**Table 2** - *CYP19* genotype distribution in Afro- and Euro-Brazilians¹.

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

¹p < 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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References

- Baxter SW, Choong DYH, Eccles, DM and Campbell IG (2001) Polymorphic variation in *CYP19* and the risk of breast cancer. *Carcinogenesis* 22:347-349.
- Gu CC, Mutch DG and Goodfellow PJ (2001) Association of the aromatase gene (*CYP19*) and endometrial cancer - Testing for Hardy-Weinberg disequilibrium in cancer patients. 50th Annual Meeting ASHG, Philadelphia, USA. *Am J Hum Genet* 68:292.
- Healey CS, Dunning AM, Durocher F, Teare D, Pharoah PDP, Luben RN, Easton DF and Ponder BAJ (2000) Polymorphisms in the human aromatase cytochrome P450 gene (*CYP19*) and breast cancer risk. *Carcinogenesis* 21:189-193.
- Kristensen VN, Andersen TI, Lindblom A, Erikstein B, Magnus P and Brresen-Dale A (1998) A rare *CYP19* (aromatase) variant may increase the risk of breast cancer. *Pharmacogenetics* 8:43-48.
- Kristensen VN, Harada N, Yoshimura N, Haraldsen E, Lonning PE, Erikstein B, Karesen R, Kristensen T and Brresen-Dale A (2000) Genetic variants of *CYP19* (aromatase) and breast cancer risk. *Oncogene* 19:1329-1333.
- Miller SA, Dykes DD and Polesky HF (1988) A simple salting out procedure for extraction DNA from human nucleated cells. *Nucl Acids Res* 16: 215.
- Miyoshi Y, Iwao K, Ikeda N, Egawa, C and Noguchi, S (2000) Breast cancer risk associated with polymorphism in *CYP19* in Japanese women. *Int J Cancer* 89:325-328.
- Probst-Hensch NM, Ingles SA, Diep AT, Haile RW, Stanczyk FZ, Kolonel LN and Henderson BE (1999) Aromatase and breast cancer susceptibility. *Endocr Relat Cancer* 6:165-173.
- Pottelbergh IV, Goemaere S and Kaufman JM. (2003) Bioavailable estradiol and na aromatase gene polymorphism are determinants of bone mineral density changes in men over 70 years of age. *J Clin Endocrinol Metab* 88:3075-3081.
- Roff DA and Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Mol Biol Evol* 16:539-545.
- Siegelmann-Danieli N and Buetow KH (1999) Constitutional genetic variation at the human aromatase gene (*CYP19*) and breast cancer risk. *Brit J Cancer* 79:456-463.
- Suspitsen EN, Grigoriev MY, Togo AV, Kuligina ES, Belogubova, KM, Pozharisski KM, Chagunava OL, Sokolov EP, Theillet C, Berstein LM, Hanson KP and Imyanitov EN (2002) Distint prevalence of the *CYP19* $\Delta 3$ (TTTA)₇ allele in premenopausal versus postmenopausal breast cancer patients, but not in control individuals. *Eur J Cancer* 38:1911-1916.

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