Analysis of the CCR5 gene coding region diversity in five South American populations reveals two new non-synonymous alleles in Amerindians and high CCR5*D32 frequency in Euro-Brazilians

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Abstract

The CC chemokine receptor 5 (CCR5) molecule is an important co-receptor for HIV. The effect of the CCR5*D32 allele in susceptibility to HIV infection and AIDS disease is well known. Other alleles than CCR5*D32 have not been analysed before, neither in Amerindians nor in the majority of the populations all over the world. We investigated the distribution of the CCR5 coding region alleles in South Brazil and noticed a high CCR5*D32 frequency in the Euro-Brazilian population of the Paraná State (9.3%), which is the highest thus far reported for Latin America. The D32 frequency is even higher among the Euro-Brazilian Mennonites (14.2%). This allele is uncommon in Afro-Brazilians (2.0%), rare in the Guarani Amerindians (0.4%) and absent in the Kaingang Amerindians and the Oriental-Brazilians. R223Q is common in the Oriental-Brazilians (7.7%) and R80S in the Afro-Brazilians (5.0%). A29S and L55Q present an impaired response to β-chemokines and occurred in Afro- and Euro-Brazilians with cumulative frequencies of 4.4% and 2.7%, respectively. Two new non-synonymous alleles were found in Amerindians: C323F (g.3729G > T) in Guarani (1.4%) and Y68C (g.2964A > G) in Kaingang (10.3%). The functional characteristics of these alleles should be defined and considered in epidemiological investigations about HIV-1 infection and AIDS incidence in Amerindian populations.

Key words: CCR5, Brazilian, Amerindian, HIV, polymorphism.

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Introduction

The human immunodeficiency virus type 1 (HIV-1) epidemic shows great variation among the different Brazilian regions. A progressive reduction in the number of deaths from acquired immunodeficiency syndrome (AIDS) was observed after the introduction of potent antiretroviral therapy in 1996, but the deceleration of the AIDS epidemic was not homogenous throughout all the Brazilian regions (Brito et al., 2005). The Southeast region has experienced the lowest increase in the AIDS epidemic from 1990 to 1996, contrasting with a steep rise in the North and South regions (Szwarcwald et al., 2000). Since 1996, the incidence rates of AIDS in Brazil as a whole and in the State of São Paulo in particular show a trend towards stability, whereas in the Brazilian Northeast the incidence rates of the disease continue to grow (Brito et al., 2005). The different spreading of the disease is due to multiple variables, including biological, behavioural, demographic and economic/political factors that influence the rate of contact between infected and susceptible individuals, as well as the individual’s infectiousness and susceptibility. Among these factors are genetic variants of host genes that facilitate or hamper viral entry into the cells and modulate immune responses against the infection.

The chemokine (C-C motif) receptor 5 gene (CCR5) comprises three exons. The polypeptide of 352 amino acid
residues is encoded by exon 3 (formerly named exon 4) (Mummidi et al., 1997). CCR5 transduces the signals of several different chemokines in phagocytes and T lymphocytes and serves as an essential co-receptor for the entry of R5-tropic HIV-1 into those cells (Blanpain et al., 2001). This is the viral form that most frequently infects people in Brazil (Ferraro et al., 2001). Therefore, CCR5 alleles that code for proteins poorly or not expressed at the cell surface are strong candidates for protection against the infection and for the delay of AIDS onset. This is the case of the truncated CCR5*D32 allele, and probably also of the Fs299 and R60S alleles (Dean et al., 1996; Shioda et al., 2001; Tama-sauskas et al., 2001). CCR5*D32 was also favourably associated with autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and type 1 diabetes mellitus, but increases the risk for abdominal aortic aneurysm and sarcoidosis (for a review, see Navratilova, 2006).

The interaction between the CCR5 receptor and its ligands can block HIV-1 entry and thus retard disease progression. The A29S and L55Q alleles encode products with a reduced affinity for (C-C motif) chemokines and might be associated with a shorter time interval from HIV infection to AIDS onset (Howard et al., 1999).

During AIDS, the acquisition of mutations in the HIV-1 gp120 envelope glycoprotein gene leads to the switch from primary R5 (CCR5-using) to highly cytopathic X4 (CXCR4-using) HIV-1 variants. According to the somatic hypermutation hypothesis, this switch takes place in the germinal center B cells, due to aberrant somatic hypermutation of the gp-120-coding region of the HIV-1 env gene (Suslov, 2004). This process seems to be more effective in CCR5*D32 heterozygotes, which were found at a 2.5 times higher risk of harbouring X4 HIV-1 variants before the onset of highly active antiretroviral therapy. The presence of X4 variants in the patients seems not to compromise the therapy outcome (Brumme et al., 2005), whereas the presence of a CCR5*D32 allele was found associated with a better response (Accetturi et al., 2000; Guerin et al., 2000).

In order to better understand the diversity of the CCR5 gene and to supply data for studies on the functional effect and epidemiological consequences of the CCR5 variants, we investigated the distribution of CCR5*D32 and other known exon 3 coding region CCR5 alleles in five populations of South Brazil. These alleles and their known functional characteristics are listed in Table 1. We also sequenced part of the coding region of the gene, in order to search for new variants.

Materials and Methods

Samples

One hundred and seventy two Afro-Brazilians, 172 Euro-Brazilians, 18 Oriental-Brazilians, 115 Guarani (89 of which belong to the M’byá sub-group) and 160

| DNA level | SNP (NCBI) database | Protein level | Common nomenclature | Gene transcription | Membrane expression | HIV-1 infection | Response to chemokines |
|-----------|---------------------|--------------|---------------------|-------------------|---------------------|----------------|-----------------------|
| rs2448596 G > T | rs2919319 | p.Ala29Ser | A29S | + | + | + | X |
| rs2448605 T > A | rs1799863 | p.Leu55Gln | L55Q | + | + | + | X |
| rs3494269 G > A | rs3494269 | p.Arg60Ser | R60S | + | + | + | X |
| rs3495330 C > T | rs3495330 | p.Arg223Gln | R223Gln | + | + | + | X |
| rs1800945 C > T | rs1800945 | p.Phe299fs | Fs299 | + | + | + | X |
| rs1800946 G > A | rs1800946 | p.Trp332Phe | W332P | + | + | + | X |
| rs1800947 C > T | rs1800947 | p.Ala335Val | A335V | + | + | + | X |
| rs1800948 A > T | rs1800948 | p.Tyr339Phe | Y339F | + | + | + | X |
| rs1800949 G > A | rs1800949 | silent | - | - | - | - | - |
| rs1801042 C > T | rs1801042 | p.Ala258Thr | A258T | + | + | + | X |
| rs1801043 A > C | rs1801043 | p.Ala335Val | A335V | + | + | + | X |
| rs1801044 G > A | rs1801044 | p.Tyr339Phe | Y339F | + | + | + | X |
| rs1801045 T > A | rs1801045 | p.Arg223Gln | R223Gln | + | + | + | X |
| rs1801046 C > T | rs1801046 | p.Phe299fs | Fs299 | + | + | + | X |
| rs1801047 G > A | rs1801047 | p.Trp332Phe | W332P | + | + | + | X |
| rs1801048 C > T | rs1801048 | p.Ala335Val | A335V | + | + | + | X |
| rs1801049 A > T | rs1801049 | p.Tyr339Phe | Y339F | + | + | + | X |
| rs1801050 G > A | rs1801050 | silent | - | - | - | - | - |
| rs1801051 T > A | rs1801051 | p.Ala258Thr | A258T | + | + | + | X |
| rs1801052 A > C | rs1801052 | p.Ala335Val | A335V | + | + | + | X |
| rs1801053 G > A | rs1801053 | p.Tyr339Phe | Y339F | + | + | + | X |
| rs1801054 T > A | rs1801054 | p.Arg223Gln | R223Gln | + | + | + | X |
| rs1801055 C > T | rs1801055 | p.Phe299fs | Fs299 | + | + | + | X |
| rs1801056 G > A | rs1801056 | p.Trp332Phe | W332P | + | + | + | X |
| rs1801057 C > T | rs1801057 | p.Ala335Val | A335V | + | + | + | X |
| rs1801058 A > T | rs1801058 | p.Tyr339Phe | Y339F | + | + | + | X |
| rs1801059 G > A | rs1801059 | silent | - | - | - | - | - |
| rs1801060 T > A | rs1801060 | p.Ala258Thr | A258T | + | + | + | X |
| rs1801061 A > C | rs1801061 | p.Ala335Val | A335V | + | + | + | X |
| rs1801062 G > A | rs1801062 | p.Tyr339Phe | Y339F | + | + | + | X |
| rs1801063 T > A | rs1801063 | p.Arg223Gln | R223Gln | + | + | + | X |
| rs1801064 C > T | rs1801064 | p.Phe299fs | Fs299 | + | + | + | X |
| rs1801065 G > A | rs1801065 | p.Trp332Phe | W332P | + | + | + | X |
| rs1801066 C > T | rs1801066 | p.Ala335Val | A335V | + | + | + | X |
| rs1801067 A > T | rs1801067 | p.Tyr339Phe | Y339F | + | + | + | X |
| rs1801068 G > A | rs1801068 | silent | - | - | - | - | - |
| rs1801069 T > A | rs1801069 | p.Ala258Thr | A258T | + | + | + | X |
| rs1801070 A > C | rs1801070 | p.Ala335Val | A335V | + | + | + | X |
| rs1801071 G > A | rs1801071 | p.Tyr339Phe | Y339F | + | + | + | X |
Kaingang were investigated. All individuals were randomly selected and live in the State of Paraná, in South Brazil, with the exception of 26 Guarani belonging to the Kaiowá and Nandeva subgroups which live in the State of Mato Grosso do Sul in Central-Western Brazil. For some CCR5 alleles, the number of individuals analysed was lower. The classification of individuals as Euro-Brazilians and Afro-Brazilians was based on morphological features. The Euro-Brazilians included 53 unrelated German-speaking individuals whose ancestors came from or joined Mennonite settlements in South Brazil. No HLA genotyping data was available for this subsample. Based on HLA allelic frequencies previously determined for all other population samples, an average European component of 34% and an average Amerindian component of 6% were estimated for the Afro-Brazilians. For the non-Mennonite Euro-Brazilians, the African and Amerindian components are approximately 9% and 5%, respectively (Braun-Prado et al., 2000; Probst CM, MSc Dissertation, Universidade Federal do Paraná, Curitiba, 2000; Probst et al., 2000). The average admixture values of the Guarani and Kaingang with the immigrants from Europe and Africa were estimated to be 4% and 7%, respectively (Petzl-Erler et al., 1993; Probst CM, MSc Dissertation, Universidade Federal do Paraná, Curitiba, 2000; Tsuneto et al., 2003). The gene flow between these two Amerindian groups is also low, being approximately 1.4% in Guarani and 0.5% in Kaingang (Petzl-Erler et al., 1993).

Typing method

DNA was extracted from peripheral blood cells using the standard phenol/chloroform/isooamy alcohol or salting-out techniques. The coding region of exon 3 of the CCR5 gene was amplified by PCR as described previously (Boldt and Petzl-Erler, 2002). The product was applied on nylon membranes in the form of dot-blots and allowed to hybridize with sequence-specific oligonucleotide probes (SSOP, Table 2), according to the protocol of the XII International Histocompatibility Workshop (Fernandez-Viña and Bignon, 1997). Part of the coding region of exon 3 was additionally sequenced using the CCR5rev internal primer in 13 Guarani and 29 Kaingang, one Euro-Brazilian and five Oriental-Brazilian samples. These samples and 59 additional Guarani and 55 additional Kaingang samples were also sequenced using the CCR5for internal primer. One Guarani M’bya individual was genotyped only by sequencing. Sequencing reactions were performed with BigDye Terminator version 1.1 chemistry (Applied Biosystems, Foster City, CA). The sequences of the primers and probes are listed in Table 2.

Statistical analysis

Genotype and allele frequencies were obtained by direct counting with the aid of the Convert program version 1.1 (Program distributed by the author, CM Probst). The Hardy-Weinberg equilibrium and population homogeneity

Table 2 - CCR5 PCR primers and sequence-specific probes.

| Sequence 5’→3’                  | Variant |
|---------------------------------|---------|
| PCR primer CCR5m                | TATGCACAGGGTTGGAACAAG | ———— |
| PCR primer CCR5jn               | CACACTCTGACTGGGTGCAC | ———— |
| Seq. primer CCR5for             | AATGAGAAGAAGAGGACAGGCT | ———— |
| Probe CCR5 9-                   | AAGCAAAATCGACGCCCC | + |
| CCR5 9+                         | AAGCAAAATCGACGCCCC | A29S |
| Probe CCR5 1-                   | CTATCCTGTATACACCAAC | + |
| CCR5 1+                         | CTATCCTGTATACACCAAC | L55Q |
| Probe CCR5 10-                  | GAAAAGGCTGAGGAAGAAGA | + |
| CCR5 10+                        | GAAAAGGCTGAGGAAGAAGA | R60S |
| Probe CCR5 2-                   | CAGATATCAATTCTTGGG | + |
| CCR5 2+                         | CAGATATCAATTCTTGGG | D32 |
| Probe CCR5 3+                   | CTCTGGTTCCGMTGTC | + |
| CCR5 3-                         | CTCTGGTTCCGMTGTC | R223Q |
| Probe CCR5 14-                  | CATCTAGCCCTTTTTGT | + |
| CCR5 14+                        | CATCTAGCCCTTTTTGT | Fs299 |
| Probe CCR5 6-                   | AGGCCTCCGAGCCGAGAG | + |
| CCR5 6+                         | AGGCCTCCGAGCCGAGAG | P332P |
| Probe CCR5 7-                   | GAGCCTGCAAGCTCACA | + |
| CCR5 7+                         | GAGCCTGCAAGCTCACA | A335V |
| Probe CCR5 8-                   | TCAGTGTACACCCGAAG | + |
| CCR5 8+                         | TCAGTGTACACCCGAAG | Y339F |

PCR: polymerase chain reaction
Seq.: sequencing; +: major allele; in bold: variant nucleotides.
Table 3 - CCR5 allelic frequencies and standard deviations in various populations.

| Population            | A29S | L55Q | R60S | D32  | R223Q | Fs299 | P332P | A335V | Y339F |
|-----------------------|------|------|------|------|-------|-------|-------|-------|-------|
| Afro-American1,2 n = 50 | 0.015 ± 0.015 (32) | 0.008 ± 0.003 (332) | 0.013 ± 0.013 (38) | 0.019 ± 0.003 (1015) | 0     | 0     | 0.01 ± 0.01 | 0.025 ± 0.007 (242) | 0.026 ± 0.015 (58) |
| Afro-Brazilian n = 37 | 0.017 ± 0.017 (29) | 0.027 ± 0.019 | 0.05 ± 0.05 (10) | 0.02 ± 0.008 (172) | 0     | 0     | 0.015 ± 0.007 (172) | 0 (11) |
| Euro-American1,2 n = 50 | nt   | 0.041 ± 0.008 (354) | nt   | 0.1 ± 0.004 (2056) | 0.016 ± 0.016 (32) | 0     | 0     | 0.006 ± 0.006 (87) | 0 (121) |
| Euro-Brazilian n = 172 | 0.007 ± 0.007 (69) | 0.02 ± 0.008 | 0 (156) | 0.093 ± 0.016 | 0     | 0     | 0.003 ± 0.003 | 0.006 ± 0.004 |
| Mennonites n = 53   | 0 (30) | 0.038 ± 0.019 | 0 (39) | 0.142 ± 0.034 | 0     | 0     | 0     | 0     |
| Non-Mennonites n = 119 | 0.013 ± 0.013 (39) | 0.013 ± 0.007 | 0 (117) | 0.071 ± 0.017 | 0     | 0     | 0.004 ± 0.004 | 0.008 ± 0.006 |
| Chinese n = 785     | 0     | 0     | 0     | 0     | 0.047 ± 0.005 | 0.005 ± 0.002 | 0     | 0     |
| Oriental-American1,2 n = 100 | nt   | 0     | nt   | 0     | 0.04 ± 0.014 | 0.04 ± 0.014 | 0     | 0     |
| Oriental-Brazilian n = 13 | nt   | 0     | 0 (11) | 0 (16) | 0.077 ± 0.053 | 0 (12) | 0 (18) | 0 (12) |
| Guarani n = 27      | 0     | 0 (34) | 0     | 0.004 ± 0.004 (115) | 0 (34) | 0     | 0.013 ± 0.008 (115) | 0     |
| Kaingang n = 31     | 0     | 0     | 0     | 0 (160) | 0     | 0     | 0 (160) | 0     |
| Hispanic n = 50     | nt   | 0.01 ± 0.01 | nt   | 0.03 ± 0.012 | 0.02 ± 0.014 | 0     | 0     |

n: number of individuals; nt: not tested; in parenthesis: number of individuals if different from “n”.

1(Ansari-Lari et al., 1997); 2(Carrington et al., 2005); shadowed in italics: this work.

Results

The CCR5 genotype distributions met the Hardy-Weinberg equilibrium expectations in all populations. The allelic frequencies of the most common CCR5 alleles varied from 88% to 100% in the population samples studied. The other alleles were seen in about 0.5% to 5% of the population samples except for D32 and R223Q.

Table 4 shows the allelic frequencies of the most common CCR5 alleles and their distributions in various populations.

We used the approach of Cueto and Thompson and the ALEQUIN software package version 3.1 (http://cmpg.unibe.ch/software/alequin3) (Excoffier et al., 2005). p = 0.05 was adopted as the significance limit.

**Reference:**

Cueto B, Thompson EA. (2002) Allele frequencies and genetic diversity in the human CCR5 gene among populations from Latin America. Hum Mol Genet 11:3325-3330.
zygote out of the 5 Oriental-Brazilians whose exon 3 was sequenced.

**Discussion**

This is the first study investigating the A29S and R60S alleles in European-derived populations. Also, alleles other than D32 have not been analysed before in Amerindians. Based on the CCR5 allelic frequencies in the Chinese, North- and South-American populations (Table 3), it is possible to infer that A29S, R60S, A335V and Y339F most likely originated in Africa; L55Q and D32 in Europe; R223Q and Fs299 in Asia. The P332P allele, found only once in one heterozygote Afro-American (Ansari-Lari et al., 1997), was not found in our population samples nor in

| Population                  | n     | D32 freq. | Region                        | Reference                                      |
|-----------------------------|-------|-----------|-------------------------------|------------------------------------------------|
| Admixed Mexican             | 212   | 0.014 ± 0.006 | ————, MX                  | (Zuniga et al., 2003)                        |
| Amerindian Mayo             | 70    | 0         |                               |                                                |
| Amerindian Teenek           | 61    | 0         |                               |                                                |
| Amerindian Mazatecan        | 61    | 0.016 ± 0.011 | ————, JM | (Hisada et al., 2002)          |
| Afro-Jamaican               | 242   | 0.01 ± 0.005 | ————, JM | (Hisada et al., 2002)          |
| Colombian                   | 150   | 0.027 ± 0.009 | Medellin, CO | (Diaz et al., 2000)          |
| Amerindian                  | 172   | 0.009 ± 0.005 | Areqqua, PE | (Calzada et al., 2001)        |
| Amerindian Tikuna           | 191   | 0         | North West Amazonas, BR       | (Leboute et al., 1999)                       |
| Amerindian Baniwa           | 46    | 0         |                               |                                                |
| Amerindian Kashinawa        | 29    | 0         | South West Amazonas, BR       | (Leboute et al., 1999)                       |
| Amerindian Kanamari         | 34    | 0         |                               |                                                |
| Amerindian Tiriyó           | 180   | 0         | North Amazonas, BR            | (Grimaldi et al., 2002)                      |
| Amerindian Waiampi          | 221   | 0         |                               |                                                |
| Six Amerindian groups       | 89    | 0         | North Pará, BR                | (De Pinho Lott Carvalhaes et al., 2004)      |
| Brazilian                   | 394   | 0.03 ± 0.006 |                             |                                                |
| Afro-Brazilian              | 67    | 0.008 ± 0.008 |                             |                                                |
| Oriental-Brazilian          | 111   | 0         |                               |                                                |
| Brazilian                   | 104   | 0.02 ± 0.01 | Recife, Pernambuco, BR        | (de Souza et al., 2006)                      |
| Admixed Brazilian           | 549   | 0.026 ± 0.005 | Northeast Bahia, BR          | (Grimaldi et al., 2002)                      |
| Afro-Brazilian              | 54    | 0.019 ± 0.013 | Rio de Janeiro, BR           | (Chies and Hutz, 2003)                      |
| Brazilian                   | 115   | 0.056 ± 0.015 | São Paulo, BR                | (Munero et al., 2003)                       |
| Brazilian                   | 100   | 0.035 ± 0.013 | Ribeirão Preto, São Paulo, BR | (Passos Jr and Picanço, 1998)                |
| Euro-Brazilian              | 102   | 0.044 ± 0.014 | Paraná, Santa Catarina and Rio Grande do Sul, BR | (Chies and Hutz, 2003) |
| Brazilian                   | 127   | 0.055 ± 0.014 |                             | (Kainen-Maciel et al., 2007)                 |
| Eight Amerindian groups     | 241   | 0.013 ± 0.005 |                             | (Hunemeier et al., 2005)                    |
| Afro-Brazilian              | 172   | 0.02 ± 0.008 | Paraná, BR                   |                                                |
| Euro-Brazilian              | 172   | 0.093 ± 0.016 |                             |                                                |
| Oriental-Brazilian          | 16    | 0         |                               | This work.                                   |
| Guarani                     | 114   | 0.064 ± 0.004 |                             |                                                |
| Kaingang                    | 160   | 0         |                               |                                                |
| Brazilian                   | 100   | 0.05 ± 0.015 | Londrina, Paraná, BR         | (Brajão de Oliveira et al., 2007)            |
| Euro-Brazilian              | 99    | 0.066 ± 0.018 | Santa Catarina, BR           | (Grimaldi et al., 2002)                      |
| Euro-Brazilian              | 59    | 0.068 ± 0.023 | Alegrete, Rio Grande do Sul, BR | (Vargas et al., 2006)                      |
| Afro-Brazilian              | 13    | 0.038 ± 0.038 |                             |                                                |
| Admixed Brazilian           | 31    | 0.064 ± 0.032 |                             |                                                |
| Afro-Brazilians             | 58    | 0.009 ± 0.009 | Rio Grande do Sul, BR        | (Chies and Hutz, 2003)                      |
| Amerindian Chiriguano       | 42    | 0.012 ± 0.012 | Northwest Argentina, AR      | (Mangano et al., 2001)                      |
| Argentinean                 | 751   | 0.02 ± 0.004 | ————, AR                    | (Gonzalez et al., 2001)                      |
| Chilean                     | 62    | 0.024 ± 0.014 | ————, CL                    | (Desgranges et al., 2001)                    |

n: number of individuals; freq.: frequency; ISO 3166-1 codes indicate countries.
HIV-1 co-receptor activity as the major
Chinese population. It is equally distributed in HIV-1
et al., 1997; Zhao et al., 2005). The presence of the D32
allele in the Guarani seems to be the result of gene flow
from Neo-Brazilians, as suggested for Mura and Kaingang
in another study (Hunemeier et al., 2005).

The high D32 frequency in Euro-Brazilians is similar
to the frequencies found in Central Europe (Stephens et al.,
1998). It is compatible with the greater European compo-
nent in the Euro-Brazilian population of the Paraná State, in
comparison to other, previously analysed Brazilian popu-
lations of predominantly European ancestry (Probst et al.,
2000). The D32 frequency in the Mennonite subsample is
two times higher than in the non-Mennonite Euro-Brazilian
subsample and equals the high D32 frequencies in North
Europe (Stephens et al., 1998; Yudin et al., 1998). The fre-
quency of L55Q, another allele with likely European origin,
is three times higher in Mennonite compared to non-
Mennonite Euro-Brazilians. The Mennonites have Friessian
origin (North of Germany and the Netherlands) and exist as
a religious Anabaptist group since the second half of the
XVI century. The majority of individuals in this subsample
are direct descendants from 200 Mennonite families that
left their villages in the Ukraine and in Siberia and arrived
in South Brazil in 1930 (Pauls Jr., 1976). Thus, a founder or
bottleneck effect associated to random genetic drift most
probably caused the rise in the D32 and L55Q allelic fre-
cuencies in this population.

The R223Q allele is the most frequent variant in the
Chinese population. It is equally distributed in HIV-1 in-
fected and non-infected Chinese groups and has similar
HIV-1 co-receptor activity as the major CCR5 allele (Zhao
et al., 2005). Other populations have thus far not been in-
vestigated. We also found this allele among the Orien-
tal-Brazilians.

The cysteine residue we found mutated to phenyl-
alanine at codon 323 (p.Cys323Phe) in two heterozygote
Guarani individuals is not conserved in CCR2, the homolo-
gous C-C chemokine-receptor protein with the highest se-
quence similarity to CCR5 (75%). The substitution of the
same residue by alanine was found to decrease the expres-
sion of the CCR5 protein on the cellular membrane by pre-
venting receptor palmitoylation (Blanpain et al., 2001). A
change in the secondary structure and function may also be
expected from the replacement of this residue by phenyl-
alanine. In the Kaingang, sequencing revealed another new
allele (g.2964A > G) causing the substitution of tyrosine by
cysteine at the otherwise conserved residue 68 (p.Tyr68Cys)
in the second transmembrane part of the protein.
This allele seems to be very common in the Kaingang
population and restricted to it. Possible protective effects of
both alleles regarding HIV-1 infection and progression to
AIDS have to be established in appropriate cohorts attend-
ing Amerindian(-derived) populations.

In summary, we studied the distribution of the CCR5
coding region alleles in various Brazilian populations and
noticed a high D32 frequency in the Euro-Brazilian popula-
tion of the Paraná State in South Brazil. The D32 frequency
is even higher among the Mennonites and is the highest
thus far reported for Latin America. We also identified two
new coding CCR5 mutations in the Amerindian popu-
lations, whose functional characteristics should be defined
and considered in epidemiological investigations about
HIV-1 infection and AIDS incidence in Amerindian popu-
lations.

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