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Short Communication

Frequencies of CCR5-Δ32, CCR2-64I and SDF1-3'A mutations in Human Immunodeficiency Virus (HIV) seropositive Subjects and seronegative Individuals from the State of Pará in Brazilian Amazonia

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Abstract

The distribution of genetic polymorphisms of chemokine receptors CCR5-Δ32, CCR2-64I and chemokine (SDF1-3'A) mutations were studied in 110 Human Immunodeficiency Virus type 1 (HIV-1) seropositive individuals (seropositive group) and 139 seronegative individuals (seronegative group) from the population of the northern Brazilian city of Belém which is the capital of the state of Pará in the Brazilian Amazon. The CCR5-Δ32 mutation was found in the two groups at similar frequencies, *i.e.* 2.2% for the seronegative group and 2.7% for the seropositive group. The frequencies of the SDF1-3'A mutation were 21.0% for the seronegative group and 15.4% for the seropositive group, and the CCR2-64I allele was found at frequencies of 12.5% for the seronegative group and 5.4% for the seropositive group. Genotype distributions were consistent with Hardy-Weinberg expectations in both groups, suggesting that none of the three mutations has a detectable selective effect. Difference in the allelic and genotypic frequencies was statistically significant for the CCR2 locus, the frequency in the seronegative group being twice that found in the seropositive group. This finding may indicate a protective effect of the CCR2-64I mutation in relation to HIV transmission. However, considering that the CCR2-64I mutation has been more strongly associated with a decreased risk for progression for AIDS than to the resistance to the HIV infection, this could reflect an aspect of population structure or a Type I error.

Key words: CCR5, CCR2, SDF-1, HIV-1 infection, allele frequency, Brazilian Amazon.

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Several relatively recent studies (Kinter *et al.,* 2000; Berger *et al.,* 1999; O'Brien *et al.*, 2000; Dragic *et al.*, 1996; Barroga *et al.,* 2000) have identified effects of genetic polymorphisms of the chemokine receptors on individual susceptibility to Human Immunodeficiency Virus type 1 (HIV-1) infection and the rate of progression to AIDS or death. The CCR5-Δ32 mutation results from a 32-basepair (bp) deletion from the coding region of the CCR5 gene that creates a premature stop codon, producing a defective receptor that is not expressed at the cell surface. Homozygotes resistant to R5-HIV-1 infection (the principal infecting HIV-1 strain) lack the requisite HIV-1 entry coreceptor CCR5 on their lymphoid cells (Dean *et al.,* 1996; O'Brien and Moore, 2000). Heterozygotes express less than half the wild-type levels of CCR5 receptors, slowing HIV-1 replication, spread and pathogenesis (Wu *et al*., 1997; Benkirane *et al*., 1997). The CCR5-Δ32 allele is found at high frequencies (12% to 15%) in northern European populations, decreasing gradually from north to south (Martinson *et al.,* 1997). Outside Europe, the mutation is found in populations of European descent. In Brazilian urban populations (HIV serological status not determined) the CCR5 variant has been found with frequencies between 0.030 and 0.065 (Passos and Picanço, 1998; Pereira *et al*., 2000; Munerato *et al*., 2003; Carvalhaes *et al*., 2004). This mutation is rare in Afro-Brazilians communities (0.008) and absent in Brazilian Amerindians (Leboute *et al*., 1999; Su *et al*., 2000; Grimaldi *et al*., 2002; Carvalhaes *et al*., 2004). Smith *et al.* (1997) states that CCR2-64I is a G to A substitution that results in the replacement of valine with

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isoleucine at position 64 of the CCR2 protein. Mellado *et al*. (1999) indicated that the CCR2-64I protein can preferentially dimerize with CXCR4 polypeptides (the HIV-1 receptor that replaces CCR5 as an entry receptor at later stages), whereas the wild-type CCR2 peptides do not. Thus, this mechanism suggests that CCR2-64I delays AIDS by limiting the transition from CCR5 to CXCR4 in infected individuals, a turning point in the collapse of the CD4-T lymphocyte cell population and a prelude to AIDS-defining disease (Berger *et al*., 1999). The CCR2-64I allele has been found at relatively high average frequencies in almost all populations studied to date, *i.e.*: European, 13%; African, 17%, Asian 13% (Su *et al*., 1999, 2000; Martinson *et al*., 2000. A previous study performed on a sample of the general population of the northern Brazilian city of Belém, the capital of the state of Pará, population around 1,4 million (Brazilian Institute of Geography and Statistics - IBGE, 2004) in the Brazilian Amazon revealed the presence of the CCR2-64I mutation at a frequency of 0.161 (Carvalhaes *et al*., 2004) but higher frequencies have been found in Afro-Brazilians (0.230), whereas in Brazilian Amerindians the frequency of this mutation varies from 0.030 to 0.300 (Su *et al*, 1999; Acosta *et al*., 2003; Carvalhae*s et al*., 2004).

Stromal-derived factor-1 (SDF-1) is the chemokine ligand of CXCR4, the coreceptor used by the more pathogenic X4HIV-1 strains for cell entry (Lu *et al.,* 1997; Björndal *et al.,* 1997; Wang *et al.,* 2003). A G-to-A substitution at position 801 in the 3' untranslated region (UTR) of the SDF-1 gene (SDF1-3'A allele) has been reported to slow disease progression (Winkler *et al.,* 1998; Dezzutti *et al.,* 2000), although this finding is still controversial (Ioannidis *et al.,* 2001). Although allele-specific cellular or virological differences have not been explicitly established, the delay in onset of AIDS observed in SDF1-3'A homozygotes might result from overproduction of SDF1 in certain tissue compartments, postponing the CCR5-CXCR4 transition. A synergistic protective effect in individuals carrying SDF1-3'A and CCR2-64I would be consistent with this model. The SDF1-3'A allele is widely distributed, and has particularly high frequencies in Oceania, especially in New Guinean Highlanders (up to 72%), but is less common (3% to 12%) in African populations and has been found with frequencies between 15% and 22% in Europeans (Su *et al.,* 1999, 2000). In urban Brazilian populations not tested for HIV infection the SDF1-3'A allele was found at frequency of 0.223 in the population of Belém (Carvalhaes *et al*. 2004). In non-urban populations this variant was found at frequency of 0.153 in Afro-Brazilian communities and at frequencies of 0.060 to 0.227 in Amerindian tribes from the Brazilian Amazon (Su *et al*., 1999; Carvalhaes *et al*. 2004).

The current article describes the frequency of the CCR5-Δ32, CCR2-64I and SDF1-3'A mutations in 110 HIV-1 seropositive individuals and 139 seronegative individuals from Belém. The survey was carried out to investigate the possible influence of these genetic mutations on HIV transmission in the population of Belém.

Blood samples were randomly and anonymously collected from 249 individuals from Belém, all samples being collected with the informed consent of the participants. The HIV-1 seronegative individuals consisted of 20 females and 119 males (seronegative group, n = 139; age range: 17-49 years) who were blood donors at the Hemotherapy and Hematology Center of the state of Pará in Belém. The HIV-1 seropositive individuals consisted of 35 females and 75 males (seropositive group, n = 110; age range: 23-82 years) who attended the Reference Unit for Special Infectious and Parasitic Diseases (URE-DIPE) in Belém between 1998 and 2002. The mode of HIV-1 transmission was clear for only 74 of the seropositive individuals (71 having been infected by sexual transmission and 03 by transfusion of HIV-1-contaminated blood or by sharing contaminated syringes and needles), the remaining 36 seropositive individuals being unable to identify the mode of HIV-1 transmission.

The CCR5Δ32 genotype was determined by PCR with primers spanning the 32 bp deletion (Martinson *et al.,* 1997). The SDF1-3'A and CCR2-64I mutations were detected by PCR with primers covering polymorphic sites, followed by *Msp*I and *Bsa*BI digestion, respectively, and restriction fragment length polymorphism (RFLP) analysis as described by Voevodin *et al.* (1999).

Allele frequencies and expected Hardy-Weinberg values were estimated by the maximum likelihood method using the TFPGA program version 3.1 (Miller, 1998). Statistical comparisons of allele and genotype frequencies between samples used the chi-squared (χ^2) test function of the same program.

Genotype and allele frequency distributions recorded for the three loci are presented in Table 1. Genotype distributions were consistent with the Hardy-Weinberg expectations for the three loci in both groups. Allele frequencies of the CCR5 locus were similar in both groups, being 2.2% in the seronegative group and 2.7% in the seropositive group ($p = 0.771$). The SDF1-3'A mutation was common in both groups but relatively more so (21%) in the seronegative group as compared to seropositive group (15.4%), although this difference was not significant at $p = 0.134$. The CCR2-64I allele was also relatively common but its frequency in the seropositive group was 5.4% and hence less than half of the 12.5% found in the seronegative group, this difference being statistically significant at $p = 0.0135$.

The fact that genotype distributions of all three loci are in Hardy-Weinberg equilibrium in both groups suggests that none of the mutations studied here has a detectable selective effect, as observed previously in other human populations. This is supported by the lack of statistically significant differences between seropositive and seronegative groups in the allele and genotype frequencies at the CCR5 ($p = 0.771$) and SDF-1 ($p = 0.134$) loci. By con-

Table 1 - Allele and genotype frequencies of the CCR5, CCR2 and SDF-1 loci in Human immunodeficiency virus (HIV) seropositive and seronegative individuals from Belém.

Locus/group	N	Genotype frequency			Allele frequency	Expected Hardy-Weinberg p-value
		wt/wt	wt/Δ32	Δ32/Δ32	Δ32	
CCR5D32						
Seronegative	139	133	6	0	0.022	0.7948
Seropositive	110	104	6	0	0.027	0.7534
CCR2-64I		wt/wt	wt/64I	64I/64I	64I	
Seronegative	112	84	28	0	0.125	0.1306
Seropositive	110	98	12	0	0.054	0.4870
SDF1-3'A		wt/wt	wt/3'A	3'A/3'A	3'A	
Seronegative	126	96	32	3	0.210	0.7584
Seropositive	110	79	28	3	0.154	0.8633

trast, the frequency of the CCR2-64I allele in the HIV-1 seropositive group was significantly lower (p = 0.014) than that of the seronegative group. In the case of the CCR5 locus, the low frequency of the CCR5-Δ32 mutation implies that the protective genotype may be too rare for confirmation. The absence of significant differences in the allelic and genotypic frequencies between HIV-1 seropositive and seronegative individuals has also been reported in other Brazilian populations. Grimaldi *et al*. (2002) reported such a lack of difference for the population of Salvador, the capital of the Brazilian state of Bahia, total population estimated at 2,631,831 (IBGE, 2004) and Munerato *et al*. (2003) in the population of Brazilian city of São Paulo, the largest Brazilian city with a population of about 11 million (IBGE, 2004).

Our results for the SDF-1 locus are consistent with findings in other human groups, which have shown that the SDF1-3'A mutation has no effect on HIV transmission, but is related to the delay in onset of AIDS. In fact, homozygosity for the SDF1-3'A allele has been reported to slow disease progression, but results obtained in an international meta-analysis of individual patient data showed that SDF1-3'A homozygosity gave no such protection (Ioannidis *et al*., 2001). With regard to the CCR2 locus, the CCR2-64I allele has been found at relatively high average frequencies in European, African and Brazilian Amerin-

Table 2 - Allele and genotype frequencies of CCR5, CCR2 and SDF-1 loci in Human Immunodeficiency Virus (HIV) seropositive and seronegative Brazilians.

Locus/group/place	N	Genotype frequency			Allele frequency	Reference
		wt/wt	wt/Δ32	Δ32/Δ32	Δ32	
CCR5						
Seronegative						
Southeast Brazil	229	205	24	0	0.052	Pereira *et al*. (2000)
Joinville (SC)	99	87	11	1	0.065	Grimaldi *et al*. (2002)
Salvador (BA)	549	520	29	0	0.026	Grimaldi *et al*. (2002)
Seropositive						
São Paulo (SP)	183	162	21	0	0.057	Munerato *et al*. (2003)
Salvador (BA)	113	103	10	0	0.044	Grimaldi *et al*. 2002)
CCR2		wt/wt	wt/64I	64I/64I	64I	
Seronegative						
Salvador (BA)	305	227	73	5	0.140	Acosta *et al*. (2003)
Joinville (SC)	89	57	32	0	0.180	Acosta *et al*. (2003)
Seropositive						
São Paulo (SP)	178	150	27	1	0.082	Munerato *et al*. (2003)
SDF		wt/wt	wt/3'A	3'A/3'A	3'A	
Seropositive						
São Paulo (SP)	62	41	21	0	0.169	Watanabe *et al*. (2003)

Key: SC = Santa Catarina State; BA = Bahia state; SP = São Paulo state.

dian populations, which indicates that the expected frequencies of individuals who would be wholly or partially resistant to the infection are usually high, with consequent lower rates of disease transmission. However, considering that the CCR2-64I is more strongly associated with a decreased risk for progression to AIDS than to resistance to the HIV infection, the suggested protective effect of this variant on HIV transmission in the population of Belém may simply reflect an aspect of the population structure of this city or to be due to a Type I error (*i.e.* the probability of falsely rejecting the hypothesis that two samples are not different in a series of comparisons). The distribution of the CCR2-64I and SDF1-3'A mutations in seropositive and seronegative individuals from Belém were not different from those of other Brazilian populations in São Paulo and Bahia (Table 2), although comparisons of allelic and genotypic frequencies among seropositive subjects and uninfected healthy individuals in these studies are not available.

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