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Research Article

Distribution of the CCR2-64I allele in three Brazilian ethnic groups

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Abstract

CCR2 is a member of the superfamily of seven transmembrane domain G protein-coupled receptors, the largest receptor superfamily in the human genome. CCR2 acts as a receptor for MCP-1 (CC chemokine) and as a co-receptor for HIV-1 cell-target entry. The gene encoding this receptor is mapped to the chromosome band 3p21. A G-to-A transition at position 190 characterizes the CCR2-64I mutation, causing valine to isoleucine substitution in codon 64. This mutation has been identified as an important factor for delaying progression to AIDS. Here, we determined the prevalence of this allele in three different Brazilian populations: 261 Amerindians inhabiting an isolated region in northern Brazil (82 samples from the Waiampi tribe, and 179 samples from the Tiriyó tribe); 89 German descendents from Joinville, a city in southern Brazil; and 305 individuals of predominantly African ancestry, from Salvador, a city in northeast Brazil. The CCR2-64I mutant allele was identified in 26% of the Tiryió and 30% of Waiampi samples, in 18% of the Joinville samples, and in 14% of the Salvador samples.

Key words: CCR2-64I mutation, Brazilian populations, HIV co-receptor.

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Introduction

Chemokine and chemotaxis cytokine-activated receptors from the superfamily of seven transmembrane domain G protein-coupled receptors, such as CCR2, consist of a single polypeptide chain with an extracellular amino-terminal domain and a cytoplasmic carboxyl-terminal domain. The amino terminal and third extracellular domain have been implicated in receptor-ligand interaction, while the carboxy-terminal and the third intracellular domain cooperate to bind and activate G proteins (Frade *et al*. 1997). Due to the importance of chemokines in inflammatory processes, special attention has been given to leukocyte receptors that mediate chemokine responses, and the cloning of these receptors has already been reported (Charo *et al*. 1994).

CCR2 acts as a receptor for MCP1 (Monocytes Chemoattractive Protein type 1), a CC chemokine of the family of chemokines which mediate the chemotaxis of leukocytes. The gene for CCR2 is located at chromosome band 3p21, and contains three exons distributed over 7 kb of the genomic sequence (Wong *et al*. 1997). Two receptors derived from alternative splicing in the carboxyl terminal-coding region of the protein have been identified (CCR2-α and CCR2-β), both of which signal a highly specific response for MCP1 (Charo *et al*. 1994; Wong *et al*. 1997).

Like other chemokine receptors (*e.g.* CCR5, CCR3, and CXCR4), CCR2 plays a role as a co-receptor for HIV-1 infection (Berger *et al*. 1999; Frade *et al*. 1997). A nucleotide transition (G to A) at position 190 of the *CCR2* gene was found to produce a substitution of valine to isoleucine in aminoacid 64 of the protein. This represents a conservative change in the protein's first transmembrane domain and, as shown by Smith *et al*. (1997), does not appear to be a significant risk factor for HIV-1 infection, since infected individuals and highly exposed uninfected individuals do not display significant differences in allelic and genotypic CCR2-64I frequencies. However, progression to AIDS was observed to be two to three years longer in patients who were homozygous or heterozygous for the CCR2-64I mutation, as compared to those with wild-type CCR2 (Smith *et*

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al. 1997). Subsequent studies investigating the distribution of the CCR2-64I mutation and allelic frequencies in different populations and ethnic groups worldwide (Martinson *et al.* 2000; Struyf *et al.* 2000; Mangano *et al.* 2000; Barbouche *et al.* 2001; Iyer *et al.* 2001) have indicated that this mutation is most common in Asian populations (0.250), least common in Europeans (0.098), and of intermediate frequency (0.151) in African populations (Smith *et al*. 1997). Subsequent studies, however, have shown contradictory values (Struyf *et al.* 2000).

Here, we investigated the frequency of CCR2-64I in three distinct Brazilian ethnic groups. This is the first work describing the distribution of these mutations in Brazilian HIV- negative populations.

Subjects and Methods

A total of 655 samples were collected from distinct populations residing in three separate regions of Brazil (North, Northeast, and South); all samples tested negative for HIV-1. The samples from the northeastern region were collected during the epidemiologic evaluation of a basic sanitation program ("Bahia Azul") currently under construction in Salvador, a city with 2.4 million inhabitants (from the Brazilian Demographic Census, IBGE 2000 - http://www.ibge.gov.br), 80% of which are of African descent or mestizos, mainly of mixed Portuguese and African ancestry. The samples from the southern region were collected from 89 German descendents at the Joinville Regional Hemocenter, and the 261 samples from the northern region came from two Indian tribes (179 Tiriyós and 82 Waiampis) which live in a secluded and rugged region along the Brazilian border with French Guyana, in the states of Amapá and Pará. All samples were collected with the informed consent of the participants.

DNA was extracted from both peripheral blood mononuclear cells (PBMCs) and whole blood, using the DNAzol commercial kit (GIBCO-BRL, Rockville, USA). PCR was performed in a Perkin-Elmer 9600 thermal cycler (Perkin-Elmer, Connecticut, USA), using 100 ng of DNA in a final reaction-mixture volume of 25 μL. Forward with the nucleotide "a" as a mismatch (5'-TTGTGGGCAACATGaTGG -3') and reverse (5'-GAGCCCACAATGGGAGAGTA -3') primers were used to amplify a 128 bp fragment. The reaction mixture contained 10x PCR buffer at 20 mM (10 mmol/L Tris HCl pH 8.3, 50 mmol/L KCl, 3 mmol/L $MgCl_2$ and 10% BSA); 1.25 mM of dNTP; 2.5 pmol of each primer, and 2.0 U of Taq DNA polymerase. Thirty-five cycles were performed, each consisting of 1 minute at 72 °C, 30 s at 94 °C, and 30 s at 59 °C. According to Smith *et al*. (1997), the amplified products were digested with 5 U *BsaB* I restriction enzyme for three hours at 60 °C, and electrophoresed on 10% acrylamide gels. A 128 pb fragment indicated a homozygous wild genotype, while 110 pb and 18 pb fragments diagnosed the homozygous mutant genotype, and the presence of the three fragments revealed the heterozygous genotype (Figure 1).

Results and Discussion

The observed allele and genotype frequencies of the CCR2-64I mutation are shown in Table 1. The values obtained are consistent with the Hardy-Weinberg equilibrium.

The homozygous wild-type and heterozygous CCR2-64I genotypes were present in all populations, and the homozygous CCR2-64I genotype was found in all populations except for the one from Joinville. The prevalence of the CCR2-64I mutation in heterozygosis was quite high in all populations studied.

The frequencies found in the indigenous populations are similar to those previously reported in populations of Asian origin (Smith *et al*. 1997; Su *et al*. 1999; Voevodin *et al.* 1999; Mangano *et al*. 2000; Iyer *et al*. 2001; Wang *et al*. 2001; Hong *et al*. 2001; Ramana *et al*. 2001). This observation is in accordance with the prevailing theory regarding the Asian origin of Amerindians (Silva *et al*. 2002), as well as with the theory that CCR2-64I is an ancient mutation, since it is both common in Asians and present in other ethnic groups.

The frequency of the CCR2-64I mutation in the sample from Salvador is consistent with other populations of

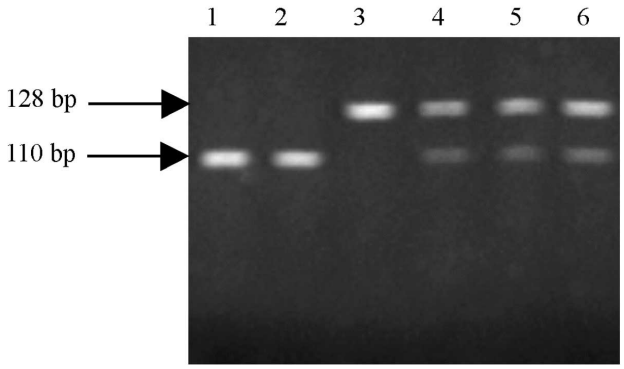

Figure 1 - CCR2-64I mutation genotyping by PCR and *BsaB* I endonuclease restriction: homozygous CCR2-64I (lanes 1 and 2), homozygous wild-type (lane 3), and heterozygous (lanes 4 to 6) genotypes.

Table 1 - CCR64I allele and genotype frequencies in four Brazilian populations:

Genotypes	Populations			
	Salvador n = 305	Joinville n = 89	Tiriyó n = 179	Waiampi n = 82
wt/wt	227 (74%)	57 (64%)	92 (51%)	39 (48%)
wt/64I	73 (24%)	32 (36%)	80 (45%)	37 (45%)
64I/64I	5 (2%)	0 (0%)	7 (4%)	6 (7%)
CCR2-64I allele	0.14	0.18	0.26	0.30

predominantly African origin (Smith *et al*. 1997; Martinson *et al*. 2000, Iyer *et al*. 2001), reflecting the widespread African/European miscegenation in this population.

Although the individuals from Joinville were considered as being of European descent, the observed mutant allele and genotype frequencies were much higher than those found in European populations. This is apparently due to the fact that, although this population descended primarily from Europeans, considerable miscegenation has probably occurred after the original German migrations to Brazil. A high frequency of CCR2-64I (14.37%) has also been observed in European-derived populations of other countries (Iyer *et al*. 2001).

The prevalence of mutations that influence AIDS pathology and progression is relevant for the establishment of strategies for the prevention and treatment of AIDS, and, as shown for the CCR64I mutation, they should be investigated in different populations, taking into account their ethnic background and genetic diversity.

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