

**ORIGINAL ARTICLE**

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**FREQUENCY OF CCR5 $\Delta$ 32 MUTATION IN BLOOD  
DONOR CANDIDATES FROM THE *FUNDAÇÃO  
HOSPITALAR DE HEMATOLOGIA E HEMOTERAPIA DO  
AMAZONAS, BRAZIL***

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*Allyson Guimarães da Costa<sup>1,2,3</sup>, Andréa Monteiro Tarragô<sup>1,4</sup>, Josilene da Silva Abranches<sup>1</sup>, Nadja Pinto Garcia<sup>1,4</sup>, Pedro Vieira da Silva Neto<sup>1,4</sup>, Hiocelson Najibe dos Santos Ibiapina<sup>2,3</sup>, Aya Sadahiro<sup>4</sup> and Adriana Malheiro<sup>1,4</sup>*

**ABSTRACT**

The chemokine receptor CCR5 is a major co-receptor for HIV-1 entry into the host cell. Deletion of 32 bp ( $\Delta$ 32) alters the receptor structure and is associated with the protection against infection. The distribution of allelic variant depends on several factors influencing the epidemiology of HIV infections. Thus, the present study sought to estimate the allelic frequency of the CCR5 gene variant / CCR5 $\Delta$ 32 in blood donor candidates with and without positive serology for HIV-1<sup>+</sup> at the HEMOAM Foundation. 239 candidates were enrolled and divided into two groups, HIV-1<sup>+</sup> (101 individuals) and HIV<sup>-</sup> controls (138 individuals). After collecting peripheral blood, DNA was extracted and allele-specific PCR for identification of CCR5 $\Delta$ 32 polymorphism, was performed. The results obtained were analyzed using Stata (v.13). The groups were of similar ages, predominantly male and the distribution of genotypes and alleles were in Hardy-Weinberg equilibrium ( $p=0.725$  and  $p=0.879$ , respectively). The highest frequency was wild genotype, followed by the heterozygous genotype in both groups (control and the HIV-1<sup>+</sup>). When the frequencies in HIV-1<sup>+</sup> subgroups were analyzed, the absence of the allelic variant CCR5 $\Delta$ 32 subgroup ELISA<sup>(+)</sup> Western Blot<sup>(+)</sup> was noted. Therefore, our data indicate that CCR5 $\Delta$ 32 polymorphism has a low frequency in the population studied.

**KEY WORDS:** Genetic polymorphism; HIV-1; Amazon.

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1. Laboratório de Genômica, Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas, Manaus, Amazonas, Brazil.
  2. Programa de Pós-Graduação em Medicina Tropical, Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil.
  3. Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Amazonas, Brazil.
  4. Programa de Pós-Graduação em Imunologia Básica e Aplicada, Universidade Federal do Amazonas, Manaus, Amazonas, Brazil.

Corresponding author: Adriana Malheiro, Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas (HEMOAM), Av. Constantino Nery, N° 4397, CEP 69050-002, Chapada, Manaus, Amazonas, Brazil. E-mail: malheiroadriana@yahoo.com.br

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## INTRODUCTION

Infection by the Human Immunodeficiency Virus (HIV) is characterized by immunosuppression associated with opportunistic infections, malignancies, weight loss, degeneration of the central nervous system (CNS) and may cause Acquired Immune Deficiency Syndrome (AIDS) (Brasil, 2015).

HIV binds to the cell surface through the CD4<sup>+</sup> receptor and the CCR5/CXCR4 co-receptors to gain entry into cells (Berger et al., 1999). There seems to be a selection of variants of HIV-1 R5 strains, which are responsible for the majority of HIV transmissions while a prevalence of HIV-1 X4 strains is present in some patients with advanced HIV-1 disease. However, HIV-1 and HIV-2 isolates can exploit alternate molecules *in vitro* as co-factors for viral entry. Several other receptors have been implicated as coreceptors (CCR2b, CCR3, CCR4, CCR6, CCR8, CCR9, CCR10, CXCR2, CXCR5, CXCR6, CX3CR1, XCR1, FPRL1, GPR1, GPR15, APJ, ChemR23, CXCR7/RDC1, D6, BLTR and US28) (Berger et al., 1999; Keele & Estes, 2011; Santos et al., 2014).

The CCR5 gene encodes a G protein transmembrane (b-chemokine receptor) and acts as a co-receptor for HIV-1 attachment and entry to T lymphocytes and mononuclear cells through the envelope glycoprotein gp120 binding to the CD4 molecule. Despite high-risk behavior with multiple exposures to HIV-1, some individuals present a rare genetic resistance to HIV-1 infection and AIDS related to the CCR5 gene (Liu et al., 1996; Alvarez et al., 1998). The deletion of 32 ( $\Delta$ 32) base pairs in the CCR5 gene leads to the suppression of CC chemokine receptor 5 presentation at the cell surface, thus impeding the HIV entry process into the host cell and protecting against HIV-1 infection (Liu et al., 1996; Samson et al., 1996; Reiche et al., 2007; Février et al., 2011; Vieira et al., 2011; Saez-Cirion et al., 2014; Li, 2015). Besides, three polymorphisms (-59029, -59353 and -2459) in the promoter region of CCR5 (-59029, -59353 and -2459) have been reported to influence its expression and may modulate the susceptibility of human cells to HIV-1 and Simian Immunodeficiency Virus (SIV) (Zare-Bidaki et al., 2015).

It is noteworthy that the only cure of HIV-1 infection was described in an adult living in Berlin, known as the Berlin patient, who developed acute myelogenous leukemia (AML), was immunosuppressed and treated with an allogeneic hematopoietic stem cell transplant from a donor who was homozygous for the CCR5 $\Delta$ 32 deletion (Hütter et al., 2009; Yukl et al., 2013).

The global distribution and ethnic factors for allele CCR5/CCR5 $\Delta$ 32 vary significantly, contributing to a variety of genetic resistance profiles to HIV-1 infection seen in different populations (Apostolakis et al., 2005). The variant allele CCR5 $\Delta$ 32 is relatively common in European populations, whose allele frequency is approximately 10% (Saez-Cirion et al., 2014), while in Asian populations, allele is absent among the Japanese, Filipino, Korean,

Chinese and Indians (Martinson et al., 1997). In Brazil, the allelic frequency observed in urban population (198 random unrelated volunteers) was 93% wild-type homozygous allele (CCR5/CCR5), 7% in heterozygous allele (CCR5/CCR5 $\Delta$ 32) and absence of the variant allele in homozygous (CCR5 $\Delta$ 32/CCR5 $\Delta$ 32), resulting in a 3.5% allele frequency (Passos & Proença-Picanço, 1998).

Thus, the aim of this study was to estimate the allelic frequency of the CCR5/CCR5 $\Delta$ 32 gene variant in candidates for blood donation with and without positive serology for HIV-1 from the *Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas* (HEMOAM).

## METHODS

### *Ethical issues*

The study protocols were approved by the Research Ethics Committee of the *Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas* (REC-HEMOAM, process #0003.0.112.000-11). The free and informed consent form (ICF) was signed by each research subject prior to enrollment, according to the Helsinki Declaration and Resolution 466/12 of the National Health Council for research involving human subjects.

### *Sampling*

The study samples were obtained from 239 candidates for blood donation at HEMOAM, from April 2006 to March 2007 and August 2012 to July 2013. All the candidates were first-time donors, without previous donation history and were subjected to a detailed serological screening, recommended for the screening of microorganisms transmitted through blood, by the Brazilian Blood Bank Authorities. Blood donor candidates who exhibited HIV reactivity in at least one serological screening test were included in the case group (101 samples), whereas the control group (138 samples), consisted of donors without positive serology for screening markers.

### *Collection of Samples*

10 mL of peripheral blood was collected using the vaccum system in tubes with gel separator (BD gel SST<sup>®</sup> Advance<sup>®</sup> II) and PPT (Tube picker K2 EDTA plasma, BD Vacutainer<sup>™</sup>) for complete serological testing for HIV as well as for biomolecular analysis.

### *Serological tests*

Serological tests were performed by 3rd generation ELISA AxSYM I/II g0® (Abbott, Wiesbaden, Germany) and HIV Ag/Ab combined® (Murex Biotech, Darford, UK). Confirmatory testing was conducted by Western Blot - Blot 2.2® (Genelabs Diagnostics, Science Park, Singapore). All the procedures and the results were conducted and evaluated in accordance with the manufacturer's specifications, fulfilling the requirements determined by decree No. 488/98 by the Brazilian Ministry of Health.

### *DNA extraction*

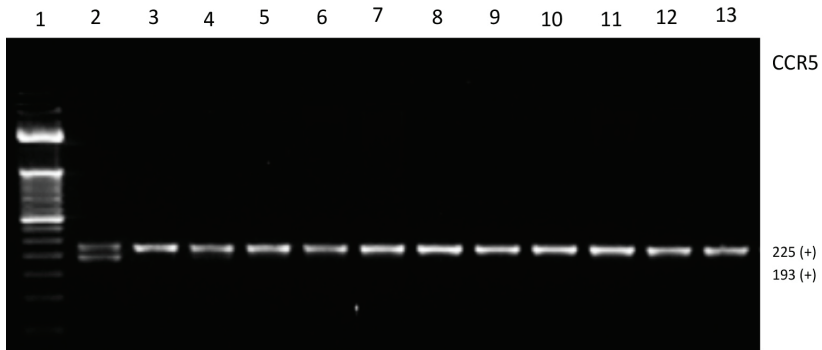
DNA was extracted with the Brazol kit® (LCG Biotechnology), second protocol developed by Chomczynski and Sacchi (1987), following the recommendations outlined by the manufacturer.

### *CCR5Δ32 polymorphism genotyping*

Amplification was performed using the PCR technique described by Chies and Hutz (2003). The following pair of primers was used: forward 5'ACCAGATCTCAAAAAGAA3' and reverse: 5'CATGATGGTGAAGATAA-GCTTCA3', derived from previously published sequences in GenBank (AF009962). A total of 25 µL of amplification primer mix was used containing 3.0 µL (100 ng) of genomic DNA, 1.5 µL (2.5 mM) of each primer, 2.0 µL (1.25 mM) dNTPs (CENT BIO®), 0.75 µL MgCl<sub>2</sub> (50 mM), 2.5 µL of 10X PCR buffer (500mM KCl and 200mM Tris-HCl, pH 8.4), 2.5 µL (2U) of Taq DNA polymerase (CENT BIO®) and 11.25 µL of ultrapure water. PCR was performed in an Applied Biosystems thermocycler (96 Veriti® Thermal Cycler) in accordance with the program described: an initial 1 cycle at 94°C for 5 min (Initial denaturation); 35 cycles: 1 min (94°C - denaturation), 1 min (58°C - annealing) and 1 min (72°C - extension); finishing with 10 min at 72°C. The fragments generated by PCR (Figure 1) were separated by agarose gel electrophoresis at 3%, stained with GelRed™ Nucleic Acid Gel Stain (Biotium®) and visualized in ultraviolet transilluminator (UV) light with photo documentation system, Gel+Doc<sup>XR</sup> System (Bio-Rad Corporation).

### *Statistical analysis*

The  $\chi^2$  two-tailed test and odds ratio (OR) with 95% confidence interval (CI) were calculated. The Hardy-Weinberg equilibrium (HWE) was estimated, compared to the frequencies of the different genotypes with expected values. Logistic regression analysis was performed using the website <https://ihg.gsf.de/cgi-bin/hw/hwa1.pl> and STATA software (version 13).



*Figure 1.* Analysis of Genotype CCR5 in candidates for blood donation at the HEMOAM for Polymerase Chain Reaction (PCR). Agarose gel electrophoresis stained with 3% GelRed™ Nucleic Acid Gel Stain. Wild-type allele of the CCR5 gene (225bp) and 32bp deletion in the gene variant CCR5Δ32 (193bp). Slots 1: Ladder 50bp. Slots 2: Heterozygous (CCR5/CCR5Δ32). Slots 3-13: Wild Homozygous (CCR5/CCR5).

## RESULTS

Subjects were separated into two groups: control (n=138) and HIV-1<sup>+</sup> (n=101). At the confirmatory tests, the HIV-1<sup>+</sup> group was subdivided into 3 subgroups: ELISA<sup>(+)</sup>Westen Blot<sup>(+)</sup> (n=29); ELISA<sup>(+)</sup>Westen Blot<sup>(Ind)</sup> (n=22) and ELISA<sup>(+)</sup>Westen blot<sup>(-)</sup> (n=50). Demographic data (age and gender) of participants are described in Table 1. The groups had similar age, median of 30 years (controls) and 36 years (HIV-1<sup>+</sup>), with a predominance of males (90 [65%] and 79 [78%], respectively).

The frequency of allelic variants of CCR5 (CCR5/CCR5Δ32) are shown in Table 2. The distribution of genotypes and alleles were according to the Hardy-Weinberg expectation (p=0.725 and p=0.879). Regarding the allelic frequencies in the control group and HIV-1<sup>+</sup>, the wild genotype (CCR5/CCR5) was the most prevalent (94% and 96%) followed by the heterozygous genotype (CCR5/CCR5Δ32) with 6% and 4%, respectively. The differences were neither statistically significant nor demonstrated association of the allele variant CCR5Δ32 with HIV-1<sup>+</sup> (OR=0.497, CI=0.129 to 1.924, p=0.3029).

On analysis of the frequencies of HIV-1<sup>+</sup> subgroups, the absence of the allelic variant CCR5Δ32 in the subgroup ELISA<sup>(+)</sup>Westen Blot<sup>(+)</sup> was noted. The frequencies of this polymorphism in the other subgroups (ELISA<sup>(+)</sup>Westen Blot<sup>(Ind)</sup> and ELISA<sup>(+)</sup>Westen Blot<sup>(-)</sup>) were lower than in the control group. However, the differences observed were not statistically significant (p=0.183, p=0.812 and p=0.627, respectively), as shown in Table 3.

*Table 1.* Demographic data of candidates for blood donation in the study.

Demographic Data	Control Group	HIV <sup>+</sup> Group			Total
		ELISA <sup>(+)</sup> <sup>a</sup> Westen Blot <sup>(+)</sup> <sup>b</sup>	ELISA <sup>(+)</sup> Westen Blot <sup>(Ind)</sup> <sup>c</sup>	ELISA <sup>(+)</sup> Westen Blot <sup>(-)</sup> <sup>d</sup>	
Number	(n=138)	(n=29)	(n=22)	(n=50)	(n=239)
Gender (M/F)*	90/48	22/7	19/3	38/12	169/70
Age (median)	30	36	35	36	

\*M: Male, F: Female. <sup>a</sup>ELISA<sup>(+)</sup>: Positive ELISA test; <sup>b</sup>Westen Blot<sup>(+)</sup>: Positive Westen Blot test; <sup>c</sup>Westen Blot<sup>(Ind)</sup>: Indetermined Westen Blot test; <sup>d</sup> Westen Blot<sup>(-)</sup>: Negative Westen Blot test.

*Table 2.* Frequency of genotypes CCR5Δ32 according to the study group.

Genotype / Allele CCR5	Control	HIV <sup>+</sup>	$\chi^2$ *	OR <sup>#</sup> (95% CI <sup>‡</sup> )	(p) value
	Group n (%)	Group n (%)			
CCR5/CCR5 <sup>a</sup>	130 (94%)	98 (96%)			
CCR5/CCR5Δ32 <sup>b</sup>	8 (6%)	3 (4%)	1,06	0.497 (0.129-1.924)	0.3029
CCR5Δ32/CCR5Δ32 <sup>c</sup>	0	0			
CCR5	268	199	1.04	0.505 (0.132-1.928)	0.3699
CCR5Δ32	8	3			

<sup>a</sup>CCR5/CCR5: Wild-type; <sup>b</sup>CCR5/CCR5Δ32: Heterozygotes; <sup>c</sup>CCR5Δ32/CCR5Δ32: Homozygotes mutant. \* $\chi^2$ : Chi-square. <sup>#</sup>OR: Odds Ratio. <sup>‡</sup>CI: Confidence Interval

Table 3. Frequency of CCR5Δ32 genotype in HIV+ subgroups.

Genotype CCR5	Control Group		HIV+ Group				
	n (%)	ELISA <sup>(+)</sup> Westen Blot <sup>(+)</sup>	n (%)	ELISA <sup>(+)</sup> Westen Blot <sup>(ind.)</sup>	OR# (95% IC*)	ELISA <sup>(+)</sup> Westen Blot <sup>(-)</sup>	OR# (95% IC*)
CCR5/CCR5 <sup>a</sup>	130 (94%)	29 (100%)	21 (95%)	0.260 (0.015-4.636)	0.774 (0.092-6.507)	48 (96%)	0.677 (0.39-3.302)
CCR5/CCR5Δ32 <sup>b</sup>	8 (6%)	0	1 (5%)			2 (4%)	
CCR5Δ32/CCR5Δ32 <sup>c</sup>	0	0	0			0	

<sup>a</sup>CCR5/CCR5: Wild-type; <sup>b</sup>CCR5/CCR5Δ32: Heterozygotes; <sup>c</sup>CCR5Δ32/CCR5Δ32: Homozygotes mutant. #OR: Odds Ratio. \*CI: Confidence Interval

## DISCUSSION

The allelic variant CCR5 $\Delta$ 32 has been widely studied in the past decades, due to its association with resistance to HIV-1 infection. Liu et al. (2004) demonstrated that both heterozygous and homozygous CCR5 $\Delta$ 32 mutations have a protective effect against HIV-1 infection, since fusion of nucleocapsid viral genetic material with the host cells does not occur. In addition, individuals exhibiting the mutation in homozygous ( $\Delta$ 32/ $\Delta$ 32) have been found to be resistant to HIV-1 infection, whereas the presence in heterozygosis slows the progression and involvement of AIDS patients (Sullivan et al., 2001).

In the state of Amazonas, as in the rest of Brazil, HIV infection is associated with sexual transmission in three main groups: men who have sex with men (MSM), sex professionals and intravenous drug users (Oliveira et al., 2015). Men aged 20 to 34 comprise the majority of those infected, being the highest number of AIDS cases (Oliveira et al., 2015). It is known that the presence of the homozygous/heterozygous CCR5 $\Delta$ 32 polymorphism may influence HIV infection and modify the epidemiological trends within a population, possibly limiting the spread of HIV-1 and reducing the number of infected individuals (Sullivan et al., 2001).

The frequency of heterozygous polymorphism was 5.7% in HIV-1<sup>+</sup> individuals in São Paulo-SP (Munerato et al., 2003) and 4.4% in Salvador-BA (Grimaldi et al., 2002). In the states of Rio de Janeiro and Rio Grande do Sul the observed frequencies of heterozygous polymorphisms were 2.1% and 2.4% in HIV-infected individuals, data similar to that observed in this study (4%) (Teixeira et al., 2009; Vieira et al., 2011).

The first Brazilian report of CCR5/CCR5 $\Delta$ 32 heterozygotes was provided by Grimaldi et al. (2002), who investigated the occurrence of this allele in different ethnic groups from different regions. They noted a variation in frequency in accordance with ethnicity. The populations described as Euro-Brazilian generally had a higher frequency (6.5% in Joinville-SC and Alegrete-RS and 9.3% in the State of Paraná). In populations described as African-Brazilian from the state of Bahia the observed frequency was 5.6% (Carvalho et al., 2004). The presence of the CCR5/CCR5 $\Delta$ 32 variant was 0.2% in Native Americans probably due to mixing with non-Indians, as noted in the Pataxó and Kaingang tribes (Vargas et al., 2006). Similar results to those observed by our group were described by Farias et al. (2012), when they studied 839 individuals in the state of Rondônia, with 2.5% total frequency observed. In a study conducted in the city of Belém-PA, the frequency of CCR5 $\Delta$ 32 seronegative individuals was 2.2% and 2.7% in seropositive individuals (Carvalho et al., 2005).



In another study, Lebouté et al. (1999), noted the absence of this polymorphism in patients with Amerindian ancestry from the Amazon region, suggesting that the CCR5 $\Delta$ 32 variant may be associated with migratory flows occurring in Brazil. In the same study, the authors describe the decreasing allele frequencies from the south (8.1%) towards the north (3.3%) of Brazil, suggesting the differential influence of European immigration in the various regions.

Although the levels of association observed are similar to other studies performed in populations of the Brazilian Amazon region, this study was limited due to the size of the population studied. Therefore these results should be validated in larger study populations to confirm these findings.

Not with standing, these results contribute to the understanding of the frequency of the CCR5/CCR5 $\Delta$ 32 variant allele in candidates for blood donation with and without HIV-1 infection from the city of Manaus-AM. In this study, heterozygous CCR5/CCR5 $\Delta$ 32 polymorphism was only noted in control subjects and in subjects in the ELISA<sup>(+)</sup>Westen Blot<sup>(Ind)</sup> and ELISA<sup>(+)</sup>Westen Blot<sup>(-)</sup> subgroups. Despite the low frequency of this mutation in this study population, the possibility that this polymorphism may be acting as a natural barrier against HIV infection cannot be excluded. However, again larger study populations need to be investigated in order to enable more accurate inferences on the influence of this polymorphism in this population.

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