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CCR5-Δ32, CCR2-64I, SDF1-3'A, and *IFNλ4* rs12979860 and rs8099917 gene polymorphisms in individuals with HIV-1, HIV/HTLV-1, and HIV/HTLV-2 in São Paulo, Brazil

Author: Adele Caterino-de-Araujo[1,*], Karoline R. Campos[1,2], Emylenne C. Cabral-de-Oliveira[1], Ana Kelly S. Rodrigues[1], Rafael X. Silva[1], Bruna V. Azevedo[1], Rosa M. N. Marcusso[3]

Abstract

Background. Chemokine and chemokine-receptor polymorphisms have been associated with protection against HIV infection and delayed progression to AIDS, whereas polymorphisms in *IFNλ4* (formerly *IL28B*) have been associated with human T-lymphotropic virus 1 (HTLV-1)-associated myelopathy (HAM) development. Evolutionary selection against ancestral genes differs among human populations, resulting in varying risks of acquiring and developing viral diseases. **Methods**. DNA samples from 434 patients infected with HIV-1 and/or co-infected with HTLV-1/-2, and samples from 74 HIV and HTLV non-infected individuals from São Paulo, Brazil, were divided into five groups: HIV-naïve, n=160; HIV-ART, n=180; HIV/HTLV-1, n=53; HIV/HTLV-2, n=41; and control, n=74. These samples were analyzed for *CCR5*-Δ32 deletion, *CCR2-64I, SDF1-3'*A, and *IFNλ4* rs12979860 and rs8099917 single nucleotide polymorphisms using PCR and PCR-RFLP techniques. These polymorphisms' genotype and allele frequencies were calculated and compared among groups using logistic regression analysis. **Results**. All polymorphism profiles described in the literature were detected in this study. The wild-type genotype predominated in all genes analyzed except for *IFNλ4* rs12979860. Statistical differences in allele frequencies among groups were detected in the *CCR5* and *CCR2* genes, with a high frequency of Δ32 in HIV-naïve vs. HIV-ART (OR 2.45, *P*=0.037) and a minus mutant allele A (*CCR2-64I*) in HIV-naïve vs. HIV/HTLV-1 (OR 1.90, *P*=0.048), HIV-ART vs. HIV/HTLV-1 (OR 2.62, *P*=0.003), and HIV/ART vs. HIV/HTLV-2 (OR 2.42, *P*=0.016). **Conclusions**. The polymorphism profiles detected in the study groups corroborate the profiles described in racial admixed populations. High *CCR2-64I* mutant allele frequencies were detected in HIV/HTLV-1/-2 co-infected individuals, and *CCR5*-Δ32 showed predictive value for ART initiation.

Key word: HIV-1, HTLV-1/2, chemokine, chemokines receptors, *IFNλ4*, polymorphisms.

[1]Instituto Adolfo Lutz, Centro de Imunologia, Laboratório de Pesquisa em HTLV, São Paulo, SP, Brasil.
[2]Instituto Adolfo Lutz, Centro de Respostas Rápidas, Laboratório Estratégico, São Paulo, SP, Brasil.
[3]Instituto de Infectologia Emilio Ribas, Serviço de Neurologia Clínica, Ambulatório de HTLV, São Paulo, SP, Brasil.
*https://orcid.org/0000-0003-0155-6580

Corresponding author:
Adele Caterino de Araujo
Address: Centro de Imunologia, Instituto Adolfo Lutz, Av. Dr. Arnaldo, 351, 11º andar, CEP 01246-000, Pacaembu, São Paulo, SP., Brasil. Telefone: +55-11-30682898.
E-mail: adele.caterino@ial.sp.gov.br

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Introduction

Studies have highlighted the importance of individual genetic backgrounds in disease outcomes by reporting the influence of genetic markers on the host immune response against viral infections, such as human immunodeficiency virus 1 (HIV-1), hepatitis C virus (HCV), and human T-lymphotropic virus 1 (HTLV-1). Evolutionary selection against ancestral genes varies across human populations(1-2), resulting in a varied risk of acquiring and developing such viral diseases(3-9).

Chemokine and chemokine-receptor polymorphisms have been associated with protection against HIV infection and progression to AIDS (*CCR5-Δ32, CCR2-64I, SDF1-3'A*), but their frequencies vary by regions/populations(10-12).

A homozygous mutation in the HIV-1 CCR5 co-receptor (deletion of 32 nucleotides in *CCR5* gene; *CCR5*-Δ32 polymorphism) blocks the entry of HIV-1 R5 strains into cells, whereas heterozygosity delays this infection(4,5).

A single nucleotide polymorphism (SNP) in the *CCR2* HIV-1 co-receptor gene (*CCR2-64I*; nucleotide transition G>A at codon 64, encoding isoleucine (ATC) instead of valine (GTC)) has been associated with protection against HIV and other inflammatory disease progressions(13,14). This is due to its ability to dimerize with CXCR4 and/or CCR5, reducing CXCR4 and CCR5 levels in peripheral blood mononuclear cells (PBMC) and delaying the progression to seroconversion in HIV-infected donors carrying this mutation(15).

Similarly, high concentrations of the chemokine SDF1 block or delay HIV entry by binding to the HIV co-receptors CCR5 and CXCR4. The G>A nucleotide change in the *SDF1* gene (*SDF1-3'A* polymorphism) has been associated with high

production of this chemokine and conferred protection against HIV infection(6,16).

Polymorphisms in other protein genes, formerly known as *IFNL3/IL28B* and presently as *IFNλ4* (nucleotide transition C>T in rs12979860 and T>G in rs8099917), in patients with hepatitis C have been associated with reduced HCV clearance and poor response to PEG-IFNα treatment(7,17,18). Interestingly, the same polymorphisms in patients infected with HTLV-1 are associated with developing HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)(19-21).

The frequencies and functions of such polymorphisms are widely known, predominantly for HIV and HCV infections and HIV/HCV coinfections(22,23); however, there is a lack of studies on HIV/HTLV coinfections. The present study aimed to determine the frequencies of these polymorphisms in HIV/HTLV-1 and HIV/HTLV-2 co-infected patients from São Paulo, Brazil, and to compare them with the frequencies obtained in HIV mono-infected, naïve, and antiretroviral therapy (ART) patients, along with HIV and HTLV-1/2 non-infected individuals from the same geographical region.

Methods

Study design, population, samples, and groups for analysis

This study was conducted as an anonymous, cross-sectional, and descriptive study using DNA samples previously used for the molecular diagnosis, characterization, and surveillance of HTLV-1 and HTLV-2 in individuals infected with HIV in São Paulo, Brazil(24-27). DNA samples were obtained from patients attending the AIDS/STD Reference and Training Center in São Paulo (CRT DST/AIDS-SP) and the HTLV Research Laboratory of the Immunology Department of Instituto Adolfo Lutz (IAL) in São Paulo, SP. Blood samples were collected from 2013 to 2016, separated into plasma and peripheral blood leukocytes (PBL), divided into aliquots, and stored at -80 °C for subsequent use. We randomly selected available DNA samples from 340 HIV-1 mono-infected patients, 94 HIV/HTLV-1/2 co-infected patients, and 74 HIV and HTLV-1/2 seronegative individuals to search for polymorphism in chemokine, chemokine receptors, and *IFNL4* genes and grouped these samples into five groups for analyses: HIV-naïve, 160 HIV-infected patients without ART; HIV-ART, 180 HIV-infected patients on ART, matched for sex/age and time of HIV infection with HIV-naïve patients; HIV/HTLV-1, 53 patients infected with HIV and HTLV-1; HIV/HTLV-2, 41 patients infected with HIV and HTLV-2; control, 74 individuals HIV and HTLV-1/2 seronegative (20 from the staff and 54 from the laboratory routine). Patient characteristics (age and sex) and color/race data were obtained from interviews, medical records (CRT DST/AIDS-SP), and routine medical requests (IAL). Unfortunately, the color/race data was unavailable for HIV-and/or HTLV-infected individuals and controls since this characteristic is not included in routine medical requests. Thus, this variable was unavailable in 38 HIV-naïve, 23 HIV-ART, 30 HIV/HTLV-1, and 26 HIV/HTLV-2 patients, as well as for all individuals in the control group (**Table 1**).

Polymorphism analyses were conducted at the HTLV Research Laboratory (IAL). Informed consent was obtained from patients and controls. The study was approved by the Ethics Committee for Research of IAL (Ministry of Health protocol numbers CAAE #55837316.0.0000.0059 and #52493316.1.0000.0059). The data were analyzed anonymously.

Table 1. Characteristics of the study groups employed for the evaluation of polymorphisms in *CCRΔ532, CCR2-64I, SDF1-3'A*, and *IFNλ4* rs12979860 and rs8099917 genes

Variables	HIV-Naïve n=160	Groups				*P* value
		HIV-ART	HIV/HTLV-1	HIV/HTLV-2	Control	
		n=180	n=53	n=41	n=74	
Gender, n (%)						
Male	131 (81.9)	135 (75.0)	30 (56.6)	21 (51.2)	31 (41.9)	<0.001[b]
Female	29 (18.1)	45 (25.0)	23 (43.4)	20 (48.8)	43 (58.1)	
Age, years (mean, 95% CI)						
Male	34.9 (33.3–36.6)	36.4 (34.7–38.1)	46.9 (43.2–50.6)	48.9 (45.6–52.2)	40.7 (33.8–47.6)	
Female	38.6 (34.9–42.4)	42.6 (40.1–45.2)	46.7 (41.8–51.6)	49.0 (45.4–52.7)	33.2 (28.1–38.3)	<0.001[c]
***P* value**	0.067[d]	<0.001[d]	0.941[d]	0.883[d]	0.069[d]	
Color/race, n (%)[a]						
White	72 (59.0)	100 (63.7)	10 (43.5)	6 (40.0)		
Black and *pardum*	48 (39.4)	54 (34.4)	11 (47.8)	9 (60.0)		0.139[b]
Amerindian	1 (0.8)	0	1 (4.35)	0		
Japanese	1 (0.8)	3 (1.9)	1 (4.35)	0		

n, number of individuals; CI, confidence interval; [a] frequencies calculated only in cases with available information; [b] *P* values calculated using X^2 of the Pearson test, [c] nonparametric Kruskal-Wallis test (three or more groups), and [d] Mann-Whitney U test (two groups)

DNA Extraction

Genomic DNA was extracted and purified from PBL using the Roche MagNA Pure RLC Robot Instrument (Mannheim, Germany) with the LC MagNA Pure Nucleic Acid Isolation Kit I (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions and eluted with 100 µL of PCR-grade water. The extracted DNA was aliquoted into four vials for subsequent analysis and thawed once.

Characterization of *CCR5*-Δ32, *CCR2-64I*, *SDF1-3'A*, and *IFNλ4* rs12979860 and rs8099917 polymorphisms

Table 2 presents the sequences of primers and restriction enzymes used in the *CCR5*-Δ32, *CCR2-64I*, *SDF1-3'A*, and *IFNλ4* rs12979860 and rs8099917 gene polymorphism analyses, the fragment size of each genotype, and the authors' reference number.

For *CCR5*-Δ32 genotype characterization, the PCR reaction mixture contained 20 pmol of each primer, 2.5 X Go*Taq* colorless master mix (Promega), 2.5 µL of genomic DNA, and H2O in a total volume of 25 µL. The cycling amplification consisted of 5 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min; 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, followed by 1 cycle of 72 °C for 10 min. A 10 µL aliquot of each 25 µL PCR reaction mixture was run on a 3 % agarose gel, electrophoresed, and the appropriately sized products were visualized under UV illumination after staining with SYBR™ Safe DNA Gel Stain (Invitrogen)(28) (**Table 2**).

The PCR conditions for *CCR2-64I* were as follows: 10 pmol of each primer, 2.5 X Go*Taq* colorless master mix (Promega), 2.5 µL of genomic DNA, H2O, in a total volume of 25 µL. The cycling conditions were as follows: 94 °C for 10 min, followed by 40 cycles at 94 °C, 56.5 °C, and 72 °C for 1 min at each temperature, followed by 72 °C for 7 min. For the *CCR2-64I* mutation detection, the amplified product was digested with the *Bsa*BI restriction endonuclease (New England, BioLabs Inc.) at 72 °C for 4 h, and then the PCR amplification product was run on a 2 % agarose gel electrophoresis stained with SYBR™ Safe DNA Gel Stain (Invitrogen)(14). For *SDF1-3'A* polymorphism detection, the PCR mix was the same as for *CCR2-64I*, except that 20 pmol of each primer was used. The cycling scheme consisted of 1 cycle at 94 °C for 10 min, followed by 35 cycles at 94 °C, 55 °C, and 72 °C for 30 s at each temperature, followed by 72 °C for 7 min. After amplification, the PCR product was digested with the restriction endonuclease *MspI* (New England, BioLabs Inc.) at 37 °C for 4 h, and then run on a 3% agarose gel electrophoresis stained with SYBR™ Safe DNA Gel Stain (Invitrogen)(29). The size products in base pairs (bp) for different *CCR2* and *SDF1* genotypes are presented in Table 2. The bands of 23 bp in *CCR2-64I* genotypes GA and AA, and bands of 30 bp in *IFNλ4* rs12979860 genotypes CT and TT were not visualized in agarose gel electrophoresis.

Regarding the polymorphisms in *IFNλ4* (*IL28B*) rs12979860 and rs8099917, the PCR reactions consisted of 5 µL of genomic DNA, 1 U of Platinum® Taq DNA polymerase (Invitrogen, São Paulo, Brazil), 200 µM of each deoxynucleoside triphosphate (GE Healthcare, Little Chalfont, UK), 2.5 mM of MgCl2 (Invitrogen), PCR buffer (20 mM Tris–HCl, pH 8.4; 50 mM KCl) and 10 pmol of each primer in a total volume of 50 µL. The reaction for rs12979860 amplification was enhanced by the addition of 5% dimethyl

Table 2. PCR primers and restriction enzymes employed for the evaluation of polymorphisms in *CCR532*, *CCR2-64I*, *SDF1-3'A*, and *IFNλ4* (IL28B) rs12979860 and rs8099917 genes

Polymorphism	Primer	Sequence 5'- 3'	Tm (°C)	Direction	Product size (bp)	Restriction enzyme, time, temperature	Recognition site	Genotype	Fragment (bp)	Ref
CCR5Δ32	P1 (2975)	AAC AGA TCT CAA AAA GAA GGT CT	55	Sense	189	NA	NA	wt/wt	189	28
	P2 (2976)	CAT GAT GGT GAA GAT AAG CCT CAC A		Antisense				wt/Δ32	189 + 157	
								Δ32/Δ32	157	
CCR2-64I	CKR2IAF	TTG TGG GCA ACA TGA TGG	56.5	Sense	183	*Bsa* BI, 4 h, 60 °C	GATNN^NN ATC	G/G	183	14
	CCR264IR	CTG TGA ATA ATT TGC ACA TTG C		Antisense				G/A	183 + 165 + 23	
								A/A	165 + 23	
SDF1-3'A	SDF1 F	CAG TCA ACC TGG GCA AAG CC	55	Sense	302	*Msp* I, 4 h, 37 °C	C^CGG	G/G	202 + 100	29
	SDF1 R	AGC TTT GGT CCT GAG AGT CC		Antisense				G/A	302 + 202 + 100	
								A/A	302	
***IFNλ4* rs12979860**	IL28B-860F	AGC AGG ACA GAT TGG CAA AG	59	Sense	694	*Hpy* 166II, 2 h, 37 °C	GTN^NAC	C/C	509 + 185	30
	IL28B-860R	CAC AAT TCC CAC CAC GAG AC		Antisense				C/T	509 + 185 + 155 + 30	
								T/T	509 + 155 + 30	
***IFNλ4* rs8099917**	IL28B-917F	CTG GAA CAA ATC GTC CCA AT	57.5	Sense	496	*Bsr* DI, 2 h, 65 °C	GCAATG^NN	T/T	496	30
	IL28B-917R	TTC CTT TAG GCC TGT GGA TG		Antisense				T/G	496 + 272 + 224	
								G/G	272 + 224	

Tm, melting temperature; bp, base pair; Ref, Reference; NA, not applicable; h, hour; wt, wild type; Δ32, 32 nucleotide deletion; A, adenine; G, guanine; C, cytosine; T, thymine.

sulfoxide. The annealing temperature was 59 °C for rs12979860 and 57.5 °C for rs8099917, and the thermal cycling scheme was as follows: 94 °C for 3 min, followed by 40 cycles at 94 °C for 30 s, 59 or 57.5 °C for 30 s, 72 °C for 1 min, and 72°C for 7 min. The amplicon corresponding to rs12979860 was digested with *Hpy*166II, and rs8099917 was digested with *Bsr*DI (both from New England BioLabs Inc.). Incubations were performed at 37 °C (*Hpy*166II) and 65 °C (*Bsr*DI) for 2 h, and the products of digestion with *Hpy*166II were separated through 3 % agarose gel electrophoresis for 2 h, and for *Bsr*DI through 1.5 % agarose gel electrophoresis for 1.5 h (protocols adapted from Moreira et al.)(30). The product sizes are presented in **Table 2**.

Statistical Analyses

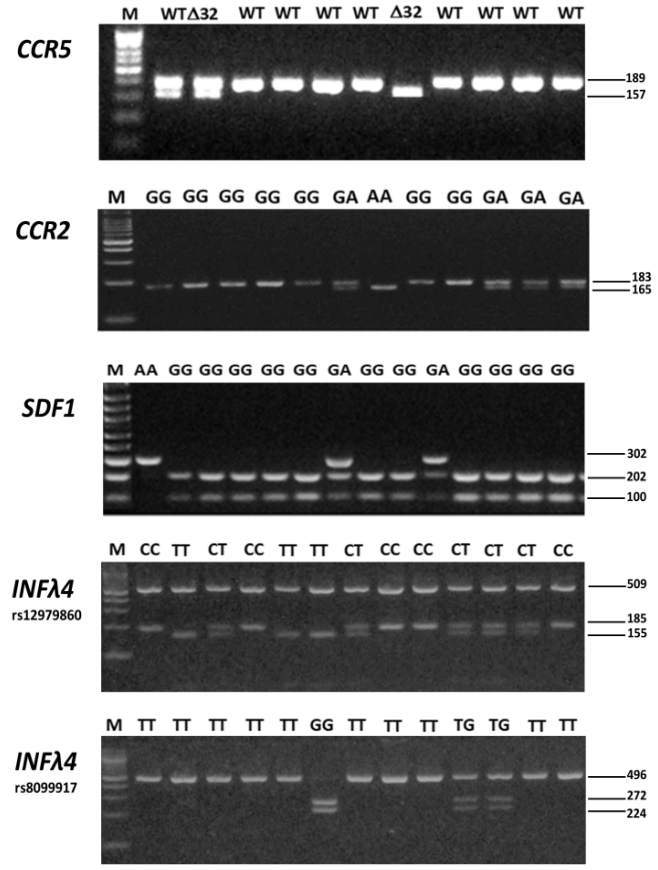
Differences in the characteristics of individuals regarding sex and race/color were evaluated using the Chi-square (X^2) of the Pearson test, and age was evaluated using the nonparametric Kruskal-Wallis test (for three or more groups) and Mann-Whitney U test (for two groups). Allele frequency was determined using the formula f = (1 × h + 2 H) / 2 N, where h is the number of heterozygotes, H is the number of homozygotes, and N is the total number of individuals. Differences in genotype and allele frequencies among groups were evaluated using the X^2 of the Pearson test and logistic regression univariate analysis by calculating the odds ratio (OR) and 95% confidence interval (CI). The level of significance was set at P <0.05. Data were analyzed using SPSS® Statistics 29 (Statistical Package for the Social Sciences, 29.0), (Statistical Software: IBM, NY, USA).

Results

Table 1 shows the characteristics of the study groups with statistically significant differences. For instance, the HIV-naïve and HIV-ART groups included more males than females, while the HIV/HTLV-1 and HIV/HTLV-2 groups included more black/*pardum* individuals. Regarding age, HIV/HTLV-1 and HIV/HTLV-2 co-infected patients were older, regardless of being male or female. In the HIV-ART group, women were older than men (P<0.001). The comparison of ages between the two groups revealed statistically significant differences: HIV-naïve vs. HIV-ART (P=0.048); HIV-naïve vs. HIV/HTLV-1 and HIV/HTLV-2 (both P<0.001); HIV-ART vs. HIV/HTLV-1 and HIV/HTLV-2 (both P<0.001); HIV-ART vs. control (P=0.017); and HIV/HTLV-1 and HIV/HTLV-2 vs. control (both P<0.001). No differences in age were detected between the HIV/HTLV-1 vs. HIV/HTLV-2 (P=0.312) groups or the HIV-naïve vs. control (P =0.408) groups.

This study detected all the polymorphism profiles described in the literature (**Figure 1**). The wild-type genotype was prevalent in all genes analyzed except for *IFNλ4* rs12979860. The genotypes and allele frequencies of the polymorphisms are shown in **Table 3**. Preliminary analyses of the polymorphisms in *CCR5* showed differences in genotype frequencies (P=0.036) and high Δ32 alleles in HIV-naïve, HIV/HTLV-2, and control groups, but without significant differences among them (P=0.154). In *CCR2* polymorphism analysis, differences in genotype (P=0.011) and allele frequencies (P<0.001) were detected, and more allele A mutants were identified in the HIV/HTLV-1 and HIV/HTLV-2 groups compared to the control group. No difference in genotype and allele frequencies in *SDF1* and *IFNλ4* rs12979860 and rs8099917 were detected (all P >0.05, **Table 3**).

Figure 1. Electrophoretic representative patterns of all genotypes related to *CCR5*Δ*32, CCR2-64I, SDF1-3'A*, and *IFNλ4* rs12979860 and rs8099917 polymorphisms detected in the present study



Legend: M: molecular size marker (100 bp Ladder) except in CCR5 (50 bp Ladder); CCR5 – WT: wt/wt wild genotype, WTΔ32: wt/Δ32 polymorph genotype, Δ32: Δ32/Δ32 polymorph genotype; *CCR2 – GG*: wild genotype, GA and AA polymorph genotypes; *SDF1- GG*: wild genotype, GA and AA polymorph genotypes; *IFNλ4* rs12979860 – CC: wild genotype, CT and TT polymorph genotypes; *IFNλ4* rs8099917 – TT: wild genotype, TG and GG polymorph genotypes.

Using logistic regression analysis, statistically significant differences among groups were confirmed only in the *CCR5* and *CCR2* genes (**Table 4**), and all analyses are presented in the **Supplementary Table**. Briefly, higher Δ32 allele frequencies were detected in the HIV-naïve, HIV/HTLV-2, and control groups than in the HIV-ART group, with statistical significance only when comparing HIV-naïve vs. HIV-ART (P=0.037). However, a tendency towards significance was observed between the HIV-ART and HIV/HTLV-2 groups (P=0.061), and no difference was observed between the HIV-ART and control groups (P=0.112). Interestingly, although Δ32 allele frequencies differ between HIV/HTLV-1 and HIV/HTLV-2 (0.029 and 0.074, respectively), no significant difference was detected using regression analysis (P=0.189). Regarding *CCR2*, high allele A mutant frequencies were detected in the HIV/HTLV-1 (0.198) and HIV/HTLV-2 (0.186) groups, a more modest frequency in the HIV-naïve group (0.115), and low

frequencies in the HIV-ART (0.086) and control groups (0.047). However, significant differences were detected between HIV-naïve vs. HIV/HTLV-1 (*P*=0.048), HIV-naïve vs. control (*P*=0.028), HIV-ART vs. HIV/HTLV-1 (P=0.003), and HIV-ART vs. HIV/HTLV-2 (*P*=0.016). All other genes displayed similar percentages of allele frequencies among groups without significant differences, except for a tendency towards significance when *IFNλ4* rs12979860 T allele was compared between HIV-ART and HIV/HTLV-2 (*P*=0.059).

Table 3. Genotypic and allelic frequencies of *CCR5Δ32, CCR2-64I, SDF1-3'A*, and *IFNλ4* rs12979860 and rs8099917 genes polymorphisms according to the study groups

	HIV-Naïve	HIV-ART	HIV/HTLV-1	HIV/HTLV-2	Control	X^2 *P* value
CCR5D32	n=156 n (%)	n=155 n (%)	n=52 n (%)	n=34 n (%)	n=71 n (%)	
Genotype						
wt/wt	137 (87.8)	148 (95.5)	50 (96.2)	30 (88.24)	63 (88.7)	
wt/Δ32	19 (12.2)	6 (3.9)	1 (4.6)	3 (8.82)	8 (11.3)	**0.036**
Δ32/Δ32	0	1 (0.6)	1 (4.6)	1 (2.94)	0	
Allele						
wt	0.939	0.974	0.971	0.926	0.934	
Δ32	0.061	0.026	0.029	0.074	0.056	0.154
CCR2-64I (G>A)	n=122 n (%)	n=157 n (%)	n=48 n (%)	n=35 n (%)	n=74 n (%)	
Genotype						
G/G	97 (79.5)	131 (83.5)	31 (64.5)	23 (65.7)	67 (90.5)	
G/A	22 (18.0)	25 (15.9)	15 (31.3)	11 (31.4)	7 (9.5)	**0.011**
A/A	3 (2.5)	1 (0.6)	2 (4.2)	1 (2.9)	0	
Allele						
G	0.885	0.914	0.802	0.814	0.953	
A	0.115	0.086	0.198	0.186	0.047	**<0.001**
SDF1-3'A (G>A)	n=104 n (%)	n=145 n (%)	n=45 n (%)	n=33 n (%)	n=69 n (%)	
Genotype						
G/G	68 (65.4)	98 (67.6)	31 (68.9)	22 (66.7)	48 (69.6)	
G/A	34 (32.7)	45 (31.0)	14 (31.1)	9 (27.3)	21 (30.4)	0.565
A/A	2 (1.9)	2 (1.4)	0	2 (6.0)	0	
Allele						
G	0.817	0.831	0.844	0.803	0.848	
A	0.183	0.169	0.156	0.197	0.152	0.907
INFλ4 /IL28B SNP rs12979 860 (C>T)	n=142 n (%)	n=158 n (%)	n=28 n (%)	n=20 n (%)	n=71 n (%)	
Genotype						
C/C	62 (43.7)	81 (51.3)	11 (39.3)	9 (45.0)	33 (46.5)	
C/T	57 (40.1)	59 (37.3)	13 (46.4)	4 (20.0)	31 (43.7)	0.131
T/T	23 (16.2)	18 (11.4)	4 (14.3)	7 (35.0)	7 (9.8)	
Allele						
C	0.637	0.699	0.625	0.55	0.683	
T	0.363	0.301	0.375	0.45	0.317	0.23
IFNλ4/IL28B SNP rs8099917 (T>G)	n=143 n (%)	n=158 n (%)	n=28 n (%)	n=20 n (%)	n=74 n (%)	
Genotype						
T/T	92 (64.3)	106 (67.1)	20 (71.4)	14 (70.0)	54 (73.0)	
T/G	47 (32.9)	48 (30.4)	7 (25.0)	4 (20.0)	18 (24.3)	0.64
G/G	4 (2.8)	4 (2.5)	1 (3.6)	2 (10.0)	2 (2.7)	
Allele						
T	0.808	0.823	0.839	0.8	0.851	
G	0.192	0.177	0.161	0.2	0.149	0.823

Note: The number of samples analyzed for each polymorphism in the same group varied owing to insufficient amounts of DNA and/or no PCR amplification.

Table 4. *CCR5Δ32* and *CCR2-64I* gene polymorphisms that were significant predictors of HIV infection/progression using logistic regression univariate analysis in the study groups

Gene locus	Allele frequency		Genotype frequency			OR (95% CI)
			n (%)	n (%)	-/- n (%)	*P* value
CCR5D32	wt	Δ32	wt/wt	wt/Δ32	Δ32/Δ32	
HIV-naïve(n=156)	0.939	0.061	137 (87.8)	19 (12.2)	0	2.45 (1.06–5.68)
HIV-ART (n=155)	0.974	0.026	148 (95.5)	6 (3.9)	1 (0.6)	0.037
CCR2-64I	G	A	GG	GA	AA	
HIV-naïve(n=122)	0.885	0.115	97 (79.5)	22 (18.0)	3 (2.5)	1.90 (1.01–3.60)
HIV-HTLV-1 (n=48)	0.802	0.198	31 (64.5)	15 (31.3)	2 (4.2)	0.048
HIV-naïve(n=122)	0.885	0.115	97 (79.5)	22 (18.0)	3 (2.5)	2.61 (1.11–6.14)
Control (n=74)	0.953	0.047	67 (90.5)	7 (9.5)	0	0.028
HIV-ART (n=157)	0.914	0.086	131 (83.5)	25 (15.9)	1 (0.6)	2.62 (1.38–4.97)
HIV-HTLV-1 (n=48)	0.802	0.198	31 (64.5)	15 (31.3)	2 (4.2)	0.003
HIV-ART (n=157)	0.914	0.086	131 (83.5)	25 (15.9)	1 (0.6)	2.42 (1.18–4.98)
HIV-HTLV-2 (n=35)	0.814	0.186	23 (65.7)	11 (31.4)	1 (2.9)	0.016
HIV-HTLV-1 (n=48)	0.802	0.198	31 (64.5)	15 (31.3)	2 (4.2)	4.97 (2.00–12.35)
Control (n=74)	0.953	0.047	67 (90.5)	7 (9.5)	0	<0.001
HIV-HTLV-2 (n=35)	0.814	0.186	23 (65.7)	11 (31.4)	1 (2.9)	4.59 (1.74–12.11)
Control (n=74)	0.953	0.047	67 (90.5)	7 (9.5)	0	0.002

n, number of DNA samples; OR, Odds ratio; CI, confidence interval.

Discussion

The heterogeneous population residing in the city of São Paulo (Southeastern Brazil) reflects the high frequency of racial inter-mixing over time, firstly between Amerindians, Europeans and Africans, and subsequently with migrants from other regions of the country and elsewhere. Thus, the ethnicity of this population varies greatly, and although it should be necessary to use more objective methods to evaluate the patients' ethnic background to measure ancestry, the genetic polymorphism results obtained in the present study corroborate the polymorphisms disclosed in an admixed population. The present study uses self-reported and phenotypic racial features to define race/color.

The age and sex of the study groups were variable (**Table 1**). The HIV/HTLV-1/2 co-infected patients had (i) high age, (ii) approximately equal proportions of males and females (except when compared with the control group), and (iii) a majority of black/*pardum* color/race individuals. These characteristics have been extensively analyzed in previous studies(24,25,31), corroborating the characteristics of HTLV-1/2 patients in Brazil(32-34).

Concerning *CCR5-Δ32* frequencies, the mutant allele is common in North America and Europe, with heterozygote frequencies ranging from 10 to 20 % in Caucasians and 2 to 5 % in the Middle East and Indian populations(4,10). This allele is not found in aboriginal populations outside Eurasia, and its presence elsewhere probably represents European gene flow into local populations(10). This is the case in Brazil, where the population is predominantly of European ancestry with significant contributions from African and Amerindian gene pools. Since the Δ32 alleles have not been identified in Africans or Amerindians, the presence of the *CCR5-Δ32* in Brazilians can be attributed to European immigration. In effect, one study conducted in Belém, PA (Northern Brazil), showed Δ32 allele frequencies of 3.04 % in the general population, 0.75 % in Afro-descendants, and 0 % in Amerindians and Japaneses(35), and

2.7 and 2.2 % in HIV positive and negative individuals, respectively(36). In Salvador, BA, among blood donors, the Δ32 alleles were detected at a frequency of 2.6 %, while in HIV-infected individuals without ART, it was 4.4 %(37). German-descendant blood donors from Joinville, SC (Southern Brazil) had a Δ32 allele frequency of 6.5 %, while among Tiriyó and Waiampi Amerindian tribes from the Amazon River basin (North region), this allele has not been found(37). In Belo Horizonte, MG (Southeast region), among unrelated healthy individuals, the Δ32 allele frequency was 5.3 %, while in blood donors screened for HTLV infection, the frequencies varied: 7.0 % in HTLV-1 seronegative, 5.7 % in HTLV-1 serum indeterminate, 4.1 % in HTLV-1 seropositive asymptomatic carriers, and 0% in HAM(38). In patients with colorectal cancer from São Paulo, SP, the Δ32 allele frequency was 5.2 %(38). A systematic review and meta-analysis of 30 different healthy populations from the north-northeast (N-NE) and south-southeast (S-SE) regions of Brazil found Δ32 allele frequencies ranging from 0 to 10 %, with an average of 3 and 4 % in the N-NE and S-SE populations, respectively (*P*=0.002)(39). Considering these data, the Δ32 allele frequencies detected in the present study (2.9 to 7.4%, **Table 3**) agree with the frequencies detected in mixed-race populations living in Brazil and with European ancestry in São Paulo.

Regarding the functional consequences of *CCR5*-Δ32 polymorphism in HIV-1 infected patients, one study from Salvador showed a minor HIV viral load (VL) and *in vitro* partial resistance to R5-HIV-1 strains in the PBMC of heterozygote individuals(37). In Rio Grande, RS (Southern Brazil), among HIV-infected patients followed for an average of 6.4 years, the presence of *CCR5*-Δ32 was associated with a reduction in the risk of CD4+ T-cell depletion and an increased risk of death after AIDS diagnosis(40). In São Paulo, Δ32 allele frequencies of 3.8 and 5.5% were detected in HIV-1-infected and healthy individuals, respectively. Multivariate regression analysis identified an association between *CCR5-D32/CCR2-V64I* polymorphisms and positive CD4+ T cell recovery after ART(41). Herein, although the cross/sectional design did not allow concluding protection against HIV infection and delayed progression to AIDS, the higher Δ32 allele frequency in HIV-naïve (6.1 %) compared to HIV-ART (2.6 %) (OR 2.45, *P*=0.0037, (**Table 4**) confirms the prognostic value of the Δ32 allele for ART (remembering that both groups are matched for sex, age, and time of HIV acquisition). Notably, a tendency toward a statistical difference was detected when comparing the HIV-ART vs. HIV/HTLV-2 groups (OR=2.99, *P*=0.061, **Supplemental Table**). Although both groups received ART, HIV/HTLV-2 co-infected subjects were older and more likely to have HIV and HTLV-2 infections than HIV-ART subjects(24,25). Furthermore, HTLV-2 in HIV-infected subjects has been associated with delayed progression to AIDS and death(42), lower HIV VL, and higher CD4+ T cell counts compared to HIV/HTLV-1 co-infected subjects(43). Thus, the Δ32 allele could be another protective factor that delayed HIV disease progression in HIV/HTLV-2 co-infected subjects.

Regarding the *CCR2-64I* polymorphism, these genetic variants are observed in almost all populations studied. The mutant (G>A) allele frequencies are the highest (approximately 35 %) in Africa and Asia, decrease in Northern Europe, in Caucasian-Americans, and are low in Pacific Islander populations(11), contrasting with *CCR5*-Δ32 polymorphism distribution(10). In Brazil, the *CCR2-64I* allele frequencies vary according to the population analyzed, reaching 14 % in HIV non-infected subjects from Salvador (80 % of which are African descendants), 18 % in German descendants from the Joinville Regional Hemocenter, 26 % in Tiriyó and 30 % in Waiampi Indian tribes from Amazonia(44). In Belém, PA, the *CCR2-64I* frequencies were 16.08 % in the general population, 22.95 % in Afro-descendants, 13.27 % in Amerindians, 24 % in Japanese(35), 5.4 % in HIV-infected, and 12.5 % in HIV uninfected subjects, the last data suggesting a protective effect of such gene polymorphism in HIV acquisition(36). In the present study, differences in *CCR2-64I* frequencies were detected, with emphasis on HIV/HTLV co-infected patients who presented the highest frequencies (19.8 % in HIV/HTLV-1 and 18.6 % in HIV/HTLV-2, **Table 3**), correlating with the percentage detected in Afro-descendants from Northern Brazil(35), and the black/*pardum* color/race of this group of patients(24). In contrast, the lowest frequency of *CCR2-64I* was detected in the control group (4.7 %, **Table 3**), specifically in the group where colors/races were not disclosed. The majority were probably white or Caucasian, similar to most employees of the IAL laboratory.

Studies on *CCR2-64I* polymorphism and HIV outcome are scarce in Brazil; one study of 6.4 years follow-up from Rio Grande, RS, found 11.3 % *CCR2-64I* allele frequency and the association of this polymorphism with a reduced risk for developing AIDS(40). In São Paulo, SP, although the frequencies of *CCR2-64I* did not differ between HIV-infected (14 %) and HIV-uninfected (11 %) subjects, the presence of both *CCR2-64I/CCR5*-Δ32 polymorphisms was associated with positive recovery of CD4+ T cells after ART(41). Unfortunately, herein, it was not possible to associate *CCR2-64I* with HIV disease progression but rather with the diversity of the genetic backgrounds of individuals residing in São Paulo. In fact, as mentioned above, the highest frequencies of such polymorphism were detected among HIV/HTLV-1/2 co-infected individuals (**Table 3**), just the groups with more black/*pardum* individuals (**Table 1**). However, it is important to emphasize that these groups of patients had more age and consequently more years of retrovirus infections (more than 20 years of HIV infection), as previously described(24).

In Brazil, in the *SDF-1-3'A* polymorphism, the mutant *3'A* allele frequencies vary according to region/population: in the North, it was detected in 22.33 % of healthy individuals from Belém, 15.33 % of Afro-descendants, 22.74 % of Amerindians, 26 % of Japaneses(35), 15.4 % of HIV-infected, and 21 % of HIV uninfected subjects(36), as well as 24 % in Tiriyó and 5 % in Waiampi Indian tribes(16). In other regions, it was detected in 17 % of healthy subjects from Salvador, 21 % from Joinville(37), 20.7 % of HIV-infected patients from Rio de Janeiro(40), and 14 % of HIV-infected patients from São Paulo(41). These frequencies agree with the frequencies detected in this study in HIV-infected and HIV/HTLV-co-infected individuals (15.6 to 19.7 %, **Table 3**). In addition, an association between *SDF1-3'A/CCR2-64I* polymorphisms and a

minor risk for developing AIDS was detected in a study conducted in Rio de Janeiro(40), but the same result was not observed in a study conducted in São Paulo(41). Notably, one longitudinal study of at least 6 years in HIV-infected patients from the Amazon region found an association of the *3'A* allele variant with a marked loss of CD4+ T lymphocytes and high plasma VL(45), emphasizing the need of more studies for this issue in Brazil.

Regarding the polymorphisms in *IFNλ4* rs12979860 (C>T) and rs8099917 (T>G) and HAM development, one study of Spanish HTLV-1 infected subjects (12 with HAM and 29 asymptomatic carriers) associated *IL28B* rs12979860 polymorphisms CT/TT with HAM and high HTLV-1 proviral load (PVL)(46). In contrast, another study conducted in São Paulo with 112 unrelated Brazilian subjects (81 HTLV-1 asymptomatic carriers, 24 with HAM/TSP, and 7 with Adult T cell leukemia/lymphoma) did not support these associations; neither the homozygote TT nor the heterozygote CT mutations nor the combination genotypes (TT/CT) were associated with high PVL and HAM/TSP(47). Similarly, no significant differences were observed in Belém, PA, when comparing 26 HAM/TSP patients, 53 asymptomatic carriers, and 300 seronegative healthy subjects(48). In contrast, two studies from São Paulo associated IL28B rs8099917 genotype GG with HAM/TSP(20,21); the first included 229 subjects (136 HTLV-1 asymptomatic carriers and 93 HAM/TSP)(20), and the other 247 subjects (160 HTLV-1 asymptomatic carriers and 87 HAM/TSP)(21). In the present study, there were no differences in *IFNλ4* rs12979860 and rs8099917 polymorphisms frequencies among groups, nor the case of HAM in HIV/HTLV co-infected patients(24). But, a tendency toward a statistical difference was detected when comparing the frequencies of *IFNλ4* rs12979860 polymorphism in HIV-ART vs. HIV/HTLV-2 groups (OR=1.90, *P*=0.059, **Supplemental Table**). Curiously, the HIV-ART group showed the highest percentage of *IFN-λ4* rs12979860 wild-type genotype (CC), while the HIV/HTLV-2 had the highest percentage of the mutant genotype (TT). Recently, one study of 30 years follow-up conducted with a cohort of 402 HIV-1-infected subjects from São Paulo disclosed an association of *IFN-λ4* rs12979860 wild-type genotype (CC) with higher mortality rate compared to CT and TT genotypes and with an increased probability of death from AIDS (*P* =0.01)(49). The authors suggested that this effect may be related to higher baseline plasma HIV VL and altered immune reconstitution, associated with lack of interferon λ expression in that population(49). Unfortunately, we do not know the HIV infection disease outcome of patients from the present study, but taking into account the *IFNλ4* rs12979860 polymorphism results, we can hypothesize worse disease outcomes in HIV-ART and better outcome in HIV/HTLV-2 co-infected patients also on ART.

Despite the limitations of the present study regarding its cross-sectional design and the lack of some information that does not allow firm conclusions, the results support the use of *CCR5-Δ32* polymorphism as a predictive marker for ART initiation in individuals without universal access to this treatment. In addition, the results show the presence of all polymorphism profiles described in the literature in the studied groups (**Figure**), corroborating data from inter-mixed racial populations residing in São Paulo and elsewhere. High *CCR2-64I* mutant allele frequencies were detected in HIV/HTLV co-infected patients, and this finding deserves more investigation. Finally, well-designed longitudinal studies are needed to know the effects of the polymorphisms detected in the present study on the clinical outcome of HIV-infected and HIV/HTLV co-infected individuals.

Author Contribution Statement

The authors confirm their contribution to the paper as follows: **study conception, design and supervision**: ACA and KRC; **data collection**: ECCO, AKSR, RXS and BVA; **analysis and interpretation of results**: ACA, KRC and RMNM; **draft manuscript preparation**: ACA, KRC, ECCO, AKSR, RXS, BVA and RMNM. **All authors** reviewed the results and approved the final version of the manuscript. **All authors** agreed to be responsible for all aspects of the work to ensure the accuracy and integrity of the published manuscript.

Ethics statement

The authors declare that the published work reflects an investigation and analysis carried out truthfully and completely. The study was approved by the Ethics Committee for Research of IAL (Ministry of Health protocol numbers CAAE #55837316.0.0000.0059 and #52493316.1.0000.0059). The data were analyzed anonymously.

Conflict of interest

The authors declare no conflict of interest.

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Availability of data

Available from the corresponding author upon request.

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References

1. Williamson SH, Hubisz MJ, Clark AG, Payseur BA, Bustamante CD, et al. Localizing recent adaptive evolution in the human genome. PLoS Genet. 2007;3(6): e90. doi:10.1371/journal.pgen.0030090
2. O'Bleness M, Searles V, Varki A, Gagneux P, Sikela JM. Evolution of genetic and genomic features unique to the human lineage. Nat Rev Genet. 2012; 13(12):853-66. doi:10.1038/nrg3336
3. Chatterjee A, Rathore A, Vidyant S, Kakkar K, Dhole TN. Chemokines and chemokine receptors in susceptibility to HIV-1 infection and progression to AIDS. Dis Markers. 2012;32:143-51. doi:10.3233/DMA-2011-0874
4. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, et al. Homozygous defect in HIV-1 co-receptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell. 1996;86(3):367-77. doi: 10.1016/S0092-8674(00)80110-5
5. Barmania F, Pepper MS. C-C chemokine receptor type five (CCR5): An emerging target for the control of HIV infection. Appl Transl Genom. 2013;2:3-16. doi: 10.1016/j.atg.2013.05.004
6. Winkler C, Modi W, Smith MW, Nelson GW, Wu X, Carrington M, et al. Genetic restriction on AIDS pathogenesis by an SDF-1 chemokine gene variant. Science. 1998; 279(5349): 389-93. doi: 10.1126/science.279.5349.389
7. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Uigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009;461:798-801. doi: 10.1038/nature08463
8. Assone T, Paiva A, Fonseca LAM, Casseb J. Genetic markers of the host in persons living with HTLV-1, HIV and HCV infections. Viruses. 2016;8:38. Available from: https://doi.org/10.3390/v8020038
9. Fang MZ, Jackson SS, Thomas R. O'Brien TR. IFNL4: Notable variants and associated phenotypes. Gene. 2020;730: 144289. Available from: https://doi.org/10.1016/j.gene.2019.144289
10. Martinson JJ, Chapman NH, Rees DC, Liu Y-T, Clegg JB. Global distribution of the CCR5 gene 32-basepair deletion. Nat Genet. 1997;16:100-3. doi: 10.1038/ng0597-100
11. Martinson JJ, Lily H, Karanicolas R, Moore JP, Kostrikis LG. Global distribution of the CCR2-64I/CCR5-59653T HIV-1 disease-protective haplotype. AIDS. 2000;14(5):483-9. doi: 10.1097/00002030-200003310-00003
12. Su B, Sun G , Lu D, Xiao J, Hu F, Chakraborty R, et al. Distribution of three HIV-1 resistance-conferring polymorphism (SDF1-3'A, CCR2-64I, and CCR5-delta32) in global populations. Eur J Hum Gen. 2000;8:975-9. doi: 10.1038/sj.ejhg.5200568
13. Mellado M, Rodríguez-Frade JM, Vila-Coro AJ, de Ana AM, C Martínez-A C. Chemokine control of HIV-1 infection. Nature. 1999;400(6746):723-4. doi: 10.1038/23382
14. Wachira D, Lihana R, Okoth V, Maiyo A, Khamadi SA. Chemokine coreceptor-2 gene polymorphisms among HIV-1 infected individuals in Kenya. Dis Markers. 2015;2015: 952067. Available from: https://doi.org/10.1155/2015/952067
15. Rafrafi A, Kaabachi S, Kaabachi W, Chahed B, Amor AB, Mbarik M, et al. CCR2-64I polymorphism is associated with non-small cell lung cancer in Tunisian patients. Hum Immunol. 2015; 76(5): 348-4. doi: 10.1016/j.humimm.2015.03.003
16. Grimaldi R, Acosta AX, Machado TMB, Bomfim TF, Galvão-Castro. Distribution of SDF1-3'A polymorphisms in three different ethnic groups from Brazil. Braz J Infect Dis. 2010;14(2):197-200. doi: 10.1016/S1413-8670(10)70039-8
17. Cavalcante LN, Abe-Sandes K, Angelo ALD, Machado TMB, Lemaire DC, Mendes CMC, et al. IL28B polymorphisms are markers of therapy response and are influenced by genetic ancestry in chronic hepatitis C patients from an admixed population. Liver Int. 2011; 476-86. doi: 10.1111/j.1478-3231.2011.02653.x
18. Ramos JA, Ramos ALA, Hoffmann L, Perez RM, Coelho HSM, Ürményi TP, et al. A single nucleotide polymorphism, rs129679860, in the IL28B locus is associated with the viral kinetics and a sustained virological response in a chronic, monoinfected hepatitis C virus genotype-1 Brazilian population treated with pegylated interferon-ribavirin. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2012;107(7):888-92. Available from: http://www.bioline.org.br/pdf?oc12189
19. Treviño A, Lopez M, Vispo E, Aguilera A, Ramos JM, Benito R, et al. Development of tropical spastic paraparesis in human T-lymphotropic virus type 1 carriers is influenced by interleukin 28B gene polymorphisms. Clin Infect Dis. 2012;55(1):e1-4. Available from: https://doi.org/10.1093/cid/cis343.
20. Assone T, de Souza FV, Gaester KO, Fonseca LA, Luiz OC, Malta FM, et al. IL28B gene polymorphism SNP rs8099917 genotype GG is associated with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in HTLV-1 carriers. PLoS Negl Trop Dis. 2014;8(9):e3199. Available from: https://doi.org/10.1371/journal.pntd.0003199
21. Assone T, Malta FM, Bakkour S, Montalvo L, Paiva AM, Smid J, et al. Polymorphisms in HLA-C and KIR alleles are not associated with HAM/TSP risk in HTLV-1-infected subjects. Virus Res. 2018;244:71-4. doi: 10.1016/j.virusres.2017.11.010
22. de Araújo ESA, Harel Dahari H, Cotler SJ, Layden TJ, Neumann AU, Melo CE, Barone AA. Pharmacodynamics of PEG-IFN alpha-2a and HCV response as a function of IL28B polymorphism in HIV/HCV co-infected patients. J Acquir Immune Defic Syndr. 2011;56(2):95-9. doi: 10.1097/QAI.0b013e3182020596
23. Ferreira PRA, Santos C, Cortes R, Reis A, Tenore SB, Silva MH, Vilhena C, Diaz RS. Association between IL28B gene polymorphisms and sustained virological response in patients co-infected with HCV and HIV in Brazil. J Antimicrob Chemother. 2012;509-10. doi: 10.1093/jac/dkr488
24. Caterino-de-Araujo A, Sacchi CT, Gonçalves MG, Campos KR, Magri MC, Alencar WK, et al. Current prevalence and risk factors associated with human T lymphotropic virus type 1 and human T lymphotropic virus type 2 infections among HIV/AIDS patients in São Paulo, Brazil. AIDS Res Hum Retroviruses. 2015;31(5):543-9. doi: 10.1089/AID.2014.0287
25. Campos KR, Gonçalves MG, Costa NA, Caterino-de-Araujo

A. Comparative performances of serologic and molecular assays for detecting human T lymphotropic virus type 1 and type 2 (HTLV-1 and HTLV-2) in patients infected with human immunodeficiency virus type (HIV-1). Braz J Infect Dis. 2017;21(3):297-305. doi: 10.1016/j.bjid.2017.02.005
26. Campos KR, Caterino-de-Araujo A. Provirus mutations of human T-lymphotropic virus type 1 and type 2 (HTLV-1 and HTLV-2) in HIV-1 co-infected individuals. mSphere. 2020;5(5):e00923-20. Available from: https://doi.org/10.1128/mSphere.00923-20
27. Gonçalves MG, Fukasawa LO, Campos KR, Higa FT, Caterino-de-Araujo A. Development and validation of multiplex quantitative real-time PCR assays for simultaneous detection and differentiation of HTLV-1 and HTLV-2, using different PCR platforms and reagent brands. Front Microbiol. 2022;13:831594. Available from: https://doi.org/ 10.3389/fmicb.2022.831594
28. de Angelis DAS, Freire WS, Cláudio Sergio Pannuti CS, Succi RCM, Machado DM. CCR5 genotypes and progression to HIV disease in perinatally infected children. Braz J Infect Dis. 2007;11(2):196-8. doi: 10.1590/S1413-86702007000200004
29. Voevodin A, Samilchuk E, Dashti S. Frequencies of SDF-1 chemokine, CCR-5, and CCR-2 chemokine receptor gene alleles conferring resistance to Human Immunodeficiency Virus Type 1 and AIDS in Kuwaitis. J Med Virol. 1999;58:54-8. Available from: https://doi.org/10.1002/(SICI)1096-9071(199905)58:1%3C54::AID-JMV8%3E3.0.CO;2-N
30. Moreira S, Garcia RFL, Gutberlet A, Bertol BC, Ferreira LE, Pinho MSL, et al. A straightforward genotyping of the relevant IL28B SNPs for the prediction of hepatitis C treatment outcome. J Virol Methods. 2012;184:93-7. doi: 10.1016/j.jviromet.2012.05.024
31. Campos KR, Gonçalves MG, Caterino-de-Araujo A. (2017a). Failures in detecting HTLV-1 and HTLV-2 in patients infected with HIV-1. AIDS Res Hum Retroviruses. 2017;33:382-5. doi: 10.1089/AID.2016.0191
32. Paiva A, Casseb J. Origin and prevalence of human T-lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2) among indigenous populations in the Americas. Rev Inst Med Trop Sao Paulo. 2015;57(1):1-13. doi: 10.1590/S0036-46652015000100001
33. Ministério da Saúde (BR). Secretaria de Vigilância em Saúde, Departamento de Doenças de Condições Crônicas e Infecções Sexualmente Transmissíveis. Guia de manejo clínico da infecção pelo HTLV [Internet]. Brasilia: Ministério da Saúde; 2021 [cited 2022 jun 24]. Available from: http://antigo.aids.gov.br/pt-br/pub/2022/guia-de-manejo-clinico-da-infeccao-pelo-htlv
34. Galvão-Castro B, Grassi MFR, Galvão-Castro AV, Nunes A, Galvão–Barroso AK, Araújo THA, et al. (2022) Integrative and Multidisciplinary Care for People Living With Human T-Cell Lymphotropic Virus in Bahia, Brazil: 20 Years of Experience. Front Med. 2022;9:884127. Available from: https://doi.org/10.3389/fmed.2022.88412
35. Carvalhaes FAPL, Cardoso GL, Hamoy IG, Liu YT, Guerreiro JF. Distribution of CCR5-[delta]32, CCR2-64I, and SDF1-3'A mutations in populations from the Brazilian Amazon region. Hum Biol. 2004;76(4):643-6. doi: 10.1353/hub.2004.0052.
36. Carvalhaes FAPL, Cardoso GL, Vallinoto ACR, Machado LF, Ishak MOG, Ishak R et al. Frequencies of CCR5-32, 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. Gen Mol Biol. 2005;28(4):665-9. Available from: https://www.scielo.br/j/gmb/a/G955MHGb5Np9XX5n7Hp76TP/?format=pdf&lang=en
37. Grimaldi R, Shindo N, Acosta A, Dourado I, Brites C, de Melo Carvalho O, et al. Prevalence of the CCR5Δ32 mutation in Brazilian populations and cell susceptibility to HIV-1 infection. Hum Genet. 2002;111(1):102-4. doi: 10.1007/s00439-002-0747-x
38. Pereira RW, Pires ER, Duarte APM, de Moura RP, Monteiro E, Torloni H, et al. Frequency of the CCR5 32 allele in Brazilians: a study in colorectal cancer and in HTLV-I infection. Genet Mol Biol. 2000;23(3):523-6. doi: 10.1590/S1415-47572000000300003
39. Silva-Carvalho WHV, de Moura RR, Coelho AVC, Crovella S, Guimarães RL. Frequency of the CCR5-delta32 allele in Brazilian populations: A systematic literature review and meta-analysis. Infect Genet Evol. 2016;43:101-7. doi: 10.1016/j.meegid.2016.05.024
40. Vieira VC, Barral MFM, Mendoza-Sassi RA, Silveira JM, Soares MA, de Martínez AMB. The effect of combined polymorphisms in chemokines and chemokine receptors on the clinical course of HIV-1 infection in a Brazilian population. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2011;106(4):408-14. doi: 10.1590/S0074-02762011000400005
41. Rigato PO, Hong MA, Casseb J, Ueda M, Castro I, Benard G, et al. Better CD4+ T cell recovery in Brazilian HIV-infected individuals under HAART due to cumulative carriage of SDF-1-3'A, CCR2-V64I, CCR5-D32 and CCR5-promoter 59029A/G polymorphisms. Curr HIV Res. 2008;6(5):466-73. doi: 10.2174/157016208785861131
42. Beilke MA. Retroviral coinfections: HIV and HTLV: Taking stock of more than a quarter century of research. AIDS Res Hum Retroviruses. 2012;28:139-47. doi: 10.1089/aid.2011.0342
43. Caterino-de-Araujo A, Campos KR, Oliveira LMS, Rigato PO. Biomarkers in a cohort of HIV-infected patients single- or co-Infected with HTLV-1, HTLV-2, and/or HCV: A cross-sectional, observational study. Viruses. 2022;14:1955. Available from: https://doi.org/10.3390/v14091955
44. Acosta AX, Grimaldi R, Spínola JL, Galvão-Castro B. Distribution of the CCR2-64I allele in three Brazilian ethnic groups. Genet Mol Biol. 2003;26(3):241-43. Available from: https://www.academia.edu/18986668/Distribution_of_the_CCR2_64I_allele_in_three_Brazilian_ethnic_groups
45. Lima ÉRG, Queiroz MAF, Lima SS, Machado LFA, Cayres-Vallinoto IMV, Vallinoto ACR, et al. CCR5 32 and SDF13'A: gene variants, expression and influence on biological markers for the clinical progression to AIDS among HIV-1 virus controllers in a mixed population of the Amazon region of Brazil. Int J Mol Sci. 2023;24:4958. Available from: https://doi.org/10.3390/ijms24054958
46. Treviño A, Lopez M, Vispo E, Aguilera A, Ramos JM, Benito R, et al. Development of tropical spastic paraparesis in human T-lymphotropic virus type 1 carriers is influenced by interleukin 28B gene polymorphisms. Clin Infect Dis.

2012;55(1):e1-4. Available from: https://doi.org/10.1093/cid/cis343

47. Sanabani SS, Nukui Y, Pereira J, da Costa AC, de Oliveira CS, Pessôa R, et al. Lack of evidence to support the association of a single IL28B genotype SNP rs12979860 with the HTLV-1 clinical outcomes and proviral load. BMC Infect Dis. 2012;12:374 Available from: http://www.biomedcentral.com/1471-2334/12/374
48. Vallinoto ACR, Santana BB, Sá KSG, Ferreira TCS, Sousa CM, Azevedo VN, et al. HTLV-1-associated myelopathy/tropical spastic paraparesis is not associated with SNP rs12979860 of the IL-28B gene. Mediators Inflamm. 2015;2015:804167. Available from: http://dx.doi.org/10.1155/2015/804167
49. da Silva Prates G, Malta FM, Toledo F, Monteiro MA, Fonseca LAM, Veiga APR, et al. AIDS incidence and survival in a hospital-based cohort of HIV-positive patients from São Paulo, Brazil: The role of IFN-λ4 polymorphisms. J Med Virol. 2021;93:3601-6. doi: 10.1002/jmv.26054