



Research Article  
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## CCR5Δ32 and HLA allele diversity in bone marrow donors from southern Brazil

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### Abstract

Transplantation of stem cells derived from donors with CCR5Δ32 homozygous genotype is a potential strategy to achieve both the control of malignant hematological disease as well as sustained remission of the HIV infection, and researchers in different countries are looking for CCR5Δ32 homozygous donors to replicate such a 'double-target' strategy. We determined the frequency of the CCR5Δ32 variant in a sample of 1,398 bone marrow donors from Rio Grande do Sul State, Brazil. This study also evaluated whether *HLA-A*, *HLA-B* and *HLA-DRB1* genotypes are homogeneously distributed between CCR5Δ32 carriers and non-carriers in a population characterized by a significant genetic admixture. The CCR5Δ32 allele frequency was 7.4% (CI<sub>0.95</sub> 6.4–8.4%), and the frequency of the Δ32/Δ32 homozygous genotype was 0.72% (CI<sub>0.95</sub> 0.34–1.31%). In general, HLA genotypes are homogeneously distributed between CCR5Δ32 carriers and non-carriers. Considering the large number of bone marrow donors in Brazil and the high CCR5Δ32 allele frequency observed in our study, our results clearly indicate the existence of a considerable amount of potential CCR5Δ32 homozygous bone marrow donors in southern Brazil, suggesting that an active search for these donors is not only feasible but an attractive and promising strategy towards effective HIV infection control and treatment.

**Keywords:** CCR5Δ32, immunogenetics, HIV, HLA, viral suppression.

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### Introduction

Despite intense research involving HIV/AIDS since the 1980s, there is still no effective cure for HIV infection. Scientific efforts are still extremely necessary to reduce HIV-associated conditions, including AIDS and the chronic inflammation observed in patients on antiretroviral therapy (ART). In the clinical course of HIV infection, there is a large depletion of CD4<sup>+</sup> T cells since the CD4 molecule is the main binding receptor which HIV uses to penetrate cells. The HIV gp120 surface protein interacts with CD4 protein. After this initial gp120/CD4 binding, the gp120 is displaced, leaving the HIV gp41 protein free, which binds to a host cellular

co-receptor, more frequently CCR5, a chemokine receptor involved in inflammatory reactions. In this sense, CCR5 is an accessory molecule needed for effective HIV infection. Because of its direct role in this pathogenic process, multiple aspects of CCR5 have been studied in the context of HIV infection and AIDS progression (Gottlieb *et al.*, 1981; Broder and Gallo, 1984; Montagnier *et al.*, 1984; Moore *et al.*, 1990; Berson *et al.*, 1996; Alkhatib, 2009).

In 1996 a 32 base-pair deletion in the *CCR5* gene was described and subsequently named as CCR5Δ32 (Liu *et al.*, 1996). The CCR5Δ32 variant consists of a frameshift mutation in the coding region of the *CCR5* gene (exon 3), which generates a premature stop codon and the consequent expression of a non-functional protein, which is not transported to the cell surface, thus preventing its function as chemokine receptor. Considering that individuals who show this variant in homozygosity do not express the CCR5 molecule in the surface of their cells, CCR5-tropic HIV-1 strains are prevented

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from successfully interacting with host cells, blocking the process of viral penetration (Dean *et al.*, 1996; Liu *et al.*, 1996; Samson *et al.*, 1996; Proudfoot, 2002; Venkatesan *et al.*, 2002; Ellwanger *et al.*, 2020a,b). Nevertheless, some HIV strains can interact with other accessory molecules (especially CXCR4) (Hoffmann, 2007). Therefore, although CCR5 $\Delta$ 32 homozygosity is a natural factor associated with HIV infection resistance, this condition *per se* is not capable to abrogate infection of non CCR5-tropic strains.

The CCR5 $\Delta$ 32 allele has a heterogeneous distribution in populations around the world. This variant has a European origin and is practically absent in African, Asian, and Amerindian populations (Martinson *et al.*, 1997; Libert *et al.*, 1998; Solloch *et al.*, 2017). The Brazilian population is highly admixed, with an important genetic contribution of African, Native-American, and European populations (Kulmann-Leal *et al.*, 2021). The presence of Euro-descendant individuals is greater in the southern region of Brazil, which reflects the higher frequencies of the CCR5 $\Delta$ 32 allele in this region within the Brazilian territory. The CCR5 $\Delta$ 32 allele frequency is around 4-6% in Brazil, but it can be as high as 9% in specific southern populations (as observed in a study performed in Paraná State with Euro-descendant population) (Boldt *et al.*, 2009; Silva-Carvalho *et al.*, 2016; Kulmann-Leal *et al.*, 2021). For example, based on studies performed with samples of healthy population ranging from  $n=103$  to  $n=334$  individuals, in Rio Grande do Sul State the CCR5 $\Delta$ 32 allele is commonly observed at frequencies between 6% to 7% (Vargas *et al.*, 2006; Ellwanger *et al.*, 2018; Ellwanger *et al.*, 2020c).

Since its discovery, the CCR5 $\Delta$ 32 variant has been subject of numerous studies in the context of HIV infection. In 2009, Hütter *et al.* (2009) published an innovative and striking study about the ‘Berlin Patient’, a 40-year-old HIV-infected man who was diagnosed with acute myeloid leukemia. As a treatment for the neoplasia, the patient underwent an allogeneic stem cell transplant from an HLA compatible donor, but also homozygous for the CCR5 $\Delta$ 32 allele. The donor was chosen in order to carry out a possible combined (‘double-target’) therapy for leukemia and HIV infection, since the donor’s cells without CCR5 expression could not be infected by the patient’s CCR5-tropic HIV strains. After the transplant, the patient no longer received ART and his viral load was followed for the next 20 months. Remarkably, active and replicating viral particles were not detected in that period. Three years after the procedure, Hütter and Thiel (2011) updated the Berlin Patient’s clinical situation, reporting the continuous absence of replicative viral particles still without ART need (Hütter *et al.*, 2009; Hütter and Thiel, 2011). The Berlin Patient eventually died in 2020 due to a relapse of the leukemia, although he was still HIV-free (Kulmann-Leal *et al.*, 2021).

Gupta *et al.* (2019) reported a second successful case of sustained remission of HIV infection. Following a similar protocol described by Hütter *et al.* (2009), a patient diagnosed with HIV-1 infection and IVb stage Hodgkin’s lymphoma underwent an allogeneic stem cell transplant with CCR5 $\Delta$ 32 homozygous cells. ART was discontinued at day 510, and in the same way of the first reported case, the presence of HIV was no longer observed after the transplant. Subsequently, Gupta *et al.* (2020) reinforced the success of the procedure 30 months after the transplant, describing more details regarding

the case. This second individual to achieve sustained remission of HIV infection is known as the ‘London Patient’ (Gupta *et al.*, 2019; 2020). Recently, another case of HIV sustainable remission after transplant with CCR5 $\Delta$ 32 homozygous cells was announced, the ‘Düsseldorf patient’ (Jensen *et al.*, 2023).

Considering the possibility of obtaining a sustained remission of HIV infection after stem cell transplant, different research groups are investigating the feasibility of this procedure in various populations. Solloch *et al.* (2017) summarized CCR5 $\Delta$ 32 variant frequencies for 87 countries, also describing the CCR5 $\Delta$ 32 genotypes for over 1.3 million individuals from Germany, Poland, and UK. Genotype data was obtained from potential hematopoietic stem cell donors from the German Bone Marrow Donor Center (DKMS). Solloch *et al.* (2017) estimated that a German HIV+ individual who needs a stem cell transplant has a 28.7% probability of finding a donor both HLA compatible and CCR5 $\Delta$ 32 homozygous. Also, Enrich *et al.* (2018) genotyped 20,236 cord blood units (CBUs) from The Spanish Bone Marrow Registry (REDMO), and found a total of 130 CBUs homozygous for the CCR5 $\Delta$ 32 variant. Both studies show that, although the CCR5 $\Delta$ 32 allele is not highly prevalent in most countries, it is possible to find CCR5 $\Delta$ 32 homozygous individuals in hematopoietic stem cell donor registries that could contribute with new attempts of sustained HIV suppression in HIV+ individuals who eventually need a stem cell transplant due to malignant hematological diseases (Solloch *et al.*, 2017; Enrich *et al.*, 2018).

The search for CCR5 $\Delta$ 32 homozygous individuals as potential donors to HIV+ patients in bone marrow transplants due to malignant hematological diseases represents a cutting-edge initiative in HIV/AIDS research. In Brazil, the National Registry of Bone Marrow Volunteer Donors (*Registro Nacional de Doadores de Medula Óssea* - REDOME) is responsible for gathering information from people willing to donate bone marrow to anyone who needs a transplant, having more than 5 million registered members. In this context, the main objective of this study was to answer this question: (I) what is the CCR5 $\Delta$ 32 allele frequency in bone marrow donors from southern Brazil? Also, considering a potential association between HLA alleles and chemokine system genes (Favorova *et al.*, 2002; Öckinger *et al.*, 2010; Javor *et al.*, 2015), the secondary objective of this study was to answer this exploratory question: (II) are the HLA genotypes homogeneously distributed between CCR5 $\Delta$ 32 carries and non-carries? The potential clinical implications of this research include benefits for HIV+ individuals who eventually need a stem cell transplant due to malignant hematological diseases, strengthening the proof-of-concept described by Hütter *et al.* (2009) and Gupta *et al.* (2019).

## Material and Methods

### DNA samples and ethical aspects

The genomic DNA samples used in the study come from bone marrow donors and were provided by the *Unidade de Imunologia de Transplantes e Medicina Personalizada* (Transplant Immunology and Personalized Medicine Unit) of *Hospital de Clínicas de Porto Alegre* (HCPA, Porto Alegre, Brazil), which is responsible for REDOME in Porto Alegre, the capital of Rio Grande do Sul, the southernmost state of Brazil.

HLA typing was performed in the Transplant Immunology and Personalized Medicine Unit of HCPA using the LABType SSO Typing Test methodology (One Lambda, CA, USA) for loci A, B and DRB1. Of note, the population of Rio Grande do Sul State is composed mostly of Euro-descendant individuals (Pena *et al.*, 2011). A total of 1,398 blood samples were investigated in this study, of whom *HLA-A*, *HLA-B* and *HLA-DRB1* alleles were determined previously. Only individuals recently registered as bone marrow donors (between 2019 to 2020) were included in the study. All potential donors signed a consent form allowing the genetic analysis of tissue compatibility antigens for transplantation purposes, in which *CCR5* genotyping is included. This study was approved by the research ethics committees of *Hospital de Clínicas de Porto Alegre* and *Universidade Federal do Rio Grande do Sul* (# 20460719.6.0000.5327).

### CCR5Δ32 genotyping

The *CCR5Δ32* variant (rs333) was genotyped according to Chies and Hutz (2003) and minor adaptations by Ellwanger *et al.* (2020c). In brief, the fragments of interest were amplified by conventional PCR and the primers used in the reaction were: *CCR5a* 5'-GGTCTTCATTACACCTGC-3'; *CCR5b* 5'-AGGATTCCCAGTAGCAGATG-3'. Genotyping was performed by analyzing the amplicons on a 3% agarose gel under UV light. A single 137 base-pair band represents the wild-type homozygous genotype; a 137 base-pair band associated with a 105 base-pair band indicates heterozygous genotype; a single 105 base-pair band indicates the variant homozygous genotype.

### Statistical analysis

*CCR5Δ32* allele and genotype frequencies were calculated, and respective Fisher's exact 95% confidence intervals (C.I.) were obtained using WinPEPI version 11.65 (Abramson, 2011). Hardy-Weinberg equilibrium was evaluated using the chi-square goodness of fit test. Due to the low frequency of *CCR5Δ32* homozygous genotypes, the frequencies of 'CCR5Δ32 carriers' (*CCR5Δ32* homozygous individuals grouped with heterozygous individuals) and 'CCR5Δ32 non-carriers' (individuals with wild-type homozygous genotype) were also calculated. The total number of individuals ( $n=1,398$ ) was included in these initial analyses used to answer our first research question.

We subsequently assessed whether HLA genotypes were homogeneously distributed between *CCR5Δ32* carriers and non-carriers (our second research question). For this, *HLA-A*,

*HLA-B* and *HLA-DRB1* genotype and allele frequencies were compared between *CCR5Δ32* carriers and non-carriers using Fisher's exact tests.  $P$ -values  $<0.05$  were considered statistically significant. We used the software GENEPOP (available at: <https://genepop.curtin.edu.au/>) (Raymond and Rousset, 1995; Rousset, 2008) to estimate *HLA* allele and genotype frequencies, test for Hardy-Weinberg equilibrium, and to compare allele and genotype frequencies between groups.

## Results

### CCR5Δ32 genotype and allele frequencies

Table 1 shows the *CCR5Δ32* genotype and allele frequencies among bone marrow donors genotyped in this study ( $n=1,398$ ). Most donors (86%) do not carry the *CCR5Δ32* allele. We observed a *CCR5Δ32* allele frequency of 7.4% (CI<sub>0.95</sub> 6.4 – 8.4%), which is higher than the average Brazilian frequency of 4–6% (Kulmann-Leal *et al.*, 2021). In total, ten Δ32/Δ32 homozygous individuals were found (0.72%). Genotypes were distributed according to the expected for the Hardy-Weinberg equilibrium ( $p=0.345$ ). Some samples showed uncertain HLA genotypes ( $n=208$ ) and were excluded from the analyses onwards.

### Distribution of CCR5Δ32 alleles in CCR5Δ32 carriers and non-carriers

Table 2 shows the allele frequencies of the three HLA loci investigated in the study ( $n=1,190$ ). Nineteen *HLA-A*, 30 *HLA-B*, and 13 *HLA-DRB1* alleles were found in blood samples of bone marrow donors. The three most frequent alleles of each HLA loci were: *HLA-A\*2* (28.24%), *HLA-A\*24* (11.09%), and *HLA-A\*3* (9.71%); *HLA-B\*44* (12.44%), *HLA-B\*35* (11.09%), and *HLA-B\*15* (8.66%); *HLA-DRB1\*7* (14.41%), *HLA-DRB1\*11* (13.28%), and *HLA-DRB1\*13* (12.77%) (Table 2).

Alleles were distributed according to the expected for the Hardy-Weinberg equilibrium for *HLA-A* and *HLA-DRB1* ( $p=0.093$  and  $p=0.187$ , respectively). On the other hand, the *HLA-B* locus showed a deviation from the Hardy-Weinberg equilibrium ( $p=0.024$ ). However, this result may be explained by the large number of tests carried out. Table 3 details the *HLA* allele frequencies among *CCR5Δ32* carriers ( $n=174$ ) and non-carriers ( $n=1,016$ ), and Table 4 shows the results of the statistical comparison of the allele frequencies of the three *HLA* loci evaluated here (*HLA-A*, *HLA-B* and *HLA-DRB1*) between *CCR5Δ32* carriers and non-carriers.

**Table 1** – *CCR5Δ32* genotype and allele frequencies ( $n$  and %) among bone marrow donors.

<i>CCR5Δ32</i>	Bone marrow donors ( $n=1,398$ )	95% Confidence Interval (C.I.)
wt/wt	1202 (85.98%)	84.05% – 87.76%
wt/Δ32	186 (13.30%)	11.57% – 15.20%
Δ32/Δ32	10 (0.72%)	0.34% – 1.31%
Δ32 carrier	196 (14.02%)	12.24% – 15.95%
Δ32 non-carrier	1202 (85.98%)	84.05% – 87.76%
Δ32 allele frequency	0.074	0.064 – 0.084

$n$ , number of individuals. wt/wt, wild homozygous genotype. wt/Δ32, heterozygous genotype, Δ32/Δ32, variant homozygous genotype.

**Table 2** – *HLA-A*, *HLA-B* and *HLA-DRB1* allele frequencies in 1,190 bone marrow donors.

<i>HLA-A</i>			<i>HLA-B</i>			<i>HLA-DRB1</i>		
Allele	(number of alleles)	Frequency (%)	Allele	(number of alleles)	Frequency (%)	Allele	(number of alleles)	Frequency (%)
<i>HLA-A*2</i>	672	28.24	<i>HLA-B*44</i>	296	12.44	<i>HLA-DRB1*7</i>	343	14.41
<i>HLA-A*24</i>	264	11.09	<i>HLA-B*35</i>	264	11.09	<i>HLA-DRB1*11</i>	316	13.28
<i>HLA-A*3</i>	231	9.71	<i>HLA-B*15</i>	206	8.66	<i>HLA-DRB1*13</i>	304	12.77
<i>HLA-A*1</i>	229	9.62	<i>HLA-B*7</i>	204	8.57	<i>HLA-DRB1*4</i>	282	11.85
<i>HLA-A*29</i>	132	5.55	<i>HLA-B*51</i>	198	8.32	<i>HLA-DRB1*15</i>	244	10.25
<i>HLA-A*68</i>	127	5.34	<i>HLA-B*8</i>	133	5.59	<i>HLA-DRB1*1</i>	241	10.13
<i>HLA-A*11</i>	122	5.13	<i>HLA-B*40</i>	130	5.46	<i>HLA-DRB1*3</i>	223	9.37
<i>HLA-A*32</i>	101	4.24	<i>HLA-B*14</i>	128	5.38	<i>HLA-DRB1*8</i>	129	5.42
<i>HLA-A*31</i>	98	4.12	<i>HLA-B*18</i>	126	5.29	<i>HLA-DRB1*14</i>	110	4.62
<i>HLA-A*23</i>	97	4.08	<i>HLA-B*38</i>	70	2.94	<i>HLA-DRB1*16</i>	80	3.36
<i>HLA-A*33</i>	78	3.28	<i>HLA-B*27</i>	66	2.77	<i>HLA-DRB1*9</i>	48	2.02
<i>HLA-A*30</i>	75	3.15	<i>HLA-B*13</i>	65	2.73	<i>HLA-DRB1*10</i>	31	1.30
<i>HLA-A*26</i>	72	3.03	<i>HLA-B*49</i>	61	2.56	<i>HLA-DRB1*12</i>	29	1.22
<i>HLA-A*25</i>	33	1.39	<i>HLA-B*57</i>	60	2.52			
<i>HLA-A*66</i>	16	0.67	<i>HLA-B*50</i>	49	2.06			
<i>HLA-A*74</i>	16	0.67	<i>HLA-B*39</i>	43	1.81			
<i>HLA-A*34</i>	8	0.34	<i>HLA-B*58</i>	43	1.81			
<i>HLA-A*36</i>	7	0.29	<i>HLA-B*45</i>	33	1.39			
<i>HLA-A*69</i>	2	0.08	<i>HLA-B*52</i>	29	1.22			
			<i>HLA-B*53</i>	28	1.18			
			<i>HLA-B*55</i>	27	1.13			
			<i>HLA-B*41</i>	27	1.13			
			<i>HLA-B*48</i>	27	1.13			
			<i>HLA-B*37</i>	24	1.01			
			<i>HLA-B*42</i>	15	0.63			
			<i>HLA-B*56</i>	12	0.50			
			<i>HLA-B*47</i>	10	0.42			
			<i>HLA-B*81</i>	4	0.17			
			<i>HLA-B*46</i>	1	0.04			
			<i>HLA-B*73</i>	1	0.04			

The total number of alleles is twice the number of individuals analyzed.

**Table 3** – *HLA-A*, *HLA-B* and *HLA-DRB1* allele frequencies in 174 ‘CCR5Δ32 carriers’ and 1,016 ‘CCR5Δ32 non-carriers’ bone marrow donors.

CCR5Δ32 carriers (n=174)								
<i>HLA-A</i>			<i>HLA-B</i>			<i>HLA-DRB1</i>		
Allele	(number of alleles)	Frequency (%)	Allele	(number of alleles)	Frequency (%)	Allele	(number of alleles)	Frequency (%)
<i>HLA-A*2</i>	103	29.60	<i>HLA-B*44</i>	27	7.76	<i>HLA-DRB1*7</i>	45	12.93
<i>HLA-A*24</i>	34	9.77	<i>HLA-B*35</i>	39	11.21	<i>HLA-DRB1*11</i>	50	14.37
<i>HLA-A*3</i>	27	7.76	<i>HLA-B*15</i>	40	11.49	<i>HLA-DRB1*13</i>	46	13.22
<i>HLA-A*1</i>	40	11.49	<i>HLA-B*7</i>	29	8.33	<i>HLA-DRB1*4</i>	42	12.07
<i>HLA-A*29</i>	18	5.17	<i>HLA-B*51</i>	33	9.48	<i>HLA-DRB1*15</i>	36	10.34
<i>HLA-A*68</i>	20	5.75	<i>HLA-B*8</i>	19	5.46	<i>HLA-DRB1*1</i>	39	11.21
<i>HLA-A*11</i>	16	4.60	<i>HLA-B*40</i>	11	3.16	<i>HLA-DRB1*3</i>	33	9.48
<i>HLA-A*32</i>	23	6.61	<i>HLA-B*14</i>	17	4.89	<i>HLA-DRB1*8</i>	22	6.32
<i>HLA-A*31</i>	10	2.87	<i>HLA-B*18</i>	19	5.46	<i>HLA-DRB1*14</i>	11	3.16
<i>HLA-A*23</i>	11	3.16	<i>HLA-B*38</i>	13	3.74	<i>HLA-DRB1*16</i>	14	4.02
<i>HLA-A*33</i>	13	3.74	<i>HLA-B*27</i>	14	4.02	<i>HLA-DRB1*9</i>	4	1.15
<i>HLA-A*30</i>	10	2.87	<i>HLA-B*13</i>	11	3.16	<i>HLA-DRB1*10</i>	4	1.15
<i>HLA-A*26</i>	10	2.87	<i>HLA-B*49</i>	9	2.59	<i>HLA-DRB1*12</i>	2	0.57
<i>HLA-A*25</i>	7	2.01	<i>HLA-B*57</i>	9	2.59			
<i>HLA-A*66</i>	2	0.57	<i>HLA-B*50</i>	12	3.45			
<i>HLA-A*74</i>	3	0.86	<i>HLA-B*39</i>	9	2.59			
<i>HLA-A*34</i>	0	0	<i>HLA-B*58</i>	4	1.15			
<i>HLA-A*36</i>	1	0.29	<i>HLA-B*45</i>	5	1.44			
<i>HLA-A*69</i>	0	0	<i>HLA-B*52</i>	3	0.86			
			<i>HLA-B*53</i>	5	1.44			
			<i>HLA-B*55</i>	4	1.15			
			<i>HLA-B*41</i>	6	1.72			
			<i>HLA-B*48</i>	2	0.57			
			<i>HLA-B*37</i>	4	1.15			
			<i>HLA-B*42</i>	2	0.57			
			<i>HLA-B*56</i>	1	0.29			
			<i>HLA-B*47</i>	1	0.29			
			<i>HLA-B*81</i>	0	0			
			<i>HLA-B*46</i>	0	0			
			<i>HLA-B*73</i>	0	0			

The total number of alleles is twice the number of individuals analyzed.

Table 3 – Cont.

CCR5A32 non-carriers (n=1,016)								
<i>HLA-A</i>			<i>HLA-B</i>			<i>HLA-DRB1</i>		
Allele	(number of alleles)	Frequency (%)	Allele	(number of alleles)	Frequency (%)	Allele	(number of alleles)	Frequency (%)
<i>HLA-A*2</i>	569	28.00	<i>HLA-B*44</i>	269	13.24	<i>HLA-DRB1*7</i>	298	14.67
<i>HLA-A*24</i>	230	11.32	<i>HLA-B*35</i>	225	11.07	<i>HLA-DRB1*11</i>	266	13.09
<i>HLA-A*3</i>	204	10.04	<i>HLA-B*7</i>	175	8.61	<i>HLA-DRB1*13</i>	258	12.70
<i>HLA-A*1</i>	189	9.30	<i>HLA-B*15</i>	166	8.17	<i>HLA-DRB1*4</i>	240	11.81
<i>HLA-A*29</i>	114	5.61	<i>HLA-B*51</i>	165	8.12	<i>HLA-DRB1*15</i>	208	10.24
<i>HLA-A*68</i>	107	5.27	<i>HLA-B*40</i>	119	5.86	<i>HLA-DRB1*1</i>	202	9.94
<i>HLA-A*11</i>	106	5.22	<i>HLA-B*8</i>	114	5.61	<i>HLA-DRB1*3</i>	190	9.35
<i>HLA-A*31</i>	88	4.33	<i>HLA-B*14</i>	111	5.46	<i>HLA-DRB1*8</i>	107	5.27
<i>HLA-A*23</i>	86	4.23	<i>HLA-B*18</i>	107	5.27	<i>HLA-DRB1*14</i>	99	4.87
<i>HLA-A*32</i>	78	3.84	<i>HLA-B*38</i>	57	2.81	<i>HLA-DRB1*16</i>	66	3.25
<i>HLA-A*33</i>	65	3.20	<i>HLA-B*13</i>	54	2.66	<i>HLA-DRB1*9</i>	44	2.17
<i>HLA-A*30</i>	65	3.20	<i>HLA-B*27</i>	52	2.56	<i>HLA-DRB1*10</i>	27	1.33
<i>HLA-A*26</i>	62	3.05	<i>HLA-B*49</i>	52	2.59	<i>HLA-DRB1*12</i>	27	1.33
<i>HLA-A*25</i>	26	1.28	<i>HLA-B*57</i>	51	2.51			
<i>HLA-A*66</i>	14	0.69	<i>HLA-B*58</i>	39	1.92			
<i>HLA-A*74</i>	13	0.64	<i>HLA-B*50</i>	37	1.82			
<i>HLA-A*34</i>	8	0.39	<i>HLA-B*39</i>	34	1.67			
<i>HLA-A*36</i>	6	0.30	<i>HLA-B*45</i>	28	1.38			
<i>HLA-A*69</i>	2	0.10	<i>HLA-B*52</i>	26	1.28			
			<i>HLA-B*48</i>	25	1.23			
			<i>HLA-B*53</i>	23	1.13			
			<i>HLA-B*55</i>	23	1.13			
			<i>HLA-B*41</i>	21	1.03			
			<i>HLA-B*37</i>	20	0.98			
			<i>HLA-B*42</i>	13	0.64			
			<i>HLA-B*56</i>	11	0.54			
			<i>HLA-B*47</i>	9	0.44			
			<i>HLA-B*81</i>	4	0.20			
			<i>HLA-B*46</i>	1	0.05			
			<i>HLA-B*73</i>	1	0.05			

The total number of alleles is twice the number of individuals analyzed.

No test showed statistical significance ( $p>0.05$ ), indicating a homogeneous distribution of *HLA* alleles among CCR5Δ32 carriers and non-carriers (Table 4).

#### Distribution of CCR5Δ32 genotypes in CCR5Δ32 carriers and non-carriers

Due to the large number of genotypes and consequent small number of individuals in each class, detailed frequencies of *HLA-A*, *HLA-B*, and *HLA-DRB1* genotypes are detailed in Tables S1, S2 and S3. Regarding *HLA* genotypes, all loci were distributed according to the expected for the Hardy-Weinberg equilibrium ( $p>0.05$  in all tests). Table 5 shows the results of the comparison of the genotype frequencies of the *HLA* loci observed between CCR5Δ32 carriers and non-carriers. As observed in the tests involving alleles, no result showed statistical significance ( $p>0.05$ ), indicating a homogeneous distribution of *HLA* genotypes among CCR5Δ32 carriers and non-carriers (Table 5).

**Table 4** – Comparison of allele frequencies of *HLA-A*, *HLA-B* and *HLA-DRB1* loci between CCR5Δ32 carriers and non-carriers.

Locus	p-value <sup>1</sup>
<i>HLA-A</i>	0.598
<i>HLA-B</i>	0.408
<i>HLA-DRB1</i>	0.783

<sup>1</sup>Fisher's exact test.

**Table 5** – Comparison of genotype frequencies of *HLA-A*, *HLA-B* and *HLA-DRB1* loci between CCR5Δ32 carriers and non-carriers.

Locus	p-value <sup>1</sup>
<i>HLA-A</i>	0.614
<i>HLA-B</i>	0.381
<i>HLA-DRB1</i>	0.782

<sup>1</sup>Fisher's exact test.

## Discussion

The works of Hütter *et al.* (2009), Gupta *et al.* (2019), and Jensen *et al.* (2023) were pioneers in achieving sustained remission of HIV infection without the need for continuous use of ART. By deliberately looking for donors of hematopoietic stem cells who were also homozygous to the CCR5Δ32 variant, they achieved the cure of HIV infection in the 'Berlin', 'London', and 'Düsseldorf' patients, respectively. Also, studies performed in Spain by Enrich *et al.* (2018) and Germany, Poland, and the UK by Solloch *et al.* (2017) reinforced the importance of searching for CCR5Δ32 homozygous individuals in hematopoietic stem cell donor registries. Here, we conducted a similar investigation with Brazilian donors.

The CCR5Δ32 variant has a very heterogeneous distribution in different populations, even in the same country, as Brazil. According to Kulmann-Leal *et al.* (2021), the Brazilian CCR5Δ32 allele frequency is around 4-6%. In the present study, we found a CCR5Δ32 frequency of 7.4%, with ten individuals (0.72%) showing homozygous genotype, and

these results answer our first research question. The increased CCR5Δ32 allele frequency observed in our samples may be due to the high density of European-descendent individuals in the south region of Brazil, as indicated by genetic and historical data (Callegari-Jacques *et al.*, 2003; Lins *et al.*, 2010). We also highlight that the CCR5Δ32 allele frequency observed in the present study is in agreement with our previous studies involving the CCR5Δ32 frequency (6-7%) in healthy sample groups from Rio Grande do Sul State (Vargas *et al.*, 2006; Ellwanger *et al.*, 2018; Ellwanger *et al.*, 2020c), highlighting the fact that this study encompassed a substantially larger sample size (more than a thousand individuals) than the previous works performed with other samples from Rio Grande do Sul (Vargas *et al.*, 2006; Ellwanger *et al.*, 2018; Ellwanger *et al.*, 2020c).

According to Solloch *et al.* (2017), the average Brazilian frequency of Δ32/Δ32 homozygous individuals is around 0.35%. It is important to highlight that Solloch *et al.* (2017) reported this frequency for only 570 individuals, and no information is given concerning the geographical or ethnic origin of this sample. Here, we found a higher frequency (0.72%). More than 5 million Brazilians are currently registered as bone marrow donors. Considering a minimum frequency of 0.35% (Solloch *et al.*, 2017) and a maximum frequency of 0.72% (this study), we would expect 17,500 to 36,000 of these voluntary donors homozygous for the CCR5Δ32 variant who could therefore participate in transplants for HIV-infected individuals with malignant hematological diseases. Nevertheless, when extrapolating these results to the Brazilian population in general, it should be noted that the frequencies of individuals carrying the CCR5Δ32 allele, in particular homozygous, vary along the country (Silva-Carvalho *et al.*, 2016; Solloch *et al.*, 2017). Standardization of the genotyping of the CCR5Δ32 variant in all Brazilian stem cell donors, as well as considerations of populations composition would be required for a better estimate of these numbers in each region. Also, it is important to highlight that the estimate cited anteriorly only considers the genotypes of the CCR5Δ32 variant, and that a bone marrow transplant would only be viable with HLA system compatibility. Therefore, the real chance of finding a compatible donor who has the homozygous genotype for Δ32 is considerably lower. However, we stress that the intention of this work is to highlight the benefits of standardizing and including genotyping of the CCR5Δ32 variant in histocompatibility panel screenings, which we believe would be viable considering technical terms, with costs covered with resources from the Brazilian Unified Health System (*Sistema Único de Saúde* – SUS).

In this study, the *HLA-B* locus was the most polymorphic, with 30 alleles. The *HLA-B* locus is indeed very polymorphic, showing allele diversity superior to other HLA class I loci (Kiepiela *et al.*, 2004). In general, in our study the evaluated *HLA-A*, *HLA-B*, and *HLA-DRB1* alleles and genotypes were homogeneously distributed between CCR5Δ32 carriers and non-carriers ( $p>0.05$ ). The absence of an association of a given HLA allele/genotype with the CCR5Δ32 variant could be interpreted as advantageous since it would suggest that CCR5Δ32 donors are highly diversified in terms of their HLA genotypes, suggesting a wide range of possibilities concerning HLA/CCR5Δ32 matchings.

Finally, we highlight that the sample size of this study, although considerably large, did not allow us to analyze the different *HLA* genotypes and alleles more comprehensively, representing a study limitation. However, as mentioned anteriorly, this is an exploratory study. Furthermore, due to the (expected) low frequency of the  $\Delta 32$  allele, the number of CCR5 $\Delta 32$  homozygotes was quite limited. Therefore, more studies with larger sample sizes are required to better understand potential interactions between *HLA* alleles and the CCR5 $\Delta 32$  variant in the Brazilian population.

## Conclusion

The genotype and allele frequencies of the CCR5 $\Delta 32$  in a sample of voluntary bone marrow donors of Rio Grande do Sul State (south Brazil) were reported in this study, as well as allele diversity of three *HLA* loci in the same sample. Considering the large number of individuals registered as bone marrow donors, here we show that it is possible to find a considerable amount of CCR5 $\Delta 32$  homozygous donors who might donate bone marrow to HIV+ patients who need bone marrow transplantation due to a malignant hematological disease. We did not detect an association between *HLA*-specific genotypes and the CCR5 $\Delta 32$  variant.

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## Conflicts of interest

The authors declare no conflicts of interest regarding this study.

## Author contributions

BKL performed the genotyping, data analysis and wrote the first version of the manuscript; BKL, JHE, RTM, and SMCJ analyzed the data and edited the manuscript; ACA, LFJJ and MJ provided the samples; LFJJ coordinated the work at Hospital de Clínicas de Porto Alegre; JABC, JHE and LCMSP conceived the study; JABC revised and edited the manuscript and coordinated the work at Universidade Federal do Rio Grande do Sul. All authors approved the final version of the manuscript.

## References

Abramson JH (2011) WINPEPI updated: Computer programs for epidemiologists, and their teaching potential. *Epidemiol Perspect Innov* 8:1.  
 Alkhatib G (2009) The biology of CCR5 and CXCR4. *Curr Opin HIV AIDS* 4:96.  
 Berson JF, Long D, Doranz BJ, Rucker J, Jirik FR and Doms RW (1996) A seven-transmembrane domain receptor involved in

fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains. *J Virol* 70:6288-6295.  
 Boldt ABW, Culpi L, Tsuneto LT, Souza IR, Kun JFJ and Petzl-Erler ML (2009) Analysis of the CCR5 gene coding region diversity in five South American populations reveals two new non-synonymous alleles in Amerindians and high CCR5\* $\Delta 32$  frequency in Euro-Brazilians. *Genet Mol Biol* 32:12-19.  
 Broder S and Gallo RC (1984) A pathogenic retrovirus (HTLV-III) linked to AIDS. *N Engl J Med* 311:1292-1297.  
 Callegari-Jacques SM, Grattapaglia D, Salzano FM, Salamoni SP, Crossetti SG, Ferreira ME and Hutz MH (2003) Historical genetics: Spatiotemporal analysis of the formation of the Brazilian population. *Am J Hum Biol* 15:824-834.  
 Chies JAB and Hutz MH (2003) High frequency of the CCR5 $\Delta 32$  variant among individuals from an admixed Brazilian population with sickle cell anemia. *Braz J Med Biol Res* 36:71-75.  
 Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E *et al.* (1996) Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene. *Science* 273:1856-1862.  
 Ellwanger JH, Leal BK, Valverde-Villegas JM, Simon D, Marangon CG, Mattevi VS, Lazzaretti RK, Sprinz E, Kuhmmer R and Chies JAB (2018) CCR5 $\Delta 32$  in HCV infection, HCV/HIV co-infection, and HCV-related diseases. *Infect Genet Evol* 59:163-166.  
 Ellwanger JH, Kaminski V de L, Rodrigues AG, Kulmann-Leal B and Chies JAB (2020a) CCR5 and CCR5 $\Delta 32$  in bacterial and parasitic infections: Thinking chemokine receptors outside the HIV box. *Int J Immunogenet* 47:261-285.  
 Ellwanger JH, Kulmann-Leal B, Kaminski V de L, Rodrigues AG, Bragatte MA de S and Chies JAB (2020b) Beyond HIV infection: Neglected and varied impacts of CCR5 and CCR5 $\Delta 32$  on viral diseases. *Virus Res* 286:198040.  
 Ellwanger JH, Kulmann-Leal B, Wolf JM, Michita RT, Simon D, Lunge VR and Chies JAB (2020c) Role of the genetic variant CCR5 $\Delta 32$  in HBV infection and HBV/HIV co-infection. *Virus Res* 277:197838.  
 Enrich E, Vidal F, Sánchez-Gordo F, Gómez-Zumaquero JM, Balas A, Rudilla F, Barea L, Castro A, Larrea L, Perez-Vaquero MA *et al.* (2018) Analysis of the Spanish CCR5- $\Delta 32$  inventory of cord blood units: Lower cell counts in homozygous donors. *Bone Marrow Transplant* 53:741-748.  
 Favorova OO, Andreevski TV, Boiko AN, Sudomoina MA, Alekseenkov AD, Kulakova OG, Slanova AV and Gusev EI (2002) The chemokine receptor CCR5 deletion mutation is associated with MS in HLA-DR4-positive Russians. *Neurology* 59:1652-1655.  
 Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA and Saxon A (1981) *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men. *N Engl J Med*. 305:1425-1431.  
 Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppia D, Salgado M, Martinez-Picado J, Nijhuis M, Wensing AMJ, Lee H *et al.* (2019) HIV-1 remission following CCR5 $\Delta 32/\Delta 32$  haematopoietic stem-cell transplantation. *Nature* 568:244-248.  
 Gupta RK, Peppia D, Hill AL, Gálvez C, Salgado M, Pace M, McCoy LE, Griffith SA, Thornhill J, Alrubayyi A *et al.* (2020) Evidence for HIV-1 cure after CCR5 $\Delta 32/\Delta 32$  allogeneic haematopoietic stem-cell transplantation 30 months post analytical treatment interruption: A case report. *Lancet HIV* 7:e340-e347.  
 Hoffmann C (2007) The epidemiology of HIV coreceptor tropism. *Eur J Med Res* 12:385-390.  
 Hütter G, Nowak D, Mossner M, Ganepola S, Müßig A, Allers K, Schneider T, Hofmann J, Kücherer C, Blau O *et al.* (2009)



- Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* 360:692-698.
- Hütter G and Thiel E (2011) Allogeneic transplantation of CCR5-deficient progenitor cells in a patient with HIV infection: An update after 3 years and the search for patient no. 2. *AIDS* 25:273.
- Javor J, Párnická Z, Michalik J, Čopíková-Cudráková D, Shawkatová I, Ďurmanová V, Gmitterová K, Klímová E, Bucová M and Buc M (2015) The +190 G/A (rs1799864) polymorphism in the C-C chemokine receptor 2 (CCR2) gene is associated with susceptibility to multiple sclerosis in *HLA-DRB1\*15:01*-negative individuals. *J Neurol Sci* 349:138-142.
- Jensen BO, Knops E, Cords L, Lübke N, Salgado M, Busman-Sahay K, Estes JD, Huyveneers LEP, Perdomo-Celis F, Wittner M *et al.* (2023) In-depth virological and immunological characterization of HIV-1 cure after CCR5Δ32/Δ32 allogeneic hematopoietic stem cell transplantation. *Nat Med* 29:583-587.
- Kiepiela P, Leslie AJ, Honeyborne I, Ramduth D, Thobakgale C, Chetty S, Rathnavalu P, Moore C, Pfafferoth KJ, Hilton L *et al.* (2004) Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. *Nature* 432:769-775.
- Kulmann-Leal B, Ellwanger JH and Chies JAB (2021) CCR5Δ32 in Brazil: Impacts of a European genetic variant on a highly admixed population. *Front Immunol* 12:758358.
- Libert F, Cochaux P, Beckman G, Samson M, Aksenova M, Cao A, Czeizel A, Claustres M, de la Rúa C, Ferrari M *et al.* (1998) The *Accr5* mutation conferring protection against HIV-1 in Caucasian populations has a single and recent origin in Northeastern Europe. *Hum Mol Genet* 7:399-406.
- Lins TC, Vieira RG, Abreu BS, Grattapaglia D and Pereira RW (2010) Genetic composition of Brazilian population samples based on a set of twenty-eight ancestry informative SNPs. *Am J Hum Biol* 22:187-192.
- Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, MacDonald ME, Stuhlmann H, Koup RA and Landau NR (1996) Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86:367-377.
- Martinson JJ, Chapman NH, Rees DC, Liu Y-T and Clegg JB (1997) Global distribution of the CCR5 gene 32-basepair deletion. *Nat Genet* 16:100-103.
- Montagnier L, Dauguet C, Axler C, Chamaret S, Gruet J, Nugeyre MT, Rey F, Barré-Sinoussi F and Chermann JC (1984) A new type of retrovirus isolated from patients presenting with lymphadenopathy and acquired immune deficiency syndrome: Structural and antigenic relatedness with equine infectious anaemia virus. *Ann Inst Pasteur Virolog* 135:119-134.
- Moore JP, McKeating JA, Weiss RA and Sattentau QJ (1990) Dissociation of gp120 from HIV-1 virions induced by soluble CD4. *Science* 250:1139-1142.
- Öckinger J, Stridh P, Beyeen AD, Lundmark F, Seddighzadeh M, Oturai A, Sørensen PS, Lorentzen AR, Celius EG, Leppä V *et al.* (2010) Genetic variants of CC chemokine genes in experimental autoimmune encephalomyelitis, multiple sclerosis and rheumatoid arthritis. *Genes Immun* 11:142-154.
- Pena SDJ, Pietro GD, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy F de SG, Kohlrausch F, Magno LAV, Montenegro RC, Moraes MO *et al.* (2011) The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS One* 6:e17063.
- Proudfoot AEI (2002) Chemokine receptors: Multifaceted therapeutic targets. *Nat Rev Immunol* 2:106-115.
- Raymond M and Rousset F (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J Hered* 86:248-249.
- Rousset F (2008) Genepop'007: A complete reimplementation of the Genepop software for Windows and Linux. *Mol Ecol Resour* 8:103-106.
- Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber C-M, Saragosti S, Lapoumèroulie C, Cognaux J, Forceille C *et al.* (1996) Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* 382:722-725.
- Silva-Carvalho WHV, de Moura RR, Coelho AVC, Crovella S and Guimarães RL (2016) Frequency of the CCR5-delta32 allele in Brazilian populations: A systematic literature review and meta-analysis. *Infect Genet Evol.* 43:101-107.
- Solloch UV, Lang K, Lange V, Böhme I, Schmidt AH and Sauter J (2017) Frequencies of gene variant CCR5-Δ32 in 87 countries based on next-generation sequencing of 1.3 million individuals sampled from 3 national DKMS donor centers. *Hum Immunol* 78:710-717.
- Vargas AE, Marrero AR, Salzano FM, Bortolini MC and Chies JAB (2006) Frequency of CCR5Δ32 in Brazilian populations. *Braz J Med Biol Res* 39:321-325.
- Venkatesan S, Petrovic A, Ryk DIV, Locati M, Weissman D and Murphy PM (2002) Reduced cell surface expression of CCR5 in CCR5Δ32 heterozygotes is mediated by gene dosage, rather than by receptor sequestration. *J Biol Chem* 277:2287-2301.

## Supplementary material

The following online material is available for this article:

Table S1 – *HLA-A* genotypes.

Table S2 – *HLA-B* genotypes.

Table S3 – *HLA-DRB1* genotypes.

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