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Prevalence of *CCR5*-Δ32 and *CCR2*-V64I polymorphisms in a mixed population from northeastern Brazil

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ABSTRACT. Chemokines are low-molecular weight proteins that play a key role in inflammatory processes. Genomic variations in chemokine receptors are associated with the susceptibility to various diseases. Polymorphisms in chemokine receptor type 5 (*CCR5*)-Δ32 and *CCR2*-V64I are related to human immunodeficiency virus infection resistance, which has led to genetic association studies for several other diseases. Given the heterogeneous distribution of these polymorphisms in different global populations and within Brazilian populations, we analyzed the prevalence of *CCR5*-Δ32 and *CCR2*-V64I polymorphisms in a mixed population from northeastern Brazil. The study included 223 individuals from the general population of the city of Parnaíba, Piauí, who had a mean age of 73 years. Of these individuals, 37.2% were men

and 62.8% were women. Polymorphisms were analyzed using DNA extracted from peripheral blood leukocytes by using polymerase chain reaction alone (*CCR5*-Δ32) or accompanied by restriction endonuclease digestion (*CCR2*-V64I). In both cases, the genotypes were determined using 8% polyacrylamide gel electrophoresis and silver nitrate staining. The population conformed to Hardy-Weinberg equilibrium for both the loci studied. No individuals were homozygous for allele-Δ32, which was present in 1.8% of the population, whereas allele-64I was present in 13.9% of the participants studied; 74.9% were homozygous for the wild-type allele, while 22.4 and 2.7% were heterozygous and homozygous for the mutant allele, respectively. Additional studies are needed to investigate the relationship between these polymorphisms and disease etiopathogenesis in reference populations.

Key words: Chemokine receptor type 5-Δ32; Northeastern Brazil; Chemokine receptor type 2-V64I; Genetic polymorphisms; Mixed population

INTRODUCTION

Chemokines are low-molecular weight proteins that, in general, mediate the migration of immune cells during inflammatory responses (Griffith et al., 2014). To drive the migration of these cells during inflammation, chemokines interact with G protein-coupled 7-transmembrane domain receptors that are differentially expressed on the surfaces of several cell types. Chemokine receptor 5 (CCR5), which recognizes CCL5, CCL3, CCL4, and CCL8 chemokine ligands, is encoded by the *CCR5* gene located on 3p21 and forms a cluster with other chemokine receptor genes. This cluster includes *CCR2*, which encodes the receptor for CCL2, CCL8, CCL7, and CCL13 (Soto-Sánchez et al., 2010; Griffith et al., 2014).

Many recent studies have shown that the susceptibility of an individual to infectious and inflammatory diseases can be modulated by genomic variations in chemokine receptors (Guergnon and Combadière, 2012). The strongest evidence comes from studies of the *CCR5*-Δ32 (rs333) polymorphism, a 32-bp deletion in the *CCR5* coding region that introduces a premature stop codon and results in the production of a nonfunctional receptor (Liu et al., 1996). In 1996, Deng et al. showed that CCR5 acts as a co-receptor for human immunodeficiency virus type 1 (HIV-1), and Huang et al. (1996) subsequently found that Δ32/Δ32 individuals are generally protected from HIV-1 infection.

Other chemokine receptor gene polymorphisms may not directly affect the susceptibility of individuals to a disease, but may affect their clinical outcomes. *CCR2*-V64I (rs1799864) polymorphism, in which adenine is substituted for guanine at *CCR2* position 190, changes the amino acid valine (V) to isoleucine (I) at position 64 within the first transmembrane domain of the protein (Rabkin et al., 1999). Although *CCR2*-V64I polymorphism does not influence HIV-1 transmissibility, studies have shown that patients carrying the mutant 64I allele have better prognoses when infected with this virus (Mahajan et al., 2010; Ammaranond et al., 2013).

Since their initial characterization, *CCR5*-Δ32 and *CCR2*-V64I polymorphisms have become attractive candidates for genetic association studies of various diseases, particularly those examining the circulatory system and neoplasms (Jones et al., 2011; Lin et al., 2012;

Kucukgergin et al., 2012; Tanyel et al., 2013; Zambra et al., 2013). However, for complex diseases, the actual clinical relevance of these polymorphisms remains inconclusive and studies have shown conflicting results (Vázquez-Lavista et al., 2009; Kucukgergin et al., 2012). The genetic variability of different populations worldwide may help explain the discrepancies observed in these studies, which reinforces the need for validation of studies in different populations.

CCR5-Δ32 polymorphism is highly prevalent in individuals of European descent and virtually nonexistent in African, Asian, and Native American populations, strongly suggesting that the allele-Δ32 originated from a single mutational event in northern Europe a few thousand years ago (Novembre et al., 2005). In contrast, the *CCR2*-V64I polymorphism is relatively common in different populations and ethnic groups worldwide (De Pinho Lott Carvalhaes et al., 2004; Zafiropoulos et al., 2004; Qian et al., 2008; Zapata et al., 2013).

The Brazilian population is one of the most racially mixed in the world. Recently, Lopes et al. (2014) evaluated the ancestry and genetic distance of the population residing in the Piauí State in northeastern Brazil using 46 informative markers of ancestry. The population of this state comprised 60% European, 21.5% African, and 18.5% indigenous peoples. The distribution of *CCR5*-Δ32 and *CCR2*-64I polymorphisms has been studied in different regions in Brazil (Acosta et al., 2003; De Pinho Lott Carvalhaes et al., 2004; Boldt et al., 2009); however, the frequencies of these polymorphisms in Piauí have not been reported. Therefore, in this study, we analyzed the prevalence of the *CCR5*-Δ32 and *CCR2*-V64I polymorphisms in a sample population in the State of Piauí and compared their frequencies to those observed in Brazil and other countries.

MATERIAL AND METHODS

Study population and DNA extraction

We analyzed a sample population representing the general population of the city of Parnaíba, Piauí, Brazil (02°54′18″ S; 41°46′37″ W). The sample population included 223 elderly individuals recruited from the largest project on frailty in the elderly population in Brazil - the Network of Research on Frailty in Elderly Brazilians. All volunteers were informed of the purpose of the study and the experimental procedures. All the participating individuals signed an informed consent statement. The study was approved by the Universidade Federal do Piauí Ethics Committee in Research. Peripheral blood samples (4 mL) were collected from each subject, from which leukocyte DNA was extracted using the Wizard® Genomic DNA Purification kit (Promega, Madison, WI, USA) according to the manufacturer instructions.

Genotyping

The polymorphisms *CCR2*-V64I and *CCR5*-Δ32I were detected in the DNA samples using polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism analysis. The sequence of the primers and interpretation of the genotypes are shown in Table 1. The 25-μL reaction contained 50 ng DNA, 0.4 mM of each primer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1.5 U Taq DNA polymerase, and distilled Milli-Q H_2O. The PCR cycling parameters, optimized for both polymorphisms, were as follows: initial denaturation at 95°C for 5 min; followed by 35 cycles at 95°C for 45 s, 58°C for 45 s, and 72°C for 45 s; and a final extension step at 72°C for 7 min. To analyze the *CCR2*-V64I polymorphism, the PCR product was

digested with *Bsa*BI restriction endonuclease (New England Biolabs, Ipswich, MA, USA) according to the manufacturer recommendations. The PCR products (*CCR5*-Δ32) and digestion fragments (*CCR2*-V64I) were visualized after separation by 8% polyacrylamide gel electrophoresis and staining with silver nitrate.

Table 1. Sequence of the primers and genotype interpretations of *CCR5*-Δ32 and *CCR2*-V64I polymorphisms.

Polymorphism	Primers (forward and reverse)	Method of analysis	Genotype (bp)
CCR5-Δ32	5'-TGT TTG CGT CTC TCC CAG-3' 5'-CAC AGC CCT GTG CCT CTT-3'	PCR	wt/wt: 233 wt/Δ32: 233, 201 Δ32/Δ32: 201
CCR2-V64I	5'-TTG GTT TTG TGG GCA ACA TGA TGG-3' 5'-CAT TGC ATT CCC AAA GAC CCA CT-3'	PCR-RFLP (*Bsa*BI)	Wt/Wt: 173 Wt/64I: 173, 149, 24 64I/64I: 149, 24

To detect possible contamination, a blank PCR (no DNA template) was performed as a negative control. As an internal control of enzymatic activity, a 347-bp PCR product of *SCN5A* exon 15, which contains a known *Bsa*BI cleavage site, was digested with each set of reactions. Finally, 5% of samples were randomly selected for repeat analysis in order to ensure the reliability of the results.

Statistical analysis

Genotypic and allelic frequencies were determined by simple counting. To test whether the population was in Hardy-Weinberg equilibrium, genotype frequencies were first calculated from allele frequencies, and then their deviation from the number of observed genotypes was determined using χ^2 tests. The Fisher exact test was used to compare allele frequencies found in this study with those observed in other populations. The level of significance was set to 5%. For statistical evaluation, the BioEstat version 5.0 software was used.

RESULTS

The study participants (N = 223) included elderly individuals aged 65-93 years (mean 73 ± 4.90 years). Of these participants, 37.2% were males and 62.8% were females. Allele and genotype frequencies of the 2 loci investigated are presented in Table 2.

Table 2. Genotypic and allelic frequencies of the *CCR5*-Δ32 and *CCR2*-V64I polymorphisms in the study population.

Population	N	Genotype and allele frequency (%)							
		CCR5-Δ32			Allele	*CCR2*-V64I			Allele
		wt/wt	wt/Δ32	Δ32/Δ32	Δ32	wt/wt	wt/64I	64I/64I	64I
Total	223	96.4	3.6	0.0	1.8	74.9	22.4	2.7	13.9
Male	83	96.4	3.6	0.0	1.8	74.7	24.1	1.2	13.5
Female	140	96.4	3.6	0.0	1.8	75.0	21.4	3.6	14.3
≤73 years old	135	97.0	3.0	0.0	1.5	75.5	23.0	1.5	12.9
>73 years old	88	95.5	4.5	0.0	2.3	73.9	21.6	4.5	15.3

The genotype frequencies of the 2 polymorphisms analyzed were in Hardy-Weinberg equilibrium (*CCR5*-Δ32: $\chi^2 = 0.074$, P = 0.785; *CCR2*-V64I: $\chi^2 = 0.895$, P = 0.344). The allele-Δ32 frequency was 1.8%; 8 of the 223 study participants (3.6%) were heterozygous (wt/Δ32),

whereas no participants were homozygous (Δ32/Δ32). In contrast, 50 (22.4%) and 6 (2.7%) of the 223 participants studied were heterozygous (wt/64I) and homozygous (64I/64I) for the *CCR2*-V64I polymorphism, respectively, corresponding to an allele frequency of 13.9%. No statistically significant differences were observed in the distribution of either polymorphism according to gender and age ($P > 0.05$).

DISCUSSION

The Brazilian population is characterized by significant genetic diversity, which is the result of a rich history of miscegenation, mainly among European, African, and native Amerindian populations. Therefore, the distribution of genomic variants in the general Brazilian population does not show a consistent pattern, as is observed in other countries whose populations are predominantly Caucasian, African, or Asian (Qian et al., 2008; Barmania et al., 2013; Zwolińska et al., 2013). Therefore, each Brazilian state should be studied to clarify the distribution of genetic polymorphisms that are relevant to public health. In this study, we report the distribution of the *CCR5*-Δ32 and *CCR2*-V64I polymorphisms in the State of Piauí, located in northeastern Brazil. Such polymorphisms are associated with HIV infection resistance and, as such, have been targets of many genetic association studies for several diseases.

The frequency of allele-Δ32 in the mixed population in this study was 1.8%, which is not significantly different from the observed frequency in the general population in other regions of Brazil, which is 3-6% (Table 3).

Table 3. Comparison of the allelic frequencies of *CCR5*-Δ32 and *CCR2*-64I from this study and other Brazilian populations.

Population/ethnicity		N (*CCR5*/*CCR2*)	Alelle-Δ32	P	Alelle-64I	P	Reference
Northeastern region							
Piauí	General population	223	1.8	Ref.	13.9	Ref.	This study
Bahia	General population	549; 305	2.6	0.500	13.5	0.885	Grimaldi et al., 2002; Acosta et al., 2003
	Afro-Brazilian	153	0.98	0.500	-	-	Carvalho et al., 2004
South region							
Rio Grande do Sul	General population	103	6.3	0.139	-	-	Vargas et al., 2005
	Euro-Brazilian	102; 118	4.4	0.341	14.0	0.580	Chies and Hutz, 2003; Zambra et al., 2013
	Afro-Brazilian	58; 87	0.8	0.500	11.0	0.334	Vargas et al., 2005
Paraná	General population	134	5.6	0.222	-	-	Reiche et al., 2008
	Euro-Brazilian	172	9.3	0.029*	-	-	Boldt et al., 2009
	Afro-Brazilian	172	2.0	0.689	-	-	
	Guarani Amerindians	115	0.4	0.248	-	-	
Santa Catarina	Euro-Brazilian	99; 89	6.5	0.085	18.0	0.549	Grimaldi et al., 2002; Acosta et al., 2003
Southeastern region							
Rio de Janeiro	Afro-Brazilian	54	1.9	0.689	-	-	Chies and Hutz, 2003
São Paulo	General population	324	5.4	0.222	-	-	Pereira et al., 2000
Northern region							
Pará	General population	394; 56	3.04	0.500	16.0	0.421	De Pinho Lott Carvalhaes et al., 2004
	Afro-Brazilian	67; 61	0.75	0.500	23.0	0.072	
	Japanese immigrants	111; 50	0.0	0.248	24.0	0.052	
	Tiryió Amerindians	180; 179	0.0	0.248	26.3	0.044*	Grimaldi et al., 2002; Acosta et al., 2003
	Waiampi Amerindians	221; 82	0.0	0.248	30.0	0.005*	

*Statistically significant difference.

However, when the analysis was stratified according to ethnic groups in the population, the frequency of allele-Δ32 varied from 0-9%. Remarkably, allele-Δ32 was more frequently found in individuals of European descent in southern Brazil. Boldt et al. (2009) analyzed the distribution of this polymorphism in different ethnic groups residing in the State of Paraná in southern Brazil and found a frequency of 9.3% in a sample of 172 Euro-Brazilian individuals, which was significantly different from the population in this study ($P < 0.05$). Moreover, the frequency of this allele increased to 14.2% in a group of 53 Euro-Brazilian individuals whose ancestors were from or joined Mennonite settlements in southern Brazil (data not shown). In contrast, the frequency of allele-Δ32 in the African-Brazilian population ranges from 0.7-2.0% and is rarely observed in Indian tribes (Table 3). Additionally, one study reported a frequency of 0% in Japanese immigrants living in the State of Pará in northern Brazil (De Pinho Lott Carvalhaes et al., 2004).

Globally, the *CCR5*-Δ32 polymorphism is more prevalent in European countries and is found in 3-12% of the healthy population (Table 4). Although this value was lower, the frequency of allele-Δ32 in this study (1.8%) did not differ from that reported in some European countries, including Greece (3.3%) and Sweden (6%) ($P > 0.05$) (Zafiropoulos et al., 2004; Zheng et al., 2006). However, allele-Δ32 is present at a significantly higher frequency in countries such as Spain (9.4%) and Poland (12.1%) ($P < 0.05$) (Soto-Sánchez et al., 2010; Zwolińska et al., 2013). In Portugal, one of the countries that has most significantly influenced the genetic background of the Brazilian population, a frequency of 8% was reported for the general population, which is at the limit of statistical significance when compared with the frequency observed in this study ($P = 0.05$) (Carvalho et al., 2014). In Middle Eastern countries, variation in the allele-Δ32 frequency was more apparent, ranging from 0.6-14%, with the highest frequencies found in Turkey (9.1%) and Israel (14.3 %) ($P < 0.05$) (Martinson et al., 2000; Khabour et al., 2013; Tanyel et al., 2013). In contrast, allele-Δ32 is rarely seen in Asian and African countries (Table 4). Finally, the frequency of the *CCR5*-Δ32 polymorphism in Latin American countries ranged from 0%, as observed in a Haitian population (Martinson et al., 2000), to 3.9%, a frequency reported in Argentina (Mangano et al., 2000).

While the *CCR5*-Δ32 polymorphism exhibits a relatively restricted distribution, the *CCR2*-V64I polymorphism is distributed globally and appears to be common in all ethnic groups studied, indicating that allele-64I is much older than Δ32 (Martinson et al., 2000). In the present study, the frequency of the *CCR2*-V64I polymorphism observed in a population from northeastern Brazil was 13.9%, which is consistent with the observed frequencies in other world populations, particularly in European countries and the Middle East (Table 4). The frequency of this polymorphism is comparatively high in some Asian countries such as Japan and South Korea, where allele-64I shows frequencies of approximately 23 and 30%, respectively (Miyagishi et al., 2003; Kim et al., 2007). However, Qian et al. (2008) assessed the frequency of allele-64I in 1036 Chinese individuals belonging to different ethnicities and found that the frequency of allele-64I ranged from 2-28% according to ethnicity, with an average frequency of 13.8%. Furthermore, in a Taiwanese population, a frequency of 14.4% for allele-64I was reported, which is consistent with the population observed in this study ($P > 0.05$) (Lin et al., 2012). Our results were also consistent with those observed in African and some American populations, although the frequency of allele-64I in these populations is relatively higher, as observed in Cameroon (19.9%), Colombia (23.2%), and Mexico (24.2%) ($P > 0.05$) (Ma et al., 2005; Vázquez-Lavista et al., 2009; Zapata et al., 2013).

Table 4. Comparison of the allelic frequencies of *CCR5*-Δ32 and *CCR2*-64I from this study and other studied populations worldwide.

Population	N	Alelle-Δ32	P	Alelle-64I	P	Reference
Americas						
Brazilians, northeastern region	223	1.8	Ref.	13.9	Ref.	This study
Colombians, different regions	112	2.2	0.689	23.2	0.072	Zapata et al., 2013
Argentine, Buenos Aires	449	3.9	0.341	17.5	0.348	Mangano et al., 2000
Mexicans, Mestizos	126	1.6	0.689	24.2	0.052	Vázquez-Lavista et al., 2009
Haitians	67	0.0	0.248	-	-	Martinson et al., 2000
Europe						
Greeks, Athens	210	3.3	0.500	13.5	0.580	Zafiropoulos et al., 2004
Swedes, Västerbotten	148; 149	6.0	0.139	13.0	0.500	Zheng et al., 2006
Spanish, Asturias region	500	9.4	0.029*	9.8	0.257	Soto-Sánchez et al., 2010
Polish, Lower Silesia region	311	12.1	0.005*	-	-	Zwolinska et al., 2013
Portugueses, Porto	230	8.0	0.050	-	-	Carvalho et al., 2014
Middle East						
Jordanians, different regions	540	0.6	0.500	-	-	Khabour et al., 2013
Iranians, south region	395	1.5	0.689	12.2	0.416	Gharagozloo et al., 2005
Turkish, Istanbul	104; 197	9.1	0.029*	10.4	0.257	Tanyel et al., 2013; Kucukgergin et al., 2012
Israelis, Ashkenazim	147	14.3	0.001*	-	-	Martinson et al., 2000
Ásia						
Japanese, Hokkaido	112	0.0	0.248	29.9	0.005*	Miyagishi et al., 2003
South Koreans, Gangnam-gu	115	0.0	0.248	23.1	0.072	Kim et al., 2007
Chinese, 17 ethnic groups	1036	0.0	0.248	13.8	0.580	Qian et al., 2008
Taiwanese, Han ethnicity	216	-	-	14.4	0.580	Lin et al., 2012
Africa						
Cameroonian, different regions	321	0.0	0.248	19.9	0.173	Ma et al., 2005
South Africans, black	124	0.0	0.248	-	-	Barmania et al., 2013

*Statistically significant difference.

Within the Brazilian territory, the frequency of the *CCR2*-V64I polymorphism observed in the Piauí population was similar to that reported for the general population in other states as well as in European subpopulations or in individuals of African descent (Acosta et al., 2003; De Pinho Lott Carvalhaes et al., 2004; Vargas et al., 2005; Zambra et al., 2013). Notably, the observed frequency in indigenous tribes in northern Brazil may be as high as 30%, a significantly higher frequency than that observed in this study ($P < 0.05$) (Acosta et al., 2003). The frequency reported in a population of Japanese immigrants was 24% ($P = 0.05$) (De Pinho Lott Carvalhaes et al., 2004).

CONCLUSIONS

In conclusion, our results did not differ from those reported for other Brazilian admixed populations. Moreover, the high frequency of the *CCR5*-Δ32 and *CCR2*-V64I polymorphisms observed in certain ethnic populations reflected the high level of genetic heterogeneity in the Brazilian population. In addition to the well-documented relationship between these polymorphisms and the susceptibility to HIV infection, studies have revealed an association between these polymorphisms and diseases such as cancer and cardiovascular disease. Therefore, further studies on this population are required to determine how these polymorphisms modulate the clinical course of these diseases.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Acosta AX, Sampaio RG, Spínola JL and Galvão-Castro B (2003). Distribution of the CCR2-64I allele in three Brazilian ethnic groups. *Genet. Mol. Biol*. 26: 241-243.

Ammaranond P, Sanguansitthianan S, Phaengchomduan P, Sae-Lee C, et al. (2013). Impact of CCR2 and SDF1 polymorphisms on disease progression in HIV-infected subjects in Thailand. *J. Clin. Lab. Anal.* 27: 38-44.

Barmania F, Potgieter M and Pepper MS (2013). Mutations in C-C chemokine receptor type 5 (CCR5) in South African individuals. *Int. J. Infect. Dis.*17: 1148-1153.

Boldt AB, Culpi L, Tsuneto LT, Souza IR, et al. (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*D32 frequency in Euro-Brazilians. *Genet. Mol. Biol.* 32: 12-19.

Carvalho C, Calvisi SL, Leal B, Bettencourt A, et al. (2014). CCR5-Delta32: implications in SLE development. *Int. J. Immunogenet*. 41: 236-241.

Carvalho MWP, Leboute APM, Oliveira SF, Sousa SMB, et al. (2004). CCR5D32 mutation in three Brazilian populations of predominantly Sub-Saharan African ancestry. *Genet. Mol. Biol*. 27: 321-325.

Chies JA and Hutz MH (2003). High frequency of the CCR5delta32 variant among individuals from an admixed Brazilian population with sickle cell anemia. *Braz. J. Med. Biol. Res*. 36: 71-75.

De Pinho Lott Carvalhaes FA, Cardoso GL, Hamoy IG, Liu YT, et al. (2004). Distribution of CCR5-delta32, CCR2-64I, and SDF1-3'A mutations in populations from the Brazilian Amazon region. *Hum. Biol.* 76: 643-646.

Deng H, Liu R, Ellmeier W, Choe S, et al. (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 381: 661-666.

Gharagozloo M, Doroudchi M, Farjadian S, Pezeshki AM, et al. (2005). The frequency of CCR5Delta32 and CCR2-64I in southern Iranian normal population. *Immunol. Lett*. 96: 277-281.

Griffith JW, Sokol CL and Luster AD (2014). Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu. Rev. Immunol*. 32: 659-702.

Grimaldi R, Shindo N, Acosta AX, Dourado I, et al. (2002). Prevalence of the CCR5Delta32 mutation in Brazilian populations and cell susceptibility to HIV-1 infection. *Hum. Genet*. 111: 102-104.

Guergnon J and Combadière C (2012). Role of chemokines polymorphisms in diseases. *Immunol. Lett*. 145: 15-22.

Huang Y, Paxton WA, Wolinsky SM, Neumann AU, et al. (1996). The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nat. Med.* 2: 1240-1243.

Jones KL, Maguire JJ and Davenport AP (2011). Chemokine receptor CCR5: from AIDS to atherosclerosis. *Br. J. Pharmacol*. 162: 1453-1469.

Khabour OF, Abu-Haweleh LJ and Alzoubi KH (2013). Distribution of CCR-5Δ32, CCR2-64I, and SDF-1-3'A alleles among Jordanians. *AIDS Res. Hum. Retroviruses* 29: 151-155.

Kim HS, Kim DS, Lee EY, Sunwoo IN, et al. (2007). CCR2-64I and CCR5Delta32 polymorphisms in Korean patients with myasthenia gravis. *J. Clin. Neurol*. 3: 133-138.

Kucukgergin C, Isman FK, Dasdemir S, Cakmakoglu B, et al. (2012). The role of chemokine and chemokine receptor gene variants on the susceptibility and clinicopathological characteristics of bladder cancer. *Gene* 511: 7-11.

Lin HL, Ueng KC, Hsieh YS, Chiang WL, et al. (2012). Impact of MCP-1 and CCR-2 gene polymorphisms on coronary artery disease susceptibility. *Mol. Biol. Rep*. 39: 9023-9030.

Liu R, Paxton WA, Choe S, Ceradini D, et al. (1996). Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86: 367-377.

Lopes TR, Santos S, Ribeiro-Dos-Santos A, Resque RL, et al. (2014). Population data of the 46 insertion-deletion (INDEL) loci in population in Piauí State, Northeastern Brazil. *Forensic Sci. Int. Genet.* 9: 13-15.

Ma L, Marmor M, Zhong P, Ewane L, et al. (2005). Distribution of CCR2-64I and SDF1-3'A alleles and HIV status in 7 ethnic populations of Cameroon. *J. Acquir. Immune Defic. Syndr.* 40: 89-95.

Mahajan SD, Agosto-Mojica A, Aalinkeel R, Reynolds JL, et al. (2010). Role of chemokine and cytokine polymorphisms in the progression of HIV-1 disease. *Biochem. Biophys. Res. Commun*. 396: 348-352.

Mangano A, Kopka J, Batalla M, Bologna R, et al. (2000). Protective effect of CCR2-64I and not of CCR5-delta32 and

SDF1-3'A in pediatric HIV-1 infection. *J. Acquir. Immune Defic. Syndr*. 23: 52-57.
Martinson JJ, Hong L, Karanicolas R, Moore JP, et al. (2000). Global distribution of the CCR2-64I/CCR5-59653T HIV-1 disease-protective haplotype. *AIDS* 14: 483-489.
Miyagishi R, Niino M, Fukazawa T, Yabe I, et al. (2003). C-C chemokine receptor 2 gene polymorphism in Japanese patients with multiple sclerosis. *J. Neuroimmunol.* 145: 135-138.
Novembre J, Galvani AP and Slatkin M (2005). The geographic spread of the CCR5 Delta32 HIV-resistance allele. *PLoS Biol*. 3: e339.
Pereira RW, Pires ER, Duarte APM, Moura RP, et al. (2000). Frequency of the CCR5Δ32 allele in Brazilians: a study in colorectal cancer and in HTLV-I infection. *Genet. Mol. Biol*. 23: 523-526.
Qian Y, Sun H, Lin K, Shi L, et al. (2008). Distribution of CCR5-Delta32, CCR2-64I, SDF1-3'A, CX3CR1-249I, and CX3CR1-280M in Chinese populations. *AIDS Res. Hum. Retroviruses* 24: 1391-1397.
Rabkin CS, Yang Q, Goedert JJ, Nguyen G, et al. (1999). Chemokine and chemokine receptor gene variants and risk of non-Hodgkin's lymphoma in human immunodeficiency virus-1-infected individuals. *Blood* 93: 1838-1842.
Reiche EM, Ehara Watanabe MA, Bonametti AM, Morimoto HK, et al. (2008). Frequency of CCR5-Delta32 deletion in human immunodeficiency virus type 1 (HIV-1) in healthy blood donors, HIV-1-exposed seronegative and HIV-1-seropositive individuals of southern Brazilian population. *Int. J. Mol. Med*. 22: 669-675.
Soto-Sánchez J, Santos-Juanes J, Coto-Segura P, Coto E, et al. (2010). Genetic variation at the CCR5/CCR2 gene cluster and risk of psoriasis and psoriatic arthritis. *Cytokine* 50: 114-116.
Tanyel CR, Cincin ZB, Gokcen-Rohlig B, Bektas-Kayhan K, et al. (2013). Effects of genetic variants of CCR5 chemokine receptors on oral squamous cell carcinoma. *Genet. Mol. Res.* 12: 5714-5720.
Vargas AE, da Silva MA, Silla L and Chies JA (2005). Polymorphisms of chemokine receptors and eNOS in Brazilian patients with sickle cell disease. *Tissue Antigens* 66: 683-690.
Vázquez-Lavista LG, Lima G, Gabilondo F and Llorente L (2009). Genetic association of monocyte chemoattractant protein 1 (MCP-1)-2518 polymorphism in Mexican patients with transitional cell carcinoma of the bladder. *Urology* 74: 414-418.
Zafiropoulos A, Crikas N, Passam AM and Spandidos DA (2004). Significant involvement of CCR2-64I and CXCL12-3a in the development of sporadic breast cancer. *J. Med. Genet*. 41: e59.
Zambra FM, Biolchi V, Brum IS and Chies JA (2013). CCR2 and CCR5 genes polymorphisms in benign prostatic hyperplasia and prostate cancer. *Hum. Immunol*. 74: 1003-1008.
Zapata W, Aguilar-Jiménez W, Pineda-Trujillo N, Rojas W, et al. (2013). Influence of CCR5 and CCR2 genetic variants in the resistance/susceptibility to HIV in serodiscordant couples from Colombia. *AIDS Res. Hum. Retroviruses* 29: 1594-1603.
Zheng B, Wiklund F, Gharizadeh B, Sadat M, et al. (2006). Genetic polymorphism of chemokine receptors CCR2 and CCR5 in Swedish cervical cancer patients. *Anticancer Res.* 26: 3669-3674.
Zwolińska K, Knysz B, Rybka K, Pazgan-Simon M, et al. (2013). Protective effect of CCR5-Δ32 against HIV infection by the heterosexual mode of transmission in a Polish population. *AIDS Res. Hum. Retroviruses* 29: 54-60.