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Frequency of CCR5Δ32 in Brazilian populations

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Abstract

A sample of 103 randomly chosen healthy individuals from Alegrete, RS, Brazil, was tested for the CCR5Δ32 allele, which is known to influence susceptibility to HIV-1 infection. The CCR5Δ32 allele was identified by PCR amplification using specific primers flanking the region of deletion, followed by electrophoresis on a 3% agarose gel. The data obtained were compared to those reported for other populations and interpreted in terms of Brazilian history. The individuals studied came from a highly admixed population. Most of them were identified as white (N = 59), while blacks and browns (mulattoes) were N = 13 and N = 31, respectively. The observed frequencies, considering the white, black and brown samples (6.8, 3.8, and 6.4%, respectively), suggest an important European parental contribution, even in populations identified as black and brown. However, in Brazil as a whole, this allele shows gradients indicating a relatively good correlation with the classification based on skin color and other physical traits, used here to define major Brazilian population groups.

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One of the most interesting characteristics of the Brazilian population is its heterogeneity. When Brazil was "discovered" by the Portuguese in 1500, the land was already inhabited by Amerindians (estimated at 2 million people). Since then, emigration of individuals from different countries and continents with diverse ethnic backgrounds has contributed to the establishment of the genetic pool of the contemporary Brazilian population. These parental contributions included a constant influx of Portuguese, 4 million Africans (mainly from West-Central Africa) and 3.9 million Europeans (other than Portuguese), who arrived here in the 19th and 20th centuries (1).

However, the distribution of these immigrants was unequal in the various Brazilian regions. In the North, the populations were formed mainly by Europeans and Amerindians; in the Southeast and Northeast, Europeans, Africans and Amerindians had different degrees of influence, while in the South, the European heritage prevails (1).

CCR5 is a chemokine receptor present mainly in cells of the immune system, such as macrophages and T lymphocytes, playing a major role in the migration of these cells to sites of inflammation. The gene encoding CCR5 (*CKR5*) is located in the p21.3 region of the human chromosome 3, forming a cluster with other chemokine receptor genes (2). Deng et al. (3) demonstrated that CCR5 serves as a co-receptor for human immunodeficiency vi-

rus-1 (HIV-1). The variant allele CCR5Δ32 described by Liu et al. (2) contains a 32-bp deletion that generates a truncated protein, which confers relative resistance to HIV-1 infection.

The study of the allelic frequency of CCR5Δ32 in 18 European populations revealed an interesting North-South gradient, with the highest frequencies of the variant allele being observed in Finnish and Mordvinian populations (16%) and the lowest in Sardinia (4%) (4). The last investigators also proposed that the CCR5Δ32 allele originated from a single mutation event in Northeastern Europe a few thousand years ago. The high frequencies of CCR5Δ32 found in Europeans have been attributed to a strong selective pressure, possibly exerted by pathogens such as *Yersinia pestis* (the bubonic plague agent), *Shigella*, *Salmonella*, and *Mycobacterium tuberculosis*, all of which target macrophages, or by some other infectious diseases such as syphilis, smallpox and influenza (5).

Thus, the prevalence of this allele is of obvious medical importance. We have investigated its distribution in a random sample of individuals from Alegrete, a town located in the western region of Rio Grande do Sul (29º53' S; 55º57' W) where the population was basically established from a mixture of Spanish, Portuguese and African individuals and native Amerindians, and compared it to those already reported for other populations, interpreting the data in terms of Brazilian history.

A sample of 103 randomly chosen unrelated healthy individuals from Alegrete, RS, Brazil, was analyzed in the present study. Most of the individuals studied were identified as white (N = 59), while blacks and browns (mulattoes) were N = 13 and N = 31, respectively. This classification was based on physical appearance as judged by the researcher at the time of blood collection, and on data about the ethnicity of parents/grandparents reported by the participants. The investigation was approved by the Brazilian National Ethics Committee (CONEP No. 1333/2002) and all donors were informed about the aims of this study and signed a written consent.

DNA was extracted from saliva or blood samples using the Nucleon DNA Extraction kit (Nucleon Bioscience, Coatbridge, UK) or a salting-out method, respectively.

Genotyping was performed by PCR amplification with specific primers. PCR samples were prepared to a final volume of 25 µL as follows: 1 µL DNA (0.2-0.5 µg), 2.5 µL 10X PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 1 µL 50 mM $MgCl_2$, 1 µL 3 mM dNTP mix, 1 µL 10 pmol primer mix, and 0.2 µL *Taq* DNA polymerase, 5 U/µL (Invitrogen Corporation, San Diego, CA, USA). Samples were submitted to 40 cycles of 1 min at 94ºC, 1 min at 55ºC, and 1 min at 72ºC. The set of specific primers used to amplify the CCR5 gene segment was described by Chies and Hutz (6). It yields a 137-bp fragment for the wild-type allele and a 105-bp fragment for the CCR5Δ32 variant. PCR products were plotted on 3% agarose gel containing ethidium bromide and submitted to electrophoresis. Fragments were visualized under UV irradiation.

Skin color is used in Brazil as the equivalent of race, and is based on a complex and subjective phenotypic evaluation. In Brazil, the emphasis is on physical appearance rather than ancestry, which is in contrast to the situation in the United States. The Brazilian Institute of Geography and Statistics (IBGE) adopts the criterion of classification of individuals into the following categories: white (in Portuguese, branco), black (preto), brown (pardo), yellow (amarelo), and Amerindian (indígena). Accordingly, in Brazil as a whole, 53, 6, and 38% of the persons are identified as white, black, and brown, respectively, the remaining 3% being distributed among yellow and Amerindian persons. In Rio Grande do Sul (~10 million inhabitants), the numbers are 87.5, 5, 7, and 0.5% for white, black,

brown, and yellow + Amerindian individuals, respectively (7). More recently, the expression Afro-descendent has been incorporated into this ethnic semantic definition (8). However, the last investigators have estimated that about 148 million Brazilians present more than 10% of African nuclear genome ancestry, and that at least 89 millions of individuals have mtDNA lineages of African origin (8). This illustrates the extension of admixture in Brazil and supports the suggestion that skin color and other phenotypic traits can be poor predictors of genomic ancestry. These results reinforce the idea that, independently of the chosen criteria, it is problematic to classify people. To facilitate reading and comprehension, the word "black" will be used here to refer to any person (or population) identified and/or self-identified with some term that reports African ancestry according to physical appearance, whereas "white" will be used to define those that, according to their physical traits, do not report admixture with non-Europeans. Brown will be used to refer to individuals with intermediate physical appearance between white and black.

It has been widely observed that most populations share alleles at any given locus and that those alleles that are most frequent in one population are also found at high frequency in others, reflecting the recent dispersion of *Homo sapiens* into continental groups (9). Due to this fact, there are few classical or DNA markers that have been demonstrated either to be population-specific or to have large frequency disparities among geographically and ethnically defined populations (9).

In the present study, no CCR5Δ32/CCR5Δ32 homozygotes were detected. The presence of the CCR5/CCR5Δ32 genotype among whites, blacks and browns was 14, 8, and 13%, indicating a CCR5Δ32 allele frequency of 6.8, 3.8, and 6.4%, respectively. The CCR5Δ32 distributions observed in

Table 1. CCR5 genotype and CCR5Δ32 allele frequencies in Brazilian populations and in their putative parental groups.

Brazilian populations	No. individuals	Genotype frequencies (%)		Δ32 allele frequency (%)[a]	References
		CCR5/Δ32	Δ32/Δ32		
Brown/unclassified urban/semi-urban					
North	203	15	1	4.2	14
Southeast	539	57	0	5.3	17-19
South	31	4	0	6.4	Present study
Black urban/semi-urban					
Northeast	549	29	0	2.6	6
Southeast	54	4	0	1.9	6
South	71	3	0	0.7	6, present study
Black					
Rural	296	11	0	1.9	14,15
White urban/semi-urban					
South	158	19	1	6.6	10, present study
Parental groups					
Amerindians	1071	5	0	0.2	10,12,14,20
Europeans	2668	492	23	10.1	4
Africans	251	0	0	0	11

[a]Weighted average allele frequencies were obtained when more than one study was considered.

whites and browns in Alegrete are similar to those reported for other white Brazilians (German descendants: 6.5% (10) and Portuguese: 6.4% (4).

For a better understanding of the scenarios of the CCR5 genotype distribution and the CCR5Δ32 allele frequency in Brazil, we grouped our data with those obtained by others, considering the sample classification into three major population groups: white, black and brown/unclassified (Table 1). Additionally, Table 1 provides information about the numbers described for the three putative parental groups (European, African, and Amerindian). The CCR5Δ32 allele frequency in Europe is ~10%, whereas in Sub-Saharan African populations this allele is absent (11). Therefore, its presence among native Americans (0.2%) is probably influenced by admixture with non-Indians, as observed in the Pataxó and Kaingang tribes (12,13).

Considering the frequencies in the major human geographical groups shown in Table 1, CCR5Δ32 can be referred to as a private European allele, and could be used along with other genetic markers in studies of genome ancestry in admixed populations.

The frequency of the CCR5Δ32 allele in the Brazilian populations identified as brown/unclassified ranges from 4.2 in the North region (14) to 6.4 in the South (present study). Our data contribute to the establishment of a scenario that shows a North-South gradient. This view is compatible with the colonization of Brazil, since the Southeast and South regions received the highest numbers of European immigrants during the 19th and early 20th centuries (1). The numbers are lower when we consider the black Brazilian populations, ranging from 0.7 to 2.6% (14,15, present study), and are indicative of an inverse gradient. Curiously, rural black populations, which are usually the descendants of "quilombos" (communities founded by fugitive slaves at colonial times), show average age CCR5Δ32 allele frequency values similar to those observed in urban black samples (14,15). These results suggest that the level of introgression of European genes in these small rural communities is not very different from that observed in urban groups. Alternatively, random drift could be evoked to explain an increase in the frequency of formerly rare alleles, a phenomenon that has been frequently described in other quilombo communities (16).

Finally, our investigation concerning a typical European private allele revealed that the CCR5Δ32 frequencies in Brazil as a whole (Table 1) show a relatively good correlation with the classification based on skin color and other physical traits, used here to define major Brazilian population groups.

The implication of these results for public health is that the contemporary Brazilian populations may be differentially susceptible to infectious diseases, and that caution is needed since, sometimes, depending on the population and genetic markers used, the association between phenotypic characters and genome ancestry is not found, whereas in others, as demonstrated here, it is.

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