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Research Article

CCR5D32 mutation in three Brazilian populations of predominantly Sub-Saharan African ancestry

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Abstract

This study reports the frequencies of the *CCR5D32* mutation of the β-chemokine 5 gene and discusses the possible effects of past and recent gene flow in three *quilombo* remnants (Brazilians communities with anthropological African ancestry whose ancestors were escaped slaves): Rio das Rãs, Mocambo, and São Gonçalo in the northeastern region of Brazil. The *CCR5D32* allele frequency of the Mocambo population was significantly higher (5.6%) than that found in the Rio das Rãs (1%) and São Gonçalo (0.9%) populations. These differences may reflect different proportions of parental populations in the founders individuals, a founder-effect and/or different histories of inter-ethnic contact. The frequency of the *CCR5D32* allele in the Mocambo sample is similar to that found in those urban Brazilian populations which have a large amount of European genetic input, indicating a European contribution to the gene pool of this population and suggesting that, perhaps since its foundation, Mocambo has had a high level of admixture or experienced a founder-effect.

Key words: CCR5, genetic polymorphism, Sub-Saharan Afro-derived Brazilian populations, ethnic-grouspecific marker.

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Introduction

Official reports estimate that about 3.5 million Africans were brought to Brazil as slaves between 1500 and 1850 (Salzano, 1986), during which time groups of fugitive slaves broke out of their workplaces and settled down in remote areas where they founded independent and isolated communities called *quilombo*s. Some of these communities, known as *quilombo* remnants and officially recognized by the Brazilian government, have kept their cultural identities and now claim the land historically occupied by them. At present, at least 743 remnant *quilombo* communities have been identified in Brazil with most of them being located in the northeastern region of Brazil. In many of these communities genetic studies have shown several degrees of admixture with indigenous groups and European descendants (*e.g.*, Arpini-Sampaio *et al*., 1999; Guerreiro *et al*., 1999).

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The β-chemokine receptor 5 protein, which acts as an entry co-receptor for the HIV-1 virus, is coded for by the CCR5 gene which sometimes presents a 32 bp deletion mutation (the CCR5D32 allele) that results in a truncated protein associated with resistance to HIV-1 infection (Dean *et al*. 1996; Berger *et al*., 1999). There are rare reports of HIV-infected individuals who are *CCR5D32* homozygous (Hogan and Hammer, 2001). Epidemiological studies have associated the *CCR5/CCR5D32* heterozygotic state with a delay in the onset of AIDS and a higher degree of resistance against HIV infection than the wild type *CCR5/CCR5* homozygous state (Venkatesan *et al*., 2002).

The frequency distribution of the *CCR5D32* allele varies considerably between different ethnic groups, being common in Europeans (±10% frequency) but quite rare or absent (< 1%) in Asian, African and Brazilian indigenous

groups (Martinson *et al.* 1997; Leboute *et al.,* 1999; Williamson *et al.*, 2000; Lucotte, 2001).

Passos and Picanço (1998) found a *CCR5D32* allele frequency of 3.5% in one urban Brazilian population, which may have resulted from a European genetic contribution. Leboute (2000) reported that there is a decreasing cline in *CCR5D32* frequencies from southern (8.1%) to northern (3.3%) Brazil which may be compatible with the differential influence of European immigration in the various regions of the country. Grimaldi *et al*. (2002) observed that *CCR5D32* allelic frequencies vary according to ethnic origin and reflect the admixture that occurred between the various ethnic immigrant groups after their arrival in Brazil. As a result of this differential distribution, *CCR5D32* frequencies may be used as an indicator of the European contribution to populations of mixed ethnic origin.

No study has yet been made to identify the frequency of the *CCR5D32* allele in *quilombo* remnant communities. The objectives of our study were to assess the frequency of the *CCR5D32* allele in three *quilombo* communities in northeastern Brazil of Mocambo, Rio das Rãs and São Gonçalo and to investigate the effect of past or recent gene flow on the allele frequencies in these communities.

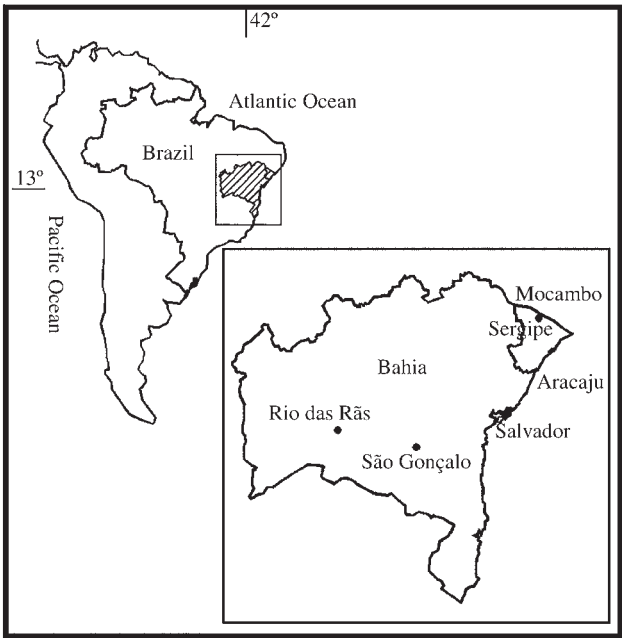

Figure 1 - Geographic locations of Rio das Rãs, Mocambo and São Gonçalo, three Afro-derived populations in northeastern Brazil.

Material and Methods

Populations and sample size

The populations studied were *quilombo* remnants situated in northeastern Brazil which were founded mainly by sub-Saharan Africans or African-derived individuals at least 150 years ago. The communities studied (Figure 1) were: (1) Rio das Rãs, a community lying near the eastern margin of the São Francisco river at 13°41' S, 43°20' W and which had at the time of sampling an estimated population of 4,000 people distributed between two large villages (Rio das Rãs and Brasileiras) and several small villages (*e.g.* Enchú, Retiro and Capão do Cedro). For our study we selected 100 individuals, 72 born locally (48 in Rio das Rãs, 13 in Brasileiras and 11 in the small villages) and 28 (28%) recent immigrants; (2) Mocambo, a community situated on the eastern bank of the São Francisco river at 9°4' S, 37°25' W with an estimated population of 500 people, of which we chose 71 individuals for our study, 53 born locally and 18 (25%) recent immigrants; (3) São Gonçalo, a community located at 13°45' S, 41°02' W which had an estimated population of 194 people, of which we sampled 53, 38 born locally and 15 (28%) recent immigrants. The population data for these sites comes from Oliveira *et al*. (2002). The total sample size was 224 people (163 born locally and 61 recent immigrants) with, in most cases, the oldest person in each family being selected for the study. The highest relationship coefficient accepted in the sample was 1/16. Recent immigrants, people who did not born in the communities which were living there, were included in the study and their data could therefore be used to analyze the impact of genetic flow on the CCR5D32 allele frequency in these communities.

DNA extraction and polymerase chain reaction conditions

We collected 4 ml of venous blood from volunteers of each community. We explained orally the purpose of this study to each donator who consent the blood collect. For the São Gonçalo samples, genomic DNA was extracted from the leukocytes contained in 300 µL of blood cell suspension according to Higuchi's protocol (1989), while for the Mocambo and Rio das Rãs populations it was extracted from the buffy coat leukocytes using a standard phenol-chloroform technique. A heterozygous individual from an urban Brazilian population of known European origin was used as a positive control. The target sequence was amplified using the polymerase chain reaction (PCR) conditions and primers described by Martinson *et al*. (1997) to produce a 193 bp product for the normal *CCR5* allele and a 161 bp product for the mutant *CCR5D32* allele, the products being separated by electrophoresis in 8% non-denaturing polyacrylamide gels and stained with silver nitrate.

Statistical analysis

Allelic frequencies were estimated by direct counting, conformity to a Hardy-Weinberg equilibrium being tested using the chi-squared (χ^2) test. To analyze the impact of genetic flow on the frequency of the *CCR5D32* mutation in each community we used Pearson's χ^2 test of heterogeneity to compare native born individuals pooled as a group to recent immigrants pooled as a group, the test being per-

formed using the BIOSYS software (Swofford and Selander, 1981). Allelic frequencies at the *CCR5* locus were compared for all population pairs using 2x2 contingency tables and Fisher's exact test (Zhang *et al*., 1998).

Results and Discussion

The *CCR5D32* allele was found in the populations studied exclusively as the *CCR5/CCR5D32* heterozygote, the *CCR5/CCR5D32* genotype ratios being 2:100 in Rio das Rãs, 8:71 in Mocambo, and 1:53 in São Gonçalo. None of these populations showed significant deviation from the Hardy-Weinberg equilibrium (Table 1).

The *CCR5*/*CCR5D32* individual detected in São Gonçalo (the smallest community) was a recent immigrant and the occurrence of the *CCR5D32* allele was therefore a new event in this population, being introduced by recent gene flow. One of the two *CCR5/CCR5D32* individuals in Rio das Rãs was born in the community (Brasileiras village), while the other was a recent immigrant. In Mocambo, three out of the eight *CCR5/CCR5D32* individuals were recent immigrants. In spite of these facts, the migration observed during the last generation did not change the frequency of the *CCR5D32* allele in Rio das Rãs or Mocambo, since we found no significant differences between the group of locally born individuals and the group of recent immigrants (Rio das Rãs $\chi^2 = 0.485$, $p = 0.486$; Mocambo $\chi^2 = 0.661$, $p = 0.416$).

In western Europe there is a north-to-south declining gradient in the frequency of the *CCR5D32* allele (Lucotte, 2001) and compared with its frequency in Europeans (9.4%) this allele is rare in Africans (0.1%) (Williamson *et al*., 2000). The IRI3.1*0 and IRI3.2*0 alleles which flank the CCR5 gene are present on most chromosomes containing the *CCR5D32* allele (95% for the IRI3.1*0 allele and 88% for the IRI3.2*0 allele) which suggests that the 32 bp deletion arose in northeastern Europe from a single recent mutational event (Libert *et al*., 1998), probably about 700 years ago (Stephens *et al*., 1998). The increase in frequency of the *CCR5D32* mutant allele in a relatively short period of time could be due to selection in favor of this allele. The high frequency of the *CCR5D32* allele in European populations and its virtual absence in African, Amerindian and Asian populations (Martinson *et al*., 1997; Leboute *et al*., 1999; Williamson *et al*., 2000; Lucotte, 2001) implies that this allele can be used as a marker of European origin. The occurrence of the *CCR5D32* allele in remnant *quilombo* populations indicates the presence of European genes, which may have entered the gene-pool of these populations either when the populations were founded or more recently due to gene flow, although it is also conceivable that both events may have happened.

Statistically, the frequency of the *CCR5D32* allele in the Mocambo population (5.6%) is significantly higher (Fisher's test, $p = 0.019$) than that observed in the Rio das Rãs population (1.0%) and similar ($p = 0.059$) to that observed in the São Gonçalo population (0.9%). This may reflect the occurrence of different proportions of the *CCR5D32* allele from the parental populations at the foundation of these communities and/or different histories of inter-ethnic contact after foundation. The frequency of the *CCR5D32* allele in Mocambo is similar to the average of 5.6% obtained by Leboute (2000) and the 3.5% average found by Passos and Picanço (1998) for urban populations in Brazil, an observation which suggests that the Mocambo community has the highest contribution of European genes of all the communities studied. However, the high fre-

Table 1 - Genotypes and allelic frequencies of the β-chemokine CCR5 gene found in three different African-derived *quilombo* communities in northeastern Brazil.

	Community					
	Rio das Rãs		Mocambo		São Gonçalo	
Genetic constitution	N#	Immigrants*	N#	Immigrants*	N#	Immigrants*
Genotypes						
CCR5/*CCR5*	98	27	63	15	52	14
CCR5/*CCR5D32*	2	1	8	3	1	1
Total	100	28	71	18	53	15
Allele						
CCR5	0.990	0.982	0.944	0.917	0.991	0.967
CCR5D3[2]	0.010	0.018	0.056	0.083	0.009	0.033
χ^2	0.013		0.255		0.009	
p	0.90		0.75 > p > 0.50		>0.99	

#Total number of analyzed individuals, compound by people born in the community plus recent immigrants (shown in the adjacent column).
*Number of recent immigrants.

quency of the *CCR5D32* allele observed in the Mocambo community could also be the result of a founder-effect.

A high frequency of one specific Y chromosome haplotype which is common in Europeans and rare or absent in Africans has been found in Mocambo (Lima, 2002). Two scenarios could have led to this situation: a) a significant European contribution during the history of the community or b) the occurrence of a founder-effect. Historic data indicates the occurrence of miscigenation in the Brazilian state of Sergipe before the end of slavery in Brazil (Mott ,1974; 1976), probably between African-derived and European-derived individuals. In addition, Lima (2002) found the presence of a low level of haplotypic diversity in Mocambo, which indicates a small number of founders. Both these sets of findings support the founder effect hypothesis, and our data also suggests a founder effect in Mocambo. The high frequency may be due to the fact that at least one of the founders in those populations presented the *CCR5D32* mutation.

The presence of the *CCR5D32* allele in these three *quilombo* remnant population supports the idea of a European contribution to the present genetic makeup of the populations studied by us. It appears, therefore, that the *CCR5D32* mutation is an efficient genetic marker for the evaluation of the European genetic contribution, and its investigation is useful for the purpose of estimating inter-ethnic admixture.

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