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Frequency of the *CCR5Δ32* allele in Brazilians: a study in colorectal cancer and in HTLV-I infection

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Abstract

The identification of a 32-bp deletion in the cc-chemokine receptor-5 gene *(CCR5Δ32* allele*)* that renders homozygous individuals highly resistant to HIV infection has prompted worldwide investigations of the frequency of the *CCR5Δ32* allele in regional populations. It is important to ascertain if *CCR5Δ32* is a factor to be considered in the overall epidemiology of HIV in individual populations. With this in mind we determined the *CCR5Δ32* allele frequency in a large sample (907 individuals) of the southeastern Brazilian urban population, stratified as follows: 322 healthy unrelated individuals, 354 unselected colorectal cancer patients, and 229 blood donors. The three groups displayed essentially identical allelic frequencies of *CCR5Δ32* and pairwise comparisons did not show significant differences. Thus, our results can be pooled to provide a reliable estimate of the *CCR5Δ32* allele frequency in the southeastern Brazil of 0.053 ± 0.005. The blood donors comprised 50 HTLV-I serologically negative individuals, 115 non-symptomatic individuals HTLV-I positive by ELISA but with indeterminate Western blot results, 49 healthy blood donors HTLV-I positive both at ELISA and Western blot and 15 patients with clinical spinal cord disease (HAM). A suggestive trend was observed, with the *CCR5Δ32* frequencies decreasing progressively in these four categories. However, when we applied Fischer's exact test no significant differences emerged. We believe that further studies in larger cohorts should be performed to ascertain whether the *CCR5Δ32* allele influences the chance of becoming infected or developing clinical symptoms of HTLV-I infection.

INTRODUCTION

The cc-chemokine receptor-5 gene protein (CCR5) is a seven-transmembrane-domain G protein coupled receptor that has as its natural ligands RANTES (regulated on activation normal T-cell expressed and secreted), MIP-alpha (macrophage inflammatory protein) and MIP-beta, which are members of the CC subfamily of chemokines (Samson *et al.*, 1996a). The CCR5 protein, in concert with CD4, is also the receptor that mediates the internalization of HIV in macrophages and monocytes (Dragic *et al.*, 1996). Studies of individuals who have been multiply exposed to HIV and yet remain uninfected led to the identification of the *CCR5Δ32* allele, that exhibits a 32-bp deletion in the *CCR5* gene (Liu *et al.*, 1996). Because of a frameshift at amino acid 185 the deleted allele codes for a truncated protein that is consequently not integrated into the cell membrane (Liu *et al.*, 1996). The homozygous state for the *CCR5Δ32* allele has been shown to be associated with a high degree of protection against HIV infection *in vivo* (Dean *et al.*, 1996; Huang *et al.*, 1996; Samson *et al.*, 1996b). This protection is not absolute as demonstrated by identification of one HIV-1-infected *CCR5Δ32* homozygote (Biti *et al.*, 1997; O'Brien *et al.*, 1997). Although there is no evidence for a strong protective effect in heterozygotes (Dean *et al.*, 1996; Huang *et al.*, 1996; Michael *et al.*, 1997), a modest reduction in HIV incidence has been reported (Samson *et al.*, 1996b). Also, the observation that cohorts of long-term survivors are enriched for *CCR5Δ32* heterozygotes suggests the existence of some protection against the AIDS progression (Dean *et al.*, 1996; Huang *et al.*, 1996; Michael *et al.*, 1997).

The *CCR5Δ32* allele is common in North America and Europe, with heterozygote frequencies varying from 10 to 20% in Caucasians (Dean *et al.*, 1996; Huang *et al.*, 1996; Liu *et al.*, 1996; Samson *et al.*, 1996b; Martinson *et al.*, 1997). In Eurasia *CCR5Δ32* frequencies show a north-south gradient with the highest allele frequencies in Finnish and Mordvinian populations (16%) and the lowest in Sardinia (4%) (Libert *et al.*, 1998). The *CCR5Δ32* allele is not found in aboriginal populations outside Eurasia (Martinson *et al.*, 1997; Leboute *et al.*, 1999; Lu *et al.*, 1999). The presence of *CCR5Δ32* allele elsewhere throughout the world probably represents recent European gene flow into local populations (Martinson *et al.*, 1997). The origin of the *CCR5Δ32* has been speculatively dated to approximately 700 years

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ago, with its frequency increasing rapidly as consequence of a strong selective pressure, possibly an ancient plague (Stephens *et al.*, 1998). A possible candidate is the bubonic plague that claimed the lives of 25-33% of Europeans in the 14th century (Stephens *et al.*, 1998).

HTLV-I is a retrovirus from the oncoretroviridae family with variable prevalence rate in human populations. The vast majority of HTLV-I-infected individuals have no associated symptoms. However, 1 in every 1000-2000 individuals with positive serology per year develop adult T-cell leukemia/lymphoma (ATLL) (Poiesz *et al.*, 1980; Tajima, 1990) and other patients may present with myelopathy (HAM) (Gessain *et al.*, 1985). The prevalence of the latter has been estimated to be 2.4% in the United States (Murphy *et al.*, 1997) but only about 0.07% among the Japanese (Osame *et al.*, 1990). Several genetic aspects, viral or human, have been tested as possible determinants of clinical course of the HTLV-I infection. Among the viral markers, *tax* mutations (Renjifo *et al.*, 1995) and the proportion of synonymous and non-synonymous mutations on the *tax* have been tested (Niewiesk and Bangham, 1996). Regarding host genetic markers, the HLA polymorphisms are the main class tested until now, mostly with weak associations (LaGrenade *et al.*, 1996; Sonoda *et al.*, 1996; Jeffery *et al.*, 1999). Recently we have tested polymorphisms of endogenous retroviral sequences (Pereira, R.W., Proietti, A.B. and Pena, S.D.J., unpublished results) but did not see any association with one of the clinical outcome of HTLV-I infection.

Inter-population differences in *CCR5Δ32* frequency may influence the pattern of HIV transmission and will need to be incorporated into future predictions of HIV levels (Martinson *et al.*, 1997). We thus considered it important to establish the frequency of *CCR5Δ32* allele in the Brazilian population to support future studies in groups at risk of HIV infection. Moreover, we evaluated two special groups: patients with colorectal cancer and patients with HTLV-I infection.

MATERIAL AND METHODS

We analyzed 322 unrelated Brazilian individuals randomly chosen among unrelated participants in paternity testing studies. Most of the patients were self-classified as "white" and originated from the southeast region of Brazil. We also studied 354 unselected colorectal cancer patients from the Hospital do Câncer A.C. Camargo in São Paulo. Finally, 229 individuals participating in a cohort study at Fundação Hemominas were distributed as follows: 50 HTLV-I seronegative individuals, 115 non-symptomatic individuals HTLV-I positive at the ELISA test but with indeterminate Western blot results, 49 healthy blood donors HTLV-I positive both at ELISA and Western blot and 15 HTLV-I patients with clinical spinal cord disease (HAM). Consent was obtained from all participants and all analyses were performed anonymously.

The deletion in *CCR5* was assayed by means of the polymerase chain reaction (PCR) with previously described primers (Samson *et al.*, 1996b). PCR was carried out with 20 pmol of each primer, 0.1 mM dNTPs, 1.5 mM $MgCl_2$ 75 mM KCl, 10 mM Tris-HCl, pH 8.3, and 1 unit of Taq polymerase in a final volume of 20 µl. Cycle conditions were: initial denaturation of 94°C for 3 min and 30 cycles of 94°C for 1 min, 62°C for 2 min, 72°C for 1 min, followed by a final extension step of 72°C for 5 min. A sample of the reaction product (5 µl) was run on 5% polyacrylamide gels and visualized by silver staining (Santos *et al.*, 1993). Typing was unambiguous, the wild type allele and the *CCR5Δ32* allele generating fragments of 735 bp and 703 bp, respectively.

The allele frequencies were calculated by gene counting. The Hardy-Weinberg equilibrium of the groups and the significance level of the genotypic and allelic frequency differences between groups were calculated using Fisher's exact test.

RESULTS

Our three groups (healthy individuals, colorectal cancer patients and blood donors) displayed essentially identical allelic frequencies of *CCR5Δ32* (Table I) and pairwise comparisons did not show significant differences. In all the groups the genotype frequencies did not deviate significantly from the Hardy-Weinberg equilibrium. Thus, our results can be pooled to provide a reliable estimate of the *CCR5Δ32* allele frequency in southeastern Brazil of 0.053 ± 0.005, which agrees with previous estimates (Martinson *et al.*, 1997).

Within the blood donor patients, we compared the allelic and genotypic frequency of *CCR5Δ32* in four groups: HTLV-I negative healthy blood donors, HTLV-I ELISA positive and Western blot negative healthy individuals, ELISA and Western blot positive non-symptomatic individuals, and ELISA and Western blot positive symptomatic patients with HAM (Table II). A suggestive trend was observed, with the *CCR5Δ32* frequencies decreasing progressively in these four categories. However, as expected from the small number of symptomatic patients and the broad confidence limits of the *CCR5Δ32* allele frequencies, no significant differences were obtained when we applied the Fischer's exact test.

DISCUSSION

The Brazilian population is predominantly of European ancestry with significant contributions from African and Amerindian gene pools (Pena *et al.*, 2000). Since the *CCR5Δ32* allele has not been identified in Africans (Martinson *et al.*, 1997) or Amerindians (Martinson *et al.*, 1997; Leboute *et al.*, 1999), the presence of the *CCR5Δ32* allele in Brazilians can be attributed to European immigration. The 0.053 frequency of *CCR5Δ32* allele that we ob-

Table I - Determination of the *CCR5Δ32* allele frequency in the urban population of southeastern Brazil.

Population	N	Genotype			*CCRΔ32* frequency (95% binomial confidence limits)
		CCR5/CCR5	*CCR5/CCR5Δ32*	*CCR5Δ32/CCR5Δ32*	
Healthy individuals	324	289	35	0	0.054 (0.038-0.074)
Cancer patients	354	318	34	2	0.054 (0.036-0.070)
Blood donors	229	205	24	0	0.052 (0.034-0.077)
Total	907	812	93	2	0.053 (0.043-0.064)

Table II - Determination of the *CCR5Δ32* allele frequency in blood donors with different status in reference to HTLV-I infection.

Blood donors	N	Genotype			*CCRΔ32* frequency (95% binomial confidence limits)
		CCR5/CCR5	*CCR5/CCR5Δ32*	*CCR5Δ32/CCR5Δ32*	
ELISA + WB negative	50	43	7	0	0.070 (0.029-0.139)
ELISA positive, WB indeterminate	115	102	13	0	0.057 (0.030-0.095)
ELISA + WB positive, non-symptomatic	49	45	4	0	0.041 (0.011-0.101)
ELISA + WB positive, symptomatic with HAM	15	15	0	0	0.000 (0.000-0.116)
Total	229	205	24	0	0.052 (0.034-0.077)

WB, Western blot.

served in our sample is lower than the average frequency in Europe (0.10), reflecting the African and Amerindian contributions. A previous estimate of the *CCR5Δ32* allele frequency among Brazilian individuals was 0.035, but only 100 individuals were studied (Passos and Picanco, 1998). At any rate, the *CCR5Δ32* allele frequency will fluctuate according to the proportion of European versus African or Amerindian ancestry in the sample.

The *CCR5Δ32* allele frequency in patients with colorectal cancer was exactly the same as in healthy individuals. When we compared the frequencies in relation to HTLV-I infection a suggestive trend was observed with the frequency decreasing progressively among the four categories of blood donors (Table II). However, when we applied Fischer's exact test no significant differences emerged. We believe that further studies in larger cohorts should be performed to ascertain whether the *CCR5Δ32* allele influences the chance of becoming infected or developing clinical symptoms of HTLV-I infection.

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RESUMO

A observação de que indivíduos homozigotos para uma deleção de 32 pares de base no gene que codifica para o receptor 5 de cc-quimiocinas apresentam um menor risco de contrair a infecção por HIV-1 levou à investigação da freqüência deste polimorfismo em várias populações mundiais. É importante investigar se o *CCR5Δ32* é um fator a ser considerado na epidemiologia do HIV em populações individuais. Com estes pressupostos em mente nós estabelecemos a freqüência do *CCR5Δ32* em uma grande amostra (907 indivíduos não-relacionados) da população urbana do sudeste brasileiro, estratificada da seguinte maneira: 322 indivíduos sadios, 354 pacientes com câncer colorretal e 229 doadores de sangue. Os três grupos apresentaram essencialmente a mesma freqüência alélica de *CCR5Δ32* e a comparação par-a-par não revelou diferenças significativas. Assim, os nossos resultados podem ser agrupados para fornecer uma estimativa confiável de 0,053 ± 0,005 da freqüência alélica de *CCR5Δ32*. Os doadores de sangue compreendiam 50 indivíduos soronegativos para HTLV-I, 115 indivíduos assintomáticos por ELISA mas com resultados indeterminados em *Western blot*, 49 indivíduos soropositivos para HTLV-I mas assintomáticos e 15 indivíduos soropositivos para HTLV-I sintomáticos com mielopatia. Foi observado um sugestivo gradiente decrescente da freqüência alélica de *CCR5Δ32* nestas categorias. Entretanto, quando aplicamos o teste exato de Fisher, não emergiram diferenças significativas. Para uma melhor avaliação da influência do alelo *CCR5Δ32* na probabilidade de infectar-se

com HTLV-I ou de desenvolver doença clínica serão necessários estudos com um maior número de doadores de sangue.

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