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Distribution of CCR5-Δ32 and CCR2-64I alleles in an Argentine Amerindian population

Key words:

Amerindian; CCR5; CCR2; polymorphisms

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Abstract: In order to evaluate the frequency distributions of CCR5-Δ32 and CCR2-64I polymorphisms in an Amerindian population, we tested a total of 42 Chiriguano individuals that are aboriginal inhabitants of the north west of Argentina. Only one carried the CCR5-Δ32 allele (0.0238), while 17 out of 35 carried the CCR2-64I mutation, including one homozygous for the mutated allele (0.2571). Although the cohort studied is considered highly endogamic, the HLA genotyping revealed that 8 out of 42 subjects had a gene flow from Caucasian populations. The only heterozygous CCR5+/Δ32 found and three heterozygous CCR2+/64I belonged to the admix group. In conclusion, the protective deletion CCR5-Δ32 is practically absent in Chiriguano whereas the CCR2-64I allele is highly frequent.

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Chemokine receptors are seven transmembrane-spanning molecules that in response to their natural ligands are involved in chemotaxis of leukocytes towards areas of inflammation. Besides their physiological functions, several of them serve as HIV-1 coreceptors mediating entry in CD4-bearing cells (1, 2). Of them, CC-chemokine receptor 5 (CCR5) is considered the main coreceptor used by HIV-1 macrophage-tropic strains (R5) recognized as the most frequent transmitter (3–7). Genetic variants of some coreceptors can modify HIV-1 transmission and/or disease progression. The first protective allele identified was the CCR5-Δ32. The mutation consists in a 32 base pair (bp) deletion in the coding region of the CCR5 gene that causes a frameshift, generating a non-functional/truncated protein. The non-expression of the receptor CCR5 on the cell surface prevents R5 HIV-1 but not X4 HIV-1 cell entry (8–10). Therefore, homozygotes for the CCR5-Δ32 allele are highly, but not absolute resistant to HIV-1 infection (8, 9, 11–16). Heterozygosity do not modify HIV-1 transmission but can confer a delay in progression to AIDS in HIV-1-infected adults (8, 10, 17–21). Thereafter, a point mutation in another R5 HIV-1 coreceptor, the CCR2b minor receptor, has been described (22, 23). This polymorphism consists in a conservative

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substitution of a guanine for adenine that changes a valine for an isoleucine (CCR2-V64I) in the first transmembrane domain of the coreceptor. The CCR2-64I allele is able to influence HIV-1 disease outcome by delaying the onset of AIDS and by extending the survival time in infected subjects (21, 22, 24–26).

The global distribution of the CCR5-Δ32 and CCR2-64I alleles according to the ethnic background is widely variable. The CCR5-Δ32 allele is more prevalent in Caucasian populations and almost absent in African and Asians groups (10). Within European populations a wide variation of 10–20% in CCR5-Δ32 frequency was observed with a gradient, uppermost at the north around the Baltic Sea down to the Mediterranean coast. In Ashkenazi Jews, a highly endogamous population of ancient Israeli and east European descent, had been recorded the highest frequency (27, 28). In Caucasian populations from U.S. and Southern Europe, CCR5-Δ32 was observed at a frequency of 5–10%, but decreased to 2–5% in Hispanic populations, throughout the Middle East or in the Indian continent. However, this allele is almost absent among African-Americans in whom admixture with people of European descents has been considerable (27).

Contrary to the CCR5-Δ32 distribution, the CCR2-64I allele is more prevalent in the Asian population with a frequency of 25% (22). Though population surveys have not been so extensive as for

CCR5 genotypes, CCR2-64I polymorphism was also commonly found among Hispanics (17%) and African-Americans (15%), whereas the frequency in Caucasian populations was the lowest reported (10%) (22).

In the present study, we analyzed the presence and distribution of CCR5-Δ32 and CCR2-64I alleles in an Argentine Amerindian cohort called Chiriguano. This population lives in isolation in the province of Salta, located north West of Argentina, and represents a highly endogamous group with little admixture with Caucasians. Genomic DNA was obtained from 42 Chiriguano using anonymously collected samples. The CCR5 and CCR5-Δ32 alleles were amplified by PCR with the flanking primers CCR5 sense GTCTTCATTACACCTGCAGCTCT and CCR5 antisense CAGCCCTGTGCCTCTT, that produce fragments of 184 bp for the wild-type allele and of 152 bp for the mutant allele (29). The amplicons were detected by 2.5% agarose gels electrophoresis. CCR2 genotypes were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) assay as reported by Smith et al. (22). The 128-bp amplified fragment was digested with a *Bsa* *BI* restriction enzyme that yields fragments of 110 and 18 bp when the CCR2-64I polymorphism is present. The digested products were separated by 4% agarose (3:1 high biotechnology resolution; Amresco, USA) gels electrophoresis.

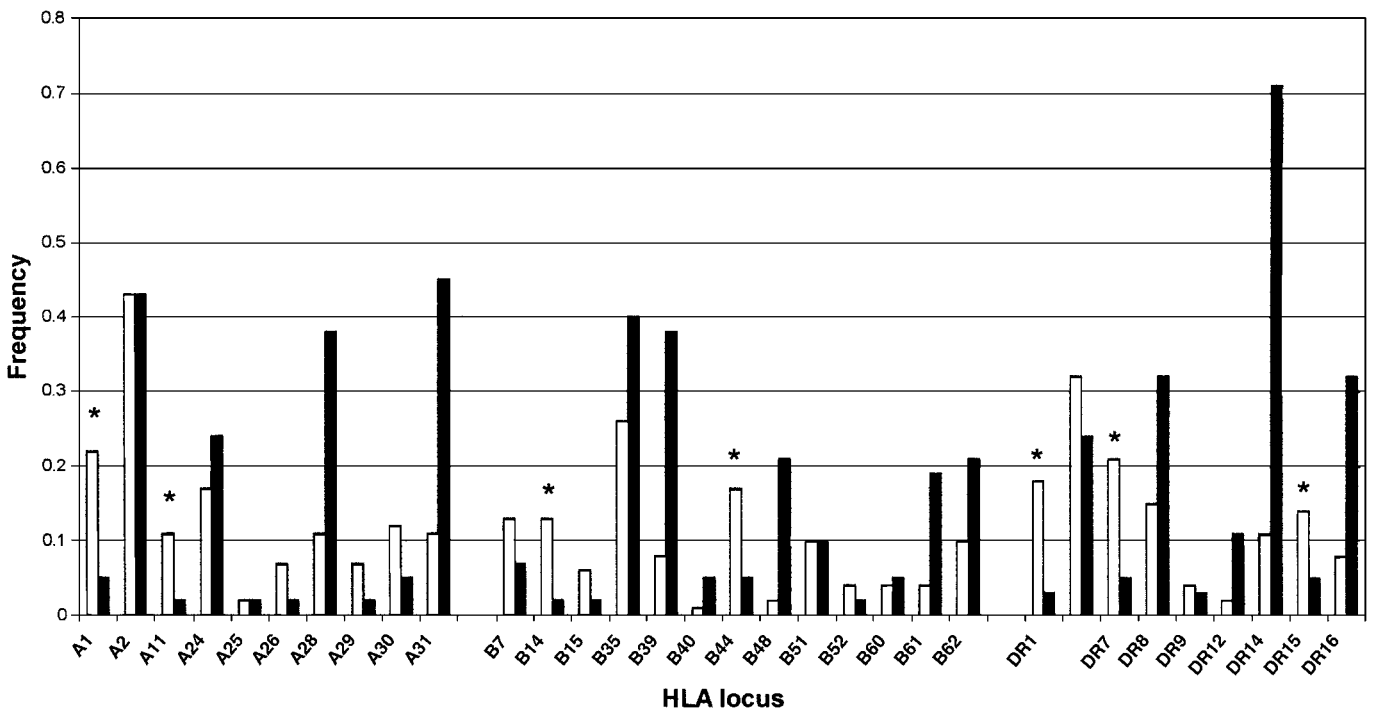


Fig. 1. Frequency distributions of HLA-A, B and DR locus in 42 Chiriguano subjects and in Argentine Caucasian subjects (HLA-A n=275; HLA-B n=272; HLA-DR n=1195). □ Argentine Caucasian

subjects, ■ Chiriguano subjects. * Chiriguano alleles considered with Caucasian origin.

As described in Table 1, we found that 41 individuals were homozygous for the wild-type CCR5 allele and one was heterozygous for the CCR5-Δ32 allele, giving a CCR5-Δ32 allelic frequency of 0.0119. In relation to CCR2 genotypes, of the total 42 Chiriguano studied, we had available DNA from 35 subjects, of whom 18 were homozygous for the CCR2 wild-type allele, 16 were heterozygous for the CCR2-64I allele and one was homozygous for the mutant allele, yielding a CCR2-64I allelic frequency of 0.2571. We typed HLA in the Chiriguano population to investigate for putative alleles originated from admixture with European ancestry individuals. We determined the frequencies of HLA class I and HLA class II alleles in this population for comparison with the HLA frequencies obtained in 272 Argentine European ancestry individuals (30). As illustrated in Fig. 1, the alleles HLA-A1, HLA-A11, HLA-B14, HLA-B44, HLA-DR1, HLA-DR7 and HLA-DR15 are poorly represented in the Chiriguano population as previously observed in other Amerindian groups (31–35), thus we considered that the presence of these alleles in this population might have arisen from European ancestry admixture. Based on the latter criteria, we established that 8 out of 42 Chiriguano studied had a gene flow from Caucasian population. CCR5 and CCR2 genotyping analysis of these individuals showed that 7 were CCR5+/+ of whom 2 were also CCR2+/+, 3 were heterozygous CCR2+/64I and in 2 individuals CCR2 genotype could not be determined (Table 1). Of note, the only Chiriguano subject that was heterozygous CCR5+/Δ32 belonged to the group of individuals with European ancestry origin by HLA typing, suggesting that the CCR5-Δ32 allele is not naturally present in the native Chiriguano population. Excluding all the subjects with European ancestry gene flow, the CCR2-64I allele frequency among Chiriguano Amerindians reached 0.2586 that was significantly higher compared with the CCR2-64I frequency of 0.1289, previously reported in an Argentine blood donor population ($P=0.038$) (26). The CCR2-64I allele distribution was not in Hardy-Weinberg equilibrium ($P<0.01$), and the high frequency observed may reflect the endogamic characteristic of this population.

In agreement with other American aborigine groups studied (Mexico, Brazil, Jamaica, etc.) (36–38), our findings support that the

CCR5 and CCR2 genotype distributions in Chiriguanos

Genotypes	Number of individuals	Allele frequency
CCR5+/+	41	0.9762
CCR5+/Δ32	1*	0.0238
Total	42	
<i>P</i> -value (HWE)	>0.1	
CCR2+/+	18*	0.5143
CCR2+/64I	16*	0.4571
CCR2-64I/64I	1	0.0286
Total	35	
<i>P</i> -value (HWE)	<0.01	

HWE: Hardy-Weinberg equilibrium
 * Number of individuals with caucasian origin indicated by HLA typing: CCR5+/+ and CCR2+/+=2, CCR5+/Δ32 and CCR2+/+=1, CCR5+/+ and CCR2+/64I=3, CCR5+/+ and CCR2 not determined=2

Table 1

CCR5-Δ32 is absent in Amerindians. In contrast, CCR2-64I allele had a high frequency in Chiriguano and this represent the first report of the presence of this allele in an Amerindian population. The greater global distribution of the CCR2-64I mutation compared with that of CCR5-Δ32 suggests that CCR2-64I is a much older mutation originated before the dispersal of modern humans (39). The existence of a complete linkage disequilibrium between CCR2-64I and CCR5-Δ32 alleles supports that CCR5-Δ32 mutation is restricted to the haplotype CCR2 wild-type, thus both mutations can not co-occurred in the same chromosome (22). These data suggest that the more ancient and highly frequent CCR2-64I allele may have contributed to obstruct the settling of the CCR5-Δ32 mutation among African, Asian and Amerindians.

In conclusion, the high frequency of the CCR2-64I and the absence of CCR5-Δ32 mutations in the Chiriguano populations favors the hypothesis of a single and relative recent origin of the CCR5-Δ32 in the Caucasian population.

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