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HIV-1 co-receptor CCR5 and CCR2 mutations among Greeks

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Abstract

The frequency of CCR5 and CCR2 alleles in human immunodeficiency virus (HIV)-positive and HIV-negative populations of Northern Greece was investigated. The frequency of the CCR5Δ*32* allele among the HIV-negative subjects was 0.052, while it was approximately two-fold lower among the seropositives, suggesting that the heterozygous genotype confers a partial resistance to the HIV infection. No significant difference in CCR2 allele frequency between the two groups was observed.

Keywords: Human immunodeficiency virus type 1; Co-receptor; CCR5; CCR2

1. Introduction

A 32-bp deletion in the CCR5 gene (CCR5Δ*32*), resulting in a truncated and non-functional receptor, has been shown to play a protective role against human immunodeficiency virus (HIV) infection [1]. CCR5Δ*32* homozygosity occurs in about 1% of the Caucasian population [2]. Furthermore, the CCR5Δ*32* allele has been associated with delayed disease progression. Individuals homozygous for this deletion are highly resistant to HIV-1 infection, whereas heterozygotes demonstrate a limited protection against disease progression [1,3]. In addition, a recent study has shown that a conservative substitution (V64I) in the first transmembrane region of the CCR2 chemokine and HIV-1 receptor gene delays disease progression, but does not prevent HIV-1 transmission [4]. This mutation has been observed in every ethnic group that has been tested. The aim of this study was to investigate the frequency of CCR5 and CCR2 alleles in the population of Northern Greece.

2. Materials and methods

Two groups of individuals were studied; the first consisted of 240 HIV-negative individuals and the second group consisted of 138 HIV-positive individuals, who were infected between 1991 and 1998. A region of the CCR5 gene was amplified from genomic DNA using the primers CCR5c and CCR5d that flank the 32-bp deletion and generate wild-type and deleted fragments of 189 bp and 157 bp, respectively [3]. Similarly, a region of the CCR2 gene was amplified using the primers CCR2B-forward and CCR2B-reverse [5]. To distinguish between the CCR2 alleles, the PCR product was digested with *Asa*BI (New England Biolabs, Beverly, MA, USA) producing a wild-type 183-bp fragment or a V64I mutant 165-bp fragment [5]. In this manner we determined the CCR5 and CCR2 genotypes and we calculated the respective allele frequencies. Statistical tests were performed using Fisher's exact test.

3. Results and discussion

Among the HIV-negative subjects, heterozygous (+/Δ*32*) CCR5 genotypes were found in 23 individuals (frequency 0.096), whereas the homozygous (Δ*32*/Δ*32*) genotype for the CCR5 deletion was found only in one woman (fre-

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quency 0.004). The allele frequencies for the wild-type allele and for the mutant allele were therefore calculated to be 0.948 and 0.052 respectively (Table 1). Among the HIV-positive subjects, the frequency of the CCR5Δ*32* allele was approximately two-fold lower, and was calculated to be 0.022 ($P = 0.055$). Thus, the heterozygous genotype appears to confer a partial resistance to the HIV infection. In the group of HIV-positive individuals no mutant homozygous genotypes were found. These results are consistent with the CCR5 allele frequencies previously found in a separate group of Greek Caucasians [6]. The frequency of the CCR5Δ*32* allele among HIV-negative subjects in Greece (0.052) is in the mid range of the values observed in Northern Europe (∼0.09) and those observed in Cyprus (0.029) [7]. The CCR5Δ*32* allele is almost absent from populations in sub-Saharan countries, indicating that there is a gradient of values from northern [2,3] to southern geographical regions.

For the CCR2B gene, the *64I* allelic frequency among the HIV-negative and -positive subjects was calculated to be 0.146 and 0.134 respectively, indicating that no significant difference in CCR2 allele frequency between the two groups was observed ($P = 0.74$) (Table 2).

Combining the results of the presence of the co-receptor genotypes from the Greek population, it was shown that 36% of the HIV-negative and 28.3% of the HIV-positive individuals have a CCR5 and/or CCR2 mutation. The combined genotype CCR5-+/Δ*32*, CCR2-+/*64I* appeared in four subjects in the HIV-negative group, and in only two subjects in the HIV-positive group, whereas three genotypes (CCR2-*64I*/*64I*, CCR5-+/Δ*32*; CCR2-+/*64I*, CCR5-Δ*32*/Δ*32*; and CCR2-*64I*/*64I*, CCR5-Δ*32*/Δ*32*) were absent in both groups. Similar results were found by Smith et al. [4] who examined the co-occurrence and genotypic independence of CCR5 and CCR2 alleles among patients from five different AIDS cohorts. They suggested that the low prevalence of these genotypes is a consequence of strong, perhaps complete, linkage disequilibrium between the mutant alleles of the two genes. Keeping in mind that the gene encoding CCR5 lies just 15 kb towards 3′ to CCR2, linked mutations in the CCR5 promoter or other regulatory sequences could explain the association of CCR2-*64I* with the delay in AIDS progression. But recently it was shown that CCR2-*64I* does not have a dominant negative effect on the CCR5 co-receptor function and that CCR2-*64I* does not act by influencing CCR5 transcription [8].

Further studies are needed in order to find out the real effect of these mutations on the human population concerning HIV transmission and progression and to detect if there is any relation of the CCR5 and CCR2 genotype to the subtype of the infecting virus, as there is evidence that exist subtype-dependent differences in frequency of usage of certain co-receptors [9]. Up to now, this relation could not be tested, as the majority of HIV-positive individuals in Greece are carrying subtype B sequences [10].

Table 1
CCR5-Δ*32* genotypes and allelic frequencies

	HIV-negative		HIV-positive	
	Number	Frequency	Number	Frequency
Genotypes				
CCR5-+/+	216	0.900	132	0.957
CCR5-+/Δ*32*	23	0.096	6	0.043
CCR5-Δ*32*/Δ*32*	1	0.004	0	0.000
Total	240	1.000	138	1.000
Alleles				
CCR5	455	0.948	270	0.978
CCR5-Δ*32*	25	0.052	6	0.022
Total	480	1.000	252	1.000

Table 2
CCR2B-*64I* genotypes and allelic frequencies

	HIV-negative		HIV-positive	
	Number	Frequency	Number	Frequency
Genotypes				
CCR2-+/+	173	0.721	103	0.746
CCR2-+/*64I*	64	0.267	33	0.239
CCR2-*64I*/*64I*	3	0.012	2	0.015
Total	240	1.000	138	1.000
Alleles				
CCR2	410	0.854	239	0.866
CCR2-*64I*	70	0.146	37	0.134
Total	480	1.000	276	1.000

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