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The Frequency of CCR5delta32 Polymorphism in the Central Black Sea Coastal Region in a Healthy Population

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Aim: Delta32 polymorphism in the chemokine receptor 5 (CCR5) gene affects human immune deficiency virus type 1 (HIV-1) entry, transmission, and outcome. The aim of this study was to determine the allelic frequencies of genetic variant CCR5delta32, which influence susceptibility to HIV-1 infection, in a northern Turkish population.

Materials and Methods: We determined the allelic frequencies of CCR5delta32 in 449 unrelated healthy individuals from the central Black Sea coastal region without any known history of HIV-1 infection. The polymorphism was analyzed using polymerase chain reaction (PCR). The population was in the Hardy-Weinberg equilibrium. Delta32 allelic frequencies were calculated.

Results: The frequency of mutant delta32 allele in this population was 5.2%.

Conclusions: Our data indicate that the low frequency of CCR5delta32 allele may be related to higher genetic susceptibility to HIV-1 infection in the central Black Sea coastal region Turkish population.

Key Words: Chemokine receptor, delta32, Turkish people

Orta Karadeniz Bölgesi Sağlıklı Türk Populasyonunda CCR5delta32 Frekansı

Amaç: Kemokin reseptör (CCR5) geninde yer alan delta32 polimorfizmi, insan immün yetmezlik sendromu virüsünün (HIV-1) giriş, bulaşma ve prognozunu etkiler. Bu çalışmada, kuzey Anadolu Türk populasyonunda HIV–1 enfeksiyonlarına yatkınlığı etkileyen CCR5delta32 gen varyantlarının allel frekanslarının hesaplanması amaçlandı.

Yöntemler: Orta Karadeniz kıyı bölgesinde yaşayan aralarında akrabalık ve HIV-1 enfeksiyonu öyküsü olmayan 449 sağlıklı bireyin CCR5delta32 allel frekansları hesaplandı. Polimorfizm, polimeraz zincir reaksiyonu (PCR) tekniği kullanılarak analiz edildi. Populasyon Hardy-Weinberg dengesindeydi. Delta32 allel frekansı hesaplandı.

Bulgular: Populasyonda mutant delta32 allelinin frekansı %5.2 olarak bulundu.

Sonuç: Bulgularımız düşük CCR5delta32 allel frekansının orta Karadeniz bölgesi Türk populasyonun, HIV-1 enfeksiyonlarına yüksek derecede yatkınlığı olduğunu göstermektedir.

Anahtar Sözcükler: Kemokin reseptörü, delta32, Türk halkı

Introduction

The chemokine receptor 5 gene (CCR5) appears to be the major co-receptor for the macrophage-tropic strains of human immunodeficiency virus type 1 (HIV-1). The chemokine receptor, CCR5, is encoded by the CMKBR5 gene located on the p21.3 region of human chromosome 3. HIV-infected individuals heterozygous for CCR5+/delta32 can significantly delay the onset of acquired immunodeficiency syndrome (AIDS) compared with HIV-infected individuals who do not carry the delta32 polymorphism. Homozygous individuals for CCR5-delta32 allele have been found to be highly resistant to HIV infection (1-3). A 32-bp deletion in the open reading frame of the CCR5 gene induces a frame shift premature stop codon within the third extracellular domain and results in a truncated protein product that is not transported to the cell surface as a HIV-1 co-receptor (4).

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One thousand nine hundred and twenty-two patients with AIDS were reported from 1998 to 2005 in Turkey. This figure is lower than those reported from Europe (5).

The aim of this study was to investigate the allelic frequencies of CCR5delta32 in a healthy northern Turkish population to assess the genetic susceptibility to HIV infection.

Materials and Methods

A total of 449 unrelated healthy individuals (324 men and 125 women; mean (range) age 43.7 (12-87) years without any known history of HIV-1 infection were enrolled from Ondokuz Mayis University between January 1998 and May 2003. All subjects were of Turkish origin and from the Black Sea coastal region and gave informed consent to participate in this study.

DNA was isolated from the heparinized peripheral blood samples from all subjects using the 'salting out' method (6). 32-bp deletion polymorphism was determined by polymerase chain reaction (PCR). PCR amplification was performed in a programmable thermal cycler (Techne Gradient, Cambridge, UK). The primers (Iontec, Bursa, Turkey) were 5'-TCA AAA AGA AGG TCT TCA TTA CAC C-3' forward and 5'-AGC CCA GAA GAG AAA ATA AAC AAT C -3' reverse.

PCR reaction was performed in 25 µl, 1xPCR buffer containing 20 pmol of each primer, 2.0 mM $MgCl_2$, 200 mM of each dNTP (MBI, Fermentas, Lithuania), 50 ng DNA, and 1.0 U Taq polymerase (Promega, Madison, WI, USA). Following initial denaturation at 94 ºC for 3 minutes, amplification was performed by 35 cycles of denaturation at 94 °C for 75 seconds, annealing at 58 °C for 75 seconds and extension at 72 °C for 1 minute. The reaction was terminated by final extension at 72 °C for 7 minutes (7). The PCR produced a 241 bp product from the wild-type allele and 209 bp product from the deleted allele (Figure).

Amplified fragments for the CCR5 locus were resolved on 2% agarose and analyzed in a video gel documentation system (Biolabs, Kyoto, Japan) after staining with ethidium bromide.

CCR5 gene allelic and genotypic frequencies were compared and the differences evaluated using the chi-square (χ^2) test (two-sided) for Hardy-Weinberg equilibrium (HWE). The expected values were compared with those observed by the χ^2 test contingency tables. A probability of less than 0.05 was required for statistical significance.

Results

The prevalence of CCR5 genotypes was investigated among 449 healthy individuals from northern Turkey. Among the healthy subjects, 402 (89.8%) had CCR5 +/+ homozygosity and 47 (10.2%) had CCR5+/delta32 heterozygosity (Table). CCR5 delta32delta32 homozygosity was not observed among the healthy controls. The frequency of the CCR5 allele was 5.2%. Genotypic frequencies were in HWE (χ^2=1.39; *df*=1; P=0.758).

Table. CCR5 genotype distribution in a Northern healthy Turkish population.

CCR5 Genotypes	No of individuals	Frequencies
CCR5+/+	402	0.895
CCR5+/delta32	47	0.105
CCR5delta32/delta32	0	
Total	449	
CCR5 Alleles		
CCR5+	851	0.948
CCR5Δ32	47	0.052
Total	898	

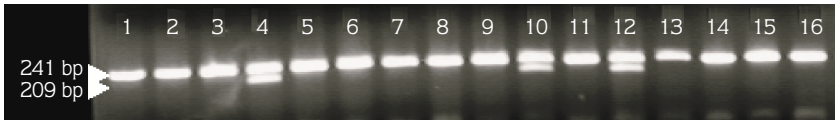

Figure. Agarose gel electrophoresis of PCR products corresponding to the delta32 mutation region. Lane 1-3, 5-9, 11, and 13-16 wild type (CCR5+/+); lane 4, 10, and 12 heterozygous (CCR5+/delta32).

Discussion

The delta32 allelic frequency of the CCR5 gene shows regional variations and differences among the population samples. This is the first report on frequency of CCR5delta32 polymorphism from northern Turkey. The frequency of the delta32 allele of CCR5 in the non-HIV-1 infected northern Turkish population was 5.2%. Lucotte et al. (11) reported that 32 allele frequency was 6.2% in a small Turkish population consisting of 104 individuals. Another study reporting from central Anatolia showed that the allelic frequency of delta32 was 2.2% (9). Yigit et al. (10) reported that all of the renal transplant patients were homozygous for CCR5delta32 wild type allele. The number of the individuals tested in present study is relatively higher in comparison with earlier studies. The differences in the reported frequencies of delta32 allele could be due to differing sample sizes, heterogeneity of ethnic composition and latitudes of the geographic areas under study. The results indicate that frequency of CCR5 heterozygotes observed in this study latitude was similar to those reported from other countries in Europe that are in the same latitude with us (10).

In conclusion, our results indicate that in relation to geographic position, CCR5delta32 allele frequencies in northern Turkey fit well in the observed north-south gradient.

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