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GAMZE KARAKAYA

FATMA AZİZE BUDAK YILDIRAN

ŞÜKRAN ÇAKIR ARICA

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Investigation of the frequency of the mutant CCR5-Δ32 allele related to HIV resistance in Turkey

Gamze KARAKAYA¹, Fatma Azize BUDAK YILDIRAN^{2*}, Şükran ÇAKIR ARICA³

¹Department of Biology, Institute of Science, Kırıkkale University, Yahşihan, Kırıkkale, Turkey

²Department of Biology, Faculty of Art and Science, Kırıkkale University, Yahşihan, Kırıkkale, Turkey

³Department of Medical Services and Techniques, Kırıkkale Vocational School, Kırıkkale University, Yahşihan, Kırıkkale, Turkey

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Aim: To determine the frequency with which the CCR5-Δ32 mutant allele caused resistance to HIV, which is main agent of AIDS in all geographic regions of Turkey.

Materials and methods: Randomly selected human blood samples from 400 healthy individuals were collected. The polymerase chain reaction method was used to determine the CCR5-Δ32 polymorphism and samples were screened using 2 primers.

Results: The frequency of wild alleles was found to be 0.9738 and the frequency of mutant alleles was found to be 0.0262. The highest frequency of the CCR5-Δ32 allele was found to be 0.0726 in the Central Anatolia region. The lowest frequency of the CCR5-Δ32 allele was found to be 0.0093 in the Southeast Anatolia and the Mediterranean regions. The mutant allele was not observed in any individuals from the Marmara region. Homozygosity was not observed in terms of this mutant allele in the individuals studied.

Conclusion: According to the data obtained from this study, the frequency of CCR5-Δ32, except for in the Marmara region, showed parallelism with a decrease consistent with a north-south direction across the world. This study was conducted to determine the frequency of the CCR5-Δ32 allele, which is important for the evaluation of samples that are representative of all the geographic regions of Turkey.

Key words: HIV, AIDS, CCR5-Δ32, Turkey, population

1. Introduction

Acquired immune deficiency syndrome (AIDS) emerges with the involvement of the immune system by a virus named human immunodeficiency virus (HIV). Intracellular replication of this virus harms the cell. HIV mostly infects T lymphocytes known as CD4+ or T-helpers. Some individuals have a mutant chemokine receptor gene that provides resistance or partial immunity against HIV. Chemokines include 70–90 amino acids weighing 8–10 kDa, which activate different cell types and are interrelated with them. Chemokine receptors that have 20%–70% common amino acid sequences are divided into 3 groups [C, CC (b), CXC (a)] according to the amount and location of cysteine in their structure. Of them, the chemokine 5 receptor (CCR5) has a 352 amino acid sequence, its molecular mass is 40,600 Da, and it is located on the third chromosome (1–5). The relationship of CCR5 and the chemokine 2 receptor (CCR2) and HIV has been put forward in studies.

The mutant CCR5 allele is related to HIV resistance in human genomes. A 32-bp deletion in the human CCR5 gene leads to this resistance (1,2). HIV should attack the CCR5 receptor in order to infect CD4+ T cells. Individuals who have this mutation either do not have the CCR5 receptor or have less than the mean number of CCR5 receptors. Presence of the CCR5-Δ32 allele provides very strong protection against the retransfer of HIV in homozygous individuals. On the other hand, the typical course of the disease has been reported to be delayed for about 2 years in heterozygous individuals (6,7).

A population that has both the highest Δ32 frequency and the highest HIV infection together is not known today. Many populations in North Europe have Δ32 allele frequency in the ratio of 0.1–0.2. Meanwhile, the Δ32 frequency is low and almost nil in African populations in which AIDS is common. The highest Δ32 allele frequency has been reported as 21% as the result of many population screenings (8–10).

* Correspondence: azhar2498@yahoo.com

In this study, it was aimed to determine CCR5-Δ32 allele frequency, which has great importance for resistance against HIV, by taking all geographical regions of Turkey into consideration.

2. Materials and methods

2.1. Collection of samples and DNA isolation

We ensured that there were blood samples representing all of the geographical regions of Turkey. Blood samples were obtained from a total of 400 healthy individuals (259 females, 141 males; Table 1). The blood samples were taken from volunteer participants and were obtained in accordance with the requirements of informed consent, and 2 cm³ of each blood sample was put into tubes containing EDTA and stored at 4 °C.

DNA isolation from the blood was performed according to QIAamp DNA Mini Kit (QIAGEN) protocol.

2.2. Optimization of polymerase chain reaction (PCR) conditions

Molecular genetic analysis of individuals was done with the PCR method. Two primers that gave the best results in trials were used for the analysis of samples (Table 2).

A PCR reaction mixture was prepared in a total volume of 15 μL in such a way as to include 50–100 ng DNA sample, 1X PCR buffer ((NH₄)₂SO₄, Fermentas), 2 mM MgCl₂, 200 μM dNTP, 3 pmol primer, and 0.5 U Taq DNA polymerase. PCR conditions for the SP4.760 primer were realized in a total of 35 cycles as 40 s at 94 °C for denaturation after a predenaturation procedure for 4 min at 94 °C, 45 s at 58 °C for primer bonding, and 1 min at 72 °C for elongation. Afterwards, it was kept at 72 °C for 10 min in order to complete the elongation procedure. Samples that completed the PCR procedure were kept at 4 °C until the elution procedure. These conditions for the AB primer used in the study were realized in a total of 35 cycles as 30 s at 94 °C for denaturation after a predenaturation procedure for 4 min at 94 °C, 40 s at 58

°C for primer bonding, and 40 s at 72 °C for elongation. Afterwards, it was kept at 72 °C for 10 min in order to complete the elongation procedure.

2.3. Agarose gel electrophoresis

PCR products to which 6X loading dye was added were eluted for approximately 2 h with 1.7% agarose gel so as to be 5 V/cm. DNA band profiles obtained with a gel imaging system were transferred to a computer environment and photographed.

2.4. Statistical analysis

Homozygous individuals were taken as 'AA' and heterozygous individuals were taken as 'AB' for the data analysis. Chi-square analysis was used for the assessment of genotype and allele frequencies. Expected and observed frequencies of genotypes were calculated. The expected heterozygosity value was assessed using the PopGene (version 1.31) computer program (11,12).

3. Results

CCR5-Δ32 allele frequency as related to resistance against HIV was determined with the analysis of blood samples obtained from a total of 400 individuals. Blood samples were collected in such a way as to represent all provinces included in the region. Twenty-one of the individuals who were screened with 2 primers were found to be heterozygous in terms of the CCR5-Δ32 allele (CCR5/CCR5-Δ32). No homozygous individuals (CCR5-Δ32/CCR5-Δ32) were determined in the study. A single band measuring 241 bp was detected in individuals with the CCR5/CCR5 genotype and 2 bands measuring 209 and 241 bp were determined in individuals with the CCR5/CCR5-Δ32 genotype as a result of screening samples with the SP4.760 primer (Figure 1). A single band measuring 225 bp was detected in individuals with the CCR5/CCR5 genotype and 2 bands measuring 193 and 225 bp were determined in individuals with the CCR5/CCR5-Δ32 genotype as a result of screening with the AB primer (Figure 2).

While the wild allele frequency was found to be 0.9738 for all individuals, the mutant allele frequency was 0.0262. In the statistical analysis done according to geographical regions, while wild allele frequency was found to be 0.9590 for the population representing the Black Sea region,

Table 1. Distribution of participants according to number and regions.

Regions	Number of participants
Black Sea region	61
Marmara region	58
Aegean region	57
Mediterranean region	54
Southeast Anatolia region	54
East Anatolia region	54
Central Anatolia region	62
TOTAL	400

Table 2. Names and sequences of primers used in the study.

Names of primers	Sequences of primers (5'-3')
SP4.760	CCTCATTACACCTGCAGCTCT CACAGCCCTGTGCTTCTTCTT
AB	ACCAGATCTCAAAAAGAAGGTCT CATGATGGTGAAGATAAG CCTCACA

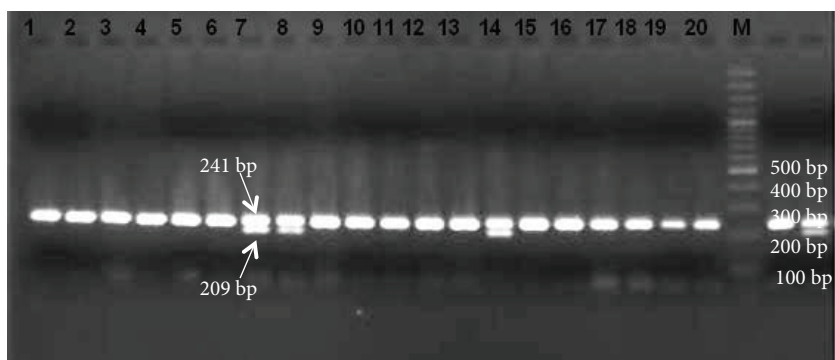


Figure 1. DNA band profiles obtained with SP4.760 primer [M: 100-bp DNA determinant; 7, 8, 14: heterozygous individuals; 1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20: wild type (CCR5/CCR5)].

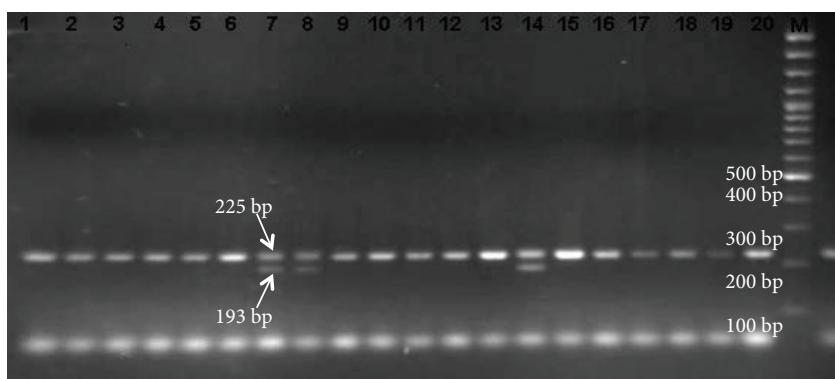


Figure 2. DNA band profiles obtained with AB primer [(M: 100-bp DNA determinant; 7, 8, 14: heterozygous individuals; 1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20: wild type (CCR5/CCR5)].

mutant allele frequency was 0.0410. No heterozygous individuals who had the mutant genotype were detected in the population representing the Marmara region. While wild allele frequency was found to be 0.9825 for the population representing the Aegean region, mutant allele frequency was 0.0175. While wild allele frequency was found to be 0.9274 for the population representing the Central Anatolia region, mutant allele frequency was 0.0726. While wild allele frequency was found to be 0.9722 for the East Anatolia region, mutant allele frequency was 0.0278. While wild allele frequency was found to be 0.9907 for the population representing the Mediterranean region, mutant allele frequency was 0.0093. While wild allele frequency was found to be 0.9907 for the Southeast Anatolia region, mutant allele frequency was 0.0093. The Central Anatolia region was found to have the population with the highest heterozygous percentage among the analyzed populations. Heterozygous statistical values for all individuals are given in Table 3. In addition, the chi-square value used in Hardy-Weinberg equilibrium analysis was estimated to be 0.276506, the freedom degree to be 1, and probability (P) to be 0.599000 (Table 4).

4. Discussion

In many studies conducted at the beginning of the 1990s, it was reported that some people were not infected although they had been repeatedly exposed to HIV, and some infected people lived longer than expected (13). Recognition of the molecular mechanism of this resistance was achieved with the definition of co-receptor molecules that enable the entrance of HIV into macrophages and T cells (14,15). In previous studies, it was shown that resistance against HIV was provided or the course of the disease was slowed down in the presence of allele variants in CCR5 and CCR2 (1,2). Research about the worldwide distribution models of variants was conducted after these studies. In a study by Martinson et al., it was reported that CCR5- Δ 32 allele frequency was quite high in North European populations, while this frequency reduced towards the South and East and almost none is seen in Africa, Oceania, the Middle East, or West Asia (8). In a large-scale sample composed of a total of 8842 noninfected individuals obtained from 40 different white populations including Europe, the Middle East, and North Africa, Lucotte et al. reported that Δ 32 allele frequency significantly reduced from north to south

Table 3. Heterozygosity statistical results for two locus of the population composed of 400 individuals.

Locus	Sample size	Observed homozygosity	Observed heterozygosity	Expected homozygosity*	Expected heterozygosity*	Nei**	The average heterozygosity
SP4.70	800	0.9475	0.0525	0.9488	0.0512	0.0511	0.0511
AB	800	0.9475	0.0525	0.9488	0.0512	0.0511	0.0511
Ort.	800	0.9475	0.0525	0.9488	0.0512	0.0511	0.0511
Standard deviation	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

*: Expected homozygous and heterozygous data were obtained using Levene's method (11).

** : Expected heterozygosity value of Nei (12).

Table 4. Chi-square and P values used for Hardy–Weinberg equilibrium analysis of 400 individuals.

Genotype	Observed number of individuals (O)	Expected number of individuals (E)	$(O - E)^2 / E$	$2 \cdot O \cdot \ln(O / E)$
(A, A)	379	379.2628	0.0002	-0.5255
(A, B)	21	20.4743	0.0135	1.0647
(B, B)	0	0.2628	0.2628	0.0000

(from North Europe to North Africa). It was reported that the frequency of the $\Delta 32$ allele was 13.4% among the populations of Sweden, Norway, Denmark, Finland, and Iceland, and this value was quite higher than mean $\Delta 32$ values; the lowest frequency was reported from the more southern countries of Morocco, Tunisia, Syria, and Iran (16).

Despite the presence of population studies in Turkey, these studies were limited to certain regions or certain populations. According to a study in 2004, a randomly selected Turkish population was analyzed and the mutant allele frequency was detected to be 2.18%. No CCR5- $\Delta 32$ homozygous individuals were detected in that study (17). In the present study, the mutant allele frequency was found to be a mean of 1.95% for all regions and no mutant homozygous individuals were encountered. However, the Central Anatolia region was different from others with a mutant allele frequency of 7.26%. This result is parallel with the mutant allele frequency (6.3%) found in the study of Libert et al., which included 18 European countries and 104 individuals from Ankara, the only province from Turkey that took part in the study (18). Similarly, in a study conducted by Güneş et al. (3), healthy individuals living in the coastal region of the Middle Black Sea region were analyzed and the mutant $\Delta 32$ allele frequency was reported to be 5.2%. In the present study, a similar frequency value (4.1%) was found for the Black Sea region.

When the chi-square and probability values calculated using the PopGene program for all individuals are taken into consideration (at $P < 0.05$ significance level),

the H_0 hypothesis is acceptable; the population is at Hardy–Weinberg equilibrium. This result is parallel with that of Güneş et al. (3).

The present study is the first conducted with subjects selected from all geographical regions of Turkey. A total of 400 individuals with 54–61 from each region were analyzed for the CCR5- $\Delta 32$ allele. The CCR5- $\Delta 32$ mutant allele frequency was found to be 0.0262 for all individuals. This frequency value is parallel with the values of Christodoulou et al. regarding the Cretan population in Greece (0.028) and Battiloro et al. regarding the Italian Sardinian population (0.021) (19,20). In this study, a difference was observed in the distribution of CCR5- $\Delta 32$ allele frequency according to regions. Central Anatolia is the region in which this allele is represented with the highest frequency (0.0726). Frequency values obtained for the Mediterranean region and Southeast Anatolia region were found to be equal (0.0093). The $\Delta 32$ allele was not encountered in the analysis of the sample selected for the Marmara region. It is thought that a reevaluation of the Marmara region with higher numbers of samples and screened primers would provide better results.

In this study, all geographical regions of Turkey were evaluated for the first time in terms of this allele. Data showed that frequency of that allele was consistent with its reduction from north to south worldwide, except for in the Marmara region.

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