

Distribution of the mutated delta 32 allele of CCR5 co-receptor gene in Iranian population

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Abstract

Background: The CCR5 is a chemokine receptor that serves as a co-receptor for HIV-1 attachment and entry to T lymphocytes. A 32bp deletion ($\Delta 32$) in this gene is believed to be associated with resistance to infection and delay disease progression. The aim of this study was to determine the $\Delta 32$ allele frequency in healthy individuals and HIV-infected individuals with AIDS.

Methods: In this experiment, 530 normal individuals from healthy Iranian population and 40 HIV-infected samples from Western Clinic of Tehran were examined for $\Delta 32$ in CCR5 gene using polymerase chain reaction (PCR) techniques followed by agarose gel electrophoresis.

Results: Allele frequencies of the CCR5 $\Delta 32$ in normal individuals were calculated to be 1.1% for heterozygous genotype and 0.19% for homozygous genotype. None of the co-receptor gene in HIV cases was found to be mutated in this study.

Conclusion: Based on the findings of this study and the literature in Iran, we could conclude that Iranian people similar to neighbor countries such as Arabs are susceptible to HIV virus infection.

Keywords: CCR5 $\Delta 32$, Chemokine receptor, HIV-1, AIDS, Iran.

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Introduction

Over the past quarter-century, the global spread of HIV virus has become one of the deadliest epidemics in modern times. In 2007 a total of 2.1 million men, women, and children died of AIDS. The death toll will remain high in the future because 33.2 million individuals are currently infected and about 2.5 million new HIV infections occur each year (1).

Among various AIDS restriction genes (ARGs) only CCR5 $\Delta 32$ prevents HIV infection (1-3, 9-12). The CCR5 chemokine receptor acts as a co-receptor on CD4⁺ T lymphocytes for HIV-1 virus attachment and penetration (4). People with a 32-bp deletion in T lymphocytes CCR5 (CCR5- $\Delta 32$) receptor have shown to be nearly re-

sistant to HIV-1 strains (5). HIV heterozygous individuals with CCR5-delta32 deletion also have better therapeutic response to highly active antiretroviral drugs (6). Nevertheless, survival analyses have shown that progression to AIDS is slower in CCR5- $\Delta 32$ heterozygous individuals (7). Various studies attributed normal function for peripheral blood (PB) T cells, homozygous for the CCR5- $\Delta 32$ mutation. Besides, no clinical abnormalities have been observed to be associated with this genotype (8). Genetic retardation of HIV progression could introduce new cellular targets for anti-HIV therapy that completes the current available antiretroviral compounds. Different inhibitors designed for blocking the CCR5 chemokine receptor have shown relatively high

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protective effect in HIV infection (13).

The aim of this study was to determine the Δ32 allele frequency in healthy individuals and HIV-infected individuals with AIDS. The present study explored the effect of the gene variants that can be useful for treatment and predicting response of drugs that target this chemokine receptor in order to block the HIV entrance to the target cells.

Methods

Sample collection

A total of 530 unrelated healthy Iranian individuals were enrolled. The healthy individuals were randomly selected among the university hospital staffs and students from different provinces. Randomized stratification was used for selection of provinces and Gilan, East Azarbaijan, West Azarbaijan, Ghazvin, Tehran, Semnan, Kurdistan, Ghom, Isfahan, Khorasan, Yazd, Lorestan, Hormozgan were selected. Forty blood samples from HIV infected individuals were collected from Tehran University of Medical Sciences clinics, Tehran, Iran. All samples were confirmed to be HIV positive or negative by western blotting and real time PCR.

DNA preparation

DNA was prepared from blood leukocytes by standard methods using QIAamp DNA Mini Kit (Biorain Company. Iran) (14).

PCR

PCR amplification was carried out in 25 µl reactions containing 50–100 ng of template DNA, 12.5 pM of each oligonucleotide primers, 0.2 mM dNTPs, in 1.5 mM MgCl₂, 100 mM Tris–HCl (pH 8.8) and 1U Taq polymerase (Qiagen supplied by Biorain company, Iran) using the Corbet CG1-96 thermocycler (Armin teb. Iran). The specific segment of CCR5 gene was amplified by PCR using the following primers:

CCR5-F (5'-ATCACTTGGGTGGCTGTGTTTGCCTCTC-3') and CCR5-R(5'-AGTAGCAGATGACCATGACAAGCAG

CGGCAG-3').

These primers were used to amplify a 193bp fragment for the normal allele, and 161bp fragment for the mutant allele. The reaction was subjected to 30 cycles: an initial denaturation of 5min at 94°C, 30s denaturation at 94°C, 45s at the annealing temperature 58°C, extension at 72°C for 30s. Following the amplification cycles a final extension was performed at 72°C for 10 min. The polymorphism was detected by electrophoresis of 2% MS agarose gel (Applied Roche).

Statistical analysis

The allele frequency was calculated by the allele-counting method. The Hardy-Weinberg equilibrium was tested by chi-square analysis, and Pearson's correlation coefficient used to find possible correlations with latitude and longitude.

Inclusion Criteria

The healthy subjects included the University hospital staffs and students of different provinces aged between 18 and 35 years, and their sexes notwithstanding. This target age had the highest prevalence of HIV-1 and AIDS infections in the country. The inclusion criteria for patients were HIV infected individuals with AIDS.

Exclusion Criteria

The individual with autoimmune liver disease, thyroid disease or diabetes and serious illness requiring systemic treatment or hospitalization for at least 14 days prior to study were excluded.

Results

We found 523 homozygous for the wild-type CCR5 allele from 530 mononuclear cells of normal Iranian population from various provinces, 6 heterozygous for the CCR5Δ32 allele, giving a CCR5-Δ32 allelic frequency of 1.1% and only one homozygous genotype for the Δ32/Δ32 allele (Fig. 1, Table 1 & 2). Almost all of the heterozygous genotypes and the only one homozygous genotype were determined in

Table 1. Samples collected from different provinces from south to the north of Iran

Province from north to south	Number of sample	$\Delta 32/\text{wt}$ frequency	$\Delta 32/\Delta 32$ frequency
Gilan	20	1	0
Azarbaijan-e- sharghy	50	1	0
Azarbaijan-e-gharbi	50	1	0
Ghazvin	45	1	0
Tehran	100	2	1
Semnan	30	0	0
Kurdistan	35	0	0
Ghom	30	0	0
Isfahan	40	0	0
Khorasan	40	0	0
Yazd	30	0	0
Lorestan	30	0	0
Hormozgan	30	0	0
Total	530	6	1

Table2. Allele and genotype frequency of CCR5 $\Delta 32$ polymorphism in Iranian healthy individuals

Chemokine	Allele	F(%)	Genotype	F(%)	E Genotype	Pearson's Chi-Square	p
Homozygous CCR5	+	1046(99.25)	wt/wt	523(98.5%)	522.03	0.03	0.99
Heterozygous CCR5	$\Delta 32$	6(0.57)	$\Delta 32/\text{wt}$	6(1.1%)	7.94		
			$\Delta 32/\Delta 32$	1(0.19%)	0.03		

individuals from the northern parts of Iran (Table 1). The frequency of wt/wt, $\Delta 32/\text{wt}$, and $\Delta 32/\Delta 32$ genotypes were determined as 523 (98.7%), 6 (1.1%) and 1 (0.19%), respectively (Table 2). The mutant allele was not detected in the HIV-positive samples. The observed genotype frequencies were also in accordance with Hardy–Weinberg equilibrium ($p=0.003$)

We found a strong positive significant correlation with latitude ($r=0.72$) and a somewhat weaker negative correlation with longitude ($r=-0.34$) of the geographic location within Iran.

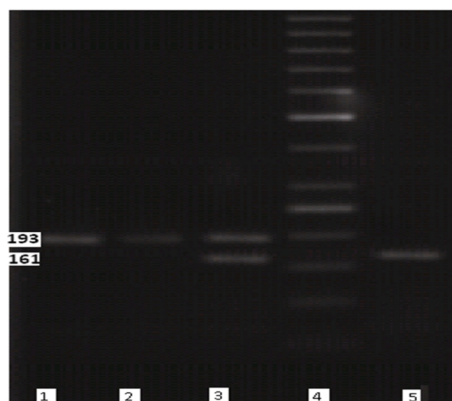


Fig. 1. Analysis of the PCR product of CCR5 alleles
Lane 1 and2: wild type (w/w), lane 3: heterozygous (CCR5 $\Delta 32/\text{wt}$), lane 4: DNA ladder, lane 5: homozygous (CCR5 $\Delta 32/\text{CCR5}\Delta 32$)

Discussion

The description of natural CCR5 polymorphisms in human populations is an approach to identify immunologic and genetic factors implicated in innate resistance to HIV-1 infection and in the delay of progression to AIDS (3). In a study by Martinson et al., it was reported that CCR5- $\Delta 32$ allele frequency was quite high in North European populations, but it reduced while moving toward the south and east of Europe and almost it becomes rare in Africa, Oceania, the Middle East, or West Asia (28). The highest frequency of heterozygous individuals (%26.6) was reported in Finland from Europe. While increasing HIV prevalence in parts of Asia and Africa may be attributed to social and demographic factors, as well as differences in the phenotype of circulating viruses (26). The racial distribution of HIV risk raises the possibility that differences in the distribution of the CCR5- $\Delta 32$ allele or other heritable host factors/mutations may influence the rate of transmission or the speed of the epidemic in different racial groups (22). The present study was the first research conducted with subjects selected from all geo-

Table 3. Comparison of CCR5+Δ32 allele frequency (%) in Iranian normal population with other locations in Asia and European populations

Population	Sample size	CCR5 Δ32 Frequency (%)	Reference	p
Iran	530	1.1	This study	0.99
South of Iran	395	1.4	[21]	0.0
North-West of Iran	376	1.05	[16]	0.9
Jordan	52	0.0	[19]	---
Kazakistan	50	3	[20]	0.2014
Kazakistan	50	6.0	[25]	----
Kuwait	393	1.2	[19]	----
Lebanon	51	0.0	[25]	-----
Pakistan	36	2.9	[18]	0.2755
Punjab	34	1.5	[18]	1.0000
Saudi Arabia	341	1	[20]	0.9193
Syria	106	3	[17]	0.9
Turkey	91	13	[23]	0.79
United Arab Emirates	26	0.0	[24]	0.170
Iraq	13	0.0	[17]	----
Uzbekistan	29	3	[18]	0.2213
China	447	0.0	[18]	0.0046
Yemen	124	1	[24]	0.7440
India	100	1	[1]	0.4772
Bahrain	304	2.8	[27]	0.2053
Egypt	200	0.5	[27]	-----
European populations	2522	9.16%.	[17]	0.001

graphical regions of Iran. A total of 530 individuals with 30–100 from each region were analyzed for the CCR5- Δ32 allele. Results of this study indicate that the CCR5-Δ32 allele exists in Iranian individuals at a very low frequency (1.1%). This result is even lower than previous reports from Iran. The Gharagozloo et al (2005) (21) reported 2.8% for CCR5-Δ32 allele in Fars province located in southern part of Iran. Though we could not find mutant alleles in that part of the country. This probably is because of studies population and limitations. The Omrani et al (2009) (16) reported 2.1% for CCR5-Δ32 allele in Uromia province in North-West of Iran. According to our finding, all of the mutant alleles were found in north and north-west of Iran. This may confirm the correlation between CCR5-Δ32 allele frequency and climate-geographical factors.

Finding very low frequency of CCR5-Δ32 allele in Iran according to this study is in agreement with the result reported from most countries of the Middle East. The rate reported from some of Middle East countries, including Saudi Arabia, and Yemen (CCR5- Δ32 Frequency= 1%) is lower than that of our result (Table 3). It is reported that the frequency of heterozygote forms of

the CCR5-Δ32 in some Middle Eastern countries including Jordan, Lebanon, United Arab Emirates and Iraq is about zero (Table 3). The highest frequencies of heterozygous individuals from this area were reported in Turkey (CCR5- Δ32 Frequency= 13%), Kazakistan (CCR5- Δ32 Frequency= 6%) Syria (CCR5- Δ32 Frequency= 3%) and Uzbekistan (CCR5- Δ32 Frequency 3%) (Table 3).

In the south part of Middle East and Arabic countries, the frequency of the mutant allele CCR5-Δ32 is very low, and very few existed are probably derived from admixture with the populations from European descent rather than as a result of parallel independent mutations (22). No significant differences in CCR5-Δ32 allele frequency between Iranian and Arab populations were detected (Table 3).

Genetically, Iranians are considered to be close to Northern Indian, Greek and some of the European populations such as Italians, German and English. Recently reported human leukocyte antigen (HLA) class II allelic and haplotypic frequency data have confirmed similarity of Iranians, Greek and Italian's genetic background (29). Historical evidences have suggested divergence of Iranian population and Europeans from a

same ancestor known as Indo-Europeans. Around the year 2000 BC, an Indo-European tribe named Aryans invaded central Asia and occupied Iran, Iraq, north of India and Afghanistan. The great difference between the western and eastern migration of the Indo-Europeans was that in the west they genetically mixed with similar populations while in the east, admixture with genetically dissimilar people created largely mixed race population (30). Moreover, extensively genetic admixture with Mongols, Greeks and Arabs gradually diluted the major traces of Indo-Aryans during centuries.

The frequency of homozygote form of the CCR5-Δ32 chemokine as reported in Garaghozoolo et al and Omrani et al (2005 & 2009) and also in present study is about zero in Iran, therefore we should consider Iranians genetically susceptible to HIV virus during any potential exposure in the future. The CCR5-Δ32 allele frequency in Iran was similar to neighbor countries (Table 3).

When the Chi-square and probability values calculated for all individuals are taken into consideration ($p = 0.003$), the H_0 hypothesis becomes acceptable, and the population would be at Hardy-Weinberg equilibrium.

In this study samples from different parts of Iran were examined. All heterozygous genotypes and the only one homozygous genotype were determined in individuals of the north and north-west of Iran (Ghazwin, Azarbaijan, Gilan, and Tehran) (6/270), and no incidence was not found in other parts (0/270) (Table 1). Our data showed that frequency of that allele was consistent with its reduction from north to south worldwide.

Conclusion

Strong positive correlation with latitude of the geographic location within Iran clarified the positive correlation between CCR5-Δ32 allele frequency and climate-geographical factors. The results presented here are consistent with the idea that climatic factors can play a certain selective

role, either directly related to the expression of the CCR5-Δ32 allele, or to the action of a pathogen against which that allele confers some degree of protection.

Based on the findings of this study, we conclude that Iranians compared to Europeans are more susceptible to HIV virus infection. The resistance to HIV virus in Iranian population is similar to neighbor countries such as Arabs. However, since the infection with HIV virus is under the influence of the several different factors, therefore, this conclusion should be assumed as a preliminary statement and further investigation should be carried out to prove this hypothesis.

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