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Frequency of CCR5Δ32 Variant in North-West of Iran

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Abstract

Chemokine and chemokine receptors show several variations which may affect resistance to infectious disease. A 32 base pair deletion in the open reading frame of the human CCR5 gene (CCR5Δ32) results in producing a truncated antigen which fails to be presented on the surface of target cells. CCR5Δ32 variant is not a functional co receptor for HIV-1 entrance and delay the onset of acquired immunodeficiency syndrome. To determine the situation of Iranian population, regarding having this protective mutation in their CCR5 gene or not, this study was carried out. Genomic DNA was extracted using the salting out method from 190 healthy subjects. Using PCR method, the allelic and genotypic frequencies were found for this locus in the CCR5 gene. The results of this study showed that the frequency of the CCR5Δ32 allele in heterozygote form is about 0.0211. Also no homozygote form of the mutated CCR5 gene (Δ32/Δ32) was identified. Based on the findings of this study it is possible to say, Iranian nation compare to European nation are more susceptible to HIV virus infection during exposure to this virus. But to confirm this hypothesis, more investigation needs to be designed and performed.

Keywords: HIV; CCR5Δ32 allele frequency; Iran; PCR

Introduction

Population's genetic variations play an important role in disease susceptibility or progression [1]. Selective loci profile provides genetic etiology of human disease [2]. C-C chemokine receptor 5 (CCR5) variations could explain why some people get infected by certain disease agent and some people do not. CCR5 or CXCR4 are co receptors for entry of human immunodeficiency virus type1 (HIV-1) in CD4+ T cells [3]. CCR5 as receptor of the CC chemokines RANTES, MIP-α and MIP-β is expressed on the surface of $CD4^+$ and $CD8^+$ T cells [4]. Chemokines exert biological functions by binding to their receptors. Chemokines contribute in inflammatory responses signaling system and leukocytes trafficking into site of inflammation [5]. It has been demonstrated that there are several polymorphisms within chemokine and chemokine receptor genes [6]. The CCR5 encoding gene is mapped on human chromosome 3p21.3 [7]. Various mutations within CCR5 gene lead to receptor dysfunction. One of the known mutant variant is CCR5Δ32. A 32 base pair deletion in the region of human CCR5 open reading frame results in truncated protein product which fails to be expressed on the

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surface of cells. However, CCR5Δ32 variant is not a functional co receptor for HIV-1 entrance [8-11]. CCR5Δ32 antigen is approximately 30 kd and contains 215 amino acids in primary structure but the wild type of CCR5 protein has 46 kd molecular weight and 352 amino acids [8-11].The frequency of CCR5Δ32 mutant allele varies by ethnicity and is more frequent among Caucasians (10%-15%) and low frequent in African Americans (2%) and almost absent in native Africans and East Asians [8-11]. In Europe, there is a CCR5Δ32 mutant allele frequency cline from north to south. The highest rates are reported in north of Europe (14%) whereas the allele is low frequent in Greece (4.4%) or absent in the south of Europe and Africa [12-14]. CCR5Δ32 has advantageous to the wild type. Homozygous individuals conferred almost complete protection against infection of HIV. Heterozygous individuals significantly delay the onset of acquired immunodeficiency syndrome. For example they show the signs of HIV 2-3 years later than homozygous individuals with the wild type allele [15-19]. In order to study the frequency of this genetic variation in North West of Iran, and possible role of this genetic susceptibility for infection with HIV this study was carried out.

Materials and Methods

After the approval of the ethical committee of the Uromia medical science university, 190 normal healthy male who were attended for annual eye check up at the Uremia's Imam Khomieni educational hospital in Uromia Iran during the period of 2007-2008 were selected. To make sure that selected controls were healthy and free of diseases, they all underwent various tests that included physical exams, questionnaires about their health and history, and serum tests for evaluating their Prostate-Specific Antigen (PSA) tumor marker. Genomic DNA was extracted from 3-5 ml of whole blood collected in tube containing EDTA as anticoagulants using the salting out method [20].

CCR5Δ32 Genotyping

A polymerase chain reaction (PCR) carried out for detection of CCR5Δ32 genotype using the primers as described previously [21].

Forward primer: 5'-TGTTTGCGTCTCTCCCAG-3' and Reverse primer: 5'-CACAGCCCTGTGCCTCTT-3'. Each PCR reaction was performed in 25 µl containing 50-100 ng DNA, 10 pmol of each primer, 175 µmol dNTPs, 1.5 mM MgCl2, 1x PCR buffer, 0.3 U Taq DNA polymerase enzyme (Cinnagen, Tehran, Iran). Thermocycling program (Eppendorf mastercycler, Germany) consisted initial denaturation at 94°C 4 min; 35 cycles: 94°C 30 s, 52°C 45 s, 72°C 1 min; final extension at 72°C for 5 min. Presence or absence of PCR products were monitored by ultraviolet transillumination in 2% gel of agarose contain ethidium bromide. The PCR reaction yields a 233-bp amplicon for the wild type and 201-bp for the mutant product (Fig. 1).

Statistical Analysis

The frequencies of alleles and genotypes were obtained by direct counting and division by the number of subjects in each group. The results were analyzed for their fit to Hardy-Weinberg equilibrium by using a chi square test. The expected frequencies of each genotype were calculated and compared with the observed results. The minimum sample size was calculated as 170 subjects for studied groups (two sided test, power (1-β): 97.5% level of significance α: 5%). Statistical analysis was performed with SPSS-12 software (Statistical Package for the social science, for Windows, Chicago). Odd ratio and confidence interval (95%) and p value were calculated for both groups. A p value less than 0.05 is considered as significant.

Normal and variant allele frequencies could be determined as follow:

$p = [2 \times \text{obs (Homozygote normal)} + \text{obs (Heterozygote)}] / [2 \times N]$,

$N = \text{obs (Homozygote normal)} + \text{obs (Heterozygote)} + \text{obs (Homozygote variant)}$; and $q = 1 - p$.

Then the Hardy-Weinberg expectation is calculated as:

$\text{Exp (Homozygote normal)} = p^2 \times N$,

$\text{Exp (Homozygote variant)} = q^2 \times N$ and

$\text{Exp (Heterozygote)} = 2 \times p \times q \times N$, and

Pearson's chi-square test state is obtained using:

$\chi^2 = \sum (\text{Observed frequency} - \text{Expected frequency})^2 / \text{Expected frequency}$

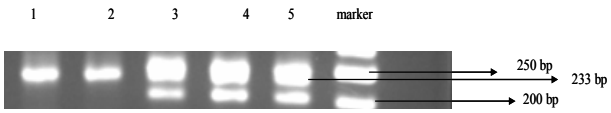


Figure 1. Presence or absences of a 233-bp amplicon for the wild type and 201-bp for the mutant product were visualized by ultraviolet transillumination in 2% gel of agarose stained with ethidium bromide. Lanes 1 and 2 show normal individual genotype and 3, 4 and 5 indicate heterozygous individual's genotype.

Table 1. Allele and genotype frequency of CCR5Δ32 polymorphism in studied group

Chemokine	Allele	F (%)	Genotype	F (%)	Expected Genotype (n)	Pearson's chi-square	P-value
CCR5	+	376(98.95)	+/+	186(97.89)	186.02	0.021	0.99
	Δ32	4(1.05)	+/ Δ32	4(2.105)	3.95		
			Δ32/ Δ32	0(0)	0.02		

Table 2. CCR5 allele frequency among different studied groups: including Asian, European (ref. 22-25). As it is apparent the mutant allele and carriers for this allele are more frequent in north of Europe and about zero in Asia. All the studied population as we see from the result were in Hardy-Weinberg equilibrium

Population and Citation	CCR5 allele frequency (p)	CCR5Δ32 allele frequency (q)	Frequency of Heterozygous Individuals (2pq)	Number of individuals	p-value	Pearson's chi-square
Syria	0.98	0.01	0.02	106	0.9	0.02
Iran	0.97	0.02	0.04	84	0.9	0.04
Cyprus	0.97	0.02	0.05	1002	0.9	0.03
Greece	0.95	0.04	0.08	143	0.8	0.27
Italy	0.94	0.05	0.09	1281	0.03	6.91
Portugal	0.94	0.05	0.09	124	0.8	0.37
Turks (ref. 24)	0.93	0.06	0.11	104	0.79	0.46
Spain	0.93	0.06	0.12	645	0.8	0.32
Hungary	0.91	0.08	0.15	99	0.2	2.64
Switzerland	0.91	0.08	0.15	64	0.7	0.70
France	0.90	0.09	0.16	1836	7.79E-05	18.92
Belgium	0.89	0.10	0.18	1014	0.2	2.70
Norway	0.89	0.10	0.18	100	0.5	1.37
Germany	0.89	0.10	0.18	99	0.6	0.88
Britain	0.88	0.11	0.19	283	0.9	0.09
Lithuanian	0.88	0.11	0.20	283	0.5	1.02
Denmark	0.87	0.12	0.21	239	0.7	0.65
Sweden	0.85	0.14	0.24	204	0.5	1.16
Iceland	0.85	0.14	0.25	102	0.8	0.39
Finland	0.84	0.15	0.26	98	0.1	3.45
South Iran	0.98	0.01	0.02	395	0.9	0.07
Asian	1	0	0	198		
Pacific Islander	0.99	0.008	0.01	461	0.9	0.03
Caucasian	0.90	0.09	0.17	176	0.5	1.12
Turks (ref. 25)	0.97	0.02	0.04	267	0.9	0.16
Present Study	0.98	0.01	0.02	190	0.9	0.02

Results

The mean age of the selected cases were 65.2 ± 6.60 (S.D.). The result of this study showed only 4 (2.105%) of the studied cases had mutant Δ32 allele in the heterozygote form. But there is no homozygote Δ32/ Δ32 genotype in our studied population. Also it was found that the frequencies of alleles in this population were in Hardy-Weinberg equilibrium (P-value = 0.99 and $\chi^2 = 0.021$) (Table 1).

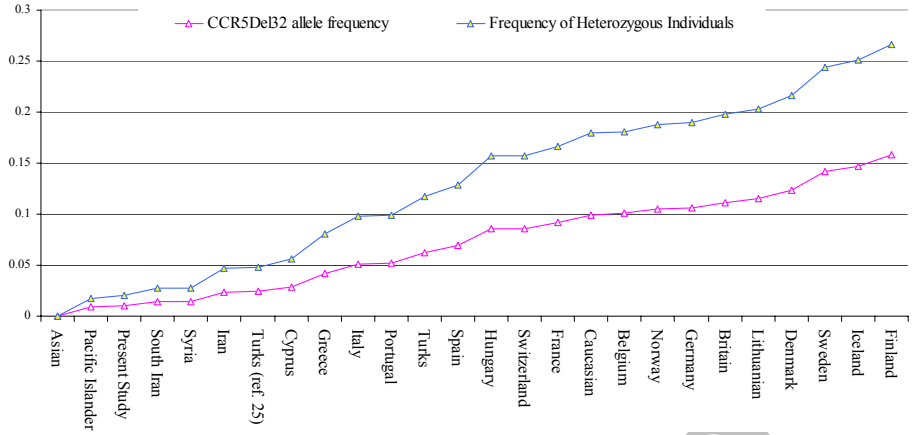
Discussion

The results of this study showed that the frequency of the heterozygote CCR5Δ32 allele in our studied population is about 2%. This result is in agreement with the finding of the Gharagozloo et al. (2005) in southern Iranian population (22) and the rate reported from Syria, Turkey and Cyprus (Table 2). Also when we compare CCR5Δ32 allele frequency in our population and others findings in the region and Europe, it was found that the

Table 3. Frequencies of homozygous and heterozygous genotypes for mutated and normal form of CCR5 chemokine receptor gene from 25 different populations were extracted and compared with present study (Ref. 22-25). The results of comparisons imply that significant differences were found among our study and others especially in north of Europe and Asia and the distribution of this allele is similar in different parts of Iran. Interestingly the distribution of mutant allele in Asia is about zero

No.	Comparisons vs.	Studied populations		Odds Ratio	Lower 95% C.I.-	Chi-square	
	our study	*W/Mu+Mu/Mu*	*W/W*		Upper 95% C.I.	*Value*	*P-Value*
		F (F%)	*F*				
1	South Iran	11(2.78)	384	1.33	0.41-4.23	0.23	0.62
2	Iran	4(4.76)	80	2.32	0.56-9.52	1.45	0.22
3	Turks (25)	13(4.87)	254	2.37	0.76-7.41	2.36	0.12
4	Turks (24)	13(12.5)	91	6.64	2.10-20.95	13.33	0.0002
5	Sweden	52(25.5)	152	15.9	5.62-44.98	44.12	3.1E-11
6	Norway	21(21)	79	12.36	4.11-37.18	29.69	5.1E-08
7	Finland	31(31.6)	67	21.5	7.32-63.23	52.8	3.7E-13
8	Denmark	54(22.6)	185	13.5	4.81-38.24	38.01	7.1E-10
9	Hungary	15(15.2)	84	8.30	2.67-25.77	18.04	2.2E-05
10	France	306(16.7)	1530	9.3	3.42-25.23	28.17	1.1E-07
11	Lithuanian	63(22.3)	220	13.31	4.75-37.27	37.99	7.1E-10
12	Spain	85(13.2)	560	7.05	2.55-19.5	18.9	1.4E-05
13	Germany	19(19.2)	80	11.04	3.64-33.5	25.94	3.5E-07
14	Switzerland	10(15.6)	54	8.61	2.59-28.55	16.8	4.1E-05
15	Portugal	13(10.5)	111	5.44	1.73-17.11	10.29	0.001
16	Italy	124(9.68)	1157	4.98	1.81-13.65	11.95	0.0005
17	Cyprus	57(5.69)	945	2.80	1.005-7.82	4.22	0.03
18	Iceland	27(26.5)	75	16.74	5.66-49.48	41.52	1.2E-10
19	Britain	60(21.2)	223	12.51	4.46-35.07	35.43	2.6E-09
20	Greece	12(8.39)	131	4.25	1.34-13.5	7.049	0.007
21	Syria	3(2.83)	103	1.35	0.29-6.16	0.155	0.69
22	Belgium	189(18.6)	825	10.65	3.90-29.04	32.5	1.2E-08
23	Asian	0(0)	198	0		4.21	0.04
24	Caucasian	32(18.2)	144	10.33	3.57-29.88	26.63	2.5E-07
25	Pacific Islander	8(1.74)	453	0.82	0.24-2.76	0.102	0.74

genotype similarity between Iranian population and Asian populations (Chinese, Japanese, Filipino, Indian and Korean) is very high and no significant differences were noticed (23). The comparison of different population in this regard summarized and outlined in Table 3. The frequency of the even heterozygote forms of the CCR5Δ32 chemokine in Asian population is about zero (23). In this regard, the highest frequency of heterozygous individuals were distributed in Iceland (0.2509) and Finland (0.2663). When the genotype frequency for this gene is studied wider, an East-West and South-North increasing cline for heterozygous individuals were noticed (24). This ascending pattern from east to west and the degree of the difference are demonstrated in Graph 1 and Table 3. Differences among populations within different regions could be due to the different environmental conditions coming across during the centuries. For example some researcher says since European community had two pandemic explosion of the plague (Black Death period), therefore they had a selective pressure for having more CCR5Δ32/Δ32 genotypes compare to Asian community (14, 26-28). Having this mutated chemokine now is counted as an advantage for these nations and can protect them from HIV virus. But since the frequency of the homozygote form of the CCR5Δ32 chemokine as



Graph 1. As we see in the graph, when we investigate the frequency of the CCR5 allele from east toward west, the mutated form of the CCR5 chemokine (Δ32) is increasing (ref. 22-25).

reported in Garaghozoolo et al., Omrani et al. reports (22, 29) and present report is about zero in our country, therefore we should consider our nation genetically susceptible to HIV virus during any potential exposure in the future.

Based on the findings of this study and other researchers in Iran, one may conclude, Iranian nation compare to European nation are more susceptible to HIV virus infection. But since the infection with HIV virus is under the influence of the several different criteria, 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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