

Frequency of CCR5 delta 32 polymorphism and its relation to disease progression in Iranian HIV-1 positive individuals

Abstract

Introduction: Homozygosity or heterozygosity for 32-nucleotide deletion (Δ 32) within CCR5 gene may influence HIV acquisition and progression in HIV-1 exposed or HIV-infected individuals. In this study, frequency of heterozygosity for CCR5 mutation in an Iranian HIV-infected population was determined and its relation with their disease progression was evaluated.

Methods: A total of 194 HIV-infected patients were enrolled. The CCR5 Δ 32 in peripheral blood mononuclear cells was detected by the polymerase chain reaction (PCR) and gel electrophoresis. The disease progression was determined based on changes in CD4+ cell counts or clinical presentation of recruited patients.

Results: Eight of all 194 patients were heterozygous for (CCR5 Δ 32) deletion 32 genotype (wt/del 32). We found no homozygosity for the mutated gene (Del 32/del 32). Of the 103 patients with clinical diagnosis of AIDS, 3 were heterozygote. Although statistically insignificant, the frequency of rapid progression to AIDS was less in heterozygous individuals than the rest (37.5% vs. 53.2%).

Discussion: CCR5 mutation is found to be more prevalent in HIV-1 infected Iranians than literature reported data of healthy Iranian cohorts. Due to small sample size, our study did not demonstrate the implication of this mutation in HIV disease progression. Our finding could be related to ethnic variation between the populations and further studies on larger populations may help clarify the role of this mutation in the progression pattern of HIV in Iran.

Keywords: Chemokine Receptor 5 Gene (Ccr5) Mutation, HIV-1 Infection, HIV Progression, Iran

Volume 2 Issue 5 - 2015

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Received: October 25, 2014 | **Published:** September 03, 2015

Introduction

Worldwide, 7400 people become infected with HIV every day.¹ A number of host genetic variants have been identified that show significant association with HIV infection and progression to AIDS.² A mutation in the chemokine receptor gene CCR5 has been shown to provide strong protection from infection with HIV-1.³⁻¹⁰ The CCR5 gene is a G-protein-coupled chemokine receptor that is the major co-receptor for the macrophage-tropic strains of HIV. The CCR5 is encoded by the CMKBR5 gene and is located on the human chromosome 3p21.¹¹ One defective allele bearing a 32-basepair (BP) Δ 32 deletion has been extensively studied. CCR5 Δ 32 is not distributed among the world's population equally and population surveys suggest an approximate 10% frequency among Caucasian.¹² The mutation Δ 32 has functional significance in determining susceptibility to HIV infection and can possibly significantly delay the onset of AIDS.^{3,11-13} Homozygous individuals for CCR5 32 allele constitute only about 1% of the general population and these individuals are found to be resistant to HIV infection. This heterogeneity has led to an interest in the Δ CCR5 allele in the HIV infected population for a better understanding of its role in disease acquisition and progression.

This study is the first study that evaluates the frequency of the CCR5 gene and its relation to disease progression in the Iranian HIV-infected population. Studies on this topic can lead us to a better understanding of the acquisition of and progression of HIV.

Methods

Of the 200 randomly selected HIV/AIDS patients, 194 accepted to participate in this study. All the patients were adults with the age of (Mean \pm SD) 36.6 \pm 8.4. The sample size was calculated to detect frequency of 5-10% of CCR5 Δ 32 in a Caucasian population, with 95% confidence interval and 4% accuracy rate.¹⁴ For this cross-sectional study, all of the 194 HIV infected subjects were recruited from the Behavioral Consultation Center of Imam Khomeini Hospital in Tehran, Iran which is the largest inner city HIV outpatient clinic in Tehran. All of these individuals had a documented positive HIV test diagnosed by ELISA and confirmed with Western blot and were tested for CD4+ counts as per clinic routine. All subjects were of Iranian origin and gave informed consent to participate in this study. The study was approved by the Iranian Research Center for HIV/AIDS, Tehran University of Medical Sciences (TUMS), Tehran, Iran. In all patients 10 mls of whole blood was obtained whereby the WBCs were isolated by the lysine of the RBCs and by centrifuging of the samples to obtain Pellet WBCs. We then lyse the WBCs by using Proteinase and eliminated the protein and lipids by using SDS. We subsequently incubated the samples to activate Pr-kinesis and free the DNA. We then added some Ezopropanol for the clearing of DNA. The dried DNA was then placed in appropriate tubes.

The extracted DNA was then washed with Ethanol, and then dried; next we added some water to it. The DNA was then dissolved in water again and incubated in Healer tubes. The CCR-delta 32

genotypes were determined by PCR and gel electrophoresis. A portion of the CCR5 gene was then amplified by PCR from genomic DNA and analyzed on a 4% Metaphoric agarose gel. Primers CCR5c, 5'-CAAAAAGAAGGTCTTCATTACACC-3', and CCR5d, 5'-CCTGTGCCTCTTCTTCATTTCG-3' that flank the 32 BP deletion were used to generate wild-type and deleted fragments of 189 BP and 157 BP, respectively. The PCR reaction was performed in 17 μ l, PCR reaction mixture contained 0.25 mM of dNTPs. Ten pmol of each prime (2 μ l forward, 2 μ l Reverse) besides 0.3 unit of Taq polymerase in 1 \times reaction Buffer. Each PCR amplification consisted of 40 cycles with the first 5 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min, followed by 35 cycles of 94°C for sec, 60°C for 30 sec and 72°C for 45 sec. In this study, we define rapid progressorsas to AIDS as those who have a 50% drop in their CD4+ value or those who acquire an opportunistic infections during two years or less. The other patients put into the non rapid progressorsas.

The statistical analysis was performed using SPSS 17 (SPSS Inc., Chicago, IL, USA). Values were tested for normality. When appropriate an independent sample t-test, Chi square test or nonparametric equivalents were used. A p-value of 0.05 or less was considered statistically significant. Last but not least, we define rapid progressorsas to AIDS as those who have a 50% drop in their CD4+ value (not necessarily <200/mm3) or those who acquire an opportunistic infection during two years or less.

Results

One hundred ninety-four HIV infected patients were enrolled in this study. Table 1 summarizes their demographic and clinical characteristics. Eight individuals or 4.1% of the sample were heterozygous for the deletion 32 genotype (wt/del32). We found no homozygosis for this mutation (i.e. del32/del32) in our sample population.

Table 1 Demographic and clinical characteristics of the study population

	CCR5		p-value
	Homozygous wild type (wt/wt) (n=186)	CCR5 Heterozygous (Δ 32/wt) (n=8)	
Gender Male, No. (%)	145 (78%)	6 (75%)	0.844
Age, year (mean \pm SD)	36.6 \pm 8.4	35.2 \pm 9.4	0.694
AIDS, No. (%)	100 (54.1%)	3 (37.5%)	0.476
Type of progression to AIDS, No. (%)			0.481
Rapid	99 (53.2%)	3 (37.5%)	
Non-Rapid	87 (46.8%)	5 (83.3%)	

Of the eight heterozygous patients, three were taking HAART. Three of these were assessed as being rapid progressorsas; five were non-rapid progressorsas as defined previously. There were no remarkable statistical difference between the CD4+ count and HIV viral loads of the rapid progressorsas vs the rest.

Discussion

The vulnerability, morbidity, and mortality related to many pathogens and infections are influenced by the host's genetic makeup. The CCR5 molecule, a chemokine receptor, is the most important co-receptor for macrophage-tropic HIV-1 entry into the cell. The rate of progression to AIDS varies among individuals infected with HIV-1 and it has been shown that CCR5 Δ 32 confers almost complete resistance to HIV-1 infection in homozygote, and partial protection against HIV disease progression in heterozygous adults. CCR5 has recently been identified as an important co-receptor for HIV-1 entry into CD4+ T cells. The frequency of CCR5 heterozygote is increased among HIV-1 infected long-term non-progressorsas compared with the rest.³⁻¹⁰ Depending on studies the frequency of CCR5 Δ 32 allele was reported to be 0-38% among Caucasians. As a perfect example the frequency of CCR5 Δ 32 allele was reported to be 0-11% in the general population depending on the different samples in different geographic location. The frequency of heterozygous of this mutant allele is around 10% to 20% among Caucasians in North America and Europe, although it is found to be lower in African or Asian and Latin American populations.¹⁴ This study is the first to evaluate CCR5 polymorphisms among Iranian HIV-1 infected individuals. Previous studies have shown the CCR5 Δ 32 allele frequency to be 1.4% in healthy Iranian individuals in the south of Iran.¹⁵ In our study we found a higher frequency of CCR5 Δ 32 in the HIV-infected Iranians. We suspect that many factors contribute to this diversity. For example, variations in ethnicity may play a large role in the Iranian population.

Frequency of alleles or genotypes that are associated with progression to AIDS is studied by Carrington et al. In a cohort study they found a heterozygous frequency of 38% in Caucasians, 32% in African Americans and 39% in Hispanic populations.³ CCR5 Δ 32 heterogeneity is not only important in susceptibility to HIV infection, but it also can affect progression to AIDS. Some studies suggest that patients with the 32 genotype (del32/del32) CCR5 progress to AIDS less rapidly than those who do not have this mutation.^{4,5,7,8,14,16-21} As expected, we did not identify any homozygous cases for the deletion 32 genotype (del32/del32) CCR5 in our small sample. In our study, the rate of progression to AIDS in CCR5 Δ 32 cases were less than in patients without it, however this finding was not statistically significant. Our study was limited by our small sample size of patients with (del32/wt). It is also to be noted that the definition of progression to AIDS varies between different studies. For instance, Visco-comandini et al.²² define progression as CD4+ cell count drop to less than 200 \times 106 in less than 9 years and Smitabh Chakraborti define progression to an average time for progression of AIDS around 8-10 years.

Conclusion

The results of the present study show that the rate of progression in Iranian HIV-infected patients is not determined by CCR5 genotype. We further recommend that similar studies be conducted in larger populations at multiple centers in Iran before any clinical conclusions are drawn.

Acknowledgments

None.

Conflicts of interest

None.

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