

Original Article

## Frequency of CCR5-Δ32, CCR2-64I and SDF1-3'A alleles in HIV-infected and uninfected patients in Istanbul, Turkey

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### Abstract

**Introduction:** Co-receptors involved in cell entry of the human immunodeficiency virus (HIV) and mutations in genes encoding their ligands may play a role in the susceptibility to infection and resistance to the progression of the infection. The best studied mutations that can exist in these genes are the CCR5-Δ32, CCR2-64I and SDF1-3'A mutations. The frequency of these mutations vary from continent to continent and even from region to region. However, there is limited information on their distribution throughout the Turkish population. Istanbul is the city with the highest number of documented HIV-infected patients in Turkey, which can be attributed to the population size. The aim of this study was to determine the distribution of three AIDS-related gene variants among HIV-infected and uninfected population in Istanbul, Turkey and to estimate the contribution of these variants to susceptibility or resistance to HIV.

**Methodology:** A total of 242 healthy individuals and 200 HIV-positive patients were included in the study. CCR5 polymorphisms were genotyped by polymerase chain reaction. CCR2 and SDF1 polymorphisms were genotyped using PCR restriction fragment length polymorphism (PCR-RFLP).

**Results:** The allelic frequencies for CCR5-Δ32, CCR2-64I and SDF1-3'A were 4.07%, 19.8% and 28.7%, respectively. No individual was found to carry the homozygous CCR5-Δ32 mutation in either cohort. No polymorphism was found to be significantly elevated in the HIV-infected cohort compared to the healthy group.

**Conclusions:** The distribution of CCR5-Δ32, CCR2-64I, and SDF1-3'A variants does not differ between HIV-infected and uninfected patients. CCR2-64I and SDF1-3'A frequencies are relatively high where as the frequency of CCR5-Δ32 is low.

**Key words:** AIDS; HIV-1; CCR5-Δ32; CCR2-64I; SDF1-3'A; genetics; epidemiology.

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### Introduction

Humans are continuously in contact with infectious pathogens. Selective pressure may cause genetic changes that may be important to avoid or fight against the infection. The interesting interaction between HIV and the chemokine system has dramatically advanced our understanding of the pathogenesis of AIDS and created new perspectives for the development of effective prophylactic and therapeutic measures [1]. In case of HIV-1 infection, the CD4 molecule and fusion co-receptors on T cells that allow the virus to enter the host cell play an important role in the acquisition of the infection. However, there are several other chemokine receptors that are critical for the entry of HIV into the cells. In particular, two chemokine receptors,

macrophage–cell-tropical chemokine receptor 5 (CCR5) with a C-C motif and the T–cell–tropical chemokine receptor 4 (CXCR4) with a C-X-C motif, play a key role in the infection. In addition, some M-tropic HIV-1 strains may use other chemokines such as chemokine receptor 2 (CCR2) or chemokine receptor 3 (CCR3) to enter into macrophages [2]. In particular, polymorphisms in stromal derived factor 1 (SDF1) genes, which are quite interestingly also known ligands of CCR5, CCR2 and CXCR4, have been reported to alter the resistance and / or pathogenic progression of individuals infected with HIV [2].

The CCR5 chemokine receptor is the major co-receptor for macrophage-tropical HIV-1 strains. In the absence of CCR5 on the cell surface, the entry of HIV-

1 into host cells is prevented. Interestingly,  $\Delta 32$  mutation - a 32-nucleotide deletion in the CCR5 gene – not only results in the synthesis of a dysfunctional protein incapable of migrating the protein to the cell surface [3], but is also noteworthy that people who are homozygous in respect to this mutation exhibit a greater resistance towards HIV-1 infection. Even people with only a heterozygous genotype will still develop AIDS 2-3 years later when compared to patients who do not have the mutation [3,4]. The distribution of CCR5- $\Delta 32$  frequencies vary in different ethnic populations. A high frequency of CCR5- $\Delta 32$  is observed in Caucasians whereas a very low frequency is reported in the Asian population [5,6]. Outside Europe, only sporadic mutational events are observed, possibly due to the Caucasian contribution to the gene pool in these populations [4,7].

Due to a nucleotide change in position 190 in the first transmembrane loop of CCR2, valine at position 64 is replaced by isoleucine [8]. CCR2-64I has been associated with reduction in the progression to AIDS [9]. CCR2-64I results in normal expression levels of the CCR2 receptor and has no effect on the incidence of HIV infection. However, in contrast to normal CCR2 peptides, the CCR2-64I mutant protein preferentially dimerizes with the CXCR4 polypeptide, isolating it in the endoplasmic reticulum [2,10]. It is also thought that the inhibitory effect is dependent on the stages of HIV-1 infection and interactions with other genetic variants [11]. The frequency of the CCR2-64I allele ranges from 0.1 to 0.25 in different ethnic populations [2].

Stromal cell-derived factor 1 (SDF-1) is the main ligand for CXCR4 that is the co-receptor for T-cell tropical HIV-1. G-A mutation at position 801 relative to the ATG initial codon in the non-coding region of the SDF-1 gene has been shown to slow down the progression of the infection [12]. This limits the occurrence of X4 HIV-1 strains, since SDF1 blocks CXCR4 receptors once it binds them [2,9,13]. Allele frequency of SDF1-3 varies significantly among

different ethnic populations. The allele frequency of 0.149-0.217 and 0.06-0.43 has been reported in Europeans and South Asians, respectively [2].

The functional interaction of CCR5 / CCR2 and SDF1-3 may explain the enhanced protection against HIV / AIDS in people harboring the aforementioned specific mutations. It is hypothesized that CCR5 / CCR2 variants may hamper progression of the disease by limiting the number of CCR5 receptors that mediate R5 HIV-1 infection. Moreover, SDF1-3'A variant has been reported to restrict the emergence of X4 HIV-1 strains [2,9].

The frequency of these genetic variants especially among HIV-1 seropositive and seronegative high-risk populations is still unknown in Turkey. Understanding how these genetic variants contribute to the susceptibility to HIV-1 infection in Turkey will be of great importance. However, limited information is available about the distribution among Anatolian populations of host genetic polymorphisms that resist or slow down the progression of HIV infection. Identification and the distribution of allelic variants CCR5- $\Delta 32$ , CCR2-64I, and SDF1-3'A may help to understand the burden and course of the disease. In this study, we investigated three chemokine variants in Turkey's largest city and analyzed the host gene polymorphisms.

## Methodology

### *Study participants and specimen collection*

A total of 442 individuals from Istanbul, Turkey, were included in the study. Of these, 242 (137 male and 105 female) were 18-60 years old healthy individuals and 200 (147 male, 53 female) were 30-55 years old HIV-positive patients. The distribution of age among healthy individuals and patients are shown in Table 1. All HIV-positive patients were on antiretroviral therapy (ART). Individuals younger than 18 years of age or with severe illness were excluded. Informed consent was obtained from all participants in the study. The study

**Table 1.** Age and gender distribution of the study population.

Age groups	HIV- infected patients		Healthy		Total	
	Female n (%)	Male n (%)	Female n (%)	Male n (%)	Female n (%)	Male n (%)
≤ 19	4 (7.84)	13 (8.72)	8 (6.56)	13 (10.83)	12 (6.94)	26 (9.67)
20-29	12 (23.53)	34 (22.82)	18 (14.75)	20 (16.67)	30 (17.34)	54 (20.07)
30-39	10 (19.61)	43 (28.86)	38 (31.15)	27 (22.50)	48 (27.75)	70 (26.02)
40-49	15 (29.41)	36 (24.16)	30 (24.59)	40 (33.33)	45 (26.01)	76 (28.25)
50-59	6 (11.77)	15 (10.07)	11 (9.02)	18 (15.00)	17 (9.83)	33 (12.27)
60-69	3 (5.88)	7 (4.70)	15 (12.29)	2 (1.67)	18 (10.40)	9 (3.35)
≥ 70	1 (1.96)	1 (0.67)	2 (1.64)	0 (0.00)	3 (1.73)	1 (0.37)
Total	51 (100)	149 (100)	122 (100)	120 (100)	173 (100)	269 (100)

was approved by the Ethics committee of the Istanbul University, Istanbul Faculty of Medicine (2017-125).

#### Genotyping of single nucleotide polymorphisms

Four mL blood was collected in EDTA (2 mg/mL of blood). Lymphocytes were separated by Ficoll-Paque PREMIUM gradient centrifugation (density 1.073 g/mL) according to the instructions of the manufacturer (Ge Healthcare, Uppsala, Sweden). Briefly, 3 mL Ficoll-Paque PREMIUM gradient was pipetted into 10 mL centrifuge tubes. Two mL blood was diluted with an equal volume of phosphate-buffered saline (PBS). Four mL PBS-diluted blood was carefully layered over the Ficoll-Paque PREMIUM gradient with pasteur pipette in tubes. The tubes were centrifuged at 450 g for 30 minutes with the brake off, after which the cell interface layer was collected. Cells were collected in tubes, decomposed with PBS and stored at -80 °C until isolation. Just before extraction, 400 µL PBS-cell suspension was manually transferred into 2 mL cryotubes. The tubes were placed into 24 tube capacity carrier racks and loaded into the instrument. Sample extraction was performed automatically on the QIASymphony SP/AS platform, using the QIASymphony DNA mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. As shown in Table 2, primers 5'-CTTCATCATCCTCCTGACAATCG-3' and 5'-GACCAGCCCCAAGTTGACTATC-3' were used in the PCR reaction for the determination of CCR5-Δ32 [14]. 25 µL reaction mixture was set up with 10 µL MyTaq™ DNA Polymerase (Bioline Ltd, London, UK) containing 0.75 unit Taq DNA polymerase, 5 mM dNTPs, 15 mM MgCl<sub>2</sub>, 25 pmol of each primer and 2 µL (150–250 ng) genomic DNA in final concentration. The reaction mixture was subjected to an initial denaturation of 95 °C for 5 minutes followed by 35 cycles of 95 °C for 45 seconds, 58 °C for 45 seconds and 72 °C for 45 seconds. Final extension was done at 72 °C for 10 minutes. The amplified products were analyzed on 3% agarose gel and bands were visualized on UV-transilluminator. The wild type gene resulted in a 262 bp fragment, while the Δ32 mutant variant resulted in a 230 bp fragment. Heterozygotes produced

both of the bands. CCR2-64I gene polymorphism was studied by PCR-RFLP following the previously described protocols [15]. A 380 bp product of CCR2 gene was amplified using the following primers: 5'-GGATTGAACAAGGACGCATTTCCCC-3' and 5'-TTGCACATTGCATTCCCAAAGACCC-3'. The 25 µL reaction mixture was set up similar to that in CCR5 Δ32 analysis. The reaction mixture was subjected to an initial denaturation of 95 °C for 5 minutes followed by 35 cycles of 95 °C for 45 seconds, 63 °C for 45 seconds and 72 °C for 45 seconds. Final extension was done at 72 °C for 10 minutes. Four µL PCR product was used for the RFLP analysis that was performed in a 15 µL reaction volume using 4 U Fast digest *FokI* enzyme (MBI Fermentas GmbH, St. Leon-Rot, Germany). After 15 minutes of incubation at 37 °C, the digested products were analyzed on 4% agarose gel. The wild type gene yielded a single 380 bp fragment, whereas *FokI* produced two bands at 215 bp and 165 bp only in the presence of an ATC triplet coding for isoleucine. For heterozygotes, three bands at 380 bp, 215 bp, and 165 bp were present. SDF1-3'A mutation was confirmed by PCR-RFLP using the primers 5'-CAGTCAACCTGGGCAAAGCC-3' and 5'-AGCTTTGGTCTGAGAGTCC-3' and *MspI* (MBI Fermentas GmbH, St. Leon-Rot, Germany) restriction endonuclease [14]. PCR reaction mixture was set up similar to that in CCR5 Δ32 analysis. The reaction mixture was subjected to an initial denaturation of 95 °C for 5 minutes followed by 35 cycles of 95 °C for 45 seconds, 58 °C for 45 seconds and 72 °C for 45 seconds. Final extension was done at 72 °C for 10 minutes. PCR was followed by digestion of 4 µL PCR amplicon with 5 U *MspI* in a 15 µL reaction volume at 37 °C for 24 hours. Digested products were analyzed on 4% agarose gel after electrophoresis. For the wild type gene, the 302 bp product yielded two bands at 100 bp and 202 bp. For heterozygous mutants, the bands were observed at 302, 202, and 100 bp whereas for homozygous mutants there was only a single band at 302 bp.

#### Statistical analysis

Allele frequencies were calculated by allele counting. Hardy-Weinberg equilibrium was tested by

**Table 2.** The sequences of primers, amplicon size, enzyme and fragment size.

Allelic genotype	Primer sequence	Amplicon size	Enzyme	Fragment size
CCR5-Δ32	CTTCATCATCCTCCTGACAATCG	262 wt	none	262 or 230
	GACCAGCCCCAAGTTGACTATC	230 mut		
CCR2-64I	GGATTGAACAAGGACGCATTTCCCC	380	FokI	215+165
	TTGCACATTGCATTCCCAAAGACCC			
SDF1-3'A	CAGTCAACCTGGGCAAAGCC	302	MspI	202+100
	AGCTTTGGTCTGAGAGTCC			

**Table 3.** Distribution of CCR5-Δ32, CCR2-64I, and SDF1-3'A gene polymorphisms.

	HIV(+) patients	HIV(-) control	OR (95% CI)	<i>p</i> value
<b>CCR5-Δ32</b>				
Wt/Wt n (%)	184 (92.0)	222 (91.0)	1	
Wt/Δ32 n (%)	16 (8.0)	20 (9.0)	0.870 (0.444-1.705)	0.684
Δ32/Δ32 n (%)	0 (0.0)	0 (0.0)		
<b>CCR2-64I</b>				
G/G	133 (66.5)	149 (61.6)	1	
G/A	62 (31.0)	83 (34.3)	1.077 (0.703-1.651)	0.733
A/A	5 (2.5)	10 (4.1)	0.721 (0.348-1.494)	0.379
<b>SDF1-3'A</b>				
G/G	108 (54.0)	120 (49.6)	1	
G/A	76 (38.0)	98 (40.5)	1.001 (0.739-1.355)	0.996
A/A	16 (8.0)	24 (9.9)	0.860 (0.553-1.338)	0.505

HIV: Human immunodeficiency virus; OR: Odds ratio; CCR5: chemokine receptor 5; Wt: wild type; CCR2: chemokine receptor 2; SDF1: Stromal cell-derived factor 1.

using the chi-square goodness-of-fit test. Allele frequencies of HIV infected and healthy individuals were compared using chi-square test. The level of statistical significance was set at 0.05.

**Results**

A total of 442 individuals from Istanbul, Turkey were enrolled to the study. Of these individuals, 200 (45.2%) were infected with HIV and 242 (54.8%) were healthy. The mean age was 37.3 for HIV-infected patients and 42.8 for healthy individuals. Thirty-six out of 442 people (8.1%) were found to have a heterozygous Δ32 genotype, representing a frequency of 4.07% for this allele (Table 3). It was found that 16 out of 200 HIV-infected patients and 20 out of 242 healthy individuals had the heterozygous CCR5-Δ32 genotype. The frequency of the Δ32 allele among HIV-infected patients and the healthy group were 4% and 4.1%, respectively. No homozygous variant (Δ32/Δ32) was found in 442 genotyped samples. The total frequency of the wild type allele (Wt/Wt) and mutant variant (Wt/Δ32) in healthy samples was found to be 0.959 and 0.041, respectively. As shown in Table 4, the frequency of deletion in CCR5-Δ32 does not follow the

Hardy-Weinberg balance in the healthy and HIV-1-infected groups (*p* > 0.05).

The GG genotype represents the homozygous wild type genotype (CCR2 +/+), whereas GA codes for the heterozygous CCR2 +/64I and AA for the homozygous recessive genotype CCR2 64I/64I. CCR2+/+ was not observed in a total of 160 patients (36.2%), including 67 (15.2%) HIV-infected patients and 93 (21%) healthy controls. The prevalence of G and A allele among HIV-infected Turkish patients was found to be 82% and 18%, respectively. In healthy individuals, the prevalence of G and A allele was 78.6% and 21.4%, respectively. The frequency of the allele A in the entire study group was 19.4%. When the data was analyzed, no significant difference was observed between HIV infected and healthy individuals. (*p* > 0.05).

SDF1-3'A polymorphism has been observed at a relatively high rate compared to other polymorphisms (48.4%). SDF1-3'A polymorphism was observed at the rate of 27% in HIV-infected individuals and 30.2% in healthy controls, while the G wild-type allele was observed in 73% of HIV-infected individuals and 69.8% of healthy individuals.

Although the frequencies of heterozygous CCR5-Δ32 and heterozygous/ homozygous CCR2-64I and

**Table 4.** Allele frequency and Hardy–Weinberg equilibrium (HWE) analysis.

Allelic genotypes	HIV-1(+) Patients			HIV(-) Controls			Total freq.(%)
	Freq (%)	$\chi^2$	<i>p</i> value	Freq (%)	$\chi^2$	<i>p</i> value	
<b>CCR5-Δ32</b>		0.34	0.55		0.44	0.5	
wt	96.0			95.9			95.93
Δ32	4.0			4.1			4.07
<b>CCR2-64I</b>		0.5	0.48		0.13	0.71	
V	82.0			78.7			80.2
I	18.0			21.3			19.8
<b>SDF1-3'A</b>		0.26	0.61		0.36	0.54	
G	73.0			69.8			71.3
A	27.0			30.2			28.7



SDF1-3'A were found slightly higher in HIV-1 seronegative group than that in seropositive group, there was no significant difference in the frequency of these genetic variants between the seronegative and seropositive groups.

## Discussion

The CCR5-Δ32 and CCR2-64I alleles have been reported to have a strong protective effect on the progression of HIV-1 infection whereas SDF1-3'A homozygosity had no such a protective effect [16]. On the contrary, there are studies which reported that the homozygous SDF1-3'A allele is also protective against HIV-1 infection [12,13]. This study describes the genotype and allele frequencies of the polymorphisms CCR5-Δ32, CCR2-64I and SDF1-3'A among HIV-1-infected patients and healthy individuals in Istanbul, Turkey. This is the first large scaled study investigating the frequencies of the polymorphisms among HIV infected patients in Turkey. The distribution of CCR5-Δ32, CCR2-64I and SDF1-3'A varies in different ethnic populations. While the CCR5-Δ32 allele frequency was found as high as 15% in Caucasians, the genetic variant was rarely detected within the Asian population [5,6,17,18]. The present study, conducted in Istanbul, Turkey's largest city, reflects the country profile due to constant migration from different locations of the country. The allelic frequency of CCR5-Δ32 was found to be 4.07% among HIV infected patients and healthy individuals. Consistent with our findings, Nkenfou *et al.* [19] and Veloso *et al.* [20] reported similar frequencies of CCR5-Δ32 in healthy and HIV-1 infected individuals in Cameroon and Spain, respectively. In our study, no significant difference was detected in the frequency of CCR5-Δ32 allele between healthy individuals and HIV-1 infected patients. In parallel with our findings, in the two aforementioned studies carried out in Cameroon and Spain, relatively higher frequency of CCR5-Δ32 was reported in HIV-1-seronegative subjects compared to seropositive individuals. The frequency of CCR5-Δ32 allele was reported to vary from 2.6% to 6.2% in Turkish population [21,22]. Similarly, in another study conducted on different populations in a different region of Turkey, the CCR5-Δ32 frequency among HCV infected patients was found to be 4.3% [23]. In a study conducted on 1.3 million blood donors from various countries, including about 36,000 donors from Turkey, the frequency of the CCR5-Δ32 allele in Turkish individuals was reported to be 3.4% [24]. Taken together, the data from Solloch *et al.* [24] and our study

suggest that the CCR5-Δ32 allele distribution in Turkey is low.

The CCR2-64I allele is associated with a reduction in the progression of AIDS [25,26]. Several recent studies carried out in different countries have shown that the CCR2-64I allele is broadly distributed [6,17,19,27]. The frequency of CCR2-64I in Turkish patients was reported to be as high as 25.7%, which was similar to that in Southeast Asians [6,17]. However, in the present study, the frequency of CCR2-64I allele among HIV-1 seropositive Turkish patients was found to be similar to that of seronegative individuals. CCR2-64I mutation in Turkey was investigated in two studies conducted among different patient groups and found to be 15.6-19.3% in healthy control groups [28,29]. In this study, the frequency of the CCR2-64I allele was found to be 19.4%. In another study carried out among Turkish women in Istanbul, the allele frequency was found to be 15.6%. These data are similar to those found in North America and Western Europe [30].

The SDF1-3'A mutation was quite common in Asia and Australia [7,13]. In the present study that included the high-risk Turkish population, the frequency of SDF1-3'A allele was determined to be 27.8%. Of 442 individuals, 40 (9%) were homozygous and 174 (39.4%) were heterozygous for SDF1-3'A. However, the distribution of the SDF1-3'A genotype and allele was almost the same in HIV-1 seropositive and seronegative patients. In Turkey, Kucukgergin *et al.* [29] analyzed CCR2-64I among women and found an allelic frequency of 32% that is similar to the frequency (27%) found in this study.

In summary, we investigated the distribution of HIV-1-related genetic variants among HIV-1 seropositive patients and seronegative individuals in Istanbul, Turkey. Not surprisingly, no homozygous CCR5-Δ32 allele was detected. On the other hand, interestingly, no polymorphism was found significantly higher neither in HIV-infected patients nor in healthy individuals. Although the advantage provided by these mutations may contribute to the resistance of human populations to the infectious disease, the mechanism that leads to the accumulation of these alterations remains unknown. Therapeutic strategies that aim to alter the receptor or block HIV and co-receptor interactions by chemokines or their synthetic analogues would be effective to combat HIV infection or retard its progression.

A potential limitation of our study is that there is no data on how these polymorphisms alter the clinical course of the disease and affect the survival of patients. The reason for this limitation is that many of the

patients in our study group were unable to attend follow-up examinations due to socioeconomic and psychological reasons.

## Conclusions

This is the first study to report the frequencies of the chemokine receptor variants in individuals living with HIV in Turkey. The distribution of CCR5-Δ32, CCR2-64I, and SDF1-3'A variants among HIV infected individuals does not differ from uninfected patients. The frequencies of CCR2-64I and SDF1-3'A are relatively high where as the frequency of CCR5-Δ32 is low. Further investigation on the frequency of these polymorphisms in Turkey is of importance to clarify the susceptibility rate of individuals to HIV infection.

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