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Short Communication

Polymorphism in the CC-chemokine receptor-5 (CCR5) gene

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Chemokine receptors and their ligands may confer resistance to HIV-1 infection and/or AIDS progression. Our aim was to study our population for the most frequently studied polymorphism CCR5-32 for evaluating their contribution to a protective genetic background against HIV infection and progression. One hundred and fifty blood samples from normal controls were recruited at random among prospective normal blood donors and forty blood samples of HIV/AIDS patients from Ethnic Kashmiri population were collected from Blood Bank and National AIDS Control Organisation of Sheri Kashmir Institute of Medical Sciences Soura, Kashmir respectively. Genotyping was performed by polymerase chain reaction (PCR) analysis followed by electrophoresis. The CCR5- 32 genotype frequency among our study group was wt/wt (93.3%), wt/mt (4%) and mt/mt (3%) from control group, revealing CCR5 32 allele frequency of 5%. The frequency of the CCR5- 32 allele among our study population seems to be remarkably higher compared to previously reported frequencies in other Asian populations. However, since this polymorphism is related with delayed progression from HIV infection to AIDS, it could be used for prognostic genotyping in HIV infected Kashmiri individuals.

Key words: CCR5- 32, HIV (Human immuno deficiency virus), mt (mutant type), wt (wild type).

INTRODUCTION

Chemokines (chemotactic cytokines) are proinflammatory cytokines that attract leukocytes to tissues, a process necessary for inflammation (Luster, 1998). Chemokine receptors act with CD4 as HIV-1 coreceptors to mediate the first step in cell entry: fusion of the viral envelope with the target-cell membrane. Although this activity has been shown *IN VITRO* for seven chemokine receptors and several related orphan receptors, only one of these, CCR5, has been shown to be important in the pathogenesis of HIV-1 (Berger et al., 1997; Doms and Peiper, 1997). This discovery came from the epidemiological analysis of a polymorphism, CCR5- 32, that contains a 32 base-pair (bp) deletion in the open reading frame (ORF), and encodes a non-functional protein (Dean et al., 1996; Huang et al., 1996; Lui et al., 1996; Samson et al., 1996; Zimmerman et al., 1997). CCR5- 32 is relatively common in Caucasians where the allele frequency is about 5 to 14%.

Homozygotes are found in 1% of white blood donors but at a much lower than- expected frequency in HIV-1-infected Caucasians. (Dean et al., 1996; Huang et al., 1996; Lui et al., 1998; Samson et al., 1996; Zimmerman et al., 1997; Wang et al., 1997; Smith et al., 1997; O'Brien et al., 1997). The *CCR5* gene product encodes a 7 transmembrane G protein– coupled chemokine receptor that, with CD4, serves as an entry port for primary human immunodeficiency virus (HIV)–1 strains that infect macrophages and monocytes (Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Doranz et al., 1996; Dragic et al., 1996). In mid-1996, several groups described a 32-bp deletion mutation that interrupts the coding region of the *CCR5* chemokine-receptor locus

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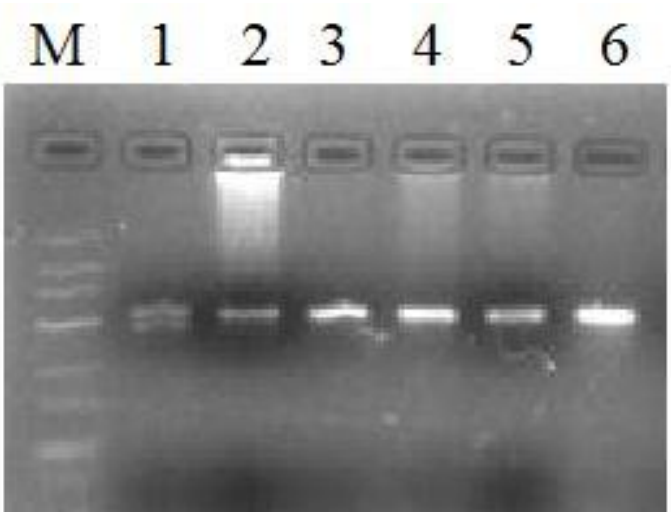

Figure 1. Amplified DNA fragments of wild-type CCR5 and CCR5- 32 mutant gene.M 25 bp ladder, 1 wt/mt , 2, 3, 4, 5, 6 wt/wt.

on human chromosome 3p21 (Dean et al., 1996; Liu et al., 1996; Samson et al., 1996). The *CCR5 32* mutation, which leads to truncation and loss of the receptor on lymphoid cells, was remarkable because homozygous individuals had nearly complete resistance to HIV-1 infection despite repeated exposure, and HIV-1 infected heterozygote's delay the onset of acquired immunodeficiency syndrome (AIDS) 2 to 3 years longer (Dean et al., 1996; Huang et al., 1996; Biti et al., 1997; Michael et al., 1997; O'Brien et al., 1997; Theodorou et al., 1997; Zimmerman et al., 1997). Chemokines are chemoattractant proteins with diverse biological functions and contribute in homeostatic processes.

In recent years, chemokines have gained significant importance, because of their involvement in inflammation, and autoimmune diseases. Now chemokines are also known to influence tumour cell's activity. Specifically, tumour cells express chemokine receptors in a non-random manner which suggests a role of chemokines in metastatic destination of tumour cells (Duell et al., 2006). *CCR5- 32/ 32* homozygotes lack *CCR5*-mediated chemokine responsiveness, probably because of the genomic redundancy of chemokine receptor functions (Premack and Schall, 1996). CCR5 32 heterozygotes may be partially protected against HIV-1 transmission by heterosexual intercourse, but may be protected minimally or not at all against perinatal transmission or transmission by homosexual intercourse (Hoffman et al., 1997; Edelstein et al., 1997; Rousseau et al., 1997). In studies of HIV-1 seroconvertors, progression to AIDS was delayed by an average of 2 years in CCR5 32 heterozygotes compared to people lacking this allele (Dean et al., 1996; Huang et al., 1996; Zimmerman et al., 1997; Michael et al., 1997).

In the present study, we investigated the potential influence of CCR5 32 polymorphism on contribution to a protective genetic background against HIV infection and progression.

MATERIALS AND METHODS

Patient recruitment

One hundred and fifty blood samples of normal controls and forty AIDS patients were recruited at random among prospective blood donors from the blood transfusion services and antenatal services of NACO (National AIDS Control Organisation) team of the Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Soura, Srinagar, Jammu and Kashmir (India), respectively. All the patients recruited in our study were having full blown AIDS and all the patients were on HAART (Highly active anti retroviral therapy) with an average age group of >40. All the donors were related Kashmiri residents. Ethnic bias within the population studied was minimized by excluding non-Kashmiri resident subjects. Informed consent was obtained from all the individuals that participated in the study.

Genotyping

Genomic DNA was extracted from 10 ml EDTA (Ethylene di amine tetracetate) treated venous blood samples using the standard phenol-chloroform extraction protocol. DNA purity was assessed by a UV–Vis spectrophotometer estimating the A260/A280 ratio or by running samples on 1% agarose. Genotyping for the CCR5- 32 was performed by PCR using pair of external primers framing the regions surrounding the polymorphic genetic sites. The reaction was conducted in a total volume of 25 µl containing 100 ng of genomic DNA and 25 pmol of each primer. Primers used were as described by Apostolakis et al. (2005).The wild-type CCR5 gene reveals a 302-bp fragment, whereas the 32 mutant results in a 270-bp fragment (Figure 1).

Statistical analysis

Statistical analysis of allele frequencies was performed using Chi-square statistics (Pearson test using SPSSv10 software). Genotype distribution for polymorphism was first compared to predictable values from Hardy–Weinberg equilibrium. In all cases, P-values less than 0.05 were considered to be statistically significant.

RESULTS

The frequencies of CCR5-delta32 alleles were surveyed in a group of 150 blood donors from the ethnic population of Kashmir, six subjects (4%) were found heterozygous and four subjects (3%) were found homozygous giving an allele frequency of 5%, with a 95% confidence interval (CI) for conformity with Hardy–Weinberg equilibrium of 1.52 to 8.48% (Table 1). All HIV/AIDS patients in our study were found to be wild type homozygous. To improve the genotyping quality and validation, 20% of samples were re-genotyped by other laboratory personnel and results were reproducible with no discrepancy in genotyping.

Table 1. Genotype frequencies of HIV/AIDS protective mutations in CC chemokine-receptors genes within Ethnic Kashmiri population.

	wt/wt[a]		wt/mt[b]		mt/mt[c]		Mutated allele frequency (%)
	n	%	n	%	n	%	
CCR5 Controls	140	93.3	6	4	4	3	5
CCR5 HIV patients	40	100	-	-	-	-	

[a] Wild type homozygotes; [b] Heterozygotes; [c] Mutant type homozygotes.

Genotyping of 10% of samples were confirmed by PAGE (Polyacrylamide gel electrophoresis).

DISCUSSION

The frequencies of CCR5- 32 alleles among ethnic population of Kashmir deduced from our study was found as, six subjects (4%) were found heterozygous and four subjects (3%) were found homozygous giving an allele frequency of 5%, with a 95% confidence interval (CI) for conformity with Hardy–Weinberg equilibrium of 1.52 to 8.48%. The CCR5- 32 allele frequency among Asians is very low in Rajasthan Indians (0.05%), Andhra-Pradesh Indians (0 to 0.03%) (Kozhekbaeva et al., 2004), North Indians (1.5%) (Verma et al., 2007) and South Indians (1 to 3%) (Ramana et al., 2001). A similar study conducted from Island of Crete, Greece showed allele frequency of 3.25%, with a 95% confidence interval (CI) for conformity with Hardy–Weinberg equilibrium of 0.74 to 5.7% (Apostolakis et al., 2005). The CCR5- 32 polymorphism is found all across Europe at different allele frequencies, with a North to South decreasing gradient and lower distribution in the regions of Southeast Mediterranean (Libert et al., 1998). However, the host protection conferred by the presence of the aforestated allele, against the viral entry has been well established, discrepancy remains concerning the significance of this polymorphism in HIV-1 disease progression. The CCR5-

32 mutation homozygote's were found from each region, one individual among Russians (frequency, 0.011) and Ukrainians (frequency, 0.009), and three individuals among Belarussians (0.024).

In our study, it was found that 4% Kashmiri population is homozygous; thus giving an idea that the individuals with such genotypes may be resistant to sexually transmitted HIV-1 infection. The frequency of the CCR5-delta32 allele among our study population seems to be remarkably higher compared to previously reported frequencies in other Asian populations. However, since this polymorphism is related with delayed progression from HIV infection to AIDS, it could be used for prognostic genotyping in HIV infected Kashmiri individuals. Since in our study, it was found that all AIDS patients were having wild type genotype so not protected for acquiring AIDS, it was also found that the patients were rapid progressers of AIDS.

Conclusion

In conclusion, this study indicates that the CCR5 32 polymorphism is associated with delayed progression from HIV infection to AIDS in the Kashmir valley. Additional studies on larger cohorts are warranted to verify the correlation and to help discern racial differences. Early identification of individuals with HIV would allow targeted and aggressive screening in the population.

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