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Original Article

Mutation in AIDS restriction gene affecting HIV infection and disease progression in a high risk group from Northeastern India

Col Partha Roy^{a,*}, Sekhar Chakrabarti^b^aProfessor, Department of Microbiology, Armed Forces Medical College, Pune 411040, India^bDirector, National Institute of Cholera and Enteric Diseases, Beliaghata, Kolkata 700010, India

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ABSTRACT

Background: HIV estimates for 2009 released by National AIDS Control Organization (NACO) reveals that 2.4 million people in India were living with HIV, 39% being female, 4.4% children and 82.4% adult males between the age group of 15 and 49 years. Persons with host genetic polymorphism CCR5Δ32 mutation are known to be partially or fully resistant to HIV infection. Persons with mutation affecting both the alleles (homozygous) are resistant to HIV infection whereas single allele (heterozygous) polymorphism leads to slower progression to AIDS. CCR5Δ32 mutation is commoner in Caucasians but less prevalent amongst Africans and Asians thereby rendering them susceptible to HIV infection.

Method: 571 HIV serologically naive subjects from a young and homogenous male population hailing from the seven northeastern states; West Bengal and Gorkha people were selected. All the subjects belonged to a special high risk group, sexually active and typically working in difficult and uncongenial terrain involved in frequent moves including overseas missions. Their family lives are severely disrupted. 181 HIV seropositive cases of which 92 cases that were admitted in a large tertiary care hospital were also included. The distribution of CCR5Δ32 polymorphism amongst both HIV seronegative (HSN) and HIV seropositive study cohorts (HSP) using molecular methods was studied.

Results and conclusion: There was failure to detect any CCR5Δ32 amongst this study group suggesting that this population from the northeastern India, West Bengal and Gorkha people are not protected by this specific host polymorphism in respect of acquisition of HIV infection as well as progression to AIDS.

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Introduction

HIV estimates for 2009 released by National AIDS Control Organization (NACO) reveal that 2.4 million people in India

were living with HIV, 39% being female, 4.4% children and 82.4% adult male between the age group of 15 and 49 years. Despite a decrease in prevalence rates from 0.36% in 2006 to 0.31% in 2009 and the progress made by India in advancing towards prescribed national targets, much remains to be done

* Corresponding author. +91 9637805666 (mobile).

E-mail address: drparthoroyafmc@hotmail.com (P. Roy).

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for halting and reversing HIV infections considering that in absolute terms; a large proportion of India's populous is infected or affected with HIV.¹

The hallmark of HIV infection is the progressive loss of CD4 + T cells that results in a dysfunctional immune system failing to combat the onslaught of opportunistic pathogens. Interestingly, there is significant heterogeneity among individuals in infection susceptibility, the time period necessary to deplete the CD4 T-lymphocytes and progress to AIDS-defining diseases.^{2–4} Various virologic and host genetic factors appear to account for a part of the discordance in infection susceptibility and in progression rates to AIDS and death.^{5–7}

Host genes collectively known as AIDS restriction genes (ARGs) have been shown to modify individual response to HIV-1 exposure, infection and pathogenesis markedly.^{6–8} CCR5, a chemokine receptor has been studied extensively. A 32 base pair deletion in the gene encoding the CCR5 receptor, resulting in CCR5Δ32 mutation has been shown to significantly affect the entry of the HIV and also the progression of the disease to AIDS.^{8–11} Individuals who do not exhibit this particular gene polymorphism are prone to the disease.¹² In contrast, individuals who are homozygous for this defect appear to be resistant to HIV infection,^{6,8} and it has therefore been assumed that individuals who are heterozygous for this deletion are partially resistant. Various other genes are known to affect HIV infection and progression to the disease.

The seven sister states of Northeastern India (NE states) are Assam, Manipur, Nagaland, Arunachal Pradesh, Mizoram, Tripura and Meghalaya. This region is primarily inhabited by Tibeto-Burman speakers who are culturally and genetically distinct from the Indo-European and Dravidian speakers present in the other geographical regions of India.^{13,14} Considering the distinct genetic makeup of this population and the high risk group involved, we decided to undertake a study on the presence of host genetic polymorphisms of the CCR5 gene within this specific high risk population. The study period was of 41 months (Aug 2007–Jan 2010). The present prevalence of HIV infection and other demographic parameters of the NE states are incorporated in Table 1.

Material and methods

Two distinct set of populations were selected:

- (a) Persons with unknown status for HIV infection.
- (b) Persons known seropositive for HIV infection.

A total of 571 males were recruited for the study whose HIV infection status was not known. All these persons belonged to a single homogenous professional background with common peculiarities identified as risk factors individually and collectively that were as follows: In the age group between 18 and 40 years, sexually active with phases of sexual deprivation due to long separation from family. Frequent movements and an inherent risk taking ethos are inbuilt into the profession. Being financially independent they are centers for attraction by sex workers and their middlemen.

All the subjects belonged to either of the following geographical locations:

- (a) The 07 sister states in northeastern India namely Assam, Manipur, Nagaland, Arunachal Pradesh, Mizoram, Tripura and Meghalaya
- (b) The state of West Bengal, Eastern India.
- (c) The Gorkha population belonging to West Bengal, Nepal (but working in India) and other parts of the country.

Subsequently 03 cases were reduced from the figure of 571 as these were detected seropositive during testing and shifted to the group, 'Persons known seropositive for HIV infection'. Therefore, a total of 568 cases were analyzed in the seronegative group.

We also included 181 known HIV seropositive patients (HSP) in this study of which 92 cases belonged to the specified high risk group admitted in a tertiary care hospital for medical management; 61 cases were male intravenous drug users (IDUs) from the state of Manipur and 28 cases were female sex workers (FSW's) from parts of West Bengal. The blood samples of these 89 cases from Manipur (IDU) and West Bengal (FSW) were processed strictly on "Unlinked and Anonymous" basis.

Samples were obtained after detailed pre-test counseling and an informed written consent. Permission to carry out the study was obtained from the institutional ethical committee. All samples were serologically tested for Anti-HIV antibodies 1&2; HBsAg; Anti-HCV antibodies by Enzyme linked immune sorbent assay (ELISA). Test for syphilis was carried out using *Treponema pallidum* hemagglutination (TPHA). Post-test counseling was done after the laboratory reports were available. The entire study population was medically followed up six monthly for 41 months to ascertain incidence of HIV infection in HSN and progression to AIDS in HSP group.

Molecular characterization of mutant CCR5Δ32 alleles was performed, using previously published PCR primers¹⁵ for CCR5Δ32 namely Forward: 5'-CTCGGATCCACCAGATCTCAAAAA GAAGGTCT-3' and Reverse: 5'-CTCGTGCACATGATGTGTAAGATAAGCCT-CAC-3' in a PTC 200 system (USA) under standard conditions and then separated by 2% agarose-gel electrophoresis. A 100 base pair ladder was incorporated. Based on the characteristics of CCR5Δ32 alleles, the expected banding patterns were as under: Homozygotes for the normal allele (wild type): 217 bp; Heterozygote for the mutant allele: 217 bp and 185 bp and Homozygote for the mutant allele: 185 bp. Appropriate positive and negative controls were incorporated. Amplified PCR products were randomly selected and sequenced to ascertain the correctness of CCR5Δ32 electrophoresis band patterns.

Results

Of the 568 HIV negative samples and the 181 HIV positive samples, all were wild types or homozygotes for the normal allele. No CCR5Δ32 mutations were detected (Fig. 2). All sequenced PCR products showed homology with the CCR5 gene.

The total statewide distribution of known HIV seronegative (HSN) subjects is depicted in Fig. 1. Out of a total of 568 HSN persons, a state-wise ethnic identity of 24 persons could not be ascertained with certainty although it was given to understand through official records that all these 24 persons did belong to

Table 1 – Statewise demographic parameters related to HIV infection (2009).¹

	Assam	Manipur	Mizoram	Nagaland	Meghalaya	Tripura	Arunachal Pradesh
Adult prevalence (15–49 yrs)	0.08	1.4	0.81	0.78	0.08	0.15	0.16
Number of HIV infection	14,244	26,773	6025	13,120	1332	2381	808
Percent distribution (15–49 yrs)	91.1	70.13	74.21	74.51	90.69	86.36	88.82
New adult infections	2540	1219	409	704	168	280	134
AIDS related deaths	471	1700	362	783	53	244	54
Adult FSW population	13,096	6417	2273	6597	3083	17,675	1208
Adults receiving ART	915	5605	705	1736	86	120	28

the geographical area under study. Therefore, these 24 individuals were grouped under the 'others' group distinct from the north-eastern states (NES).

Over a follow up period of 41 months 2 persons each from Manipur and Nagaland seroconverted for HIV-1 infection.

Fig. 1 – Statewise distribution of 568 HIV seronegative subjects (HSN).

Fig. 2 – PCR gel documentation picture in 2% agarose gel for CCR5Δ32 polymorphism. Lane no 1 shows a single band at 185 bp depicting homozygous mutant allele. Lane 3 shows bands at 185 and 217 bp indicating heterozygous mutation. Lane 2, 4, 5 and 6 shows only wild type alleles depicted by a single band at 217 bp. A 100 bp ladder has been used, marked at 100 bp, 200 bp and 500 bp for reference.

Of the 92 HIV positive cases at our hospital 25 cases progressed to AIDS (27%) as per the AIDS definitions of 1993, 53 persons (57.6%) were exhibited highly active antiretroviral therapy (HAART) and 5 cases expired during this period (5.4%).

In the absence of CCR5Δ32 mutations in our study population, we could not carry out statistical evaluation of effects of gene polymorphism towards acquisition of HIV-1 infection and progression of disease in HSN and HSP individuals respectively.

Discussion

The CCR5Δ32 mutation results from a 32-base pair (bp) deletion from the coding region of the CCR5 gene that creates a premature stop codon that results in a defective receptor that is not expressed at the cell surface. Homozygotes to the mutant allele resistant to R5 HIV-1 infection (the principal infecting HIV-1 strain) lack the requisite HIV-1 entry coreceptor CCR5 on their lymphoid cells.^{5,7} Heterozygotes to the mutant allele express less than half the wild-type levels of CCR5 receptors, slowing HIV-1 replication, spread and pathogenesis.^{8,13}

Table 2 – CCR5Δ32 genotype frequency distribution among different populations.

Population	Wt/wt	Wt/mut	Mut/mut
Egypt-Ismailia ²²	98.70%	1.29%	0%
Syria ²²	98.55%	1.44%	0%
Lebanon ²²	100%	0%	0%
Saudi Arabia ¹⁴	95.85%	4.14%	0%
United Arab Emirates ²³	100%	0%	0%
Yemen ¹⁴	100%	0%	0%
Sudan ²³	100%	0%	0%
Kenya ¹⁴	100%	0%	0%
Nigeria ¹⁴	99.09%	0.90%	0%
African-American ²²	97.70%	2.29%	0%
German European ²²	78.37%	17.56%	4.05%
Turkey ¹⁴	87.5%	12.5%	0%
Cyprus (Greek) ²²	98.21%	1.75%	0%
Kuwait ²²	97.96%	2.03%	0%
Syria ²²	97.16%	2.83%	0%
Jordan ²²	91.22%	3.50%	0%
Iran ²²	95.23%	4.76%	0%
Russia ²²	75.56%	24.43%	0%
North India ²⁴	100%	0%	0%
North India ²⁵	97%	3%	0%
North India ²⁶	100%	0%	0%
Present study	100%	0%	0%

Table 3 – Allele frequencies for AIDS restriction gene in three racial groups (Adapted from Winkler et al).⁴

	Allele or factor	Genetic model	African American (AA)	European American (EA)	Delta EA vs AA	Han Chinese ¹⁵
Resistant factors						
CCR5	D32	Recessive	0.02	0.10	0.08	Not observed
CCR2	64I	Dominant	0.15	0.10	0.05	0.25
SDF1	3'A	Recessive	0.02	0.21	0.19	0.26
MCP1,MCP3, Eotaxin	Hap 7	Dominant	0.031	0.192	0.16	ND
CXCR6	E3K	Dominant	0.44	0.006	0.43	0.133
HLA	B*27	Co-dominant	0.01	0.041	0.03	ND
HLA	B*57	Co-dominant	0.06	0.04	0.02	ND
KIR-HLA	SDS1-Bw4-80I	Epistatic	0.08	0.12	0.04	
Susceptible factors						
IL10	5'A	Dominant	0.4	0.24	0.16	0.6
IFNG	179T	Dominant	0.02	0.001	0.02	Not observed
RANTES	In1.IC	Dominant	0.20	0.14	0.06	0.30
HLA	B*35	Dominant	0.07	0.09	0.02	ND
HLA	B*35*Px	Dominant	0.09	0.03	0.06	ND
HLA Class I	1 locus homozygous	Co-dominant	0.16	0.22	0.08	ND
HLA Class II	2/3 loci homozygous	Co-dominant	0.03	0.06	0.03	ND

The CCR5Δ32 allele is found at high frequencies (12%–15%) in northern European populations, decreasing gradually from north to south.¹⁴ Outside Europe, the mutation is found in populations of European descent. On the other hand, higher frequencies of the CCR5Δ32 allele have been reported in 9.6% in the Spanish,¹⁶ up to 15% in the Caucasians in America⁵ and Estonia.¹⁷ In contrast, the CCR5Δ32 allele is virtually absent among native African ethnic group.

Amongst Indians the CCR5Δ32 allele was found to be rare in South Indians (1.3%)¹⁸ and in other Asian population¹⁹ (Table 2). The marginal presence of the CCR5Δ32 allele seen in Asian population could plausibly be due to gene flow from Caucasians population. The uneven distribution of the genotype across geographic and racial groups may be related to some previous epidemic, restricted to Europe that led to a survival advantage among persons homozygous or heterozygous for the CCR5Δ32 genotype and resulted in a concentration of the CCR5Δ32 genotype in persons of European ancestry. The bubonic plague, which claimed the lives of 25–33% of Europeans during the Black Death from 1346 to 1352 and other large epidemics such as small pox have been used as examples as supportive observation.^{20,21}

Studies conducted on Tibeto-Burman speakers and amongst the Han Chinese earlier have established the absence of CCR5Δ32 mutation in these populations and therefore a lack of protection against HIV infection^{4,15} (Table 3).

We failed to detect any CCR5Δ32 polymorphism in our study group both amongst the HSN and the HSP group. The absence of this particular mutation indicates that the HSN group possibly lacks resistance to HIV infection and no delay in progression to AIDS could be anticipated in the HSP group. Furthermore, Manipur and Nagaland are the two high prevalence states while Mizoram too has moderate prevalence rates,¹ the complete absence of CCR5Δ32 polymorphism does appear to be a survival disadvantage to HIV/AIDS.

In view of the absence of this important genetic advantage, preventive methods like condom use, Prevention of mother to child transmission (PMTCT) program etc shall remain the cornerstone towards the control of HIV infection. Though the prevalence rate of HIV in India is declining gradually, mainly

due to the strict implementation of the national programs like National AIDS Control Program (NACP III), newer challenges like resistance to first line antiretroviral drugs are emerging rapidly. There is a need to monitor and identify drug resistance quickly as second line drugs are expensive. Despite intense efforts, vaccine development has been of limited success. Social and rehabilitative efforts need to be strengthened nationally to reduce stigmatization.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. NACO. *Technical report India HIV estimates*. 2012.
2. Centers for Disease Control. *Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. Morbidity and mortality weekly report* 1987.
3. Buchbinder SP, Katz MH, Hessel NA, O'Malley PM, Holmberg SD. Long-term HIV-1 infection without immunologic progression. *AIDS*. 1994;8:1123–1128.
4. Winkler C, Ping AN, Stephen J, Brien O'. Patterns of ethnic diversity among the genes that influence AIDS. *Hum Mol Genet*. 2004;13: Review.
5. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene. Hemophilia growth and development study, multicenter AIDS Cohort study, multicenter Hemophilia Cohort study, San Francisco city Cohort, ALIVE study. *Science*. 1996;273:1856–1862.
6. Samson M, Libert F, Doranz BJ, et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature*. 1996;382:722–725.
7. O'Brien SJ, Nelson GW. Human genes that limit AIDS. *Nat Genet*. 2004;36:565–574.
8. Liu R, Paxton WA, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell*. 1996;86:367–377.

9. Martin MP, Dean M, Smith MW, et al. Genetic acceleration of AIDS progression by a promoter variant of CCR5. *Science*. 1998;282:1907–1911.
10. Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. *Nature*. 1996;381:661–666.
11. Dragic T, Litwin V, Allawa GP, et al. HIV-1 entry into CD4^b cells is mediated by the chemokine receptor CC- CKR-5. *Nature*. 1996;381:667–673.
12. Bradbury J. HIV-1-resistant individuals may lack HIV-1 coreceptor [letter]. *Lancet*. 1996;348:466.
13. Wu L, Paxton WA, Kassam N, et al. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. *J Exp Med*. 1997;185:1681–1691.
14. Martinson JJ, Chapman NH, Rees DC, Liu YT, Clegg JB. Global distribution of the CCR5 gene 32-basepair deletion. *Nat Genet*. 1997;16:100–103.
15. Fusheng W, Jin L, Lei Z, et al. Genotypes and polymorphisms of mutant CCR5-Δ32, CCR2-64I and SDF1-3'A HIV- 1 resistance alleles in indigenous Han Chinese. *Chin Med J*. 2001;114:1162–1166.
16. Alvarez V, López-Larrea C, Coto E. Mutational analysis of the CCR5 and CXCR4 genes (HIV-1 co-receptors) in resistance to HIV-1 infection and AIDS development among intravenous drug users. *Hum Genet*. 1998;102:483–486.
17. Kalev I, Mikelsaar AV, Beckman L, Tasa G, Parlist P. High frequency of the HIV-1 protective CCR5 delta 32 deletion in native Estonians. *Eur J Epidemiol*. 2000;16:1107–1109.
18. Ramana GV, Vasanthi A, Khaja M, et al. Distribution of HIV-1 resistance-conferring polymorphic alleles SDF-1-3'A, CCR2-64I and CCR5-delta32 in diverse populations of Andhra Pradesh, South India. *J Genet*. 2001;80:137–140.
19. Voevodin A, Samilchuk E, Dashti S. Frequencies of SDF-1 chemokine, CCR-5, and CCR-2 chemokine receptor gene alleles conferring resistance to human immunodeficiency virus type 1 and AIDS in Kuwaitis. *J Med Virol*. 1999;58:54–58.
20. Galvani AP, Slatkin M. Evaluating plague and smallpox as historical selective pressures for the CCR5-Delta 32 HIV-resistance allele. *Proc Natl Acad Sci USA*. 2003;100:15276–15279.
21. Lenski RE. Evolution of plague virulence. *Nature*. 1988;334:473–474.
22. Salem AH, Batzer MA. Distribution of the HIV resistance CCR5-delta32 allele among Egyptians and Syrians. *Mutat Res*. 2007;616:175–180.
23. Su B, Guangyan S, Daru , et al. Distribution of three HIV-1 resistance-conferring polymorphisms (SDF1-3'A, CCR2-64I, and CCR5-D32) in global populations. *Eur J Hum Genet*. 2000;8:975–979.
24. Kaur G, Singh P, Rapthap CC, et al. Polymorphism in the CCR5 gene promoter and HIV-1 infection in North Indians. *Hum Immunol*. 2007;68:454–461.
25. Verma R, Gupta RB, Singh K, et al. Distribution of CCR5delta32, CCR2-64I and SDF1-3'A and plasma levels of SDF-1 in HIV-1 seronegative North Indians. *J Clin Virol*. 2007;38:198–203.
26. Suresh P, Wanchu A, Sachdeva RK, Bhatnagar A. Gene polymorphisms in CCR5, CCR2, CX3CR1, SDF-1 and RANTES in exposed but uninfected partners of HIV-1 infected individuals in North India. *J Clin Immunol*. 2006;26:476–484.

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