7. Summary & Conclusions
7.1 Summary

HIV/AIDS represents the deadliest emergency and the greatest social, economic and health crisis of modern times. More than 26 years since the first AIDS case was diagnosed in 1981, nearly 21 million people have died worldwide and nearly 33.2 million people are living with HIV-1 infection globally. The HIV is spreading at an alarming rate killing over 2.1 million people and causing over 6800 new HIV infections daily in 2007. The latest estimates released by the National AIDS Control Organization (NACO), supported by UNAIDS and WHO, indicate that national adult HIV prevalence in India is approximately 0.36%, which corresponds to an estimated 2.5 million people living with HIV in the country.

The natural course of HIV-1 infection shows considerable inter-individual variability, with some individuals progressing to AIDS rapidly following primary infection, known as 'fast progressors' (succumb to AIDS in 1-3 years), while others remain clinically asymptomatic and maintain their normal CD4 counts for more than 10 years with no evidence of immune dysfunction. There is still another group of individuals that are HIV-1 exposed seronegative (HES) who do not get infection even after repeated sexual exposures. This group includes discordant couples who practice unprotected sexual intercourse. The HES groups of individuals are indeed uncommon, but possess 'natural resistance' to infection.

Genetic studies of these groups of people have suggested that there are host genetic variants that could prevent or hinder HIV-1 infection. Moreover, the inter-individual variability observed in terms of HIV-1 susceptibility and disease progression is governed by multiple host genetic factors. These factors are directly involved in HIV-1 cell entry, immune recognition and antigen presentation, and include chemokine receptors and their ligands, intermediary receptors and others.

The present study objectives was to analyze the impact of the genetic variants of CCR5 (CCR5delta32, CCR5 59029 A/G), CCL3L1, RANTES (RANTES-403 A/G, RANTES -28 C/G, RANTES In1.1 T/C), DC-SIGN, DC-SIGNR and APOBEC3G genes on HIV-1 susceptibility, resistance and disease progression. Association of genetic variants was analyzed with the central question which genetic variants are responsible for the HIV-1 susceptibility, resistance and development of HIV/AIDS disease. Gene-gene interactions were evaluated to
determine the role of combined action of two alleles together in conferring HIV-1 resistance/susceptibility and HIV/AIDS progression.

For the present study, a total of 196 patients with HIV-1 infection (HSP), 47 HIV-1 exposed seronegative (HES) and 315 age gender matched HIV-1 seronegative (HSN) healthy controls were enrolled after considering the inclusion and exclusion criteria. Information regarding demography and risk exposures was taken through personnel interview. Blood samples were collected from all the study subjects and genomic DNA was extracted for genotyping of candidate gene variants using PCR, RFLP and Real-Time PCR (Taqman) assay methods. The statistical analysis of genotyped data was done through chi-square test. A relative comparison between HSP subjects and HSN healthy controls was carried out to assess their affect on risk of HIV-1 acquisition while, HES individuals were compared with HSP subjects to assess their affect on resistance to HIV-1 infection. To assess the impact of genetic variants on development of AIDS, analysis was done among HIV/AIDS patients (HSP) stratified on the basis of disease severity (Stage I, II and III).

A brief study design is given below:

![Study Design Diagram](image-url)
The findings of present study are summarized below:

1. The frequency of CCR5 delta 32 mutation was very low in the studied population and was absent among HES individuals. Therefore we were not able to evaluate protection associated with this mutation. Alternative mechanism might be operating among HES individuals in conferring resistance to HIV-1 infection.

2. Genotype frequency of CCR5-59029 AG genotype was higher in HES (57.44%; P=0.014) as compared to HSP (37.24%) and imparted partial resistance for acquisition of HIV-1 infection (O.R.=0.440, 95% C.I.=0.230-0.839). None of the CCR5 59029 genotypes were associated with disease progression.

3. Copy number of CCL3L1 gene was not associated with HIV-1 susceptibility/resistance or with development of HIV/AIDS. Similarly, lower or higher than median CCL3L1 copy number was also not associated with HIV-1 susceptibility/resistance and disease progression.

4. Gene-gene interaction study of CCR5 59029 A/G and CCL3L1 copy number polymorphism showed the higher frequency of CCR5 59029AG*CCL3L1>2 genotype among HES (31.91%) when compared with HSP group (15.81%; P=0.021, O.R. =0.401, C.I. =0.194-0.826). This observation suggested the combined effect of CCR5*CCL3L1 in providing partial resistance to HIV-1 infection among HES individuals. Furthermore, CCR5*CCL3L1 genotypes were not associated with disease progression.

5. None of the RANTES -403 genotypes or alleles were associated with HIV-1 susceptibility/resistance. However, the frequency of RANTES -403 AA genotype was higher in stage III (8.16%) as compared to stage I (1.16%; OR=7.556; CI=0.820-69.629) and was modestly associated with higher risk of disease progression (P=0.058).

6. Genotypes of RANTES -28 were not found to be associated with HIV-1 susceptibility and course of disease progression.

7. Genotype frequency of RANTES In1.1 TT was higher in HSP (84.69%) as compared to HSN (73.01%; OR=2.045, P=0.002) and HES (59.5 %; OR=3.755; P=<0.001) and higher risk for HIV-1 infection was observed. The frequency of RANTES In1.1 TC genotype was significantly lower in HSP (13.26%) as compared to HSN (26.34%; OR=0.427; P=<0.001) and HES
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(38.20%: OR=0.246; P=<0.001), and imparted reduced risk for HIV-1 infection. RANTES Inl.1 TT genotype frequency was lower in stage III (73.46%) as compared to stage I (88.37%; OR=0.364; P=0.033) and stage II (88.52%; OR=0.359; P=0.050) and reduced risk for HIV-1 infection was observed, however it was marginally significant. The frequency of RANTES Inl.1 TC genotype was higher in stage III (24.48%) as compared to stage I (10.46%; OR=2.775; P=0.047) and stage II (8.19%; OR=3.632; P=0.032) and imparted higher risk for HIV-1 infection which was also marginally significant.

8. Haplotype II (RANTES -403/-28/Inl.1; ACT) was found at significantly higher frequency in HSP (9.69%) compared with HSN (1.58%; O.R. =6.655; P=<0.001) and risk for HIV-1 infection due to haplotype II (ACT) was six fold in patients. None of the haplotypes were found to be associated with HIV-1 disease progression.

9. Genetic polymorphism in the DC-SIGN neck region was very low and was not associated either reduced or enhanced susceptibility to HIV-1 infection or disease progression.

10. The frequency of DC-SIGNR 5/5 homozygous genotype was significantly lower in HSP (3.57%) as compared to HSN (8.25%; OR=0.412; P=0.041) and HES (12.7%; OR=0.253; P=0.022) and imparted reduced risk of HIV-1 infection. None of the other genotypes and alleles was significantly associated with risk of HIV-1 disease progression.

11. Genotyping of APOBEC3G (H186R) revealed that the codon-changing variant APOBEC3G H186R was absent among North Indians.

7.2 Conclusions

In conclusion, this is the first study from India which explored the influence of CCR5 delta 32, CCR5 promoter 59029 G/A, CCL3L1 copy number, RANTES promoter -403G/A and 28C/G, RANTES Inl.1T/C, DC-SIGN/R neck repeat and APOBEC3G H186R genotypes on HIV-1/AIDS susceptibility and disease progression. The present study suggest that –

1. Low frequency of CCR5 delta 32 allele observed in our study may be related to a higher genetic susceptibility to the HIV-1 infection in North Indians.
2. CCR5 59029 AG genotype alone and in combination with CCL3L1 median copy number greater than 2, and DC-SIGNR 5/5 homozygous genotype may be associated in imparting partial resistance to HIV-1 infection among HIV-1 exposed seronegative North Indians.

3. RANTES -403 and RANTES ln1.1 genotypes were found to modulate HIV-1 susceptibility and disease progression while, CCL3L1 copy number, RANTES -28 C/G, DC-SIGN neck repeat and APOBEC3G H186R polymorphisms do not influence HIV-1/AIDS susceptibility/resistance or disease progression.

4. The baseline data emerging from this study would be useful in assessing vulnerability of our population to HIV/AIDS epidemic and in designing therapeutic strategies to combat HIV infection.