



REVIEW ARTICLE

Genetic variation in the chemokine receptor 5 gene and course of HIV infection; review on genetics and immunological aspect

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Abstract Chemokines are small protein molecules associated with various physiological events precisely in immune modulation via chemokine receptors. The chemokine receptors are G-protein coupled receptors express mainly on the cell surface of immune cells. Retroviruses, including HIV in the early stage of infection, primarily target chemokines receptors and get internalized easily into immune cells; T cell and escape from immune surveillance. HIV glycoprotein selectively develops an affinity for the extracellular domain of chemokines receptors and allows the pathogen to internalize via CCR-5. Now, CCR-5 remains a crucial signaling pathway that can be translated into the therapeutic target by changing the receptor protein environment. Many populations have a mutation in coding and promoter regions of CCR-5, tuning a resistance for HIV infection. Natively, there are several mechanisms where the human genome remains in the dynamic state by changing its composition and acquiring variations. Single nucleotide polymorphism is spontaneous phenomenon responsible for precise and point mutation at the genome. Several studies have demonstrated that European and African American populations are enriched in significant CCR5 promoter SNP (CCR5Δ32) in the coding and promoter region as well. Now, such SNP can be an early-stage biomarker in studying HIV and other similar infections. Here, in this study, we have elucidated the role of SNP (both the promoter and coding region) and the fate of HIV infections. We also empathized with the genetics of such SNPs, mostly frequency and its immunological impact.

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Introduction

Over the last 40 years since the discovery of HIV and associated disease, i.e., AIDS in the 1980s, the death rate remains high in developing countries, and more than 32 million people died worldwide. Among retroviruses, human immune deficiency virus (HIV) remains a major global health issue and infects millions of people worldwide annually.¹ HIV selectively target immune cells; T cells (CD4 T helper cell) and reside there for a long time; as a result, innate immune surveillance mechanism fail to identify infection and pathogen entry.² The unique ability of HIV to bypass immune surveillance entirely relies on glycoprotein mimic ligand for CCR-5 (early stage) and CXCR in advance stage.^{3,4} In the advanced stage, HIV starts copying itself using reverse transcription as it comprises of RNA as genetic material. HIV-infected CD-4 T cell undergoes a rapid cell death via programmed cell death mechanism and at the same time decreased the production of immune cells further weakens immunity.⁵ However, HIV loaded CD 4 T cell programmed cell death mechanism remains unclear. Still, several reports have demonstrated that such cells are much prone to programmed cell death via an indirect mechanism, i.e., the formation of syncytia and apoptosis subsequently.^{6,7} Hence, the gradual decrease in CD4 T cells in untreated HIV infection patients affects both adaptive and acquired immunity leading to severe malignancy called AIDs.⁸ In a chronic condition, patients become prone to other infections, including bacterial and fungi, as a pathological outcome from decreased CMI.⁹

HIV is a retrovirus, and its genome is comprised of RNA. Retroviruses also contain an enzyme called reverse transcriptase, which allows the retrovirus's genetic material to imitate the host cell's genetic material.^{10,11} Human immunodeficiency virus (HIV) grouped to the genus lentivirus within the family of Retroviridae, subfamily Orthoretrovirinae. Epidemiologic and phylogenetic analyses currently available imply that HIV was introduced into the human population around 1920 to 1940.^{12,13} HIV-1 evolved from nonhuman primate immunodeficiency viruses from Central African chimpanzees (SIVcpz) and HIV-2 from West African sooty mangabeys.¹⁴ The HIV genome is nearly 9 kb (9.2 kb in HIV-1 and 9.6 kb in HIV-2) with significant genes within the genome of HIV lies in between LTR (3' and 5') are polymerase (RT), gag (group-specific antigens) and env (genes coding for envelope).¹⁵ Further, HIV also possesses *tat* (trans-activation of transcription), *rev* (regulator of virion), *nef* (negative factor), *vpr* (viral protein r), *vif* (viral infectivity factor), and *vpu* (viral protein u).¹⁶ The HIV genes are highly specific and robust, encode several proteins essential for viral replication and infection. The pol gene encodes three proteins; protease, RT, and integrase, while gag is responsible for condign all the protein for capsid.¹⁷ Additionally, HIV acquired extra protection by encoding protective lipids by env.

Chemokines receptors and physiological significance

Chemokines are small protein molecules with immense physiological significance precisely in immunomodulatory

activities.¹⁸ Chemokine receptors are G-protein coupled receptor containing seven transmembrane domains. These seven domains span plasma membrane where C-terminal is attached to G protein (cytosolic), and the N-terminal region lies in the extracellular region.¹⁹ The chemokines receptors are primarily express over the cell surface of macrophages, dendritic cells, and memory T cells. However, these receptors are also typical in the endothelium, epithelium, vascular smooth muscle and fibroblasts, and microglia, neurons, and astrocytes in the central nervous system.²⁰ Here, the unique pattern of Asp-Arg-Tyr (DRY motif) plays critical roles in the determination of receptor conformations for signaling and intracellular trafficking of GPCR by forming intermolecular interactions.²¹ The C-terminal domain of the Chemokine receptor contains serine and threonine acts as a potential site for phosphorylation during receptor regulation.²² Further, the N-terminal domain acts as a ligand-binding site to target by many pathogens, including retroviruses such as HIV. Its cysteine defines chemokines and classified as C, CC, CXC, and CX3C, while chemokines receptors are denoted with additional R with a chemokine.²³

G Protein-Coupled Receptor (GPCR) represents one of the most crucial signal transduction receptor families associated with many physiological processes. Chemokines receptor is one of a group of GPCR family, i.e., rhodopsin-like GPCR.²⁴ Chemokines receptors are further subclassified, including CCR1, CXCR1, CCR5, XCR1, etc. Over the last few decades, nearly 20 chemokines receptors have been identified and characterized for their involvement in different physiological signaling pathways.²⁵ If we examine proteomics of chemokines, it's a single peptide of 352 amino acids with a molecular weight of 40.6 kDa.²⁶ Considering chemokines receptor signaling, it purely depends on G proteins in the cytosolic region. A few retroviruses, including HIV, selectively utilized the unique pattern of CCR-5 for its internalization into T cells.²⁷ The HIV two glycoprotein receptors, gp120 and gp41 (designated gp160), possess a high affinity for CCR-5 and allow pathogen binding and internalization^{28,29} (Fig. 1).

Chemokine receptor-mediated signaling

HIV finds an opportunity to get internalize via chemokines receptor (CCR 5 in early-stage and CXCR4 in advance stage) in conjugation with CD4.³⁰ In the course of the first infection, the human macrophage expressing CCR5 and CXCR4 are primarily targeted by HIV. The primary interaction between viral envelope surface glycoprotein gp120, the cell-surface protein CD4, and a chemokines receptor CCR5 or CXCR4 start the complex process involved in the internalization of the pathogen in the cell.³¹ It has been demonstrated that HIV-1 gp120 and chemokines activate ion channels in primary macrophages through CCR5 and CXCR4 stimulation.³² The HIV gp120, a dynamic envelope protein utilizes chemokines receptors as co-receptor for pathogen binding, and several conformational changes occur during the interaction.³³ The gp120 and CD4 interaction lead to conformational changes in the viral envelope and facilitate membrane fusion.³⁴ Further, gp120 binds to integrin $\alpha_4\beta_7$ activating LFA-1, the central integrin involved in the

establishment of virological synapses, which facilitate efficient cell-to-cell spreading of HIV-1.^{35,36} A detailed mechanism is shown in Fig. 2.

Single nucleotide polymorphism

The human genome is highly dynamic, and genetic variations are driving force for molecular evolution.³⁷ The human genome comprised of several modifying events, and single nucleotide polymorphism remains one major spontaneous phenomenon among.³⁸ The SNPs are changes in a single nucleotide in the genome that happen spontaneously, leading to a change in ORF and amino acid arrangement in protein. The site and frequency of SNPs can vary among different populations. However, in general, one in 1000 bp remains a widespread pattern of SNP in each population. This is most important to note here that the SNP site, either in the coding region and or promoter region directly associated with gene regulations.³⁹ As a result, SNPs associated changes among a given populations ranges from normal phenotypes to developmental processes. It is also necessary to understand the type of SNPs before making concluding their significance. The SNPs are classified into four categories based on site of occurrence; Random SNPs (*rSNPs*), gene-associated SNPs; (*gSNPs*), coding SNPs: (*cSNPs*) and phenotype-relevant SNPs (*pSNPs*).⁴⁰ A small percentage of the human genome is functional (coding region) and undergoes for replication and transcription.

The majority of SNPs are, therefore, located within the genome as silent regions of the human genome.⁴¹ SNPs in introns or other non-coding sequences such as those for microRNAs may affect gene expression.

From the beginning, these variations have attracted researchers worldwide to translate polymorphism as a diagnostic tool.⁴² There are several SNPs are used as markers in the mapping of genes within the genome. Many SNPs are situated alongside genes or in introns; the regions of a gene that do not code for a gene product, i.e., do not form part of the template for a protein.⁴³ The fact that they are inherited with these genes makes gSNPs useful for the study of associations between the gene (and its variants) and specific phenotypes. Mapping gSNPs may be functionally relevant if they influence essential control elements of the gene and thereby decrease or increase the transcription of a gene.⁴⁴ Exons are the coding regions of a gene, i.e., the sequences of a gene that are translated into the gene products i.e., protein. SNPs that are present in exons can have a significant influence on the function of the protein concerned if they result in the incorporation of alternative amino acid. Both gSNPs and cSNPs can influence a person's phenotype: the former primarily via the amount, and the latter usually via the form of the protein for which the gene codes.⁴⁵ Phenotype-relevant SNPs are the essential type of SNP from medicine. Wang et al, in 2012 profiled pSNPs associated with Alzheimer's disease.⁴⁶ In their study, using a computational approach Wang et al, have proposed a useful method/tool for a precise finding of phenotype-

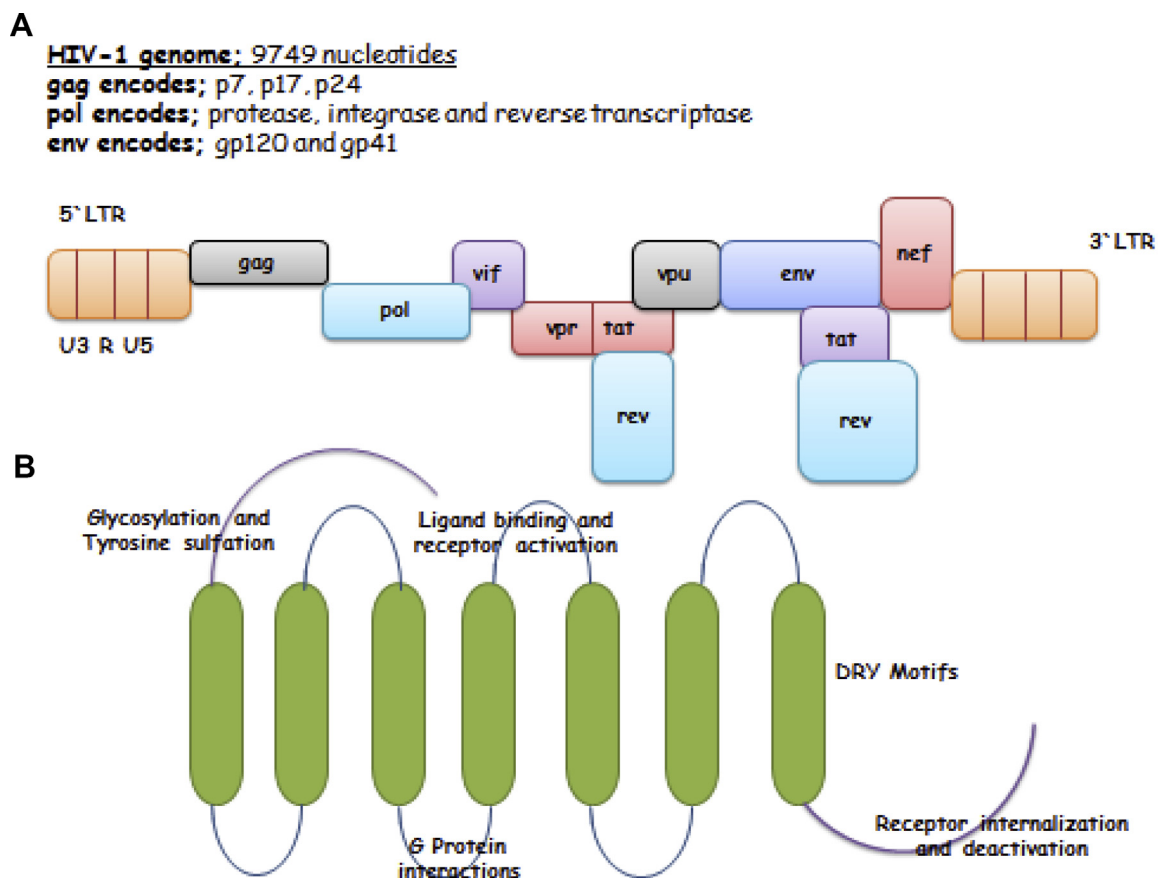


Figure 1 (A) HIV genome (HIV-1); figure schematically represents the position of various genes associated with HIV replication and infection. (B) A typical cartoon depicts chemokines co-receptor 5 involve in HIV infection.

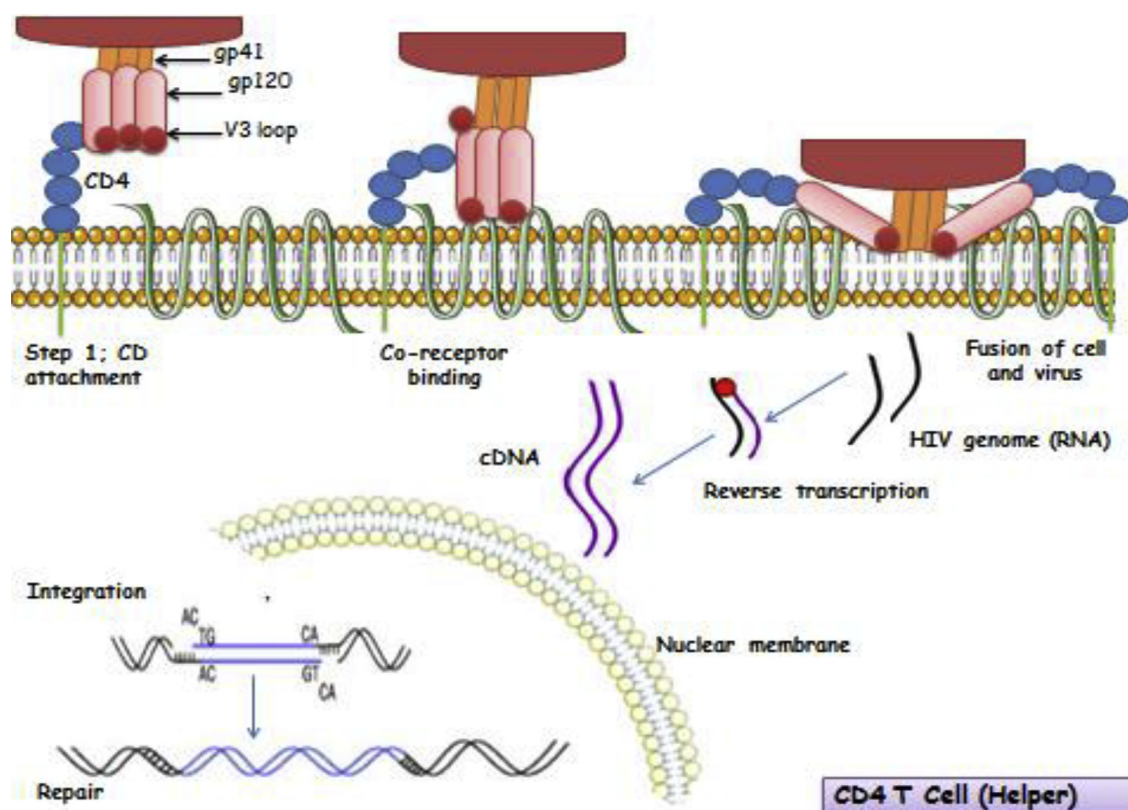


Figure 2 The figure demonstrates the detailed mechanism and the role of chemokine signaling in HIV infection. Here, a chemokine receptor that acts of co-receptor for binding of HIV envelope using gp120 and V3 loop mainly stabilizes interaction. While the CD4 molecule act as primarily attachment molecule present on the cell surface of T cells (T helper cells).

specific single nucleotide polymorphism (<http://ranger.uta.edu/~heng/Longitudinal/>). Likewise, several online tools and software are available to identify polymorphism in the genome and their link for particular diseases (<https://omictools.com/statistical-analysis-category>).

Significance of SNPs

The SNP is a key biomarker in the early diagnosis of various diseases and metabolic disorders. As reported in the study, SNP leads to change in disease outcome as rs333 (Decreased susceptibility), rs1799987 (Fast progression), rs1800023 (Slow progression), rs1800024 (Slow progression), rs2734648 (Slow progression) and rs1799988 (Slow progression).⁴⁷ Many SNPs have also been shown to affect the development and predisposition of disease.⁴⁸ The site of SNP remains crucial; one if SNPs in the coding region alters mRNA transcription and amino acid in translating protein, which could be a marker for identifying a disease in the early stage.⁴⁹ Second and most important, SNP in the promoter region has a significantly larger impact on protein expression, either up-regulation and or down-regulation.⁵⁰ The SNPs which are associated with diseases precisely are key markers brought to pharmacogenomics studies for therapeutic interventions.⁵¹ The functional genomics largely utilizes SNPs as a tool for the study of drug response (pharmacodynamics studies). There is a preponderance of data suggesting the role of SNPs in cancer (all major cancer

and tumor including breast, colon, colorectal, myeloma, thyroid and lung etc.), vascular disorders, immunological disorders and metabolic disorders.^{52–54} Considering intensive research findings in the last decade, SNPs have a direct impact on the pathophysiology of various infectious diseases, including hepatitis, leprosy, tuberculosis, AIDs, malaria, and meningitis etc.^{55–57} As a result, the SNPs database has been built after complete genome analysis demonstrate the role of SNPs in the onset of disease and metabolic disorders in human.⁵⁸

SNPs in chemokine gene

Within human genome, two significant clusters of chemokine receptor genes were identified on chromosome 3 encoding all major chemokine receptor proteins including CCR 2, CCR 3, CCR 4, CCR 5, CCR 7, CCR 8, CCR 10, CCR 11, CCR 12, CCR 20 and CCR 21.⁵⁹ Among these genes, CCR 5 reported as a significant co-receptor in HIV infection. The CCR 5 gene contains four exon and two introns with two promoters; one-week promoter (upstream) and one strong promoter (downstream).⁶⁰ The most phenomenon genetic variation in CCR5 gene; polymorphism at CCR5 promoter region as Delta 32 ($\Delta 32$) mutation was reported (rs333) (CCR5 $\Delta 32$) protect the individual group.^{61,62} In both cases, either SNP lies at the promoter and or at the coding region of the CCR5 gene, the expression of the receptor protein (CCR5) will be altered and or inhibited completely.⁶³ The

altered protein (CCR5) will not be active ultimately and simultaneously, affect (minimize) HIV infections.⁶⁴ Looking more precisely, SNPs in the coding region (A77G, G316A, T532C, C921T, and G668A) (mutation) will primarily change protein structure (due to the incorporation of wrong amino acid) that further affects localization, transport, chemokines binding and signaling of the CCR5 receptor.^{65,66} However, if polymorphism will be reported in the promoter region, it primarily affects binding DNA transcription factors and aberration in mRNA formation.⁶⁷ In both cases, binding of HIV gp120 to the host cell will be altered based on the site of polymorphism.

The CCR 5 single nucleotide polymorphism remains high throughout research concern worldwide parallel with HIV infections.⁵⁹ Over a period of time, several racial and ethnic populations were profiled for CCR5 polymorphism. These studies include polymorphism in the coding and promoter region of CCR5.⁶⁸ Gonzalez, E *et al*, 2001 have conducted a global survey for genetic variations in CCR5 and possible ligands, more precisely RANTES, and MIP-1alpha. Their finding suggested a different distribution of RANTES haplotypes (AC, GC, and AG).⁶⁹ They have identified an association of RANTES haplotype in the selected population (European Americans) with an increased risk of acquiring HIV-1 as well as accelerated disease progression in European Americans. However, African Americans were reported in acquiring resistance to HIV-1 infection due to a particular AC haplotype. Further, the Japanese cohort, with an AC haplotype, has shown a distinct phenotype associated with delayed HIV-1 infection. Similarly, Mikawa *et al*, 2002 studied Brazilian populations for CCR5 polymorphism and plasma β Chemokines in HIV-1 infection.⁷⁰ Their finding differs from Gonzalez *et al*, in correlating a link between plasma β Chemokines (RANTES, and MIP-1alpha). Such variations could be due to multifactorial HIV infections and dependence on different racial and ethnic populations.

India is the third-largest home for HIV-1 patients and has significant HIV growth within the Indian population. The increased HIV-1 infection and subsequent AIDS linked genetics as driving force.⁷¹ In a study, Gupta and Harish (2015) profiled CCR5 and SDF1 genetic variations in the Indian population and analyzed links in HIV-1 infections.⁷² In the study, Gupta and Harish targeted SDF1-3'A, CCR5 Δ 32, and CCR5 promoter polymorphism at selected positions, including 58934 G/T, 59029 G/A, 59353 T/C, 59356 C/T, 59402 A/G and 59653 C/T within Indian population. They conclude Indian people probably more susceptible to HIV-1 infection as the most common CCR Δ 32 mutation was utterly absent. Further, nine haplotypes with more than 1% frequency identified were not significant in their protective role against HIV-1.⁷³ However, within Indian populations, southern India has a comparative larger impact of HIV-1 infection. Four states, including Tamil Nadu, Andhra Pradesh, Telangana, and Karnataka, collectively account for half (50%) cases of HIV -1 infection in India.⁷⁴ There are limited studies in the Indian population precisely looking haplotype screening of CCR5 and role in the onset and progression of HIV-1 infection. The similar patterns of CCR5 polymorphism were reported in native African people.

Joshi *et al*, 2017 studied CCR 5 promoter activity in different haplotypes under polymorphism, considering CD 4 T cell apoptotic activity in HIV infected patients.⁷⁵ The

study concludes a relative CCR 5 promoter activity (CRPA) in HIV patients regulates CD4 cell loss. Gonzalez, E. *et al*, 1999 has identified 8 CCR5 promoter haplotypes as a result of single nucleotide polymorphisms in CCR 5 promoter region.⁷⁶ They have estimated CCR5 haplogroup frequencies in different racial and ethnic groups, including African, African American, Asian Caucasian, and Hispanic Americans considering HIV infected and uninfected patients. The major CCR5 haplotypes reported are HHA, HHC, HHD, HHE, HHF1, HHF 2, HHG1, and HHG2. Now, the study demonstrates all these haplotypes varies in different race and ethnic populations towards HIV infections.⁷⁷ In general, haplogroup C (HHC) and haplogroup E (HHE) were identified comparatively higher in HIV infected patients in all the races, and ethnic populations studied. These findings further support Joshi *et al*, work concludes different CCR5 promoter transcriptional activity in different haplotypes.

CCR5 Δ 32 mutations and HIV resistance

CCR5 delta 32 SNPs and association with the HIV susceptibility remain a key area of research and scientific debates. Grown scientific findings and conclusive outcomes suggest SNPs/mutations CCR5 delta 32 differ in the human ethnic population. In a meta-analysis, Ni *et al* 2018 demonstrated that CCR5 delta 32 homozygous offer resistance against HIV infection over heterozygous genetic orientation.⁷⁸ The CCR5 delta 32 mutations vary in different human populations. Novembre *et al* 2005 studied patterns and frequency of CCR5 delta 32 HIV resistance alleles in different populations.⁷⁹ The study also concludes such mutations are more common in Europe and western Asia. Homozygous carriers of the Delta32 mutations remain associated with higher resistance against HIV infections with lower expression of CCR5 chemokine receptors. The expression of CCR5 chemokine receptor and SNP/mutations in CCR5 Delta 32 are closely associated. It is also important to note here mutations are random, and selection of such mutations among various human populations defines resistance against HIV. Adojaan *et al* 2007 investigated the higher frequency of CCR5Delta32 HIV-resistance mutations in the northern European population.⁸⁰ Targeting CCR5 chemokine receptors has shown a promising result in HIV infection management. Hutter *et al*, 2015 have demonstrated that allogeneic transplantation of CCR5-delta 32 homozygous stem cells in HIV patients offers a practical and robust approach to control HIV infection and disease burden.⁸¹ This could be achieved via either using CCR5 negative stem cells and or CCR5 knockout cells. However, such studies require validation in larger populations of different human ethnic groups. The second approach altering CCR5 availability for HIV gp120 protein attachment via inhibitors and or antibodies might not be feasible as CCR5 associated with several vital cellular processes.⁸²

Genetics and immunological attributes of polymorphism in CCR5

The CCR5 Δ 32 allele containing a homozygous individual completely lacks a functional CCR5 receptor and hence

provides resistance to HIV infection.⁸³ On the country, heterozygous individuals will have a moderate activity of HIV infection as lower expression and activity of CCR 5. The Delta 32 variant of rs333 is also associated with a higher risk of abdominal aortic aneurysm (AAA).⁸⁴ The population genetic studies have demonstrated that CCR5Δ32 is a universal and most predominant genetic variation in different population groups acquire and develop resistance towards HIV infection. The encoded CCR 5 protein (functional CCR5) N-terminal region is enriching in tyrosine and acidic amino acids that play an essential role in both HIV interaction and chemokines binding.⁸⁵ A polymorphism in the CCR5 coding region will have a more substantial impact not only in binding with HIV HIV-1 gp120 but also natural ligand (MIP-1α, RANTES).⁸⁶ Now, under polymorphism at CCR5 will be unable to bind its ligands and disable several critical immunological events such as recruitment of leukocytes into inflammatory sites.⁸⁷ The patients containing such phenotype will be unable to activate granulocytes (neutrophils, eosinophils, and basophils).⁸⁸ Such phenotype will also be unable to synthesis and release of other pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α from fibroblasts and macrophages.⁸⁹

The diagnostic and therapeutic relevance

Genomic instability has been characterized as a critical trigger toward the onset and progression of several human diseases and metabolic disorders.⁹⁰ Further, it is the signature patterns of a sequence such as alterations, frequency, site (regulatory, coding, and or noncoding region), etc. resulting in an allelic imbalance. Several reports have demonstrated how useful SNPs are in correlating with a particular phenotype. Erichsen and Chanock, 2002 confirmed risk assessment in breast cancer based on SNPs analysis, and subsequent meta-analysis further validated their findings.⁹¹ The presence of CCR5 promoter haplotype can be used as the first biomarker for susceptibility and resistance to HIV-1 infection. However, finding a therapeutic relevance of CCR5 promoter polymorphism may need a large scale population study to ensure the exact pattern of haplotype and common conserved haplotypes.⁵⁹ There are growing pieces of evidence that the use of natural CCR5 ligand mimics could find an alternate approach provided immunological functions remain unaffected. Such competitive natural CCR5 ligand inhibitors effectively compete with HIVgp120 for receptors with the least immune damage.

Conclusions

Chemokines signal are vital for many physiological events, including immune and inflammatory responses, wound healing, and angiogenesis. The chemokine receptors with appropriate ligand play a crucial role in the activation and mobilization of immune cells. HIV pathogenesis is multifactorial, involving both CD4 and CCR5 as a coreceptor for the infection. The single nucleotide polymorphism results in the aberrations in CCR 5 receptor protein disable

binding of gp120 and hence the entry of the virus into the host cell. It's noteworthy to understand the effective inhibition of HIV entry relies on the site single nucleotide polymorphism. Several approaches have been made over some time to utilize such genomic stability (polymorphism) as a potential target against HIV infections. It is evident considering extensive studies on population genetics that the rise in CCR5Δ32 mutations at the promoter region of CCR 5 gene offers resistance against HIV infection. It is evident that CCR5Δ32 mutations differ in the human population, and the selection of such mutations can lead to an HIV infection resistant population. On the contrary, these studies required validation on a large scale to find a close relation between CCR5Δ32 mutations and resistance to HIV infection. Research finding has demonstrated CCR5 knockdown CCR5 stem cells could be a possible cure for HIV. There is growing concern and emphasis on developing CCR5 deficient stem cells and clinical uses.

Conflict of Interests

The author declares no conflict of interest.

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Abbreviations

AIDs	Acquired Immunodeficiency Syndrome
HIV	Acquired immune deficiency viruses
CCR	Chemokines receptor
G-PCRs	G-protein coupled receptor
gp	Glycoprotein
SNPs	Single nucleotide polymorphism
CMI	Cell-mediated Immunity
HI	Humoral Immunity
RT	Reverse transcriptase
CD	Cluster of differentiation
CRPA	CCR5 Relative Promoter Activity
RANTES	Macrophage inflammatory protein (MIP)-1α (MIP) and Regulated on Activation Normal T Cell Expressed and Secreted
rSNPs	Random SNPs
gSNPs	gene-associated SNPs
coding SNPs	(cSNPs) and phenotype-relevant SNPs (pSNPs)

References

1. Fetti J, Swaminathan M, Murrill CS, Kaplan JE. Global epidemiology of HIV. *Infect Dis Clin*. 2014;28(3):323–337.
2. Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet*. 2014;384(9939):258–271.

3. Quagliarello V. The acquired immunodeficiency Syndrome: current status. *Yale J Biol Med.* 1982;55(5-6):443–452.
4. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature.* 1995;373(6510):123–126.
5. Lackner AA, Lederman MM, Rodriguez B. HIV pathogenesis: the host. *Cold Spring Harbor Persp Med.* 2012;2, a007005.
6. Picker LJ, Hagen SL, Lum R, et al. Insufficient production and tissue delivery of CD4+ memory T cells in rapidly progressive simian immunodeficiency virus infection. *J Exp Med.* 2004;200(10):1299–1314.
7. Gougeon ML, Laurent-Crawford AG, Hovanessian AG, Montagnier L. Direct and indirect mechanisms mediating apoptosis during HIV infection: contribution to in vivo CD4 T cell depletion. *Semin Immunol.* 1993;5(3):187–194.
8. Doms RW. Chemokine receptors and HIV entry. *AIDS.* 2001;15(Suppl 1):S34–S35.
9. Veazey RS, Mansfield KG, Tham IC, et al. Dynamics of CCR5 expression by CD4 (+) T cells in lymphoid tissues during simian immunodeficiency virus infection. *J Virol.* 2000;74(23):11001–11007.
10. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science.* 1997;278(5341):1295–1300.
11. Koppensteiner H, Brack-Werner R, Schindler M. Macrophages and their relevance in human immunodeficiency virus type I infection. *Retrovirology.* 2012;9, e82.
12. Vergis EN, Mellors JW. Natural history of HIV-1 infection. *Infect Dis Clin.* 2000;14(4):809–825.
13. Paul M. Sharp and Beatrice H. Hahn, origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med.* 2011;1(1), a006841.
14. Sharp Paul M, Hahn Beatrice H. The evolution of HIV-1 and the origin of AIDS. *Philos Trans R Soc Lond B Biol Sci.* 2010;365(1552):2487–2494.
15. Watts Joseph M, Dang Kristen K, Gorelick Robert J, et al. Weeks architecture and secondary structure of an entire HIV-1 RNA genome. *Nature.* 2009;460(7256):711–716.
16. Al-Hashimi HM. Structural biology: aerial view of the HIV genome. *Nature.* 2009;460(7256):696–698.
17. Watts JM, Dang KK, Gorelick RJ, et al. Architecture and secondary structure of an entire HIV-1 RNA genome. *Nature.* 2009;460(7256):711–716.
18. McDermott DH, Zimmerman PA, Guignard F, Kleeberger CA, Leitman SF, Murphy PM. CCR5 promoter polymorphism and HIV-1 disease progression. Multicenter AIDS Cohort Study (MACS). *Lancet.* 1998;352(9131):866–870.
19. David T. Lodowski and Krzysztof Palczewski, chemokine receptors and other GPCRs. *Curr Opin HIV AIDS.* 2009 Mar;4(2):88–95.
20. Park PS, Lodowski DT, Palczewski K. Activation of G protein-coupled receptors: beyond two-state models and tertiary conformational changes. *Annu Rev Pharmacol Toxicol.* 2008;48:107–141.
21. Vaidehi N, Bhattacharya S, Larsen AB. Structure and dynamics of G-protein coupled receptors. *Adv Exp Med Biol.* 2014;796:37–54.
22. Allen SJ, Crown SE, Handel TM. Chemokine: receptor structure, interactions, and antagonism. *Annu Rev Immunol.* 2007;25:787–820.
23. Higueta Edwin A, Jaimes Fabián A, Rugeles Maria T, Montoya Carlos J. In vivo effect of statins on the expression of the HIV co-receptors CCR5 and CXCR4. *AIDS Res Ther.* 2013;10, e10.
24. Worth CL, Kleinau G, Krause G. Comparative sequence and structural analyses of G-protein-coupled receptor crystal structures and implications for molecular models. *PLoS One.* 2009;4(9), e7011.
25. Ray N, Doms RW. HIV-1 co-receptors and their inhibitors. *Curr Top Microbiol Immunol.* 2006;303:97–120.
26. Alkhatib G, Berger EA. HIV co receptors: from discovery and designation to new paradigms and promise. *Eur J Med Res.* 2007;12(9):375–384.
27. Eckert DM, Kim PS. Mechanisms of viral membrane fusion and its inhibition. *Annu Rev Biochem.* 2001;70:777–810.
28. Gallo SA, Finnegan CM, Viard M, et al. The HIV Env-mediated fusion reaction. *Biochim Biophys Acta.* 2003;1614(1):36–50.
29. Alkhatib G. The biology of CCR5 and CXCR4. *Curr Opin HIV AIDS.* 2009;4(2):96–103.
30. Corbeau P, Reynes J. CCR5 antagonism in HIV infection: ways, effects, and side effects. *AIDS.* 2009;23:1931–1943.
31. Mueller A, Kelly E, Strange PG. Pathways for internalization and recycling of the chemokine receptor CCR5. *Blood.* 2002;99:785–791.
32. Achour L, Scott MG, Shirvani H, et al. CD4-CCR5 interaction in intracellular compartments contributes to receptor expression at the cell surface. *Blood.* 2009;113(9):1938–1947.
33. Issafras H, Angers S, Bulenger S, et al. Constitutive agonist-independent CCR5 oligomerization and antibody-mediated clustering occurring at physiological levels of receptors. *J Biol Chem.* 2002;277(38):34666–34673.
34. Benkirane M, Jin DY, Chun RF, Koup RA, Jeang KT. Mechanism of transdominant inhibition of CCR5-mediated HIV-1 infection by ccr5delta32. *J Biol Chem.* 1997;272(49):30603–30606.
35. Quillent C, Oberlin E, Braun J, et al. HIV-1-resistance phenotype conferred by combination of two separate inherited mutations of CCR5 gene. *Lancet.* 1998;351(9095):14–18.
36. Lehner T. The role of CCR5 chemokine ligands and antibodies to CCR5 co-receptors in preventing HIV infection. *Trends Immunol.* 2002;23(7):347–351.
37. Pikor L, Thu K, Vucic E, Lam W. The detection and implication of genome instability in cancer. *Canc Metastasis Rev.* 2013;32(3–4):341–352.
38. Soerensen M. Genetic variation and human longevity. *Dan Med J.* 2012;59(5), B4454.
39. Zhao Z, Fu YX, Hewett-Emmett D, Boerwinkle E. Investigating single nucleotide polymorphism (SNP) density in the human genome and its implications for molecular evolution. *Gene.* 2003;312:207–213.
40. Miller RD, Phillips MS, Jo I, et al. High-density single-nucleotide polymorphism maps of the human genome. *Genomics.* 2005;86(2):117–126.
41. Kim KS, Kim GS, Hwang JY, et al. Single nucleotide polymorphisms in bone turnover-related genes in Koreans: ethnic differences in linkage disequilibrium and haplotype. *BMC Med Genet.* 2007;8, e70.
42. Strauss KA, Puffenberger EG, Craig DW, et al. Genome-wide SNP arrays as a diagnostic tool: clinical description, genetic mapping, and molecular characterization of Salla disease in an Old Order Mennonite population. *Am J Med Genet.* 2005;138(3):262–267.
43. Alwi ZB. The use of SNPs in pharmacogenomics studies. *Malays J Med Sci.* 2005;12(2):4–12.
44. Katsanis SH, Katsanis N. Molecular genetic testing and the future of clinical genomics. *Nat Rev Genet.* 2013;14(6):415–426.
45. Karki R, Pandya D, Elston RC, Ferlini C. Defining “mutation” and “polymorphism” in the era of personal genomics. *BMC Med Genom.* 2015;8, e37.
46. Ma LY, Hong KX, Lu XZ, et al. Single nucleotide polymorphisms of HIV coreceptor CCR5 gene in Chinese Yi ethnic group and its

- association with HIV infection. *Zhonghua Yixue Zazhi*. 2005; 85(45):3181–3185.
47. Wang H, Nie F, Huang H, et al. Identifying quantitative trait loci via group-sparse multitask regression and feature selection: an imaging genetics study of the ADNI cohort. *Bioinformatics*. 2012; 28(2):229–237.
 48. Taylor JG, Choi EH, Foster CB, Chanock SJ. Using genetic variation to study human disease. *Trends Mol Med*. 2001;7(11): 507–512.
 49. Erichsen HC, Chanock SJ. SNPs in cancer research and treatment. *Br J Canc*. 2004;90(4):747–751.
 50. Chanock S. Genetic variation and hematology: single-nucleotide polymorphisms, haplotypes, and complex disease. *Semin Hematol*. 2003;40(4):321–328.
 51. Carracedo A. Finding genes that underlie cancer using genetic tools. *Clin Transl Oncol*. 2006;8(11):771–772.
 52. Pharoah PD, Dunning AM, Ponder BA, Easton DF. Association studies for finding cancer-susceptibility genetic variants. *Nat Rev Canc*. 2004;4(11):850–860.
 53. Gabriel S. Population genetic tools: application to cancer. *Semin Oncol*. 2007;34(2 Suppl 1):S21–S24.
 54. Wang Z, Moutl J. SNPs, protein structure, and disease. *Hum Mutat*. 2001;17(4):263–270.
 55. Capriotti E, Calabrese R, Casadio R. Predicting the insurgence of human genetic diseases associated to single point protein mutations with support vector machines and evolutionary information. *Bioinformatics*. 2006;22(22):2729–2734.
 56. Bernig T, Chanock SJ. Challenges of SNP genotyping and genetic variation: its future role in diagnosis and treatment of cancer. *Expert Rev Mol Diagn*. 2006;6(3):319–331.
 57. Rieder MJ, Livingston RJ, Stanaway IB, Nickerson DA. The environmental genome project: reference polymorphisms for drug metabolism genes and genome-wide association studies. *Drug Metab Rev*. 2008;40(2):241–261.
 58. Carlson CS, Eberle MA, Kruglyak L, Nickerson DA. Mapping complex disease loci in whole-genome association studies. *Nature*. 2004;429(6990):446–452.
 59. Galvani AP, Novembre J. The evolutionary history of the CCR5-Delta32 HIV-resistance mutation. *Microb Infect*. 2005;7(2): 302–309.
 60. Sheppard HW, Celum C, Michael NL, et al. HIV-1 infection in individuals with the CCR5-Delta32/Delta32 genotype: acquisition of syncytium-inducing virus at seroconversion. *J Acquir Immune Defic Syndr*. 2002;29(3):307–313.
 61. Paxton WA, Kang S, Koup RA. The HIV type 1 coreceptor CCR5 and its role in viral transmission and disease progression. *AIDS Res Hum Retrovir*. 1998;14(Suppl 1):S89–S92.
 62. Picton Anabela CP, Paximadis Maria, Tiemessena Caroline T. Genetic variation within the gene encoding the HIV-1 CCR5 coreceptor in two South African populations. *Infect Genet Evol*. 2010;10(4):487–494.
 63. Zapata W, Jimenez WA, Trujillo NP, Rojas W, Estrada H, Rugeles MT. Influence of CCR5 and CCR2 genetic variants in the resistance/susceptibility to HIV in serodiscordant couples from Colombia. *AIDS Res Hum Retrovir*. 2013;29(12):1594–1603.
 64. Lopalco L. CCR5: from natural resistance to a new anti-HIV strategy. *Viruses*. 2010;2(2):574–600.
 65. Novembre J, Galvani AP, Slatkin M. The geographic spread of the CCR5 $\Delta 32$ HIV-resistance allele. *PLoS Biol*. 2005;3(11), e339.
 66. de Silva E, Stumpf MP. HIV and the CCR5-Delta32 resistance allele. *FEMS Microbiol Lett*. 2004;241(1):1–12.
 67. Henrich TJ, McLaren PJ, Rao SS, et al. Genome-Wide association study of human immunodeficiency virus (HIV)-1 coreceptor usage in treatment-naïve patients from an AIDS clinical trials group study. *Open Forum Infect Dis*. 2014;1(1), ofu018.
 68. Hedrick PW, Verrelli BC. “Ground truth” for selection on CCR5-Delta32. *Trends Genet*. 2006;22(6):293–296.
 69. Gonzalez E, Dhanda R, Bamshad M, et al. Global survey of genetic variation in CCR5, RANTES, and MIP-1 α : impact on the epidemiology of the HIV-1 pandemic. *Proc Natl Acad Sci U S A*. 2001;98(9):5199–5204.
 70. Mikawa AY, Tagliavini SA, Costa PI. CCR5 genotype and plasma β -chemokine concentration of Brazilian HIV-infected individuals. *Braz J Med Biol Res*. 2002;35(11):1333–1337.
 71. Pandve Harshal T, Giri Purushottam A. HIV/AIDS prevention and control in India: achievements and future challenges. *N Am J Med Sci*. 2015;7(12):575–576.
 72. Gupta A, Padh H. Analysis of CCR5 and SDF-1 genetic variants and HIV infection in Indian population. *Int J Immunogenet*. 2015;42(4):270–278.
 73. Bharti D, Kumar A, Mahla RJ, et al. Low prevalence of CCR5- $\Delta 32$, CCR2-64I and SDF1-3'A alleles in the Baiga and Gond tribes of Central India. *SpringerPlus*. 2015;4, e451.
 74. Hollen CV. HIV/AIDS and the gendering of stigma in Tamil Nadu, South India. *Cult Med Psychiatry*. 2010;34(4):633–657.
 75. Joshi A, Punke EB, Sedano M, et al. CCR5 promoter activity correlates with HIV disease progression by regulating CCR5 cell surface expression and CD4 T cell apoptosis. *Sci Rep*. 2017;7(1), e232.
 76. Gonzalez E, Bamshad M, Sato N, et al. Race-specific HIV-1 disease-modifying effects associated with CCR5 haplotypes. *Proc Natl Acad Sci Unit States Am*. 1999;96(21): 12004–12009.
 77. Al-Mahruqi Samira H, Zadjali Fahad, Beja-Pereira Albano, Koh Crystal Y, Balkhair Abdullah, Al-Jabri Ali A. Genetic diversity and prevalence of CCR2-CCR5 gene polymorphisms in the Omani population. *Genet Mol Biol*. 2014;37(1):7–14.
 78. Ni J, Wang D, Wang S. The CCR5-delta32 genetic polymorphism and HIV-1 infection susceptibility: a meta-analysis. *Open Med*. 2018;13:467–474.
 79. Novembre J, Galvani AP, Slatkin M. The geographic spread of the CCR5 Delta32 HIV-resistance allele. *PLoS Biol*. 2005;3(11), e339.
 80. Adojaan M, Mölder T, Männik A, et al. High prevalence of the CCR5Delta32 HIV-resistance mutation among Estonian HIV type 1-infected individuals. *AIDS Res Hum Retrovir*. 2007;23(2):193–197.
 81. Jilzi A, Edouard J, Fadhlaoui-Zid K, et al. Identification of the CCR5-Delta32 HIV resistance allele and new mutations of the CCR5 gene in different Tunisian populations. *Hum Immunol*. 2007;68(12):993–1000.
 82. Hütter G, Bodor J, Ledger S, et al. CCR5 targeted cell therapy for HIV and prevention of viral escape. *Viruses*. 2015;7(8): 4186–4203.
 83. Vanegas NT, Zapata W, Rugeles MT. Genetic and immunological factors involved in natural resistance to HIV-1 infection. *Open Virol J*. 2011;5:35–43.
 84. Lopalco L. CCR5: from natural resistance to a new anti-HIV strategy. *Viruses*. 2010;2(2):574–600.
 85. Morawetz RA, Rizzardi GP, Glauser D, et al. Genetic polymorphism of CCR5 gene and HIV disease: the heterozygous (CCR5/delta ccr5) genotype is neither essential nor sufficient for protection against disease progression. *Eur J Immunol*. 1997;27(12):3223–3227.
 86. Carrington M, Dean M, Martin MP, O'Brien SJ. Genetics of HIV-1 infection: chemokine receptor CCR5 polymorphism and its consequences. *Hum Mol Genet*. 1999;8(10):1939–1945.
 87. Huttenrauch F, Nitzki A, Lin FT, Honing S, Oppermann M. Beta-arrestin binding to CC chemokine receptor 5 requires multiple C-terminal receptor phosphorylation sites and involves a conserved

- Asp-Arg-Tyr sequence motif. *J Biol Chem.* 2002;277(34):30769–30777.
88. Farzan M, Mirzabekov T, Kolchinsky P, et al. Tyrosine sulfation of the amino terminus of CCR5 facilitates HIV-1 entry. *Cell.* 1999;96(5):667–676.
89. Kostrikis LG, Huang Y, Moore JP, et al. A chemokine receptor CCR2 allele delays HIV-1 disease progression and is associated with a CCR5 promoter mutation. *Nat Med.* 1998;4(3):350–353.
90. Aguilera A, Garcia-Muse T. Causes of genome instability. *Annu Rev Genet.* 2013;47:1–32.
91. Erichsen HC, Chanock SJ. SNPs in cancer research and treatment. *Br J Canc.* 2004;90(4):747–751.