

Genetic Redundancy and Chemokines: CCR5 Δ 32 HIV-Resistance Allele

Abdullahi I Uba^{1*}, Sani S Usman², Musbahu M Sani³, Umar A Abdullahi⁴, Mustapha G Muhammad⁵ and Umar S Abdussalam⁶

¹Center for Biotechnology Research, Bayero University, Kano, Nigeria/Department of Bioinformatics and Genetics, Graduate School of Science and Engineering, Kadir Has University, Istanbul, Turkey

²Department of Biochemistry and Molecular Biology, Jordan University of Science and Technology, Irbid, Jordan

³Department of Biochemistry, Faculty of Basic Medical Sciences, CoMAHP Federal University Dutse, Jigawa, Nigeria

⁴Department of Biological Science, Faculty of Science, Federal University Dutse, Jigawa, Nigeria

⁵Department of Science Laboratory Technology, Kano State Polytechnic, Kano, Nigeria

⁶Department of Pharmacology, Faculty of Clinical Sciences, Bayero University, Kano, Nigeria

*Corresponding author: Abdullahi Ibrahim Uba, Center for Biotechnology Research, Bayero University, Kano, P.M.B 3011, Nigeria/Department of Bioinformatics and Genetics, Graduate School of Science and Engineering, Kadir Has University, Istanbul, Turkey, Tel: +90 212 533 65 32; E-mail: abdulurgeon@yahoo.com

Received date: April 17, 2015, Accepted date: July 31, 2015, Published date: August 5, 2015

Copyright: © 2015 Uba AI, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Abstract

Gene mutation is a change in nucleotide sequence of DNA which results in an impaired or loss of functions of the associated gene. Mutation can occur spontaneously or be induced by mutagenic agent. It is considered deleterious when it affects the phenotypic expression of the gene products. However, some mutations, such as CCR5 gene mutation turns out to be beneficial. HIV virus uses the gene product, CCR5, as a co-receptor along with CD4 receptor to enter the host's cell. The product of CCR5 mutant gene does not interact with HIV surface antigen, hence blocks the primary entry of the virus and thus provides immunity to AIDS for homozygous carriers and greatly slows the progress of the disease in heterozygous carriers. How about the critical role of the gene, being the gene encoding a member of the beta chemokine receptors, which in turn play an important role in the immune response? This is probably compensated by genomic redundancy of chemokine-receptor functions. Genetic redundancy refers to the situation where the loss of a gene can be completely or partially compensated by one or more other genes. Taken together, CCR5 Δ 32 protein product is of clinical significance in conferring resistance to HIV infection and is thought to reduce the surface expression of wild type CCR5. In this review we highlight the origin of CCR5 Δ 32 HIV-Resistance Allele and discuss chemokine receptors' functional redundancy as the phenomenon compensating for the normal function the allele in individuals carrying the mutation.

Keywords: Mutation; Chemokines; CCR5 Δ 32 HIV-Resistance Allele; Genetic redundancy

Introduction

In CCR5- Δ 32 mutation thirty-two base pairs that correspond to the second extracellular loop of the receptor, CCR5, are deleted. This mutated receptor is non-functional and non-perfect fit for the surface antigen presented by M-tropic HIV-1 virus resulting in infection resistance [1-4]. The CCR5 Δ 32 mutation is considered a good example of an advantageous allele which plays an important role in HIV resistance. The mutation is prevalent in Europe and western Asia, with average frequencies of about 10%. History shows that the age of Δ 32 allele has been estimated to be between 700 and 3,500 years based on linkage disequilibrium data [5,6]. Recent ancient DNA evidence suggests that the allele can be at least 2,900 years old [7]. It has also been estimated that up to 20% of ethnic western Europeans carry this mutation, which can be rare or even absent in other ethnic groups [8-10]. This estimate suggests that the CCR5- Δ 32 mutation was strongly selected for sometime during European history. It has been proposed by some researchers that the plague epidemics that repeatedly swept Europe during the Middle Ages were responsible for this selection [11].

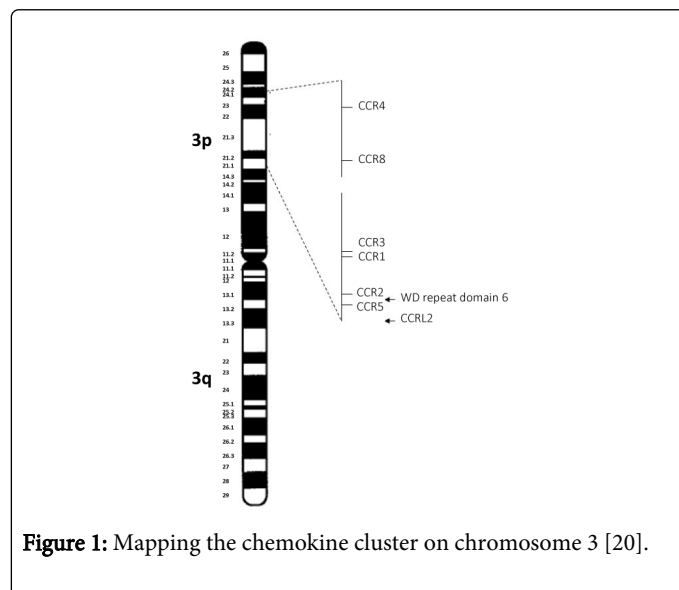
The HIV virus enters a cell with the aid of its surface antigen, HIV-1 gp120 protein which binds specifically to a receptor jointly

made by CD4 and multi-CC and CXC chemokine receptors in the initial stage of infection [12]. However, in people with CCR5- Δ 32 mutation, the primary entry of HIV by this means is blocked, thus providing immunity to AIDS for homozygous carriers and greatly slowing progress of the disease in heterozygous carriers [13,14]. Dr. Stephen J. O'Brien of the National Institutes of Health in Washington D.C. was reported to have said "It's highly unusual, 'Most genes, if you knock them out, cause serious diseases like cystic fibrosis or sickle cell anemia or diabetes. But CCR5-delta32 is rather innocuous to its carriers. The reason seems to be that the normal function of CCR5 is redundant in our genes; that several other genes can perform the same function" [15]. It was reported that one of the best documented genetic polymorphisms which regulates disease susceptibility is the CCR5- Δ 32 HIV resistance allele [16]. In this review we highlight the origin of the allele and discuss genetic redundancy in chemokines as the phenomenon compensating for the normal function of the allele in individuals carrying the mutation.

Physiological role of chemokines and chemokine receptor functions

All chemokines have a number of conserved cysteine residues that are involved in intramolecular disulfide bond formation. While some chemokines are pro-inflammatory molecules induced during an immune response to recruit cells of the immune system to a site of infection, others are homeostatic and are involved in controlling the

migration of cells during normal processes of tissue maintenance or development [17-19]. They are the only cytokines known to interact with seven-transmembrane-domain-G-protein-coupled receptors, proteins that respond to an external signals and transmit them to an intracellular compartment [20]. Chemokines are labelled according to the structure at conserved sites of their cysteine residues such that CC chemokines have neighbouring cysteines while CXC chemokines have an additional amino acid separating cysteine residues. So far seven CC (of which CCR5 is one) and four CXC receptors are known. It is thought that a primordial chemokine gene that underwent duplication and divergence is responsible for the different chemokine groups. The chemokine CCR5 plays an important role in the immune response, where it is highly expressed on macrophages and CD4 T cells [18,19].



Understanding the mechanics of chemokine action is complicated considering the fact that chemokine receptors can bind to number of chemokines, while chemokines in turn can bind to number of chemokine receptors as well. Chemokine receptors are expressed on cells of the immune system; lymphocytes, macrophages, manocytes, eosinophils, basophils and platelets. Some chemokines are unique to certain cell, for instance CXCR4 receptors are expressed on T cells and CXCR5 receptors on B cells [21]. CC chemokines have a complex and diverse role, for example monoclonal antibodies such as MIP-1 α have been used in inflammatory models to understand the function of chemokines in inflammation. CCR5 plays a role in the inflammatory response by directing cells to sites of inflammation. Other chemokine receptors work in synergy with CCR5 to mediate T-cell functions [22]. The receptors enhance T-cell co-stimulation and cytokine release from CD4+ T-cells. Ligands for CCR5 augment the activation of T-cell responses and enhance the production of antigen specific T-cells [23]. The level of CCR5 expression is up-regulated in CD8+ cells during inflammation which allows the cells to move towards sites of CD4+ T-cell and dendritic interactions [24]. By this, the chance of CD8+ cells encountering antigen specific cells increases. CCR5 generally, enhances the adaptive immune response.

CCR5 protein is expressed by T cells and macrophages; an important co-receptor for macrophage-tropic virus including HIV, for entry into host cells. Defective alleles of this gene confers HIV infection resistance on the host. The ligands for this receptor include macrophage inflammatory protein 1 alpha, macrophage inflammatory

protein 1 beta, monocyte chemoattractant protein 2 and regulated on activation normal T expressed and secreted protein. Expression of this protein was also detected in a promyeloblastic cell line, implying its role in granulocyte lineage proliferation and differentiation. The role of CCR5 is to allow entry of chemokines into the cell; chemokines are involved in signalling the body's inflammation response to injuries [21]. The observation that its congenital absence does not lead to any overt pathology suggested that CCR5 might be a valid target for pharmacological blockade. CXCR4, on the other hand, and its single known ligand SDF-1/CXCL12 are both highly conserved; they play an essential role during embryonic development and in several major processes in the adult, including hematopoiesis, trafficking of leukocytes to the target site in the adaptive immune system, and vascularization [25].

Genetic redundancy in CCR5 Δ 32 HIV-resistance allele

Genetic redundancy refers to the situation where the loss of a gene can be completely or partially compensated by one or more other genes. Cases of genetic redundancies are well documented in the literature and can be roughly categorized into two types. The first type of genetic redundancy occurs at the individual gene level such as that between isoenzymes. Isoenzymes are generated by gene duplication and differ in protein sequence, yet catalyze the same biochemical reactions in an organism. The second type of redundancy occurs at the systems level, as a result of distributed properties of networks [25,26]. The consequence of genetic redundancy is robustness against genetic perturbations such as mutation that are deleterious in nature. Genetic robustness is a characteristic of cellular life, observed at many levels of biological organizations, from DNA replication and gene expression to metabolism, cell cycle, and embryonic development [27-29].

Cytokines and chemokines are redundant secreted proteins with growth, differentiation, and activation functions that regulate and determine the nature of immune responses and control immune cell trafficking and the cellular arrangement of immune organs. Which cytokines are produced in response to an immune attacks determines initially whether an immune response develops and subsequently whether that response is to be cytotoxic, humoral, cell-mediated, or even allergic. A cascade of responses operates in response to cytokines, and often several cytokines are required in synergy to express optimal function [30]. Chemokines are functionally redundant, have synergetic effects, and can behave as antagonists. These properties enable the formation of a complex and appropriate network that activates the immune response. The human CC chemokine receptor family includes 10 genes (designated as CCR1 through CCR10). Most of the human CC chemokine receptor genes, with the exception of CCR7 are concentrated on the short arm of chromosome number 3. Receptors CCR1 through CCR5 are very similar in gene structure, suggesting that all five receptors share a recent common ancestor and originated through gene duplications [30]. Two pairs of loci, CCR1-CCR3 and CCR2- CCR5 are more closely related based on sequence similarity and physical distance than to the other members of the cluster (Figure 1) [20].

"CCR5- Δ 32/ Δ 32 homozygotes lack CCR5-mediated chemokine responsiveness but do not show immunological pathology, probably because of the genomic redundancy of chemokine-receptor functions. It is reported that the programmed induction of chemokine receptor expression on CD4+ leukocytes is of clear advantage to the host in mounting an appropriate immune response, although the subsequent use of these receptors by HIV is likely be the result of evolution and a

millennium of genetic pressures". Apparently, these genetic pressures causing 32 base-pair deletion in the CCR5 gene on human leukocytes (Figure 2) has allowed for some resistance to infection without impairing the host's immune response [31-33]. The absence of any apparent immunological deficits of significance among individuals with naturally occurring loss-of-function mutations provides some certainty that pharmacological blockade of CCR5 in normal individual, will relatively provide protection against the viral entry [34]. One can raise questions about the normal function of the receptor. It is thought that redundancy in the chemokine network is thought to allow other chemokine receptors to perform the function of CCR5 [35].

However, some researchers have argued that the blockage of the CCR5 pathway does not compromise the 'robust' role of the chemokine system in the ontogeny of innate and acquired immunity. They put forward an argument that blocking certain chemokines or receptors can definitely affect the product of the system. This is evidently illustrated by eotaxin and its receptor CCR3, being clearly essential for full expression of allergic inflammation, and for CCR2 and MCP-1 Gene targeting. The latter molecules clearly affect monocyte recruitment under certain conditions of stimulation. However, lack of these molecules does not compromise ontogeny [36-39].

Researchers corroborating studies in mice [35] with studies in humans have shown that CCR5Δ32 heterozygotes have a sixfold increased risk for severe West Nile virus (WNV) infection and a five-fold increased risk of mortality [40]. Similarly, meta-analysis of the four patient cohorts in the United States confirmed that CCR5 deficiency is a strong and consistent risk factor for symptomatic WNV infection [41]. Association between the lack of functional CCR5 and tick-borne encephalitis has also been reported [42]. However, the available data currently suggest that pharmacological blockage of CCR5 is likely to be largely well tolerated; adequate safety studies required to monitor the long-term safety of CCR5 blockage while paying attention to the consequences of infection by certain pathogens [43,44]. There are several approaches undertaken to pharmaceutically target CCR5, such as the use of small molecule co-receptor inhibitors, chemokine analogues, anti-CCR5 antibodies, gene knockdown and knockout strategies [35,43].

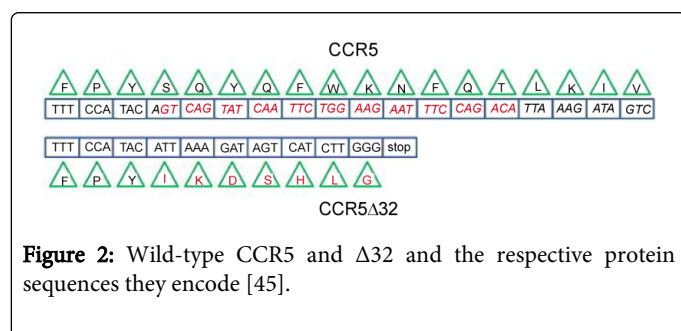


Figure 2: Wild-type CCR5 and Δ32 and the respective protein sequences they encode [45].

Some experts in the area consider the use of receptor antagonist drugs as problematic in view of drug toxicity; possibility of drug binding to up-target chemokine receptors and the emergence of escape mutants. Better approach is gene therapy which can provide a long-term protection against HIV infection and progression. Transducing hematopoietic stem cells ensures continued self-renewal and differentiation ability of HIV resistant cells while ultimately replacing and reconstituting the individual's HIV depleted immune system [46].

Invasion of HIV-1 into the host cell requires binding of HIV-1 envelope protein gp120 to the cellular receptor CD4 which orients the viral spike, stabilize the bridging sheet and exposes the V3 loop of gp120. This new orientation allows for viral interactions with the cellular co-receptor CCR5 [47]. Thus genetic therapies disrupting the CCR5 proteins are promising avenues of research for developing HIV resistance.

Similarly, in our previous article entitled "Genetic approach to protecting infants to be breast-fed by HIV positive mothers against HIV infection," we proposed CCR5 genetic modification at stem cell level considering the redundancy of chemokine receptor network [1]. Thus, CCR5 Δ32 protein product is of clinical significance in conferring resistance to HIV infection and is thought to reduce the surface expression of wild type CCR5 as shown by Benkirane et al. and Agrawal et al., [48,49]. To further express our view here, genetic redundancy can be the driving force compensating for the normal functions of CCR5 gene considering the fact that CCR1 through CCR5, among chemokines clusters, are very similar in gene structure, suggesting that all five receptors emanated from the same phylogenetic tree and originated through gene duplications [20].

Conclusion

CCR5 Δ32 HIV-Resistance Allele is beneficial to its host, the mutation originated from Europe and Western Asia. Redundancy in chemokine genes can be the driving force compensating for the normal functions of the CCR5 gene.

Recommendation

CCR5 Δ32 mutation provides an important hint on how to prevent HIV primary entry to the host both pharmacologically and by genetic manipulation. The latter can be promising to ensure a lifelong protection if carefully performed. However, analysis of this mutation needs to be performed both *in silico* and *in vitro* to explore other means of clinical intervention.

Acknowledgement

We acknowledge The Center for Biotechnology Research, Bayero University, Kano for their guidance on the preparation of this manuscript.

References

1. Uba AI, Abubakar H, Atiku MK, Wudil AM (2014) Proposed genetic approach to protecting infants to be breastfed by HIV positive mothers against HIV infection. Int J Infect Trop Dis 1: 73-76.
2. Buseyne F, Janvier G, Teglas J, Ivanoff S, Burgard M, et al. (1998) "Impact of heterozygosity for the chemokine receptor CCR5 32-bp-deleted allele on plasma virus load and CD4 T lymphocytes in perinatally human immunodeficiency virus-infected children at 8 years of age" J Infect Dis 178: 1019-23.
3. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, et al. (1996) Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 86: 367-377.
4. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, et al. (1996) Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 382: 722-725.
5. Stephens JC, Reich DE, Goldstein DB, Shin HD, Smith MW, et al. (1998) Dating the origin of the CCR5-Delta 32 AIDS-resistance allele by the coalescence of haplotypes. Am J Hum Genet 62: 1507-1515.

6. Slatkin M (2001) Simulating genealogies of selected alleles in a population of variable size. *Genet Res* 78: 49-57.
7. Hummel S, Schmidt D, Kremeyer B, Herrmann B, Oppermann M (2005) Detection of the CCR5-Delta32 HIV resistance gene in Bronze Age skeletons. *Genes Immun* 6: 371-374.
8. Zimmerman PA, Buckler-White A, Alkhatib G, Spalding T, Kubofcik C, Combadiere J, et al. (1997) Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5: studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. *Molecular Medicine* 3: 23-36
9. Stephens JC, Reich DE, Goldstein DB, Shin HD, Smith MW, et al. (1998) Dating the origin of the CCR5-Delta32 AIDS-resistance allele by the coalescence of haplotypes. *Am J Hum Genet* 62: 1507-1515.
10. Majumder PP, Dey B (2001) Absence of the HIV-1 protective Delta ccr5 allele in most ethnic populations of India. *Eur J Hum Genet* 9: 794-796.
11. Duncan SR, Scott S, Duncan CJ (2005) Reappraisal of the historical selective pressures for the CCR5-Delta32 mutation. *J Med Genet* 42: 205-208.
12. Kawamura T, Forrest O, Gulden, Sugaya M, David TM, Debra LB, et al. (2003) R5 HIV productively infects Langerhans cells and infection levels are regulated by compound CCR5 polymorphisms. *Proceedings of the National Academy of Sciences of the USA* 100 (14).
13. Zagury D, Lachgar A, Chams V, Fall LS, Bernard J, et al. (1998) C-C chemokines, pivotal in protection against HIV type 1 infection. *Proc Natl Acad Sci U S A* 95: 3857-3861.
14. Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, et al. (1996) Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86: 367-377.
15. *Secrets of the Dead* (2006) *Mystery of the Black Death*, 9
16. Mummidi S, Ahuja SS, Gonzalez E, Anderson SA, Santiago EN, et al. (1998) Genealogy of the CCR5 locus and chemokine system gene variants associated with altered rates of HIV-1 disease progression. *Nat Med* 4: 786-793.
17. Kurihara T, Bravo R (1996) Cloning and functional expression of mCCR2, a murine receptor for the C-C chemokines JE and FIC. *J Biol Chem* 271: 11603-11607.
18. Berger EA, Murphy PM, Farber JM (1999) Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu Rev Immunol* 17: 657-700.
19. Rollins BJ (1997) Chemokines. *Blood* 90: 909-928.
20. Hütter G, Neumann M, Nowak D, Klein S, Klüter H, et al. (2011) The effect of the CCR5-delta32 deletion on global gene expression considering immune response and inflammation. *J Inflamm (Lond)* 8: 29.
21. Wells TN, Power CA, Proudfoot AE (1998) Definition, function and pathophysiological significance of chemokine receptors. *Trends Pharmacol Sci* 19: 376-380.
22. Contento RL, Molon B, Boularan C, Pozzan T, Manes S, et al. (2008) CXCR4-CCR5: a couple modulating T cell functions. *Proc Natl Acad Sci U S A* 105: 10101-10106.
23. Taub DD, Ortaldo JR, Turcovski-Corrales SM, Key ML, Longo DL, et al. (1996) Beta chemokines costimulate lymphocyte cytolysis, proliferation, and lymphokine production. *Journal of Leukocyte Biology* 59: 81-89.
24. Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Scheincker C, et al. (2006) Chemokines enhance immunity by guiding naive CD8+ T cells to sites of CD4+ T cell-dendritic cell interaction. *Nature* 440: 890-895.
25. Hartman JL 4th, Garvik B, Hartwell L (2001) Principles for the buffering of genetic variation. *Science* 291: 1001-1004.
26. Wagner A (2005) Distributed robustness versus redundancy as causes of mutational robustness. *Bioessays* 27: 176-188.
27. de Visser JA, Hermisson J, Wagner GP, Ancel Meyers L, Bagheri-Chaichian H, et al. (2003) Perspective: Evolution and detection of genetic robustness. *Evolution* 57: 1959-1972.
28. Lenski RE, Barrick JE, Ofria C (2006) Balancing robustness and evolvability. *PLoS Biol* 4: e428.
29. Wagner A (2005) *Robustness and Evolvability in Living Systems*. Princeton University Press, Princeton, NJ 6
30. Borish LC, Steinke JW (2003) 2. Cytokines and chemokines. *J Allergy Clin Immunol* 111: S460-475.
31. Rowland-Jones SL (1998) Survival with HIV infection: good luck or good breeding? *Trends Genet* 14: 343-345.
32. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, et al. (1996) 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* 273: 1856-1862.
33. Huang Y, Paxton WA, Wolinsky SM, Neumann AU, Zhang L, et al. (1996) The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nat Med* 2: 1240-1243.
34. de Silva E, Stumpf MP (2004) HIV and the CCR5-Delta32 resistance allele. *FEMS Microbiol Lett* 241: 1-12.
35. Tsibris AM, Kuritzkes DR (2007) Chemokine antagonists as therapeutics: focus on HIV-1. *Annu Rev Med* 58: 445-459.
36. Kuziel WA, Morgan SJ, Dawson TC, Griffin S, Smithies O, et al. (1997) Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. *Proc Natl Acad Sci U S A* 94: 12053-12058.
37. Kurihara T, Warr G, Loy J, Bravo R (1997) Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J Exp Med* 186: 1757-1762.
38. Boring L, Gosling J, Chensue SW, Kunkel SL, Farese RV Jr, et al. (1997) Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. *J Clin Invest* 100: 2552-2561.
39. Lu B, Rutledge BJ, Gu L, Fiorillo J, Lukacs NW, et al. (1998) Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *J Exp Med* 187: 601-608.
40. Glass WG, McDermott DH, Lim JK, Lekhong S, Yu SF, et al. (2006) CCR5 deficiency increases risk of symptomatic West Nile virus infection. *J Exp Med* 203: 35-40.
41. Lim JK, Louie CY, Glaser C, Jean C, Johnson B, et al. (2008) Genetic deficiency of chemokine receptor CCR5 is a strong risk factor for symptomatic West Nile virus infection: a meta-analysis of 4 cohorts in the US epidemic. *J Infect Dis* 197: 262-265.
42. Kindberg E, Mickiene A, Ax C, Akerlind B, Vene S, et al. (2008) A deletion in the chemokine receptor 5 (CCR5) gene is associated with tickborne encephalitis. *J Infect Dis* 197: 266-269.
43. Kuhmann SE, Hartley O (2008) Targeting chemokine receptors in HIV: a status report. *Annu Rev Pharmacol Toxicol* 48: 425-461.
44. Telenti A (2009) Safety concerns about CCR5 as an antiviral target. *Curr Opin HIV AIDS* 4: 131-135.
45. Barmania F, Pepper MS (2013) C-C chemokine receptor type five (CCR5) An emerging target for the control of HIV infection. *Applied & Translational Genomics* 2: 3-16
46. Hütter G, Nowak D, Mossner M, Ganepola S, Müssig A, et al. (2009) Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* 360: 692-698.
47. Huang CC, Lam SN, Acharya P, Tang M, Xiang SH, et al. (2007) Structures of the CCR5 N terminus and of a tyrosine-sulfated antibody with HIV-1 gp120 and CD4. *Science* 317: 1930-1934.
48. Benkirane M, Jin DY, Chun RF, Koup RA, Jeang KT (1997) Mechanism of transdominant inhibition of CCR5-mediated HIV-1 infection by ccr5delta32. *J Biol Chem* 272: 30603-30606.
49. Agrawal L, Lu X, Qingwen J, VanHorn-Ali Z, Nicolescu IV, et al. (2004) Role for CCR5Delta32 protein in resistance to R5, R5X4, and X4 human immunodeficiency virus type 1 in primary CD4+ cells. *J Virol* 78: 2277-2287.