

Prevalence of CCR2 gene polymorphisms among HIV infected individuals from different regions in Kenya

Dorcas Wachira¹, Samoel Khamadi¹, Alex Wamachi¹, Jones Mueke², Zipporah Ng'ang'a³, Moureen Maraka¹

¹Kenya Medical Research Institute, ²Kenyatta University, ³Jomo Kenyatta University of Agriculture and Technology, ⁴Kenya Methodist University

Email: Dorcas Wachira*-dorcas.wangui@kemu.ac.ke, Moureen Maraka- maumoen@yahoo.com, Alex Wamachi-awamachi@kemu.ac.ke, Jones Mueke-mueke.jones@ku.ac.ke, Zipporah Ng'ang'a- zipnganga@gmail.com, Samoel Khamadi- skhamadi@gmail.com

*Corresponding author

Abstract

Background: Chemokine receptor-2 (CCR2) is a co-receptor for the entry of human immunodeficiency virus-1 (HIV-1) into the target cells. Patients with the CCR2 mutations may progress to AIDS at least 2-4 years later than individuals carrying the normal gene. A G-to-A transition at position 190 characterizes the CCR2-64I mutation. This mutation has been identified as an important factor for delaying progression to AIDS. The effect of CCR2 polymorphism on HIV-1 disease progression has not been explored in depth within Africa. The status of CCR2 gene polymorphisms among HIV infected individuals in the Kenyan population is unknown. The objective of this study was to determine the existence and distribution of CCR2 gene mutations and to identify the different polymorphic groups of the coreceptor gene among HIV infected individuals in the Kenyan population.

Methods: Blood samples were collected from HIV screening centers and analyzed for the presence of CCR2-64I mutation. One hundred and eighteen samples were genotyped for the CCR2-64I mutation by PCR-RFLP.

Results: Among the 118 samples genotyped, 4 (3.4%) were homozygous mutants (I/I) and 21 (17.8%) were heterozygous (V/I) for the CCR2-64I polymorphism, with the remaining 93 subjects (78.8%) being wild-type homozygote (V/V) giving a 21.1% frequency of the CCR2-64I allele in this sample population.

Conclusion: The presence of CCR-2 polymorphism among the sample population contribute to the growing evidence that host genetic factors are important in predicting susceptibility to HIV-1 infection.

IJPP 2012 3 (3): 270 – 274

Background

Entry of HIV-1 into target cells requires both CD4 and one of the chemokine receptors. The most common co-receptors are CCR5 and CXCR4. Individuals with CCR5 mutation

may be partially be protected against infection with NSI strains of HIV. Non-progressors have also been found to have higher levels of certain variations in parts of the DNA that control the amount of CCR5 receptor that the cells

SELECTED CONFERENCE PAPERS

produce. These 'promoter polymorphisms', such as CCR5 59353-C, may also delay disease progression (Easterbrook *et al.*, 1999).

Genetic polymorphisms in Chemokine receptor CCR2 protein (CCR2-64I) have been identified which influence the rate of disease progression in AIDS. Natural mutations have been found to delay progression to AIDS. Chemokine receptor-2 is a chemoattractant for chemokines. These are molecules that are part of the body's inflammatory response located on immune cells. These receptors, however, are also used as a gateway by the HIV virus to enter and infect the immune cells. Polymorphisms in the CCR-2 system are known to modulate the natural history of HIV-1 infection (Anzala *et al.*, 1998). Individual variants in the coding or promoter and regulatory regions of the Chemokine 5 receptors (CCR5) and 2 (CCR2) have often been associated with various events in the pathogenesis of HIV-1/AIDS (Easterbrook *et al.*, 1999). It has been suggested that stable CCR haplotypes, alone or paired as genotypes, may influence the course of HIV-1 infection differentially according racial distribution. Variations in genotype frequencies among populations could have a sizeable impact on the course and burden of disease (Capoulade-Métay *et al.*, 2006).

While the CCR5-Δ32 mutation is mostly seen in Caucasian people, significant numbers of non-Caucasian people in Asia and Africa are known to resist HIV infection despite repeated exposure. One study in Vietnam identified at least five additional CCR5 mutations in as many as 1% of Vietnamese and Cambodian individuals. Two of these mutations, which were only found in HIV-negative people, altered the way CCR5 functions, suggesting that these other mutations in CCR5 may play a role in protection from HIV infection or disease progression in non-Caucasian people (Gonzalez *et al.*, 2001). The CCR5 protein is believed normally to act as a receptor for three chemokines: RANTES, MIP-1 alpha and MIP-

1 beta. Studies have shown that HIV-positive people who do not progress, and HIV-negative people who have been repeatedly exposed to the virus through unprotected sex yet do not become infected, often have unusually high levels of these chemokines. Increased chemokine production correlates with a greater proliferation of HIV-specific T-cells (Michael NL and Moore JP, 1999).

Forty percent of non-progressors carry the CCR5-Δ32 mutation, 60% of non-progressors carry the normal CCR5 gene, and similarly, 80% of non-progressors carry the CCR2 wild type gene. This suggests that a number of host and viral factors may combine to determine disease progression in any individual (An *et al.*, 2000). Polymorphisms in the CCR2 gene involve a point mutation that results in a switch from valine to isoleucine at transmembrane protein position 64, described as CCR2-64I. Generating more information on CCR-2 gene polymorphism in different regions of Kenya will shade more light on disease progression.

Methods

Study samples

HIV-1 positive blood samples were collected in EDTA tubes from consenting adults in different provincial and district hospitals in Kenya through venipuncture. A total of 118 samples were collected and analyzed. The research work was approved by the Graduate School Board of Kenyatta University (Ref I56/13034/05). The study was conducted according to the National and International regulations governing the use of human subjects in biomedical research.

Laboratory procedures

Peripheral blood mononuclear cells (PBMCs) and genomic DNA was extracted from whole blood as

SELECTED CONFERENCE PAPERS

previously described (Khamadi *et al.*, 2005). Amplification of a 128-base pair fragment of the CCR2 gene was carried out using the CCR2 gene primers CKR2_1A (sense): 5' TTG TGG GCA ACA TGA TGG and CKR2_1Z (antisense): 5' GAG CCC ACA ATG GGA GAG TA. The forward primer had the nucleotide "a" as a mismatch. The reaction mixture contained 10 μ mol of each primer, 2 μ l of extracted DNA and a commercial PCR master mix. After an initial denaturation step at 94°C for 5 minutes, PCR was run for 40 cycles (94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute) in a thermal cycler. The resulting amplicons were digested with *BsaBI* at 60°C for 2 hours. The resulting products were electrophoresed on a 4% agarose gel and stained with ethidium bromide for visualization. A 128bp fragment indicated a homozygous wild genotype, while 110bp and 18bp fragments were indicative of the homozygous mutant genotype. The presence of three fragments revealed the heterozygous genotype.

Statistical analysis

Genotype frequencies were evaluated using the Hardy–Weinberg equilibrium test. Allele frequencies and the prevalence of genotypes were determined using the χ^2 test. Statistical significance was defined as $p < 0.05$.

Results

From the 118 samples successfully analyzed, 4 (3.4%) had the CCR2-V64I homozygous mutation A/A while 21 (17.8%) had the heterozygous mutation G/A. Majority of the samples (78.8%) had the wild type allele.

Distribution of the wild type gene in the provinces ranged from 93.3% in Nairobi to

69.2% in Coast province. Nyanza province had the highest heterozygous mutations (25%) while Nairobi province had the lowest (6.7%). A single case of homozygous mutations was present in only four provinces (Table 1). There were however no significant differences in the distribution of CCR2-V64I mutations across the provinces ($p=0.98$). Within the provinces, there was a significant difference in the distribution of G/A mutations compared to A/A mutations ($p < 0.001$). Nyanza province had the highest homozygous mutation (25%) followed by Central, Coast, North Eastern and Rift Valley provinces with all three having approximately 22%. Nairobi Province had the lowest occurrence of homozygous mutation with just a single occurrence of 6.7%. Nairobi had the highest number of wild type and lowest number of mutants which made it the province with the highest range between the wild type and mutant alleles. The allelic genotypic frequency of the CCR2-V64I in all the eight provinces was below 8% of the samples analyzed.

Discussion

The prevalence of the mutant homozygous and heterozygous CCR2-64-I alleles was significant considering the small number of samples that were analyzed. This is as expected considering that mutations occur at low levels in populations. The significantly high CCR2-64-I mutations in the sampled population could be an indication that the mutation is common in the population. It could also suggest that this is an established mutation that has been passed down through generations, even before the advent of the HIV/AIDS scourge. Similar studies have been carried out in other populations that show a significantly high distribution of the CCR2-64-I allelic mutations (Ryabov *et al.*, 2002). In a study in Moscow in 2001, for example, the wild-type CCR2 gene alleles were 77.87% with the rest being CCR2-64-I heterozygotes without any CCR2-64-I mutant homozygotes (Michael NL

SELECTED CONFERENCE PAPERS

and Moore JP, 1999). In a similar study in Brazil in 2002, it was observed that the frequency of the CCR2 wild type genotype was 60% while that of the heterozygous mutant genotype was 44% while the homozygous mutant genotype 3% [10]. In a similar study carried out in Kenya in 1998, it was observed that frequency of the CCR2-64I allele was 23% in a cohort of commercial sex workers (Anzala *et al.*, 1998). This is comparable to what was seen in this study though there were variations within the different provinces of Kenya.

The CCR2-V64I mutation affects the gene that encodes the CCR2 receptor on the outside of cells, and is more common than the CCR5 mutation, between 10 and 25% of the population are believed to have at least one mutant CCR2 gene (Smith *et al.*, 1997).

In a study of over 3000 HIV-positive people, those who had one mutant CCR2 gene developed AIDS two to four years later than people who had two normal copies of the CCR2 gene. The results were even more striking when the data on the effects of CCR2 and CCR5 mutations were combined. About 30% of long-term survivors who had been infected with HIV for at least 16 years or more without developing AIDS had at least one CCR2 or CCR5 mutant gene (Smith *et al.*, 1997). The protective effect of CCR2B-64I has also been demonstrated by other studies (Anzala *et al.*, 1998). However, as with the CCR5-Δ32 mutation, the CCR2b mutation did not affect disease progression among injecting drug users.

Conclusions

This study survey focused on a relatively small number of individuals. However, the findings contribute to the growing evidence that the presence and effects of genetic variants in the understudied African population are still important when predicting hosts susceptibility to HIV-1 and progression to AIDS within

the sub-Saharan African and more so in our Kenyan population. The knowledge of this mechanism of HIV entry into cells has resulted in the development of a new class of ARVs entry inhibitors, aimed at blocking the CCR5, CCR2 or CXCR4 co-receptors.

Competing interest

The authors declare that they have no competing interests

Authors' contributions

DW, SK and AW conceived and designed the study. MM and JM discussed and provided interpretation of the results. DW, AW and ZW wrote and revised the manuscript. All authors gave final approval of the manuscript to be published.

Acknowledgments

I would like to acknowledge and extend my heartfelt gratitude to the following persons who have made the completion of this work possible. Dr M. Warigia, J. Khamala, R Omollo and A. Maiyo

References

1. Anzala, AO, Ball, TB, Rostron T, O'Brien SJ, Plummer FA, Jones SL (1998), CCR2-64I allele and genotype association with delayed AIDS progression in African women. University of Nairobi Collaboration for HIV Research. *Lancet* 351, 1632-1633.
2. An PM, Martin P, Nelson GW, Carrington , Smith MW, Gong K, Vlahov D, O'Brien SJ, Winkler CA (2000), Influence of CCR5 promoter haplotypes on AIDS progression in African-Americans. *AIDS* 14, 2117-2122.
3. Easterbrook PJ, Rostron T, Ives N, Troop M, Gazzard BG, Rowland-Jones SL (1999), Chemokine receptor polymorphisms and human immunodeficiency virus disease progression. *Journal of Infectious Diseases*

SELECTED CONFERENCE PAPERS

180, 1096-1105.

4. Capoulade-Métay C, Ayoub A, Kfutwah A, Lole K, Pêtres S, Dudoit Y, Deterre P, Menu E, Barré-Sinoussi F, Debré P, Theodorou I (2006), A natural CCL5/RANTES variant antagonist for CCR1 and CCR3. *Immunogenetic* 58, (7):533-41.
5. Gonzalez, ER, Dhanda M, Bamshad S, Mummidi R, Geevarghese G, Catano SA, Anderson EA, Walter KT, Stephan MF, Hammer A, Mangano LSen, Clark RA, Ahuja SS, Dolan MJ, Ahuja SK (2001), Global survey of genetic variation in CCR5, RANTES, and MIP-1: impact on the epidemiology of the HIV-1 pandemic. *Proceedings of the National Academy of Sciences* 98, 5199-5204.
6. Garzino-Demo A, Moss RB, Margolick, JB, Cleghorn F, Sill A, Blattner WA, Cocchi F, Carlo DJ, DeVico AL, Gallo RC (1999), Spontaneous and antigen-induced production of HIV-inhibitory beta-chemokines are associated with AIDS-free status. *Proceedings of the National Academy of Sciences* 96, 11986-11991.
7. Kaslow RA, McNicholl JM (1999), Genetic determinants of HIV-1 infection and its manifestations. *Physicians* 111, 299-307.
8. Khamadi SA, Ochieng W, Lihana RW, Kinyua J, Muriuki J, Mwangi J, Lwemba R, Kiptoo M, Osman S, Lagat N, Pelle R, Muigai A, Carter JY, Oishi I, Ichimura H, Mwaniki DL, Okoth FA., Mpoke S, Songok EM (2005), HIV type 1 subtypes in circulation in northern Kenya. *AIDS Research and Human Retroviruses* 21, 810-814.
9. Michael NL, Moore JP (1999), HIV-1 entry inhibitors: evading the issue. *National Medicine* 5, 740-741.
10. Marmor M, Hertzmark K, Thomas SM, Halkitis PN, Vogler M (2006), Resistance to HIV infection. *Journal of Urban Health* 83, 5-17.
11. Ryabov GS., Kazennova EV. and Bobkov AF (2002), Frequencies of the CCR2-64I and SDF1-3'A Alleles Associated With Progression of the HIV-1 Disease in Healthy Individuals from Moscow. *A Journal of Virology* 38, 278-20.
12. Smith M W, Dean M, Carrington M, Winkler C, Huttley GA, Lomb DA, Goedert JJ, O'Brien TR, Jacobson LP, Kaslow R, Buchbinder, Vittinghoff E, Vlahov D, Hoots K, Hilgartner MW, O'Brien SJ (1997), Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. *Science* 277, 959-965.