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Null Single Nucleotide Polymorphism in Chemokine Receptor 5 (CCR5) Genes among the Ijaw Ethnic Population of Nigeria

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Abstract

Background: A deletion of 32 bp in the nucleotide sequence of CCR5 gene results in a defective CCR5 which confers protection from HIV infection in the homozygous state, while reducing the rate of disease progression to AIDS and death in the heterozygous state. The status of the CCR5Δ32 gene has not been reported in Nigeria. **Aim:** This study was aimed at analyzing single nucleotide polymorphism of CCR5 gene among the Ijaws resident in Yenagoa, Nigeria. **Methods:** 100 subjects (75 HIV negative and 25 HIV positive control) were recruited for this study. The CCR5 genes were amplified by 2 Stage PCR reaction using GeneAmp 9700 PCR system utilizing specific primers that would flank 32 bp deletion, followed by agarose gel electrophoresis, DNA sequencing of 20 subjects was done followed by phylogenetic and polymorphism analysis. **Results:** The results showed that 75 (100%) of the HIV negative subjects had 189 base pair in their CCR5 gene. Nucleotide of the 20 (100%) of the sequenced samples were conservatively same and no SNP was observed. **Conclusion:** This study documented no SNPs in CCR5 gene of the study population hence; the study population has no protection from HIV infection.

Keywords

CCR5, Single Nucleotide Polymorphism (SNP), PCR Amplicon

1. Introduction

Cystein-Cystein Chemokine Receptor 5 (CCR5) is a co-receptor for human immunodeficiency virus (HIV). It facilitates the virus entry into cells and mediates infection [1] [2]. CCR5 is expressed in memory or effect or T cells, monocytes or macrophages and

immature dendritic cells [3]. It is important in physiological and pathological conditions because of its ability to bind chemokines and regulate the migration of leukocytes throughout the body [4].

In humans, CCR5 gene is situated in position 21 of the p arm of chromosome 3 and consists of four exons and two introns [5]. Two promoters have been reported in the gene, P1, an upstream promoter results in two full length transcript variants, while P2, a weak downstream promoter do not have exon 1 and results in many truncated transcripts, but give rise to same CCR5 protein [5] [6] [7].

A32-bp deletion in the Open Reading Frame (ORF) of the gene (CCR5*Δ*32), has been reported to result in a premature shut down of translation and this gives a non-functional receptor [8]. Research showed that, this non-functional CCR5 protein (CCR5Δ 32), protects from HIV infection in the homozygous state, while offering protection from infection or reducing the rate of progression of the disease to AIDS and death in the heterozygous state [9]. CCR2 is another chemokine receptor located close to the CCR5 gene region in chromosome 3 (**Figure 1**). A CCR2V64I (which reads, valine to isoleucine amino acid substitution at position 64 of CCR2) has been reported to be closely linked to certain single nucleotide polymorphisms (SNPs) in the CCR5*cis*-regulatory region [10] [11] [12].

CCR5*Δ*32 alleles have been reported to have variable distributions along geographic lines. A frequency of 10% - 20% among European populations, with the highest found in northern Europe, and a marked decrease from the European figure to 2% - 5% in the Middle East and the Indian subcontinent and is rare (1%) in populations of African origin [5] [13] [14] [15].

CCR5 has been documented as a co-receptor in HIV infection. A sequence deletion of this gene is believed to reduce the risk of HIV infection. However, this deletion is reported in a lower percentage of the African population. No study on the nucleotides sequence of this gene has been reported in Nigeria. This study was therefore conducted among the adult population in Yenagoa, Nigeria to bridge this knowledge gap.

2. Materials and Methods

2.1. Study Design and Area

A cross-sectional observational study design was used. The study was carried out in Yenagoa, Bayelsa, South Nigeria.

2.2. Ethical Considerations

Ethical clearance was sought and gotten from the Bayelsa State Ministry of Health Ethi-

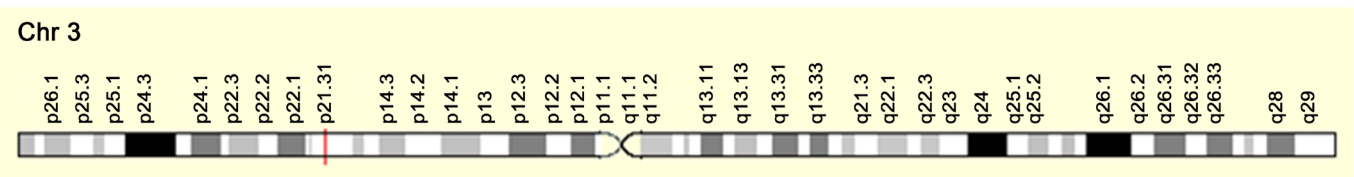

Figure 1. Chromosome 3 showing position 21 where CCR5 gene is located.

cal Committee in line with Helsinki Declaration. Subjects were informed on the objectives, benefits and procedure of the study and they were reassured of confidentiality. Written Informed consent was obtained from each subject. The investigations were carried out at no cost to the subjects.

3. Study Population

Adult residents of Yenagoa within the ages of 19 to 30 years who attended Voluntary HIV Counselling and Testing Centre, HIV/STI Division, Department of Public Health, Bayelsa State Ministry of Health, Yenagoa. HIV negative subjects were used while, HIV positives subjects served as control.

3.1. Sample Size

The sample size was determined using W.G Cochran's method [16]; Sample size = $z^2 \times p \times q/d^2$ Where p = Prevalence in Africa which is 1% Q = 1 − p, z = confidence level (z-score) at 95% is 1.96, d = acceptable p-value given as 0.05. Gives a sample size of 15 which is statically significant but, to expand the study scope 100 subjects was recruited while only 20 samples were sent for DNA sequencing and bioinformatics.

3.2. Sample Collection

Blood was collected aseptically from the median cubital vein of subjects into 4 ml EDTA Vacuumtainer tubes using USAID/FHI360 method [17].

3.3. Molecular Studies

DNA Extraction

Genomic DNA was isolated from the EDTA anti-coagulated whole blood samples using Quick gDNA Mini Prep kits supplied by Zymos Research Inqaba, South Africa. Procedure for extraction was according to the manufacturer's Protocol on the extraction of gDNA from whole blood. Extracted DNAs were stored at −20˚C elsius until used.

3.4. PCR Reaction Mixture

A total of 100 polymerase chain reactions were performed in a 20 ul PCR reaction mix as shown below;

Master mix = 10.0 ul,
Forward Primer = 0.20 ul,
Reverse Primer = 0.20 ul,
Template = 3.0 ul,
PCR Water = 6.6 ul,
Total = 20 ul.

A portion of the CCR5 gene from gDNA was amplified by 2 Stage PCR reaction using GeneAmp 9700 PCR system utilizing primers that would flank the 32-bp deletion (**P1(2975)**, 5'CAAAAAGAAGGTCTTCATTACACC-3' and **P2(2976)**, 5'-CCTGTGCCTCTTCTTCTCATTTCG-3'. Steps of PCR were:

1) Initial Denaturation,
2) Denaturation,
3) Annealing,
4) Extension.

Steps 2 to 4 were repeated in 5 cycles and 35 cycles for each DNA sample in stages 1 and 2 respectively.

4. Agarose Gel Electrophoresis

This was done on a portion of the CCR5 gene PCR amplicons. The 2.5% agarose gel contained ethidium bromide 1 µg/ml as stain. A 10 µl aliquot of each 20 µl PCR reaction mixture ran on a 2.5% agarose gel alongside a GeneRuler DNA Ladder Mix (ready-to-use; Thermo Fischer Scientific, Waltham, MA) to determine the lengths of the PCR products and the appropriately sized products was visualized under UV illuminator and the bands were micro-photographed.

4.1. Sequencing

The CCR5-PCR amplicons were sequenced using the Bigdye Terminator Kit on an ABI 3510 sequencer by Inqaba Biotechnological, Pretoria South Africa.

4.2. Phylogenetic Analysis

Sequences obtained were edited by the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using ClustalX. Evolutionary lineage was inferred using the Neighbour-Joining method [18] in MEGA 6.0. The bootstrap [19] consensus tree inferred from 500 replicates was taken to represent the evolutionary lineage of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [20].

Polymorphism Analysis

The aligned sequences of the DNA fragment of the isolates were translated to protein sequences using the bioinformatics software MEGA 6.

4.3. Results

A total of 100 subjects, were recruited for the study, among them 75 (75%) were HIV negative and 25 (25%) were HIV positive controls. Sequencing was done for only 20 samples. The allelic frequencies of these genes were: 0% and 100% respectively for CCR5-Delta32 and CCR5 (**Table 1**).

Phylogenetic analysis of sequences obtained from the 20 CCR5 gene PCR amplicons when compared with similar sequences from the NCBI data base using BLASTN showed that subjects were homozygous for the CCR5 gene (**Table 2**). Sequences were aligned using ClustalX. Bioinformatics for polymorphism on the 20 CCR5 gene PCR amplicons using MEGA 6.0 showed no single nucleotide polymorphisms (SNPs)

Table 1. Showing demography of study population.

Age Range in Years	No.	% Male	% Female	Group % of Population
19 - 21	31	25.8	74.2	31
22 - 24	23	26.1	73.9	23
25 - 27	21	19.0	81.0	21
28 - 30	25	36.0	64.0	25

Mean age 24.3 Male 27% Female 73%.

Table 2. Showing CCR5 distribution in the study population.

HIV Status	Number of Base Pairs	Homozygous CCR5 %	Homozygous CCR5Δ32 %	Heterozygous CCR5Δ32 %
Negative	189	75	0	0
Positive	189	25	0	0

among the 20 sequenced samples, since their protein sequences were conservatively the same (**Table 3**) when compared to standard CCR5 gene from NCBI. Gene.

Figure 2 shows the agarose gel electrophoresis of CCR5 gene PCR amplicons for the 75 HIV negative and 25 HIV positive subjects.

5. Discussion

In Nigeria, the HIV prevalence recorded in Bayelsa State in 2007 was ranked the third highest and thus became a flash point [21]. It therefore became necessary for deliberate research to be carried out to explain why this HIV prevalence could assume such an alarming proportion. It has been reported that a 32 base pair deletion (CCR5Δ32) in the coding region is a non functional receptor that confers resistance to HIV and some degree of protection from HIV infection. [8] [9] and CCR5 is a co-receptor for human immunodeficiency virus (HIV) that mediates HIV infection [1] [2]. However, there is paucity of information relating to studies done on SNPs and the nucleotide sequences of this gene in Nigeria. Hence, this study was aimed at investigating SNPs of CCR5 gene if any, among the Ijaws of the Niger Delta region of Nigeria. .

Our study revealed that 75 (100%) of the HIV negative subjects had 189 base pair in their CCR5 gene. The agarose gel electrophoresis of CCR5 gene PCR amplicons for the 75 HIV negative and 25 HIV positive subjects showed no genetic deletion on the CCR5 genes of the subjects, as it read 189bp for both the HIV negative subjects and HIV positive controls when compared with a Gene Ruler DNA Ladder Mix.

These results obtained in this study corroborate some earlier findings that CCR5Δ32 confers some degree of protection from HIV. [6] [7] [8]. However, one study found SNP of CCR5 gene to be absent (0/268) in Central Africa [15].

In 1997, a man homozygous for CCR5Δ32 was found to be infected with HIV-1 after receiving several pints of infected blood. This finding was uncommon and exceptional and raised so much questions to the authenticity of CCR5Δ32 homozygosity providing

Table 3. Translated nucleotide sequences of the amplified CCR5 segment from the subjects.

Seq Name	Protein sequence
1_R5F_H10_22	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
2_R5F_A11_02	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
3_R5F_B11_05	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
5_R5-F_D05_11	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
7_R5-F_F05_17	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
8_R5-F_G05_20	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
9_R5-F_H05_23	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
10_R5-F_A06_03	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
11_R5-F_B06_06	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
12_R5-F_C06_09	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
13_R5-F_D06_12	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
14_R5-F_E06_15	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
15_R5-F_F06_18	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
16_R5-F_G06_21	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
17_R5-F_H06_24	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
18_R5-F_A07_01	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
19_R5-F_B07_04	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR
20_R5-F_C07_07	FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNEK KRHR

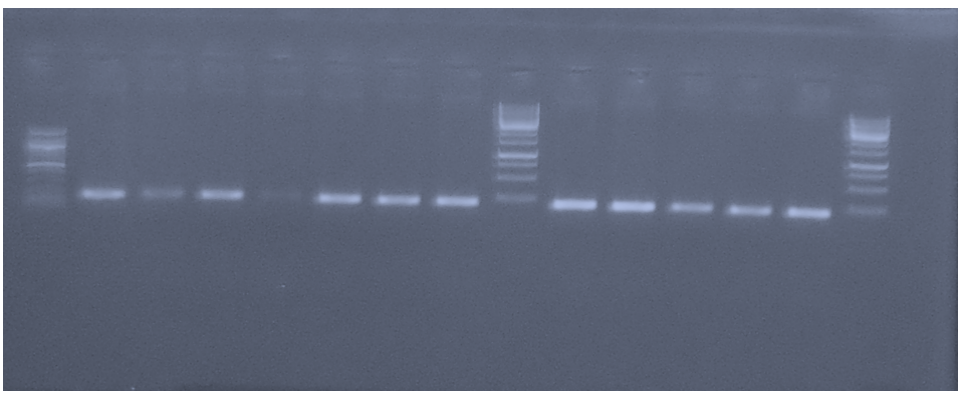

Figure 2. Photomicrograph of the agarose gel electrophoresis of the CCR5 gene (189bp). Lane 1 - 12 represent the CCR5 bands while M, N and L represent 100 bp, 1 kb and 1kb ladder respectively.

strong relative resistance to parenterally acquired HIV-1 infection [22]. However, in this exceptional case, it was thought that the subject might have been infected with HIV-1 strain that can use an alternative receptor.

The results of periodic national surveys among ante-natal clinic attendees in Nigeria

has shown a progressive decrease in the adult HIV sero-prevalence rate from 6.5% in 2007 [21] to 2.7% in 2012 [23]. This low prevalence could be as a result of concerted HIV education from both the State Ministry of Health and non-governmental agencies in the fight against HIV/AIDS in the State. In fact, the prevalence rate of 2.7% in Bayelsa State as it is now is far lower than the National prevalence of 3.4% [23]. It could be deduced that the large health sector response, and now a multi-sectoral response that focuses on prevention, treatment and mitigation of impact interventions has paid off in reducing the HIV prevalence. Such efforts need to be sustained especially in regions like this where there is nothing in our genetic makeup that confers resistance to the dreaded HIV infection.

6. Conclusion

We concluded that the nucleotide sequences of CCR5 gene are highly conserved and are all homozygous among HIV negative individuals living in this part of the globe. Hence, no protection is guaranteed if exposed to HIV CCR5 tropic strain. In other words, infection of such individuals with HIV predisposes them to rapid progression towards AIDS.

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