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Innate Resistance to HIV by CCR5-delta 32 in Serodiscordant Couples in Kinshasa Hospitals

Isaac Woto Tshikuma*
Molecular Biology Service, Department of Basic Sciences,
Faculty of Medicine, University of Kinshasa, Kinshasa,
Democratic Republic of Congo

Simplice Makoka Kivuvu
Faculty of Medicine, Université Technologique Bel Campus,
Kinshasa, Democratic Republic of Congo

Charlotte Tshinguta Londji
Central Veterinary Laboratory, Kinshasa,
Democratic Republic of Congo

Berry Bongenya Ikolango
Faculty of Medicine, Université Technologique Bel Campus,
Kinshasa, Democratic Republic of Congo

Erick Kamangu Ntambwe*
Molecular Biology Service, Department of Basic Sciences,
Faculty of Medicine, University of Kinshasa, Kinshasa,
Democratic Republic of Congo

ABSTRACT

Background: Worldwide, around 1% of people remain free of Human Immunodeficiency Virus infection without receiving Antiretroviral Therapy. This innate resistance is genetic or immunological in origin, and one of the main mechanisms reported to date is the homozygous Δ32 mutation of the CCR5 gene. Objective: Search for the delta 32 mutation of the CCR5 gene in HIV discordant couples in Kinshasa. Methods: Descriptive cross-sectional study of different HIV serodiscordant heterosexual couples followed at CHME Monkole and CS Sainte Anne in Kinshasa, DRC, from November 2021 to June 2022. Data were collected from information sheets drawn up by the research team and from computerized patient records in the Infectious Diseases Unit of these centers. Blood was collected from a peripheral vein in an EDTA tube. It was used for biomolecular analyses. Conventional PCR was performed to detect the CCR5-delta 32 mutation. Results: Of a total of 514 heterosexual couples followed for HIV, 472 were sero-concordant and 42 were HIV-serodiscordant, representing a discordance frequency of 8.2%. Eighteen discordant couples (3.5%) consented to participate in the present study. The heterozygous CCR5-delta 32 mutant genotype was found in 11.1% of HIV-negative partners. The chi-square test did not show a significant relationship between serodiscordance and the CCR5-delta 32 mutation. Conclusion: The frequency of HIV-discordant couples and of heterozygous CCR5-delta 32 mutation

is significant. CCR5-delta 32 may account for the serodiscordance of couples in the DRC.

Keywords: CCR5 delta-32, Discordant couples, Innate HIV resistance, Kinshasa.

INTRODUCTION

Despite intensive research into Human Immunodeficiency Virus (HIV) infection, there is neither a vaccine to prevent the disease nor a treatment to completely eradicate this viral infection [1]. Various preventive and curative approaches are available to reduce the risk of transmission of the virus, in an attempt to curb the epidemic [2]. Although modern anti-retrovirals (ARVs) provide sufficient control of viral replication to achieve high-quality immune restoration and reduce the number of AIDS-related deaths, some viruses are not neutralized and remain within immune cells (T lymphocytes and macrophages), where they constitute virus reservoirs [3,4]. As a result of ARV treatment, HIV/AIDS has become a chronic disease [3]. However, around 1% of people, despite being highly exposed to HIV, remain free of infection without receiving antiretroviral treatment. These are called exposed seronegatives (ESN). Among these groups are HIV-negative people in serodiscordant couples, sex workers [5,6], homosexuals whose partners had died of AIDS, haemophiliacs who received contaminated blood [7], exposed healthcare workers, and infants of HIV-1-infected mothers who were exposed in utero [5].

Studies carried out in various ESN cohorts have not led to the establishment of a single mechanism responsible for protection against HIV infection [1], rather, they have demonstrated that the mechanisms of natural resistance to HIV are varied, essentially genetic and immunological in particular [1,6]: deletion of 32 base pairs of the gene coding for the CCR5 or R5 co-receptor (CCR5-delta 32) [8], heterozygosity or discordance of HLA class I alleles (HLA B27 and B57) [9,10], RNases with anti-HIV-1 activity [11], Tripartite motif-containing 5α (TRIM-5alpha) [12], Apolipoproteins B mRNA Editing enzyme Catalytic polypeptide-like 3 (APOBEC3) [13–15], Secretory Leukocyte Protease Inhibitor (SLPI) [16] including Trappin-2 [17], α-defensins [18] and β-defensins 2 and 3 [19], β-chemokines, including Regulated on Activation Normal T-cell Expressed and Secreted (RANTES) and Macrophage Inflammatory Protein (MIP-1 α et MIP-1 β) [20,21], Stromal-cell-Derived Factor 1 (SDF-1) [22], Interferons Alpha (IFN α) [23] and Gamma (IFN γ) [24], CAF [25], Leukemia Inhibitory Factor (LIF) [26], lysozymes, Alloantigen-Stimulated Factor (ASF) [27], HIV-specific cytotoxic T lymphocytes [6], HIV-1-neutralizing IgA antibodies [28], as well as high levels of spontaneous and HIV-1-induced apoptosis [1]. This suggests that the determinants of resistance depend on a complex association of several genes affecting the immune response and viral sensitivity of host cells [29].

In doing so, the main mechanism so far reported to be responsible for absolute resistance to infection by M-tropic strains of HIV-1 is the homozygous Δ32 mutation in the ccr5 gene [1,30]. Some studies had shown that this mutation was absent in sub-Saharan African populations, and could not be designated as the reason for the resistance found in these populations [30]. ther findings have also limited the existence of the CCR5-delta 32 mutation to the regions of Northern Europe, Western Asia and North Africa [31]. However, recent studies in sub-Saharan Africa have shown the opposite [32,33].

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A study of samples taken from people living with HIV (PLHIV) between 1987 and 2019 in the DRC has revealed that the DRC group is the largest detected in any country, with between 2.7% and 4.3% of HIV-positive people able to suppress the virus naturally. A fact that has never been reported elsewhere; and no one knows how these people neutralize their HIV infection. This means that, instead of a small number of people as in previous studies, thousands of "elite HIV controllers" in the DRC could be used to demonstrate how to design a treatment or develop better vaccines to wipe out HIV/AIDS [34].

The phenomenon of discordance among HIV-infected couples in the DRC needs to be untangled [33]. Discordant couples are those in which one partner is infected with HIV while the other is not [35]. Discordance among infected couples is a common phenomenon in sub-Saharan Africa and has always been a challenge in the fight to prevent and cure HIV in the population [36,37]. The cysteine-cysteine chemokine receptor type 5 (C-C chemokine receptor type 5 or CCR5), also known as CD195, CC-CKR5, CKR-5, CMKBR5, belongs to the beta chemokine receptor family of integral membrane proteins, in the Cysteine-Cysteine chemokine group; as such, it participates in the immune system [38–41]. CCR5 is a G protein-coupled receptor [42,43]. The wild-type CCR5 protein (CCR5-wt) consists of 352 amino acids and is folded into seven transmembrane domains linked by three extracellular and three intracellular loops [44]. Several forms of the HIV virus use this receptor to enter host cells [38]. In interaction with its natural ligands MIP-1α, MIP-1β and RANTES , CCR5-wt is involved in leukocyte recruitment to sites of inflammation [39]. CCR5 receptors are found on the surface of several types of cells, in particular on certain leukocytes such as lymphocytes, monocytes, macrophages and eosinophils, on lymphoid tissue, on dendritic cells, on neurons, astrocytes and glial cells in the central nervous system, on endothelial cells, smooth muscle cells and fibroblasts, and on a subpopulation of breast and prostate cancer cells [40,42,43,45]. In humans, the ccr5 gene, which codes for the CCR5 protein, is located on the short arm (p) of chromosome 3 at position 21 [46]. CCR5-delta 32 (CCR5-Δ32 or CCR5-D32) is a CCR5 allele with a 32-base-pair (bp) deletion in the coding region that introduces a premature stop codon and whose product is a truncated protein resulting in a non-functional receptor that cannot be detected at the cell surface [39,44,47]. CCR5 Δ32 has a heterozygous allele frequency of 10% in Europe and a homozygous frequency of 1% [48]. In homozygous individuals (Δ32/Δ32), the truncated protein product cannot undergo post-translational modifications, and therefore remains retained in the endoplasmic reticulum [47]. They are naturally protected against HIV (resistant to M-tropic strains) [49]. Heterozygous individuals (+/Δ32) have a greater than 50% reduction in cell surface CCR5 receptors as the CCR5-Δ32 mutant confines itself to the endoplasmic reticulum and dimerizes or forms heteroplexes with the normal CCR5 product, also retaining it in the endoplasmic reticulum [47,50]. Thus, normal CCR5 can no longer mediate HIV-1 infection, which explains the reduced viral loads and the 2 to 3-year delay in progression to AIDS compared with wild types [44,47,51,52]. They have a late onset of AIDS symptoms [49].

This study aims to detect for the first time the CCR5-delta 32 mutation rate in serodiscordant couples with HIV infection in Kinshasa.

METHODS

Collection and Storage

An average of 8 cc of blood was collected from a peripheral vein in the forearm of each subject, and stored on EDTA tubes at -20°C. 4 spots of blood per couple (2 spots of 200 µL for each

partner) were placed on filter paper and air-dried. The rest of the sample was used for other analyses. The well-labelled dried filter paper was placed in the individual zyploc bag with silicagel; it was stored at -20°C, and used for bio-molecular analyses (PCR) carried out after DNA extraction.

Preparing the PCR Mix (Master Mix or Premix)

PCR was performed to amplify CCR5-delta 32 DNA using two pairs of primers (Integrated DNA Technologies, BVBA, Interleuvenlaan 12A, 3001 Leuven Belgium):

Table 1: CCR5-delta 32 primers

CCR5-delta 32 primers	Sequences
Forward 1	5'-CTT CAT TAC ACC TGC AGC T-3'
Reverse 1	5'-TGA AGA TAA GCC TCA CAG CC-3'
Forward 2	5'-ACC AGA TCT CAA AAA GAA GGT CT-3'
Reverse 2	5'-CAT GAT GGT GAA GAT AAG CCT CAC A-3'

As some data (such as fragment size in post-PCR) were not provided by the supplier, we resorted to bioinformatics to search for primer characteristics. We used the primer Blast tool from the National Center for Biotechnology Information (NCBI) [53]. The analyses focused on the ccr5 gene, not on the transcripts. The sequence used to search for primers was obtained in fasta format from NCBI data sets [54]. We considered that the deletion on the variant transcript *NM_001394783.1* (CCR5) occurs in the CCR5 gene sequence from nucleotide position 3315 to nucleotide position 3346, a position we identified by aligning the deletion sequence (GTCAGTATCAATTCTGGAAGAATTTCCAGACA) with the previously obtained CCR5 gene sequence. We used the "Naïve Exact Matching" algorithm with the Python 3 language to do this. The results were as follows:

Table 2: Primer characteristics

Primers	Length (number of nucleotides)	Start	Stop	GC %	Frag. Δ32	Frag. wt
F1	19	3280 bp	3299 bp	47.37	164 bp	196 bp
R1	20	3444 bp	3424 bp	50.00		
F2	23	3259 bp	3282 bp	39.13	193 bp	225 bp
R2	25	3452 bp	3427 bp	44.00		

Frag.: Fragment, bp: base pair, F: Forward, R: Reverse

F1 and R1 are sufficiently stable given their GC content which is about 50%.

The 32 bp deletion is located from nucleotide 3314 to nucleotide 3345.

Freeze-dried primers were prepared according to the manufacturer's instructions. Concentrations were multiplied by 10 to obtain the volume of PCR water to be added and to make up the 100 µM stock solution. The working solution was 2.5 µM, obtained after a 40-fold dilution of the stock solution.

The dNTPs were already available in liquid form at a concentration of 100 mM, which we reduced to 2 mM by making a 50-fold dilution of the stock solution.

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The amplification buffer was Mg Cl2 10 X concentrate.

We used Taq DNA polymerase 250 U (5 U/µL) from Roche Diagnostics (Indianapolis, IN, USA). Sold in liquid form.

Amplification of the ccr5-delta 32 Gene

As no previously published protocol had worked, we set out to develop one that would work under our conditions.

Samples, controls and reagents were allocated as follows:

N = 48 (36 samples + 2 positive controls + 2 negative controls + 8 extra or excess), which were divided into 2, for HIV- and HIV+ respectively.

Table 3: PCR Mix

Batch no	#µL/sample	#µL/sample +8 = 40+8=48	TOTAL (µL)
H2O	6.9 µL	X 48	331.2
Primer F1 (2.5 µM)	2 µL	X 48	96
Primer R1 (2.5 µM)	2 µL	X 48	96
dNTP's (2 mM)	2 µL	X 48	96
Amplification buffer (MgCl2)	2 µL	X 48	96
Taq DNA Pol. (5U/µl)	0.1 µL	X 48	4.8
TOTAL	15 µlL	X 48	720
Ajouter 5 µL d'ADN du patient			

Total reaction volume was 20 µL (15 µL mix + 5 µL DNA).

The mix (720 µL) was mixed (vortexed and centrifuged) and 15 µL was dispensed into each PCR tube or strip (40 strips in total).

5 µL of patient DNA and 5 µL of water (negative control) were added to the corresponding strips and labeled.

Strips were sealed with caps (covered with strip) and placed in the thermal cycler.

As we had no positive control, we used only the negative control.

The reaction was then carried out using an Appliedbiosystems by Thermo Fisher Scientific Veriti 96 Well Thermal Cycler, following the program below:

Table 4 : PCR program

Stages	Temperatures	Duration	Number of cycles
Initial denaturation	95 °C	5 min	
Denaturation	95 °C	30 sec	32 cycles
Hybridization	56 °C	30 sec	
Elongation	72 °C	30 sec	
Final elongation	72 °C	5 min	

At the end of these steps, the holding temperature was 4°C.

Agarose Gel Preparation

The 2% agarose gel was prepared with 1X TAE buffer and 2.5 µL of GelRed ;
It is important to use an appropriate DNA ladder that covers lengths of 100 bp. The agarose gel was poured into the mold with a comb, resulting in 16 x 1 mm wells.

Electrophoretic Migration on Agarose Gel:

The following steps were followed:

- Place 10 µL of each PCR product (amplicons) and 2 µL of Bromophenol blue in the corresponding microtubes;
- Place 2 µL of molecular weight marker (Leadder), 5 µL of water and 2 µL of Bromophenol Blue in its microtube;
- Place the mixture in the respective wells;
- Allow to migrate by agarose gel electrophoresis (2%) at 60 V for 2 hours.

Gel Reading and Detection of CCR5-delta 32 Mutation

Reading was performed on a transilluminator with UV illumination. Photographs were taken (Figures 3 and 4).

Two primers were used to detect the CCR5-delta 32 mutation by PCR as previously described. Only the primer pair F1 and R1 was found to be efficient in amplifying the CCR5-delta 32 gene fragment, and was therefore selected for the study (Figure 3).

To verify the results (that these were indeed the two bands), PCR of the amplicons was performed with primer pair A1 (F1 and R1), and the two bands were visualized via the 2% agarose gel by UV transillumination (Figure 4).

As this protocol has given results even for extracted DNA of lesser quality and on multiple occasions, it can work under all conditions.

Setting, Type and Period of Study

This is a descriptive, cross-sectional study. The Centre Hospitalier Mère et Enfant Monkole (CHME Monkole) and the Centre de Santé Sainte Anne (CS Sainte Anne) were selected on the basis of record keeping, couple management and accessibility. The inclusion period was from November 2021 to June 2022, i.e. 8 months.

Study Populations

All heterosexual couples discordant for HIV followed at CHME Monkole and CS Sainte Anne were included in the present work after reading and signing an informed consent. Couples were selected in pairs for data compliance.

Sample

Our sampling was based on convenience. We considered all serodiscordant couples identified during the study period, followed up at CHME Monkole and CS Sainte Anne, and who met the inclusion criteria.

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Selection Criteria

Inclusion Criteria:

- All discordant couples after reading and signing an informed consent form;
- Couples must be paired for data conformity.

Non-inclusion Criteria:

- All HIV sero-concordant couples;
- All PLHIV or HIV-negative people not living in couples;
- All sero-negative couples.

Parameters of Interest

Apart from couple serology, the biomolecular parameter of interest is the detection of the CCR5-delta 32 mutation.

Data Collection and Analysis

Data were collected from information sheets drawn up by the research team, and from computerized patient records at the Infectious Diseases Units of CHME Monkole and CS Sainte Anne. A database was created using Excel (Microsoft 2016), then exported to SPSS version 25.0 for descriptive analysis. Variables were presented as proportions (%). Our results are presented in tabular form (containing absolute values and percentages). The Chi-square test was used to look for associations between the variables studied and serological status.

Ethical Considerations

The present study was approved by the research ethics committee of the School of Public Health, Faculty of Medicine, University of Kinshasa (Ref: ESP/E/115/2021). Authorization to access CHME Monkole and CS Sainte Anne was obtained from the competent authorities of these Centers. Prior to inclusion, informed consent was obtained from each patient. Blood samples for the couples were taken by the technical teams of these two institutions. The results of the bio-molecular analyses were returned to them at the end of the work.

RESULTS

Frequency

Out of a total of 514 couples followed for HIV, 42 were HIV serodiscordant, i.e. a frequency of 8.2%, of whom 18 couples, i.e. 3.5% of discordant couples out of all couples followed, consented to participate in the present study. The wild-type CCR5 genotype was identified in all seropositive patients (100% or 18/18 cases) and in 88.9% (16/18 cases) of seronegative patients. The homozygous CCR5-delta 32 mutant genotype was not identified (0%), while the heterozygous CCR5-delta 32 mutant genotype was found in **11.1%** (2/18 cases) of HIV-negative patients. The Chi-square test showed no significant relationship between serodiscordance and CCR5-delta 32 mutation (p=0.146) (Table 5).

Table 5 : Frequency of CCR5-delta 32 mutation in HIV discordant couples

Variables	VIH – n (%)	VIH+ n (%)	TOTAL n (%)	p-value
CCR5 wild type	16 (88.9%)	18 (100.0%)	34 (94.4%)	0.146
CCR5 Δ 32 homozygote	0 (0%)	0 (0%)	0 (0%)	
CCR5 Δ 32 heterozygotes	**2 (11.1%)**	0 (0%)	2 (5.6%)	
TOTAL	18 (100.0%)	18 (100.0%)	36 (100.0%)	

n = number, % = percentage

Detection of the CCR5-delta 32 Mutation

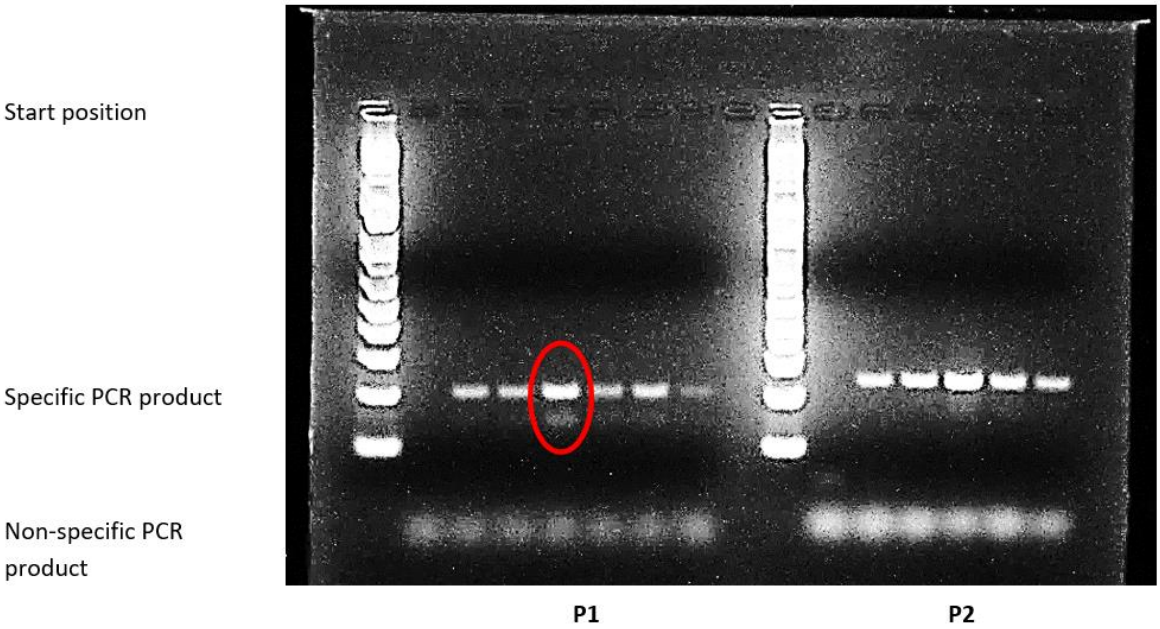

Figure 1 : Agarose gel electrophoresis of DNA isolated from HIV serodiscordant pairs.

This figure shows a 16-well gel, where two molecular weight markers (100 bp DNA ladder) were placed in the 1st and 10th wells, respectively. In the 2nd and 11th wells were placed the negative controls (PCR water). In wells 8 and 16 were placed the DNA of seropositive patients, and in the remaining wells were placed the DNA of seronegative patients. A1 represents the part concerning the 1st pair of primers (F1 and R1) and A2 represents the part concerning the 2nd pair of primers (F2 and R2). For the same sample, A1 gave a result (5th well) and A2 gave nothing (14th well). Well 5 revealed 2 bands (at 196 bp and at 164 bp), which is characteristic of the heterozygous CCR5-delta 32 mutation. The remaining wells gave a 196 bp band, characteristic of wild-type CCR5.

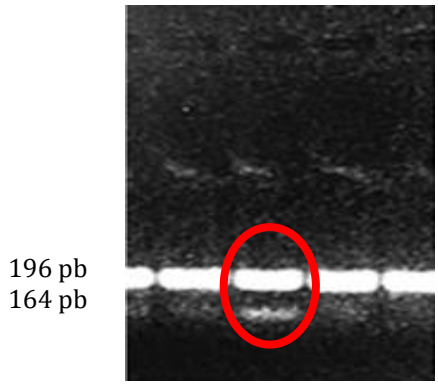

Figure 2: Agarose gel electrophoresis of isolated DNA from HIV serodiscordant pairs derived from double PCR.

This figure shows the visualization of 2 bands, one at 196 bp and the other at 164 bp for the same DNA sample from well 5 in Figure 3.

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DISCUSSION

This study investigated the CCR5-delta 32 mutation in HIV serodiscordant couples in Kinshasa. Eighteen (18) couples out of 42 responded to inclusion in the two medical units selected for the study.

In the present study, the frequency of serodiscordant couples was 8.2% of all couples followed for HIV. This high frequency corroborates the 8.4% found in Ethiopia in 2015 [55]. This frequency, although significant, is also low compared to the results of other authors who have found a serodiscordance prevalence of 20 to 30% in sub-Saharan African countries [56]. However, it remains higher than the 5.8% and 5.9% found in Kenya in 2007, respectively [57] and Ethiopia in 2011 [58].

In Africa, the recorded frequency of HIV serodiscordance varies widely. In Central Africa, it has been estimated at 5% in the Republic of Congo [59] ; in East Africa, it was 8.4% in Ethiopia [55], 12 % in Rwanda [60], 14% in Tanzania et 13.6% in Kenya [61] in West Africa, it was estimated at 21.6% in Togo [62] and 20% in Burkina Faso [63] ; in Southern Africa, it was 27% in South Africa, 14.2% and 20.1% respectively in Zambia and Botswana [61]. These divergent results could be attributed to the difficulties in the sub-region, border permeability, socio-economic problems, level of knowledge or education; but also to non-acceptance of one's serological status, stigmatization and sample size.

The wild-type CCR5 genotype was identified in all seropositive patients (100%) and in 88.9% of seronegative patients. The homozygous CCR5-delta 32 mutant genotype was not identified (0%), while the heterozygous CCR5-delta 32 mutant genotype was found in 11.1% of HIV-negative patients. Our results contradict data showing that the mutated CCR5 allele either does not exist or is rare in Africa. This frequency of 11.1% is significantly higher than those reported in Sudan (3.5%) in 2022 among sex workers [32] and in Nigeria (1.3%) in 2022 among HIV-negative partners of discordant couples [33], although they concerned the homozygous CCR5-delta 32 mutation. We believe that the presence of heterozygous CCR5-D 32 individuals proves the existence of individuals homozygous for this mutation. Furthermore, the predominance of the heterozygous CCR5-delta 24 mutation has been found to be greater in HIV-resistant African populations [64].

The Chi-square test showed no significant relationship between serodiscordance and the CCR5-delta 32 mutation (p=0.146), which is likely given that heterozygosity of CCR5-delta 32 alleles can be found in both HIV- and HIV+ patients. Heterozygous carriers (+/Δ32) have been shown to have a delayed onset of AIDS symptoms [49].

In view of our results, we wondered how likely it was that the virus would evolve to overcome the constraint imposed by the CCR5-Δ32 allele? Quite unlikely, we thought. The reason is that the allele prevents the virus from establishing a massive presence inside a host. The adaptive evolution of viruses depends in a complex way on the extent of selection pressure and the evolvability of the virus, which in turn depends on the number of viral genomes and the mutation rate [65]. A small viral population is much less likely to give rise to escape mutants than a larger one. Moreover, the virus is highly dependent on a fully functional CCR5 receptor. For this reason, it is thought that a potential strain of HIV that does not depend on CCR5 must differ from the normal wild-type by several nucleotides. For this reason, the evolution of an

escape mutant seems highly unlikely (even if intermediate states were fully viable). Finally, as the viral population size in carriers of the CCR5-Δ32 allele appears to be reduced, these individuals are less likely to infect others [46].

It has recently been shown that CCR5-Δ32 confers resistance not only because CCR5 does not reach the cell surface, but also because the truncated CCR5-Δ32 protein is able to down-regulate the expression of any CCR5 and CXCR4 on the cell surface by trapping them [66].

There is evidence that CCR5 downregulation rather than CCR5 detour in CCR5-Δ32 heterozygotes is responsible for the measured lower levels of surface CCR5 [67].

Study Limitations

Due to the rare nature of the study, it was difficult to enroll a large number of couples. The exclusion of people with multiple sexual partners had an influence on obtaining a sufficient number of couples. Consequently, the small sample size is a limitation of this study.
In addition, cross-sectional studies do not allow us to determine the sequence of cause and effect, which complicates the interpretation of associations.

CONCLUSION

The frequency of HIV-discordant couples and of heterozygous CCR5-delta 32 mutation is significant. Prevention of sexual transmission of HIV in serodiscordant couples is a major challenge, given the risky sexual behavior and the relatively low frequency of discordant couples compared with concordant HIV couples. In addition to other factors, CCR5-delta 32 may account for the serodiscordance of couples in the DRC.

Conflict of Interest

The authors declare no conflict of interest in this work.

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List of Abbreviations and Acronyms

APOBEC3: Apolipoproteins B mRNA Editing enzyme, Catalytic polypeptide-like 3,
ARV: AntiRetrovirals,
ASF: Alloantigen-Stimulated Factor,
CCR5: (also called CD195 or CC-CKR5, CKR-5, CMKBR5) Cysteine-Cysteine Chemokine Receptor Type 5 or C-C Chemokine Receptor Type 5 or CC-Chemokine Receptor Type 5,
CCR5 delta 32 (CCR5Δ32): Cysteine-cysteine chemokine receptor type 5 with a 32-base-pair deletion,

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CD: clusters of differentiation
CHME: Centre Hospitalier Mère et Enfant (Mother and Child Hospital)
CS: Centre de Santé,
CXCR4: CXC chemokine receptor type 4,
ESN: exposed seronegatives
HLA: Human Leucocyte Antigen,
IFN: Interferons,
IL: interleukin,
LIF: Leukemia Inhibitory Factor,
MIP: Macrophage Inflammatory Protein,
p: p-value,
PCR: Polymerase Chain Reaction,
PLHIV: People Living with Human Immunodeficiency Virus
RANTES: Regulated on Activation Normal T-cell Expressed and Secreted,
DRC: Democratic Republic of Congo,
SDF-1: Stromal-cell-Derived Factor 1,
AIDS: Acquired Immunodeficiency Syndrome,
SLPI: Secretory Leukocyte Protease Inhibitor,
SPSS: Statistical Package for the Social Sciences,
TALEN: Transcription Activator-Like Effectors Nuclease,
TNF-α: Tumor Necrosis Factor Alpha,
TRIM-5α: Tripartite motif-containing 5 α,
UNIKIN: University of Kinshasa,
UV: Ultraviolet,
HIV: Human Immunodeficiency Virus,
Wt: Wild type.

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