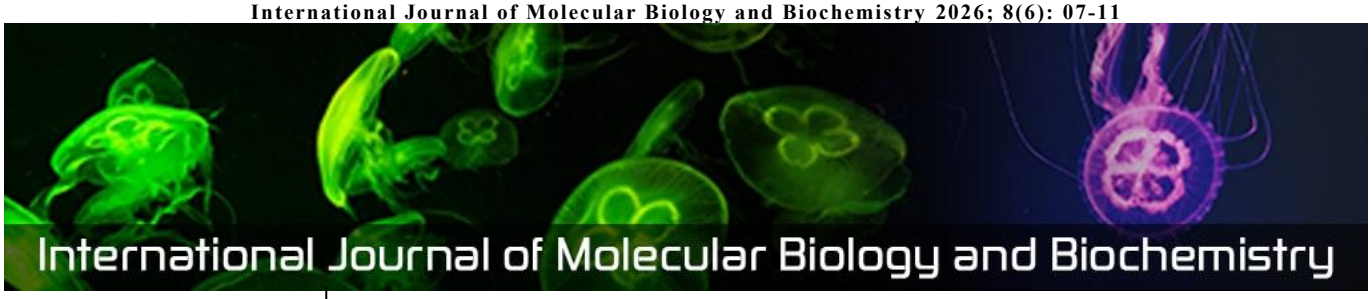

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Discriminating HIV-1 mother-to-child transmission genotypes using correspondence analysis

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Abstract
Background: Correspondence Analysis (CA) is a graphical method that help to analyse categorical variables. Biologists frequently use Odd Ratio (OR) as association measure on data collected from multiple variables. CA seems to be an underused method in biology. The objective of this study was to discriminate the mutated genotype of the CCR2 gene implicated to the increased risk of HIV transmission from mother to child using CA.
Materials and Methods: A case-control study was carried out in the North Region of Cameroon. Genotyping was carried out using biological techniques like Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP). Statistical analyses were performed using the IBM-SPSS version 26 and STATA version 19. Odd Ratios (OR) with 95% Confidence Interval (CI) were used as measure of association. CA was used to graphically discriminate whether a potential effect of mutation on HIV transmission was due to mutant homozygous or heterozygous genotype.
Results: Mothers carrying the mutated phenotype were 1.2 times more likely to have HIV-infected children compared to those without mutation (OR = 1.2, 95% CI: [0.5, 3.0]); thus, CCR2 mutated phenotype in mothers increased the risk of transmission. Nevertheless, the use OR can't permit to discriminate the genotype that was more responsible. Correspondence analysis showed that GA genotype (heterozygous) in mothers was closer to HIV+ compared to AA genotype which was more distant. This implied that the heterozygous genotype of the CCR2-64I gene was mainly responsible for the increased risk to have an HIV-infected child.
Conclusion: CA was able to discriminate genotypes mainly responsible to HIV MTCT. In biological studies involving many categorical variables, we recommend CA as complementary method to find associations between gene mutations and HIV transmission after using odd ratio as measure of association.

Keywords: Genotypes, HIV, correspondence analysis, Cameroon

Introduction
Statistical analysis is a critical component that support any finding whether from a clinical trial or other data collection [1]. Correspondence Analysis (CA) is a statistical and graphical method that help to analyse categorical variables. Multiple Correspondence Analysis (MCA) is an extension of CA that allow researchers to investigate associations among several categorical dependant data [2]. It is the multivariate extension of CA that help to analyse tables containing three or more variables [3] and it is easy to learn [4]. The main benefits of CA are: it shows relations and their strength between categorical variables, it is objective, it can be used on all types of categorical variables, it provides simple visualization of data [2]. This technique has been beneficial in areas ranging from health and medicine to social sciences, archeology, ecology, software development and market research [5-8]. However, CA is not yet widely used in biological research. Odds Ratios (ORs) are most widely used in case-control studies to identify risk factors associated with specific outcome measures; meanwhile, they can also be used in other types of studies, following a few adjustments, such as logistic regression analysis and cross-sectional studies. OR is best used when the proportion of a particular outcome is quite low. As this statistical measure provides information that helps doctors and their patients assess the benefits of a given treatment, it is increasingly used when the prevalence of a particular health outcome is not rare [9]. In the

case of HIV infection in children, the OR has been largely used as a measure of association. Among children, Mother-to-Child Transmission (MTCT) of HIV remains the primary route of infection [10]. Without treatment, the rate of transmission of HIV from a mother living with HIV to her child during pregnancy, labour, delivery or breastfeeding ranges from 15% to 45% [11]. Early Infant Diagnosis **(**EID) is a key way to prevent and manage MTCT, as it enables early detection of HIV in infant born to HIV-infected mothers [12]. Many factors have been associated to HIV MTCT, like host genetic factors. C-C motif chemokine receptor 2 (CCR2) is one of the co-receptors of HIV found on the surface of the target cell, studied as genetic factor associated with MTCT [13].

In biological research, large data are often collected on study participants. Much of these data are collected through questionnaires. However, the use of OR presents some limitations in the discrimination of genotypes and phenotypes related to HIV MTCT. For this reason, the CA technique was used in this study to better discriminate the genotypes of CCR2 gene. The objective of this study was to discriminate the mutated genotype of the CCR2 gene implicated to the increased risk of HIV transmission from mother to child using CA.

Materials and Methods

Study participants

This was a case-control study conducted in the North region of Cameroon from July 2015 to October 2016. The participants were the mother-child couples. Overall, 113 couples participated. The mothers were all HIV infected whereas 88 children were HIV-uninfected and 25 were HIV-infected. The study protocol was approved by the Cameroon National Ethical Committee for Research on Human Health under the number N°2013/11/375/L/CNERH/SP. Mothers freely consented by signing the informed consent form as well as parental consent and assent, when indicated.

Genotyping

Blood sample was collected in EDTA tube and HIV status determined. Serological tests named Determine HIV ½ and OraQuick HIV1/2 were used in mothers and children aged 18 months and more. In children under 18 months, the Abbott qualitative that directly detect the nucleic acid was used to detect the HIV status. For genotyping analysis, genomic DNA was extracted from the buffy coat followed with the amplification of the fragment of interest by Polymerase Chain Reaction (PCR) using the following pair of primers: forward: 5'-GGATTGAACAAGGACGCATTTCCCC-3' and reverse: 5'- TTGCACATTGCATTCCCAAAGACCC-3' [14]. On the amplificated PCR fragment, a restriction fragment length polymorphism (RFLP) test was conducted. The amplified fragment was first digested with restriction enzyme *Fok I*. The product of digestion was then migrated in an agarose gel and the genotypes were determined based on the number and length of the fragments visualized.

Statistical analyses

To look for the polymorphism and HIV acquisition, statistical analyses were performed using the IBM-SPSS version 26 and STATA version 19. Odd Ratios (OR) with 95% Confidence Interval (CI) were used as measure of association. Correspondence analysis (CA) was used to graphically discriminate whether a potential effect of mutation on child HIV acquisition was due to mutant homozygous or heterozygous genotype.

Results

Socio-demographic characteristics of the participants

A total of 113 HIV-infected mothers aged 16 to 43 years, and their 113 children (49.6% female) were enrolled. Children were aged under 15 years, amongst which 25 were HIV- infected and 88 HIV-uninfected.

Maternal CCR2 genotypes/phenotypes and children's HIV status using the odd ratio

The association between CCR2 genotypes, CCR2 phenotypes and HIV status is presented in the Table 1. The link between maternal genotypes children HIV status was that the frequency of heterozygous genotype GA was 4.9% higher in mothers who transmitted HIV to their infants compared to non-transmitters (95% CI: [-0.29, 0.20]). In terms of phenotypes, mothers carrying the mutated phenotype were 1.2 times more likely to have HIV-infected children compared to those without mutation (OR = 1.2, 95% CI: [0.5, 3.0]). This mean that the CCR2 mutated phenotype in mothers increased the risk of transmission. Nevertheless, we were unable to discriminate the genotype that was more responsible.

Table 1: Maternal CCR2 genotypes/phenotypes and children's HIV status

Gene variants	Children HIV-non infected n (%)	Children HIV- infected n (%)	Total n (%)	
Genotypes				Genotype frequency difference (95% CI)
G//G	43 (48.9)	11 (44.0)	54 (47.8)	-4.9[-0.20, 0.29]
G//A	38 (43.1)	12 (48.0)	50 (44.2)	4.9[-0.29, 0.20]
A//A	7 (8.0)	2 (8.0)	9 (80.0)	0[-0.12, 0.12]
Phenotypes				OR (95% CI)
Wild type	43 (48.9)	11 (44.0)	54 (47.8)	1
mutant	45 (51.0)	14 (56.0)	59 (52.2)	1.2[0.5, 3.0]

G//G: homozygous wild type genotype
G//A: heterozygous genotype
A//A: homozygous mutant genotype
Wild type phenotype includes G//G genotype
Mutant phenotype includes G//A and A//A genotypes
N: number of participants presenting the genotype

Association between maternal CCR2 genotypes and children's HIV status using CA

The result of CA showed that GA genotype (heterozygous) in mothers was closer to HIV+ compared to AA genotype which was more distant. This implied that the heterozygous genotype of the *CCR2-64I* gene was mainly responsible for the increased risk to have an HIV-infected child. CA was able to discriminate the CCR2 genotype more implicated with the transmission.

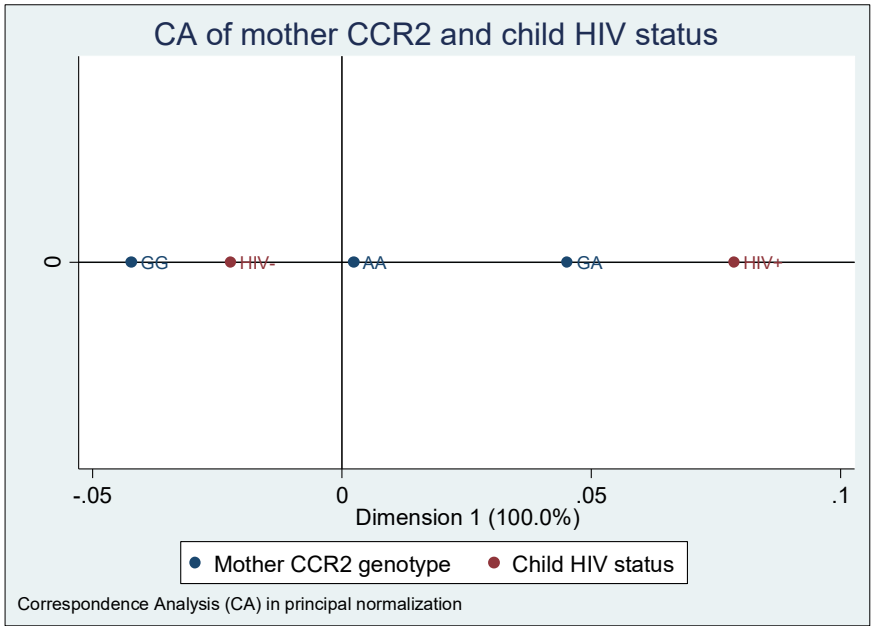

Fig 1: Association between maternal CCR2 genotypes and children's HIV status

Association between children's CCR2 genotypes and their HIV status

Figure 2 presents the relationship between children's CCR2 genotypes and their status. The result of CA showed that AA and GA genotypes of CCR2-64I gene in children were equidistant to HIV+. Therefore, both genotypes increased the risk of HIV acquisition in these children.

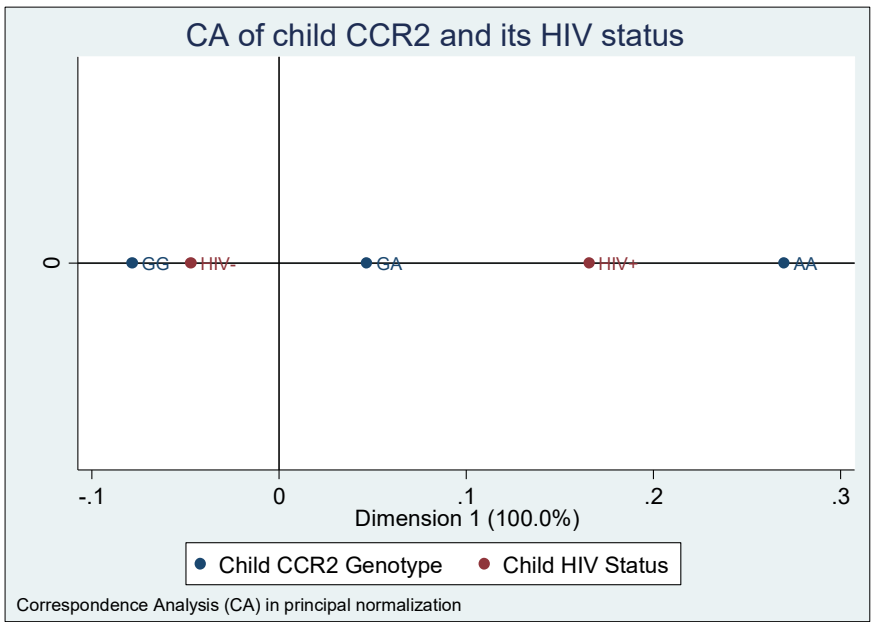

Fig 2: Association between children's CCR2 genotypes and their HIV status

Association between maternal and child CCR2 genotypes and children's HIV status using MCA

The relationship between maternal and child CCR2 genotypes and children's HIV status is presented in Figure 3. GA genotypes in mothers and in children were grouped around HIV+ than AA genotype. This indicates that the heterozygous genotype of CCR2-64I gene was implicated in HIV transmission.

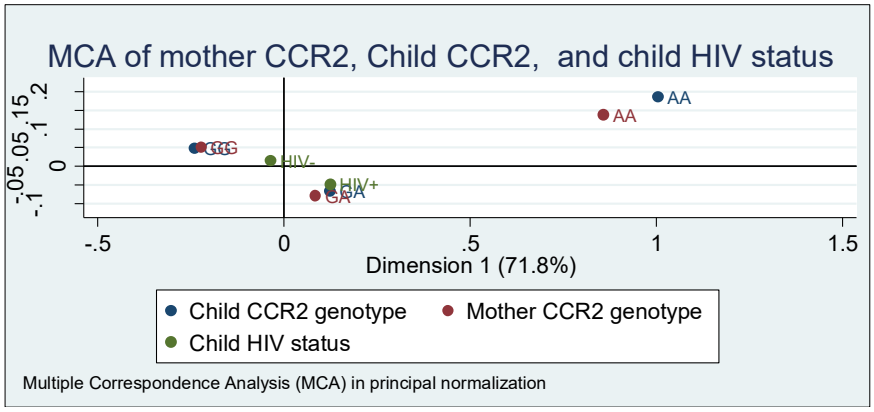

Fig 3: Association between maternal and child CCR2 genotypes and children's HIV status

Discussion

The objective of this study was to discriminate the mutated genotype responsible for the increased risk of HIV-1 mother-to-child transmission. The association using Correspondence Analysis (CA) gives the relationship among coded variables and their associations. The technique allows the analysis of the relationships between the variables and different levels of one variable. Furthermore, the results of the analysis can be seen analytically and visually [7].

In the present study, we firstly used odd ratio to find association between CCR2 genotypes/phenotypes and transmission of HIV-1 from an infected mother to her child. We found that mothers carrying the mutated phenotype were 1.2 times more likely to have HIV-infected children compared to those without mutation (OR = 1.2, 95% CI: [0.5, 3.0]). This mean that mutation increases the risk of transmission. Some studies also reported that CCR2 64I mutation was associated with the risk of MTCT [15], although others did not find any association [16-18]. In contrary, the mutation had a protective role in some researches [19-20].

The odd Ratio method used in this study did not help to determine the genotype responsible. We further used the CA and MCA methods to graphically represent and interpret the relations between genotypes and HIV transmission. The result of CA showed that the heterozygous genotype of the *CCR2-64I* gene in mother was mainly responsible for the increased risk to have an HIV-infected child. CA was able to discriminate the CCR2 genotype more implicated with the transmission. In children, both AA and GA genotypes of *CCR2-64I* gene increased the risk of HIV acquisition. The two genotypes were graphically equidistant to HIV+. By the used of MCA method, it was found in mothers and children that, the heterozygous genotype of *CCR2-64I* gene was implicated in HIV transmission. The graphic indicated that the GA genotypes were grouped around HIV+ than AA genotype. The result obtained clearly demonstrate that CA and MCA are useful techniques that can be used in biological studies. One study in Africa reported from multiple correspondence analysis that there was association between malaria RDT result and different socio-economic, demographic and geographic variables [3].

Conclusion

The present study indicates that MCA represents an important complementary technique that can be used to identify genotypes that are associated with HIV mother-to-child transmission. Odd ratio, as measure of association helped to determine phenotypes related to HIV-1 MTCT. Correspondence Analysis was able to discriminate genotypes mainly responsible to HIV-1 MTCT. In biological studies involving many categorical data, we recommended to use correspondence analysis statistical method to look for the association, as a complementary to odd ratio.

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