Scientific Research Publishing

American Journal of Molecular Biology, 2025, 15(3), 262-275
https://www.scirp.org/journal/ajmb
ISSN Online: 2161-6663
ISSN Print: 2161-6620

# Diversity of KIR Genes and Their Association with Human Papillomavirus (HPV) Infection in West Africa

**Mah Alima Esther Traore[1,2], Marius Ayaovi Setor[1], Teega-Wendé Clarisse Ouedraogo[1], Théodora Mahoukèdè Zohoncon[1,3], Dorcas Obiri-Yeboah[4], Régine Ilboudo[3], Abel Pegdwendé Sorgho[1,5], Prosper Bado[1], Abdoul Karim Ouattara[1,5], Albert Théophane Yonli[1,5], Apollinaire Horo[6], Moutawakilo Gomina[7], Mady Nayama[8], Simon Akpona[7], Simplice Damintoti Karou[9], Charlemagne Ouedraogo[10], Florencia Wendkuuni Djigma[1,5*], Jacques Simpore[1,3,5]**

[1]Université Joseph KI-ZERBO, Laboratory of Molecular Biology and Genetics (LABIOGENE), Ouagadougou, Burkina Faso
[2]National Center for Scientific and Technological Research (CNRST) Institute for Research in Applied Sciences and Technologies Ouagadougou, Burkina Faso
[3]Faculty of Medicine, University Saint Thomas d'Aquin, Ouagadougou, Burkina Faso
[4]Department of Microbiology and Immunology, School of Medical Sciences, University of Cape Coast, Cape Coast, Ghana
[5]Pietro Annigoni Biomolecular Research Centre (CERBA), Ouagadougou, Burkina Faso
[6]Department of Obstetrics and Gynecology, University Félix Houphouët Boigny, UFR Des Sciences Médicales D'Abidjan, Abidjan, Ivory Coast
[7]Faculty of Medicine, University of Parakou, Parakou, Benin
[8]Faculty of Health Sciences, University Abdoul Moumouni, Niamey, Niamey
[9]Regional Center of Excellence in Poultry Science, University of Lomé, Lomé, Togo
[10]University Joseph KI-ZERBO, Health Sciences Training and Research Unit (UFR/SDS) Ouagadougou, Burkina Faso
Email: *florencia.djigma@gmail

**How to cite this paper:** Traore, M.A.E., Setor, M.A., Ouedraogo, T.-W.C., Zohoncon, T.M., Obiri-Yeboah, D., Ilboudo, R., Sorgho, A.P., Bado, P., Ouattara, A.K., Yonli, A.T., Horo, A., Gomina, M., Nayama, M., Akpona, S., Karou, S.D., Ouedraogo, C., Djigma, F.W. and Simpore, J. (2025) Diversity of KIR Genes and Their Association with Human Papillomavirus (HPV) Infection in West Africa. *American Journal of Molecular Biology*, **15**, 262-275.
https://doi.org/10.4236/ajmb.2025.153018

**Received:** April 2, 2025
**Accepted:** June 27, 2025
**Published:** June 30, 2025

## Abstract

**Background**: Cervix cancer is the leading cause of cancer death in women in many developing countries, and most commonly the result of human papillomavirus (HPV) infection. Most often, this infection does not lead to injury or cancer - there is viral clearance. We therefore proposed to study the polymorphism of certain genes such as KIR, which seems to have a strong association with HPV infection. We aimed to contribute to the fight against HPV virus infection, through better knowledge of circulating high risk-HPV (HR-HPV) genotype, and the involvement of KIR genes in the progression to cervical cancer. **Methods**: A total of 1419 samples, taken from the endocervix, were collected. The women came from 6 countries in West Africa (Burkina Faso, Benin, Côte d'Ivoire, Niger, Nigeria, and Togo). These samples were analyzed by multiplex real-time PCR to search for fourteen HR-HPV genotypes, and the

Copyright © 2025 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/
Open Access

KIR genes were characterized using the SSP-PCR method. **Results**: The results showed that 35.94% (510/1419) of women were infected with HR-HPV. KIR genes were significantly more common in HPV-infected subjects than in controls. The inhibitory KIR genes were, *KIR* 2*DL*3 [OR = 2.24, CI (95%) (1.48 - 3.39) p < 0.001], *KIR* 2*DL*4 [OR = 3.82, CI (95%) (1.77 - 8.23) p < 0.001], *KIR* 2*DL5A* [OR = 1.50, CI (95%) (1.05 - 2.15) p = 0.02], *KIR* 2*DL5B* [OR = 1.52, CI (95%) (1.07 - 2.17) p = 0.02], *KIR* 3*DL*2 [OR = 3.30, CI (95%) (1.29 - 8.47) p = 0.01] and the pseudogene *KIR* 2*DP*1 [OR = 1.97, CI (95%) (1.36 - 2.85) p < 0.001]. **Conclusion**: This study showed that the KIR genes 3*DL3,* 2*DL4,* 2*DL5,* 3*DL*2 and 2*DP*1 seems to be associated with the risk of developing HPV infection.

## Keywords

HR-HPV, KIR, SSP-PCR, West Africa

## 1. Introduction

Natural killer cells are innate immune cells that control certain microbial infections and tumors. The function of natural killers (NK) is regulated by a balance between the signals transmitted by the activating receptors, which recognize the ligands located on cells infected by the virus or on cancer cells, and the inhibitory receptors specific to the molecules of the major histocompatibility complex [1]. NK cells exhibit a variety of surface receptors that can enhance or decrease their response against the target cell. This includes the killer cell immunoglobulin-like receptors (KIRs), encoded by a region of chromosome 19 called the Leukocyte Receptor Complex (LRC) [2]. They are required for natural killer cell function against virus-infected cells or tumor cells [3]. The polymorphism of these KIR receptors has been linked to several diseases, including infection, autoimmunity, and cancer [4]. KIR molecules are a family of receptors expressed on the surface of natural killer (NK) cells. They constitute one of the first line defense mechanisms of innate immunity against microorganisms and neoplastic cells [4] [5]. The KIR polymorphism has been studied in several populations around the world [4]-[9]. Genotypes have been described in more than 200 diverse populations worldwide [10]. KIR receptors have been associated with pathologies induced by HPV infection around the world [1] [11]-[13], but very few studies, to our knowledge, have been carried out on African populations. This study is the first to be carried out in west Africa on KIR genes in relation to HPV infection. Studies in Africa on this gene are very few and generally concern HIV or HBV infections [14]-[18]. Due to their function, KIRs may contribute to the clearance of HPV-infected cells and/or dysplastic cells and some studies confirm the association of KIR genes with cervical cancer in women with HPV infection [3]. However, since KIR controls the activity of NK cells, variation in the KIR gene can determine the course of the infection [13]. It would therefore be interesting to study the KIR

genes in relation to HPV infection because this knowledge can be exploited to identify potential immunological biomarkers that can help select patients for suitable therapy [19].

## 2. Materials and Methods

### 2.1. Study Site, Type and Population

The study population consisted of 1419 endocervical samples collected from the cervix. These were two distinct populations at the start, namely 200 samples from a population of sex workers from the city of Ouagadougou and a population of 1219 samples from five countries in West Africa. They are: Côte d'Ivoire (Abidjan), Togo (Kara), Burkina Faso (Ouagadougou), Niger (Niamey), Nigeria (Abuja) and Benin (Parakou). Among the 200 sex workers, there were Beninese, Burkinabè, Ivorian, Nigerian and Togolese women. This was a case-control study. We considered high-risk HPV-positive samples to be the cases and high-risk HPV negative samples to be controls. So, we had a total of 510 cases and 909 controls.

### 2.2. Collection of Cervical Samples and Screening for Precancerous Lesions

Samples were collected by endocervical swabbing. The fresh cytological samples from sex workers were collected at the Saint Camille Hospital in Ouagadougou (Burkina Faso). The other samples were collected from a previous study, the project of the Agence universitaire de la Francophonie. These samples were collected in different countries in West Africa. Sex workers were added to this cohort because they are a group of sexually active and predominantly young women [20]. The samples obtained were immersed in a transport medium obtained from the DNA-Sorb-A kit (Sacace Biotechnologies, Como, Italy) and stored at −20˚C in the laboratory in the local sites. They were then sent to the Pietro Annigoni Biomolecular Research Center (CERBA) in Ouagadougou and stored there until DNA extraction and characterization of high-risk HPV genotypes. Women were screened for precancerous lesions by visual inspection with acetic acid and Lugol (VIA/VIL) immediately after swabbing.

### 2.3. DNA Extraction

For HPV genotyping, DNA from samples was extracted using the commercial kit called 'DNA-Sorb-A' from sacace biotechnology® according to the manufacturer's protocol. For KIR genotyping, human DNA was extracted using the RIDA® Xtract Art 'R-Biopharm' kit. No. PGZ001 (R-Biopharm AC, Germany). The DNA thus obtained were stored at −20˚C awaiting further analysis.

### 2.4. HR-HPV Detection

The detection of HR-HPV was carried out with the "HPV 14 genotypes Real-TMQuant" kit (SACACE Biotechnologies®, Italy) by real-time multiplex PCR.

This kit enabled the detection of 14 high-risk HPV genotypes (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). For PCR amplification each sample was allowed 4 tubes. Each tube contained 15 µl of the reaction mixture and 10 µl of the DNA to be amplified. The mixture consisted of Hot Start DNA polymerase, PCR-buffer-FRT and respectively the E6 and E7 primers of the target regions of 3 to 4 HR-HPV (PCR-mix-1 16, 18, 31, IC; PCR-mix-2 39, 45, 59, IC; PCR-mix-3 33, 35, 56, 68; PCR-mix-4 51, 52, 58, and 66). The amplification program was 1 cycle of 95˚C for 15 minutes, followed by 5 cycles of 95˚C for 5 sec, 60˚C for 20 sec and 72˚C for 15 sec and finally 40 cycles of 95˚C for 5 sec, 60˚C for 30 sec and 72˚C for 15 sec. The results were interpreted with the Microsoft Excel software program named HPV Genotypes 14 Real-TM.xls (SACACE Biotechnologies®, Italy) according to the manufacturer's protocol.

## 2.5. KIR Genotyping

The SSP PCR technique was used to type the fifteen KIR genes (KIR-2DL1, -2DL 2, -2 DL3, -2DL 4, -2 DL5 A, -2 DL 5 B, -2DS 1, -2DS 2, -2DS 3, -2DS 4/1D, -2DS 5, -3DL 1, -3DL 2, -3 DL3, -3DS 1) and the pseudogene KIR-2DP1. This technique allows the amplification of each KIR gene and/or allele using specific primers that bind to the exons of the genes. DNA extracts with a concentration of at least 50 ng/µL were used for the characterization of KIR genes. The characterization of the KIR genes was carried out according to the SSP-PCR method described by Kulkarni *et al.* in 2010 using the GeneAmp PCR system 9700 device (Applied Biosystem, USA). The PCR was carried out by preparing a reaction mixture of 60 µL per sample as follows: a variable volume of DNA so as to have a quantity of DNA beyond 50 ng, 7.5 µL of 10 × PCR buffer, 2.25 µL of $MgCl_2$; 0.6 µL of dNTPs and 0.375 µL of DNA platinum Taq polymerase; the mixture was made up with nuclease-free water to make a volume of 60 µL. Four (4 µL) of the reaction mixture then 1 µL of each mixture of primers or mix was respectively distributed in each well of the PCR 96 plate for a total of 12 wells per sample. Twelve mixes necessary to process each sample were prepared according to the method of Kulkani *et al.* (Kulkarni *et al.*, 2010) or each mix included two to three pairs of primers. The classic PCR Thermocycler 'Gene Amp PCR System 9700 (Applied Biosystem, USA)' was programed for the activation of Taq polymerase at 94˚C for 3 minutes followed by 5 cycles at 94˚C for 15 sec, 65˚C for 15 sec, 72˚C for 30 sec; 21 cycles at 94˚C for 15 sec, 60˚C for 15 sec, 72˚C for 30 sec; 4 cycles at 94˚C for 15 sec, 55˚C for 1 mn, 72˚C for 2 mn and finally a final extension step at 72˚C for 7min. Then PCR products were run by electrophoresis on 3% agarose gel and visualized under UV light at 312 nm.

## 2.6. Data Analysis

Statistical analysis and interpretation of data were performed using Statistical Package for Social Sciences (SPSS) software version 20.0, Epi Info 7 and Microsoft Office Excel 2013. Chi-square test was used for comparison of proportions. The

difference was significant for $p < 0.05$. The estimate of carrier frequencies for each KIR gene was determined by direct counting. The genotypic profiles were used to assess haplotypes A and B. We were thus able to determine the homozygous AA and BB frequencies and the heterozygous AB frequencies, then deduce the frequencies of the haplotypes.

### 2.7. Ethical Considerations

This study received the agreement of the Ethics Committee for Health Research of Burkina Faso (CERS) (deliberation n˚ 2018-01-012). Respect of confidentiality and anonymity in relation to the information provided was essential.

## 3. Results

### 3.1. Socio-Demographic Characteristics of Study Participants

The socio-demographic characteristics of the population studied are given in Table 1. Of the 1419 participants, 510 (35.94%) were tested positive for HPV-HR and 909 (64.06%) were HR-HPV negative. The age of the women ranged from 16 to 67 years with an average of 34.50 ± 10.14 years. Sixty seven (67.25)% were between 25 and 45 years old. More than half of the women (54.12%) were married and most had reached the secondary school level. The majority of women in the study were Burkinabe which is the country where the study was conducted (32.55%), followed by women from Togo (17.25%), Côte d'Ivoire (17.06%), Benin (16.86%), Nigeria (10%) and finally Niger (6.28%).

Table 1. Socio-demographic characteristics Representation of characteristics of HPV-infected and HPV-uninfected women in the study population, according to age, country, marital status, education level and Lugol's test results IVA IVL.

| Variable | HPV+ | HPV− | Total | p-value |
|---|---|---|---|---|
| | N = 510 (%) | N = 909 (%) | N = 1419 (%) | |
| **Age (years)** | | | | |
| **< 25** | 99 (19.41) | 122 (13.42) | 221 (15.57) | 0.010 |
| **25 - 45** | 343 (67.25) | 658 (72.39) | 1001 (70.52) | |
| **>45** | 68 (13.34) | 129 (14.19) | 197 (13.88) | |
| **Country** | | | | |
| **Benin** | 86 (16.86) | 167 (18.37) | 253 (17.83) | <0.001 |
| **Burkina** | 166 (32.55) | 161 (17.71) | 327 (23.04) | |
| **Côte d'Ivoire** | 87 (17.06) | 168 (18.48) | 255 (17.97) | |
| **Niger** | 32 (6.28) | 222 (24.42) | 254 (17.90) | |
| **Nigéria** | 51 (10) | 35 (3.85) | 86 (6.06) | |
| **Togo** | 88 (17.25) | 156 (17.17) | 244 (17.20) | |
| **Marital status** | | | | |
| **Single** | 211 (41.37) | 193 (21.23) | 404 (28.47) | |

Continued

| | | | | |
|---|---|---|---|---|
| **Divorced** | 7 (1.37) | 5 (0.55) | 12 (0.85) | |
| **Married** | 276 (54.12) | 686 (75.47) | 962 (67.80) | <0.001 |
| **Widow** | 16 (3.14) | 25 (2.75) | 41 (2.88) | |
| **Educational level** | | | | |
| **Illiterate** | 135 (26.47) | 296 (32.56) | 431 (30.37) | 0.010 |
| **Educated** | 375 (73.53) | 613 (67.44) | 988 (69.63) | |
| **VIA** | | | | |
| **Positif** | 51 (10) | 48 (5.28) | 99 (6.97) | |
| **Négatif** | 458 (89.8) | 857 (94.28) | 1315 (92.67) | 0.003 |
| **Unrealized** | 1 (0.2) | 4 (0.44) | 5 (0.35) | |
| **VIL** | | | | |
| **Positif** | 31 (6.08) | 44 (4.84) | 75 (5.28) | |
| **Négatif** | 478 (93.72) | 861 (94.72) | 1339 (94.36) | 0.400 |
| **Unrealized** | 1 (0.2) | 4 (0.44) | 5 (0.35) | |

### 3.2. Prevalence of HR-HPV

HPV research results showed that 35.94% (510/1419) of women were infected with HR HPV. Figure 1 shows the distribution of these genotypes. The most frequent was HPV 68 (11.33%), HPV 52 (10.86%), HPV 45 (9.22%) and HPV 66 (8.86%). HPV 16 and HPV 33 were the least represented in the women in this study with 2.24% and 1.30% respectively.

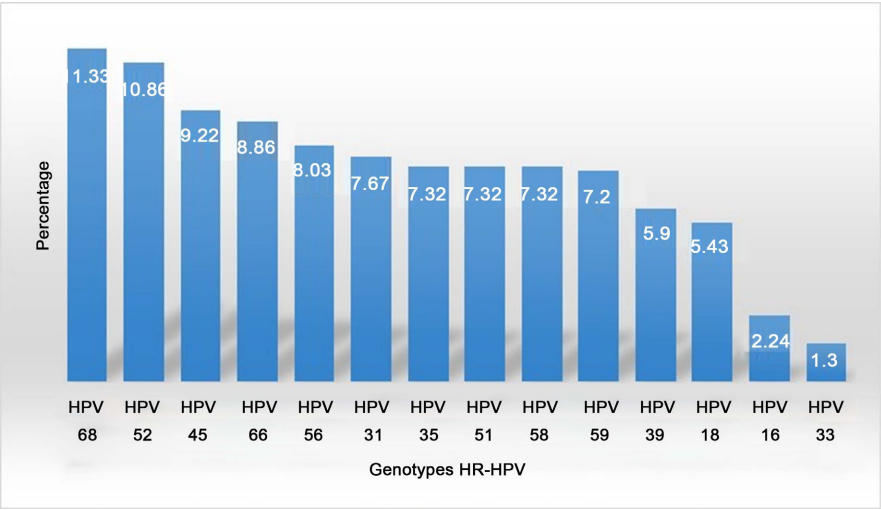


Figure 1. HR-HPV genotypes distribution.

Percentage of circulating HR-HPV in the study population from highest to lowest

### 3.3. KIR Gene Distribution in Study Population

We report the frequencies of 15 KIR genes and one pseudogene in a West African

population of sexually active women. Analysis showed that the KIR inhibitory KIR genes 2*DL*1, 2*DL*4, 3*DL*2, 3*DL*3 and the *KIR* 2*DS*4 activator gene were present in over 90% of subjects (cases and controls). The *KIR* 3*DS*1 gene was the least prevalent with 7.20% in HPV infected subjects and 7.14% in controls. By comparing the frequencies of KIR genes between groups infected with HPV and groups not infected with HPV, in order to determine the association between the presence of KIR genes and HPV status (Table 2), we found that some KIRs genes were significantly more common in HPV infected subjects than in controls. These are the inhibitory KIR genes, *KIR* 2*DL*3 [OR = 2.24, CI (95%) (1.48 - 3.39) p < 0.001], *KIR* 2*DL*4 [OR = 3.82, CI (95% ) (1.77 - 8.23) p < 0.001], *KIR* 2*DL*5*A* [OR = 1.50, CI (95%) (1.05 - 2.15) p = 0.02], *KIR* 2*DL*5*B* [OR = 1.52, CI (95%) (1.07 - 2.17) p = 0.02], *KIR* 3*DL*2 [OR = 3.30, CI (95%) (1.29 - 8.47) p = 0.01] and the pseudogene *KIR* 2*DP*1 [OR = 1.97, CI (95%) (1.36 - 2.85) p < 0.001]. We have grouped the KIR genes into AA, AB or BB haplotypes. These haplotypes were determined according to criteria adopted by Middleton on the Interactive Allele Frequency website (http://www.allelefrequencies.net). Analyzes showed that the AB haplotype was statistically more frequent in cases than in controls [OR = 1.75, CI (95%) (1.23 - 2.50) p = 0.001]. The BB haplotype, on the other hand, was statistically more frequent (p = 0.003) in women not infected with HPV than in those infected with HPV [OR = 0.50, CI (95%) (1.23 - 2.55) p = 0.001].

Table 2. KIR Frequency and their association with HPV status.

| KIR | HPV⁺ | HPV⁻ | OR | (95% CI) | p-value |
|---|---|---|---|---|---|
| | N = 264 (%) | N = 252 (%) | | | |
| **Inhibitors** | | | | | |
| 2*DL*1 | 260 (98.48) | 248 (98.41) | 1.04 | 0.25 - 4.23 | 0001 |
| 2*DL*2 | 118 (44.7) | 106 (42.06) | 1.11 | 0.78 - 1.57 | 0.500 |
| 2*DL*3 | 218 (82.58) | 171 (67.86) | 2.24 | 1.48 - 3.39 | **<0.001** |
| 2*DL*4 | 255 (96.59) | 222 (88.1) | 3.82 | 1.77 - 8.23 | **<0.001** |
| 2*DL*5*A* | 117 (44.32) | 87 (34.52) | 1.5 | 1.05 - 2.15 | **0.020** |
| 2*DL*5*B* | 120 (45.45) | 89 (35.32) | 1.52 | 1.07 - 2.17 | **0.020** |
| 3*DL*1 | 263 (99.62) | 251 (99.6) | 1.04 | 0.06 - 16.84 | 0001 |
| 3*DL*2 | 258 (97.73) | 234 (92.86) | 3.3 | 1.29 - 8.47 | **0.010** |
| 3*DL*3 | 261 (98.86) | 242 (96.03) | 3.59 | 0.97 - 13.21 | 0.050 |
| **Activators** | | | | | |
| 2*DS*1 | 36 (13.64) | 21 (8.33) | 1.73 | 0.98 - 3.06 | 0.060 |
| 2*DS*2 | 122 (46.21) | 116 (46.03) | 1 | 0.71 - 1.42 | 0001 |
| 2*DS*3 | 80 (30.3) | 58 (23.02) | 1.45 | 0.98 - 2.15 | 0.070 |
| 2*DS*4 | 256 (96.97) | 242 (96.03) | 1.32 | 0.51 - 3.40 | 0.600 |
| 2*DS*5 | 88 (33.33) | 71 (28.17) | 1.27 | 0.87 - 1.85 | 0.200 |
| 3*DS*1 | 19 (7.2) | 18 (7.14) | 1.01 | 0.51 - 1.96 | 0001 |

**Continued**

| | | | | | |
|---|---|---|---|---|---|
| **Pseudogene** | | | | | |
| 2*DP*1 | 193 (73.11) | 146 (57.94) | 1.97 | 1.36 - 2.85 | **<0.001** |
| **Haplotype** | | | | | |
| AA | 90 (34.09) | 89 (35.32) | 0.94 | 0.65 - 1.36 | 0.700 |
| AB | 127 (48.11) | 87 (34.52) | 1.75 | 1.23 - 2.50 | **0.001** |
| BB | 47 (17.8) | 76 (30.16) | 0.5 | 0.33 - 0.75 | **0.001** |

### 3.4. KIRs Gene and their Association with HR-HPV Genotypes

A separate analysis of samples on the basis that induced HPV 16/18 infections may be particularly resistant to NK cell cytotoxicity was conducted. HR-HPV infections were divided into two distinct groups: those induced by genotypes 16/18 that are 38 (14.40%) on the one hand and infections induced by other genotypes that are 226 (85.60%) on the other hand. Possible associations were sought between KIR genes and different HR-HPV genotypes. Our results showed a significant association of the KIR2DL5 gene with induced HPV 16/18 infection compared to other HPV genotypes [OR = 0.43, CI (95%) (0.20 - 0.92) p = 0.03] (Table 3).

Table 3. Association between KIR genes and HR-HPV genotypes.

| KIR | HPV16/18+ | Other HR-HPV | OR | (95% CI) | p-value |
|---|---|---|---|---|---|
| | N = 38 (%) | N = 226 (%) | | | |
| **Inhibitors** | | | | | |
| 2*DL*1 | 38 (100) | 220 (97.35) | - | - | 0.500 |
| 2*DL*2 | 12 (31.58) | 90 (39.82) | 0.69 | 0.33 - 1.45 | 0.300 |
| 2*DL*3 | 31 (81.58) | 169 (74.78) | 1.49 | 0.62 - 3.57 | 0.400 |
| 2*DLA* | 32 (84.21) | 197 (87.17) | 0.78 | 0.30 - 2.04 | 0.600 |
| 2*DL5A* | 11 (28.95) | 109 (48.23) | 0.43 | 0.20 - 0.92 | **0.030** |
| 2*DL5B* | 11 (28.95) | 109 (48.23) | 0.43 | 0.20 - 0.92 | **0.030** |
| 3*DL*1 | 36 (94.74) | 215 (95.13) | 0.92 | 0.19 - 4.32 | 0001 |
| 3*DL*2 | 35 (92.11) | 207 (91.59) | 1.07 | 0.30 - 3.81 | 0001 |
| 3*DL*3 | 38 (100) | 220 (97.35) | 0.82 | 0.22 - 3.01 | 0.700 |
| **Activators** | | | | | |
| 2*DS*1 | 6 (15.79) | 30 (13.27) | 1.22 | 0.47 - 3.17 | 0.600 |
| 2*DS*2 | 11 (28.95) | 96 (42.48) | 0.55 | 0.26 - 1.16 | 0.100 |
| 2*DS*3 | 7 (18.42) | 61 (26.99) | 0.61 | 0.25 - 1.45 | 0.300 |
| 2*DS*4 | 37 (97.37) | 204 (90.27) | 3.99 | 0.52 - 30.51 | 0.200 |
| 2*DS*5 | 7 (18.42) | 70 (30.97) | 0.5 | 0.21 - 1.19 | 0.100 |
| 3*DS*1 | 1 (2.63) | 15 (6.64) | 0.38 | 0.04 - 2.96 | 0.400 |
| **Pseudogène** | | | | | |
| *2*DP *1* | 30 (78.95) | 163 (72.12) | 1.44 | 0.63 - 3.33 | 0.400 |

## 4. Discussion

The objective of this study was to investigate the involvement of the KIR genes in Human Papillomavirus (HPV) infections in women in West Africa. Given the sample size, we combined the results for better understanding. The most prevalent genotypes found were HPV 68 (11.33%), HPV 52 (10.86%), HPV 45 (9.22%), followed by genotypes HPV 66 (8.86%), HPV 56 (8.03%), HPV 31 (7.67%), HPV 35, 51, 58, (7.32%), HPV 59 (7.20%), HPV 39 (5.90%); finally, HPV 18 (5.43%), HPV 16 (2.24%) and HPV 33 (1.30%). The HPV 16 and HPV 18 genotypes are among the lowest prevalence. Similar results were found in previous studies in Burkina Faso [21]-[27]. The high prevalence of high-risk genotypes other than 16 and 18 had been found in cases of precancerous and cancerous lesions in Burkina Faso and Benin [28] [29]. In this study, the prevalence of HR-HPV was 35.94%. These results were higher than those found by Traore *et al.* in Bobo-Dioulasso which was 25.40% [25]. While recently, Salambanga *et al.* in 2019 found a high prevalence of HR-HPV of 52.56% in the city of Ouagadougou [30]. This high prevalence can also be explained by the fact that the cohort of women included sex workers where there was a high rate of HPV [20]. Fortunately, not all of these HPV-HR infections systematically progress to cervical cancer because of the human factors that regulate clearance. Knowing that the KIR genes are essential in the regulation of the activation of NK cells, it is therefore interesting to ask whether they would potentially have an influence on the infection due to HPV and on the development of precancerous lesions and even of Cancer, because NK cells are the first line of defense against any viral infection and newly formed tumor cells [31]. Our results found that the frequency of KIR genes seem quite close to those found in the literature. The KIR 3DL1 gene is found in virtually all subjects; this is a gene that seems to be unique to West Africa [18]. Contreras had already encountered it in a population of black Mexicans with a frequency of 100% [32]. This was confirmed by Augusto who showed that the KIR 3DL1 gene has a high frequency in Africans [33]. Denis *et al.* Also found that the KIR 2DS4 activator KIR gene is overrepresented in the Senegalese population [34]. Next to these genes, which have a high frequency, we notice the KIR 3DS1 gene which is less prevalent in most black African populations, corroborating our results [35]. A study of 492 subjects from Kampala, Uganda, indicating that African populations have lower frequencies of telomeric region B and higher frequencies from centromeric region B compared to European populations. This is also the case in South Africa, were in a study involving 167 blacks, the KIR 2DS1 and 3DS1 genes were infrequent [36]. These different data on the KIR genes comfort us because it shows that our results agree with the mapping that already exists around the world. Black Africa has its peculiarities, as does white Africa [6] [33] [34] [37]. We also found that the KIR 2DL5 gene was less prevalent ($P = 0.03$) in the HPV 16/18 group than in the group made up of other HR-HPV. Also the KIR genes 2DL3, 2DL4, 2DL5, 3DL2 and the pseudogene 2DP1 were more prevalent among cases than in subjects not infected with HPV. This could indicate possible protection against HPV infection. In a

cohort study of 65 women with CIN and 150 controls in northern Sweden, Arnheim *et al.*, found 70 different genotypes and only one genotype A was associated with increased risk of CIN including KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3 and KIR2DS4 (OR 6.7; 95% CI 1.7 - 26.3) and the KIR 2DL5 B gene appears to be related to the disease. And there was no association between the KIR genes and the type of HPV [11]. As for the Brestovac team in Western Australia, it had enrolled 147 CIN patients and 187 controls. CIN 2 and 3 were weakly associated with the absence of KIR 2DL2 AND KIR 2DS2, but this value was insignificant. And no difference was observed between KIR frequencies, CINs and HPV genotypes [13]. Tembhurne found that the number of activating KIR genes was higher in women with cancer, as in our study in infected women. The fact that there are high numbers of activating KIR genes may translate into the possibility of a genetic predisposition to chronic inflammatory disease [3]. This chronic inflammation over the years can lead to cancerous lesions and, with persistent HPV infection, These two previous interesting studies have given us information on the absence and presence of certain KIR genes, but it should be remembered that these are cases of precancerous lesions (CIN 1 and CIN 2), whereas our cases were HPV infectious status, which does not necessarily lead to the CIN stage or even cancer due to viral clearance [11] [13]. In Brazil, 79 women infected with HPV and 150 uninfected took part in a study to see the association between HPV and the genes of the immune response [38]. It was found that the KIR genes are not associated with HPV, although certain pairs of inhibitory KIR ligands are more common in patients, which supports the role of NK in harmful inflammation and chronic carcinogenesis. Our results are also different from those of Song in Korean women. No relationship between KIR genes and HPV-related cervical disease was found but a significant relationship was found between HLA-C alleles as ligands in KIR and HPV-related cervical disease [39]. HLA-C * 0303 was found to confer susceptibility to HPV-related cervical disease, while HLA-C * 01 was found to confer a protective effect against HPV-related cervical disease [39] while Rizzo showed that HLA-C and KIR typing is a marker for the risk of HPV infection and the progression of the lesion [40]. Indeed, as in our study, Rizzo *et al.* found an increase in the KIR 2DL3 gene in cases compared to controls. But it was in association with HLA-C1 [40].

## 5. Conclusion

This study shows that the HR-HPV genotypes circulating in our regions are not dominated by genotypes 16 and 18 among asymptomatic women. This has implications for vaccine use to prevent infection. It would be interesting to get pharmaceutical companies to develop polyvalent broad-spectrum anti-HPV vaccines taking into account all the HPV-HR genotypes found in studies from the West Africa region. Studies on KIR genes give a first insight into their distribution in this West African population. The inhibitory KIR genes, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL2 and the pseudogene KIR2DP1 were statistically associated

with HPV infection. KIR/HLA studies combined with additional genotyping are needed to investigate the molecular mechanisms by which KIR genes contribute to infection or elimination of HPV.

## Acknowledgements

We thank the "International Center for Genetic Engineering and Biotechnology (ICGEB)" for funding this research work through the project: "Implication of the host genetic factor in Human Papillomavirus Infection and its Associated Cervical Lesions and cancer in West African Women". Ref. No. CRP/BFA17-01. We also thank the Agence Universitaire de la Francophonie (AUF) for the financial support. We thank also the UNESCO chaire in "Génie génétique et Biologie Moléculaire" for the technical support.

## Data Availability

The authors declare that there is no conflict of interest regarding the publication of this article.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Cerwenka, A. and Lanier, L.L. (2001) Natural Killer Cells, Viruses and Cancer. *Nature Reviews Immunology*, **1**, 41-49. https://doi.org/10.1038/35095564

[2] Wilson, M.J., Torkar, M., Haude, A., Milne, S., Jones, T., Sheer, D., *et al.* (2000) Plasticity in the Organization and Sequences of Human KIR/ILT Gene Families. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 4778-4783. https://doi.org/10.1073/pnas.080588597

[3] Tembhurne, A.K., Maheshwari, A., Warke, H., Chaudhari, H., Kerkar, S.C., Deodhar, K., *et al.* (2022) Killer Cell Immunoglobulin-like Receptor (KIR) Gene Contents: Are They Associated with Cervical Cancer? *Journal of Medical Virology*, **95**, e27873. https://doi.org/10.1002/jmv.27873

[4] Augusto, D.G. (2016) The Impact of KIR Polymorphism on the Risk of Developing Cancer: Not as Strong as Imagined? *Frontiers in Genetics*, **7**, Article 121. https://doi.org/10.3389/fgene.2016.00121

[5] Augusto, D.G., Lobo-Alves, S.C., Melo, M.F., Pereira, N.F. and Petzl-Erler, M.L. (2012) Activating KIR and HLA Bw4 Ligands Are Associated to Decreased Susceptibility to Pemphigus Foliaceus, an Autoimmune Blistering Skin Disease. *PLOS ONE*, **7**, e39991. https://doi.org/10.1371/journal.pone.0039991

[6] Augusto, D.G., Zehnder-Alves, L., Pincerati, M.R., Martin, M.P., Carrington, M. and Petzl-Erler, M.L. (2011) Diversity of the KIR Gene Cluster in an Urban Brazilian Population. *Immunogenetics*, **64**, 143-152. https://doi.org/10.1007/s00251-011-0565-1

[7] Hollenbach, J.A., Augusto, D.G., Alaez, C., Bubnova, L., Fae, I., Fischer, G., *et al.* (2012) 16th IHIW: Population Global Distribution of Killer Immunoglobulin-Like Receptor (KIR) and Ligands. *International Journal of Immunogenetics*, **40**, 39-45. https://doi.org/10.1111/iji.12028

[8] Norman, P.J., Carrington, C.V.F., Byng, M., Maxwell, L.D., Curran, M.D., Stephens, H.A.F., *et al.* (2002) Natural Killer Cell Immunoglobulin-Like Receptor (KIR) Locus Profiles in African and South Asian Populations. *Genes & Immunity*, **3**, 86-95. https://doi.org/10.1038/sj.gene.6363836

[9] Chandanayingyong, D., Vaughan, R.W., Verity, D.H., Norman, P.J. and Stephens, H.A.F. (2001) Distribution of Natural Killer Cell Immunoglobulin-Like Receptor Sequences in Three Ethnic Groups. *Immunogenetics*, **52**, 195-205. https://doi.org/10.1007/s002510000281

[10] González-Galarza, F.F., Takeshita, L.Y.C., Santos, E.J.M., Kempson, F., Maia, M.H.T., Silva, A.L.S.D., *et al.* (2014) Allele Frequency Net 2015 Update: New Features for HLA Epitopes, KIR and Disease and HLA Adverse Drug Reaction Associations. *Nucleic Acids Research*, **43**, D784-D788. https://doi.org/10.1093/nar/gku1166

[11] Arnheim, L., Dillner, J. and Sanjeevi, C.B. (2005) A Population-Based Cohort Study of *kir* Genes and Genotypes in Relation to Cervical Intraepithelial Neoplasia. *Tissue Antigens*, **65**, 252-259. https://doi.org/10.1111/j.1399-0039.2005.00359.x

[12] Brestovac, B., Harnett, G.B., Smith, D.W., Shellam, G.R. and Frost, F.A. (2005) Human Papillomavirus Genotypes and Their Association with Cervical Neoplasia in a Cohort of Western Australian Women. *Journal of Medical Virology*, **76**, 106-110. https://doi.org/10.1002/jmv.20330

[13] Brestovac, B., Wong, M.E., Tjendera, R., Costantino, P.J., Mamotte, C. and Witt, C.S. (2013) Human Papillomavirus, High-Grade Intraepithelial Neoplasia and Killer Immunoglogulin-Like Receptors: A Western Australian Cohort Study. *Infectious Agents and Cancer*, **8**, Article No. 33. https://doi.org/10.1186/1750-9378-8-33

[14] Mhandire, K., Zijenah, L.S., Yindom, L., Duri, K., Mlambo, T., Tshabalala, M., *et al.* (2016) *KIR* Gene Content Diversity in a Zimbabwean Population: Does *KIR2DL2* Have a Role in Protection against Human Immunodeficiency Virus Infection? *OMICS*: *A Journal of Integrative Biology*, **20**, 727-735. https://doi.org/10.1089/omi.2016.0154

[15] Naranbhai, V., de Assis Rosa, D., Werner, L., Moodley, R., Hong, H., Kharsany, A., *et al.* (2015) Killer-Cell Immunoglobulin-Like Receptor (KIR) Gene Profiles Modify HIV Disease Course, Not HIV Acquisition in South African Women. *BMC Infectious Diseases*, **16**, Article No. 27. https://doi.org/10.1186/s12879-016-1361-1

[16] Sorgho, A.P., Martinson, J.J., Djigma, F.W., Yonli, A.T., Nagalo, B.M., Compaore, R.T., *et al.* (2018) Insights into the Interplay between KIR Gene Frequencies and Chronic HBV Infection in Burkina Faso. *Mediterranean Journal of Hematology and Infectious Diseases*, **10**, e2018060. https://doi.org/10.4084/mjhid.2018.060

[17] Yindom, L., de Silva, T.I. and Rowland-Jones, S.L. (2015) Identification of a Novel Three-Domain KIR Allele: *KIR3DL1**087 Using High-Resolution Molecular Techniques. *Tissue Antigens*, **85**, 153-154. https://doi.org/10.1111/tan.12502

[18] Yindom, L., de Silva, T.I. and Rowland-Jones, S.L. (2015) *KIR3DL1**0250102: A Novel Three-Domain KIR Subtype Identified in West Africa. *Tissue Antigens*, **85**, 151-152. https://doi.org/10.1111/tan.12501

[19] Leone, P., De Re, V., Vacca, A., Dammacco, F. and Racanelli, V. (2017) Cancer Treatment and the KIR-HLA System: An Overview. *Clinical and Experimental Medicine*, **17**, 419-429. https://doi.org/10.1007/s10238-017-0455-4

[20] Ilboudo, R., *et al.* (2020) Prevalence and Characterization of High-Risk Human Papilloma-Virus Genotypes among a Group of Sex Workers in Ouagadougou, Burkina Faso. *EC Gynaecology*, **9**, 1-9.

[21] Djigma, F.W., Ouédraogo, C., Karou, D.S., Sagna, T., Bisseye, C., Zeba, M., *et al.*

(2011) Prevalence and Genotype Characterization of Human Papillomaviruses among HIV-Seropositive in Ouagadougou, Burkina Faso. *Acta Tropica*, **117**, 202-206. https://doi.org/10.1016/j.actatropica.2010.12.007

[22] Ouedraogo, C.M., *et al.* (2015) [Epidemiology and Characterization of High-Risk Genotypes of Human Papillomavirus in a Population of Sexually Active Adolescents in Ouagadougou]. *Journal de Gynécologie Obstétrique et Biologie de la Reproduction* (*Paris*), **44**, 715-722.

[23] Ouedraogo, R.A., Zohoncon, T.M., Guigma, S.P., Angèle Traore, I.M., Ouattara, A.K., Ouedraogo, M., *et al.* (2018) Oncogenic Human Papillomavirus Infection and Genotypes Characterization among Sexually Active Women in Tenkodogo at Burkina Faso, West Africa. *Papillomavirus Research*, **6**, 22-26. https://doi.org/10.1016/j.pvr.2018.09.001

[24] Prosper, B., Florencia, D.W., Mahoukèdè, Z.T., Dorcas, O., Alima, T.E.M., Karim, O.A., *et al.* (2020) Polymorphism of MMP1 and MMP3 Promoter Regions and HR-HPV Infection in Women from Burkina Faso and Côte d'Ivoire. *Biomolecular Concepts*, **11**, 116-124. https://doi.org/10.1515/bmc-2020-0010

[25] Traore, I.M.A., Zohoncon, T.M., Dembele, A., Djigma, F.W., Obiri-Yeboah, D., Traore, G., *et al.* (2016) Molecular Characterization of High-Risk Human Papillomavirus in Women in Bobo-Dioulasso, Burkina Faso. *BioMed Research International*, **2016**, Article ID: 7092583. https://doi.org/10.1155/2016/7092583

[26] Traore, I.M.A., Zohoncon, T.M., Ndo, O., Djigma, F.W., Obiri-Yebo, D., Compaore, T.R., *et al.* (2016) Oncogenic Human Papillomavirus Infection and Genotype Characterization among Women in Orodara, Western Burkina Faso. *Pakistan Journal of Biological Sciences*, **19**, 306-311. https://doi.org/10.3923/pjbs.2016.306.311

[27] Zohoncon, T.M. and Simpore, J. (2013) Prevalence of HPV High-Risk Genotypes in Three Cohorts of Women in Ouagadougou (Burkina Faso). *Mediterranean Journal of Hematology and Infectious Diseases*, **5**, e2013059. https://doi.org/10.4084/mjhid.2013.059

[28] Zohoncon, T.M., Ouedraogo, T.C., Brun, L.V.C., Obiri-Yebo, D., Djigma, W.F., Kabibou, S., *et al.* (2016) Molecular Epidemiology of High-Risk Human Papillomavirus in High-Grade Cervical Intraepithelial Neoplasia and in Cervical Cancer in Parakou, Republic of Benin. *Pakistan Journal of Biological Sciences*, **19**, 49-56. https://doi.org/10.3923/pjbs.2016.49.56

[29] Zohoncon, T.M.B., *et al.* (2016) Molecular Characterization of High-Risk Human Papillomavirus Genotypes Involved in Invasive Cervical Cancer from Formalin-Fixed, Paraffin-Embedded Tissues in Ouagadougou, Burkina Faso. *International Journal of Current Research*, **8**, 39314-39318.

[30] Salambanga, C., *et al.* (2019) High Prevalence of High-Risk Human Papillomavirus (HPV) Infection among Sexually Active Women in Ouagadougou. *Médecine et Santé Tropicales*, **29**, 302-305.

[31] Lanier, L.L. (2005) NK CELL Recognition. *Annual Review of Immunology*, **23**, 225-274. https://doi.org/10.1146/annurev.immunol.23.021704.115526

[32] Contreras, G., Aláez, C., Murguía, A., García, D., Flores, H. and Gorodezky, C. (2007) Distribution of the Killer Cell Immunoglobulin-Like Receptors in Mexican Mestizos. *Tissue Antigens*, **69**, 125-129. https://doi.org/10.1111/j.1399-0039.2006.76212.x

[33] Augusto, D.G., Norman, P.J., Dandekar, R. and Hollenbach, J.A. (2019) Fluctuating and Geographically Specific Selection Characterize Rapid Evolution of the Human KIR Region. *Frontiers in Immunology*, **10**, Article 989. https://doi.org/10.3389/fimmu.2019.00989

[34] Denis, L., Sivula, J., Gourraud, P.-., Kerdudou, N., Chout, R., Ricard, C., *et al.* (2005) Genetic Diversity of KIR Natural Killer Cell Markers in Populations from France, Guadeloupe, Finland, Senegal and Réunion. *Tissue Antigens*, **66**, 267-276. https://doi.org/10.1111/j.1399-0039.2005.00473.x

[35] Nakimuli, A., Chazara, O., Farrell, L., Hiby, S.E., Tukwasibwe, S., Knee, O., *et al.* (2013) Killer Cell Immunoglobulin-Like Receptor (KIR) Genes and Their HLA-C Ligands in a Ugandan Population. *Immunogenetics*, **65**, 765-775. https://doi.org/10.1007/s00251-013-0724-7

[36] Gentle, N.L., Loubser, S., Paximadis, M., Puren, A. and Tiemessen, C.T. (2017) Killer-cell Immunoglobulin-Like Receptor (KIR) and Human Leukocyte Antigen (HLA) Class I Genetic Diversity in Four South African Populations. *Human Immunology*, **78**, 503-509. https://doi.org/10.1016/j.humimm.2017.05.006

[37] Bani, M., Seket, J., Kaabi, H., Cherif, G., Chaabane, M., Bellali, H., *et al.* (2015) Killer Cell Immunoglobulin-Like Receptor (KIR) Locus Profiles in the Tunisian Population. *Human Immunology*, **76**, 355-361. https://doi.org/10.1016/j.humimm.2015.03.002

[38] Marangon, A.V., Guelsin, G.A.S., Visentainer, J.E.L., Borelli, S.D., Watanabe, M.A.E., Consolaro, M.E.L., *et al.* (2013) The Association of the Immune Response Genes to Human Papillomavirus-Related Cervical Disease in a Brazilian Population. *BioMed Research International*, **2013**, Article ID: 146079. https://doi.org/10.1155/2013/146079

[39] Song, M.J., Lee, C.W., Kim, J.H., Lee, S.J., Kim, C.J., Hur, S.Y., *et al.* (2013) Association of KIR Genes and HLA-C Alleles with HPV-Related Uterine Cervical Disease in Korean Women. *Tissue Antigens*, **81**, 164-170. https://doi.org/10.1111/tan.12068

[40] Rizzo, R., Gentili, V., Rotola, A., Bortolotti, D., Cassai, E. and Di Luca, D. (2014) Implication of HLA-C and KIR Alleles in Human Papillomavirus Infection and Associated Cervical Lesions. *Viral Immunology*, **27**, 468-470. https://doi.org/10.1089/vim.2014.0017