BMC Immunology

RESEARCH

Open Access

KIR2DL1 gene is a surrogate marker of protection against infection-related hospitalization among HIV-1 unexposed versus exposed uninfected infants in Cameroon

Luc-Aimé Kagoué Simeni[1,2*], Gabriel Loni Ekali[8†], Clauvis Kunkeng Yengo[3†], Rodrigue Kamga Wouambo[2,4†], Janett Fischer[4†], Oumarou M'rikam A. Bessong[5†], Joseph Fokam[1,6†], Louis-Marie Yindom[9†] and Jules Clément Assob Nguedia[7†]

Abstract

Background HIV-exposed uninfected infants (HEU) appear more vulnerable to infections compared to their HIV-unexposed uninfected (HUU) peers, generally attributed to poor passive immunity acquired from the mother. This may be due to some genetic factors that could alter the immune system. We thus sought to determine the distribution of Killer Cell Immunoglobulin-Like Receptors (KIRs) genes in HEU versus HUU and study their associations with the occurrence of infection-related hospitalization.

Methods A cohort study was conducted from May 2019 to April 2020 among HEU and HUU infants, including their follow-up at weeks 6, 12, 24, and 48, in reference pediatric centers in Yaoundé-Cameroon. The infant HIV status and infections were determined. A total of 15 KIR genes were investigated using the sequence-specific primer polymerase chain reaction (PCR-SSP) method. The KIR genes that were significantly associated with HIV-1 status (HEU and HUU) were analyzed for an association with infection-related hospitalizations. This was only possible if, and to the extent that, infection-related hospitalizations varied significantly according to status. Multivariate logistic regression analyses were conducted to determine the association between KIR gene content variants and HIV status, while considering a number of potential confounding factors. Furthermore, the risk was quantified using relative risk, odds ratio, and a 95% confidence interval. The Fisher exact test was employed to compare the frequency of occurrences. A *p*-value of less than 0.05 was considered statistically significant.

Results In this cohort, a total of 66 infants participated, but only 19 acquired infections requiring hospitalizations (14.81%, 04/27 HUU and 38.46%, 15/39 HEU, $p=0.037$). At week 48 (39 HEU and 27 HUU), the relative risk (RR) for infection-related hospitalizations was 2.42 (95% CI: 1.028–5.823) for HEU versus HUU with OR 3.59 (1.037–12.448). *KIR2DL1* gene was significantly underrepresented in HEU versus HUU (OR = 0.183, 95%CI: 0.053–0.629; $p=0.003$),

†Gabriel Loni Ekali, Janett Fischer, Joseph Fokam, Louis-Marie Yindom and Jules Clément Assob Nguedia share senior authorship.

†Clauvis Kunkeng Yengo, Rodrigue Kamga Wouambo, Oumarou M'rikam A Bessong contributed equally to this work.

*Correspondence:
Luc-Aimé Kagoué Simeni
kagouesimeni@gmail.com
Full list of author information is available at the end of the article

and the absence of *KIR2DL1* was significantly associated with infection-related hospitalization ($p < 0.001$; aOR = 0.063; 95%CI: 0.017–0.229).

Conclusion Compared to HUU, the vulnerability of HEU is driven by *KIR2DL1*, indicating the protective role of this KIR against infection and hospitalizations.

Keywords KIR, HIV-1, HIV-1 exposed uninfected, HIV-1 unexposed uninfected, Infection-related hospitalizations

Introduction

The prevalence of HIV (human immunodeficiency virus) in sub-Saharan Africa is significantly higher than in other regions of the world, with an estimated 65% of the population infected [1]. Consequently, the number of HIV-exposed, uninfected (HEU) children is high in this region [2]. Studies have demonstrated that HEU children exhibit a 37–46% higher mortality rate than HIV-unexposed, uninfected (HUU) children [3]. One of the most effective strategies for controlling and reducing new infections in infants and children is the prevention of mother-to-child transmission (MTCT) of the virus. This transmission can occur in utero during pregnancy, during delivery, or through breastfeeding (BF) [4]. In the absence of any intervention, including antiretroviral treatment, pediatric HIV can reach a prevalence of up to 25%. This contributes to a reduction in the infection rate to below 2% [5, 6]. In Cameroon, the prevalence of HEU was 3.2% in 2020. A number of studies have demonstrated that children exposed to HIV and antiretroviral treatment, although not infected with the virus themselves, have nevertheless been affected by it. This condition is manifested in HEU children by increased clinical signs of immune dysfunction, susceptibility to multiple infections in infancy and childhood, and differences in cell-mediated and humoral immunity compared to HUU children [2, 7, 8]. Natural killer (NK) cells play a pivotal role in antiviral and antitumor innate immunity. To fulfill their functions, NK cells interact through killer cell immunoglobulin-like receptors (KIR) present on their surface. These receptors are polymorphic and recognize human leukocyte antigens-1 (HLA-1), thus enabling the interaction to elicit a more robust immune response [9]. In humans, KIRs are a family of activating and inhibitory receptors that are predominantly expressed in natural killer (NK) cells, as well as in subsets of late-stage differentiated T cells [10]. The genes encoding these receptors are located on chromosome 19q13.4 of the leukocyte receptor complex family (LRC) and are clustered in a region of 150 to 200 kb. A total of 17 KIR genes have been identified, including two pseudogenes (*2DP1* and *3DP1*) [11]. The primary function of KIRs is to regulate NK cell-mediated immunity. This role is of significant importance, as evidenced by immunogenetic studies in which the presence of KIR genes in conjunction with HLA-I genes has been statistically linked to the control of viral infections [12]. In addition to their well-documented role in modulating NK cell-mediated immunity, there are multiple, non-exclusive pathways by which KIRs could potentially modulate T cell-mediated immunity. These pathways can be divided into two categories: direct and indirect. In the direct pathway, KIR expression on a T cell directly impacts that T cell's function and survival. In the indirect pathway, KIR expression on another cell (e.g., an NK cell or a different T cell) indirectly affects T cell function [10].

KIR genes exhibit a high degree of polymorphism, with everyone displaying a unique distribution of these genes. The same is true for genotypes and haplotypes, whose repertoire varies from one individual to another and within different populations [13, 14]. A number of studies have demonstrated that exposure to antiretroviral therapy and HIV is a significant factor contributing to high mortality, morbidity, and susceptibility to diseases in HEU compared to HUU [2, 3, 15–18]. In addition, several recent reviews have identified social, parental, and domestic conditions, the role of poverty, maternal and environmental risk factors as explanatory factors for high mortality and morbidity among HEU children [2]. However, beyond these confounding factors, there may also be variations in immunity that are genetically caused [19, 20]. Consequently, KIR genes may be considered a host immunogenetic factor. The diversity of KIR genes is primarily attributed to the evolution and adaptation of the immune system's response to pathogens [21–23]. Consequently, numerous studies have demonstrated that KIR genes are associated with a multitude of diseases. The *KIR2DL2* gene was associated with a decreased risk of HBV (hepatitis B virus) infection in the overall population. In contrast, the *KIR2DS3* gene was associated with an increased risk of HBV infection in the overall population. Furthermore, the *KIR2DS2* and *KIR2DS3* genes were associated with an increased risk of HBV infection in Asians, as evidenced by studies [24, 25]. Conversely, individuals carrying the *KIR3DS1* gene may be at an increased risk of HCV (hepatitis C virus) infection compared to uninfected controls. Furthermore, *KIR3DL2* and *KIR2DS1* expression may be associated with a more rapid progression of CHCV (chronic hepatitis C virus) to fibrosis in patients [25–27]. *KIR2DL3* may be associated with an increased risk of developing COVID-19 disease

[28]. Conversely, in Burkina Faso, *KIR2DL2*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, and *KIR3DS1* were found to be significantly associated with HIV-1 infection, whereas *KIR3DL1* was associated with protection against HIV-1 infection [29]. In Chinese, *KIR2DL3* was significantly less common in the HIV-1 infected group [30]. In Cameroon, the frequencies of *KIR2DS1*, *KIR2DS5*, and *KIR2DL5* were significantly different between infants perinatally infected with HIV-1 [31]. There is a paucity of literature on how the KIR diversity informs the perspectives on HIV disease states in understudied African settings. A more comprehensive understanding of the impact of KIRs on the host's immune response to HIV in African settings is crucial to inform the development of more effective therapies and vaccines to improve health outcomes among people living with HIV (PLWH) or those exposed to HIV [32]. Cameroon is home to over 200 distinct ethnic groups [33]. However, there has been a paucity of research on KIR genes, which has resulted in a dearth of data on KIR frequency and diversity. Several studies have examined the impact of HIV exposure on the immune system of adults [7, 8, 34, 35], but no study has yet examined the immunogenetic aspect involving KIR genes in HIV-exposed uninfected children and the relation with other infection-related hospitalizations.

Hence, this study aimed to determine the distribution of KIR genes in HEU versus HUU and study the association between KIR profiling and occurrence of infection-related hospitalizations.

Materials and methods

Study design

All infants were selected within the frame of the already existing "PREVENT-IT cohort" previously established during the CIPHER project, aimed at investigating the impact of in-utero exposure to Tenofovir on the occurrence of neonatal tubulopathies in Cameroon. For this reason, following the acquisition of parental consent, all HEU and HUU individuals who were not undergoing antiretroviral therapy (ART) were enrolled in May 2019 at week 6 postpartum (which coincided with the initial HIV infection testing visit, in accordance with the HIV screening protocol in Cameroon at that time) and subsequently monitored at weeks 12, 24, and 48 (up to April 2020). Additionally, an additional 2 weeks were allotted for late subjects at each time point. The study population was divided into two groups: infants born to HIV-1 negative mothers (Unexposed Uninfected: HUU) and infants born to HIV-1 positive mothers (Exposed Uninfected: HEU). Maternal blood samples from both groups of infants tested negative for malaria, hepatitis B, and hepatitis C virus (HBV and HCV) infections.. This procedure was approved by the Institutional Ethics Committee for Research on Human Health, of the University of Douala (Ethical clearance No. 1639IEC-UD/06/2018/T). Moreover, Administrative authorization was equally obtained from all sentinel sites in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Clinical characteristics of mothers and children

Clinical information from the mother and child was obtained using a structured questionnaire. This included feeding practices of the infants (artificial or breast), the type of delivery (full-term vaginal delivery or cesarean section), age, weight, and height of each mother and infant. In addition, infection-related hospitalizations of each recruited child and information on antiretroviral treatment, and HIV viral load of HEU-infant's mother during pregnancy were also recorded. All mothers of HEU infants were on first-line antiretroviral treatment with the majority on Tenofovir-Lamivudine-Efavirenz (TELE) and the rest on Zidovudine-Lamivudine-Efavirenz.

Specimen collection, storage, DNA extraction, and HIV screening

Six weeks after delivery (1st mother's visit), up to 5ml of whole blood was collected from both mother and child into ethylene diamine tetra acetic acid (EDTA) anticoagulant tubes. Each sample was processed to isolate the peripheral blood mononuclear cells (PBMCs), buffy coat, and plasma isolated within 6 h of collection. PBMCs were stored immediately at -20°C for 2 h and transferred to -80°C until required for further analysis. Each participant was tested for HIV-1, and their viral load was quantified using the Amplicor HIV-1 DNA PCR assay (Roche Diagnostics, Branchburg, NJ) as previously described [36].

Genotyping of KIR genes

Genomic DNA was extracted from buffy coat using the QIA amp DNA Blood Mini kit (Qiagen Ltd, Germany) according to the manufacturer's instructions and quantified using NanoDrop spectrophotometer. KIR genotyping was carried out using sequence specific primer polymerase chain reaction (SSP PCR) as previously described [37]. The mix was composed of 2µl of DNA polymerase (PrimeStar GXL, Takara Bio Europe, France), 270µl of αQH2O, 60µl of 5X buffer, and 9µl of dNTPs (10mM) per sample. Briefly, two pairs of sequence specific primers were used to amplify each of 14 functional KIR genes: *2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5*, *2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5*, *3DS1*, *3DL1*, *3DL2*, *3DL3*, and the pseudogene *2DP1*. Amplicons were electrophoresed in 2% agarose gel and visualized under ultraviolet light for the presence or absence of each gene as previously reported [31]. The quality of the KIR genotyping data was verified and ensured by the presence of a control band for each

well, and by the position of each gene in accordance with the bands of the low DNA mass ladder in the electrophoresis gel (Fig. 1).

KIR genotypes and haplotypes

KIR genotypes were assigned according to the allele frequency net database (http://www.allelefrequencies.net). In the assessment of the KIR genotypes, group B genotypes were defined by the presence of one or more of the following genes: *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS5*, and *KIR3DS1*. Conversely, the stable group A genotype was defined by the absence of all the above-mentioned genes and the presence of *KIR3DL1*, *KIR2DL1*, *KIR2DL3*, and *KIR2DS4* genes. KIR haplotypes AA (A) and Bx were assigned as previously described [38]. Briefly, individuals carrying *KIR2DL1*, *KIR2DL3*, *KIR2DL4*, *KIR2DS4*, and *KIR3DL1* genes in addition to the framework genes were assigned the AA haplotype. Individuals carrying all AA haplotype genes and any one of the following genes, *KIR2DL2*, *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS5*, and *KIR3DS1*, were denoted as AB haplotype while individuals lacking any of the following, *KIR2DL1*, *KIR2DL3*, *KIR3DL1*, and *KIR2DS4*, were BB haplotype. Given the difficulties in distinguishing between AB and BB haplotypes, we coded all AB and BB carriers as Bx [39].

Infection-related hospitalization

Whenever the children enrolled in our study had a clinically proven infection or illness, it was reported to us so that we could check and manage it. Therefore, each infection was noted on the child's observation form.

In a triangularization procedure, only genes that were significantly associated with HIV-1 status (HEU and HUU) were analyzed for an association with infection-related hospitalizations. This was possible only if, and to the extent that, infection-related hospitalizations varied significantly according to status (Fig. 2).

Statistical analysis

The statistical analyses were conducted using the SPSS software (IBM SPSS, version 21.0, Chicago). The frequencies of genes and variables were determined through direct counting. The relative risk (RR) was calculated for infants with complete follow-up to 12 months, while an incidence rate ratio was calculated for all infants with any duration of follow-up, with the denominator being the number of months of follow-up. Additionally, an odds ratio (OR) and a 95% confidence interval (95% CI) were estimated to assess the risk.. The association between KIR genes and HIV status groups (HEU and HUU) was determined using either the Chi-squared or Fisher exact test, as appropriate. The Student's t-test and Man Whitney U

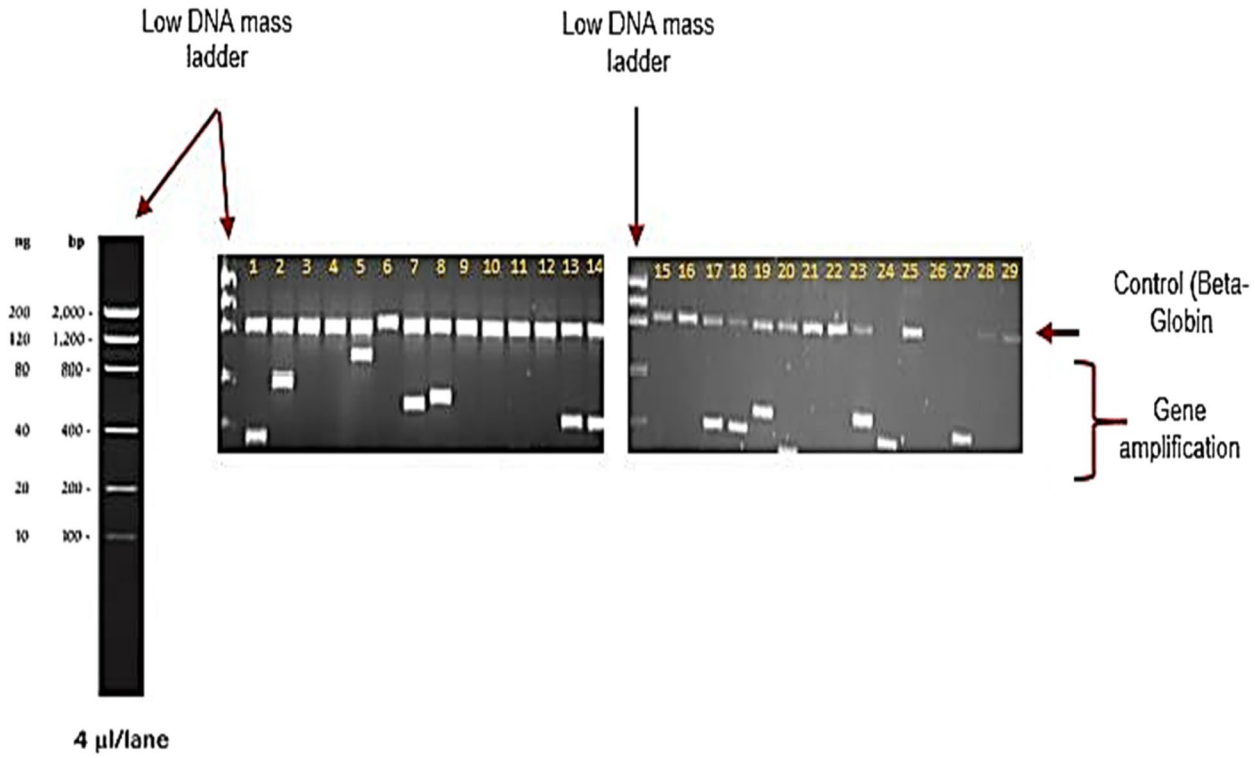

Fig. 1 Electrophoresis gel results for the KIR genes

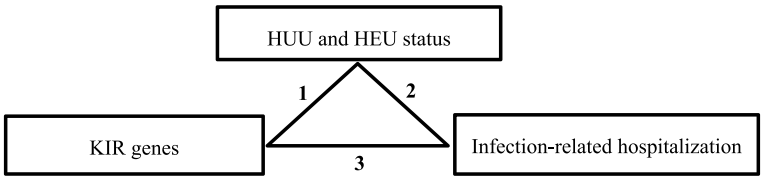

Fig. 2 Triangularization procedure of data analysis

test were employed to assess the statistical significance of the differences between the means of continuous variables between HIV-unexposed uninfected and HIV-exposed uninfected groups. The association between KIR genes and infection-related hospitalization was evaluated using Fisher exact test. Multivariate logistic regression analyses were used to employed to ascertain the association between KIR gene content variants and HIV status, taking into account various factors, including the infant's weight, gender, kinds of feeding, mother's age, and type of delivery. A p-value < 0.05 was considered statistically significant.

Results

Clinical and demographic details

In all, 66 infants (27 unexposed uninfected and 39 exposed uninfected) were enrolled at the baseline and followed up in this study for 12 months. No loss to follow-up was recorded during the entire study period. Demographic and Clinical Characteristics of mothers and infants are presented in Table 1. We noticed that females were more represented in the HEU group and HUU group (56.4% vs. 55.6%). Moreover, 92.3% of HIV-1 positive mothers with uninfected infants were on antiretroviral therapy, and vaginal delivery was more frequentin both HUU and HEU groups [92.6% (25/27) and 89.7% (35/39) respectively]. Breastfeeding was the most represented type of feeding [81.5% (22/27) in HUU and 76.9% (30/39) in HEU. The mean weight was not significantly different in HUU and HEU infants (5196.3 ± 525.1 g vs. 5061.5 ± 771.7 g; $p = 0.433$).

The trend of new infections-related hospitalizations during 1 year of follow-up of HUU and HEU

We noticed that the kinetics of new infections-related hospitalization was likely higher in HEU as compared to HUU over time. In all, 19 acquired infections requiring hospitalizations were recorded in this study within 12 months as follows: 04 in HUU [14.81% (4/27)] and 15 in HEU [38.46% (15/39)], $p = 0.037$) with the majority ($n = 13$) most likely occurring during the first 24 weeks of follow-up (10 in HEU versus 03 in HUU, $p = 0.14$). On the other side, only 6 infection-related hospitalizations appeared in the second semester: 5 in HEU [12.82% (5/39)] and 1 in HUU [3.7% (1/27)] (Fig. 3). Furthermore, lower respiratory tract infections accounted in all

Table 1 Demographic and clinical characteristics of both mothers and infants of the study

Variables	HUU ($n = 27$)	HEU ($n = 39$)	*P*
Gender			
• Female	15 (55. 6%)	22 (56. 4%)	1.000
• Male	12 (44. 4%)	17 (43. 6%)	
Weight (m ± SD)	5196.3 ± 525.1	5061.5 ± 771.7	0.433
BMI	1.7 ± 0.2	1.8 ± 0.3	0.180
Maternal age at delivery (years) m ± SD	27.9 (6.2)	28.7 (6.3)	0.211
Mothers median viral load (copies/ml)	/	466	**/**
-Mothers on ART (%)	/	36 (92.3%)	**/**
Type of delivery			1.000
• Vaginal delivery (%)	25 (92.6%)	35 (89.7%)	
• Caesarean delivery (%)	2 (7.4%)	4 (10.3%)	
Feeding options			0.821
• Artificial feeding (%)	4 (14.8%)	8 (20.5%)	
• Breastfeeding (%)	22 (81.5%)	30 (76.9%)	
• Mixed feeding (%)	1 (3.7%)	1 (2.6%)	

ART Anti-retroviral therapy, *%* Percentage, *BMI* Body Mass Index, *SD* Standard deviation

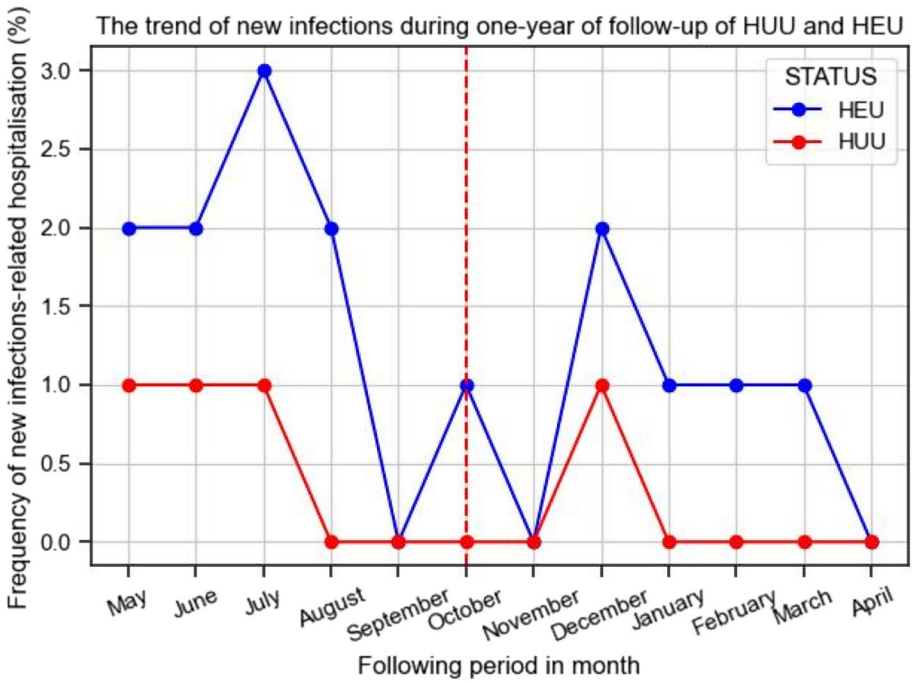

Fig. 3 The trend of new infections during 1-year of follow-up of HUU and HEU. Only 2 Lower respiratory infections, 2 severe gastroenteritis and one urinary tract infections appeared in the second semester (after October)

Table 2 Infection-related hospitalization during 12 months of follow-up

Events	*HEU*	*HUU*	*RR*	*OR*	*Incidence rate*
Urinary tract infections	*2*	*1*	*/*	*/*	*/*
Severe gastroenteritis	*4*	*1*	*/*	*/*	*/*
Lower respiratory infections	*8*	*1*	*/*	*/*	*/*
Neonatal sepsis	*1*	*1*	*/*	*/*	*/*
Total infection-related hospitalization	*15*	*4*	*2.42*	*3.59*	*3.2*

for 47.36% (9/19) of hospitalizations (8 in HEU and 1 in HUU). Other events were severe gastroenteritis (4 HEU and 1 HUU), culture confirmed urinary tract infections (2 HEU and 1 HUU), and neonatal sepsis (2 HEU and 03 HUU). However, only 2 lower respiratory tract infections, 2 severe gastroenteritis, and one urinary tract infection were recorded in the second semester.

Infection-related hospitalization during 12 months of follow-up

Among infants who completed follow-up to month 12 (39 HEU and 27 HUU), the RR for hospitalization was 2.42 (1.03–5.82) fold greater for HEU than HUU with OR 3.59 (1.04–12.45). Moreover, HEU infants experienced an incidence rate of 3.2 (1.63–7.14) hospitalized infants per 100 infant-months, compared to 1.2 (0.57–3.60) in HUU infants for a RR of 2.22 (0.50–9.39) (Table 2). Lastly, two infants with gastroenteritis belonged to the group on artificial feeding.

KIR gene frequencies in HUU and HEU infants

A total of 39 infants born to HIV-1 infected mothers who were on a combination of antiretroviral therapy (ART) to prevent mother-to-child transmission (MTCT) of HIV in-utero were included in the study. However, infants' exposure to ART and viruses in-utero can be affected by multiple infections in their first years of life. To investigate the role of KIR genes, the study first presents the distribution of KIR genes between HEU and HUU infants. The KIR2DL1 gene was found to be significantly more-frequent in the HUU group compared to HEU group (85.2% vs. 51.3%, $p=0.003$) (Fig. 4).

KIR2DL1 gene and infection-related hospitalization

The study revealed that individuals who were not hospitalized due to infections had a higher prevalence of the *KIR2DL1* gene. Therefore, there is a significant correlation between KIR2DL1 and hospitalization related to infections (p value <0.001; OR $=0.06$; 95% CI: 0.02–0.23) (Table 3).

Centromeric/telomeric KIR locus

To determine possible associations with HIV-exposure status, the frequencies of centromeric and telomeric KIR loci between the two groups of infants were

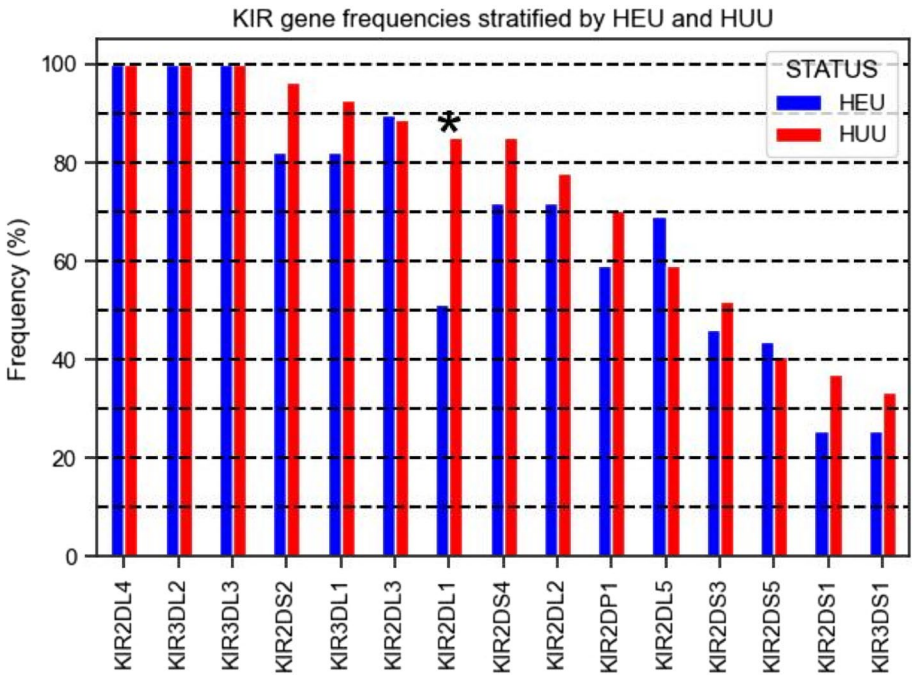

Fig. 4 KIR gene frequencies stratified by HEU and HUU. *p* is the *p*-values comparing HIV unexposed to HIV exposed children using Chi-squared or Fisher exact test as may be appropriated. Multivariate logistic regression analysis were used to determine association between *KIR2DL1* gene and HIV status considering different factors such as the infant's weight, gender, kinds of feeding, mother's age and type of delivery. The symbol (*) represents the gene with a significant *p* value

Table 3 Relation between KIR2DL1 gene and infection-related hospitalization

KIR2DL1			*P*. value	ORa	95%CI	
No		Yes			Lower	Upper
Hospitalization						
No	7 (14.9%)	40 (85.1%)				
Yes	14 (73.7%)	5 (26.3%)				
Total	21 (31.8%)	45 (68.2%)	< 0.001	0.06	0.02	0.23

compared (Table 4), only taking into account KIR locus with at least 5% frequency in the general population. Globally, the frequency of centromeric KIR motif 2 (Ce2) was most represented in the study population with Ce2 prevalent in both HUU and HEU infants (55.6% vs. 43.60% respectively). Moreover, Ce3 motif was more predominant in HUU than HEU infants [55.6% (15/27) vs. 2.6% (1/39) respectively, $p = 0.003$)]. Te1 was equally overrepresented in the study population with 37.04% (10/27) and 30.8% (12/39) in HUU versus HEU respectively. Lastly, Ce/Te2 motif was significantly associated with the HIV-exposure status ($p = 0.035$).

Discussion

Natural Killer cells are known to play an important role by acting as the first line of protection against cells infected by virus and tumor cells. Killer cell immunoglobulin receptors regulate Natural Killer cells cytotoxic activity in innate response against viral infections by interacting with their corresponding HLA class I molecules (ligands) expressed on target cells. This study aimed to compare the diversity of KIR genes in HIV-1 unexposed uninfected (HUU) and HIV-1 exposed uninfected (HEU) infants from Yaoundé, Cameroon. The objective was to elucidate their association with multiple infections. At the onset, it was reported that many

Table 4 Association of KIR haplotypes locus with HIV status

Centromeric					Status	P	
	2DS2	2DL2	2DL3	2DL1	HUU n(%)	HEUn(%)	
Ce1			■	■	1(3.7%)	1(2.6%)	0.741
Ce2	■	■	■	■	15(55.6%)	17(43.6%)	0.588
Ce3		■	■	■	15(55.6%)	1(2.6%)	**0.003**
Ce4	■		■		1(3.7%)	5(12.8%)	0.424
Ce5	■	■		■	2(7.4%)	1(2.6%)	0.428
Ce6	■	■			1(3.7%)	3(7.7%)	0.796
Ce7	■	■	■		1(3.7%)	4(10.4%)	0.055
Ce8			■		1(3.7%)	5(12.8%)	0.424
Ce/Te		2DL5	2DS3	2DS5			
Ce/Te1		■	■	■	4(14.8%)	6(15.4%)	0.730
Ce/Te2		■		■	3(11.1%)	13(33.3%)	**0.035**
Ce/Te3		■	■		5(18.5%)	11(28.2%)	0.645
Ce/Te4			■	■	2(7.4%)	1(2.6%)	0.428
Ce/Te5				■	2(7.4%)	1(2.6%)	0.428
Ce/Te6					4(14.8%)	4(10.3%)	0.425
Ce/Te7		■			4(14.8%)	2(5.1%)	0.171
Ce/Te8			■		3(11.1%)	1(2.6%)	0.246
Telomeric	3DS1	2DS1	3DL1	2DS4			
Te1			■	■	10(37%)	12(30.8)	0.436
Te2	■	■	■	■	5(18.5%)	3(7.7%)	0.138
Te3		■	■	■	5(18.5%)	7(17.9%)	0.223
Te4	■		■	■	2(7.4%)	2(5.1%)	0.536
Te5				■	1(3.7%)	2(5.1%)	0.890
Te6	■				1(3.7%)	1(2.6%)	0.741
Te8			■		0(0.0%)	2(5.1%)	0.427
Te9	■		■		2(7.4%)	3(7.7%)	0.998
Te10					0(0.0%)	2(5.1%)	0.427
Te11		■	■		0(0.0%)	3(7.7%)	0.192

Shaded box: Gene present. clear box: Gene absent

HEU HIV exposed uninfected, *HUU* HIV unexposed uninfected, *Min* Minimum, *Max* Maximum, *CI* Confidence interval, *Ce* Centromeric, *Te* Telomeric, *p P* value < 0.05

factors contributing to the disproportionately higher risk of infectious disease in HEU infants compared to HIV-unexposed infants were investigated. (i) The severity of maternal HIV infection is associated with higher mortality rates in infants born to mothers with high HIV viral loads and low CD4+ lymphocyte counts. (ii) Infants are at increased risk of exposure to maternal bacterial and viral infections. (iii) Malnutrition is a risk factor. (iv) Avoiding breastfeeding is also a risk factor. (v) ARV treatment and (vi) immune aberrations, some of which may be interrelated, are also risk factors [16]. We found that HUU infants had a significantly higher frequency of the inhibitory gene KIR2DL1 ($p=0.008$) compared to HEU infants (Fig. 4). Additionally, the results indicate that the absence of specific KIR genes may be independently associated with multiple diseases in HEU infants.

The mean weight was higher in HUU infants than in HEU infants, but no difference was found (Table 1). These results are similar with those of Slogrove, Afran and Moseholm, and Ekali [2, 7, 8]. The lack of significance may be due to the effects of perinatal/postnatal exposure to HIV and ART on growth outcomes in HEU children [2, 8]. Gastroenteritis was more prevalent in HEU infants who were not breastfed, whereas respiratory infections were more common (Table 2). This suggests that breastfeeding may provide protection against gastroenteritis. It has been suggested that breastfeeding provides greater protection against diarrhoea than respiratory infections in early infancy [40].

The frequency of inhibitory gene *KIR2DL1* was found to be significantly higher in HUU infants compared to HEU infants ($p=0.008$) (Fig. 4), indicating a stronger association with HUU status. The language used is clear, objective, and value-neutral, with a formal register and precise word choice. Differences were noted when comparing different NK subsets. Activated NK cells (CD38 + CD69 +) were higher in HEU infants < 6 months of age [41, 42]. Although inhibitory genes were more frequent in HUU status, the majority of activating genes were represented at a high frequency in HUU compared to HEU, but the differences were not significant.

KIR2DL1 was found to be more closely associated with HUU status ($p=0.008$), even when the *KIR2DL2* and *KIR2DL3* genes were also present (Cent 3) with $p=0.003$ (Table 4). In a study on ligand-instructed models of NK-cell education, it was demonstrated that the recognition of HLA by an inhibitory *KIR2DL2* receptor suppressed the subsequent expression of a second *KIR2DL1* receptor [43]. We propose that NK cell inhibition resulting from the presence of the *KIR2DL1*, *KIR2DL2* and *KIR2DL3* genes may have a lower inhibitory effect than that resulting from NK cells with the *KIR2DL1* gene. We suggest that this reduction in NK cell inhibition is associated with a normal HUU status.

The analysis revealed that the region including both the *KIR2DL5* and *KIR2DS5* genes (Ce/Te2) was significantly associated with HEU ($p=0.035$), while the association of HEU with *KIR2DL5* alone was not significant ($p=0.052$) (Table 4). This suggests that *KIR2DS5* may enhance the inhibitory effect of *KIR2DL5*. The combination of *2DL5/2DS5* may explain why NK cells are activated but have low cytolytic function in the HEU group, which is associated with multiple infections.

Although there is no statistically significant difference in the incidence of different infections between HEU and HUU infants, we observed that HEU infants had a higher incidence rate of hospitalization at 3.2 (1.63–7.14) per 100 infant-months compared to 1.2 (0.57–3.60) in HUU infants, resulting in a RR of 2.22 (0.50–9.39) (Table 2). The study found that the presence of the *KIR2DL1* gene was more common in individuals who were not hospitalized due to infections, suggesting that it may provide protection against infection-related hospitalization (Table 3).

KIR2DL1 also plays a crucial role in HIV disease progression [29]. Furthermore, Studies have shown that the absence of *KIR2DL1* is significantly associated with infection-related hospitalization in HIV-exposed uninfected infants (HEU) compared to HIV-unexposed uninfected (HUU) infants, indicating a protective role of *KIR2DL1* against infections [29], further supporting its protective role against infections. Additionally, the interaction between *KIR2DL1* and *HLA-Bw4-80I* gene has been linked to lower viral loads and higher CD4+ T-cell counts, suggesting a potential impact on HIV control and disease progression [44]. The data indicates that *KIR2DL1* plays a significant role in influencing susceptibility to infections and potentially modulating HIV disease outcomes.

The research project concentrated on the KIR genes with the objective of identifying differences between HUU and HEU infants, with a particular focus on the reasons behind the latter group's increased susceptibility to multiple infections. The study's strength lies in its target population of 6-week-old infants, chosen prior to any hospitalization. Investigating the impact of the presence and expression profiles of KIR genes on the function of NK cells in relation to disease susceptibility represents an intriguing avenue for further inquiry. Furthermore, it would be beneficial to ascertain the influence of NK cells on the functionality of other immune cells, such as dendritic cells and monocytes. A number of studies have demonstrated that KIR genes and HLA class I molecules can act in concert to influence disease progression in adults [23, 45, 46]. Our findings may prove useful in the field of precision medicine, particularly in the context of multiple infections in HIV-1 exposed uninfected patients. Nevertheless, further research is required to elucidate the molecular mechanisms underlying the interactions between KIR genes and HIV-1 exposure in HEU infants in Cameroon. The limitations of this study include the small sample size, which precludes the possibility of performing statistical analysis to verify the link between infection type and status (HUU and HEU). Additionally, the study is unable to adopt a comparative multinational approach to investigate the impact of geographic and genetic diversity on KIR gene expression and infection susceptibility.

Conclusion

Our study which aimed to determine KIR genes and their association to multiple infections in HEU in Yaoundé-Cameroon is the first to our knowledge to be done.

Compared to HUU, the vulnerability of HEU is driven by *KIR2DL1*, indicating a possible protective role of this KIR gene against infection and hospitalizations.

Acknowledgements
We thank all the study participants for willingly participating in the study. We would also like to thank the staff of Green City, Efoulan, Mbiyem-Assi, and CASS Nkoldongo hospitals for their tremendous support and collaborations.

Authors' contributions
LAK and JCA designed this study. JCA supervise the work, reviewed data and manuscript. LAK, CYK, OM and RKW performed the search and collected data. LAK and GLE, re-checked data. LMY co-supervised the work, reviewed data and manuscript. LAK and CYK performed analysis. LA wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

Funding
No funding.

Availability of data and materials
All the data are available in the manuscript.

Declarations

Ethics approval and consent to participate
The Institutional Ethics Committee for Human Health at the University of Douala conducted an ethical review of the protocol (No: 1639IEC-UD/06/2018/T). Administrative approval was obtained from the directors of various health facilities. Mothers of infants provided written informed consent to participate with their children. Data was processed using specific identifiers to ensure privacy and confidentiality. Clinical data generated during the study was provided free of charge to all participants.
Written informed consent including assent for infants were obtained for all the recruited study participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Buéa, Buéa, Cameroon. [2]American Association of Microbiology (ASM), ASM Cameroon, Bangangté, Cameroon. [3]Department of Biochemistry, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon. [4]Division of Hepatology, Leipzig University Medical Centre, Leipzig, Germany. [5]SOA Health District, Ministry of Public Health, Yaoundé, Cameroon. [6]Virology Laboratory, Chantal BIYA International Reference Centre for Research on HIV/AIDS Prevention and Management, Yaoundé, Cameroon. [7]Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon. [8]ICAP, Columbia University, Yaoundé, Cameroon. [9]Nuffield Department of Medicine (NDM), University of Oxford, Oxford, UK.

Received: 17 February 2024 Accepted: 23 July 2024
Published online: 01 August 2024

References

1. van Schalkwyk C, Mahy M, Johnson LF, Imai-Eaton JW. Updated data and methods for the 2023 UNAIDS HIV estimates. J Acquir Immune Defic Syndr 1999. 2024;95 1 Suppl:e1–4.
2. Slogrove AL, Goetghebuer T, Cotton MF, Singer J, Bettinger JA. Pattern of infectious morbidity in HIV-exposed uninfected infants and children. Front Immunol. 2016;7:164.
3. Brennan AT, Bonawitz R, Gill CJ, Thea DM, Kleinman M, Useem J, et al. A meta-analysis assessing all-cause mortality in HIV-exposed uninfected compared with HIV-unexposed uninfected infants and children. AIDS Lond Engl. 2016;30:2351–60.
4. Lorestani RC, Rostamian M, Akya A, Rezaeian S, Afsharian M, Habibi R, et al. Prevention of mother-to-child transmission of HIV in Kermanshah, west of Iran from 2014 to 2021. BMC Pediatr. 2023;23:29.
5. Society CP. HIV in pregnancy: identification of intrapartum and perinatal HIV exposures. Canadian Paediatric Society. https://cps.ca/documents/position/hiv-in-pregnancy. Accessed 28 May 2024.
6. Brocklehurst P. Interventions for reducing the risk of mother-to-child transmission of HIV infection. Cochrane Database Syst Rev. 2002. https://doi.org/10.1002/14651858.CD000102.
7. Afran L, Garcia Knight M, Nduati E, Urban BC, Heyderman RS, Rowland-Jones SL. HIV-exposed uninfected children: a growing population with a vulnerable immune system? Clin Exp Immunol. 2014;176:11–22.
8. Ekali GL, Jesson J, Enok PB, Leroy V. Effect of in utero exposure to HIV and antiretroviral drugs on growth in HIV-exposed uninfected children: a systematic review and meta-analysis protocol. BMJ Open. 2019;9:e023937.
9. Chavan VR, Ahir S, Ansari Z, Samant-Mawani P, Nanavati R, Mehta P, et al. Diversity in KIR gene repertoire in HIV-1 exposed infected and uninfected infants: a study from India. J Med Virol. 2016;88:417–25.
10. Mora-Bitria L, Asquith B. Innate receptors modulating adaptive T cell responses: KIR-HLA interactions and T cell-mediated control of chronic viral infections. Immunogenetics. 2023;75:269–82.
11. Downing J, D'Orsogna L. High-resolution human KIR genotyping. Immunogenetics. 2022;74:369–79.
12. Moretta L, Moretta A. Killer immunoglobulin-like receptors. Curr Opin Immunol. 2004;16:626–33.
13. Yin C, Hu L, Huang H, Yu Y, Li Z, Ji Q, et al. Genetic polymorphism and evolutionary differentiation of Eastern Chinese Han: a comprehensive and comparative analysis on KIRs. Sci Rep. 2017;7:42486.
14. Dubreuil L, Chevallier P, Retière C, Gagne K. Relevance of polymorphic KIR and HLA class I genes in NK-cell-based immunotherapies for adult leukemic patients. Cancers. 2021;13:3767.
15. Desmonde S, Goetghebuer T, Thorne C, Leroy V. Health and survival of HIV perinatally exposed but uninfected children born to HIV-infected mothers. Curr Opin HIV AIDS. 2016;11:465–76.
16. Dzanibe S, Jaspan HB, Zulu MZ, Kiravu A, Gray CM. Impact of maternal HIV exposure, feeding status, and microbiome on infant cellular immunity. J Leukoc Biol. 2019;105:281–9.
17. Gabriel B, Medin C, Alves J, Nduati R, Bosire RK, Wamalwa D, et al. Analysis of the TCR repertoire in HIV-exposed but uninfected infants. Sci Rep. 2019;9:11954.
18. Ruck C, Reikie BA, Marchant A, Kollmann TR, Kakkar F. Linking susceptibility to infectious diseases to immune system abnormalities among HIV-exposed uninfected infants. Front Immunol. 2016;7:310.
19. McLaren PJ, Fellay J. HIV-1 and human genetic variation. Nat Rev Genet. 2021;22:645–57.
20. Durovic B, Gasser O, Gubser P, Sigle J, Hirsch HH, Stern M, et al. Epstein-Barr virus negativity among individuals older than 60 years is associated with HLA-C and HLA-Bw4 variants and tonsillectomy. J Virol. 2013;87:6526–9.
21. Benton ML, Abraham A, LaBella AL, Abbot P, Rokas A, Capra JA. The influence of evolutionary history on human health and disease. Nat Rev Genet. 2021;22:269–83.
22. Bruijnesteijn J, de Groot NG, Bontrop RE. The genetic mechanisms driving diversification of the KIR gene cluster in primates. Front Immunol. 2020;11:582804.
23. Pollock NR, Harrison GF, Norman PJ. Immunogenomics of killer cell immunoglobulin-like receptor (KIR) and HLA class I: coevolution and consequences for human health. J Allergy Clin Immunol Pract. 2022;10:1763–75.
24. Mohammadhosayni M, Aslani S, Norouzi M, Jazayeri SM, Ahmadi M, Ghazanfari T. A systematic review and meta-analysis of *killer-cell immunoglobulin-like receptor* (*KIR*) family genes association with risk of hepatitis B virus (HBV). Gene Rep. 2021;23:101096.
25. Torimiro J, Yengo CK, Bimela JS, Tiedeu AB, Lebon PA, Sake CS, et al. Killer cell immunoglobulin-like receptor genotypes and haplotypes contribute to susceptibility to hepatitis B Virus and hepatitis C virus infection in Cameroon. Omics J Integr Biol. 2020;24:110–5.
26. Podhorzer A, Dirchwolf M, Machicote A, Belen S, Montal S, Paz S, et al. The clinical features of patients with chronic hepatitis C virus infections

are associated with killer cell immunoglobulin-like receptor genes and their expression on the surface of natural killer cells. Front Immunol. 2018;8:1912.
27. Abdelmaguid W, Maher D, Kohla MAS, Ezzat S, Moaz I, Abdel-Mageed WS, et al. KIR genotypes impact progression to hepatocellular carcinoma in patients with chronic hepatitis C infection. Livers. 2023;3:354–68.
28. Teshnizi SH, Mirzazadeh S, Mashhadi N, Meri S, Kabelitz D, Kalantar K. Association study between killer immunoglobulin-like receptor polymorphisms and susceptibility to COVID-19 disease: a systematic review and meta-analysis. Immunol Res. 2024;72:175–84.
29. Sorgho PA, Djigma FW, Martinson JJ, Yonli AT, Nagalo BM, Compaore TR, et al. Role of Killer cell immunoglobulin-like receptors (KIR) genes in stages of HIV-1 infection among patients from Burkina Faso. Biomol Concepts. 2019;10:226–36.
30. Wang L, Zhang Y, Xu K, Dong T, Rowland-Jones S, Yindom L-M. Killer-cell immunoglobulin-like receptors associate with HIV-1 infection in a narrow-source Han Chinese cohort. PLoS One. 2018;13:e0195452.
31. Luc-Aimé KS, Louis-Marie Y, Gabriel LE, Yengo CK, EsemuLivo F, Assob NJC. Killer-cell immunoglobulin-like receptors (KIR) in HIV-exposed infants in Cameroon. J Immunol Res. 2021;2021:9053280.
32. Mukisa J, Amujal M, Sande OJ, Joloba ML, Jjingo D, Kateete DP, et al. Killer cell immunoglobulin receptor diversity and its relevance in the human host's response to HIV infection in African populations. Transl Med Commun. 2023;8:8.
33. Présidence de la république du Cameroun. Cameroon. https://www.prc.cm/en/cameroon. Accessed 28 May 2024.
34. Sanjeeva G, Sathenahalli VB. HIV-exposed uninfected children: a vulnerable and neglected population. Indian Pediatr. 2019;56(6):461–2.
35. Djoba Siawaya JF, Maloupazoa Siawaya AC. HIV-exposed uninfected infants' immune defects: From pathogen sensing, oxidative burst to antigens responses. iScience. 2023;26:108427.
36. Torimiro JN, Mafopa NG, Ndongo A, Moyo ST, Lobe E, Sosso SM, et al. Evaluation of virologic methods for early detection of HIV-1 in a resource-limited setting: performance and cost Analysis. Health Sci Dis. 2013;14(3):7.
37. Carrington M, Martin MP, van Bergen J. KIR-HLA intercourse in HIV disease. Trends Microbiol. 2008;16:620–7.
38. González-Galarza FF, Takeshita LYC, Santos EJM, Kempson F, Maia MHT, da Silva ALS, et al. Allele frequency net 2015 update: new features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. Nucleic Acids Res. 2015;43:D784–8.
39. Mhandire K, Zijenah LS, Yindom L-M, Duri K, Mlambo T, Tshabalala M, et al. KIR gene content diversity in a Zimbabwean population: does KIR2DL2 have a role in protection against human immunodeficiency virus infection? Omics J Integr Biol. 2016;20:727–35.
40. Juharji H, Albalawi K, Aldwaighri M, Almalki A, Alshiti H, Kattan W, et al. Impact of breastfeeding on low birthweight infants, weight disorders in infants, and child development. Cureus. 2022;14:e32894.
41. Slyker J, Lohman Payne B, John-Stewart G, Dong T, Mbori-Ngacha D, Tapia K, et al. The impact of HIV-1 infection and exposure on natural killer (NK) cell phenotype in Kenyan infants during the first year of life. Front Immunol. 2012;3:399.
42. Jalbert E, Ghosh T, Smith C, Amaral FR, Mussi-Pinhata MM, Weinberg A. Impaired functionality of antigen presenting cells in HIV- exposed uninfected infants in the first six months of life. Front Immunol. 2022;13:960313.
43. Chang K-C, Wu Y-Y, Hung C-H, Lu S-N, Lee C-M, Chiu K-W, et al. Clinical-guide risk prediction of hepatocellular carcinoma development in chronic hepatitis C patients after interferon-based therapy. Br J Cancer. 2013;109:2481–8.
44. Błachowicz O, Zwolińska K. The role genes encoding of killer cell immunoglobulin-like receptors (KIRs) and their ligands in susceptibility to and progression of HIV infection. Postepy Hig Med Dosw. 2016;70:1409–23.
45. Umemura T, Joshita S, Saito H, Yoshizawa K, Norman GL, Tanaka E, et al. KIR/HLA genotypes confer susceptibility and progression in patients with autoimmune hepatitis. JHEP Rep. 2019;1:353–60.
46. HematianLarki M, Ashouri E, Barani S, Ghayumi SMA, Ghaderi A, Rajalingam R. KIR-HLA gene diversities and susceptibility to lung cancer. Sci Rep. 2022;12:17237.

Publisher's Note