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Original Article

KIR2DL2, KIR2DL5A and KIR2DL5B Genes Induce Susceptibility to Dengue Virus Infection, while KIR3DL3 and KIR2DS5 Confer Protection

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Abstract. *Background and Objectives*: Dengue fever (DF), an emerging and re-emerging viral disease, is a major public health problem. The aim of this study was to investigate the influence of *KIRs* genes polymorphism and *KIRs* genotypes in susceptibility to dengue virus infection and disease severity in a population from Burkina Faso through a case-control study.
***Methods*: *KIRs* genes determination was performed using PCR-SSP in 50 patients infected by dengue virus (DENV) and 54 Healthy controls (HC) subjects who had never been infected.**
***Results*: Data analysis showed significant association between frequencies of three KIR genes and dengue virus infection (DF): *KIR2DL2* (OR: 7.32; IC: 2.87-18.65; P < 0.001); *KIR2DL5A* (OR: 15.00, IC: 5.68-39.59; P < 0.001) and *KIR2DL5B* (OR: 11.43; IC: 4.42-29; P < 0.001). While, *KIR3DL3* (OR: 0.13, IC: 0.052-0.32; P < 0.001) and *KIR2DS5* (OR: 0.12; IC: 0.04-0.30; P < 0.001) were associated with protection against DF. *KIR2DL4* (OR: 9.75; IC95%: 1.33-70.97; p: 0.03) and *KIRD3DL1* (OR: 12.00; IC95%: 1.60-90.13; p: 0.02) were associated with an increased risk in the development of secondary dengue infection (SDI).**
***Conclusion*: The results suggest a contribution of *KIR2DL2, KIR2DL5A,* and *KIR2DL5B* genes in the susceptibility of DF development. In contrast, *KIR3DL3* and *KIR2DS5* were associated with protection against DF development by enhancing both innate and acquired immune responses.**

Keywords: Dengue infection, *KIRs* genes, Haplotype, SSP-PCR, Burkina Faso.

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Introduction. Dengue fever is widespread in the tropics and subtropical regions; it is the first public health problem caused by arboviruses. According to the World Health Organization,[1] around 40-50% or 3.9 billion people in 128 countries are exposed to the dengue virus (DENV); each year, there are 390 million cases of dengue fever with 96 million presenting symptoms and more than 3,000 deaths in the world.[1] Recently, outbreaks of the Dengue Fever (DF) epidemic were reported in many European countries and Africa, including Burkina Faso where 1061 probable cases and 15 deaths were reported in 2016.[2] In August 2019, Burkina Faso once again experienced cases of DF observed in hospitals of Ouagadougou and its surroundings.[3] In their study, Ouattara *et al.* (2017) reported, in Burkina Faso, that the prevalence of dengue virus infection was 23.5% in 2016 and 13.3% in 2017.[4] Dengue virus (DENV) is a member of the flavivirus family comprising at least four distinct serotypes. Transmitted by the mosquito *Aedes aegypti*, DENV is endemic in the tropics/subtropics and causes an acute febrile illness known as dengue fever (DF). However, a small percentage of individuals experience a more severe syndrome known as dengue hemorrhagic fever (DHF). The key features of DHF are plasma leakage and a bleeding tendency, which develop as the fever subsides with clearance of viremia.[5,6] There are four serotypes of dengue viruses (DENV-1, DENV-2, DENV-3 and DENV-4) which share 65–70% sequence homology.[1,7]

The onset of the severe form of Dengue is due to increased endothelial dysfunction and vascular leakage. It could be explained by an increase in viremia but also by the phenomenon of antigenic sin linked to the genetics of the host.[8] As the vaccine or effective antiviral therapy is not yet available to everyone to prophylactically or therapeutically treat DENV infection, Dengue's incidence is increasing globally, worldwide, especially in the endemic area.[9]

Many studies have shown the influence of the KIR genes on the host's susceptibility and resistance to infectious diseases, such as AIDS, Hepatitis B, C, and leprosy.[10-12]

Studies conducted in many countries revealed the importance of KIR and HLA ligands in innate immune responses to Dengue viral infections and, in particular, their effect on clinical outcomes and disease severity.[13-16]

The human KIR gene locus is located on chromosome19q13.4 and extends approximately 150KB, encoding more than 15 KIR genes.[17] The KIR genes are grouped into two major haplotypes, namely haplotype A consisting of the KIR3DL3, 2DL3, 2DL1, 2DP1, 3DP1, 2DL4, 3DL1, 2DS4, 3DL2 genes, and haplotype B, the composition of which is variable including several genes and alleles which are not part of haplotype A. Each haplotype (A or B) consists of four framework genes (KIR3DL3, 3DP1, 2DL4, and 3DL2) which, with very rare exceptions, are present in each individual (18, 19). All human populations have haplotypes of groups A and B with varying frequencies. Individuals with only the genes of the group A KIR haplotypes (KIR3DL3, 2DL3, 2DL1, 2DP1, 3DP1, 2DL4, 3DL1, 2DS4, 3DL2) were considered to be homozygous for haplotype A and received the AA genotype of KIR. Individuals without one of the four genes associated with haplotype A (KIR2DL1, 2DL3, 3DL1, and 2DS4), which have a known function and vary from one individual to another, are considered to be homozygous for haplotypes of group B and have received the KIR BB genotype. All other individuals considered heterozygous for haplotypes A and B were assigned the KIR genotype AB.[19,20] Either AB or BB genotypes were referred to as KIR genotype Bx, which contains more activating KIR genes.[21] The human leukocyte antigen (HLA) class I molecules on target cells are ligands for some KIRs. The presence or absence of KIR genes and their HLA class I ligands are associated with susceptibility to or protection against infectious diseases.[22] In Burkina Faso, there are yet no studies in the literature showing the influence of KIRs genes on the development of dengue fever. Therefore, the aim of this study was to investigate the impact of *KIRs* genes polymorphisms on susceptibility and resistance to dengue virus infections and disease severity from a population of Burkina Faso.

Material and Methods.

Type and Population of the Study. This is a case-control study that was conducted from June to December 2018. A total of 104 individuals were included in this study, which consisted of 50 patients of Dengue virus and 54 Healthy Controls recruited at the laboratories of Saint Camille Hospital in Ouagadougou (HOSCO), National Center for Blood Transfusion (CNTS) and Pietro Annigoni Biomolecular Research Center (CERBA/LABIOGENE) respectively. All subjects were seronegative for Human Immunodeficiency Virus (HIV), hepatitis B (HBV), and C (HCV) infections and had not also other pathology history reported. Patients of all ages, including children and blood donors, came from all professions and social categories. All patients seen for consultations during the sample collection period and presenting at least two signs suggestive of dengue fever were included after giving their free consent. In addition, voluntary blood donors received during the collection period were also included with no known history of Dengue. The subjects with no contact with DENV from the same geographical area were included as Healthy Controls after giving their free consent. Healthy controls were screened for exposure to DENV (AgNS1, IgM, and IgG).

Ethical Consideration. The present study received the approval of the Ministry of Health of Burkina-Faso through its Ethics Committee for Health Research (CERS) (Deliberation N°2017-01-004), and the institutional ethics committee of CERBA/LABIOGENE approved this study. According to the Helsinki declarations, written informed consent was obtained from the study participants for adult persons and tutors for children.

Dengue Virus Diagnostic. Serological markers for DENV were detected using Dengue Duo Comb Test Kits (Abon Biopharm Guangzhou, Co., Ltd. China). The AgNS1, IgM and IgG were detected directly from blood samples obtained by taking venous blood from the bend of the elbow. The results were read between 15 and 20 minutes.

Definition of Primary and Secondary Dengue Infection. There are four distinct serotypes of the dengue virus which infect humans. An individual infected with one of them is immunized for life against this serotype but only acquires transient and partial immunity against the other serotypes. Consequently, this disease has no cross-protective immunity,[35] so a single person can have up to four episodes of dengue fever in their lifetime. Primary dengue fever is thus distinguished from secondary Dengue through the analysis or diagnostics of the kinetics of anti-IgM and anti-IgG antibodies and of viremia.[36]

Primary infection of DENV is defined as the cases where we have the immuno-serological AgNS1 (+) / IgM (+/-) / IgG (-), and secondary infection of DENV is the cases of reinfection by another serotype, therefore on the immune-serological level we translate it by AgNS1 (+)/ IgM (+/-) IgG (+).

Genomic DNA Extraction and Determination of KIR Genes by SSP-PCR. Genomic DNA was extracted from the serum or plasma using the commercial kit called "DNA-Sorb-B" from Sacace Biotechnologies®, Italy, according to the manufacturer's protocol. DNA purity and concentration were determined using a Biodrop (Isogen Life Science, NV/S.A, Temse, Belgium). Approximately 100 ng/µl of DNA was used to amplify the subset of 12 targeted KIR genes using the SSP-PCR method as previously described.[22] The PCR reactions were performed in 60 µL of the reaction mixture containing 100 ng/µL of DNA (variable volume), 7.5 µL of 10 × PCR buffer, 2.25 µL MgCl2; 0.6 µL of dNTPs and 0.375 µL of Platinum™ DNA Taq polymerase in nuclease-free water. The PCR reactions were performed as follows: after initial denaturation for 3 minutes at 94°C, the amplifications were carried out respectively for 5 cycles, 21 cycles and 4 cycles of denaturation at 94°C, annealing at primer-specific temperature for 15 seconds (65°C and 60°C) or 1 minute (55°C for 4 cycles), and extension at 30 seconds at 72°C or 2 minutes for 4 cycles step with a final extension at 72°C for 7 minutes. The PCR products were separated on 3% agarose gel and visualized under UV light at 312 nm using the Gene flash apparatus (Gene Flash syngenge Bio-Imaging, USA). PCR products were validated against a positive internal control corresponding to the DRB1 gene fragment.

Prediction of KIR Haplogroups from Genotypes. The KIR gene content of a given individual is conventionally called "KIR genotype", which is variable among individuals. The KIR gene content was used to infer group A and B KIR Haplotypes and to assign each person to one of three genotypes: AA, BB, and AB. Individuals having only genes of the group A KIR haplotypes (KIR3DL3-2DL3-2DL1-2DP1-3DP1-2DL4-3DL1-2DS4-3DL2) were considered to be homozygous for the A haplotype and assigned the KIR genotype AA. Individuals lacking any of the four A haplotype-associated genes (KIR2DL1, 2DL3, 3DL1, and 2DS4) that have a known function and vary among individuals in their existence were regarded to be homozygous for group B haplotypes and assigned the KIR genotype BB. All other individuals were considered heterozygous for A and B haplotypes and assigned the KIR genotype AB. The individuals with AB genotypes had all nine genes on the A haplotype and one or more B haplotype-specific genes (2DL2, 2DL5, 2DS1, 2DS2, 2DS3, 2DS5, and 3DS1).[19,20] Therefore, the AB genotypes were considered heterozygous, carrying both haplogroup genes. However, due to the difficulty in differentiation between AB and BB genotypes, the current system annotated them as Bx genotypes according to Allele Frequency Net Database (AFND).

Statistical Analysis. The data was analyzed using the standard Statistical Package for Social Sciences (SPSS) version 20.0. The $\chi 2$ test was used to compare variant frequencies between groups. The risk was estimated with an Odds Ratio (OR) and 95% of confidence interval (95% CI). P-values < 0.05 were considered statistically significant. Association between KIRs genes and dengue virus infection was established by comparing frequencies between cases and controls using the $\chi 2$ test.

Results. The study population consisted of 104 subjects, with 50 patients of DENV presenting clinical signs of dengue fever, which were confirmed by diagnostic, and 54 Healthy Controls who had never been infected by the DENV. The percentage of men was 40.38% (42/104) and 59.62% (62/104) for women. Among 50 dengue virus patients, women represented the most (52%). The sex ratio of the study population was 0.68 (42/62). In the study population, the youngest was 4 years old, and the

Table 1. Sociodemographic characteristics of the study population.

Variables	AgNS1 (+) *n* (%)	AgNS1 (-) *n* (%)	Total *n* (%)	OR	95% CI	p-value
Gender						
Men	24 (48.00)	18 (33.33)	42 (40.38)	**Ref**		
Women	26 (52.00)	36 (66.67)	62 (59.62)	**0.54**	**0.24-1.20**	**0.16**
Age (Year)						
0-19	12 (24.00)	8 (14.81)	20 (19.23)	**0.51**	**0.18-1.39**	**0.21**
20-39	32 (64.00)	42 (77.78)	74 (71.15)	**Ref**		
≥40	6 (12.00)	4 (7.70)	10 (09.61)	**0.51**	**0.13-1.95**	**0.34**

+: positive to dengue virus; -: negative to dengue virus; AgNS1: nonstructural 1 Antigen.
This table shows the distribution of the study population by gender and age.

Table 2. Serological diagnostic of dengue infection**.**

Variable	Negative		Positive	
	N	%	N	%
AgNS1	54	100.00	50	100.00
IgM (-)/ IgG (-)	54	100.00	5	10.00
IgM (+/-) IgG (+)	0	00.00	45	90.00

Results of different markers of dengue virus infection.

majority had an age between 20 to 39 years. The average age of the patients was 26.58 ± 12.01 years. The highest frequency of dengue fever (64.00%) was noted in patients aged between 20 to 39 years **(Table 1).**

The serological diagnostic of Dengue virus revealed 10.00% (5/50) of primary infection with dengue virus and 90% (45/50) of secondary infection to DENV in the study population. The proportion of dengue fever was 48.08% (50/104), with a rate of at least one contact with DENV **(Table 2).**

A total of 16 KIR genes were genotyped by using the SSP-PCR method. The results showed the different frequencies of KIR genes between dengue patients (DF) and Healthy Controls. The frequencies of *KIR2DL2* (OR: 7.32; IC: 2.87-18.65; P < 0.001); *KIR2DL5A* (OR: 15.00, IC: 5.68-39.59; P < 0.001); *KIR2DL5B* (OR: 11.43; IC: 4.42-29; P < 0.001); *KIR2DS2* (OR: 2.40; IC: 1.06-5.41; P = 0.04) were more frequent in dengue patients (DF) while the frequencies of *KIR3DL3* (OR: 0.13, IC: 0.052-0.32; P < 0.001) and *KIR2DS5* (OR: 0.12; IC: 0.04-0.30; P < 0.001) were more frequent in Healthy controls subjects **(Table 3).**

When the DENV primary infection group was compared to DENV secondary infection group, we found that *KIR2DL4* (OR: 9.75; IC95%: 1.33-70.97; p: 0.03), *KIRD3DL1* (OR: 12.00; IC95%: 1.60-90.13; p: 0.02) were associated with an increased risk in the development of dengue secondary infection. In contrast *KIRD2DLB* (OR: 0.08; IC95%: 0.08-0.62; P: 0.02) was associated in the protection of secondary dengue development (**Table 4).**

The content of the KIR genes from our study population was used to infer the different KIR haplotypes and assign a genotype to each person. Three genotypes, notably the AA, AB, and BB genotypes, were identified from the study population. The AB and BB genotypes were both referred to as KIR genotype Bx which contains more activating KIRs genes. In the general population study, we found 5.77% of AA genotypes and 92.23% of Bx genotypes. In DENV patients, we recorded an AA genotype frequency of 8.00% and a Bx genotype frequency of 92.00%. The AA and Bx genotypes frequencies were 3.70% and 96.30%, respectively, in Healthy Controls subjects. No association was established between the frequencies of AA and Bx genotypes in DENV patients and Healthy controls. However, the Bx genotype was the predominant genotype in the total population study **(Table 5).**

Discussion. This study identified *KIRs* genes and haplotypes in dengue patients and healthy control subjects for the first time in a population of Burkina Faso. Given the damage caused by this arbovirus in the country's health system with its share of deaths as well as psychosis in the previous years, we did not hesitate to carry out this investigation despite the modest size of our sample compared to the general population of the Burkina Faso an endemic country. This pilot study will help to understand how human genetic factors are involved in cases of viral dengue infection.

Many previous studies have shown that KIRs receptors, a group of Natural Killer receptors, play an important role in controlling the severity of viral diseases and infections in humans.[12,23-26] Previous studies have already established a relationship between *KIRs* genes and certain infectious diseases and cancers, such as

Table 3. Association between KIRs genes and dengue virus infection (DF).

GENES KIR n (%)		Dengue Fever (DF)	Healthy Controls (HC)	OR (95% CI)	p- value
Inhibitors					
KIR2DL1	-	25 (50,00)	32(59,26)	Ref.	
	+	25 (50,00)	22 (40,74)	1,45 (0,67-3,16)	0,45
KIR2DL2	-	22 (44,00)	46 (85,19)	Ref.	
	+	28 (56,00)	8 (14,81)	7,32 (2,87-18,65)	**<0,001**
KIR2DL3	-	22 (44,00)	34 (62,96)	Ref	
	+	28 (56,00)	20 (37,04)	2,16 (0,98-4,74)	0,08
KIR2DL4	-	41 (82,00)	47 (87,04)	Ref.	
	+	9 (18,00)	7 (12,96)	1,47 (0,50-4,31)	0,66
KIR2DL5A	-	8 (16.00)	40 (74,07)	Ref.	
	+	42 (84,00)	14 (25,93)	15,00 (5,68-39,59)	**<0,001**
KIR2DL5B	-	8 (16.00)	37 (68,52)	Ref.	
	+	42 (84,00)	17 (31,48)	11,43 (4,42-29,52)	**<0,001**
KIR3DL1	-	42 (84,00)	40 (74,07)	Ref.	
	+	8 (16.00)	14 (25,93)	0,54 (0,21-1,44)	0,24
KIR3DL2	-	43 (86,00)	50 (92,59)	Ref.	
	+	7 (14,00)	4 (7,41)	2,03 (0,56-7,42)	0,35
KIR3DL3	-	41 (82,00)	20 (37,04)	Ref.	
	+	9 (18,00)	34 (62,96)	0,13 (0,052-0,32)	**<0,001**
Activators					
KIR2DS1	-	32(64,00)	26 (48,15)	Ref.	
	+	18(36,00)	28 (51,85)	0,52 (0,23-1,14)	0,12
KIR2DS2	-	26 (52,00)	39 (72,22)	Ref.	
	+	24 (48,00)	15 (27,78)	2,40 (1,06-5,41)	**0,04**
KIR2DS3	-	36 (72,00)	37 (68,552)	Ref.	
	+	14(28,00)	17 (31,48)	0,85 (0,36-1,96)	0,83
KIR2DS4	-	32 (64,00)	29 (53,70)	Ref.	
	+	18 (36,00)	25 (46,30)	0,65 (0,29-1,43)	0,32
KIR2DS5	-	41(82,00)	19 (35,19)	Ref.	
	+	9 (18,00)	35 (64,81)	0,12 (0,04-0,30)	**<0,001**
KIR3DS1	-	43 (86,00)	49 (90,74)	Ref.	
	+	7 (14,00)	5 (9,26)	1,59 (0,47-5,39)	0,54
Pseudogene					
KIR2DP1	-	40 (80,00)	40 (74,07)	Ref.	
	+	10 (20.00)	14 (25,93)	0,71 (0,28-1,80)	0,49

+: presence of kir gene; -: absence of kir gene. These results describe the presence or absence of kir genes according to dengue virus infection or not.

hepatitis B,[12,23,26] hepatitis C with hepatocellular carcinoma (27-29), and AIDS with Lymphomas.[11,24] The 20-39 year age group of patients had the highest frequency of dengue fever (64.00%) **(table 1),** and 90% (45/50) of patients had a secondary dengue infection in our population of the study **(Table 2).** This proportion of young people who contracted the dengue virus and the rate of secondary dengue infection in the study population justifies that dengue infection represents a major health problem in tropical areas, according to WHO.[1]

KIR receptors influence susceptibility or protection from certain diseases through a balance between the signals of activation or inhibition that regulate the function of NK cells. These cells interact with target cells that express HLA class I molecules on their surface, which are ligands for KIR ().[14]

The study showed that *KIR2DL2, KIR2DL5A, KIR2DL5B, and KIR2DS2* were susceptibility genes associated with DF development, while *KIR3DL3* and

Table 4. Implication of KIRs genes in primary and secondary dengue infection.

GENES KIR n (%)		Dengue Primary Infection	Dengue secondary Infection	OR (95% CI)	p-value
Inhibitors					
KIR2DL1	-	03 (60.00)	22 (48.89)	Ref.	
	+	02 (40.00)	23 (51.11)	0.64(0.10-4.19)	1.00
KIR2DL2	-	3 (60.00)	19 (42.22)	Ref.	
	+	2 (40.00)	26 (57.78)	0.49 (0.07-3.21)	0.64
KIR2DL3	-	02(40.00)	20 (44.44)	Ref.	
	+	03 (60.00)	25(55.56)	1.20(0.18-7.89)	1.00
KIR2DL4	-	02 (40.00)	39 (86.67)	Ref.	
	+	03 (60.00)	06 (13.33)	9.75(1.33-70.97)	**0.03**
KIR2DL5A	-	2 (40.00)	06 (13.33)	Ref.	
	+	3 (60.00)	39 (86.67)	0.23 (0.03-1.68)	0.17
KIR2DL5B	-	3 (60.00)	05 (11.11)	Ref.	
	+	2 (40.00)	40 (88.89)	0.08 (0.08-0.62)	**0.02**
KIR3DL1	-	02 (40.00)	40 (88.89)	Ref.	
	+	03 (60.00)	05 (11.11)	12.00(1.60-90.13)	**0.02**
KIR3DL2	-	04 (80.00)	39 (86.67)	Ref.	
	+	01 (20.00)	06 (13.33)	1.62(0.15-17.10)	0.54
KIR3DL3	-	4 (80.00)	37 (82.22)	Ref.	
	+	1 (20.00)	08 (17.78)	1.15 (0.04-10.72)	**1.00**
Activators					
KIR2DS1	-	03 (60.00)	29(64.44)	Ref.	
	+	02 (40.00)	16 (35.56)	1.20(0.1-8.00)	1.00
KIR2DS2	-	2 (40.00)	24 (53.33)	Ref.	
	+	3 (60.00)	21 (46.67)	1.71 (0.26-11.26)	**0.66**
KIR2DS3	-	04 (80.00)	32 (71.11)	Ref.	
	+	01 (20.00)	13 (28.89)	0.61(0.06-6.04)	1.00
KIR2DS4	-	04 (80.00)	28 (62.22)	Ref.	
	+	01(20.00)	17 (37.78)	0.41(0.04-3.99)	0.64
KIR2DS5	-	4 (80.00)	37 (82.22)	Ref.	
	+	1 (20.00)	08 (17.78)	1.15 (0.11-11.77)	**1.00**
KIR3DS1	-	04 (80.00)	39 (86.67)	Ref.	
	+	01(20.00)	06 (13.33)	1.62(0.15-17.10)	0.54
Pseudogene					
KIR2DP1	-	04(80.00)	36 (80.00)	Ref.	
	+	01 (20.00)	09 (20.00)	1.00(0.10-10.07)	1.00

These results describe the presence or absence of KIR genes according to dengue virus primary of secondary infection or not.

Table 5. Frequencies of KIR genotypes considering the haplotypes.

		HC (N=54)	DEN (N=50)	Total (N=104)	OR (95%CI)	p-value
		n (%)	n (%)	n (%)		
Haplogroups	**AA**	02(3.70)	04(8.00)	06 (5.77)	0.44 (0.07-2.52)	0.42
	Bx	52(96.30)	46(92.00)	98(92.23)	2.26 (0.39-12.92)	0.42

Different genotypes of KIR and dengue virus infection.

KIR2DS5 were associated with protection from DF development. These susceptibility genes were present in greater number in the group of dengue patients; it would seem that these genes are potential factors of susceptibility to infection by the dengue virus; many additional studies are needed to confirm this observation. Among these genes, we observed that *KIR2DL2, KIR2DL5 and KIR2DL5B* were inhibitory, and *KIR2DS2*

was an activator. Inhibitory and activators *KIRs* genes act in complementary, non-infected, healthy cells expressing HLA class I proteins are preserved through inhibitory "self-recognition" mechanisms that prevent their lysis. In contrast, infected cells and cancer cells, lacking the HLA class I molecules on their surfaces, are recognized and destroyed by lysis activating receptors;[30] this could be justified by assuming that the infected cells do not lack their HLA ligands, thus allowing the inhibitory KIR receptors to protect the infected cells. A study conducted in India found that *KIR3DL1/KIR3DS1* locus might be associated with the risk of developing DF;[13] in our investigation, this gene *KIR3DL1* was associated with the development of secondary dengue infection. Another study conducted in Southern Brazil found that inhibitory *KIR2DL5* and activator *KIR2DS5* were associated with the development of DF;[14] in our study, we found *KIR2DL5* associated with the development of DF and *KIR2DS5* associated with the protection of DF development. The *KIR2DL1* and its related ligand HLA-C2 were significantly associated with susceptibility to infection with CHIKV arbovirus transmitted by the same mosquitoes in Gabon;[15] we do not find *KIR2DL1* associated with DF development in the study. In our study population, *KIR2DL4 and KIR3DL1* were associated with the development of secondary dengue infection, and *KIRD2L5B* was associated with the protection of secondary dengue infection. Furthermore, in their study,[26] Zhi-Ming *et al.* suggested that the *KIR2DS3* gene favors infection by inducing a persistent inflammatory reaction and chronic hepatitis in the case of the hepatitis B virus. However, the *KIR2DS1* and *KIR2DL5* genes may contribute to protection against this virus.[26]

The extensive polymorphism of the KIR genes may suggest the possibility of pleiotropic effects in different diseases, i.e., a KIR gene that confers protection against one disease may predispose the organism to another.[31] Activating KIR receptors, which stimulate the secretion of cytokines and the lysis of target cells by NK cells, might be beneficial in response to infectious diseases and tumors. However, these diseases have a variety of etiologies, so immune activation is not necessarily beneficial in all phases of the disease process. KIR genotypes that stimulate strong activation may increase the risk of developing tumors associated with localized inflammation, as in the case of cervical cancer. They have also been connected to the pathogenesis of autoimmune diseases.[32] It emerges from this work that the inhibitory *KIR* genes are more numerous among those associated with the development of dengue fever, with the *KIR2DL5* genes showing very high frequencies in dengue cases compared to controls. Indeed, the expression of these genes would cause an inhibition on the NKs, hence their inaction and the progression of the disease. The paradox is that in this group, there is an activator gene whose frequency is statistically significant; it is *KIR2DS2*; this could be explained by the fact that a ligand defect or a difficulty of recognition could make null the action of this gene and consequently have an effect contrary to what is expected: that of activating the NKs against the pathogen. In the group of genes associated with protection against the development of dengue fever there is an activator *KIR* gene which is *KIR2DS5*; the receptors resulting from the latter activate the NK cells, which in turn act in the form of a cytolytic action against DENV. In the Dengue Fever group, the Bx (AB+BB) genotype frequency was 92%, and the AA genotype was 8%. There was not any association between Healthy Controls and dengue patients. In Brazil, Beltrame *et al.*, in their study, showed a possible protective factor against dengue fever in individuals with the AA genotype.[14] In a study on Ebola infection, Wauquier *et al.* showed that the AA profile was more frequent in survivors and a control group compared to fatal cases.[33] Based on these findings, it could be that an inhibitory KIR repertoire, represented in this case by the AA genotype, is conferring a protective effect on the individuals that possess it against such infections. According to Lu et al. (2008), genotypes and haplotypes containing more activating genes may play an important role in the infection or clearance of certain viruses.[34]

The main limitation of this study is that we only characterized the KIR genes, but not the KIR/HLA combination, and we did not notify Dengue Hemorrhagic Fever cases (DHF).

This study showed the implication of the KIRs genes in the immune pathogenicity of dengue fever in Burkina Faso. For the first time in Burkina Faso, it has been demonstrated that the susceptibility to dengue fever is related to the individual's KIR genotype; there is also a significant association between certain KIR genes and dengue fever. KIR inhibitors genes such as *KIR2DL2*, *KIR2DL5A*, and *KIR2DL5B* and the activating *KIR2DS2* were associated with a risk of development and progression of the disease, while *KIR2DS*5 and *KIR3DL3* would confer protection against this disease. However, KIR/HLA/cytokine studies combined with further genotyping of DENV are needed to investigate the molecular mechanisms by which KIR genes contribute to infection or clearance or even progression to severe forms of the disease dengue fever.

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