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Killer cell immunoglobulin-like receptor alleles influence susceptibility to occult hepatitis B infection in West African population

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Abstract. Occult hepatitis B infection (OBI) is a public health problem in Burkina Faso. OBI represents a risk factor for the development of cirrhosis and hepatocellular carcinoma (HCC). OBI could be due to mutant viruses undetectable by HBsAg assays or a strong suppression of viral replication and gene expression under the pression of the host immune system. To investigate the role of killer cell immunoglobulin-like receptor (KIR) gene polymorphisms in patients with OBI in Burkina Faso compared to healthy and chronic hepatitis B subjects. A total of 286 participants was recruited, including 42 cases of OBI, 110 cases of chronic hepatitis B and 134 HBV negative subjects. SSP-PCR was performed to search for the presence of KIR genes. The HBV viral load was determined by qPCR. The frequencies of the activator gene *KIR2DS5* (P=0.045) and the pseudogene *KIR2DP1* (P<0.001) in patients with OBI were higher than those in patients with chronic hepatitis B. These genes are associated with susceptibility of occult hepatitis B infection. The frequencies of the inhibitory KIR gene *KIR2DL3* (P=0.01) of patients with occult hepatitis B were lower than those in chronic hepatitis B patients. This gene *KIR2DL3* is associated with protection against occult hepatitis B infection. Also, the frequencies of the inhibitory KIR genes *KIR2DL2* (P<0.001), *KIR2DL3* (P<0.001) and activators *KIR2DS2* (P<0.001) in chronic hepatitis B patients were higher compared to the frequencies of the KIR genes in healthy subjects. These genes *KIR2DL3*, *KIR2DL5* (*A*, *B*), *KIR3DL3*, *KIR3DS1*, *KIR2DL2* and *KIR2DS2* are thought to be genes associated with the susceptibility to OBI. The *KIR2DS5* and *KIR2DP1* genes could be associated with susceptibility to OBI. As for the KIR gene *KIR2DL3* could be associated with protection against occult hepatitis B infection.

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Abbreviations: KIR, Killer cell immunoglobulin-like receptors; OBI, occult hepatitis B infection; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; SSP-PCR, single specific primer-polymerase chain reaction

Key words: OBI, KIR, SSP-PCR, Burkina Faso

Introduction

Occult hepatitis B or occult hepatitis B infection (OBI) is an entity described in the early 1980s that corresponds to the persistence of hepatitis B virus DNA (HBV) in serum and/or in the liver of patients with undetectable HBs antigen (HBsAg) by standard serological tests, generally with an anti-HBc antibody (anti-HBc Ab). It is usually asymptomatic and is characterized by a very low level of HBV DNA (DNA <200 IU/ml when it is detectable in serum) (1-3). Apart from certain cases in which the absence of detection of HBsAg is due to the genetic heterogeneity of HBV, in most cases, OBI is related to replication competent viruses that are strongly suppressed in their activities (replicative and transcriptional) by the host's defense mechanisms (4).

HBV remains a major public health problem. Indeed, WHO (2020) estimates that in 2015, 257 million people were living with chronic hepatitis B infection. In 2015, hepatitis B resulted in an estimated 887 000 deaths, mostly from cirrhosis and hepatocellular carcinoma. Hepatitis B prevalence is highest in the Western Pacific Region and the African Region, where 6.2 and 6.1% of the adult population is infected respectively (5).

In Burkina Faso, the prevalence rates of 32.8 and 7.3% were found by Somda (2016) in blood donors, and by Diarra (2018) in the general population (6,7). The prevalence of OBI and its clinical consequences are debated in the literature (1,8). It is also known that the molecular mechanisms underlying the onset of OBI play a direct role in the development of hepatocellular carcinoma (HCC) (9,10). In addition to the classic risk factors for progression from hepatitis B infection to cirrhosis or HCC, the host genetic host factors also play an important role to the disease progression.

The NK cells (Natural killers) of the innate immune system are well known for their important role in defense against viral infections and tumor transformation and could therefore contribute to the protection against occult hepatitis B infection. The actions of NK cells are mediated by direct cytotoxicity and the secretion of cytokines. Cytotoxicity is controlled by closely opposed signals from the inhibitory and activating KIRs present on NK cells surface (11). The KIR genes coding for the KIR receptors are located in a 100-200 Kb region of the Leukocyte Receptor Complex (LRC) which is a complex grouping of other genes coding for receptors expressed by the NKs and which is located on chromosome 19q13. 4 (12). The nomenclature of KIR genes is according to the number of domains of extracellular immunoglobulins (D) which can be double (2D) or triple (3D), the length of the intra cytoplasmic tail will be either long (L), or short (S) (13-15). To date, 16 human KIR genes have been identified (16,17), including six (06) genes encoding signal activating receptors activation through the short cytoplasmic tail (*KIR2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5* and 3DS1), seven (07) genes encoding inhibitory receptors with long cytoplasmic tails (*KIR2DL1*, *2DL2*, *2DL3*, *2DL5*, *3DL1*, *3DL2* and *3DL3*) and two pseudo genes *KIR2DP1* and *KIR3DP1*. *KIR2DL4* can act as an activating or inhibiting receptor for NK cell activity (17,18). 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 (16,19) 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 a 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 (19-22).

Several studies have highlighted the relationship between KIR genes and diseases (17). Sorgho *et al* (2018) associated the genes *KIR3DL1*, *KIR3DL2* and *KIR2DS1* with protection against chronic hepatitis B infection in the population of Burkina. Zhi-Ming *et al* (2007) concluded that the *KIR3DS1*, *KIR2DS1* and *KIR2DL5* genes were protective genes for HBV infection in the Han population in China. Kibar *et al* (2014) made the same observation with the *KIR2DL3* and *KIR3DS1* genes in the Turkish population. These authors have shown that the *KIR2DL5* and *KIR3DS1* genes are associated with protection against occult HBV infection (23,24).

The aim of this study was to characterize the polymorphism of the KIR genes in patients with occult hepatitis B infection. In Burkina Faso, few studies have been carried out on occult hepatitis B infection (OBI) and even less on genetic factors like as KIR genes which could be as-sociated with this infection. This pioneering study on the characterization of KIR genes in patients with occult hepatitis B will also allow us to clarify the contribution of biology and molecular genetics in the diagnosis and monitoring of occult hepatitis B in Burkina.

Materials and methods

Ethical considerations. The subjects recruited gave their free and informed written consent to participate in the study. The protocol for this research was approved by the Ethics Committee for Health Research (CERS) of Burkina Faso by deliberation number 2017-01-004 of January 11, 2017.

Study type and population. This was a prospective case-control study conducted from June to December 2018 which in-volved 286 people aged from 14 to 73 years divided into three (03) groups. The first group, which is the case group consisted of fourty-two (42) carriers of occult hepatitis B infection recruited from the Pietro Annigoni Center for Biomolecular Research (CERBA/LABIOGENE). These cases were diagnosed by a doctor specializing in gastroenterology and confirmed through serological and molecular examinations. These patients had no history of chronic hepatitis B, cirrhosis, or HCC. The second group included one hundred thirty-four (134) HBV, HCV and HIV negative control subjects recruited from the Ouagadougou Regional Blood Transfusion Center (CRTS/O). The third group was that of the positive control (controls) made up of one hundred and ten (110) subjects carrying chronic HBV (HBsAg positive >6 months) recruited at the Center for Biomolecular Research Pietro Annigoni (CERBA/LABIOGENE). The sociodemographic data of the patients such as sex, age, residency, and some viral serological markers were collected by questionnaire.

Case and control definitions. An individual was considered to be a carrier of OBI if the HBs antigen (HBsAg) was undetectable in his serum by the usual serological tests whereas the viral load indicates the presence of a very low level of the hepatitis B virus DNA (DNA <200 IU/ml) and generally with a positive anti-HBc antibody (anti-HBc Ab) (1,8).

An individual was considered to be chronic HBV if the HBsAg persisted in their blood beyond 6 months after acute hepatitis (25). The diagnosis of hepatitis was made by serology testing for hepatitis B virus surface antigen (HBsAg). These

patients are followed by a hepato-gastroenterologist. Healthy patients are patients with negative HBsAg as well as all hepatitis B markers (anti-HBs Ab, HBeAg, anti-HBe Ab and anti-HBc Ab).

Sampling. Five (05) milliliters of venous blood were taken from EDTA and dry tubes. After centrifugation at 3,500 rpm for 15 min, the serum was collected from the dry tube, the plasma, and the pellet from the EDTA tubes and stored at -20°C until using. The plasma was used for the determination of the HBV viral load and the pellet for the research of the KIR genes.

Serology. Serum samples were tested for serological markers for HBV (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb) using HBV 5-in-1 Hepatitis B Markers Rapid Test Panel (Prechek Bio, Inc.).

Viral DNA extraction and determination of HBV viral load. Viral DNA was extracted from 200 μl of plasma using the PureLink® Genomic DNA Kits Extraction Kit (Life Technologies, Van Allen WayCarlsbad, CA USA) as per the manufacturer instructions. The plasma viral load was determined by Real-Time PCR using the 7500 Fast Real time PCR device with the Genesig HBV Real Time Quantitative Kit Primer design (Southampton, United Kingdom) by amplification of the Core Protein Region according to the following program: a cycle of 95°C for 10 min and 50 cycles consisting of 95°C for 10 sec and 60°C for 1 min. The quantity of the viral load of the samples is relative to the standard straight line which is obtained by diluting the positive control (supplied) to the tenth (1/10) five times in cascade (Tube 1: Positive controls 1=2.10^7; Tube 2=2.10^6; Tube 3=2.10^5; Tube 4=2.10^4; Tube 5=2.10^3; Tube 6=2.10^2).

Genomic DNA extraction and characterization of KIR genes by sequences specific primer PCR (SSP-PCR). Genomic DNA was extracted from the blood pellet using the manual 'Rapid Salting-Out' extraction technique described by Miller *et al* (1988) and stored at -80°C until use (26). The concentration and purity of the DNA were determined using the Biodrop device (Isogen Life Science, NV/S.A, Temse, Belgium).

The characterization of the KIR genes was carried out by SSP-PCR using the GeneAmp PCR system 9700 (Applied Biosystem, USA) according to the method described by Kulkarni *et al* (2010). DNA extracts with a concentration of at least 50 ng/μl were used for the characterization of the KIR genes. The PCR was carried out by preparing a mixture of 60 μl per sample containing: a variable volume of DNA so as to have a DNA concentration above 50 ng/μl, 7.5 μl of 10 x PCR buffer, 2.25 μl of $MgCl_2$; 0.6 μl of dNTPs and 0.375 μl of DNA platinium Taq polymerase; the mixture was completed with water (nuclease-free) to make a volume of 60 μl (27). The thermocycler was programmed as follows: a cycle of 94°C for 3 min for the activation of Taq polymerase; five (05) cycles consisting of 94°C for 15 sec, 65°C for 15 sec, 72°C for 30 sec; twenty-one (21) cycles consisting of 94°C for 15 sec, 60°C for 15 sec, 72°C for 30 sec; four (04) cycles consisting of 94°C for 15 sec, 55°C for 1 min, 72°C for 2 min and 72°C for 7 min for the final extension.

The PCR products were subjected to an electrophoresis on a 3% agarose gel and viewed un-der UV light at 312 nm. The PCR products were validated against a positive internal control corresponding to the DRB1 gene fragment.

Data analysis. The data were entered in Excel 2013 and then analyzed with Standard Statistical Package for Social Sciences (SPSS) version 20.0 software. Changes were considered statistically significant at $P \leq 0.05$, using the Cochran-Mantel-Haenszel test. Odds ratio (OR) and confidence intervals (CI) at 95% were calculated to estimate the associations using Epi Info 7.

Results

Socio-demographic characteristics of the study population. During the study period, 286 individuals aged from 14 years to 73 years with a mean age of 34.21±11.25 years were enrolled. Twenty (20) men (47.62%) and 22 women (52.38%) had occult HBV infection with a sex ratio of 0.90. The average age of occult hepatitis B cases was 32.59±9.30 years and the predominant age group was 25 to 39 years of age comprising 20 people (47.62%). Among HBV negative subjects, the sex ratio was 0.81 with 44.78% men and 55.22% women with an average age of 31.62±9.11 years. Fifty-three point sixty-four percent (53.64%) of men and 46.36% of women had chronic hepatitis B, a sex ratio of 1.15 with an average age of 38.23±13.25 years. The 25-39 age group was more affected by chronic hepatitis B compared to OBI.

No statistically significant difference was found between the age groups of the occult hepatitis B group compared to that of the occult hepatitis B (Table I).

Frequencies of KIR genes in occult and chronic hepatitis B. The frequencies of all inhibitory KIR genes and the activator KIR genes *KIR2DS1*, *KIR2DS2*, and *KIR3DS1* in patients with OBI were lower than in patients with chronic hepatitis (Table II). However, the activator KIR genes *KIR2DS3*, *KIR2DS4*, *KIR2DS5* and the pseudo gene *KIR2DP1* were found to be more common in patients with OBI than in chronic hepatitis B cases. A statistically significant association was established between the inhibitory KIR gene *KIR2DL3* (P=0.01), activators *KIR2DS5* (P=0.045) and the *KIR2DP1* pseudogene (P<0.001) of patients with occult hepatitis B compared to the KIR genes of chronic hepatitis B patients. Fig. 1 shows a comparison between the frequencies of the KIR genes in patients with OBI and the negative and positive controls.

Frequencies of KIR genes in chronic hepatitis B and negative subjects. The frequencies of KIR inhibitory genes *KIR2DL1*, *KIR2DL4*, *KIR2DL5* (*A*, *B*), *KIR3DL1*, *KIR3DL2*, *KIR3DL3*, activators *KIR2DS1*, *KIR2DS3*, *KIR2DS4*, *KIR2DS5* and pseudogene *KIR2DP1* in healthy patients were high than the frequencies of chronic hepatitis B patients (Table III). An association has been found between the inhibitory KIR genes *KIR2DL2* (P<0.001), *KIR2DL3* (P<0.001), *KIR3DL1* (P=0.04), the activators KIR genes *KIR2DS1* (P=0.002), *KIR2DS2* (P<0.001) and the pseudo gene *KIR2DP1* (P=0.014).

Prediction of haplogroups from genotypes. The content of the KIR genes from our study population was used to infer the different KIR haplotypes and assign a genotype to each

Table I. Socio-demographic characteristics of the study population.

Variable	OBI n=42 (%)	Control n=134 (%)	cHBV n=110 (%)	Total 286 (%)	p_{i-t}-P-value	p_{i-c}-P-value
Sex						
Male	20 (47.62)	60 (44.78)	59 (53.64)	139 (48.60)	0.75	0.51
Female	22 (52.38)	74 (55.22)	51 (46.36)	147 (51.40)		
Age (years)						
<25[a]	11 (26.19)	38 (28.36)	11 (10)	60 (20.98)		
25-39	20 (47.62)	70 (52.24)	55 (50)	145 (50.70)	0.37	0.38
>39	11 (26.19)	26 (19.40)	44 (40)	81 (28.32)		
Serological status						
HBV+	0 (0)	0 (0)	110	110 (100)	-	-
HBV-	42 (23.86)	134 (76.14)	0 (0)	176 (100)	-	-
HIV-/HCV-	42 (14.69)	134 (46.85)	110 (38.46)	286 (100)	-	-

OBI, occult hepatitis B infection; cHBV, chronic infection by HBV; p_{i-t}-P-value for the comparison between the OBI group and the negative control group; p_{i-c}-P-value for the comparison between the OBI group and the positive control group (HBVc). [a]taken as reference (Ref) for comparisons.

Table II. Frequencies of KIR genes in occult and chronic hepatitis B infections.

KIR GENES	n (%)	OBI n=42	cHBV n=110	crude OR (95% CI)	adj. OR (95% CI)	P-value
Inhibitors						
KIR2DL1	-	16 (38.1)	30 (27.3)	1	1	0.971
	+	26 (61.9)	80 (72.7)	0.61 (0.29-1.29)	1.02 (0.31-3.32)	
KIR2DL2	-	30 (71.4)	63 (57.3)	1	1	0.759
	+	12 (28.6)	47 (42.7)	0.54 (0.25-1.16)	0.86 (0.32-2.29)	
KIR2DL3	-	25 (59.5)	35 (31.8)	1	1	0.01
	+	17 (40.5)	75 (68.2)	0.32 (0.15-0.66)	0.25 (0.09-0.74)	
KIR2DL4	-	15 (35.7)	33 (30.0)	1	1	0.269
	+	27 (64.3)	77 (70.0)	0.77 (0.36-1.64)	1.88 (0.6-5.93)	
KIR2DL5A	-	34 (81.0)	70 (63.6)	1	1	0.446
	+	8 (19.0)	40 (36.4)	0.41 (0.17-0.98)	0.63 (0.19-2.09)	
KIR2DL5B	-	34 (81.0)	61 (55.5)	1	1	0.101
	+	8 (19.0)	49 (44.5)	0.29 (0.12-0.69)	0.39 (0.12-1.23)	
KIR3DL1	-	15 (35.7)	36 (32.7)	1	1	0.595
	+	27 (64.3)	74 (67.3)	0.88 (0.42-1.85)	1.33 (0.46-3.83)	
KIR3DL2	-	17 (40.5)	33 (30.0)	1	1	0.894
	+	25 (59.5)	77 (70.0)	0.63 (0.3-1.32)	1.07 (0.38-3.05)	
KIR3DL3	-	20 (47.6)	29 (26.4)	1	1	0.562
	+	22 (52.4)	81 (73.6)	0.39 (0.19-0.82)	0.71 (0.22-2.25)	
Activators						
KIR2DS1	-	38 (90.5)	88 (80.0)	1	1	0.274
	+	4 (9.5)	22 (20.0)	0.42 (0.14-1.31)	0.5 (0.14-1.81)	
KIR2DS2	-	30 (71.4)	65 (59.1)			0.095
	+	12 (28.6)	45 (40.9)	0.58 (0.27-1.25)	0.45 (0.18-1.18)	
KIR2DS3	-	32 (76.2)	92 (83.6)	1	1	0.074
	+	10 (23.8)	18 (16.4)	1.6 (0.67-3.82)	2.75 (0.9-8.37)	
KIR2DS4	-	9 (21.4)	43 (39.1)	1	1	0.189
	+	33 (78.6)	67 (60.9)	2.35 (1.03-5.4)	1.86 (0.73-4.75)	

Table II. Continued.

KIR GENES	n (%)	OBI n=42	cHBV n=110	crude OR (95% CI)	adj. OR (95% CI)	P-value
KIR2DS5	-	18 (42.9)	66 (60.0)	1	1	0.045
	+	24 (57.1)	44 (40.0)	2 (0.97-4.11)	2.39 (1.01-5.65)	
KIR3DS1	-	41 (97.6)	98 (89.1)	1	1	0.078
	+	1 (2.4)	12 (10.9)	0.2 (0.03-1.58)	0.17 (0.02-1.69)	
Pseudogène						
KIR2DP1	-	3 (7.1)	51 (46.4)	1	1	<0.001
	+	39 (92.9)	59 (53.6)	11.24 (3.28-38.55)	10.98 (3.13-38.47)	

+, Presence of KIR gene; -, Absence of KIR gene; OBI, Occult hepatitis B Infection; cHBV, chronic infection by HBV; taken as reference (Ref) for comparisons.

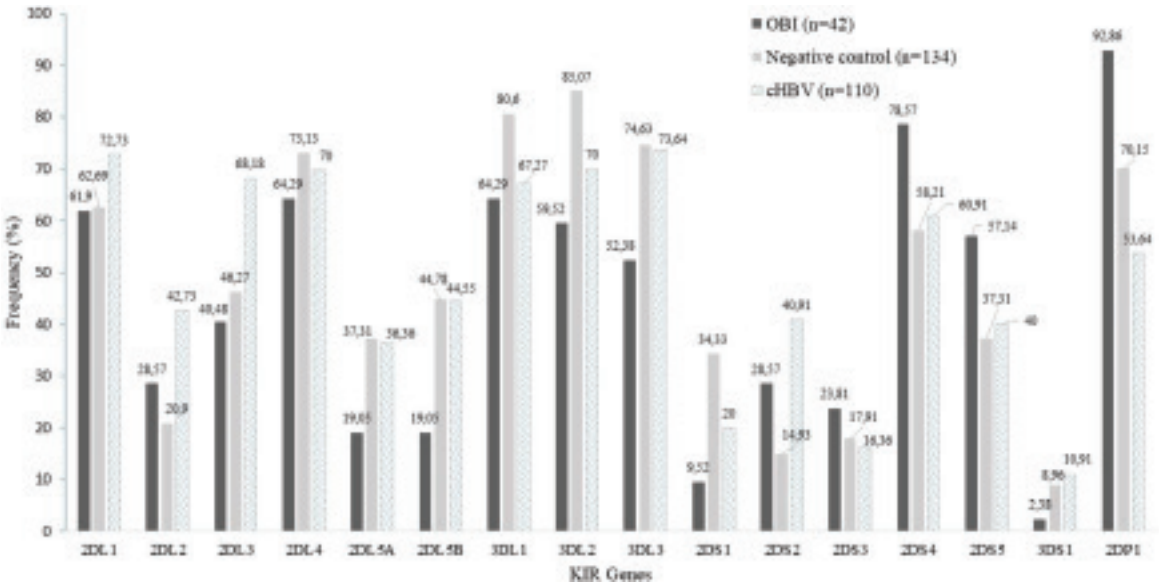

Figure 1. Comparison between the frequencies of the KIR genes in patients with OBI and the negative and positive controls.

patient. From the entire study population, two genotypes were identified, notably the AB and BB genotypes. In occult hepatitis B patients, we recorded an AB genotype frequency of 33.33% and a BB genotype frequency of 66.67%. The frequencies of the AB and BB genotypes were 23.88 and 76.12% respectively in the negative controls. As for the positive controls, the frequencies of the AB and BB genotypes were 29.09 and 70.91% respectively (Table IV). No association was established between the frequencies of the genotypes of patients with occult hepatitis B and the negative and positive controls. However, the AA genotype has not been identified.

Discussion

Burkina Faso is a country of high endemicity for hepatitis B (28-31), where the prevalence varies between 9 and 15%, (29-31) and that of occult hepatitis B infection varying between 7.3 and 32.8% (6,7). Our study is a pioneering study in the sense that it involved characterizing KIR genes in a population with OBI in Burkina Faso.

The average age of our study population was 32.59±9.30 years and the majority age group was that of 25 to 39 years comprising 20 people (47.62%). Compared to the Burkinabe working population, the age group 25 to 39 is the highest (32), prone to many infections including OBI. This could explain our results because it is at this age that several young people know their HIV status for the first time either during screening campaigns or during prenuptial assessments. In addition, the introduction of hepatitis B vaccination into expanded newborn vaccination programs on the recommendation of WHO in countries with high endemicity of hepatitis B (28), and applied to Burkina Faso since 2006 has immunized the young segment (<15 years). However, the immunization rate remains to be estimated.

Our data clearly showed that there were statistically significant differences between the frequencies of the KIR genes of the OBI case and those in chronic hepatitis B. The high frequency of the activator KIR genes *KIR2DS5* and the pseudo gene *KIR2DP1* of the OBI group compared to that of

Table III. Frequency of KIR genes in chronic HBV patients and controls subjects.

KIR GENES	n (%)	Control n=134	cHBV n=110	crude OR (95% CI)	adj. OR (95% CI)	P-value
Inhibitors						
KIR2DL1	-	50 (37.3)	30 (27.3)	1	1	0.06
	+	84 (62.7)	80 (72.7)	1.59 (0.92-2.74)	1.99 (0.96-4.12)	
KIR2DL2	-	106 (79.1)	63 (57.3)	1	1	<0.001
	+	28 (20.9)	47 (42.7)	2.82 (1.61-4.96)	3.62 (1.78-7.36)	
KIR2DL3	-	72 (53.7)	35 (31.8)	1	1	<0.001
	+	62 (46.3)	75 (68.2)	2.49 (1.47-4.21)	3.58 (1.77-7.24)	
KIR2DL4	-	36 (26.9)	33 (30.0)	1	1	0.465
	+	98 (73.1)	77 (70.0)	0.86 (0.49-1.5)	0.73 (0.31-1.71)	
KIR2DL5A	-	84 (62.7)	70 (63.6)	1	1	0.448
	+	50 (37.3)	40 (36.4)	0.96 (0.57-1.62)	1.38 (0.6-3.14)	
KIR2DL5B	-	74 (55.2)	61 (55.5)	1	1	0.347
	+	60 (44.8)	49 (44.5)	0.99 (0.6-1.65)	0.66 (0.28-1.57)	
KIR3DL1	-	26 (19.4)	36 (32.7)	1	1	0.004
	+	108 (80.6)	74 (67.3)	0.49 (0.28-0.89)	0.31 (0.14-0.71)	
KIR3DL2	-	20 (14.9)	33 (30.0)		1	0.066
	+	114 (85.1)	77 (70.0)	0.41 (0.22-0.77)	0.46 (0.2-1.07)	
KIR3DL3	-	34 (25.4)	29 (26.4)	1	1	0.641
	+	100 (74.6)	81 (73.6)	0.95 (0.53-1.69)	1.21 (0.55-2.66)	
Activators						
KIR2DS1	-	88 (65.7)	88 (80.0)	1	1	0.002
	+	46 (34.3)	22 (20.0)	0.48 (0.27-0.86)	0.34 (0.16-0.69)	
KIR2DS2	-	114 (85.1)	65 (59.1)			<0.001
	+	20 (14.9)	45 (40.9)	3.95 (2.15-7.25)	5.94 (2.88-12.22)	
KIR2DS3	-	110 (82.1)	92 (83.6)	1	1	0.049
	+	24 (17.9)	18 (16.4)	0.9 (0.46-1.75)	0.43 (0.18-1.01)	
KIR2DS4	-	56 (41.8)	43 (39.1)	1	1	0.622
	+	78 (58.2)	67 (60.9)	1.12 (0.67-1.87)	0.86 (0.47-1.58)	
KIR2DS5	-	84 (62.7)	66 (60.0)	1	1	0.346
	+	50 (37.3)	44 (40.0)	1.12 (0.67-1.88)	1.37 (0.71-2.61)	
KIR3DS1	-	122 (91.0)	98 (89.1)	1	1	0.114
	+	12 (9.0)	12 (10.9)	1.24 (0.54-2.89)	2.32 (0.81-6.61)	
Pseudogène						
KIR2DP1	-	40 (29.8)	51 (46.4)	1	1	0.014
	+	94 (70.2)	59 (53.6)	0.49 (0.29-0.83)	0.50 (0.29-0.87)	

+, Presence of KIR gene; -, Absence of KIR gene; cHBV, chronic infection by HBV; taken as reference (Ref) for comparisons.

the positive control group associates them with susceptibility of occult hepatitis B infection. These results were different from those of Zhi-Ming *et al* (2007) and Kibar *et al* (2014). Kibar *et al* (2014) did not find an association between the KIR genes studied in occult and chronic hepatitis B cases in the Turkish population, while Zhi-Ming *et al* (2007) found that the *KIR2DL5*, *KIR2DS1* and *KIR3DS1* genes were associated with spontaneous remission of HBV infection in the Han population in China. Our results show that the KIRs expressed on the surface of NK and T cells play a role in the regulation of immune responses following occult hepatitis B infection by transducing inhibitory or activating signals.

As for the KIR inhibitor gene *KIR2DL3*, it is associated with protection against occult hepatitis B.

Regarding the frequencies of KIR genes between patients with chronic hepatitis B and healthy controls, the frequency of inhibitory genes *KIR2DL2*, *KIR2DL3* and activator gene *KIR2DS2* was high in patients with chronic HBV compared to the control group, while the frequency of the inhibitory gene *KIR3DL1*, the activator gene *KIR2DS1* and the pseudogene *KIR2DP1* were higher in the control group than in patients with chronic HBV. The multivariate analysis of these frequencies implies that, the genes KIR, *KIR2DL2*, *KIR2DL3*, *KIR2DS2*, are associated with chronic infection with HBV and *KIR3DL1*, *KIR2DS1*, *KIR2P1*

Table IV. Frequencies of KIR genotypes considering the haplotypes.

		OBI n=42 (%)	Control n=134 (%)	cHBV n=110 (%)	p_{i-t}-P-value	p_{i-c}-P-value
Genotypes	AA	-	-	-		
	AB	14 (33.33)	32 (23.88)	32 (29.09)	0.22	0.61
	BB	28 (66.67)	102 (76.12)	78 (70.91)		

OBI, occult hepatitis B infection; cHBV, chronic infection by HBV; p_{i-t}-P-value, for comparison between OBI group and negative controls group; p_{i-c}-P-value, for comparison between OBI group and positive controls group (cHBV).

are associated with protection against chronic infection with HBV. Our results are similar to those of Sorgho *et al* (2018) in Burkina Faso in a previous study of cases of chronic hepatitis B correlated with healthy controls shows that the inhibitory genes *KIR2DL2*, *KIR2DL3* and activator *KIR2DS2* were associated with chronic HBV infection while the inhibitory gene *KIR3DL1*, the activator gene *KIR2DS1* and the pseudo gene *KIR2DP1* were associated with protection against chronic HBV infection (22). *KIR2DP1* is the only gene in our study that is both associated with protection against infection occult hepatitis B and chronic HBV infection. *KIR2DP1* Also known as *KIRY*, *KIRZ*, *KIR15* and *KIR2DL6* is strongly related to KIR inhibitors *KIR2DL2*, *KIR2DL3* and more than 97% to *KIR2DL1* (33). During occult infection with HBV or chronic infection, this causes a modification of the expression of class 1 molecules of the MHC so as not to be recognized by the T lymphocytes. In this way the inhibition of the cells NK will be lifted and the signal is transmitted by DAP-12 allowing lysis of the target cell (34).

The major genotypes from the KIR haplotypes of our study population were the AB and BB genotypes. The AA genotype has not been identified.

Respectively the frequencies of the AB and BB genotypes were 33.33 and 66.67%, in patients with occult hepatitis B; 23.88 and 76.12% in the negative controls and 29.09 and 70.91% in the positive controls.

B haplotypes have previously been shown to be more prevalent in non-Caucasian populations, such as Australian Aborigines and Asian Indians (35), while approximately 55% of the Caucasian population would have haplotypes A and 30% of both haplotypes A and B (36). It is believed that populations with higher frequencies of B haplotypes will be those under high pressure from infectious diseases (36). Several genetic studies have revealed that the frequency and distribution of KIR genes and haplotypes vary according to ethnicity (19,36-38). This could explain the disparity of our results compared to the results of other studies carried out in other continents.

The main limitation of our study is that we only characterized the KIR genes, but not the KIR/HLA association.

Conclusions

This study highlighted that the *KIR2DS4* and *KIR2DP1* genes are associated with susceptibility of occult hepatitis B infection. *KIR2DL3* is associated with protection against occult hepatitis B infection. As for the KIR genes *KIR2DL2*, *KIR2DL3*, *KIR2DL5*, *KIR3DL3*, *KIR2DS2* and *KIR3DS1*, they are associated with chronic hepatitis B infection.

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Ethics approval and consent to participate

The protocol for this research was approved by the Ethics Committee for Health Research (CERS) of Burkina Faso by deliberation number 2017-01-004 of January 11, 2017. Written informed consent was obtained from all the patients.

Contributions

FWD, MS, JKS, study concept and design; MMB, PAS, NK, HKS, PB, ETY, ITK, sampling and laboratory analysis; AKO, PAS, MMB, statistical analysis and interpretation of data; FWD, MMB, MBN, ATY, drafting of the manuscript; FWD, MBN, DOY, JKS, critical revision of the manuscript for important intellectual content; ATY, JS, administrative, technical, and material support; FWD, JS, study supervision. All authors have read and approved the manuscript.

Informed consent

All the participants gave informed consent for data sharing and the data presented in this paper are anonymized.

Availability of data and materials

All the data supported our finding are contained within this work. The datasets analyzed during the current study are available from the corresponding author upon request.

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Conflict of interest

The authors declare no potential conflict of interest.

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References

1. Raimondo G, Pollicino T, Cacciola I and Squadrito G: Occult hepatitis B virus infection. J Hepatol 46:160-170, 2007.
2. Allain J and Candotti D: Diagnostic algorithm for HBV safe transfusion. Blood Transfus 7: 174-182, 2009.
3. Feld J, Janssen HLA, Abbas Z, Elewaut A, Ferenci P, Isakov V, Khan AG, Lim SG, Locarnini SA, Ono SK, *et al*: World Gastroenterology Organisation Global Guideline Hepatitis B. J Clin Gastroenterol 50: 691-703, 2016.
4. Pollicino T and Saitta C: Occult hepatitis B virus and hepatocellular carcinoma. World J Gastroenterol 20: 5951-5961, 2014.
5. OMS. Hepatitis B [Internet]. pp1-9, 2020. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b.
6. Somda KS, Sermé AK, Coulibaly A, Cissé K, Sawadogo A, Sombié AR and Bougouma A: Hepatitis B surface antigen should not be the only sought marker to distinguish blood donors towards Hepatitis B virus infection in high prevalence area. Open J Gastroenterol 6: 362-372, 2016.
7. Diarra B, Yonli AT, Sorgho PA and Compaore TR: Occult Hepatitis B Virus infection and associated genotypes among HBsAg-negative subjects in burkina faso. Mediterr J Hematol Infect Dis 10: 1-9, 2018.
8. Vallet-Pichard A and Pol S: L 'Hépatite B occulte. Virologie 12: 87-94, 2008.
9. Xu R, Zhang X, Zhang W, Fang Y, Zheng S and Yu X: Association of Human APOBEC3 cytidine deaminases with the generation of hepatitis Virus B x antigen mutants and hepatocellular carcinoma. Hepatology 46: 1810-1820, 2007.
10. Vivekanandan P, Daniel HD, Kannangai R, Martinez-murillo F and Torbenson M: Hepatitis B virus replication induces methylation of both host and viral DNA. J Virol 84: 4321-4329, 2010.
11. Yindom LM, Mendy M, Bodimeade C, Chambion C, Aka P, Whittle HC, Rowland-Jones SL and Walton R: KIR content genotypes associate with carriage of hepatitis B surface antigen, e antigen and HBV viral load in Gambians. PLoS One 12: e0188307, 2017.
12. Trowsdale J: Genetic and functional relationships between MHC and NK receptor Genes HLA class I and NK receptors are encoded within. Immunity 15: 363-374, 2001.
13. Kulkarni S, Martin MP and Carrington M: The Ying and Yang of HLA and KIR in Human disease. Semin Immunol 20: 343-352, 2008.
14. Marsh SGE, Parham P, Dupont B, Geraghty DE, Trowsdale J, Middleton D, Vilches C, Carrington M, Witt C, Guethlein LA, *et al*: Killer-cell immunoglobulin-like receptor (KIR) nomenclature report, 2002. Immunogenetics 55: 220-226, 2003.
15. Vilches C and Parham P: KIR: Diverse, rapidly evolving receptors of innate and adaptive immunity. Annu Rev Immunol 20: 217-251, 2002.
16. Robinson J, Halliwell JA, McWilliam H, Lopez R and Marsh SGE: IPD-the immuno polymorphism database. Nucleic Acids Res 41: 1234-1240, 2013.
17. González-Galarza FF, Takeshita LYC, Santos EJM, Kempson F, Maia MHT, Da Silva AL, Teles e Silva AL, Ghattaoraya GS, Alfirevic A, Jones AR and Middleton D: Allele frequency net 2015 update: New features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. Nucleic Acids Res 43: D784-D788, 2015.
18. Faure M and Long EO: KIR2DL4 (CD158d), an NK Cell-activating receptor with inhibitory potential. J Immunol 4: 6208-6214, 2002.
19. Uhrberg M, Parham P and Wernet P: Definition of gene content for nine common group B haplotypes of the Caucasoid population: KIR haplotypes contain between seven and eleven KIR genes. Immunogenetics 54: 221-229, 2002.
20. Mcqueen KL, Dorighi KM, Guethlein LA, Wong R and Parham P: Donor-recipient Combinations of Group A and B KIR haplotypes and HLA Class I ligand affect the outcome of HLA-matched, sibling donor hematopoietic cell transplantation. Hum Immunol 68: 309-323, 2007.
21. Ashouri E, Farjadian S, Reed EF, Ghaderi A and Rajalingam R: KIR gene content diversity in four Iranian populations. Immunogenetics 61: 483-492, 2009.
22. Sorgho PA, Martinson JJ, Djigma FW, Yonli AT, Nagalo BM, Compaore TR, Obiri-Yeboah D, Diarra B, Sombie HK, Zongo AW, *et al*: Insights into the interplay between KIR gene frequencies and chronic HBV infection in Burkina Faso. Mediterr J Hematol Infect Dis 10: e2018060, 2018.
23. Zhi-Ming L, Yu-Lian J, Zhao-Lei F, Chun-Xiao W, Zhen-Fang D, Bing-Chang Z and Yue-Ran Z: Polymorphisms of killer cell immunoglobulin-like receptor gene: Possible association with susceptibility to or clearance of hepatitis B virus infection in chinese han population. Croat Med J 48: 800-807, 2007.
24. Kibar F, Ozturk OG, Ulu A, Erken E, Inal S, Dinkci S, Kurtaran B, Tasova Y, Aksu HS and Yaman A: Role of KIR genes and genotypes in susceptibility to or protection against hepatitis B virus infection in a Turkish cohort. Med Sci Monit 20: 28-34, 2014.
25. Hu K: A practical approach to management of chronic hepatitis B. Int J Med Sci 2: 17-23, 2005.
26. Miller SA, Dykes DD and Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1, 1988.
27. Kulkarni S, Martin MP and Carrington M: KIR Genotyping by Multiplex PCR-SSP. Methods Mol Biol 612: 365-375, 2010.
28. WHO. Lignes directrices pour la prévention, les soins et le traitement en faveur des personnes atteintes d'une infection à hépatite B chronique [Internet]. pp176, 2015. Available from: http://apps.who.int/iris.
29. Simpore J, Savadogo A, Ilboudo D, Nadambega MC, Esposito M, Yara J, Pignatelli S, Pietra V and Musumeci S: Toxoplasma gondii, HCV, and HBV seroprevalence and co-infection among HIV-positive and -negative pregnant women in Burkina Faso. J Med Virol 78: 730-733, 2006.
30. Tao I, Compaoré TR, Diarra B, Djigma F, Zohoncon TM, Assih M, Ouermi D, Pietra V, Karou SD and Simpore J: Seroepidemiology of hepatitis B and C viruses in the general population of burkina faso. Hepat Res Treat 2014: 781843, 2014.
31. Sanou AM, Benkirane K, Tinto B, Cissé A, Sagna T, Ilboudo AK, Dording C, Tarnagda Z, Muller CP and Hübschen JM: Prevalence of hepatitis B virus and hepatitis D virus coinfection in western burkina faso and molecular characterization of the detected virus strains. Int J Infect Dis 70: 15-59, 2018.
32. INSD. Emploi et chômage: Enquête multisectorielle continue (EMC) [Internet]. 2014. Available from: www.insd.bf.
33. Carrington M and Norman P: The KIR Gene Cluster [Internet]. National Center for Biotechnology Information (US). pp227, 2003. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10135/
34. Delves PJ and Roitt IM: The immune system First of Two Parts. N Engl J Med 343: 37-49, 2000.
35. Norman P, Carrington CVF, Byng M, Maxwell LD, Curran MD, Stephens HA, Chandanayingyong D, Verity DH, Hameed K, Ramdath DD and Vaughan RW: Natural killer cell immunoglobulin-like receptor (KIR) locus profiles in African and South Asian populations. Genes Immun 3: 86-95, 2002.
36. Parham P: Immunogenetics of killer-cell immunoglobulin-like receptors. Tissue Antigens 62: 194-200, 2003.
37. Single RM, Martin MP, Gao X, Meyer D, Yeager M, Kidd JR, Kidd KK and Carrington M: Global diversity and evidence for coevolution of KIR and HLA. Nat Genet 39: 1114-1119, 2007.
38. Lu Z, Zhang B, Chen S, Gai Z, Feng Z, Liu X, Liu Y, Wen X, Li L, Jiao Y, *et al*: Association of KIR genotypes and haplotypes with susceptibility to chronic hepatitis B virus infection in chinese han population. Cell Mol Immunol 5: 457-463, 2008.