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MAJOR ARTICLE

Role of the Killer Immunoglobulin-like Receptor and Human Leukocyte Antigen I Complex Polymorphisms in Kaposi Sarcoma–Associated Herpesvirus Infection

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Background. Kaposi sarcoma, caused by the pathogen Kaposi sarcoma–associated herpesvirus (KSHV), is the most common neoplasm for patients with AIDS. Susceptibility to KSHV has been associated with several different genetic risk variants. The purpose of this study was to test whether variants of killer cell immunoglobulin-like receptors (KIRs) and their human leukocyte antigen (HLA-I) ligands influence the risk of KSHV infection.

Methods. A case-control study was performed in Xinjiang, a KSHV-endemic region of China. We recruited 299 individuals with HIV, including 123 KSHV-seropositive persons and 176 KSHV-seronegative controls. We used logistic regression and the MiDAS package to evaluate the association between *KIR/HLA-I* polymorphisms and KSHV infection.

Results. *HLA-A*31:01*, *HLA-C*03:04*, and *HLA-C*12:03* were found to be associated with KSHV infection, with *A*31:01* showing a protective effect under 3 different models (dominant: 0.30 [95% confidence interval {CI}, .08–.82], $P = .031$; additive: 0.30 [95% CI, .09–.80], $P = .030$; overdominant: 0.31 [95% CI, .09–.88], $P = .042$). The effect of *A*31:01* might cause the variants of amino acid at HLA-A position 56, with individuals carrying an arginine having a lower KSHV infection risk. The increased homozygous *KIR2DL3* was associated with a relatively high KSHV viral load (16.30% vs 41.94%, $P = .010$).

Conclusions. This study provides further insight into the link between *HLA-I* alleles and *KIR* genes and KSHV infection, highlighting KSHV-susceptible variants of *HLA-I* and KSHV replication caused by specific *KIR* genotype, and revealing a potential role of KIR-mediated natural killer cell activation in anti-KSHV infection.

Keywords. human leukocyte antigen; infection; Kaposi sarcoma–associated herpesvirus; killer cell immunoglobulin-like receptor; polymorphism.

Kaposi sarcoma–associated herpesvirus (KSHV; also known as human herpesvirus 8) is the causative agent of Kaposi sarcoma (KS), the most common neoplasm and the major cause of death for patients with AIDS [1, 2]. Regardless of the wide application of antiretroviral therapy (ART), KS continues to affect people with human immunodeficiency virus (PWH), posing a great challenge to the 2022–2030 global health sector strategy of the World Health Organization (WHO) on human immunodeficiency virus (HIV) [3–5]. As reported in a newly published study [6], the overall infection rate of KSHV in Xinjiang was 25.60%, with a higher infection rate of 29.79% in the Uyghur population. Liu et al have reported that KSHV seroprevalence among PWH was 48.9% [7]. The disproportionately higher prevalence of KSHV infection and KS incidence in PWH compared to immunocompetent individuals indicates the importance of host immunity in KSHV infection and restricting viral pathogenesis [8, 9].

Human natural killer (NK) cells, an integral part of innate immunity, can rapidly provide defenses against herpesvirus infection [9]. Killer cell immunoglobulin-like receptors (KIRs) are a polymorphic family of receptors on the surface of NK cells that regulate NK-mediated cytotoxicity when ligated to requisite human leukocyte antigen (HLA) class I molecules [10, 11]. Both KIR and the specific HLA ligands are indispensable to regulating NK cell activity, such that one without the other is functionally inert [12]. When KSHV invades, the K3 and K5 proteins encoded by KSHV can downregulate HLA-I molecules, thereby disrupting the balance of KIRs and activating NK cells to recognize and clear infected cells [13].

Previous studies have shown that KSHV susceptibility is caused by virus-host-environment interactions [14]. The characteristics of KSHV infection in China are quite distinct from those in the Mediterranean region, suggesting that genetic background plays an important role [2, 7]. Thus, the genes

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encoding KIR and HLA-I are centrally involved in the immunological response to KSHV infection, and the *KIR/HLA-I* polymorphisms would be expected to affect the host susceptibility to KSHV infection. However, only tenuous support for this hypothesis has been reported [15–20], and most focused on Mediterranean populations and classic KS. For example, *KIR2DS1* binding to *HLA-C2* was prevalent in Italian patients with classical KS [16], whereas *KIR3DS1*-specific binding to *HLA-B Bw4-80I* and homozygous *HLA-C1* might prevent KSHV infection [17, 18].

Considering that the extensive polymorphisms of *KIR/HLA-I* are closely related to the immune function against viral infections and the risk genes of KSHV infection vary substantially across different ethnic groups and individuals, the present study focused on AIDS-related KSHV infection among participants with HIV in Xinjiang, an endemic area for KSHV/KS in China [7], to examine the possible association between *KIR/HLA-I* polymorphisms and KSHV infection. Findings would have important implications for the development of targeted prevention and control measures in areas with a high prevalence of KSHV and the precision protection of KSHV-susceptible populations.

MATERIALS AND METHODS

Study Design and Sample Collection

This study was conducted in the Kazakh Autonomous Prefecture of Ili, Xinjiang, China. From May to December 2019, individuals from the Center for Disease Control and Prevention (CDC) in Ili were consecutively recruited. Participants were all aged 18 years or older and receiving regular ART for HIV infection. The HIV-positive status of all participants was confirmed by the CDC. Participants were interviewed in person by trained staff from local health facilities. A standard questionnaire was used to collect information on demographic characteristics and information related to HIV infection. Then, participants were stratified based on their ethnicity, sex, age, and KSHV infection status. We further performed random sampling from different subgroups to select individuals for this study to ensure the representativeness of the selected participants. All recruited individuals agreed to participation in the study with written informed consent and provided blood samples. This study was approved by the research ethics review committee of Fudan University, Shanghai, China (approval number IRB#03-0506).

Laboratory Testing

KSHV Serology

Antibodies against KSHV were detected using an enzyme-linked immunosorbent assay (ELISA) that employs the most immunogenic lytic antigens ORF65 and K8.1 and latent antigen ORF73. When used together, serological assays against KSHV lytic and latent antigens show the best combination of sensitivity (89.1%) and specificity (94.9%) [21–23]. The ORF65, K8.1, and ORF73 coding sequences were recombined into the pQE-80L vector to express the respective proteins in competent *Escherichia coli* cells, followed by the purification of the 3 proteins using a nickel column, as antigens. Three target proteins were used as antigens to coat the three 96-well plates, and alkaline phosphatase–labeled sheep antihuman immunoglobulin G was used as the secondary antibody. Detailed information has been previously described [6, 24]. Each serum to be tested was subjected to an ELISA for each of the 3 target proteins, with detection based on positive wells, negative wells, and blank wells. The absorbance value of each plasma sample was measured at 405 nm using a microplate reader, and the average optical density value of the negative control sample plus 2 times the standard deviation was used as the cutoff value. Participants who were positive for any of the 3 KSHV antibodies were considered KSHV seropositive.

HIV and KSHV Viral Load

The HIV viral load was quantified by Cobas AmpliPrep Cobas TaqMan HIV-1 Test, version 2.0 (Roche). HIV type 1 (HIV-1) RNA was extracted from plasma and reverse transcribed into complementary DNA for detection. Using real-time polymerase chain reaction (PCR) to amplify the gag region of HIV-1, samples were quantified using the internal standard method, with a linear range of 20–10^7 copies/mL.

KSHV viral loads were detected using real-time fluorescence quantitative PCR (qPCR) among KSHV-seropositive individuals. Primer pairs used were KS-1 and KS-2 (forward, 5′-AGCCGAAAGGATTCCACCAT-3′; reverse, 5′-TCCGTG TTGTCTACGTCCAG-3′), amplifying the KS330Bam233 region of ORF26 (a viral capsid protein). Each 20-μL PCR reaction contained 10 μL SYBR 2× mix (NO ROX), 2 μL DNA template, 7 μL double-distilled water, and 0.5 μL of each primer. The PCR conditions were 95°C for 3 minutes, followed by 40 cycles at 95°C for 5 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and a final step at 72°C for 5 minutes. A cutoff value of ≥1000 copies/μL was used to identify participants positive for KSHV DNA, which also indicated a high KSHV viral load.

KIR-HLA *Genotyping*

DNA was extracted using the TIANGEN blood genomic DNA extraction kit DP318-03 (centrifugal column type). DNA samples were stored at −80°C before genotyping.

KIR *Typing*

Genotyping for the 11 *KIR* genes (excluding the framework genes *KIR3DL3*, *2DL4*, and *3DL2* and pseudogenes *2DP1* and *3DP1*), including 6 activating loci (*KIR2DS2*, *3DS1*, *2DS3*, *2DS5*, *2DS1*, and *2DS4*) and 5 inhibitory loci (*KIR2DL2*, *2DL3*, *2DL1*, *3DL1*, and *2DL5*), was conducted by PCR sequence-specific primer [25]. The primer sequences of these 11 *KIR* genes are shown in Supplementary Table 1. PCR

conditions were 95°C for 2 minutes, 30 cycles at 94°C for 20 seconds, 63°C for 30 seconds, 68°C (*2DS3-2*, *2DS5*, *3DS1*, *3DL1*, and *2DL3* for 1.5 minutes; *2DS2-1*, *2DL1-1*, *2DL2-1*, *2DS1*, *2DS4*, and *2DL5* for 2.5 minutes; *2DS2-2*, *2DL2-2*, *2DS3-1*, and *2DL1-2* for 10 minutes), and 72°C for 10 minutes. The amplification products were analyzed by stained agarose gel electrophoresis. The genotypes were classified based on the criteria adopted by Middleton (Allele Frequency Net Database: http://www.allelefrequencies.net/kir6001a.asp) [26], and 2 broad haplotypes, termed A and B, were defined.

HLA-I *Typing*

HLA-I alleles were detected via multiplex PCR amplification using the FastTarget system. Sequencing libraries were generated using the FastTarget Custom Panel following the manufacturer's recommendations, with index codes being added to each sample. The fragment length distribution of the library was verified using Agilent 2100 Bioanalyzer. Following the accurate quantification of the molar concentration of the library, FastQ data were obtained using the Illumina HiSeq platform for high-throughput sequencing using the 2×250 bp double-terminal sequencing mode. Consequently, 4-digit HLA-A/B/C allele typing results were acquired.

KIR/HLA-I *Complex Genotypes*

KIR2DL1 and *2DS1* bind the *HLA-C2* epitope (asparagine at position 77, lysine at position 80), and *KIR2DL2*, *2DL3*, and *2DS2* bind the *C1* epitope (serine at position 77, asparagine at position 80) [27]. *HLA-B*46* allotype also was a ligand of *2DL2* and *2DL3* [28]. *HLA-Bw4*80I* (isoleucine at position 80) subset was considered the ligand for *KIR3DL1* and *3DS1* [29, 30]. In addition, *HLA-A*23*, *-*24*, and *-*32* molecules (all carrying *Bw4* epitope) were included among the ligands of *3DL1* [31, 32].

Statistical Analysis

All data were double-entered using EpiData version 3.1 and subsequently transferred to R version 3.5.2 for further statistical analysis. Comparisons between different groups was performed by the Mann–Whitney *U* test for continuous variables and χ^2 analysis or Fisher exact test (each when adequate) for categorical variables. The detection rate (F) of each KIR gene was determined as F = number of gene detections/number of individuals; and the gene frequency (GF) of each KIR gene was determined as $GF = 1-\sqrt{(1-F)}$. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) under different genetic patterns (dominant, additive, and overdominant models). The association analysis of *KIR/HLA-I* polymorphisms with KSHV infection was performed using the MiDAS package [33], in which Hardy-Weinberg equilibrium (HWE) testing for *HLA-I* alleles can be performed. MiDAS is based on the Immuno Polymorphism Database-ImMunoGeneTics (IPD-IMGT)/HLA database (http://www.allelefrequencies.net/hla.asp) to perform *HLA* sequence alignment and can obtain the variable amino acid residues of individuals from *HLA* allele data and then determine the variable amino acid residues associated with KSHV infection in the HLA protein by the likelihood ratio test. ORs and their 95% CIs were calculated for all of the coefficients of regression. Differences were considered statistically significant at $P < .05$, and *P* values were calculated for Bonferroni method corrected for multiple testing.

RESULTS

A total of 299 PWH were finally recruited into the present study, including 123 KSHV-seropositive persons and 176 KSHV-seronegative controls. There were 162 (54.2%) males and 201 (67.2%) Uyghurs, with a mean age of 44.35 years (range, 19–89 years). Sex, age, and ethnic distribution did not differ between the infected and uninfected groups. We found that compared with KSHV-seronegative participants, individuals infected with KSHV had significantly lower $CD4^+$ T-cell counts (593.28 ± 303.55 cells/μL vs 505.54 ± 234.13 cells/μL, $P = .006$). However, no significant difference between the 2 groups was detected in terms of HIV stage, HIV viral load, or duration of HIV infection (Supplementary Table 2). Moreover, among the KSHV-seropositive individuals, there were 92 persons with KSHV viral load <1000 copies/μL and 31 persons with high KSHV viral load (≥1000 copies/μL).

HLA Alleles and *KIR* Genes With KSHV Seroprevalence

HLA alleles and *KIR* genes were successfully determined in all 299 participants. We compared each *HLA-A*, *HLA-B*, and *HLA-C* allele and *KIR* gene in different KSHV infection groups to explore their possible associations with KSHV infection.

HLA-I alleles with frequencies >2.5% were screened, and alleles strongly deviating from the HWE equilibrium were filtered out (removing alleles with $P_{HWE} < 3.5 \times 10^{-4}$). We obtained 40 alleles, including 13 *HLA-A*, 13 *HLA-B*, and 14 *HLA-C* alleles. Since the inheritance pattern of *HLA* alleles was not determined, each allele was discussed separately under common genetic models (recessive model was not applicable here). The association between *HLA-A/-B/-C* and KSHV infection was analyzed under dominant, additive, and overdominant models. Three *HLA-I* alleles (*HLA-A*31:01*, *HLA-C*03:04*, and *HLA-C*12:03*) were nominally associated with KSHV infection (Table 1). Among them, *HLA-A*31:01* showed a protective effect against KSHV infection under all 3 models (dominant: OR, 0.30 [95% CI, .08–.82], $P = .031$; additive: OR, 0.30 [95% CI, .09–.80], $P = .030$; overdominant: OR, 0.31 [95% CI, .09–.88], $P = .042$), and *HLA-C*03:04* showed protection against KSHV infection only in the additive model (OR, 0.45 [95% CI, .20–.95], $P = .047$). In contrast, individuals

Table 1. Associations Between *HLA-A*, *HLA-B*, and *HLA-C* Alleles and Kaposi Sarcoma–Associated Herpesvirus Infection Under the Dominant, Additive, and Overdominant Models

	Dominant Model[a]		Additive Model[b]		Overdominant Model[c]	
Allele	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
*A*01:01*	1.05 (.52–2.08)	.897	1.12 (.57–2.15)	.746	0.97 (.47–1.95)	.937
*A*02:01*	0.91 (.51–1.61)	.750	1.00 (.61–1.63)	.995	0.81 (.44–1.48)	.499
*A*02:06*	1.23 (.52–2.85)	.626	1.32 (.59–2.94)	.487	1.11 (.46–2.61)	.812
*A*02:07*	0.95 (.43–2.03)	.894	0.90 (.42–1.86)	.779	1.01 (.45–2.19)	.978
*A*03:01*	1.51 (.77–2.97)	.223	1.51 (.77–2.97)	.223	1.51 (.77–2.97)	.223
*A*11:01*	1.35 (.77–2.35)	.294	1.18 (.72–1.95)	.504	1.50 (.85–2.67)	.164
*A*24:02*	0.92 (.54–1.56)	.771	1.03 (.65–1.62)	.886	0.80 (.46–1.39)	.438
*A*26:01*	0.79 (.32–1.82)	.586	0.76 (.32–1.66)	.499	0.85 (.35–1.97)	.706
*A*30:01*	0.81 (.36–1.75)	.600	0.83 (.40–1.62)	.591	0.83 (.35–1.85)	.650
*A*31:01*	0.30 (.08–.82)	**.031**	0.30 (.09–.80)	**.030**	0.31 (.09–.88)	**.042**
*A*32:01*	0.85 (.28–2.36)	.761	0.85 (.28–2.36)	.761	0.85 (.28–2.36)	.761
*A*33:03*	1.63 (.67–4.04)	.281	1.50 (.68–3.39)	.310	1.64 (.64–4.26)	.297
*A*68:01*	1.29 (.47–3.47)	.610	1.41 (.56–3.56)	.459	1.12 (.39–3.09)	.827
*B*07:02*	0.95 (.36–2.37)	.915	0.95 (.36–2.37)	.915	0.95 (.36–2.37)	.915
*B*13:02*	0.79 (.40–1.52)	.487	0.81 (.43–1.46)	.483	0.80 (.40–1.57)	.524
*B*14:02*	0.87 (.34–2.13)	.769	0.82 (.33–1.90)	.653	0.95 (.36–2.37)	.915
*B*35:01*	0.50 (.14–1.51)	.251	0.50 (.14–1.51)	.251	0.50 (.14–1.51)	.251
*B*35:03*	2.06 (.81–5.48)	.132	2.06 (.81–5.48)	.132	2.06 (.81–5.48)	.132
*B*40:01*	0.61 (.24–1.41)	.265	0.69 (.30–1.46)	.349	0.56 (.21–1.35)	.219
*B*40:06*	1.46 (.52–4.08)	.461	1.46 (.52–4.08)	.461	1.46 (.52–4.08)	.461
*B*44:02*	1.27 (.43–3.62)	.656	1.40 (.53–3.73)	.490	…	…
*B*46:01*	1.16 (.51–2.57)	.714	0.96 (.46–1.91)	.904	1.48 (.63–3.44)	.360
*B*50:01*	0.68 (.32–1.40)	.312	0.67 (.32–1.33)	.268	0.72 (.33–1.48)	.382
*B*51:01*	0.97 (.49–1.87)	.925	0.97 (.49–1.87)	.925	0.97 (.49–1.87)	.925
*B*52:01*	2.14 (.80–6.04)	.135	2.14 (.80–6.04)	.135	2.14 (.80–6.04)	.135
*B*58:01*	1.47 (.61–3.55)	.383	1.56 (.68–3.61)	.289	1.33 (.54–3.25)	.532
*C*01:02*	1.25 (.70–2.24)	.446	1.25 (.70–2.24)	.446	1.25 (.70–2.24)	.446
*C*03:02*	1.33 (.54–3.25)	.532	1.28 (.58–2.84)	.535	1.31 (.51–3.35)	.569
*C*03:03*	1.11 (.46–2.61)	.812	1.11 (.46–2.61)	.812	1.11 (.46–2.61)	.812
*C*03:04*	0.46 (.20–.98)	.053	0.45 (.20–.95)	**.047**	0.48 (.20–1.03)	.070
*C*04:01*	0.95 (.50–1.75)	.858	0.95 (.50–1.75)	.858	0.95 (.50–1.75)	.858
*C*06:02*	0.86 (.52–1.42)	.567	0.78 (.50–1.21)	.276	1.03 (.62–1.72)	.907
*C*07:01*	0.49 (.16–1.32)	.183	0.49 (.16–1.32)	.183	0.49 (.16–1.32)	.183
*C*07:02*	0.96 (.52–1.76)	.903	0.96 (.52–1.76)	.903	0.96 (.52–1.76)	.903
*C*08:01*	1.79 (.75–4.39)	.189	1.79 (.75–4.39)	.189	1.79 (.75–4.39)	.189
*C*08:02*	0.87 (.34–2.14)	.769	0.87 (.34–2.14)	.769	0.87 (.34–2.14)	.769
*C*12:02*	2.14 (.80–6.04)	.135	2.14 (.80–6.04)	.135	2.14 (.80–6.04)	.135
*C*12:03*	2.50 (1.18–5.49)	**.019**	1.94 (.98–3.99)	.062	3.03 (1.38–7.03)	**.007**
*C*14:02*	1.18 (.46–2.95)	.717	1.30 (.54–3.06)	.552	1.04 (.39–2.66)	.929
*C*15:02*	0.95 (.40–2.17)	.904	0.95 (.40–2.17)	.904	0.95 (.40–2.17)	.904

Statistically significant associations are marked in bold. *P* values were calculated for uncorrected.

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Dominant model: comparing individuals who carry 1 allele against individuals who do not carry it (CA + AA vs CC).

[b]Additive model: comparing pure individuals carrying 1 allele and pure sum individuals not carrying that allele (AA vs CC).

[c]Overdominant model: comparing individuals carrying pure alleles and individuals carrying heterozygous alleles (CC + AA vs CA).

carrying *HLA-C*12:03* were more prevalent in the KSHV-positive group, suggesting that it might be a risk allele for KSHV infection under the dominant and overdominant models (ORs, 2.50 [95% CI, 1.18–5.49], $P = .019$ and 3.03 [95% CI, 1.38–7.03], $P = .007$, respectively). However, none of these 3 alleles was statistically different after Bonferroni correction ($P = 0.05/40/3 = 4.2 \times 10^{-4}$).

Genotyping for 11 *KIR* genes showed that the frequency of *KIR* genes was similar in the KSHV-infected group versus controls (Supplementary Table 3). Consequently, we classified the *KIR* genotypes and found a total of 28 genotypes, which were all the Bx (AB or BB genotypes) (Supplementary Table 4). Previous research has highlighted that *KIR2DL2/DS2* (considered together because of their strong linkage disequilibrium)

and *KIR2DL3* genotypes are the most significant *KIR* variations for the risk of developing herpesvirus-associated diseases [34, 35]. In our study, we observed differences in *KIR2DL2/DS2* and *KIR2DL3* genotypes between the KSHV infection status groups. Specifically, there was a slight increase in *KIR2DL2/2DL3* heterozygosity (76.42% vs 73.30%) in the KSHV-infected group, although this difference was not statistically significant ($P = .636$). Intriguingly, subdividing the KSHV-infected group accordingly to KSHV viral load, the results evidenced a significantly different distribution of the *KIR2DL2/DS2* and *KIR2DL3* genotypes in these 2 groups. Individuals with KSHV carrying the homozygous *KIR2DL3* had a relatively high viral load (16.30% vs 41.94%, $P = .010$) (Figure 1).

KIR/HLA-I Complex With KSHV Seroprevalence

In contrast to single *HLA* alleles and *KIR* genes, the joint classification of participants according to their KIR and HLA-I ligand pairs was more beneficial to understand the pathogenesis of KIR-mediated NK cytotoxicity in KSHV infection. The analysis of the *KIR/HLA-I* complex genotypes, summarized in Table 2, suggested that although statistical significance was not reached, 5 complex genotypes appeared to be protective against KSHV infection, while others were expressed as risk genotypes for infection. Specifically, participants with *C1-KIR2DL3* (OR, 1.85 [95% CI, .98–3.65], $P = .064$) and *C1_KIR2DS2* (OR, 1.86 [95% CI, .97–3.75], $P = .069$) genotypes showed a marginal increase in the risk of KSHV infection.

HLA Association Fine Mapping on the Amino Acid Level

Corresponding to the results of our allele-level associations, we found the strongest associated amino acid positions in the *HLA-I* region, which can help to finely map the relevant variants to peptide binding regions or other functionally distinct parts of the protein. As shown in Table 3, a variety of amino acids exhibited an association with KSHV seropositivity, with position 56 of HLA-A having a significant association under all models. Individuals carrying an arginine had a lower risk of KSHV infection, whereas individuals carrying a glycine at the same position had an elevated risk of infection.

Mapping the significant amino acid residues back to the respective *HLA* alleles, the protective effect of arginine in position 56 of HLA-A may be due to the different *HLA-A* alleles, which include the protective allele *HLA-A*31:01* that we previously found.

DISCUSSION

Although the cause of KSHV susceptibility is still unclear, the previous studies showed that in addition to differences in exposure chance, genetic background also plays an important impact [14, 36]. Furthermore, KIRs present in the surface of NK

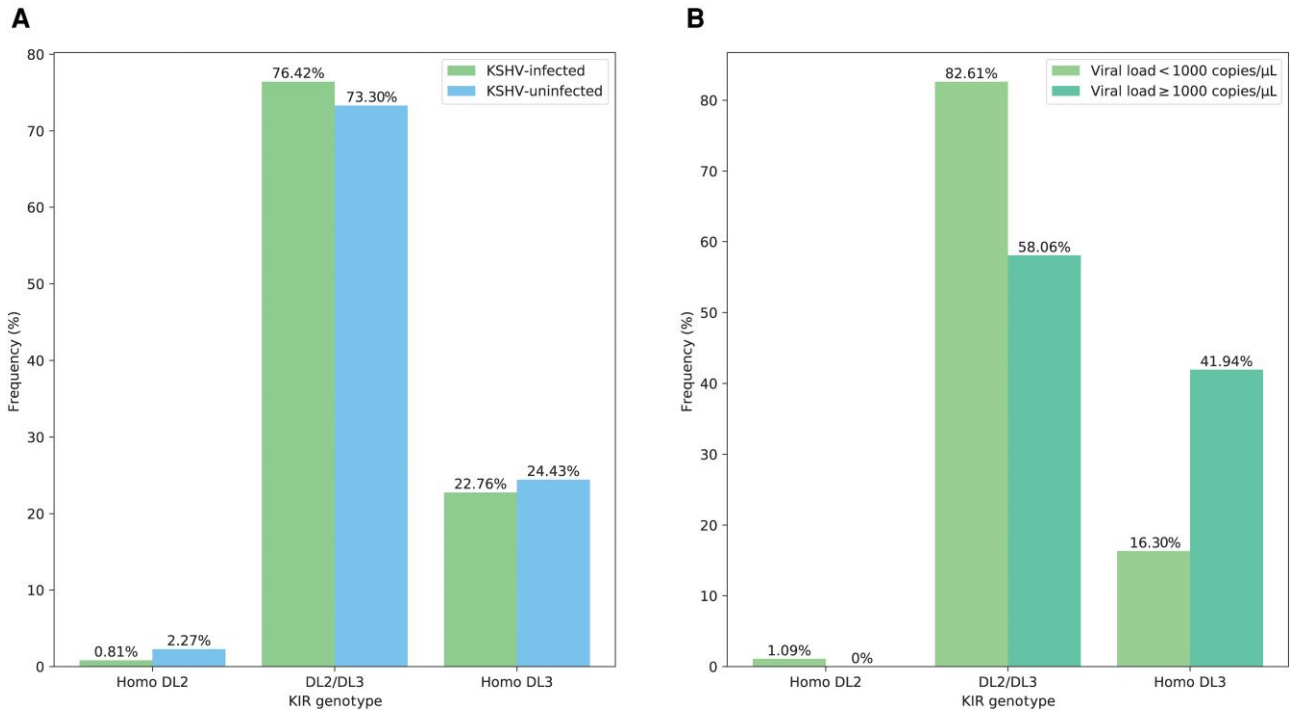

Figure 1. *A*, Frequency of *KIR* genotypes in different Kaposi sarcoma–associated herpesvirus (KSHV) serum infection groups. *B*, Frequency of *KIR* genotypes in different KSHV viral load groups. Abbreviations: Homo DL2, *KIR2DL2/DS2* homozygosity; DL2/DL3, *KIR2DL2/KIR2DL3* heterozygosity; Homo DL3, *KIR2DL3* homozygosity.

Table 2. Associations Between *KIR/HLA-I* Complex Genotypes and Kaposi Sarcoma–Associated Herpesvirus infection

	KSHV Positive (n = 123)		KSHV Negative (n = 176)			
KIR/HLA-I	No.	(%)	No.	(%)	OR (95% CI)	*P* Value
*A*03_KIR3DL2*	25	(20.33)	25	(14.20)	1.54 (.84–2.84)	.165
*A*11:01_KIR2DS2*	30	(24.39)	34	(19.32)	1.35 (.77–2.35)	.294
*A*11_KIR3DL2*	32	(26.02)	36	(20.45)	1.37 (.79–2.36)	.260
*A*11_KIR2DS4*	24	(19.51)	29	(16.48)	1.23 (.67–2.23)	.499
*A*23_KIR3DL1*	2	(1.63)	9	(5.11)	0.31 (.04–1.22)	.135
*A*24_KIR3DL1*	33	(26.83)	47	(26.70)	1.01 (.60–1.69)	.981
*A*32_KIR3DL1*	6	(4.88)	10	(5.68)	0.85 (.28–2.36)	.761
Bw4 80I_KIR3DL1	76	(61.79)	99	(56.25)	1.26 (.79–2.02)	.339
*B*46:01_KIR2DL2*	9	(7.32)	10	(5.68)	1.31 (.51–3.35)	.569
*B*46:01_KIR2DL3*	12	(9.76)	15	(8.52)	1.16 (.51–2.57)	.714
*B*51_KIR3DS1*	19	(15.45)	25	(14.20)	1.10 (.57–2.10)	.765
C1_KIR2DL3	108	(87.80)	140	(79.55)	1.85 (.98–3.65)	.064
C1_KIR2DS2	109	(88.62)	142	(80.68)	1.86 (.97–3.75)	.069
C1_KIR2DL2	83	(67.48)	107	(60.80)	1.34 (.83–2.18)	.238
C2_KIR2DL2	65	(52.85)	83	(47.16)	1.26 (.79–2.00)	.334
C2_KIR2DS1	66	(53.66)	88	(50.00)	1.16 (.73–1.84)	.534
C2_KIR2DL3	75	(60.98)	104	(59.09)	1.08 (.68–1.74)	.744
C2_KIR2DL1	75	(60.98)	108	(61.36)	0.98 (.61–1.58)	.946
*C*01:02_KIR2DS4*	20	(16.26)	30	(17.05)	0.95 (.50–1.75)	.858
*C*04:01_KIR2DS4*	17	(13.82)	20	(11.36)	1.25 (.62–2.50)	.526
*C*05:01_KIR2DS4*	4	(3.25)	5	(2.84)	1.15 (.28–4.43)	.838
*C*14:02_KIR2DS4*	8	(6.50)	10	(5.68)	1.15 (.43–3.02)	.769

C1 denotes *HLA-C*01, C*03, C*07*, and *C*08*, and *C2* denotes *HLA-C*02, C*04, C*05*, and *C*06* [27]. *HLA-B*46* allotype was a ligand of *2DL2* and *2DL3* [28]. *HLA-B Bw4 80I* denotes *HLA-B*27:02, B*38:01, B*49:01, B*51, B*57*, and *B*58* [29, 30]. *HLA-A*23, -*24*, and *-*32* molecules (all carrying *Bw4* epitope) are included among the ligands of *3DL1* [31, 32].

Abbreviations: CI, confidence interval; KSHV, Kaposi sarcoma–associated herpesvirus; OR, odds ratio.

cells and their specific HLA-I ligands have been shown to predispose both to susceptibilities to herpesvirus infection [37] and to the development of herpesvirus-associated diseases, such as multiple sclerosis [35]. In addition, the invasion of KSHV downregulates the expression of HLA-I antigens, which can be recognized by KIR and then delivers activation signals to NK cells [13]. Thus, the polymorphisms of *KIR/HLA-I* influence the response of NK cells against herpesvirus infection [12, 38]. We conducted the current study based on the population of PWH in Xinjiang, which avoids confounding by *HLA* polymorphisms on the HIV infection, to elucidate the association between *KIR/HLA-I* polymorphisms and the risk of KSHV infection.

We found that 3 alleles (*HLA-A*31:01*, *HLA-C*03:04*, and *HLA-C*12:03*) in *HLA-I* were associated with KSHV infection, in which *HLA-A*31:01* causes the variants of amino acid in position 56 of HLA-A, with individuals carrying arginine having a lower risk of KSHV infection. We also found homozygous *KIR2DL3* may affect KSHV viral load in the infected hosts. These results reveal a potential role of KIR-mediated NK cell activation in anti-KSHV infection.

In our study, the risk of KSHV infection was reduced in people with *HLA-A*31:01* and *HLA-C*03:04*, and it was increased for those with *HLA-C*12:03*. Among them, the protective effect of *A*31:01* was shown significantly under all 3 models (dominant: OR, 0.30 [95% CI, .08–.82], $P = .031$; additive: OR, 0.30 [95% CI, .09–.80], $P = .030$; overdominant: OR, 0.31 [95% CI, .09–.88], $P = .042$). In addition, the analysis of the molecular basis revealed that arginine at position 56 of HLA-A also reduces the risk of KSHV infection. It is logical to hypothesize that the protective effect of the arginine at position 56 of HLA-A against KSHV infection was due to the association basis of *HLA-A*31:01*. Similar to other research, this rare allele was also shown to be a protective allele against nasopharyngeal carcinoma caused by Epstein-Barr virus [39], which, like KSHV, is a γ-herpesvirus. This allele was also found to be associated with macular papular erythema caused by human herpesvirus reactivation in the Chinese population [40]. This implies that *A*31:01* may have the same manner of effective control of the herpesvirus, especially γ-herpesvirus infection.

The significant effects of the inhibitory KIR receptors on KSHV viral load also merit special attention. The *KIR* genotype of KSHV-seropositive individuals with different viral loads was characterized for the frequency of the *KIR2DL2* and *KIR2DL3* genes, to evidence any potential influence of NK receptor allotype in the risk of KSHV replication. The observed increased homozygous *KIR2DL3* and a relatively high KSHV viral load in participants with KSHV suggest higher inhibitory

Table 3. Associations Between Amino Acids of HLA-A and Kaposi Sarcoma–Associated Herpesvirus Infection Under the Dominant, Additive, and Overdominants Model

Position of Amino Acid	Amino Acid Residues	KSHV Infection Group		OR (95% CI)	*P* Value
		KSHV Positive (n = 123)	KSHV Negative (n = 176)		
Dominant model					
A_56	Glycine (Gly, G)	120 (48.78%)	169 (48.01%)	1.66 (.45–7.81)	.471
	Arginine (Arg, R)	14 (5.69%)	40 (11.36%)	0.44 (.22–.83)	.014
A_149	Alanine (Ala, A)	123 (50.00%)	173 (49.15%)	…	.986
	Threonine (Thr, T)	10 (4.07%)	26 (7.39%)	0.51 (.23–1.07)	.087
B_41	Alanine (Ala, A)	111 (45.12%)	142 (40.34%)	2.21 (1.12–4.64)	.027
	Threonine (Thr, T)	60 (24.39%)	100 (28.41%)	0.72 (.46–1.15)	.171
Additive model					
A_56	Glycine (Gly, G)	229 (93.09%)	303 (86.08%)	1.93 (1.15–3.42)	.017
	Arginine (Arg, R)	17 (6.91%)	45 (12.78%)	0.55 (.31–.94)	.036
A_144	Lysine (Lys, K)	191 (77.64%)	239 (67.90%)	1.58 (1.11–2.30)	.013
	Glutamine (Gln, Q)	55 (22.36%)	109 (30.97%)	0.66 (.45–.95)	.026
Overdominant model					
A_56	Glycine (Gly, G)	11 (4.47%)	35 (9.94%)	0.37 (.18–.79)	.012
	Arginine (Arg, R)	11 (4.47%)	35 (9.94%)	0.37 (.18–.79)	.012
C_94	Isoleucine (Ile, I)	31 (12.60%)	64 (18.18%)	0.59 (.35–.98)	.042
	Threonine (Thr, T)	31 (12.60%)	64 (18.18%)	0.59 (.35–.98)	.042
C_95	Isoleucine (Ile, I)	27 (10.98%)	62 (17.61%)	0.52 (.31–.87)	.014
	Leucine (Leu, L)	30 (12.20%)	67 (19.03%)	0.52 (.31–.87)	.014

Only statistically significant amino acids are shown.

Abbreviations: CI, confidence interval; KSHV, Kaposi sarcoma–associated herpesvirus; OR, odds ratio.

capabilities of *KIR2DL2* in comparison with the *KIR2DL3* receptor. This result was consistent with a study in Italy [15], which found that this specific *KIR* genotype may impair NK cell function in controlling KSHV infection and proliferation, leading the virus to exert its angioproliferative activity. There is clinical evidence to suggest that lytic KSHV replication is important for the progression to KS [41]. Thus, it can be hypothesized that the modulation of NK cell–mediated cytotoxicity by KIR may be beneficial against infectious agents.

However, no statistically significant *KIR/HLA*-I complex genotypes associated with KSHV infection were found in our study, which was consistent with some results of other studies in the Mediterranean region [16]. *KIR/HLA*-I polymorphisms may have more impact on the development of KS. The significance of the *HLA* single allele and nonsignificance of the *KIR/HLA*-I complex genotypes suggest that there are other inflammatory effects against KSHV infection in addition to KIR-mediated NK cell cytotoxicity. HLA could present herpesvirus-related antigenic epitopes to cytotoxic T lymphocytes, resulting in effective control of the virus in the lytic phase [42]. Furthermore, the extensive polymorphisms in the *KIR/HLA-I* and the relatively small sample size included in the study may also influence the results.

Certain limitations also should be considered in this study. First, there are extensive polymorphisms in *KIR/HLA-I*, and the relatively small sample size of the study might have reduced the statistical power of the findings. Second, the association between the identified alleles and genes and KSHV infection was not significant after Bonferroni correction. Given the conservative results generated by the multiple correction methods and because this was a preliminary study, multiple comparisons could not be considered in the association analysis. Finally, KSHV infection was measured by serology, which may be confounded by exposure. Despite the inclusion of relevant covariates, it remains challenging to fully control for these exposure-related confounders, which may affect the interpretation of the results. The participants included were all HIV-infected with no KS occurring after ART, and there was a lack of association studies between *KIR/HLA-I* polymorphisms and KS progression. The role of NK cells and KIR in the development of KS has not been studied in depth. Further functional studies are warranted to clarify the findings described herein and to understand their role in the development of KS.

In conclusion, even considering the exploratory characteristics of the study, our results provide further insight into the link between *HLA-I* alleles and *KIR* genes and KSHV infection, highlighting the potential role of KIR-mediated NK cells in anti-KSHV infection. Further studies should aim at completing the picture of the interplay among KSHV, genetic background, and immunosuppression.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the

posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. All authors contributed to the study's conception and design. X. Z., Y. L., and X. H. were responsible for data collection. Y. X. and H. W. were responsible for sorting and cleaning the data. X. Z. and T. W. performed data analysis. X. Z. was responsible for interpreting the results and drafting the article. T. Z. was responsible for revising the manuscript critically for important intellectual content and final approval.

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