***KIR* gene content diversity in a Zimbabwean population: Does *KIR2DL2* have a role in protection against HIV infection?**

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Abstract

Killer Immunoglobulin-like Receptors (KIR) mediate Natural Killer (NK) cell function through interaction with their cognate human leukocyte antigen (HLA) ligand. Thus, KIR gene variants have been implicated in resistance or susceptibility to viral infections. However, research on the role of these variants remains contradictory and inconclusive. In the present study, we investigated *KIR* gene content diversity and its association with Human Immunodeficiency Virus (HIV) infection in an adult Black Zimbabwean population. Presence or absence of 15 KIR genes was determined in 189 HIV-infected adults and 97 HIV-uninfected blood donors using sequence specific primer polymerase chain reaction (SSP-PCR). Frequencies of KIR genes, genotypes and haplotypes were compared between the cases and controls to identify putative associations between *KIR* gene variants and HIV status. We report here the frequencies of 15 KIR genes and 43 KIR genotypes (40 known and three novel) among Zimbabweans. Importantly, the frequency of the inhibitory *KIR2DL2* gene was significantly higher in the uninfected group (62%) compared to the HIV infected group (47%) (OR=0.55, 95%CI: 0.33-0.90, P=0.019). *KIR2DL2/2DL2* homozygosity was also significantly higher in the uninfected group (35%) compared to HIV infected group (53%) (OR=0.33, 95%CI: 0.16-0.72, P=0.005) under a recessive model. We conclude that the *KIR2DL2* gene may be involved in protection against HIV infection. It may be possible that inhibitory KIR genes may have an important role to play in HIV acquisition among populations of African origin in whom the activating KIR genes are less frequent compared to among Caucasians.

Introduction

Resistance to HIV infection despite repeated exposure among a minority of the world population is partially explained by variation in genes that encode host immune factors. Among the immune factors, variants of genes encoding chemokine receptors, the HLA and KIR are strong correlates of resistance/susceptibility to HIV infection (Singh and Spector, 2009; Martin and Carrington, 2013). KIR are a diverse family of transmembrane activating and inhibitory proteins expressed on NK cells and some T lymphocytes. Activating and inhibitory KIRs (aKIRs and iKIRs, respectively) regulate the function of NK cells through binding their cognate HLA class I ligands (Lanier, 2005). NK cells form the front line of defense against HIV and other viruses through cytotoxic destruction of virus infected cells and release of cytokines that regulate the adaptive immune response (Vivier et al., 2008). NK cells can directly recognise stress ligands expressed on HIV-infected host cells leading to autolysis of the infected cell. Furthermore, secretion of chemokines by NK cells in response to HIV infection can block new cycles of infection (Scully and Alter, 2016). The critical role of NK cells in HIV infection control is also evidenced by the expansion of the NK cell population and the change in distribution of NK cell subsets in acute HIV infection (Mavilio et al., 2005). The KIR and HLA loci are highly diverse, hence antiviral functions of NK cells are heterogeneous among individuals and populations. Reported research findings on the effect of KIR gene variants on HIV infection are contradictory and inconclusive.

The KIR locus on chromosome 19 comprises of 14 functional genes, six encoding activating receptors (*KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, *KIR2DS5*, *KIR3DS1*), seven encoding inhibitory receptors (*KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL5*, *KIR3DL1*, *KIR3DL2* and *KIR3DL3*), one encoding a receptor with both inhibitory and activation activity (*KIR2DL4*) and two pseudogenes (*KIR2DP1* and *KIR3DP1*) (Takeshita et al., 2013). Activating receptors carry short cytoplasmic tails while inhibitory receptors carry long cytoplasmic

tails, hence the S and L designations, respectively (Marsh et al., 2003). Genetic variation in KIR occurs at three levels; (1) presence or absence of any of the 12 non-framework genes, (2) combination of genes that make up KIR genotypes and haplotypes and (3) single nucleotide polymorphisms in the individual KIR genes (Carrington and Norman, 2003). Currently greater than 600 alleles, 50 haplotypes and 398 genotypes have been identified (González-Galarza et al., 2015).

KIR genes and KIR/HLA class I gene variants are linked to outcomes of HIV disease course largely, but there is little evidence on how they influence HIV acquisition (Alter et al., 2007; Singh et al., 2016; Hens et al., 2016; Naranbhai et al., 2016). The activation role of *KIR3DS1* justifies its candidature as an agent for resistance to HIV infection (Carrington et al., 2008; Hong et al., 2013; Hens et al., 2016). However, the *KIR3DS1* gene is infrequent in Africans (generally below 25%) relative to Caucasians (>50%) and Asians (>30%) (Hollenbach et al., 2012). There is emerging evidence of the influence of iKIR genes in differential outcomes of viral acquisition (Jennes et al., 2006; Jennes et al., 2013; Petitdemange et al., 2014; De Re et al., 2015). Whether iKIR genes have greater influence on HIV acquisition among populations with low *KIR3DS1* frequency remains unclear. Heterogeneity in frequencies and distribution of KIR genes, genotypes and haplotypes among populations may explain the failure to establish the role of KIR genes in HIV acquisition.

The current study describes the frequencies and distribution of KIR genes, genotypes and haplotypes among HIV-infected and HIV-uninfected adult Black Zimbabweans. Findings indicate a potential role of an iKIR gene *KIR2DL2* in protection against HIV infection. This information may help predict risk of HIV infection.

Materials and Methods

Study participants

HIV-infected, antiretroviral therapy naïve Zimbabweans (of Bantu origin) aged ≥ 18 years were enrolled under the Immunological and Virological Investigations of HIV-infected individuals with CD4 counts above 350 cells/ μL (IVI HIV) Study at the Opportunistic Infections Clinic at Parirenyatwa Groups of Hospitals in Harare, Zimbabwe. Written informed consent was obtained from each participant. The study was conducted from 2010 to 2013. At enrolment, a questionnaire was administered to collect demographic data and medical history, whole blood was collected in ethylene diaminetetraacetic acid (EDTA) anti coagulant and peripheral blood mononuclear cells (PBMCs) and plasma isolated within six hours of collection. The PBMCs and plasma were immediately stored at -80°C until further use. In the current study, DNA was isolated from the enrolment archived PBMCs of 189 randomly selected HIV-infected individuals. EDTA anti coagulated blood and demographic information were also collected from 97 consenting unmatched HIV-uninfected Black Zimbabwean blood donors aged ≥ 18 years attending the National Blood Service of Zimbabwe (NBSZ) in Harare. The study was approved by the Medical Research Council of Zimbabwe (MRCZ/A/2016).

KIR typing

Genomic DNA was isolated from PBMC or whole blood at the University of Zimbabwe College of Health Sciences, Harare, Zimbabwe using the Quick-gDNATM MiniPrep kit (Zymo Research Corporation, Irvine, CA, USA) according to the manufacturer's instructions. KIR genotyping was carried out at the University of Oxford, Oxford, United Kingdom using sequence specific primer polymerase chain reaction (SSP-PCR) according to a previously described method (Martin and Carrington, 2008). Briefly, two pairs of sequence

specific primers were used to amplify each of 14 functional KIR genes; *2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5*, *2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5*, *3DS1*, *3DL1*, *3DL2*, *3DL3* and the pseudogene *2DPI*. The ethidium bromide stained amplicons were electrophoresed on 2% agarose gel and visualized under ultraviolet light for determination of presence/absence of each gene. The allelic variants of the *KIR2DS4* gene were also determined by gel electrophoresis since the deleted version of the gene (*KIR2DS4v*) is 22 base pairs (bp) shorter in length than the full gene (*KIR2DS4v*).

Assignment of KIR genotypes and haplotypes

The combination of KIR genes occurring in an individual were used to assign a KIR genotype identity (GID) according to the Allele Frequency Net Database (AFND) (Gonzalez-Galarza et al., 2015). KIR haplotypes AA (A) and Bx were assigned according to a previously described method (González-Galarza et al., 2015). In summary, four genes, *KIR3DL2*, *KIR3DP1*, *KIR2DL4* and *KIR3DL3* are present in almost all individuals, thus, form the framework genes. Individuals carrying *KIR2DL1*, *KIR2DL3*, *KIR2DL4*, *KIR2DS4* and *KIR3DL1* genes in addition to the framework genes were assigned the AA haplotype. Individuals carrying all AA haplotype genes and any one of the following genes; *KIR2DL2*, *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS5* and *KIR3DS1* were denoted as AB haplotype whilst individuals lacking any of the following; *KIR2DL1*, *KIR2DL3*, *KIR3DL1* and *KIR2DS4* were BB haplotype. Due to difficulties in distinguishing AB and BB haplotypes, the two groups form the Bx haplotype. Centromeric and telomeric haplotypes were assigned according to a previously described method (De Re et al., 2015). Briefly, *KIR3DL3* and *KIR3DP1* delimit the centromeric part of the KIR locus, whereas *KIR2DL4* and *KIR3DL2* delimit the telomeric part. Thus, the centromeric haplotypes were inferred from combinations of *KIR2DS2*, *KIR2DL2*, *KIR2DL3* and *KIR2DL1*, whereas combinations

of *KIR3DS1*, *KIR2DS1*, *KIR3DL1* and *KIR2DS4* formed telomeric haplotypes. *KIR2DL5*, *KIR2DS3* and *KIR2DS5* genes may occur in either centromeric or telomeric regions.

Statistical analysis

Statistical analysis was done using Stata version 13.0 (StataCorp, Texas, USA). The Student's t-test was used to compare differences between means of continuous variables between HIV-infected and HIV-uninfected groups. Frequencies of genes, alleles, genotypes and haplotypes were determined using direct counting. Pairwise linkage disequilibrium (LD) between KIR genes was calculated using Cramer's V statistic and Lewontin's D' on the MASSKIR software (Pandey et al., 2015). Heterogeneity in frequencies of genes, alleles, genotypes and haplotypes between HIV-infected and HIV-uninfected groups was determined using Chi-squared or Fisher's exact test as may be appropriate. A value of $P < 0.05$ was considered statistically significant. P-values were adjusted for multivariate comparisons using Bonferroni correction method. Univariate logistic regression was used to determine association between KIR gene content variants and HIV status. Gene variants showing significant difference between HIV-infected and HIV-uninfected groups in univariate analysis were adjusted for gender and weight in a multivariate regression.

Results

Demographic characteristics of participants

The mean weight of the HIV-infected participants (66.9 ± 12.7 kg standard deviation (SD)) was significantly lower than that of the HIV-uninfected participants (74.8 ± 9.4 kg SD), $P < 0.001$. Females were overrepresented in the HIV-infected group than in the HIV-uninfected group (72% v 49%, $P = 0.003$) because women are more likely than men to seek

healthcare in most African settings. The mean age was not significantly different between the HIV-infected (35.2 ± 9.4 years SD) and HIV-uninfected (37.5 ± 11.4 years SD), $P=0.074$) participants.

Frequencies of single KIR genes and their association with HIV infection

We report frequencies of 15 single KIR genes in an adult Zimbabwean population (Table 1). As expected, three framework genes analysed, *KIR3DL3*, *KIR3DL2* and *KIR2DL4* were present in all 97 HIV-uninfected controls. Four other genes, two inhibitory genes (*KIR2DL1* and *KIR3DL1*), a pseudogene (*KIR2DP1*) and an activation gene (*KIR2DS4*) were present in >97% of the controls. *KIR3DS1*, a gene previously implicated in HIV acquisition and disease progression (reviewed by Hens et al., 2016) was the least prevalent at 17% followed by *KIR2DS1* which occurred in 22% of the control population. Three pairs of KIR genes exist as allelic variants; *KIR3DS1/KIR3DL1*, *KIR2DL2/KIR2DL3* and *KIR2DS3/KIR2DS5* and will be referred to as *KIR3DS1L1*, *KIR2DL2L3* and *KIR2DS3S5*, respectively. Either one or both of allelic variants at *KIR3DS1L1* and *KIR2DL2L3* loci were present in all participants. However, 40% of the control population had neither *KIR2DS3* nor *KIR2DS5* at the *KIR2DS3S5* locus.

When frequencies of the KIR genes were compared between the HIV-infected and HIV-uninfected groups to determine association between presence of single KIR genes with HIV status (Table 1), we found that the *KIR2DL2* gene was significantly more frequent in the HIV-uninfected controls than in the HIV-infected cases (62% v 47%), suggesting that *KIR2DL2* may protect against acquisition of HIV (OR=0.55, 95%CI: 0.33-0.90, $P=0.019$). The association between *KIR2DL2* and the HIV uninfected status remained significant after multivariate analysis (adjusted OR=0.52, 95%CI: 0.30-0.56, $P=0.017$), but was not statistically significant after correcting for multiple comparisons ($p=0.209$). None of the other 14 genes were associated with HIV infection. When allelic variants at the *KIR3DS1L1*,

KIR2DL2L3 and *KIR2DS3S5* loci were considered, frequency of the *KIR2DL2/KIR2DL2* genotype was significantly higher in the HIV-uninfected controls (53%) than the HIV-infected group (35%) (OR=0.49, 95%CI: 0.30-0.81, P=0.006) under a recessive model. The association between *KIR2DL2* homozygosity and the HIV-uninfected status remained significant after multivariate analysis (adjusted OR=0.46, 95%CI: 0.27-0.79, P=0.005) and showed borderline significance after correction for multiple comparisons (P=0.055).

Pairwise LD between 12 KIR genes (framework genes were excluded from analysis) was calculated in HIV-infected and HIV-uninfected groups separately to determine patterns of co-inheritance in the KIR locus (Table 2). Three pairs of genes were in strong positive LD in both groups; *KIR2DL2* and *KIR2DS2* (0.86 and 0.75, respectively); *KIR2DL5* and *KIR2DS5* (0.78 and 0.69, respectively); and *KIR2DL1* and *KIR2DP1* (0.71 and 1.00). *KIR3DL1* and *KIR2DS4* (0.71) showed strong LD in HIV-infected, while *KIR3DS1* and *KIR2DS1* (0.68) were likely to be co-inherited in the HIV-uninfected population.

KIR genotypes and haplotype, and their association with HIV infection

Single KIR genes were combined to form KIR genotypes (Table 3). A total of 43 genotypes were observed in the whole study population, 40 previously reported in other populations and three unique to the Zimbabweans; (1) *KIR3DL1-KIR2DL1*, *KIR2DL2-KIR2DL3-KIR2DS4-KIR3DS1-KIR2DS1-KIR2DP1*, (2) *KIR3DL1-KIR2DL1-KIR2DL2-KIR2DL5-KIR2DS4-KIR2DS1-KIR2DS2-KIR2DS5*, (3) *KIR3DL1-KIR2DL2-KIR2DL5-KIR2DS4-KIR2DS2-KIR2DS5*). 26 (60%) genotypes occurred in two or more study participants while the remainder 17 (40%) occurred once. 17 (40%) genotypes were common to both HIV-infected and HIV-uninfected groups, 15 (35%) restricted to HIV-infected participants, while 11 (26%) occurred in the HIV-uninfected controls group only. None of the genotypes observed was associated with HIV status. Genotype 1 which belongs to haplotype AA, comprising six inhibitory genes and a single activation gene was the most prevalent

genotype in both HIV-infected (34%) and HIV-uninfected (27%) groups. However, the Bx haplotype which comprises many genotypes each containing at least two activation genes was predominant in both HIV-infected (66%) and HIV-uninfected (73%) groups. There were no significant differences in frequencies of AA and Bx haplotypes between the HIV-infected and HIV-uninfected groups.

We compared frequencies of centromeric and telomeric haplotypes between the HIV-infected and HIV-uninfected groups to determine their possible association with HIV infection (Table 4). The frequency of centromeric haplotype 1 (Ce1) which lacks the *KIR2DL2*, but carries the *KIR2DL3* was significantly higher in the HIV-infected than the HIV-uninfected (53% v 38%, $P=0.02$) when compared to non-Ce1 haplotypes. Thus, individuals carrying the Ce1 haplotype were 1.82 times more likely to be HIV-infected than individuals carrying any other centromeric haplotype. In contrast, Ce3 which carries both the *KIR2DL2* and *KIR2DL3* allelic variants was associated with an HIV-uninfected status (OR=0.44, 95%CI: 0.17-0.90, $P=0.028$) as the haplotype was significantly over-represented in the HIV-uninfected group than HIV-infected group (14% v 7%, $P=0.028$) when compared to non-Ce haplotypes.

Discussion

The current study highlights a possible role of KIR gene content diversity in HIV infection. KIR are critical in the regulation of NK cell activation, hence potentially influence HIV acquisition through NK cell based innate anti HIV immune activity. The *KIR2DL2* gene is associated with an HIV-uninfected status evidenced by over-representation of *KIR2DL2* gene carriers, *KIR2DL2/KIR2DL2* homozygotes and the Ce3 haplotype carriers in the HIV-uninfected group compared to the HIV-infected group. Activating genes *KIR2DS1* and

KIR3DS1 were infrequent in the Zimbabwean study population. We observed 43 KIR genotype profiles, 40 previously reported and three unreported. However, we found no association between KIR genotypes and HIV status. Pair wise LD analysis of KIR genes reproduced the co-inheritance of *KIR2DL2* and *KIR2DS2* genes that has been previously reported (Moesta and Parham, 2012, Nakimuli et al., 2013). Patterns of KIR gene pair co-inheritance were mostly similar between the HIV-infected and HIV-uninfected groups.

The antimicrobial activity of NK cells is a function of balance between stimulation of activating and inhibitory KIRs on NK cells by their MHC ligands on target cells [Lanier 2005]. However, KIRs are not restricted to inhibition or activation roles as previously thought. Resistance to HIV infection largely correlates to the presence of activating KIR profiles such as the *KIR3DS1* and haplotype Bx (Bashirova, et al., 2011; Hong et al., 2013; Hens et al., 2016) because activating KIR profiles are expected to enhance NK cell function. Nevertheless, we found inhibitory gene, *KIR2DL2* more frequent among HIV uninfected controls than HIV-infected individuals suggesting a protective function against HIV infection. “Licensing” or “education” of NK cells by KIRs during NK cell maturation provides the most credible mechanism of *KIR2DL2* protection against HIV infection. “Licensing” allots functionality to NK cells that possess iKIRs which recognise self-HLA ligands during maturation to prevent uncontrolled immune activation [Hens et al., 2016]. Receptors of the KIR2DL motif KIR2DL1/2/3 are recognised by ligands of the HLA-C allotype. While KIR2DL1 binds to HLA-C2 specifically, KIR2DL2 and KIR2DL3 bind to HLA-C1 strongly, but also cross-react with HLA-C2 (Moesta and Parham, 2012). Thus, expression of KIR2DL2 and/or KIR2DL3 receptors on NK cells in presence of HLA-C1 on other nucleated immune cells during licensing lowers the threshold of NK cell activation by signals from virus infected cells. This facilitates a strong and quick immune response in the face of HIV infection (Hens et al., 2016). KIR2DL2-associated polymorphisms in the HIV

genomes from chronic HIV patients suggest another mechanism of anti-HIV activity where KIR2DL2 receptors on NK cells bind HIV peptides presented on HLA-C (Alter et al., 2011).

Our study findings are consistent with reports from earlier studies involving both vertical and horizontal exposure to HIV (Jennes et al., 2006; Paximadis et al., 2011; Jennes et al., 2013). *KIR2DL2/3* genotypes alone and/or in absence of their cognate HLA-C1 ligands were associated with protection against HIV acquisition among Senegalese HIV discordant couples (Jennes et al., 2013) and also among commercial sex workers in Abidjan Cote d'Ivoire (Jennes et al., 2006). Low NK cell activation threshold was a plausible explanation for the protective effect of the iKIRs *2DL2/3* in both studies. Increased function and frequency of KIR2DL1/2/3 positive NK cells in primary HIV infection (Korner et al., 2014) make a case for antiviral activity of iKIRs. It appears that iKIR/HLA-C mismatches are critical in prevention of both vertical (Hong et al., 2013) and horizontal transmission of HIV. However, our study demonstrates that the *KIR2DL2* gene may influence HIV acquisition although the role of HLA-C was not investigated. Whether presence of iKIRs may overcloud the role of HLA-C alone remains unclear.

Despite growing evidence of the influence of *KIR2DL2/3* genes in HIV acquisition, a study among South Africans dismisses association between KIR gene variation and HIV acquisition (Naranbhai et al., 2016). South Africa is geographically close to Zimbabwe hence the two populations are assumed to have similar KIR gene frequencies. However, the South African study involved HIV- negative females who were followed up until they seroconverted whereas our study involved both men and women with an already known HIV status. In contrast, the *KIR2DL2* gene increases risk of HIV acquisition while the *KIR2DL3* protects against HIV infection among Polish Caucasians (Zwolińska et al., 2016).

Differences in distribution of KIR genes between Africans and Caucasians possibly account for the contrast between our findings and those from the Polish study. Higher prevalence of

the *KIR3DS1* gene and other gene variants such as *CCR5* $\Delta 32$ deletion among Caucasians compared to Africans perhaps overshadow the antiviral effect of the *KIR2DL2* gene among Caucasians. The *CCR5* $\Delta 32$ deletion is perhaps the single most important genetic determinant of HIV acquisition, but it is rare among African populations with an allele frequency of 0.1% compared to up to 9.8% in Caucasians (Williamson et al., 2000). The distribution of several other genes implicated in HIV acquisition and disease course have been queried to reflect the HIV map among African and world populations (Skelton et al., 2014, Mhandire et al., 2014). Beyond HIV acquisition, presence of *KIR2DL2* gene is associated with slow HIV disease progression (Naranbhai et al., 2016). The antiviral properties of *KIR2DL2/3* are not restricted to HIV as presence of *KIR2DL2/3* genes is also associated with protection against human lymphotropic virus type 1 and hepatitis C virus (Seich Al Basatena et al., 2011; De Re et al., 2015). Paradoxically, *KIR2DL2* gene may also predispose to chronic HCV infection (Kusnierczyk et al., 2015).

We speculate that the antiviral influence of *KIR2DL2* genes may be pertinent to African populations. The distribution of KIR genes, genotypes and haplotypes is broadly similar, with minor variation among African populations (Yindom et al., 2010; Nakimuli et al., 2013). The *KIR3DS1* gene, which is largely implicated in resistance to HIV infection and slow HIV disease progression, is infrequent among Zimbabweans (17%) and African populations in general (Nakimuli et al., 2013). This infrequency of the *KIR3DS1* gene could limit its antiviral function among Africans. Perhaps, this facilitates function of iKIRs such as *KIR2DL2* in activation of NK cells in response to viral infection. However, we found *KIR2DL2* in strong LD with *KIR2DS2* in both HIV-infected (0.86) and HIV-uninfected groups (0.75). This finding is consistent with earlier reports (Moesta and Parham, 2012; De Re et al., 2015; Naranbhai et al., 2016). The two genes are in close proximity, thus tend to be inherited together. Whether the *KIR2DS2* gene influences NK cell activity independently or

through epistatic interaction with *KIR2DL2* remains unclear. There is evidence that the *KIR2DS2* can activate NK cells through an unknown mechanism (Moesta and Parham, 2012). The *KIR2DS2* gene was more frequent in the HIV-uninfected than the HIV-infected participants, but the difference was not statistically significant, perhaps downplaying the independent role of the *KIR2DS2* gene in HIV infection.

Diversity of KIR genotypes presents a challenge in determination of association between KIR genotypes and outcomes of disease. This study observed 43 KIR genotypes in 286 individuals, 40% of which occurred only once. Other populations have shown more genotypes (Chavan et al., 2016; Zwolińska et al., 2016). With growing literature and advances in genotyping technology, the list of KIR genotypes continues to grow as more novel genotypes are reported. Genotype 1 consisting of AA haplotype is the most prevalent genotype among African populations ranging from 12% to greater than 50% (Williams and Middleton, 2004; Yindom et al., 2010). The A haplotype is predominantly inhibitory, but contrary to expectation is linked to both protection and susceptibility to viral infection. No association was observed between KIR genotypes or KIR haplotypes with HIV status, until centromeric and telomeric haplotypes were considered separately.

Our findings require careful interpretation as the study has a few limitations. Firstly, the statistical power of the study may have been limited by the sample size. Determination of association between KIR genotypes and risk of HIV infection requires a large sample size due to the high diversity of KIR gene content and genotypes profiles. Secondly, association between *KIR2DL2* gene variants and the HIV-uninfected status was only observed before Bonferroni correction for multiple comparisons, hence further studies are required to substantiate our findings. However, it must be noted that Bonferroni correction is a very stringent test which may lead to rejection of pertinent findings. Thirdly, other host genetic and immunological factors that were not investigated in this study may have a background

effect on individuals' susceptibility to HIV infection. Such factors include variation in chemokine, chemokine receptor, cytokine and HLA encoding genes. Although the current study was limited to KIR genotyping, the findings strongly argue that KIR gene diversity may have a role in HIV acquisition. The study demonstrates the diversity of the KIR gene loci in a Zimbabwean adult population. To the authors' knowledge, this is the first study to report KIR gene, genotype and haplotype frequencies in this population. The *KIR2DL2* gene may be associated with protection against HIV acquisition. Inhibitory KIRs may have a critical role in viral infection among populations of African origin in whom the *KIR3DS1* gene is infrequent.

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Author Disclosure Statement

The authors declare that there are no conflicting financial interests.

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Abbreviations used

aKIR = activating KIR

HLA = human leukocyte antigen

iKIR = inhibitory KIR

KIR = killer cell immunoglobulin-like receptor

LD = linkage disequilibrium

NBSZ = National Blood Service Zimbabwe

NK = natural killer

PBMC = peripheral blood mononuclear cells

SSP-PCR = sequence specific primer polymerase chain reaction

TABLE 1. FREQUENCIES OF *KIR* GENES AND THEIR ASSOCIATION WITH HIV STATUS

Gene	HIV+ (n=189)	HIV- (n=97)	*O/Ratio	*95% CI	P-value
	Present	Present			
<i>KIR2DL1</i>	188 (0.99)	96 (0.99)	1.96	0.12-31.65	0.636
<i>KIR2DL2</i>	89 (0.47)	60 (0.62)	0.55	0.33-0.90	0.019
			^a 0.52	^a 0.30-0.88	^a 0.017
<i>2DL3/2DL3</i>	100 (0.53)	37 (0.38)	1		
<i>2DL2/2DL3</i>	22 (0.12)	9 (0.09)	1.28	0.56-2.91	0.544
<i>2DL2/2DL2</i>	67 (0.35)	51 (0.53)	0.49	0.30-0.81	0.006
			^a 0.46	0.27-0.79	0.005
<i>KIR2DL3</i>	167 (0.88)	88 (0.91)	0.78	0.34-1.75	0.342
<i>KIR2DL4</i>	188 (0.99))	97 (1.00)	N/A	N/A	N/A
<i>KIR2DL5</i>	107 (0.57)	58 (0.60)	0.87	0.53-1.44	0.44
<i>KIR2DS1</i>	35 (0.19)	21 (0.22)	0.82	0.48-1.51	0.521
<i>KIR2DS2</i>	76 (0.40)	46 (0.47)	0.75	0.46-1.22	0.244
<i>KIR2DS3</i>	39 (0.21)	27 (0.28)	0.67	0.38-1.18	0.165
<i>2DS3/2DS3</i>	24 (0.13)	18 (0.19)	1		
<i>2DS3/2DS5</i>	15 (0.08)	9 (0.09)	0.84	0.35-2.00	0.699
<i>2DS5/2DS5</i>	68 (0.36)	31 (0.32)	1.19	0.71-2.01	0.499
<i>KIR2DS5</i>	83 (0.44)	40 (0.41)	1.11	0.68-1.83	0.671
<i>KIR2DS4</i>	187 (0.99)	96 (0.99)	0.97	0.08-10.8	0.6
<i>2DS4f</i>	84/188 (0.45)	41/93 (0.44)	1.02	0.62-1.68	0.925
<i>2DS4f/v</i>	65/188 (0.35)	33 (0.35)	0.96	0.57-1.61	0.88
<i>2DS4v</i>	36/188 (0.19)	18/93 (0.19)	0.98	0.52-1.85	0.967
<i>KIR3DL1</i>	188 (0.99)	97 (1.00)	N/A	N/A	N/A
<i>3DL1/3DL1</i>	162 (0.86)	80 (0.82)	1.27	0.65-2.47	0.473
<i>3DL1/3DS1</i>	26 (0.14)	17 (0.18)	0.75	0.38-1.46	0.4
<i>3DS1/3DS1</i>	1 (0.005)	0	N/A	N/A	N/A
<i>KIR3DS1</i>	27 (0.14)	17 (0.18)	0.78	0.40-1.52	0.404
<i>KIR3DL2</i>	189 (1.00)	97 (1.00)	N/A	N/A	N/A
<i>KIR3DL3</i>	189 (1.00)	97 (1.00)	N/A	N/A	N/A
<i>KIR2DP1</i>	187 (0.99)	96 (0.99)	0.97	0.08-10.8	0.373

KIR: Killer Immunoglobulin-like receptor, O/Ratio: Odds Ratio, 95% CI: 95% confidence interval, *Univariate logistic regression, ^aadjusted for gender, age and other genes

HIV+: HIV-infected, HIV-: HIV-uninfected

TABLE 2. PAIRWISE LINKAGE DISEQUILIBRIUM BETWEEN KIR GENES IN THE HIV POSITIVE AND HIV NEGATIVE GROUPS

Genes	<i>KIR3DL1</i>	<i>KIR2DL1</i>	<i>KIR2DL3</i>	<i>KIR2DS4</i>	<i>KIR2DL2</i>	<i>KIR2DL5</i>	<i>KIR3DS1</i>	<i>KIR2DS1</i>	<i>KIR2DS2</i>	<i>KIR2DS3</i>	<i>KIR2DS5</i>	<i>KIR2DP1</i>
<i>KIR2DL1</i>	-0.005		0.325	-0.011	-0.083	0.124	0.059	0.052	-0.115	0.066	0.083	1
	NS		<0.005	NS	NS	NS	NS	NS	NS	NS	NS	<0.001
<i>KIR2DL3</i>	0.200	0.200		-0.032	0.262	-0.206	-0.042	-0.093	-0.344	-0.286	-0.325	0.319
	<0.01	<0.01		NS	<0.025	NS	NS	NS	<0.001	<0.01	<0.005	<0.005
<i>KIR2DS4</i>	0.705	-0.007	0.123		-0.081	-0.094	-0.221	-0.197	-0.113	-0.167	-0.128	-0.010
	<0.001	NS	NS		NS	NS	<0.055	NS	NS	NS	NS	NS
<i>KIR2DL2</i>	-0.076	-0.076	-0.381	-0.108		0.497	0.204	0.275	0.755	0.367	0.316	-0.081
	NS	NS	<0.001	NS		<0.001	<0.058	<0.01	<0.001	<0.001	<0.005	NS
<i>KIR2DL5</i>	-0.064	-0.064	-0.318	-0.090	0.559		0.176	0.244	0.445	0.523	0.695	0.121
	NS	NS	<0.001	NS	<0.001		NS	<0.025	<0.001	<0.001	<0.001	NS
<i>KIR3DS1</i>	-0.178	0.029	0.053	-0.253	-0.023	0.297		0.685	0.224	0.204	0.185	0.047
	<0.025	NS	NS	<0.001	NS	<0.001		<0.001	<0.05	NS	NS	NS
<i>KIR2DS1</i>	-0.153	0.034	0.002	-0.217	0.093	0.336	0.545		0.213	0.123	0.285	0.053
	<0.05	NS	NS	<0.005	NS	<0.001	<0.001		<0.05	NS	<0.01	NS
<i>KIR2DS2</i>	-0.089	-0.089	-0.409	-0.126	0.860	0.567	-0.024	0.110		0.483	0.214	-0.109
	NS	NS	<0.001	NS	<0.001	<0.001	NS	NS		<0.001	<0.05	NS
<i>KIR2DS3</i>	-0.145	0.036	-0.353	-0.206	0.447	0.440	0.060	-0.067	0.531		-0.088	0.063
	<0.05	NS	<0.001	<0.005	<0.001	<0.001	NS	NS	<0.001		NS	NS
<i>KIR2DS5</i>	-0.082	-0.082	-0.311	-0.117	0.354	0.775	0.370	0.430	0.320	-0.068		0.083
	NS	NS	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001	NS		NS
<i>KIR2DP1</i>	-0.00	0.705	0.285	-0.010	-0.108	-0.090	0.041	-0.084	-0.126	0.051	-0.117	
	NS	<0.001	<0.001	NS	NS	NS	NS	NS	NS	NS	NS	

Pairwise LD plot for HIV positive group (unshaded) and for HIV negative group (shaded) calculated using Cramer's V statistic.

Significance of LD at 0.05 confidence interval using Chi-square test without Yates Correction; NS: Non significant P-value

TABLE 3. FREQUENCIES OF KIR GENOTYPES IN HIV POSITIVE AND HIV NEGATIVE GROUPS

HID	GID	3DL1	2DL1	2DL2	2DL3	2DL5	2DS4	3DS1	2DS1	2DS2	2DS3	2DS5	2DP1	HIV+ (n=189)	HIV- (n=97)
AA	1													65(0.34)	26(0.27)
Bx	5													17 (0.089)	12 (0.12)
Bx	21													14 (0.074)	7 (0.07)
Bx	32													13 (0.07)	6 (0.06)
Bx	2													10 (0.053)	2 (0.02)
Bx	4													7 (0.037)	5 (0.05)
Bx	9													7 (0.037)	3 (0.03)
Bx	112													7 (0.037)	3 (0.03)
Bx	19													6 (0.032)	3 (0.03)
Bx	20													5 (0.026)	7 (0.07)
Bx	228													5 (0.026)	1 (0.01)
Bx	3													4 (0.021)	2 (0.02)
Bx	30													2 (0.011)	2 (0.02)
Bx	6													1 (0.005)	2 (0.02)
Bx	91													1 (0.005)	1 (0.01)
Bx	93													1 (0.005)	1 (0.01)
Bx	401													1 (0.005)	1 (0.01)
Bx	15													3 (0.016)	0
Bx	71													3 (0.016)	0
Bx	14													2 (0.011)	0
Bx	17													2 (0.011)	0
Bx	18													2 (0.011)	0
Bx	22													2 (0.011)	0
Bx	35													2 (0.011)	0
Bx	328													0	2 (0.021)
Bx	N													0	2 (0.021)
Bx	N													1 (0.005)	0
Bx	N													1 (0.005)	0

HID: haplotype identification, GID: genotype identification, N: novel genotype; HIV+: HIV positive, HIV-: HIV negative

Shaded box: Gene present, clear box: Gene absent

Only genotypes with frequencies >1% and unique genotypes are shown.

TABLE 4. ASSOCIATION OF KIR HAPLOTYPES WITH HIV STATUS

Haplotype	<i>2DS2</i>	<i>2DL2</i>	<i>2DL3</i>	<i>2DL1</i>	HIV+	HIV-	#Odds Ratio	#95% CI	#p- value
Centromeric									
Ce2					55 (0.29)	37 (0.38)	1		
Ce1					100 (0.53)	37 (0.38)	1.82	1.10-3.00	0.019
Ce3					13 (0.07)	14 (0.14)	0.44	0.17-0.90	0.028
Ce5					1 (0.005)	0	NA	NA	NA
Ce6					20 (0.11)	8 (0.08)	1.39	0.52-3.5	0.44
Ce7					1 (0.005)	1 (0.01)	0.76	0.08-9.2	0.76
Ce/Te	<i>2DL5</i>	<i>2DS3</i>	<i>2DS5</i>						
Ce/Te1					15 (0.08)	9 (0.09)	1		
Ce/Te2					68 (0.36)	31 (0.32)	1.19	0.71-2.01	0.499
Ce/Te3					24 (0.13)	18 (0.19)	0.63	0.33-1.24	0.187
Ce/Te6					83 (0.44)	39 (0.40)	1.14	0.69-1.87	0.606
Telomeric	<i>3DS1</i>	<i>2DS1</i>	<i>3DL1</i>	<i>2DS4</i>					
Te2					17 (0.09)	13 (0.13)	1		
Te1					147 (0.77)	73 (0.75)	1.1	0.62-1.97	0.707
Te3					16 (0.08)	7 (0.07)	1.1	0.47-2.99	0.712
Te4					8 (0.04)	3 (0.03)	1.38	0.35-5.3	0.636
Te6					1 (0.005)	0	NA	NA	0.477
Te9					1 (0.005)	1 (0.01)	NA	NA	0.99
Te10					1 (0.005)	1 (0.01)	NA	NA	0.99

HIV+: HIV positive, HIV-: HIV negative, Ce: Centromeric, Te: Telomeric

Shaded box: Gene present, clear box: Gene absent

Chi-squared test (unadjusted p-value)