JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

*HLA-C**18:01 and *KIR2DL2+C1* genetic variants are associated with low viral load in cART naïve HIV-infected adult Zimbabweans

Kudakwashe Mhandire[1], Mqondisi Tshabalala[2], Lynn Sodai Zijenah[2], Louis-Marie Yindom[3], Tommy Mlambo[2], Doreen Zvipo Mhandire[4], Cuthbert Musarurwa[1], Kerina Duri[2], Hilda Tendisa Matarira[1], Collet Dandara[4], Sarah L. Rowland-Jones[3], Babill Stray-Pedersen[5]

[1] Department of Chemical Pathology, University of Zimbabwe College of Health Sciences, Harare, Zimbabwe
[2] Department of Immunology, University of Zimbabwe College of Health Sciences, Harare, Zimbabwe
[3] Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom
[4] Division of Human Genetics, Department of Clinical Laboratory Sciences, University of Cape Town, Cape Town, South Africa
[5] Institute of Clinical Medicine, University of Oslo and Womens' Clinic, Rikshospitalet, University Hospital, Oslo, Norway

Abstract

Introduction: Polymorphisms in killer cell immunoglobulin-like receptor (*KIR*) and human leukocyte antigen (*HLA*) gene families are implicated in differential outcomes of HIV infection. However, research findings on the influence of *KIR* and *HLA-C* polymorphism on HIV disease progression remain inconclusive. We thus investigated the association of *KIR* and *HLA-C* gene polymorphisms with plasma HIV load (VL) and CD4+ T lymphocyte (CD4) count in 183 chronically HIV-infected, combination antiretroviral therapy (cART) naïve Zimbabweans of Bantu origin.

Methodology: The presence or absence of 15 *KIR* genes were determined using sequence specific primer polymerase chain reaction while *HLA-C* typing was performed using chain termination DNA sequencing. Plasma VL was determined using the Cavidi Exavir viral load version 3 assay while CD4+ T lymphocytes were enumerated using flow cytometry. VLs and CD4 counts were compared between gene/genotype carriers and non-carriers using Mann-Whitney ranksum test.

Results: *HLA-C**18:01 allele carriers had a significantly lower median $\log_{10}$ VL (2.87copies/mL [IQR;2.3-3.2]) than the non-C*18:01 carriers (3.33copies/mL [IQR; 2.74-3.9]), p = 0.018. Further, median $\log_{10}$ VL was significantly lower in *KIR2DL2+C1* carriers (2.745 [IQR; 2.590-2.745]) than non-*KIR2DL2+C1* carriers (3.4 [IQR; 2.746-3.412]), p = 0.041. Comparison of CD4 + T lymphocyte counts between C*08:02 allele carriers and non-C*08:02 carriers showed a significantly higher median CD4 count in C*08:02 carriers (548cells/µL [IQR;410-684]) than in non-carriers (428cells/µL [IQR;388-537]), p = 0.034.

Conclusion: We conclude that the *HLA-C**18:01 and *KIR2DL2+C1* genetic variants are associated with low VL while the C*08:02 is associated with high CD4+ T lymphocyte count among cART naïve Zimbabwean adults with chronic HIV infection.

Key words: HLA; KIR; natural killer cells; HIV; viral load; Zimbabweans.

J Infect Dev Ctries 2018; 12(12):1105-1111. doi:10.3855/jidc.10848

(Received 10 September 2018 – Accepted 20 November 2018)

Introduction

Host genetic polymorphisms are strongly linked with differential outcomes of HIV infection, disease progression and response to treatment [1,2], but research findings remain inconclusive. Gene association studies of markers of HIV disease progression (viral load [VL] and CD4+ T lymphocyte count [CD4 count]) show that polymorphisms in the human leukocyte antigen (*HLA*) gene family are among the most potent determinants of HIV disease progression [3]. Carriage of *HLA-B**57, B*58:01, B*27 and B*81 variants is associated with slow HIV disease progression and low VL while in contrast B*35 and B*53 variants are associated with accelerated disease progression and high VL [3-5]. HLA class I molecules, expressed on nucleated cells are involved in the presentation of HLA bound HIV derived peptides to cytotoxic T-lymphocytes (CTLs) [6]. This antigen presentation stimulates cytotoxic activity of the CD8+ T lymphocytes which consequently destroys the HIV infected cells. In addition, HLA also act as ligands for killer cell immunoglobulin-like receptors (KIR) that are expressed on natural killer (NK) cells [7].

KIR exist as activating (aKIR) or inhibitory (iKIR) receptors depending on whether they stimulate or dampen NK cell activity [8]. The interaction of HLA ligands with their cognate KIRs inhibit or stimulate NK cell activity accordingly. As such, polymorphisms in *KIR* gene content have been implicated in control of HIV disease progression [9]. The *KIR3DS1* gene is widely reported to associate with low viraemia among Caucasian populations [10,11]. Studies have also linked *KIR3DL1, KIR2DL2, KIR2DL3,* and *KIR2DS4* genes with differential outcomes of HIV infection [12-15]. Moreover, the epistatic interaction of *HLA* and *KIR* variants is reported to have a stronger effect on HIV infection outcomes than individual *HLA* and *KIR* variants, perhaps due to the combined effect of *HLA* and *KIR* variants co-carriage on NK cell education and function. For example, the compound genotype of *KIR3DS1* and *HLA*-Bw4-80I (*KIR3DS1-Bw4 80I*) variants is associated with resistance to HIV, slow disease progression and protection against opportunistic infection [16,17].

While *HLA-B* variants are well reported determinants of HIV disease progression, *HLA-C* variants have largely been overlooked. Where associations of *HLA-C* variants with HIV infection outcomes are reported, linkage disequilibrium of the *HLA-C* alleles with protective or detrimental *HLA-B* variants is often cited as the plausible mechanism of action [18,19]. However, with the discovery of HLA-KIR interaction *HLA-C* is emerging as an important host genetic determinant of HIV infection outcomes [9,20]. HLA-C molecules form arguably the most important KIR ligands because inhibitory (i) receptors of the KIR2DL motif (KIR2DL1/2/3) are recognized by ligands of the HLA-C allotype [7]. KIR2DL1 binds to allotypes with amino acid lysine at position 80 of the HLA-C heavy chain called HLA-C2. KIR2DL2 and KIR2DL3 bind to allotypes with asparagine in place of lysine called HLA-C1. All human nucleated cells express either the C1 or C2 allotype in homozygotes, or both in heterozygous state [21]. Further, a Genome Wide Association Study (GWAS) completed in 2007 reported that a SNP located in the 5′ region of the *HLA-C* gene, 35 kb away from transcription initiation (rs9264942) could independently explain 6.5% variation in set point VL in the study population [22]. High HLA-C expression on T lymphocytes is inversely proportional to VL suggesting a direct role in the control of viral replication [23].

There is a dearth of studies on the role of *KIR, HLA-C* and *KIR+HLA-C* genetic polymorphisms in HIV disease progression among African populations. We have previously reported an overrepresentation of *KIR2DL2* gene containing profiles in HIV-uninfected adult group compared to the HIV infected group suggesting the possible role of KIR2DL2 in HIV infection control [14]. We hypothesise that *KIR2DL+HLA C1/C2* profiles are potent determinants of VL and CD4+ T lymphocytes among Africans in whom the well documented favourable *KIR3DS1* gene is infrequent. The current study therefore, investigates the association of *KIR* gene content, *HLA-C* alleles and *KIR+HLA-C* compound genotypes with traditional markers of HIV disease progression in combined antiretroviral therapy (cART) naïve adults with chronic HIV infection seeking care at a central hospital in Harare, Zimbabwe.

Methodology

Study participants and specimen collection

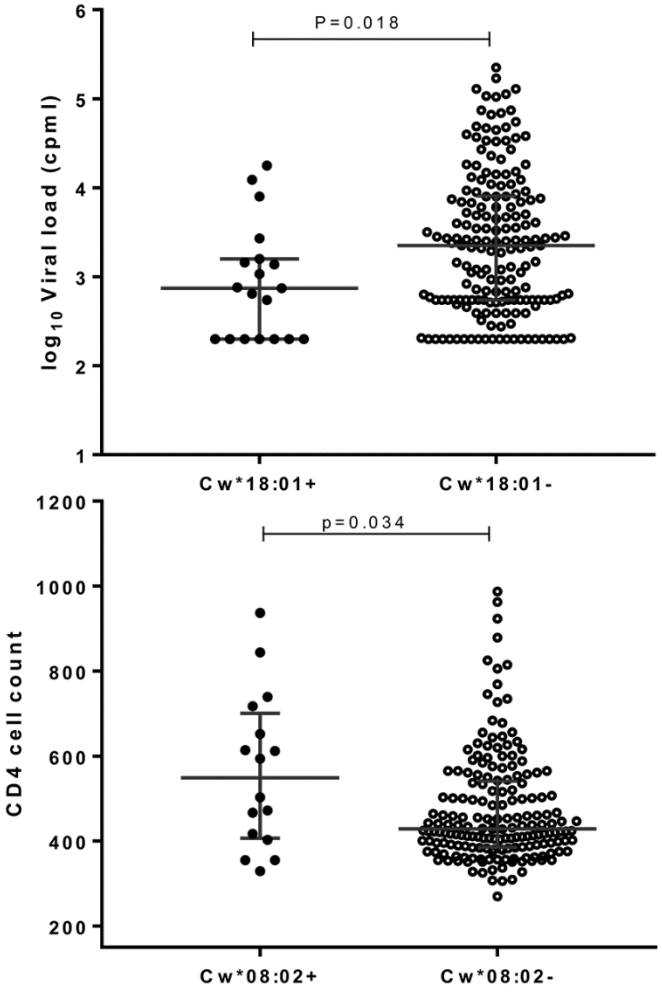
We conducted a cross sectional study which was nested in the "Immunological and Virological Investigations of HIV-infected individuals with CD4 counts above 350 cells/μL (IVIHIV)" prospective cohort study as described elsewhere [24]. Briefly, the IVIHIV study was conducted at the Parirenyatwa Group of Hospitals Opportunistic Infections Clinic, in Harare, Zimbabwe. HIV-infected, antiretroviral therapy naïve Zimbabweans (of Bantu origin) aged ≥ 18 years were enrolled after written informed consent. In the current study, DNA was isolated for *KIR* and *HLA-C* genotyping from the archived PBMCs of 183 HIV-infected individuals. The median time since the diagnosis of HIV infection in the cohort was 6.3 years, hence the patients were considered as chronically HIV-infected. The study was approved by the Medical Research Council of Zimbabwe (MRCZ/A/2016).

KIR and HLA-C typing

KIR typing was carried at the University of Oxford, United Kingdom using sequence specific primer polymerase chain reaction according to a method that we fully described elsewhere [14]. Briefly, 2 pairs of sequence specific primers were used to amplify each of one pseudogene (*KIR2DP1*) and 14 functional KIR genes: 6 encoding activating receptors (*KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1*), 7 encoding inhibitory receptors (*KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL5, KIR3DL1, KIR3DL2)* and 1 encoding a receptor with both inhibitory and activation activities (*KIR2DL4*). *HLA-C* typing was done by Sanger's chain termination sequencing using the BigDye Terminator v3.1 Cycle Sequencing kit (ThermoFisher Scientific, Waltham, USA) at the

University of Oxford, Oxford, United Kingdom. Briefly, fragments of exons 2 and 3 were simultaneously amplified in a multiplex PCR using an in-house protocol. Primers used for amplification are protected under intellectual property agreement and have not yet been published. *HLA-C* traces were analyzed to determine *HLA-C* alleles using the HLASeq software (JSI Medical Systems, Ettenheim, Germany). The software was accessed through a generous donation from JSI Medical Systems. Electrophoregrams of *HLA-C* traces were manually inspected to resolve any discrepant nucleotide calls. *HLA-C* alleles were assigned to either HLA-C1 or HLA-C2 KIR ligands based on the amino acid residues of the HLA-C molecules encoded by the alleles, according to a previously described method [25].

Figure 1. Comparison of viral load and CD4+ T lymphocyte count between *HLA-C* allele carriers and non-carriers using the Mann-Whitney rank sum test. Panel A: Carriage of the C*18:01 allele was associated with low viral load when compared to non-carriage of the allele. Panel B: Carriage of the C*08:02 allele was associated with high CD4+ T lymphocyte count when compared to non-carriage of the allele.



Measurement of CD4 counts and plasma viral load

CD4+ T lymphocytes were enumerated in EDTA anti coagulated whole blood within 6 hours of blood collection in the IVIHIV study using the Partec platform (Sysmex, Görlitz, Germany) as we described elsewhere [24]. Plasma HIV load was also estimated in the IVIHIV study from the archived plasma using the Cavidi Exavir viral load version 3 assay (Cavidi AB, Uppsala Science Park, Sweden) following the manufacturer's instruction. The VL assay has a lower detection limit of 200 copies/mL.

Statistical analysis

Statistical analyses were done using Stata version 13.1 (StataCorp, Texas, USA) and GraphPad Prism 7 (GraphPad Software Inc, California, USA). Univariate linear regression was used to detect any association between $\log_{10}$ VL/CD4+ T-lymphocyte count and; *KIR, HLA-C* and *KIR+HLA-C* gene variants. Where univariate linear regression analysis showed a significant association, $\log_{10}$ VL and CD4+ T-lymphocyte counts were then compared between gene variant carriers and non-carriers using Mann-Whitney test. A value of $P < 0.05$ was considered statistically significant. P-values were adjusted for multivariate comparisons using Bonferroni correction for multivariate comparisons (BCMC) method. Gene variants showing significant association with VL in univariate analysis and gender were included in a multivariate regression analysis.

Results

HLA-C and KIR gene variants and their association with viral load and CD4 counts

The demographic and clinical characteristics of the study participants have been reported elsewhere [24]. A total of 36 *HLA-C* alleles were present in the study population, 18/36 (50%) with frequencies >1%. Frequencies of *KIR* genes and genotypes of the participants are described elsewhere [14]. Of the 18 *HLA-C* alleles detected in >1% of the study population, carriage of the C*18:01, an allele that is restricted to Africans, was associated with low VL compared to non-C*18:01 carriage (Supplementary Table 1). When we compared VLs between C*18:01 allele carriers and non-C*18:01 carriers using the Mann-Whitney rank sum test, carriers had a significantly lower median $\log_{10}$ VL (2.87copies/mL [IQR;2.3-3.2]) than the non-carriers (3.33copies/mL [IQR; 2.74-3.9]), p = 0.018 (Figure 1) suggesting a favourable role against HIV disease progression. However, the C*18:01 allele was not associated with CD4+ T-lymphocyte count.

Simple linear regression analysis of *HLA-C* alleles with CD4+ T-lymphocyte count showed an association of the C*08:02 allele carriage with a high CD4+ T lymphocyte count compared to non-C*08:02 carriage (Supplementary Table 2). Comparison of CD4 + T lymphocyte counts between C*08:02 allele carriers and non-C*08:02 carriers using the Mann-Whitney rank sum test (Figure 1) showed a significantly higher median CD4+ T lymphocyte count in C*08:02 carriers (548cells/μL [IQR;410-684]) than in non-carriers (428cells/μL [IQR;388-537]), p = 0.034. The associations C*18:01 with VL and C*08:02 with CD4+ T-lymphocyte both could not withstand strict BCMC (p = 0.306 and p = 0.612, respectively). None of the individual *KIR* genes were significantly associated with either VL or CD4 + T lymphocyte count.

KIR+HLA-C genotypes and their association with VL and CD4+ T lymphocyte count

We also carried out simple univariate linear regression analysis of *KIR+HLA*-C1/C2 compound genotypes to determine the possible influence of the epistatic interaction of *KIR* genes and *HLA*-C1/C2 allotypes on VL and CD4+ T-lymphocyte count. The *KIR2DL2+C1* genotype was weakly linked with a favourable HIV outcome as carriage of the genotype was associated with lower VL compared to non-carriage. Using the Mann-Whitney rank sum test, median $\log_{10}$ VL was significantly lower in *KIR2DL2+C1* carriers (2.745 [IQR; 2.590-2.745]) than non-*KIR2DL2+C1* carriers (3.4 [IQR; 2.746-3.412]), p = 0.041. The association could not withstand BCMC (p = 0.720). None of the other *KIR+HLA-C* genotypes was significantly associated with either VL or CD4+ T lymphocyte count.

Finally, to account for possible confounding effect of variables, we carried a stepwise multivariate regression analysis of VL against (1) genetic factors that were significantly associated with VL in the univariate analyses (HLA-C*18:01 and KIR2DL2+C1), (2) C*08:02 due to its association with CD4+ T lymphocyte count and (3) gender due to the high representation of females in the study population (Table 1). The C*18:01 allele (p = 0.003) and *KIR2DL2+C1* compound genotype (p = 0.027) were significant determinants of VL under the model.

Discussion

The *HLA-C*18:01 allele, as previously reported [18,19,26,27] was associated with a favourable HIV infection control as C*18:01 carriers had lower VL compared to non-carriers. The favourable effect of the C*18 variant is largely attributed to its co-existence with the well documented B*57 as the two variants are in strong LD [19]. *HLA-C*18 is also in strong LD with the B*81:01, an allele whose carriers also exhibited lower VL compared to non-carriers among South African blacks [28]. All HIV controllers in a study among Brazilians who were C*18 carriers also carried the B*57 variant [27] showing the strength of the co-existence of the two alleles in HIV control. The protein products of the B*57 and B*81:01 variants effectively present HIV Gag epitopes to CTLs hence render effective antiviral activity in carriers of the alleles [29,30].

A study among Zambians with chronic untreated HIV infection reported that the association between B*57:03 and low VL was strong in the presence of C*18 only, but was entirely lost in its absence [26]. This finding suggests an independent effect of C*18 on HIV viraemia. Moreover, in the same study, VL was higher in B*57:03 carrying individuals who lacked the C*18 variant. In a study among sero-discordant Zambian adults, Tang et al., concluded that C*18 is a major predictor of low VL since B*57 and B*81 variants fail to influence VL in the absence of C*18 [18]. However, both B*57-C*18 and B*81-C*18 haplotypes were associated with low VL, thus making a strong case for the favourable effect of C*18 variant. It is important to further investigate the contribution of Cw*18:01 in HIV infection control as the allele is restricted to people of African origin and may have an important yet neglected role in HIV control in the population.

Table 1. Stepwise multivariate regression analysis of determinants of plasma viral load.

Variable	Coefficient	Standard error	p-value
*HLA-C*18:01	-0.599	0.199	**0.003**
*KIR2DL2+HLA-C*1	-0.286	0.140	**0.043**
*HLA-C*08:02	-0.147	0.215	0.494
Male gender	0.119	0.138	0.39
Removing variables with p > 0.05			
*HLA-C*18:01	-0.598	0.197	**0.003**
*KIR2DL2+HLA-C*1	-0.301	0.134	**0.027**

Viral loads were log transformed to fit the regression model, p < 0.05 were considered statistically significant.

The C*18:01 allele did not have an effect on CD4+ T lymphocyte count. Instead, carriage of the C*08:02 allele was associated with higher CD4+ T lymphocyte count when compared to non-carriage of the allele suggesting a favourable role. Interestingly, a study among Zimbabwean adolescents reported a significant enrichment of the C*08:02 allele among the long term survivor group compared to an HIV-uninfected control group [31]. Thus carriage of the C*08:02 allele was associated with longevity in the HIV infected adolescents perhaps due to the allele conferring a favourable immunological profile. Further, a study among African American adolescents reported a significant overrepresentation of the Cw*08 variant among HIV controllers (VL < 1000 copies/mL and CD4+ T lymphocyte count >450 cells/μL) compared to non-controllers (VL>16000 copies/mL and CD4+ T lymphocyte count < 450 cells/μL) and intermediates (1000 ≤ VL copies/mL ≤ 16000, regardless of CD4+ T lymphocyte count, VL < 1000 and CD4+ T lymphocyte count < 450cells/μL, VL>16000 copies/mL and CD4+ T lymphocyte count>450cells/μL) [19]. Our study among these therefore supports a case of Cw*08 allele influencing HIV disease course in a favourable way. Like most *HLA-C* alleles, the effect of the Cw*08 variant can be explained by its strong LD with an *HLA-B* variant, B*14 which is associated with favorable HIV disease control [32]. In the African American adolescents study [19], the Cw*08 and B*14 variants could only account for CD4+ T lymphocytes count when they occurred together with neither showing any significant association with CD4+ T lymphocyte count on its own.

It is noteworthy that the study showed associations of *HLA-C* variants with either VL (C*18:01) or CD4+ T lymphocyte counts (C*08:02) independently and not both. Both measures are used as independent predictors of HIV infection outcomes, VL indicating viralogical activity and CD4+ T lynphocyte count immunological status. Whilst an inverse relationship is expected between the two measures, discrepancies have been reported [19] hence VL and CD4+ T lymphocyte counts may be utilised independently. Also, CD4+ T lymphocyte absolute counts may be harder to interpret in non-Caucasian populations where normal values may vary from Western populations, which is why some groups use CD4%.

KIR2DL1+C1 compound genotype is associated with low viral load

Our study also demonstrates a significant association between the *KIR2DL2+C1* genotype and low VL. While neither *KIR2DL2* gene nor the C1 allotype were independently associated with VL or CD4+ T lymphocyte counts, *KIR2DL2+C1* carriers experienced significantly lower VL than non-carriers therefore supporting the importance of the epistatic interaction between KIR2DL2 and C1 in HIV control. The role of the *KIR2DL2+C1* compound genotype in the control of HIV viraemia can be explained by the low NK cell activation threshold programmed during NK cell "education/licensing" [11].

''Education'' apportions functionality to NK cells that possess iKIRs, which recognize self-HLA ligands during maturation to prevent uncontrolled immune activation [11]. Thus, the threshold of NK cell activation following stimulation from virus-infected cells is lowered by expression of KIR2DL2 receptors on NK cells in the presence of HLA-C1 on other nucleated cells during "licensing". This facilitates an enhanced immune response following HIV infection. Lin et al., have provided an alternative explanation in a study of ART-naive Japanese patients who like our study participants also had chronic HIV infection [33]. They showed that KIR2DL2+ NK cells suppress viral replication in C*12:03+ and C*14:03+ (both C1) cells to a greater extent than did KIR2DL2- NK cells in vitro. This is because binding of HIV-derived peptide to the C1 molecules reduces expression of the peptide-HLA complex on the HIV-infected cells hence activating KIR2DL2+ NK cells by reducing inhibitory KIR2DL2 ligand expression and not KIR2DL2 affinity for C1 as is usually expected.

An opposing opinion is that the KIR2DL2 receptor is capable of identifying and subsequently binding to HIV-derived peptides presented on C1 molecules from HIV-infected cells [34]. This binding stimulates the inhibitory pathway that will in turn dampen NK cell activation. KIR2DL2 and KIR2DL2+C1 induced HIV mutations that enhance binding of HIV peptide-HLA complex to KIR2DL2 favor the inhibition of KIR2DL2+ NK cells and the associated antiviral activities [35]. The mechanism of immune action in *KIR2DL2+C1* carriage therefore remains unclear. Perhaps, the low threshold of activation set during KIR2DL2+ NK cell "education" in presence of C1+ host cell allows for prompt NK cell activation following peptide-HLA complex binding to KIR2DL2.

Our study adds to the growing knowledge of the possible role of *KIR* and *HLA-C* genetic polymorphisms in HIV infection outcomes. However, the study had a few limitations, chiefly the cross-sectional design. While cross-sectional studies are a useful assessment for association of host genetic factors with markers of

disease progression, the ideal approach is the follow-up of recently sero-converted individuals, taking note of changes in virological and immunological profiles of the participants over time. However, longitudinal studies involve high costs hence are limited in resource limited settings such as Africa. Other host genetic, virological and immunological factors that were not investigated in this study may have confounding effects on our findings. Such factors include HIV genetic diversity and variation in chemokine, chemokine receptors, cytokines, and other HLA encoding genes, among others. A model accounting for all factors would accurately address the potential discrepancies.

Conclusions

We conclude that the C*18:01 and C*08:02 alleles are associated with low VL and high CD4+ T lymphocyte count respectively in cART naïve adult Zimbabweans with chronic HIV-infection. Further, the *KIR2DL2+HLA-C1* compound genotype is associated with low VL in the same population. Thus, carriage of the C*18:01, C*08:02 and *KIR2DL2+C1* variants may have a favourable effect against HIV disease progression in the study population. Further work is required to ascertain the role of *KIR+HLA-C* polymorphisms in HIV infection outcomes.

Acknowledgements

The authors thank the Letten Foundation Research House, Norway; HIV Research Trust, United Kingdom; and the European and Developing Countries Clinical Trials Partnership for funding. The authors are also indebted to the Parirenyatwa Groups of Hospital, University of Zimbabwe College of Health Sciences, and Professor Sarah Rowland-Jones' laboratory at the Nuffield Department of Medicine, University of Oxford for facilitating the research activities. None of this would have been possible without the voluntary participation of the IVIHIV study participants.

References

1. Casado C, Colombo S, Rauch A, Martinez R, Gunthard HF, Garcia S, Rodríguez C, DelRomero J, Telenti A, López-Galíndez C (2010) Host and viral genetic correlates of clinical definitions of HIV-1 disease progression. PLoS ONE 5: e11079.
2. Naranbhai V, Carrington M (2017) Host genetic variation and HIV disease: from mapping to mechanism. Immunogenetics 69: 489-498.
3. Martin MP, Carrington M (2013) Immunogenetics of HIV disease. Immunol Rev 254: 245-264.
4. Carrington M, Nelson GW, Martin MP, Kissner T, Vlahov D, Goedert JJ, Kaslow R, Buchbinder S, Hoots K, O'Brien SJ (1999) HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science 283: 1748-1752.
5. Gao X, Bashirova A, Iversen AK, Phair J, Goedert JJ, Buchbinder S, Hoots K, Vlahov D, Altfeld M, O'Brien SJ, Carrington M (2005) AIDS restriction HLA allotypes target distinct intervals of HIV-1 pathogenesis. Nat Med 11: 1290.
6. Shiina T, Hosomichi K, Inoko H, Kulski JK (2009) The HLA genomic loci map: expression, interaction, diversity and disease. J Hum Genet 54: 15-39.
7. Moesta AK, Parham P. Diverse functionality among human NK cell receptors for the C1 epitope of HLA-C: KIR2DS2, KIR2DL2, and KIR2DL3 (2012) Front Immunol 3: 336.
8. Lanier LL (2005) NK cell recognition. Annu Rev Immunol 23: 225-274.
9. Carrington M, Martin MP, van Bergen J (2008) KIR-HLA intercourse in HIV disease. Trends Microbiol 16: 620-627.
10. Zipperlen K, Gallant M, Stapleton S, Heath J, Barrett L, Grant M (2015) Protective genotypes in HIV infection reflect superior function of KIR3DS1+ over KIR3DL1+ CD8+ T cells. Immunol Cell Biol 93: 67-76.
11. Hens J, Jennes W, Kestens L (2016) The role of NK cells in HIV-1 protection: autologous, allogeneic or both? AIDS Res Ther 13: 15.
12. Hong HA, Paximadis M, Gray GE, Kuhn L, Tiemessen CT (2013) KIR2DS4 allelic variants: differential effects on in utero and intrapartum HIV-1 mother-to-child transmission. Clin Immunol 149: 498-508.
13. Jiang Y, Chen O, Cui C, Zhao B, Han X, Zhang Z, Liu J, Xu J, Hu Q, Liao C, Shang H (2013) KIR3DS1/L1 and HLA-Bw4-80I are associated with HIV disease progression among HIV typical progressors and long-term nonprogressors. BMC Infect Dis 13: 405.
14. Mhandire K, Zijenah LS, Yindom LM, Duri K, Mlambo T, Tshabalala M, MazengeraLR, Mhandire DZ, Musarurwa C, Dandara C, Rowland-Jones S, Matarira HT, Stray-Pedersen B (2016) KIR gene content diversity in a Zimbabwean population: Does KIR2DL2 have a role in protection against human immunodeficiency virus infection? OMICS 20: 727-735.
15. Naranbhai V, de Assis Rosa D, Werner L, Moodley R, Hong H, Kharsany A, Mlisana K, Sibeko S, Garrett N, Chopera D, Carr WH, Abdool Karim Q, Hill AV, Abdool Karim SS, Altfeld M, Gray CM, Ndung'u T (2016) Killer-cell immunoglobulin-like receptor (KIR) gene profiles modify HIV disease course, not HIV acquisition in South African women. BMC Infect Dis 16: 27.
16. Martin MP, Gao X, Lee J-H, Nelson GW, Detels R, Goedert JJ, Buchbinder S, Hoots K, Vlahov D, Trowsdale J, Wilson M, O'Brien SJ, Carrington M (2002) Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nature Genet 31: 429-434.
17. Qi Y, Martin MP, Gao X, Jacobson L, Goedert JJ, Buchbinder S, Kirk GD, O'Brien SJ, Trowsdale J, Carrington M (2006) KIR/HLA pleiotropism: protection against both HIV and opportunistic infections. PLoS Pathog 2: e79.
18. Tang J, Shao W, Yoo YJ, Brill I, Mulenga J, Allen S, Hunter E, Kaslow RA (2008) Human leukocyte antigen class I genotypes in relation to heterosexual HIV type 1 transmission within discordant couples. J Immunol (Baltimore, Md : 1950) 181: 2626-2635.
19. Lazaryan A, Song W, Lobashevsky E, Tang J, Shrestha S, Zhang K, McNicholl JM, Gardner LI, Wilson CM, Klein RS, Rompalo A, Mayer K, Sobel J, Kaslow RA, HIVEpidemiology Research Study Group, Reaching for Excellence in Adolescent Care and Health Study Group (2011)

The influence of HLA class I alleles and their population frequencies on human immunodeficiency virus type 1 control among African Americans. Hum Immunol 72: 312.
20. Kulpa DA, Collins KL (2011) The emerging role of HLA-C in HIV-1 infection. Immunology 134: 116-122.
21. Wagtmann N, Rajagopalan S, Winter CC, Peruzzi M, Long EO (1995) Killer cell inhibitory receptors specific for HLA-C and HLA-B identified by direct binding and by functional transfer. Immunity 3: 801-809.
22. Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, Zhang K, Gumbs C, Castagna A, Cossarizza A, Cozzi-Lepri A, De Luca A, Easterbrook P, Francioli P, Mallal S, Martinez-Picado J, Miro JM, Obel N, Smith JP, Wyniger J, Descombes P, Antonarakis SE, Letvin NL, McMichael AJ, Haynes BF, Telenti A, Goldstein DB (2007) A whole-genome association study of major determinants for host control of HIV-1. Science 317: 944-947.
23. Thomas R, Apps R, Qi Y, Gao X, Male V, O'HUigin C, O'Connor G, Ge D, Fellay J, Martin JN, Margolick J, Goedert JJ, Buchbinder S, Kirk GD, Martin MP, Telenti A, Deeks SG, Walker BD, Goldstein D, McVicar DW, Moffett A, Carrington M (2009) HLA-C cell surface expression and control of HIV/AIDS correlate with a variant upstream of HLA-C. Nat Genet 41: 1290-1294.
24. Mhandire K, Mlambo T, Zijenah LS, Duri K, Mateveke K, Tshabalala M, Mhandire DZ, Musarurwa C, Wekare PT, Mazengera LR, Matarira HT, Stray-Pedersen B (2017) Plasma IP-10 concentrations correlate positively with viraemia and inversely with CD4 counts in untreated HIV infection. Open AIDS J 11: 24.
25. Yabe T, Matsuo K, Hirayasu K, Kashiwase K, Kawamura-Ishii S, Tanaka H, Ogawa A, Takanashi M, Satake M, Nakajima K, Tokunaga K, Inoko H, Saji H, Ogawa S, Juji T, Sasazuki T, Kodera Y, Morishima Y; Japan Marrow Donor Program (2008) Donor killer immunoglobulin-like receptor (KIR) genotype-patient cognate KIR ligand combination and antithymocyte globulin preadministration are critical factors in outcome of HLA-C-KIR ligand-mismatched T cell-replete unrelated bone marrow transplantation. Biol Blood Marrow Transplant 14: 75-87.
26. Lazaryan A, Lobashevsky E, Mulenga J, Karita E, Allen S, Tang J, Kaslow RA (2006) Human leukocyte antigen B58 supertype and human immunodeficiency virus type 1 infection in native Africans. J Virol 80: 6056-6060.
27. da Silva EM, Acosta AX, Santos EJM, Netto EM, Lemaire DC, Oliveira AS, BarbosaCM, Bendicho MT, Galvão-Castro B, Brites C (2010) HLA-Bw4-B* 57 and Cw* 18 alleles are associated with plasma viral load modulation in HIV-1 infected individuals in Salvador, Brazil. Braz J Infect Dis 14: 468-475.
28. Kiepiela P, Leslie AJ, Honeyborne I, Ramduth D, Thobakgale C, Chetty S, Chetty S, Rathnavalu P, Moore C, Pfafferott KJ, Hilton L, Zimbwa P, Moore S, Allen T, BranderC, Addo MM, Altfeld M, James I, Mallal S, Bunce M, Barber LD, Szinger J, Day C, Klenerman P, Mullins J, Korber B, Coovadia HM, Walker BD, Goulder PJ (2004) Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. Nature 432: 769-775.
29. Ferrari G, Currier JR, Harris ME, Finkelstein S, de Oliveira A, Barkhan D, Cox JH, Zeira M, Weinhold KJ, Reinsmoen N, McCutchan F, Birx DL, Osmanov S, Maayan S (2004) HLA-A and -B allele expression and ability to develop anti-Gag cross-clade responses in subtype C HIV-1-infected Ethiopians. Hum Immunol 65: 648-659.
30. Stewart-Jones GB, Gillespie G, Overton IM, Kaul R, Roche P, McMichael AJ, Rowland-Jones S, Jones EY (2005) Structures of three HIV-1 HLA-B*5703-peptide complexes and identification of related HLAs potentially associated with long-term nonprogression. J Immunol 175: 2459-2468.
31. Shepherd BL, Ferrand R, Munyati S, Folkard S, Boyd K, Bandason T, Jallow S, Rowland-Jones SL, Yindom LM (2015) HLA Correlates of Long-Term Survival in Vertically Infected HIV-1-Positive Adolescents in Harare, Zimbabwe. AIDS Res Hum Retroviruses 31: 504-507.
32. Hendel H, Caillat-Zucman S, Lebuanec H, Carrington M, O'Brien S, Andrieu J-M, Schächter F, Zagury D, Rappaport J, Winkler C, Nelson GW, Zagury JF (1999) New class I and II HLA alleles strongly associated with opposite patterns of progression to AIDS. J Immunol 162: 6942-6946.
33. Lin Z, Kuroki K, Kuse N, Sun X, Akahoshi T, Qi Y, Chikata T, Naruto T, Koyanagi M, Murakoshi H, Gatanaga H, Oka S, Carrington M, Maenaka K, Takiguchi M (2016) HIV-1 Control by NK Cells via Reduced Interaction between KIR2DL2 and HLA-C *12:02/C *14:03. Cell Rep 17: 2210-20.
34. Fadda L, Korner C, Kumar S, van Teijlingen NH, Piechocka-Trocha A, Carrington M, Altfeld M (2012) HLA-Cw*0102-restricted HIV-1 p24 epitope variants can modulate the binding of the inhibitory KIR2DL2 receptor and primary NK cell function. PLoS Pathog 8: e1002805.
35. van Teijlingen NH, Holzemer A, Korner C, Garcia-Beltran WF, Schafer JL, Fadda L, Suscovich TJ, Brander C, Carrington M, Evans DT, van Baarle D, Altfeld M (2014) Sequence variations in HIV-1 p24 Gag-derived epitopes can alter binding of KIR2DL2 to HLA-C*03:04 and modulate primary natural killer cell function. AIDS 28: 1399-1408.

Corresponding author
Kudakwashe Mhandire, PhD
University of Zimbabwe, College of Health Sciences
P. O Box A178 Avondale, Harare, Zimbabwe
Email: mhandiremhandire@gmail.com

Conflict of interests: No conflict of interests is declared.

Annex – Supplementary Items

Supplementary Table 1. Simple linear regression of *HLA-C* alleles and viral loads at enrollment

***HLA-C* allele**	**Coefficient**	**Standard Error**	**p-value**
Cw*02:02	0.05	0.22	0.788
Cw*02:10	0.29	0.45	0.517
Cw*03:03	0.40	0.59	0.495
Cw*03:04	-0.44	0.30	0.138
Cw*03:113	0.13	0.36	0.707
Cw*04:01	0.18	0.20	0.374
Cw*05:01	-0.01	0.31	0.975
Cw*06:02	**-0.39**	**0.22**	**0.077**
Cw*07:01	-0.20	0.21	0.341
Cw*07:02	-0.08	0.26	0.748
Cw*07:04	-0.66	0.48	0.177
Cw*08:02	-0.36	0.25	0.161
Cw*08:04	-0.16	0.38	0.678
Cw*12:03	0.03	0.38	0.928
Cw*14:02	-0.10	0.59	0.859
Cw*16:01	0.36	0.26	0.174
Cw*17:01	-0.24	0.20	0.246
Cw*18:01	**-0.59**	**0.24**	**0.017**
Age	0.01	0.01	0.232
Gender	0.12	0.14	0.39
Weight	-0.01	0.01	0.34

VL was $\log_{10}$ transformed to fit the regression model.

Supplementary Table 2. Simple linear regression model of *HLA-C* allelic variants and CD4+T lymphocyte count.

Variable	**Coefficient**	**Standard error**	**p-value**
Cw*02:02	0.73	0.82	0.377
Cw*02:10	-0.29	1.69	0.860
Cw*03:03	-1.85	2.22	0.404
Cw*03:04	0.43	1.11	0.699
Cw*03:113	-0.78	1.35	0.563
Cw*04:01	-0.57	0.77	0.457
Cw*05:01	0.27	1.21	0.823
Cw*06:02	-0.72	0.82	0.383
Cw*07:01	-0.03	0.79	0.966
Cw*07:02	0.66	0.99	0.506
Cw*07:04	0.59	1.81	0.741
Cw*08:02	**2.04**	**0.95**	**0.034**
Cw*08:04	1.66	1.44	0.249
Cw*12:03	0.17	1.44	0.906
Cw*14:02	-0.05	2.20	0.981
Cw*16:01	-0.42	1.00	0.669
Cw*17:01	0.11	0.77	0.886
Cw*1801	0.55	0.91	0.546
Age	-0.02	0.02	0.395
Gender	-0.88	0.52	0.095
Weight	0.01	0.01	0.273

CD4+ T lymphocyte counts were square root transformed to fit the model.