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RESEARCH ARTICLE

Killer immunoglobulin-like receptor (KIR) genes are associated with the risk of episodes of high-level and detectable viremia among HIV controllers [version 1; peer review: awaiting peer review]

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Abstract

Background: HIV controllers (HICs) constitute a heterogeneous group of HIV-1 individuals able to suppress plasma viremia to low or undetectable levels in the absence of antiretroviral therapy. Host genetic factors may be involved in the sustained control of viral replication observed. We investigated the distribution and the potential impact of human leukocyte antigens (HLA)-B and -C alleles, killer immunoglobulin-like receptor (KIR) genes, single nucleotide polymorphisms (SNPs) of the NLRP3, CARD8 and IL-1β inflammasome genes, and CCR5Δ32 mutation on the viral control among HICs.
Methods: In total, 28 HICs were categorized as persistent elite controllers (PECs, n = 7), ebbing elite controllers (EECs, n = 7), and viremic controllers (VCs, n = 14) according to the level of natural suppression of viremia. HLA alleles were assigned by sequencing-based typing, KIR alleles by polymerase chain reaction (PCR) sequence-specific amplification, SNPs by real-time PCR, and the CCR5 Δ32 mutation by PCR.
Results: Significant differences were observed in the pairwise comparisons of protective HLA-B alleles, KIR Bx genotype, KIR2DL3 + C1 pair, KIR2DL5, and KIR2DS5 allelic carrier frequencies among the HIC groups. Multivariate models showed that HICs without the KIR2DL3 allele or without KIR2DL3 + C1/C2 pair, with the HLA-C*08 allele or with the NLRP3 rs10754558-G SNP had a higher mean hazard of a viral load above 2,000 copies/mL, while a lower mean hazard of this event was observed for HICs with KIR2DL5, KIR2DS1, KIR2DS5,

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and KIR3DS1 alleles. Moreover, HICs with the KIR2DS5 allele had less risk of undergoing viral load (VL) blips within the same normalized period than those participants without this allele, while HICs without the KIR2DL3 allele had a mean higher risk of experiencing VL blips.
Conclusions: These results indicate that innate immune mechanisms may play an essential role in modulating the sustained control of viral replication in HICs.

Keywords
HIV controllers, viral load, HLA, KIR, CCR5 Δ32, Inflammasome SNPs.

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Introduction

Different levels of viremia control are observed among human immunodeficiency virus (HIV)-1-infected individuals. Approximately 1% of the HIV-1-seropositive population suppresses viral replication to extremely low or undetectable levels in the absence of antiretroviral therapy and are termed HIV controllers (HICs) or elite controllers (ECs) (Deeks & Walker, 2007; Lambotte *et al*., 2005). HICs constitute a heterogeneous group of HIV-1-infected individuals, even when compared to persons with low-level viremia, they exhibit marked genetic and immunologic heterogeneity (Pereyra *et al*., 2008; Côrtes *et al*., 2015). Heterogeneity in viral load (VL), classification criteria and follow-up time to define the EC profile is also observed among the studies (Gurdasani *et al*., 2014; Navarrete-Muñoz *et al*., 2020). The mechanisms involved in the control of viral replication remain to be fully elucidated. Some studies point to the role of viral features in this phenomenon, while others focus on the role of host genetic in the efficient control of HIV-1 observed among HICs (Côrtes *et al*., 2015; Balasubramaniam *et al*., 2019).

Human leukocyte antigens (HLA) class I molecules have a crucial role in the cytotoxic T-lymphocyte (CTL) response. Genes that encode these molecules have been consistently associated with distinct patterns observed in the dynamics of HIV-1 infection (McLaren & Carrington, 2015), among which we can highlight the protective role attributed to the HLA-B*27 and B*57 alleles in EC cohorts (Lécuroux *et al*., 2014; Migueles *et al*., 2000; Fellay *et al*., 2007; Pereyra *et al*., 2010). However, the presence of HLA-B*27 and B*57 cannot be directly related to viral control, since some HIV-1-infected individuals who harbor these alleles present absence of viremia control (Pereyra *et al*., 2008; Emu *et al*., 2008). While HLA-B alleles are well-reported markers of HIV disease progression and control (McLaren & Carrington, 2015), HLA-C alleles have been neglected. HLA-C alleles were generally considered inferior in restricting CTLs responses compared to HLA-A and –B (Kulpa & Collins, 2011). On the other hand, they act as excellent ligands for killer immunoglobulin-like receptor (KIR) receptors on natural killer (NK) cells and protect target cells from lysis mediated by NK cells (Kulpa & Collins, 2011). In this context, the HLA-C locus emerged as an important host genetic determinant of HIV infection outcomes. The HLA-C*08 and HLA-C*18 alleles have already been associated with better disease control in a study of HIV controllers (Lazaryan *et al*., 2011), and the HLA-C*15 allele was a protective factor in a cohort of exposed uninfected infants (Bardeskar *et al*., 2018). Moreover, a single-nucleotide polymorphism 35 kb upstream the HLA-C locus (rs9264942) showed the most significant association with viral load (VL) control (Fellay *et al*., 2007; Thørner *et al*., 2016; Malnati *et al*., 2017). This variant has been associated with high HLA-C mRNA (messenger ribonucleic acid) levels and higher surface expression of HLA-C alleles (Fellay *et al*., 2007; Thørner *et al*., 2016; Malnati *et al*., 2017).

Inflammasomes are cytosolic multiprotein complexes of the innate immune system responsible for activating inflammatory responses (Rathinam & Fitzgerald, 2016). These complexes regulate the maturation of cytokines of the IL-1 family, including IL-1β and IL-18 (Rathinam & Fitzgerald, 2016). Activation of the inflammasome during HIV-1 infection mainly contributes to immune hyperactivation, which is the main pathogenic mechanism of HIV-1 progression (Marín-Palma *et al*., 2018). However, the relationship between inflammasome single nucleotide polymorphisms (SNPs) and viral load control in the context of HIV-1 infection is still unclear. The genetic restriction to HIV-1 infection caused by polymorphisms in the chemokine receptor CCR5 is another important factor that can modulate HIV-1 dynamics. Homozygosity for a 32 base-pair deletion in the *CCR5* gene (Δ32 mutation) is considered as a resistant phenotype leading to the protection of individuals against infection with HIV-1 R5 tropic lineages (Dean *et al*., 1996; Liu *et al*., 1996; Samson *et al*., 1996; Marmor *et al*., 2011), while heterozygosity may result in in slower progression to acquired immune deficiency syndrome (AIDS) (Huang *et al*., 1996; Sullivan *et al*., 2001).

Innate immunity mechanisms also participate in the sustained control of viral replication observed among ECs, mainly effector responses mediated by NK cells (Tomescu *et al*., 2012). The activity of these cells contributes to reduce viral replication in acute infection, thereby cooperating to the ability of ECs to control viremia (O'Connell *et al*., 2009). Consistent associations between KIR genes — expressed on the surface of NK cells — and viral control have already been described, highlighting the protective role conferred by KIR3DL1 associated with HLA-B molecules carrying the Bw4 motif (Martin *et al*., 2007). The inhibitory KIR2DL1, 2DL2, and 2DL3 recognize HLA-C ligands (Kulkarni *et al*., 2008), but no consistent association between HLA-C/KIR alleles and viral control has been described so far. Other innate immunological mechanisms have also been studied in ECs cohorts, including the ability of NK cells to induce cell death by antibody-dependent cellular cytotoxicity (ADCC) (Lambotte *et al*., 2013).

Studies with HICs may provide novel insights regarding host mechanisms of virus control (Hatano *et al*., 2009). Different from previous studies with HICs from the United States and Europe (Lambotte *et al*., 2005; Pereyra *et al*., 2008; Walker & Yu, 2013; Pernas *et al*., 2018), here, we have the opportunity to examine the role of these genetic markers in a HICs cohort that contains a high degree of miscegenation, which characterizes the Brazilian population. Besides this, other genetic-related particularities can be observed among Brazilians, such as the association of HLA-B*52 with the non-progression

to AIDS (Teixeira *et al.*, 2014). Therefore, in this study, we analyzed the distribution of HLA-B and -C, KIR, CCR5 genes, as well as selected SNPs to investigate their impact on the viral control observed in a cohort of Brazilian HICs.

Methods

Study subjects and ethical issues

The individual description of the HICs analyzed in this study had already been published (de Azevedo *et al.*, 2017; Côrtes *et al.*, 2018; Caetano *et al.*, 2020). Individuals who fulfilled the criteria of HICs were identified in a cohort of HIV-1 seropositive individuals followed at the Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz (INI/FIOCRUZ), Rio de Janeiro, Brazil. These individuals were defined as subjects over 18 years old with documented HIV-1 infection for >3 years and RNA viral load below the detection limit (40 or 80 copies/mL, depending on the year of inclusion) in the absence of antiretroviral treatment. As this is a longitudinal study, these individuals were/have been followed up and classified under one of the categories of viral control.

After 12 years of follow up, a cohort of 28 HICs could be composed including HIV-1-infected individuals classified in three categories according to the plasmatic viral load (VL): (1) persistent elite controllers (PECs) if 100% of VL measures were below the limit of detection (< LD; 50-80 copies/mL) for the respective available commercial assays (n = 7); (2) ebbing elite controllers (EECs) if subjects had occasional (≤ 30%) episodes of transient low-level (> LD to 400 copies/mL) viremia (n = 7); and (3) viremic controllers (VCs) if most (≥ 70%) of VL determinations were between 51 and 2,000 copies/mL (n = 14). Occasional VL measurements above the upper limits were accepted for the EECs and VCs. Participants were followed at least once every 6-12 months, starting in November 2008, to perform HIV-RNA VL quantification and $CD4^+$ T lymphocytes counts. In each visit, whole blood was collected into an ethylenediaminetetraacetic acid (EDTA)-containing tube, and 1 mL was stored at -20 °C until use. At this moment, from the 28 individuals [median follow-up = 9.02 years (interquartile range (IQR) = 6.46)], 11 are currently being followed up and 17 are no longer study participants due to cART entry (n = 09) or loss of follow up (n = 08).

All participants provided written informed consent, and the ethical committee of Instituto Nacional de Infectologia Evandro Chagas (INI-FIOCRUZ) approved the study (CAAE 1717.0.000.009-07). The corresponding documents can be found as extended data (de Sá & Teixeira, 2021).

Genomic DNA extraction

DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Nordrhein-Westfalen, Germany) according to the manufacturer's instructions. The DNA concentration was determined using the Thermo Scientific Nanodrop 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and the filtrates containing the isolated DNA were stored at −20 °C until used for genomic analysis.

HLA typing

High-resolution HLA-B and -C alleles typing was performed by automated nucleotide sequencing (sequencing-based typing — SBT) according to the manufacturer's instructions on the ABI platform using commercial kits (SECORE Sequencing kit, Invitrogen by Life Technologies, Brown Deer, Wisconsin, USA). HLA-B and -C alleles were assigned using a four-digit designation utilizing uTYPE® v6.0 SBT software (Invitrogen by Life Technologies, Brown Deer, Wisconsin, USA). The grouping of HLA-B alleles in HLA Bw4 and/or Bw6 epitopes associated specificities followed the Immuno Polymorphism Database (IPD)-International Immunogenetics Project (IMGT)/HLA nomenclature guidelines (Robinson *et al.*, 2013). The grouping of HLA-C genes in C1 (HLA-C*01/*03/*07/*08/*12/*14/*16) and C2 (HLA-C*02/*04/*05/*06/*15/*17/*18) epitope-associated specificities was based in the classification currently used in the literature (Mandelboim *et al.*, 1996; Faridi & Agrawal, 2011; de Sá *et al.*, 2020).

KIR genotyping

Qualitative analysis of KIR genes was performed using a commercial kit based on sequence-specific primer amplification methods — SSP (SSP KIR Genotyping Kit, Invitrogen, Brown Deer, Wisconsin, USA). A total of 14 KIR genes and 2 KIR pseudogenes (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, and 3DP1) were screened using this approach. KIR AA and Bx genotypes designation followed the current working definition, which characterizes these genotypes based on the combinations of haplotype A (absence of all the activating genes, except KIR2DS4) and haplotype B (presence of one or more of the activating genes) (Uhrberg *et al.*, 1997; Fernandes-Cardoso *et al.*, 2016).

Single nucleotide polymorphism selection and genotyping

We selected four single nucleotide polymorphisms (SNPs) in 3 inflammasome genes based on previously published data (Pontillo *et al.*, 2013) and considering the relevance of each gene in the inflammasome pathway: CARD8 (Caspase Activation and Recruitment Domains-8) rs2043211 and rs6509365; NLRP3 (Nucleotide oligomerization domain–Like

Receptor family, Pyrin domain-containing protein-3) rs10754558; and IL-1β rs1143634. Besides that, we selected HLA-C rs9264942 C>T (TaqMan® genotyping assay C_29901957_10) based on the association of this SNP with a lower HIV-1 viral load (Fellay *et al.*, 2007, 2009; Wei *et al.*, 2015). SNP genotyping was performed using commercially available TaqMan assays (Applied Biosystems/AB and Life Technologies) using the ABI7500 Real-Time platform (AB). Allelic discrimination was carried out by means of the Thermo Fisher Connect Software® version 1.

CCR5 genotyping

DNA samples were PCR amplified to determine the presence of the CCR5 Δ32 mutation. Primers (CCR5-F: GCT GTC TTT GCG TCT CTC CCA GGA and CCR5-R: CTC ACA GCC CTG TGC CTC TTC TTC) were used to amplify a 239 base-pair (bp) fragment covering the Δ32 mutation region. The cycling conditions were: 1 cycle 94°C 5 minutes; 30 cycles 94°C 1 minute, 60°C 30 seconds, 72°C 2 minutes; 1 cycle 72°C 10 minutes. The PCR amplified products (5 ul) were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. A single band of 239 bp indicated the CCR5/CCR5 wild-type genotype, while the heterozygous genotype CCR5/Δ32 was detected by 239 and 207 bp bands, and the homozygous genotype Δ32/Δ32 by a single band of 207 bp.

Statistical analyses

Direct count estimated the frequencies of HLA, KIR, SNPs and CCR5 alleles and genotypes. Kruskal-Wallis ANOVA by Ranks test was used to compare the sociodemographic, clinical, and laboratory characteristics among the different HICs groups for continuous numerical variables. In contrast, for categorical nominal variables, Fisher's exact test was used in the evaluation of frequencies among the different HICs groups. Tests of equal proportions were used to evaluate the allele carriers' relative frequencies among the HICs groups. For comparative purposes, HLA-B genomic distribution data from the general Brazilian population were extracted from REDOME (National Registry of Bone Marrow Donors, Brazilian Ministry of Health) data (Rede Brasil de Imunogenética, 2018), and for HLA-C we used data available at Allele Frequencies Net Database.

To estimate the hazard of a transitory loss of virological control, defined as the observation of the first VL determination above 2,000 copies/mL, we calculated person-years (pY) at risk for each patient between HIV diagnosis and the occurrence of an event. Individuals were censored either at the time of final observation, death, cART (combined antiretroviral therapy) initiation, or the last follow-up visit/exam, whichever occurred first. The effect of various risk factors on the outcome was assessed using hazard ratios (HR) and corresponding 95% confidence intervals (CI), which were estimated through the Cox proportional hazard model (Therneau & Grambsch, 2000).

To evaluate the occurrence of multiple events of VL blips, herein defined as the number of detectable viral loads counted after HIV diagnosis and until cART initiation, we calculated the incidences/rates of the number of viral loads per pY for each patient, and 95% CI were estimated according to asymptotic standard errors calculated from a Gamma distribution (Lehmann & Casella, 1998). The effect of various risk factors on the outcome was assessed by relative risks (RR) and corresponding 95% CI were estimated employing the Negative Binomial (NB) models. Individual exposure time (in years) was used as an offset in the NB models (Hilbe, 2011).

Two-tailed levels of significance $\leq$ 0.01, 0.05 and 0.1 were considered "highly significant", "significant" and "suggestive", respectively. All statistical analysis was performed using the R statistical software package, version 3.4.1 (R Development Core Team, 2017).

Results

Clinical and epidemiological characteristics of HICs

Table 1 depicts the main clinical and epidemiological characteristics of the HICs cohort distributed according to the viral control groups (de Sá & Teixeira, 2021). Overall, we found a similar frequency of females and males (16 [57.1%] and 12 [42.9%], respectively), while 21 (75%) of the individuals have identified themselves as heterosexual and 6 (21.4%) as men who have sex with men (MSM). Most heterosexual male (75%) and MSM subjects (83.3%) belonged to the VCs group. The ethnic background composition was diverse, with an unequal distribution of White, Brown, and Black participants among groups of HICs ($P = 0.04$). Participants had a median age of 32 (IQR 10.4) years old, with a similar median age observed among the PECs (33 years old; IQR 6.9), EECs (37 years old; IQR 13.9), and VCs (30 years old; IQR 9.8) groups.

All HICs groups displayed a median $CD4^+$ T cells count above 800 cells/μL (IQR 431). Further details about immunological and virological characteristics of most HICs of this study were depicted in previous studies from our group (Bello *et al.*, 2009; Côrtes *et al.*, 2015, 2018; de Azevedo *et al.*, 2017; Caetano *et al.*, 2020).

Table 1. Clinical and epidemiological data of HIV controllers (HICs).

Features	Overall	PECs (N = 7)	EECs (N = 7)	VCs (N = 14)	*P*-value[a]
Age (years)[b] (IQR)	32.72 (10.4)	33.7 (6.9)	37.2 (13.9)	30.1 (9.8)	0.5291
Gender; n (%)					
Female	16 (57.1)	5 (17.9)	6 (21.4)	5 (17.9)	0.084
Male	12 (42.9)	2 (7.1)	1 (3.6)	9 (32.1)	
Skin color; n (%)					
Black	7 (25)	0 (0)	1 (3.6)	6 (21.4)	**0.0431**
Brown	11 (39.3)	6 (21.4)	2 (7.1)	3 (10.7)	
White	10 (35.7)	1 (3.6)	4 (14.3)	5 (17.9)	
Education[c]; n (%)					
Illiterate	1 (3.6)	1 (3.6)	0 (0)	0 (0)	0.2544
Early childhood education	1 (3.6)	0 (0)	1 (3.6)	0 (0)	
Primary	6 (21.4)	0 (0)	3 (10.7)	3 (10.7)	
Lower-secondary	6 (21.4)	2 (7.1)	2 (7.1)	2 (7.1)	
Upper-secondary	11 (39.3)	3 (10.7)	1 (3.6)	7 (25)	
Bachelor	3 (10.7)	1 (3.6)	0 (0)	2 (7.1)	
HIV transmission route; n (%)					
Heterosexual	21 (75.0)	6 (21.4)	7 (25)	8 (28.6)	0.2301
MSM	6 (21.4)	1 (3.6)	0 (0)	5 (17.9)	
Unknown	1 (3.6)	0 (0)	0 (0)	1 (3.6)	
Clinical parameters					
CD4 Count (cells/μL)[d] (IQR)	994 (431)	1219 (246)	1071 (597)	825 (465)	0.1533
Viral Load (copies/mL)[d] (IQR)	120 (492)	49 (0)	82 (32)	548 (507)	**< 0.0001**

[a]For categorical nominal variables, *P*-values were calculated using Fisher's exact test. For continuous numeric variables, *P*-values were calculated using Kruskal-Wallis ANOVA by Ranks test. Differences were considered significant with a value of $P < 0.05$.
[b]Age at HIV diagnosis.
[c]Classification according to the International Standard Classification of Education (ISCED) maintained by the United Nations Educational, Scientific, and Cultural Organization (UNESCO).
[d]Middle cumulative $CD4^+$ T Cells Count and HIV-1 Viral Load.
Abbreviations: HICs = HIV controllers; PECs = persistent elite controllers; EECs = ebbing elite controllers; VCs = viremic controllers. IQR = interquartile range.

Distribution of HLA-B, -C, KIR, and CCR5 genes among HICs

Table 2 describes the relative frequencies of HLA-B and -C, KIR, and CCR5Δ32 alleles carriers found in the different HICs groups. Due to the dependence between the genotypes, we did not perform this comparative analysis for the SNPs included in this study. However, the complete genetic characterization of the analyzed markers is depicted in Table 3. We observed significant differences between PECs and EECs for protective HLA-B alleles, KIR Bx genotype, KIR2DL5, and KIR2DS5 allelic carrier frequencies. Among EECs and VCs, the frequencies of KIR Bx genotype, KIR2DL3 + C1 pair, KIR2DL2, KIR2DL5, and KIR2DS2 alleles carriers were also significantly distinct. The only genetic marker with significantly different frequencies between PECs and VCs was KIR2DS5. It is noticeable that HLA-B protective alleles (HLA-B*27, B*52, and/or B*57) were found in 71% of the PECs, 43% of the VCs, and only 14% of the EECs.

To check for an enrichment of protective HLA alleles in our cohort, we compared the classical protective HLA-B and -C frequencies here obtained with those from the general Brazilian population, and with HIV-1-infected individuals who progressed to AIDS (Teixeira *et al.* 2014; Biberg-Salum *et al.*, 2018), considered as HIV non-controllers (HIV-NC) (Table 4). For HLA-B, we noted that the frequency of carriers of these alleles in our HICs cohort (0.464; n = 13) is on average 3.34-fold higher ($P < 0.001$) than in the overall Brazilian population (0.139; n = 396,740) and 3.22-fold higher ($P = 0.014$) in the HIV non-controllers group (0.144; n = 29). Similarly, for HLA-C we noted that the frequency of carriers of these alleles in our HICs cohort (0.571; n = 16) is on average 3.60-fold higher ($P < 0.001$) than in the overall Brazilian population (0.159; n = 42) and 3.50-fold higher ($P < 0.001$) than in the HIV non-controllers group (0.161; n = 26). Only

Table 2. Relative frequencies of HLA-B, HLA-C, KIR, and CCR5Δ32 alleles carriers' among HIV controllers (HICs).

Features	PECs N=7		EECs N=7		VCs N=14		PECs *vs.* EECs	VCs *vs.* EECs	PECs *vs.* VCs
	n	Freq	n	Freq	n	Freq	*P*-value[a]	*P*-value[a]	*P*-value[a]
Any protective HLA-B[b]	5	0.714	1	0.143	6	0.429	**0.002**	0.071	0.289
HLA-B*27	1	0.143	0	0.000	1	0.071	NC	NC	0.992
HLA-B*52	1	0.143	1	0.143	2	0.143	0.983	0.992	0.992
HLA-B*57	4	0.571	0	0.000	3	0.214	NC	NC	0.139
Any protective HLA-C[c]	2	0.286	4	0.571	8	0.571	0.861	0.999	0.288
HLA-C*08	0	0.000	2	0.286	2	0.143	0.802	0.944	NC
HLA-C*15	0	0.000	1	0.143	4	0.286	0.984	0.832	NC
HLA-C*18	2	0.286	2	0.286	3	0.214	0.996	0.998	0.998
KIR Bx genotype	6	0.857	2	0.286	11	0.786	**0.037**	**0.001**	0.992
KIR3DL1 + Bw4	0	0.000	0	0.000	2	0.143	NC	NC	NC
KIR3DL1 +Bw4/Bw6	5	0.714	4	0.571	9	0.643	0.998	0.999	0.998
KIR3DL1 + Bw6	1	0.143	2	0.286	3	0.214	0.996	0.998	0.992
KIR3DS1 + Bw4	1	0.143	1	0.143	2	0.143	0.984	0.992	0.992
KIR3DS1 + Bw4/Bw6	1	0.143	0	0.000	2	0.143	NC	NC	0.992
KIR3DS1 + Bw6	1	0.143	0	0.000	0	0.000	NC	NC	0.832
KIR2DL1 + C2	3	0.429	1	0.143	3	0.214	0.548	0.992	0.754
KIR2DL1 + C1/C2	4	0.571	3	0.429	11	0.786	0.998	0.139	0.754
KIR2DL2 + C1	0	0.000	0	0.000	0	0.000	NC	NC	NC
KIR2DL2 + C1/C2	3	0.429	0	0.000	6	0.429	NC	NC	0.999
KIR2DL3 + C1	0	0.000	3	0.429	0	0.000	0.386	**0.033**	NC
KIR2DL3 + C1/C2	4	0.571	3	0.429	8	0.571	0.998	0.964	0.999
KIR2DS1 + C2	2	0.286	0	0.000	1	0.071	NC	NC	0.667
KIR2DS1 + C1/C2	2	0.286	1	0.143	4	0.286	0.984	0.832	0.998
KIR2DS2 + C1	0	0.000	0	0.000	0	0.000	NC	NC	NC
KIR2DS2 + C1/C2	2	0.286	0	0.000	6	0.429	NC	NC	0.944
KIR2DL2	4	0.571	1	0.143	7	0.500	0.066	**0.007**	0.999
KIR2DL3	7	1.000	7	1.000	11	0.786	NC	NC	NC
KIR2DL5	5	0.714	1	0.143	7	0.500	**0.002**	**0.007**	0.667
KIR2DS1	4	0.571	1	0.143	5	0.357	0.066	0.363	0.754
KIR2DS2	3	0.429	1	0.143	7	0.500	0.548	**0.007**	0.999
KIR2DS3	1	0.143	0	0.000	4	0.286	NC	NC	0.832
KIR2DS4	6	0.857	6	0.857	14	1.000	0.984	0.832	0.832
KIR2DS5	6	0.857	1	0.143	5	0.357	**<0.001**	0.363	**<0.001**
KIR3DL1	6	0.857	6	0.857	14	1.000	0.984	0.832	0.832
KIR3DS1	4	0.571	1	0.143	4	0.286	0.066	0.832	0.398
CCR5Δ32	1	0.143	1	0.143	2	0.143	0.984	0.992	0.992

[a]*P*-values were calculated using Tests of Equal Proportions. Differences were considered significant with a value of $P < 0.05$.
[b]Protective HLA-B alleles included HLA-B*27, B*52, and B*57 alleles.
[c]Protective HLA-C alleles included HLA-C*08, C*15, and C*18 alleles.
Abbreviations: PECs = persistent elite controllers; EECs = ebbing elite controllers; VCs = viremic controllers. NC = not calculated. N = number of individuals in each HIC group. n = observed number of an allele/genetic marker carriers. Freq = relative frequency of carriers for each allele/genetic marker, calculated as the number of carriers/N.

Table 3. **Genetic characterization of HIV controllers (HICs) (N = 28).**

HICs Groups	Patient ID	HLA-B alleles	HLA-B serological epitope	HLA-C alleles	HLA-C serological epitope	KIR genotype	CCR5 genotype	CARD8 rs6509365	CARD8 rs2043211	NLRP3 rs10754558	IL-1β rs1143634	HLA-C rs9264942
PECs	PEC02	48:02 X 52:01	Bw4/Bw6	04:01:01 X 12:02:03	C1/C2	AA	WT/WT	AA	AA	CC	GA	CT
	PEC26	07:02:01 X 57:02:01	Bw4/Bw6	07:56 X 18	C1/C2	Bx	WT/WT	AA	AA	CC	GG	TT
	PEC30	48:02:01 X 48:01	Bw6	04:01:01 X 04:01:01	C2	Bx	WT/WT	AA	AA	CC	GG	TT
	PEC35	27:05:02 x 57:01:01	Bw4	01 X 06	C1/C2	Bx	WT/WT	AG	AT	CG	AA	CC
	PEC38	44:03:01 X 48:02:01	Bw4/Bw6	04 X 04	C2	Bx	WT/WT	AG	AT	CC	GG	TT
	PEC39	50:01:01 x 57:01:01	Bw4/Bw6	06:02:01 X 06:02:01	C2	Bx	WT/Δ32	AA	AA	CG	GG	CC
	PEC52	45:02 x 57:03	Bw4/Bw6	16:01:01 X 18:02	C1/C2	Bx	WT/WT	AG	AT	CG	GG	TT
EECs	EEC07	48:02:01 X 81:01	Bw6	04 X 18	C2	Bx	WT/WT	GG	TT	CC	GG	TT
	EEC11	49:01:01 X 81:01	Bw4/Bw6	07:01:01 X 08:04:01	C1	AA	WT/Δ32	AG	AT	CG	GA	CT
	EEC17	07:05:01 X 40:02:01	Bw6	03:04:01 X 15:05:02	C1/C2	AA	WT/WT	AG	AT	CC	GG	TT
	EEC42	15:01 X 51:01	Bw4/Bw6	03:04 X 14:46	C1	AA	WT/WT	AG	AA	CC	GG	CT
	EEC18	07:02:01 X 52:01:01	Bw4/Bw6	07 X 12	C1	AA	WT/WT	AG	AA	GG	GG	CT
	EEC19	44:03:01 X 35:03:01	Bw4	07 X 17	C1/C2	Bx	WT/WT	AA	AA	CC	GA	CT
	EEC36	53:39 X 81:01	Bw4/Bw6	08:02:01 X 18:01	C1/C2	AA	WT/WT	AG	AT	CC	GG	CT

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Table 3. *Continued*

HICs Groups	Patient ID	HLA-B alleles	HLA-B serological epitope	HLA-C alleles	HLA-C serological epitope	KIR genotype	CCR5 genotype	CARD8 rs6509365	CARD8 rs2043211	NLRP3 rs10754558	IL-1β rs1143634	HLA-C rs9264942
VCs	VC05	15:03 X 52:01	Bw4/Bw6	02:02 X 04:01	C2	AA	WT/WT	AG	AT	CG	GG	TT
	VC06	15:01 X 48:02	Bw6	03:04:01 X 04	C1/C2	Bx	WT/Δ32	AG	AT	CG	GG	TT
	VC09	49:01 X 52:01	Bw4	07 X 15	C1/C2	Bx	WT/WT	AA	AA	CC	GG	TT
	VC14	42:01 X 44:03	Bw4/Bw6	07 X 17	C1/C2	Bx	WT/WT	AA	AA	CG	GG	CT
	VC15	56:01:01 X 57:03:01	Bw4/Bw6	01 X 18	C1/C2	Bx	WT/WT	AG	AT	GG	GA	TT
	VC16	14:02:01 X 57:03:01	Bw4/Bw6	08:02 X 18	C1/C2	Bx	WT/WT	AA	AA	CG	GG	CT
	VC23	55:01:01 X 81:01	Bw6	07:04 X 18	C1/C2	Bx	WT/WT	AA	AA	CC	GA	TT
	VC27	08:01 X 27:03	Bw4/Bw6	03:04:01 X 15:05	C1/C2	Bx	WT/WT	AG	AT	CC	GG	TT
	VC31	35:02:01 X 44:02:01:01	Bw4/Bw6	04:01:01 X 05	C2	AA	WT/Δ32	AG	AT	CG	GG	CT
	VC32	53:01:01 X 57:02:01	Bw4	04 X 07	C1/C2	Bx	WT/WT	AG	AT	CC	GA	CT
	VC34	42:01:01 X 44:03:01	Bw4/Bw6	02 X 12	C1/C2	Bx	WT/WT	AG	AA	CC	GG	CT
	VC41	35:01:01:01 X 40:04	Bw6	05 X 15	C2	Bx	WT/WT	AA	AA	CG	GA	CT
	VC43	15:01:27 X 51:01:01:01	Bw4/Bw6	12:03:01 X 15:03	C1/C2	Bx	WT/WT	AG	AT	CG	GA	CT
	VC47	13:02:01 X 48:02:01	Bw4/Bw6	04:01:01 X 08:04:01	C1/C2	AA	WT/WT	AA	AA	CG	GG	CT

Abbreviations: WT = wild type allele. PECs = persistent elite controllers; EECs = ebbing elite controllers; VCs = viremic controllers.

Table 4. Distribution of protective HLA-B and HLA-C alleles of HICs, HIV non-controllers and the Brazilian general population.

HLA alleles	Brazilian general population (BGP)[a] N = 2.847.869		HICs N = 28		HIV-NC[b] N = 201		BGP *vs.* HICs	HICs *vs.* HIV-NC	BGP *vs.* HIV-NC
HLA-B	**n**	**Freq**	**n**	**Freq**	**n**	**Freq**	***P*-value**	***P*-value**	***P*-value**
Any protective HLA-B[c]	396,740	0.139	13	0.464	29	0.144	**< 1e-12**	**0.013**	0.999
HLA-B*27	126,605	0.044	2	0.071	4	0.02	**< 1e-12**	0.700	0.946
HLA-B*52	110,700	0.039	4	0.143	11	0.055	**< 1e-12**	0.976	0.998
HLA-B*57	159,435	0.056	7	0.25	14	0.07	**< 1e-12**	0.801	0.999
	Brazilian general population (BGP)[d] N = 264		**HICs N = 28**		**HIV-NC[e] N = 161**		**BGP *vs.* HICs**	**HICs *vs.* HIV-NC**	**BGP *vs.* HIV-NC**
HLA-C	**n**	**Freq**	**n**	**Freq**	**n**	**Freq**	***P*-value**	***P*-value**	***P*-value**
Any protective HLA-C[f]	42	0.159	16	0.571	26	0.161	**3,1 e-06**	**0.0008**	0.999
HLA-C*08	20	0.076	4	0.143	10	0.062	0.964	0.984	0.995
HLA-C*15	22	0.083	5	0.179	11	0.068	0.919	0.978	0.998
HLA-C*18	0	0	7	0.25	5	0.031	NC	0.985	NC

P-values were calculated using the unconditional logistic regression model. Differences were considered significant with a value of * $P < 0.05$.
[a]Data from Brazilian Registry of Bone Marrow Donors (REDOME).
[b]Data from Teixeira *et al.*, 2014.
[c]Protective HLA-B alleles included HLA-B*27, B*52, and B*57 alleles.
[d]Data from Allele Frequencies Net Database (http://www.allelefrequencies.net).
[e]Data from Biberg-Salum *et al.*, 2018.
[f]Protective HLA-C alleles included HLA-C*08, C*15, and C*18 alleles.
Abbreviations: HICs = HIV controllers; HIV-NC = HIV non-controllers; BGP = Brazilian general population; N = number of individuals; n = observed number of an allele carriers; Freq = relative frequency of carriers for each allele, calculated as the number of carriers/N; NC = not calculated.

two PECs carried the CC genotype in the HLA-C rs9264942 polymorphism. The KIR Bx genotype was found in 86% of PECs, 79% of the VCs, and 29% of EECs. The KIR3DL1+Bw4 genotype was detected in only two VCs, and the KIR3DS1+Bw4 genotype in four participants (one PEC, one EEC, and two VCs). All PECs and EECs had the KIR2DL3 allele. The CCR5Δ32 mutation was found in four heterozygous (WT/Δ32) participants (one PEC, one EEC, and two VCs), resulting in an overall allelic frequency of 7.1%, and no homozygous (Δ32/Δ32) participants were identified in this cohort. Therefore, we cannot assign any relation between this genetic marker and the distinct patterns of viremia control observed in these HICs.

Episodes of high-level and detectable viremia among HICs

To test the potential relevance of genetic host factors in the risk of a transitory loss of virological control in some HICs, we conducted Cox proportional hazard multivariate analysis to identify factors associated with the occurrence of the high-level viremia (> 2,000 copies/mL) (Table 5). A higher mean hazard of this event was observed for HICs without the KIR2DL3 allele [aHR = 79.098 (3.236-1933.264); $P = 0.007$], HICs with the HLA-C*08 allele [aHR = 8.379 (1.181-59.472); $P = 0.034$], HICs with the C/G genotype [aHR = 7.462 (2.294-2505.478); $P = 0.042$] and G/G genotype [aHR = 75.817 (2.294-2505.478); $P = 0.015$] in the NLRP3 polymorphism (rs10754558), and HICs without the KIR2DL3 + C1/C2 pair [aHR = 11.78 (1.602-86.619); $P = 0.015$] than for their counterparts. In the other way, a lower mean hazard of this event was observed for HICs with the following alleles: KIR2DL5 [aHR = 0.131 (0.023-0.738); $P = 0.021$], KIR2DS1 [aHR = 0.109 (0.014-0.867); $P = 0.036$], KIR2DS5 [aHR = 0.058 (0.007-0.466); $P = 0.007$], and KIR3DS1 [aHR = 0.108 (0.013-0.876); $P = 0.037$] when compared with their counterparts.

The relative risk of occurring any event of detectable viremia (VL blip) was also evaluated by means of the negative binomial (NB) multivariate models (Table 6). Overall, HICs with the KIR2DS5 allele [aRR = 0.294 (0.122-0.705);

Table 5. Effect of risk/protective factors associated with a transitory loss of virological control estimated by Cox proportional hazard models.

Features	Levels[a]	Event[b]	pY[c]	HR (95% CI); *P*-value	aHR (95% CI)[d]; *P*-value
Overall		10	304,61		
Gender	Female	3	191,09	Reference	Reference
	Male	7	113,51	6.42 (1.281-32.189); **0.024**	4.644 (0.772-27.926); 0.093
KIR2DL3	1	8	291,62	Reference	Reference
	0	2	12,99	9.647 (1.556-59.798); **0.015**	79.098 (3.236-1933.264); **0.007**
KIR2DL5	0	7	160,04	Reference	Reference
	1	3	144,56	0.487 (0.125-1.895); 0.299	0.131 (0.023-0.738); **0.021**
KIR2DS1	0	8	198,98	Reference	Reference
	1	2	105,62	0.528 (0.11-2.533); 0.425	0.109 (0.014-0.867); **0.036**
KIR2DS5	0	8	163,41	Reference	Reference
	1	2	141,2	0.294 (0.062-1.389); 0.122	0.058 (0.007-0.466); **0.007**
KIR3DS1	0	8	207,63	Reference	Reference
	1	2	96,98	0.598 (0.125-2.853); 0.519	0.108 (0.013-0.876); **0.037**
HLA-C*08	0	7	278,82	Reference	Reference
	1	3	25,79	6.3 (1.398-28.398); **0.017**	8.379 (1.181-59.472); **0.034**
KIR2DL3 + C1/C2	1	5	187,93	Reference	Reference
	0	5	116,67	2.239 (0.596-8.411); 0.232	11.78 (1.602-86.619); **0.015**
NLRP3 (rs10754558)	C/C	3	177.62	Reference	Reference
	C/G	6	112.91	3.415 (0.842-13.843); 0.085	7.462 (1.074-51.847); **0.042**
	G/G	1	14.08	6.509 (0.602-70.387); 0.123	75.817 (2.294-2505.478); **0.015**
	C	12	468.14	Reference	Reference
	G	8	141.07	2.462 (0.682-8.888); 0.169	3.294 (0.773-14.032); 0.107

[a]Levels = 0 (absent); 1 (present).
[b]First HIV-1 VL determination above 2,000 copies/mL.
[c]pY: person-years at risk were calculated for each patient between HIV diagnosis and the occurrence of an event.
[d]Hazard ratios were adjusted by gender and education whenever applicable, which were variables associated with an uncontrolled viremic event in bivariate analysis ($P < 0.2$).
Abbreviations: HR = hazard ratios; aHR = adjusted hazard ratios.

$P = 0.006$] had less risk of undergoing VL blips within the same normalized period than those participants without this allele. Conversely, HICs without the KIR2DL3 allele [aHR = 4.987 (1.174-21.196); $P = 0.03$] had a mean higher risk of experiencing VL blips within the same normalized period than those with this allele. The results of Cox's proportional hazard multivariate analysis and Negative Binomial (NB) models of the HLA-C and inflamassome SNPs are depicted in Tables 7 and 8.

Our results point to a protective role for KIR2DL3 and KIR2DS5 alleles since the absence of the former was associated both with a higher risk of a transitory loss of virological control and to a higher risk of any event of detectable viremia (VL blip); while the presence of the latter was associated to a lower risk of these events. Epidemiological features were

Table 6. Effect of risk/protective factors associated with multiple events of viral load blips estimated by Negative Binomial (NB) models.

Features	Levels[a]	Number of events[b]	pY[c]	RR (95% CI); *P*-value	aRR (95% CI)[d]; *P*-value
Overall		308	313,83		
KIR2DL3	1	260	289,49	Reference	Reference
	0	48	24,34	2.669 (0.616-11.563); 0.189	4.987 (1.174-21.196); **0.03**
KIR2DS5	0	210	175,5	Reference	Reference
	1	98	138,33	0.508 (0.199-1.298); 0.157	0.294 (0.122-0.705); **0.006**

[a]Levels = 0 (absent); 1 (present).
[b]Number of events: number of viral load blips (defined as the number of detectable HIV-1 viral loads after HIV diagnosis).
[c]pY: person-years at risk were calculated from HIV diagnosis until a cART treatment initiation.
[d]Relative risks were adjusted by gender, age at HIV diagnosis, education, and skin color whenever applicable, which were variables associated with multiple events of viral load blips in the bivariate analysis ($P < 0.2$).
Abbreviations: RR = relative risk. aRR = adjusted relative risk.

introduced in the multivariate models for controlling bias in the statistical analysis. The limited sample size and the study design do not allow us to explore the associations involving these epidemiological features depicted in Tables 5 and 6.

Discussion

In the present study, we characterized the distribution of HLA-B, HLA-C, KIR, SNPs of the NLRP3, CARD8, IL-1β, and CCR5Δ32 mutation in a cohort of HICs with different levels of virological control. Our results show that host genes classically associated with protective mechanisms are present in our HICs cohort. Despite the small number of participants analyzed, we observed a higher frequency of HLA-B protective alleles in this cohort than in the general Brazilian population and Brazilian HIV non-controllers (Teixeira *et al.*, 2014), confirming the results of many studies showing enrichment of protective B*27 and B*57 alleles in HICs (Migueles *et al.*, 2000; Fellay *et al.*, 2007; Pereyra *et al.*, 2010; Lécuroux *et al.*, 2014). We also observed that B*27 and B*57 allele carriers' frequencies were not different among HICs groups. However, when considered together with HLA-B*52 composing the 'protective HLA-B alleles' group, a significant difference of these alleles frequencies between PECs and EECs was observed ($P = 0.002$, Table 2). In this study, multivariate models showed that some genetic markers were associated with a transitory loss of virological control and/or of an event of detectable viremia (VL blip). A lower mean hazard for the risk of transitory loss of virological control was observed for KIR2DL5, KIR2DS1, KIR3DS1, and KIR2DS5 alleles. In contrast, the HLA-C*08 allele and the absence of KIR2DL3 and KIR3DL3+C1/C2 alleles were associated with an increased risk of this event (Table 5). Additionally, KIR2DL3 allele absence was also associated with a higher risk of an event of detectable viremia (VL blip), and KIR2DS5 was also associated with a lower risk of these events (Table 6), pointing to a protective role of these alleles. Interestingly, no association was found for the CC genotype in the HLA C rs9264942 polymorphism with either the hazard of an uncontrolled viremic event or the risk of undergoing VL blips. This variant (CC genotype) has been associated with a higher viral load control (VL) in previous studies (Fellay *et al.*, 2007; Thørner *et al.*, 2016; Malnati *et al.*, 2017).

Studies have been associated KIR2DL3 receptor and its ligand HLA-C1 with a lower VL and a higher $CD4^+$ T cell count (Körner *et al.*, 2014). In agreement with these studies, we observed an increased HR of a high-level viremic event and a higher risk of experiencing VL blips in HICs without KIR2DL3. Inhibitory KIR receptors (KIR2DL2/KIR2DL3 heterozygosity) were associated with resistance to HIV-1 infection among female sex workers in the absence of their respective HLA ligands. These results indicate that absence of these ligands may reduce the threshold for NK cell activation via activating KIR, resulting in NK cytotoxic activity and early elimination of HIV-1 infected cells (Jennes *et al.*, 2006).

KIR2DS5 allele carriers had a lower mean hazard of high-level viremia (> 2,000 copies/mL) events and, in the mean, less VL blip events than those without the allele (Table 5). This activating gene was associated with the protection against HIV-1 infection of exposed uninfected infants (Chavan *et al.*, 2016) and reducing HIV-1 transmission from mother to child (Omosun *et al.*, 2018). It remains unclear what mechanisms at the KIR2DS5 allele might result in this protection. We speculate that the presence of activating mechanisms driven by this gene could impact viral control. This mechanism may be due to the activation signal delivered to NK cells when activating receptors bind their ligand on the target cell surface, leading to the activation of these cells and eliminating the pathogen (Srivastava *et al.*, 2003).

Table 7. Cox's proportional hazard multivariate analysis of the HLA-C and inflamassome single nucleotide polymorphisms (SNPs).

Features	Alleles/ Genotypes	Event[a]	pY[b]	HR (95% CI); *P*-value	aHR (95% CI)[c]; *P*-value
Overall		10	304,61		
HLA-C (rs9264942)	T/T	5	134.17	Reference	Reference
	C/C	0	14.99	NC	NC
	T/C	5	155.44	0.87 (0.252-3.009); 0.826	0.763 (0.187-3.116); 0.706
	T	15	423.79	Reference	Reference
	C	5	185.43	0.79 (0.188-3.317); 0.748	0.818 (0.186-3.609); 0.791
CARD8 (rs2043211)	A/A	4	151.33	Reference	Reference
	A/T	6	140.99	1.566 (0.441-5.561); 0.488	1.751 (0.352-8.712); 0.494
	T/T	0	12.28	NC	NC
	A	14	443.66	Reference	Reference
	T	6	165.56	1.173 (0.302-4.545); 0.818	1.273 (0.298-5.444); 0.745
CARD8 (rs6509365)	A/A	3	103.19	Reference	Reference
	A/G	7	189.14	1.267 (0.326-4.919); 0.732	1.477 (0.35-6.235); 0.596
	G/G	0	12.28	NC	NC
	A	13	395.51	Reference	Reference
	G	7	213.71	1.024 (0.279-3.756); 0.972	1.191 (0.316-4.494); 0.797
IL-1β (rs1143634)	G/G	7	221.07	Reference	Reference
	A/A	0	7.04	NC	NC
	G/A	3	76.49	1.309 (0.334-5.13); 0.699	1.026 (0.22-4.777); 0.974
	G	17	518.64	Reference	Reference
	A	3	90.58	1.079 (0.188-6.19); 0.932	0.937 (0.15-5.866); 0.945

[a]First HIV-1 VL determination above 2,000 copies/mL.
[b]pY: person-years at risk were calculated for each patient between HIV diagnosis and the occurrence of an event.
[c]Hazard ratios were adjusted by gender and education whenever applicable, which were variables associated with an uncontrolled viremic event in bivariate analysis.
Abbreviations: HR = hazard ratios; aHR = adjusted hazard ratios; NC = not calculated.

The relevance of NK cell function regarding the HIC status has been suggested by genetic studies (Martin *et al.*, 2002, 2007). The effect of the combination of KIR3DS1$^+$ NK cells with the Bw4 epitope reduces the susceptibility to HIV-1 infection, improves viremia control, and decreases CD4$^+$ T cells depletion (Martin *et al.*, 2002; Alter *et al.*, 2007, 2009). Moreover, in agreement with our results, the KIR3DS1 gene has been indicated as a protective factor in the resistance to HIV in different routes of exposure (Boulet *et al.* 2008; Chavan *et al.* 2014; Habegger de Sorrentino *et al.* 2013; Tallon *et al.* 2014). KIR2DL5 has already been associated with a reduced risk of HIV-1 infection and reduced HIV-1 transmission among Africans (Jennes *et al.*, 2006; Omosun *et al.*, 2018), but there is no previous report associating this allele with virological control. The KIR2DS1 gene bind HLA-C molecules of the C2 group (Folley *et al.*, 2008). It has already been significantly associated with a lower viral load in HIV-1 patients from India (Chavan *et al.*, 2014) and South Africa (Wong *et al.*, 2010).

The HLA-C*08 allele and the rs10754558 G allele were associated with an increased risk of transitory loss of virological control in this study. Several researchers have reported the positive influence of these alleles in HIV disease course (Lazaryan *et al.*, 2011; Shepherd *et al.*, 2015; Mhandire *et al.*, 2018; Pontillo *et al.*, 2010, 2012). The presence of the C*08 allele has already been associated with a high CD4$^+$ T cell count (Mhandire *et al.*, 2018), and this allele was enriched

Table 8. Negative binomial (NB) models of the HLA-C and inflammassome single nucleotide polymorphisms (SNPs).

Features	Alleles/ Genotypes	Number of events[a]	pY[b]	RR (95% CI); *P*-value	aRR (95% CI)[c]; *P*-value
Overall		308	114626		
HLA-C (rs9264942)	T/T	141	54208	Reference	Reference
	C/C	0	5434	NC	NC
	T/C	167	54984	1.015 (0.405-2.544); 0.974	1.671 (0.669-4.176); 0.272
	T	449	163400	Reference	Reference
	C	167	65852	0.785 (0.271-2.274); 0.656	1.054 (0.382-2.906); 0.919
NLRP3 (rs10754558)	C/C	114	58743	Reference	Reference
	C/G	175	48388	1.791 (0.653-4.908); 0.257	1.978 (0.762-5.135); 0.161
	G/G	19	7495	1.167 (0.167-8.16); 0.877	3.714 (0.578-23.878); 0.167
	C	403	165874	Reference	Reference
	G	213	63378	1.321 (0.443-3.938); 0.617	1.612 (0.582-4.463); 0.358
CARD8 (rs2043211)	A/A	137	53667	Reference	Reference
	A/T	169	57697	1.328 (0.495-3.563); 0.573	0.8 (0.274-2.331); 0.682
	T/T	2	3262	0.204 (0.011-3.846); 0.289	0.075 (0.004-1.428); 0.085
	A	443	165031	Reference	Reference
	T	173	64221	1.067 (0.348-3.277); 0.909	0.814 (0.271-2.441); 0.713
CARD8 (rs6509365)	A/A	98	38696	Reference	Reference
	A/G	208	72668	1.459 (0.536-3.972); 0.46	1.179 (0.446-3.121); 0.74
	G/G	2	3262	0.224 (0.012-4.273); 0.32	0.115 (0.006-2.169); 0.149
	A	404	150060	Reference	Reference
	G	212	79192	1.108 (0.383-3.207); 0.85	1.001 (0.368-2.724); 0.998
IL-1β (rs1143634)	G/G	222	81499	Reference	Reference
	A/A	0	2542	NC	NC
	G/A	86	30585	0.92 (0.322-2.626); 0.876	1.8 (0.691-4.684); 0.229
	G	530	193583	Reference	Reference
	A	86	35669	0.746 (0.203-2.741); 0.659	1.23 (0.369-4.103); 0.736

[a]Number of events: number of viral load blips (defined as the number of detectable HIV-1 viral loads after HIV diagnosis).
[b]pY: person-years at risk were calculated from HIV diagnosis until a cART treatment initiation.
[c]Relative risks were adjusted by gender, age at HIV diagnosis, education and skin color whenever applicable, which were variables associated with multiple events of viral load blips in the bivariate analysis ($P < 0.2$).
Abbreviations: RR = risk ratios or relative risk; aRR = adjusted relative risk; NC = not calculated.

among HIV controllers (Lazaryan *et al.*, 2011) and long-term non-progressors (LTNPs) (Shepherd *et al.*, 2015). The NLRP3 rs10754558-G SNP has already been described as a protective factor against HIV-1 infection in different ethnic groups (Pontillo *et al.*, 2010, 2012). Moreover, *in vitro* assays showed that rs10754558 was associated with NLRP3 mRNA stability since the PBMCs isolated the CG and GG patients increase the NLRP3 expression approximately 2-fold

when compared to CC patients (Ravimohan *et al*., 2018). These results indicate that the protection to the disease exerted by the variant possibly depends on the stable expression of the NLRP3. Although our findings do not corroborate previous studies, it is relevant to note that the current study has some limitations, as the lack of a functional NK cell characterization of HICs and the limited number of participants enrolled in our cohort. EC cohorts are difficult to establish and maintain, given that they represent a rare group of individuals. Therefore, to obtain more reliable results, additional studies with larger populations and functional studies are needed to better understand the importance and role of the HLA-C*08 and the rs10754558 G allele in the context of HIV infection.

In conclusion, our results showed an association between the absence and presence of host immunogenetic markers with the frequencies of high-level viremia episodes and the frequency of VL blips in our HICs cohort, highlighting the protective role of KIR2DL3 and KIR2DS5. Besides, we confirmed that protective HLA-B and HLA-C allelic frequencies are higher in HICs than in HIV-1 non-controllers and in the general population. Although the limitations of this study, the results presented here provide an essential contribution to understanding the genetic factors that can modulate viral control in these individuals. Additional studies aiming to identify the role of host genetics in persistent *vs.* transient control of HIV-1 replication in HICs will undoubtedly help a more efficient clinical management of this particular cohort.

Data availability

Underling data

Open Science Framework: Killer immunoglobulin-like receptor (KIR) genes are associated with the risk of episodes of high-level and detectable viremia among HIV controllers. https://doi.org/10.17605/OSF.IO/ZGQPA (de Sá & Teixeira, 2021).

This project contains the following underlying data:

- CCR5 Raw Data.pdf (PDF containing CCR5 raw gel images)
- Epidemiological data of HICs.csv (Epidemiological characteristics of HICs)
- Clinical data of HICs.csv (Immunological and virological characteristics of HICs)
- Genetic characterization of HICs.csv (HLA-B, HLA-C, KIR and SNPs of the NLRP3, CARD8, IL-1β genes raw data of HICs)
- KIR Results.pdf (PDF containing KIR gels raw images of HICs)

Extended data

Open Science Framework: Killer immunoglobulin-like receptor (KIR) genes are associated with the risk of episodes of high-level and detectable viremia among HIV controllers. https://doi.org/10.17605/OSF.IO/ZGQPA (de Sá & Teixeira, 2021).

This project contains the following extended data:

- Consent Form.pdf (PDF containing the original and English version)
- Project consent form (PDF containing the ethical committee approval)

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

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