Genetic variations in loci relevant to natural killer cell function are affected by ethnicity but are generally not correlated with susceptibility to HIV-1

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Abstract
Polymorphisms in cell surface receptors of natural killer cells and their ligands on target cells can affect susceptibility to viral infections including human immunodeficiency virus (HIV)-1. We found that the carriage of the human leukocyte antigen (HLA)-G minus 14-bp polymorphism, LILRB1 single nucleotide polymorphism rs1061680, and activating and inhibitory killer immunoglobulin-like receptors (KIRs) were different when data were compared between Caucasian, African Americans and Asian populations. However, carriage was similar when HIV-1 patients were compared with control donors, with the exception of the African American cohort.

Natural killer (NK) cells are crucial in controlling viral infections. For example; individuals without functional NK cells are particularly susceptible to cytomegalovirus (CMV) disease (1). In human immunodeficiency virus (HIV)-1 infected patients, NK cells are reduced in numbers and show diminished function as HIV-1 disease progresses (2). NK cells express activating and inhibitory receptors, which recognize host cells via classical and non-classical major histocompatibility complex class I molecules (MHC-I), which affect the progression of HIV-1 disease (3).

Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1, LIR-1, CD85j) is an inhibitory receptor that recognizes MHC-I molecules, including HLA-G (4). LILRB1 is expressed on NK cells, monocytes, macrophages, dendritic cells and subsets of B cells and T cells. A non-synonymous single nucleotide polymorphism (SNP) in the putative ligand-binding domain of LILRB1 protein (rs1061680) associates with susceptibility to rheumatoid arthritis (5). rs1061680 is found in LILRB1 haplotypes present in Japanese, American Caucasian, French and Venezuelan populations (5).

NK cell activity is also regulated by killer immunoglobulin-like receptors (KIR) that interact with HLA-B, HLA-C or HLA-G on targeted cells. The 16 known KIR genes encode both inhibitory and activating receptors (6). Inhibitory KIRs possess long cytoplasmic tails bearing immunoreceptor tyrosine-based inhibitory motif residue and upon dephosphorylation by SHP-1, leads to recruitment of Src family kinase and decrease downstream NK cell activation signals (7). The presence of KIR inhibitory and activating receptors can influence the outcome of bone marrow
transplantation and diseases such as HIV-1 infection, CMV end-organ disease, Hepatitis C virus and Hepatitis B virus (8). Carriage of the KIR3DS1 gene and a variant of HLA-B (Bw4 with isoleucine 80) associates with slower progression to acquired immune deficiency syndrome (9).

HLA-G is a non-classical HLA-class 1b molecule thought to regulate the function of NK cells, T cells and dendritic cells (10). HLA-G is expressed in inflamed skin and muscle, and tissues affected by multiple sclerosis, tumours, allografts and HIV-1 infection (11). HLA-G may exert its inhibitory functions by interacting with LILRB1, LIR-2 or KIR2DL4. Over-expression of HLA-G is linked with up-regulation of these molecules on NK cell lines and may facilitate immune evasion (10). A 14-bp insertion/deletion polymorphism in HLA-G (rs16375) associates with susceptibility to CMV disease (12), idiopathic dilated cardiomyopathy (13) and other conditions (13).

This study addressed SNPs and genes affecting NK cell function that may influence the outcome of HIV-1 disease across multiple ethnicities. It is feasible that populations with genotypes promoting NK activity may be protected against HIV infection. This has never been addressed. We examined carriage of polymorphisms in HLA-G and LILRB1 and carriage of activating and inhibitory KIR genes in Asian, Caucasian and African American donors and evaluated whether carriage differed between individuals with confirmed HIV-1 infection (HIV+ ) and unselected healthy donors. We also compared KIR frequencies from all HIV+ cohorts with healthy controls (14, 15). This study addresses the distribution of these polymorphisms across diverse ethnic groups.

Samples were available from population-based cohorts from Busselton, Western Australia (WA), Australia (n = 193, ethnically Caucasian) and Kubang Kerian, Kelantan, Malaysia (n = 78, ethnically Chinese). It was assumed that these donors were HIV-1 negative. Samples were also collected from HIV-1 patients attending the Infectious Disease Clinic at University Malaya Medical Centre, Kuala Lumpur, Malaysia (n = 61), Royal Perth Hospital, Perth, WA, Australia (n = 25) and University Hospitals/Case Medical Center in Cleveland, Ohio, USA (n = 218). Demographic details were not recorded, but all diagnoses were confirmed by serological and/or virological assessments. All individuals were required to nominate their ethnicity during sample collection. Nominated ethnicities were consistent with geographic location. The study was approved by the human subjects institutional review boards of participating institutions, and informed consent was given by the individuals and/or their guardians.

DNA samples were extracted from saliva or blood leukocytes using QIAamp DNA Mini Kits (QIAGEN, Valencia, CA) and subjected to multiplex polymerase chain reaction (PCR)-sequence-specific primers for KIR genotyping (except KIR framework loci 3DL3, 2DL4 and 3DL2) (16), Taqman® allelic discrimination assays (Applied Biosystems assay C-9491145-10, Foster City, CA) for LILRB1 SNP rs1061680 and PCR amplification with gel electrophoresis to screen for the HLA-G 14-bp polymorphism (rs16375) (12, 13, 17). HLA-G 14-bp polymorphism and LILRB1 SNP rs1061680 frequencies were in Hardy–Weinberg equilibrium in every ethnic group studied (P > 0.05). Allele frequencies were compared using Fisher exact test. Numbers of activating and inhibitory KIR were calculated for each donor (13), presented as mean ± standard error (Table 1) and compared using student’s t-test. P < 0.05 was accepted as indicating a significant difference.

The HLA-G -14bp allele is defined as allele 1 as it was the more common allele in all populations tested, as expected (12, 13, 17, 18). Carriage of allele 1 was not associated with HIV-1 status among Chinese or Australian Caucasian donors. Carriage differed between Caucasians and Chinese control donors (Figure 1; Fisher exact test, P = 0.003), but was similar in all HIV+ populations tested. A Spanish study showed higher expression of HLA-G among HIV+ patients and suggested this may be an immune evasion mechanism (19). Several studies suggest a role for rs16375. Individuals with the HLA-G* G1,1 had significantly higher soluble HLA-G levels (12, 20). Soluble HLA-G may exert immune suppressive properties by binding inhibitory NK cell receptors such as KIR2DL4 and LILRB1 (21). However, HIV+ African Americans showed lower carriage of HLA-G* G1,1 when compared with published data for healthy African Americans (22) (P < 0.0001).

In a Japanese study, rs1061680 was part of a haplotype shown to affect rheumatoid arthritis (5). Here homozgyous carriage of allele 1 (T/T) of the LILRB1 SNP rs1061680 was similar in HIV+ donors and controls (Australian, P = 0.6; Chinese, P = 0.5; Fisher exact test). Comparison of HIV+ African Americans with healthy Nigerians (www.HapMap.org) showed lower carriage of allele 1 in the patients (P < 0.001). Amongst the controls, Australians

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Chinese HIV-1 patients carry less activation KIR than Caucasians*</th>
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<tr>
<td>HIV+ Australian Caucasian</td>
<td>Healthy Australian Caucasian controls</td>
</tr>
<tr>
<td>Activating</td>
<td>2.6 ± 0.24*</td>
</tr>
<tr>
<td>KIR</td>
<td>(n = 53)</td>
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<tr>
<td>Inhibitory</td>
<td>3.6 ± 0.11</td>
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<tr>
<td>KIR</td>
<td>(n = 53)</td>
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HIV, human immunodeficiency virus; KIR, killer immunoglobulin-like receptors.

*Data are presented as mean ± standard error.

*P = 0.01 Student’s t-test: HIV+ Chinese vs HIV+ Caucasian.
carried allele 1 more often than did Chinese ($P = 0.0004$). Carriage of allele 1 was similar in HIV$^+$ Chinese and African Americans ($P = 0.36$), but higher in HIV$^+$ Caucasians (Australian, $P = 0.01$; American, $P < 0.0001$).

All 11 KIR genes were detected in all cohorts tested (Figure 2) with more inhibitory than activating KIR genes (Table 1) (15, 23). Associations with HIV-1 infection in the Australian Caucasians displayed no significant differences in the average numbers of activating and inhibitory genes ($P > 0.05$; Table 1) or the carriage of individual genes (except 3DL1, Figure 2). Average numbers of activating KIR were higher in HIV$^+$ Australian Caucasians compared with Chinese ($P = 0.01$; Table 1), but similar when HIV$^+$ Australian Caucasians were compared with HIV$^+$ African Americans ($P = 0.12$). Carriage of inhibitory KIR was similar in all HIV$^+$ donor groups ($P > 0.05$). Individual KIR genotypes showed that HIV$^+$ Australian Caucasians carried more 2DL2, 2DS2, 2DS3, 2DS5 and 2DS1 compared with HIV$^+$ Chinese and more 2DS2, 2DS1 and 2DS1 compared with HIV$^+$ African Americans ($P < 0.05$; Figure 2). Carriage of 3DL1 was higher in Australian Caucasians controls compared with HIV$^+$ Australian Caucasians ($P < 0.001$). This was consistent with Martin et al. where high expressing 3DL1 alleles combined with HLA-B*57 was the most protective combination against progression of HIV-1 (24). In addition, 99% carriage of 3DL1 among HIV$^+$ African Americans was consistent with similar levels in HIV$^+$ Ugandans (25).

We noted significant differences in KIR2DS4 gene frequencies between HIV$^+$ patients and healthy controls; for example, Chinese HIV$^+$ patients and African American HIV$^+$ patients carried lower 2DS4 compared with their healthy control counterparts ($P < 0.001$). Although this may suggest a protective role of 2DS4, other publications have associated 2DS4 as a risk factor for HIV-1 transmission (26). Further studies is required especially association studies which includes 2DS4 ligands; HLA-A11 and HLA-C.

All polymorphisms assessed may affect the cytolytic function and/or cytokine production of NK cells. rs1061680 may alter the binding domain of LILRB1 (5), while the −14 bp polymorphism genotype may confer higher expression of HLA-G (27). In theory, co-inheritance of genes for any NK cell receptor-ligand pair that weakens NK cell inhibition in...
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**Figure 2.** Killer immunoglobulin-like receptors gene frequencies display differences associated with ethnicity when compared between human immunodeficiency virus (HIV)+ Australian Caucasians, healthy Australian Caucasians, HIV+ African Americans, healthy African Americans (14), HIV+ Chinese donors and healthy Singaporean Chinese (15). *P value of 0.01–0.05; **P value of 0.001–0.01; ***P value of <0.001.

Inhibitory KIR genes

Activating KIR genes

In summary, the allele frequencies related to the HLA-G −14 bp polymorphism, LILRB1 SNP rs1061680, activating and inhibitory KIRs varied among ethnic populations tested. This establishes the importance of tight control of ethnicity in any future studies. Whilst the genotypes did not associate consistently with presence of HIV-1 infection, interesting associations were evident in African Americans. These warrants follow-up in other African populations. Importantly, this study did not address a role for these genotypes in disease progression, as has been shown for HLA-G*0105N (28) and carriage of KIR 3DL1 with HLA-Bw4 (9). Analysis of other polymorphisms affecting LILRB1, HLA-G and KIR genes in a larger cohort may illuminate the protective role of NK cells against HIV-1 disease.

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Conflict of interests

The authors have declared no conflicting interests.
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