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Killer-cell immunoglobulin-like receptor (KIR) and human leukocyte antigen (HLA) class I genetic diversity in four South African populations

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ABSTRACT

Killer-cell Immunoglobulin-like Receptor (KIR) and *Human Leukocyte Antigen (HLA)* genotypes vary considerably between individuals and populations due to *KIR*/*HLA* allelic variation and variable haplotype configurations of *KIR*. HLA mediate natural killer cell activity by serving as KIR ligands. *KIR*/*HLA* polymorphisms associate with both disease susceptibility and severity. We determined the frequencies of *KIR*, *KIR* genotypes and *KIR-HLA* combinations in 364 healthy individuals from four South African populations. Study participants included black African (n = 167), Caucasian (n = 97), Mixed ancestry (n = 50) and Indian (n = 50) individuals. We identified 48 *KIR* genotypes that included two genotypes not previously reported. Based on *KIR* gene content, Indian individuals represented the most distinct group, showing the highest frequencies of *KIR2DL2, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3* and *KIR3DS1,* the lowest frequencies of *KIR2DL3, KIR2DS4* and *KIR3DL1;* and a *KIR2DL4*-negative individual. *KIR2DS1* and *KIR3DS1* were infrequent in black African populations. *HLA-C2* was more common in black African individuals, while *HLA-C1* predominated in the other populations. Indian individuals were more likely to possess *KIR2DL2* paired with *HLA-C1*, while Caucasian individuals exhibited the highest frequencies of *KIR2DL3* paired with *HLA-C1*. This report provides comprehensive reference data for further study of the roles of *KIR*/*HLA* in non-communicable and infectious diseases in South African populations.

1. Introduction

The killer cell immunoglobulin-like receptors (KIR) are a family of glycoproteins encoded by 14 functional genes (*KIR2DL1*, *KIR2DL2*, *KIR2DL3*, *KIR2DL4*, *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, *KIR2DS5*, *KIR3DL1*, *KIR3DL2*, *KIR3DL3* and *KIR3DS1*) and two pseudogenes (*KIR2DP1* and *KIR3DP1*) located on chromosome 19 [1]. These genes exhibit extensive haplotypic diversity with respect to gene content, copy number and allelic representation within individual genes [2–4]. Variability in terms of *KIR* gene content is determined by the presence of two *KIR* haplotypes [5], whose distributions are maintained within all human populations through balancing selection [6–8].

Natural killer (NK) cell activation is regulated in part, by the interaction between inhibitory/activating KIR and specific human leukocyte antigen (HLA) class I ligands [9]. HLA-C ligands are divided into two allotype subsets that express either the HLA-C1 epitope that recognises KIR2DL2 and KIR2DL3 [10] or the HLA-C2 epitope that recognises KIR2DL1 [11] and KIR2DS1 [12]. HLA-Bw4 epitopes with isoleucine at position 80 (Bw4-80I) serve as ligands for KIR3DL1 and KIR3DS1, while KIR3DL1 additionally recognises HLA-Bw4 epitopes with a threonine at position 80 (Bw4-80T), albeit with a weaker affinity [13–16]. Both KIR2DS2 [17] and KIR3DL2 [18] recognise HLA-A*11 ligands, while KIR2DS4 binds HLA-A*11 and a subset of HLA-C molecules [19]. KIR3DL2 additionally binds to HLA-A*03 ligands [18]. However, some KIR-HLA interactions, like that between KIR3DS1 and HLA-Bw4-80I [15,20] as well as KIR3DL2 with HLA-A*03/*11 have been shown to be peptide-dependent [18].

The association between genetic variability within the loci of *KIR* and *HLA* class I ligands and differential disease susceptibility and outcome has been established [21,22]. Of particular interest in a South African context, is the association observed between specific KIR-HLA receptor-ligand interactions and HIV-1 transmission [23,24] and disease progression [25]. However, the distributions of both these gene families are known to vary, often dramatically, between geographically and ethnically distinct popu-

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lations [6,8,26,27]; a factor, which if unrecognised, could confound later association studies.

Due to its history of colonisation and situation along intercontinental trade routes, South Africa is a highly culturally diverse country. The population is composed of four predominant ethnic groups, each with their own distinct demographic history. The black South African population is heterogeneous, comprising individuals from nine culturally distinct linguistic groups [28]. South African Caucasians are the descendants of English, Dutch, French and German settlers who began colonising the area from around the mid 1600s [29]; while the Mixed ancestry or "Coloured" population arose as a consequence of the interaction of these settlers with the local indigenous people of the Western Cape, as well as slaves from Malaysia, Indonesia and Asia and political exiles from Indonesia [30]. The South African Indian population has a more recent origin, arising from the arrival of indentured labourers and "passenger" Indians from mainland India approximately 150 years ago [31]. Given this rich demographic history, we examined genetic variability within the *KIR* and *HLA* gene complexes in individuals recruited from these four population groups, in order to better understand patterns of genetic diversity within these genes in the South African population.

2. Materials and methods

2.1. Study population

A total of 364 healthy, unrelated individuals from four ethnically diverse South African populations (i.e. black African, Caucasian, Indian and Mixed ancestry), were used to describe patterns of genetic variation within the *KIR* and classical *HLA* class I loci. The 167 black African and 97 Caucasian South Africans included in this study were selected from a larger previously described Electricity Supply Commission (ESKOM) cohort on the basis of a non-reactive HIV-1 enzyme-linked immunosorbent assay (ELISA) test (Genscreen HIV1/2 version 2; Bio-Rad, Marnes-La-Coquette, France) [32]. An additional 22 Indian and 40 Mixed ancestry South Africans were also available from the ESKOM cohort and these numbers were augmented by the prospective recruitment of a further 28 Indian and 10 Mixed ancestry South Africans from staff and students at the National Institute for Communicable Diseases and the University of the Witwatersrand in Johannesburg, bringing the total number of individuals included in these two study populations to 50. Informed consent was obtained from all study participants and the study was approved by the University of the Witwatersrand Committee for Research on Human Subjects.

2.2. DNA extraction

Genomic DNA for genotyping was isolated from either stored buffy coat or fresh whole blood samples and quantified using a nanodrop spectrophotometer (Thermo Scientific, Waltham, USA). DNA from buffy coat samples was extracted using the PEL-FREEZ DNA isolation kit (DYNAL Invitrogen Corporation, Carlsbad, California, USA), while DNA from whole blood samples was extracted using the QiaAmp DNA blood mini kit (Qiagen, Hilden, Germany).

2.3. KIR genotyping

Individuals were genotyped for the presence/absence of the 16 *KIR* genes using a previously described real-time PCR assay [33], with slight modifications. Namely, a second set of primers were designed for the detection of *KIR2DL3*, *KIR2DS1* and *KIR3DP1*, in order to ensure coverage of allelic variants not captured by the original assay (Table S1). The new primer sets were evaluated alongside the published primers for *KIR2DL3*, *KIR2DS1* and *KIR3DP1* during data collection and shown to be in agreement with the presence/absence of the gene of interest. Reactions were performed in a 5 μl volume, containing 2× Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Burlington, Canada), 0.2 μM of *KIR*-specific primers, 0.2 μM of *galactosylceramidase*-specific primers and 5 ng of DNA. Thermocycling was performed using the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Foster City, California, USA) under the following conditions: 95 °C for 10 min, followed by 30 cycles of 95 °C for 15 s and 60 °C for 1 min, with subsequent melt curve analysis.

2.4. KIR genotype assignment

Individuals were assigned either AA or Bx *KIR* genotypes, according to the guidelines and nomenclature stipulated by the Allele Frequencies Database (www.allelefrequencies.net). AA *KIR* genotypes were defined as consisting of the four framework genes (*KIR2DL4*, *KIR3DL2*, *KIR3DL3* and *KIR3DP1*) in combination with *KIR2DL1*, *KIR2DL3*, *KIR2DS4*, *KIR2DP1* and *KIR3DL1*, while Bx genotypes were defined by the presence of the framework genes and any one or more of the following genes: *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS5* and *KIR3DS1*.

2.5. HLA class I genotyping

Published high resolution *HLA-A*, *-B* and *-C* genotype data were available for all black African and Caucasian South Africans [32,34] and South African Indian [50] and Mixed ancestry populations [51]. Comparison of *HLA-A*, *-B* and *-C* allele frequencies in all four populations are listed in Supplementary Tables S2, S3 and S4.

2.6. Statistical analyses

KIR gene and *HLA* class I allele frequencies were determined by direct counting. The significance of differences in the frequencies observed between population groups was assessed using a two-sided Fisher's exact test, as implemented in GraphPad Prism v4.02 (GraphPad software, San Diego, USA). Deviations from Hardy-Weinberg equilibrium were assessed using the Markov chain exact test implemented using Arlequin v3.5.1.2 [35]. All statistical measures were considered significant at $p < 0.05$.

3. Results

3.1. KIR gene diversity

We examined *KIR* gene diversity in 364 South African individuals from four ethnically distinct populations (Table 1). The four framework *KIR* genes (*KIR2DL4*, *KIR3DL2*, *KIR3DL3* and *KIR3DP1*) were present in all individuals, other than a single Indian individual who was found to lack *KIR2DL4*. The pseudogene, *KIR2DP1* and inhibitory receptor *KIR2DL1* were also found to occur at frequencies greater than 94% in all four populations (Table 1). The frequencies of *KIR2DL3*, *KIR2DS4* and *KIR3DL1* were found to be lowest in the Indian population group, while the frequencies of *KIR2DL2*, *KIR2DL5*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3* and *KIR3DS1* were highest in this group. *KIR2DS2* and *KIR2DS5* frequencies in the black African and Caucasian populations were lower than those seen in the Indian and Mixed ancestry groups, while the frequencies of *KIR2DS1* and *KIR3DS1* were found to be lowest in the black African population and highest in the Indian population group.

We further compared our results with those from similar studies conducted in other population groups (Tables S5–S8). The frequencies of *KIR2DS1* and *KIR3DS1* observed in the black South