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KIR2DS4 allelic variants: Differential effects on in utero and intrapartum HIV-1 mother-to-child transmission

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Abstract *KIR2DS4* is the only activating gene within the A haplotype, and alleles of *KIR2DS4* can encode either functional (KIR2DS4-f) or non-functional (KIR2DS4-v) variants. To establish the role of *KIR2DS4* in the context of HIV-1 mother-to-child transmission, we *KIR* genotyped 145 HIV-1 non-transmitting mothers (NT) and their exposed uninfected infants (EU), and 72 HIV-1 transmitting mothers (TR) and their infected infants [intrapartum (IP), in utero (IU) or IU2 (an IU-enriched infected group)]. The frequency of *KIR2DS4-v* was significantly higher in IU2 infants compared to EU infants (P = 0.022, OR = 2.88); this association was more significant amongst AA haplotypes (P = 0.004, OR = 18.4). Possession of *KIR2DS4-f* in the mother with absence in the infant (M+I− discordance) was associated with a higher risk of IP transmission (P = 0.005, OR = 3.84); whilst in M−I+ discordance, infant possession of *KIR2DS4-v* was associated with increased risk of IU acquisition (P = 0.002; OR = 6.40). This study highlights the importance of *KIR2DS4* in HIV-1 transmission/acquisition.

1. Introduction

Natural killer (NK) cells are cytotoxic lymphocytes crucial to the innate immune system in that they are able to provide rapid response to tumour and viral infected cells. NK cells are able to bridge the gap between the innate and adaptive immune systems through the release of cytotoxic granules and pro-inflammatory cytokines that signal to other immune cells such as dendritic cells and CD4+ T cells [1–4]. Several studies have described the importance of NK cells in HIV-1 infection [5–12] and strategies evolved by HIV-1 to specifically evade NK cell-mediated immune responses [1,3,13–15]. Furthermore, there is increasing evidence for a role of NK cells in

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protection from acquisition of HIV-1 infection in HIV-1 exposed uninfected adult cohorts [16–19] and in HIV-1 mother-to-child transmission (MTCT) [20,21].

NK cell function is regulated by the fine balance of cell surface inhibitory and activating receptors including the C-type lectin receptors and killer cell immunoglobulin-like receptors (KIRs) [2,4,22]. The *KIR* gene family encodes cell surface receptors that are found on NK cells and certain T-cell subsets. Fourteen functional *KIR* genes have been identified, of which seven are inhibitory (*KIR2DL1–3*, *KIR2DL5*, *KIR3DL1–3*), six are activating (*KIR2DS1–5* and *KIR3DS1*), and *KIR2DL4* has both inhibitory and activating potential [23]. Based on the number and type of *KIR* genes, two broad haplotype groups have been defined: Group-A haplotypes have a fixed *KIR* gene content of *KIR2DL1*, *-2DL3*, *-2DL4*, *-3DL1*, *-3DL2*, *-3DL3*, *-2DS4* and pseudogenes *KIR2DP1* and *KIR3DP1*. Haplotypes carrying any other combination of *KIR* are classified as Group-B. In general, Group-B haplotypes have a more activated *KIR* gene profile, whilst Group-A have a more inhibited *KIR* gene profile with *KIR2DS4* as the only activating *KIR* gene.

Known *KIR2DS4* alleles can be divided into those encoding full-length functional cell surface receptors (*KIR2DS4*0010101-00103*) and alleles encoding truncated non-functional variants (*KIR2DS4*003*, **004*, **006*, **007*, **008*, **009*), designated as *KIR2DS4-f* and *KIR2DS4-v*, respectively. Based on affinity assays, KIR2DS4-f variants have been reported to interact with a select group of HLA-C molecules, namely three encoded by HLA-C alleles with the C1 epitope (C1) defined by asparagine at position 80 (*C*01:02*, *C*14:02*, *C*16:01*) and three C2 epitope (C2) alleles (*C*02:02*, *C*04:01*, *C*05:01*) defined by lysine at position 80 [24], as well as two HLA-A molecules encoded by alleles *A*11:01* and *A*11:02* [25–27]. *KIR2DS4-v* alleles differ from the *KIR2DS4-f* alleles, in that they have a 22-bp deletion in exon 5 which causes a frame shift that produces a premature stop codon, preventing the formation of a functional membrane-bound receptor [17,25,28–30]. Therefore individuals who are homozygous for the Group-A haplotype and that harbour *KIR2DS4-v* alleles, will not have a membrane-bound *KIR2DS4* protein but rather a soluble form of KIR2DS4 [30].

Globally *KIR2DS4-f* and *KIR2DS4-v* frequencies vary amongst different population groups, with *KIR2DS4-v* alleles accounting for a substantial proportion (up to 89%) in Caucasians and approximately 30% in Asians of the total *2DS4* alleles in human populations [30]. In Caucasians the ratio of *KIR2DS4-f* to *KIR2DS4-v* is 1:2 [31], whilst in Asian populations (Japanese and Koreans) it is 2:1; thus in AA haplotypic individuals the overall percentage of Koreans without any activating gene is 3.9% lower than that of Caucasians (16.5%) [32]. In a Zambian population, the frequency of *KIR2DS4-f* and *KIR2DS4-v* was found to be 79% and 57%, respectively [17]. Similarly, in a South African Xhosa population study, the frequency of *KIR2DS4-v* was found to be 54% [30].

There has been increasing attention paid to the role of *KIR* gene content and *KIR* allelic variation in infectious diseases such Hepatitis C and HIV [12,33–36], with some studies focussed more specifically on *KIR2DS4* [17,37–40]. In Zambian serodiscordant couples, Merino et al. [17], showed that carriage of *KIR2DS4-f* was associated with relatively higher viral load (VL) for HIV-1 in index (HIV-1 seroprevalent) partners compared with index partners without the allele, and even after statistical adjustment for gender, VL was significantly higher in *KIR2DS4-f* index partners. Additionally, carriage of *KIR2DS4-f* was associated with accelerated transmission of HIV-1 to cohabiting seronegative partners.

These observations have raised the question of how allelic variants of *KIR2DS4* might influence the likelihood of HIV-1 infection through other modes of transmission. This current study uses the MTCT model to address this question in the context of infant HIV-1 infection acquired during pregnancy (in utero, IU) or during labour and delivery (intrapartum, IP). Importantly, having data on both transmitter (mother) and recipient (infant) allowed us to assess concordant and discordant possession of *KIR2DS4* alleles amongst matched mother–infant pairs. Findings confirm a greater likelihood of transmission in the presence of full-length *KIR2DS4*, suggesting similarities between adult sexual transmission and maternal–infant intrapartum transmission. In contrast, acquisition of infection in utero was characterized by the absence of full-length *KIR2DS4* in the infant, suggesting that a less activated NK cell phenotype in the infant might be deleterious upon HIV-1 exposure during pregnancy.

2. Materials and methods

2.1. Cohort

This study was a retrospective analysis of 217 Black South African mother and infant pairs recruited as part of four mother-to-infant HIV-1 transmission cohorts in Johannesburg, South Africa, as described by Kuhn et al. [41] and Paximadis et al. [20].

HIV-1 transmitting (TR) mothers and their HIV-1 infected infants were compared with HIV-1 non-transmitting (NT) mothers and their exposed uninfected (EU) infants. All available TR/infected mother–infant pairs (n = 72) were matched in a 1:2 ratio with two random samples of two NT/EU mother–infant pairs (n = 145) from each cohort using a case-cohort design.

Infected infants were further characterized according to their time of HIV-1 acquisition as determined by a HIV-1 DNA PCR test (Roche Amplicor version 1.5) at birth and at 6 weeks of age: 19 were in utero (IU) infected (PCR positive at birth and at 6 weeks), 29 were intrapartum (IP) infected (PCR negative at birth but positive at 6 weeks). The remaining 24 infants were found to be infected at 6 weeks but had no birth sample available (i.e. unknown mode of infection).

All infants received single-dose Nevirapine (sdNVP) and approximately half the mothers received sdNVP during labour (NT: 81/145, 56% and TR: 41/72, 57%). Thus in analyses where we corrected for the influence of maternal sdNVP, all mothers and infants that received other antiretroviral drugs were excluded from this part of the analysis. Since maternal sdNVP administration is known to only reduce IP-transmission, it was expected that there would be significantly less IP-infants from the sdNVP-treated TR mothers compared to the untreated TR mothers (10/41, 24% vs. 16/23, 70%, respectively, $P < 0.001$). Using this information we further assessed the 24 infants of unknown mode of transmission based on the administration of maternal sdNVP. Within this subgroup, there was a far greater proportion of mothers who received sdNVP compared to those without (19/24, 79.2% vs. 1/24, 4.2%, respectively, $P < 0.0001$), whilst the remaining 4 mothers either received other antiretroviral drugs or had no