Impact of a Functional *KIR2DS4* Allele on Heterosexual HIV-1 Transmission among Discordant Zambian Couples

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Killer cell immunoglobulin-like receptors (KIRs) and their HLA ligands interact to regulate natural killer (NK) cell function. KIR gene content and allelic variations are reported to influence human immunodeficiency virus (HIV)-1 infection and pathogenesis. We investigated the impact of KIR genes on heterosexual HIV-1 transmission among 566 discordant couples from Lusaka, Zambia. KIR2DS4*001, the only allele of *KIR2DS4* known to encode a functional activating receptor, was associated with relatively high viral load for HIV-1 in index (HIV-1 seroprevalent) partners (β [standard error (SE)], .17 [.8] $\log_{10}$; $P = .04$) and with accelerated transmission of HIV-1 to cohabiting seronegative partners (relative hazard [RH], 2.00; $P = .004$). The latter association was independent of the direction of transmission (male-to-female or female-to-male), genital ulcers, and carriage of the putative ligand (HLA-Cw*04). No KIR-gene variant in the initially seronegative partners was associated with HIV-1 acquisition or early viral load following seroconversion. Further analysis of NK cell function should clarify the role of KIR2DS4*001 in HIV-1 transmission.

The products of multiple killer cell immunoglobulin-like receptor (KIR) genes are present predominantly on the surface of natural killer (NK) cells and occasionally on subsets of T cells [1]. KIRs influence the killing state of NK cells, often via interaction with human leukocyte antigen (HLA) class I proteins, and KIR-gene products show several distinctive patterns of binding to HLA alleles. The leukocyte-receptor complex is formed by 15 KIR genes and 2 pseudogenes clustered at chromosome 19q13.4. KIR-complex polymorphism is characterized by haplotypes that differ by the contents of their individual KIR genes and by moderate to extensive allelic variations within a number of the loci. As with HLA diversity KIR-gene polymorphisms likely arose from balancing selection in the context of evolving immune function [2].

A KIR can inhibit or activate NK-cell function depending on the type of signaling it transduces through its cytoplasmic tail [3]. Inhibitory KIRs are characterized by long cytoplasmic tails (designated by "L" in the gene name) that carry an immunoreceptor tyrosine-based inhibition (ITIM) motif, which interacts with Src homology 2–containing tyrosine phosphatases [4]. Activating KIRs with short (S) cytoplasmic tails have a charged residue in the transmembrane region, which mediates interaction with DnaX-activating protein 12 (DAP12), a cytoplasmic protein with an immunoreceptor tyrosine-based activation (ITAM) motif [5]. The extracellular portion of the KIR molecule consists of 2 (2D) or 3 (3D) Ig-like domains that typically interact with HLA-C or HLA-B molecules, respectively [6].

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Variations in KIR-gene content have been associated with autoimmunity, transplantation success, and infectious diseases such as hepatitis C and human immunodeficiency virus (HIV)/AIDS [7–9]. Recently, increasing attention to KIR gene allelic effects on HIV/AIDS has focused on analyses of *KIR3DS1* and *KIR3DL1* alleles and the variants of the ligand HLA-Bw4 [9–11]. In addition to KIR, other genetic and nongenetic factors have been associated with HIV/AIDS. Various HLA markers, most notably alleles of *HLA-B*, affect progression to AIDS. Alleles like HLA-B*57 and B*27 and the Bw4 epitope are associated with slowed progression, whereas at least some HLA-B*35 alleles may be associated with accelerated progression to AIDS [12]. Genetic variability in the HIV coreceptor *CCR5* has been associated with significant protection from acquisition of the virus. Several nongenetic factors influence the transmissibility of HIV-1. High plasma viral load and herpes simplex co-infection (in the absence of ulceration) are associated with increased HIV-1 shedding in semen [13, 14]. Genital ulceration (whether related to herpes simplex or of unknown etiology) causes direct shedding of HIV-1 from the ulcer surface [15]. The presence of other sexually transmitted infections, such as syphilis and gonorrhea, has been associated with accelerated HIV-1 acquisition [16, 17].

The Zambian cohort of serodiscordant couples is among the largest under study. Because of the prospective nature of the study, events related to transmission are observed in both partners over time. We have previously described the influences of several HLA class I alleles and haplotypes on 3 related outcomes of HIV-1 infection: transmission by index partners, acquisition by seronegative partners, and virologic control of HIV-1 infection in seroprevalent and seroconverted partners, who were enrolled and observed as cohabiting, serodiscordant couples (one partner HIV-1 seropositive and the other seronegative) [18–21]. In this article, we report associations between KIR-gene distribution and those 3 HIV-1–related outcomes in the Zambian cohort.

PARTICIPANTS AND METHODS

Study Population

Between 1995 and 2006, 616 HIV-1 serodiscordant couples were enrolled in Lusaka, Zambia, as part of the Rwanda/Zambia HIV-1 Research Group. We considered all couples with ⩾9 mo (274 d) of follow-up without antiretroviral treatment eligible for inclusion. We described initial screening, testing, and quarterly medical examinations in a previous article [22]. We determined HIV-1 transmission status at quarterly medical examinations by dipstick HIV-1/HIV-2 Ab screening assay with Capillus latex aggregation for confirmation and Uni-Gold Recombigen HIV test (Trinity Biotech). We established the viral subtype and intracouple linkage of virus by viral sequencing and phylogenetic analysis for both seropositive partners after transmission [23]. Data on couples were censored at the time of seroconversion, on withdrawal from the study, or on 31 December, 2006 (whichever occurred first). All participants were naive to antiretroviral treatment and provided written, informed consent. For this study, we included 566 couples based on known viral linkage (identity of the virus between the index and seroconverting partners), sufficient follow-up, and adequate biologic specimens (for HLA and KIR genotyping). Depending on whether transmission had occurred by the censoring date, we classified index partners as transmission-pair index partners (TPIs) or non-transmission-pair index partners (NTIs); nonindex partners were classified as exposed seronegatives (ESNs) or seroconverters (SCs) [21]. This study conforms to (1) the procedures for informed consent approved by institutional review boards at all sponsoring organizations, and (2) human-experimentation guidelines set forth by Department of Health and Human Services, United States of America.

HIV-1 Viral Load Measurement and Analysis

HIV-1 RNA copies in patient plasma were quantified by Roche Amplicor 1.0 assay (Roche Diagnostics Systems) in laboratories certified by the Virology Quality Assurance Program of the AIDS Clinical Trials Group. The lower detection limit was 400 copies per mL of plasma. Previous analyses [22] indicated that index partners with medium (10^4–10^5 copies/mL) and high ($>10^5$ copies/mL) levels of HIV-1 RNA were more likely to transmit the infection than those with low viremia ($<10^4$ copies/mL). Viral load (VL) was analyzed as a categorical variable (high, medium, and low) and as a continuous variable ($\log_{10}$ VL).

Genotyping of Killer Cell Immunoglobulin-like Receptor and HLA Class I Genes

We extracted genomic DNA from whole blood or buffy coat using QIAamp blood kits (Qiagen). We determined KIR gene content and certain alleles of *KIR2DS4* and *KIR3DP1* by polymerase chain reaction with sequence-specific primers (PCR-SSP; Invitrogen). We performed HLA genotyping using PCR with sequence-specific primers (Dynal/Invitrogen), automated reference-strand conformation analysis (Dynal/Invitrogen), automated sequence-specific oligonucleotide probe hybridization (Innogenetics), and automated sequencing-based typing (Abbott Molecular). With these techniques, we achieved medium to high resolution of HLA class I alleles. We resolved ambiguities by sequencing-based typing with capillary electrophoresis and an ABI 3130 ×1 DNA analyzer (Applied Biosystems).

Haplotype Assignment

We assigned KIR haplotypes using HAPLO-IHP, a program that combines a greedy algorithm with an expectation-maximization method based on previously identified haplotype patterns [24]. For the analysis, we retained participants whose haplotypes were assigned with ⩾.70 probability for the first assigned match, or ⩾.50 probability for the first match and ⩽.20 probability for

the second match. Haplotypes from 733 of 1132 participants were resolved (64.8%).

Statistical Analyses

We used statistical routines in SAS (Statistical Analysis Software, version 9.2) for the following analyses. First, we compared patient characteristics in transmitting and nontransmitting partners by χ^2 (categorical variables) or *t* test (continuous variables). Using logistic regression models, we tested the relationships of KIR genotypes and KIR/HLA combinations in index partners to HIV-1 transmission events within the study period. We used the Bonferroni correction to adjust *P* values for the KIR genes/alleles screened initially for transmission [25]. We treated nongenetic factors including age, genital ulcers/inflammation (GUI) in index and nonindex partners, and VL in index partners as covariates in appropriate models. Third, we analyzed the relationship of KIR genotypes to VL as both a categorical and a continuous variable in chronically infected index partners and the relationship to GUI in all partners. Fourth, with Kaplan–Meier plots we compared time-to-transmission by index partners according to their KIR genotypes (using log-rank and Wilcoxon tests). We included genetic and nongenetic factors with suggestive effects on HIV-1 transmission in multivariable Cox proportional hazards models. Fifth, in a similar way, we tested KIR and KIR/HLA combinations in nonindex partners for possible relationships to HIV-1 acquisition. Lastly, we examined the impact of haplotypes with a minimum cohort frequency of 1.0% on HIV-1 transmission and VL using the same procedures outlined for single-gene analysis.

RESULTS

Characteristics of Zambian Couples Available to This Study

Of 566 couples analyzed, 240 were transmission pairs with viral linkage and 326 were nontransmission pairs (Table 1). All transmission pairs were infected with HIV-1 subtype C. For both TPIs and NTIs, intracouple age differences were comparable (age Δ [standard deviation (SD)], 6.8 [4.7] years in TPI; 7.1 [5.1] years in NTI; $P > .10$). On average both female and male partners were younger in the TPI group compared with those in the the NTI group ($P = .01$). Male-to-female transmission (MTF) was more common ($n = 147$) than female-to-male (FTM) transmission ($n = 93$). Mean and median follow-up times in the nontransmission pairs were nearly double the mean and median times to seroconversion in the transmission pairs ($P < .001$).

For the entire follow-up period, GUI in the 6 mo preceding partner seroconversion occurred in 50.5% of TPIs compared with 22.6% of NTIs ($P < .001$). The proportion of SCs who had GUI was higher than that among the ESNs (45.7% vs 10.6%; $P < .001$). These differences were unchanged after stratification for FTM and MTF transmission. GUI was included as a covariate in subsequent analyses.

Distribution of Killer Cell Immunoglobulin-like Receptor Genes

All 17 KIR genes and the 4 alleles tested were present in the Zambian population. Their frequencies did not differ significantly between index and nonindex partners ($P > .05$). The 4 so-called framework loci (*KIR3DL3*, *KIR3DP1*, *KIR2DL4*, and *KIR3DL2*), ostensibly present in all individuals, as well as the inhibitory gene *KIR3DL1* and the pseudogene *KIR2DP1* were present at >98% (Table 2). The frequency of *KIR3DS1* in Zambians (10.4%) was significantly lower than that in either whites (22.5%) or Chinese (37.6%) [26].

Primary Analyses of Killer Cell Immunoglobulin-like Receptor Genes in Relation to HIV-1 Transmission by Index Partners

In univariate comparisons, the frequencies of KIR2DS4*001 and *KIR2DP1* were significantly higher in TPIs than in NTIs ($P < .05$) (Table 2). These 2 markers were not in strong linkage disequilbrium ($r^2 = .28$). The association of *KIR2DP1* with

Table 1. Characteristics of 566 Couples and Their Constituent Partners Included in the Study of Zambian Serodiscordant Couples

	By Individual						By Couple			
	Index partners			Nonindex partners			Transmission		Nontransmission	
	TPIs	NTIs	*P*	SCs	ESNs	*P*	FTM	MTF	F+M-	M+F-
No. of participants	240	326	NA	240	326	NA	93	147	178	148
Men age, years (SD)	33.5 (7.5)	35.7 (8.0)	.01	32.3 (7.6)	34.8 (8.1)	.01	32.3 (7.6)	33.5 (7.5)	34.8 (8.1)	35.7 (8.0)
Women age, years (SD)	26.1 (5.9)	28.1 (5.9)	.01	26.2 (6.2)	28.6 (7.2)	.003	26.2 (5.9)	26.3 (6.2)	28.1 (5.9)	28.6 (7.2)
Age Δ, years (SD)	6.8 (4.7)	7.1 (5.1)	.76	6.8 (4.7)	7.1 (5.1)	.75	6.2 (4.9)	7.2 (4.6)	7.1 (5.5)	7.1 (4.9)
FUT, days	757/537	1317/954	<.001	757/537	1317/954	<.001	737/456	769/545	1386/1080	1234/799
GUI Index	50.5	22.6	<.001	NA	NA	NA	56.3	46.7	26.6	19.2
GUI NI	NA	NA	NA	45.7	10.6	<.001	40.7	48.8	11.2	10.1
VL, copies/mL (SD)	5.0 (.7)	4.5 (.9)	<.001	NA	NA	NA	4.8 (.7)	5.1 (.6)	4.3 (.9)	4.9 (.8)

NOTE. TPIs, transmission-pair index partners; NTIs, nontransmission-pair index partners; SCs, seroconverters; ESNs, exposed seronegatives; FTM, female-to-male; MTF, male-to-female; F+M-, seropositive female, seronegative male; M+F-, seropositive male, seronegative female; SD, standard deviation; FUT, follow-up time expressed as mean/median; GUI, genital ulcers or inflammation; NI, nonindex; VL, viral load; NA, not applicable.

Table 2. Killer Cell Immunoglobulin-like Receptor Gene Frequencies in Transmitting and Nontransmitting Index Partners among Zambian Serodiscordant Couples

KIR	Total	Index	TPI	NTI	*P*	Adjusted *P*
No. of participants	1132	566	240	326	NA	NA
2DL1	99.0	98.9	99.2	98.8	.64	1.0
2DL2	61.4	61.2	61.4	61.1	.93	1.0
2DL3	88.5	88.1	89.4	87.1	.39	1.0
2DL4	99.8	99.8	99.6	100	.24	1.0
2DL5A	10.1	9.9	8.0	11.3	.21	1.0
2DL5B	53.4	52.7	51.9	53.3	.74	1.0
2DP1	98.8	98.0	99.6	96.9	.02	.36
2DS1	18.8	17.9	16.7	18.8	.52	1.0
2DS2	48.5	48.5	48.7	48.3	.92	1.0
2DS3	25.4	25.1	24.9	25.3	.91	1.0
2DS4*001	79.0	81.2	86.9	76.9	.003	.05
2DS4*003	56.6	55.7	53.2	57.6	.29	1.0
2DS5	46.4	46.7	44.1	48.6	.29	1.0
3DL1	99.0	99.1	99.6	98.8	.30	1.0
3DL2	99.6	99.6	99.2	100	.10	1.0
3DL3	99.9	99.8	99.6	100	.24	1.0
3DP1*001, *002, *004	9.9	10.4	8.8	11.5	.30	1.0
3DP1*003	98.9	99.3	99.2	99.4	.76	1.0
3DS1	10.4	10.0	8.0	11.6	.16	1.0

NOTE. KIR, killer cell immunoglobulin-like receptors; Total, both index and nonindex partners; TPIs, transmission-pair index partners; NTIs, nontransmission-pair index partners; adjusted *P*, *P* after Bonferroni correction for multiple comparisons.

HIV-1 transmission was not significant in univariate or multivariable logistic regression models or in Cox proportional hazards models. The association of KIR2DS4*001 was at the threshold of statistical significance ($P = .057$) after Bonferroni correction.

By multivariable analysis, we tested the association of selected genetic and nongenetic factors on HIV-1 transmission (Table 3). We included the only HLA class I allele, A*36, shown in our Zambian TPIs to be associated with transmission (18). Of the included factors, only KIR2DS4*001, GUI, and index partner VL were statistically significant, and these were included in the reduced model. In the multivariable Cox proportional hazards model, KIR2DS4*001 was associated with more rapid HIV-1 transmission (relative hazard [RH], 2.00; 95% confidence interval [CI], 1.24–3.22; $P = .004$), and a logistic regression model showed a similar effect (odds ratio [OR], 2.40; 95% CI, 1.31–4.39; $P = .003$). The VL of the index partner and GUI (in either partner) showed independent associations with increased transmission ($P < .001$ for both).

The population frequency of individuals who carry neither KIR2DS4*001 or *003 (KIR2DS4 -/-) was 3.2%. Our use of PCR-SSP for genotyping did not allow us to distinguish between individuals carrying 1 or 2 copies of KIR2DS4*001 (except in the case of KIR2DS4*001/*003). Although we could not test for a true allele dose-response relationship (ie, an additive model), the relative hazard of HIV-1 transmission for heterozygous KIR2DS4*001/*003 was similar to that for KIR2DS4*001 only (RH, 1.68; 95% CI, 1.16–2.10; $P = .01$ and RH, 1.72; 95% CI, 1.11–2.29; $P = .03$; respectively). The deletion mutant of *KIR2DS4* was not associated with HIV-1 transmission by Cox proportional hazards model (RH, 1.01; 95% CI, .79–1.31; $P = .92$).

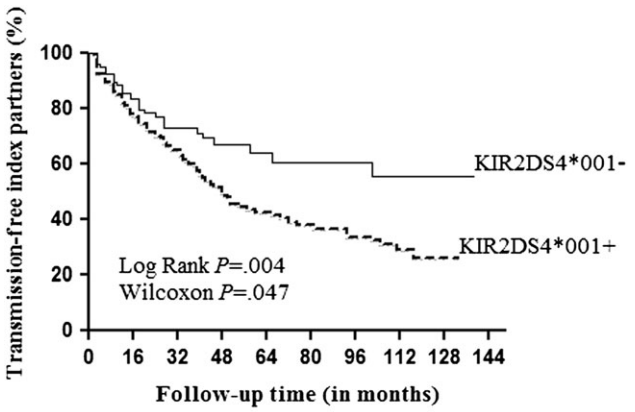
During the study period, 45.6% of index partners with KIR2DS4*001 transmitted HIV-1 (median transmission-free time, 1440 d; 95% CI, 1230–1710 days) compared with only 29.5% of index partners without that allele (median transmission-free time, >1980 days) (Figure 1). Estimates of

Table 3. Genetic and Nongenetic Factors as Independent Contributors in Multivariable Models of HIV-1 Transmission among Zambian Serodiscordant Couples

		Cox Proportional Hazards Model (Time to Transmission)			Logistic Regression Model (Transmission Status)		
Selected Factors Tested		RH	95% CI	Adjusted *P*	OR	95% CI	Adjusted *P*
	KIR2DS4*001	1.74	1.09–2.74	.02	2.07	1.10–3.90	.02
	HLA-Bw4	.98	.70–1.36	.89	.70	.41–1.18	.18
	C1/C2 heterozygosity	.91	.68–1.22	.52	.72	.46–1.13	.16
	A*36	1.48	.97–2.32	.07	1.39	.90–2.22	.11
	KIR3DS1	.64	.37–1.11	.11	.64	.27–1.54	.32
	VL	1.41	1.14–1.74	.002	1.93	1.42–2.62	<.001
	GUI	3.32	2.42–4.56	<.001	5.38	3.43–8.42	<.001
Best Reduced Model	KIR2DS4*001	2.00	1.24–3.22	.004	2.40	1.31–4.40	.003
	VL	1.53	1.24–1.88	<.001	1.88	1.42–2.51	<.001
	GUI	2.05	1.54–2.74	<.001	2.78	1.80–4.31	<.001

NOTE. RH, relative hazards; CI, confidence interval; OR, odds ratio; Adjusted *P*, *P* after Bonferroni correction for multiple comparisons; VL, viral load; GUI, genital ulcers or inflammation.

RH, OR, CI, and *P* are computed with adjustments for all covariates as shown in Table 1.



	Transmission Events: n (%)	Transmission-free couples remaining at various intervals (months) 0	16	32	64	128
KIR2DS4*001−	206 (45.6)	452	285	177	63	4
KIR2DS4*001+	31 (29.5)	105	69	40	18	5

Figure 1. Kaplan–Meier plot of transmission-free time in index partners with and without the KIR2DS4*001 allele. Log-rank and Wilcoxon tests of significance are shown, along with numbers of transmission-free couples at various follow-up intervals.

transmission-free time differed significantly between the 2 groups by log-rank ($P = .004$) and Wilcoxon ($P = .047$) tests.

KIR2DS4*001 in Relation to HIV-1 Viral Load in Index Partners

A higher proportion of index partners with KIR2DS4*001 had high VL ($>10^5$ copies/mL) compared with index partners without the allele (OR, 1.62; 95% CI, 1.09–2.60; $P = .04$). There was a significant association of KIR2DS4*001 with higher VL across the 3 VL categories in a test for trend ($P = .04$) (Table 4). Even after statistical adjustment for age and sex, VL was significantly higher in KIR2DS4*001-positive index partners (β [SD] = .17 [.8] $\log_{10}$; $P = .04$). KIR2DS4*001 was not associated with VL in SCs either as a continuous variable ($P = .67$) or in a test for trend across VL categories ($P = .32$).

KIR2DS4*001 with Genital Ulcers and Inflammation

We analyzed both genital ulcers and genital inflammation as transient outcomes preceding HIV-1 transmission. In transmission pairs, we identified inflammation or genital ulcers in the 6 months prior to seroconversion; in nontransmission pairs, we measured these outcomes within the 6 months prior to the last visit or the censoring date. KIR2DS4*001 was associated with genital ulcers in index partners by logistic regression (OR, 2.50; 95% CI, 1.24–5.06; $P = .01$) (Table 5) but not with ulcer-free genital inflammation ($P = .84$). KIR2DS4*001 was not associated with the outcome of either genital ulcers or genital inflammation in nonindex partners ($P = .34$ and .64, respectively). Analysis of only the seroconverting partners did not reveal an association of KIR2DS4*001 with genital ulcers or inflammation ($P = .92$ and .51, respectively).

KIR2DS4*001in Combination with Putative HLA Ligands

KIR2DS4*001 has previously been reported to directly interact with HLA-Cw*04 [3], a member of the HLA-C2 group, which is recognized as the set of ligands for KIR [27]. More recently, alleles of the HLA-C1 subgroup were shown to bind weakly to the KIR2DS4 receptor [28]. We analyzed the joint effects of KIR2DS4*001 with HLA-Cw*04, with C1, with C2, and with C1/C2 heterozygosity. The combination of KIR2DS4*001 with HLA-Cw*04 showed an association with increased rate of HIV-1 transmission ($P = .02$) (Table 6). HLA-Cw*04 alone was not associated with HIV-1 transmission (RH, 1.04; 95% CI, .47–2.30; $P = .92$). The combination of KIR2DS4*001 and HLA-Cw*04 was not associated with VL in index partners ($P = .41$).

Killer Cell Immunoglobulin-like Receptor Haplotypes and HIV-1 Transmission

Haplotypes were resolved in 733 participants; 362 of these were index partners. Haplotypes were distributed as follows: 42% A/A, 44% A/B, and 14% B/B. Of the dozens of possible haplotypes, only 35 (2 type A and 33 type B) were identified with a frequency of 1% or higher. The 2 A haplotypes differed only in the content of their *KIR2DS4* alleles: Haplotype 1 carried KIR2DS4*003 and haplotype 2 carried KIR2DS4*001.

Only haplotype 2 in the index partner was associated with HIV-1 transmission (RH, 1.70; 95% CI, 1.12–2.21; $P = .004$). This haplotype was present in 59.1% of the study population.

Table 4. Association of KIR2DS4*001 with HIV-1 Viral Load in 517 Index Partners (226 Transmission-Pair Index Partners and 296 Nontransmission-Pair Index Partners) and 193 Seroconverters among Zambian Serodiscordant Couples

		Distribution across VL Categories				Generalized Linear Model	
Index partners		Low ($<10^4$ copies/mL)	Medium (10^4–10^5 copies/mL)	High ($>10^5$ copies/mL)	*P*	β, mean (SE)	*P*
	KIR2DS4*001+	78 (18.5%)	163 (38.7%)	180 (42.8%)	.04	.17 (.08 $\log_{10}$)	.04
	KIR2DS4*001−	23 (24.2%)	43 (44.2%)	30 (31.6%)	NA	REF	NA
Seroconverters							
	KIR2DS4*001+	38 (24.5%)	74 (47.7%)	43 (27.7%)	.32	−.06 (.15 $\log_{10}$)	.67
	KIR2DS4*001−	5 (13.2%)	21 (55.3%)	12 (31.6%)	NA	REF	NA

NOTE. TPIs, transmission-pair index partners; NTIs, nontransmission index partners; VL, viral load; SE, standard error; NA, not applicable; REF, reference group. Plasma viral load is expressed as RNA copies/mL; 44 participants (14 TPIs and 30 NTIs) did not have viral load data.

Table 5. Association of KIR2DS4*001 with Genital Ulcers and Inflammation in Index and Nonindex Partners among Zambian Serodiscordant Couples

		Logistic Regression Model (Outcome GU)				Logistic Regression Model (Outcome GI)			
Index	(n = 440)	GU +	OR	95% CI	P	GI +	OR	95% CI	P
	KIR2DS4*001+	100	2.50	1.24–5.06	.01	66	1.07	.56–2.06	.84
	KIR2DS4*001−	10	NA	NA	REF	13	NA	NA	REF
Nonindex	(n = 473)								
	KIR2DS4*001+	53	1.38	.71–2.70	.34	60	1.15	.63–2.10	.64
	KIR2DS4*001−	12	NA	NA	REF	16	NA	NA	REF
Seroconverters	(n = 210)								
	KIR2DS4*001+	43	1.04	.47–2.24	.92	45	1.28	.62–2.64	.51
	KIR2DS4*00−	11	NA	NA	REF	12	NA	NA	REF

NOTE. GU, genital ulcers; GI, genital inflammation; OR, odds ratio; CI, confidence interval; NA, not applicable; REF, reference group; TPIs, transmission-pair index partners; NTIs, nontransmission index partners; SCs, seroconverters; ESNs, exposed seronegatives.

GU and GI were assessed quarterly by physical examination; 126 index partners (38 TPIs and 88 NTIs) and 93 nonindex partners (30 SCs and 63 ESNs) did not have clinical data for 1 of these outcomes.

Multivariable modeling with VL and GUI confirmed its independent association with transmission (RH, 1.78; 95% CI, 1.30–2.19; $P = .003$). Haplotype 2 was not associated with VL by linear regression (β [SD], .13 [.4] $\log_{10}$; $P = .51$) or in the test for trend across VL categories ($P = .39$). Among the 29 index partners carrying a B haplotype with KIR2DS4*001, no association was found with HIV-1 transmission ($P = .60$).

DISCUSSION

There is growing experimental [11, 30] and epidemiologic [31–33] evidence that polymorphisms in multiple KIR genes modulate the acquisition and control of HIV-1 infection. The effects may be due to KIR-gene or allelic products alone or may be dependent on their interactions with various ligands in the HLA class I system [32, 34]. In particular, *KIR3DS1* has been associated with delayed progression to AIDS [9], although its exact role in HIV-1 disease has not been fully defined [10, 33]. Whether *KIR3DS1* requires its putative ligand, HLA-Bw4 80I, to influence HIV-1 disease progression is under contention because the reported synergy between *KIR3DS1* and HLA-Bw4 80I [9, 11] has not seemed to operate invariably in early HIV-1 infection [10].

Our analyses of one of the largest cohorts of HIV-1 serodiscordant heterosexual couples under study did not confirm any of the previously reported associations with HIV-1 acquisition or altered VL and pathogenesis. *KIR3DS1* showed no impact on HIV-1 transmission or acquisition, or on control of VL, either in the presence or absence of HLA-Bw4 80I (data not shown Merino, 2010), possibly due to the relatively low *KIR3DS1* frequency in the Zambian population or to interaction with other genetic factors unique to this population. As for *KIR3DL1*, resolution to allelic specificity will be required to test the hypothesis that high- and low-expressing alleles of this gene differentially alter the HIV-1 outcomes of interest [28].

The associations of KIR2DS4*001 observed with both elevated VL in transmitting partners and accelerated HIV-1 transmission to susceptible heterosexual partners have not previously been reported. *KIR2DS4* encodes a unique and activating KIR thought to interact with both HLA and non-HLA ligands, through low-affinity binding to HLA-Cw*04, a C2 group molecule [3], as well as functional binding to HLA-A*1102 [28]. Interaction of KIR2DS4 with HLA-deficient melanoma cells has been shown to activate NK cytolytic activity, which could be abrogated by application of a KIR2DS4-directed antibody [35].

Table 6. Association of KIR2DS4*001 and Selected *HLA-C* Alleles with HIV-1 Transmission

				Cox Proportional Hazards			Logistic Regression		
	n	TPI	NTI	RH	95% CI	P	OR	95% CI	P
KIR2DS4*001 + HLA-Cw4	138	67	71	1.38	1.04–1.84	.02	1.39	.95–2.05	.09
KIR2DS4*001 + C1	286	128	158	1.20	.93–1.55	.16	1.22	.87–1.70	.25
KIR2DS4*001 + C2	367	162	205	1.22	.93–1.60	.15	1.23	.86–1.74	.26
KIR2DS4*001 + C1 + C2	201	84	117	1.05	.81–1.38	.69	.96	.68–1.36	.83

NOTE. TPIs, transmission-pair index partners; NTIs, nontransmission index partners; RH, relative hazards; OR, odds ratio; CI, confidence interval; C1, HLA-C C1 group; C2, HLA-C C2 group [29].

Alleles of the *KIR2DS4* gene can be divided into full-length receptors and truncation mutants [36, 37]. KIR2DS4*001 is the only known allele of *KIR2DS4* that encodes a full-length receptor; the other known alleles have a 22-bp deletion in exon 5 that generates a premature stop codon, preventing formation of a functional (membrane-bound) receptor [38].

The KIR2DS4*001 association with both index partner VL and accelerated transmission was consistent for both MTF and FTM transmission. Although VL can change over time, our use of VL as a categorical variable is less sensitive to modest fluctuations. Conversely, the independent effects of VL and KIR2DS4*001 on time to HIV-1 transmission (Table 3) raise the possibility that whatever the KIR association signifies, its effect is not operating exclusively through its association with high VL.

One alternative explanation would be that the implicated KIR allele might predispose to genital ulceration. Several studies have indicated that HIV-1 shedding from genital mucosa and in semen may be independent of plasma VL [39–41]. Inflammation and ulceration of genital tissues increases viral shedding [39, 42]. Also, stimulatory KIR genes have been associated with inflammation and inflammatory diseases. Examples include the apparent involvement of the activating KIRs *KIR2DS1* or *KIR2DS2* with psoriatic arthritis [43], even in the absence of their *HLA-C* ligands [42]; higher frequency of both full-length and truncated alleles of *KIR2DS4* in Taiwanese patients with rheumatoid arthritis than in controls; and increased risk of acute graft-versus-host disease following bone marrow transplant in patients with KIR2DS4*001 [44].

Expression of activating KIR might promote mucosal inflammation, viral shedding and transmission. *KIR2DS2* and its closely related *KIR2DL2* [45] have been associated with recurrent, inflammatory herpetic lesions [46]. The positive association we observed between KIR2DS4*001 and genital ulcers in seroprevalent index partners but not in seroconverters raises the intriguing possibility that functional *KIR2DS4* might promote tissue inflammation and increased shedding of HIV-1, particularly in chronically HIV-1 infected individuals with progressive immunodeficiency. KIR2DS4*001 was not associated with genital ulcers in nonindex partners or with early viral load in newly infected seroconverters, suggesting that immune function mediated by KIR2DS4 may operate in later stages of HIV-1 infection. NK cells from HIV-1/HSV-2 co-infected individuals displayed alterations in degranulation abilities compared with NK cells from individuals singly infected with either HSV or HIV-1 [47]. The association of KIR2DS4*001 with index partners' genital ulcers (a surrogate marker for HSV-2 infection) may further reflect the complexity of immune responses induced by co-infections with HIV-1 and HSV-2.

In our analyses, the association of KIR2DS4*001 with HIV-1 transmission was only weakly enhanced by the presence of its reported ligand, HLA-Cw*04, a member of the C2 group of *HLA-C* alleles [3]. However, it is unlikely that the weak synergy is dependent on HIV-derived antigenic peptides because the known HLA-Cw4 binding residues on *KIR2DS4* are distant from the peptide-binding groove [48].

Most KIR2D molecules can be classified according to whether they bind C1 or C2 molecules. Recent evidence suggests that KIR2DS4 binds weakly with molecules encoded by alleles of both C1 and C2 subgroups, albeit not with uniformly strong subsequent NK cell activation [28]. KIR2DS4 has some residues that would tend to bind with both of these groups in addition to other residues with unique binding affinities [49]. Moreover, KIR2DS4 may bind molecules other than HLA-C alleles. KIR2DS4*001 has also been found to functionally bind HLA-A*1102 [28] although that *HLA-A* allele occurs too infrequently in our Zambian cohort (<1%) to have contributed meaningfully to the association. Because KIR2DS4*001 does not functionally bind the more common A*1101 [26], we excluded A*1101 from our analysis. Because the KIR2DS4 receptor also appears to bind specifically and functionally to a protein expressed on melanoma cell lines (through different residues from those that bind HLA epitopes [35]), an as yet unknown ligand or intermediary factor could enhance receptor binding.

Among the 2 broad KIR gene haplotype groups (A and B) [50], group A carries only the single stimulatory gene *KIR2DS4*. Because our Zambian population is 42% homozygous A/A, *KIR2DS4* represents their only stimulatory KIR. Further, of the 2 variant A haplotypes, only haplotype 2 carrying KIR2DS4*001 was associated with HIV-1 transmission. The association of this haplotype with viral load was not statistically significant, most likely because of the loss of power with haplotype assignment. Haplotype 2 differs from haplotype 1 only in its carriage of a functional *KIR2DS4*. Therefore, the associations observed here most likely arises from KIR2DS4*001 itself rather than another gene or combination of genes in the haplotype. Alternatively, KIR2DS4*001 may be in linkage disequilibrium with still unrecognized polymorphisms in the leukocyte-receptor complex. Group B haplotypes carry a variable number of stimulatory KIR genes, including *KIR2DS4* [26], but few B haplotypes in Zambians carry KIR2DS4*001, and it was impossible to determine whether these haplotypes exert a similar effect on HIV-1 transmission. Overall, it is encouraging that the association of KIR2DS4*001 with viral transmission survived correction for multiple comparisons; confidence in its causal relationship will await replication in representative populations and direct evidence from experimental studies of KIR2DS4*001 and its ligands.

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