

# Chemokine Receptor Gene (CCR5 and CCR2) Haplotypes as Risk Factors for HIV-1 Acquisition and Pathogenesis

Poster # 305

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## ABSTRACT:

**Background:** Polymorphisms in *CCR2* and *CCR5*, adjacent genes encoding chemokine receptors 2 and 5, respectively, influence occurrence of HIV-1 infection and disease progression in various settings, but little is known about the impact of these variants in HIV-1 discordant couples with a seropositive (index) and a seronegative (exposed) partner. We investigated the impact of *CCR2-CCR5* haplotypes on HIV-1 viral load and heterosexual transmission in cohabiting discordant Zambian couples. **Methods:** Between 1995 and 2003, 337 initially discordant couples from Lusaka, Zambia, were enrolled for quarterly voluntary counseling and serologic testing to assess factors associated with change in serostatus. For HIV-1 acquired by the exposed partners, viral sequencing documented its phylogenetic linkage to the index virus. PCR-based genotyping at 8 polymorphic sites capable of resolving 9 previously recognized haplotypes was performed. To assess haplotypic effects on viral load (VL) among index partners, we used beta values from linear regression models. The risk of infection in exposed was assessed with Kaplan-Meier curves and Cox proportional hazard methods including multivariable analysis of genetic and non-genetic factors.

**Results:** Of the 337 couples, serostatus and linkage of transmitted virus were unequivocal for 281 at the time of analysis. By December 2006, transmission had occurred within 157 of those 281 couples. The *CCR2-CCR5* HHE haplotype (containing *CCR2-64I*) was associated with high VL in the index ( $\beta = -0.26 \log_{10}$ ,  $p < 0.01$ ). In multivariable analyses of transmitting couples the HHE/HHD diplotype and the HHF\*2 haplotype were associated with more rapid infection of the exposed ( $p \leq 0.010$ ), with a stronger association in females exposed to index males. After statistical adjustment for other known risk factors, including index partner VL and genital ulcer/inflammation in either partner, adjusted hazard ratios for these genotypes ranged from 1.80 to 2.35 ( $p \leq 0.016$ ).

**Conclusion:** At least three common *CCR2-CCR5* haplotype variants (HHE, HHE/HHD, and HHF\*2) appeared to modulate VL in index partners or to alter the rate of acquisition of exposed partners among Zambian discordant couples. These findings indicate that patterns in which *CCR2-CCR5* polymorphism alter the development and progression of HIV-1 infection are similar but not identical between populations of African and European ancestry.

## INTRODUCTION:

Heterosexual transmission is highly variable, and a number of viral, host and environmental factors may modify transmission (infectiousness), acquisition (susceptibility) or both. Donor HIV-1 viral load (VL), age, sex, history of sexually transmitted infection (STI), unprotected sex, and possible HIV-1 subtype are among the factors identified. In southern Africa unusual biological features of the predominant C subtype of HIV-1 and absence of the human CC chemokine receptor 5 (*CCR5*)  $\Delta 32$  mutations as a major resistance marker may contribute to relatively high transmission rates.

In Zambia, over 1500 cohabiting discordant couples have been enrolled by the voluntary counseling testing (VCT) services and followed for more than 10 yrs beginning in 1995. Despite counseling and behavioral interventions the rate of transmission among these couples has remained comparatively high. We describe association of *CCR5* genetic variation with intra-couple transmission documented by phylogenetic techniques.

## STUDY METHODS:

### Study Participants:

The subjects selected from RZHRG between 1995 and 2003 were initially HIV-1 serodiscordant and cohabiting heterosexual couples with subsequent intra-couple (virologically linked) HIV-1 transmission, as defined by the phylogenetic analysis of HIV-1 subgenomic sequences corresponding to gag, env(gp120 and gp41), and long terminal repeat regions. Of the 337 HIV-1 discordant couples enrolled between 1995 and 2003, 281 were included in the final analysis. Exclusions were based on unlinked transmission, lack of DNA samples for either partner and lack of follow-up information on either partner. The majority of HIV-1 sequences derived from the Zambian transmission pairs were subtype C (HIV-1C), with other subtypes detected only occasionally.

## DNA extraction and CCR typing:

PCR-based typing differentiated the dimorphic variants at eight sites: the SNP encoding V641 in *CCR2*, six SNPs (rs2856758, rs1799987, rs1799988, -2132, rs1800023 and -1835) in or adjacent to the cis-regulatory or promoter region of *CCR5*, and the 32-bp deletion in *CCR5*. Haplotyping was facilitated by PCR with 12 combinations of sequence-specific primers (SSP) along with four additional SSP reactions in conjunction with rs1799988-specific primers to define the rs2856758 variants. Combinations of variants at all the eight sites form nine haplotypes (haplogroups) according to the nomenclature of the Tri-Service HIV-1 Natural History Study (TSS).

## Statistical Methods

General characteristics of transmitting and non-transmitting couples were compared using  $\chi^2$  and t-tests. HWE and CCR haplotype distribution among the cohort was assessed using SAS genetics. Associations of frequent CCR haplotypes/genotypes with HIV-1 VL in the index partners were assessed using general linear model (GLM) with adjustment for gender and age. Time to HIV-1 infection among the exposed was plotted using the Kaplan-Meier method. Between-group analysis was done using log-rank tests. Genetic markers with suggestive effects on HIV-infection among exposed ( $p < 0.10$ , or (RH)  $\geq 2.0$  or  $\leq 0.5$ ) and other covariates were included in multivariable models. We adjusted for potentially confounding non-genetic variables using Cox proportional hazard analysis with date of enrollment as the zero time point and HIV-1 infection in the exposed as the event. Tests of statistical significance included estimation of RH, 95% confidence interval (CI) of RH, and two-sided p-values.

## RESULTS: Table 1. General characteristics of the Zambian cohort

Characteristics	Non-Transmitting Couples	Transmitting couples	P*
Number of Couples	144 (124)	163 (157)	
Male/Female (Index Partner)	55/69	95/62	0.0001
Age of Partners (yrs)			
Index	30.9 ± 8.9	29.8 ± 7.8	0.29
Exposed	31.8 ± 9.6	28.1 ± 6.9	0.0001
Follow-up Time (median [IQR], days)	1743 (823-2844)	540 (186-1098)	<0.0001
Genital Ulcers*			
Index	18.26%	36.62%	0.00124
Exposed	2.44%	28.86%	<0.0001
Genital inflammation*			
Index	13.91%	26.06%	0.0167
Exposed	9.76%	28.19%	0.0001
HIV-1 RNA level (log10)			
Index	4.59 ± 0.84	5.03 ± 0.61	<0.0001

a In the 3-6 months before HIV-1 transmission (transmitting couples) or latest follow-up visit (non-transmitting couples).

## Table 2. CCR2-CCR5 haplotype distribution in the Zambian couples

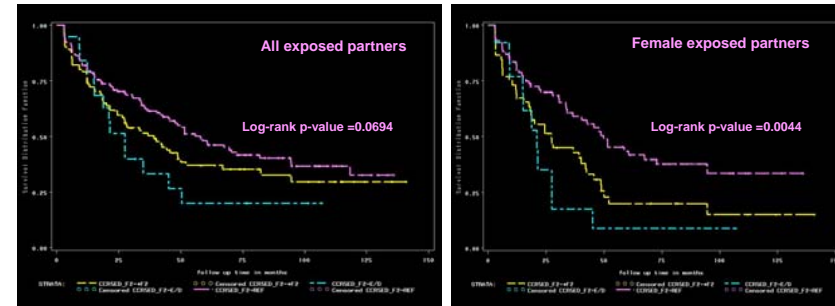
	All		Index Partner		Exposed Partner	
	N(%)	All	Transmitters	Non-transmitters	All	Seroconverters
HH						
A	281 (25.0)	137 (24.3)	77 (24.5)	67 (27.0)	144 (25.6)	70 (22.3)
B	32 (2.8)	21 (3.7)	7 (2.2)	9 (3.6)	11 (1.96)	12 (3.8)
C	99 (8.8)	40 (7.1)	30 (9.5)	18 (7.3)	59 (10.5)	22 (7.0)
D	170 (15.1)	81 (14.4)	50 (15.9)	36 (14.5)	89 (15.8)	45 (14.3)
E	138 (12.3)	66 (11.7)	42 (13.4)	28 (11.3)	72 (12.8)	38 (12.1)
F1	55 (4.9)	32 (5.6)	14 (4.5)	12 (4.8)	23 (4.1)	20 (6.4)
F2	269 (23.9)	150 (26.7)	71 (22.6)	67 (27.0)	119 (21.2)	83 (26.4)
G1	80 (7.1)	35(6.2)	23 (7.3)	11(4.4)	45 (8.0)	24 (7.6)
HWE: p*	0.0001*	0.0005*	0.0139*	0.0227*	0.1402	0.0356*
						0.493

a P values for tests of Hardy-Weinberg equilibrium (HWE) across patient groups in each column. Deviation from equilibrium among all seropositive groups (index partners and seroconverters) suggests selection pressure by HIV-1 on the distribution of *CCR5* haplotypes, particularly F\*2.

## Table 3. Age- and gender-adjusted association of CCR2-CCR5 with VL in index partners

Parameter Estimates					
Variable	Label	Parameter Estimate	Standard Error	t Value	Pr >  t
Intercept	Intercept	5.09118	0.28084	18.13	<.0001
HHE	haplotype E	0.26424	0.09635	2.85	0.0048
gender	gender	-0.33245	0.09791	-3.4	0.0008
age	age	0.0057	0.00592	0.96	0.3362

Figure 1. Transmission-modifying *CCR2-CCR5* determinants in exposed Zambian partners



1a. Kaplan-Meier plots for the HHF\*2 haplotype and the HHE/HHD diplotype in all exposed partners. The KM plot above shows the time to seroconversion among the 281 exposed initially seronegative. 1b. Kaplan-Meier plots for the HHF\*2 haplotype and the HHE/HHD diplotype in female exposed partners. The KM plot above shows the time to seroconversion among the 150 exposed initially seronegative.

Table 4. Multivariable model of *CCR2-CCR5* haplotypes significantly associated with acquiring HIV-1 infection, adjusted for index partner VL

Variable	All Exposed (N=281)				Females Exposed (N=150)			
	Hazard Ratio	95% Hazard Ratio Confidence Limits	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	Pr > ChiSq		
ONE	-	-	-	-	-	-		
HHF*2	1.371	0.97	1.92	0.066	1.793	1.15	2.78	0.009
HHE/D	2.02	1.14	3.57	0.015	2.665	1.35	5.24	0.004
Donor VL	1.737	1.35	2.22	<.001	1.517	1.06	2.15	0.020

## CONCLUSIONS:

- Homozygosity or heterozygosity for HHE was associated with higher viral RNA among the index partner, compared with those lacking HHE haplotype ( $\beta = -0.26 \log_{10}$ ,  $p = 0.004$ ).
- Deviation from Hardy-Weinberg equilibrium seen among each group of seropositive partners (index and seroconverters) supported the hypothesis that the distribution of *CCR2-CCR5* haplotypes observed among the Zambian couples is under selective pressure from HIV-1 infection.
- In multivariable analyses the HHF\*2 haplotype was associated with more rapid acquisition of infection by exposed partners (RH= 1.80, 95% CI [1.15-2.81],  $p = 0.01$ ), with a stronger association in exposed females. This corroboration of a HHF\*2 disadvantage for those exposed uninfected despite its widely reported advantage in those already infected illustrates the potential for dissociation of co-receptor gene relationships in uninfected and infected individuals.
- The diplotype HHE/HHD was associated with increased rate of transmission among the exposed uninfected (RH=2.28, 95% CI [1.14-4.55],  $p < 0.01$ ), with stronger association in females exposed to index males as seen with haplotype HHF\*2. This effect among the exposed has not been previously reported.

## KEY REFERENCES:

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