

POSTER #905 IL-10-1082-G/A Promoter Variants Alter HIV-Mother-To-Child-Transmission in African Mother-Infant Pairs Exposed to Zidovudine at Delivery

Justine K. Wong^{1*}, Ping K. Ruan², Kumud K. Singh¹, Yan Wang², Terry Fenton², Christine Rousseau³, Louise Kuhn⁴, Anna Coutsooudis⁵, J. Brooks Jackson⁶, Laura A. Guay⁶, Philipa Musoke⁷, Francis Mmiro⁷, Richard D. Semba⁶, Rodney N. Trout¹, and Stephen A. Spector¹.

¹Univ. of California San Diego, La Jolla, CA, ²Harvard Sch. of Publ. Health, Boston, MA, ³Univ. of Washington, Seattle, WA, ⁴Columbia University, New York, NY, ⁵Univ. of Natal, Durban, South Africa, ⁶Johns Hopkins Univ., Baltimore, MD, ⁷Makerere University, Kampala, Uganda.

Abstract

Background: IL-10 secreting T cells from HIV-1 infected pregnant women have been found to inhibit HIV-1 replication and this effect is increased in women receiving antiretrovirals. However, other findings suggest that an increase in IL-10 producing CD8+ cells play a detrimental role and are associated with HIV-1 immune dysfunction. In this study, we examined the association of *IL-10* genetic variants linked to higher IL-10 expression on HIV-1 MTCT.

Methods: *IL-10*-1082-G/A, -819-C/T and -592-C/A variants were detected by real-time PCR in 980 children born to antiretroviral (ARV) naïve HIV-1 infected mothers from Malawi (n=322) and South Africa (n=300); and to ARV exposed mothers from Uganda (HIVNET-012, n=358) where intrapartum and neonatal single-dose NVP was compared with intrapartum ZDV plus 7 days after birth for preventing MTCT. The Chi-Squared test and logistic regression were used to evaluate the association of *IL-10* polymorphisms with the risk of HIV-1 MTCT.

Results: Overall 21% of infants were HIV-1 infected [ARV naïve: 145/637 (22.7%), NVP-exposed: 18/171 (10.5%), ZDV-exposed: 46/169 (27.2%)]. *IL-10* variants did not alter MTCT for ARV-naïve mother-infant pairs either for early MTCT (within 6 wks of birth) or through breastfeeding (after 6 wks of birth). Presence of *IL-10*-1082-G/G or G/A variants (linked with higher IL-10 expression) had a modest, non-significant association with overall MTCT in NVP exposed infants: G/G vs. A/A (OR=0.31, 95%CI:0.04,2.53, P=0.27); G/A vs. A/A (OR=0.60, 95%CI:0.22,1.68, P=0.33). However, *IL-10*-1082-G/G or G/A genotypes were associated with increased MTCT in ZDV exposed infants: G/G vs. A/A (OR=3.22, 95%CI:1.09,9.48; P=0.034); G/A vs. A/A (OR=3.24, 95%CI:1.46,7.18, P=0.004). These findings remained after adjusting for mothers CD4+ count, and log HIV-1 RNA. *IL-10* haplotypes for -1082, -819 and -592 variants showed a modest, but non-statistical significant association with overall MTCT: GCC vs. ATA for NVP exposed (OR=0.20, 95%CI:0.02,1.80, P=0.15) and ZDV exposed mother-infant pairs (OR=2.74, 95%CI:0.75,10.04, P=0.13).

Conclusions: Presence of *IL-10*-1082-G/G or G/A genotype was associated with higher MTCT in ZDV exposed infants. These findings suggest that in the presence of ZDV administered shortly before delivery the immunosuppressive effects of IL-10 appear to dominate over its HIV-1 inhibitory effects leading to an association with increased MTCT. Validation cohort studies are needed to confirm these findings.

Objective. To examine the impact of genetic variants of IL-10 promoter on the risk of HIV-1 MTCT in sub-Saharan African infants.

Hypothesis. We hypothesized that the presence of genetic variants modulating the expression and/or function of IL-10 would affect MTCT. Additionally, antiretroviral therapy used to prevent MTCT might alter genetic associations with risk of MTCT, particularly early MTCT, and that these altered associations might differ according to the antiviral potency of the antiretroviral regimen used.

Subjects and Methods. **Study subjects.** Studied samples included dry blood spot samples from children born to ARV naïve HIV-1 infected mothers from Malawi (n=322, involved in vitamin A intervention trials to reduce MTCT of HIV-1), PBMC samples from 300 South Africa children (born to ARV naïve HIV-1 infected mothers involved in vitamin A intervention trials to reduce MTCT of HIV-1) and dry blood spot samples from children from Uganda (HIVNET-012 cohort, n=358, where intrapartum and neonatal single-dose nevirapine was compared with ZDV for preventing MTCT). Mothers were randomly assigned NVP 200 mg orally at onset of labor and 2 mg/kg to babies within 72 hours of birth, or ZDV 600 mg orally to the mother at onset of labor and 300 mg every 3 hours until delivery, and 4mg/kg orally twice daily to babies for 7 days after birth. Early transmission of HIV-1 in infants was defined as transmission within 6 weeks of birth, as confirmed by HIV-1 DNA PCR. All transmissions included both early and late (after 6 weeks of birth) MTCT. Overall 210 of 980 children (21%) were HIV-1-infected (20% in Malawi cohort, 25% in South Africa cohort and 20% in Uganda cohort).

Genotyping by real-time PCR. Genomic DNA was extracted from dried blood spots and frozen PBMCs from all cohorts using QIAamp DNA Mini Kit (Qiagen, Valencia, CA). Samples were genotyped for *IL-10*-1082-G/A, -819-C/T, -592-C/A promoter polymorphisms.

Statistical Analyses: Chi-square test and the Kruskal-Wallis test were used to compare categorical and continuous variables, respectively, between cohorts. The Cochran-Mantel-Haenszel test with stratification by study and randomized intervention and logistic regression were used to evaluate the association of genotypes with risk of HIV infection. All P-values are two-tailed and are unadjusted for multiple comparisons.

Characteristics of Subjects and Genotype Frequency

Characteristics	Malawi cohort (n=322)	S. Africa cohort (n=300)	Uganda cohort (n=358)	Total (n=980)	P value 3 cohorts
Sex: Female	176 (54.7%)	130 (43.3%)	188 (52.5%)	494 (50.4%)	0.027
Twin birth	3 (0.93%)	5 (1.7%)	0 (0%)	8 (0.82%)	0.027
Mother's CD4 count (cells/μl): median (10th, 90th percentiles)	399 (174,733) [n=306]	440 (188, 720) [n=285]	447 (148,910) [n=354]	428 (170,802) [n=945]	0.049
Mother's HIV-1 RNA (log₁₀ copies /ml):median (10th, 90th percentiles)	4.33 (3.31,5.15) [n=314]	4.46 (3.49, 5.47) [n=105]	4.38 (3.36,5.3) [n=340]	4.37 (3.36,5.3) [n=759]	0.090
HIV-1-infected	65 (20.2%)	74 (24.7%)	71 (19.8%)	210 (21.4%)	0.28

Genotypes studied	Malawi cohort	South Africa Cohort	Uganda cohort	Total	Comparison of 3 cohorts	
<i>IL-10-592</i>	A/A	54 (17%)	42(14%)	63 (18%)	159 (16%)	P=0.187
	C/A	166 (52%)	138 (46%)	158 (44%)	462 (47%)	
	C/C	102 (28.9%)	102 (32%)	135 (38%)	354 (36%)	
<i>IL-10-819</i>	C/C	100 (31%)	114 (39%)	133 (37%)	347 (36%)	P=0.193
	C/T	166 (52%)	136 (47%)	160 (45%)	462 (48%)	
	T/T	55 (17%)	42 (14%)	64 (18%)	161 (17%)	
<i>IL-10-1082</i>	A/A	168 (53%)	143 (48%)	152 (43%)	463 (47%)	P=0.037
	G/A	126 (39%)	134 (45%)	162 (45%)	422 (43%)	
	G/G	26 (8%)	21 (7%)	43 (12%)	90 (9%)	

IL-10-1082-G/A Genotypes and Mother-to-Child HIV-1 Transmission Rates

<i>IL-10</i> -1082	ARV-naïve	NVP-exposed	ZDV-exposed
Overall transmissions			
A/A	68/318 (21.4%)	10.71 (14.1%)	11/73 (15.1%)
G/A	65/268 (24.2%)	7/78 (9.0%)	27/74 (36.5%)
G/G	12/47 (25.5%)	1/21 (4.8%)	8/22 (36.4%)
Early transmissions			
A/A	60/318 (18.9%)	4/71 (5.6%)	5/73 (6.8%)
G/A	50/268 (18.6%)	5/78 (6.4%)	12/74 (16.2%)
G/G	9/47 (19.1%)	0/21 (0%)	1/22 (4.5%)
Late transmissions			
A/A	8/245 (3.3%)	6/71 (8.5%)	6/73 (8.2%)
G/A	15/203 (7.4%)	2/78 (2.6%)	15/74 (20.3%)
G/G	3/35 (8.6%)	1/21 (4.8%)	7/22 (31.8%)

Effects of *IL-10*-1082-G/A Polymorphisms on the Risk of HIV-1 MTCT by Breastmilk.

Of the 764 infants at risk for HIV infection through breast milk (all infants not infected by 6 weeks of age), 36 (4.7%) acquired HIV infection through this route. Because of the relatively small number of infants identified as infected through breastfeeding, there was limited power to detect differences by specific genetic variants. However, the trends went in the same direction as those identified for early transmissions (data not shown).

Association of Overall HIV-1 MTCT with *IL-10*-1082-G/A Variants in ARV-Exposed Ugandan Mother-Infant Pairs

<i>IL-10</i> -1082	Comparisons	Unadjusted for study and randomized intervention (SRI)	P-value	Adjusted for SRI, mother's CD4 count and log HIV-1 RNA	P-value adjusted for Mother's CD4 count and log HIV-1 RNA
Overall Transmissions					
NVP-exposed pairs	G/A vs. A/A	0.60 (0.22,1.68)	0.331	0.47 (0.15,1.49)	0.201
ZDV-exposed pairs		3.24 (1.46,7.18)	0.004	3.25 (1.35,7.84)	0.009
	P* (NVP vs.ZDV)		0.011		0.009
NVP-exposed pairs	G/G vs. A/A	0.31 (0.04,2.53)	0.272	0.18 (0.02,1.63)	0.128
ZDV-exposed pairs		3.22 (1.09,9.48)	0.034	3.36 (1.04,10.86)	0.043
	P**(NVP vs.ZDV)		0.052		0.02
NVP-exposed pairs	G/A+G/G vs. A/A	0.54 (0.20,1.44)	0.215	0.39 (0.13,1.16)	0.089
ZDV-exposed pairs		3.23 (1.51,6.94)	0.003	3.28 (1.42,7.58)	0.006
	P*** (NVP vs.ZDV)		0.005		0.002
Early transmissions					
NVP-exposed pairs	G/A vs. A/A	1.15 (0.30,4.45)	0.843	1.22 (0.30,4.91)	0.782
	G/G vs. A/A	NA	0.991	NA	NA
	G/A+G/G vs. A/A	0.89 (0.23,3.44)	0.867	0.85 (0.21,3.38)	0.813
	G/A+A/A vs. G/G	NA	NA	NA	NA
ZDV-exposed pairs	G/A vs. A/A	2.63 (0.88,7.90)	0.084	2.51 (0.80,7.87)	0.115
	G/G vs. A/A	0.65 (0.07,5.86)	0.699	0.60 (0.06,5.55)	0.653
	G/A+G/G vs. A/A	2.13 (0.72,6.27)	0.17	1.97 (0.65,6.01)	0.232
	G/A+A/A vs. G/G	2.75(0.35,21.73)	0.338	2.82 (0.35,22.89)	0.332

* P-value (NVP vs. ZDV) is for testing that association between risk of MTCT and genetic variant (G/A vs. A/A) that differs between NVP- and ZDV-exposed mother-infant pairs.

**P value (NVP vs. ZDV) is for testing that association between risk of MTCT and genetic variant (G/G vs. A/A) that differs between NVP- and ZDV-exposed mother-infant pairs.

***P value (NVP vs. ZDV) is for testing that association between risk of MTCT and genetic variant (G/A+G/G vs. A/A) that differs between NVP- and ZDV-exposed mother-infant pairs.

Associations of HIV-1 MTCT with *IL-10* Haplotypes in ARV-Exposed Ugandan Mother-Infant Pairs

HIV-1 Transmission	Comparisons	OR (95%CI) Unadjusted	P-value Unadjusted	OR (95%CI) Adjusted for Mother's CD4 Count and log HIV-1 RNA	P-value Adjusted for Mother's CD4 Count and log HIV-1 RNA
NVP-exposed pairs					
	GCC vs. ATA	0.20 (0.02,1.80)	0.151	0.14 (0.01,1.45)	0.099
	ACC vs. ATA	0.37 (0.12,1.13)	0.081	0.44 (0.13,1.41)	0.167
	ACC vs.GCC	1.87 (0.23,15.42)	0.561	3.10 (0.35,27.25)	0.308
ZDV-exposed pairs					
	GCC vs. ATA	2.74 (0.75,10.04)	0.127	4.16 (0.83,20.87)	0.083
	ACC vs. ATA	1.86 (0.66,5.29)	0.243	1.94 (0.63,5.97)	0.247
	ACC vs.GCC	0.68 (0.26,1.77)	0.429	0.67 (0.23,1.93)	0.46

Summary and Conclusions

1. Our data suggest that polymorphisms in *IL-10*-1082-G/A are associated with HIV-1 MTCT in mother-infant pairs exposed to zidovudine. When all transmissions were considered: G/G was associated with higher risk of transmission versus A carriers for antiretroviral-exposed mother/infant pairs, but not with antiretroviral-naïve mother-infant pairs.

2. Antiretroviral treatment altered the impact of host genetics on MTCT, specifically with respect to polymorphisms in *IL-10*-1082. This finding suggests that whereas single dose nevirapine to mother and baby had a significant impact on transmission and reduced maternal viral load at birth, the administration of zidovudine to mothers shortly before delivery leads to the immunosuppressive effects of IL-10 dominating over its HIV inhibitory effects leading to an association with increased MTCT.

3. We recognize that the differences in associations between antiretroviral-exposed infants and antiretroviral-naïve infants could be confounded by geographic location of cohorts studied (Uganda versus Malawi/South Africa) and hence by factors such as population background, genetic differences or viral subtypes.

4. Validation cohort studies are needed to confirm these findings.

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For further information, please contact jkw001@ucsd.edu, kusingh@ucsd.edu, or saspector@ucsd.edu