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Maternal Human Leukocyte Antigen - A*2301 Is Associated with Increased Mother-to-Child HIV-1 Transmission

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Abstract

We examined associations between maternal HLA and vertical HIV-1 transmission in a perinatal cohort of 277 HIV-infected women in Nairobi. HLA class I genes were amplified using sequence-specific oligonucleotide probes and analyses were performed using logistic regression. Maternal A*2301 was associated with increased transmission risk before and after adjusting for maternal viral load (odds ratio [OR]=3.21; 95% CI: 1.42, 7.27, p=0.005, p_{corr}=0.04; adjusted OR=3.07; 95% CI: 1.26, 7.51, p=0.01, p_{corr}=NS). That maternal HLA-A*2301 was associated with transmission independent of plasma HIV-1 RNA levels, suggests that HLA may alter infectivity through mechanisms other than influencing HIV-1 viral load.

Keywords

Human immunodeficiency virus; vertical HIV-1 transmission; human leukocyte antigen

Introduction

Not all infants born to HIV-1-infected women become infected, possibly due to human leukocyte antigen (HLA) molecules of the mother and child. HLA class I alleles have been associated with HIV-1 transmission and progression and this may be due to differential peptide presentation to CD8+ T cells [1, 2].

Effects of infant HLA and mother-child HLA concordance on vertical HIV-1 transmission have been demonstrated in several cohorts [2-4]. Maternal alleles may also alter transmission risk by modifying maternal HIV-1 progression via adaptive or innate immune responses, which may select for easily transmitted viral isolates or may modify the amount of maternal virus to be transmitted [5]. Alternatively, maternal HLA effects may reflect

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shared infant alleles that alter susceptibility to HIV-1 acquisition. The importance of HLA alleles of the HIV-infected person in a potential transmission pair is supported by a recent study of HIV-discordant couples demonstrating associations between class I alleles of the infected partner and transmission that were not associated with susceptibility among uninfected partners [1]. We examined associations of common maternal HLA class I alleles and haplotypes with vertical transmission in a perinatal cohort in Kenya.

Methods

Study setting and subjects

HIV-1 infected women were recruited from antenatal clinics in Nairobi as previously described [2]. Participants took zidovudine from 34-36 weeks of gestation until delivery [6]. Maternal blood and genital swabs were collected at 32 weeks of gestation and maternal blood was collected at delivery to assess HIV-1 RNA viral load, quantified by the Gen-Probe HIV-1 viral load assay (Gen-Probe, Inc., San Diego, USA), which is sensitive for Kenyan subtypes A, C and D [7]. All women provided written informed consent and the study received ethical approval from the institutional review boards at University of Washington and University of Nairobi.

HLA typing

DNA was extracted from maternal peripheral blood mononuclear cells using Gentra Purgene reagents (Qiagen, Valencia, California). High resolution maternal HLA class I typing was conducted at the 4-digit level following a sequence specific oligonucleotide probe (SSOP) typing protocol developed by the 13th International Histocompatibility Workshop (http://www.ihwg.org/protocols/protocol.htm) in which alleles were determined by SSOP hybridization patterns following locus-specific polymerase chain reaction (PCR) amplification. Because maternal HLA typing was part of a secondary study aim, we were only able to obtain HLA data for a subset of the full cohort.

Determination of infant HIV-1 infection

Infant HIV-1 gag DNA in filter blood specimens was detected using PCR at birth and at 1 month after delivery to determine infant infection status [8]. Infants were considered to be HIV-1-infected if two consecutive filter paper specimens were positive for HIV-1 DNA or if the last available filter assay was positive. In order to determine more precise timing of infection, HIV-1 RNA levels were measured on plasma samples obtained prior to the first positive HIV-1 DNA filter paper using the Gen-Probe assay.

Data analysis

To analyze maternal HLA associations with HIV-transmission, we used logistic regression with infant infection at month 1 as the outcome. We restricted the analysis to transmissions occurring before 1 month of age because the mechanisms influencing *in utero* and intra-partum transmission may be different from transmission via breastfeeding and there were too few late transmission events to analyze associations between HLA alleles and breast milk transmission. Multivariable regression models were adjusted for maternal HIV-1 plasma RNA viral loads at 32-weeks of gestation. Because many alleles were uncommon, odds ratios were computed only for alleles found in >10% of mothers in order to limit spurious associations. HLA haplotype analyses were performed using the Haplo Stats package available in S-Plus/R. We inferred haplotypes using an expectation-maximization (EM) algorithm and assessed haplotype-transmission associations using score statistics for haplotypes found at frequency ≥0.01 as previously described [1, 9].

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Results

Cohort characteristics

HLA typing was performed for 277 mothers of infants for whom HIV-1 transmission was assessed. Among these mothers the median CD4+ T cell count and plasma HIV-1 RNA viral load at 32 weeks of gestation were 469 cells/μl (interquartile range [IQR]=295-608) and 4.7 $\log_{10}$ copies/ml (IQR=4.2-5.3), respectively. At delivery, the median maternal plasma HIV-1 RNA viral load was 4.1 $\log_{10}$ copies/ml (IQR=3.5-4.8) and was strongly correlated with maternal viral load antenatally (Pearson's correlation coefficient=0.67). Between birth and 1 month of age, 9 (3%) infants were lost to follow-up and 4 deaths occurred among uninfected children. By 1 month of age, 47 infants acquired HIV-1, with 21 (46%) infections being detected within 48 hours of birth. Overall, 88 unique HLA alleles were identified, of which, 20 were expressed by at least 10% of participants. Overall, 21 three-locus and 23 two-locus haplotypes were found at frequencies ≥0.01 using an EM algorithm (Table 1).

Maternal HLA alleles associated with HIV-1 transmission

HLA-A*2301 was associated with increased transmission risk, even after adjusting for maternal HIV-1 RNA levels (odds ratio [OR]=3.21, 95% CI: 1.42, 7.27, p=0.005, p_{corr}=0.04 and adjusted OR [aOR]=3.07, 95% CI: 1.26, 7.51, p=0.01) (Figure 1). Prior to 1 month of age, 11 of 31 (35%) infants of mothers carrying A*2301 versus only 36 of 246 (15%) infants of mothers without this allele were infected. Mothers with A*2301 also had higher mean plasma HIV-1 RNA viral loads at 32 weeks of gestation than non-carriers (5.03 versus 4.65 $\log_{10}$ copies/ml, p=0.03). We observed a protective trend for A*7401 (OR=0.31, 95% CI: 0.09, 1.06, p=0.06 and aOR=0.35, 95% CI: 0.10, 1.17, p=0.08). After using a Bonferroni correction for multiple comparisons at the A locus (8 alleles), only the unadjusted association between A*2301 and transmission remained significant (p<0.006).

HLA-B*1503 was not associated with higher early transmission risk before adjusting for maternal viral load but became statistically significant after adjustment (OR=1.73, 95% CI: 0.82, 3.64, p=0.15 and aOR=2.36, 95% CI: 1.05, 5.32, p=0.04). The association between B*1503 and transmission risk may be due to the presence of this allele on a haplotype with A*2301 (see below). We also observed a protective trend when mothers carried B*1801 (OR=0.19, 95% CI: 0.03, 1.49, p=0.09). No HLA-C alleles were associated with transmission.

Maternal HLA haplotypes associated with HIV-transmission

Evidence that three-locus maternal HLA haplotypes were associated with HIV-transmission comes from the global test of association (p=0.04) (Table 1). Specifically, the A*2301-B*1503-C*0202 haplotype was enriched among HIV-1 transmitting mothers (p=0.0001, p_{corr}=0.002). The corresponding B*1503-C*0202 haplotype was not significantly associated with transmission and the global test for local haplotypes was non-significant (p=0.47). The A*3001-B*4201-C*1701 and B*4403-C*0401 haplotypes were also enriched among transmitting mothers.

Multiple allele models

Because of associations between the A*2301 and B*1503 alleles, and the A*2301-B*1503-C*0202 haplotype with HIV-1 transmission, we also evaluated associations between these alleles and transmission using regression models that included both alleles. In a model that included A*2301 and B*1503, A*2301, but not B*1503, was significantly associated with transmission (OR=3.0, 95% CI: 1.3, 6.8, p=0.01 and OR=1.5, 95% CI: 0.7, 3.1, p=0.3, respectively).

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Discussion

In this perinatal cohort, infants of women carrying HLA-A*2301 had a higher risk of HIV-1 acquisition before 1 month of age than infants of women without this allele. Maternal A*2301 was also associated with higher plasma HIV-1 RNA viral loads. Additionally, the A*2301-B*1503-C*0202 haplotype, but not the B*1503-C*0202 haplotype, was associated with increased transmission, suggesting that A*2301 may have driven the observed association between B*1503 and transmission.

Little is known about distinct immunologic features of A*2301 that might explain its association with increased vertical HIV-1 transmission. However, the potential biological significance of these results is supported by other population-based studies which found A*23(01) to be associated with HIV-1 related outcomes. In a Kenyan commercial sex worker cohort, women carrying A*2301 had a 3.6-fold increased risk of HIV-1 seroconversion[10]. Among HIV-1 infected children in the US, 57% of rapid progressors versus 4% of long-term surviving children carried A*2301 [11]. The role of A*2301 in transmission and progression is further supported by a Zambian study in which the A*23-B*07-Cw*07 haplotype was enriched in transmitters and studies that found the serologically defined A*23 to be associated with disease progression and Kaposi's sarcoma [1, 12-14].

Part of the effect of A*2301 on transmission may simply be due to greater levels of maternal virus. However, significance and strength of the association after adjusting for maternal plasma viral load indicates that other mechanisms may contribute to the influence of A*2301 on transmission. Given the consistency of A*23-associated susceptibility across studies, it is possible that immune pressure conferred by this allotype selects for viral isolates that are particularly infectious and virulent in infants/children. Other possible mechanisms to explain this association include linkage disequilibrium with another gene, that A*2301 is a ligand for the KIR3DL1 natural killer cell receptor, and latent associations between A*23 and viral shedding that are independent of plasma viral load (such as local flora and co-infection with other pathogens such as herpes simplex virus (HSV)-2) [1].

We also found evidence that maternal B*1801 might be associated with protection from transmission. Fewer mothers carrying B*1801 were transmitters than non-carriers, although B*1801 was uncommon (8%), resulting in limited statistical power. This observation is in agreement with our group's previous finding that infant B*18 protected against acquisition [2]. Vertical HIV-1 transmission occurs in an environment with strong correlation between maternal and infant HLA alleles. Furthermore, HIV-1 exposed uninfected infants frequently have HIV-1 specific IFN-γ responses that may be protective against HIV-1 infection [15]. Thus, another potential explanation for associations between maternal HLA and HIV-1 transmission is that maternal alleles are proxies for infant alleles that are associated with acquisition. HLA alleles may be differentially associated with maternal infectivity and infant susceptibility, thus, defining the role of maternal and infant alleles concurrently in larger studies will be useful.

Previous studies, including an analysis of this cohort, have suggested that HLA concordance and homozygosity at any allele are associated with increased transmission [3, 4]. It is also possible that concordance or homozygosity for a specific allele may be associated with increased transmission risk but we were not able to evaluate this hypothesis because doing so would require a much larger cohort. However, few mother-child pairs shared A*2301 and few mothers carried two copies of the allele in our study, therefore, it is unlikely that A*2301 was associated with increased transmission because of these factors. Another limitation of this study is that there was a diverse array of alleles and that relatively few

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transmissions occurred among mothers with any particular allele. Thus, we had limited statistical power to examine associations for rare alleles.

In summary, we found that maternal HLA was associated with early HIV-transmission. Generally, studies of HLA in HIV-1 transmission have focused on susceptibility of infants or adults in vertical and sexual transmission, respectively. However, the current study and another recent study of HIV-1 discordant couples contribute to evidence that HLA alleles of infected mothers and partners also influence transmission risk [1]. In sum, these data emphasize the complex roles immunogenetic factors play in HIV-1 infectiousness, and strengthen evidence that HLA must be considered when developing vaccines and other interventions.

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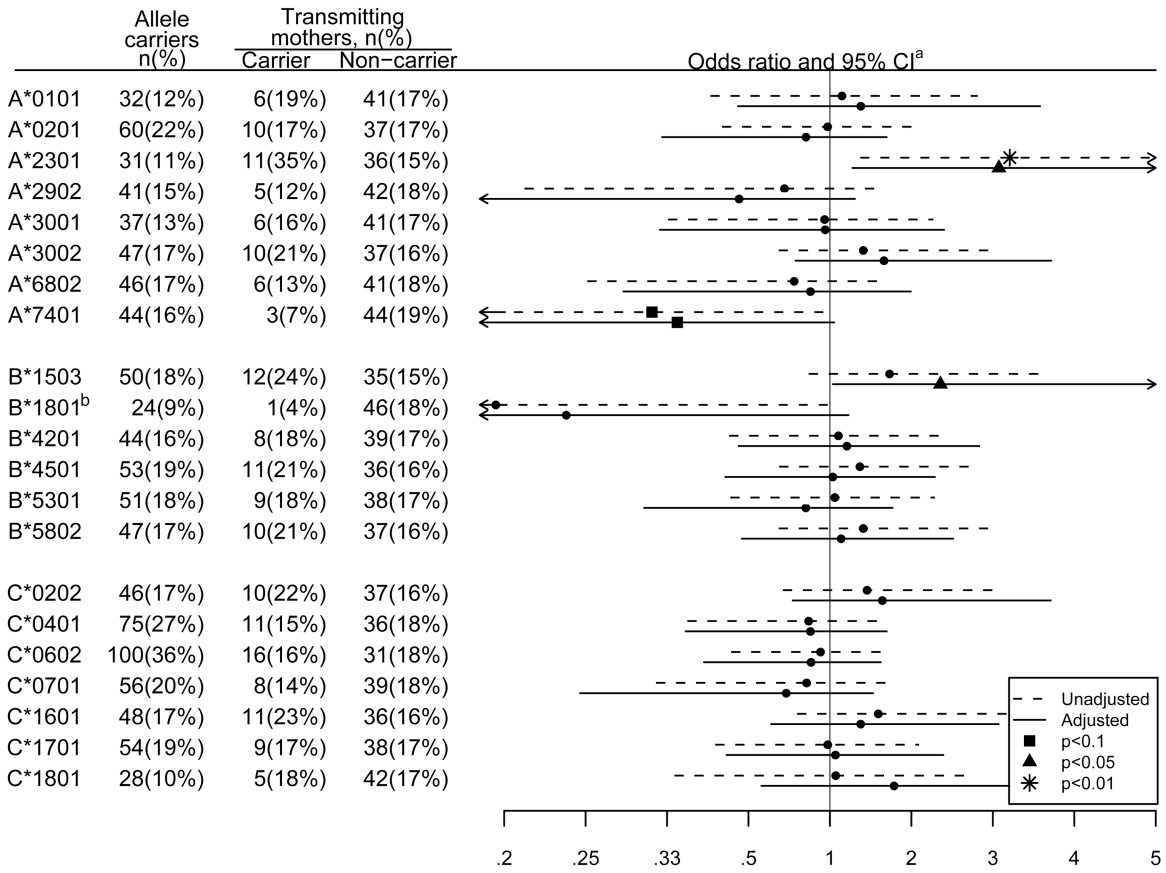

Figure 1. Associations between maternal HLA class I alleles and vertical HIV-1 transmission

[a] Unadjusted odds ratios are for all 277 mothers with HLA data, of whom, 47 transmitted HIV-1 to their infants by 1 month postpartum. Plasma viral load adjusted odds ratios are for 263 mothers for whom HIV-1 viral load data from 32 weeks gestation were available. Unadjusted and adjusted odds ratios were estimated using logistic regression.

[b] While only prevalent in 9% of the women in this cohort, B*1801 was included in these an alyses because infant B*18 was protective against HIV-1 infection in a previous study with in this cohort.

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Table 1

Major HLA class I haplotypes among transmitting and non-transmitting mothers (n=277).

Three locus (A, B, C) Haplotypes[a]					Two locus (B, C) Haplotypes[a]				
21 Haplotypes[b]	**Overall Freq.**	**TM[c] Freq.**	**NTM[d] Freq.**	**Score[e]**	**23 Haplotypes[b]**	**Overall Freq.**	**TM[c] Freq.**	**NTM[d] Freq.**	**Score[e]**
A*7401-B*3501-C*0401	0.02	–	0.02	-1.4	B*3501-C*0401	0.02	–	0.03	-1.7
A*3002-B*1801-C*0704	0.01	–	0.02	-1.3	B*1402-C*0802	0.04	–	0.02	-1.3
A*7401-B*5802-C*0602	0.01	–	0.02	-1.2	B*1801-C*0704	0.03	0.01	0.03	-1.3
A*0201-B*4201-C*1701	0.01	–	0.01	-1.2	B*5801-C*0602	0.01	–	0.02	-1.2
A*2902-B*4201-C*1701	0.03	0.01	0.03	-1.1	B*4901-C*0701	0.03	0.01	0.03	-1.2
A*3002-B*1402-C*0802	0.01	–	0.02	-1.1	B*1302-C*0602	0.02	0.01	0.02	-0.7
A*0201-B*4501-C*1601	0.03	–	0.03	-0.6	B*5801-C*0701	0.02	0.01	0.02	-0.6
A*3601-B*5301-C*0401	0.03	0.02	0.03	-0.6	B*5301-C*0401	0.08	0.06	0.07	-0.5
A*7401-B*1503-C*0202	0.03	0.01	0.03	-0.5	B*5703-C*0701	0.02	–	0.02	-0.5
A*6802-B*0702-C*0702	0.01	0.01	0.01	-0.3	B*1503-C*0401	0.02	0.01	0.02	-0.5
A*0101-B*8101-C*1801	0.01	–	0.01	-0.2	B*0702-C*0702	0.03	0.02	0.03	-0.5
A*6802-B*1510-C*0304	0.02	0.01	0.01	-0.1	B*5703-C*1801	0.01	–	0.01	-0.1
A*0101-B*4901-C*0701	0.01	0.01	0.02	-0.1	B*8101-C*1801	0.03	0.03	0.03	0.1
A*3002-B*4501-C*1601	0.02	0.04	0.01	0.2	B*4501-C*1601	0.06	0.06	0.06	0.1
A*0201-B*5301-C*0401	0.02	0.02	0.02	0.3	B*1510-C*0304	0.03	0.03	0.03	0.1
A*2902-B*4501-C*0602	0.01	0.02	0.01	0.9	B*5301-C*0602	0.02	0.02	0.02	0.4
A*6601-B*5802-C*0602	0.02	0.03	0.02	1.4	B*4201-C*1701	0.09	0.11	0.08	0.7
A*0201-B*1503-C*0202	0.02	0.01	0.01	1.5	B*0801-C*0701	0.01	0.02	0.01	0.7
A*0202-B*5802-C*0602	0.02	0.05	0.02	1.5	B*4501-C*0602	0.04	0.05	0.03	0.9
A*3001-B*4201-C*1701[f]	**0.04**	**0.09**	**0.03**	**2.3**	B*5802-C*0602	0.09	0.12	0.09	1.1
A*2301-B*1503-C*0202[g]	**0.01**	**0.07**	**0.01**	**3.8**	B*1503-C*0202	0.07	0.11	0.07	1.4
					B*5101-C*1601	0.02	0.04	0.02	1.5
					B*4403-C*0401[f]	**0.01**	**0.03**	**0.01**	**2.2**

[a] Three and two-locus haplotypes were inferred using an expectation-maximization algorithm.

[b] Three and two locus haplotypes with overall frequencies ≥0.01 were included in this analysis (global p-values = 0.03 and 0.47, respectively).

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[c] Transmitting mothers

[d] Non-transmitting mothers

[e] Haplotypes are sorted by ascending order of score statistics. Haplotype-specific scores allow the evaluation of which haplotypes are most strongly associated with HIV-1 transmission. Negative scores are given for haplotypes that are protective against transmission and positive scores are given for haplotypes that are enhanced among HIV-1 transmitters.

[f] $p \leq 0.05$, p_{corr}=NS

[g] $p=0.0001$, $p_{corr}=0.002$