

Host Genetic and Viral Determinants of HIV-1 RNA Set Point among HIV-1 Seroconverters from Sub-Saharan Africa

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ABSTRACT

We quantified the collective impact of source partner HIV-1 RNA levels, human leukocyte antigen (HLA) alleles, and innate responses through Toll-like receptor (TLR) alleles on the HIV-1 set point. Data came from HIV-1 seroconverters in African HIV-1 serodiscordant couple cohorts. Linear regression was used to determine associations with set point and R^2 to estimate variation explained by covariates. The strongest predictors of set point were HLA alleles (B*53:01, B*14:01, and B*27:03) and plasma HIV-1 levels of the transmitting partner, which explained 13% and 10% of variation in set point, respectively. HLA-A concordance between partners and TLR polymorphisms (*TLR2* rs3804100 and *TLR7* rs179012) also were associated with set point, explaining 6% and 5% of the variation, respectively. Overall, these factors and genital factors of the transmitter (i.e., male circumcision, bacterial vaginosis, and use of acyclovir) explained 46% of variation in set point. We found that both innate and adaptive immune responses, together with plasma HIV-1 levels of the transmitting partner, explain almost half of the variation in viral load set point.

IMPORTANCE

After HIV-1 infection, uncontrolled virus replication leads to a rapid increase in HIV-1 concentrations. Once host immune responses develop, however, HIV-1 levels reach a peak and subsequently decline until they reach a stable level that may persist for years. This stable HIV-1 set point represents an equilibrium between the virus and host responses and is predictive of later disease progression and transmission potential. Understanding how host and virus factors interact to determine HIV-1 set point may elucidate novel mechanisms or biological pathways for treating HIV-1 infection. We identified host and virus factors that predict HIV-1 set point in people who recently acquired HIV-1, finding that both innate and adaptive immune responses, along with factors that likely influence HIV-1 virulence and inoculum, explain ~46% of the variation in HIV-1 set point.

Following HIV-1 acquisition, plasma HIV-1 concentrations reach a peak level and then decline to a stable set point within 3 to 4 months after infection. This HIV-1 set point level is a critical determinant of later disease progression and infectivity (1–4) and is thought to be a measure of the balance between the replicative fitness of the transmitted virus and the antiviral responses of the new host. The importance of viral characteristics in determining set point is supported by multiple studies reporting a strong correlation between plasma HIV-1 RNA levels in HIV-1-transmitting (source) partners with the HIV-1 set point of their seroconverting partner (5–10). The host contribution is exemplified by the importance of variation in the human leukocyte antigen (HLA) class I (HLA-A, HLA-B, and HLA-C) genes, which are strongly associated with set point by virtue of the essential role for HLA in presenting HIV-1 peptides to CD8⁺ T cells (11). Furthermore, HLA allele sharing within an HIV-1 transmission pair may lead to a higher seroconverter set point by limiting recognition of transmitted HIV-1 escape mutations by the seroconverter's HLA-mediated immune response (12–14). These host factors may elicit

changes in HIV-1 characteristics that impact HIV-1 replicative capacity (15–17).

Studies of HIV-1 serodiscordant couples are particularly useful for evaluating host and virus determinants of HIV-1 set point, since they facilitate longitudinal analysis of viral and host data from both partners in a sexual transmission dyad. In this context,

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we recently reported that factors contributing to set point in seroconverters included HIV-1 level of the source partner as well as other factors influencing the inoculum of HIV-1 from the source partner's genital compartment (5). Specifically, bacterial vaginosis among source partners was associated with a higher seroconverter HIV-1 set point, whereas male circumcision and use of acyclovir to suppress genital herpes simplex virus type 2 (HSV-2) were associated with a lower set point. However, it remains unclear if unattributed variation in HIV-1 set point reflects as-yet unevaluated host response factors through alternative arms of the host response. For example, the Toll-like receptors (TLR) are critical to initiating innate host responses through the recognition of specific pathogen-associated molecular patterns (18–23). Here, we seek to augment our prior evaluation of determinants of HIV-1 set point among African HIV-1 serodiscordant couples by quantifying variation in HIV-1 viral set point specifically attributable to major host genetic mediators of the host adaptive (HLA genes) and innate (TLR genes) responses in the context of virus and source partner characteristics.

MATERIALS AND METHODS

Ethics statement. Human subject research review and approval was obtained from the University of Washington Institutional Review Board (IRB) and at the following study sites and affiliated institutions: University of Cape Town Research Ethics Committee, Moi University Institutional Research and Ethics Committee, Botswana Ministry of Health Research and Development Committee, Harvard School of Public Health Human Subjects Committee, Uganda National Council for Science and Technology, Uganda National HIV/AIDS Research Committee, UCSF Committee on Human Research, Kenya Medical Research Institute Ethics Review Committee, Kilimanjaro Christian Medical University College Ethics Review Committee, Kenyatta National Hospital Ethics Review Committee, and University of Witwatersrand Johannesburg Human Research Ethics Committee. All participants provided written informed consent.

Participant selection. This analysis included data from 510 participants with both seroprevalent HIV-1 (unknown date of HIV-1 infection) and seroincident HIV-1 (HIV-1 seroconversion observed during follow-up) who were enrolled in cohort studies of HIV-1 serodiscordant couples. Specifically, all HIV-1-infected participants were included in an analysis of HLA allele associations with HIV-1 set point, whereas only data from HIV-1 seroconverters and their HIV-1-infected transmitting heterosexual partners were included when modeling the impact of host and virus factors on set point. Data were collected from two African cohorts of HIV-1 serodiscordant couples: (i) the Partners in Prevention HSV/HIV Transmission Study evaluated the efficacy of genital herpes (HSV-2) suppression, with acyclovir provided to 3,408 HIV-1/HSV-2-infected partners to reduce HIV-1 transmission to their heterosexual HIV-1-uninfected partner (24), and (ii) the Couples Observational Study, an observational study of 485 couples (25). For both cohorts, HIV-1 serostatus in the initially HIV-1-uninfected partner was assessed prospectively by HIV-1 rapid assays, with HIV-1 infection confirmed by enzyme-linked immunosorbent assay (ELISA), Western blotting, or reverse transcription-PCR (RT-PCR). The date of infection for HIV-1 seroconverters was estimated by combining HIV-1 serology and plasma HIV-1 RNA PCR results as follows (5). (i) For participants that had HIV-1 RNA detected in a plasma sample collected at a visit prior to HIV-1 seroconversion (e.g., HIV-1 RT-PCR positive but seronegative), the date of infection was estimated to occur 17 days before the date of the first plasma-positive visit (26, 27). (ii) If HIV-1 RNA was detected first at the HIV-1 seroconversion visit (e.g., HIV-1 RT-PCR positive and seropositive), the date of infection was calculated as the midpoint between the last HIV-1 seronegative and first HIV-1 seropositive visit or 45 days before the first HIV-1 seropositive visit for participants who missed the previous quarterly visit and had a

long gap since the previous visit. Transmission linkage among couples was assessed through sequence comparison of a limited region of HIV-1 *env* and/or *gag* in plasma HIV-1 from both partners (28). Prevalent HIV-1-infected partners whose virus sequence was linked to their seroconverting partner's virus are referred to as source partners.

Assessment of plasma HIV-1 RNA concentrations and HIV-1 set point. Plasma HIV-1 RNA levels were determined for all HIV-1-infected participants using the COBAS AmpliPrep/COBAS TaqMan HIV-1 RNA assay, version 1.0 (Roche Diagnostics, Indianapolis, IN), with a limit of quantification of 240 copies/ml. A plasma HIV-1 RNA level below the limit of detection (240 copies/ml) was set to the midpoint (e.g., 120 copies/ml). For seroconverters, HIV-1 RNA levels were determined using plasma collected at the first HIV-1-seropositive visit and at visits <1 month and 3, 6, 9, and 12 months after seroconversion. Plasma HIV-1 RNA measurements taken 4 or more months after the estimated date of infection were considered to have occurred after the establishment of the HIV-1 set point (1). For participants with seroprevalent HIV-1, set point was defined using plasma HIV-1 RNA collected at 0, 3, 6, and 12 months after enrollment and at the final study visit. We excluded RNA measurements that varied from the previous measurement by $>1 \log_{10}$ copies/ml, measurements when the participant reported being on antiretroviral therapy (ART) during the preceding interval, and measurements from participants whose CD4 cell count dropped below 200 cells/mm³.

Genotyping. HLA class I genes were amplified from genomic DNA using locus-specific PCR primers flanking exons 2 and 3. HLA alleles were assigned based on patterns of hybridization of PCR products to sequence-specific oligonucleotide (SSO) probes. Ambiguous SSO results were resolved by sequencing as described elsewhere (29).

TLR single-nucleotide polymorphism (SNP) genotypes were defined by an Illumina custom oligonucleotide pool assay (OPA) as previously described (30). Data for one *TLR2* SNP (rs3804100) and one *TLR7* SNP (rs179012) were included in this analysis based on previously reported associations with set point (30).

Statistical analysis. Multiple HIV-1 RNA measurements representing HIV-1 set point were analyzed for HIV-1-infected participants. Therefore, associations of host factors with set point were determined using linear mixed models (LMM), with *P* values and 95% confidence intervals (CI) estimated using Markov chain Monte Carlo (MCMC) methods. The initial selection of HLA alleles was based on univariate LMM of HLA class I allele associations with set point among all HIV-1-infected participants, including seroprevalent and seroincident participants. These univariate models were restricted to alleles found in $>2\%$ of participants.

We evaluated the effects of host genetic, virus, and transmitting partner characteristics on HIV-1 set point among HIV-1 seroconverters with virus that was genetically linked with the HIV-1-infected study partner's virus using univariate and multivariable LMM models. To accomplish this, we added HLA alleles identified as significantly impacting the set point in the analysis described above and TLR alleles previously found to be associated with set point in a larger group of HIV-1-infected participants from the same cohorts (30). We incorporated these alleles into the previously described model for predictors of set point that included HIV-1 RNA levels most proximate to the estimated date of infection, male circumcision, bacterial vaginosis, and acyclovir use (5). In order to maximize the power of this model to define predictors of set point, in the multivariable model we included only those host genetic factors (HLA alleles and TLR SNPs) that were previously associated with set point in prior univariate analyses. For this analysis, HLA class I allele sharing (concordance) within a transmission dyad was scored at the HLA-A, HLA-B, and HLA-C loci separately based on the number of identical alleles shared by both partners at the 4-digit level (0 to 2). In addition, a cumulative concordance score was generated by counting the number of shared alleles across the three HLA class I loci (0 to 6). In the multiple-variable model, the overall amount of variation explained was determined based on the coefficient of determination (R^2) from an ordinary least-squares regression model using the mean set point level of each seroconverter as

TABLE 1 Quantitative associations between HLA class I alleles and log₁₀ plasma HIV-1 RNA levels among all HIV-1-infected participants, including recent seroconverters and those with seroprevalent HIV-1 infection

Allele	No. (%) with allele	Association between allele and RNA level ^a					
		Unadjusted			Adjusted		
		Mean difference	95% CI	<i>P</i>	Mean difference	95% CI	<i>P</i>
A.2301	71 (14)	0.4	(0.2, 0.5)	<0.001	0.3	(0.2, 0.5)	<0.001
A.3001	66 (13)	0.3	(0.2, 0.4)	<0.001	0.3	(0.2, 0.4)	<0.001
A.3303	12 (2)	-0.6	(-0.9, -0.3)	<0.001	-0.7	(-1, -0.4)	<0.001
B.1401	11 (2)	-0.6	(-1.0, -0.3)	<0.001	-0.5	(-0.9, -0.2)	0.001
B.2703	11 (2)	-0.7	(-1.2, -0.5)	<0.001	-0.7	(-1.2, -0.5)	<0.001
C.0202	17 (3)	-0.5	(-0.8, -0.3)	0.0002	-0.5	(-0.8, -0.3)	<0.001
B.5301	66 (13)	0.2	(0.1, 0.4)	0.001	0.2	(0.1, 0.4)	0.003

^a Linear mixed models were used to estimate effects of HLA alleles on longitudinal HIV-1 set point measurements. Results report mean differences in set points between allele carriers and noncarriers. 95% CI and *P* values were estimated using Markov chain Monte-Carlo methods for linear mixed models. Models were adjusted for population stratification.

the outcome variable. Statistical analyses were conducted in the R software environment (31).

RESULTS

Description of participants. Among the HIV-1-infected participants included in the HLA analysis ($n = 510$), 385 (76%) were from east Africa. Participants had a median of 4 HIV-1 RNA set point measurements (interquartile range [IQR], 3, 5); the median set point was slightly higher among those with seroprevalent HIV-1 (4.7 copies/ml; IQR, 4.1, 5.2) than linked and unlinked seroconverters (4.5 [IQR, 3.6, 5.0] and 4.6 [IQR, 4.1, 5.0] copies/ml, respectively). HIV-1 *gag* and *env* subtype data were available for 270 participants from both partners in the couples associated with HIV-1 transmission. Among these individuals, subtype A (*env*, 48%; *gag*, 47%) was most common, followed by subtypes C (*env*, 33%; *gag*, 27%) and D (*env*, 16%; *gag*, 12%). Recombinant forms were found in 15% of HIV-1 *gag* sequences and 3.4% of *env* sequences. As expected, subtype C predominated among participants from southern Africa, whereas subtypes A and D predominated among east Africans.

Host genetic associations with HIV-1 set point. Among these 510 HIV-1-infected participants, 125 HLA alleles were identified, including 58 found in >2% of study participants. We first evaluated the 58 HLA alleles found in >2% of all HIV-1-infected participants for associations with HIV-1 set point. HLA-A*23:01 was most significantly associated with set point. Individuals with 1+ copies of this allele had mean set point levels that were 0.4 log₁₀ copies/ml (95% CI, 0.2, 0.5; $P < 0.001$) higher than those of participants without this allele (Table 1). A*30:01 and B*53:01 also were associated with increased HIV-1 set point among all participants (for A*30:01, mean differences of 0.3 log₁₀ copies/ml; 95% CI, 0.2, 0.4; $P < 0.001$; for B*53:01, 2 log₁₀ copies/ml; 95% CI, 0.1, 0.4; $P = 0.008$). Four HLA alleles were associated with decreased HIV-1 set point levels. Most notably, B*27:03 was associated with a 0.7 log₁₀ copies/ml (95% CI, 0.5, 1.2; $P < 0.001$) decrease. A*3303, B*14:01, and C*02:02 were associated with 0.6 (95% CI, 0.4, 1.0, $P < 0.001$), 0.6 (95% CI, 0.3, 1.0, $P < 0.001$), and 0.5 (95% CI, 0.3, 0.8, $P = 0.0002$) log₁₀ copies/ml decreases in HIV-1 set point, respectively. The B*27:03-C*02:02 haplotype was associated with a decreased set point ($P = 0.02$), suggesting that the association for C*02:02 was driven by linkage disequilibrium with B*27:03. B*57:03, B*58:01, and B*81:01, which have been

associated with protection in previous studies, were at low frequency in this population (2%, 5%, and <1%, respectively) and not associated with differences in HIV-1 set point among all HIV-1 participants. When only HIV-1 seroconverters were considered, B*57:03 was associated with a reduction of 0.9 log₁₀ copies/ml (95% CI, 0.9, 1.6; $P = 0.004$), although only 3 seroconverters carried this allele.

In order to model effects of host and source partner factors on HIV-1 set point among recent HIV-1 seroconverters, we next restricted the analysis of HLA allele associations with set point to HIV-1 seroconverters. Among the HLA alleles associated with HIV-1 set point among all HIV-infected participants, three (B*53:01, B*14:01, and B*27:03) remained statistically significant after restricting the analysis to the 90 linked HIV-1 seroconverters. The strongest of these was B*53:01, with a 0.7 log₁₀ copies/ml reduction in set point (95% CI, 0.4, 1.0; $P < 0.001$), while B*14:01 and B*27:03 were associated with increased HIV-1 set point concentrations.

Among transmission pairs with genetically linked HIV-1 sequences, 50 (57%) shared 1+ alleles at the HLA-A, HLA-B, or HLA-C locus, including 18 (21%) with 1+ shared HLA-A alleles, 18 (21%) with 1+ shared HLA-B alleles, and 31 (36%) with 1+ shared HLA-C alleles. Having one or more shared alleles at any HLA locus was associated with a 0.6 log₁₀ copies/ml increase (95% CI, 0.4, 0.9; $P < 0.001$) in plasma HIV-1 RNA set point (Fig. 1A). When evaluated independently, HLA-A concordance was associated with a 0.7 log₁₀ copies/ml increase in set point (95% CI, 0.4, 1.0; $P < 0.001$), and HLA-C concordance was associated with a 0.5 log₁₀ copies/ml increase (95% CI, 0.2, 0.8; $P = 0.002$). HLA-B concordance was not associated with altered HIV-1 set point concentrations (mean difference, 0.2 log₁₀ copies/ml; 95% CI, -0.2, 0.6; $P = 0.5$) (Fig. 1B).

Finally, we evaluated whether two TLR SNPs previously identified as being associated with HIV-1 RNA set point (30) also were associated with the set point among the subgroup of HIV-1 seroconverters with linked transmission included in this analysis. In this group, the *TLR7* rs179012 SNP was associated with a 0.4 log₁₀ copies/ml decreased set point (95% CI, -0.6, -0.2; $P < 0.001$), and the *TLR2* rs3804100 SNP was associated with a 0.7 log₁₀ copies/ml increased set point (95% CI, 0.1, 1.3; $P = 0.02$) (Fig. 1C).

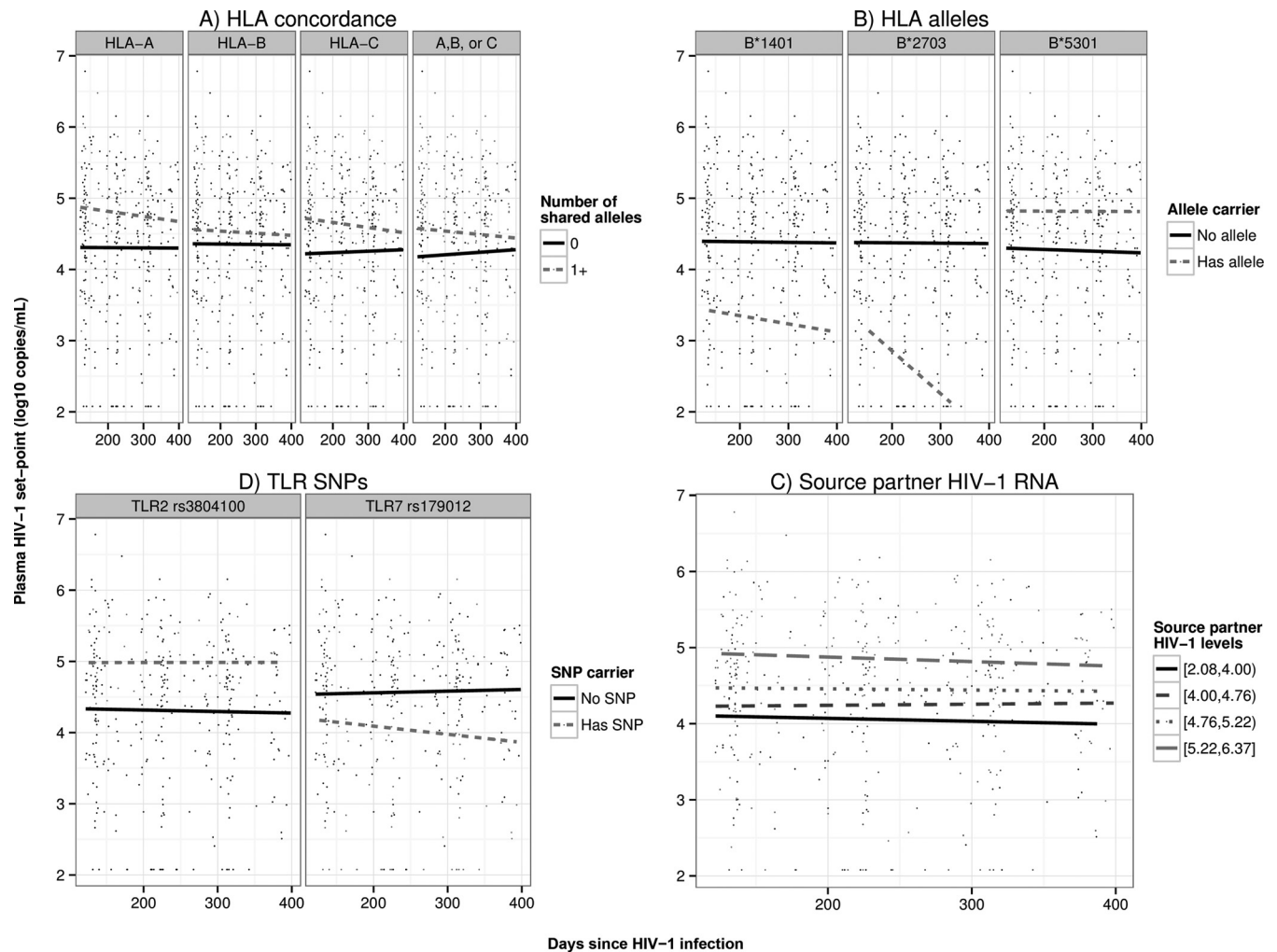


FIG 1 Longitudinal HIV-1 RNA set point measurements by HLA concordance, HLA alleles, source partner HIV-1 RNA, and TLR polymorphisms. Points represent individual HIV-1 set point measurements, and lines were created by fitting seroconverter set point measurements for the indicated group against time. Infection for participants were subgrouped according to HLA concordance between source partner and seroconverter (A), the presence or absence of the specific HLA allele (B*1401, B*2703, or B*5301; the dashed line indicates the presence of at least one allele) (B), the quartile of the source partner's plasma HIV-1 RNA level (in \log_{10} copies/ml) (C), and the presence or absence of a specific TLR variant (*TLR2* rs3804100 or *TLR7* rs179012, with red indicating the absence and blue indicating the presence of at least 1 allele) (D).

Associations of source partner characteristics with HIV-1 set point. In univariate analyses applied to all linked seroconverters, each 1 \log_{10} copies/ml increase in the last known plasma HIV-1 RNA level of the HIV-1 source partner was associated with a 0.4 \log_{10} copies/ml increased HIV-1 set point in seroconverters (95% CI, 0.3, 0.6; $P < 0.001$) (Fig. 1D). Additionally, male seroconverters whose female source partner had bacterial vaginosis at the time of transmission also had a 0.4 \log_{10} copies/ml higher set point (95% CI, 0.2, 0.7; $P = 0.001$). Male circumcision and acyclovir use among HIV-1 source partners both were associated with a decreased set point (for circumcision, $-0.5 \log_{10}$ copies/ml; 95% CI, $-0.9, -0.2$; $P = 0.01$; for acyclovir use, $-0.3 \log_{10}$ copies/ml; 95% CI, $-0.6, -0.1$; $P = 0.01$).

Integrating host and virus characteristics in a prediction model for plasma HIV-1 RNA set point. We next evaluated the impact of adding host genetic characteristics of the HIV-1 seroconverter to a prediction model of plasma HIV-1 set point we previously developed based on epidemiologic characteristics of

seroconverters and source partners (5) (Table 2). Overall, the new model accounted for 46% of the variation in HIV-1 seroconverter set point, as determined by the coefficient of determination (R^2) (Fig. 2). HLA alleles and source partner HIV-1 RNA levels had the strongest influence on set point levels. The removal of HLA alleles from the multivariate model while retaining all other variables resulted in a 13% decrease in R^2 , and removal of source partner HIV-1 RNA levels resulted in a 10% decrease in the overall amount of explained variation. HLA-A concordance explained 6% of set point variation, and two TLR SNPs explained 5% of variation in set point. Associations of acyclovir, bacterial vaginosis, and male circumcision of the source partner were diminished in terms of strength and significance compared to univariate models; however, the removal of all these variables from the model still resulted in an $\sim 7\%$ decrease in R^2 . Since the effect of each variable on R^2 of the multivariate model partially depends on the other variables in the model, the sum of the change in R^2 after removing each predictor individually does not equal R^2 for the full model.

TABLE 2 Multiple-variable model of quantitative associations between host/source partner characteristics and plasma HIV-1 RNA set-point in HIV-1 seroconverters

Model parameter	Mean difference in set point (95% CI)	P	Change in R^{2a}
HLA-A concordance	0.47 (0.16, 0.75)	0.003	0.06
Seroconverter HLA alleles			
HLA-B*14:01	-1.14 (-1.88, -0.45)	0.002	0.13
HLA-B*27:03	-0.72 (-1.55, 0.09)	0.08	
HLA-B*53:01	0.63 (0.28, 0.9)	<0.001	
Seroconverter TLR SNPs			
<i>TLR2</i> rs3804100	0.38 (-0.29, 1.08)	0.2	0.05
<i>TLR7</i> rs179012	-0.39 (-0.69, -0.17)	0.001	
Source partner characteristics			
Acyclovir use	-0.24 (-0.47, 0.04)	0.1	0.09
Bacterial vaginosis	0.26 (0, 0.54)	0.05	
Male circumcision	-0.26 (-0.61, 0.14)	0.2	
Plasma HIV-1 RNA of source partner (\log_{10} copies/ml)	0.36 (0.21, 0.51)	<0.001	0.10

^a Change in R^2 from removing groups of variables from multiple variable model.

DISCUSSION

In this study, we found that a combination of seroconverter genetic and source partner characteristics (including source partner viral HIV-1 RNA level) explained nearly 46% of the variation in seroconverter plasma HIV-1 RNA set point concentrations. The strongest contributors to set point were HLA alleles from the HIV-1 seroconverters and HIV-1 RNA levels of the transmitting partner. Additionally, in this model, HLA concordance within transmission pairs and TLR SNPs of the seroconverter also were associated with changes in set point. In a previous study of Zambian HIV-1 serodiscordant couples, male sex, age, HLA alleles, HLA concordance, and source partner HIV-1 RNA level explained ~37% of the variation in set point (9). The increased variation explained by our current model may be partly attributed to variation in the *TLR* genes, which previously have been shown to be associated with innate host control of HIV-1 (30, 32–39). However, in both studies a substantial portion of variation in seroconverter HIV-1 set point remains unexplained, suggesting that there still are factors influencing set point that have yet to be identified. One potential source of unexplained variation may be identified by explicitly incorporating into this model both the specific seroconverter HLA alleles and the viral epitopes that are thought to interact with HLA to elicit cytotoxic T-lymphocyte (CTL) responses. An analysis that incorporates other host and viral factors (both HLA and non-HLA related) may help dissect the specific factors in the source partners' viral genome that contribute to predicting seroconverter set point. However, that analysis requires a more detailed evaluation of HIV-1 *gag* sequences than is currently available from this cohort. Furthermore, unexplained variation in seroconverter set point may be contributed through variation in additional host genetic factors that have not been included in this model, such as those that restrict HIV-1 replication (e.g., APOBEC3G, TRIM5 α , and tetherin). Finally, variation

in set point also may arise from variation in laboratory assays or other unexplained sources.

Our finding that HLA-A*23:01, B*53:01, B*27:03, and B*14:01 are associated with HIV-1 set point is consistent with previously reported associations (11, 29, 40–60). Similarly, our finding that HLA concordance between the transmitting and seroconverting partner also was associated with increased set point among seroconverters is consistent with a previous study of Zambian couples (9). This association could result from HLA concordance leading to similar immunological environments in both the transmitter and seroconverter that prevent seroconverter CTL responses to HIV-1 escape mutations. In our data, concordance in HLA-A and HLA-C, but not HLA-B, was associated with increased set point. However, data from the study of Zambian couples also reported that HLA-B concordance was associated with increased seroconverter set point (9). Possible reasons for these discrepant results include different distributions in HLA alleles and HIV-1 subtypes between cohorts, although we did not detect effect modification by geographic region.

Our study also confirms a series of recent studies showing that, among couples with HIV-1 transmission, the HIV-1 RNA level of the source partner is associated with the HIV-1 RNA set point among the seroconverters. It should be noted, however, that results from these studies have been reported in different ways, potentially leading to confusion (61, 62). In this analysis we report that, based on the coefficient of determination (R^2), 10% of the variation in seroconverter HIV-1 set point is explained by the source partner's HIV-1 RNA level. This statistic measures how well the data fit the regression line and measures the amount of

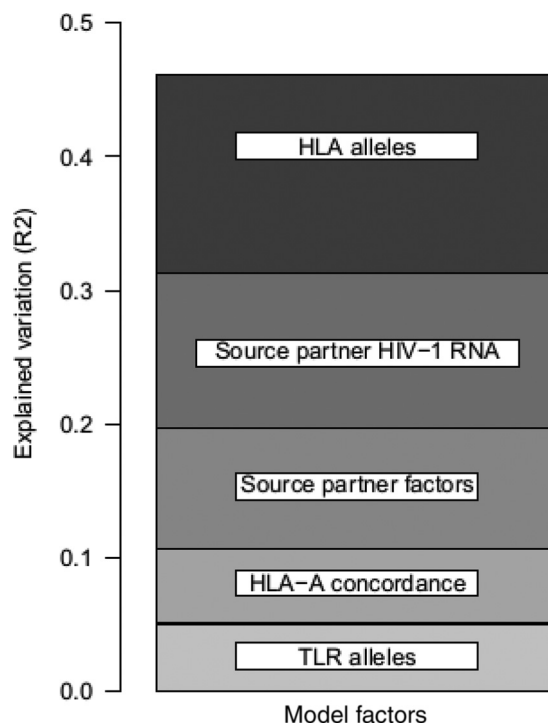


FIG 2 Contributions of virus and host/source partner characteristics to explained variation in HIV-1 seroconverter set point levels. Variation explained by each factor was determined as the decrease in the coefficient of determination (R^2) when the variable or group of variables was removed from a multiple-variable ordinary least-squares regression model.

explained variation in the context of prediction models or hypothesis testing. Other studies have assessed the relationship between source partner and seroconverter HIV-1 RNA levels as a measure of heritability, a quantitative genetics statistic measuring the proportion of variance in the HIV-1 RNA levels within a population that is attributable to factors shared by the couple (e.g., viral genetics as well as shared environmental factors) (62). Fraser et al. evaluated several studies and estimated heritability of HIV-1 virulence to be 26 to 44% across all studies (62). It is important to distinguish between R^2 and population heritability, because the former allows comparison of effects of virus and host factors for predicting set point within a person, whereas heritability does not.

A strength of our study design is that we were able to analyze characteristics of both partners in HIV-1 transmission pairs. This allowed us to evaluate effects of both HLA concordance and HIV-1 source partner virus RNA levels on seroconverter set point. Furthermore, having multiple HIV-1 RNA measurements enabled us to be certain that participants were actually at a stable set point and increased our statistical power. One potential source of unexplained variation is identified by explicitly incorporating into this model the specific seroconverter HLA alleles with the viral epitopes that are thought to interact with HLA to elicit CTL responses (63–67). Future analyses that incorporate these detailed host and viral factors may help to further dissect the specific contribution of source partners' viral genome and seroconverter host factors predicting seroconverter set point.

In sum, we found that both host genetic and viral characteristics, as well as source partner characteristics influencing virus inoculum, contributed to determination for HIV-1 set point in individuals who had recently seroconverted. Together, our model was able to explain 46% of the variation in set point, with the strongest contribution coming from HLA alleles of the seroconverter and HIV-1 RNA levels of the HIV-1 source partner. HLA-A concordance and host innate immune variation in the form of TLR SNPs also were important contributors to plasma HIV-1 set point. The remaining unexplained variation in plasma HIV-1 set point may arise due to additional host, viral, or environmental factors that remain to be defined.

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