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Cumulative Impact of Host and Viral Factors on HIV-1 Viral-Load Control during Early Infection

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In HIV-1 infection, the early set-point viral load strongly predicts both viral transmission and disease progression. The factors responsible for the wide spectrum of set-point viral loads are complex and likely reflect an interplay between the transmitted virus and genetically defined factors in both the transmitting source partner and the seroconverter. Indeed, analysis of 195 transmission pairs from Lusaka, Zambia, revealed that the viral loads in transmitting source partners contributed only ~2% of the variance in early set-point viral loads of seroconverters ($P = 0.046$ by univariable analysis). In multivariable models, early set-point viral loads in seroconverting partners were a complex function of (i) the viral load in the source partner, (ii) the gender of the seroconverter, (iii) specific HLA class I alleles in the newly infected partner, and (iv) sharing of HLA-I alleles between partners in a transmission pair. Each of these factors significantly and independently contributed to the set-point viral load in the newly infected partner, accounting for up to 37% of the variance observed and suggesting that many factors operate in concert to define the early virological phenotype in HIV-1 infection.

The HIV-1 set-point viral load (VL) in infected individuals directly affects the transmission rate of the virus (1–3) and early disease progression (4–6). Recent studies have shown a trend of increasing set-point VLs over the last 30 years of the HIV-1 epidemic (7, 8), raising the possibility of both increasing transmission rates and virulence in treatment of naïve HIV-1 infection. A plausible explanation for this observation is viral adaptation to the host at the population level over time (9, 10), providing a further challenge for HIV-1 vaccine design. The determinants of set-point VL in newly infected individuals include viral genetic factors (11–13) and host genetic factors (14–16). Thus, to comprehensively define the role of underlying factors in determining early set-point VL, it is necessary to understand the complexity of the interaction between the transmitted founder virus, with its embedded footprints of the immune response of the transmitting source partner (TSP), and the *de novo* immune defense of the seroconverting partner (SC).

We previously observed that cytotoxic T-lymphocyte (CTL) epitope escape mutations selected by immune pressure in the TSP can modulate the early set-point VL in the SC (11, 17), suggesting that mutations that would be expected to positively impact VL in the TSP can negatively impact VL in the seroconverter. This observation is consistent with our previous studies of the ZEHCP cohort and with other studies of heterosexual cohorts of limited size. These studies demonstrated a relatively weak correlation between VLs in TSPs and SCs in linked heterosexual transmission partners (18–20). In contrast, a more significant VL correlation was shown for a transmission pair cohort of men who have sex with men (MSM) in San Francisco (21), and a phylogenetic analysis of MSM in the Swiss HIV Cohort Study concluded that up to half of the variance in set-point VL can be heritable (22). These observations reinforced the hypothesis that the genetic characteristics of the transmitted founder virus could be an important determinant for defining the early set-point VL in the linked SC (18, 19, 21, 22). The contribution of the virus may, however, be mod-

ulated by human genetic factors in both the SC and the TSP. In the newly infected SC, HIV-specific CD8⁺ T-cell responses, facilitated by specific HLA class I (HLA-I) molecules, clearly have a substantial immediate impact on VL, as documented for recently and chronically infected individuals (16, 23–25). The effects of favorable and unfavorable HLA-I alleles (23, 26, 27) and their additive effect (28) on VL control have been reported. Gender has also been observed to modulate VL in both early and chronic infections in antiretroviral-naïve and -treated populations (29–31), although the exact mechanism for this is not known (32). Finally, sharing of HLA-I alleles between the TSP and the SC can lead to reduced control of virus following transmission because of the existence of CTL escape mutations selected in the TSP that reduce the efficacy of the *de novo* immune response (11, 19, 33). In our current study of 195 phylogenetically linked heterosexual transmission couples, we had a sufficiently large population to document for the first time that while the phenotype of the transmitted founder virus had a significant impact on early set-point VL, the gender of the SC as well as the immunogenetic characteristics of both the TSP and the SC significantly and independently modulated this effect. Taken together, we show that the early set-point VL established by a particular transmitted founder virus in a newly infected individual will be influenced by a complex interplay of virus and host factors.

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TABLE 1 Characteristics of HIV SC partners in entire study population and in phylogenetically linked couples only^c

Characteristic	All SCs			Linked SCs		
	Linked	Unlinked	<i>P</i> value	Male-to-female transmission	Female-to-male transmission	<i>P</i> value
No. of SCs	195	23		117	78	
No. (%) of individuals aged >40 yr	13 (6.7)	2 (8.7)	0.663	3 (2.6)	10 (12.8)	0.005
Sex ratio (no. of males/no. of females)	0.7 (78/117)	1.6 (14/9)	0.055	NA	NA	
Index partner log ₁₀ VL ^a (mean ± SD)	5.0 ± 0.7	4.3 ± 1.2	0.009	5.1 ± 0.7	4.8 ± 0.6	0.004
DOI (days) until set-point VL (median [IQR])	231 (134–339)	222 (130–251)	0.218	210 (131–322)	268 (140–378)	0.058
DOF (days) for index partner until SC EDI (median [IQR])	408 (157–972)	688 (292–1596)	0.059	421 (224–963)	379 (142–972)	0.037
No. (%) of individuals with Zambian HLA-I marker set score ^b			0.002			0.176
≥2	5 (2.6)	4 (17.4)		1 (0.8)	4 (5.1)	
1	35 (17.9)	0 (0.0)		20 (17.1)	15 (19.2)	
0	155 (79.5)	19 (82.6)		96 (82.0)	59 (75.6)	
No. (%) of individuals with <i>HLA-B</i> sharing with TSP	47 (24.1)	NA		21 (18.0)	26 (33.3)	0.014

^a VL measurement taken on the visit date closest to the estimated date of infection of the SC.

^b Zambian-specific HLA-I markers (favorable markers A*74, B*13, and B*57) were given a value of +1 if present, and the scores were added. The sums for individual SC partners ranged from 0 to ≥2. *P* values for differences in distributions of scores between linked and unlinked groups are shown.

^c VL, viral load; TSP, transmission source partner; SD, standard deviation of the mean; DOI, duration of infection; DOF, duration of follow-up from baseline visit to VL collection date; EDI, estimated date of infection; IQR, interquartile range; NA, not applicable.

MATERIALS AND METHODS

Study population. We studied 218 HIV-concordant couples from the Zambia-Emory HIV Research Project, among which 195 had phylogenetically linked and 23 had unlinked viruses based on viral sequencing (34). More than 95% of couples were infected with HIV-1 subtype C (34). This study was approved by the institutional review boards at Lusaka, Emory University, and the University of Alabama at Birmingham; all subjects gave written informed consent for participation.

Virologic assessment. Plasma VL was measured as the number of HIV RNA copies/ml, using the Roche Amplicor 1.0 assay (Roche Diagnostics Systems Inc., Branchburg, NJ), with a lower limit of detection of 400 copies/ml. Phylogenetic analysis and criteria for establishing identity between viruses from the two partners have been documented previously (34). In all 195 SCs available for analysis, set-point VL was the first VL measured ≥9 weeks after the estimated date of infection (EDI), which was defined as the midpoint between the last seronegative and first seropositive visits, resulting in a median interval of 231 days for linked couples and 222 days for unlinked couples. In a subset of SCs (*n* = 143) with multiple VL measurements, set-point VL was calculated as the geometric mean of all VLs collected between 3 and 12 months after the EDI. In TSPs, the VL measured at the visit date closest to the SC's EDI was analyzed (median interval, 89 days for linked couples and 113 days for unlinked couples).

Genotyping. HLA-I genotyping resolved alleles to the first four digits by use of a combination of PCR-based methods, including PCR with sequence-specific primers (Dyna/Invitrogen, Brown Deer, WI), automated sequence-specific oligonucleotide probe hybridization (Innogenetics, Alpharetta, GA), and sequencing-based typing (Abbott Molecular, Inc., Des Plaines, IL) tailored to capillary electrophoresis and an ABI model 3130xl DNA analyzer (Applied Biosystems, Foster City, CA).

Favorable and unfavorable HLA-I markers. The Zambian-specific HLA-I marker set included three population-specific markers previously identified (27, 35) as favorable soon after seroconversion (A*74, B*13, and B*57); the A*30-C*03 combination was excluded because of a lack of statistical significance in this subset of SCs, and HLA-C*18 was excluded due to its strong linkage disequilibrium (LD) with B*57. No HLA-I marker has been confirmed unequivocally as unfavorable in Zambian SCs with early infection. In order to summarize the impact of the Zambian HLA-I set on set-point VL for each SC, each favorable marker was given a value of +1 and then summed, for a total set score ranging from 0 to ≥2.

HLA class I allele sharing. HLA-I allele sharing and SC set-point VLs were assessed for the phylogenetically linked population only. Sharing was

defined as carriage by both the TSP and the SC of one or both alleles at *HLA-A*, *HLA-B*, or *HLA-C*. Because two-digit alleles at *HLA-B* tend to be rather homogeneous in our Zambian population, we considered sharing at the two-digit level of genotyping closely equivalent to sharing at the four-digit level, with two exceptions: the more heterogeneous B*15 and B*58 alleles, whose effects on VL have shown differences at the four-digit level (26). Although tight linkage disequilibrium between *HLA-B* and *HLA-C* alleles produces very similar associations for B and B-C haplotype sharing, analyses were performed with B allele sharing, since the evidence for involvement of *HLA-B* is stronger.

Statistical analysis. Differences in characteristics between partners of epidemiologically linked and unlinked couples were assessed using Student's *t* test (for continuous variables) and the χ^2 test or Fisher exact test (for categorical variables). All VL data were log₁₀ transformed. Generalized linear models (GLMs) were applied separately to linked and unlinked couples. Multivariable models included adjustments for other known factors associated with set-point VL, including an SC age of >40 years and SC female gender (19), as well as TSP log₁₀ VL, the Zambian-specific HLA-I score, and HLA-I allele sharing. A significant trend (not shown) of decreasing SC set-point VL with increasing Zambian-specific HLA score was detected; therefore, the score was treated as an ordinal variable in regression analysis. Normality of the multivariable linear model was assessed by the Kolmogorov-Smirnov test. For factors with statistical significance (*P* < 0.05) in two-sided tests, the effect size was measured by the beta estimate ± standard error and by % variance in GLMs. All data analyses were done using SAS statistical software, version 9.2 (SAS Institute, Cary, NC). Graphs were drawn using GraphPad Prism, version 5.0.

RESULTS

Study population and virus phylogeny. The study population consisted of 195 heterosexual transmission pairs in which the HIV-1 subtype C viruses in the TSPs and SCs were phylogenetically linked using methods described previously (34). An additional 23 couples in which the SC was infected with viruses clearly unrelated to those of the chronically infected index partner were available as controls. Among the linked pairs, male-to-female (MTF) transmissions outnumbered female-to-male (FTM) transmissions by about 50%, and male SCs were significantly older than female SCs (Table 1). The TSPs of linked pairs had a significantly higher mean VL than that of the index partners of the 23 unlinked

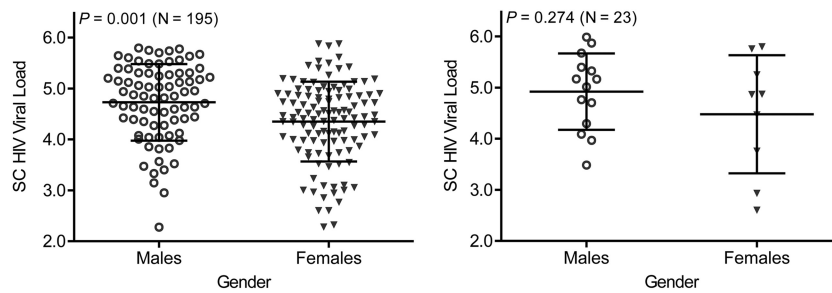


FIG 1 HIV-1 set-point viral loads in male versus female seroconverters (SCs) with (left) and without (right) confirmed transmitting source partners. Within each group, the horizontal bars connected to a vertical line represent the mean value \pm standard deviation.

SCs, consistent with the finding from prior studies that the likelihood of transmission in discordant couples increases with VL in the chronically infected partner (1, 3). The fraction of individuals with HLA-I markers reported previously to downmodulate VL (23, 27, 36) did not differ significantly between linked and unlinked SCs or between linked male and female SCs, although the distribution was significantly different, since in this small number of unlinked SCs all four individuals carried two protective alleles ($P = 0.002$) (Table 1).

For all 195 SCs, at least one VL determination from more than 9 weeks postinfection was available to establish the early set-point VL (VL1), while for 143 SCs sufficient longitudinal samples were available to determine the geometric mean early set-point VL (VL2). Univariable and multivariable analyses are presented for both early set-point VL calculations. We first analyzed the impact of host genetic factors on early set-point VL in the SC and then, in this context, the impact of TSP VL on this parameter.

Effects of age and gender. To quantify the impact of each suspected host factor on SC VL, we first tested each one individually in univariable models. Both the age and gender of infected individuals have repeatedly been associated with VL differences (19). For the 195 SCs analyzed here, only gender showed a strong statistically significant association (Fig. 1), with males exhibiting higher VLs ($P = 0.001$; $\beta = 0.38 \log_{10}$ VL). Similar results were

observed with the 143 SCs with geometric mean VLs ($P = 0.003$; $\beta = 0.41 \log_{10}$ VL), and the significance and effect of gender remained in multivariable models (Table 2). However, because age and gender have both previously been implicated as determinants of VL, regardless of source partner (19, 27), they were included in a later multivariable analysis in this study to be consistent.

Effects of host genetic factors. Evidence for favorable or unfavorable relationships of certain HLA-I markers to virologic and immunologic outcomes of HIV infection is conclusive (19, 27, 28, 33, 35, 37–39). Here we evaluated a set of these markers chosen on the basis of their documented impact and population frequencies in this Zambian HIV-discordant heterosexual transmission cohort. The Zambian-specific markers included three “favorable” HLA alleles (A^*74 , B^*13 , and B^*57) that were significantly associated with lower VLs within the early weeks of infection (Table 2). The effect on early set-point VL control for each favorable HLA class I marker in the group of 195 SCs is shown in Fig. 2A (left panel) and Table 2. In univariable analyses, the presence of each of these protective alleles had a large negative impact on early set-point VL ($\beta = -0.57$ to $0.84 \log_{10}$) (Table 2) in the SCs, and in the case of A^*74 and B^*57 , this was highly statistically significant. In multivariable models, all three alleles exhibited highly significant and similarly large effects on SC set-point VL ($P \leq 0.003$; $\beta = -0.53$ to $0.87 \log_{10}$) (Table 2). Very similar results were observed

TABLE 2 Multifactorial contributions to set-point VLs in SCs with confirmed TSPs, as revealed by GLMs^a

Host/viral factors used as independent variables (IVs)	Univariable models for VL1		Multivariable model for VL1 ^b		Univariable models for VL2		Multivariable model for VL2 ^b	
	Mean $\beta \pm$ SE	<i>P</i> value	Mean $\beta \pm$ SE	<i>P</i> value	Mean $\beta \pm$ SE	<i>P</i> value	Mean $\beta \pm$ SE	<i>P</i> value
IV1 (age of >40 yr of SC)	0.30 \pm 0.23	0.189	0.29 \pm 0.20	0.154	0.44 \pm 0.26	0.094	0.32 \pm 0.23	0.172
IV2 (SC is male)	0.38 \pm 0.11	0.001	0.47 \pm 0.11	<0.001	0.41 \pm 0.13	0.003	0.53 \pm 0.13	<0.001
IV3 (HIV-1 VL [\log_{10}] in TSP ^c)	0.17 \pm 0.08	0.046	0.28 \pm 0.07	<0.001	0.13 \pm 0.10	0.180	0.26 \pm 0.09	0.003
IV4 (HLA-A*74 in SC)	-0.59 \pm 0.17	<0.001	-0.53 \pm 0.15	<0.001	-0.84 \pm 0.19	<0.001	-0.72 \pm 0.17	<0.001
IV5 (HLA-B*13 in SC)	-0.57 \pm 0.33	0.081	-0.84 \pm 0.29	0.003	-0.54 \pm 0.37	0.142	-0.79 \pm 0.31	0.012
IV6 (HLA-B*57 in SC)	-0.84 \pm 0.20	<0.001	-0.87 \pm 0.19	<0.001	-0.77 \pm 0.24	0.001	-0.75 \pm 0.21	<0.001
IV7 (HLA-B allele sharing by SC and TSP ^d)	0.36 \pm 0.13	0.007	0.26 \pm 0.11	0.023	0.43 \pm 0.16	0.007	0.37 \pm 0.14	0.010
Individual HLA variants 1 (IV4, IV5, and IV6) ^e	-0.61 \pm 0.11	<0.001	-0.69 \pm 0.10 ^f	<0.001	-0.72 \pm 0.12	<0.001	-0.74 \pm 0.11 ^f	<0.001

^a First in models using the 9-week set-point VL in 195 SCs and second in models using the alternative geometric mean set-point VL (geometric mean in the 3 to 12 months after infection) in 143 SCs.

^b Testing of all factors (IV1 through IV7) in one model.

^c VL measurement taken at the visit date closest to the estimated date of infection of the SC.

^d HLA sharing was defined as positive if the TSP and SC partners shared at least one HLA-B allele by 2-digit designations, except for B^*15 or B^*58 , which required sharing by 4-digit designations.

^e A^*74 , B^*13 , and B^*57 were given a score of +1 if present, and the scores were summed into a single score variable. The sums in individual seroconverting partners ranged from 0 to ≥ 2 .

^f Beta result from multivariable model including IV1, IV2, IV3, IV7, and HLA variants.

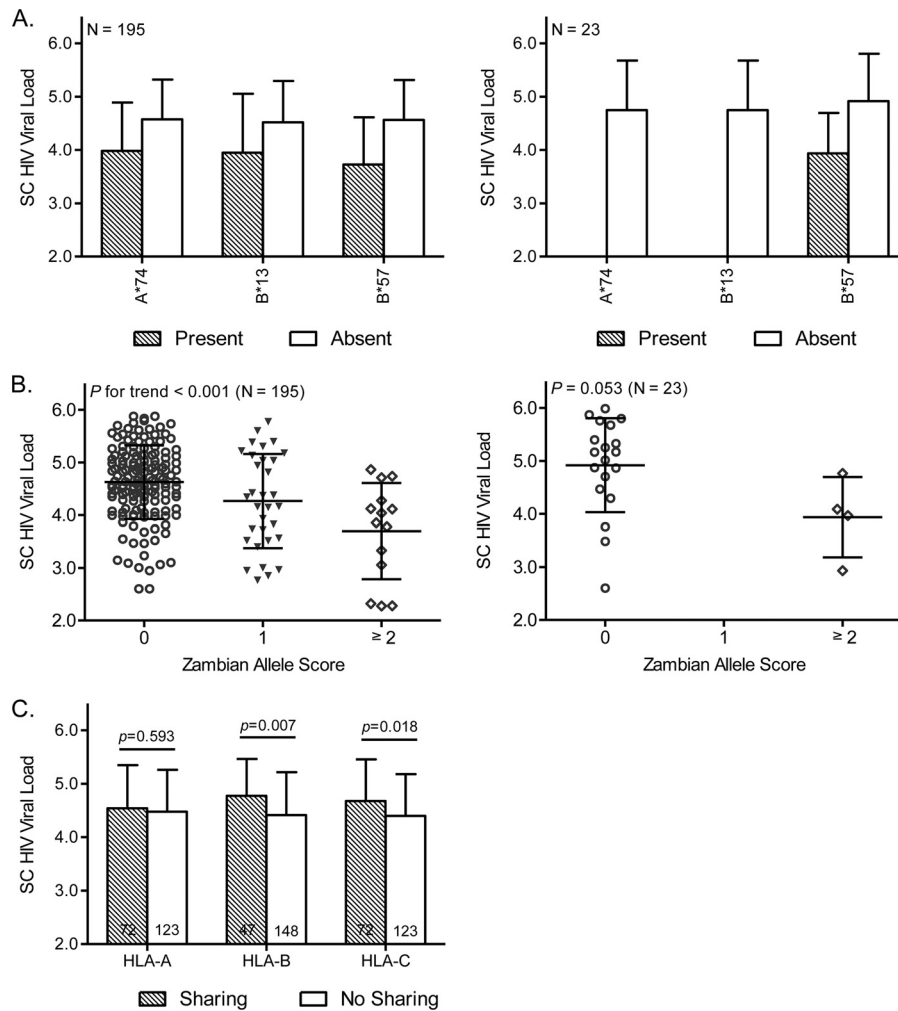


FIG 2 Association of HLA class I variants with HIV-1 set-point viral loads in seroconverters with confirmed transmitting source partners. Analyses were done for individual variants alone (A), their composite scores (additive model) (B), and allele sharing (C).

for the cohort of 143 individuals with geometric mean VLs (Table 2).

Combinations of HLA-I markers associated with more favorable outcomes would be predicted to enhance control of viremia (28, 40). Therefore, in order to summarize the impact of this Zambian-specific favorable HLA-I set on set-point VL for each SC, each of the favorable markers was given a value of +1, and the values were summed to define an overall HLA score for each individual. The additive effect shows a statistically significant inverse relationship between the sum of these values and set-point VL in SCs from the 195 linked pairs (Fig. 2B and Table 2) (univariable analysis P value of <0.001 ; $\beta = -0.61$), with similar results in multivariable analyses and in the subset of individuals with VL2. A broadly comparable relationship was also observed in the small number of SCs from unlinked pairs (Fig. 2A and B, right panels).

A major advantage of a cohort of linked transmission pairs is the capacity to examine the effect of sharing HLA markers between partners, and we were able to document the unequivocal disadvantage for the SCs of sharing *HLA-B* alleles with their partner. Early set-point VLs were significantly higher in SCs who

shared these markers with their respective TSPs (Fig. 2C and Table 2) (univariable analysis P value = 0.007; $\beta = 0.36$). Sharing of *HLA-A* alleles alone showed no effect (Fig. 2C), and strong LD precluded demonstrating a deleterious effect of sharing *HLA-C* alleles that is independent of the B allele sharing effect (Fig. 2C). Furthermore, neither homozygosity at *HLA-A*, *HLA-B*, or *HLA-C* nor carriage of specific *HLA-A* or *HLA-B* supertypes, previously implicated in HIV disease control (41), showed a significant independent association with early set-point VL in the SCs (data not shown).

Effect of transmitted virus on early set-point VL. We next determined the impact of the replicative phenotype of the virus, as assessed by VL in the TSP, on the early set-point VL in the SC. In a univariable Pearson correlation analysis of the 195 Zambian pairs, we observed a statistically significant correlation ($P = 0.046$) (Fig. 3, left panel). With an r^2 value of 0.02, this indicates that only a small fraction of the effect on SC VL could be related to TSP VL. However, when host factors in both TSPs and SCs were examined jointly with TSP VL in a multivariable analysis, the impact of the latter on early set-point VL in the SC was highly significant (Table 2) ($P < 0.001$; $\beta = 0.28$). For the smaller VL2 cohort,

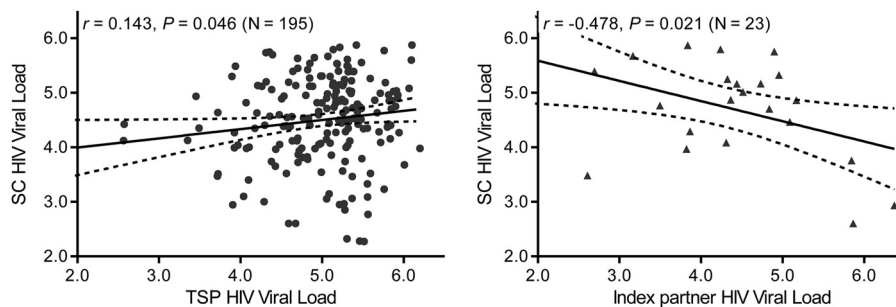


FIG 3 Correlation of HIV set-point VLs in seroconverters with VLs in confirmed transmitting source partners (TSPs) (left) or cohabiting index partners who were not the TSPs (right). Solid and dotted lines correspond to the slope and 95% confidence intervals, respectively.

VL in the TSP lost significance in univariable analyses ($P = 0.180$; $\beta = 0.13$) (Table 2), but it once again became highly significant ($P = 0.003$; $\beta = 0.26$) (Table 2) in multivariable models adjusted for the effects conferred by gender, favorable HLA alleles, and HLA sharing.

Phylogenetically unlinked cohabiting SC partners showed a negative correlation between VL in the nontransmitting index partner and that in the SC (Fig. 3, right panel). Since transmission correlates strongly with VL in the TSP (1, 3), this observation likely reflects the greater probability that SCs will be infected by nonspousal partners with higher VLs than by spousal partners with lower VLs. This explanation is supported by the fact that the mean VL in phylogenetically unlinked Zambian index partners was significantly lower than the mean VL in TSPs (4.3 versus 5.0 \log_{10} ; $P = 0.009$) (Table 1).

Complexity of early set-point VL determination. Finally, the joint impact of host and viral factors in both TSPs and SCs on the variance in early set-point VL was evaluated for the 195 SCs (Table 3). In these analyses, gender and the presence of either the A*74 or B*57 HLA-I allele individually had the greatest impact on early set-point VL (5.5, 6.1, and 8.0% variance in VL, respectively), while HLA-B allele sharing and TSP VL had a more modest impact (3.8 and 2.0% variance, respectively). In multivariable models that

take into account all of the host and viral factors, the combined impact on VL was highly significant and explained 29% of the variance observed. Similarly highly significant results were obtained for the 143 SCs with VL2 values, where the overall effect on variance was 36.9%.

DISCUSSION

This study has quantified the relative and cumulative contributions of host and viral factors in each partner of a transmission pair to early set-point VL in the SC partner. Specifically, we have demonstrated that both the VL and HLA-I profile of the TSP, as well as demographic factors and the HLA-I profile of the SC, contribute significantly to early set-point VL, a highly predictive determinant of HIV disease progression.

It is striking that in both the current study and previous analyses of heterosexual transmission pairs (18–20), the significance of the contribution of the TSP VL to SC early set-point VL in univariable analyses (Pearson correlation P value of 0.046; $r = 0.14$) was much lower than that reported for a similar study in MSM from San Francisco (Pearson correlation P value of 0.006; $r = 0.55$) (21). While the fact that HIV-1-infected females present lower VLs than their male counterparts may in part act as a confounder in the VL correlation between TSPs and SCs, it seems unlikely that this completely explains the difference. The other possible difference between these studies is that in the studies of heterosexual transmission pairs, the transmitting partner was already identified as HIV positive prior to inclusion of the couple in discordant couple prevention studies, and in most linked transmission pairs we have studied phylogenetically, the diversity of the TSP virus population is consistent with chronic infection (42–44). In a study by Hecht et al. (21), the majority of TSPs were identified posttransmission, and 9 of the 24 TSPs represented individuals with recent infection. High frequencies of recently/acutely infected source partners were also observed in phylogenetic analyses of similar transmission pairs in San Diego, CA (45). In such transmission pairs, it is likely that only limited adaptation to immune pressures was occurring in the TSP, thereby introducing fewer potential confounding components into the analysis. Moreover, in the study of Hecht et al., no correlation was observed between SC VL and SC age, race, or favorable/unfavorable HLA-I alleles, in contrast to the consistent observations from other cohorts (19, 38, 46–50). A second study of MSM, which examined the molecular phylogenetic basis for set-point VL, concluded that up to half of the variance in this parameter could be heritable, even though the stage of infection and host factors were not taken into account (22). By our estimate, host and viral factors in this heterosexual

TABLE 3 Variability in HIV-1 set-point VL attributable to host and viral factors

Host/viral factors used as independent variables (IVs) ^a	Variance in VL1 (R ²)		Variance in VL2 (R ²)	
	%	P value	%	P value
IV1 (age of >40 yr of SC)	0.9	0.189	2.0	0.094
IV2 (SC is male)	5.5	0.001	6.2	0.003
IV3 (HIV-1 VL [\log_{10}] in TSP)	2.0	0.046	1.3	0.180
IV4 (HLA-A*74 in SC)	6.1	<0.001	12.7	<0.001
IV5 (HLA-B*13 in SC)	1.6	0.081	1.5	0.142
IV6 (HLA-B*57 in SC)	8.0	<0.001	7.1	0.001
IV7 (HLA-B allele sharing by SC and TSP)	3.8	0.007	5.1	0.007
Demographic factors only (IV1 + IV2)	5.7	0.003	6.7	0.008
Individual HLA-I variants (IV4, IV5, and IV6) ^b	13.8	<0.001	19.4	<0.001
Host and viral factors combined ^c	29.1	<0.001	36.9	<0.001

^a As evaluated in generalized linear models (Table 2).

^b A*74, B*13, and B*57 were given a score of +1 if present, and the scores were summed into a single score variable. The sums in individual seroconverting partners ranged from 0 to ≥ 2 .

^c Results from multivariable model including IV1, IV2, IV3, IV7, and HLA variants.

cohort collectively accounted for up to 37% of the variance in early set-point VL. Thus, it is possible that very different factors can determine set-point VL in MSM versus heterosexual transmission pairs, but that hypothesis requires further evaluation in regions where sufficient numbers of MSM and heterosexual transmission pairs can be assembled. As we have pointed out elsewhere (51), the ongoing search for host and viral factors associated with the dynamics of HIV-1 VL should benefit from close attention to the timing of phenotypic measurements as well as careful application of statistical models.

One result of the HLA class I allele-restricted CTL response to HIV infection is the promotion of viral evolution at the population level. CTL epitope escape mutations in the virus that are selected under immune pressure can allow higher levels of virus replication in the selecting host due to reduced immune suppression, despite negatively affecting virus replicative capacity (11, 52). In contrast, upon transmission to an individual with different HLA-I alleles, such mutations can negatively affect virus fitness through decreased replicative capacity (11, 47, 53, 54). Indeed, we have shown previously an inverse correlation between the number of transmitted CTL escape mutations in *gag* and early set-point VL in the newly infected partner (17), thereby providing an additional source of variation between TSP and SC VLs. This variation may be more pronounced in our cohort, where transmission occurred from a chronically infected partner (with adapted viruses) to a cohabiting seronegative partner. Cohorts with acutely or recently infected source partners might display a different pattern of host-virus interaction.

The strong independent effect of *HLA-B* allele sharing on early set-point VL observed in the current study ($P = 0.007$; $\beta = 0.36$ to 0.43) implies that escape from HLA-I-mediated CTL recognition results in elevated viremia not only in a chronically infected individual but can also confer a replicative advantage when the virus is transmitted from that individual to a new host displaying a similar HLA-I profile. The reduced immune pressure on the virus in such transmission pairs may also reduce the impact of the CTL escape mutations on overall viral fitness (11). These observations demonstrate that the absence of information about the transmitted virus and its previous host can obscure the pathogenic potential of the virus in a recently infected individual. Such missing information likely accounts for some of the inconsistency in studies of host and viral determinants of control of viremia in recently infected individuals, and it may explain a portion of the observed variability in responses to similar antiretroviral drug regimens at the individual and population levels. Although partners who are the sources of infection have rarely been assessed in populations, our work attests to the potential value of the discordant couple paradigm for investigating host and microbial interactions, not only for HIV but for other infectious agents as well.

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