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Why does age at HIV infection correlate with set point viral load? An evolutionary hypothesis

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Abstract

Background: Set-point viral load (SPVL) correlates with the age at which people acquire HIV. Although immunosenescence may seem like a parsimonious explanation for this, it does not easily explain the observation that the relationship between age and SPVL attenuates when accounting for source partner SPVL. Here we propose an alternative explanation that encompasses this latter

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Declaration of Competing Interest

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.epidem.2022.100629.

finding: that decreasing risk of acquisition with older age generates a selection bottleneck that selects for more virulent strains with age.

Methods: We adapted a previously published model of HIV transmission and evolution (EvoNetHIV), parameterized here for men who have sex with men (MSM). We conducted a series of simulation experiments that vary seven behavioral or clinical parameters that affect exposure risk as people age. We conducted regressions to determine the mean increase in SPVL per 10-year increase in seroconversion age, with and without source SPVL in the model.

Results: All runs generated significant relationships between seroconversion age and SPVL when not including source SPVL. All saw attenuated relationships, most to near 0, with source SPVL included. Four of our behavioral measures (relational duration, age-related homophily, coital frequency, and mean age at relationship formation) had clear effects on this relationship, all in the hypothesized direction. Combining multiple forms of behavioral heterogeneity yielded an increase of 0.056 log10 copies/mL SPVL per 10-year increase in seroconversion age, nearly as large as that seen in two empirical studies of age-SPVL correlations in MSM.

Conclusion: The higher virulence of HIV among those infected later in life may be partly explained by a combination of selective bottlenecks and behavioral heterogeneity by age. Variation in the strength of this effect across populations may be in part due to different behavioral, epidemiological and clinical conditions, and not require assumptions about differences in patterns of immunosenescence among populations.

Keywords

HIV-1; Set point viral load; Evolution; Age; Networks; Modeling

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HIV set-point viral load (SPVL) – the viral load around which an individual stabilizes after acute infection – partly determines rates of HIV progression and survival in untreated infection, as well as risk of onward transmission (Fideli et al., 2001; Modjarrad et al., 2008; Quinn et al., 2000). The factors that determine SPVL for individuals or mean SPVL for populations are thus of key clinical and epidemiological importance. They are also of interest to evolutionary biologists: higher SPVL has contrasting effects on overall transmission potential (higher probability per sexual exposure, but shorter overall time during which an individual may transmit due to faster disease progression), creating the conditions for evolutionary trade-offs to produce intermediate values of SPVL. These values of SPVL may vary by population depending on the exact fitness landscape (Fraser et al., 2007; Goodreau et al., 2018; Stansfield et al., 2019). Many individual-level predictors of SPVL have been identified (Blanpain et al., 2002; Gray et al., 2004; Hollingsworth et al., 2010; Lingappa et al., 2013; McLaren et al., 2015; Roberts et al., 2012; Touloumi et al., 2013), including SPVL of the viral source partner. Multiple estimates for SPVL heritability (i.e., the proportion of SPVL variation explainable by variation in source SPVL) cluster around 30–40 % (Fraser et al., 2014; Hollingsworth et al., 2010; Lingappa et al., 2013; Yue et al., 2013).

Age at HIV seroconversion has also been considered as a predictor of SPVL in multiple studies. In general, it appears that individuals who acquire HIV at older ages average higher SPVL. At least three studies have found a significant effect (Table 1), and three

others have found a non-significant effect in the same direction. We know of no study that has reported this relationship with a point estimate at or below 0. The studies cover a range of designs (e.g. serodiscordant couple studies, seroconverter cohorts) and populations (predominantly heterosexual, MSM, African, European, and American). The two predominantly heterosexual studies with significant effects found similar effect sizes to each other when comparing those with a seroconversion age > 40 to younger groups – 0.63 higher mean SPVL for Tang et al. (2004) and 0.66 for Lingappa et al. (2013); (these and all other measures of SPVL in this paper are in units of log10 copies/mL). Studies among MSM generally show lower effect sizes, although parameterization differences make direct comparisons challenging. For example, Touloumi et al. (2013) found an average SPVL increase of 0.07 for every 10 additional years of seroconversion age, and Herbeck et al. (2008) found 0.092 for the same measure. Two studies with non-significant findings, one predominantly heterosexual (Hollingsworth et al., 2010) and one MSM (Blanquart et al., 2017), had intermediate effect sizes.

Despite the repeated documentation of increasing SPVL with older seroconversion age, the reasons for this relationship are not well understood. Like each proximate determinant of SPVL, seroconversion age likely sits within a complex set of causal pathways. Nevertheless, previous studies have primarily considered seroconversion age as a control variable, without focusing on why it may be predictive of SPVL. This is understandable, given that researchers are generally most interested in identifying predictors of SPVL that are modifiable. The individual determinants of SPVL are important to understand, however, as they fit together into a single causal framework, and can provide additional clues as to the nature of that framework as it collectively shapes this crucial feature of HIV pathogenesis, transmission, and evolution.

Perhaps the most straightforward, default explanation for the correlation of seroconversion age and SPVL is that it is caused by age-related host effects. For example, declines in CD4 counts with age may be a marker of gradual immunosenescence (De Paoli et al., 1988; Dorshkind et al., 2009; Rea, 2010; Wikby et al., 2008); and lower pre-seroconversion CD4 + count may in turn be associated with higher SPVL (Lyles et al., 2000). However, several studies have failed to replicate the finding of significant declining CD4 + count with age among HIV-negative individuals (Maini et al., 1996; Mair et al., 2008; Menard et al., 2003; Uppal et al., 2003).

One alternative explanation relates to a recent line of research on differential selection pressures at the transmission bottleneck. Carlson et al. (2014) found that amino acid residues associated with higher fitness were disproportionately transmitted from females to males rather than vice versa, suggesting differential selection due presumably to the difference in transmission probabilities between females and males (Patel et al., 2014). Building on this idea, Stansfield et al. (2019) considered insertive and receptive men who have sex with men (MSM), and presented a simple model of predicted incident SPVL that depended on both the sexual role of the seroconverting partner and the number of sex acts per relationship. In both cases, lower overall risk (insertive role, fewer acts) translated into higher SPVL for the seroconverting partner, due to the greater selective pressure at transmission. These effects interacted, with the difference by role disappearing in the context of one-time contacts or

other short relationships. These patterns were then confirmed in the same paper using a more realistic dynamic model, and in data from the Multicenter AIDS Cohort Study.

Although none of this work was focused on age, its insights may be conceptually extended to any attribute across which acquisition risk varies. This leads to the hypothesis, then, that the effect of seroconversion age on SPVL could result at least in part from people having declining rates of exposure with age, primarily as a result of fewer sexual acts, but perhaps also because their partners are less likely to be a source of infection (e.g., if most are virally suppressed). This would lead older individuals to acquire HIV at lower rates than younger ones, but (given the stronger selection bottleneck at transmission) to acquire disproportionately from those with higher SPVL. Note that the mechanism here is not about permanent behavioral heterogeneity across individuals, but changes in behavior over time within individuals.

One piece of evidence that may help to tease apart the immunosenescence and selection bottleneck hypotheses is the intriguing finding of Lingappa et al. (2013), in which the relationship between seroconversion age and SPVL disappeared when source SPVL was added to a multivariate model. This suggests that source SPVL acts as a mediating variable in the causal relationship of interest. In considering the immunosenescence hypothesis, SPVL and source SPVL are connected in two ways (Fig. 1). One is the direct pathway created by the heritability of SPVL. The other is a more indirect path involving age homophily, i.e. that older individuals also tend to have older partners. In this case, the correlation in ages between partners could also cause a correlation in their SPVL. However, neither causal pathway fulfills the laws required for statistical mediation (Baron and Kenny, 1986; Valeri and Vanderweele, 2013), with source SPVL (C) outside the direct casual chain from seroconversion age (A) to SPVL (B), such that this hypothesis should not predict the results observed by Lingappa and colleagues. On the other hand, the selection bottleneck hypothesis relies on the idea that heterogeneous exposure causes those infected later in life to have higher SPVL precisely because they are disproportionately infected by source partners with higher SPVL. Here source SPVL is on the pathway of interest (A to B), and fulfills the conditions that predict statistical mediation of that relationship.

The selection bottleneck explanation has to contend with an additional form of selection bias: assuming that there is some level of age concordance in partnerships, then those who get infected while older would also have older partners in general; and those would disproportionately comprise the set of people who had not died from HIV infection at younger ages, i.e. who had lower SPVL. This could be counteracted through effective treatment, which suppresses VL and thus increases survival, but the same viral suppression would also make those partners unlikely transmission sources. Periods of suppression interspersed by some windows of non-suppression could in theory enable transmission at older ages while minimizing the selection effect created by homophily by age. Alternatively, lower levels of age concordance or the presence of age discordance might reduce or eliminate this selection bias. It is thus worth noting that, while all of the studies showing a relationship between seroconversion age and SPVL excluded index cases who had begun antiretroviral treatment, the set of study locations and timings suggest that the roll-out of treatment, and thus the potential for source partners to have been previously suppressed,

likely ranged from quite low (Hollingsworth et al., 2010; Lingappa et al., 2013) to relatively high (Blanquart et al., 2017; Touloumi et al., 2013).

Given these complex potential causal pathways, involving a mix of biological, clinical, behavioral, and demographic factors and their interactions, with additional unidentified confounders likely, it might not come as a surprise that studies have varied widely in the magnitude of relationships identified between seroconversion age and SPVL, even as the overall pattern has generally supported a positive correlation between the two. This situation also points to mathematical modeling as an ideal tool to help isolate the potential magnitude and direction of hypothesized causes, as it allows for investigations in which different features are varied in turn while holding others fixed.

In this paper, we explore the potential for selective transmission from high SPVL sources caused by a greater transmission bottleneck for older individuals to explain the relationship between seroconversion age and SPVL. We adapt an existing modeling toolkit that integrates behavioral, demographic, biological, and clinical determinants of SPVL evolution among MSM to a new series of experiments, modeling the relationship between seroconversion age and SPVL. We vary individual potential contributing factors in turn, considering how each alters the relationship between seroconversion age and SPVL. We measure this relationship in a univariate fashion, then consider its change when also accounting for source SPVL, in order to determine whether this causal framework can account for attenuation of the relationship as seen in Lingappa et al. (2013). Note that we do not directly test whether immunosenescence may also contribute to these relationships, but focus on estimating the potential of these less intuitive explanations. Finally, we combine the measures from each of our individual experiments into a single model, to estimate the maximum effect size attributable to all of these together.

1. Methods

We adapted *EvoNetHIV,* a model for SPVL evolution on networks described in detail in previous studies (Goodreau et al., 2018; Herbeck et al., 2018; Stansfield et al., 2019). We provde an overview here, and additional detail in the Online Supplement. *EvonetHIV* is a discrete-time, stochastic model written in R; a code release and scripts specific to this manuscript can be found at https://github.com/EvoNetHIV/Age_and_SPVL. *EvonetHIV* uses the *EpiModel* API for epidemic modeling (Jenness et al., 2016), which in turn employs the statnet suite of packages for dynamic network estimation and simulation, which are based in exponential-family random graph models (Handcock M et al., 2016; Hunter et al., 2008; Krivitsky and Handcock, 2014). These allow users to set targets for an arbitrary number and set of network statistics, generating a probability model that has those statistics as their means during dynamic simulations.

Individual SPVL was determined by summing an inherited component and random component, such that the heritability (proportion of the variation in SPVL determined by source SPVL) was 0.36, equal to the estimate in Hollingsworth et al. (2010) and very similar to a subsequent meta-analysis estimate of 0.33 (Fraser et al., 2014). Mean SPVL in the initial population was 4.5, with variance 0.8 (Fraser et al., 2007; Herbeck et al., 2012).

SPVL governed the rate of transition among four CD4 + cell categories, which in turn governed rate of progression to AIDS and HIV-related death. VL varied through the acute phase and achieved SPVL at the beginning of the chronic phase, with a slow linear increase over this phase, followed by a rapid rise beginning with AIDS. Transmission probabilities were governed by current VL as well as sexual role, using a functional form derived from Hughes et al. (2012), reparametrized for MSM. Treatment was included, with individual treatment trajectories determined by a coverage rate and discontinuation rate; treatment reduced VL, and thus reduced transmission, and also increased CD4 + category, thus delaying progression and mortality. We assumed no age-related immunosenescence related to viral load—i.e. acquisition probability was constant by age conditional on exposure and source SPVL; we did so in order to isolate the potential effects of alternative explanations for increasing SPVL by age. However, background (non-AIDS) mortality rates increased with age (following US life tables), as did progression and AIDS-specific mortality rates.

The population comprised men aged 18–75, with initial population size 10,000. Parameters governing sexual behavior and network structure were adapted from our previous studies of SPVL evolution in MSM (Goodreau et al., 2018; Stansfield et al., 2019), which were ultimately derived from behavioral data from Atlanta-area MSM (Hernandez-Romieu et al., 2015; Sullivan et al., 2015). Exceptions included those which were systematically altered or varied as part of the various experiments, listed in Table 2. For these parameters, values were not drawn from specific citations, but were selected to cover a representative range of meaningful variation based on prior modeling experience, with the intent of demonstrating how variation in each predictor altered the relationship between seroconversion age and SPVL. The components of the experiments were generally drawn from the theoretical considerations laid out above, focusing either on declining risk of exposure with age (declining coital frequency, declining relationship formation, declining rates of relational concurrency), or the issue of source partner survival bias (age concordance, levels of treatment coverage and cessation). The exception is our first experiment, mean relational duration, which our previous work found to have a large impact on SPVL in some contexts, and which thus represents a behavioral substrate whose impact we wish to capture using the same age-by-SPVL regression metric found in the previous literature and in the rest of this paper.

Simulations were run for 20 years, in one-day time-steps; all incident cases had their SPVL and seroconversion age recorded, along with the SPVL of their source partner. We ran 112 random replicates of each scenario (based on our computing cluster architecture of 28 cores per node). We conduct linear regressions on $\log_{10}$ SPVL copies/mL by seroconversion age; consistent with some previous studies cited above (Herbeck et al., 2008; Touloumi et al., 2013), we report the output in terms of the effect size per 10-year increase in seroconversion age in all of our numerical and graphical results. Since we use linear models, this is simply 10 times the unit (1-year) increase. We then examine the 10-year regression coefficient for seroconversion age on SPVL when also including source SPVL. We present the mean of each 10-year coefficient across runs, and define confidence intervals (CIs) for that mean based on the standard error of the mean across the 112 replicates.

2. Results

Fig. 2 shows the results of our baseline model (all parameters at defaults), along with our first experiment, exploring different levels of mean relational duration. Here, as in all subsequent figures, black dots represent the mean value of the univariate regression coefficient for seroconversion age on SPVL and black lines represent the CI for the mean; gray dots and lines represent the same quantities for the coefficient of seroconversion age on SPVL when source SPVL is also included in the model. We see that our baseline model (with mean relational duration of 1000 days) does indeed predict a positive coefficient for the univariate analysis. The point estimate for the size of the effect is a 0.020 increase in SPVL per 10-year increase in seroconversion age, with a CI fully above 0. For comparison, the values from the two studies that measure the effect in this same way were 0.07 (Touloumi et al., 2013) and 0.092 (Herbeck et al., 2008). Controlling for source SPVL (i.e. including it as an additional predictor to remove its role as a confounder; see gray lines) essentially eliminates the effect, with a point estimate of 0.0037 and CI encompassing 0. This supports our prediction, in line with the findings of Lingappa et al. (2013), that source SPVL will appear as a mediating variable in the relationship between seroconversion age and SPVL when that relationship is caused by selection bottleneck effects.

We also see that relational duration affects this relationship, corroborating our previous work (Stansfield et al., 2019) that very short relationships show the least amount of opportunity for the selection bottleneck to generate differences in SPVL between those potentially at high risk and those at low. At mean relationship durations at around 250 days or more, we see the point estimate for the univariate analysis begin to plateau. Again, including source SPVL in the regression removes the effect of seroconversion age, supporting the former's role as a mediating variable. Note that medians here and in all subsequent scenarios were very similar to means, with the largest absolute difference in the univariate regression between the two for any scenario equal to 0.0036.

In Fig. 3, we see that the magnitude of age homophily also has a strong effect, with the largest opportunities for variation in SPVL occurring with the *weakest* age homophily. (Strong homophily means a small expected difference in age, which appears towards the left of the plot). Recall that we anticipated countervailing effects here, given the additional form of selection bias potentially induced by differential partner survival by SPVL, and thus did not have a clear hypothesis. We again see a plateau of effect sizes around 0.02, all disappearing when we include source SPVL in the model. It appears that when older individuals partner mostly with other older individuals, the survival bias towards low SPVL among their infecting partners may indeed work against the opportunities for selection by age, at least for our default values of treatment coverage and discontinuation rates.

Next we consider the rate at which coital frequency within relationships declines by age (Fig. 4). As hypothesized, the greater this decline (i.e. the greater the exposure difference by age), the higher the difference in SPVL by age. Effect sizes for the greatest differential considered rise to 0.029, and again effects are all eliminated when controlling for source SPVL. This is the first case where we may not see a plateauing of effect size, i.e. had our range of exploration included even steeper declines in coital frequencies, we may have

seen even greater effect sizes. Similarly, Fig. 5 considers variation in the rates of relational formation by age – that is, who starts new relationships, as opposed to how often coital acts occur within those relationships. Note that the mean rate of relational formation in the population as a whole is fixed across scenarios here, with lower mean age at relational formation meaning that there is a higher slope in the rate at which relational formation probabilities decline by age (and thus presumably in the heterogeneity in exposure risk by age). The variation in effect size here covers a similar range as in Fig. 4, and again moves in the hypothesized direction.

Three additional experiments yielded relatively little variation across the range of parameters explored, and are shown in the online supplement. One (Fig. S1) is another behavioral scenario (differences in the prevalence of relational concurrency by age), while the other two involve treatment. We anticipated that greater treatment coverage and lower cessation rates would each increase the effect size of SPVL variation by age, although neither hypothesis appears to be upheld (Figs. S2 and 3, respectively).

Finally, we ran an experiment that combines parameter values from each previous experiment that had generated a strong effect size in order to identify the overall magnitude of effect we can explain by the phenomena of interest (Fig. 6). The resulting effect size reaches 0.056, as compared to the empirical estimates of 0.07 (Touloumi et al., 2013) and 0.092 (Herbeck et al., 2008). Note that this value is greater than what one would get by adding together the coefficient for the baseline model (0.020), plus the change to that coefficient from each of the individual experimental runs whose components we include in our “all significant effects” model (an additional 0.019, for a combined coefficient of 0.039). This implies some synergistic effects among the phenomena we explore. Controlling for source SPVL attenuates the estimate considerably, to 0.016, but this figure remains well above the comparable value from all previous scenarios, and with a CI entirely above 0.

3. Discussion

People infected with HIV later in life display higher SPVL on average, for reasons that are not fully understood. In this paper, we hypothesized that this relationship may arise from selection bias, based on two principles: that those with lower acquisition risk who do become infected acquire more virulent variants, as previously reported (Carlson et al., 2014; Stansfield et al., 2019), and that in general acquisition risk among the still-HIV-negative population declines with age through behavioral change. We conducted simulation experiments in an MSM model and demonstrated that multiple forms of behavioral variation across age could generate a significant relationship between seroconversion age and SPVL. Moreover, we found that relationship was highly attenuated when one controlled for source SPVL, which is consistent with at least one empirical study (Lingappa et al., 2013).

Including multiple forms of behavioral heterogeneity together led to an overall effect size of 0.056 log10 copies/mL per 10-year increment in seroconversion age. This value approaches that of the two previous studies who measured SPVL heterogeneity in this way, both of which were among MSM (0.07 in Touloumi et al., 2013; 0.092 in Herbeck et al. 2008). Another predominantly MSM analysis also had similar effect sizes (Blanquart et al., 2017),

although the difference in parameterization makes the values difficult to compare directly. One commonly cited metric for defining clinically significant differences in SPVL is any difference over 0.3 $\log_{10}$ units (Modjarrad et al., 2008); this difference would occur over 43 and 33 years for the two empirical studies just mentioned, respectively, and 54 years for our model. Two prior studies with considerably larger effect studies were both among populations with predominantly heterosexual transmission (Lingappa et al., 2013; Tang et al., 2004). This suggests the possibility that these effects might often be larger among heterosexuals than MSM for at least two possible reasons: (1) the much-lower per-act risk for heterosexuals creates a stronger bottleneck with greater possibilities for selection pressure; and (2) heterosexuals may experience greater levels of behavioral risk reduction across the life span than MSM. The latter is consistent with empirical data (Glick et al., 2012). Heterosexual populations add in additional sex-by-age interactions, e.g., that age mixing is often asymmetric (older men with younger women) and that concurrency prevalence has different levels and age patterns by sex (Glick et al., 2012; Maher et al., 2011; Morris et al., 2010). Future work should aim to compare models with heterosexual transmission probabilities and behavioral parameters to those explored here to see if the former yield stronger correlations between age at seroconversion and SPVL. We also note that our models suggested considerable synergy among the phenomena explored, further emphasizing that the relationships here are complex and interacting; additional causal factors and/or confounders, both related to risk heterogeneity by age or not, are certain to exist and might help to further explain the observed relationships.

While four forms of behavioral heterogeneity by age did show notable and largely monotonic effects, one did not: the level of relational concurrency by age. This may not, in fact, be too surprising. Relational concurrency is distinct from many other behaviors that increase HIV epidemic potential: it does so by increasing the probability of acquisition among the partners of the person practicing it, not among that person themselves (Morris, 2010; Morris and Kretzschmar, 1997). Although our models exhibited some age homophily, the indirect effect of concurrency means that those put most at risk by the practice were not necessarily the same age as those practicing it, dampening its potential role in generating our relationship of interest. Similarly, patterns of treatment – either levels of coverage or rates of discontinuation – did not show much impact either. These were explored as they were expected to ameliorate a different form of selection bias: that HIV-positive individuals living to older age (and thus having older partners) would, in the absence of treatment, be biased towards those with lower SPVL due to survival differences. The absence of a strong effect of treatment patterns may mean that this additional form of selection bias was not as strong as we anticipated.

A major motivation for this analysis was the finding of Lingappa et al. (2013) that the relationship of seroconversion age and SPVL disappeared when source SPVL was considered as a mediating variable. We believe that this would not be consistent with the parsimonious explanation of immunosenescence. It is important to note, however, that two other studies did not find similar attenuation with the inclusion of source SPVL (Blanquart et al., 2017; Tang et al., 2004). While it is difficult to know the reasons for this difference, our results suggest that the nature and strengths of the relationship between age and SPVL are likely multifaceted, and can be strongly influenced by local behavioral conditions. Both

immunosenescence and behavioral heterogeneity by age may play a role, with one or the other being more or less important in different specific settings, based not only on behavioral heterogeneity by age but on overall HIV burden and other clinical factors. In that vein, it is crucial to reiterate that our analyses do not rule out the possibility of immunosenescence as an additional explanation, as it was not explored at all; our goal was simply to ascertain the potential for alternative explanations.

Our study has numerous limitations. Many of our behavioral parameters were exploratory, and we did not conduct a thorough analysis of data sources to determine what observed ranges for each are. This path was chosen since such a task would be extensive and imperfect, as studies vary widely in how they parameterize some of these measures, and the analysis here was considered exploratory, to determine whether and under what conditions the predicted relationships might appear. Moreover, these were in general guided by our familiarity with overall patterns in MSM sexual network data. Other parameters were derived from a study of Atlanta-area MSM specifically, an area with higher HIV prevalence among MSM than the country as a whole (Centers for Disease Control and Prevention, 2019); these were chosen since they were well-tailored for the specific features of our modeling framework (since they were developed by members of our research team), and because they would ensure a robust epidemic that would still allow for estimation as we altered parameters up and down in our sensitivity analyses. However, this choice limits the generalizability of our results, at least in terms of the specific quantitative findings; we suspect that the overall qualitative patterns would still hold in a setting with lower overall transmission risk behavior and HIV prevalence. Similarly, we made multiple choices in the functional forms of relationships in our model (e. g. between age and coital frequency) whose selection was aided by empirical studies but rarely known with great detail. Changing these would also likely change our quantitative results, although perhaps not the direction of relationship or general magnitude.

There are many additional phenomena that we could have included that may have increased the correlation between seroconversion age and SPVL, such as MSM switching from predominantly receptive to predominantly insertive roles as they age (Tieu et al., 2013). We also did not systematically co-vary all of the different factors across ranges, exploring how their effects interact with a multivariate regression model; this is a logical next step. We used standard errors of means as measures of uncertainty, knowing that in simulation studies these have a degree of arbitrariness to them, as sample sizes can easily be expanded; consistent with our previous work, then, we sought to limit this arbitrariness by pre-determining the number of simulation replicates at a level (~100) commonly used in simulation studies.

HIV set point viral load (SPVL) exhibits large variation, and is a major determinant of disease progression and mortality. We hypothesized that differences found in mean SPVL by seroconversion age may be due to selection bottlenecks, rather than (or in addition to) immunosenescence. We show the potential for these bottlenecks to produce a sizeable relationship between seroconversion age and SPVL as a result of declining exposures with age. Our finding that many different behavioral factors can impact this relationship, independently and in combination, should make it less surprising that different empirical

studies have found different magnitudes of effects in different populations. Viral evolution occurs on a complex landscape of pathogen and host biology intersecting with host behavior and clinical practice, often with non-intuitive outcomes for key clinical metrics like SPVL. In the search to understand how and why HIV virulence varies among individuals, it is important to incorporate these behavioral components into studies of this landscape.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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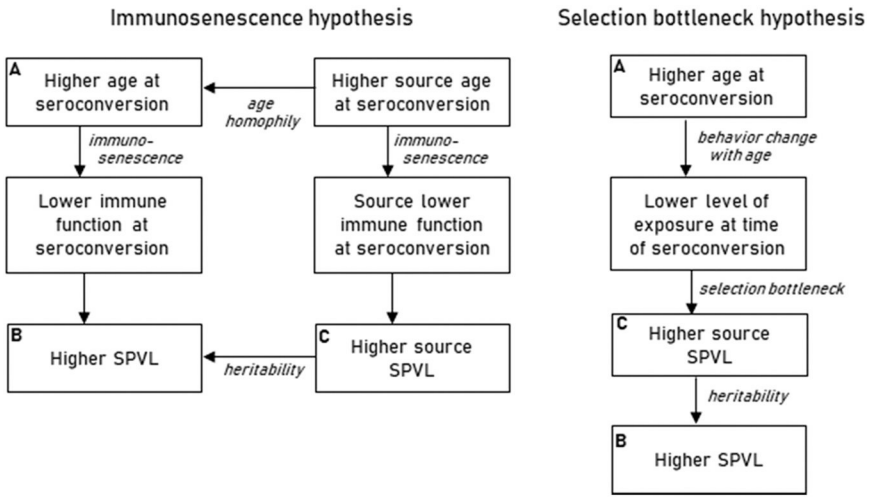

Fig. 1.

Causal diagrams linking seroconversion age (A), SPVL (B) and source SPVL (C) for each hypothesis.

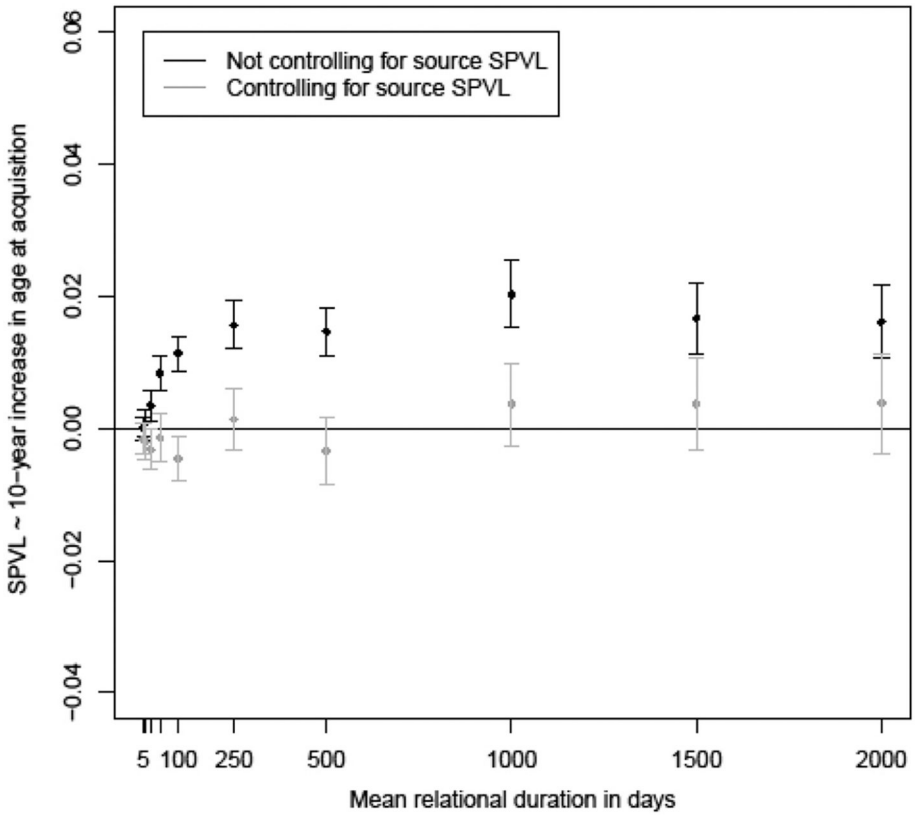

Fig. 2.
Regression coefficient for seroconversion age on SPVL, by mean relational duration.

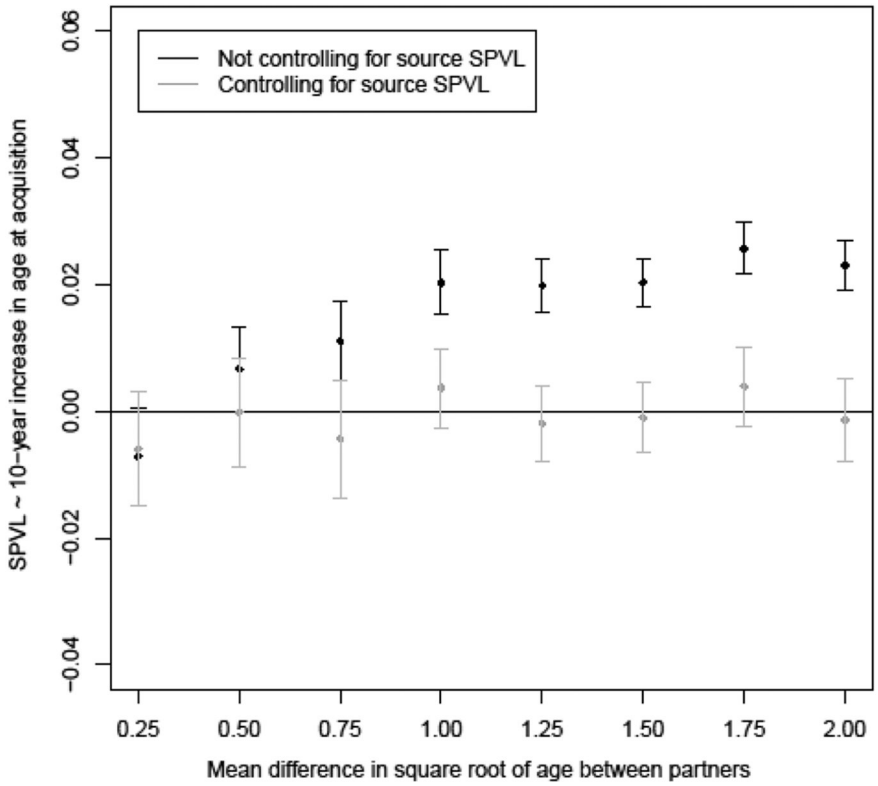

Fig. 3.

Regression coefficient for seroconversion age on SPVL, by level of age homophily.

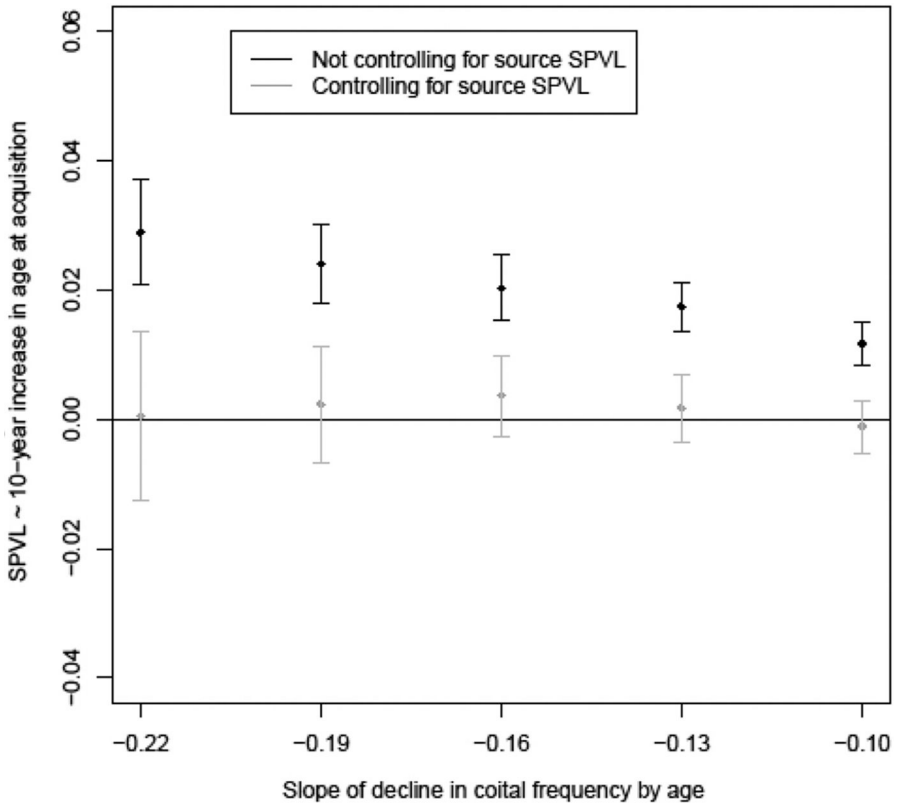

Fig. 4.

Regression coefficient for seroconversion age on SPVL, by slope of coital frequency decline by age.

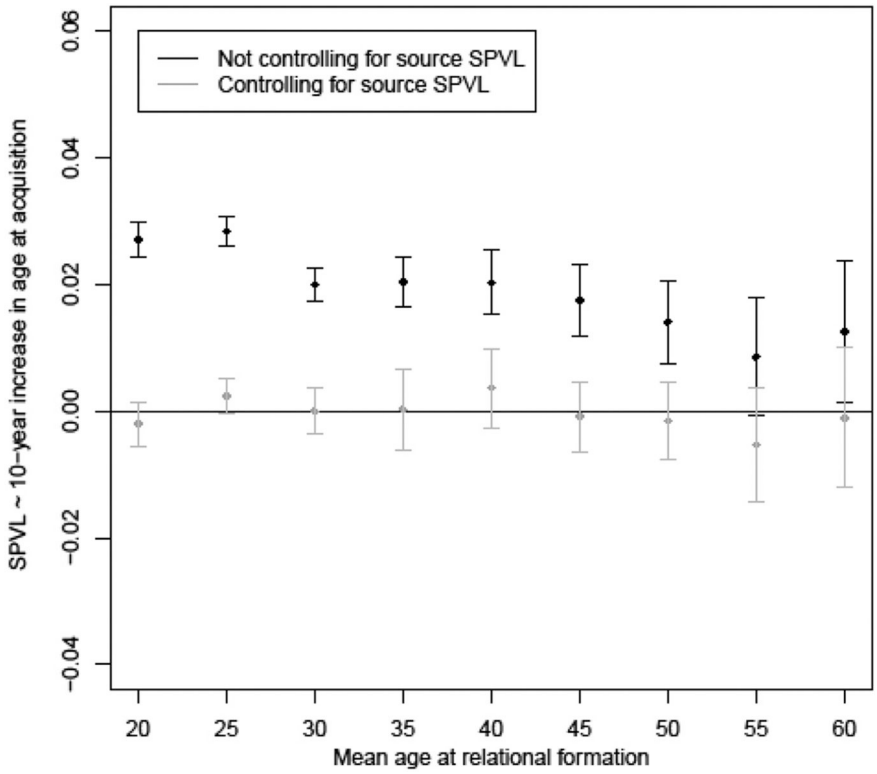

Fig. 5.
Regression coefficient for seroconversion age on SPVL, by mean age at relational formation.

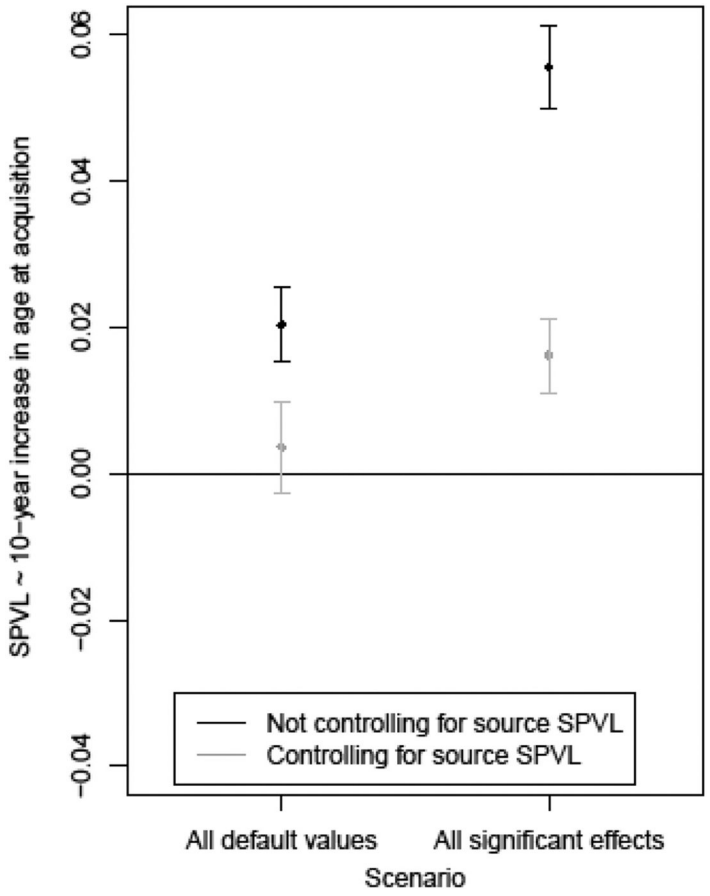

Fig. 6.

Regression coefficient for seroconversion age on SPVL, combining multiple previous effects (see Table 2).

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Table 1

Studies showing positive relationships between age at HIV seroconversion and set-point viral load (SPVL).

	Citation	Data set and population	Relationship between seroconversion age and SPVL when source SPVL not included	Effect of controlling for source SPVL via inclusion in a multivariate model
Significant findings	Tang et al. (2004)	Zambian cohort of serodiscordant couples (n = 115)	• Ref. category: age < 40 • Age 40 + averaged 0.63 $\log_{10}$ copies/mL higher than ref. (p = 0.019)	Effect remained almost the same (0.59, p = 0.020)
	Lingappa et al. (2013)	Partners in Prevention trial (n = 141)	• Ref. category: age < 25 • Ages 25–39 averaged 0.26 $\log_{10}$ copies/mL higher than ref. • Ages 40 + averaged 0.66 $\log_{10}$ copies/mL higher than ref • Overall p = 0.04	Strong attenuation: • Effect for ages 25–39 becomes –0.11 • Effect for ages 40 + becomes 0.15 • Overall p = 0.3
	Touloumi et al. (2013)	CASCADE data set (a collection of 28 seroconverter cohorts, mostly MSM, n = 2526)	• 0.07 increase in median SPVL per 10-year increase in seroconversion age (p < 0.001)	• N/A
Suggestive findings	Hollingsworth et al. (2010)	Rakai serodiscordant couples (n = 97)	• Ref. category: age 15–25 • Ages 40 + averaged 0.18 $\log_{10}$ copies/mL higher than ref. • differences among all age categories were not significant once the missing value category was excluded	• N/A
	Herbeck et al. (2008)	MACS (n = 384)	• Increase of 0.092 in mean SPVL per 10-year increase in seroconversion age (p = 0.07) • Details vary depending on which study visit was used (either the average of the VL obtained ~9 and ~15 months after the estimated date of seroconversion, or a single visit if only one was available)	• N/A
	Blanquart et al. (2017)	Five European cohorts collaborating in BEEHIVE, subtype B participants (n = 1581)	• Ref. category: age < 20 • Ages 20–39 averaged 0.088 $\log_{10}$ copies/mL higher than ref. • Ages 40–59 averaged 0.19 log10 copies/mL higher than ref. • Ages 60–80 averaged 0.24 $\log_{10}$ copies/mL higher than ref. • Overall p = 0.14 • See theirsupplementary data files, “NULL method”	• Effect remains similar: • Ages 20–39: value becomes 0.12 • Ages 40–59: value becomes 0.21 • Ages 60–80: value becomes 0.27 • See theirsupplementary data files, “OU method”

Table 2

List of experiments with associated parameter values.

Fig.	Phenomenon varied	Parameterization	Values
2	Mean relational duration	Days	5, 10, 25, 50, 100, 250, 500, **1000**, 1500, 2000
3	Age homophily in relationships	Mean absolute difference in square root of partner ages	0.25, 0.50, 0.75, **1.00**, 1.25, 1.50, 1.75, 2.00
4	Decline in coital frequency by age	μ in the expression for per-day probability of coital act $1.55\ (e^{\mu(a-18)}/1+e^{\mu(a-18)})$ where a is the harmonic mean of the ages of the individuals in a relationship. Harmonic mean was chosen to create a non-linear pattern with age (Tanfer and Cubbins, 1992)	−0.10, −0.13, **−0.16**, −0.19, −0.22
5	Decline in relational formation by age	Mean age of individuals at age of relationship formation	20, 25, 30, 35, **40,** 45, 50, 55, 60
S1	Decline in probability of relational concurrency by age	Proportion of those age <=25 with concurrent partners, vs. proportion of those age >25 with concurrent partners	**[0.15, 0.15]**, [0.20, 0.05], [0.25, 0.02], [0.30, 0]
S2	Treatment coverage	Proportion of those diagnose who are taking antiretroviral treatment at any point in time	0.20, 0.30, 0.40, **0.50**, 0.60, 0.70, 0.80
S3	Treatment cessation rates	Per-year probability of stopping treatment	0, 0.05, **0.10**, 0.15, 0.20, 0.25
6	Multiple	Values for each of the above parameters in order	1000, 1.00, −0.22, 25, [0.25, 0.02], 0.50, 0.10

Bold = default value used in experiments varying other parameters