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Approval Sheet

Defining the Effects of Virus Characteristics and Host Genetic Factors on HIV-1 Viral Fitness and Virulence After HIV-1 Transmission

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Abstract Cover Page

Defining the Effects of Virus Characteristics and Host Genetic Factors on HIV-1 Viral Fitness and Virulence After HIV-1 Transmission

By

Ling Yue
M.D. Harbin Medical University 1984

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An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
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Abstract

Defining the Effects of Virus Characteristics and Host Genetic Factors on HIV-1 Viral Fitness and Virulence After HIV-1 Transmission

By Ling Yue

Introduction: Previous studies suggest that viral load in newly infected individuals is dependent on viral characteristics in the chronically infected donors. Further evidence has confirmed that host genetic factors, such as human leukocyte antigen (HLA) molecules, play an important role in immune control of HIV-1 infection. The main goal of this study is to determine the effects of virus characteristics and host genetic factors on HIV-1 viral fitness and virulence after HIV-1 transmission in a Zambian heterosexual transmission cohort.

Methods: Nested case-control studies were conducted within a Zambian HIV-1 heterosexual transmission cohort. The 195 epidemiologically linked transmission couples who comprised the case group and the 23 unlinked couples who comprised the reference group were enrolled from 1995-2006. Favorable and unfavorable HLA alleles were selected based on previous studies both in this Zambian cohort and other cohort studies in the general population. Multivariable generalized linear models (GLM) were used to compare the simultaneous, independent contributions of individual genetic and non-genetic factors to the variability in HIV-1 viremia. The partial correlations between donor viral load at the time of transmission and recipient viral load at set-point were assessed by Pearson Correlation analysis.

Results: In the epidemiologically linked transmission group, seroconverters were younger than transmitters and the male/female sex ratio of Transmitters/Seroconverters was >1. Favorable and unfavorable HLA markers in both partners were found in a relatively equal distribution. The Final GLM model revealed in linked transmission couples that donor viral load is able to modestly increase the recipients VL set-point; however, the presence of favorable HLA markers and being female are the major effectors for lowering VL set-point in an HIV-1 newly infected recipient. When controlling for age, gender, and favorable/unfavorable HLA markers, the correlation between donor VL at the time of transmission and linked-recipient set-point VL is highly significant ($P < 0.001$). The VL correlation between unlinked couples in the reference group was not statistically significant.

Conclusion: Our studies show that protective HLA alleles are the major driving force in establishing set-point VL in individuals newly infected with HIV-1. However, donor VL at the time of transmission also has a modest effect on establishing set-point VL in linked recipients, and females generally have lower set-point viral loads than males.

Cover Page

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INTRODUCTION

After transmission, the course of HIV-1 infection is a dynamic process defined by the complex interactions between the transmitted viral variant and the newly infected host's immune system [1]. The rapid viral replication to peak of viral load (VL) at the acute infection phase is thought to be mainly modulated by the replicative fitness of the transmitted donor virus that establishes infection in the linked recipient. However, the remarkable VL decline following the peak viremia in early stage of infection is responsible by HIV-specific CD8+ cyto-toxic T lymphocyte (CTL) responses [2] [3]. More direct evidence was collected by the studies of SIV-macaque model of CD8-specific monoclonal antibodies abrogated the VL decline from its peak level [4] [5]. This specific immune response is restricted by host genetic factor human leukocyte antigen (HLA) genotype [6].

The VL set-point in a newly infected individual is gradually reached during the first year of infection, which is thought to be a correspondence of the interaction between the virus and host immune response. The clinical outcome of HIV-1 infection is highly variable and determined by the complex interplay between virus and host [7]. The set-point of VL is a surrogate measure of viral fitness and virulence. Thus, it is one of the most important indicators in determining clinical outcome and progression to AIDS. A number of studies in varying HIV-1 transmission cohorts have shown the positive correlation between donor and linked recipient viral loads in epidemiologically linked heterosexual transmission pairs when adjusted for non-genetic factors such as age and gender. This correlation indicates that characteristics of the transmitted founder

virus are the major determinant of VL set-point in the newly infected individual [8] [9]. In addition, one of the human genetic factors HLA class I alleles having a great impact on HIV-1 viral load control and disease progression has been reported [10] [11] [12].

We hypothesize that the human leukocyte antigen (HLA) genotype of the epidemiologically linked recipient is another independent factor contributing to set-point viral load in newly infected individuals. To test our hypothesis, we performed a nested case-control study evaluating the relationship between Donor VL at the time of transmission and VL set-point in the linked recipient among 195 epidemiologically linked, heterosexual Zambian transmission pairs as well as in 23 unlinked couples (used as a reference group). Specifically, the results of this study determined the effect of host genetic and non-genetic factors (age and gender) on viral load set-point after HIV-1 transmission; defined the difference between favorable HLA alleles and unfavorable HLA alleles associated with immune suppression in this specific Zambian cohort; and measured the effect of transmitted viral genetic characteristics in HIV-1 acute infection.

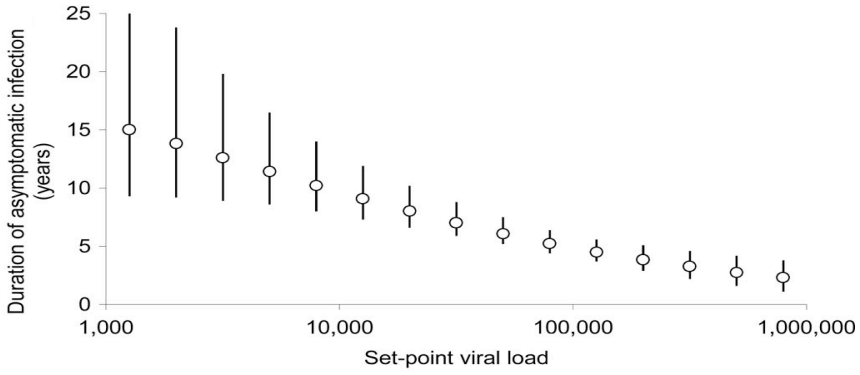
The findings of this study indicate host genetic factors such as HLA genotype can play an important role in combination with transmitted founder virus characteristics to predict the clinical outcome and disease progression for HIV-1 infected patients. The transmitted founder virus genome evolution during HIV-1 infection results from this unique virus-host interaction in each genetically distinct

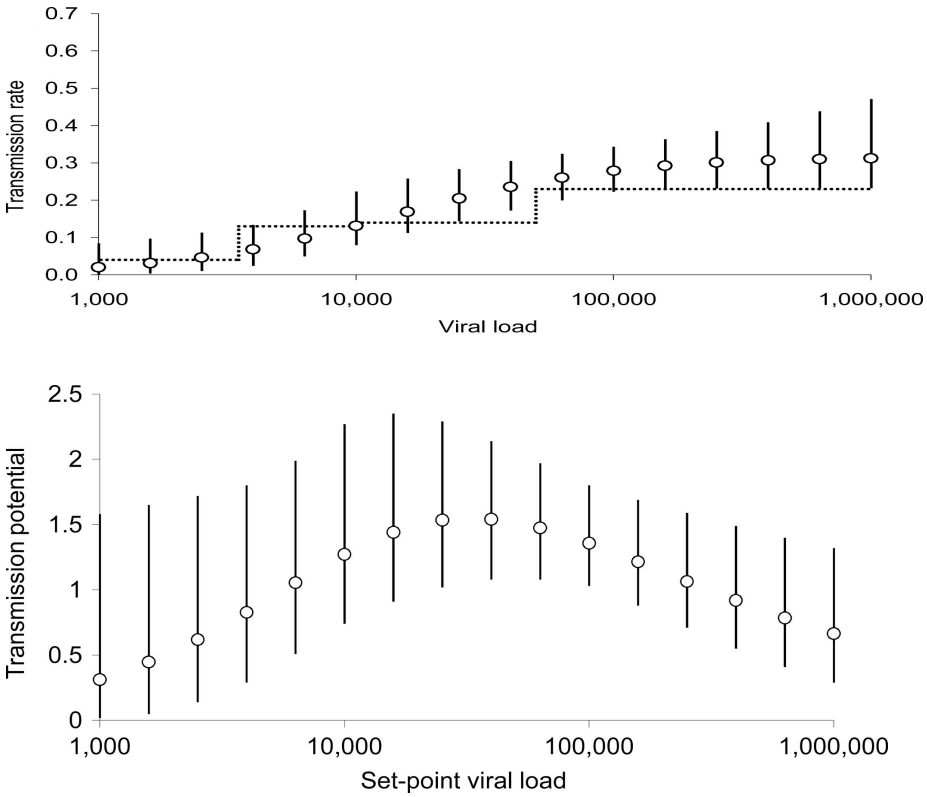
individual. We believe that analyzing HIV-1 viral evolution and defining the benefit of host genetics in HIV controllers will provide critical insights for HIV-1 vaccine design.

BACKGROUND

HIV-1 Transmission

Since 1981, almost 60 million people worldwide have been infected with the human immunodeficiency virus (HIV) and 25 million people have died of HIV-related causes (2009 AIDS epidemic update). More than 6,800 people become newly infected and 5,700 people die of AIDS each day. Viral load set-point play a very important role to determine HIV-1 transmission rate. Higher VL, especially at acute and late stage of infection [13] will increase transmission rate greatly. The duration of asymptomatic chronic infection is another key factor to predict the rate of transmission. Longer time of disease progression is more potential for HIV-1 transmission. Taking both effects into consideration, HIV-1 transmission potential is dependent on viral load set-point and the duration of asymptomatic chronic infection in the chronically infected transmitter [14] and median level of VL set-point has the greatest potential of transmission. This finding is significant and may have great epidemiological impact for defining the public health consequences of HIV-1 prevention.





Fraser et al. *PNAS* 2007

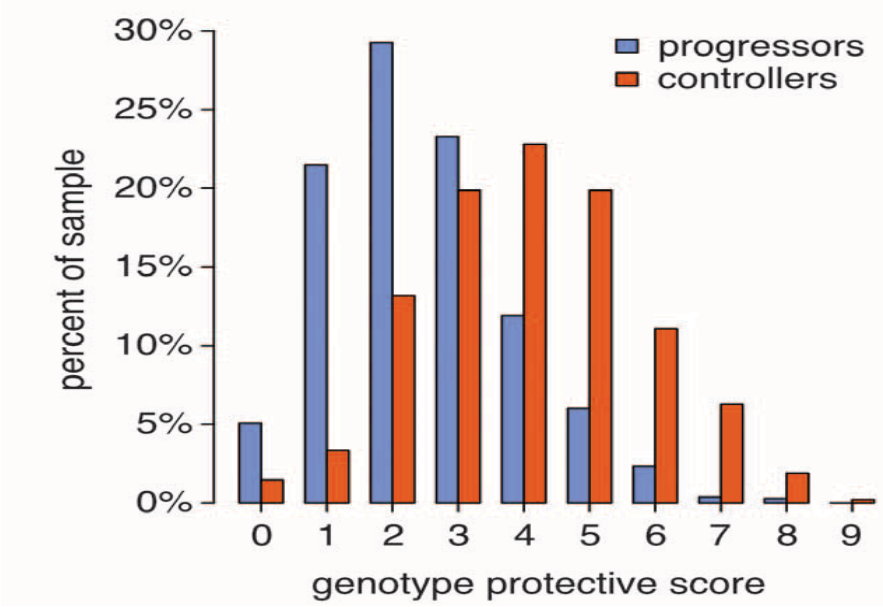
Host genetics

To date, human genes have been shown to account for 10% of variability in disease progression rates [15]. The translational impact of these genes on individual and population sensitivity to HIV-1 transmission and disease prevention and progression must be considered. As we have documented, a fraction of the human population is resistant to HIV-1 infection [16] [17]. Perhaps the most striking example is the case of hemophilia. A vast majority of severe hemophilia A patients born before 1979 became HIV-1 seropositive due to virtually universal exposure to contaminated batches of factor VIII concentrates. Interestingly, about 5% remained seronegative [17]. Another exceptional case of

resistance to HIV-1 infection can be seen in female sex workers in Nairobi, Kenya [18]. The ability to control HIV-1 infection spontaneously is highly variable between individuals [19] [20] [21]. Many host genetic factors have been shown to influence virus adaptation [22] [23] [24] and disease progression [25] [26] [10]. In particular, several genome-wide association (GWA) studies confirmed the important role of HLA in immune control of HIV-1 infection [12] [27] [28] [26] [10] [29, 30] [21].

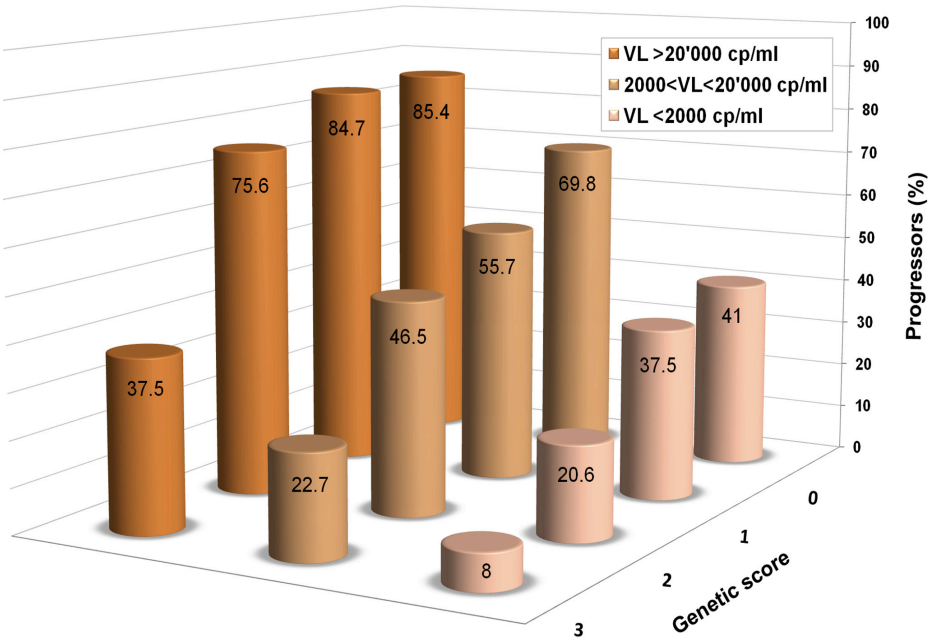
The GWA study conducted by "The International HIV Controllers Study" team [12] compared 974 controllers to 2648 progressors from multiple populations (European, African American and Hispanic). For HIV controllers enrolled in this study, median VL was 241 copies/ml, average CD4 count was 699 cells/mm3 and disease duration was 10 years. For treatment-naïve chronically infected HIV progressors studied as a reference group, median VL was 61,695 copies/ml and average CD4 was 224 cells/mm3. Data was obtained for 1,384,048 single nucleotide polymorphisms (SNPs). In the largest European group, 1712 individuals of European ancestry were compared and 313 SNPs were identified with genome-wide significance. All significant SNPs were located in the major histocompatibility complex (MHC) region on chromosome 6. A similar result was obtained from other two ethnic groups. Further closer examination of the SNPs within the MHC and concentrated around class I HLA genes, there were only 4 independent markers reached with association. Among these 4 SNPs, rs9264942, a putative variant is associated with HLA-C expression level; and rs2395029, a proxy for HLA-B*5701, which was previously

reported to associate with VL set-point following acute infection [26] [25]. These 4 SNPs accounted for 19% of the observed variance of host control in European group and 23% when coupled with those found in the gene that responsible for CCR5 coreceptor. From this figure [12] reported from this article, we clearly see the difference of genetic score between the HIV controllers and progressors.



The International HIV Controllers Study November 2010 *Sciencexpress Report*.

The GWA studies review by Fellay et al shows the role of the host genetic variation in controlling HIV-1 infection beyond the information provided by viral load can be used to refine the prediction of disease progression and clinical outcome [10] (Fig of Host Genetics and HIV-1).



Host Genetics and HIV-1: The Final Phases Jacques Fellay et al. PLOS Pathogens 2010

HLA class I alleles and HIV-1 specific CD8+ T cell

Human leukocyte antigens (HLA) molecules regulate the function of CD4 and CD8 T cells as well as natural killer (NK) cells. In the context of HIV-1 infection, HLA class I restricted CD8+ T cells play a key role in decreasing peak viremia during acute infection and maintaining long-term suppression of viral replication during chronic infection [6]. Except quickly reduced peak of VL at early stage of infection, CD8+ T cells have additional three ways to influence viral replication during chronic infection phase. First, specific HLA class I molecules are consistently associated with disease control by inducing specific CTL immune response that restricted by HLA class I molecules [31]. Second, we have observed the rapid disease progression in patients who carried with HLA class I

homozygosity [32] [33]. Third, the CTL epitope escape mutations evidenced by studying HIV-1 viral evolution indicate the selection is temporally associated with loss of immune control of infection [34] [35]. Taking together, the findings have revealed there is differences amount CD8+ T-cell specificities and amount the HLA class I molecules that are critically effective for HIV-1 control individually. Therefore, favorable and unfavorable HLA alleles or haplotypes can be consistently associated with different clinical, immunological and virological features of HIV disease. Certain specific HLA alleles (Favorable markers) have been demonstrated relatively successful control of viral replication and slower disease progression; the other particular HLA alleles (unfavorable markers) have been shown weaker effect on VL control in all stages of infection. Most prominently, the favorable alleles HLA-B*57 and HLA-B*27 have been significantly associated with disease control in the ways of either due to strong specific CTL response or increasing viral fitness cost. [36] [37] [34] [38] [39] [40]. In other documentations, the other specific HLA alleles have acted relatively inefficient control of viral replication and disease progression, such as the unfavorable alleles B*35 (px), B5802, B18 and A36 [11] [38] [40] [32] [41]. Additionally, full heterozygosity at HLA class I loci [32] or inherited rare alleles at the population level [42] can be an advantageous for controlling viremia and delaying disease progression. Recently, the surface expression level of HLA-Cw alleles was significantly correlated to the control of viremia and slower disease progression [43] [26] .

Among the different HLA class I loci, the HLA-B alleles which are thought to act the central role in determining clinic outcome in HIV-1 infection. To date, evidence has shown that majority of detectable HIV-specific CTL responses are restricted by HLA-B alleles [44] [38] [5]. Importantly, a more effective polyfunctional phenotype is seen in CD8+ T-cells restricted by HLA-B alleles [45]. Typically, the strongest HLA associations with either slow or rapid disease progression are linked to HLA-B alleles [32] [46] [26] [38], and HLA-B-restricted CD8+ T cells exert the most effective selection pressure on the virus [38] [47] [48], which is a major force driving HIV-1 viral evolution.

Zambia discordant couple cohort and HIV-1 transmission and infection

HIV discordant couples represent a significant fraction of more than 60,000 couples tested in the capital cities of Zambia (20%) and Rwanda (12%). Despite counseling and condom provision, low levels of transmission still occur (7%/yr in Zambia, 3%/yr Rwanda). Approximately 80-85% of transmissions are epidemiologically linked (i.e. within the couple) allowing comparison of donor and recipient viruses and follow-up studies of the newly infected partner.

The Lusaka Cohort was established in Zambia in 1994 to provide voluntary HIV testing and counseling, long-term monitoring, health care, and preventative measures to cohabitating couples in the capital city of Lusaka [49]. HIV discordant couples currently return at three-month intervals where they are counseled and the HIV-negative partner is tested to confirm that they remain uninfected using a rapid-test for anti-HIV antibodies. In a *2004 Science* paper, we

examined a subset of HIV-discordant cohabiting couples in the Lusaka Cohort to determine the nature of heterosexually acquired HIV-1 infection [50]. The pattern of transmission in this cohort indicates a severe genetic bottleneck during HIV-1 heterosexual transmission [50]. Comparison of donor and recipient viral sequences showed that in 90% of linked transmission pairs, a single viral variant is responsible for infections in the new host [51].

Initial work in the Zambian discordant couples cohort has yielded important clues regarding the role of HLA class I alleles both in transmission of the virus from the HIV-positive donor to the susceptible HIV-negative partner as well as the their role in disease progression as measured by viral load in newly infected individuals. We have documented associations of partner sharing of *HLA-B* alleles with transmission independent of other known risk factors [8]. The *A*6802* allele has been associated with increased transmission, which is in contrast to its apparent protective effect in populations infected with HIV-1 subtypes A or B [52] [53], implying that certain class I effects on transmission are population-specific. For viral load control and disease progression, A*3601 impacts chronically infected individuals by showing a weak control of VL, but no such effect on newly infected seroconverter [11]. Cw*18 is the most favorable allele for decreasing both of male to female and female to male transmission [54] and controlling VL [11]; B*57 alleles with inordinate protection of infected individuals, as indicated by low viral load, in the absence of any effect on transmission [55] [56] [57] and other distinctively African class I alleles with either higher or lower viral load [57] [11]. Taking together, inherit different HLA alleles

could reveal different outcome accordingly in combating of HIV-1 infection. The evidence clearly shows the great impact of HLA in HIV-1 transmission and disease control.

SIGNIFICANCE

The importance of CTL in control of HIV-1 infection is clear and significant. The studies based on this Zambian cohort [58] and a Ugandan cohort [9] show that virulence can be partially defined by the characteristics (often modified by donor HLA genotype) of the donor virus transmitted to linked recipients - regardless of the HLA genotype in donors or recipients. As we know from numerous previous studies, host genetics, especially HLA genotype, can exert specific and strong immune responses capable of controlling HIV-1 viremia. In this study, we used the same Zambian cohort with larger numbers of linked transmission couples as our case study and unlinked transmission couples as our reference group to conducted a nested case-control study to re-examined the correlation and relationship between Donor VL at the time of transmission and Recipient set-point VL in consider of the effort of the host HLA genotype in linked recipient. We conclude that the host HLA genotype is the major force for controlling VL set-point in HIV-1 newly infected individuals. Carrying favorable or unfavorable HLA allele can significantly modulate the clinical outcome of infected individuals and further influence HIV-1 transmission potential. These findings highlight the role of host genetic factors, specifically HLA, in controlling HIV-1 infection after transmission and provide an insight to further understanding the complex interactions between HIV-1 and its human host.

METHODS

The main purpose of this study was to assess whether the host HLA genotype of a newly infected epidemiologically linked recipient influences the control of HIV-1 infection after transmission. This was accomplished by performing a set of data analysis in a nested case-control designed study in a specific heterosexual transmission Zambian cohort. In addition, we also reevaluated the VL correlation between donor and linked recipient by considering both genetic and non-genetic factors.

Null hypothesis

VL set-point in linked recipients will correlate with the VL in chronically infected donors regardless of the human leukocyte antigen (HLA) genotypes in the newly infected partners.

Alternative hypothesis

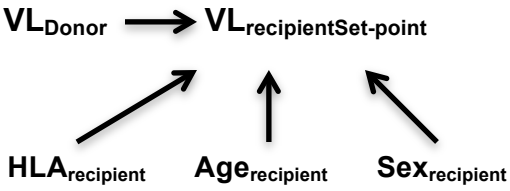
The HLA genotype of an epidemiologically linked recipient is another independent effector of viral load set-point in a newly infected individual.

Study Design

A retrospective nested case-control study was designed based on a specific Zambian HIV-1 heterosexual transmission cohort. Cases in this cohort were epidemiologically linked heterosexual transmission couples, while controls

were epidemiologically unlinked heterosexual transmission couples. Both cases and controls were selected from an 11-year period between 1995 and 2006.

Since the aim of the null hypothesis is to test whether the donor VL can be the major predictor for clinical outcome of the linked recipient, we used VL set-point to represent virulence. Therefore, the time of transmission is considered to be when exposure to the donor VL set-point occurs. To test the alternative hypothesis that HLA genotype of the linked recipient could be another factor affecting the VL set-point in linked recipient, the following parameters were considered. The first potential modifier is the linked recipient's host genetic factors (Human Leukocyte Antigens, HLA genotype), while the second potential modifier is the host's non-genetic factors (age and gender) since it is known that VL positively correlates with age, and HIV-infected men exhibit higher VL than HIV-infected women [8] [59] [21]. The primary outcome is: Linked recipient's viral load set-point. The second outcome is the clinical outcome and disease progression based on the interpretation of the primary outcome. The third outcome is based on the findings of this study, which affects future research about virus-host interaction and vaccine design.

Causal Diagrams: 

Final full model: Log10(VLR)= Log10(VLD)+ HLA(R-alleles)+ ageR + sexR

Since VL data do not distribute normally, Log10 conversion has been used for analysis.

Study population

The patients enrolled in this study are from the HIV-1 discordant couple cohorts in Lusaka, Zambia, and include both the chronically infected donors and the newly infected recipients. The Lusaka cohort was established in Zambia in 1994 to provide voluntary HIV testing and counseling, long term monitoring, health care, and preventative measures to cohabitating couples. HIV discordant couples currently return at three-month intervals when they are counseled, and the HIV-negative partner is tested using a rapid-test for anti-HIV antibodies and an ELISA for p24 to confirm that they are uninfected. Despite the preventative counseling measures, approximately 7% of the seronegative partners become infected each year. In the event that transmission is detected, blood samples are collected simultaneously from both donor and recipient at that time. The longitudinal blood samples are collected every 3 months in follow up. It is important to note that greater than 95% of the transmission pairs are infected by HIV-1 subtype C viruses [60].

Patient selection

The patient selection was based on inclusion and exclusion criteria. Inclusion criteria for patient selection involves the following: 1) Epidemiologically-linked transmission couples and epidemiologically-unlinked transmission couples; 2) HLA genotype has been defined; 3) for donors, VL is measured around transmission date; for recipients, the VL set-point has been determined about 9

months after transmission occurred; 4) non-genetic factors (age and sex) have been documented. Exclusion criteria: Non-paired HIV-1 infected individuals.

In this particular cohort, the total number of HIV-1 positive patients from the1995-2006 period is 1564 subjects. There are 231 linked couples and 29 unlinked couples in total 542 coupled subjects. Then, we exclude those without HLA typing and VL record (Appendix A). The final number of subjects enrolled for the case study group is 390 subjects (195 epidemiologically linked pairs), for the control group is 46 subjects (23 pairs unlinked couples).

Ethics Statement

This study complied with the human experimentation guidelines of the United States Department of Health and Human Services, and all enrolled patients provided written informed consent. The Institutional Review Boards at Emory University and University of Alabama at Birmingham further approved the work presented here.

Viral load and HLA typing

Measurements of plasma HIV-1 VL (RNA copies) were made in most cases with the Roche Amplicor 1.0 assay (Roche Diagnostics Systems Inc., Branchburg, NJ), which had a lower limit of detection (LLD) of 400 RNA copies per ml of plasma. HLA genotyping relied on a combination of PCR-based methods, including PCR with sequence-specific primers (SSP) (Dynal/Invitrogen,

Brown Deer, WI), automated sequence-specific oligonucleotide (SSO) probe hybridization (Innogenetics, Alpharetta, GA), and sequencing-based typing (SBT) (Abbott Molecular, Inc., Des Plaines, IL) using capillary electrophoresis and the ABI 3130xl DNA Analyzer (Applied Biosystems, Foster City, CA) [54] [61]. HLA alleles were resolved to the first two or four digits, which correspond to distinct protein products designated by the World Health Organization Nomenclature Committee for Factors of the HLA System [62] [63].

Definition of Favorable and Unfavorable HLA alleles:

In this study, we compared and analyzed the 2 groups of favorable/unfavorable alleles according to the results from previous studies involving this particular Zambian cohort [8] [11] [64] and in other ethnic or different geographic cohorts [9] [65].

Group 1 includes the most common favorable/unfavorable HLA alleles in this particular Zambian cohort. Zambian_Favorable_ alleles: A*74, B*13, B*42, B*57, B*8101, Cw*18 and A*30Cw*03; Zambian_Unfavorable_allele: A*36. The 2nd group of favorable/unfavorable HLA alleles includes those that are common in the Zambian cohort, and also some common alleles in other cohorts and in the Zambian cohort that may not emerge significantly. General_Favorable_alleles: A*74, B*13, B*42, B*5703, B*5702, Cw*18, A*30Cw*03, B*51, B*5801, B*14Cw*0802 and B*81; General_Unfavorable_alleles: B*5802, B*45, A*36 and B*18.

Statistical analysis

Statistical routines in SAS (Statistical Analysis Software, version 9.2, SAS Institute, Cary, NC) were applied to (1) describe and compare patient age and mean log10 HIV-1 VL using *t*-tests and *F* tests; (2) estimate the duration of HIV-1 infection based on the interval from seroconversion to subsequent plasma sampling, with seroconversion time being the midpoint between the last seronegative and first seropositive visits; (3) examine the Pearson correlation coefficient (*r*) between HIV-1 VL in donors (Log10(VLD)) and recipients (Log10(VLR) set-point); and (4) analyze the effects of donor VL and recipient genetic and non-genetic factors on recipient VL set-point using generalized linear model (GLM) statistics. (5) Multivariable GLM models will be used to compare the simultaneous, independent contributions of individual genetic and non-genetic factors to the variability in HIV-1 viremia. Both adjusted and unadjusted *p* values are shown wherever deemed necessary.

RESULTS

General characteristics of Zambian couples with linked and unlinked HIV-1 viruses

Based on data censored in October 2006, complete *HLA* class I genotyping was available for 195 transmission pairs with linked (phylogenetically related) HIV-1 viruses and 23 transmission pairs with unlinked HIV-1 viruses, and these were primarily (95%) the subtype C virus. In the case group (195 transmission couples), the age of transmitters was on average, older than that of seroconverters. (Table 1) Almost two-thirds of these couples involved male-to-female transmission (Table 1). While the duration of HIV-1 infection in seroprevalent individuals (all donors) could not be accurately established, the duration in seroconverters averaged at 270 (range 90–451) days at the time when HIV-1 VL was measured. Log10 HIV-1 RNA level (VL) in donors is statistically and significantly higher than in recipients, which also reflects the sex ratio (67% of donors are male) and the observation that male partners usually have higher VL that have been reported previously. Both Zambian cohort specific favorable *HLA* markers (A*74, B*13, B*42, B*57, B*8101, Cw*18, and A*30Cw*03) and Zambian unfavorable *HLA* markers (*A*36*), both general population favorable *HLA* markers (A*74, B*13, B*42, B*5703, B*5702, Cw*18, A*30Cw*03, B*51, B*5801, B*14Cw*0802 and B*81) and general population unfavorable *HLA* markers (B*5802, B*45, A*36 and B*18) were identified and distributed almost equally among transmitters and seroconverters (Table 1), except that the general population favorable HLA markers are slightly higher in

donors than recipients. In the control group (23 unlinked transmission couples), the data did not show any significant difference between transmitters and seroconverters (Table 2).

Confirming a multifactorial influence on HIV-1 VL in seroconverters

Single early VL measures taken mostly (90%) within the first 9 months of infection were evaluated in the 195 linked seroconverters and 23 unlinked seroconvertors. In comparison to previous reports, men do have higher VL than women between linked transmission couples (univariate $p = 0.001$, multivariable analysis $p < 0.0001$) but we have not found older patients (>40 years old) who have statistically significant higher VL than younger ones (univariate $p = 0.268$) (Table 3 and Table 5). Variability in VL was associated with *HLA* markers between linked transmission couples. The analyses of both Zambian cohort specific HLA markers and general population HLA markers have shown that inheriting favorable HLA markers can significantly decrease VL set-point in newly infected individuals even in unlined couples (in linked group univariate $p <0.0001$; in unlinked group univariate $p < 0.03$) (Table 3, 4, 5 and 6). This suggests that the host genetic marker such as HLA can independently be a strong and specific influence on virulence especially during the first year of infection. The VL in seroconverters strongly correlates to that in transmitters between epidemiologically linked transmission pairs ($p < 0.0005$ with Zambian specific HLA markers, $p = 0.0013$ with general population HLA markers) (Table 9 and 10, Fig. 1 A and 2 A) after adjustment of host genetic factor HLA and non-genetic

factors (age and gender), but this analysis did not reveal a significant correlation between VL in donor and VL set-point in recipient solely (Table 9). Being female (p = 0.001) or carrying favorable HLA markers (p < 0.001) have shown strong negative correlations with VL set-point in newly infected linked recipients (Table 9). Carrying unfavorable HLA markers positively correlated with VL set-point in linked recipient (p = 0.023) (Table 9). There is no statistically significant correlation between unlinked transmission couples (Fig 1B, 2B), which is fairly reasonable given that the infections were initiated by different viruses. By multivariable linear regression final reduced model (table 7 and table 8), the VL set-point variability in linked recipient can be reduced 0.5 log by being female (p < 0.0001), or carrying favorable HLA markers (p < 0.0001) respectively (Table 7 and Table 8). Transmitter VL accounted for VL set-point in linked recipient is giving a increase about 0.3 log. Overall, the relative impact of non-genetic host factors on VL was comparable to that of *HLA* class I markers (Tables 3, 4, 5, 6).

DISCUSSION

At the beginning of acute infection phase, the characteristics of the transmitted founder virus presumably plays a critical role in viral replication and virulence as the virus has not yet fully adapted to the new infected host's immune response. Early in infection, we observed virus genome evolution in response to a strong immune response comprised of CTL and neutralizing antibodies (Nab). CTL escape mutations can be detected 1 month post infection and Nab escape mutations can be detected 2 months post transmission. After peak viremia is reached, a gradual balance between viral replication and immune control is attained, as indicated by the establishment of set-point VL within the first year of infection. Similar studies conducted in other cohorts and within this same cohort using a smaller sample size concluded that the characteristics of the transmitted founder virus are the major determinant influencing VL set-point in the newly infected partner. Our approach differs from these studies in that we take HLA class I alleles, an important host genetic factor in modulating HIV viral load and pathogenesis, into consideration and our results show that the HLA class I genotype plays a central role in defining this balance (VL set-point) (Table 7 and 8). In addition, non-genetic factors, especially gender, play a significant role in defining VL set-point as we have known before. The characteristics of transmitted variant from the epidemiologically linked donor have only modest effects on defining the VL set-point in newly infected individual. The correlation between the donor VL close to transmission and VL set-point of linked recipient is only established under the consideration of gender and HLA markers (Table 9,

10, and fig 1, 2). Furthermore, the duration of asymptomatic chronic infection as well as the set-point VL contribute to the potential of HIV-1 transmission. Therefore, our data emphasize that understanding host genetics is an integral factor in comprehensively defining the complex interplay between virus and host [10]. Fully characterizing the host genetic factors that influence HIV acquisition and pathogenesis may be a direct way to explain different clinical outcomes in different patients and provide insight for HIV-1 vaccine design and disease control. Here we show that the HLA genotype is convincingly associated with viral control and disease progression. A greater effort towards attaining a greater depth of understanding in this field is needed to gain a more precise picture, such as how sharing HLA alleles in different level and favorable or unfavorable alleles between donor and recipient may influence transmission and disease control; how the outcome of the transmission between the donor carrying specific favorable HLA marker and the linked recipient carrying specific unfavorable HLA marker in a particular cohort and vice versa; how the effect of certain transmitted founder virus in different host by analyzing virus genome evolution in the population level in order to understand the trans evolution of favorable and unfavorable HLA alleles in a particular cohort, because the CTL escape mutation can abolish some certain HLA allele protection by transmit the mutated virus. Further more, since HLA genotype variation can only provide 19% explanation in HIV-1 infection in European ethnic, what is responsible for the large variability in HIV-1 control still remains unclear [12].

Within HIV-1 subtype C virus infection, the Zambian cohort has documented some of the first evidence that HLA host genetic factors enable to mediate HIV-1 virulence as well as heterosexual transmission [29] [6] [37]. Identification of favorable HLA factors in this particular cohort has made possible to significantly comprehensive the interaction between virus characteristics and host immune response. Few favorable HLA class I alleles confirmed in the Zambian cohort, several of them have known or predicted HIV-1 epitopes [66, 67] [68]. For example, epitope-specific CTL function correlation is well documented for B*57 [69] [70]. B*13 (all B*1302 in Zambians) as a favorable allele is another class I allele associated better clinical outcome repeatedly [71] [72] [73, 74] [46, 75]. The impact of the favorable HLA class I alleles and haplotypes on viral replication control and slower disease progression was consistently more noticeable in seroconvertors than in transmitters, suggesting that specific CTL immune response that restricted by favorable alleles like B*57 and B*13 diminish with time, perhaps due to CTL epitope escape mutations and compensatory mutations accumulation [35] [66] [76]. Reducing rate of heterosexual HIV-1 transmission in the Zambian cohort by inherited favorable HLA alleles (B*57 and Cw*18) was also defined during the early follow-up period [54]. Continuing efforts to evaluate the evolution of viral epitopes targeted by specific CTL that restricted by favorable HLA variants are critical for providing guidance in regard to HIV-1 vaccine design. In addition, focusing on elucidation of another important genetic factor for the

comprehension of innate immune response in HIV-1 disease control, that killer cell immunoglobulin-like receptor (KIR) genotypes should be a thorough, systematic evaluation of HLA-KIR interaction, which is another potentially critical pathway regulated by HLA class I alleles related with HIV-1 infection.

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Table 1. General Characteristics of Epidemiologically Linked HIV-1 Transmitters and Seroconverters

	Transmitters[a] (n=195)	Seroconverters[a] (n=195)	P value[b]
Age (years) (mean ± SD)	30.68 ± 7.75	28.72 ± 7.60	**0.0116**
Sex ratio	117 males, 78 females	78 males, 117 females	**<0.0001**
Log_{10} HIV-1 RNA level (VL)[c]	4.97 ± 0.65	4.50 ± 0.79	**<0.0001**
Duration (days) of infection[d]	NA	270.5 ± 180.5	NA
Favorable HLA Markers[e]	80 (41.03%)	70 (35.90%)	0.2980
Unfavorable HLA Markers[e]	33 (16.92%)	29 (14,87%)	0.5796
Favorable HLA Markers[f]	116 (59.49%)	96 (49.23%)	**0.0420**
Unfavorable HLA Markers[f]	80 (41.03%)	91 (46.67%)	0.2616

[a]Based on phylogenetic analyses of subgenomic HIV-1 sequences.
[b]unadjusted univariate analyses.
[c]A single measure of virus load (per milliliter of plasma).
[d]Duration of infection refers to the interval between seroconversion and VL measurement.
[e]The favorable HLA markers (a74 b13 b57 b8101 b42 c18 a30c03) and one unfavorable HLA marker a36 according to Zambia cohort.
[f]The favorable HLA markers (a74 b13 b5702 b5703 b42 c18 a30c03 b51 b5801 b14c0802 b81) and the unfavorable HLA markers (b5802 b45 a36 b18) according to Zambia cohort and general population.

Table 2. General Characteristics of Epidemiologically Unlinked HIV-1 Transmitters and Seroconverters

	Transmitters[a] (n=23)	Seroconverters[a] (n=23)	P value[b]
Age (years) (mean ± SD)	27.57 ± 5.99	29.26 ± 6.25	0.3529
Sex ratio	14 males, 9 females	9 males, 14 females	0.1404
Log_{10} HIV-1 RNA level (VL)[c]	4.23 ± 1.01	4.75 ± 0.93	0.0790
Duration (days) of infection[d]	NA	198.2 ± 149.3	NA
Favorable HLA Markers[e]	13 (56.52%)	8 (34.78%)	0.1389
Unfavorable HLA Markers[e]	4 (17.39%)	3 (13.04%)	0.6814
Favorable HLA Markers[f]	15 (65.22%)	10 (43.48%)	0.1389
Unfavorable HLA Markers[f]	9 (39.13%)	15 (65.22%)	0.2616

[a]Based on phylogenetic analyses of subgenomic HIV-1 sequences.
[b]unadjusted univariate analyses.
[c]A single measure of virus load (per milliliter of plasma).
[d]Duration of infection refers to the interval between seroconversion and VL measurement.
[e]The favorable HLA markers (a74 b13 b57 b8101 b42 c18 a30c03) and one unfavorable HLA marker a36 according to Zambia cohort.
[f]The favorable HLA markers (a74 b13 b5702 b5703 b42 c18 a30c03 b51 b5801 b14c0802 b81) and the unfavorable HLA markers (b5802 b45 a36 b18) according to Zambia cohort and general population.

Table 3. The Relative Impact of Transmitter and Seroconverter Host Factors on Log_{10} HIV-1 RNA Level (Viral Load) in 195 Zambia **Linked** Seroconverters Analyzed by **Zambian Cohort specific** HLA Genetic Markers

	Univariate analyses[a]			Multivariable analyses[a]		
Covariates	mean VL change	SE[b]	P	mean VL change	SE[b]	P
Age >40	+ 0.24	0.22	0.268	+ 0.18	0.20	0.3865
Being female	- 0.38	0.11	**0.001**	- 0.46	0.11	**<0.0001**
Favorable HLA markers[c]	- 0.54	0.11	**<0.0001**	- 0.57	0.11	**<0.0001**
Unfavorable HLA markers[c]	+ 0.36	0.16	**0.0233**	+ 0.14	0.15	0.3302
Transmitter viral load	+ 0.17	0.09	0.059	+ 0.29	0.08	**0.0004**

[a]Based on GLM (generalized linear model).
[b]SE, standard error of the mean.
[c]The favorable HLA markers (a74 b13 b57 b8101 b42 c18 a30c03) and one unfavorable HLA marker a36 according to Zambian cohort.

Table 4. The Relative Impact of Transmitter and Seroconverter Host Factors on Log_{10} HIV-1 RNA Level (Viral Load) in 23 Zambia **Unlinked** Seroconverters Analyzed by **Zambian Cohort specific** HLA Genetic Markers

	Univariate analyses[a]			Multivariable analyses[a]		
Covariates	mean VL change	SE[b]	P	mean VL change	SE[b]	P
Age >40	- 0.03	0.70	0.9609	+ 0.30	0.56	0.5932
Being female	- 0.44	0.39	0.2738	- 0.51	0.43	0.2478
Favorable HLA markers[c]	- 1.20	0.32	**0.0014**	- 1.08	0.36	**0.0086**
Unfavorable HLA markers[c]	+ 0.84	0.56	0.1466	+ 0.58	0.52	0.2802
Transmitter viral load	- 0.40	0.18	**0.0405**	- 0.05	0.22	0.8328

[a]Based on GLM (generalized linear model).
[b]SE, standard error of the mean.
[c]The favorable HLA markers (a74 b13 b57 b8101 b42 c18 a30c03) and one unfavorable HLA marker a36 according to Zambian cohort.

Table 5. The Relative Impact of Transmitter and Seroconverter Host Factors on Log_{10} HIV-1 RNA Level (Viral Load) in 195 Zambia **Linked** Seroconverters Analyzed by **general population** HLA genetic Markers

	Univariate analyses[a]			Multivariable analyses[a]		
Covariates	mean VL change	SE[b]	P	mean VL change	SE[b]	P
Age >40	+ 0.24	0.22	0.268	+ 0.17	0.20	0.3971
Being female	- 0.38	0.11	**0.001**	- 0.48	0.11	**<0.0001**
Favorable HLA markers[c]	- 0.45	0.11	**<0.0001**	- 0.47	0.11	**<1.0001**
Unfavorable HLA markers[c]	+ 0.26	0.11	**0.0230**	+ 0.12	0.11	0.2434
Transmitter viral load	+ 0.17	0.09	0.059	+ 0.27	0.08	**0.0013**

[a]Based on GLM (generalized linear model).
[b]SE, standard error of the mean.
[c]The favorable HLA markers (a74 b13 b5702 b5703 b42 c18 a30c03 b51 b5801 b14c0802 b81) and the unfavorable HLA markers (b5802 b45 a36 b18) according to Zambia cohort and general population

Table 6. The Relative Impact of Transmitter and Seroconverter Host Factors on Log_{10} HIV-1 RNA Level (Viral Load) in 23 Zambia **Unlinked** Seroconverters Analyzed by **general population** HLA genetic Markers

	Univariate analyses[a]			Multivariable analyses[a]		
Covariates	mean VL change	SE[b]	P	mean VL change	SE[b]	P
Age >40	- 0.03	0.70	0.9609	+ 0.00	0.03	0.9058
Being female	- 0.44	0.39	0.2738	- 0.34	0.49	0.4955
Favorable HLA markers[c]	- 0.83	0.36	**0.0294**	- 0.75	0.43	0.1008
Unfavorable HLA markers[c]	+ 0.42	0.41	0.3170	+ 0.38	0.38	0.3303
Transmitter viral load	- 0.40	0.18	**0.0405**	- 0.19	0.26	0.4913

[a]Based on GLM (generalized linear model).
[b]SE, standard error of the mean.
[c]The favorable HLA markers (a74 b13 b5702 b5703 b42 c18 a30c03 b51 b5801 b14c0802 b81) and the unfavorable HLA markers (b5802 b45 a36 b18) according to Zambia cohort and general population

Table 7. The Relative Impact of Transmitter and Seroconverter Host Factors on Log_{10} HIV-1 RNA Level (Viral Load) in 195 Zambia Linked Seroconverters Analyzed by Zambian Cohort specific HLA Genetic Markers (Reduced Model)

	Multivariable analyses		
Covariates	**mean VL change**	**SE**	**P**
Being female	**- 0.50**	**0.11**	**<0.0001**
Favorable HLA markers	**- 0.58**	**0.11**	**<0.0001**
Transmitter viral load	**+ 0.29**	**0.08**	**0.0005**

Final Reduced Model:

$$Log_{10}(VL_R) = 0.29Log_{10}(VL_D) - 0.58HLA_{(R\text{-alleles})} - 0.50sex_R$$

Table 8. The Relative Impact of Transmitter and Seroconverter Host Factors on Log_{10} HIV-1 RNA Level (Viral Load) in 195 Zambia Linked Seroconverters Analyzed by general population HLA genetic Markers (Reduced Model)

	Multivariable analyses		
Covariates	**mean VL change**	**SE**	**P**
Being female	**- 0.51**	**0.11**	**<0.0001**
Favorable HLA markers	**- 0.49**	**0.10**	**<0.0001**
Transmitter viral load	**+ 0.26**	**0.08**	**0.0017**

Final Reduced Model:

$$Log_{10}(VL_R) = 0.26 Log_{10}(VL_D) - 0.49 HLA_{(R\text{-alleles})} - 0.51\ sex_R$$

Table 9. Pearson Correlation of Transmitter Log_{10} VL, Seroconverter Host Factors and Log_{10} VL in 195 Zambia Linked Seroconverters

	Logvl_d	>age40_r	females_r	fav_zam_r	fav_gen_r	unfav_zam_r	unfav_gen_r
Logvl_r	0.135	0.080	-0.236	-0.326	-0.286	0.162	0.163
P value	0.0592	0.2680	**0.0010**	**<0.0001**	**<0.0001**	**0.0233**	**0.0230**

logvl_d: log donor VL;
logvl_r: log recipient VL set-point;
>age40_r: age > 40;
females_r: female recipient ;
fav_zam_r: recipient carrying favorable Zambian HLA marker;
fav_gen_r: recipient carrying favorable general population HLA marker;
unfav_zam_r: recipient carrying unfavorable Zambian HLA marker;
unfav_gen_r: recipient carrying unfavorable general population HLA marker.

Table 10. Pearson Partial Correlation of Transmitter Log_{10} VL, Seroconverter Host Factors and Log_{10} VL in 195 Zambia Linked Seroconverters

4 Partial Variables : age40_r gender_r fav_zam_r unfav_zam_r
2 Variables: logvl_r logvl_d
Pearson Partial Correlation Coefficient r= **0.25363** p-value= **0.0004**

4 Partial Variables : age40_r gender_r fav_gen_r unfav_gen_r
2 Variables: logvl_r logvl_d
Pearson Partial Correlation Coefficient r= **0.23161** p-value= **0.0013**

logvl_d: log donor VL;
logvl_r: log recipient VL set-point;
>age40_r: age > 40;
females_r: female recipient ;
fav_zam_r: recipient carrying favorable Zambian HLA marker;
fav_gen_r: recipient carrying favorable general population HLA marker;
unfav_zam_r: recipient carrying unfavorable Zambian HLA marker;
unfav_gen_r: recipient carrying unfavorable general population HLA marker.

Fig. 1A.

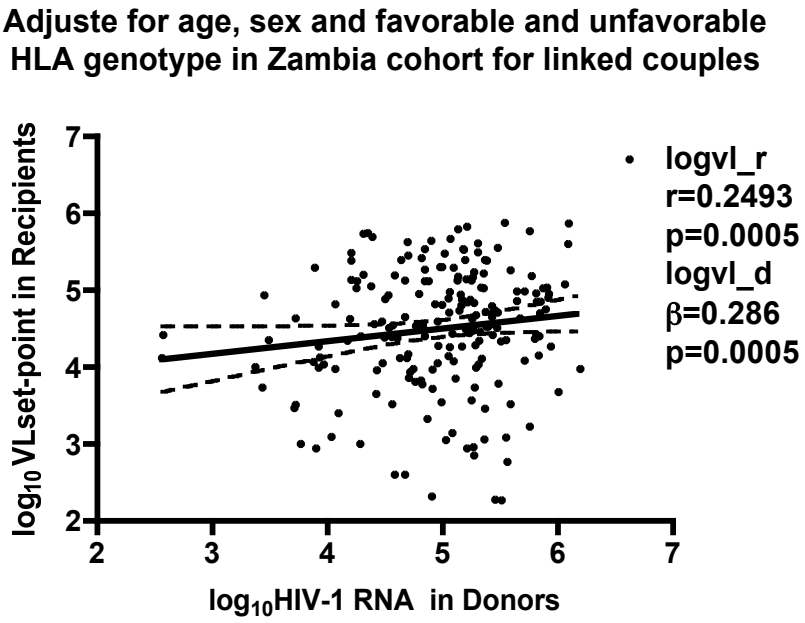

Adjuste for age, sex and favorable and unfavorable HLA genotype in Zambia cohort for linked couples
log10 VLset-point in Recipients
log10HIV-1 RNA in Donors
logvl_r
r=0.2493
p=0.0005
logvl_d
β=0.286
p=0.0005

Fig. 1B.

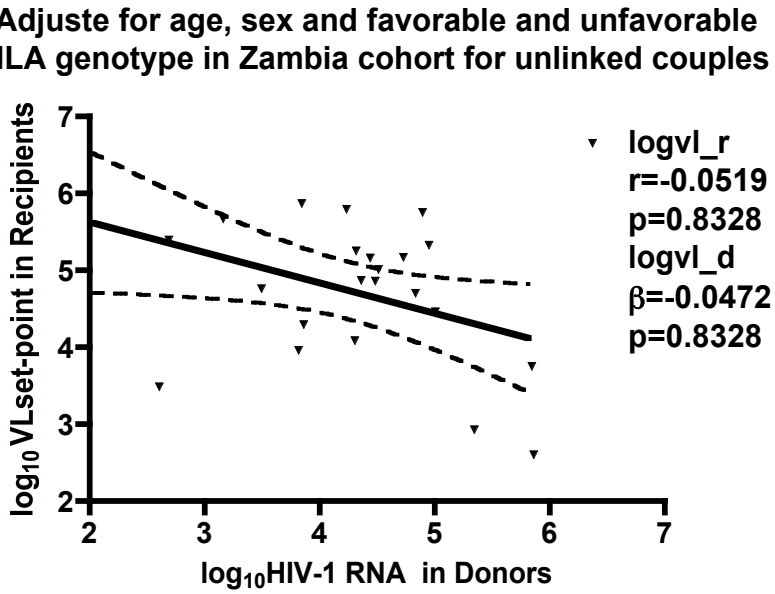

Adjuste for age, sex and favorable and unfavorable HLA genotype in Zambia cohort for unlinked couples
log10 VLset-point in Recipients
log10HIV-1 RNA in Donors
logvl_r
r=-0.0519
p=0.8328
logvl_d
β=-0.0472
p=0.8328

Fig. 2A.

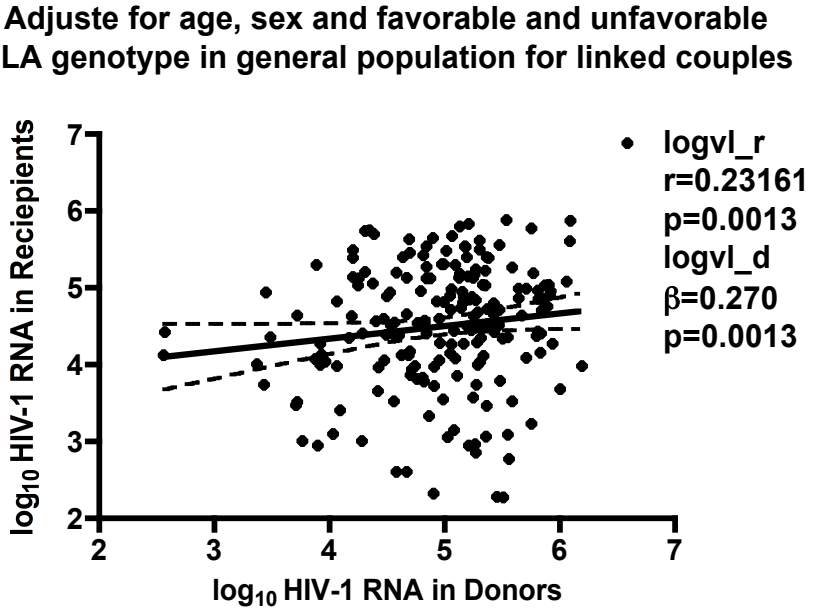
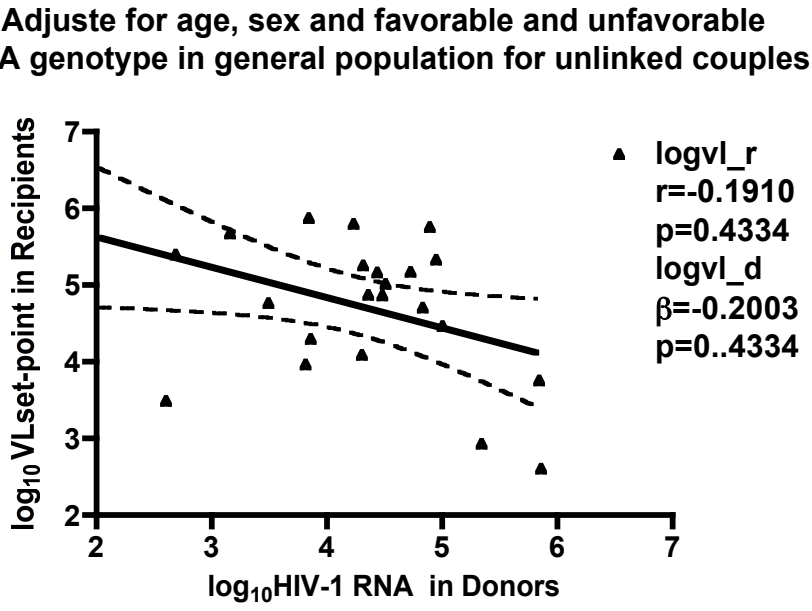

Adjuste for age, sex and favorable and unfavorable
HLA genotype in general population for linked couples
log10 HIV-1 RNA in Reciepients
log10 HIV-1 RNA in Donors
logvl_r
r=0.23161
p=0.0013
logvl_d
β=0.270
p=0.0013

Fig. 2B.


Adjuste for age, sex and favorable and unfavorable
HLA genotype in general population for unlinked couples
log10 VLset-point in Recipients
log10HIV-1 RNA in Donors
logvl_r
r=-0.1910
p=0.4334
logvl_d
β=-0.2003
p=0..4334

Appendix: Sample selection flow chart: