Host genetics and viral load in primary HIV-1 infection: clear evidence for gene by sex interactions

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Abstract Research in the past two decades has generated unequivocal evidence that host genetic variations substantially account for the heterogeneous outcomes following human immunodeficiency virus type 1 (HIV-1) infection. In particular, genes encoding human leukocyte antigens (HLA) have various alleles, haplotypes, or specific motifs that can dictate the set-point (a relatively steady state) of plasma viral load (VL), although rapid viral evolution driven by innate and acquired immune responses can obscure the long-term relationships between HLA genotypes and HIV-1-related outcomes. In our analyses of VL data from 521 recent HIV-1 seroconverters enrolled from eastern and southern Africa, HLA-A*03:01 was strongly and persistently associated with low VL in women (frequency = 11.3 %, \( P < 0.0001 \)) but not in men (frequency = 7.7 %, \( P = 0.66 \)). This novel sex by HLA interaction (\( P = 0.003, q = 0.090 \)) did not extend to other frequent HLA class I alleles (\( n = 34 \)), although HLA-C*18:01 also showed a weak association with low VL in women only (frequency = 9.3 %, \( P = 0.042, q > 0.50 \)). In a reduced multivariable model, age, sex, geography (clinical sites), previously identified HLA factors (HLA-B*18, B*45, B*53, and B*57), and the interaction term for female sex and HLA-A*03:01 collectively explained 17.0 % of the overall variance in geometric mean VL over a 3-year follow-up period (\( P < 0.0001 \)). Multiple sensitivity analyses...
of longitudinal and cross-sectional VL data yielded consistent results. These findings can serve as a proof of principle that the gap of “missing heritability” in quantitative genetics can be partially bridged by a systematic evaluation of sex-specific associations.

**Introduction**

In the era of genome-wide association studies (GWAS) on human traits and diseases, one overarching issue is “missing heritability,” as thousands of GWAS (http://www.genome.gov/gwastudies/) have readily identified and confirmed quantitative trait loci (QTLs) based on statistical significance, but these QTLs typically explain little or rather limited phenotypic variance (Brookfield 2013). Proponents of quantitative genetics have called for close attention to study design (Putter et al. 2011), phenotypic robustness (Queitsch et al. 2012), and the effects of rare (including de novo) variants, haplotypes (combinations of variants that are inherited as a single unit), gene by gene interaction (epistasis), gene by environment interaction, as well as epigenetics (Eichler et al. 2010; Gianola et al. 2013; Keller et al. 2012; Lee et al. 2011; Mahachie John et al. 2011). For complex traits with evolving and multifactorial mechanisms, the journey ahead for finding the missing heritability can be long and bumpy.

During the natural course of human immunodeficiency virus type 1 (HIV-1) infection, viremia and time from infection to development of severe immunodeficiency or AIDS are often used as quantitative traits to gauge HIV-1 pathogenesis and/or rates of disease progression. In particular, plasma viral load (VL) set-point during chronic HIV-1 infection offers a relatively steady and widely available outcome measure with both clinical and epidemiological implications (Fideli et al. 2001; Lyles et al. 2000; Mellors et al. 1995; Quinn et al. 2000; Saag et al. 1996). Predictors of set-point VL range from viral characteristics (e.g., subtypes and replicative capacity) (Prentice et al. 2014a; Prince et al. 2012; Yue et al. 2013) to host genotypes (QTLs) that govern innate and adaptive immune responses (Apps et al. 2013; Fellay et al. 2009; Leslie et al. 2010; Prentice and Tang 2012). Depending on the study population and definition of set-point VL (single or multiple measurements), the proportion of VL variance explained by any single host or viral factor is often less than 4% (Fellay et al. 2007; Prentice et al. 2014a; Yue et al. 2013). The most promising model that incorporates genetic and non-genetic features of epidemiologically linked HIV-1 transmission pairs (source and recipient partners) can account for nearly 37% of early set-point VL variance (Yue et al. 2013).

Our recent data from a large cohort of HIV-1 seroconverters (SCs) suggest that host and viral factors associated with set-point VL can evolve as the infection progresses (Prentice et al. 2014a), even during the early chronic phase when complications by coinfections and comorbidities are infrequent. The correlates of longitudinal and cross-sectional VL in this cohort include four HLA-B variants (B*18, B*45, B*53, and B*57) that encode polymorphic cell surface glycoproteins specializing in antigen presentation (Prentice et al. 2014). While these observations are consistent with the well-documented hypothesis that viral epitopes bound to HLA-B molecules can dominate the induction of HIV-1-specific, cytotoxic T-lymphocyte responses (Kiepiela et al. 2004, 2007; Rajapaksa et al. 2012) and further dictate viral evolution or adaptation (Goulder and Walker 2012; Kawashima et al. 2009; Leslie et al. 2004; Moore et al. 2002; Rolland et al. 2010), the VL variance explained by individual HLA-B variants is also limited (ranging from 0.7 to 1.6%). Our new objective is to refine the analytical approaches and to identify potential interaction terms between sex and HLA variants.

**Subjects and methods**

**Study population**

Recent HIV-1 seroconverters (SCs) were enrolled from Kenya, Rwanda, Uganda, and Zambia between 2005 and 2011 (Table 1), under a uniform study protocol sponsored by the International AIDS Vaccine Initiative (IAVI) (Amornkul et al. 2013; Price et al. 2011). The procedures for written informed consent and multidisciplinary research activities were approved by institutional review boards at all clinical research centers and participating institutions.

**Follow-up strategies, genotyping, and outcome measures**

SCs in this study were identified by frequent (monthly to quarterly) testing of HIV-1 seronegative subjects at high risk of HIV-1 infection through heterosexual and homosexual exposure, with the majority being seronegative partners in HIV-1 discordant couples and/or individuals reporting multiple heterosexual partners or diagnosed with sexually transmitted infections (85% of the SC cohort). The subjects included for this study were SCs with sufficient longitudinal data, and the visit intervals were expanded from 3 to 24 months (Prentice et al. 2014a) to 2 to 36 months beyond estimated dates of infection (EDI). All study visits considered were before the initiation of antiretroviral therapy under national guidelines (Ngongo et al. 2012). Viral sequencing, molecular HLA genotyping, and quantification of plasma VL followed procedures described in detail elsewhere (Amornkul et al. 2013; Prentice et al. 2014a; Price et al. 2011; Tang et al. 2011). Identification of HLA-B*18
Central hypothesis and analytical procedures

Our study aimed to test a central hypothesis that gene (HLA class I) by sex (viral microenvironment) interaction can be uncovered by separate analyses of men and women, especially when longitudinal VL measurements (with log10-transformation) are evaluated in mixed models. Data analyses began with the screening of potential interaction terms, with a focus on common HLA variants (population frequencies \( \geq 4 \% \)).

Descriptive statistics

HIV-1-infected men and women were compared for their overall baseline characteristics, including (a) Wilcoxon’s rank-sum test for quantitative variables lacking a normal distribution, (b) \( t \) test for quantitative variables with a normal distribution, and (c) \( \chi^2 \) and Fisher exact tests for categorical variables (Table 1). These and other analytical procedures (summarized below) were done using SAS, version 9.3 (SAS Institute, Cary, NC, USA).

**Table 1** Characteristics of HIV-1 seroconverters stratified by sex: demographic features, viral subtypes, outcome measures, and major HLA variants of interest

| Characteristicsa | Men       | Women     | \( P^b \) |
|------------------|-----------|-----------|----------|
| No. of subjects  | 327       | 194       | NA       |
| Age: mean ± SD (year) | 32.5 ± 8.8 | 29.5 ± 7.3 | <0.0001 |
| Age ≥40: no. (%)         | 59 (18.0)  | 21 (10.8) | 0.027    |
| Enrolment site          |            |           | <0.0001 |
| Kenya                    | 89 (27.2)  | 14 (7.2)  | <0.0001 |
| Rwanda                   | 50 (15.3)  | 35 (18.0) | 0.412    |
| Uganda                   | 69 (21.1)  | 58 (29.9) | 0.024    |
| Zambia                   | 119 (36.4) | 87 (44.9) | 0.057    |
| EDIs                     |            |           |          |
| Earliest                 | 3/15/2005  | 2/4/2005  | NA       |
| Latest                   | 10/12/2011 | 6/29/2011 | NA       |
| Length of follow-up (months): median (IQR) | 29 (21–31) | 29 (20–31) | 0.376    |
| Eligible visits per person: median (IQR) | 10 (8–11)  | 9 (7–11)  | 0.370    |
| HIV-1 subtypes          |            |          | 0.040    |
| Subtype A1               | 123 (37.6) | 52 (26.8) | 0.012    |
| Subtype C                | 118 (36.1) | 80 (41.2) | 0.242    |
| Others (B, D, recombinants, or unknown) | 86 (26.3)  | 62 (32.0) | 0.167    |
| Person-visits with eligible viral load (2–36 months) | 3,002 | 1,732 | NA |
| Person-visits with eligible CD4+ T-cell counts | 3,000 | 1,777 | NA |
| First eligible viral load (log10)c: mean ± SD | 4.49 ± 1.03 | 4.30 ± 1.09 | 0.048 |
| HLA-A*03: no. (%)        |            |           |          |
| Kenya                     | 5 (5.6)    | 1 (7.1)   | –        |
| Rwanda                    | 5 (10.0)   | 5 (14.3)  | –        |
| Uganda                    | 5 (7.3)    | 9 (15.5)  | 0.140    |
| Zambia                    | 10 (8.4)   | 7 (8.1)   | –        |
| HLA-B*18: no. (%)         |            |           |          |
| Kenya                     | 23 (7.0)   | 11 (5.7)  | –        |
| Rwanda                    | 58 (17.7)  | 25 (12.9) | 0.144    |
| HLA-B*53: no. (%)         | 60 (18.4)  | 41 (21.1) | 0.437    |
| HLA-B*57: no. (%)         | 30 (9.2)   | 18 (9.3)  | –        |
| HLA-C*18: no. (%)         | 26 (8.0)   | 18 (9.3)  | –        |

(a) Non-standard abbreviations: IQR interquartile range (25th to 75 % percentile), SD standard deviation of the mean, NA not applicable
(b) The \( P \) values \( >0.50 \) are omitted (−)
(c) First eligible outcome beyond the acute phase of infection (>9 weeks after EDI)
distributed visits (Prentice et al. 2014a), (2) stepwise elimination of subjects representing individual countries or geographic regions (e.g., eastern versus southern Africa), and (3) elimination of subjects infected with rare or unknown HIV-1 subtypes. In the final multivariable models, age, sex, duration of infection (DOI, measured quarterly), and previously identified (generalizable) HLA variants (B*18, B*45, B*53, and B*57) (Prentice et al. 2014) were treated as covariates. The performance of individual statistical models was gauged by their overall $R^2$ values (corresponding to variance explained by factors in the model), while the impact of individual factors was measured by the regression beta (adjusted mean beta difference, $\Delta \beta$, and standard error, SE). Associations with borderline statistical significance ($P \leq 0.050$, FDR = 0.20–0.50) were exempt from multivariable models or sensitivity analyses.

Refinement through evaluation of linkage disequilibrium (LD) profiles and extended haplotypes

Using SAS Genetics (SAS Institute, Cary, NC, USA), HLA genotyping data for eastern and southern African SCs were analyzed separately for LD and extended haplotypes, with additional reference to fully resolved haplotypes in other populations (Cao et al. 2001). Association analyses based on 2- and 3-locus haplotypes were deemed informative if the adjusted effect sizes improved over those attributable to the component alleles.

Bioinformatics

Several public databases were surveyed for existing evidence of function mechanisms pertinent to HLA/MHC gene expression and immune surveillance. First, HLA-restricted HIV-1 epitopes were retrieved from the HIV Molecular Immunology Database (http://www.hiv.lanl.gov/content/immunology/ctl_search, last accessed on May 18, 2014). Second, MHC SNPs known to tag-specific HLA class I alleles in Africans (de Bakker et al. 2006) were queried in HaploReg (Ward and Kellis 2012) for additional LD information uncovered by The 1000 Genomes Project or functional properties annotated by the ENCODE project (Encode Project Consortium et al. 2012; Rosenbloom et al. 2010). Third, previous associations with immune disorders and/or gene expression QTLs (eQTLs) (Fairfax et al. 2012) were checked in the NCBI Global Cross-database (http://www.ncbi.nlm.nih.gov/) and the SCAN database (http://www.scandb.org/newinterface/index.html, last accessed on May 20, 2014), with close attention to cis- and trans-acting eQTLs (Nicolae et al. 2010).

Results

Characteristics of men and women in the study population

A total of 521 subjects had sufficient prospective data (three or more visits) during the 2-36-month interval after EDI (Table 1). The overall baseline data differed between 327 men and 194 women in terms of (1) age ($P < 0.0001$), country of origin ($P < 0.0001$), HIV-1 subtype ($P = 0.040$), and first available VL ($P = 0.048$). HLA alleles of interest had similar distribution in men and women ($P = 0.14–0.97$) (Table 1).

Screening for interaction terms between sex and HLA factors

When 35 common HLA variants (2- or 4-digit resolution levels, whenever possible) were screened in mixed models with adjustment for demographic factors (age and geography), only HLA-A*03:01 showed a clear interaction with sex ($P = 0.003$, FDR = 0.09). LOESS curves supported this finding, as women with (+) and without (−) HLA-A*03:01 persistently differed in VL over the study intervals (1,732 person-visits, $P < 0.0001$) (Fig. 1).

In contrast, HLA-A*03:01+ and A*03:01− men (3,002 person-visits) had highly comparable VL trajectories ($P = 0.66$).

Multivariable models for longitudinal VL data

For the 2- to 36-month intervals, the interaction term between female sex and HLA-A*03:01 was independent of other known factors pertinent to the study population (Table 2), with an adjusted $P$ value of 0.005. On average, VL differed by $-0.67 \pm 0.24 \log_{10}$ between HLA-A*03:01+ and A*03:01− women after adjusting for other known factors. Analyses of data over the 3–24 months intervals yielded almost identical results ($-0.71 \pm 0.25 \log_{10}$, $P = 0.005$ for the interaction term) (Table 2).

Sensitivity analyses

In separate analyses of subjects representing individual countries or major geographic regions (eastern versus southern Africa), the interaction term for female sex and HLA-A*03:01 was persistently favorable, with adjusted effect sizes ($\Delta \beta$) ranging from $-0.30 \pm 0.35 \log_{10}$ ($P = 0.378$) to $-0.90 \pm 0.32 \log_{10}$ ($P = 0.005$) (Table 3), well within the 95% confidence intervals established by the overall cohort. Data analyses restricted to subjects with different infecting HIV-1 subtypes led to similar observations as well (Table 3).
alternative multivariable models for cross-sectional VL data

In a reduced multivariable model, age, sex, geography (clinical sites), previously identified HLA variants (HLA-B*18, B*45, B*53, and B*57), and the interaction term for female sex and HLA-A*03:01 collectively explained 17.0 % of the total variance in the overall geometric mean VL during the 2- to 36-month period \( (P < 0.0001) \) (Table 4). Statistical adjustments for the number of eligible visits or the length of follow-up for each subject did affect the model (data not shown). The summary statistics remained unchanged in analysis of geometric mean VL during the 3- to 24-month period (Table 4). In this case, the joint model explained 16.2 % of the overall VL variance (Fig. 1).

Other HLA variants of interest

In addition to the main observations on HLA-A*03:01, HLA-C*18:01 showed a trend for favorable interaction with female sex \( (P = 0.042, FDR > 0.50) \) (Fig. 2). Lack of LD between A*03:01 and C*18:01 \( (D' = -0.06, r^2 < 0.001, P = 0.90) \) ruled out the possibility of mutual tagging. Stratification by country did not reveal LD between A*03:01 and C*18:01 either \( (P = 0.34–0.99) \). Meanwhile, a previously reported, sex-specific effect for HLA-A*74:01 and HIV-1 VL (Koehler et al. 2010) could not be substantiated \( (P > 0.50) \) for the interaction term, although HLA-A*74:01+ and A*74:01− men did differ slightly in longitudinal VL \( (\Delta \beta = -0.22 \pm 0.13 \log_{10} \) for HLA-A*74:01+ men, \( P = 0.131) \).

HLA-A*03:01-related haplotypes

In contrast to earlier observations based on five North American populations (Cao et al. 2001), HLA-A*03:01 is not in strong LD with B*07:02-C*07:02 in our study cohort. The only statistically meaningful LD profiles for A*03:01 \( (P \leq 0.02) \) involved two haplotypes \( (B^*49:01-C^*07:01 \) and \( B^*58-C^*06) \) present in the eastern African SCs. Neither \( B^*49:01-C^*07:01 \) nor \( B^*58-C^*06 \) had differential impact on VL in women (adjusted \( P = 0.17 \) and 0.64, respectively). The observed 3-locus haplotypes containing A*03:01 were too diverse \( (9–11 \) per geographic region) to allow separate testing.

HLA-A*03-restricted HIV-1 epitopes

In the context of antigen presentation and CTL responses, multiple studies have identified HLA-A*03-restricted HIV-1 epitopes, especially a conserved epitope \( (KK9/rK9) \) in Gag (p17) (Balamurugan et al. 2010; Brumme et al. 2008; Dinges et al. 2010; Goulder et al. 1997; Heath et al. 2011; Peretz et al. 2011; Schneidewind et al. 2009). Other HLA-A*03-restricted CTL epitopes have been mapped to Env/gp120 (TW9 and VE12) (McKinnon et al. 2007; Peretz et al. 2011; Schneidewind et al. 2009), Nef (AK9, GL9, QK10, RK9, and RK10) (Almeida et al. 2011; Balamurugan et al. 2010; Brumme et al. 2008; Peretz et al. 2011; Schneidewind et al. 2009), Pol (AK11, ATK9, DI11, KA9, and SK11) (Balamurugan et al. 2010; Brumme et al. 2008; Chen et al. 2009; Peretz et al. 2011; Turnbull et al. 2009), Rev (TY9) (Yang et al. 2005), and a cryptic antigen (RR9) encoded by an alternative open reading frame (Berger et al. 2010). None of these existing immunologic data have been stratified by sex.
Further findings from bioinformatics

In populations of African ancestry (e.g., Yoruba), HLA-A*03:01 is tagged by one intergenic SNP (rs2524024), which is in strong LD ($r^2 = 0.81–1.0$) with 63 other intergenic SNPs distributed along a 45.1 kb region (5.9–51 kb upstream of HLA-A). The rs2524024 SNP is also a known eQTL for the integral membrane protein 2a gene (ITM2A) at Xq13.3-Xq21.2. When ranked by $P$ values, rs2524024 ($8.0 \times 10^{-6}$) falls out of the top 20 eQTLs (SNPs) associated with ITM2A gene expression in lymphoblastoid cell lines (Nicolae et al. 2010).

Discussion

By focusing on generalizable findings that are applicable to eastern and southern Africa with multiple circulating HIV-1 subtypes, our analyses yielded clear evidence that female sex can be an important environmental factor to facilitate HLA class I-mediated immune control of HIV-1 infection. Because women typically have lower VL than men after acquiring HIV-1 (Fideli et al. 2001; Prentice and Tang 2012; Tang et al. 2002), our hypothesis about gene by sex interaction may offer some explanation.

In the context of HIV-1 infection, at least two earlier studies have alluded to sex-specific findings with HLA-A*74:01 and HLA-DRB1*11 (Hendel et al. 1999; Kohler et al. 2010). In our analysis, HLA-A*74:01 (a frequent allele) was weakly associated with relatively low VL in men. However, there was no evidence for interaction

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**Table 2** Multivariable models for longitudinal viral load (VL) at two overlapping intervals of early HIV-1 infection

| Factors in model | Analyses of VL$^a$ in the 2–36 months interval | Analyses of VL$^a$ in the 3–24 months interval$^b$ |
|------------------|-----------------------------------------------|-----------------------------------------------|
|                  | $n$   | $\Delta \beta$ | SE  | Adjusted $P$ | $n$   | $\Delta \beta$ | SE  | Adjusted $P$ |
| Age (if >40)$^c$ | 80    | -0.06          | 0.10 | 0.536        | 75    | -0.10          | 0.10 | 0.309        |
| Region (if Zambia) | 206   | 0.36           | 0.07 | <0.0001      | 199   | 0.38           | 0.07 | <0.0001      |
| DOI (every 3 months) | 521   | -0.02          | 0.01 | <0.0001      | 503   | -0.02          | 0.01 | 0.007        |
| HLA-B*18           | 34    | 0.34           | 0.14 | 0.015        | 34    | 0.33           | 0.14 | 0.023        |
| HLA-B*45           | 83    | 0.25           | 0.09 | 0.009        | 82    | 0.22           | 0.10 | 0.027        |
| HLA-B*53           | 101   | 0.22           | 0.09 | 0.013        | 93    | 0.19           | 0.09 | 0.047        |
| HLA-B*57           | 48    | -0.48          | 0.12 | <0.0001      | 46    | -0.48          | 0.12 | 0.0001       |
| Female sex         | 194   | -0.27          | 0.07 | <0.001       | 187   | -0.29          | 0.08 | <0.001       |
| Female sex × HLA-A*03 | 22   | -0.67          | 0.24 | 0.005        | 22    | -0.71          | 0.25 | 0.005        |

$^a$ Repeated measurements, with log$_{10}$-transformation before analysis. Summary statistics: $\Delta \beta$ regression beta (mean deviation, $\Delta$, from the reference group), SE standard error of the mean $\Delta \beta$

$^b$ As part of the sensitivity analyses (see text)

$^c$ For consistency with earlier work, age is retained as a covariate regardless of its statistical significance

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**Table 3** Alternative models for evaluating the interaction term between female sex and HLA-A*03

| Six alternative models$^a$ | $n$ | Impact on longitudinal VL$^b$ | Adjusted $P$ |
|---------------------------|----|------------------------------|-------------|
| a) Removing Kenyan subjects |    | $\Delta \beta \pm$ SE | Adjusted $P$ |
| Female sex               | 180 | -0.29 ± 0.08 | <0.001 |
| Female sex × HLA-A*03     | 21  | -0.79 ± 0.25 | 0.002 |
| b) Removing Ugandan subjects |    | $\Delta \beta \pm$ SE | Adjusted $P$ |
| Female sex               | 136 | -0.31 ± 0.09 | <0.001 |
| Female sex × HLA-A*03     | 13  | -0.39 ± 0.28 | 0.175 |
| c) Removing Zambian subjects$^c$ |    | $\Delta \beta \pm$ SE | Adjusted $P$ |
| Female sex               | 107 | -0.22 ± 0.10 | 0.039 |
| Female sex × HLA-A*03     | 15  | -0.90 ± 0.32 | 0.005 |
| d) Zambian subjects only$^d$ |    | $\Delta \beta \pm$ SE | Adjusted $P$ |
| Female sex               | 87  | -0.34 ± 0.10 | 0.001 |
| Female sex × HLA-A*03     | 7   | -0.30 ± 0.35 | 0.378 |
| e) Removing rare HIV-1 subtypes$^e$ |    | $\Delta \beta \pm$ SE | Adjusted $P$ |
| Female sex               | 132 | -0.27 ± 0.08 | 0.001 |
| Female sex × HLA-A*03     | 13  | -0.76 ± 0.28 | 0.006 |
| f) Removing HIV-1 subtype A1 |    | $\Delta \beta \pm$ SE | Adjusted $P$ |
| Female sex               | 142 | -0.33 ± 0.09 | 0.0001 |
| Female sex × HLA-A*03     | 15  | -0.53 ± 0.29 | 0.064 |

$^a$ Part of the sensitivity analyses

$^b$ Repeated measurements in the 2–36 months interval, with log$_{10}$-transformation before analysis. The summary statistics are adjustment for other factors shown in Table 2. $\beta$ regression beta (mean deviation, $\Delta$, from the reference group), SE standard error of the mean ($\Delta$)

$^c$ The remaining subjects correspond to eastern Africans

$^d$ Corresponding to southern Africans

$^e$ Defined as others (not A1 and not C) in Table 1

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Further findings from bioinformatics

In populations of African ancestry (e.g., Yoruba), HLA-A*03:01 is tagged by one intergenic SNP (rs2524024), which is in strong LD ($r^2 = 0.81–1.0$) with 63 other intergenic SNPs distributed along a 45.1 kb region (5.9–51 kb upstream of HLA-A). The rs2524024 SNP is also a known eQTL for the integral membrane protein 2A gene (ITM2A) at Xq13.3-Xq21.2. When ranked by $P$ values, rs2524024 ($8.0 \times 10^{-6}$) falls out of the top 20 eQTLs (SNPs) associated with ITM2A gene expression in lymphoblastoid cell lines (Nicolae et al. 2010).
between HLA-A*74:01 and sex. The second hypothesis about HLA-DRB1*11 being unfavorable in women was derived from a French cohort (Hendel et al. 1999), but analyses of HIV-1-infected Zambians did not replicate that finding (Tang et al. 2010). Unlike earlier studies that did not account for potential false discoveries from random, multiple testing, the interaction term seen here for female sex and HLA-A*03:01 was accompanied by a low FDR (<0.10). A series of sensitivity analyses established that other potential confounders, including age, geography, and viral subtypes, did not obscure or compromise our analytical approaches. Data from the Multicenter AIDS Cohort Study may provide anecdotal evidence to support our key findings, as analyses of viral load and disease progression have never detected differential effects for HLA-A*03 in HIV-1-infected men (Kaslow et al. 1996; Mann et al. 1998).

Statistical significance aside, the threshold for a biologically significant difference in HIV-1 VL is around 0.30 log10 after accounting for intra- and inter-assay variability (Modjarrad et al. 2008; Saag et al. 1996). By our estimates, female sex by HLA-A*03:01 interaction was independently associated with ~0.70 log10 reduction in VL (Tables 2, 3, 4), which should impact disease progression and vertical or horizontal HIV-1 transmission.

The condition for analyzing gene by sex interactions in our study population was somewhat suboptimal. First, men and women eligible for analyses differed in several non-genetic (and potentially confounding) features (Table 1), which mandates the application of multivariable models and sensitivity analyses. As such, the effect sizes (regression beta and $R^2$ values) attributable to specific interaction terms often differed by statistical models and complicated the interpretation process. Second, HLA profile and genetic backgrounds can differ by country and geographic region, suggesting that our emphasis on generalizable findings might have come at the expense of country-specific phenomena. Third, sample size was not equal between men and women, so the statistical power was somewhat compromised in analyses of female-specific associations. As such, the modest trend seen with HLA-C*18:01 in women (Fig. 2) is still worth noting. In the long term, statistical models for gene by sex interactions should continue to improve when homogeneous cohorts with unbiased sex ratios are available for follow-up studies.

HLA alleles that have early influences on HIV-1 viral load tend to impose a strong selection pressure for viral immune escape mutations, as often seen in individuals with HLA-B*57 and related alleles (Bansal et al. 2007; Crawford et al. 2009; Leslie et al. 2004; Novitsky et al. 2010; Wang et al. 2009). In HIV-1-infected African women, the VL trajectory associated with HLA-A*03:01 was relatively steady in the first 3 years of follow-up (Fig. 1). Further evaluation of immune responses and HIV-1 immune escape mutations in HIV-1-infected women with HLA-A*03:01 may provide new insights about durable immune protection against a broad spectrum of HIV-1 subtypes.

Although HLA-A*03:01 itself can play an important role in inducing immune responses to a variety of CTL epitopes, it is also possible that the interaction term seen with A*03:01 actually reflects the function of other variants that operate in a sex-specific fashion. Such genetic variations can be either upstream (telomeric) or downstream (centromeric) from the HLA-A locus (Vandiedonck and Knight 2009). The LD profiles in our study cohort strongly suggested that genes downstream from the HLA-A locus, including HLA-C and HLA-B, could not explain the A*03:01 effect. Two alternative hypotheses can relate to other genomic regions. First, through strong LD with rs2524024, a trans-acting eQTLs for the ITM2A gene at Xq13.3-Xq21.2, HLA-A*03:01 can
tag various functionally relevant SNPs. The ITM2A product has been shown to regulate CD8 T-cell selection and activation in mice (Kirchner and Bevan 1999). This biological connection can offer a probable mechanism for the observed interaction between HLA-A*03:01 and female sex. The other alternative hypothesis points to a long-range (~4 Mb) LD between A*03 and the C282Y mutation in HFE, which is a recessive causal variant for hereditary haemochromatosis (iron overload) in Caucasians (Cardoso and de Sousa 2003; de Bakker et al. 2006; Hanson et al. 2001). However, this is an unlikely explanation as fine mapping using the ImmunoChip array (Illumina, San Diego, CA, USA) has confirmed that haplotype blocks in the MHC region are relatively short in Africans (Prentice et al. 2014b).

Potential interactions between HLA alleles and sex have been reported for several autoimmune disorders and human malignancies (Davis and Dorak 2010; Dorak et al. 1999; Morrison et al. 2010). For HLA-A variants alone, evidence of sex-specific effect further points to a short sequence motif corresponding to polymorphic amino acid residues 161, 163, and 165 of the HLA-A protein product (Song et al. 2009). This particular sequence motif does not match the ones highlighted in a recent fine-mapping of HLA class I amino acid sequences in HIV-1-infected African Americans (in the absence of stratification by sex) (McLaren et al. 2012). Nonetheless, the HLA-A locus is often overshadowed by HLA-B and HLA-C in studies of HIV/AIDS (Apps et al. 2013; Fellay et al. 2009; Leslie et al. 2010; Prentice and Tang 2012). If environmental factors indeed dictate how HLA-A alleles are expressed or regulated, close attention to gene × environment or gene × sex interaction should provide a deeper understanding of “missing heritability” in quantitative genetics.

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Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Almeida CA, Bronke C, Roberts SG, McKinnon E, Keane NM, Chopra A, Kadi C, Carlson J, Haas DW, Riddler SA, Haubrich R, Heckerman D, Mallal S, John M (2011) Translation of HLA-HIV associations to the cellular level: HIV adapts to inflate CD8

Fig. 2 Additional evidence for gene × sex interactions. Prospective viral load measurements are plotted for HLA-C*18:01-positive and HLA-C*18:01-negative subjects. The thick and thin lines correspond to the expected mean value and 95 % confidence intervals for each stratum. Arrows indicate plasma viral load measurements that are <400 RNA copies/ml (transformed to 1.30 log 10)
T cell responses against Nef and HLA-variant epitopes. J Immunol 187:2502–2513. doi:10.4049/jimmunol.1100691

Ammorkul PN, Karita E, Kamali A, Rida WN, Sanders EJ, Lakhi S, Price MA, Kilembwe W, Cormier E, Anzola O, Latka MH, Bekker LG, Allen SA, Gilmour J, Fast PE, Partnership IAHP (2013) Disease progression by infecting HIV-1 subtype in a seroconverter cohort in sub-Saharan Africa. AIDS 27:2775–2786. doi:10.1097/QAD.0000000000000012

Apps R, Qi Y, Carlson JM, Chen H, Gao X, Thomas R, Yuki Y, Del Prete GQ, Goulder P, Brumme ZL, Brumme CJ, John M, Mallal S, Nelson G, Bosch R, Heckerman D, Stein JL, Soderberg KA, Moody MA, Denny TN, Zeng X, Fang J, Moffett A, Lisson JD, Goedert JT, Buchbinder S, Kirk GD, Fellay J, McLaren P, Deeks SG, Pereyra F, Walker B, Michael NL, Weintraub A, Wolinsky S, Liao W, Carrington M (2013) Influence of HLA-C expression level on HIV control. Science 340:87–91. doi:10.1126/science.12326853406128/87

Balamurugan A, Ng HL, Yang OO (2010) Rapid T cell receptor delineation reveals clonal expansion limitation of the magnitude of the HIV-1-specific CD8+ T cell response. J Immunol 185:5935–5942. doi:10.4049/jimmunol.1002236

Bansal A, Yue L, Conway J, Yusim K, Tang J, Kappes J, Kaslow RA, Wilson CM, Goepfert PA (2007) Immunological control of chronic HIV-1 infection: HLA-mediated immune function and viral evolution in adolescents. AIDS 21:2387–2397

Berger CT, Carlson JM, Brumme CJ, Hartman KL, Brumme ZL, Henry LM, Rosato PC, Piechocka-Trocha A, Brockman MA, Harrigan PR, Heckerman D, Kaufmann DE, Brander C (2010) Viral adaptation to immune selection pressure by HLA class I-restricted CTL responses targeting epitopes in HIV frameshift sequences. J Exp Med 207:61–75. doi:10.1084/jem.20091808

Brookfield JF (2013) Quantitative genetics: heritability is not always missing. Curr Biol 23:R276–R278. doi:10.1016/j.cub.2013.02.040

Brunme ZL, Brumme CJ, Strick J, John M, Eichbaum Q, Block BL, Baker B, Kadie C, Markowitz M, Jessen H, Kelleher AD, Rosenberg E, Kalder J, Yuki Y, Carrington M, Allen TM, Mallal S, Alfheid M, Heckerman D, Walker BD (2008) Marked epitope- and allele-specific differences in rates of mutation in human immunodeficiency type 1 (HIV-1) Gag, Pol, and Nef cytotoxic T-lymphocyte epitopes in acute/early HIV-1 infection. J Virol 82:9216–9227. doi:10.1128/Jvi.01041-08

Cao K, Holleben J, Shi X, Shi W, Chopek M, Fernandez-Vina MA (2001) Analysis of the frequencies of HLA-A, B, and C alleles and haplotypes in the five major ethnic groups of the United States. J Immunol 166:1009–1030

Cardoso CS, de Sousa M (2003) HFE, the MHC and hemochromatosis: paradigm for an extended function for MHC class I. Tissue Antigens 61:263–275

Chen H, Piechocka-Trocha A, Miura T, Brockman MA, Juld BD, Baker BM, Rothchild AC, Block BL, Schweindewind A, Kobuchti CH, Pereyra F, Allen TM, Walker BD (2009) Differential neutralization of human immunodeficiency virus (HIV) replication in autologous CD4 T cells by HIV-specific cytotoxic T lymphocytes. J Virol 83:3138–3149. doi:10.1128/JVI.02073-08

Crawford H, Lummm W, Leslie A, Schaefer M, Boeras D, Prado JG, Tang J, Farmer P, Nduong’t, Lakhi S, Gilmour J, Goepfert P, Walker BD, Kaslow R, Mulenga J, Allen S, Goulder PJ, Hunter E (2009) Evolution of HLA-B*5703 HIV-1 escape mutations in HLA-B*5703-positive individuals and their transmission recipients. J Exp Med 206:909–921

Davis CF, Dorak MT (2010) An extensive analysis of the hereditary hemochromatosis gene HFE and neighboring histone genes: associations with childhood leukemia. Ann Hematol 89:375–384. doi:10.1007/s00277-009-0839-y

de Bakker PI, McVeay G, Sabeti PC, Miretti MM, Green T, Marchini J, Ke X, Monsuur AJ, Whittaker P, Delgado M, Morrison J, Richardson A, Walsh EC, Gao X, Galver L, Hart J, Hafler DA, Pericak-Vance M, Todd JA, Daly MJ, Trowsdale J, Wijmenga C, Vsyje TJ, Beck S, Murray SS, Carrington M, Gregory S, Deloukas P, Rioux JD (2006) A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. Nat Genet 38:1166–1172. doi:10.1038/ng1885

Dinges WL, Richard J, Friedrich D, Jalbert E, Liu Y, Stevens CE, Maenza J, Collier AC, Geraghty DE, Smith J, Moodie Z, Mullins JJ, McElrath MJ, Horton H (2010) Virus-specific CD8+ T-cell responses better define HIV disease progression than HLA genotypes. J Virol 84:4461–4468. doi:10.1128/JVI.02438-09

Dorak MT, Lawson T, Machulla HK, Darke C, Mills KL, Burnett AK (1999) Unravelling an HLA-DR association in childhood acute lymphoblastic leukemia. Blood 94:694–700

Eichler EE, Flint J, Gibson G, Kong A, Leal SM, Moore JH, Nadeau JH (2010) Missing heritability and strategies for finding the underlying causes of complex disease. Nat Rev Genet 11:446–450. doi:10.1038/nrg2809

ENCODe Project Consortium, Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489:57–74. doi:10.1038/nature11247

Fairfax BP, Makino S, Radhakrishnan J, Plant K, Leslie S, Dillhey A, Ellis F, Langford C, Vannberg FO, Knight JC (2012) Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. Nat Genet 44:502–510. doi:10.1038/ng.2205

Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, Zhang K, Gumbs C, Castagna A, Cossarizza A, Cozzi-Lepri A, De Luca A, Easterbrook P, Francioli P, Mallal S, Martinez-Picado J, Miro JM, Obel N, Smith JP, Wyniger J, Descombes P, Antonarakis SE, Letvin NL, McMichael AJ, Haynes BF, Teleni A, Goldstein DB (2007) A whole-genome association study of major determinants for host control of HIV-1. Science 317:944–947

Fellay J, Ge D, Shianna KV, Colombo S, Ledergerber B, Cirilli ET, Urban TJ, Zhang K, Gumbs CE, Smith JP, Castagna A, Cozzi-Lepri A, De Luca A, Easterbrook P, Gunthard HF, Mallal S, Mussini C, Dalmui J, Martinez-Picado J, Miro JM, Obel N, Wolinsky SM, Martinson JJ, Detels R, Margolick JB, Jacobson LP, Descombes P, Antonarakis SE, Beckmann JS, O’Brien SJ, Letvin NL, McMichael AJ, Haynes BF, Carrington M, Feng S, Teleni A, Goldstein KB (2009) Common genetic variation and the control of HIV-1 in humans. PLoS Genet 5:e1000791

Fideli US, Allen SA, Musonda R, Trask S, Hahn BH, Weiss H, Mulenga J, Kasolo F, Vermund SH, Aldrovandi GM (2001) Virologic and immunologic determinants of heterosexual transmission of human immunodeficiency virus type 1 in Africa. AIDS Res Hum Retrovir 17:901–910. doi:10.1089/089822201750290023

Gianola D, Hospital F, Verrier E (2013) Contribution of an additive locus to genetic variance when inheritance is multi-factorial with implications on interpretation of GWAS. Theor Appl Genet 126:1457–1472. doi:10.1007/s00122-013-2064-2

Goulder PJ, Walker BD (2012) HIV and HLA class I: an evolving relationship. Immunity 37:426–440. doi:10.1016/j.immuni.2012.09.005

Goulder PJ, Sewell AK, Laloo DG, Price DA, Whelan JA, Evans JS, Taylor GP, Luzzi G, Giangrande P, Phillips RE, McMichael AJ (1997) Patterns of immunodominance in HIV-1-specific cytotoxic T lymphocyte responses in two human histocompatibility leukocyte antigens (HLA)-identical siblings with HLA-A*0201 are influenced by epitope mutation. J Exp Med 185:1423–1433
Nicolae DL, Gamazon E, Zhang W, Duan S, Dolan ME, Cox NJ (2010) Trait-associated SNPs are more likely to be eQTLs: annotation to enhance discovery from GWAS. PLoS Genet 6:e1000888. doi:10.1371/journal.pgen.1000888

Novitsky V, Wang R, Margolin L, Baca J, Moyo S, Musonda R, Essex M (2010) Dynamics and timing of in vivo mutations at Gag residue 242 during primary HIV-1 subtype C infection. Virology 403:47–46. doi:10.1016/j.virol.2010.04.001

Peretz Y, Marra O, Thomas R, Legault D, Cote P, Boulazzel MR, Rousseau CM, Bernard NP (2011) Relative contribution of HIV-specific functional lymphocyte subsets restricted by protective and non-protective HLA alleles. Viral Immunol 24:189–196. doi:10.1089/vim.2010.0117

Prentice HA, Tang J (2012) HIV-1 dynamics: a reappraisal of host and viral factors, as well as methodological issues. Viruses 4:2080–2096. doi:10.3390/v4102080

Prentice HA, Price MA, Porter TR, Cormier E, Mugavero MJ, Kamali A, Karita E, Lakhi S, Sanders JS, Prentice HA, Tang J, Pajewski NM, He D, Zhang K, Brown EE, Kilembe W, Yue L, Vishwanathan SA, Kilembe W, Goepfert P, Price MA, Wallis CL, Lakhi S, Karita E, Kamali A, Anzala O, Amorok BN, Allen S, Hunter E, Kaslow RA, Mangel F, Meehan MO, Lutalo T, Gray RH (2000) Viral load and heterosexual transmission of human immunodeficiency virus type 1. J Virol 74:8267–8284. doi:10.1128/JviL.74.11.8267-8284.2000

Prentice HA, Price MA, Porter TR, Cormier E, Mugavero MJ, Kamali A, Karita E, Lakhi S, Sanders JS, Prentice HA, Pajewski NM, He D, Zhang K, Brown EE, Kilembe W, Allen S, Hunter E, Kaslow RA, Tang J (2014b) Host genetics and immune control of HIV-1 infection: fine mapping for the extended human MHC region in an African cohort. Genes Immun. doi:10.1038/gen.2014.16

Price MA, Wallis CL, Lakhi S, Karita E, Kamali A, Anzala O, Sanders EJ, Bekker LG, Twesige-Rwaka R, Hunter E, Kaleebu P, Kayitenkore K, Allen S, Razagia E, Mwangome M, Mutia G, Amorok BN, Stevens G, Pond SL, Schaefer M, Papathanasopoulos MA, Stevens W, Gilmour J (2011) Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and southern Africa. AIDS Res Hum Retrovir 27:5–12. doi:10.1089/aid.2010.0030

Rosenbloom KR, Dreszer TR, Pheasant M, Barber GP, Meyer LR, Pohl A, Raney BJ, Wang T, Hinrichs AS, Zweig AS, Fujita PA, Learned K, Rhead B, Smith KE, Kuhn RM, Karolchik D, Haussler D, Kent WJ (2010) ENCODE whole-genome data in the UCSC Genome Browser. Nucleic Acids Res 38:D620–D625. doi:10.1093/nar/gkp961

Saag MS, Holodniy M, Kutzitzke DS, O'Brien WA, Coombs R, Poscher ME, Jacobsen DM, Shaw GM, Richman D, Volberding PA (1996) HIV viral load markers in clinical practice. Nat Med 2:625–629

Schneidewind A, Tang Y, Brockman MA, Ryland EG, Dunkley-Thompson J, Steel-Duncan JC, St John MA, Conrad JA, Kalans SA, Noel F, Allen TM, Christie CD, Feeney ME (2009) Maternal transmission of human immunodeficiency virus escape mutations subverts HLA-B57 immunodominance but facilitates viral control in the haploidentical infant. J Virol 83:8616–8627. doi:10.1128/JviL.00730-09

Song W, Ruder AM, Hu L, Li Y, Ni R, Shao W, Kaslow RA, Butler M, Tang J (2009) Genetic epidemiology of glioblastoma multiforme: confirmatory and new findings from analyses of human leukocyte antigen alleles and motifs. PLoS ONE 4:e7157. doi:10.1371/journal.pone.0007157

Tang J, Tang S, Lobashevsky E, Myracle AD, Fideli U, Aldrovandi G, Allen S, Musonda R, Kaslow RA (2002) Favorable and unfavorable HLA class I alleles and haplotypes in Zambians predominately infected with clade C human immunodeficiency virus type 1. J Virol 76:8276–8284

Turnbull EL, Wong M, Wang S, Wei X, Jones NA, Conrad KE, Eldam A, Turner J, Pellegrino P, Keele BF, Williams I, Shaw GM, Borrow P (2009) Kinetics of expansion of epitope-specific T cell responses during primary HIV-1 infection. J Immunol 182:7131–7145. doi:10.4049/jimmunol.0803565