The Influence of host genetic factors on HIV-1 infection

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RISING HIV-1 VIRAL LOAD SET-POINT AT A POPULATION LEVEL COINCIDES WITH A FAADING IMPACT OF HOST GENETIC FACTORS ON HIV-1 CONTROL

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ABSTRACT

Heterozygosity for a 32 base pair deletion in the CCR5 gene (CCR5wt/Δ32), and the minor alleles of a single nucleotide polymorphism (SNP) in the HCP5 gene (rs2395029) and in the HLA-C gene region (-35HLA-C; rs9264942) have been associated with a lower viral load set-point. Recent studies have shown that over calendar time, viral load set-point has significantly increased at a population level. Here we studied whether this increase coincides with a fading impact of above mentioned host genetic markers on HIV-1 control.

We compared the association between viral load set-point and HCP5 rs2395029, -35HLA-C rs9264942, and the CCR5wt/Δ32 genotype in HIV-1-infected individuals in the Netherlands who had seroconverted between 1982 and 2002 (pre-2003 seroconverters, n=459) or between 2003 and 2009 (post-2003 seroconverters, n=231).

Viral load set-point in post-2003 seroconverters was significantly higher than in pre-2003 seroconverters (P= 4.5x10⁻⁵). The minor alleles for HCP5 rs2395029, -35HLA-C rs9264942 and CCR5wt/Δ32 had a similar prevalence in both groups and were all individually associated with a significantly lower viral load set-point in pre-2003 seroconverters. In post-2003 seroconverters, this association was no longer observed for HCP5 rs2395029 and CCR5wt/Δ32. The association between viral load set-point and HCP5 rs2395029 had significantly changed over time while the change in impact of the CCR5wt/Δ32 genotype over calendar time was not independent from the other markers under study.

The increased viral load set-point at a population level coincides with a lost impact of certain host-genetic factors on HIV-1 control.
INTRODUCTION

Disease progression after infection with human immunodeficiency virus type 1 (HIV-1) is strongly associated with the amount of virus in blood [1]. In most infected individuals virus production and clearance reach a balance approximately 18-24 months after seroconversion, reflecting a temporary relatively stable level of HIV-1 RNA in plasma, the so called viral load set-point. This viral load set-point varies between individuals and is a strong and independent predictor of subsequent disease progression [2]. In several cohorts certain host genetic factors, such as having a HLA-B57 or HLA-B27 type, have been associated with a lower viral load set-point or a delayed clinical course of infection [3-5]. Heterozygosity for a 32 base-pair deletion in CCR5, the gene that is coding for one of the HIV-1 coreceptors, is also associated with delayed disease progression and a lower viral load early in infection [6-8]. In the first genome-wide association study (GWAS) on HIV-1 control, the minor alleles of SNP rs9264942 35 kbp upstream of HLA-C and of SNP rs2395029 in HCP5, which is in high linkage disequilibrium (LD) with HLA-B57, were associated with a lower viral load set-point [9], which was confirmed in other cohorts [10-12].

We and others have recently reported a rising trend over time of the viral load set-point at a population level, which could signal an accelerating clinical course of infection [13-15]. The underlying mechanism for this increase is unclear but may reflect an adaptation of the virus to its environment. Indeed, due to its high replication rate, error-prone reverse transcription, and lack of proof reading, HIV-1 is known to rapidly accumulate mutations which can be positively selected, allowing rapid escape from host immune surveillance [5,16-21] and escape from antiretroviral drugs [22,23]. In addition to intrapatient escape from host defense mechanisms, adaptation of HIV-1 at the population level has been observed as well [24,25]. Continuous pressure by host genetic factors may result in viral adaptation at the population level and thus changed virus replication. Here we studied whether the increasing viral load set-point over calendar time at a population level could be explained, at least partially, by a fading impact of host genetic factors on HIV-1 control.

METHODS

Study populations

The SHM-HIV-1 Host Genetics study (SHM is the abbreviation of “Stichting HIV monitoring” which is the Dutch translation for Netherlands HIV-monitoring foundation) included patients with an accurately estimated date of seroconversion (midpoint of a period of maximum 180 days between last HIV-negative and first HIV-positive), an age of >18 years at enrollment, with viral load data available or anticipated to become available at around 18-24 months after seroconversion (viral load set point), and with information on treatment history available. Patients who initiated combination antiretroviral therapy (cART) before a viral load set-point measurement was obtained
were excluded from study participation and sample collection. The patients were selected from existing cohort studies which allowed us to create two groups of HIV-1 infected individuals that differed for calendar period of seroconversion (pre-2003 and post-2003 seroconverters). This date was chosen according to the study by Gras et al who were a significant and substantial difference of HIV-1 RNA concentration at set-point was identified when patients who seroconverted between 2003 and 2007 were compared with individuals seroconverting between 1996 and 2002 and before 1996 [15].

Pre-2003 seroconverters:
Individuals who seroconverted before 2003 (n=459) were selected from the Amsterdam Cohort Studies (ACS, n=335) [26] and from the AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort (n=124) [15]. The ACS enrolled participants between October 1984 and February 1988 and a total of 1013 asymptomatic men who were living in the Amsterdam area and who reported at least two homosexual contacts in the preceding six months were included in the prospective ACS on the prevalence and incidence of HIV-1 infection and risk factors for AIDS. At entry in the ACS, 239 men tested positive for HIV antibodies, five of whom refused to participate further. For these seroprevalent individuals, an imputed seroconversion date (on average 18 months before entry into the ACS) was used [27]. Of the 774 HIV-1 negative men, 131 subsequently seroconverted during active follow-up until 1996. Seroconversion date for these seroconverters was estimated as midpoint between last HIV-negative and first HIV-positive test result. None of the 365 seropositive men received treatment in their first 30 months post infection, thus excluding an effect of therapy on viral load set-point. DNA for genotyping was available from a random sample of 335 (88%) cohort participants who subsequently were included in this study. Data on HLA type obtained by serology and/or PCR were available for all 335 individuals. All individuals with HLA-B*5701 (n=17) carried the minor rs2395029 HCP5 allele, confirming the nearly complete LD between these variants. Of these 17 individuals, 8 fulfilled our definition of LTNP (>10 years of therapy naïve asymptomatic follow-up with stable CD4+ T-cell counts and >400 CD4+ T-cells/µl in the 9th and 10th year after seroconversion. This study population has been described previously [10].

The ATHENA observational cohort [15] includes anonymized data from 1998 onwards, of all HIV-infected patients living in the Netherlands who visited one of the 25 HIV treatment centers. Twenty of the 25 participating hospitals were willing to ask their patients to participate in the SHM-HIV-1 Host Genetics study. From a total of 217 individuals with a known or accurately estimated date of seroconversion based on last HIV-negative and first HIV-positive measurement, a total of 124 individuals fulfilled all entry criteria for the SHM-HIV-1 Host Genetics study and gave informed consent and blood for DNA isolation and genetic studies.

Post-2003 seroconverters:
Individuals who seroconverted after 2003 were selected from the Primo-SHM cohort, a multicenter prospective cohort study in the Netherlands with 13 participating hospitals,
which from 2003 onwards started to enroll individuals with laboratory evidence of primary HIV infection, defined as having a negative or indeterminate Western blot in combination with detectable plasma HIV-1 RNA, or a period of less than 180 days between their last HIV-negative and first HIV-positive test result [28]. For this latter group, the midpoint between last HIV-negative and first HIV-positive test result was used as estimated date of seroconversion.

All 13 participating hospitals were willing to ask their patients to participate in the SHM HIV-1 Host Genetics study. For 24 individuals (10.3%) who were enrolled later in the epidemic, HLA-typing was performed. One of these individuals carried an HLA*B5701 allele, and also the minor HCP5 rs2395029 allele. For these post-2003 seroconverters cART was available according to the Netherlands treatment initiation guidelines based on CD4 count and viral load (http://www.nvab.org/richtlijnhiv), precluding categorization based on the clinical course of infection. None of these individuals fulfilled the definition of elite controller (more than 1 year follow-up with viral load < 5 copies HIV-1 RNA/ml plasma).

In the end, 231 patients, from a total of 304 individuals, who seroconverted after 2003 and who fulfilled all entry criteria gave informed consent for genetic studies.

All studies are conducted in accordance with the ethical principles set out in the declaration of Helsinki and were approved by the institutional Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam and the Boards of Directors and Medical Ethics Committees of participating hospitals in this multicenter study.

Genotyping

For this study we analyzed SNPs rs2395029 (HCP5), rs9264942 (HLA-C) and the CCR5wt/Δ32 genotype. For the 335 pre-2003 seroconverters, enrolled in the ACS, genotype data for SNP rs2395029 (HCP5) was available from a GWAS that used the Illumina Infinium HumanHap300 BeadChip (Illumina, San Diego, California, USA) (van Manen et al, manuscript in preparation). For the remaining 124 pre-2003 seroconverters and the 231 post-2003 seroconverters, SNP rs2395029 was analyzed by PCR amplification using Taq DNA polymerase (Invitrogen, Carlsbad, California, USA) and primer pair FW: 5’-CGAACTGCTCCTACCCCTCATTTGTG-3’ and RV: 5’-CGTGGGTCCAGATACCAAGG-3’. Restriction digestion with XcmI (1h 37°C; New England Biolabs, Ipswich, Massachusetts, USA) results in a undigested PCR product of 327 base pairs (bp) or a 133 bp and a 194 bp product after restriction digestion, depending on nucleotide G or T, respectively, at SNP position. Genotyping of rs2395029 HCP5 with the Illumina platform was confirmed for six samples (3 MAJ and 3 HZ) by the PCR as described above. SNP rs9264942 (-35HLA-C) was determined in all individuals using the predeveloped ABI TaqMan allelic discrimination-based technology with an ABI7900 Sequence Detection System (ABI, Foster City, CA), and the CCR5wt/Δ32 genotype was determined by PCR analysis of the CCR5 gene region as described previously [29].
Virological assays

Samples collected prior to 1997 were tested in the nucleic acid sequence-based amplification (NASBA) HIV-1 RNA QT (NASBA, bioMerieux, Boxtel, Netherlands). Samples with a viral load below the quantification threshold of the NASBA assay (1,000 copies/ml, n=68), were re-tested in 2008 using the RealTime HIV-1 assay (Abbott Laboratories, Abbott Park, IL), which has a threshold of 40 copies/ml. Details on the assays used after 1997 for viral load measurements in individuals from the ATHENA cohort and the primo-SHM study have been described previously [15]. Viral load set-point was defined as the stable level of HIV RNA load around 18-24 months after seroconversion (using the mean of all measurements in that period) after establishing that all viral load measurements in the period of 15-27 months after seroconversion were within a maximum 0.5 log10 bandwidth. Viral load measurements for 180 of 870 initially selected study participants did not match one or more of the above criteria and no viral load set-point was calculated. Individuals with viral load set-point information who chose not to participate in this study did have a similar viral load set-point when compared to individuals who were included in this study (data not shown). Furthermore, was the viral load set-point in the post-2003 seroconverters who refused to participate in this study (n=89) significantly higher when compared to pre-2003 seroconverters who refused to participate (n=64) (4.44 (95% CI; 4.26-4.62) log10 copies/ml versus 4.10 (95% CI; 3.98-4.20) log10 copies/ml, P= 1.1 x10^{-3}).

Statistical analyses

Viral load data were analyzed after log10 transformation. A student’s T-test was used to test for association of the SNP rs2395029 and the CCR5wt/Δ32 genotype with viral load set-point. To test for association of SNP rs9264942 with viral load set-point, we used a one-way analysis of variation (ANOVA) test. We used unadjusted (including seroconversion period and each SNP separately) and adjusted linear regression models to test the association of the 3 SNPs and seroconversion period with viral load set-point. Interaction terms between each SNP and each seroconversion period were included to test whether the effect of each SNP on viral load set-point had changed over time. Analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) and SAS version 9.1 (SAS Institute, Cary, North Carolina, USA). Power calculations for association analysis were performed using G*Power 3.1.2 [30].

RESULTS

Patients

Baseline characteristics of all study participants are summarized in Table 1. The pre-2003 seroconverters (seroconversion date between 1982 and January 1, 2003) were mainly men (95.6%), of whom 95.7% were men who have sex with men (MSM)
and 3.2% heterosexual. Most of the females were infected via heterosexual contact (80%), 10% of the females were assumed to be infected via injecting drug use. The pre-2003 seroconverters originated mainly from Western Europe (96.3%) and 80.8% were known to be infected with a subtype B HIV-1 variant. In the group of post-2003 seroconverters (seroconversion between January 1, 2003 and 2009), 92.6% were male of whom 89.7% and 7.9% were MSM or heterosexual, respectively. All females were infected via heterosexual contact. The infecting HIV-1 subtype was B in 50.6% of individuals, another subtype in 3.0% of individuals, and not determined in 46.3% of the individuals; 86.1% were born in Western-Europe.

Genotype distribution for -35HLA-C rs9264942, HCP5 rs2395029 and CCR5wt/Δ32 in pre-2003 and post-2003 seroconverters.

We determined the prevalence of the minor allele of SNP rs9264942 in the HLA-C gene region and rs2395029 in the HCP5 gene region, and of the CCR5Δ32 allele in our study population. No significant differences were observed in genotype frequencies between the groups of pre-2003 and post-2003 seroconverters, or with the non-infected Hapmap
population of European descent (Table 2). No deviations from Hardy-Weinberg Equilibrium were observed (data not shown).

Table 2. Prevalence of genotypes in pre-2003 seroconverters, post-2003 seroconverters and healthy Hapmap population.

<table>
<thead>
<tr>
<th></th>
<th>rs9264942 -35HLA-C</th>
<th>rs2395029 HCP5</th>
<th>CCR5wt/Δ32</th>
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<tbody>
<tr>
<td></td>
<td>MAJ</td>
<td>HZ</td>
<td>MIN</td>
</tr>
<tr>
<td>SC 1982-2002</td>
<td>47.7</td>
<td>42.3</td>
<td>10.0</td>
</tr>
<tr>
<td>SC 2003-2009</td>
<td>47.2</td>
<td>43.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Hapmap</td>
<td>42.4</td>
<td>47.5</td>
<td>10.1</td>
</tr>
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MAJ, homozygotes for the major allele; HZ, heterozygotes; MIN, homozygotes for the minor allele; SC, seroconversion. Prevalence of genotypes was not significantly different between pre- and post-2003 seroconverters (Fisher’s exact test).

Impact of host genetic factors on viral load in pre-2003 and post-2003 seroconverters

We first analyzed whether the observed increase in viral load set-point at a population level [13-15] could be confirmed in our study population. The viral load set-point, defined as the stable HIV RNA load around 18-24 months after seroconversion, was indeed higher in the post-2003 seroconverters as compared to the pre-2003 seroconverters (4.44 (95% CI; 4.33-4.55) log₁₀ copies/ml versus 4.17 (95% CI; 4.10-4.25) log₁₀ copies/ml, P= 4.5x10⁻⁵).

Previous studies have demonstrated a strong association between a lower viral load early in infection and the minor alleles of rs9264942 (-35HLA-C) and rs2395029 (HCP5), or with heterozygosity for a 32 bp deletion in the CCR5 gene (CCR5wt/Δ32) [6,8,9,31]. The same associations were found for our pre-2003 seroconverters: set-point viral load was highest in -35HLA-C wild-type homozygotes, and significantly lower in individuals who were heterozygous, or homozygous for the minor allele (P=7.7x10⁻³; Table 3). Also, the viral load set-point was significantly lower in pre-2003 seroconverters carrying the minor allele of SNP rs2395029 in HCP5 (n=27) than in those who were homozygous for the major allele (n=432, P= 5.4x10⁻⁴; Table 3). Finally, pre-2003 seroconverters with a CCR5Δ32 allele (either with a heterozygous (n=88) or homozygous (n=1) genotype) had a significantly lower viral load set-point than those carrying two CCR5 wild-type alleles (n=370, P= 5.2x10⁻³).

In post-2003 seroconverters, viral load set-point in individuals homozygous for the minor allele of rs9264942 (-35HLA-C) was also significantly lower than the viral load set-point in heterozygotes and homozygotes for the wild-type allele (P= 9.9x10⁻⁴). Surprisingly, however, the protective effect associated with the minor allele of rs2395029 in HCP5 or a CCR5wt/Δ32 genotype was absent in post-2003 seroconverters. The viral load set-point in post-2003 seroconverters was not significantly different in carriers
of the minor allele of rs2395029 in HCP5 (n=15) when compared to carriers of two major alleles (n=216, P= 4.7x10^-1), and no difference was present in viral load set-point between CCR5wt/Δ32 heterozygotes (n=34) and individuals with a CCR5wt/wt homozygous genotype (n=197) (P= 6.3x10^-1).

Adjusted linear regression model for the analysis of fading impact of host genetic markers on viral load set-point over calendar time

To test whether the associations of the 3 genotypes with viral load set-point where independent of each other adjusted linear regression analysis was performed. None of the associations between the three genetic markers and viral load set-point changed significantly as compared to the unadjusted analyses (Table 4).

Additionally we tested whether the observed changes in the association between each genetic marker and viral load set-point between pre-2003 and post-2003 seroconverters were independent from the effect of the other two genetic markers by including interaction terms between seroconversion period and all of the 3 genotypes
in the adjusted model. This model found no significant evidence that the association between rs9264942 -35HLA-C and viral load set-point had significantly changed over time (P= 1.7x10⁻¹; data not shown). However the association between viral load set-point and SNP rs2395029 in HCP5 had significantly changed, also after adjusting for a potential effect of the other two genetic markers under study (MAJ-HZ in pre-2003 versus post-2003 seroconverters: 0.51 log₁₀ copies/ml versus -0.06 log₁₀ copies/ml; P= 4.0x10⁻²). Although the association between CCR5wt/Δ32 and viral load set-point was only observed in pre-2003 seroconverters and absent in post-2003 seroconverters, there was no evidence that the effect had changed significantly over time (P= 2.6x10⁻¹; data not shown).

DISCUSSION

At a population level, HIV-1 viral load set-point seems to be increasing over calendar time [13-15], albeit not confirmed by others [32-34].

In our study in HIV-1-infected individuals in the Netherlands, we observed a significantly higher viral load set-point in individuals who became HIV-1 infected in the period 2003-2009 as compared to individuals who became infected in the period 1982-2002. The observed rise in viral load set-point over calendar time in the Dutch population is in agreement with the results of the CASCADE study [13] and a recent study of the epidemic in Italy [14]. Three other studies found no evidence for the increase in viral load set-point [32-34], and differences in patient selection, study period, and outcome definitions across these studies might explain the discrepancies.

Moreover, we observed that associations between certain host genetic factors and a lower viral load set-point were restricted to pre-2003 seroconverters. In this group,
the minor alleles of rs9264942 -35HLA-C and rs2395029 in HCP5, and a CCR5wt/Δ32 genotype were all associated with a lower viral load set-point as compared to the group with the respective wild-type genotypes. In post-2003 seroconverters the protective effect of the minor allele of rs9264942 -35HLA-C was preserved, but the protective effect of the minor allele of rs2395029 in HCP5 and of a CCR5wt/Δ32 genotype was no longer present in this group. The effect of carrying a minor allele of SNP rs2395029 in HCP5 had significantly changed over time, independent from the other two markers under study, while the change in impact of the CCR5wt/Δ32 genotype on HIV-1 control over calendar time was not significant.

Assuming an effect size for HCP5 rs2395029 of 0.67 (like in the pre-2003 seroconverters), a MAF of 0.1 and type 1 error rate of 0.05, we had 83% power to detect a significant association in the 231 post-2003 seroconverters. However, assuming an effect size for CCR5wt/Δ32 of 0.7, a MAF of 0.2 and type 1 error of 0.05, we had 73% power to detect a significant association in this group. Thus the post-2003 seroconverter group size may have been too small to observe this effect, which could also explain why we did not observe a significant change in the association of the CCR5wt/Δ32 genotype on viral load set-point over calendar time.

Although the original observation of the rise in viral load set-point was made using the year 2003 to divide the two groups [15], we also explored using other years. Viral load set-point did not differ significantly between individuals seroconverting before or after 1996 (4.22 (95% CI; 4.14-4.31) log_{10} copies/ml versus 4.30 (95% CI; 4.20-4.30) log_{10} copies/ml, P= 2.1 x10^{-1}), confirming the absence of a rise in viral load set-point between 1996 and 2002. Dividing the groups based on seroconversion before or after years later than 2003 was not feasible because of sample size limitations in the late group.

Individuals who started cART after January 1st 1996 before a viral load set-point measurement was obtained were excluded from further analysis which occurred more often in the group of post-2003 seroconverters. Although this might have introduced a potential bias in our study, the fact that these individuals had to initiate cART implies that their viral load was most likely high. In other words, the mean viral load in the group of post-2003 seroconverters may have been even higher had these individuals been included with a pre-therapy viral load measurement. Moreover, the distribution of genotypes under study was not different between pre- and post-2003 seroconverters, or between our seroconverter cohorts and the Hapmap population, also arguing against a bias in our patient selections.

HIV-1 subtype, region of origin, and mode of transmission were less homogeneous in the post-2003 seroconverters. These factors may have influenced disease progression [35], SNP prevalence, and/or viral load set-point [33]. However, when the analysis was limited to the post-2003 subgroup of MSM from Western-European descent infected with a documented subtype B HIV-1, the protective effect of the minor allele of HCP5 rs2395029 and CCR5wt/Δ32 heterozygosity on VL set-point remained absent. Moreover, SNP genotype distributions were not significantly different between the pre- and post-2003 seroconverters (Table 2).
Several different assays were used to determine HIV-1 RNA plasma load and batch-wise retesting was not performed. Samples with a viral load below the quantification threshold of the NASBA RNA QT assay (1,000 copies/ml, n=68), were re-tested in 2008 using the Real-Time Abbott HIV-1 assay. It has been reported that the mean HIV-1 RNA concentration at set-point was slightly higher when measured with older assays that have a lower limit of detection of 1000 or 400 copies/ml as compared to the more sensitive assays with a lower limit of detection of 50 copies/ml [36]. This would imply that the difference in viral load set-point between pre-2003 and post-2003 seroconverters would have been even more pronounced had all samples been retested with the more sensitive Abbott Real-Time assay. Furthermore, adjustment for assay sensitivity did not appreciably change viral load set-point results in the study by Gras et al [15].

Since 48% of the post-2003 seroconverters presented themselves with an acute infection, there might be a bias in this group for symptomatic infections that have been associated with a higher viral load [37]. Unfortunately, no data is available on symptoms during acute infection for post-2003 seroconverters. Although, differences in the severity of acute infection between pre-2003 and post-2003 seroconverters cannot be excluded, similar minor allele prevalence was found for all three host genetic factors under study in both groups, indicating that the post-2003 seroconverters are unlikely to be an a priori genetically more susceptible group.

The minor allele variant of SNP rs2395029 in HCP5 is in strong LD with HLA-B57, which has been associated with long-term asymptomatic survival [3-5,38] and data is accumulating that HLA-B57 is in fact the causal genotype associated with HIV-1 control tagged by HCP5 rs2395029 [31,39]. Interestingly, adaptation of HIV-1 to HLA has been described [24] and in contemporary seroconverters in the Netherlands, we have evidence for a selective loss of epitopes presented by more protective HLA-types such as HLA-B57 and HLA-B27 as compared to epitopes presented by HLA alleles that have not been associated with relative protection from disease progression [25]. These studies indicate that population-level adaptation of HIV-1 to host defense mechanisms has indeed occurred, probably explaining the fading impact of rs2395029 in HCP5 on HIV-1 viral load set-point.

The absence of an association between a CCR5wt/Δ32 genotype and a lower viral load set-point in post-2003 seroconverters may point to adaptation of HIV-1 to its host. We and others have indeed reported the intrapatient evolution of CCR5 using (R5) HIV-1 variants towards improved usage of coreceptor CCR5, which is reflected by a decreasing level of resistance of HIV-1 to inhibition by RANTES in the course of the infection [40-42]. It is tempting to speculate that the HIV-1 variants with increased ability to use CCR5 are more successfully transmitted and that this explains why the lower CCR5 expression levels in individuals with a CCR5wt/Δ32 genotype are no longer rate-limiting. Interestingly, a more efficient use of CCR5 may also explain why the replication rate of HIV-1 obtained early after transmission has increased over calendar time in the Dutch epidemic, thus possibly contributing to the observed
increase in viral load set-point [43]. Alternatively, an unusually high prevalence of CXCR4-using variants in the post-2003 seroconverters may have accounted for the absence of the protective effect of the CCR5wt/Δ32 genotype. While we do not have data on coreceptor use for all post-2003 seroconverters, a low prevalence of CXCR4-using viruses (4-6%) was observed in three recent cohorts of seroconverters including a subgroup of the post-2003 seroconverters (n=46) during the period 2003-2008 [44-46]. Since this low prevalence of CXCR4-using viruses in recent seroconverters is similar to that reported for historical seroconverters, it is unlikely that increased CXCR4-use explains the absence of the protective effect.

The association between -35HLA-C rs9264942 and viral load set-point has remained the same over calendar time. The -35HLA-C minor allele is associated with high HLA-C cell surface expression, which may account for an overall better antigen presentation to cytotoxic T-cells or improved recognition by natural killer cells [47]. The preserved protective effect of this genotype suggests that to date HIV-1 cannot escape from the mechanism associated with it. It is tempting to speculate that escape would come at a too large fitness cost to the virus [48-50].

Our study focused on an HIV-1 subtype B infected population in the Netherlands, which may limit the implications of our findings. However, the adaptation of HIV-1 to HLA, which was demonstrated in multiple cohorts [24] seems to imply that the host-adaptation of HIV-1 is a more general phenomenon. Furthermore, we recently reported that early HIV-1 variants from people who seroconverted in the beginning of the epidemic were more resistant to neutralizing antibodies than early HIV-1 variants from individuals who became infected more recently [51], suggesting adaptation of HIV-1 at a population level also to the humoral immune response.

Importantly, our findings also imply that associations from GWAS on HIV-1 control that are not replicated in cohorts that differ in the age of their HIV-1 epidemic are not necessarily false positives in the discovery cohort, but may be due to adaptations of the pathogen to its host over calendar time.

The consequences of a higher viral load set-point for the epidemic may be serious as it facilitates transmission [52,53]. The reduction of HIV-1 transmission which may be achieved by reducing viral load by cART may be counteracted by a higher viral load prior to the initiation of therapy due to the fading impact of host genetic factors that originally controlled the virus. From this point of view, even earlier initiation of cART may be warranted, as has been suggested recently [54,55].

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