

Host genomics of the HIV-1 reservoir size and its decay rate during suppressive antiretroviral treatment

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## **ABSTRACT**

**Background.** The primary hurdle for the eradication of HIV-1 is the establishment of a latent viral reservoir early after primary infection. Here we investigated the potential influence of human genetic variation on the HIV-1 reservoir size and its decay rate during suppressive antiretroviral treatment (ART).

**Setting.** Genome-wide association study and exome sequencing study to look for host genetic determinants of HIV-1 reservoir measurements in patients enrolled in the Swiss HIV Cohort Study (SHCS), a nation-wide prospective observational study.

**Methods.** We measured total HIV-1 DNA in peripheral blood mononuclear cells from study participants, as a proxy for the reservoir size, at three time points over a median of 5.4 years, and searched for associations between human genetic variation and two phenotypic readouts: the reservoir size at the first time point and its decay rate over the study period. We assessed the contribution of common genetic variants using genome-wide genotyping data from 797 patients with European ancestry enrolled in the Swiss HIV Cohort Study and searched for a potential impact of rare variants and exonic copy number variants using exome sequencing data generated in a subset of 194 study participants.

**Results.** Genome- and exome-wide analyses did not reveal any significant association with the size of the HIV-1 reservoir or its decay rate on suppressive ART.

**Conclusions.** Our results point to a limited influence of human genetics on the size of the HIV-1 reservoir and its long-term dynamics in successfully treated individuals.

**Key words:** HIV, genetics, ART, latent reservoir, GWAS, exome sequencing

## INTRODUCTION

Combination antiretroviral treatment (ART) has turned the previously lethal infection by human immunodeficiency virus type 1 (HIV-1) into a chronic disease. Despite this significant achievement, HIV-1 as retrovirus, self-integrating its genome into the host chromosome, persists indefinitely in infected individuals during treatment,<sup>1–4</sup> and life-long ART is required to control the infection.

A major hurdle to HIV-1 eradication is the establishment, already during primary infection, of a latent viral reservoir of HIV-1 DNA persisting as provirus in resting memory CD4<sup>+</sup> T cells.<sup>1,2,5–8</sup> At the molecular level, chromatin remodeling, epigenetic modifications, transcriptional interference, and availability of transcription factors have been considered as possible mechanisms contributing to HIV-1 latency.<sup>9</sup> The viral reservoir is measurable through different methods, including viral outgrowth assay and intracellular HIV-1 DNA quantification.<sup>10,11</sup> Currently, there is no consensus on the best HIV-1 reservoir biomarker. Viral outgrowth assays only measure replication-competent proviruses and have therefore been considered to be the gold standard to measure the latent reservoir.<sup>12</sup> Total cell-associated HIV-1 DNA measurements quantify both integrated and nonintegrated viral genomes coding for intact or defective viruses,<sup>13</sup> thus detecting higher infected-cell frequencies compared to those obtained with viral outgrowth assays.<sup>12,14</sup> Importantly, HIV-1 DNA measurement is easy to measure in different cell and tissue samples and is applicable to large populations, and has been shown to be a good proxy for the reservoir size.<sup>15</sup> Total HIV-1 DNA levels have been shown to correlate with viral outgrowth,<sup>16</sup> and to predict the time to viral rebound at treatment interruption.<sup>17</sup> Moreover, the substantial loss of nonintegrated HIV-1 DNA genomes following ART initiation suggests that total HIV-1 DNA after prolonged suppression is largely accounted for by integrated viral genomes.<sup>18</sup>

After an initial rapid decay following ART initiation, changes of the viral reservoir size over time display wide inter-individual variability. By limiting dilution culture assay, the half-life of the viral reservoir was first estimated to be 44 months (95% confidence interval 27.4–114.5) in individuals

with undetectable viremia.<sup>4</sup> A more recent study, based on total HIV-1 DNA quantification, showed a slow decline with a half-life of 13 years after the first four years of suppressive ART.<sup>19</sup> Generally, different culture-based and PCR-based studies show a broad variability of the average decay rate, from 2.5 months to no measurable decay.<sup>20-28</sup> One study even reported an increase in the viral reservoir size, as measured by total HIV-1 DNA quantification, in as much as 31% of patients in the 4-7 years following ART initiation,<sup>29</sup> and recent data from our group confirm this observation, reporting an increase in the reservoir size in 26.8% of individuals in the 1.5-5.5 years after ART initiation.<sup>30</sup>

Several factors are known to influence the decay rate of the viral reservoir: initiation of ART during acute HIV-1 infection substantially accelerates the decay rate, while viral blips and low-level viremia during ART slow it down, as shown in previous studies and in recent data from our cohort.<sup>24,30</sup> Conversely, treatment intensification, i.e. treating with additional drugs, does not appear to influence the decay rate, suggesting that residual replication is not the main driver of the viral reservoir or that it may happen in sanctuary sites.<sup>31</sup>

Human genetic variants have been shown to influence the outcome of various infections, including HIV. Previous genome-wide association studies (GWAS) addressed the role of common genetic polymorphisms in several HIV-related phenotypes, including plasma viral load (HIV-1 RNA) at set point, exceptional capacity to control viral replication, pace of CD4+ T lymphocyte decline, time to clinical AIDS, rapid progressor status or long-term non-progressor status (LTNP),<sup>32-39</sup> and, in one single study, the amount of intracellular HIV-1 DNA, measured at a single time point during the chronic phase of infection.<sup>40</sup> Rare genetic variants that are detectable through DNA sequencing technologies have been investigated far less. However, a large exome sequencing study did not reveal any convincing association of such variants with the natural history of HIV disease.<sup>41</sup>

To date, no studies have addressed the role of human genetic variation in determining the initial viral reservoir size and the reservoir decay rate over time. In the current study, we searched for host

genetic factors associated with the HIV-1 reservoir size and its long-term dynamics in a cohort of 797 HIV-1 positive individuals on suppressive ART for at least five years.

## METHODS

### Ethics statement

The Swiss HIV Cohort Study (SHCS) was approved by the local ethical committees of the participating centres: Kantonale Ethikkommission Zürich (KEK-ZH-NR: EK-793); Ethikkommission beider Basel (“Die Ethikkommission beider Basel hat die Dokumente zur Studie zustimmend zur Kenntnis genommen und genehmigt.”); Kantonale Ethikkommission Bern (21/88); Comité departmental d'éthique des spécialités médicales et de médecine communautaire et de premier recours, Hôpitaux Universitaires de Genève (01–142); Commission cantonale d'éthique de la recherche sur l'être humain, Canton de Vaud (131/01); Comitato etico cantonale, Repubblica e Cantone Ticino (CE 813); Ethikkommission des Kantons St. Gallen (EKSG 12/003), and written informed consent was obtained from all participants.

### Study participants

The SHCS is an ongoing, nation-wide cohort study of HIV-positive individuals, including more than 70% of all persons living with HIV in Switzerland. Clinical and laboratory information has been prospectively recorded at follow-up visits every 3-6 months since 1988.<sup>42</sup> The general enrolment criteria have been described previously.<sup>30</sup> Additionally, availability of genome-wide genotyping data from previous studies or of a DNA sample for genotyping was required for inclusion in this study (Figure 1).

## **Quantification of total HIV-1 DNA**

The collection of longitudinal cryopreserved peripheral blood mononuclear cells (PBMCs) from eligible participants and the quantification of total HIV-1 DNA by droplet digital PCR has been described previously along with the calculation of the reservoir decay rate by linear regression.<sup>30</sup> Briefly, this study utilized total HIV-1 DNA quantifications from three time points at a median of ~1.5 years, ~3.5 years, and ~5.4 years after initiation of ART.

## **Genotyping and genome-wide association analyses**

Genome-wide genotyping data were obtained from previous GWAS generated using various microarrays, or generated from DNA extracted from peripheral blood mononuclear cells using the HumanOmniExpress-24 BeadChip (Illumina Inc., San Diego, CA, USA). Genotypes from each genotyping array were filtered and imputed separately, study participants were filtered based on European ancestry as determined by principal component analysis (PCA) and imputed variants were filtered by minor allele frequency (MAF) < 5%, missingness > 10%, deviation from Hardy-Weinberg equilibrium ( $P_{HWE} < 1e-6$ ) and imputation quality score (INFO < 0.8). The remaining genotypes were then combined using PLINK (v1.90b5) prior to analyses.<sup>43</sup>

To carry out the GWASs, genome-wide genotypes were tested for association with each of the two study phenotypes (reservoir size or reservoir decay rate) in two separate genome-wide association analyses. Statistical significance was set to the standard genome-wide significance threshold of  $P < 5e-8$  to correct for multiple testing, and covariates were included to assess the contribution of variables previously shown to be associated with either reservoir size or decay rate. Classical HLA alleles at the four-digit level and variable amino acids within HLA proteins were imputed and studied for association with the study phenotypes. With our sample size, we had 80% power to detect variants with a MAF of 10% explaining at least 5% of the variance in HIV-1 reservoir size or decay rate.<sup>44</sup>

Genotypes at specific loci, i.e. the CCR5 $\Delta$ 32 deletion (rs333) and the HLA-B\*57:01 allele, known to influence the setpoint viral load (spVL),<sup>45,46</sup> were also tested for association with the reservoir size and its decay rate in 797 patients. A detailed description of genotyping and GWAS is available in the online methods in the Supplemental Digital Content,<http://links.lww.com/QAI/B521>.

### **Exome sequencing and analysis**

After capture and sequencing of all coding exons, sequence reads were processed according to genome analysis toolkit (GATKv3.7) best practices, quality filtered and annotated with SnpEff (v4.3T).<sup>47</sup>

The multi-sample VCF file was used for single variant association analyses using PLINK for common variants and optimal sequence kernel association tests (SKAT-O) for rare variants.<sup>48</sup> For the decay rate, individuals were split into two groups due to the non-normal distribution, one exhibiting a very high decay over time (< -0.03 -log10(DNA)) and another with a stable reservoir size ( $\geq$  -0.03 and  $\leq$  0.03 -log10(DNA)). For this case-control analysis we used the SKATbinary function with linear weighted variants as implemented in the SKAT R package. For association analyses, the 4-digit HLA alleles were extracted and analyzed using PyHLA.<sup>49</sup> A detailed description of exome sequencing and analysis is available in the online methods in the Supplemental Digital Content,<http://links.lww.com/QAI/B521>.

### **Copy number variation**

Copy number variations (CNVs) were called from exome sequencing data using CLAMMS and studied for association with the study phenotypes.<sup>50</sup> A detailed description of CNV analysis is available in the online supplemental digital content,<http://links.lww.com/QAI/B521>.

## Statistical analyses

All statistical analyses were performed using the R statistical software (v3.5.2), unless otherwise specified.

## RESULTS

### Host genetic determinants of the reservoir size and long-term dynamics

To investigate the effects of host genetic variation on the size of the HIV-1 reservoir 1.5 years after ART initiation and its long-term dynamics under ART over a median duration of 5.4 years, we performed a GWAS, including 797 well-characterized HIV-1 positive individuals. All study participants were enrolled in the SHCS and were of European ancestry with longitudinal total HIV-1 DNA measurements available (Table 1). The median HIV-1 reservoir size was 2.76 (IQR: 2.48-3.03) log<sub>10</sub> total HIV-1 DNA copies/1 million genomic equivalents measured ~1.5 years after initiation of ART (IQR: 1.3–1.7) (Figure S2A, Supplemental Digital Content,<http://links.lww.com/QAI/B521>). The median decay rate between 1.5-5.4 years after initiation of ART was -0.06 (IQR: -0.12-0.00) log<sub>10</sub> total HIV-1 DNA copies/1 million genomic equivalents per year (Figure S2B, Supplemental Digital Content,<http://links.lww.com/QAI/B521>), which is equivalent to a half-life of 5 years on a linear scale. We found no significant sex differences in the reservoir size 1.5 years after initiation of ART or in the decay rate (Figure S3, Supplemental Digital Content,<http://links.lww.com/QAI/B521>).

First, we performed GWAS using age and sex as covariates. We did not observe any genome-wide significant variant ( $P < 5e-8$ ) associated with either HIV-1 reservoir size or long-term dynamics (Figure 2 and S4, Supplemental Digital Content,<http://links.lww.com/QAI/B521>). However, as we have previously determined, multiple factors are associated with the HIV-1 reservoir size and its decay rate,<sup>30</sup> some of which are correlated (Figure S5, Supplemental Digital Content,<http://links.lww.com/QAI/B521>). Thus, we performed additional analyses iteratively

including these factors to test whether they could mask genetic associations. We ran multiple GWAS each adjusting for age, sex, plus one of the associated covariates, as well as all of the covariates together. The addition of the covariates did not have any significant effect on the results nor the genome-wide inflation factor (lambda) (Table S1, Supplemental Digital Content,<http://links.lww.com/QAI/B521>).

Genetic variation in the HLA region has previously been associated with multiple HIV-related outcomes, including spVL.<sup>46</sup> To test whether specific HLA variants were associated with reservoir size or long-term dynamics, we imputed the HLA alleles and amino acids for all 797 individuals from the genotyping data. In line with the previous results, we did not observe any genome-wide significant associations with any HLA allele or amino acid.

### **Impact of protein-coding and rare variants**

To assess the impact of rare variants as well as protein-coding variants missed by genotyping arrays on the HIV-1 reservoir size and long-term dynamics, we performed exome sequencing in 194 of the 797 study participants. Patients were selected at the two extremes of the observed reservoir decay rate: either very rapid, or absent (no change in reservoir size over ~5.4 years), while individuals with increasing HIV-1 reservoir sizes were excluded (N=12). Thus, the long-term dynamics phenotype was binarized for subsequent analyses of the decay rate, while the HIV-1 reservoir size phenotype remained normally distributed (Figure S6, Supplemental Digital Content,<http://links.lww.com/QAI/B521>).

To ensure that no common variants, missed by the genotype chips, were associated with the HIV-1 reservoir size or long-term dynamics, we performed a GWAS for common variants using age and sex as covariates. As with the genotyping data, we observed no genome-wide significant variants for either phenotype (Figure S7, Supplemental Digital Content,<http://links.lww.com/QAI/B521>).

We then examined the potential role of rare variants ( $MAF < 5\%$ ) with a functional impact defined as either missense, frameshift, stop gained, splice acceptor or donor. Since HIV-1 primarily infects CD4+ T cells, we only included variants within genes expressed in these cells as determined by Gutierrez-Arcelus *et al.*<sup>51</sup> The significance threshold after correcting for the number of tests performed was  $P = 1.21e-5$ . We did not observe any significant associations for either the HIV-1 reservoir or the decay rate. The *AMBRA1* gene showed the strongest association with HIV-1 reservoir size ( $P = 4.15e-5$ , not significant) (Figure S8, Supplemental Digital Content,<http://links.lww.com/QAI/B521>).

To confirm the lack of HLA association seen with the genotyping data, we imputed the HLA haplotypes from the exome data using HLA\*LA. Again, we did not observe any significant HLA association with the study outcomes.

### **Copy number variations**

To examine the role of large exonic CNVs not captured by standard genotyping and exome pipelines, we called CNVs from the mapped sequencing reads of the exome samples using the software CLAMMS. The contribution of common CNVs to HIV-1 reservoir size and long-term dynamics was analyzed by association analyses including age, sex and the first principal component as covariates. No significant association was observed after Bonferroni correction (Figure S9, Supplemental Digital Content,<http://links.lww.com/QAI/B521>). We also searched for rare CNVs in curated immune-related genes from Immpo<sup>rt</sup> but did not discover any suggestive immune-related CNVs.<sup>52</sup>

## **Influence of HLA-B\*57:01 and the CCR5Δ32 deletion on reservoir size and long-term dynamics**

We have previously shown that pre-ART RNA viral load levels are associated with the HIV-1 reservoir size and the occurrence of blips.<sup>30</sup> The HLA-B\*57:01 allele and the CCR5Δ32 deletion are well known genetic variants influencing HIV-1 spVL,<sup>45,46</sup> and could thus also be associated with the with the HIV-1 reservoir size or its decay rate. However, we did not observe any nominal association (all P > 0.05) with either reservoir size or its long-term dynamics for HLA-B\*57:01 and CCR5Δ32 (Figure S10, Supplemental Digital Content, <http://links.lww.com/QAI/B521>).

## **DISCUSSION**

We used a combination of genomic technologies to assess the potential role of human genetic factors in determining both the HIV-1 reservoir size and its long-term dynamics in a well-characterized, population-based cohort. We studied 797 HIV-1-positive individuals of European origin under suppressive ART over a median of 5.4 years, for whom extensive clinical data are available, allowing detailed characterization and correction for potential confounders.<sup>30</sup> We measured the HIV-1 reservoir size at ~1.5 years after ART initiation and the slope of the reservoir decay rate calculated over the three time points. Previous HIV host genetic studies mostly focused on phenotypes reflecting the natural history of HIV-1 infection, prior to ART initiation, including spontaneous viral control and disease progression.<sup>32-39</sup> A single study specifically tested for associations between common genetic variants and the amount of intracellular HIV-1 DNA, measured at a single time point during the chronic phase of infection prior to initiation of any antiretroviral therapy.<sup>40</sup> Here, in contrast, we longitudinally assessed samples collected from

patients under suppressive ART to search for human genetic determinants of the long-term dynamics of the HIV-1 reservoir during treatment.

We first conducted a GWAS on 797 individuals to test for association between common genetic variants and the phenotypes. The SHCS predominately consists of individuals of European ancestry (66.1%)<sup>42</sup>, which, in our cohort, accounted for 80% (797 out of 995) of individuals fulfilling the eligibility criteria and successfully genotyped (Figure 1). Given the small proportion of non-European subjects in the initial study cohort, we only included patients of European ancestry to avoid any false positive associations or masking of true positive associations due to different allele frequencies in small proportions of individuals belonging to different subpopulations (Figure 1).<sup>53</sup> Our results are therefore applicable primarily to individuals of European ancestry.

Regardless of including or not independent covariates other than the standard ones (i.e., sex and age), no genetic variant reached the genome-wide significance threshold for association with any of the two phenotypes. This may reflect a small effect size of genetic variants on the HIV-1 reservoir size and decay rate. We acknowledge that a larger sample size and thus increased statistical power may allow detecting genetic variants with a smaller effect size associated with the phenotypes. However, it should be noted that this study is by far the largest today that has investigated the size and decay of the HIV-reservoir in well characterized and well suppressed HIV-positive individuals over a longer time period. Alternatively, the control of the HIV-1 reservoir size and its long-term dynamics may be under the control of viral or host factors other than the individual germline genetic background. A previous report from our group had shown a correlation between viral blips during the first 1.5 years of suppressive ART and the HIV-1 reservoir size 1.5 years after ART initiation, and between viral blips after 1.5-5.4 years of suppressive ART or low-level viremia and a slower decay rate.<sup>30</sup> Importantly, viral blips are generally thought to reflect transient increases in viral replication, and probably occur under multifactorial influence from viral and host factors,<sup>54-61</sup> with these latter possibly including, but not being limited to, germline genetic variation. The

biological relations between viral reservoir, decay rate, viral blips, and the contribution of the individual genetic background still need full elucidation.

Standard GWAS is designed to detect associations with common genetic variants (i.e., with a MAF of at least 0.05), with little power to investigate the role of rare variants. Thus, to further assess the contribution of rare variants in individuals at the extreme of the decay rate distribution, we used exome sequencing in a selected subset of 194 study participants with very high decay rate, or conversely, a stable reservoir size over time (Figure S6, Supplemental Digital Content,<http://links.lww.com/QAI/B521>). Here again, our analyses did not detect significant associations with the phenotypes. Although not reaching statistical significance, a rare genetic variant with potential functional impact in *AMBRA1* had a p-value for association just below the corrected threshold. The expression of *AMBRA1*, a core component of the autophagy machinery, has previously been associated with long-term viral control in HIV-1 non-progressors.<sup>62</sup> Future studies may further elucidate whether genetic variation in *AMBRA1* may account for inter-individual differences in the long-term dynamics of the HIV-1 reservoir.

Large deletions or duplications of genomic material may be implicated in human phenotypes, with CNVs impacting the exonic regions being more likely to have a functional role. Thus, we further investigated whether any common or rare CNV spanning exonic regions was associated with the phenotype. Again, no CNV was statistically associated with the phenotypes both in the exome-wide analyses and in analyses focused on immune-related genes.

An inherent limitation of our exome-based association analyses was their inability to detect rare variants outside the coding or splice-site regions. The exonic regions account for approximately 1-2% of the whole human genome. Because many regulatory sequences are located in extra-genic sites, our analysis did not fully investigate the role of highly conserved, non-coding genetic regions in influencing the phenotypes linked to HIV-1 latency.

Additionally, we focused on specific genetic variants, i.e., the HLA haplotypes and the CCR5Δ32 deletion, previously demonstrated to have a role in HIV-1 related phenotypes.<sup>32,46</sup> Indeed, previous studies unraveled a robust association between variation in the HLA region and the HIV-1 spVL.<sup>32</sup> Likewise, heterozygosity for the CCR5Δ32 deletion has been shown to influence spontaneous HIV-1 control.<sup>46</sup> Thus, we imputed HLA genotypes from genotyping and exome data, and studied the CCR5Δ32 deletion, without, however, detecting any significant associations with the phenotypes or the covariates (Figure S9, Supplemental Digital Content,<http://links.lww.com/QAI/B521>). Specifically, we found no correlation between HLA genotypes and HIV-1 RNA plasma levels prior to ART initiation, apparently contrasting with the previous findings of an association between HLA-B\*57:01 haplotype and spVL. This probably reflects historical changes in the therapeutic approach following a diagnosis of HIV-1 infection, given that ART is currently initiated soon after clinical diagnosis, before most patients reach a stable plateau of plasma viral load.

In our study, the quantification of the reservoir size at different time points may have been influenced by factors as, for example, blips and low-level viremia, which may have reduced our ability to detect significant genetic effects. It is also possible that, in the future, novel methods to assess the viral reservoir will allow the detection of significant contributions of genetic factors, as there is still ongoing debate on the optimal method to quantify the HIV-1 reservoir.<sup>13,63</sup> PCR-based methods, measuring both replication-competent and defective proviruses,<sup>13</sup> have been considered by some to overestimate the reservoir size compared to culture-based viral outgrowth assays, detecting replication-competent proviruses only.<sup>12,14</sup> Despite the practical difficulties in determining the exact reservoir size at a given time point in an HIV-1 infected individual, and the inherent limitations of each quantification method, several aspects make total cell-associated HIV-1 DNA from PBMCs a reliable proxy. In our previous study, total cell-associated HIV-1 DNA independently correlated with time to initiation of ART, CD4+ cell counts, and viral blips,<sup>30</sup> and the decay of the total HIV-1 DNA that we observed was consistent with that observed in smaller studies using either viral

outgrowth assays or total HIV-1 DNA.<sup>1,4</sup> In addition, defective genomes have been shown to be capable of transcribing novel unspliced forms of HIV-RNA during cART<sup>64</sup>, suggesting a biological relevance for both replication-competent and defective proviruses. A recent study, published after the completion of our analyses, proposed a novel method to differentially quantify replication-competent and defective proviruses,<sup>13</sup> paving the way for novel studies investigating the different roles of the two components of the viral DNA.

So far, it remains unanswered whether the initial response to acute infection, the containment of ongoing replication, and the control of latently infected cells are under the influence of the same or different molecular networks. It needs to be noted that in previous work we have shown that host genetic factors as defined by GWAS did not explain the severity of symptoms during acute HIV-infection, although severity of symptoms correlated well with viral load and CD4 cell counts.<sup>65</sup>

In conclusion, our study suggests that human individual germline genetic variation has little, if any, influence on the control of the HIV-1 viral reservoir size and its long-term dynamics. Complex, likely multifactorial biological processes govern HIV-1 viral persistence. Larger genomic studies, taking into account defined biological phenotypes and the differential biological importance of replication-competent and defective proviruses, will possibly clarify the role of common or rare genetic variants explaining small proportions of the variability of the phenotypes related to viral latency.

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### Author contributions

N.B., J.B., V.R., R.D.K., H.F.G., K.J.M., and J.F. contributed to the conception and design of the study. C.v.S., V.V., K.N., Y.I.K., and K.J.M. contributed to the acquisition of data. A.B., C.W.T., N.B., T.T., S.P., M.W., R.D.K., and J.F. contributed to the analysis and interpretation of data. J.B., M.P., T.K., S.Y., M.B., A.R., P.S., E.B., M.C., H.F.G., and the members of the Swiss HIV Cohort

Study (SHCS) conceived and managed the cohort, collected and contributed patient samples and clinical data. A.B., C.W.T., and J.F. contributed to the drafting the article. All authors read and approved the final manuscript.

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## Data availability

The datasets generated during and/or analyzed during the current study are not publicly available due to privacy reasons, the sensitivities associated with HIV infections, and the representativeness of the dataset, but is available on request.

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## FIGURE CAPTIONS

**Figure 1.** Patient selection flowchart. Specific inclusion and exclusion criteria are listed for each selection step. ART (antiretroviral therapy); PBMCs (peripheral blood mononuclear cells); PI (protease inhibitor); PCA (principal component analysis).

**Figure 2.** Association results with HIV-1 reservoir size. Manhattan plot with association p-values ( $-\log_{10}(P)$ ) per genetic variant plotted by genomic position. Dashed line indicates the threshold for genome-wide significance ( $P = 5e-8$ ). No variants were found to be genome-wide significant.

**TABLE 1**

| <b>Table 1. Patient characteristics</b>                      |                      |
|--------------------------------------------------------------|----------------------|
| <b>Total number of individuals</b>                           |                      |
| Genotyped                                                    | 797                  |
| Genotyped + exome sequenced                                  | 194                  |
| <b>Age at first HIV-1 DNA sample in years</b>                |                      |
| median (IQR)                                                 | 44 (38, 50)          |
| <b>Sex</b>                                                   |                      |
| Female                                                       | 123 (15.4%)          |
| Male                                                         | 674 (84.6%)          |
| <b>Transmission group</b>                                    |                      |
| HET                                                          | 241 (30.2%)          |
| IDU                                                          | 77 (9.7%)            |
| MSM                                                          | 448 (56.2%)          |
| Other                                                        | 31 (3.9%)            |
| <b>HIV-1 subtype</b>                                         |                      |
| B                                                            | 550 (69.0%)          |
| Non-B                                                        | 128 (16.1%)          |
| Unknown                                                      | 119 (14.9%)          |
| <b>Occurrence of blips or low-level viremia</b>              |                      |
| Blips                                                        | 200 (25.1%)          |
| Low-level viremia                                            | 68 (8.5%)            |
| None                                                         | 529 (66.4%)          |
| <b>Time on ART</b>                                           |                      |
| median (IQR)                                                 | 1.50 (1.28, 1.69)    |
| <b>Infection stage</b>                                       |                      |
| Acute                                                        | 140 (17.6%)          |
| Chronic                                                      | 657 (82.4%)          |
| <b>Time to viral suppression</b>                             |                      |
| median (IQR)                                                 | 0.34 (0.23, 0.51)    |
| <b>Log<sub>10</sub> HIV-1 plasma RNA pre-ART per mL</b>      |                      |
| median (IQR)                                                 | 480 (248, 684)       |
| <b>CD4+ cell count pre-ART cells/<math>\mu</math>L blood</b> |                      |
| median (IQR)                                                 | 186 (90, 270)        |
| <b>HIV-1 reservoir size</b>                                  |                      |
| median (IQR)                                                 | 2.76 (2.48, 3.03)    |
| <b>HIV-1 reservoir decay rate</b>                            |                      |
| median (IQR)                                                 | -0.06 (-0.12, -0.00) |

Transmission group indicates the self-reported route of infection (heterosexual (HET),

intravenous drug usage (IDU), men who have sex with men (MSM), and other (including

transfusions and unknown)). The occurrence of viral blips was defined by measurements of  $\geq$  50 HIV-1 RNA copies/mL plasma within a 30-day window. Individuals with consecutive measurements of  $\geq$  50 HIV-1 RNA copies/mL plasma for longer durations were classified as exhibiting low-level viremia. Time to viral suppression was the time from initiation of ART to the first viral load measurement below 50 copies/mL HIV-1 plasma RNA. HIV-1 reservoir size was measured in  $\log_{10}$  total HIV-1 DNA/1 million genomic equivalents  $\sim$ 1.5 years after initiating ART. The HIV-1 reservoir decay rate was based on the three measurements of total HIV-1 DNA levels taken at the median of 1.5, 3.5 and 5.4 years after initiation of ART.

**18,688 individuals** (Swiss HIV Cohort Study, 19/12/2014)

Inclusion criteria:

- HIV-1 infection
- Receiving ART for  $\geq 5$  years
- No treatment interruption of  $> 7$  days
- No virological failure defined as two consecutive measurements  $> 200$  HIV-1 RNA copies/ml plasma
- PBMCs available for three time points:  
1.5 +/- 0.5 years, 3.5 +/- 0.5 years, and  
5.5 +/- 1 year, after initiating first-line ART

**1932 individuals**

Exclusion criteria:

- Start on less potent ART regimens, i.e., mono/ or dual therapy, less potent/unboosted PI (NFV, SQV etc.)

**1382 individuals**

- Received  $\geq 3$  PBMC samples (mandatory 1<sup>st</sup> - 3<sup>rd</sup> time point)

**1166 individuals**

- Successful total HIV-1 DNA quantification in  $\geq 3$  PBMC samples (mandatory 1<sup>st</sup> - 3<sup>rd</sup> time point)

**1057 individuals**

- Successfully genotyped

**995 individuals**

- European ancestry (by PCA)

**797 individuals**

+ Exome sequencing

**194 individuals**

