

## Increased CHIP Prevalence Amongst People Living with HIV

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58 **Abstract**

59

60 People living with human immunodeficiency virus (PLWH) have significantly increased risk for  
61 cardiovascular disease in part due to inflammation and immune dysregulation. Clonal  
62 hematopoiesis of indeterminate potential (CHIP), the age-related acquisition and expansion of  
63 hematopoietic stem cells due to leukemogenic driver mutations, increases risk for both  
64 hematologic malignancy and coronary artery disease (CAD). Since increased inflammation is  
65 hypothesized to be both a cause and consequence of CHIP, we hypothesized that PLWH have a  
66 greater prevalence of CHIP. We searched for CHIP in multi-ethnic cases from the Swiss HIV  
67 Cohort Study (SHCS, n=600) and controls from the Atherosclerosis Risk in the Communities  
68 study (ARIC, n=8,111) from blood DNA-derived exome sequences. We observed that HIV is  
69 associated with increased CHIP prevalence, both in the whole study population and in a subset of  
70 230 cases and 1002 matched controls selected by propensity matching to control for  
71 demographic imbalances (SHCS 7%, ARIC 3%, p=0.005). Additionally, unlike in ARIC, *ASXL1*  
72 was the most commonly implicated mutated CHIP gene. We propose that CHIP may be one  
73 mechanism through which PLWH are at increased risk for CAD. Larger prospective studies  
74 should evaluate the hypothesis that CHIP contributes to the excess cardiovascular risk in PLWH.

## 75 Introduction

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77 As current treatments have rendered human immunodeficiency virus (HIV) a chronic  
78 condition, coronary artery disease has emerged as a major source of morbidity in people living  
79 with human immunodeficiency virus (PLWH). Inflammation and immune dysregulation likely  
80 accelerate CAD risk among PLWH.<sup>1</sup> Recently, ‘clonal hematopoiesis of indeterminate potential’  
81 (CHIP), the age-related acquisition and expansion of leukemogenic mutations (primarily in  
82 *DNMT3A*, *TET2*, *ASXL1*, *JAK2*) in white blood cells, was found to increase risk for both  
83 hematologic malignancy<sup>2,3</sup> and CAD<sup>4,5</sup> among asymptomatic individuals in the general  
84 population. The proatherogenic mechanisms for CHIP included heightened inflammation.<sup>4,6</sup>  
85 Given converging mechanisms promoting CAD risk and increased hematologic malignancy risk  
86 among PLWH, we tested the hypothesis that HIV-infected individuals have heightened  
87 prevalence of CHIP.

88

## 89 Methods

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91 We identified CHIP in a multi-ethnic sample of 600 PLWH who had available exome  
92 sequences from the Swiss HIV Cohort Study (SHCS), aged 21-83. The SHCS is a multicenter,  
93 prospective observational study for interdisciplinary HIV research<sup>7</sup>. Established in 1988, the  
94 SHCS currently comprises more than 20,000 PLWH with median 51 years of age. Samples of  
95 600 patients, used for exome sequencing, were chosen randomly in terms of gender, age,  
96 category of transmission, as well as HIV management and control.<sup>8</sup>

97 We utilized a set of 8111 individuals with available exome sequences from the  
98 Atherosclerotic Risk in the Community study (ARIC), aged 45-84 years, as population controls.<sup>9</sup>  
99 The ARIC study is a prospective longitudinal investigation of the development of atherosclerosis  
100 and its clinical sequelae which enrolled 15,792 individuals aged 45 to 64 years at baseline.<sup>10</sup> At  
101 study enrollment (1987-1989), the participants were selected by probability sampling from four  
102 United States communities: Forsyth County, North Carolina; Jackson, Mississippi; the  
103 northwestern suburbs of Minneapolis, Minnesota; and Washington County, Maryland.

104 CHIP was called in both exome sequenced cohorts using a previously described  
105 pipeline.<sup>4,11</sup> Briefly, short read sequence data was aligned to the hg19 reference genome using  
106 the BWA-mem algorithm and processed with the Genome Analysis Toolkit MuTect2 tool to  
107 detect somatic variants.<sup>12</sup> Identification of individuals with CHIP, used a pre-specified list of  
108 variants in 74 genes known to be recurrent drivers of myeloid malignancies.

109 As CHIP prevalence depends strongly on age, we performed a 1:5 case/control  
110 propensity matching on age, sex and self-reported ethnicity using nearest neighbor matching<sup>13</sup>  
111 and requiring an exact match on age as implemented by the MatchIt package version 3.0.2 in R.  
112 Univariate Fisher’s exact test and multivariate logistic regression tested the association between  
113 HIV status and CHIP prevalence. Multivariate models were adjusted for age, sex, self-reported  
114 ethnicity, and smoking status. Analyses were performed in R version 3.6. A threshold of  $p < 0.05$   
115 was considered statistically significant.

116 Written informed consent was obtained from all human participants by each of the  
117 studies with approval of study protocols by ethics committees at participating institutions.  
118 Secondary analysis of the data in this manuscript was approved by the Mass General Brigham  
119 Institutional Review Board. All relevant ethics committees approved this study and this work is  
120 compliant with all relevant ethical regulations.

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## Results

We began by considering the fraction of CHIP across the entire SHCS PLWH cohort (N=600) and ARIC cohort (N=8111) (Figure 1). SHCS PLWH and ARIC participants had mean (SD) age 44 (11) and 57 (6) years ( $p=1.8 \times 10^{-167}$ ), were 25% and 56% female ( $p=1.9 \times 10^{-46}$ ), and were 95% and 74% of European ancestry ( $p=5.2 \times 10^{-36}$ ) respectively. With adjustment for age, age<sup>2</sup>, sex and ethnicity, we observed a significant association between HIV case status and CHIP (OR: 1.77, 95% CI: 1.33-2.21,  $p=0.02$ ).

Given the overall demographic imbalances, we pursued a propensity matching strategy and matched datasets by age, gender and ethnicity. Propensity matching analyses yielded a set of 230 PLWH cases and 1002 ARIC population controls. Neither age nor sex, differed significantly between the matched cohorts (Table 1) and the standardized mean difference across age, sex and self-reported ethnicity were all less than 0.1 indicative of adequate matching. In this subset, CHIP was detected in 7% of exomes from PLWH, but only 3% of the controls (Table 1, univariate  $p=0.005$ ; multivariate  $p=0.004$ ). Of note, the statistical association strengthened despite a significantly decreased sample size, likely due to the exclusion of younger SHCS PLWH, who are less likely to have CHIP. Depth of coverage of the four most common CHIP genes (*DNMT3A*, *TET2*, *ASXL1*, *JAK2*), when incorporated into the multivariate logistic regression model, did not affect the results.

The limited sample size precluded inference on the association of HIV status with specific CHIP driver genes, however we observed differences in the genes most likely to carry CHIP mutations between PLWH and population controls. The most common CHIP gene in the SHCS was *ASXL1* (13 out of 27 CHIP mutations, 48%) followed by *TET2* (8 out of 27 CHIP mutations, 30%) and *DNMT3A* (5 out of 27 CHIP mutations, 19%). Overall this distribution was inverted from the control cohort where CHIP mutations were more frequent in *DNMT3A*, followed by *TET2* and *ASXL1*. In total, 22 PLWH had a single CHIP mutation, while one individual had 2 mutations and one individual had 3 mutations.

Within the full PLWH cohort (N=600) we considered additional phenotypes, which might be a cause or consequence of CHIP. First, we observed a trend toward an increase in CAD among CHIP carriers (Fisher's exact test OR: 2.99,  $p = 0.068$ ). Second, we observed that duration of antiretroviral therapy (ART) was twice as long in CHIP carriers versus non-carriers (ART mean (st. dev.) ART 2675 [1850] days vs 1322 [1454] days in carriers vs non-carriers respectively ;  $p = 0.0004$ , Mann-Whitney U test). This association was directionally concordant after adjusting for patient age in multiple logistic regression ( $p = 0.066$ ) or and remained significant after matching of 24 CHIP carriers with 24 non-carriers by age ( $p = 0.042$  paired Mann-Whitney U test). It is important to note that although ART duration positively correlates with the total duration of HIV infection (Spearman's  $\rho = 0.58$ ,  $p=2.0 \times 10^{-54}$ , N = 600), the total duration of HIV infection is not associated with CHIP  $p = 0.452$ ; paired Mann-Whitney U test on matched CHIP carriers and non-carriers,  $p = 0.22$ ]

## Discussion

We here report that HIV associates with increased prevalence of CHIP, a recently recognized risk factor for blood cancer and CAD. In the present samples, we identify at least 2-

166 fold enrichment of CHIP among PLWH versus controls when considering known factors  
167 predisposing to CHIP.

168 HIV infection is linked to accelerated biologic aging and chronic low-grade  
169 inflammation, providing a fertile substrate for CHIP development. Our study is consistent with  
170 another recent study that showed that HIV leads to a greater risk of myelodysplastic syndrome  
171 (MDS), a downstream consequence of CHIP and precursor to myeloid malignancy.<sup>14</sup>  
172 Furthermore, similar to the gene distribution in MDS, we find a greater relative prevalence of  
173 *ASXL1* mutations among PLWH compared to controls. Of note, while cigarette smoking selects  
174 for *ASXL1* clonal hematopoiesis<sup>15</sup>, our cohort of PLWH still had an increased prevalence of  
175 *ASXL1* mutations compared to the control cohort despite being well balanced for smoking status  
176 across cohorts.

177 HIV infection may promote CHIP development through various mechanisms, including  
178 induced immunodeficiency, chronic immune activation from antigenic stimulation, as well as  
179 increased prevalence of tobacco smoking and other co-morbid conditions. HIV may induce  
180 CHIP also through the ART which can either increase the rate of somatic mutagenesis or change  
181 the fitness landscape of hematopoietic stem cells or decrease effective population size of blood  
182 cells. HIV may also modify the selective coefficients of specific CHIP mutations. A recent  
183 model proposed that many of the CHIP mutations increase cell fitness, ensuring their  
184 proliferation with age.<sup>16</sup> The relative contribution of these factors to CHIP risk, including in the  
185 context of spontaneous viral control and antiretroviral therapy, will require larger studies. An  
186 important limitation of the present study is its cross-sectional nature, but CHIP is highly unlikely  
187 to be a risk factor for HIV acquisition. The relative contribution of these factors to CHIP risk,  
188 including in the context of spontaneous viral control and antiretroviral therapy, will require  
189 larger studies. An important limitation of the present study is its cross-sectional nature, but CHIP  
190 is highly unlikely to be a risk factor for HIV acquisition.

191 We propose that CHIP may be one mechanism that elevates risk for CAD in PLWH.  
192 Further studies are required to evaluate the hypothesis that CHIP contributes to the excess  
193 cardiovascular risk associated with long-term HIV infection. CHIP may represent a unique  
194 opportunity for precision identification and targeting of CAD risk with particular relevance for  
195 HIV medicine.

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### 198 **Data Availability**

199 CHIP genetic variant callsets and associated participant level phenotype data used in this study  
200 are available to qualified investigators by application to the SHCS and ARIC.

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### 202 **Acknowledgments**

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204 SHCS data are gathered by the Five Swiss University Hospitals, two Cantonal Hospitals, 15  
205 affiliated hospitals and 36 private physicians (listed in <http://www.shcs.ch/180-health-care-providers>). The authors acknowledge the effort and commitment of SHCS participants,  
206 investigators, study nurses, laboratory personnel, and administrative assistance by the SHCS  
207 coordination and data center.  
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209

### 210 **Financial support**

211 This study has been financed within the framework of the Swiss HIV Cohort Study, supported by



212 the Swiss National Science Foundation (grant #177499), by SHCS project #860 and by the  
213 SHCS research foundation. This work was also supported by the Swiss National Science  
214 Foundation grant #175603 to JF. AGB is supported by a Burroughs Wellcome Foundation career  
215 award for medical scientists and a grant from the National Institute of Health Common Fund  
216 (DP5 OD029586). SG is supported by P30 DK040561 and U01HL123336. PN is supported by a  
217 Hassenfeld Scholar Award from the Massachusetts General Hospital, and grants from the  
218 National Heart, Lung, and Blood Institute (R01HL1427, R01HL148565, R01HL148050, and  
219 R01HL151283) and Fondation Leducq (TNE-18CVD04). Dr. Libby receives funding support  
220 from the National Heart, Lung, and Blood Institute (1R01HL134892), the American Heart  
221 Association (18CSA34080399), the RRM Charitable Fund, and the Simard Fund.

222  
223 The Atherosclerosis Risk in Communities study has been funded in whole or in part with Federal  
224 funds from the National Heart, Lung, and Blood Institute, National Institutes of Health,  
225 Department of Health and Human Services (contract numbers HHSN268201700001I,  
226 HHSN268201700002I, HHSN268201700003I, HHSN268201700004I and  
227 HHSN268201700005I). The authors thank the staff and participants of the ARIC study for their  
228 important contributions. Funding support for “Building on GWAS for NHLBI-diseases: the U.S.  
229 CHARGE consortium” was provided by the NIH through the American Recovery and  
230 Reinvestment Act of 2009 (ARRA) (5RC2HL102419). Sequencing was carried out at the Baylor  
231 College of Medicine Human Genome Sequencing Center (U54 HG003273 and R01HL086694).

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### 233 **Competing Interests**

234 Dr. Libby is an unpaid consultant to, or involved in clinical trials for Amgen, AstraZeneca, Baim  
235 Institute, Beren Therapeutics, Esperion, Therapeutics, Genentech, Kancera, Kowa  
236 Pharmaceuticals, Medimmune, Merck, Norvo Nordisk, Merck, Novartis, Pfizer, Sanofi-  
237 Regeneron. Dr. Libby is a member of scientific advisory board for Amgen, Corvidia  
238 Therapeutics, DalCor Pharmaceuticals, Kowa Pharmaceuticals, Olatec Therapeutics,  
239 Medimmune, Novartis, and XBiotech, Inc. Dr. Libby’s laboratory has received research funding  
240 in the last 2 years from Novartis. Dr. Libby is on the Board of Directors of XBiotech, Inc. Dr.  
241 Libby has a financial interest in Xbiotech, a company developing therapeutic human antibodies.  
242 Dr. Libby's interests were reviewed and are managed by Brigham and Women's Hospital and  
243 Partners HealthCare in accordance with their conflict of interest policies.

244 Dr Natarajan reported grants from Amgen during the conduct of the study and grants from  
245 Boston Scientific; grants and personal fees from Apple; personal fees from Novartis and  
246 Blackstone Life Sciences; and other support from Vertex outside the submitted work.

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258 **References**

259  
260 Zanni, M. v., Schouten, J., Grinspoon, S. K. & Reiss, P. Risk of coronary heart disease in  
261 patients with HIV infection. *Nature Reviews Cardiology* vol. 11 728–741 (2014).  
262 Jaiswal, S. *et al.* Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. *New*  
263 *England Journal of Medicine* **371**, 2488–2498 (2014).  
264 Genovese, G. *et al.* Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA  
265 Sequence. *New England Journal of Medicine* **371**, 2477–2487 (2014).  
266 Jaiswal, S. *et al.* Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease.  
267 *New England Journal of Medicine* **377**, 111–121 (2017).  
268 Bick, A. G. *et al.* Genetic Interleukin 6 Signaling Deficiency Attenuates Cardiovascular Risk in  
269 Clonal Hematopoiesis. *Circulation* **141**, 124–131 (2020).  
270 Fuster, J. J. *et al.* Clonal hematopoiesis associated with TET2 deficiency accelerates  
271 atherosclerosis development in mice. *Science* **355**, 842–847 (2017).  
272 Schoeni-Affolter, F. *et al.* Cohort profile: The Swiss HIV cohort study. *International Journal of*  
273 *Epidemiology* **39**, 1179–1189 (2010).  
274 McLaren, P. J. *et al.* Evaluating the impact of functional genetic variation on HIV-1 Control. in  
275 *Journal of Infectious Diseases* vol. 216 1063–1069 (Oxford University Press, 2017).  
276 Li, A. H. *et al.* Analysis of loss-of-function variants and 20 risk factor phenotypes in 8,554  
277 individuals identifies loci influencing chronic disease. *Nature Genetics* **47**, 640–642 (2015).  
278 INVESTIGATORS, T. A. THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC)  
279 STUDY: DESIGN AND OBJECTIVES. *American Journal of Epidemiology* **129**, 687–702  
280 (1989).  
281 Bick, A. G. *et al.* Inherited causes of clonal haematopoiesis in 97,691 whole genomes. *Nature*  
282 1–7 (2020) doi:10.1038/s41586-020-2819-2.  
283 Benjamin, D. *et al.* Calling Somatic SNVs and Indels with Mutect2. *bioRxiv* 861054 (2019)  
284 doi:10.1101/861054.  
285 Ho, D. E., Imai, K., King, G. & Stuart, E. A. Matching as nonparametric preprocessing for  
286 reducing model dependence in parametric causal inference. *Political Analysis* **15**, 199–236  
287 (2007).  
288 Kaner, J. D. *et al.* HIV portends a poor prognosis in myelodysplastic syndromes. *Leukemia and*  
289 *Lymphoma* **60**, 3529–3535 (2019).  
290 Dawoud, A. A. Z., Tapper, W. J. & Cross, N. C. P. Clonal myelopoiesis in the UK Biobank  
291 cohort: ASXL1 mutations are strongly associated with smoking. *Leukemia* **34**, 2660–2672  
292 (2020).  
293 Watson, C. J. *et al.* The evolutionary dynamics and fitness landscape of clonal hematopoiesis.  
294 *Science* **367**, 1449–1454 (2020).  
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298 **Table 1: Demographics and CHIP association in matched samples**

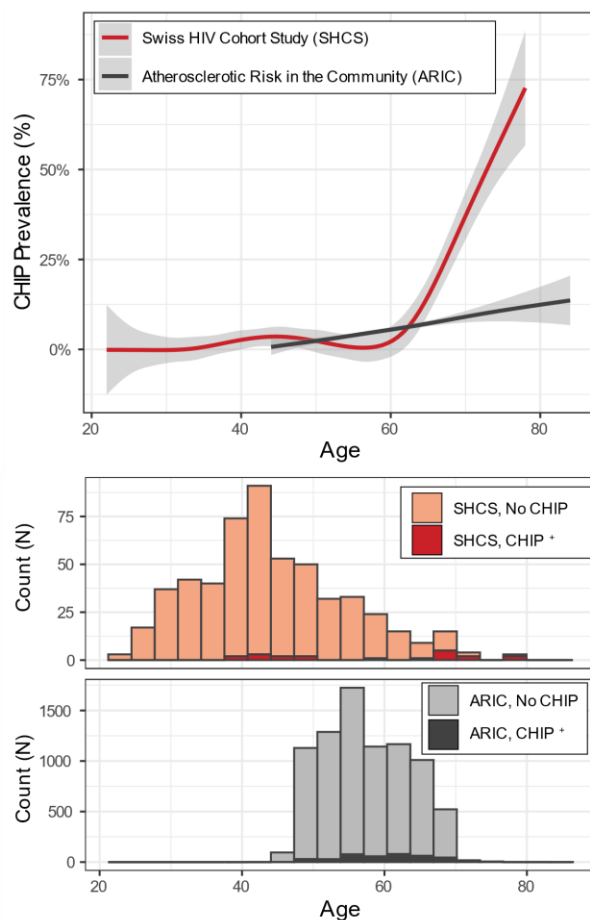
	<b>HIV+ Individuals (SHCS)</b>	<b>Population Controls (ARIC)</b>	<b>p-value</b>
n	230	1002	
Age at blood draw, mean (st. dev.)	54.2 (7.4)	55.0 (6.8)	0.12
Female, N (%)	44 (19%)	240 (24%)	0.086
Ever smoker, N (%)	143 (62%)	651 (65%)	0.408
Diabetes mellitus, N (%)	18 (8%)	80 (8%)	0.936
Black, N (%)	7 (3%)	80 (8%)	0.017
CHIP carrier, N (%)	16 (7%)	30 (3%)	0.005

299 P-value derived from Fisher's exact test for counts and t-test for continuous variables.

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**Figure 1: CHIP prevalence in Swiss HIV Cohort Study and Atherosclerotic Risk in the Community Study**

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Upper panel: fraction of cohort observed to have CHIP over time fit with a general additive model spline. 95% confidence interval displayed as shaded area. Lower panel: Count of number of individuals with and without CHIP binned by age of time of blood sampling across entire sequenced cohort.