CHIP is associated with Plasma Inflammatory Biomarkers and Vascular Events in People with HIV on ART

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BACKGROUND

- CHIP (clonal hematopoiesis of indeterminate potential mutations) are acquired somatic DNA mutations (e.g. in cell cycles genes *DNMT3A, TET2, ASXL1, etc.*) that are associated with increased risk of cancer, mortality, and vascular disease.[1]
- People with treated and suppressed HIV (PWH) have higher levels of inflammation and immune dysfunction compared to people without HIV (PWoH).[2]
- PWH have also been shown to higher frequencies of CHIP than PWoH,[3] but the underlying mechanisms linking CHIP and clinical events remains unclear among PWH or PWoH.
- We studied PWH on antiretroviral therapy (ART) to determine whether the presence of CHIP mutations was associated with (1) to incident vascular events, adding to the limited literature and (2) specific plasma biomarkers of inflammation and immune function.

METHODS

- Leveraging previously extracted DNA from 975 PWH on ART from the U.S Military HIV Natural History Study from a case-cohort study of PWH on ART, we performed targeted sequencing of 23 previously described CHIP mutations.
- As described previously, incident atherogenic vascular event (AVE) was defined as a composite outcome including new onset coronary artery disease, myocardial infarction, cerebrovascular accident, or peripheral artery disease.
- We also defined a broader composite outcome, vascular event (VE), which also included incident deep vein thrombosis and pulmonary embolism.
- Plasma cytokine levels were quantified using a custom multiplex assay, as previously described (MesoScale).
- We fit linear mixed effects models to estimate fold changes in plasma cytokine levels by the presence or absence of CHIP mutations and Cox proportional hazard models to estimate the risk of incident AVE or VE.
- Covariates for cardiovascular risk (e.g., age, sex, hyperlipidemia, hypertension, etc.), other demographics, and HIV progression (e.g., nadir CD4+ T cell count) factors were considered for inclusion.

- CHIP mutations have been associated with increased risk of cancer, vascular disease, and mortality.
- Among 975 PWH on ART, ~6% had CHIP mutations; most were in known CHIP-associated genes (e.g., *DNMT3A, TET2, ASXL1*).
- CHIP mutations were associated with incident vascular events, but this was largely seen in the middle tertile age group.
- Individuals with CHIP mutations had higher levels of several proinflammatory cytokines (IL-6, IP-10, MCP-3, MIP-3a).

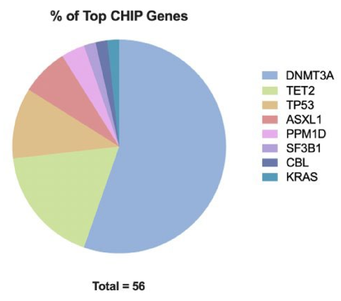
RESULTS

Table 1: Descriptive Statistics of PWH on ART from the U.S. Military HIV Natural History Study (N=975)

	PWH with CHIP (N=56)	PWH without CHIP (N=919)	Total (N=975)
Age at sample (years)	51.6 (41.7 – 59.2)	38.8 (31.8 – 47.0)	39.3 (32.1-47.9)
Race/ethnicity (self-reported)			
Caucasian	29 (52.8%)	307 (41.0%)	392 (43.2%)
African-American	25 (44.6%)	325 (43.4%)	378 (41.7%)
Hispanic/Puerto Rican/Mexican	1 (1.79%)	76 (10.1%)	92 (10.1%)
Other	1 (1.79%)	41 (5.4%)	45 (5%)
Sex at birth (% male)	51 (91.1%)	842 (91.6%)	893 (91.6%)
Cardiovascular risk factors			
Type II Diabetes	18 (32.1%)	152 (16.6%)	170 (17.5%)
Hyperlipidemia	48 (85.7%)	603 (65.7%)	651 (66.8%)
Hypertension	36 (64.3%)	427 (46.5%)	463 (447.5%)
HIV clinical parameters			
Nadir CD4+ T cell count (cells/mm³)	232 (161-301)	290 (201-388)	290 (195-382)

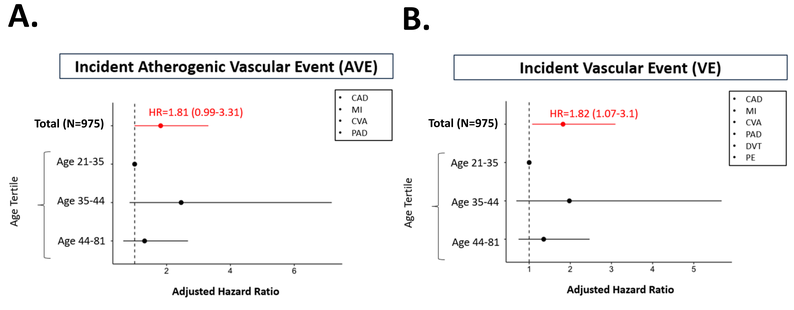
Medians with interquartile ranges or frequencies with percentages are shown.

Figure 1: Top CHIP mutations were distributed similarly to previously published work



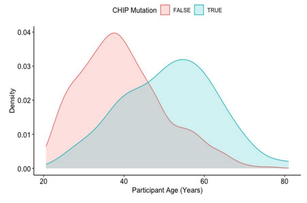
CHIP genes with mutations were identified among N=56 PWH. Most of the CHIP mutations were in DNMT3A and TET2. The majority of PWH with CHIP had only one CHIP mutation (N = 3 had more than one CHIP mutation).

Figure 2: CHIP mutations were associated with increased risk of incident vascular events, but this was strongly influenced by the age distribution of our cohort (i.e., the strongest effect was in the middle age tertile).



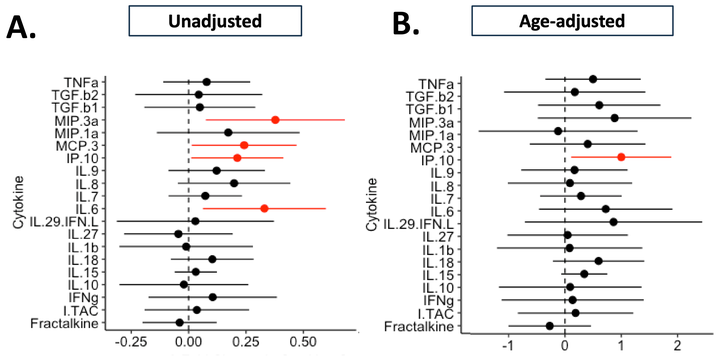
Adjusted hazard ratios for incident atherogenic vascular event (AVE) or vascular event (VE) among 975 PWH on ART, as well as by age tertiles. Potential covariates for traditional risk factors (gender, race/ethnicity, hypertension, hyperlipidemia, type II diabetes, smoking, alcohol use, family history) and HIV disease progression (nadir CD4+ T cell count, pre-ART viral load, timing of ART initiation, and duration of ART suppression) were tested for inclusion. Final adjusted HRs included terms for hypertension and hyperlipidemia at time of sample.

C.



Density plots of the age of participants with and without CHIP mutations. Both CHIP mutation prevalence and vascular event risk increase with age. There are increased competing risks among older participants.

Figure 3: CHIP mutations may influence plasma cytokine levels



Linear mixed effects models were fit to estimate fold-change differences in plasma cytokine levels between PWH on ART with and without CHIP mutations (A). The presence of CHIP mutations were associated with several cytokines (IL-6, IP-10, MCP-3, MIP-3a), of which IP-10 remained statistically significant after adjustment for age (B).

CONCLUSIONS

- These data are the first to identify potential immune pathways involved in CHIP-mediated pathogenesis, especially in PWH.
- For example, IP-10 is a critical proinflammatory factor responding to IFN-γ in HIV disease;[4] further studies are needed to determine whether there is a direct role of IP-10 on CHIP mutations.
- Our data also support prior work demonstrating that CHIP mutations are associated with incident VE (even in models adjusted for hyperlipidemia and hypertension).
- However, the lower incidence of CHIP and VE in younger participants, as well as the presence of competing risk bias in older participants (i.e., non-CHIP-associated VE), likely attenuated our overall findings.
- Further studies are needed to functionally validate the immune pathways identified in our CHIP analyses and whether these immune mechanisms may be unique to PWH.

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- R01A133031

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