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Inflammation-related genes are associated with epigenetic aging in HIV

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Abstract

Chronic inflammation is characteristic of both HIV and aging ("inflammaging") and may contribute to the accelerated aging observed in people living with HIV (PLWH). We examined whether three inflammation-related single-nucleotide polymorphisms (SNPs) were risk factors for accelerated aging and HIV-associated, non-AIDS (HANA) conditions among PLWH. We examined 155 postmortem cases with HIV (mean age = 47.3, 81% male, 68% self-reported White) from the National NeuroAIDS Tissue Consortium who had pre-mortem neurobehavioral/medical/virologic data and epigenomic data from occipital cortex tissue. Accelerated aging was measured according to the Epigenetic Clock; an aging biomarker based on DNA methylation levels. Past or current age-associated HANA conditions including cerebrovascular, liver and kidney disease, chronic obstructive pulmonary disease, cancer, and diabetes were determined via self-report. Epigenetic Aging *Z*-scores and likelihood of past/current HANA conditions were compared between major allele homozygotes and minor allele carriers for each SNP (IL-6 − 174G>C, IL-10 − 592C>A, TNF-α − 308 G>A) separately. Analyses were adjusted for relevant demographic/clinical factors. Epigenetic aging (e.g., higher *Z*-scores) was significantly greater in IL-6 C allele carriers ($p = .002$) and IL-10 CC homozygotes ($p = .02$) compared to other genotype groups. The likelihood of any past/current HANA condition did not differ by IL-10 genotype but was 3.36 times greater in IL-6 C allele carriers versus others (OR = 3.36, 95%CI = 1.09–10.34, $p = .03$). TNF-α genotype was not associated with epigenetic aging or HANA conditions. IL-6 and IL-10 SNPs may help to identify PLWH who are at high risk for accelerated aging. These insights into pathophysiological pathways may inform interventional approaches to treat rapid aging among PLWH.

Keywords HIV · Inflammation · Aging · Single nucleotide polymorphisms · Interleukin · HANA conditions

Introduction

Due to advances in antiretroviral therapy (ART), the lifespan of persons living with HIV (PLWH) has increased. Approximately 50% of PLWH are greater than 50 years of age, and this proportion is predicted to rise to 70% in 2020 (Centers for Disease Control and Prevention 2017). Although a longer lifespan among PLWH is beneficial, an unfortunate

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consequence is that older PLWH are at risk for age-associated health outcomes including HIV-associated, non-AIDS (HANA) conditions such as kidney, liver, and cardiovascular disease, and frailty. Concurrently, the determinants of morbidity and mortality among PLWH in the post-ART era have shifted from AIDS-related opportunistic infections to aging-related, HANA conditions (Warriner et al. 2014; Deeks 2011). Furthermore, HANA conditions appear approximately 5 to 10 years earlier among PLWH compared to the general population (Deeks 2011; Pathai et al. 2014; Deeks and Phillips 2009; Durand et al. 2017) suggesting a "premature aging" phenomenon in PLWH.

At the molecular level, aging is linked to epigenetic alterations, subsequent genomic instability, chromatin remodeling, and accumulation of DNA mutations (Lagathu et al. 2017), which, in turn, leads to stimulation of the epigenomic maintenance system and its protective actions such as the regulation of gene expression through DNA methylation (Horvath 2013). Using an epigenetic prediction of age based on DNA methylation (DNAm) levels, Horvath and Levine (2015) found that PLWH demonstrated accelerated epigenetic aging of 7.4 years in brain tissue and 5.2 years in peripheral blood mononuclear cells compared to HIV-seronegative controls. Similarly, Gross and colleagues found that both chronic and recent HIV infection were associated with accelerated epigenetic aging of 4.9 years, and an increased mortality risk of 19% despite most participants being virally-suppressed (Gross et al. 2016). The mechanism by which HIV infection leads to accelerated epigenetic aging in HIV remains unclear; however, evidence suggests that HIV infection provokes damage and double-strand breaks in chromosomal DNA (Tachiwana et al. 2006) by activating Rad3-related or ataxia-telangiectasia–mutated proteins and by inducing phosphorylation of their downstream substrates (Lau et al. 2005; Zimmerman et al. 2004). Thus, the accelerated epigenetic aging in HIV likely reflects the protective actions (e.g., DNA methylation) taken by the epigenomic maintenance system in the face of this DNA damage (Horvath and Levine 2015). While an accelerated aging phenomenon seems evident among PLWH, it is unknown whether (1) certain HIV-related disease processes underlie or contribute to the accelerated aging or whether (2) certain genetic factors render some PLWH more at risk for accelerated aging and its clinical consequences.

Chronic, low-grade immune activation or inflammation is characteristic of HIV infection (Nixon and Landay 2010; Nasi et al. 2017; Vera et al. 2016), even in the post-ART era where viral suppression is common (Vera et al. 2016). Chronic, low-grade inflammation is also characteristic of aging and is implicated in the pathology of many age-associated conditions (Bruunsgaard et al. 2001; Harris et al. 1999). Thus, chronic inflammatory activity may represent a mechanism that underlies or contributes to the effect of HIV infection on epigenetic aging. Chronic inflammation stimulates the generation of reactive oxygen/nitrogen species (ROS/RNS) from inflammatory and epithelial cells (Ohshima et al. 2003) which can cause DNA damage (Kawanishi and Hiraku 2006; Ohnishi et al. 2013). Clinically, chronic inflammation and the associated molecular and cellular changes may increase susceptibility to comorbid conditions among PLWH. Concordantly, inflammation is associated with most age-related comorbidities and mortality in PLWH (Durand et al. 2017; Neuhaus et al. 2010; So-Armah et al. 2016; Hunt et al. 2016).

Pro- or anti-inflammatory cytokines such as interleukin (IL)-6, IL-10, and tumor necrosis factor-alpha (TNF-α) play a critical role in the inflammatory response. Whereas evidence suggests an anti-inflammatory role for IL-10 and a pro-inflammatory role for TNF-α, IL-6 has been attributed both pro- and anti-inflammatory functions. Levels of the IL-6, IL-10, and TNF-α cytokines are altered in both HIV-seronegative older adults and PLWH (Rezer et al. 2018). These cytokines are also closely linked with several chronic diseases including cardiovascular disease, diabetes, osteoporosis, physical disability, and cognitive impairment among older adults within the general population (Pradhan et al. 2001; Ershler et al. 1993; Fagiolo et al. 1993; Cohen et al. 1997; Ferrucci et al. 1999; Barbieri et al. 2003; Singh and Newman 2011), as well as longevity (Albani et al. 2011; Giovannini et al. 2011).

There are common single-nucleotide polymorphisms (SNP) in the promoter regions of the IL-6, IL-10, and TNF-α genes that influence transcription levels of these cytokines. The C allele of the IL-6 -174G>C SNP (rs1800796) leads to lower levels of IL-6 production (Jones et al. 2001; Fishman et al. 1998; Bonafe et al. 2001). The − 592C>A SNP (rs1800872) is one of three IL-10 promoter SNPs (rs1800872, rs1800896, and rs1800871) that are in linkage disequilibrium (LD), while the − 592A allele is associated with lower IL-10 levels (Turner et al. 1997). The A allele of the TNF-α − 308 G>A SNP (rs1800629) is associated with enhanced production of TNF-α (Kroeger et al. 1997).

All three SNPs have been associated with age-related diseases and/or longevity in the general population. The frequency of the G allele or GG genotype of the IL-6 − 174G>C SNP has been found to increase with age in community-based, aging populations suggesting a protective effect of the G allele on longevity (Christiansen et al. 2004; Hurme et al. 2005; Cederholm et al. 2007); however, conflicting results have been reported with (Bonafè et al. 2001). The IL-10 − 592A allele is associated with decreased prevalence of hypertension (Yu et al. 2012), acute coronary syndrome (Fragoso et al. 2011), gastric cancer (Sugimoto et al. 2007), hyperuricemia (Posadas-Sánchez et al. 2018), and small and dense low-density lipoproteins (Posadas-Sánchez et al. 2018) in general aging populations, although opposite effects of this SNP have been reported (Altun et al. 2005; Trompet et al. 2007). IL-10 − 592C>A is also in linkage disequilibrium (LD) with the IL-

10 -1082 SNP ($R^2 = 3.7$, $D' = 1.0$), which has been associated with longevity in samples of men from Japan, Italy, and Jordan (Khabour and Barnawi 2010; Lio et al. 2002; Okayama et al. 2005) and in a sample of both men and women from Bulgaria (Naumova et al. 2004). The TNF-α − 308A allele is associated with doubled three-year survival among older women (Cederholm et al. 2007) and is part of a three SNP haplotype associated with protection against Alzheimer's disease (Collins et al. 2000; Culpan et al. 2003). These findings suggest that the IL-6 − 174G, the IL-10 − 592A, and the TNF-α − 308A alleles are beneficial for longevity (Christiansen et al. 2004; Hurme et al. 2005; Cederholm et al. 2007) and/or protection against age-associated diseases. However, these effects may differ by sex (Cederholm et al. 2007) and have not been found consistently (Kayaaltı et al. 2011; Bonafè et al. 2001; Ross et al. 2003; Khabour and Barnawi 2010; Alvarez et al. 2002).

The evidence of associations among inflammation-related genes and aging in the general population warrants their investigation among clinical conditions characterized by chronic inflammation and accelerated aging. These investigations can provide important insights into biological mechanisms underlying accelerated aging in HIV and identify those at the highest risk who may benefit the most from interventions. Using the epigenetic clock established by Horvath (2013), we examined the association between each of the three aforementioned SNPs (i.e., IL-6 − 174G>C, IL-10 − 592C>A and TNF-α − 308 G>A) with accelerated aging, as well as prevalence rates of age-associated HANA conditions among postmortem cases with HIV. We hypothesized that alleles associated with mortality or age-associated conditions in the general population (i.e., -174 C, -592 C and -308 G) would be associated with accelerated aging and a higher prevalence of HANA conditions among postmortem cases with HIV.

Methods

Participants

Participants included 155 deceased cases with HIV from one of two sites within the National NeuroAIDS Tissue Consortium (NNTC, www.nntc.org; Morgello et al. 2001), the National Neurological AIDS Bank (NNAB), or California NeuroAIDS Tissue Network (CNTN). Inclusion criteria included existing pre-mortem neurobehavioral/medical/virologic data, post-mortem tissue samples and epigenomic data. As a result of a previous study examining genetic predictors of HIV-associated neurocognitive disorders (HAND), epigenomic data were generated in a subset of participants that had (1) no pre- or post-mortem evidence of non-HIV related neurological diseases (e.g., stroke, neoplasm, multiple sclerosis, traumatic brain injury, and neurodegenerative illness), (2) no history or evidence of toxoplasmosis or progressive multifocal leukoencephalopathy, (3) no diagnosis of substance dependence, and (4) completed a neuropsychological evaluation within a year before death. Exclusionary criteria included postmortem evidence of non-HIV-related neurological disease and diagnosed substance dependence within 1 year of death.

The past or current age-associated HANA conditions that we examined were cerebrovascular disease (e.g., cardiac disease, coronary artery disease, hyperlipidemia, myocardial infarction, stroke, hypertension), liver disease, kidney disease, chronic obstructive pulmonary disorder (COPD), cancer, and diabetes. These conditions were self-reported and were available in a subset of participants ($N = 104$). During in-life study visits, trained research personnel conduct a standardized neurological examination and a standardized interview to ascertain comprehensive medical history. Participants were dichotomized (yes vs. no) based on whether or not they reported any past or current HANA condition. This study was conducted in accordance with the Institutional Review Boards of the University of California, Los Angeles (UCLA) and the University of California, San Diego (UCSD).

DNA-based methods

SNP selection and genotyping

The three SNPs were selected for analyses because: (1) they are SNPs in inflammation-related genes that have been associated with longevity or age-associated diseases in the general population and (2) their genotype data was available. Brain samples from the occipital cortex were frozen and shipped from the NNAB and CNTN to the Biological Samples Processing Core at UCLA. DNA was extracted from these brain samples using the Autopure LS nucleic acid purification instrument. Samples were quantified using OD 260/280. DNA quality was checked using agarose gel electrophoresis. The extracted DNA was genotyped at the BC Children's Hospital Research Institute using the Agena BioSciences MassArray Platform (San Diego, CA). DNA sequences used to amplify and sequence amplicons containing each SNP of interest were designed by the Agena Bioscience's Assay Design Suite V2.0, and purchased from IDT (San Jose, CA). Primers were grouped based on their amplicon mass into three separate pools, which allow for simultaneous detection of several SNPs in each reaction. Multiplexing was done following the iPLEX genotyping protocol as per manufacturer's instructions in 384-well plates. Briefly, regions of interest containing each SNP were PCR-amplified, then treated with shrimp alkaline phosphatase to neutralize unincoporated dNTPs in the PCR reactions. Extension PCRs reading into each SNP loci

was carried out using the respective extension primers. The final PCR products were desalted with the Clean Resin ion exchange resin at room temperature for 20 min and centrifuged at 3200 ×*g* for 5 min. The resulting analytes were transferred onto SpectroCHIPs using the Nanodispenser RS1000 and analyzed with the Agena MassArray MALDI-TOF mass spectrometer. The generated genotyping spectra were imported into the TYPER software (Agena BioSciences, San Diego, CA) for automated allele calling. Where necessary, the status of each genotype call was further verified by manual inspection of each spectra. All clinical lab work was performed by CLIA-certified clinical labs.

DNA processing for methylation analysis

Methylation analysis was performed with the Illumina Infinium HumanMethylation450 BeadChip, which measures bisulfite-conversion-based, single-CpG resolution DNAm levels at 485577 CpG sites in the human genome. The standard protocol of Illumina methylation assays quantifies methylation levels by the β-value using the ratio of intensities between methylated (signal A) and unmethylated (signal B) alleles. Specifically, the β-value is calculated from the intensity of the methylated (M corresponding to signal A) and unmethylated (U corresponding to signal B) alleles, as the ratio of fluorescent signals $\beta = \mathrm{Max}(M,0)/[\mathrm{Max}(M,0) + \mathrm{Max}(U,0) + 100]$. Thus, β-values range from 0 (completely unmethylated) to 1 (completely methylated) (Dunning et al. 2008). Methylation procedures have been previously described in detail (Clifford et al. 2017).

Bioinformatics: the epigenetic clock

The epigenetic clock was calculated from DNA methylation levels of 353 CpGs (Horvath 2013). Predicted age (i.e., DNAm age) was associated with chronological age in certain cell types (i.e., CD4+ cells, monocytes, B cells, glial cells, neurons), tissues, and organs (i.e., whole blood, brain, breast, kidney, liver, lung, and saliva). Data generated using the Illumina 450 K was used in creating the epigenetic clock. Detailed information about the epigenetic clock have been previously described (Horvath 2013), and an online age calculator can be found at http://labs.genetics.ucla.edu/horvath/htdocs/dnamage/. The epigenetic clock software uses a data normalization step that is adapted from the Beta Mixture Quantile dilation normalization method (Teschendorff et al. 2013), in order to automatically compare each sample to a gold standard. Epigenetic age acceleration was defined using the residuals that resulted from regressing DNAm age on chronological age for each case. The residuals were then converted to standardized *Z*-scores that were used in statistical analyses.

Statistical analyses

Based on previous studies of IL-6, IL-10 and TNF-α alleles and health outcomes (Christiansen et al. 2004; Hurme et al. 2005; Posadas-Sánchez et al. 2018), and due to sample size considerations, analyses were conducted assuming a dominant genetic model that compared major allele homozygotes (IL-6 − 174 G/G, IL-10 − 592 C/C, TNF-α − 308 G/G genotype) to minor allele carriers (IL-6 − 174 C, IL-10 − 592 A, TNF-α − 308 A allele carriers). Differences in sample characteristics between IL-6, IL-10 and TNF-α genotype groups were examined using chi-square tests for categorical variables, *t* tests for normally distributed continuous variables, and Kruskal-Wallis one-way analyses of variance (ANOVA) for non-normally-distributed continuous variables as determined by the Shapiro-Wilk test (i.e., nadir CD4, premortem CD4, viral load levels, duration of disease). Separate one-way analyses of covariance (ANCOVAs) and the resulting F-statistic (denoted as *F* (degrees of freedom = *F*-statistic) were used to compare epigenetic aging *Z*-scores between genotype groups for each SNP and effect sizes were calculated using Cohen's *d*. Separate logistic regressions and the resulting odds ratios (OR) and 95% confidence intervals (CI) were used to compare the odds of any past/current HANA condition between genotype groups for each SNP. Major allele homozygotes served as the reference group.

Demographics (age, sex, education, self-reported race [White, Black, Asian, Other], and ethnicity [Hispanic versus non-Hispanic]), non-age-associated comorbid conditions (HIV-associated neurocognitive disorders [HAND], lifetime major depressive disorder (MDD), substance use disorders, hepatitis C virus positivity), and HIV disease characteristics (duration of disease, ART status, pre-mortem plasma CD4 and viral load levels and nadir CD4), study site and DNA methylation assay batch were considered as covariates. HAND classification was performed within 18 months of death according to Frascati criteria (Antinori et al. 2007). Data were missing in a subset of participants for the following covariates: lifetime diagnosis of MDD and substance abuse/dependence (missing in 29 participants), hepatitis C virus positivity (missing in 59 participants), and nadir CD4 level (missing in 45 participants). ANCOVA and regression analyses were adjusted for any covariate that either related to the outcome in univariate analyses or differed between genotype groups at $p \leq .10$. Any covariate that was not significant in the multivariable model at $p \leq .10$ was removed from the final model.

Statistical significance was defined as $p < .05$ (two-sided). Analyses were performed using SPSS (version 24.0, SPSS Inc., Chicago, Illinois). Because our analyses focused on three a priori-selected SNPs with specific hypotheses regarding the direction of effects for each SNP, we decided that a multiple comparison statistical correction was not necessary; however, we reported the change in results when a Bonferroni correction was applied.

Results

Sample characteristics

See Table 1 for genotype distributions, sample characteristics, and the aging-associated outcomes of interest. Genotype distributions were in Hardy-Weinberg equilibrium for the IL-10 SNP, but not for the IL-6 and TNF-α SNPs (see Supplementary Table 1) suggesting that genetic distributions in our sample differed from the general population possibly due to gene associations with HIV acquisition, selection bias, or other unknown factors. Others have previously reported an association between interleukin SNPs and HIV acquisition and disease progression (Pontillo et al. 2012; Naicker et al. 2009).

The sample was 78.1% male and 66.5% self-reported White with a mean age of 47.8 years [SD = 9.4] and a mean years of education of 12.6 [SD = 2.7]. Consistent with evidence of race- and ethnicity-based differences in the distribution of polymorphic alleles in the interleukin genes (Hoffmann et al. 2002; Rady et al. 2004), self-reported Hispanics were more prevalent among IL-6 C allele and IL-10 A allele carriers compared to IL-6 GG and IL-10 CC homozygotes, respectively (p values < .05). Additionally, compared to IL-10 CC homozygotes, more IL-10 A allele carriers were self-reported Blacks and fewer IL-10 A allele carriers were self-reported Whites (p values < .05). Because of race and/or ethnicity differences in the frequency of the IL-10 SNP and the sufficient number of IL-10 CC homozygotes and A allele carriers, we repeated analyses involving this SNP in race-specific and ethnicity-specific subgroups in order to minimize population stratification bias. TNF-α minor allele carriers had a lower prevalence of lifetime substance abuse/dependence compared to TNF-α major allele homozygotes (p = .02).

SNPs and epigenetic aging

Among potential covariates, greater epigenetic aging was associated in univariate analyses with HAND (vs. no HAND) diagnosis, male sex (p = .10), lifetime MDD (p = .09), and a longer duration of HIV infection (p = .04). Assay batch (p < .001) and study site (p = .002) were also associated with epigenetic aging. Because of their univariate association with the outcome at $p \leq .10$, these covariates were included in all initial models. Given that they significantly differed by genotype group, initial models with the IL-6 and IL-10 SNPs additionally adjusted for ethnicity, and initial models with the TNF-α SNP additionally adjusted for lifetime substance abuse/dependence. Due to their significance in the multivariable model ($p \leq .10$), covariates retained in final models included ethnicity, study site, assay batch, and HAND status for IL-6 analyses; assay batch and HAND status for IL-10 analyses; and assay batch, HAND status, and sex for TNF-α analyses. There were no missing data for the covariates included in the final models.

As hypothesized, epigenetic aging Z-scores were significantly higher in IL-6 C allele carriers versus IL-6 GG homozygotes ($F(1,150) = 9.66$, p = .002), and in IL-10 CC homozygotes versus IL-10 A allele carriers ($F(1,150) = 5.83$, p = .02; Fig. 1). In contrast, epigenetic aging Z-scores did not differ significantly by TNF-α genotype ($F(1,150) = 3.87$, p = .07). When applying a Bonferroni correction for three statistical comparisons, the IL-6, but not the IL-10, SNP remained significantly associated with epigenetic aging Z-scores (α level = .017). In race-stratified analyses with the IL-10 SNP, the difference in epigenetic aging Z-scores between genotype groups was no longer significant among self-reported Whites (p = .33; Table 2). Conversely, the higher epigenetic aging Z-scores in IL-10 CC homozygotes versus A allele carriers was a statistical trend among self-reported Blacks (p = .07). In ethnicity-stratified analyses with the IL-10 SNP, IL-10 CC homozygotes showed a higher mean epigenetic aging Z –score compared to A allele carriers in both ethnicity groups; however, this difference was significant among Hispanics (p = .04) and not significant among self-reported non-Hispanics (p = .12).

SNPs and HANA conditions

Among potential covariates, presence of any HANA condition was associated with older age at death (p < .001), hepatitis C infection (p = .01), lower pre-mortem CD4 cell count (p = .002), lower nadir CD4 cell count (p = .006), and higher pre-mortem log plasma viral load (p < .001) in univariate analyses. Study site was also associated with presence of any HANA condition (p = .006). Because of their univariate association with the outcome at $p \leq .10$, these covariates were adjusted for in all initial models. Given that they significantly differed by genotype group, initial models with the IL-6 and IL-10 SNPs additionally adjusted for ethnicity and initial models with the TNF-α SNP additionally adjusted for lifetime substance abuse/dependence. Due to their significance ($p \leq .10$) in the multivariable models, covariates retained in final models included study site and age at death for all SNPs. There were no missing data for the covariates included in the final models.

The odds of any past/current HANA condition were over three times greater in IL-6 C allele carriers compared to IL-6 GG homozygotes (OR = 3.36, 95%CI = 1.09–10.34, p = .03; Table 2), although this association was no longer significant when applying a Bonferroni correction (α level = .02). When examining specific HANA conditions, this association appeared to be driven by renal disease such that past/current renal disease was over three times more likely in IL-6 C allele carriers versus IL-6 GG homozygotes (OR = 3.53, 95%CI = 1.05–11.8, p = .04; see Supplementary Table 2). Although associations between other HANA conditions and the IL-6 SNP

Table 1 Sample characteristics by IL-6, IL-10 and TNF-α genotype

Variables	IL-6 G/G allele homo-zygotes ($n = 123$)	IL-6 C/G or C/C genotypes ($n = 32$)	p value	IL-10 C/C homo-zygotes ($n = 79$)	IL-10 A/C or A/A genotypes ($n = 76$)	p value	TNF-α G/G homo-zygotes ($n = 125$[a])	TNF-α A/G and A/A genotypes ($n = 28$[a])	p value
Demographic/clinical variables									
Minor allele homozygotes, %	NA	15.6	NA	NA	19.7	NA	NA	14.3	NA
Age at death, *M* (SD)	48.0 (9.6)	46.8 (8.6)	.54	47.8 (8.7)	47.8 (10.1)	.99	48.2 (8.9)	46.6 (11.5)	.41
Male gender, %	76.4	84.4	.33	83.5	72.3	.09	75.2	89.3	.11
Self-reported race									
White, %	67.5	62.5	.59	77.2	55.3	.004	64.	71.4	.50
Black, %	26.8	28.1	.88	19.0	35.5	.02	28.8	21.4	.43
Self-reported Hispanic, %	17.1	46.9	<.001	16.5	30.3	.04	22.4	25.0	.76
Education, *M* (SD)	12.8 (2.7)	12.0 (2.9)	.13	12.9 (2.7)	12.3 (2.7)	.19	12.7 (2.6)	12.3 (3.0)	.48
Lifetime diagnosis of MDD[b]	40.4	37.0	.75	37.5	41.9	.61	42.0	25.0	.13
Lifetime diagnosis of substance abuse/dependence[b]	47.0	53.8	.53	56.1	40.0	.07	53.5	28.0	.02
Hepatitis C virus[c], %	36.5	40.9	.71	35.4	39.6	.67	35.4	39.6	.97
HIV disease characteristics									
HAND, %	77.2	75.0	.79	79.7	73.7	.37	75.2	82.1	.43
Nadir CD4 count[d] (cells/μl), *M* (SD)	59.8 (71.2)	41.6 (64.3)	.21	59.6 (71.3)	51.9 (68.9)	.48	59.3 (74.2)	42.7 (46.1)	.74
Pre-mortem CD4 count (cells/μl), *M* (SD)	131.7 (178.5)	104.8 (134.9)	.72	101.7 (138.8)	151.0 (195.2)	.08	120.7 (151.0)	155.8 (241.1)	.94
Pre-mortem log plasma viral load, *M* (SD)	3.8 (1.5)	3.7 (1.6)	.68	4.0 (1.6)	3.6 (1.5)	.17	3.5 (1.6)	3.8 (1.5)	.22
Duration of HIV disease (years), *M* (SD)	12.2 (6.7)	10.7 (5.2)	.27	12.3 (5.6)	11.5 (7.2)	.28	11.9 (6.6)	12.0 (5.7)	.84
Pre-mortem ART status, % prescribed	82.9	84.4	.84	82.3	85.5	.45	84.0	78.6	.49
Aging-associated outcomes									
Epigenetic aging z-score, *M* (SD)	− 0.05 0.07	0.20 (0.14)	.002 (0.28[e])	0.16 (0.09)	− 0.17 (0.09)	.02 (0.35[e])	0.05 (0.07)	− 0.22 (0.15)	.07 (0.35[e])
Past/current HANA condition, %	53.9	72.4	.03	60.7	57.1	.38	59.4	58.3	.91

[a] The sample size for the TNF-α genotype was reduced to $n = 153$ due to two missing TNF-α genotypes

[b] Lifetime diagnosis of MDD and lifetime diagnosis of substance abuse/dependence are missing in 29 participants

[c] Presence of hepatitis C virus positivity is missing in 59 participants

[d] Nadir CD4 level is missing in 45 participants

[e] Cohen's d is an effect size used to indicate the standardized difference between two means: 0.2 = small, 0.5 = medium, 0.8 = large. MDD = major depressive disorder.

Note: HAND = HIV-associated neurocognitive disorder. ART = antiretroviral therapy

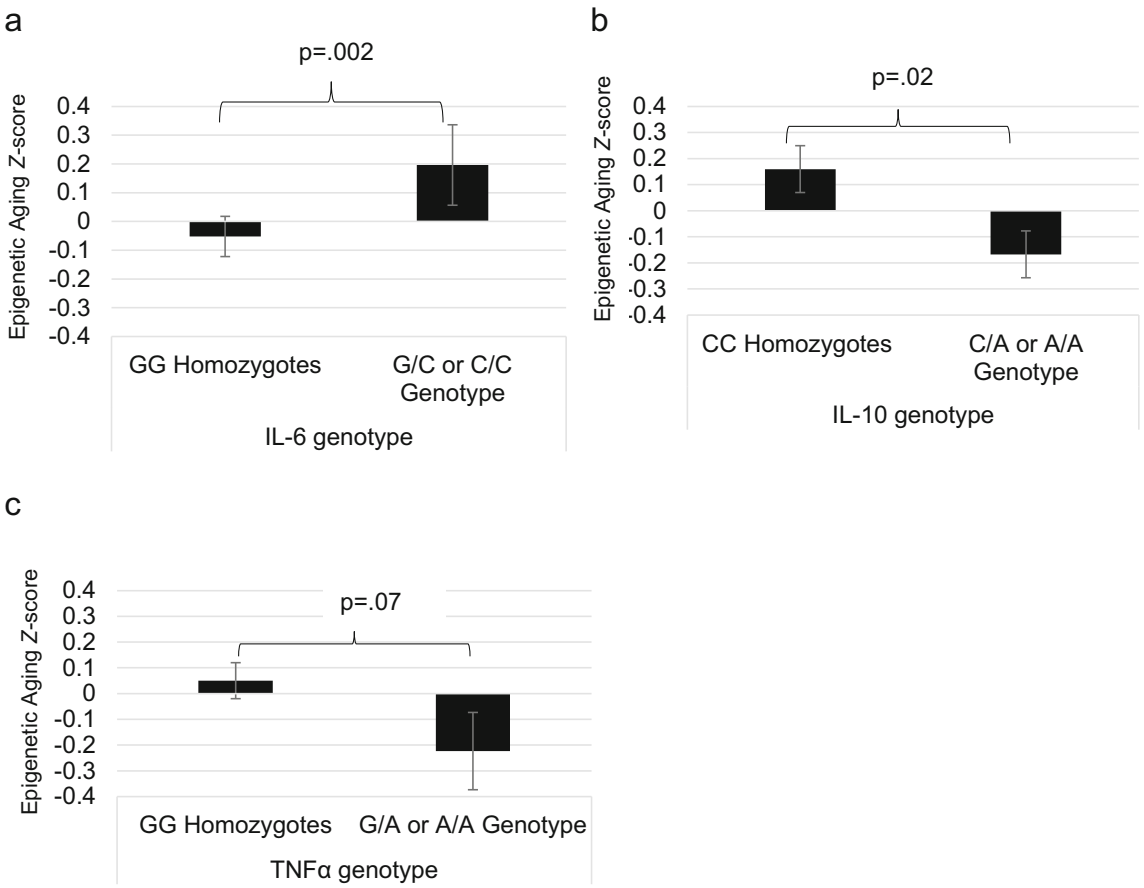

Fig. 1 Mean epigenetic aging Z-scores by genotype group for the IL-6 (a), IL-10 (b) and TNF-α (c) SNPs. Note. *p*-values represent mean differences between epigenetic aging *Z*-scores in ANCOVAS that adjusted for relevant covariates

Table 2 Self-reported race- and ethnicity-stratified comparisons of epigenetic aging Z-scores and the prevalence of HANA conditions between IL-10 genotype groups

	IL-10 C/C Homozygotes	IL-10 C/A or A/A genotypes	F statistic from ANCOVA, *p* value
Self-reported Whites			
n (subset with HANA condition data)	61 (35)	42 (31)	–
Epigenetic aging Z-score, M (SD)	0.14 (0.83)	0.00 (0.86)	F(1,99) = 0.93, *p* = .34 (Cohen's *d* = 0.16)
% Past/current HANA condition	48.6	58.1	OR = 0.44, (95%CI = 0.13–1.48), *p* = .19
Self-reported Blacks			
n (subset with HANA condition data)	15 (10)	27 (19)	–
Epigenetic aging Z-score, M (SD)	0.21 (1.02)	− 0.22 (1.06)	F(1,38) = 3.45, p = .07 (Cohen's *d* = 0.41)
Self-reported non-Hispanics			
n (subset with HANA condition data)	66 (39)	53 (39)	–
Epigenetic aging Z-score, M (SD)	0.13 (0.85)	− 0.12 (1.01)	F(1,115) = 2.46, p = .12 (Cohen's *d* = 0.27)
% Past/current HANA condition	53.8	59.0	OR = 0.74, (95%CI = 0.26–2.07), *p* = .56
Self-reported Hispanics			
n (subset with HANA condition data)	13 (9)	23 (17)	–
Epigenetic aging Z-score, M (SD)	0.31 (0.96)	− 0.28 (0.90)	F(1,32) = 4.73, p = .04 (Cohen's *d* = 0.63)

Note. The size of IL-10 genotype groups among self-reported Blacks and Hispanics with HANA data lacked sufficient statistical power to examine differences in prevalence rates of past/current HANA conditions according to Peduzzi 1996. Cohen's d is an effect size used to indicate the standardized difference between two means: 0.2 = small, 0.5 = medium, 0.8 = large. ANCOVA = analysis of covariance. M = mean, SD = standard deviation, OR = odds ratio, CI = confidence interval

were not significant, they were in the same direction and likely contributed to the association of IL-6 with the overall HANA outcome. The odds of past/current HANA conditions did not significantly differ by IL-10 or TNF-α genotype groups (IL-10 OR = 0.67, 95%CI = 0.27–1.65, $p = .38$; TNFα: OR = 0.94, 95%CI = 0.30–2.88; $p = .91$). As in the overall sample, there was no difference in the prevalence of HANA conditions between IL-10 genotype groups among self-reported Whites and among self-reported non-Hispanics. Given the small number of self-reported Black and Hispanic participants with data on IL-10 genotype and HANA conditions (≤ 10 within a genotype group), we lacked sufficient power to compare HANA conditions within these race and ethnicity groups (Peduzzi et al. 1996).

In order to assess for selection bias, we also examined whether mean epigenetic Z-scores significantly differed between those with ($n = 104$) versus without ($n = 51$) self-reported HANA data, but found no difference, $F(1,153) = 0.03$, $p = .86$.

Discussion

We examined the association between the IL-6 -174G>C, IL-10 -592C>A and TNF-α -308G>A SNPs and accelerated aging, as measured by the epigenetic clock, and HANA conditions among PLWH. We found that the IL-6 C allele and the IL-10 CC homozygous genotype were associated with accelerated aging among PLWH; however, the association between the TNF-α SNP and accelerated aging did not reach significance ($p = .07$). These significant associations were in the hypothesized direction in that the IL-6 C allele and the IL-10 CC homozygous genotype were previously linked with mortality and age-associated conditions in older adults of the general population (Christiansen et al. 2004; Hurme et al. 2005; Cederholm et al. 2007; Yu et al. 2012; Fragoso et al. 2011; Sugimoto et al. 2007; Posadas-Sánchez et al. 2018; Posadas-Sánchez et al. 2018; Okayama et al. 2005; Naumova et al. 2004); although some findings have been inconsistent and illustrated sex-dependent associations (e.g., IL-6: Albani et al. 2011; Bonafè et al. 2001; IL-10: Khabour and Barnawi 2010; Lio et al. 2002; Ross et al. 2003; TNF-α: Khabour and Barnawi 2010). Our findings help to elucidate the possible biological underpinnings for accelerated aging in HIV and to identify which PLWH may be particularly at risk of accelerated aging and HANA conditions.

Notably, only the IL-6 SNP and accelerated aging association remained significant after applying a Bonferroni correction for multiple comparisons. The IL-6 C allele was also associated with over three times greater likelihood of any HANA condition compared to the IL-6 GG homozygous genotype, with renal disease demonstrating the strongest association. This suggests that renal dysfunction and associated vascular pathology may be particularly vulnerable to inflammatory mechanisms in HIV. In a previous demonstration of the clinical significance of renal disease in HIV, glomerular filtration rate (eGFR), a measure of renal function, was the strongest predictor of neurocognitive decline over a three-year period among 191 virally-suppressed PLWH (Yuen et al. 2017).

The IL-6 SNP may have the strongest association with accelerated aging given that associations were seen at the clinical level (increased likelihood of HANA conditions), in addition to the molecular level. As previously mentioned, IL-6 has both pro- and anti-inflammatory properties and, therefore, may represent a factor that strives for balance between pro- and anti-inflammatory mechanisms (Jones et al. 2001; Jones 2005). In demonstration of this dual role, IL-6 had been found to down-regulate expression of pro-inflammatory cytokines while concurrently stimulating expression of the pro-inflammatory soluble p55 TNF-α receptor (Xing et al. 1998; Schindler et al. 1990; Tilg et al. 1994). We found that the IL-6 C allele, which is associated with lower levels of IL-6, was associated with accelerated aging according to the epigenetic clock and with a higher likelihood of HANA conditions. Our findings may suggest that a multifunctional role in the inflammatory response may be more beneficial to inflammatory-based outcomes than an exclusive pro- or anti-inflammatory role. In line with this speculation, the inflammatory system is double-edged in that it protects the body from noxious stimuli and can repair damaged tissue; however, when the response becomes dysregulated and/or chronic, it can become detrimental and cause tissue damage. Thus, the IL-6 − 174 GG homozygous genotype and the associated higher levels of IL-6 may play a pivotal role in disease risk by coordinating and optimizing the balance between pro- and anti-inflammatory actions. Conversely, the IL-6 − 174C minor allele may lead to accelerated aging through a more dysregulated inflammatory response; however, molecular-based studies are needed to test this speculation.

The IL-10 cytokine is known to exert an inhibitory effect on the inflammatory response by downregulating synthesis of pro-inflammatory cytokines. The A allele of the − 592C>A SNP is associated with lower levels of the IL-10 anti-inflammatory cytokine (Turner et al. 1997), which may seem counter-intuitive given that this allele was associated with less accelerated aging. However, similar to IL-6, IL-10 has multifactorial effects. Studies indicate a protective role of IL-10 in the development of atherosclerotic plaques (Pinderski-Oslund et al. 1999; Mallat et al. 1999); yet, IL-10 expression in advanced atherosclerotic plaques is associated with three-fold reduction in inducible nitric oxide synthase (iNOS) levels, a key contributor to the inflammatory response (Mallat et al. 1999; Smith et al. 2001). Additionally, the C allele of the − 592C>A SNP is associated with higher prevalence of small and low-density lipoproteins (Posadas-Sánchez et al. 2018), which are considered more atherogenic. Consequently, IL-10

expression may increase as a defense mechanism in response to greater inflammation and, thus, higher IL-10 levels are a proxy for greater inflammation (Posadas-Sánchez et al. 2018).

Although the IL-10 SNP was associated with accelerated epigenetic aging, it was not associated with HANA conditions. It is possible that an association between the IL-10 SNP and age-associated clinical outcomes is less robust than that of the IL-6 SNP and, thus, would only be observed in those at a more advanced age than those who comprised the current sample. IL-10 may also be associated with age-associated conditions that were not assessed herein or may be associated with rapidly progressing conditions not captured in the last assessment before death.

Although the IL SNPs were associated with accelerated aging, the age at death did not differ between these genotype groups. Thus, accelerated aging may not equate to earlier death in PLWH although that remains to be determined in a larger sample. We suggest that accelerated aging is associated with increased rate of HANA conditions which, in turn, may lead to earlier death. Although we do not know the cause of death for the NNTC cases, we know that drug overdose, AIDS, and other non-HANA conditions were common causes in this cohort (for which year of death spans 1994 to 2014). The mean age at death of our sample was 10–30 years younger than that of the general population (Kochanek et al. 2017). Associations among inflammation-related SNPs, HANA conditions, and age at death may manifest more robustly as the lifespan of PLWH continues to approach that of the general population and more PLWH experience age-related comorbidities.

Although there was a difference in epigenetic aging scores between the TNF-α − 308G>A genotype groups, it was not significant. The lack of significance may be due to low statistical power given that TNF-α minor allele carriers were the smallest genotype group across SNPs ($n = 28$). We also found no association between the TNF-α SNP and the likelihood of HANA conditions. Although this finding was contrary to our hypotheses, others have reported no association between the TNF-α − 308G>A SNP and acute ischemic stroke (Tuttolomondo et al. 2012), coronary artery disease (Koch et al. 2001), acute myocardial infarction (Hermann et al. 1998), and COPD (Seifart et al. 2005) in HIV-seronegative samples. It is possible that the effect of the SNP varies among different population subgroups depending on gene-gene and gene-environment interactions. Unlike previous studies that have reported an association between the TNF-α − 308G>A SNP and aging-associated outcomes, our sample consisted of postmortem cases with HIV. Perhaps, in the context of HIV, other environmental or gene-environment factors may be more important in determining TNF-α levels, or other inflammatory markers may play a larger role in inflammaging. For instance, the effects of TNF-α levels on inflammaging may be more subtle than interleukin levels such that the effect is masked by the effect of HIV infection itself.

In race-stratified analyses, the higher epigenetic aging Z-scores in IL-10 CC homozygotes compared to IL-10 A allele carriers was not significant among self-reported Whites but was a statistical trend among self-reported Blacks with a small-medium effect size (Cohen's $d = 0.4$). In ethnicity-stratified analyses, the higher mean epigenetic aging Z-score in IL-10 CC homozygotes versus A allele carriers was not significant among self-reported non-Hispanics but was significant among self-reported Hispanics with a medium effect size (Cohen's $d = 0.6$). This suggests that despite the smaller subsets of self-reported Blacks and Hispanics, the association between the IL-10 SNP and accelerated aging in the overall sample may have been driven by these race and ethnicity groups. The reason behind the race/ethnicity differences in our findings is currently unclear but could be due, in part, to gene-gene or gene-environment interactions with race- or ethnicity-specific genetic background or cultural factors.

This study has limitations. Given the cross-sectional study design, these associations do not indicate causality and may be influenced by confounders that were either not measured, or had limited data within our sample (e.g., hepatitis C virus positivity, nadir CD4 level). We were also unable to verify self-reported HANA conditions with medical records. Although participants with history or evidence of toxoplasmosis or progressive multifocal leukoencephalopathy were excluded, not all cases of opportunistic infections were excluded. However, among the participants with data on opportunistic infections (missing in 14), these infections were rare (1 case of cryptococcal, 1 case of lymphomatous and 2 cases of HIV-associated aseptic meningitis) and the results were unchanged when excluding these cases. Our sample lacked HIV-uninfected controls given the difficulty to obtain brain samples from relatively healthy, young, deceased subjects with in-life characterization. Thus, it remains to be determined whether the IL-6 and IL-10 risk alleles have additive or synergistic effects with HIV infection on inflammaging. Our small sample size, particularly for genetic studies, may have restricted statistical power in comparisons and precluded examination of an allele dose effect. Nonetheless, significant associations were found with the IL-6 and IL-10 SNPs despite the small sample size signifying the robustness of these associations. Given some prior literature showing sex-specific relationships between inflammation-related SNPs and longevity, such as IL-6 (Albani et al. 2011; Bonafè et al. 2001) and IL-10 (Khabour and Barnawi 2010; Lio et al. 2002; Okayama et al. 2005), it would have been ideal to conduct sex-stratified analyses; yet, our male-dominant cohort (75%) precluded such analyses. The availability of genetic data also precluded us from examining haplotypes within the IL-6, IL-10 and TNF-α genes and other inflammation-related genes; however, Christiansen et al. (2004) examined the IL-6 haplotype (− 597G>A, − 572G>C, − 174G>C, − 373(A)n(T)m) and found that due to LD, the − 174G > C polymorphism is sufficiently informative. Due to budget restraints and NNTC regulations on amount

of requested tissue, we were only able to measure DNA methylation levels in brain tissue of the occipital lobe; however, methylation levels may vary by brain region. Finally, it would be ideal to have measures of circulating cytokines to bridge the gap between genotype and outcome, although many others have demonstrated the effects of these SNPs on cytokine levels.

To our knowledge, this study is the first to identify genotypes associated with accelerated aging and age-associated conditions among PLWH. Importantly, health span, or the presence or absence of major chronic diseases or cognitive or physical impairments, has not kept pace with increases in lifespan among PLWH, although it is critical for quality of life. Our results aid in determining which genes and biological pathways may benefit from more detailed analyses in subsequent studies to further characterize complex genetic relationships with important aging outcomes in PLWH. To that end, our results suggest that greater inflammation and, more specifically, alterations in the production/degradation of IL-6 and IL-10 cytokines may be culprits in accelerated aging in HIV. Consequently, genetic polymorphisms in the interleukin pathway may be used to identify PLWH at high risk for age-associated diseases, as well as early mortality, and/or to inform therapeutic strategies to improving health span in PLWH.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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