Clinical Infectious Diseases

MAJOR ARTICLE

Epigenetic Associations With Estimated Glomerular Filtration Rate Among Men With Human Immunodeficiency Virus Infection

Junyu Chen,[1] Yunfeng Huang,[1] Qin Hui,[1] Raina Mathur,[1] Marta Gwinn,[1] Kaku So-Armah,[2] Matthew S. Freiberg,[3] Amy C. Justice,[4,5] Ke Xu,[4,6] Vincent C. Marconi,[7,8,9] and Yan V. Sun[1,9,10]

[1]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, Georgia; [2]Boston University School of Medicine, Massachusetts; [3]Cardiovascular Medicine Division, Vanderbilt University School of Medicine and Tennessee Valley Healthcare System, Nashville; [4]Connecticut Veteran Health System, West Haven, [5]Yale University School of Medicine, New Haven, and [6]Department of Psychiatry, Yale School of Medicine, New Haven, Connecticut; and [7]Hubert Department of Global Health, Rollins School of Public Health, and [8]Division of Infectious Diseases, Emory University School of Medicine, Atlanta, [9]Atlanta Veterans Affairs Healthcare System, Decatur, and [10]Department of Biomedical Informatics, Emory University School of Medicine, Atlanta, Georgia

Background. People living with human immunodeficiency virus (HIV) infection have higher risk for chronic kidney disease (CKD), defined by a reduced estimated glomerular filtration rate (eGFR). Previous studies have implicated epigenetic changes related to CKD; however, the mechanism of HIV-related CKD has not been thoroughly investigated.

Methods. We conducted an epigenome-wide association study of eGFR among 567 HIV-positive and 117 HIV-negative male participants in the Veterans Aging Cohort Study to identify epigenetic signatures of kidney function.

Results. By surveying more than 400 000 cytosine guanine dinucleotide (CpG) sites measured from peripheral blood mononuclear cells, we identified 15 sites that were significantly associated with eGFR (false discovery rate Q value < 0.05) among HIV-positive participants. The most significant CpG sites, located at *MAD1L1*, *TSNARE1/BAI1*, and *LTV1*, were all negatively associated with eGFR (cg06329547, $P = 5.25 \times 10^{-9}$; cg23281907, $P = 1.37 \times 10^{-8}$; cg18368637, $P = 5.17 \times 10^{-8}$). We also replicated previously reported eGFR-associated CpG sites including cg17944885 ($P = 2.5 \times 10^{-5}$) located between *ZNF788* and *ZNF20* on chromosome 19 in the pooled population.

Conclusions. In this study we uncovered novel epigenetic associations with kidney function among people living with HIV and suggest potential epigenetic mechanisms linked with HIV-related CKD risk.

Keywords. VACS; EWAS; eGFR; renal function; HIV infection.

Human immunodeficiency virus (HIV) infection continues to be a major cause of morbidity and mortality around the world [1]. In 2016, approximately 36.7 million people worldwide were living with HIV, 1.8 million cases of incident HIV infection occurred, and 1.0 million people died from HIV-related diseases [2]. Although antiretroviral therapy (ART) is effective at suppressing viral replication and improves life expectancy, people with HIV (PWH) [2] are at increased risk for chronic inflammation, premature aging, and metabolic disorders [3] that are associated with renal impairment [4].

Chronic kidney disease (CKD), which emerged as a common complication of both HIV infection and its treatment, has been a critical cause of shortened life span in PWH [3]. The pathogenesis of HIV-related CKD is often multifactorial, linked to direct exposure to HIV viremia, superinfections, and the systemic immune response to infection [3, 5], as well as to traditional CKD risk factors such as increased body mass index (BMI), hypertension, diabetes, and cigarette smoking [6–9]. ART regimens may partially explain the higher burden of kidney dysfunction in PWH [10]. Apart from these observations, the underlying molecular mechanisms and pathophysiologic pathways of developing HIV-related CKD remain largely unknown. To further improve health outcomes for PWH, we need to better understand the molecular mechanisms that contribute to the progression and onset of HIV-related CKD [11] and identify useful biomarkers.

In addition to genetic factors, including common and rare genetic variants, epigenetic modifications have a role in disease susceptibility [12]. DNA methylation (DNAm), the epigenetic modification most studied at the population level, has been associated with kidney disease traits [13]. Previous epigenome-wide association studies (EWAS) identified numerous DNAm sites associated with estimated glomerular filtration rate (eGFR) [14–17], which is widely used to assess the filtration function of the kidneys and to diagnose CKD. However, the results were not consistent across studies, which included populations with

Received 9 December 2018; editorial decision 11 March 2019; accepted 19 March 2019; published online March 20, 2019.

Correspondence: Y. V. Sun, Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Road NE Room 3049, Atlanta, GA, 30322 (yvsun@emory.edu).

Clinical Infectious Diseases® 2020;70(4):667–73

DOI: 10.1093/cid/ciz240

CKD of varying causes and degrees of severity. Epigenetic associations with eGFR and CKD have not been investigated among PWH.

HIV infection has also been associated with epigenetic variants and DNAm. Several EWAS have identified and replicated DNAm sites associated with HIV infection and viremia [18]. A recent study also reported epigenetic associations with diabetes among PWH [19]. Epigenetic clock, an emerging aging biomarker based on age-related DNAm [20], has been linked with HIV infection and suggested accelerated epigenetic aging among PWH [21–23].

Characterizing DNAm patterns related to CKD caused by HIV infection and antiretroviral therapy may offer insights into its pathogenesis and the potential for prevention or treatment. We utilized the EWAS approach to identify differential methylation related to the risk for HIV-associated CKD. We examined previously identified eGFR-associated DNAm sites and discovered novel epigenetic associations with eGFR among PWH.

METHODS

Samples and Phenotypes

The phenotypic and epigenetic data were from the Veterans Aging Cohort Study (VACS), which is an observational, prospective study of veterans in care at the Department of Veterans Affairs medical centers across the United States [18]. VACS was approved by the human research protection program of Yale University and the institutional research board committee at the Connecticut veteran healthcare system, West Haven campus. All VACS participants provided written consent.

We included 567 HIV-positive and 117 HIV-negative male participants who had both phenotypic and epigenome-wide DNAm data available in the VACS. A questionnaire was completed by each participant at the baseline in order to collect clinical information, including the presence of chronic health conditions, information on cigarette smoking, use of medication, and antiretroviral treatment [23]. Total white blood cell counts and CD4 counts were enumerated at the time of peripheral whole blood sample collection [18, 23]. The eGFR was calculated from standardized creatinine levels, sex, race, and age using the CKD epidemiology collaboration equation [24]. All individuals with an eGFR less than 60 mL/min/1.73 m^2 are classified as having CKD regardless of other evidence of kidney damage.

Epigenomic Data Generation, Processing, and Quality Control

Genomic DNA was extracted from whole blood samples using PAXGene collection tubes (QIAGEN, Hilden, Germany) and FlexiGene DNA extraction kits (QIAGEN). The blood samples for epigenetic analysis and estimation of eGFR were obtained at the same time point. The Illumina Infinium HumanMethylation450 Beadchip (450K; Illumina, San Diego, CA), which targets more than 480 000 DNAm sites (ie, cytosine guanine dinucleotide [CpG]) in the human genome, was utilized for epigenome-wide profiling at the Yale Center for Genomic Analysis. All samples were randomly placed on each array and across arrays to reduce batch effects.

Quality control procedures were performed to exclude problematic samples and CpG sites from analysis. The following steps were taken: (1) intensity values with a detection P value ≥ .001 were set to missing for each CpG site, and 927 CpG sites missing in more than 5% of the samples (ie, site-level missing rate >5%) were removed; (2) 35 605 probes were removed because they were within 10 base pairs from a single-nucleotide polymorphism (SNP); (3) 24 749 CpG sites were removed as they mapped to multiple genomic locations; and (4) all samples passed a call rate >95%. Following Illumina's control probe scaling procedure, all raw intensity values were quantile normalized using *limma* package in R. Normalized intensity values were then used to generate a methylation score (β value = methylated allele intensity/(unmethylated allele intensity + methylated allele intensity + 100) for each CpG site, ranging from 0 representing unmethylation to 1 for complete methylation.

DNAm age was calculated using a web-based calculator (https://dnamage.genetics.ucla.edu/) with preselected age-related CpG sites and an algorithm developed by Horvath [25]. A t test was performed to assess the mean difference in DNAm age (ΔDNAm age = DNAm age – chronological age) between the HIV-positive and HIV-negative groups. Linear regressions were modeled to examine the association between DNAm age and chronological age, as well as eGFR.

Heterogeneous cell-type proportions across individuals is a well-established confounder in epigenetic epidemiological studies [26]. We calculated proportions of 6 cell types (CD4+ T cells, CD8+ T cells, natural killer T cells, B cells, monocytes, and granulocytes) in blood using an algorithm developed by Houseman et al [27] (R *minfi* package). The proportions of these 6 leukocyte subtypes were projected based on the top 100 cell-type–specific DNAm sites from a reference panel of known proportions. These estimated cell-type proportions were subsequently adjusted in the epigenetic association analyses.

Assessment and Adjustment of Potential Confounding Factors

We conducted multivariate linear regression to examine quantitative variables such as DNAm age, race, smoking status, diabetes, antihypertension drug usage, and 6 calculated cell types as predictors of eGFR, controlling for chronological age [28]. Variables with a P value < .05 were subsequently adjusted as covariates in the EWAS of eGFR. Previously established predictors of eGFR (eg, smoking status, diabetes) were controlled in the EWAS model regardless of their P values.

Principle Components Analysis

To adjust for population stratification, we performed a principal components (PC) analysis using an analytical approach developed by Barfield et al [29]. We created a pruned dataset that

kept only CpG sites within 50 base pairs of a SNP to approximate the genome-wide genetic variants. We calculated the PCs to measure population stratification using the *prcomp* function in R package *Factoextra*. Then, we included the top 10 PCs as covariates in the EWAS of eGFR.

Statistical Methods

For autosomal analysis among PWH, we used linear mixed regression models for the effect of methylation status at individual CpG sites on eGFR with random effect for chip. The final adjusted models for the EWAS included age, race, BMI, average systolic blood pressure (SBP), hepatitis C virus (HCV) infection status, smoking status (current smoking vs noncurrent smoking), diabetes status, antihypertensive medication usage, calculated cell-type proportions, and top 10 PCs. For comparison, we performed epigenetic association analyses among the HIV-positive and HIV-negative groups separately using the same covariates. Also, we estimated the interaction between HIV status and CpG sites by running the same regression model with an additional interaction term (HIV *β value) in the combined study population. To correct for multiple testing, we applied a false discovery rate (FDR) *Q* value of 0.05 and the conservative Bonferroni-corrected *P* value of .05 (nominal *P* value < approximately 10^{-7} given 480 000 CpG sites) as significance thresholds. We compared our findings with those from 2 previously reported populations without HIV infection [15, 17]. We also separately examined DNAm of 11 232 CpG sites on the X chromosome within the pooled sample using the statistic model as previously described [30].

RESULTS

After quality control, the analysis dataset included 412 583 unique CpG sites in 567 HIV-positive and 117 HIV-negative participants. Characteristics of these groups are summarized in Table 1. All participants were males, with an average age of 52.0 ± 8.0 years. More participants in the HIV-positive group self-reported black race than in the HIV-negative group (85% vs 62%). HIV-positive participants had a higher rate of HCV infection (58% vs 28%) but lower BMI (25.5 vs 30.7 kg/m^2) and

Table 1. Demographic and Clinical Characteristics of the Veterans Aging Cohort Study Participants

Characteristic	Total (N = 684)	HIV Positive (n = 567)	HIV Negative (n = 117)	*P* Value
Estimated glomerular filtration rate (mL/min/1.73 m^2)	98.4 ± 32.9	99.0 ± 34.0	95.6 ± 26.9	.243
Chronic kidney disease	56 (8%)	47 (8%)	9 (8%)	.964
Age (years)	52.0 ± 8.0	52.0 ± 7.8	53.2 ± 8.8	.169
Body mass index (kg/m^2)	26.4 ± 5.5	25.5 ± 4.7	30.7 ± 7.1	<.0001
Race/Ethnicity	…	…	…	<.0001
White	88 (13%)	58 (10%)	30 (26%)	…
Black	554 (81%)	481 (85%)	73 (62%)	…
Hispanic	24 (3.5%)	15 (3%)	9 (8%)	…
Other	18 (2.6%)	13 (2%)	5 (4%)	…
Smoking status	…	…	…	.028
None	142 (20%)	115 (20%)	27 (23%)	…
Current	376 (55%)	324 (57%)	52 (44%)	…
Past	166 (24%)	128 (23%)	38 (23%)	…
Antihypertensive medication	497 (73%)	402 (71%)	95 (81%)	.207
Hepatitis C virus	362 (53%)	329 (58%)	33 (28%)	<.001
Diabetes	156 (23%)	115 (20%)	41 (35%)	.001
Systolic BP	129.3 ± 14.5	129.3 ± 14.7	129.3 ± 13.1	.107
Diastolic BP	79.1 ± 9.5	79.4 ± 9.4	77.7 ± 10.3	.169
CD4	…	425 ± 278	…	…
Nadir CD4 counts	…	217 ± 7	…	…
Antiretroviral therapy[a]	…	472 (83%)	…	…
Nonnucleoside reverse transcriptase inhibitor–based regimen	…	205 (36.2%)	…	…
Protease inhibitor–based regimen	…	273 (48.1%)	…	…
Ritonavir	…	93 (16.4%)	…	…
Tenofovir	…	124 (21.9%)	…	…
HIV viral load (copies/mL)	…	75 (75–3400)[b]	…	…
Undetectable viral load (<200 copies/mL)	…	329 (58%)	…	…
DNA methylation age	53.1 ± 8.6	53.6 ± 8.6	51.1 ± 8.2	.003

Continuous variables were summarized as mean ± standard deviation, and categorical variables were summarized as count (%).

Abbreviations: BP, blood pressure; HIV, human immunodeficiency virus.

[a]Nonnucleoside reverse transcriptase inhibitors–based regimen includes efavirenz, nevirapine, rilpivirine, doravirine; protease inhibitor–based regimen includes indinavir, nelfinavir, lopinavir, fosamprenavir, tipranavir, darunavir, atazanavir.

[b]Median (interquartile range).

lower prevalence of diabetes than HIV-negative participants. The eGFR was, on average, 3.4 mL/min/1.73 m^2 higher in the HIV-positive group (Table 1 and Supplementary Figure 1), although this result was not statistically significant ($P = .24$).

Race, BMI, antihypertensive medication use, SBP, and cell-type proportions were significantly associated with eGFR (Table 2). Because of previous evidence of eGFR association and epigenetic modification, we also controlled for diabetes, current smoking, and HIV infection status as potential confounders in the EWAS of eGFR.

DNAm age was correlated with chronological age (r = 0.72, $P < 2.2 \times 10^{-6}$) (Table 1). DNAm age was, on average, 1 year older than chronological age among the pooled study population ($P = .0002$). The difference between chronological age and DNAm age (ie, ΔDNAm age) was significantly associated with HIV infection ($P = 8.87 \times 10^{-9}$), but the significance diminished after controlling for diabetes, current smoking, HCV infection, and cell-type proportions ($P = .34$). The ΔDNAm age was not significantly associated with eGFR before and after adjusting for all potential confounders in the pooled sample ($P = .17$ and .67).

Supplementary Figure 2a (Manhattan plot) and Table 3 present 15 CpG sites significantly associated with eGFR among PWH at the FDR <0.05 level after adjusting for covariates. A quantile–quantile (QQ) plot (Supplementary Figure 1b) comparing the observed *P* values to the expected *P* values for this EWAS indicates a low level of global inflation (inflation factor = 1.02); therefore, we did not further control for inflation. Manhattan plots and QQ plots of the EWAS among HIV-negative group and the pooled study population are shown in Supplementary Figure 2c–2f. Supplementary Figure 2d and 2f indicate a low level of global inflation for both the HIV-negative group and the whole study population (inflation factor = 1.00 and 1.03). The test statistics and annotations of 15 epigenome-wide significant CpG sites among PWH are summarized in Table 3. Thirteen of these 15 significant CpG sites were negatively associated with eGFR (ie, hypermethylation was associated with reduced renal function). However, the significance of these sites was absent among the HIV-negative group (Table 3). Thirteen of the 15 eGFR-associated CpG sites identified within the HIV-positive group were also statistically significant among the pooled samples (Table 3). At an alpha level of 0.05, only 2 of 13 CpG sites had significant interactions with HIV (cg06329547, $P = .04$; cg07796977, $P = .04$; cg07857040, $P = .03$).

Using a more stringent Bonferroni-corrected cutoff (corrected $P < .05$, nominal $P <$ approximately 10^{-7}), 3 CpG sites, cg06329547, cg23281907, and cg07796977, remained significant. These 3 CpG sites (Supplementary Figure 3a–3c) are located within genic regions of mitotic arrest deficient like 1 (*MAD1L1*), T-SNARE domain containing 1/ brain-specific angiogenesis inhibitor 1 (*TSNARE1/BAI1)*, and DLG associated protein 2 (*DLGAP2*), respectively. CpG site cg06329547 (*MAD1L1*) was hypermethylated among patients with lower eGFR, with a 1% increase in β value associated with a 2.61 unit decrease in eGFR (95% confidence interval [CI], −3.48, −1.74; $P = 5.25 \times 10^{-9}$). CpG site cg23281907, located between *TSNARE1* and *BAI1*, was inversely associated with eGFR, with a 1% increase in β value associated with a 2.29 unit decrease in eGFR (95% CI, −3.08, −1.49; $P = 1.37 \times 10^{-8}$). CpG site cg07796977 (*DLGAP2*) was also inversely associated with eGFR, with a 1% increase in β value associated with a 4.56 unit decrease in eGFR (95% CI, −6.51, −2.97; $P =$ of 3.12×10^{-8}). Such significant associations were absent in the HIV-negative group. The regional plots (Supplementary Figure 3a–3c) showed all tested CpG sites in the neighboring regions of these 3 sites and additional association signals with eGFR within these regions. There were 35 (6.2%), 14 (4.8%), and 13 (4.2%) CpG sites with *P* values < .05 in regions for cg06329547, cg23281907, and cg07796977, respectively. No statistically significant CpG sites were identified in the X chromosome.

We attempted to replicate eGFR-associated CpG sites identified in previous EWAS. Smyth et al [15] reported 23 genes with at least 1 CpG site significantly associated with eGFR. However, Chu et al [17] reported a lack of replication of Smyth's findings and 18 novel eGFR-associated sites in a much larger EWAS of eGFR, including 2264 participants from the Atherosclerosis Risk in Communities Study and 2595 participants from the Framingham Heart Study. Only

Table 2. Summary Statistics of Associations Between Covariates and Estimated Glomerular Filtration Rate Controlled for Age

Variable	Effect[a]	Standard Error	*P* Value
Age[b]	−0.50	0.16	<.001
Black race	13.4	3.71	<.001
Body mass index	−0.35	0.04	.129
Current smoking	2.3	3.11	.467
Antihypertensive drug use	−8.5	2.89	.003
HIV	2.2	3.40	.521
Hepatitis C virus	5.2	2.55	.043
Diabetes	−2.0	3.01	.508
CD4	<0.01	<0.01	.573
CD4 nadir	<0.01	<0.01	.615
HIV viral load	<0.01	<0.01	.851
Systolic blood pressure	−0.2	0.09	.035
Diastolic blood pressure	−0.1	0.13	.506
Cell-type proportion			
CD8T cell	48.2	15.77	.002
CD4T cell	19.0	19.79	.338
Natural killer cell	29.4	23.07	.203
B cell	110.6	25.71	<.001
Monocyte	−28.0	34.12	.413
Granulocyte	−41.2	9.98	<.001
Antiretroviral therapy	−0.9	2.74	.751
DNA methylation age	0.1	0.21	.649

Abbreviation: HIV, human immunodeficiency virus.

[a]Effect is the beta coefficient from the linear regression model.

[b]This regression model did not control for chronological age.

Table 3. Top-ranked Cytosine Guanine Dinucleotide Sites Exhibiting Differential DNA Methylation for Estimated Glomerular Filtration Rate

Cytosine Guanine Dinucleotide Sites	Chromosome	Position	Gene	HIV Positive			HIV Negative			Total			Interaction *P* Value
				β	SE	*P* Value	β	SE	*P* Value	β	SE	*P* Value	
cg06329547	7	1937739	*MAD1L1*	–260.6	44.4	8.28×10^{-9}	358.6	482.5	.46	–253.8	42.9	5.25×10^{-9}	.04
cg23281907	8	143509556	*TSNARE1/BAI1*	–228.5	40.6	3.1×10^{-8}	–133.3	148.9	.37	–219.9	38.2	1.37×10^{-8}	.19
cg07796977	8	962467	*DLGAP2*	–456.2	81.1	3.12×10^{-8}	488.6	319.7	.13	–414.7	77.4	1.20×10^{-7}	.04
cg17355919	5	1331538	*CLPTM1L*	–438.2	84.3	2.93×10^{-7}	–65.4	244.6	.79	–403.2	79.0	4.40×10^{-7}	.21
cg14088090	1	43738485	*TMEM125*	–408.0	78.8	3.3×10^{-7}	–72.8	75.3	.34	–211.7	55.8	1.62×10^{-4}	.18
cg10657250	4	156136570	*NPY2R*	–531.0	103.3	3.94×10^{-7}	389.4	250.6	.12	–459.8	95.0	1.62×10^{-6}	.10
cg08198773	8	1697536	*DLGAP2/CLNB*	–490.7	97.2	6.2×10^{-7}	–77.6	213.9	.72	–429.5	86.8	9.55×10^{-7}	.09
cg18368637	6	144179905	*LTV1*	–181.4	36.4	8.93×10^{-7}	–166.0	95.0	.09	–185.8	33.7	5.17×10^{-8}	.17
cg00296643	2	234371504	*DGKD*	–416.9	84.1	9.93×10^{-7}	219.0	373.7	.56	–378.5	79.5	2.39×10^{-6}	.28
cg03658275	6	129285274	*LAMA2*	–162.0	33.2	1.39×10^{-6}	–88.0	58.8	.14	–150.7	28.9	2.53×10^{-7}	.09
cg03012642	2	21346892	*APOB*	–439.0	90.0	1.44×10^{-6}	49.5	390.8	.90	–432.6	85.2	5.12×10^{-7}	.15
cg11796565	19	13205681	*NFIX*	89.8	18.5	1.52×10^{-6}	28.4	37.7	.45	79.9	16.6	1.74×10^{-6}	.07
cg17344906	19	13202507	*NFIX*	115.3	23.7	1.61×10^{-6}	46.9	49.0	.34	101.1	21.1	2.06×10^{-6}	.36
cg17461641	1	164023978	…	–322.7	66.6	1.68×10^{-6}	–232.8	124.4	.07	–309.3	59.0	2.19×10^{-7}	.03
cg07857040	16	1582219	*IFT140*	–349.4	72.3	1.77×10^{-6}	7.1	165.8	.97	–285.3	65.2	1.40×10^{-5}	.10

The epigenetic association model was adjusted for age, race, body mass index, average systolic blood pressure, HIV-infection, hepatitis C virus, smoking, diabetes, antihypertensive medication use, cell-types proportions, and top 10 principle components.

False discovery rate $Q < 0.05$. Interaction *P* value is the *P* value for the cytosine guanine dinucleotide × HIV status term in the multiple regression model.

Abbreviation: HIV, human immunodeficiency virus; SE, standard error.

1 site reported by Smyth et al, cg17500228 in gene exocyst complex component 3 (*EXOC3*), showed an association with $P < .05$ (Smyth, $P = 1.57 \times 10^{-14}$; Chu, $P = .0002$; VACS, $P = .0095$). We evaluated 17 CpG sites reported by Chu et al (1 CpG site was excluded after quality control) in our study of HIV-positive and HIV-negative participants. We replicated cg17944885 located between zinc finger family member 788 (*ZNF788*) and zinc finger protein 20 (*ZNF20*; $P = 2.5 \times 10^{-5}$) after multiple testing correction in the pooled population (Table 4). There were 5 additional sites with *P* values < .05

Table 4. Replication Analysis of Estimated Glomerular Filtration Rate–associated DNA Methylation Identified in Non–human Immunodeficiency Virus Samples

Cytosine Guanine Dinucleotide Sites	Chromosome	Position	Gene	Meta-analysis of ARIC and FHS	VACS HIV Positive		VACS HIV Negative		VACS Total	
				P Value	β	*P* Value	β	*P* Value	β	*P* Value
cg17944885	19	12225735	*ZNF788/ ZNF20*	1.228×10^{-23}	–169.00	5×10^{-4}	–165.10	.050	–176.9	2.5×10^{-5}
cg23597162	7	28102341	*JAZF1*	2.82×10^{-19}	–135.07	8×10^{-4}	–181.78	.004	–130.9	1×10^{-4}
cg04036920	11	33562503	*C11orf41*	2.387×10^{-15}	–38.29	.309	10.97	.894	–42.0	.211
cg00501876	3	39193251	*CSRNP1*	3.623×10^{-15}	13.92	.827	–240.05	.045	–20.7	.703
cg00994936	19	1423902	*DAZAP1*	3.259×10^{-11}	76.82	.131	10.07	.917	70.7	.112
cg16428517	16	3317428	*MEFV/ ZNF263*	1.166×10^{-10}	–193.11	.016	–248.22	.058	–224.2	.001
cg11950754	1	53782077	*LRP8*	2.006×10^{-10}	–23.88	.630	–20.63	.775	–30.1	.460
cg12065228	1	19652788	*PQLC2*	2.196×10^{-10}	33.58	.242	33.21	.602	33.9	.186
cg10750182	10	73497514	*C10orf105*	4.962×10^{-10}	–4.99	.943	–297.96	.056	–23.2	.706
cg09022230	7	5457225	*TNRC18*	5.222×10^{-10}	14.70	.744	28.57	.727	13.6	.727
cg19942083	12	7070562	*PTPN6/ PHB2*	7.174×10^{-10}	75.67	.017	45.80	.595	80.9	.005
cg06158227	15	43662311	*TUBGCP4*	8.482×10^{-10}	–230.16	.008	–3.75	.978	–196.4	.007
cg27660627	16	89461803	*ANKRD11*	9.939×10^{-10}	–175.70	.002	45.92	.618	–125.6	.010
cg12116137	17	1576449	*PRPF8*	5.277×10^{-9}	–8.32	.755	91.34	.028	5.8	.796
cg04460609	4	16532808	*LDB2*	7.854×10^{-9}	–27.98	.401	–64.95	.343	–31.5	.283
cg19497511	2	238609807	*LRRFIP1*	3.451×10^{-8}	–14.49	.772	16.16	.888	–2.7	.951
cg22515589	17	79426432	*BAHCC1*	6.437×10^{-8}	25.79	.748	43.31	.713	26.9	.682

Abbreviations: ARIC, Atherosclerosis Risk in Communities Study; FHS, Framingham Study; HIV, human immunodeficiency virus; VACS, Veterans Aging Cohort Study.

(cg23591762, cg16428517, cg19942083, cg06158227, and cg27660627). All 17 associations in our study showed directionality consistent with those reported by Chu et al [17].

DISCUSSION

HIV type 1 viremia and its corresponding treatment are known to be responsible for the development of CKD and decreased eGFR [4]. Previous reports demonstrated that HIV infection was associated with DNAm age [31]. We hypothesized that epigenetic aging may contribute to aging-related pathologies of CKD. DNAm age, a marker of biological aging, was significantly older than the chronological age ($P = .0002$). However, the difference between DNAm age and chronological age was not associated with either HIV or eGFR after adjustment for covariates, suggesting that epigenetic aging might not be an independent path between HIV and eGFR.

For the EWAS of eGFR among PWH, we identified 15 significant CpG sites, including 3 significant sites (located in or close to *MAD1L1*, *TSNARE1*/*BAI1*, and *DLGAP2*) that passed Bonferroni correction. *MAD1L1* functions as a homodimer and codes mitotic-arrest deficient 1 (MAD1) protein, which inhibits maturation and expansion of T-lymphocytes, and is targeted by human T-cell leukemia virus type 1 during virus transformation [32]. Defects in *MAD1L1* are involved in the development and/or progression of various types of cancer including kidney carcinomas [33]. Reduction of MAD1 may play a role in modulating specific immune responses to HIV, which might be involved in the mechanisms of HIV-associated immune-mediated kidney diseases. Interestingly, both *MAD1L1* and *TSNARE1* were estimated to be associated with susceptibility to schizophrenia and bipolar disorder [34, 35]. Schizophrenia has been associated with a nearly 25% increase in the risk of developing CKD [36]. Both *MAD1L1* and *TSNARE1* might be key effectors in the development of schizophrenia-related CKD, which might have pathogenetic pathways that are similar to those of other types of CKD. *BAI1* appears to be a mediator of the p53 signal in suppression of glioblastoma [37]. Coherently, the tumor suppressor protein p53 strongly alters HIV type 1 replication [38].

Though the cause–consequence relationships among methylation of these genes, kidney function, and neurological diseases are unclear, it is possible that some of the observed epigenetic associations are consequences of CKD rather than predictors. On the other hand, DNAm of several identified genes, including laminin subunit alpha 2 (*LAMA2*), apolipoprotein B (*APOB*), and neuropeptide Y receptor Y2 (*NPY2R*), are associated with metabolic diseases [39–41] that can contribute to renal impairment.

Similar to the recent study by Chu et al [17], we had limited success in replicating the CKD-associated CpG sites reported by Smyth et al, except for cg17500228 in gene *EXOC3*. *EXOC3* was previously established to be a key effector in HIV-1 Nef protein-mediated enhancement of nanotube formation [42]. It has been suggested that the Nef protein plays a pathogenetic role in HIV-associated nephropathy [3]. Of 17 significant sites implicated in the EWAS by Chu et al, cg17944885 (*ZNF788*/*ZNF20*) showed a significant association with eGFR in our study. Five other candidate sites showed associations with a P value < .05 in our study, lending support to protein tyrosine phosphatase, nonreceptor type 6 (*PTPN6*) and ankyrin repeat domain 11 (*ANKRD11*) as promising candidate genes for further experimental evaluation [17]. In our replication analysis, the lack of significant association but with consistent directionality of previously implicated CpG sites could be due to lack of statistical power (particularly among the HIV-negative group), differences in the severity or causes of CKD, differences in confounding, or a combination of these factors.

A potential limitation of our study is that we measured DNAm in peripheral blood rather than in kidney tissues. Direct evaluation of DNAm in kidney tissues is not feasible in human population–based studies [15]. Recently, similar trait-associated methylation of CpG sites in blood and target tissues has been found, supporting the use of DNAm in blood as an indicator of methylation in other tissues [17]. The 450K array platform restricts the epigenome-wide coverage compared to the newer array (eg, EPIC850K array) or whole methylome sequencing and is limited to measure 5-mC, not other types of modifications such as 5-hmC. Additionally, the etiology among HIV-associated CKD can be heterogeneous, which may increase false negatives in EWAS.

Our study is a first step in investigating the relationships among HIV infection, DNAm, and CKD. The VACS provided a unique opportunity to investigate the epigenomic association with eGFR among PWH. We replicated previously reported associations of eGFR with methylation of *EXOC3*, *PTPN6*, and *ANKRD11*, which supports the belief that CKD in PWH may have similar causes as in the HIV-negative population. The identified epigenetic associations of *MAD1L1*, *TSNARE1/BAI1*, and *DLGAP2* suggest that there may be some unique mechanism for HIV-related CKD. Further studies to examine the role of these epigenetic changes in the complex mechanism for development and progression of HIV-related CKD are warranted.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank all veterans who participated in this project.

Financial support. This work was supported in part by the Emory University Center for AIDS Research (P30AI050409) and National Institutes of Health grants (K01HL134147, R03DA039745, and K12DA000167).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Wang H, Wolock TM, Carter A, et al. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980–2015: the Global Burden of Disease Study 2015. Lancet HIV **2016**; 3:e361–87.
2. UNAIDS fact sheet 2017. UNAIDS 2017 Available at: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf. Accessed 02 July 2018.
3. Cohen SD, Kopp JB, Kimmel PL. Kidney diseases associated with human immunodeficiency virus infection. N Engl J Med **2017**; 377:2363–74.
4. Nadkarni GN, Konstantinidis I, Wyatt CM. HIV and the aging kidney. Curr Opin HIV AIDS **2014**; 9:340–5.
5. Nobakht E, Cohen SD, Rosenberg AZ, Kimmel PL. HIV-associated immune complex kidney disease. Nat Rev Nephrol **2016**; 12:291–300.
6. De Wit S, Sabin CA, Weber R, et al; Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the data collection on adverse events of anti-HIV drugs (D:A:D) Study. Diabetes Care **2008**; 31:1224–9.
7. Seaberg EC, Munoz A, Lu M, et al. Association between highly active antiretroviral therapy and hypertension in a large cohort of men followed from 1984 to 2003. Aids **2005**; 19:953–60.
8. Míguez-Burbano MJ, Wyatt C, Lewis JE, Rodríguez A, Duncan R. Ignoring the obvious missing piece of chronic kidney disease in HIV: cigarette smoking. J Assoc Nurses AIDS Care **2010**; 21:16–24.
9. Nishijima T, Kawasaki Y, Mutoh Y, et al. Prevalence and factors associated with chronic kidney disease and end-stage renal disease in HIV-1-infected Asian patients in Tokyo. Sci Rep **2017**; 7:14565.
10. Mocroft A, Kirk O, Reiss P, et al; EuroSIDA Study Group. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. AIDS **2010**; 24:1667–78.
11. Keller BJ, Martini S, Sedor JR, Kretzler M. A systems view of genetics in chronic kidney disease. Kidney Int **2012**; 81:14–21.
12. McCaughan JA, McKnight AJ, Courtney AE, Maxwell AP. Epigenetics: time to translate into transplantation. Transplantation **2012**; 94:1–7.
13. Geisel J, Schorr H, Heine GH, et al. Decreased p66Shc promoter methylation in patients with end-stage renal disease. Clin Chem Lab Med **2007**; 45:1764–70.
14. Wing MR, Devaney JM, Joffe MM, et al; Chronic Renal Insufficiency Cohort (CRIC) Study. DNA methylation profile associated with rapid decline in kidney function: findings from the CRIC Study. Nephrol Dial Transplant **2014**; 29:864–72.
15. Smyth LJ, McKay GJ, Maxwell AP, McKnight AJ. DNA hypermethylation and DNA hypomethylation is present at different loci in chronic kidney disease. Epigenetics **2014**; 9:366–76.
16. Sapienza C, Lee J, Powell J, et al. DNA methylation profiling identifies epigenetic differences between diabetes patients with ESRD and diabetes patients without nephropathy. Epigenetics **2014**; 6:20–8.
17. Chu AY, Tin A, Schlosser P, et al. Epigenome-wide association studies identify DNA methylation associated with kidney function. Nat Commun **2017**; 8:1286.
18. Zhang X, Justice AC, Hu Y, et al. Epigenome-wide differential DNA methylation between HIV-infected and uninfected individuals. Epigenetics **2016**: 1–11.
19. Mathur R, Hui Q, Huang Y, et al. DNA methylation markers of type 2 diabetes mellitus among male veterans with or without human immunodeficiency virus infection. **2019**.
20. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol **2013**; 14: R115.
21. Horvath S, Levine AJ. HIV-1 Infection accelerates age according to the epigenetic clock. J Infect Dis **2015**; 212: 1563–73.
22. Rickabaugh TM, Baxter RM, Sehl M, et al. Acceleration of age-associated methylation patterns in HIV-1-infected adults. PloS One **2015**; 10: e0119201.
23. Nelson KN, Hui Q, Rimland D, et al. Identification of HIV infection-related DNA methylation sites and advanced epigenetic aging in HIV-positive, treatment-naive U.S. veterans. AIDS **2017**; 31: 571–5.
24. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med **2009**; 150: 604–12.
25. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol **2013**; 14: R115–R.
26. Adalsteinsson BT, Gudnason H, Aspelund T, et al. Heterogeneity in white blood cells has potential to confound DNA methylation measurements. PLoS One **2012**; 7: e46705.
27. Houseman EA, Kelsey KT, Wiencke JK, Marsit CJ. Cell-composition effects in the analysis of DNA methylation array data: a mathematical perspective. BMC Bioinformatics **2015**; 16: 95.
28. Bekaert B, Kamalandua A, Zapico SC, Van de Voorde W, Decorte R. Improved age determination of blood and teeth samples using a selected set of DNA methylation markers. Epigenetics **2015**; 10: 922–30.
29. Barfield RT, Almli LM, Kilaru V, et al. Accounting for population stratification in DNA methylation studies. Genetic Epidemiol **2014**; 38: 231–41.
30. Klebaner D, Huang Y, Hui Q, et al. X chromosome-wide analysis identifies DNA methylation sites influenced by cigarette smoking. Clinical Epigenet **2016**; 8: 20.
31. Horvath S, Levine AJ. HIV-1 infection accelerates age according to the epigenetic clock. J Infect Dis **2015**; 212: 1563–73.
32. Jin DY, Spencer F, Jeang KT. Human T cell leukemia virus type 1 oncoprotein Tax targets the human mitotic checkpoint protein MAD1. Cell **1998**; 93: 81–91.
33. Pinto M, Vieira J, Ribeiro FR, et al. Overexpression of the mitotic checkpoint genes BUB1 and BUBR1 is associated with genomic complexity in clear cell kidney carcinomas. Cellular Oncol **2008**; 30: 389–95.
34. Ruderfer DM, Fanous AH, Ripke S, et al. Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia. Molecular psychiatry **2014**; 19: 1017–24.
35. Sleiman P, Wang D, Glessner J, et al. GWAS meta analysis identifies TSNARE1 as a novel Schizophrenia / Bipolar susceptibility locus. Sci Rep **2013**; 3: 3075.
36. Tzeng N-S, Hsu Y-H, Ho S-Y, et al. Is schizophrenia associated with an increased risk of chronic kidney disease? A nationwide matched-cohort study. BMJ Open **2015**; 5.
37. Mathema VB, Na-Bangchang K. Regulatory roles of brain-specific angiogenesis inhibitor 1(BAI1) protein in inflammation, tumorigenesis and phagocytosis: A brief review. Crit Rev Oncol Hematol **2017**; 111: 81–6.
38. Duan L, Ozaki I, Oakes JW, Taylor JP, Khalili K, Pomerantz RJ. The tumor suppressor protein p53 strongly alters human immunodeficiency virus type 1 replication. J Virol **1994**; 68: 4302–13.
39. Andrade RC, Nevado J, de Faria Domingues de Lima MA, et al. Segmental uniparental isodisomy of chromosome 6 causing transient diabetes mellitus and merosin-deficient congenital muscular dystrophy. Am J Med Genet A **2014**; 164a: 2908–13.
40. Kristiansson K, Perola M, Tikkanen E, et al. Genome-wide screen for metabolic syndrome susceptibility Loci reveals strong lipid gene contribution but no evidence for common genetic basis for clustering of metabolic syndrome traits. Circ Cardiovasc Genet **2012**; 5: 242–9.
41. Wei Z, Zhang K, Wen G, et al. Heredity and cardiometabolic risk: naturally occurring polymorphisms in the human neuropeptide Y(2) receptor promoter disrupt multiple transcriptional response motifs. J Hypertens **2013**; 31: 123–33.
42. Mukerji J, Olivieri KC, Misra V, Agopian KA, Gabuzda D. Proteomic analysis of HIV-1 Nef cellular binding partners reveals a role for exocyst complex proteins in mediating enhancement of intercellular nanotube formation. Retrovirology **2012**; 9: 33.