Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world-wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Junyu Chen Date

Approval Sheet

Epigenetic Association with Estimated Glomerular Filtration Rate (eGFR) among HIV-infected Individuals

By

Junyu Chen

Master of Public Health

Epidemiology

_____

Yan Sun

Committee Chair

Abstract Cover Page

Epigenetic Association with Estimated Glomerular Filtration Rate (eGFR) among HIV-infected Individuals

By

Junyu Chen

B.S., Nanjing University, 2016

Faculty Thesis Advisor: Yan Sun, PhD

An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Public Health in Epidemiology, 2018

Abstract

Epigenetic Association with Estimated Glomerular Filtration Rate (eGFR) among HIV-infected Individuals

By Junyu Chen

People living with human immunodeficiency virus (HIV) infection have higher risk for chronic kidney disease (CKD), defined by reduced estimated glomerular filtration rate (eGFR). Previous studies have implicated epigenetic changes contributing to CKD, however, mechanism of HIV-related CKD has not been thoroughly investigated. We conducted an epigenome-wide association studies of eGFR among 567 HIV-infected and 117 HIV-uninfected Veterans Aging Cohort Study (VACS) participants to identify epigenetic signatures of kidney function. By surveying over 400,000 CpG sites measured on peripheral blood, we identified 15 and 16 sites significantly associated with eGFR (false discovery rate q-value < 0.05) among the HIV-infected and total study population, respectively. The most significant CpG sites, located at *MAD1L1*, *TSNARE1/BAI1*, and *LTV1*, were all negatively associated with eGFR (cg06329547: p-value of 5.25×10^{-9}; cg23281907: p-value of 1.37×10^{-8}; cg18368637: p-value of 5.17×10^{-8}). These identified associations were not significant among HIV-uninfected participants. We also replicated previously reported eGFR-associated CpG sites including cg17944885 located between *ZNF788* and *ZNF20* on chromosome 19 (p-value of 2.5×10^{-5}). Our findings highlighted novel epigenetic associations with kidney function among people living with HIV, and suggested potential epigenetic mechanism linked with HIV-related CKD risk.

Cover Page

Epigenetic Association with Estimated Glomerular Filtration Rate (eGFR) among HIV-infected Individuals

By

Junyu Chen

B.S., Nanjing University, 2016

Faculty Thesis Advisor: Yan Sun, PhD

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Public Health in Epidemiology, 2018

Literature Review

Chronic Kidney Disease (CKD) is a significant public health concern with increasing numbers of individuals affected in the world(1). CKD affected an estimated 16.8% of U.S. adults aged 20 years and older in the period from 1999 to 2004(2) and caused 956,000 deaths globally in 2013(3). Individuals with an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 are diagnosed CKD because this level or lower represents loss of at least half of the adult level of normal kidney function.

Human immunodeficiency virus (HIV) infected individuals are at increased risk of getting CKD, which is associated with higher mortality(4, 5). HIV-related renal dysfunction has been reported across several populations. In South Africa, it is estimated that 650,000 patients developed human immunodeficiency virus (HIV)-related renal disease(6). Clinically meaningful renal injury developed in HIV-infected Koreans receiving Tenofovir disoproxil fumarate(7). Among middle-aged individuals from the HIV outpatient clinic of the Academic Medical Center in Amsterdam, HIV infection was independently associated with renal impairment(8).

The pathogenesis of HIV-related CKD can be multifactorial: exposure to HIV viremia as well as traditional risk factors such as hypertension, diabetes and smoking may be independent predictors(9). Also, Tenofovir, ritonavir-boosted atazanavir, and ritonavir-boosted lopinavir use may partially explain the higher burden of kidney dysfunction compared with uninfected men(10). Moreover, HIV infection can increase histone methylation (H3K4 trimethylation) at the vitamin D receptor (VDR) promoter, which would display enhanced expression of *SNAIL*, a repressor of *VDR* in renal cells(11). However, the epigenetic markers and other types of molecular mechanisms for HIV-related CKD haven't been

investigated thoroughly.

Previous studies suggested that epigenetic changes were associated with CKD. Differential methylation of 389 CpG sties in 187 genes were significantly associated with end-stage renal disease (ESRD) among patients with diabetes(12). Recent epigenome-wide association study (EWAS) demonstrated that DNA methylation sites in more than 40 genes were associated with eGFR(13-15).

Besides CKD, previous researches have connected DNA methylation with diverse kinds of kidney diseases. Reports indicate that DNA methylation patterns of kidney fibroblasts differ between control and diseased kidneys and hypermethylation of the RAS Protein Activator Like 1 (*Rasal1*) promoter contributes to activation of fibroblasts and progression of kidney fibrosis(16-18). KLOTHO methylation is associated with uremic toxins and uremic toxin-induced CDK(19). Decreased methylation of p66Shc promoter, a member of the ShcA (Src homologous- collagen homologue) adaptor protein family, has been observed in patients with ESRD(20). Significant hypomethylation of two CpG sites within Interferon Induced Protein 44 Like (*IFI44L*) promoter were associated with renal damage among systemic lupus erythematosus (SLE) patients(21).

Introduction

Human immunodeficiency virus (HIV) infection continues to be a global pandemic with high prevalence, incidence and mortality(22). Approximately 36.7 million people living with HIV (PLWH) and 1.8 million cases of incident HIV infection with 1.0 million people died from HIV-related diseases globally by the end of 2016(23). In mid-2017, approximately 20.9 million PLWH were receiving antiretroviral therapy (ART) globally(23). Although ART is proven to suppress viral replication and improve the life expectancy of persons with HIV infection(23-26), PLWH have increased risk for chronic inflammation, premature aging, and metabolic disorders(27, 28) (e.g., diabetes, hyperlipidemia, and abnormal body fat composition), which are associated with renal impairment(29, 30).

Chronic kidney disease (CKD), which emerged as a common complication of both HIV infection and its treatment, has been a critical cause of shortening the life span of PLWH(5, 28, 30-34). The pathogenesis of HIV-related CKD can be multifactorial: it can be linked to direct exposure to HIV viremia, superinfections and the systematic immune response to infection(28, 34-38), as well as traditional CKD risk factors such as increased body mass index (BMI), hypertension, diabetes and cigarette smoking (9, 39-43); ART regimens may partially explain the higher burden of kidney dysfunction compared with uninfected men(10, 44, 45); the genetic predisposition to HIV-associated CKD is largely due to variants in the *APOL1* gene among PLWH with African ancestry(46, 47). However, our understanding of the underlying molecular mechanisms and pathophysiologic pathways of developing HIV-related CKD remains largely unknown. To further improve the health outcomes of PLWH, we need

to identify novel biomarkers and to better understand molecular mechanisms contributing to the progression and onset of HIV-related CKD(48).

While genetic factors including common and rare genetic variants, copy number variation, or mitochondrial mutations can confer a disease risk, epigenetic modifications can be another molecular mechanism regulating disease susceptibility (49, 50). Epigenetics refers to a heritable change in the pattern of gene expression that is mediated by a mechanism specifically not due to alterations in the primary nucleotide sequence(51, 52). Epigenetics risk profiles can be used as potential drug targets for clinical use(53). Recent advances in genomic technologies and the epigenome-wide association study (EWAS) design have enabled large-scale studies of epigenomic signatures associated with human diseases(54). In fact, DNA methylation, the most studied epigenetic modification at the population level, has been associated with kidney disease traits(16-21). Previous EWAS identified several DNA methylation sites associated with estimated Glomerular Filtration Rate (eGFR)(12-15), which is widely used to assess the excretory function of the kidneys and to diagnose CKD. However, these EWAS results were not consistent across studies and populations with different degree of severity or causes of CKD. The epigenetic association with eGFR and CKD has not been investigated among PLWH.

Characterizing DNA methylation patterns related to CKD caused by HIV infection and its treatments is essential for assessing treatment strategies and relieving the burden for this population. We utilized the EWAS approach to identify differential DNA methylation related

to the risk for HIV-associated CKD. We applied a stringent quality control procedure and carefully controlled for confounders to examine previously identified CKD-associated CpG sites and to discover novel epigenetics changes associated with eGFR among PLWH.

Methods

Samples and phenotypes

The phenotypic and epigenetic data were from the Veterans Aging Cohort Study (VACS), which is an observational, prospective study of HIV-infected and age, race/ethnicity, sex, and site-matched uninfected veterans in care at the Department of Veterans Affairs (VA) medical centers across the US. The VACS was approved by the Human Research Protection Program of Yale University and Institutional Research Board committee at the Connecticut Veteran Healthcare System, West Haven Campus(55, 56). All VACS participants provided written consent.

We included 567 HIV-infected and 117 HIV-uninfected participants with both phenotypic and epigenome-wide DNA methylation data for this study. A questionnaire was completed by each subject at the baseline to collect clinical information, including the presence of chronic health conditions such as diabetes, information on cigarette smoking, anti-hypertension medication and antiretroviral treatment(57). Laboratory values such as total white blood cell counts and CD4 counts were enumerated at the time of peripheral whole blood samples collection(56, 57). The eGFR was calculated from standardized creatinine levels, sex, race, and age using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation(58). All individuals with an eGFR less than 60 ml/min/1.73 m^2, are classified as having CKD regardless of the presence of kidney damage because reduction to this level or lower represents loss of half or more of the adult level of normal kidney function. VACS index score, a HIV mortality risk score introduced in VACS study, was enumerated by

summing preassigned points for age, routinely monitored indicators of HIV disease and general indicators of organ system function(59).

Epigenomic data generation, quality control and processing

Genomic DNA was extracted from whole-blood samples using PAXGene collection tubes (QIAGEN, Hilden, Germany) and FlexiGene DNA extraction kits (QIAGEN), and stored using standard techniques. The Illumina Infinium HumanMethylation450 Beadchip (450K; Illumina, San Diego, CA, USA), which targets more than 480,000 CpG sites in the human genome, was utilized for genome-wide DNA methylation profiling at the Yale Center for Genomic Analysis. All samples were randomly placed on each array and across arrays to reduce batch effect by confounding.

Quality control procedures were performed to exclude problematic samples and CpG sites for analysis: 1. Intensity values with detection P ≥0.001 were set to missing for each site, and 927 CpG sites with a missing rate over 5% were removed; 2. All samples passed a call rate > 95% (i.e., missing rate < 5%); 3. 35,605 probes were removed because they were within 10 base pairs from a single-nucleotide polymorphism (SNP); 4. 24,749 CpG sites were removed as they mapped to multiple genomic locations. Following Illumina's control probe scaling procedure, all raw intensity values were quantile normalized using limma in R package. 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(60).

DNA methylation age (DNAm age) was calculated by a web-based calculator (https://dnamage.genetics.ucla.edu/) using preselected age-related CpG sites and an algorithm developed by Horvath (2013)(61) . A t-test were performed to assess the difference of DNAm age difference (DNAm age – chronological age) between 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 in blood and other tissues is a well-established confounder in epigenetic epidemiological studies. (62). Using probes from the 450K array that were highly correlated with six cell types ($CD4^+$ T cells, $CD8^+$ T cells, NK T cells, B cells, monocytes, and granulocytes) in the blood and estimated the cell type proportions, and an algorithm developed by Houseman, et al(63) (implemented in the R minfi package), we calculated cell type proportions for each participant. 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 quantitative variables, such as DNAm age, race, smoking status, diabetes, antihypertension drug usage and six methylation-estimated cell types, as a function of eGFR, controlling for chronological age, which are known epigenetic modifiers(64, 65). Variables with $p < 0.05$, along with previously established function of eGFR, were subsequently adjusted as covariates in the EWAS model.

Principle components analysis

The population stratification is a potential confounder in EWAS(66). To address population stratification in the VACS sample(60), we performed a principal component analysis (PCA), using an analytical approach developed by Barfield et al.(67). We created a pruned dataset that kept only CpG sites within 50 bp of a genetic variant and used prcomp function in Factoextra R package to calculate the principal components (PCs). We included the top 10 PCs as covariates in the EWAS of eGFR.

Statistical methods

For autosomal analysis, mixed linear regression models for the effect of methylation status at individual CpG sites on eGFR included random effect for chip regardless of HIV-infection status were regressed out using nlme R package. The final adjusted models for the EWAS included age, race, BMI, average systolic blood pressure (SBP), HIV-infection status, HCV-infection status, smoking status (current smoking vs. noncurrent smoking), diabetes status, anti-hypertensive drug usage, 6 cell type proportions and top 10 PCs. We also performed epigenetic association analyses stratified by HIV infection status using the same covariates excluding HIV-infection status. To corrected for multiple testing in the EWAS, we applied false discovery rate (FDR) q-value of 0.05, and the conservative Bonferroni Corrected p-value of 0.05 as significance thresholds. To replicate previous findings in non-HIV population (Smyth, 2014 (13)and Chu, 2017(14)), we analyzed and compared their associations with eGFR in the VACS samples. In this VACS sample of male veterans, we also separately examined DNA

methylation of 11,232 CpG sites on the X chromosome within pooled sample using the same statistic model as above(68).

Results

After quality control, the analysis dataset included 412,583 unique CpG sites in 567 HIV-infected and 117 HIV-negative participants. The characteristics of the phenotypic variables were summarized in table 1. All subjects were males with an average age of 52.0 years. More participants self-reported black race in the HIV positive group than that in the HIV negative group (85% vs. 62%). HIV-infected participants had a higher rate of HCV infection (58% vs. 28%) and lower BMI (25.5 vs 30.7 kg/m^2). However, the prevalence of diabetes was lower among HIV-infected participants than that in the uninfected controls. The eGFR was, on average, 3.4 mL/min/1.73 m^2 higher among the HIV-infected compared to the controls, but not statistically significant (p-value of 0.24). Additional clinical and biochemical characteristics of the study population are presented in Table 1.

To determine the covariates for adjustment, we first examined the associations of each variable with the eGFR. Because age was used to calculate eGFR and was a known epigenetic modifier, we always controlled for age while testing the association of the other variables. Race, BMI, anti-hypertensive medication use, SBP and cell type proportions were significantly associated with eGFR (Table 2). In addition, VACS index score and SCD14 were associated with eGFR among HIV-positive group. Diabetes, current smoking status, HIV-infection status and ART were not significantly associated with eGFR in this study, showing heterogeneous

characteristics comparing to non-HIV studies(23, 69, 70). Because of previous evidence of eGFR association and epigenetic modification, we 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-value $< 2.2 \times 10^{-6}$) as previously reported(57) (Table 1). DNAm age was, on average, one-year older than chronological age ($P = 0.0002$). The difference between chronological age and DNAm age is not significantly associated with HIV infection after controlling for diabetes, current smoking, HCV infection and 6 cell type proportions ($P = 0.34$). Also, the difference of chronological age and DNAm age was not significantly associated with eGFR after adjusting for all potential confounders in the whole study population that was previously discussed ($P = 0.67$).

Figure 1a (Manhattan plot) and table 3 presented 16 CpG sites significantly associated with eGFR at the FDR less than 0.05 among the whole study population adjusted for covariates. A Quantile-Quantile plot (QQ plot, Figure 1b) comparing the observed p-values to the expected p-values for this EWAS indicated a low level of global inflation (genomic control inflation factor = 1.02). As a result, we did not further control for inflation as the covariate adjustment including the PCs sufficiently control for spurious associations. The test statistics and annotations of 16 epigenome-wide significant CpG sites were summarized in Table 3. Fifteen of these 16 significant CpG sites were negatively associated with eGFR (i.e., hypermethylation among patients with declined renal function) while only one CpG site was positively associated with eGFR. Fifteen out of 16 eGFR- associated CpG sites identified in the pooled samples were

also statistically significant among the HIV-infected group (Table 3). However, the significance of these sites was largely inconsistent among HIV-negative group (Table 3). Corresponding Manhattan plots and QQ plots of EWAS stratified by HIV-infection status were shown in figure 1c-f. Figure 1d and 1f indicated a low level of global inflation for both PIWH and HIV-negative group (genomic control inflation factor = 0.996 and 1.03).

Using a more stringent threshold of Bonferroni corrected p-value of 0.05 (i.e., $P_{nominal} < 10^{-7}$), three CpG sites, cg06329547, cg23281907 and cg18368637, remained significant. These three CpG sites 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 LTV1 Ribosome Biogenesis Factor (*LTV1)*, respectively. One CpG site in *MADA1L1*, cg06329547, showed higher methylation levels among patients with lower eGFR, with 1% increase in β value associated with 2.54 unit decrease of eGFR (95% CI: -3.38, -1.70; p-value of 5.25×10^{-9}). One significant CpG site, cg23281907, located between genes *TSNARE1* and *BAI1* (position: 143509372-143509630), was also negatively associated with eGFR, with 1% increase of beta-value associated with 2.20 unit decrease of eGFR (95% CI: -2.95, -1.45; p-value of 1.37×10^{-8}). Another significant CpG site in *LTV1*, cg18368637, was associated reversely with eGFR, with 1% increase in β value associated with 1.85 unit decrease of eGFR (95% CI: -2.52, -1.20; p-value of 5.17×10^{-8}). These three CpG sites showed similar effect size on eGFR within HIV-infected patients and the associations remained statistically significant, but such significant associations were absent in HIV-negative group. The regional plots (Figure 2) showed all tested CpG sites in the neighboring regions of these three sites, and additional

association signals with eGFR within these regions. There are 11(12.5%), 34 (6.0%) and 16 (5.4%) CpG sites with p-value less than 0.05 in regions for cg06329547, cg23281907 and cg18368637 respectively. No statistically significant CpG sites were identified in the X chromosome.

Previous studies have identified several CpG sites that showed differential DNA methylation associated with eGFR. Smyth, et al(13) reported 23 genes with at least one CpG site significantly associated with eGFR. However, Chu 2017(14) reported lack of replication in a much larger EWAS of eGFR including 2,264 participants from the Atherosclerosis Risk in Communities Study and 2,595 participants from the Framingham Heart Study. They reported 18 CpG sites significantly associated eGFR. We sought to replicate these findings in our study of HIV-infected and uninfected participants. We successfully found significant differential methylation of cg17944885 located between Zinc Finger Family Member 788 (*ZNF788*)/ Zinc Finger Protein 20 (*ZNF20*) on chromosome 19 ($P = 2.5 \times 10^{-5}$) (Table 4). In addition, there were 5 sites with p-values less than 0.05 (cg23591762, cg16428517, cg19942083, cg06158227 and cg27660627). The replicated association between all 19 CpG sites and eGFR showed consistency with Chu, et al in directionality(14).

Discussion

HIV-1 viremia and its corresponding treatments are known to be responsible for development of CKD and decrease of eGFR(30, 34). However, the pathogenesis has not been studied thoroughly. Previous reports demonstrated that HIV infection could accelerate epigenetic aging

in blood tissues(71). We hypotheses that epigenetic aging may contribute to aging-related pathologies of CKD. Therefore, we explored the relationship between chronological age and DNAm age, HIV status and eGFR in the hope of building the bridge between HIV infection and CKD. DNAm age, a marker of biological aging, was approximately, on average, one-year older than chronological age (P = 0.0002), illustrating accelerated epigenetic aging among our study population. However, the difference between DNAm age and chronological age was not associated with neither HIV or eGFR, suggesting that epigenetic aging might not be a path between HIV and eGFR.

Using an EWAS approach, we detected 16 significant CpG sites after correction for multiple testing. The most significant CpG site cg06329547 is located in *MAD1L1*, which is a key component of the mitotic spindle-assembly checkpoint. *MAD1L1* is known to serve as an important checkpoint that prevents the onset of anaphase until all chromosome are properly aligned at the metaphase plate during mitotic spindle-assembly(72). *MAD1L1* was recently suspected to contribute to prostate cancer, gastric cardia carcinoma, bipolar disorder and schizophrenia(73-76). Precisely how epigenetic changes in *MAD1L1* is causing different eGFR remains unknown, however, *MAD1L1* functions as a homodimer to code Mitotic-Arrest Deficient 1 (MAD1) protein, which inhibits maturation and expansion of T-lymphocytes by modulating expression of growth-regulating genes(77). MAD1 was reported to be targeted by Human T cell leukemia virus type 1 during virus transformation(78). Reduction of MAD1 may play a role in modulating specific immune responses to HIV epitopes and polyclonal gammopathy, both of which result in the formation of circulating immune complexes that are

deposited in the glomerular capillary tuft. Additionally, defects in *MAD1L1* are involved in the development and/or progression of various types of cancer including kidney carcinomas(79), suggesting that *MAD1L1* is a target for cancer immune evasion. Regulation of *MAD1L1* expression modulates host defense, which involves in the mechanisms of HIV-associated immune-mediated kidney diseases.

Another significant CpG site is cg23281907 in genes *TSNARE1* and *BAI1*. Gene *TSNARE1* participates in SNARE binding and SNAP receptor activity and may therefore have a vertebrate-specific function in intracellular protein transport and synaptic vesicle exocytosis. Interestingly, Both the top 2 significant genes *MAD1L1* and *TSNARE1* were estimated to be associated with susceptibility of Schizophrenia / Bipolar Disorder(75, 76, 80). Schizophrenia is associated with a nearly 25% increase in the risk of developing CKD(81). We speculate that both genes *MAD1L1* and *TSNARE1* might be key effectors in the development of schizophrenia-related CKD, which might have similar pathways as other types of CKD. *BAI1 (ADGRB1)* is associated with transmembrane signaling receptor activity(82). A function of gene *ADGRB1* is to mediates bacterial killing(83). *BAI1* is likely to play a significant role as a mediator of the p53 signal in suppression of glioblastoma(82). Coherently, the tumor suppressor protein p53 strongly alters human immunodeficiency virus type 1 replication(84). Also, deletion of p53 was reported to protect kidneys from functional and histologic deterioration by decreasing necrosis, apoptosis, and inflammation. *BAI1* might have regulated the impact of p53 in protection from kidney diseases during HIV infection, but further studies are needed to clarify this.

Another significant CpG site in *LTV1*, cg18368637, was differentially methylated associating with eGFR. Diseases associated with *LTV1* include Spinal Cord Disease. *LTV1* was established to be overexpressed in lung and breast, and it was linked to Spinal Cord Disease(85). However, the association with HIV-related CKD has not been investigated. Several differential CpG sites that reached FDR $q < 0.05$ failed to retain significance after Bonferroni Correction, but they may be biologically relevant. Some of them were also located on or between genes involved in the development of neural diseases. Our study points out increased DNA methylation associating with eGFR in genes DLG Associated Protein 2 (*DLGAP2*) and LIM Homeobox Transcription Factor 1 Alpha (*LMX1A*), which were reported to contribute to development of Northern Epilepsy, Parkinson's disease(86, 87). Patients with CKD are frequently afflicted with neurological complications that can potentially affect both the central and peripheral nervous systems(88). Even though the cause-consequence relationships between methylation of these genes, kidney failure and neural diseases are unknown, we speculate some of the significant CpG sites from our study are consequences of eGFR rather than predictors, and they are likely to involves in formation of neurological disorders caused by CKD. In other hand, a set of candidate genes including Laminin Subunit Alpha 2 (*LAMA2*), Apolipoprotein B (*APOB*), Neuropeptide Y Receptor Y2 (*NPY2R*) and PQ Loop Repeat Containing 2 (*PQLC2*) are associated distinct types of metabolic diseases(89-92), which can be responsible to renal impairment. The results raised a possibility that low values of eGFR in some of our study subjects might be due to different causes other than HIV infection and its treatments, and the above genes might involve in these mechanisms.

EWAS results among patients with HIV infection showed a similar set of CpG sites that were associated with eGFR changes. However, we cannot replicate these results in the EWAS among HIV negative participants. This heterogeneity might prove that indicated CpG sites in our study are associated with CKD caused only by HIV infection and its treatments. However, there is concern that this difference might be due to the small amount of HIV negative participants and the lack of power to detect true significant CpG sites among this group. Further study recruited more HIV negative subjects is needed to confirm this result.

Because all subjects in VACS were male, we separately analysis epigenetic changes in X Chromosome. There were no norminal significance signals found in this analysis. Further study is needed to confirm this result.

One smaller EWAS of eGFR were conducted using the HM450K array on whole blood to compare DNA methylation of 255 CKD cases vs.152 controls and more than 50 candidate sites were reported. Chu 2017, an EWAS of eGFR based on 2264 ARIC Study subjects and 2595 Framingham Heart Study participants, performed replication analysis those CpG sites and most of the results could not be successfully replicated. We also tried to analyze these CpG sites separately and we got similar results as Chu 2017. Only one site, cg17500228 in gene Exocyst Complex Component 3 (*EXOC3*), showed association with $P < 0.05$ (Smyth 2014: $P = 1.57\times10^{-14}$, Chu 2017: $P = 0.0002$, VACS: $P = 0.0095$). *EXOC3*, which is essential for protein binding, exocytosis and protein transport, was established to be a key effector to HIV-1 Nef protein-

mediated enhancement of nanotube formation(93). Nef protein was suggested to play a pathogenesis role in HIV-associated nephropathy(28). Of 19 significant sites implicated across Chu 2017, cg17944885 in between *ZNF788*/*ZNF20* showed significant association with eGFR in our study ($P = 2.5\times10^{-5}$). Genes *ZNF788* and *ZNF20* were both be linked to transcriptional regulation(94). Five other candidate sites showed associations with $P < 0.05$, from which we confirm that Protein Tyrosine Phosphatase, Non-Receptor Type 6 (*PTPN6*) and Ankyrin Repeat Domain 11 (*ANKRD11*) may be a promising candidate gene for further experimental evaluation(14). The poor associations of the other previously implicated CpG sties might be due to differences in the severity or causes of CKD or differences in confounders adjustment, or a combination of the above.

A limitation of our study is that we selected the sample based on HIV status, thus, the sample size might not be well-powered enough to detect true associations for eGFR. We found that known determinants of CKD such as current smoking and diabetes were not significantly correlated with eGFR in our sample population, which may be due to restricted number of CKD cases. However, in order to avoid potential confounding, we controlled current smoking and diabetes in our analysis models. HIV infection and its treatments, respectively, had a reverse relationship with eGFR, but the linkages were weak, which may attribute to the lack of patients with CKD as well. Therefore, we were unable to distinguish between epigenetic changes due to decreasing eGFR that were caused by HIV infection, HIV therapy regimens, or other factors. Further studies with larger number of HIV-related CKD cases are needed to fully understand the impact of HIV-related CKD on the DNA methylation patterns.

A potential limitation of VACS is that blood derived DNA were used rather than DNA obtained from affected and unaffected kidneys. Direct evaluation of methylation chances in kidney tissues is not readily available for human population-based studies(13). Therefore, we identify epigenetic changes from peripheral blood leucocytes, a readily accessible source that may have relevant function in a routine clinical setting. Recent publications showed similar trait-associated differentially CpG sites in blood and in the target tissues, suggesting that DNA methylation in blood can serve as an indicator of methylation in other tissues(14, 95, 96). Another limitation, inherent to the use of the 450K array, is that the discovery potential of DNA methylation screening is restricted because of the inability to detect 5-hmC modifications and low coverage rate of total human DNA methylation. Additionally, we suspect that anti-hypertension drug use might not be an ideal proxy for hypertension status: some patients without hypertension might use anti-hypertension drug to prevent hypertension and some patients with hypertension might not be taking any treatments. Further investigations are needed to evaluate this relation. We were also unable to examine the function of roles of indicated CpG sites for eGFR. Further studies performing pathway analysis to investigate the regulatory mechanism of methylation alteration in reported genes will provide insight into the methylation mechanisms.

Despite the limitations of this study, there are some undeniable strengths of our study: 1) This is the first attempt to investigate the relationship between HIV infection and its treatment, DNA methylation aging and CKD using a unique dataset; 2) This the first EWAS of eGFR that

examine the impact of HIV infection as an effect modification; 3) Previously indicated and biologically meaningful methylations of *EXOC3*, *PTPN6* and *ANKRD11* were replicated in our study sample. The highlighted methylation signals illustrate the key role of epigenetic changes in the complex mechanism for development and progression of HIV-related CKD. Identification of a genetic-epigenetic profile that contribute to HIV-related CKD is a crucial step towards early diagnosis and health benefits for affected individuals.

Table 1. Demographic and clinical characteristics of the samples

Characteristic	Total (N=684)	HIV Positive (N=567)	HIV Negative (N = 117)	P-value
eGFR (mL/min/1.73 m²)	98.4 (32.90)	99.0 (34.00)	95.6 (26.90)	0.243
Chronic Kidney Diseases	56 (8%)	47 (8%)	9 (8%)	0.9643
Age(years)	52.0 (8.01)	52.0 (7.82)	53.2 (8.83)	0.169
BMI (kg/ m²)	26.4 (5.51)	25.5 (4.67)	30.7 (7.08)	<0.0001
Race/Ethnicity				<0.0001
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				0.0283
None	142 (20%)	115 (20%)	27 (23%)	
Current	376 (55%)	324 (57%)	52 (44%)	
Past	166 (24%)	128 (23%)	38 (23%)	
Anti-hypertensive medication	497 (73%)	402 (71%)	95 (81%)	0.2067
Hepatitis C	362 (53%)	329 (58%)	33 (28%)	<0.0001
Diabetes	156 (23%)	115 (20%)	41 (35%)	0.0008
Laboratory values				
CD4*	-	425.0 (277.67)	-	-
Systolic BP	129.3 (14.46)	129.3 (14.73)	129.3 (13.13)	0.107
Diastolic BP	79.1 (9.53)	79.4 (9.35)	77.7 (10.29)	0.169
VACS index score	-	34.5 (21.27)	-	-
ART	-	472 (83%)	-	-
DNA methylation age	53.1 (8.62)	53.6 (8.64)	51.1 (8.24)	0.0033

Estimated Glomerular Filtration Rate (eGFR); Body mass index (BMI); human immunodeficiency virus (HIV); Veterans Aging Cohort Study Index score (VACS index score); Antiretroviral therapy (ART)

*: **Among HIV negative group, only two people had data on CD4 count.**

Table 2. Summary statistics of associations between covariates and eGFR.

Tested variable	Effect	SE	P-value
Black-Race*	13.4	3.71	0.0003
BMI	-0.35	0.04	0.1284
Current Smoking	2.3	3.11	0.4665
Anti-Hypertensive Drug*	-8.5	2.89	0.0033
HIV	2.2	3.40	0.5211
Hepatitis C *	5.2	2.55	0.0426
Diabetes	-2.0	3.01	0.5079
CD4	<0.01	<0.01	0.5730
HIV Virus load	<0.01	<0.01	0.8506
SBP*	-0.2	0.09	0.0350
DBP	-0.1	0.13	0.5056
CD8T*	48.2	15.77	0.0023
CD4T	19.0	19.79	0.3382
NK	29.4	23.07	0.2030
B cell*	110.6	25.71	<0.0001
Mono	-28.0	34.12	0.4125
Gran*	-41.2	9.98	<0.0001
ART	-0.9	2.74	0.7506
DNAm age - Age	0.1	0.21	0.6485

Estimated Glomerular Filtration Rate (eGFR); Body mass index (BMI); human immunodeficiency virus (HIV); systolic blood pressure (SBP); diastolic blood pressure (DBP); Antiretroviral therapy (ART); DNA methylation age (DNAm age)

***Significantly correlated with eGFR.**

Table 3. Top ranked CpG sites exhibiting differential DNA methylation for eGFR, controlled for Age, Race, BMI, Average SBP, HIV, HCV, Smoking, Diabetes, Anti-hypertension medication use, Cell types proportion and top ten principle components (FDR q<0.05)

CpG sites	CHR	Position	Closest Gene	Total			HIV positive			HIV negative		
				Effect	SE	P-value	Effect	SE	P-value	Effect	SE	P-value
cg06329547	7	1937739	*MAD1L1*	-253.8	42.85	5.25×10^{-9}	-260.6	44.45	8.28×10^{-9}	358.6	482.47	0.4599
cg23281907	8	143509556	*TSNARE1/BAI1*	-219.9	38.22	1.37×10^{-8}	-228.5	40.63	3.10×10^{-8}	-133.3	148.91	0.3739
cg18368637	6	144179905	*LTV1*	-185.8	33.70	5.17×10^{-8}	-181.4	36.45	8.93×10^{-7}	-166.0	95.02	0.0852
cg07796977	8	962467	*ERICH1-AS1*	-414.7	77.42	1.20×10^{-7}	-456.2	81.12	3.12×10^{-8}	488.6	319.73	0.1311
cg17461641	1	164023978	-	-309.3	59.02	2.19×10^{-7}	-322.7	66.57	1.68×10^{-6}	-232.8	124.43	0.0657
cg03658275	6	129285274	*LAMA2*	-150.7	28.90	2.53×10^{-7}	-162.0	33.15	1.39×10^{-6}	-88.0	58.75	0.1388
cg17355919	5	1331538	*CLPTM1L*	-403.2	78.97	4.40×10^{-7}	-438.2	84.28	2.93×10^{-7}	-65.4	244.59	0.7901
cg03012642	2	21346892	*APOB*	-432.6	85.24	5.12×10^{-7}	-439.0	89.99	1.44×10^{-6}	49.5	390.81	0.8996
cg14220251	1	19653075	*PQLC2*	303.9	59.96	5.31×10^{-7}	276.7	68.59	6.33×10^{-5}	325.3	113.28	0.0054
cg05267756	1	165329719	*LMX1A/RXRG*	-238.9	47.50	6.43×10^{-7}	-244.2	51.90	3.29×10^{-6}	-127.2	119.68	0.2916
cg08198773	8	1697536	*DLGAP2/CLNB*	-429.5	86.76	9.55×10^{-7}	-490.7	97.16	6.2×10^{-7}	-77.6	213.91	0.7181
cg04525441	10	1509792	*ADARB2*	-443.8	89.85	1.01×10^{-7}	-449.7	96.44	4.00×10^{-6}	-77.5	287.44	0.7883
cg21875401	17	12541750	*LINC00670*	-284.3	58.06	1.25×10^{-7}	-285.7	63.03	7.30×10^{-6}	-194.6	159.61	0.2271
cg10657250	4	156136570	*NPY2R*	-459.8	94.96	1.62×10^{-7}	-531.0	103.29	3.94×10^{-7}	389.4	250.59	0.1248
cg11796565	19	13205681	*NFIX*	79.9	16.55	1.74×10^{-7}	89.8	18.46	1.52×10^{-6}	28.4	37.73	0.4535

Table 4. Replication analysis of eGFR-DNA methylation in ARIC and FHS study.

CpG Sites	CHR	Position	Gene	Meta-analysis of ARIC and FHS	VACS Total		VACS HIV positive		VACS HIV negative	
				P-value	β	P-value	β	P-value	β	P-value
cg17944885	19	12225735	*ZNF788/ZNF20*	1.228×10^{-23}	-176.9	2.5×10^{-5}	169.00	0.0005	-165.10	0.0499
cg23597162	7	28102341	*JAZF1*	2.82×10^{-19}	-130.9	0.0001	135.07	0.0008	-181.78	0.0043
cg04036920	11	33562503	*C11orf41*	2.387×10^{-15}	-42.0	0.2114	-38.29	0.3085	10.97	0.8941
cg00501876	3	39193251	*CSRNP1*	3.623×10^{-15}	-20.7	0.7033	13.92	0.8266	-240.05	0.0445
cg00994936	19	1423902	*DAZAP1*	3.259×10^{-11}	70.7	0.1122	76.82	0.1307	10.07	0.9170
cg16428517	16	3317428	*MEFV/ZNF263*	1.166×10^{-10}	-224.2	0.0008	193.11	0.0159	-248.22	0.0581
cg11950754	1	53782077	*LRP8*	2.006×10^{-10}	-30.1	0.4604	-23.88	0.6296	-20.63	0.7748
cg12065228	1	19652788	*PQLC2*	2.196×10^{-10}	33.9	0.1859	33.58	0.2424	33.21	0.6021
cg10750182	10	73497514	*C10orf105*	4.962×10^{-10}	-23.2	0.7059	-4.99	0.9428	-297.96	0.0561
cg09022230	7	5457225	*TNRC18*	5.222×10^{-10}	13.6	0.7268	14.70	0.7438	28.57	0.7274
cg19942083	12	7070562	*PTPN6/PHB2,*	7.174×10^{-10}	80.9	0.0050	75.67	0.0165	45.80	0.5951
cg06158227	15	43662311	*TUBGCP4*	8.482×10^{-10}	-196.4	0.0065	230.16	0.0075	-3.75	0.9784
cg27660627	16	89461803	*ANKRD11*	9.939×10^{-10}	-125.6	0.0104	175.70	0.0022	45.92	0.6181
cg12116137	17	1576449	*PRPF8*	5.277×10^{-9}	5.8	0.7964	-8.32	0.7545	91.34	0.0284
cg04460609	4	16532808	*LDB2*	7.854×10^{-9}	-31.5	0.2826	-27.98	0.4010	-64.95	0.3433
cg19497511	2	238609807	*LRRFIP1*	3.451×10^{-8}	-2.7	0.9508	-14.49	0.7723	16.16	0.8875
cg22515589	17	79426432	*BAHCC1*	6.437×10^{-8}	26.9	0.6820	25.79	0.7484	43.31	0.7129
cg13296238	3	127323965	*MCM2*	1.453×10^{-5}	34.8	0.5834	25.45	0.7291	160.12	0.2666

Figure 1. Autosome Epigenome-wide association with eGFR in pooled sample (N=684, 1a, 1b), HIV positive group (N=567, 1c, 1d) and HIV negative group (N=117, 1e, 1f).

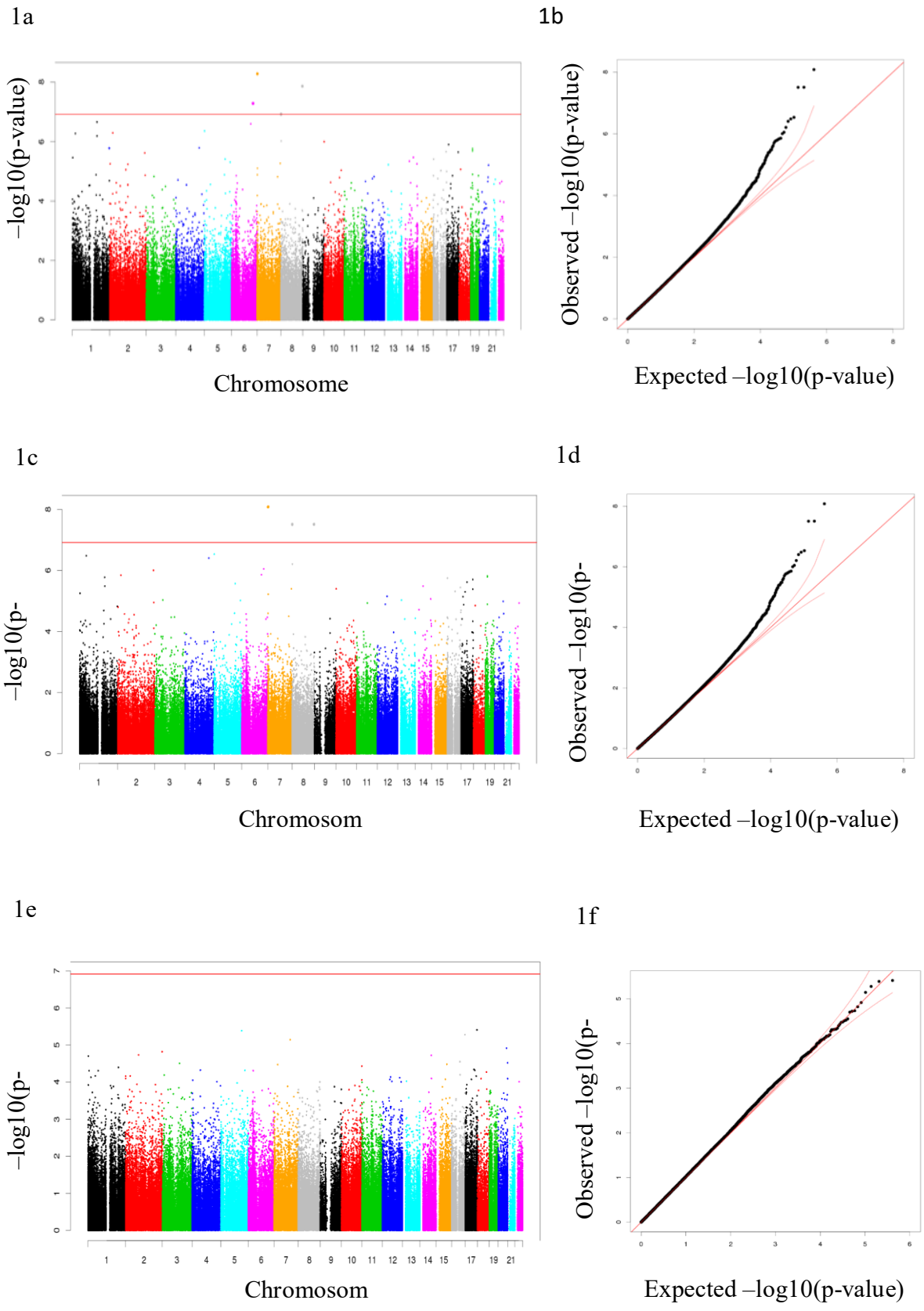
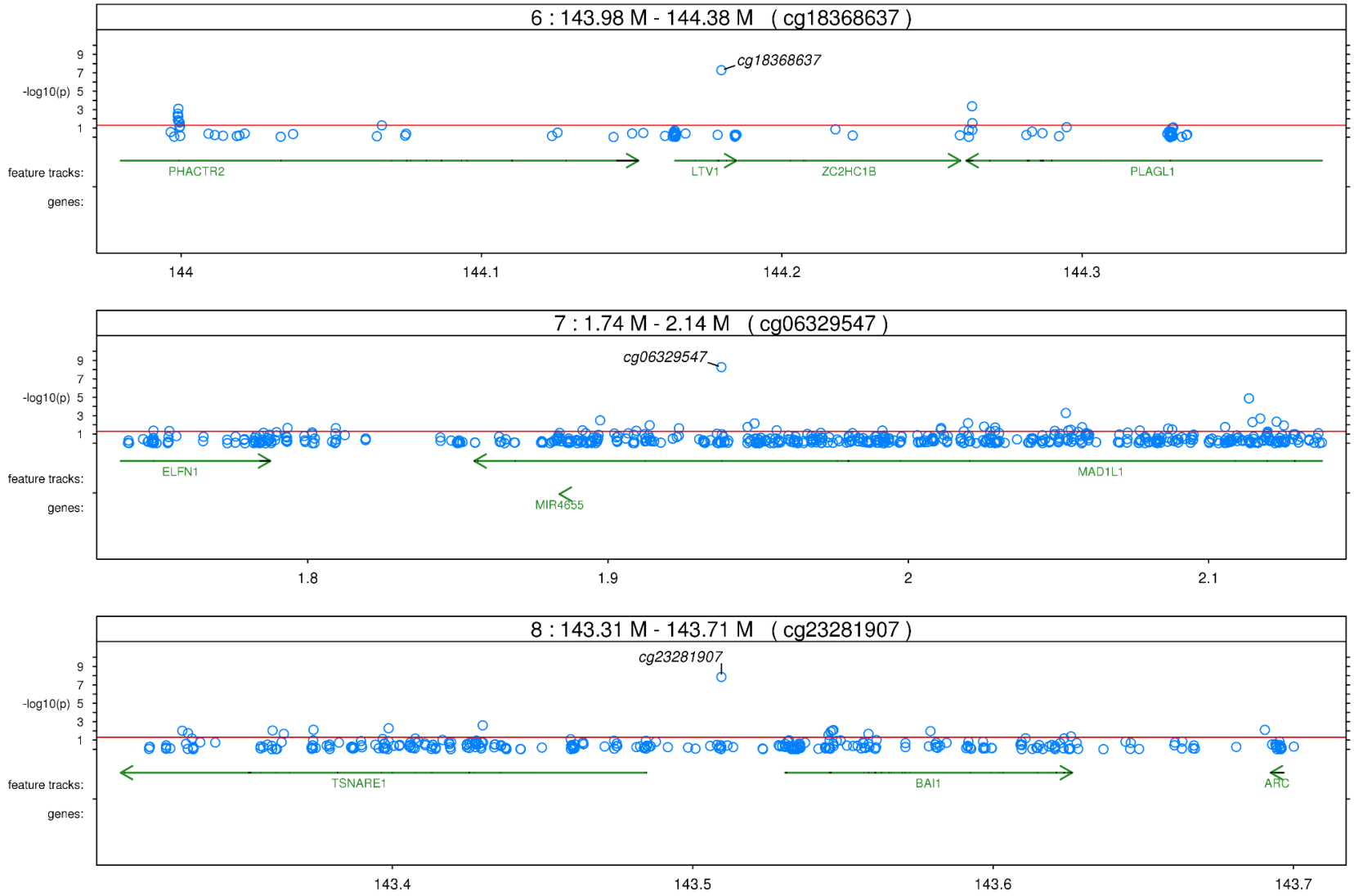


Figure 2. Regional Plots of DNA methylation site of cg06329547, cg23281907 and cg18368637.



Reference

1. Levey AS, Coresh J. Chronic kidney disease. Lancet. 2012; 379:165-80.

2. Prevalence of chronic kidney disease and associated risk factors--United States, 1999-2004. MMWR Morb Mortal Wkly Rep. 2007; 56:161-5.

3. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet. 2015; 385:117-71.

4. Wyatt CM, Arons RR, Klotman PE, Klotman ME. Acute renal failure in hospitalized patients with HIV: risk factors and impact on in-hospital mortality. Aids. 2006; 20:561-5.

5. Gardner LI, Holmberg SD, Williamson JM, Szczech LA, Carpenter CC, Rompalo AM, et al. Development of proteinuria or elevated serum creatinine and mortality in HIV-infected women. J Acquir Immune Defic Syndr. 2003; 32:203-9.

6. Assaram S, Magula NP, Mewa Kinoo S, Mashamba-Thompson TP. Renal manifestations of HIV during the antiretroviral era in South Africa: a systematic scoping review. Systematic reviews. 2017; 6:200.

7. Lee KH, Lee JU, Ku NS, Jeong SJ, Han SH, Choi JY, et al. Change in Renal Function among HIV-Infected Koreans Receiving Tenofovir Disoproxil Fumarate-Backbone Antiretroviral Therapy: A 3-Year Follow-Up Study. Yonsei Med J. 2017; 58:770-7.

8. Kooij KW, Vogt L, Wit FWNM, van der Valk M, van Zoest RA, Goorhuis A, et al. Higher Prevalence and Faster Progression of Chronic Kidney Disease in Human Immunodeficiency Virus–Infected Middle-Aged Individuals Compared With Human Immunodeficiency Virus–Uninfected Controls. The Journal of Infectious Diseases. 2017; 216:622-31.

9. Jotwani V, Li Y, Grunfeld C, Choi AI, Shlipak MG. Risk factors for ESRD in HIV-infected individuals: traditional and HIV-related factors. Am J Kidney Dis. 2012; 59:628-35.

10. Ryom L, Mocroft A, Kirk O, Worm SW, Kamara DA, Reiss P, et al. Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. J Infect Dis. 2013; 207:1359-69.

11. Chandel N, Ayasolla KS, Lan X, Sultana-Syed M, Chawla A, Lederman R, et al. Epigenetic Modulation of Human Podocyte Vitamin D Receptor in HIV Milieu. J Mol Biol. 2015; 427:3201-15.

12. Sapienza C, Lee J, Powell J, Erinle O, Yafai F, Reichert J, et al. DNA methylation profiling identifies epigenetic differences between diabetes patients with ESRD and diabetes patients without nephropathy. Epigenetics. 2014; 6:20-8.

13. 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.

14. Chu AY, Tin A, Schlosser P, Ko YA, Qiu C, Yao C, et al. Epigenome-wide association studies identify DNA methylation associated with kidney function. Nat Commun. 2017; 8:1286.

15. Wing MR, Devaney JM, Joffe MM, Xie D, Feldman HI, Dominic EA, et al. DNA methylation profile associated with rapid decline in kidney function: findings from the CRIC study. Nephrol Dial Transplant. 2014; 29:864-72.

16. Tampe B, Tampe D, Muller CA, Sugimoto H, LeBleu V, Xu X, et al. Tet3-mediated hydroxymethylation of epigenetically silenced genes contributes to bone morphogenic protein 7-induced reversal of kidney fibrosis. J Am Soc Nephrol. 2014; 25:905-12.

17. Ko YA, Mohtat D, Suzuki M, Park AS, Izquierdo MC, Han SY, et al. Cytosine methylation changes in enhancer regions of core pro-fibrotic genes characterize kidney fibrosis development. Genome

biology. 2013; 14:R108.

18. Bechtel W, McGoohan S, Zeisberg EM, Muller GA, Kalbacher H, Salant DJ, et al. Methylation determines fibroblast activation and fibrogenesis in the kidney. Nat Med. 2010; 16:544-50.

19. Young GH, Wu VC. KLOTHO methylation is linked to uremic toxins and chronic kidney disease. Kidney Int. 2012; 81:611-2.

20. Geisel J, Schorr H, Heine GH, Bodis M, Hubner U, Knapp JP, et al. Decreased p66Shc promoter methylation in patients with end-stage renal disease. Clin Chem Lab Med. 2007; 45:1764-70.

21. Zhao M, Zhou Y, Zhu B, Wan M, Jiang T, Tan Q, et al. IFI44L promoter methylation as a blood biomarker for systemic lupus erythematosus. Ann Rheum Dis. 2016; 75:1998-2006.

22. Wang H, Wolock TM, Carter A, Nguyen G, Kyu HH, Gakidou E, et al. Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980–2015: the Global Burden of Disease Study 2015. The Lancet HIV. 2016; 3:e361-e87.

23. UNAIDS fact sheet 2017. Available: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf. Accessed: 02/07/2018. UNAIDS. 2017.

24. Barnighausen T. The HIV treatment cascade and antiretroviral impact in different populations. Curr Opin HIV AIDS. 2015; 10:391-4.

25. Simonetti FR, Kearney MF. Review: Influence of ART on HIV genetics. Curr Opin HIV AIDS. 2015; 10:49-54.

26. Fauci AS, Folkers GK. Toward an aids-free generation. JAMA. 2012; 308:343-4.

27. Lake JE, Currier JS. Metabolic disease in HIV infection. The Lancet Infectious Diseases. 2013; 13:964-75.

28. Cohen SD, Kopp JB, Kimmel PL. Kidney Diseases Associated with Human Immunodeficiency Virus Infection. N Engl J Med. 2017; 377:2363-74.

29. Kooman JP, van der Sande FM, Leunissen KM. Kidney disease and aging: A reciprocal relation. Exp Gerontol. 2017; 87:156-9.

30. Nadkarni GN, Konstantinidis I, Wyatt CM. HIV and the aging kidney. Curr Opin HIV AIDS. 2014; 9:340-5.

31. Rosenberg AZ, Naicker S, Winkler CA, Kopp JB. HIV-associated nephropathies: epidemiology, pathology, mechanisms and treatment. Nat Rev Nephrol. 2015; 11:150-60.

32. Estrella MM, Fine DM. Screening for chronic kidney disease in HIV-infected patients. Adv Chronic Kidney Dis. 2010; 17:26-35.

33. Johnson JR. Hospital-onset infections: A patient safety issue. Annals of Internal Medicine. 2003; 139:233-4.

34. Naicker S, Rahmanian S, Kopp JB. HIV and chronic kidney disease. Clin Nephrol. 2015; 83:32-8.

35. Tsui J, Vittinghoff E, Anastos K, Augenbraun M, Young M, Nowicki M, et al. Hepatitis C seropositivity and kidney function decline among women with HIV: data from the Women's Interagency HIV Study. Am J Kidney Dis. 2009; 54:43-50.

36. Szczech LA, Hoover DR, Feldman JG, Cohen MH, Gange SJ, Gooze L, et al. Association between renal disease and outcomes among HIV-infected women receiving or not receiving antiretroviral therapy. Clin Infect Dis. 2004; 39:1199-206.

37. Lucas GM, Jing Y, Sulkowski M, Abraham AG, Estrella MM, Atta MG, et al. Hepatitis C viremia and the risk of chronic kidney disease in HIV-infected individuals. J Infect Dis. 2013; 208:1240-9.

38. Nobakht E, Cohen SD, Rosenberg AZ, Kimmel PL. HIV-associated immune complex kidney

disease. Nat Rev Nephrol. 2016; 12:291-300.

39. De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, et al. 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.

40. El-Sadr WM, Mullin CM, Carr A, Gibert C, Rappoport C, Visnegarwala F, et al. Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naive cohort. HIV Med. 2005; 6:114-21.

41. Seaberg EC, Munoz A, Lu M, Detels R, Margolick JB, Riddler SA, 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.

42. Miguez-Burbano MJ, Wyatt C, Lewis JE, Rodriguez 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.

43. Nishijima T, Kawasaki Y, Mutoh Y, Tomonari K, Tsukada K, Kikuchi 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.

44. Mocroft A, Kirk O, Reiss P, De Wit S, Sedlacek D, Beniowski M, et al. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. AIDS. 2010; 24:1667-78.

45. Ryom L, Mocroft A, Kirk O, Ross M, Reiss P, Fux CA, et al. Predictors of advanced chronic kidney disease and end-stage renal disease in HIV-positive persons. AIDS. 2014; 28:187-99.

46. Kasembeli AN, Duarte R, Ramsay M, Mosiane P, Dickens C, Dix-Peek T, et al. APOL1 Risk Variants Are Strongly Associated with HIV-Associated Nephropathy in Black South Africans. J Am Soc

Nephrol. 2015; 26:2882-90.

47. Kopp JB, Nelson GW, Sampath K, Johnson RC, Genovese G, An P, et al. APOL1 Genetic Variants in Focal Segmental Glomerulosclerosis and HIV-Associated Nephropathy. Journal of the American Society of Nephrology : JASN. 2011; 22:2129-37.

48. Keller BJ, Martini S, Sedor JR, Kretzler M. A systems view of genetics in chronic kidney disease. Kidney International. 81:14-21.

49. Ekstrom TJ, Stenvinkel P. The epigenetic conductor: a genomic orchestrator in chronic kidney disease complications? J Nephrol. 2009; 22:442-9.

50. McCaughan JA, McKnight AJ, Courtney AE, Maxwell AP. Epigenetics: time to translate into transplantation. Transplantation. 2012; 94:1-7.

51. Dwivedi RS, Herman JG, McCaffrey TA, Raj DS. Beyond genetics: epigenetic code in chronic kidney disease. Kidney Int. 2011; 79:23-32.

52. Feinberg AP. Epigenetics at the epicenter of modern medicine. Jama. 2008; 299:1345-50.

53. Liakopoulos V, Georgianos PI, Eleftheriadis T, Sarafidis PA. Epigenetic mechanisms and kidney diseases. Curr Med Chem. 2011; 18:1733-9.

54. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-Wide Association Studies for common human diseases. Nature reviews Genetics. 2011; 12:529-41.

55. Fultz SLMDMPH, Skanderson MMSW, Mole LAP, Gandhi NMD, Bryant KP, Crystal SP, et al. Development and Verification of a "Virtual" Cohort Using the National VA Health Information System. Medical Care Alcohol in HIV Infection: Insights from the Veterans Aging Cohort Study and the Veterans Affairs National Health Information System. 2006; 44:S25-S30.

56. Zhang X, Justice AC, Hu Y, Wang Z, Zhao H, Wang G, et al. Epigenome-wide differential DNA

methylation between HIV-infected and uninfected individuals. Epigenetics. 2016:1-11.

57. Nelson KN, Hui Q, Rimland D, Xu K, Freiberg MS, Justice AC, 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.

58. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009; 150:604-12.

59. Justice AC, Modur SP, Tate JP, Althoff KN, Jacobson LP, Gebo KA, et al. Predictive accuracy of the Veterans Aging Cohort Study index for mortality with HIV infection: a North American cross cohort analysis. J Acquir Immune Defic Syndr. 2013; 62:149-63.

60. Lehne B, Drong AW, Loh M, Zhang W, Scott WR, Tan S-T, et al. A coherent approach for analysis of the Illumina HumanMethylation450 BeadChip improves data quality and performance in epigenome-wide association studies. Genome biology. 2015; 16:37.

61. Horvath S. DNA methylation age of human tissues and cell types. Genome biology. 2013; 14:R115-R.

62. Adalsteinsson BT, Gudnason H, Aspelund T, Harris TB, Launer LJ, Eiriksdottir G, et al. Heterogeneity in white blood cells has potential to confound DNA methylation measurements. PLoS One. 2012; 7:e46705.

63. 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.

64. Garagnani P, Bacalini MG, Pirazzini C, Gori D, Giuliani C, Mari D, et al. Methylation of ELOVL2 gene as a new epigenetic marker of age. Aging cell. 2012; 11:1132-4.

65. 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.

66. Birney E, Smith GD, Greally JM. Epigenome-wide Association Studies and the Interpretation of Disease -Omics. PLoS Genet. 2016; 12:e1006105.

67. Barfield RT, Almli LM, Kilaru V, Smith AK, Mercer KB, Duncan R, et al. Accounting for Population Stratification in DNA Methylation Studies. Genetic epidemiology. 2014; 38:231-41.

68. Klebaner D, Huang Y, Hui Q, Taylor JY, Goldberg J, Vaccarino V, et al. X chromosome-wide analysis identifies DNA methylation sites influenced by cigarette smoking. Clinical epigenetics. 2016; 8:20.

69. Dabla PK. Renal function in diabetic nephropathy. World Journal of Diabetes. 2010; 1:48-56.

70. Yamada Y, Noborisaka Y, Ishizaki M, Yamazaki M, Honda R, Yokoyama H, et al. Different association of cigarette smoking with GFR estimated from serum creatinine and that from serum cystatin C in the general population. Clin Exp Nephrol. 2015; 19:669-77.

71. Horvath S, Levine AJ. HIV-1 Infection Accelerates Age According to the Epigenetic Clock. The Journal of Infectious Diseases. 2015; 212:1563-73.

72. Malmanche N, Maia A, Sunkel CE. The spindle assembly checkpoint: Preventing chromosome mis-segregation during mitosis and meiosis. FEBS Letters. 2006; 580:2888-95.

73. Karsli-Ceppioglu S, Ngollo M, Adjakly M, Dagdemir A, Judes G, Lebert A, et al. Genome-wide DNA methylation modified by soy phytoestrogens: role for epigenetic therapeutics in prostate cancer? Omics. 2015; 19:209-19.

74. Zali H, Rezaei-Tavirani M, Vafaee R, Rezaei-Tavirani M. Gastric cardia adenocarcinoma pathway analysis. Gastroenterology and Hepatology From Bed to Bench. 2013; 6:S11-S8.

75. Trost S, Diekhof EK, Mohr H, Vieker H, Kramer B, Wolf C, et al. Investigating the Impact of a Genome-Wide Supported Bipolar Risk Variant of MAD1L1 on the Human Reward System. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2016; 41:2679-87.

76. Ruderfer DM, Fanous AH, Ripke S, McQuillin A, Amdur RL, Gejman PV, et al. Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia. Molecular psychiatry. 2014; 19:1017-24.

77. Iritani BM, Delrow J, Grandori C, Gomez I, Klacking M, Carlos LS, et al. Modulation of T-lymphocyte development, growth and cell size by the Myc antagonist and transcriptional repressor Mad1. The EMBO Journal. 2002; 21:4820-30.

78. 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.

79. Pinto M, Vieira J, Ribeiro FR, Soares MJ, Henrique R, Oliveira J, et al. Overexpression of the Mitotic Checkpoint Genes BUB1 and BUBR1 is Associated with Genomic Complexity in Clear Cell Kidney Carcinomas. Cellular Oncology : the Official Journal of the International Society for Cellular Oncology. 2008; 30:389-95.

80. Sleiman P, Wang D, Glessner J, Hadley D, Gur RE, Cohen N, et al. GWAS meta analysis identifies TSNARE1 as a novel Schizophrenia / Bipolar susceptibility locus. Sci Rep. 2013; 3:3075.

81. Tzeng N-S, Hsu Y-H, Ho S-Y, Kuo Y-C, Lee H-C, Yin Y-J, et al. Is schizophrenia associated with an increased risk of chronic kidney disease? A nationwide matched-cohort study. BMJ Open. 2015; 5.

82. ADGRB1 Gene GeneCards. Available at: http://www.genecards.org/cgi-bin/carddisp.pl?gene=ADGRB1. Accessed at 03/26/2018. Human Gene Database.

83. Nijmeijer S, Vischer HF, Leurs R. Adhesion GPCRs in immunology. Biochem Pharmacol. 2016; 114:88-102.

84. 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.

85. LTV1 Gene GeneCards. Available at: http://www.genecards.org/cgi-bin/carddisp.pl?gene=LTV1&keywords=LTV1. Accessed at 03/26/2018. Human Gene Database.

86. DLGAP2 Gene GeneCards. Available: http://www.genecards.org/cgi-bin/carddisp.pl?gene=DLGAP2. Accessed: 03/26/2018. Human Gene Database.

87. LMX1A Gene GeneCards. Available: http://www.genecards.org/cgi-bin/carddisp.pl?gene=LMX1A&keywords=LMX1A. Accessed: 03/26/2018. Human Gene Database.

88. Arnold R, Issar T, Krishnan AV, Pussell BA. Neurological complications in chronic kidney disease. JRSM cardiovascular disease. 2016; 5:2048004016677687.

89. Andrade RC, Nevado J, de Faria Domingues de Lima MA, Saad T, Moraes L, Chimelli L, et al. Segmental uniparental isodisomy of chromosome 6 causing transient diabetes mellitus and merosin-deficient congenital muscular dystrophy. American journal of medical genetics Part A. 2014; 164a:2908-13.

90. Kristiansson K, Perola M, Tikkanen E, Kettunen J, Surakka I, Havulinna AS, 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. Circulation Cardiovascular genetics. 2012; 5:242-9.

91. Wei Z, Zhang K, Wen G, Balasubramanian K, Shih PA, Rao F, et al. Heredity and cardiometabolic risk: naturally occurring polymorphisms in the human neuropeptide Y(2) receptor promoter disrupt

multiple transcriptional response motifs. Journal of hypertension. 2013; 31:123-33.

92. Jezegou A, Llinares E, Anne C, Kieffer-Jaquinod S, O'Regan S, Aupetit J, et al. Heptahelical protein PQLC2 is a lysosomal cationic amino acid exporter underlying the action of cysteamine in cystinosis therapy. Proc Natl Acad Sci U S A. 2012; 109:E3434-43.

93. 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.

94. Kimura K, Wakamatsu A, Suzuki Y, Ota T, Nishikawa T, Yamashita R, et al. Diversification of transcriptional modulation: large-scale identification and characterization of putative alternative promoters of human genes. Genome research. 2006; 16:55-65.

95. Wahl S, Drong A, Lehne B, Loh M, Scott WR, Kunze S, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature. 2017; 541:81-6.

96. Demerath EW, Guan W, Grove ML, Aslibekyan S, Mendelson M, Zhou YH, et al. Epigenome-wide association study (EWAS) of BMI, BMI change and waist circumference in African American adults identifies multiple replicated loci. Human molecular genetics. 2015; 24:4464-79.