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14. ABSTRACT

This study was designed to evaluate the effect of valproic acid on a mouse model of proteinuric kidney disease and to examine several large human databases for protective signals of valproic acid among patients with and without proteinuria in terms of long-term kidney function. Over the reporting period, we have elucidated several mechanisms that may explain the observed benefit of VPA in mice and have confirmed a significant reduction in the rate of kidney function decline among humans being treated with valproic acid. These finding have been submitted to a major medical journal and are currently in peer-review.

15. SUBJECT TERMS
Focal Segmental Glomerulosclerosis, Proteinuria, Valproic Acid, Longitudinal, Veterans Affairs, Electronic Health Record, kidney, kidney function, chronic kidney disease

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Introduction

Our prior research in a mouse model of proteinuric kidney disease revealed upregulation of histone deacetylase. Inhibition of this protein with valproic acid resulted in improved kidney function and survival in mice. The purpose of this research is to understand the mechanism linking histone deacetylase activity to worsening kidney function in mice, and to confirm that such effects might be present in humans using several large national databases.

Keywords

Focal Segmental Glomerulosclerosis, Proteinuria, Valproic Acid, Longitudinal, Veterans Affairs, Electronic Health Record, kidney, kidney function, chronic kidney disease, histone deacetylase

Accomplishments

What were the major goals of the project?

Aim1: To test the hypothesis that Vorinostat, reduces the progression of mouse models of FSGS.

Aim 2: To test the hypothesis that inhibition of HDAC1 and 2 in mice by VPA targets podocytes specifically.

Aim 3: To test the hypothesis that a common set of podocyte target genes is regulated by Hdac1 and 2 inhibition following glomerular injury.

Aim 4: To evaluate the impact of HDAC exposure on incidence and progression of CKD in two large cohorts.

Aim 5: To determine patient characteristics that best predict benefit from HDAC inhibition.

What was accomplished under these goals?

SAHA prevents progression kidney failure in *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice (Ishibe)

Our preliminary results during our grant submission demonstrated the importanceof VPA in mitigating focal segmental glomerulosclerosis progression in our genetic mouse model *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice.

Because of the compelling effect of VPA on progression of glomerulosclerosis in the *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice, we administered Vorinostat (suberanilohydroxamic acid (SAHA) (20 mg/kg BW) -another FDA approved HDAC inhibitor for the treatment of cutaneous T cell lymphoma to examine whether there was a class effect with HDAC inhibitors. Similarly to VPA, treatment of the mutant mice with SAHA for 4 weeks following the completion of doxycycline induction, resulted in stabilized albuminuria, mitigated the rise of serum creatinine, and inhibited glomerulosclerosis and interstitial fibrosis (Figure 1a, 1b, 1c, 1d, and 1e and quantified in Figure 1g, and 1h). Ultrastructural examination of podocytes after SAHA treatment also demonstrated a reduction in foot process effacement (Figure 1f and quantified in Figure 1i).

Figure 1

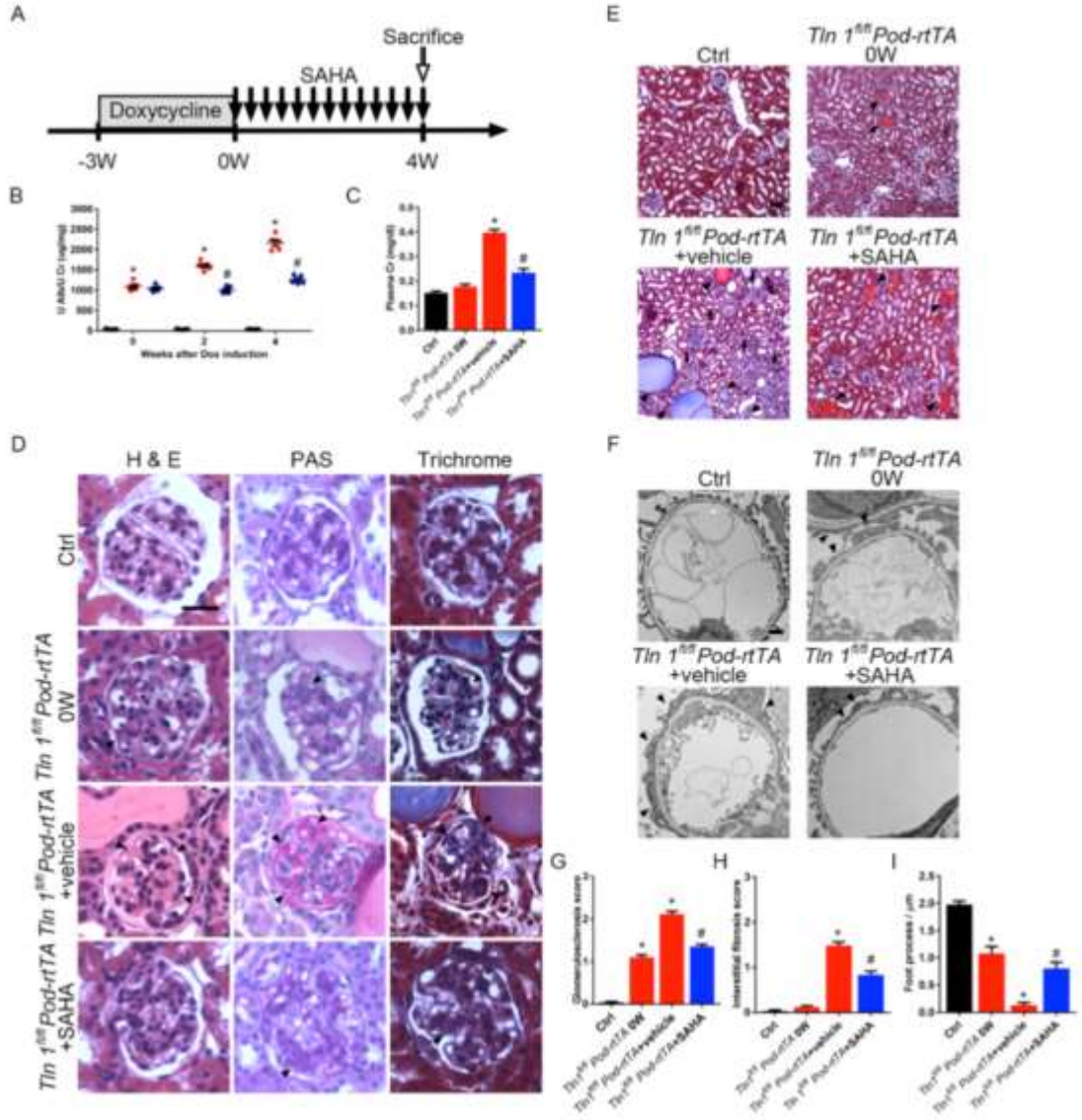


Figure 1 Vorinostat (suberanilohydroxamic acid (SAHA)) reduces podocyte injury in Doxycycline-inducible podocyte specific *Tln1* KO mice. (A) A cartoon schematic of the time course of *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice treated +/- SAHA after completing Dox induction. (B) Quantification of urine albumin/ creatinine ratio in control (black) and *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice treated +/- SAHA (red: -SAHA, blue: +SAHA) 0, 2, and 4 weeks after completing Dox induction *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice. N=5. (C) Plasma creatinine in control and *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice treated +/- SAHA at 0 and 4 weeks after completing Dox induction. *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice. N=5. (D) Representative H & E, PAS, and trichrome staining in control and *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice glomerulus treated +/- SAHA at 0 and 4 weeks after completing Dox induction. Arrowheads show mesangial matrix deposition and mesangial cell proliferation. Scale bar: 25 μm. (E) Representative trichrome staining in control and *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice kidney with treated +/- SAHA, at 0 and 4 weeks after completing Dox induction. Arrowheads show dilated tubules and proteinaceous casts, and arrows display interstitial fibrosis. Scale bar: 50 μm. (F) Representative TEM in control and *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice treated +/- SAHA at 0 and 4 weeks after completing Dox induction. Arrowheads depict

podocyte foot process effacement. Scale bar: 1 μm. (G) Quantification of glomerulosclerosis in (D). *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice. (H) Quantification of interstitial fibrosis in (E). *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice. (I) Quantification of foot process in (F). *p <0.05 compared with control mice, and #p <0.05

VPA and SAHA treatment in toxin induced mouse glomerular injury models (Ishibe)

To also examine toxin-induced mouse models of podocyte injury, we treated wild type C57BL/6 mice with NTS, and wild type BALB/c mice with Adriamycin, which induced glomerular total HDAC activity (Figure 2a), as these models can induce glomerulosclerosis. Treatment of these mice with VPA or SAHA reduced albuminuria and improved glomerular lesions that were provoked following NTS or Adriamycin administration (Figure 2b, 2c, 2d, and 2e).

Figure 2

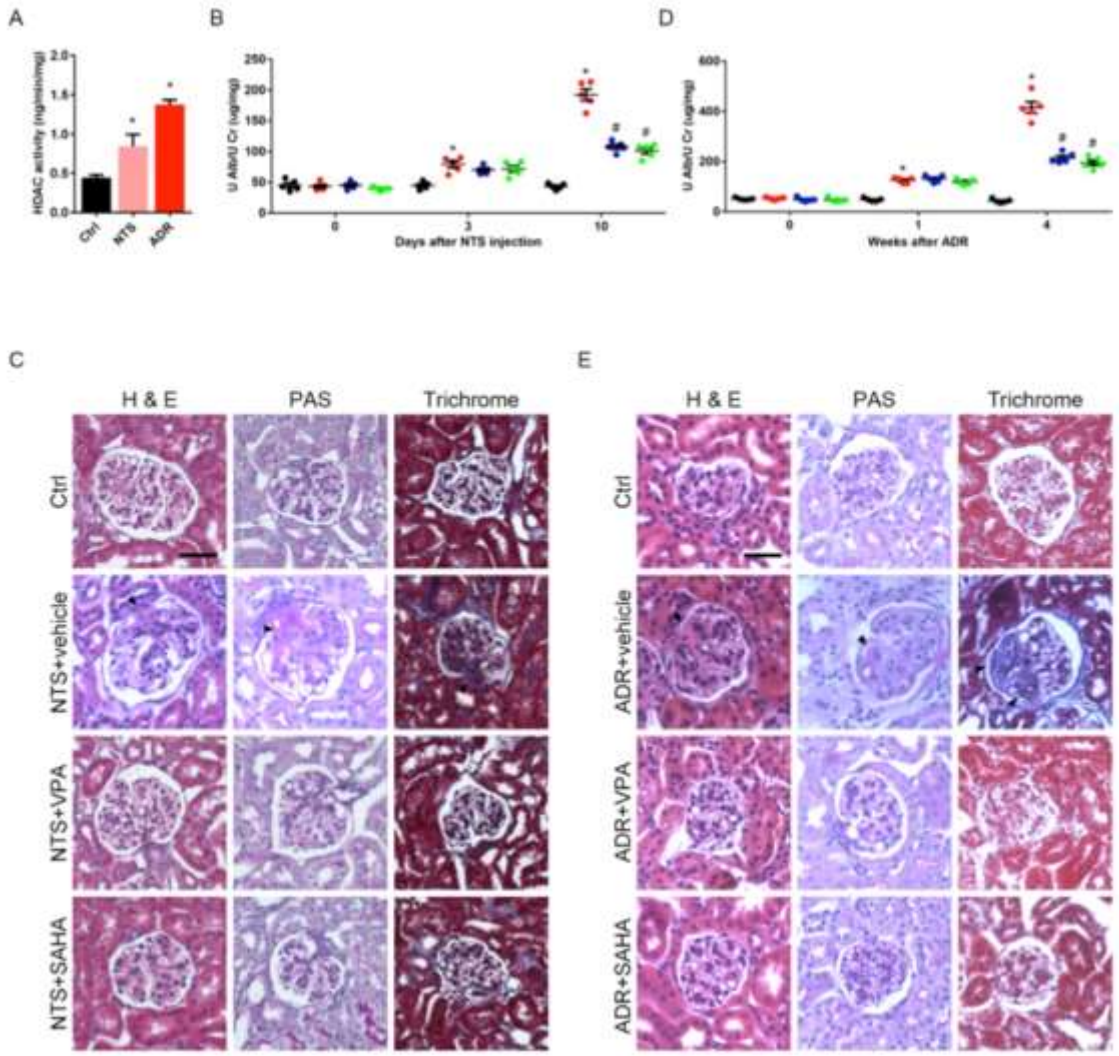


Figure 2. VPA or SAHA treatment reduces urine albumin and glomerular injury induced by NTS or Adriamycin. (A) Total HDAC activity in NTS or Adriamycin (ADR)-injected mice glomerulus. *p <0.05 compared with control mice. N=3. (B) Quantification of urine albumin/creatinine ratio at 0, 3, and 10 days after NTS injection treated +/- VPA, or SAHA (black: -NTS, red: +NTS, blue: +NTS+VPA, green: +NTS+SAHA). *p <0.05 compared with control mice, and #p <0.05 compared with NTS-injected control mice. N=5. (C) Representative light microscope images (H&E, PAS, and trichrome) of glomerulus from NTS-injected mice treated +/- VPA. Arrowheads show mesangial matrix deposition and mesangial cell proliferation. Scale bar: 25 μm. (D) Quantification of urine albumin/creatinine ratio at 0, 1, and 4 weeks after ADR injection with vehicle, VPA, or SAHA (black: -ADR, red: +ADR, blue: +ADR+VPA, green: +ADR+SAHA). *p <0.05 compared with control mice, and #p <0.05 compared with ADR-injected control mice. N=5. (E) Representative light microscope images (H&E, PAS, and trichrome) of glomerulus from ADR-injected mice treated +/- VPA and SAHA. Arrowheads show mesangial matrix deposition and mesangial cell proliferation. Scale bar: 25 μm.

Generation and Characterization of *Hdac1*$^{fl/fl}$ *Hdac2*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice (Ishibe)

Because HDAC1 and HDAC2 activity were increased in enriched podocytes obtained from *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice, and due to the beneficial effect of HDAC inhibitors in the mice, we next wanted to identify whether podocyte HDAC1 and HDAC2 activation contribute to progression of glomerular injury. As the deletion of only *Hdac1* or *Hdac2* alone in podocytes did not significantly improve glomerular lesion (data not shown), we tested whether loss of both HDAC1 and 2 in podocytes mitigates the progression of glomerular injury. We initially confirmed that tissue specific excision of HDAC1 and HDAC 2 in the Dox-inducible podocyte specific *Hdac1* and *Hdac2* KO mice (*Hdac1* $^{fl/fl}$ *Hdac2* $^{fl/fl}$ *Pod-rtTA TetO-Cre* mice) (Figure 3a), displayed no kidney phenotype after completing doxycycline induction, as demonstrated by kidney histology, urine albumin and plasma creatinine (l Figure-3b, 3c, 3d, and 3e).

Figure 3

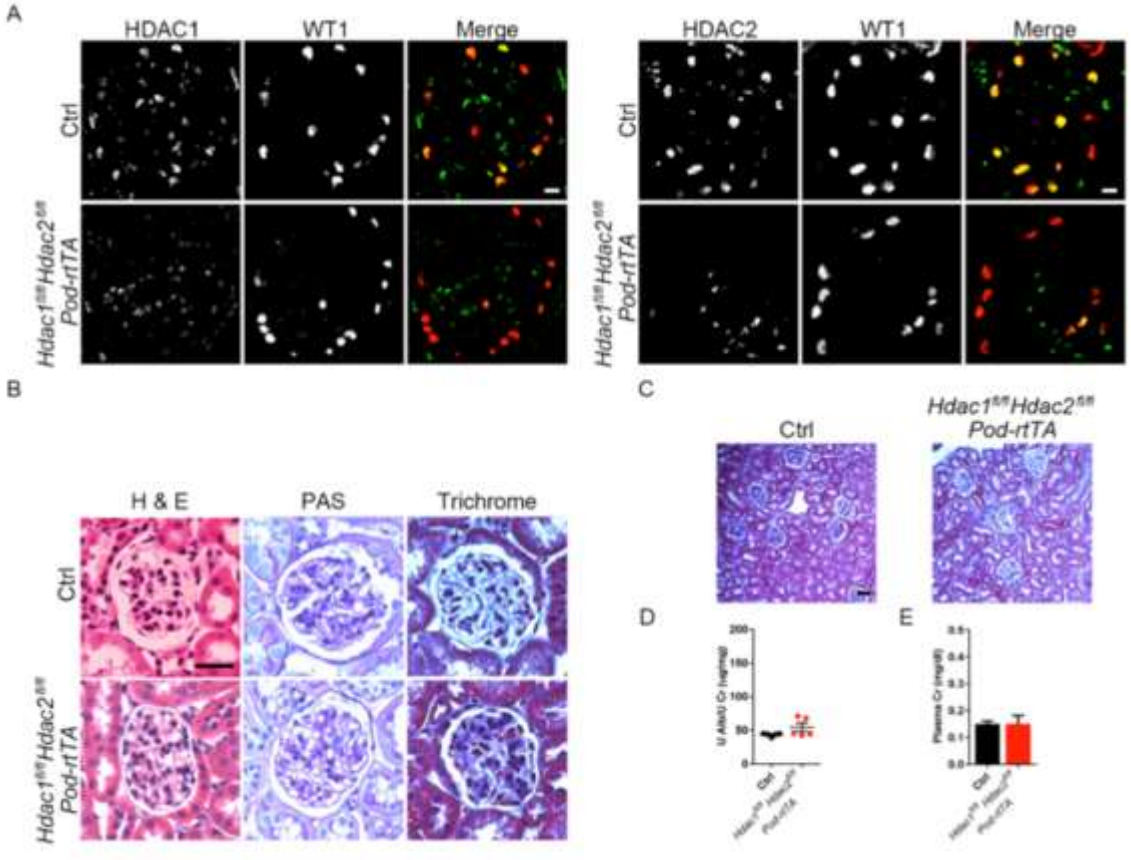


Figure 3. Doxycycline-inducible podocyte specific *Hdac1 and Hdac2* DKO mice reduce NTS-induced podocyte injury. (A) Quantification of urine albumin/creatinine at 0 and 7 days after NTS injection in control (black) and *Hdac1*$^{fl/fl}$*, Hdac2*$^{fl/fl}$*, Pod-rtTA TetO-Cre* mice (blue). *p <0.05 compared with control mice before NTS injection (day 0), and #p <0.05 compared with control mice with NTS (day 7). N=5. (B) Representative

light microscopy images (H&E, PAS, and trichrome) of glomerulus from control and *Hdac1$^{fl/fl}$, Hdac2$^{fl/fl}$, Pod-rtTA TetO-Cre* mice 7 days after NTS injection. Arrowheads show mesangial matrix deposition and mesangial cell proliferation. Scale bar: 25 μm. (C) Representative trichrome staining in control and *Hdac1$^{fl/fl}$, Hdac2$^{fl/fl}$, Pod-rtTA TetO-Cre* mice kidney 4 weeks after completing Dox induction. Scale bar: 50 μm. (D) Quantification of urine albumin/creatinine ratio in control and *Hdac1$^{fl/fl}$, Hdac2$^{fl/fl}$, Pod-rtTA TetO-Cre* mice 4 weeks after completing Dox induction. N=5. (E) Plasma creatinine in control and *Hdac1$^{fl/fl}$, Hdac2$^{fl/fl}$, Pod-rtTA TetO-Cre* mice 4 weeks after completing Dox induction. N=5.

Generation and Characterization of *Hdac1$^{fl/fl}$ Hdac2$^{fl/fl}$ Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice (Ishibe)

We next examined whether podocyte specific loss of *Hdac1* and *Hdac2* would provide protection in the *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice. Four weeks after completing doxycycline induction in the *Hdac1$^{fl/fl}$ Hdac2$^{fl/fl}$ Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice, we observed stable urinary albumin/creatinine ratio (ACR) and markedly improved kidney function compared to the *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice (Figure 4a and 4b). Histological analysis also displayed reductions in glomerulosclerosis, and interstitial fibrosis in the *Hdac1$^{fl/fl}$ Hdac2$^{fl/fl}$ Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice (Figure 4c and 4d quantified in Figure 4f and 4g). Ultrastructural examination of foot processes demonstrated reduced foot process effacement in the *Hdac1$^{fl/fl}$ Hdac2$^{fl/fl}$ Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice (Figure 4e, quantified in Figure 4h).

Figure 4

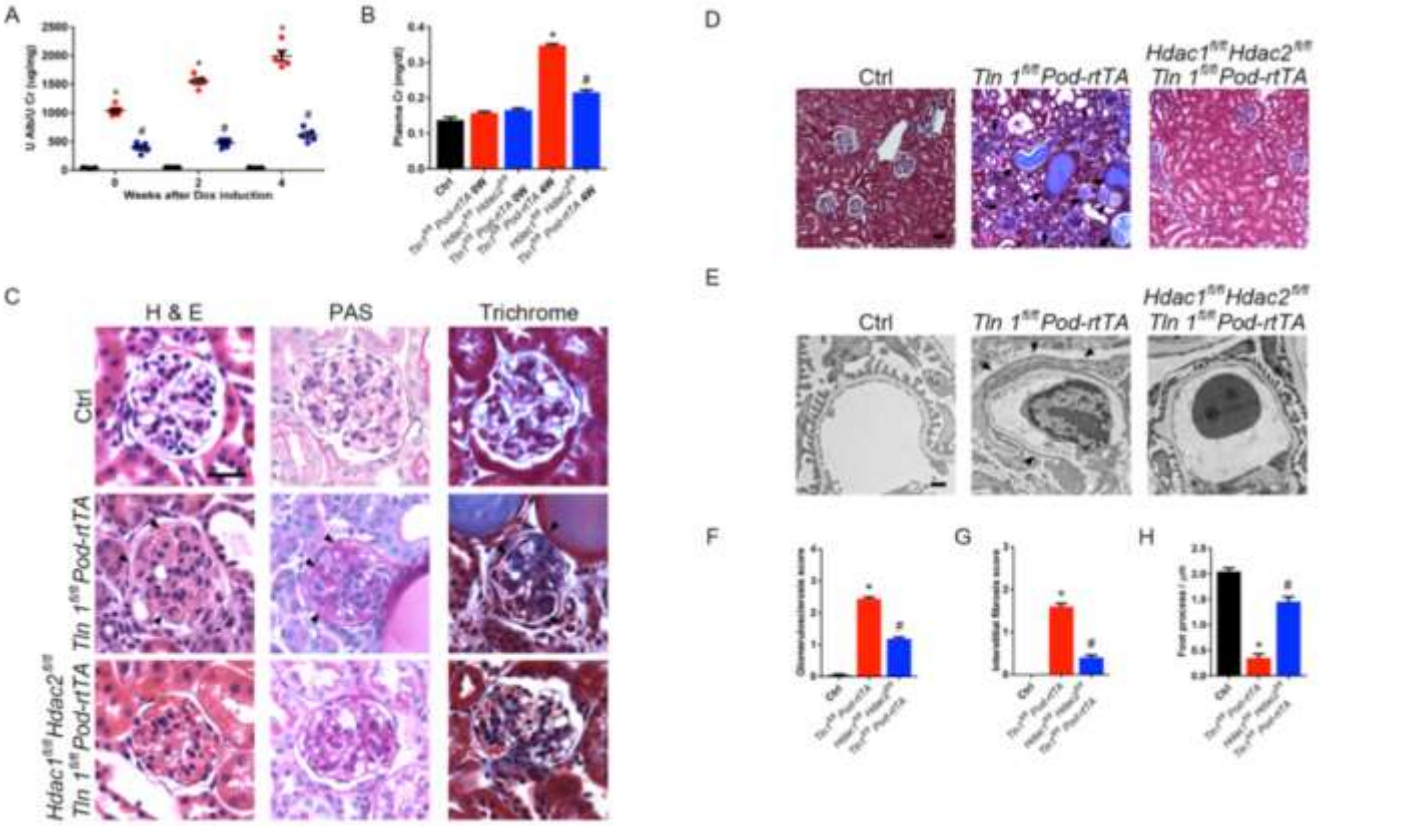
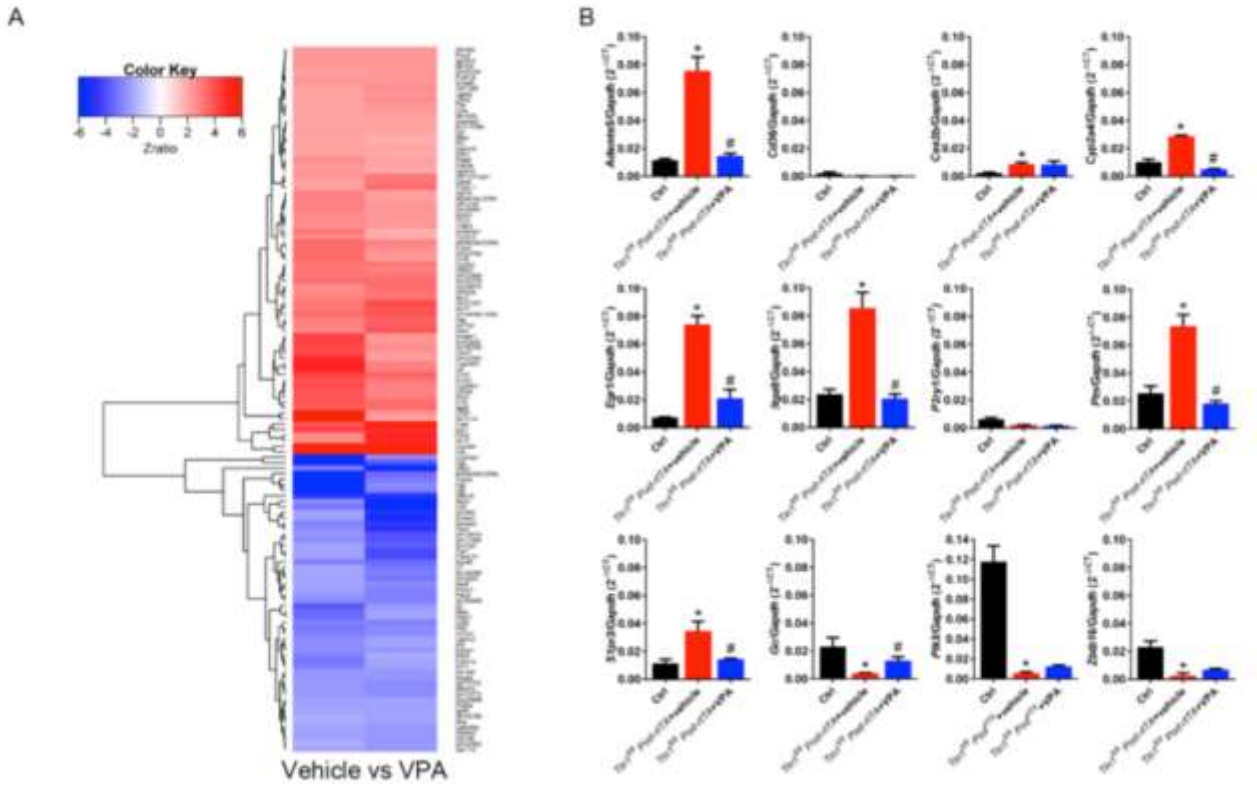


Figure 4. Podocyte deletion of *Hdac1* and *Hdac2* improves albuminuria and glomerulosclerosis in *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice induced with Dox. (A) Quantification of urine albumin/creatinine ratio in control (black), *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* (red) and *Hdac1* $^{fl/fl}$ *Hdac2* $^{fl/fl}$ *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* (blue) mice at 0, 2, and 4 weeks after completing Dox induction. *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice. N=5. (B) Plasma creatinine in control, *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* and *Hdac1* $^{fl/fl}$ *Hdac2* $^{fl/fl}$ *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice after completing Dox induction. *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice. N=5. (C) Representative light microscopy images (hematoxylin-eosin [H&E], periodic acid-Schiff [PAS], and trichrome) of control, *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice and *Hdac1* $^{fl/fl}$ *Hdac2* $^{fl/fl}$ *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice glomerulus 4 weeks after completing Dox induction. Arrowheads show mesangial matrix deposition and mesangial cell proliferation. Scale bar: 25 μm. (D) Representative trichrome staining in control, *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* and *Hdac1* $^{fl/fl}$ *Hdac2* $^{fl/fl}$ *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice kidney 4 weeks after completing Dox induction. Arrowheads show dilated tubules and proteinaceous casts, and arrows display interstitial fibrosis. Scale bar: 50 μm. (E) Representative transmission electron micrograph (TEM) in control, *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* and *Hdac1* $^{fl/fl}$ *Hdac2* $^{fl/fl}$ *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice kidney 4 weeks after completing Dox induction. Arrowheads depict podocyte foot process effacement. Scale bar: 1 μm. (F) Quantification of glomerulosclerosis in (C). *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice. (G) Quantification of interstitial fibrosis in (D). *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice. (H) Quantification of foot process in (E). *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice

Identifying increased early growth response 1 (EGR1) expression in *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice and is reversed by VPA and SAHA. (Ishibe)

Because HDAC inhibitors appear to play a critical role in maintaining the integrity of the glomerular filtration barrier following podocyte injury, we examined microarray from glomeruli of *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice with VPA in comparison with the original *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* glomeruli microarray (Figure 5a), and identified 28 genes, which were potentially reversible following VPA treatment. We confirmed by RT-PCR the group of 28 genes that were ascertained from this second microarray (Figure 5a). Of these 28 genes, transcription factor, early growth response 1 (*Egr1*), a gene shown to modulate the actin cytoskeleton and cell death (31-33), was detected to be the most highly expressed.

Figure 5



Examining VPA in patients from the Veterans Aging Cohort Study (Wilson)

Specific Aim 4: Evaluate the effect of HDAC inhibition in three large human cohorts.

Several subtasks are associated with this aim:

Local IRB approval was obtained early during the reporting period. (Completion: 100%)

Subtask 1: Obtain and clean data from VACS, VABC, and Geisinger (months 1-15) (Completion: 66%)

We have obtained and cleaned data from VACS and Geisinger. However the Veterans Aging Birth Cohort has not yet completed its initial data freeze, limiting our ability to interrogate this dataset. However the data structure is designed to be highly similar to VACS. As such, we are confident that once we gain access to this data, we can run our primary analysis expeditiously.

Subtask 2: Validate primary exposure and outcome metrics (months 3-15) (Completion: 100%)

We have validated valproic acid exposure in the two available datasets in several ways. First, we engaged in direct medical record review on a subset of patients in each dataset to confirm that valproic acid was indeed prescribed as indicated in the master dataset. Second, we examined those patients with measurable valproic acid levels to confirm that our exposure definition included them as having received valproic acid. These analyses demonstrated that our capture of VPA exposure was highly accurate.

We have validated several outcomes in the cohorts as well including: slope of estimated glomerular filtration rate, time to chronic kidney disease, time to dialysis, time to doubling of creatinine, and time to death.

Subtask 3: Model incidence of chronic kidney disease by valproate exposure in time-varying Cox proportional hazards analysis (*months 6-24)*

We are currently conducting this analysis in the VACS and Geisinger datasets.

Subtask 4: Model progression of chronic kidney disease by valproate exposure in mixed-effects analysis (months 6-24).

We have completed this analysis in the VACS dataset and the results are included in our manuscript submission that is currently under review. We are actively conducting this analysis in the Geisinger dataset.

Specific Aim 5: Determine patient characteristics that predict benefit from HDAC inhibition

Subtask 1: Validate subgroup identification measures (months 6-15)

We have defined and validated the following subgroup identification measures:

-Heavy proteinuria

-Diabetes

-Diagnosis of Focal Segmental Glomerulosclerosis (based on administrative coding)

-Chronic Kidney Disease

Subtask 2: Validate natural language processing for biopsy-based diagnoses of primary FSGS (months 6-24).

We have requested biopsy report data from VACS and Geisinger, but have not yet received it. Thus we have not yet been able to work on natural language processing approaches to assess biopsy-proven FSGS. However our initial analysis based on administrative coding (described below) suggests that the number of cases who are also receiving VPA may be too small to make robust inferences about VPA effects.

Subtask 3: Compare magnitude of benefit of VPA exposure across pre-defined subgroups (months 15-36).

We have completed the assessment of effect modification by various subgroups within the VACS cohort and are completing this work in the Geisinger cohort. In the VACS cohort, we

have demonstrated that the use of VPA preserves kidney function much more strongly among those with proteinuria, compared to individuals without proteinuria. This aligns with the mouse model and adds credibility to our approach. We were not able to demonstrate specificity of the VPA effect to patients with FSGS however, which we believe may be due to the small number of patients with both an FSGS diagnosis and exposure to VPA.

We have made substantial progress in understanding the important relationships between comorbidities, valproic acid exposure, and longitudinal kidney function trajectory in the VACS cohort. We have created a standardized longitudinal methodology that can be applied to additional cohorts as we progress further through the funding period.

To support the hypothesis that HDAC inhibition may also be a potent therapeutic strategy against human proteinuric kidney disease, we next interrogated the Veterans Aging Cohort Study. Among 122,870 veterans participating in the Study and eligible for analysis, the median (IQR) duration of follow-up was 9.0 (4.7 - 13.2) years. The mean rate of decline in eGFR was -0.94 (standard error 0.007) ml/1.73m2/year, which is consistent with several large, US population-based studies. (39, 40). Veterans exposed to VPA were slightly younger than those who were not exposed. They also had higher baseline eGFR and were less likely to be HIV or HCV infected. They were more likely to be diabetic and to have hypertension, and bore a strikingly higher rate of psychiatric comorbidities with fully 76.5% of those in the VPA group carrying a diagnosis of bipolar disorder compared to 21.4% of those in the unexposed group (Supplementary Table 3). Exposure to valproic acid (n=2,269) was associated with a significantly attenuated rate of decline in eGFR, with the unadjusted mean annual change in eGFR of -0.61 (0.07) ml/1.73m2/year among those who received VPA compared to -0.94 (0.007) ml/1.73m2/year among those who did not receive the agent – a 35% reduction in the rate of decline. The fully-adjusted difference was 0.16 (0.07) ml/min/1.73m2/year, p=0.02 (Figure 6a). Within-patient analyses (restricted to patients who

initiated VPA while under observation) revealed that, prior to initiation of VPA, the average decline in eGFR was -0.93 (SE 0.05) ml/1.73m2/year compared to -0.32 (SE 0.09) ml/1.73m2/year after the initiation of VPA ($p<0.0001$) (Figure 6b).

Next, we analyzed the patients stratified by proteinuria. Among the patients with no or mild proteinuria (1+ or less on urine dipstick), the unadjusted mean yearly decline in eGFR was -0.92 (0.007) ml/1.73m2/year in the patients not receiving VPA, compared to -0.61 (0.07) ml/1.73m2/year in those receiving VPA (Figure 6c). In the patients with heavy proteinuria (2+ or more on urine dipstick), the unadjusted mean yearly decline in eGFR was -2.5 (0.05) ml/1.73m2/year in patients not receiving VPA, compared to -0.56 (0.66) ml/1.73m2/year in those receiving VPA (Figure 6d). After full adjustment, this effect modification by proteinuria was statistically significant at $p=0.02$, suggesting that the beneficial effect of VPA is more pronounced in patients with worse proteinuria.

These results were robust to multiple sensitivity analyses. After excluding all patients who ever received lithium (a known nephrotoxic agent that is used for similar indications as VPA in this population), the protective effect of VPA and the interaction between VPA benefit and proteinuria was maintained ($p=0.008$ and $p=0.07$ respectively). Analyses that examined the effects of the comparator agents, lamotrigine, carbamazepine, and levetiracetam revealed no protection of eGFR associated with exposure (Figure 7 a-d).

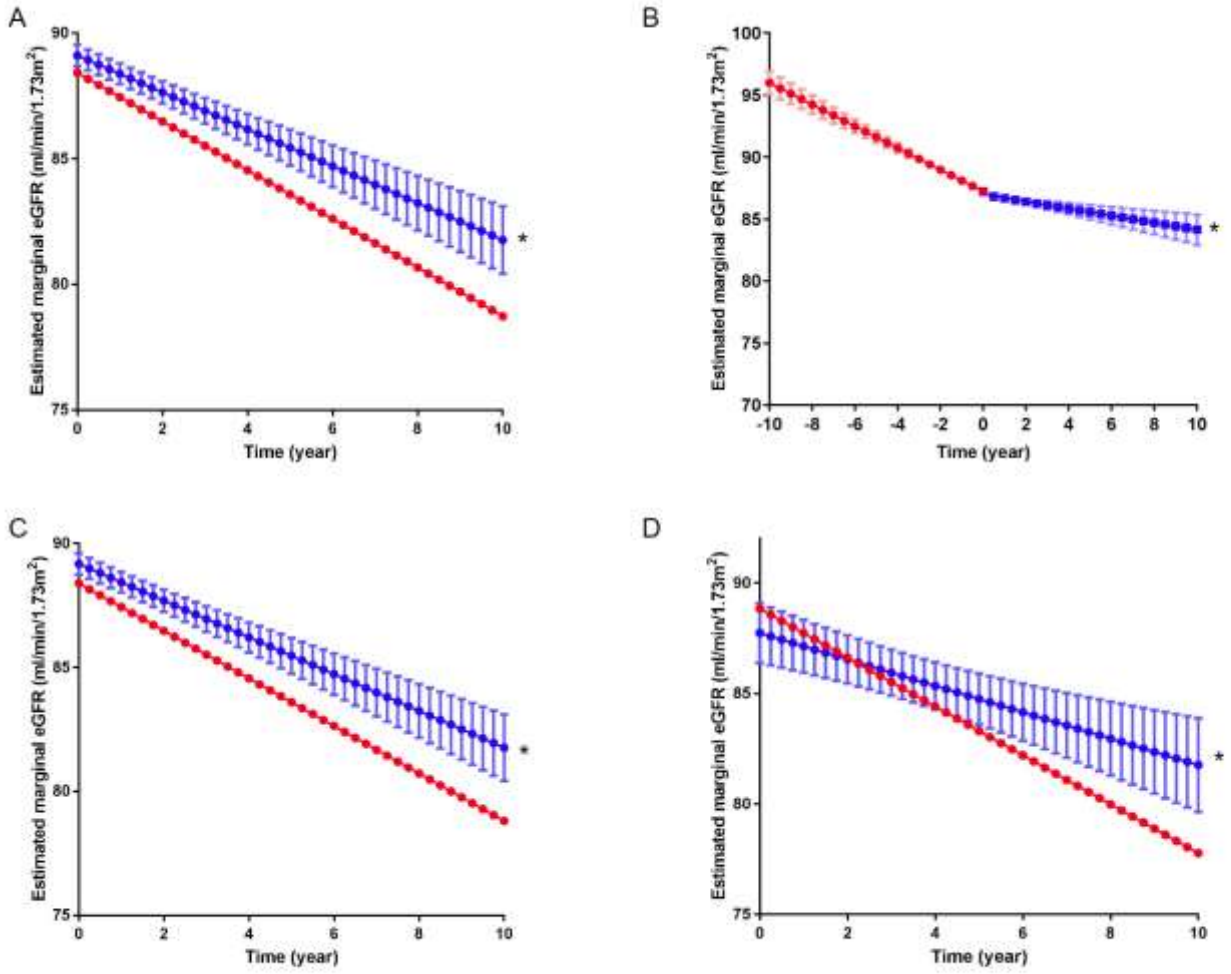


Figure 6: VPA usage is associated with slower declines in eGFR in a Veterans Affairs population cohort. A) The slope of eGFR decline among patients treated with VPA compared to controls not treated with VPA. *p=0.001. B) The slope of eGFR decline inpatients before and after initiation of VPA. *p<0.001. C and D) The slope of eGFR decline among patients with no or mild proteinuria (1+ or below on urine dipstick – C) and with heavy proteinuria (>2+ on urine dipstick – D) with or without VPA. *p-for-interaction=0.02. All graphs reflect eGFRs adjusted for age, sex, race, baseline eGFR, HCV, and HIV status and display slopes at the average values of these covariates.

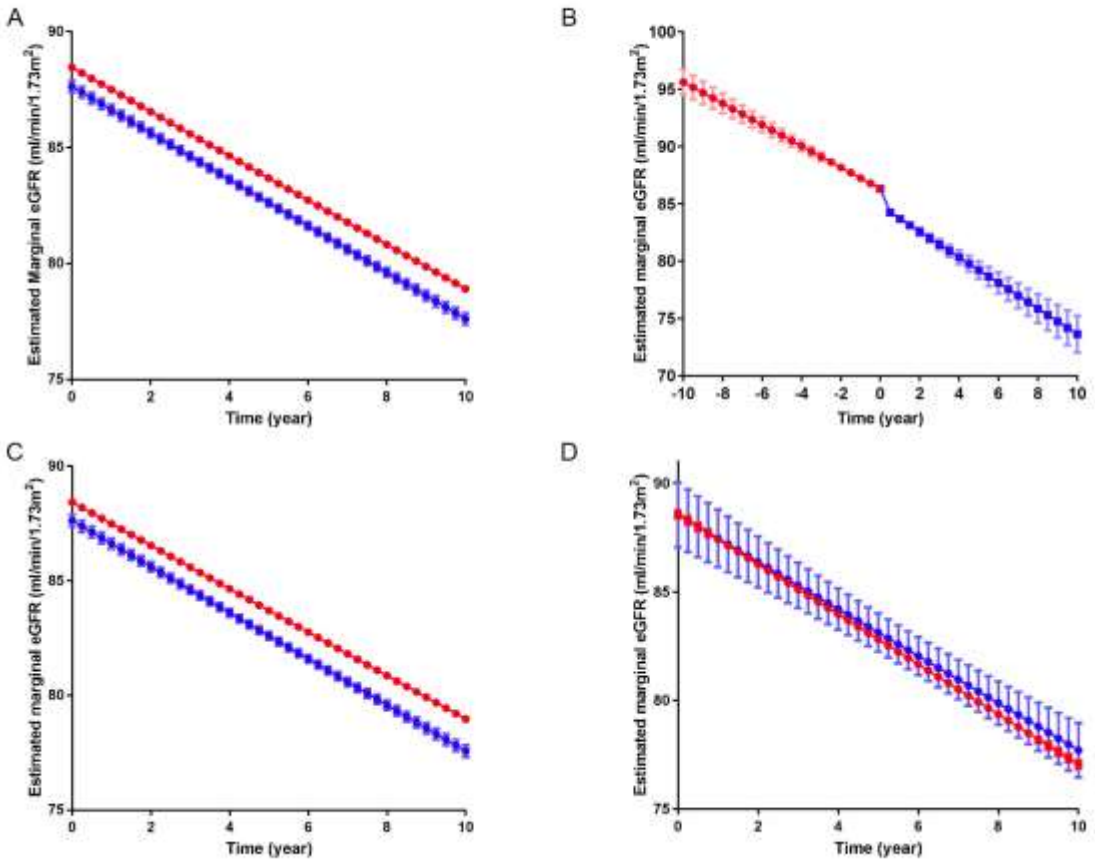


Figure 7: Comparator drugs (lamotrigine, carbamazepine, and levetiracetam) usage is not associated with declines in eGFR in a Veterans Affairs population cohort. (A) The slope of eGFR decline among patients treated with comparator drugs compared to controls not treated with comparator drugs. (B) The slope of eGFR decline in patients before and after initiation of comparator drugs. (C and D) The slope of eGFR decline among patients with no or mild proteinuria (1 + or below on urine dipstick) (C) and with heavy proteinuria (>2+ on urine dipstick) (D). with or without comparator drugs. All graphs reflect eGFRs adjusted for age, sex, race, baseline eGFR, HCV and HIV status and display slopes at the average values of these covariates.

What opportunities for training and professional development has the project provided?

Geliang Gan, a masters-level biostatistician worked closely with biostatistics faculty member Maria Ciarleglio to analyze this data and gain greater experience with longitudinal data modeling and pharmacoepidemiology.

Dr. Kazunori Inoue, who conducted research on this project obtained a position as Instructor at Osaka University School of Medicine, Japan. His American Society of Nephrology abstract has been selected as an oral presentation.

Elizabeth Cross conducted research on this project from her NIH funded summer fellowship.

How were the results disseminated to communities of interest?

These data have been presented at Veterans Affairs research-in-progress conferences as well as sectional and departmental meetings. Results from this project will be disseminated in an oral presentation at the 2018 American Society of Nephrology meeting and a manuscript is currently in revision at the Journal of Clinical Investigation.

What do you plan to do during the next reporting period to accomplish the goals?

Over the next reporting period, we will complete analysis on the Geisinger data and obtain the Veterans Birth Cohort data for analysis. We will additionally analyze time-to-event outcomes within the various datasets using time-varying Cox proportional hazards modeling. We will also work to obtain biopsy reports to evaluate the use of natural-language processing in identifying biopsy-proven cases of FSGS that may augment our ability to detect any VPA effects in this population.

Additionally, during the next reporting period, in conjunction with Dr. Ishibe we will validate from our microarray results that the protein expression of EGR1 is increased in Tln1fl/fl Pod-

rtTA TetO-Cre mice glomerulus. We shall also determine EGR1 expression in VPA or SAHA treated Tln1fl/fl Pod-rtTA TetO-Cre mice glomerulus, or in mice lacking podocyte associated Hdac1 and Hdac2 to determine whether there is a reduction in expression. EGR1 expression will also be tested in glomeruli isolated from Pod-Dnm DKO mice, which develop FSGS. To confirm the importance of EGR1 in human proteinuric disease, kidney biopsy samples from patients with FSGS will be examined for podocyte EGR1 expression. Furthermore, in vitro experiments using isolated primary podocytes from Pod-Cre Rosa-DTRflox mice will be treated with lipopolysaccharide (LPS) or protamine sulfate (PS), two agents that induce podocyte injury and EGR1 expression will be examined. To further elucidate how EGR1 expression is regulated following podocyte injury, we shall examine cAMP response element binding protein (CREB) and serum response factor (SRF) which have been previously shown to bind to the Egr1 promoter. A chromatin immunoprecipitation (ChIP) assay using CREB and SRF antibodies will be performed to determine CREB binding to CRE or SRF binding to serum response element within the Egr1 promoter in LPS or PS treated primary podocytes +/- treatment with VPA or SAHA

To next determine the importance of EGR1 in vivo, we plan to generate Egr1-/- Tln1fl/fl Pod-rtTA TetO-Cre mice, to assess whether loss of this gene could rescue the Tln1fl/fl Pod-rtTA TetO-Cre mice. We will examine changes in albuminuria, kidney failure, glomerulosclerosis, and interstitial fibrosis.

Third to elucidate a role of EGR1 upregulation in podocyte injury, we will examine F-actin staining patterns in primary podocytes, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining in Tln1fl/fl Pod-rtTA TetO-Cre mice with Dox as increased EGR1 expression has been shown to regulate the actin cytoskeleton, and induce cell apoptosis.

Impact

What was the impact on the development of the principal discipline(s) of the project?

While our results are preliminary, they suggest that a readily-available and generally well-tolerated drug might be repurposed to treat certain patients with kidney disease. Drugs like valproic acid, which are no long under patent-protection, are often not targeted for research by large pharmaceutical companies. Nevertheless, this new potential use for the agent might open a new field of therapies for patients with kidney disease.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

These findings have the possibility to qualify for use patents for proteinuric patients with FSGS as an adjuvant therapy. We have obtained a provisional patent and hope to license this to a company who can modify the drugs to improve efficacy and reduce side effects with the goal of bringing a drug candidate to clinical trials, besides VPA.

What was the impact on society beyond science and technology?

The early results of our study suggest that increased resources be devoted to “repurposing” existing medications to new uses. This study may encourage other disciplines to re-examine the common paradigm of developing new molecules to treat certain disease and instead to consider the public health benefit and efficiency of development that comes with repurposing existing agents. Broad investigation of our existing pharmaceutical

Changes/Problems

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

The Veterans Birth Cohort study comprises approximately 4.5 million individuals. We had initially tried to work with the data on VINCI (the VA internal server system) and found that the system was often down or that jobs would time out. In the past 6 months we have been downloading all data to local servers and optimizing it for use. We are now nearly at the point where we have meds and lab data ready to use.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Products

Publications, conference papers, and presentations

"Podocyte associated histone deacetylase activity regulates glomerular diseases in mice and humans". Kazunori Inoue, Geliang Gan, Maria Ciarleglio, Yan Zhang, Christopher E. Pedigo, Xuefei Tian, Corey Cavanaugh, Janet Tate, Ying Wang, Elizabeth Cross, Marwin Groener, Nathan Chai, Zhen Wang, Amy Justice, Zhenhai Zhang, Chirag R. Parikh, Francis P. Wilson, and Shuta Ishibe. Journal of Clinical Investigation (Submitted, *in revision*).

Federal Support acknowledged.

Website(s) or other Internet site(s)

We have published the following website to allow the public to keep track of papers, presentations, and new data that has resulted from this study:

https://medicine.yale.edu/intmed/patr/projects/deacetylase.aspx

Technologies or techniques

We developed and validated an operation definition of proteinuria based on clinically-collected urinalysis results within a large VA cohort. This is notable in that the values are inconsistently coded across various VA centers. We have shared this code with the VACS executive committee and will make it available to anyone who wishes to assess for clinically-detected proteinuria in a Veterans Affairs dataset.

Inventions, patent applications, and/or licenses

A provisional patent application, Composition and Method for Treating Kidney Disease has been accorded U.S. Application Serial No. 62/717,024 for use of HDAC inhibitors for treatment of kidney disease has been filed by Drs'. Kazunori Inoue, Shuta Ishibe,Chirag Parikh, and Francis Perry Wilson.

Other Products

Nothing to report

Participants and Other Collaborating Organizations

What individuals have worked on the project?

Name:	Francis Perry Wilson, MD
Project Role:	Co-Principal Investigator

Researcher Identifier (e.g. ORCID ID):	0000-0002-2633-2412
Nearest person month worked:	4
Contribution to Project:	Dr. Wilson has been involved in all areas of the project
Funding Support:	

Name:	Geliang Gan, MS
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	0000-0001-6984-754X.
Nearest person month worked:	6
Contribution to Project:	Mr. Gan has engaged in data cleaning, model building and evaluation, and manuscript preparation.
Funding Support:	

Name:	Maria Ciarleglio, PhD
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	0000-0002-6591-3674.
Nearest person month worked:	3

Contribution to Project:	Dr. Ciarleglio is overseeing all aspects of the statistical analysis of the project.
Funding Support:	

Name:	Janet Tate, PhD
Project Role:	Database Manager / Biostatistician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Tate manages the VACS database and has performed data cleaning and database creation for the project.
Funding Support:	

Name:	Amy Justice, MD
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-0139-5502
Nearest person month worked:	1
Contribution to Project:	Dr. Justice has aided with protocol development, analysis, interpretation, and manuscript preparation.

Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Geisinger Health in Danville, PA is collaborating on this project by evaluating their clinical database to validate the findings from VACS.

Special Reporting Requirements

This is a COLLABORATIVE AWARD. An independent report from BOTH the initiating PI and Collaborating PI will be provided. This current report is from the Collaborating PI (Francis Wilson). Given the collaborative nature of the work, experiments that involve materials and expertise provided by both investigators are included in this report. The reports are therefore very similar. Throughout the report, the responsible PI is shown.

Appendices

None