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14. ABSTRACT

Glomerular diseases account for approximately 80% of end stage kidney disease (ESKD). Nearly 600,000 US residents have end-stage kidney disease (ESKD) at an annual Medicare expenditure of 28 billion dollars. Through RNA profiling in our models of FSGS, we have found that HDAC activity is increased in the glomerulus and blocking with HDAC inhibitor, valproic acid or suberanilohydroxamic acid mitigates progression of kidney disease. During the last funding period, we have generated podocyte specific knockout mice for HDAC1 and 2, which also appears protective against glomerular injury in toxin mediated and genetic mouse models. In parallel, our collaborator for this study, Perry Wilson have continued to examine Veterans Affairs Cohort and discerned protective benefit for proteinuric patients who happened to be on VPA.

15. SUBJECT TERMS
Proteinuria, Chronic kidney disease, focal segmental glomerulosclerosis, nephrotic syndrome, Veterans Affairs

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Introduction

The fundamental goal of this proposal is to integrate cellular, animal models, and human databases to examine how inhibition of histone deacetylase (HDAC) activity may alter progression of proteinuric kidney disease through our partnering PI, Francis Perry Wilson, at Yale School of Medicine. We are examining the role of different HDAC inhibitor, namely suberanilohydroxamic acid (SAHA), a FDA approved drug for Cutaneous T-Cell Lymphoma, in our mouse models of focal segmental glomerulosclerosis (FSGS). Further aims were to focus on genetically deleting HDAC specifically in the podocytes to determine whether podocyte specific HDAC activation is what is responsible to drive disease progression in FSGS and to identify and characterize novel pathways downstream of HDAC. The other major focus of this grant is to determine the impact of valproic acid (VPA) exposure on the incidence and progression of CKD in humans by leveraging two Veterans Affairs cohort and to identify patient characteristics on who would benefit most from VPA inhibition.

During the last funding period, we have examined SAHA, which shows great promise for inhibiting progression of mice proteinuric disease. We have also successfully generated and phenotyped a podocyte specific HDAC 1 and 2 knockout mouse and retrospective cohort data on VA patients with proteinuria receiving valproic acid had reduced loss of kidney function.

Keywords

Focal segmental glomerulosclerosis, proteinuria, chronic kidney disease, end stage kidney disease, histone deacetylase inhibitor, valproic acid

Accomplishments

What are the major goals of the project?

1. Elucidate HDAC1 and 2's role in proteinuric kidney disease following podocyte injury by performing hypothesis driven experiments, which investigate the likely site of action of VPA in the kidney
2. Determine the critical pathways regulated by the HDAC1 and 2.
3. Assess not only if such an effect of VPA treatment is observed in FSGS patients but also if these findings extend beyond this disease by examining its effects on other causes of nephrotic syndrome such as diabetic nephropathy examining the VA cohort study, Veterans Aging Birth Cohort, and Geisinger

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 importance of 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

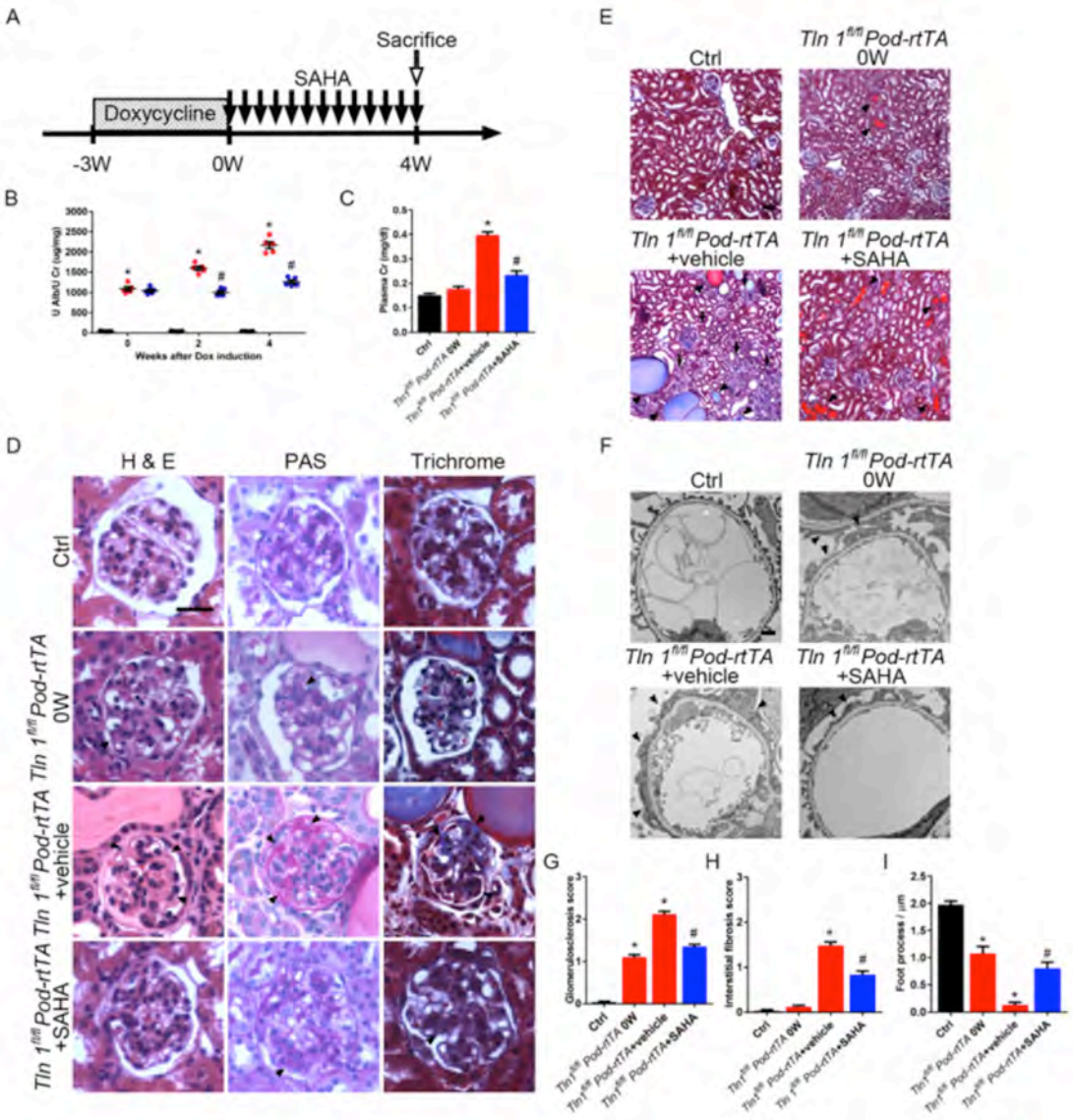

Figure 1 Vorinostat (suberanilohydroxamic acid (SAHA)) reduces podocyte injury in Doxycycline-inducible podocyte specific *Tln1* KO mice. (A) A cartoon schematic of the time course of *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice treated +/- SAHA after completing Dox induction. (B) Quantification of urine 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

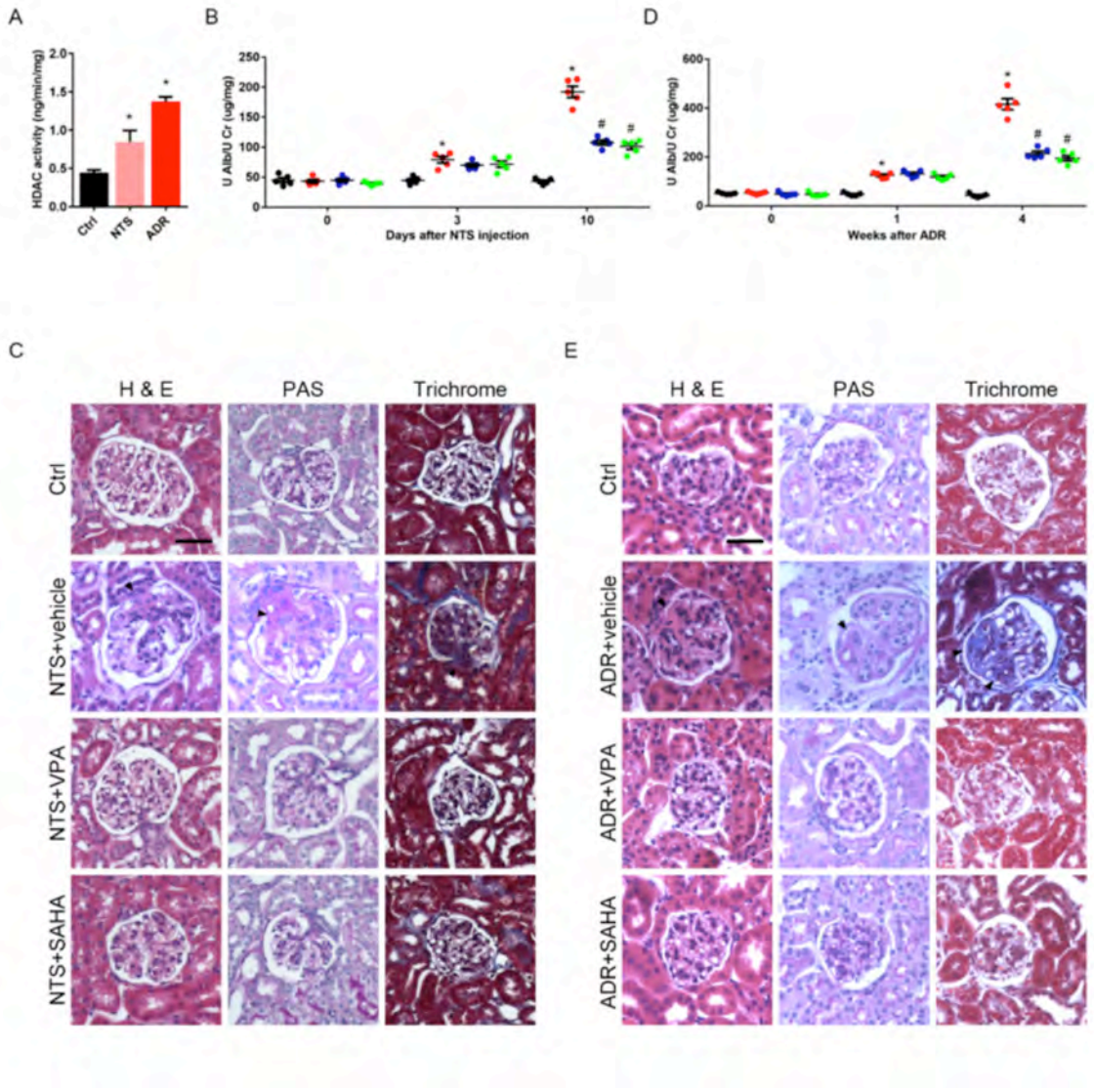

Figure 2. VPA or SAHA treatment reduces urine albumin and glomerular injury induced by NTS or Adriamycin. (A) Total HDAC activity in NTS or Adriamycin (ADR)-injected mice glomerulus. *p <0.05 compared with control mice. N=3. (B) Quantification of urine albumin/creatinine ratio at 0, 3, and 10 days after 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

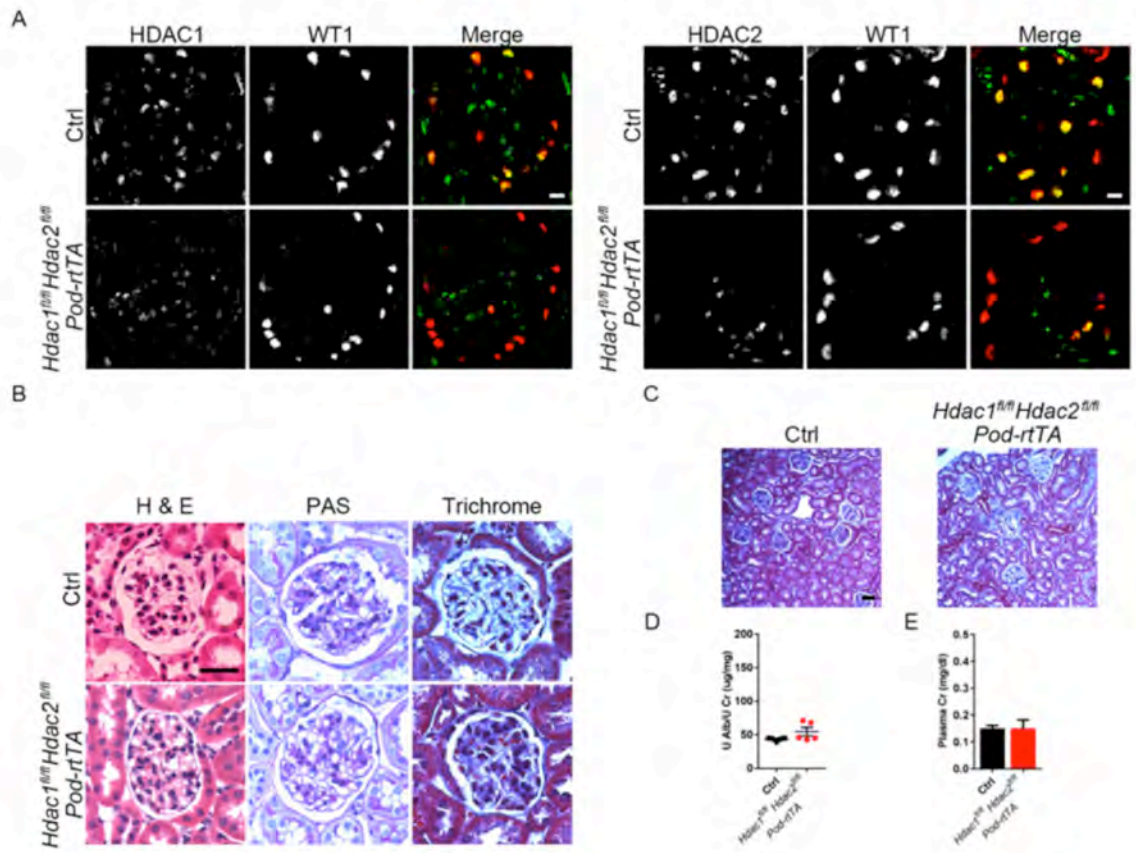

Figure 3. Doxycycline-inducible podocyte specific *Hdac1 and Hdac2* DKO mice reduce NTS-induced podocyte injury. (A) Quantification of urine albumin/creatinine at 0 and 7 days after NTS injection in control (black) and *Hdac1*$^{fl/fl}$, *Hdac2*$^{fl/fl}$, *Pod-rtTA TetO-Cre* mice (blue). *p <0.05 compared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

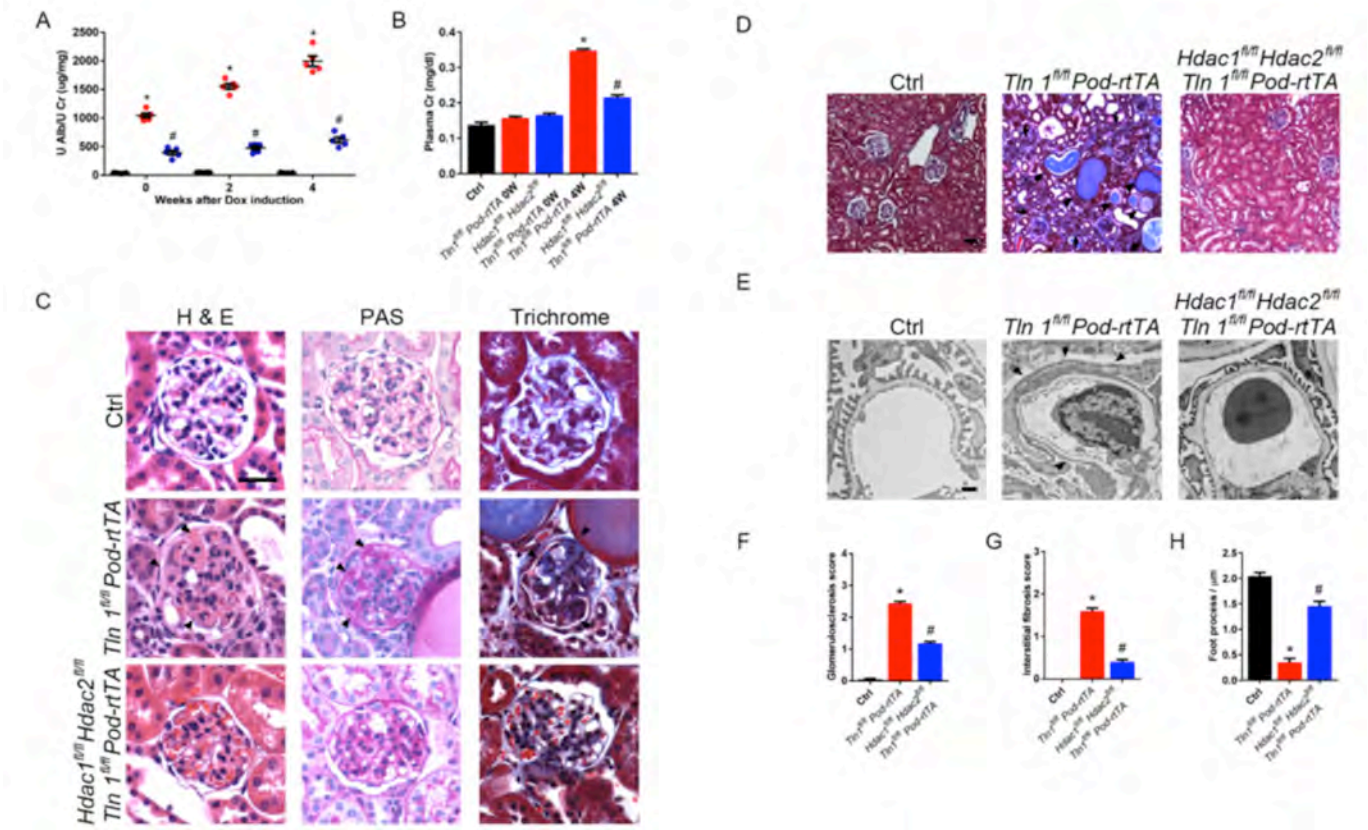

Figure 4. Podocyte deletion of *Hdac1* and *Hdac2* improves albuminuria and glomerulosclerosis in *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice induced with Dox. (A) Quantification of urine albumin/creatinine ratio in control (black), *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* (red) and *Hdac1* $^{fl/fl}$ *Hdac2* $^{fl/fl}$ *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

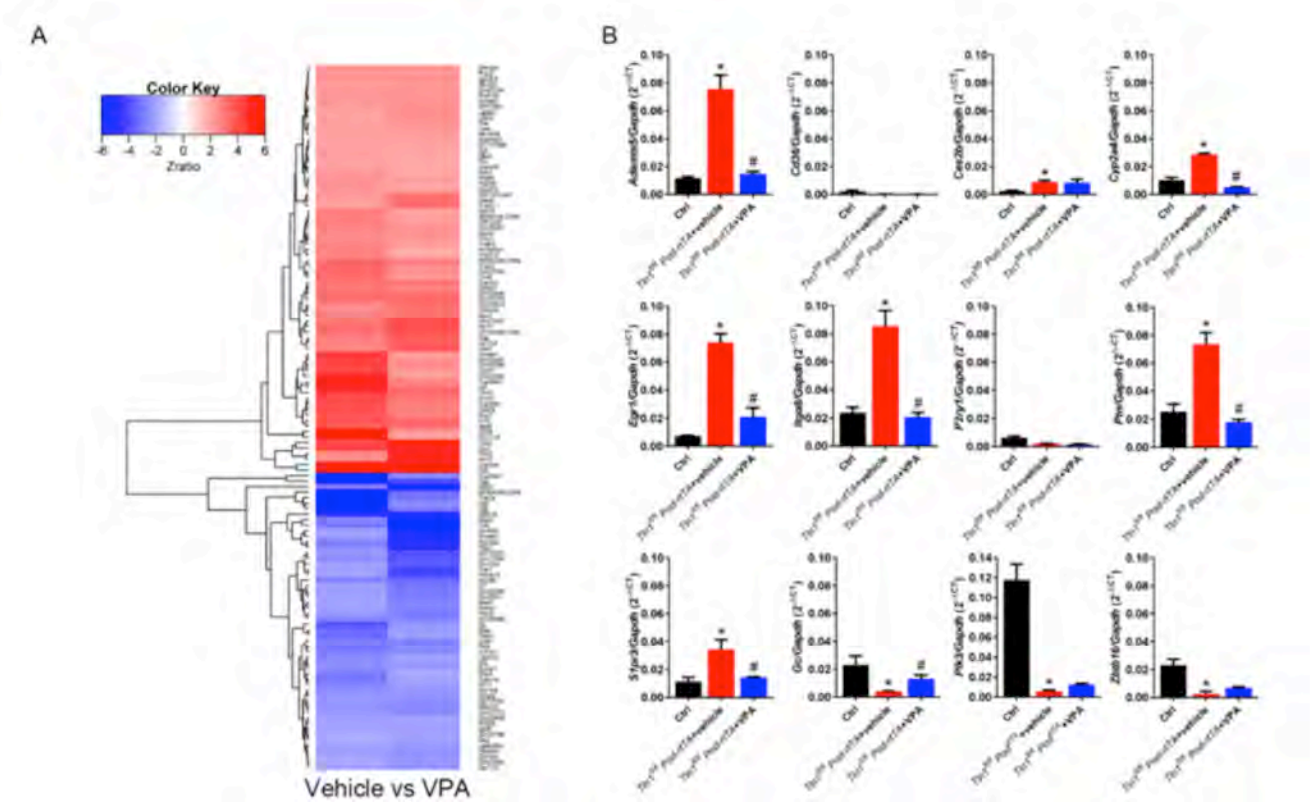

Figure 5. VPA treatment mitigates CREB-mediated EGR1 upregulation in *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice glomerulus (A) Heat map representing color-coded differential glomerular genes analyzed by Z ratio of *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice with vehicle to those with VPA, 2 weeks after completing Dox induction. (B) RT-PCR of the candidate genes in control, and *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice treated +/- VPA 2 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=3

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

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/1.73m^2/year, which is consistent with several large, US population-based studies. (40, 41). 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/1.73m^2/year among those who received VPA compared to -0.94 (0.007) ml/1.73m^2/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/1.73m^2/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/1.73m^2/year compared to -0.32 (SE 0.09) ml/1.73m^2/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/1.73m^2/year in the patients not receiving VPA, compared to -0.61 (0.07) ml/1.73m^2/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/1.73m^2/year in patients not receiving VPA, compared to -0.56 (0.66) ml/1.73m^2/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.

Figure 6

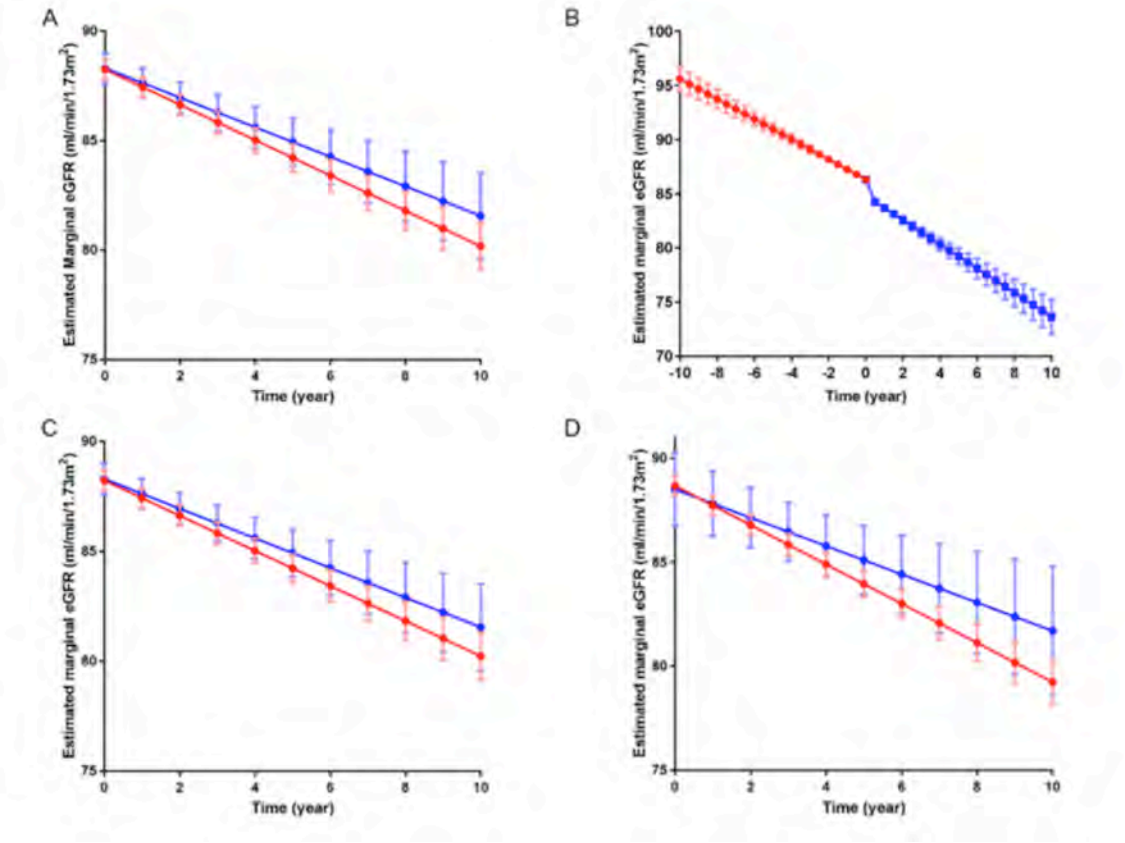

Figure 6. VPA usage is associated with slower declines in eGFR in a Veterans Affairs population cohort. (A) The slope of eGFR decline among patients treated with VPA compared to controls not treated with VPA. *p=0.001. (B) The slope of eGFR decline in patients before and after initiation of VPA. *p <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.

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

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?

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. The titles of the abstracts/oral presentation and manuscript are included in Section 6. Data were also presented in Nephrology Renal conferences at Yale and Veterans Affairs research in progress conferences.

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

During the next reporting period, first we will validate from our microarray results that the protein expression of EGR1 is increased in *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice glomerulus. We shall also determine EGR1 expression in VPA or SAHA treated *Tln1*$^{fl/fl}$ *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-DTR^{flox}* 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$^{-/-}$ Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice, to assess whether loss of this gene could rescue the *Tln1$^{fl/fl}$ 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 *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre* mice with Dox as increased EGR1 expression has been shown to regulate the actin cytoskeleton, and induce cell apoptosis.

Fourth, Dr. Perry Wilson will analyze the Veterans Affairs Birth Cohort and Geisinger Health System Cohort to examine the impact of HDAC inhibitor exposure on incidence and progression of CKD, which will serve as external validation. He 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

4. **Impact**: Describe distinctive contributions, major accomplishments, innovations, successes or any change in practice or behavior that has come about as a result of the project relative to:

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

This project has potential to make impact in the therapy of FSGS and proteinuric diseases, which often progress to ESKD where there is a current lack of treatment short of blocking angiotensin. We have not only shown the effectiveness of HDAC inhibitors in mouse glomerular diseases but also retrospectively in humans with proteinuric diseases, this motivates further studies examining whether this class of drug can be tested prospectively in humans with FSGS.

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?

Nothing to Report

5. CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Nothing to Report

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

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report

Publications, conference papers, and presentations

Journal Publications.

Podocyte histone deacetylase activity regulates mice and human glomerular diseases
Kazunori Inoue,[1] Geliang Gan, [2] Maria Ciarleglio, [2] Yan Zhang,[4,5,6] Christopher E. Pedigo,[1] Xuefei Tian,[1] Corey Cavanaugh,[1,3] Janet Tate, [3] Ying Wang,[1] Elizabeth Cross,[1] Marwin Groener,[1] Nathan Chai,[1] Zhen Wang,[1] Amy Justice, [1,7] Zhenhai Zhang, [4,5,6] Chirag R. Parikh,[1,3] Francis P. Wilson,[1,3] and Shuta Ishibe,[1]
In revision at *Journal of Clinical Investigation*. Federal Support Acknowledged.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations
American Society of Nephrology- 2018 abstract for oral presentation
The role of HDAC activation in proteinuric kidney disease progression in Mice and Humans
Kazunori Inoue, Chirag E. Parikh, Francis P. Wilson**,** and Shuta Ishibe

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

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.

7. **PARTICIPANTS and OTHER COLLABORATING ORGANIZATIONS**

Name:	Ishibe, Shuta
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	3.6
Contribution to Project:	Overall supervisory responsibility for the Yale site providing oversight of the project progress for the basic science arm of the grant along with collaboration with Dr. Francis Perry Wilson, who directs the clinical arm of the grant. Reviewing results, experimental design and quality control weekly and discussion of results on toxin and mouse models of glomerular disease
Funding Support:	

Name:	Inoue, Kazunori
Project Role:	Post doctoral associate
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	6
Contribution to Project:	Performs experiments in-vitro (podocyte cell culture) and in-vivo (mice)examining HDAC activation in glomerular disease.
Funding Support:	

Name:	Elizabeth Cross
Project Role:	Post graduate
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	6
Contribution to Project:	Mouse colony management of podocyte associated HDAC and Tln KO mice.
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?

Updated Other Support Pages are included in Appendix 1.

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.

This is a COLLABORATIVE AWARD. Our collaboration partner is Dr. Francis Perry Wilson. Details are below:
Organization Name: Yale School of Medicine
Location of Organization: New Haven, CT, USA.
Partner's contribution to the project clinical arm of the grant
Financial support: None
In-kind support: Analysis of human cohorts
Facilities: None
Collaboration: Examines the role of valproic acid in large cohorts of patients with kidney disease and proteinuria
Personnel exchanges: None
Other: None

8. 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 Initiating PI (Shuta Ishibe). 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.

9. APPENDICES

Appendix 1: Shuta Ishibe Updated Other Support Pages

2R01 DK083294-08 (PI: Ishibe) 07/01/2015 – 06/30/2020 3.60 calendar months
NIH/NIDDK $225,000
Role of Calpain in Podocyte Injury
The major focus of this grant is to investigate the role of focal adhesion proteins and the activation of calpain induced ER stress.
Overlap-None

R25-DK101408-01 (PI: Ishibe) 04/01/2014 – 03/31/2019 0.3 calendar months
NIH/NIDDK $82,171
KUH Undergraduate Summer Research Program at Yale University
The major goal of this grant is to provide undergraduate students an opportunity to perform kidney, urology, and hematology research during the summer months.

Overlap-None

2R01 DK093629-05A1 (PI: Ishibe) 09/01/2017 – 08/31/2022 2.40 calendar months
NIH/NIDDK $225,000
Role of Clathrin Mediated Endocytosis in Podocyte
The major goal of this grant is to examine the role of endocytic process in podocyte biology.

Overlap-None