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TITLE: Targeting Histone Deacetylase in Focal Segmental Glomerulosclerosis- From Mice to Patients

PRINCIPAL INVESTIGATORS: Shuta Ishibe and Francis P Wilson

CONTRACTING ORGANIZATION: Yale University

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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, the co-PI of this study, F. Perry Wilson has continued to examine the Veterans Affairs Cohort and added multiple other cohorts to examine the stability of preliminary results that suggested a protective role of VPA in proteinuric kidney disease.

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

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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 the Co-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 using several large national databases.

During the last funding period, we have examined how HDAC inhibition shows great promise for inhibiting progression of mice proteinuric disease. We had 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 which was most profound in the patients with proteinuria. These findings were published in *The Journal of Clinical Investigation*, Inoue et al. in March 2019 in a collaborative work from both our groups.

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 **80% complete**

2. Determine the critical pathways regulated by the HDAC1 and 2. **40% complete**

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 **60% complete**

What was accomplished under these goals?

During the past year, we have examined the downstream mechanism after revealing the salutary effects of VPA and SAHA in mitigating the progression of proteinuric kidney disease in the *Pod-rTTA Tln1* KO mice. In the last update we had performed RNA profiling revealing increased EGR1 expression in the glomeruli isolated from the *Pod-rTTA Tln1* KO mice upon development of proteinuria which was reduced following VPA treatment. We further validated that the protein expression of EGR1 was increased in *Tln1*$^{fl/fl}$ *Pod-rtTA TetO-Cre* mice glomerulus, which was mitigated in VPA or SAHA treated mice, or in mice lacking podocyte associated *Hdac1* and *Hdac2* (Figure 1a, quantified in Figure 1b). To confirm the importance of EGR1 in human proteinuric disease, kidney biopsy samples from patients with FSGS demonstrated increased podocyte EGR1 expression (Figure 1c, quantified in Figure 1d). Furthermore, in vitro experiments using isolated primary podocytes from *Pod-Cre Rosa-DTR*^{flox} mice treated with lipopolysaccharide (LPS) or protamine sulfate (PS), two agents that induce podocyte injury, resulted in increased EGR1 expression, and reduction by VPA or SAHA (Figure 1e, quantified in Figure 1f and 1g) To further elucidate how EGR1 expression is regulated following podocyte injury, we examined 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 demonstrated increased CREB binding to CRE within the *Egr1*

promoter in LPS or PS treated primary podocytes, which were decreased by VPA or SAHA (Figure 1h and 1i, SRF binding to serum response element (SRE) within the *Egr1* promoter was unchanged in LPS or PS treated primary podocytes (data not shown). Previous studies have shown CRE binding in the target gene promoter can be increased by phosphorylating CREB at Ser133 residue [1]. In primary podocytes treated with LPS for 3 hours or PS for 1 hour, we observed a robust increase in phosphorylated CREB (Ser 133), which could be reversed by either VPA or SAHA treatment, suggesting that HDAC inhibitors may reduce CREB phosphorylation (Figure 4j, quantified in Figure 4k and 4l).

Figure 1

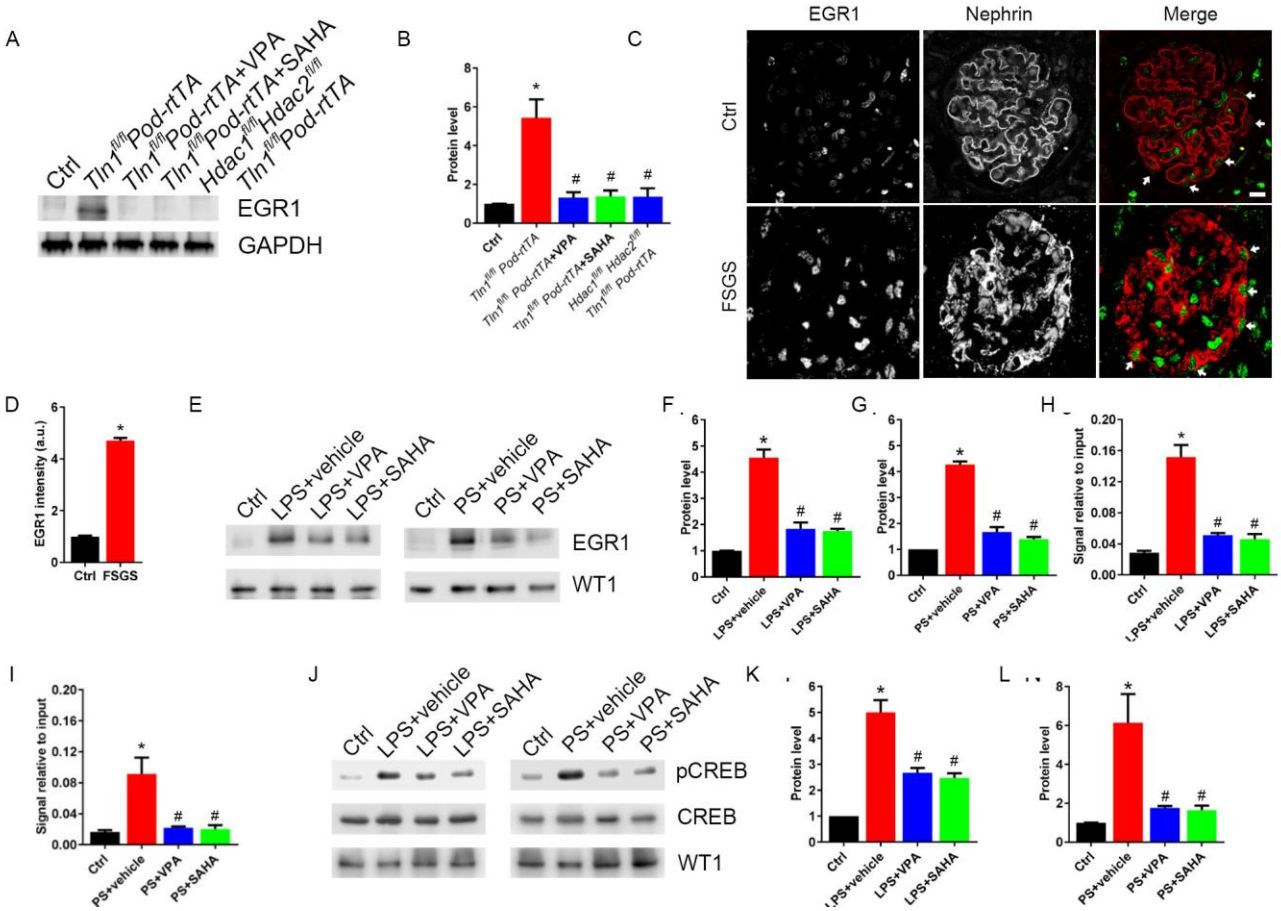

Figure 1- (A) Representative immunoblots of EGR1 and GAPDH in control, *Tln1^{flox} Pod-rtTA TetO-Cre* mice treated +/- VPA or SAHA, and *Hdac1^{flox} Hdac2^{flox} Tln1^{flox} Pod-rtTA TetO-Cre* mice glomerulus. (B) Quantification of EGR1 immunoblots in (A) *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1^{flox} Pod-rtTA TetO-Cre* mice. N=3. (C) Representative immunofluorescence images of EGR1 (green) and nephrin (red) in control and FSGS patient glomerulus. Arrows display podocyte EGR1 staining. Scale bar: 20 µm. (D) Quantification of podocyte EGR1 immunofluorescence intensity in (C). *p <0.05 compared with control. N=3 (E) Representative immunoblots of EGR1 and WT1 in primary podocytes +/- lipopolysaccharide (LPS) or protamine sulfate (PS), treated +/- VPA or SAHA. (H and I) Quantification of EGR1 immunoblots in primary podocytes with LPS (F) or PS (G) treated +/- VPA or SAHA. *p <0.05 compared with control, and #p <0.05 compared with LPS or PS treated podocytes. N=3. (J and K) ChIP-assay using CREB antibody and primer sets for Egr1 promoter 1 in LPS (H) or PS (I) treated primary podocytes treated +/- VPA, or SAHA. DNA binding was determined by PCR. *p <0.05 compared with control, and #p <0.05 compared with LPS or PS treated podocytes. N=3. (J) Representative immunoblots of phosphorylated CREB, CREB, and WT1 in LPS or PS treated primary podocytes +/- VPA, or SAHA. (K and L) Quantification of pCREB immunoblots in primary podocytes with LPS or PS (J) treated +/- VPA or SAHA. *p <0.05 compared with control, and #p <0.05 compared with LPS or PS treated podocytes. N=3. (B), (F), (G), (H), (I), (K), and (L) Statistically analyzed by one-way ANOVA with Dunnett's correction. (D) Statistically analyzed by 2-tailed Student's t test.

Loss of Egr1 in Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre mice reduces progressive albuminuria and glomerulosclerosis.

To next determine the importance of EGR1 in-vivo, we generated 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 found a striking reduction in albuminuria, kidney failure, glomerulosclerosis, and interstitial fibrosis, (Figure 2a, 2b, 2c, and 2d,

quantified in Figure 2e and 2f) similar to what was observed following VPA treatment. Next, to elucidate a role of EGR1 upregulation in podocyte injury, we examined 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 [2-5]. Loss of podocyte associated EGR1 expression resulted in stabilization and maintenance of actin stress fibers after LPS (for 6 hours) and PS (for 1 hour) treatment when compared to controls. (Figure 2g, quantified in figure 2h, 2i, and 2j). However, TUNEL staining in the Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre mice kidneys, 2 weeks after completing Dox revealed, apoptotic cells (TUNEL positive cells) in the tubular segments but not in podocytes (stained with WT1) (data not shown).

Figure 2

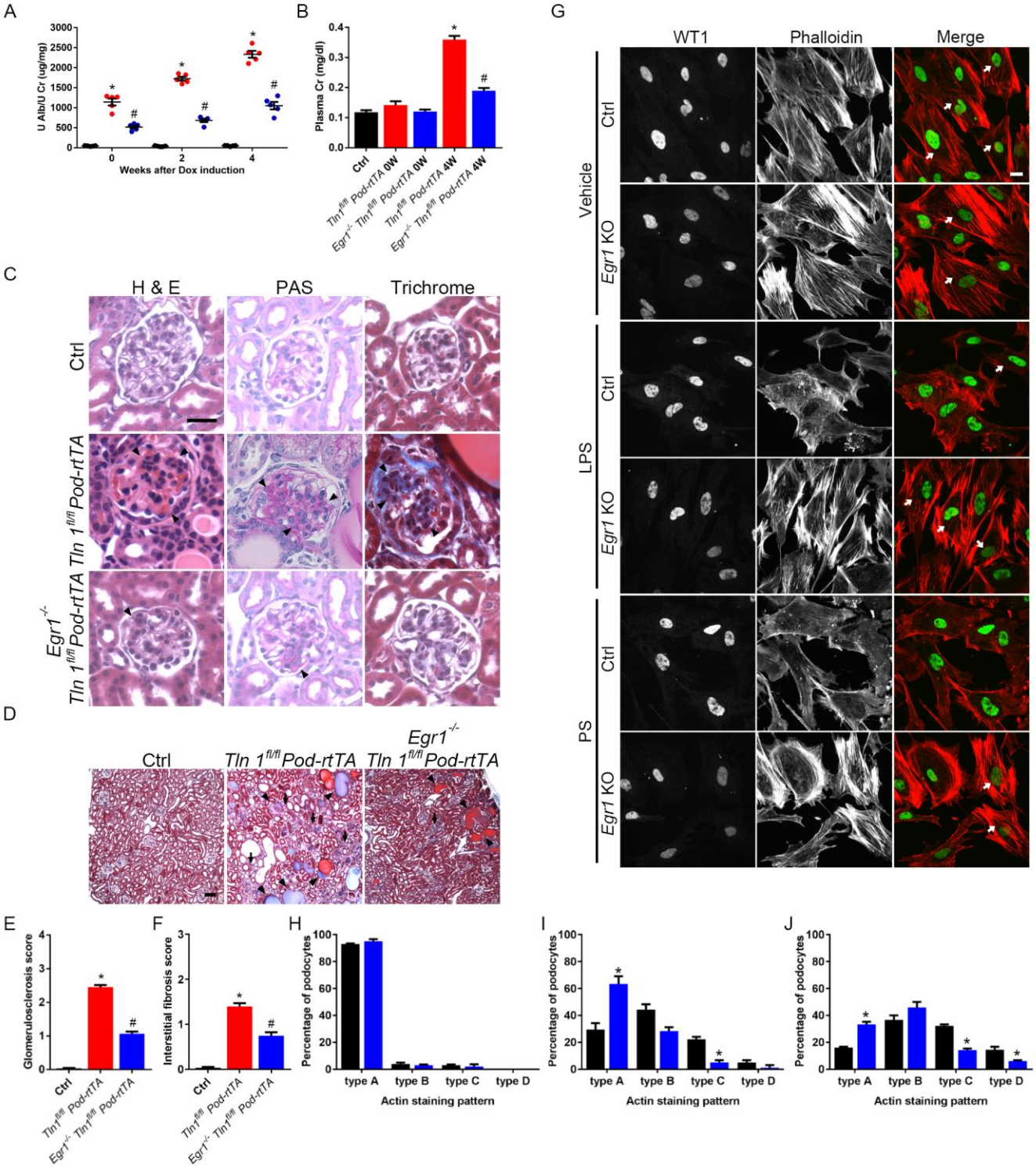

Figure 2 Loss of EGR1 in *Tln1^{flox} Pod-rtTA TetO-Cre* mice improves glomerulosclerosis and interstitial fibrosis. (A) Quantification of urine albumin/creatinine ratio in control (black), *Tln1^{flox} Pod-rtTA TetO-Cre* (red), and *Egr1$^{-/-}$ Tln1^{flox} Pod-rtTA TetO-Cre* (blue) mice at 0, 2, and 4 weeks after completing Dox induction. *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1^{flox} Pod-rtTA TetO-Cre* mice. N=5. (B) Plasma creatinine in control, *Tln1^{flox} Pod-rtTA TetO-Cre*, and *Egr1$^{-/-}$ Tln1^{flox} Pod-rtTA TetO-Cre* mice 0 and 4 weeks after completing Dox induction. *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1^{flox} Pod-rtTA TetO-Cre* mice. N=5. (C) Representative light microscope images (H&E, PAS, and trichrome) of glomerulus from control, *Tln1^{flox} Pod-rtTA TetO-Cre* mice, and *Egr1$^{-/-}$ Tln1^{flox} Pod-rtTA TetO-Cre* mice 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^{flox} Pod-rtTA TetO-Cre* mice, and *Egr1$^{-/-}$ Tln1^{flox} 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) Quantification of glomerulosclerosis in (C). *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1^{flox} Pod-rtTA TetO-Cre* mice. (F) Quantification of interstitial fibrosis in (D). *p <0.05 compared with control mice, and #p <0.05 compared with *Tln1^{flox} Pod-rtTA TetO-Cre* mice. (G) Representative immunostaining for phalloidin (red) and WT1 (gren) in control *Pod-Cre Rosa-DTR^{flox}* or *Egr1$^{-/-}$ Pod-Cre Rosa-DTR^{flox}* mice podocytes treated with LPS or PS. Scale bar: 10 μm. (H to J) Quantification of phalloidin staining in control or *Egr1$^{-/-}$ Pod-Cre Rosa-DTR^{flox}* mice podocytes with vehicle (H), LPS (I), and PS (J). *p <0.05 compared with control mice primary podocytes. N=3. (A), (B), (E), and (F) Statistically analyzed by one-way ANOVA with Dunnett's correction. (I) and (J) Statistically analyzed by 2-tailed Student's t test.

Loss of Egr1 mice reduces albuminuria in Nephrotoxic Serum Induced Glomerulonephritis

To validate the effect of EGR1 in a toxin related podocyte injury model, NTS was treated in Egr1$^{-/-}$ mice. Compared with control mice, Egr1$^{-/-}$ mice had reduced NTS-induced albuminuria and glomerular lesion. (Figure 2a and 2b)

Figure 3

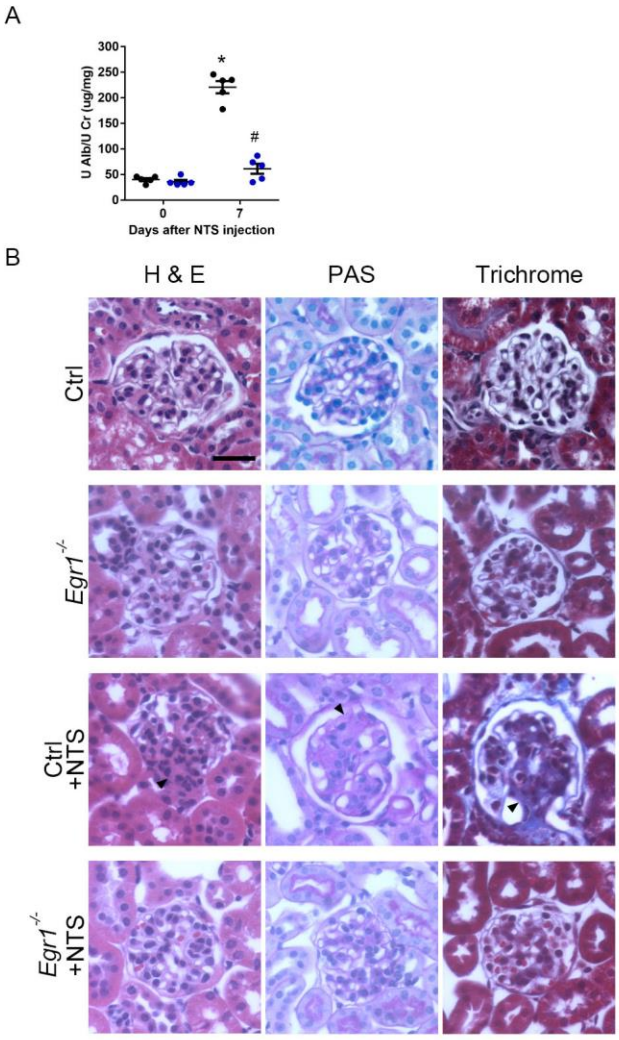

Figure 3 A) Quantification of urine albumin/creatinine ratio at 0 and 7 days after NTS injection in control (black) and *Egr1$^{-/-}$* (blue) mice. *p <0.05 compared with control mice before NTS injection (day 0), and #p <0.05 compared with NTS-injected control mice (day 7) Statistically analyzed by 2-tailed Student's t test. N=5. (B) Representative light microscope images (H&E, PAS, and trichrome) of glomerulus from NTS-injected control and *Egr1$^{-/-}$* mice. Arrowheads show mesangial matrix deposition and mesangial cell proliferation. Scale.bar: 25 μm.

Human Cohort Studies Give a Mixed Picture of VPA Effect on Renal Outcomes

Results from the Veterans Aging Cohort Study (VACS)

To support the hypothesis that HDAC inhibition may also be a potent therapeutic strategy against human proteinuric kidney disease, we 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. [6,7]. 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. 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 4B). The effects were more marked among veterans with proteinuria (Figures 4C and 4D).

Figure 4

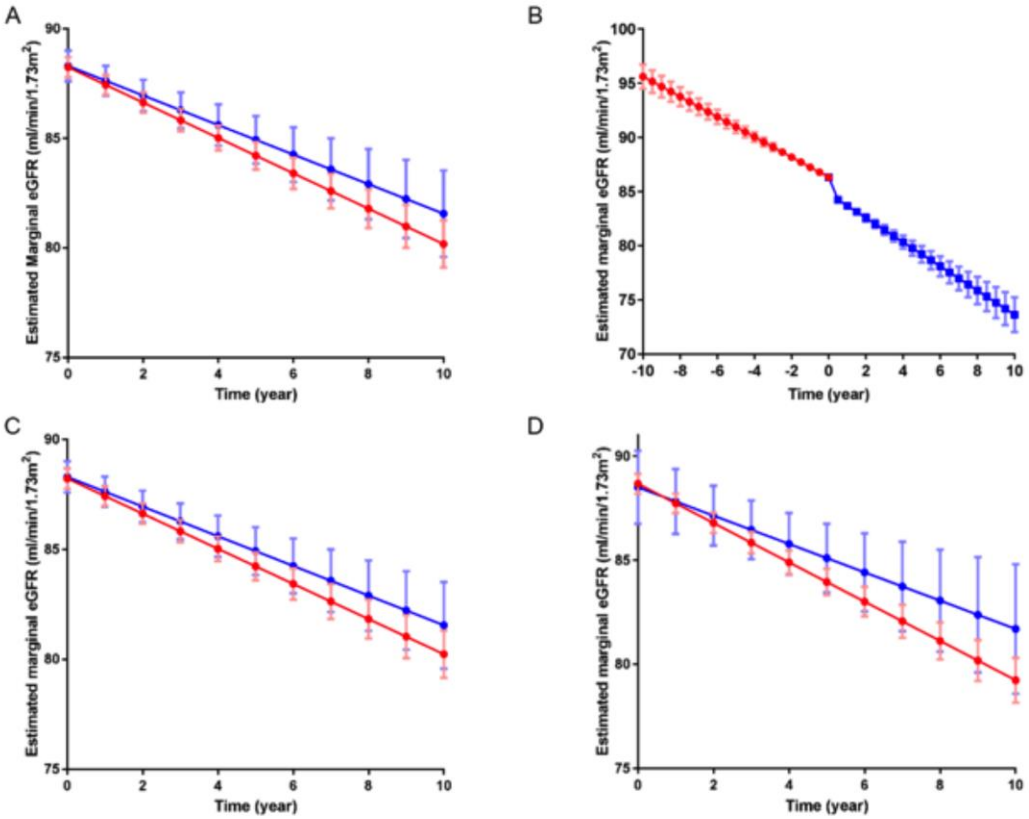

Figure 4. VPA usage is associated with slower declines in eGFR in a Veterans Affairs population cohort. (A) The slope of eGFR decline among patients treated with VPA compared to controls not treated with VPA. *p=0.001. (B) The slope of eGFR decline in patients before and after initiation of VPA. *p <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.

Since the last reporting period, we have expanded our initial restrospective pharmacoepidemiology study from the Veterans Aging Cohort Study (VACS) to the Veterans Birth Cohort Study and a Geisinger-based cohort.

Additionally, given the increased power the larger sample sizes bring, we examined clinical outcomes as opposed to differences in slope of eGFR. In this regard, our results are mixed.

In all 3 cohorts, we performed a propensity-matched cohort study. Briefly, each individual initiated on valproic acid while under observation was matched to two non-initiators based on a propensity score which included age, duration of follow-up, baseline eGFR, bipolar disorder, depression, diabetes, epilepsy, HIV status, hepatitis status, heart failure, hypertension, liver disease, migraine, PTSD, stroke, gender, and race.

We then followed these matched groups of individuals for the development of three clinical outcomes: incident chronic kidney disease (defined as an eGFR <60 ml/min/1.73m^2 with at least a 20% reduction from baseline), doubling of creatinine, dialysis, or death. A summary of these analyses appears below:

Cohort	N (VPA)	N (Matched Controls)	Incident CKD	Doubling of Creatinine	Death
VACS	2,369	4,738	0.84 (0.71, 0.98)	0.58 (0.40, 0.84)	1.05 (0.94, 1.18)
Veterans Birth Cohort	68,962	137,924	0.98 (0.95, 1.02)	0.81 (0.74, 0.88)	0.99 (0.98, 1.03)
Geisinger Cohort	1215	2517	1.06 (0.81 – 1.39)	1.01 (0.69 – 1.47)	2.52 (2.14 – 2.97)

Cohort	N (Comparator Drug)	N (Matched Controls)	Incident CKD	Doubling of Creatinine	Death
VACS	2,654	5,308	0.98 (0.86, 1.12)	0.85 (0.63, 1.15)	0.87 (0.78, 0.97)
Veterans Birth Cohort	106,372	212,744	1.10 (1.07, 1.13)	0.87 (0.81, 0.93)	0.80 (0.78, 0.82)
Geisinger Cohort	Analyses Pending*				

The results show conflicting effects of VPA on renal outcomes that vary by cohort. Of note, we found a protective effect of VPA in terms of doubling of creatinine in veterans cohorts, but not in the non-veteran predominant Geisinger cohort. We are currently exploring the reasons for this discrepancy. There are substantial differences in the composition of the cohorts, the most notable being that the VA studies are dominated by men, but there does not appear to be a sex-by-VPA interaction in the Geisinger cohort. Broadly, however, the largest of the cohorts (the birth cohort) continues to suggest a potentially protective effect of VPA in this population. In the next phase of this award, we will integrate United States Renal Data System (USRDS) data into the Veterans Birth Cohort to examine the impact of VPA on the rates of dialysis in the cohort.

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

Dr. Kazunori Inoue, who conducted research on this project is now an Assistant Professor at Osaka University School of Medicine, Japan. He was selected to present these findings at a Japan Society of Nephrology Young Investigator Symposium in August 2019.

Elizabeth Cross conducted research on this project from her NIH funded summer fellowship, and now matriculated Medical School as of August 2019.

How were the results disseminated to communities of interest?

Results from this project has been provided 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. The HDAC 1 and 2 floxed mice were distributed to Jianning Tao PhD, at the University of South Dakota following completion of a MTA

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

We have started generating the *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre TRAP* mice which is a podocyte specific process to affinity purification of translating ribosome. In this method the L10a ribosomal protein is fused with EGFP and inserted into the ROSA26 locus with a flox-STOP cassette (*R26Rpl10a*; JAX #024750). Upon Cre activation *Tln1* is deleted, along with the flox-STOP and allows expression of the L10a-EGFP fusion protein. Anti-EGFP pulldown purifies ribosomes that have incorporated L10a-EGFP and with them, the actively translating mRNA only from cells where Cre has been active. We will perform RNA profiling to determine podocyte-specific transcripts during proteinuria. These mice will be compared to *Tln1$^{fl/fl}$ Pod-rtTA TetO-Cre TRAP* mice following treatment after three weeks of VPA treatment. The rationale of the experiment is compared to the entire

glomeruli which we have performed, the podocyte enrichment should identify specific genes for this cell type. Following analysis of the differential gene expression, we will further validate by qPCR and western blotting of isolated podocytes. After successful identification and validation, we plan determine whether inhibitors or conditional knockout ice are available to further rescue the phenotype

We will further expand our above analyses within the Veterans Affairs Birth Cohort and Geisinger Health System Cohort to examine the impact of HDAC inhibitor exposure on incidence and progression of CKD among those we predict to have the most favorable response to VPA exposure – namely those with proteinuric kidney disease and comorbidities associate with significant proteinuria (like diabetes). 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. Finally, we will integrate the Veterans Birth Cohort with the USRDS database to examine the effect of VPA on end-stage renal disease 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 suggesting of repurposing of a drug for broader use

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.

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 murine and human glomerular diseases
Kazunori Inoue,[1] Geliang Gan, [2] Maria Ciarleglio, [2] Yan Zhang,[4,5,6] Xuefei Tian,[1] Christopher E. Pedigo,[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

Japan Society of Nephrology Young Investigator Symposium 2019

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.

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 th 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:	Aoki, Satoshi
Project Role:	Post doctoral associate
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	3
Contribution to Project:	Performs experiments in-vitro (podocyte cell culture) and in-vivo (mice)examining HDAC activation in glomerular disease.
Funding Support:	

Name:	Wei Li
Project Role:	Post graduate
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	1
Contribution to Project:	Mouse colony management of podocyte associated HDAC and Tln KO mice.
Funding Support:	

Name:	Geliang Gan
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	6
Contribution to Project:	Longitudinal analysis of cohort data
Funding Support:	

Name:	Maria Ciarleglio
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	3
Contribution to Project:	Longitudinal analysis of cohort data
Funding Support:	

Name:	Morgan Grams
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	1
Contribution to Project:	Study design / implementation at Geisinger
Funding Support:	

Name:	Alex Chang
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	1
Contribution to Project:	Study design / implementation at Geisinger
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 and the Veterans Birth Cohort.

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. This represents a joint report from the Initiating PI (Shuta Ishibe) and collaborating PI (F. Perry Wilson). 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

Appendix 2: F. Perry Wilson Updated Other Support Pages

R01 DK113191-01A1
WILSON, FRANCIS PERRY (PI)
02/01/18-01/31/23
Optimizing Electronic Alerts for Acute Kidney Injury
Role: PI

Overlap: None

K23 DK097201-06
WILSON, FRANCIS PERRY (PI)
07/15/13-04/30/20
Mediators & prognostic value of muscle mass & function in chronic kidney disease
Role: PI

Overlap: None

2P30DK079310-11, NIH/NIDDK
WILSON, FRANCIS PERRY (PI)
08/01/18-07/31/23
George M. O'Brien Kidney Center at Yale
This Kidney Center provides an administrative core and three research cores whose specific objectives are to provide small animal physiology services to allow detailed characterization of renal function at the level of the tubule, the kidney, and the intact organism; provide mouse genetics and cell line services to develop new animal models and kidney cell lines to elucidate the molecular mechanisms underlying the pathophysiology of kidney diseases; and provide human genetics and clinical research services to apply genetic and genomic technologies to the study of human kidney diseases. There is also a pilot and feasibility program of small grants
Role: Co-Investigator

Overlap: None

1UG3DK114866-01 , John Hopkins University
Parikh (PI)
07/01/18-06/30/20
AKI Matched Phenotype Linked Evaluation with Tissue (AMPLE-Tissue)
The major goal is to obtain biopsies from 90-100 participants and closely monitor patient safety.
Role: Co-Investigator

Overlap: None

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4. Koldamova R, Schug J, Lefterova M, et al. Genome-wide approaches reveal EGR1-controlled regulatory networks associated with neurodegeneration. *Neurobiol Dis.* 2014;63:107-114.
5. He F, Zhou M, Yu T, et al. Sublytic C5b-9 triggers glomerular mesangial cell apoptosis in rat Thy-1 nephritis via Gadd45 activation mediated by Egr-1 and p300-dependent ATF3 acetylation. *J Mol Cell Biol.* 2016;8(6):477-491.
6. Grams ME, Rebholz CM, Chen Y, et al. Race, APOL1 Risk, and eGFR Decline in the General Population. *J Am Soc Nephrol.* 2016;27(9):2842-2850.
7. Baba M, Shimbo T, Horio M, et al. Longitudinal Study of the Decline in Renal Function in Healthy Subjects. *PLoS One.* 2015;10(6):e0129036.