AWARD NUMBER: W81XWH-17-1-0202

TITLE: RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies

PRINCIPAL INVESTIGATOR: Ravichandran Ramasamy

CONTRACTING ORGANIZATION: NEW YORK UNIVERSITY GROSSMAN SCHOOL OF MEDICINE

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Fort Detrick, Maryland 21702-5012

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5d. PROJECT NUMBER

5e. TASK NUMBER

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New York University Grossman School of Medicine
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
In Year Three of the funded grant, we have substantial progress in the following critical areas: 1). As noted in the project narrative, we generated four different lines of mice to directly test the hypothesis that RAGE and DIAPH1 contribute to the pathogenesis of diabetes-associated nephropathy in the podocytes and/or in myeloid cells/macrophages. All of the mouse lines are now generated and largely completed (mice sacrificed) and samples being evaluation by Dr D'Agati. There are no new pending mice to generate - all are generated and on time course. 2). We have determined that the small molecule RAGE/DIAPH1 antagonist is best administered orally and that the RAGE antagonist survives the medicated chow pelleting, heating and irradiation. Our first data on treated vs. untreated male and female diabetic mice illustrates reduction in mesangial sclerosis, reduced thickening of the glomerular basement membrane and reduction in podocyte effacement in diabetic mice receiving RAGE229 medicated chow (vs vehicle). Additional mice are on study and time course at this time to complete the indicated enrollment.3). For transcriptomics and metabolomics/lipidomics assay, Dr. Ramasamy will be testing the macrophages from the mice through the time course and he has verified all of his experimental systems for the performance of the outlined studies. Dr. Ramasamy identifies substantial progress in the development and validation of metabolomics and lipidomics assays here at NYU and in transcriptomic data (all on macrophages) in order to understand detailed mechanisms of the role of these molecules in the diabetic kidney. Taken together, despite the >3 month shutdown due to COVID19 our work in Year 3 has been productive and we await tissue and other analyses, as above, to render final conclusions.

15. SUBJECT TERMS
Diabetes; DIAPH1; Floxed Mice; Glomerulosclerosis; Glomerular basement membrane; Inflammation; Macrophage; Nephropathy; Podocyte; RAGE; Small Molecule Antagonist

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Ann Marie Schmidt, MD – Initiating PI

Ravichandran Ramasamy, PhD – Partnering PI

Ann Marie Schmidt, MD – Initiating PI

1. INTRODUCTION:

Our laboratory discovered the receptor for advanced glycation endproducts (RAGE) and we identified that the cytoplasmic domain (tail) of RAGE binds to the formin, DIAPH1, and that this interaction is critical for RAGE ligand-mediated signal transduction and modulation of gene expression linked to cellular perturbation. DIAPH1 mediates actin cytoskeleton functions, cellular migration and activation of the Rho GTPases. DIAPH1 is expressed by immune and vascular cells; we reported that deletion of *Diaph1* in murine macrophages protects against hypoxia-mediated upregulation of proinflammatory (*Egr1* and *Ccl2*) and prothrombotic (*Tf*) and that this protection is analogous to that observed in macrophages devoid of *Ager.* Furthermore, with Dr. Alexander Shekhtman, we have identified the precise mechanism by which the cytoplasmic domain of RAGE binds DIAPH1. Critically, we have now published that DIAPH1, like RAGE, is highly expressed in human diabetic podocytes. **The goal if this grant is to determine the specific mechanisms by which RAGE/DIAPH1 contribute to the pathogenesis of diabetes associated nephropathy and we to explore novel RAGE/DIAPH1-directed therapeutic opportunities.**

2. KEYWORDS:

Diabetes
DIAPH1
Floxed Mice
Glomerulosclerosis
Glomerular basement membrane
Inflammation
Macrophage
Nephropathy
Podocyte
RAGE
Small Molecule Antagonist

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**
  There are three specific aims of the funded grant:

  AIM 1 will test the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules. We will generate mice in which podocyte-specific deletion of *Ager* or *Diaph1* is accomplished via breeding *Ager* or *Diaph1* floxed mice with podocin (*Nphs2*) cre recombinase mice.

  AIM 2 will test the hypothesis that RAGE and DIAPH1-expressing macrophages contribute to structural and functional derangements in DN through upregulation of

tissue-destructive and profibrotic mediators. We will generate mice in which myeloid cell deletion of *Ager* or *Diaph1* is accomplished by breeding *Ager* or *Diaph1* floxed mice with *Lysm* cre recombinase mice.

AIM 3 will determine if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.

- **What was accomplished under these goals?**

1) Major Activities
In the third year of the funded grant, we focused on the following major activities indicated below. There were delays during the year because of COVID19 shutdown that closed the lab
1A). As noted in the project narrative, we generated four different lines of mice to directly test the hypothesis that RAGE and DIAPH1 contribute to the pathogenesis of diabetes-associated nephropathy in the podocytes and/or in myeloid cells/macrophages. All of the mouse lines are now generated and largely completed (mice sacrificed) and samples being evaluation by Dr D'Agati. There are no new pending mice to generate – all are generated and on time course.
1B). We have determined that the small molecule RAGE/DIAPH1 antagonist is best administered orally and that the RAGE antagonist survives the medicated chow pelleting, heating and irradiation. We just completed the first set of animals in which RAGE229 medicated chow and the data are shown below.
1C). For metabolomics/lipidomics and parallel transcriptomics assays, Dr. Ramasamy will be testing the macrophages from the mice through the time course and he has verified all of his experimental systems for the performance of the outlined studies. This work commenced in Year 3.
1D). All of the colleagues and collaborators are in place, with roles and timing defined, in order to execute the outlined studies as expertly and efficiently as possible.

2) Specific objectives
Our objectives in year one were to execute the above six activities in order to be certain that the aims of the study would be completed according to the three Specific Aims outlined in Item #1 above.

3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative):
3A). Generation of Mouse Models for Study
At this time, we have generated all four of the mouse lines for study; the time course for study once diabetes (or control state) is induced is 6 months – at this time, the four lines of mice are in various stages of completion or in progress to complete the aims. All have been born, typed, made diabetic or control and are in last stages of time course. No new mice need to be generated.

Per line, we have generated the following mice and these mice are on time course (Power calculations as described in the Narrative indicated 10-12 final mice/condition were needed for statistical significance).

Note that the investigators handling/working with the mice are naïve to the genotype; although they know the diabetes/non diabetes status and the sex of the mice, they are not aware of the genotype until after data are entered post sacrifice.

Aim 1:

Ager flox flox *Nphs2* (+/wt) cre recombinase mice
To date the following number of mice have been completed (sacrificed already and tissue sample analysis completed or underway):

Diabetes:	Male (N=10)
	Female (n=12)
Non-Diabetes	Male (N=9)
	Female (N=10)

Ager flox flox *Nphs2* (wt/wt) cre recombinase mice
To date the following number of mice have been completed (sacrificed already and tissue sample analysis completed or underway):

Diabetes:	Male (N=11)
	Female (n=10)
Non-Diabetes	Male (N=10)
	Female (N=10)

Diaph1 flox flox *Nphs2* (+/wt) cre recombinase mice
To date the following number of mice have been completed (sacrificed already and tissue sample analysis completed or underway):

Diabetes:	Male (N=10)
	Female (N=12)
Non-diabetes:	Male (N=9)
	Female (N=10)

Diaph1 flox flox *Nphs2* (wt/wt) cre recombinase mice
To date the following number of mice have been completed (sacrificed already and tissue sample analysis completed or underway):

Diabetes:	Male (N=10)
	Female (N=10)
Non-diabetes:	Male (N=10)
	Female (N=9)

Final numbers of mice are all begun on time course or completing time course, with final sacrifice due to be complete by mid-December 2020. Plan is to sacrifice the mice @ 6 months diabetes or control and perform the studies indicated in the Narrative of the funded grant. The lab was shut down Mid-March to Mid-June and hence no experiments or anything more than basic mouse husbandry (no breeding or purchases) was allowed.

Aim 2:

Ager flox flox *lysm* (+/+) cre recombinase mice
To date the following number of mice have been completed (sacrificed already and tissue sample analysis completed or underway):

Diabetes:	Male (N=1)
	Female (N=10)
Non-diabetes:	Male (N=2)
	Female (N=10)

Ager flox flox *lysm* (wt/wt) cre recombinase mice
To date the following number of mice have been completed (sacrificed already and tissue sample analysis completed or underway):

Diabetes:	Male (N=10)
	Female (N=7)
Non-diabetes:	Male (N=10)
	Female (N=11)

Diaph1 flox flox *lysm* (+/wt) cre recombinase mice
To date the following number of mice have been completed (sacrificed already and tissue sample analysis completed or underway):

Diabetes:	Male (N=13)
	Female (N=13)
Non-diabetes:	Male (N=11)
	Female (N=10)

Diaph1 flox flox *lysm* (wt/wt) cre recombinase mice
To date the following number of mice have been completed (sacrificed already and tissue sample analysis completed or underway):

Diabetes:	Male (N=12)
	Female (N=11)
Non-diabetes:	Male (N=10)
	Female (N=11)

Final numbers of mice are all begun on time course or completing time course, with final sacrifice due to be complete by mid-December 2020. Plan is to sacrifice the mice @ 6 months diabetes or control and perform the studies indicated in the Narrative of the funded grant. The lab was shut down Mid-March to Mid-June and hence no experiments or anything more than basic mouse husbandry (no breeding or purchases) was allowed.

Interim Results:

Aim 1: Testing the effects of podocyte *Ager* and podocyte *Diaph1*

We have completed the first set of mice endpoints with Dr. D'Agati and the data are shown in Figures 1-2. In diabetic female and male Cre- control mice, there were significant increases in mesangial sclerosis compared to respective female and male non-diabetic mice. However, both female and male diabetic Cre+ mice (podocyte deletion of *Ager*) displayed significantly lower mesangial sclerosis vs. the respective diabetic Cre- mice (podocytes expressing *Ager*). Additional pathological endpoint studies, including electron microscopy (for assessment of glomerular basement membrane thickness (GBM) and the percent podocyte effacement) are underway. Additional animals will be included by end of study.

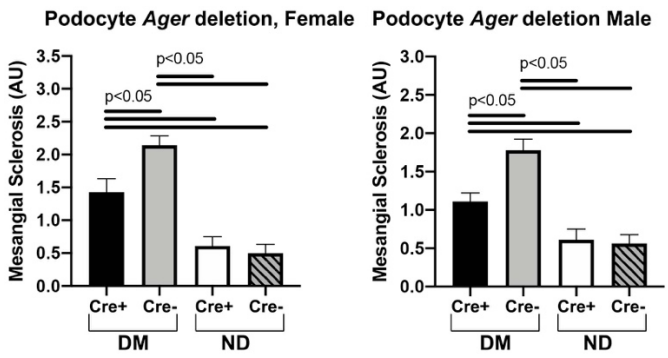

***Figure 1*. *Effect of podocyte RAGE on mesangial sclerosis in T1D mice**. Female and male mice with podocyte deletion of Ager (Cre+) vs. podocyte Ager expression (Cre-) were rendered T1D and after 6 mo. of diabetes or control nondiabetic state, mice were sacrificed for assessment of mesangial sclerosis. Mean±SEM is shown in 7-9 mice/group.*

Deletion of podocyte *Diaph1* (Figure 2) in non-diabetic female or male mice had no effect on mesangial sclerosis. In the diabetic mice, there were trends to lower mesangial sclerosis in both female and male Cre+ vs. Cre- diabetic mice. Analysis of additional mice and additional pathological endpoints, including electron microscopy (for assessment of GBM thickness and the percent podocyte effacement) is underway. Additional animals will be included by end of study.

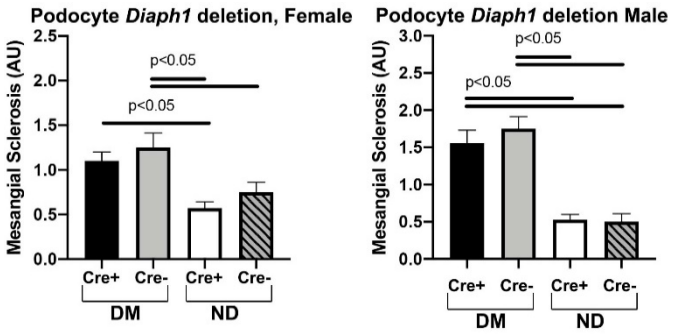

Figure 2. Effect of podocyte DIAPH1on mesangial sclerosis in T1D mice*. Female and male mice with podocyte deletion of Diaph1 (Cre+) vs. podocyte Diaph1 expression (Cre-) were rendered T1D and after 6 mo of diabetes or control nondiabetic state, mice were sacrificed for assessment of mesangial sclerosis. Mean±SEM is shown in 6-10 mice/group*

Aim 2: Testing the effects of myeloid *Ager* and *Diaph1 1*

We generated mice with myeloid deletion of *Diaph1*. We studied "Cre+" mice (that is, myeloid knockout of *Diaph1*) and "Cre-" mice (that is, *Diaph1*-expressing controls for the Cre+ mice). Mice were rendered type 1 diabetic (T1D) at age 6-7 weeks and sacrificed after 6 months of diabetes or were maintained as non-diabetic controls and sacrificed at the same age. Not shown here, by real time PCR in bone marrow-derived macrophages retrieved from these mice, in Cre+ mice, there was near absence of macrophage *Diaph1* vs. the Cre- mice. (Note that in mice with myeloid deletion of *Ager*, compared to Cre- mice, the Cre+ mice demonstrated a near total absence of *Ager* in bone marrow-derived macrophages, not shown).

As shown in Figure 3, myeloid deletion of *Diaph1* in non-diabetic female or male mice had no effect on mesangial sclerosis. In the case of *Diaph1*-expressing or deleted mice with diabetes, both female and male diabetic Cre+ mice (myeloid deletion of *Diaph1*) displayed significantly lower mesangial sclerosis vs. the respective Cre- mice (myeloid expression of *Diaph1*). Additional pathological endpoints, including electron microscopy (for assessment of glomerular basement membrane thickness and the percent podocyte effacement) are underway. Additional animals are being added to complete the groups.

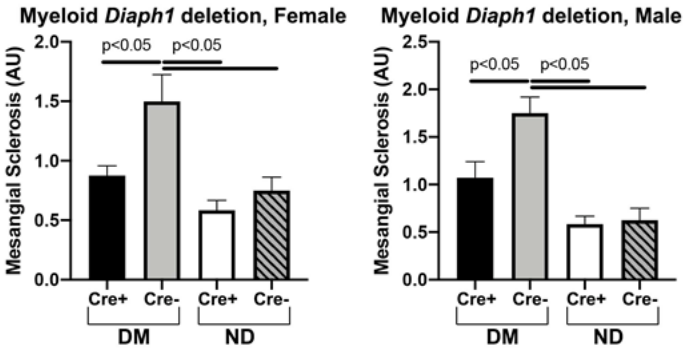

Figure 3. Effect of myeloid DIAPH1 on mesangial sclerosis in T1D mice*. Female and male mice with myeloid deletion of Diaph1 (Cre+) vs. myeloid Diaph1 expression (Cre-) were rendered T1D*

and after 6 mo of diabetes or control nondiabetic state, mice were sacrificed for assessment of mesangial sclerosis. Mean±SEM is shown in 4-8 mice/group.

Note that mice for testing myeloid deletion of *Ager* have all been generated and are at various stages in the time course and sample analysis.

Aim 3:

C57BL/6 mice were rendered diabetic and then randomized to treatment with one of three doses of the RAGE/DIAPH1 antagonist, RAGE229. Beginning at 8 weeks, mice were begun on vehicle chow or RAGE229 delivering 30 mg/kg/mouse/day, 10 mg/kg/mouse/day or 3 mg/kg/mouse/day based on pharmacokinetic data showing that doses of 30 or 10 mg/kg/mouse/day resulted in statistically significant attenuation of inflammation in a delayed type hypersensitivity (DTH) model. In contrast, 3 mg/kg/mouse/day resulted in trends to significant benefit in DTH mice. Three different endpoints have been analyzed to date: by electron microscopy, we examined GBM thickness or % podocyte effacement and mesangial sclerosis by light microscopy.

In male mice, administration of RAGE229 to diabetic mice at 30 mg/kg/day or 10 mg/kg/day but not 3 mg/kg/day resulted in significantly reduced GBM thickness vs. vehicle. In female mice, administration of all three doses resulted in significant reductions in GBM thickness vs. vehicle. These data are shown in Figure 4A. In male and female mice, administration of RAGE229 to diabetic mice at 30 mg/kg/day or 10 mg/kg/day but not 3 mg/kg/day resulted in significant attenuation of mesangial sclerosis and podocyte effacement vs. vehicle. These data are shown in Figure 4B-C.

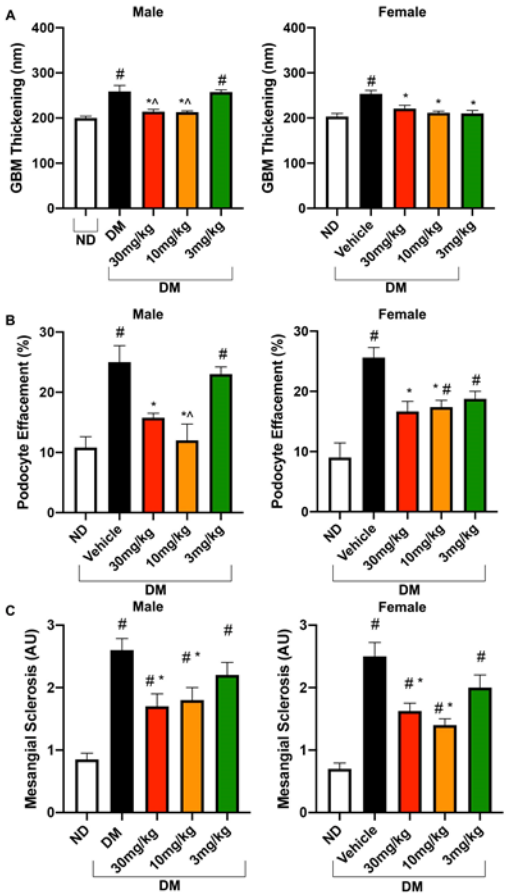

Figure 4. Effect of RAGE229 on diabetes-associated nephropathy in T1D mice*. Male and female T1D mice were treated for 6 mo with the indicated dose of RAGE229, commencing immediately after the induction of diabetes. At sacrifice, GBM thickness (A), % podocyte effacement (B) and mesangial sclerosis (C) were assessed. Mean±SEM is shown in 3-5 mice/group. #vs ND; * vs DM vehicle*

The unmet goals include completing all of the mice to the final time points and completing the molecular and pathological analyses. We have generated all of the needed mice successfully; we have characterized them; and we have assembled all of the needed collaborators to successfully execute the studies in the outlined Narrative of the funded grant.

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

Dr. NARA SZOSTACZUK under Dr. Schmidt's mentorship learned a great deal during her work on this project. She learned the complexities of working with floxed/cre mice and how to understand their characterization. She learned how to induce and monitor diabetes in the animals and she learned how to keep careful monitoring records of the mice per the protocol and to prepare for their sacrifice and post-mortem studies. Dr. Szostaczuk learned how to isolate podocytes and bone marrow derived macrophages from mice and how to properly test antibodies for specificity and how to perform real time quantitative PCR with these tissues.

How were the results disseminated to communities of interest?

NOTHING TO REPORT

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

As we have indicated throughout the narrative above, we plan to:
1). Add the mice to completion in the four groups of animals; sacrifice them at 6 months and continue the tissue/urine analysis (Aim 1-2-3).
2). With Dr. Ramasamy, we plan to perform metabolomics/lipidomics and transcriptomics on the isolated macrophages in order to discern mechanisms of action in these models once we identify the optimal conditions from studies in Aim 1 (Aim 1-2)
4). We plan to complete the analysis of pharmacological studies (Aim 3) as outlined in the Narrative.

4. IMPACT

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

Despite years of research on diabetic kidney disease, the precise cells that mediate the damage in diabetes are not fully clarified vis-à-vis RAGE and DIAPH1. We know that mice globally devoid of *Ager* or *Diaph1* (new publication 2018) are protected from diabetes associated nephropathy but we do not know the cell specific

mechanism. This work as outlined in this funded grant holds great promise to uncover new insights into the mechanisms by which diabetes causes nephropathic changes in the kidney.

- **What was the impact on other disciplines?**
  NOTHING TO REPORT AT THIS TIME

- **What was the impact on technology transfer?**
  NOTHING TO REPORT AT THIS TIME

- **What was the impact on society beyond science and technology?**
  NOTHING TO REPORT AT THIS TIME

5. CHANGES/PROBLEMS

- **Changes in approach and reasons for change**
  There are no changes in approach.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  As above the laboratory was shut down from Mid-March to Mid-June 2020. Upon return to the laboratory, mouse and molecular analysis studies needed to re commence accordingly. We are now continuing with our work and anticipating that no generalized shut down will delay completion of the studies.

- **Changes that had a significant impact on expenditures**
  As above the laboratory was shut down from Mid-March to Mid-June 2020. Upon return to the laboratory, mouse and molecular analysis studies needed to re commence accordingly. We are now continuing with our work and anticipating that no generalized shut down will delay completion of the studies.

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

  No human subjects
  No select agents
  No significant changes in the use of vertebrate animals – any amendments were first submitted at NYU and then submitted to DOD ACURO. These amendments had NO impact on the plans of the funded work but involved staffing and amendments to ensure that the aims were carried out as written in the funded grant.

  Approval Date of the IACUC:
  Effective Date: 06/17/2020
  Final Expiration Date: 6/13/2023

  ACURO
  Approval Date: 9/2/20

Final Expiration Date: 30 days after 6/13/2023

6. PRODUCTS

Publication:
Manigrasso MB, Friedman RA, Ramasamy R, D'Agati V, Schmidt AM. Deletion of the formin Diaph1 protects from structural and functional abnormalities in the murine diabetic kidney. Am J Physiol Renal Physiol 315:F1601-F1612, 2018

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Ann Marie Schmidt
Project Role:	PI (Initiating)
Researcher Identifier (e.g. ORCID ID):	SCHMIDTAM (eRA Commons ID)
Nearest person month worked:	0.4
Contribution to Project:	PI, oversight of project and all administrative work with respect to use of animals and the budgetary requirements
Funding Support:	DOD

Name:	Ravichandran Ramasamy
Project Role:	PI (Partnering)
Researcher Identifier (e.g. ORCID ID):	RAVIRAMASAMY (eRA Commons ID)
Nearest person month worked:	1
Contribution to Project:	Dr. Ramasamy developed the methods for the metabolomics and lipidomics analyses of the mice under study.
Funding Support:	DOD

Name:	Raquel Lopez-Diez
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	DIEZR01 (eRA Commons ID)
Nearest person month worked:	6
Contribution to Project:	Contributed to podocyte isolation processes and characterization with immunostaining strategies
Funding Support:	DOD

Name:	Michael MacLean
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	MM8848
Nearest person month worked:	12
Contribution to Project:	Mr. MacLean assisted in data analysis.
Funding Support:	DOD Vivette D'Agati

Name:	
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	VDA1234 (eRA Commons ID)
Nearest person month worked:	1
Contribution to Project:	Pathological analysis of kidney tissues
Funding Support:	DOD

Name:	Richard A. Friedman
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	FRIEDMANR (eRA Commons ID)
Nearest person month worked:	1
Contribution to Project:	Statistical and bioinformatics analyses
Funding Support:	DOD

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

Schmidt, Ann Marie

ACTIVE

1R01DK109675 04/01/16-03/31/21 0.91 calendar
NIH
RAGE/mDia1, Macrophage Trafficking and Inflammation in High Fat Feeding
Major goal of this application is to understand macrophage-adipocyte interactions in high fat feeding and obesity.
Role: PI

1R01HL132516 12/09/16-11/30/20 0.46 calendar
NIH
RAGE/mDia1, Macrophage Trafficking and Inflammation in Regression of Diabetic Atherosclerosis
The major goal of this grant is to probe the mechanisms by which macrophage (Mø) RAGE impairs regression of atherosclerosis in diabetic or IR mice.
Role: Multi-PIs (Schmidt & Ramasamy-Contact-PI)

1P01HL131481 (Fisher, PI) P01 05/01/17-04/30/22 2.86 calendar
NIH

Macrophage Dysfunction in Obesity, Diabetes and Atherosclerosis
Major goal of this application is to determine mechanisms of macrophage trafficking, metabolism and inflammation in the context of RAGE/DIAPH1 in obesity
Role: Co-I

USAMRAA Dept. of the Army 07/01/17-06/30/21 (NCE)
Receptor for AGE (RAGE) Signal Transduction in Amyotrophic Lateral Sclerosis: In Vivo Imaging and Novel Therapeutic Approaches
Major goals of this grant includes testing the hypothesis that microglia RAGE, through ligand-driven upregulation of inflammatory and pro-oxidative stress and suppression of reparative processes in the ALS spinal cord, mediates neuronal death and loss of motor function and probing the hypothesis that PBMM-specific deletion of Ager attenuates neuronal stress, accumulation of Aβ and amyloid plaques, synaptic dysfunction and cognitive impairment in APPswe/PS1 mice.
Role: PI

(THIS AWARD)
USAMRAA Dept. of the Army 09/30/17-09/29/21 (NCE)
W81XWH-17-1-0201/0202
RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies
Major goals for this grant involves (a) testing the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules (b) testing the hypothesis that RAGE and DIAPH1-expressing macrophages contribute to structural and functional derangements in DN through upregulation of tissue-destructive and profibrotic mediators and (c) determining if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.
Role: PI (Ramasamy-Partnering PI)

American Heart Association 04/01/17-03/31/21 3.6 calendar

Braking Inflammation in Obesity & Metabolic Dysfunction: Translational and Therapeutic Opportunities
The major goal of this grant is to investigate the novel hypothesis that impaired adipocyte, macrophage and other inflammatory cell signal transduction thwarts weight loss and its anti-inflammatory and metabolic benefits, at least in part through the activation of the receptor for advanced glycation endproducts, or RAGE pathway, which has been shown to regulate a unique repertoire of inflammatory and metabolic processes.
Role: Center Director, Project 1 Leader

1P01HL146367-01 08/01/19- 07/31/24 3.0 calendar
NHLBI
Macrophages, Cell-Cell Communication, Ischemic Injury in Diabetes and the RAGE/DIAPH1 Signaling Axis
The major goal of this grant application is to probe the mechanisms and identify new therapies for untoward monocyte and macrophage responses in ischemia, which, together with cellular

perturbation in the microenvironment, amplify damage in myocardial infarction and peripheral arterial disease, especially in diabetes.
Role: PI

(NEW)
1R01DK122456-01A1 07/01/20-03/31/25 0.91 calendar
NIDDK
Targeting RAGE/DIAPH1: Novel Therapeutic Strategy for Diabetic Complications
The major goal of this grant is to develop small molecule antagonists to treat diabetic complications.
Role: MPI (Schmidt (contact PI)/Ramasamy)

INACTIVE
(ENDED)
1R24DK103032 08/01/14-07/31/20 0.06 calendar
NIH (NCE)
Targeting RAGE-mDia1 in Diabetic Complications: Mechanisms & Therapeutics
Major goal of this application is to develop small molecule inhibitors of the interaction of the RAGE cytoplasmic domain with DIAPH1.
Role: PI

(ENDED)
Alzheimer's Association – Zenith Award 03/01/17-02/29/20 0.24 calendar ZEN-17-440472
RAGE, Diaph1, Microglia and Alzheimer's disease
Major goal of this grant is to probe the hypothesis that microglial-specific Ager deletion modulates neuronal stress, accumulation of Aβ and amyloid plaques, synaptic and cognitive dysfunction in APPswe/PS1 mice.
Role: PI

(ENDED)
P01HL60901 07/15/11-11/30/18 0.12 calendar
NIH No Cost Extension
RAGE and Mechanisms of Vascular Dysfunction
This grant focuses on the mechanisms by which diabetes accelerates atherosclerosis via RAGE.
Role: Project 1 and Core A Leader, Core C Co-Leader

(ENDED)
1R01 HL118565 06/01/13-04/30/18 0.9 calendar
NIH
RAGE, Macrophages & HDL Biology
This grant examines the molecular mechanisms by which RAGE regulates cholesterol transport.
Role: PI

(ENDED)

Harrington Discovery Institute 01/01/16-12/31/17 0.12 calendar

Targeting RAGE/mDia1 for the Prevention and Treatment of Diabetic Complications
The goal of the Harrington Discovery Institute project is to develop LOCAL intraocular and transdermal treatments for diabetic retinopathy and diabetic wound healing, respectively.
Role: PI

OVERLAP
None

D'Agati, Vivette

ACTIVE
2 PO1 DK 56492 (Klotman) 05/15/17-04/30/22 1.8 calendar
NIH NIDDK (Core B)
Long-term Consequences of HIV Infection of the Kidney
The aim of this grant is to explore the long-term consequences of HIV infection of the kidney beyond HIVAN. The role of Core B will be to provide technical and interpretative support in light microscopy, immunohistochemistry, immunofluorescence and electron microscopy in mouse and human kidney tissues for all projects, including study of the mechanisms of ApoL1 induced kidney injury, the kidney as long-term reservoir for HIV, the interactions between gut microbiome and HIVAN in the Tg26 model, and the effects of chronic HIV infection on progression of kidney disease through enhanced pro-inflammatory responses.
Role: Core B Leader

(NEW)
2U01 DK100876-07 (Gharavi) 07/01/19-06/30/24 0.60 calendar
NIH/NIDDK
The Columbia PCC for CureGN: the Cure Glomerulonephropathy network
The major goal of this project is to develop a longitudinal observational cohort of patients with different forms of glomerular diseases.
Role: Co - Investigator

R01 DK109544-01A1 (Lee) 04/01/17-03/31/22 0.6 calendar
NIH
Paneth cells and acute kidney injury
This proposal seeks to elucidate the mechanisms as well as therapies for remote organ dysfunction after renal ischemia and reperfusion injury.
Role: Co-I

1R01 DK115694-01 (Lee) 09/13/17–07/31/22 0.6 calendar
NIH
Peptidylarginine deiminase-4 and acute kidney injury

This project seeks to elucidate the mechanisms as well as therapies for inflammation and injury after renal ischemia and reperfusion.
Role: Co-I
1UG3DK114926-01 (Kiryluk, Barasch, Bomback) 07/01/17 - 06/30/22 0.9 calendar
NIH/NIDDK
Kidney Precision Medicine Program (KPMP): Columbia AKI Recruitment Site
The national Kidney Precision Medicine Project (KPMP) aims to reduce the significant global health burden of acute kidney injury (AKI) by elucidating mechanisms and effective therapies through precision medicine technologies.
Role: Co-I

(THIS AWARD)
USAMRAA Dept. of the Army (Schmidt, Ramasamy, MPI) 09/30/17-09/29/21 (NCE)
W81XWH-17-1-0201/0202
RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies
This project will test the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules and will determine if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.
Role: Co-I

INACTIVE
(ENDED)
1R24DK103032-01 (Schmidt) 08/01/14/-07/31/20 0.12 calendar
NIH (NCE)
Targeting RAGE-mDia1 in Diabetic Complications: Mechanisms & Therapeutics
This application focuses on the role of the receptor for advanced glycation endproducts (RAGE) and its cytoplasmic domain binding partner, mammalian form of diaphanous1, mDia1, which is essential for RAGE signaling as a fundamental therapeutic target for diabetic complications.
Role: Co-I

(ENDED)
1R01DK106436-01A1 (Winchester) 01/25/16-12/31/19 0.6 calendar
NIH/NIDDK
Significance of Intrarenal T Cells in SLE Nephritis
The overall goal of this study is to define the role of T cells in the development and progression of chronic lupus nephritis. It addresses the clinical problem of why nearly half of lupus nephritis cases do not adequately respond to therapy and much progress to chronic glomerulonephritis. SLE kidneys have a variable but often extensive infiltrate of predominantly clonally expanded CD4 and/or CD8 T cells with features that suggest they could drive the inflammatory process. We hypothesize that while acute glomerulitis is driven by immune complexes, chronic SLE nephritis is driven by the development of CD4 and especially CD8 T cell clonal recognition of self-peptides, resulting in glomerular and tubular cell injury. In the first aim we will delineate the extent and character of the renal CD4 or CD8 T cell infiltration in new onset nephritis and correlate these findings with short- and long-term pathological and clinical outcomes that predict poor response

to therapy and progressive renal disease. In the second part of this aim we will also determine the T cell characteristics of cases with worsening renal involvement requiring repeat biopsy, comparing current and prior biopsies for T cell features that predict progression. In the second aim we will define the properties of the infiltrating T cells and their potential mechanisms to mediate renal injury, discriminating between the phenotype of clonally expanded CD4 or CD8 T cells that potentially drive renal injury and that of the polyclonal T cells secondarily recruited by inflammation.
Role: Co-I

(ENDED)
Dept. of the Army – USAMRAA (Gharavi) 08/15/16 – 08/14/19 0.84 calendar
Grant number: PR151419
Multispecies, integrative GWAS for focal segmental glomerulosclerosis
Goals: The major goal of this project is to devise new targeted therapies that will impact and benefit treatment for chronic kidney disease at large in the general population as well as in the active duty personnel, veterans and their families. Aims: - Specific aim 1: A Genome-wide association study for common single nucleotide polymorphisms and rare copy number variations in 7,559 FSGS and over 50,000 controls - Specific aim 2: A GWAS for FSGS in a mouse leveraging the power of the newly developed DO strains - Specific aim 3. Cross annotation between human and mouse GWAS and identification of downstream dysregulated pathways and networks.
Role: Co-I

(ENDED)
1R01MD009223-01 (Multi-PI: Gharavi & Bomback) 07/01/14-06/30/19 0.6 calendar
NIH/NIMHD
Ancestry, Genetic Risk and Health Disparities in Immune-Mediated Nephritis
The major goal of this project is to investigate the role of shared and distinct genetic factors among Europeans, Asians, Hispanics, and African-Americans in the onset, course, and ultimate outcomes of IgA nephropathy, membranous nephropathy and lupus nephritis.
Role: Co-I

(ENDED)
1 UM1 DK100876-01 (Gharavi) 09/16/13-05/31/18 1.2 calendar
NIH/NIDDK
Advancing Clinical Research in Primary Glomerular Diseases
The major goals of this project are to develop a longitudinal observational cohort of patients with biopsy-documented forms of major glomerular diseases, including minimal change disease, focal segmental glomerulosclerosis, membranous glomerulopathy and IgA nephropathy.
Role: Co-I

OVERLAP
None

Friedman, Richard A.

ACTIVE

P30CA013696-45S1 (Rustgi, PI) 07/01/20 - 06/30/25 1.08 calendar
NCI
Cancer Center Support Grant
This grant supports the leadership of Columbia University's lab, clinical & population-based cancer research programs & the shared resources serving the University's Cancer Center members. Dr. Friedman's role as a member of the Biomedical Informatics Shared Resource is to provide the bioinformatics and statistical component of cancer research projects.
Role: Staff Member of the Biomedical Informatics Shared Resource.

1R35CA210088-04 (Wang-PI) 12/09/16-11/30/23 1.08 calendar
NIH/NCI
The Role of Stem Cells and the Microenvironment in Gastrointestinal Cancer
This project seeks to investigate the role of nerves and other stromal cells in the development of digestive cancers, including stomach, esophageal, colon and pancreas. The project builds on previous work that suggests that these elements can regulate stem cells and that inhibiting stromal cells in the microenvironment, it may be possible to inhibit the development of tumors. Dr. Friedman's role is to design and analyze RNASeq and other genomic experiments and perform other statistical analyses.
Role: Co-I

1U54CA163004-09 (Wang, PI) 05/12/17-4/30/22 0.6 calendar
NIH/NCI
The Role of the Microenvironment in Barrett's Esophagus
The major goal of this project is to characterize the role of the microenvironment in Barrett's esophagus and esophageal cancer. Dr. Friedman's role is to design and analyze RNASeq experiments to measure gene expression, to ascertain the role of the microenvironment and in response to drug treatment.
Role: Co-I

(NEW)
5U01DK103155-07 (Wang, PI) 09/15/14-8/31/24 0.84 calendar
NIH/NIDDK
Understanding stem cell heterogeneity and niche function in intestinal regeneration after irradiation
To investigate the molecular determinants of stem cell function in response to irradiation. Dr. Friedman's role is to analyze gene expression and other experiments.
Role: Co-I

(NEW)
3U54CA163004-09S1 (Wang, PI) 05/01/20-04/30/22 0.6 calendar
NIH/NCI
SARS-COV-2, ACE2 and Esophageal Neoplasia

The major goal of this project is to characterize how SARS-COV-2 increases the risk of Barrett's esophagus and esophageal cancer. Dr. Friedman's role is to design and analyze single cell RNASeq experiments to measure gene expression, to ascertain the role of the microenvironment and in response to drug treatment.
Role: Co-I

1R01CA208711-06 Sepulveda (PI) 09/01/2016-08/31/21 0.84 calendar
NIH/NCI
Genomics and Mechanisms of Esophageal Carcinogenesis
The goal of this project is to investigate the role CDKN1A/P16 mutations the genesis of esophageal cancer; to use expression, polymorphism, and methylation data to predict progression to cancer, and to test various drugs for their ability to prevent this progression from occurring. Dr. Friedman's role is to perform statistical analyses.
Role: Co-I

1R01DK109675-01 (Schmidt) 04/01/16-03/31/21 0.84 calendar
NIH/NIDDK
RAGE/MDIA1, Macrophage Trafficking and Inflammation in High Fat Feeding
To characterize the mechanism by which macrophage Receptor for Advanced Glycation Endproducts regulates obesity, adiposity and metabolic dysfunction in high fat feeding, both inherently and via cross-talk with the adipocyte.
Role: Co-I

1R01HL132516-01-A1 (Ramasamy, Schmidt-MPI) 12/09/16-11/30/20 1.08 calendar
NIH/NHLBI
RAGE/mDia1, Macrophage Trafficking and Inflammation in Regression of Diabetic Atherosclerosis
The goal of this project is to characterize how RAGE and mDia1 signaling macrophages affect atherosclerotic regression. Dr. Friedman's role is to perform genomic and statistical analyses.
Role: Co-I

(THIS AWARD)
USAMRAA Dept. of the Army (Schmidt, Ramasamy, MPI) 09/30/17-09/29/21 (NCE)
W81XWH-17-1-0201/0202
RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies
To characterize the mechanism by which macrophage Receptor for Advanced Glycation Endproducts helps cause diabetic kidney disease and develop therapies for the treatment of this disease. Dr. Friedman's role is to analyze RNASeq and other experiments.
Role: Co-I

(NEW)
Internal Pilot (Lieberman, PI) 04/01/19-3/31/20 0.6 calendar
Identification of Precision Diagnostic and Therapeutic Targets for Advanced Prostate Cancer Patients Based on Mechanistic RNA Landscape
Role: Co-I

(NEW)
1P01 HL146367-01 (Schmidt, PI) 08/01/19-06/30/24 0.6 calendar
NIH/NHLBI
Macrophages, Cell-Cell Communication, Ischemic Injury in Diabetes and the RAGE/DIAPH1 Signaling Axis
To elucidate the mechanism of the role of the RAGE/DIAPH1 signaling pathway in ischemic injury and diabetes. Dr. Friedman's role is to design and analyze RNASeq experiments to measure gene expression.
Role: Co-I

(NEW)
R01ES030481-01A1 (Bickers/Kim) 04/07/20-01/31/25 1.2 calendar
NCI
Molecular Mechanisms Underlying the Prevention of BCC Resistance
To test the hypothesis that dysregulation of the epigenetic regulators BRD7 and BRD9 are drivers of BCC resistance by investigating the mechanisms responsible for these effects and test the preclinical utility of pharmacological BRD9 blockade in overcoming vismodegib resistance, potentially reducing the side-effect profile and improving the therapeutic index of pharmacologic inhibitors of Sonic Hedgehog signaling in these tumors. Dr. Friedman's role is to design, analyze, and integrate ChIPSeq and RNASeq data.
Role: Co-I

INACTIVE
(No Longer on Grant)
2R01DK048077-23 (Wang, PI) 09/01/16-8/31/21 0.84 calendar
NIH/NIDDK
The Function and Regulation of Histidine Decarboxylase
To investigate the role of histidine decarboxylase in digestive cancer and the immune response. Dr. Friedman's role is to analyze gene expression and other experiments.
Role: Co-I

(ENDED)
1R01CA178445-04 (Su, PI) 07/01/15-06/30/20 0.6 calendar
NIH/NCI
The Role of wild-type Kras in the context of tumor progression and metastasis
To elucidate the mechanism of the role of Kras in human pancreatic ductal adenocarcinoma by means of a mouse model. Dr. Friedman's role is to design and analyze RNASeq and pcr experiments to measure gene expression.
Role: Co-I

(No Longer on Grant)
1P01HL131481-01A1 (E. Fisher PI) 05/01/17-04/30/19 1.8 calendar
NIH/NHLBI
Macrophage Dysfunction in Obesity Diabetes and Atherosclerosis

The goal of this project is to characterize how macrophages affect atherosclerosis in patients who are diabetic, and/or obese. Dr. Friedman's role is to perform genomic and statistical analyses.
Role: Co-I

(ENDED)
W81XWH-15-1-0296 Broustas (PI) 08/31/2015-08/30/2018 0.24 calendar
DOD
Targeting MEK5 Enhances Radiosensitivity in Human Prostate Cancer
The goal of this project is to test whether inhibition of MEK5 signaling enhances the response of human prostate cancer cell lines to radiation therapy. Dr. Friedman's role is to perform statistical analyses
Role: Co-I

(ENDED)
1R01HL118565-04 (Schmidt/Friedman, PI) 06/01/13-4/30/18 1.2 calendar
NIH/NHLBI
RAGE, Macrophages, and HDL Biology
To link RAGE (Receptor for Advanced Glycation Endproducts) function to that of High Density Lipoproteins. Dr. Friedman's role is to design and analyze RNASeq experiments to discover molecular mechanisms underlying obesity.
Role: Co-I

(ENDED)
5R03CA186218-02 (Abrams, PI) 07/01/15-06/30/17 0.3 calendar
NIH/NIDCR
Randomized placebo-controlled trial of a gastrin receptor (Abrams: PI)
The goal of this project is to test the effect of a netazepide (YF476), a gastrin receptor antagonist, on biomarkers associated with progression to esophageal adenocarcinoma. Dr. Friedman's role is to design and analyze RNASeq and PCR experiments to measure gene expression.
Specific Aims:
Role: Co-I

OVERLAP
None

- **What other organizations were involved as partners?**
  Organization Name: Columbia University
  Location of Organization: New York, NY
  Collaborating Investigators: Drs. Vivette D'Agati and Richard A. Friedman
  Collaboration: Dr. D'Agati performed the pathological analysis of kidney tissues.
  Dr. Friedman performed statistical and bioinformatics analyses.

8. SPECIAL REPORTING REQUIREMENTS

Dr. Ravichandran Ramasamy, PhD (Partnering PI) progress report follows Dr. Schmidt's section.

9. APPENDICES:

Nothing to report

Ravichandran Ramasamy – Partnering PI

1. INTRODUCTION:

Our laboratory discovered the receptor for advanced glycation endproducts (RAGE) and we identified that the cytoplasmic domain (tail) of RAGE binds to the formin, DIAPH1, and that this interaction is critical for RAGE ligand-mediated signal transduction and modulation of gene expression linked to cellular perturbation. DIAPH1 mediates actin cytoskeleton functions, cellular migration and activation of the Rho GTPases. DIAPH1 is expressed by immune and vascular cells; we reported that deletion of *Diaph1* in murine macrophages protects against hypoxia-mediated upregulation of proinflammatory (*Egr1* and *Ccl2*) and prothrombotic (*Tf*) and that this protection is analogous to that observed in macrophages devoid of *Ager*. Furthermore, with Dr. Alexander Shekhtman, we have identified the precise mechanism by which the cytoplasmic domain of RAGE binds DIAPH1. Critically, Dr. D'Agati's data reveal that DIAPH1, like RAGE, is highly expressed in human diabetic podocytes. **The goal of this grant is to determine the specific mechanisms by which RAGE/DIAPH1 contribute to the pathogenesis of diabetes associated nephropathy and we to explore novel RAGE/DIAPH1-directed therapeutic opportunities. The goal of the studies by Partnering PI is to determine is to elucidate transcriptomic and substrate metabolic mechanisms driven by RAGE/DIAPH1 in diabetic nephropathy and to explore if these specific metabolic changes can serve as RAGE/DIAPH1 target biomarkers.**

2. KEYWORDS:

Diabetes
DIAPH1
Floxed Mice
Glomerulosclerosis
Glomerular basement membrane
Inflammation
Macrophage
Nephropathy
Podocyte
RAGE
Small Molecule Antagonist

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**
  There are three specific aims of the funded grant:

  AIM 1 will test the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules. We will generate mice in which podocyte-

specific deletion of *Ager* or *Diaph1* is accomplished via breeding *Ager* or *Diaph1* floxed mice with podocin (*Nphs2*) cre recombinase mice.
AIM 2 will test the hypothesis that RAGE and DIAPH1-expressing macrophages contribute to structural and functional derangements in DN through upregulation of tissue-destructive and profibrotic mediators. We will generate mice in which myeloid cell deletion of *Ager* or *Diaph1* is accomplished by breeding *Ager* or *Diaph1* floxed mice with *Lysm* cre recombinase mice.

AIM 3 will determine if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.

- **What was accomplished under these goals?**

1) Major Activities
In the third year of the funded grant, along with Dr. Schmidt we have focused on the following major activities:

PI, Dr. Schmidt and her team have already generated the four different lines of mice to directly test the hypothesis that RAGE and DIAPH1 contribute to the pathogenesis of diabetes-associated nephropathy in the podocytes and/or in myeloid cells/macrophages. In the section prepared by Dr. Schmidt, the numbers of animals that have completed studies are indicated, as are the data analyses (from three of the four lines) available to date.

Dr. Schmidt and her team are isolating macrophages from the kidneys of the mouse models using described techniques. The goal of the Partnering PI and his team is to obtain these cells from Dr. Schmid's team for transcriptomic and metabolomic measurements to determine RAGE/DIAPH1 specific changes in macrophage properties.

For transcriptomic, metabolomics and lipidomics assays, we have set up the validation for all of the measurements to be performed. We will be testing the tissues/cells from the mice through the time course of lipid and intermediary metabolite changes. We have optimized and fine-tuned the metabolite measurements using spectroscopic approaches as in the outlined studies.

All of the colleagues and collaborators are in place, with roles and timing defined, in order to execute the outlined studies as expertly and efficiently as possible.

2) Specific objectives
Our objectives in year one were to execute the above metabolism studies in order to be certain that the aims of the study would be completed according to the three Specific Aims outlined in Item #1 above.

3) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative):

3A). Generation of Mouse Models for Study

At this time, Dr. Schmidt and her team have made extensive progress in three of the four mouse lines for study; the time course for study once diabetes (or control state) is induced is 6 months – multiple cohorts of mice have been sacrificed and data are available as outlined in the Narrative of the funded grant. In three of the four lines of mice to be tested (all but myeloid deletion of *Ager*), preliminary data are shown in the Main Section of this progress report.

Note that the investigators handling/working with the mice are naïve to the genotype; although they know the diabetes/non diabetes status and the sex of the mice, they are not aware of the genotype until after data are entered post sacrifice.

See the Section in Dr. Schmidt's progress report for the current status of the mouse numbers.

We have been obtaining tissue and cells from these mice for the outlined transcriptomic and metabolism experiments.

3B). Optimization of metabolite measurements: We have used ^{13}C heavy isotope (non-radioactive) and known lipid and intermediary metabolite standards to establish calibration and sensitivity limits for metabolomics/lipidomics measurements using mass spectrometry.

We have used primary macrophages and HEK cells to optimize lipids and intermediary metabolite extraction efficiency. Using ^{13}C heavy isotope labeled lipid standards and ^{13}C heavy isotope labeled TCA cycle metabolites we have been able to get a consistent extraction efficiency in the 75-80% ranges for lipids and 88-90% range for intermediary metabolites. Intra sample and inter sample variation of less than 5% for detection of known standards have also been established.

Comparison of lipids in WT DM and DM RKO macrophages and in PO (Palmitate-Oleate treated) RKO vs WT Macrophages:

Preliminary findings indicate that levels of PS, lysoPE, PC, phosphatidyl inositol (PI) were lower, while omega-6 fatty acids (C22:2) was higher in RKO vs WT macrophages. Some of the ceramide species also appear to be reduced in RKO macrophages. More samples are being analyzed prior to performing comprehensive statistical analysis.

Overall, the lipid changes in RKO macrophages suggest that deletion of RAGE may help in promoting anti-inflammatory effects in macrophages.

Next steps in our work include the analysis of the effects of RAGE and DIAPH1 on lipidome and aqueous metabolites in diabetic and non-diabetic macrophages. Integration of metabolomics and lipidomics with the transcriptomic data is also underway.

3C). Transcriptomic analyses.

To parallel studies of the metabolome/lipidome in the context of RAGE/DIAPH1, we employed fluorescence-activated cell sorting (FACS) techniques to sort macrophages (CD45+/F4/80+/CD11b+) from the kidney cortices (excluded kidney medulla) from three genotypes of mice (and 2 metabolic conditions, non-diabetic and diabetic states). The groups included: (1) wild-type (WT) WT non-diabetic (wtndm), WT diabetic (wtdm), (2) global RAGE knockout (ko) non-diabetic (rkondm), global RAGE ko diabetic (rkodm), and (3) global DIAPH1 ko non-diabetic (dkondm) and global DIAPH1 ko diabetic (dkodm) (n=3 mice/group). From the sorted macrophages, we performed RNA sequencing. Differential expression was estimated with weighted Limma-Voom. With Dr Friedman (Columbia University), we have made the following observations:

Comparison WT DM vs WT NDM: We used different programs to probe the relationships found among the differentially-expressed genes using strict FDR cut-off criteria (<0.05):
1). REACTOME: Reactome analysis identified 43 pathways that indicate very strong effects on **innate immune signaling** and **inflammation** including: Immune system, Innate immune system, cytokine signaling in immune system, MyD88-independent TLR4 cascade, TRIF (TICAM1)-mediated TLR4 signaling, TLR4 cascade, TLR cascades, TLR3 cascade, Signaling by Interleukins, TLR7/8 cascade, Myd88 dependent cascade initiated on endosome, TLR9 cascade, TLR10 cascade, TLR5 cascade, MyD88 cascade initiated on plasma membrane, MyD88:MAL (TIRAP) cascade initiated on plasma membrane, TLR1:TLR2 cascade, TLR6:TLR2 cascade, TLR2 cascade TRAF6 mediated induction of NFKB and MAP kinases upon TLR7/8 or TLR9 activation, Nucleotide binding domain, leucine rich repeat containing receptor (NLR) signaling pathway and Cellular responses to external stimuli, Cellular response to stress, Neutrophil degranulation, MAP kinase activation, Attenuation Phase, IL6 family signaling. IL17 signaling, Cellular response to heat stress, NOD1/2 signaling pathway, Regulation of HSF-1 mediated heat shock response, IL1 family signaling, DDX58'IF1H1 mediated induction of interferon alpha/beta, FCERI mediated MAP kinase activation, Cell surface interactions at the vascular wall, IL1 signaling, Chemokine receptors bind chemokines, Metal Ion SLC transporters, Death receptor signaling, HSF1 dependent transactivation, IL6 signaling, Interferon signaling, TAK1 activates NFKB by phosphorylation and activation of IKKs complex

2). KEGG: KEGG pathway analysis identified 81 pathways (FDR <0.05). The main themes of these 81 pathways included those such as: **inflammation** (cytokine/chemokine/NOD like receptors/IL17/TNF signaling/T and B cell receptor signaling pathway/Complement and others), and **cellular signaling** (HIF-1, mTOR, AGE-RAGE, TLR, TNF, NF-kB, JAK/STAT, adipocytokine, MAPK, PI3K/AKT, and FoxO signaling).

3). NETWORK ANALYSIS: Network analysis uncovered the **NFKB signaling pathway** as being differentially regulated in the WT DM vs WT NDM data set; the following 17 genes were differentially regulated (adj pvalue <0.05): *Birc3, Tnf, Tnfaip3, Trim25, CD40, Ddx58, Ccl4, Cxcl2, Syk, Plau, Bcl2a1b, Il1b, Nfkbia, Icam1, Vcam1, Ptgs2,* and *Cflar.*

Collectively, these data strongly support that diabetes induce an inflammatory signature in the macrophages that populate the cortex during diabetes vs the non-diabetic state.

Comparison RKO DM vs WT DM. To complete this analysis, the same programs as above were employed to compare the patterns of differentially expressed genes comparing RKO DM macrophages vs. WT DM macrophages (FDR<0.05).

1). REACTOME: There were 35 pathways uncovered by Reactome analysis. Most of these were identical to the pathways differentially regulated between WT DM and WT NDM macrophages such as Immune System, Innate Immune System, multiple pathways linked to TLR pathways, IL1, and chemokine receptors and chemokines. These data suggest that KO of RAGE attenuates expression of these highly pro-inflammatory pathways.

2). KEGG: KEGG pathway analysis identified 69 pathways (FDR <0.05). The main themes of these 69 pathways included those very similar to the comparison of WTDM vs WTNDM, including: **inflammation** (cytokine/chemokine/NOD like receptors/IL17/TNF signaling/T and B cell receptor signaling pathway/Complement and others), and **cellular signaling** (HIF-1, mTOR, AGE-RAGE, TLR, TNF, NF-kB, JAK/STAT, adipocytokine, MAPK, and FoxO signaling).

3). NETWORK ANALYSIS: Network analysis uncovered the **NFKB signaling pathway** as being differentially regulated in the RKO DM vs WT DM data set; the following 20 genes were significantly down-regulated in RKO DM vs WT DM, indicating a highly reduced activation of the NFKB network (adj pvalue <0.05): *Malt1, Map3k14, Bcl2a1d, Gadd45b, Rela, Relb, CD14,* ***Birc3, Tnf, Tnfaip3****,* ***Trim25****,* ***Ccl****4,* ***Cxcl2****,* ***Syk****,* ***Plau****,* ***Bcl2a1b****,* ***Il1b****,* ***Nfkbia****,* ***Ptgs2,*** and ***Cflar.*** **Note that those italicized in bold type are the same as those identified in the Network analysis for WT DM vs WT NDM differentially expressed genes within the NFKB network.**

Our team continues to work on the multiple additional analyses, including the analysis of the effects of DIAPH1 on the kidney macrophages and the multiple individual / new comparisons. At this time, our data support that diabetes increases macrophage inflammatory signaling (likely through NFKB, at least in part) and that in diabetes this is attenuated by deletion of RAGE.

4) Other achievements.
There are no other achievements to report at this time. Due to the COVID19 basic science shutdown (Mid-March to Mid-June) there were delays in this project. To date, we have generated all of the needed mice successfully; we have characterized them; we have established the spectroscopic methodology for metabolites/lipid measurements; we have made new discoveries on the effect of diabetes in the kidney macrophage properties and we have assembled all of the needed collaborators to successfully execute the studies in the outlined Narrative of the funded grant.

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

NOTHING TO REPORT

- **How were the results disseminated to communities of interest?**

NOTHING TO REPORT

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

As we have indicated throughout the narrative above, we plan to work with Dr. Schmidt to obtain indicted cells from various mice to complete transcriptomic and metabolomics/lipidomics assays to discern mechanisms of action in these models.

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  Despite years of research on diabetic kidney disease, the precise cells that mediate the damage in diabetes are not fully clarified vis-à-vis RAGE and DIAPH1. We know that mice globally devoid of *Ager* or *Diaph1* (DIAPH1 manuscript, publication 2018) are protected from diabetes associated nephropathy but we do not know the cell specific mechanism. This work as outlined in this funded grant holds great promise to uncover new insights into the mechanisms by which diabetes causes nephropathic changes in the kidney.

- **What was the impact on other disciplines?**
  NOTHING TO REPORT AT THIS TIME

- **What was the impact on technology transfer?**
  NOTHING TO REPORT AT THIS TIME

- **What was the impact on society beyond science and technology?**
  NOTHING TO REPORT AT THIS TIME

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
  There are no changes in approach.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  As above the laboratory was shut down from Mid-March to Mid-June 2020. Upon return to the laboratory, mouse and molecular analysis studies needed to commence accordingly. We are now continuing with our work and anticipating that no generalized shut down will delay completion of the studies.

- **Changes that had a significant impact on expenditures**
  As above the laboratory was shut down from Mid-March to Mid-June 2020. Upon return to the laboratory, mouse and molecular analysis studies needed to re commence accordingly. We are now continuing with our work and anticipating that no generalized shut down will delay completion of the studies.
  are being generated and studied as outlined.

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

No human subjects
No select agents
No significant changes in the use of vertebrate animals – any amendments were first submitted at NYU and then submitted to DOD ACURO. These amendments had NO impact on the plans of the funded work but involved staffing and amendments to ensure that the aims were carried out as written in the funded grant.

Approval Date of the IACUC:
Effective Date: 06/17/2020
Final Expiration Date: 6/13/2023

ACURO
Approval Date: 9/2/20
Final Expiration Date: 30 days after 6/13/2023

Cells and tissue from mice will be obtained from Dr. Schmidt's team for metabolite analysis. As in outlined studies for the partnering portion of the grant, no independent studies will be conducted on mice.

6. PRODUCTS:

Publication:

Manigrasso MB, Friedman RA, Ramasamy R, D'Agati V, Schmidt AM. Deletion of the formin Diaph1 protects from structural and functional abnormalities in the murine diabetic kidney. Am J Physiol Renal Physiol 315:F1601-F1612, 2018

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

- **What individuals have worked on the project?**

Name:	Ravichandran Ramasamy
Project Role:	PI (Partnering)
Researcher Identifier (e.g. ORCID ID):	RAVIRAMASAMY (eRA Commons ID)
Nearest person month worked:	1
Contribution to Project:	Dr. Ramasamy developed the methods for the metabolomics and lipidomics analyses of the mice under study
Funding Support:	DOD

Name:	Ann Marie Schmidt
Project Role:	PI (Initiating)
Researcher Identifier (e.g. ORCID ID):	SCHMIDTAM (eRA Commons ID)
Nearest person month worked:	0.4
Contribution to Project:	PI, oversight of project and all administrative work with respect to use of animals and the budgetary requirements
Funding Support:	DOD

Name:	Charlotte Detremmerie
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Performed *in vitro* experiments, data interpretation and analysis
Funding Support:	DOD

Name:	Qing Li
Project Role:	Associate Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Worked on optimizing assay conditions for metabolite extraction in cells from diabetic mice.
Funding Support:	DOD

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

Ramasamy, Ravichandran

ACTIVE

1R01DK109675 04/01/16-03/31/21 1.37 calendar
NIH
RAGE/mDia1, Macrophage Trafficking and Inflammation in High Fat Feeding
Major goal of this application is to understand macrophage-adipocyte interactions in high fat feeding and obesity.
Role: Multiple PIs (Schmidt (Contact PI) & Ramasamy)

1R01 HL132516-01 (Al) 12/09/16-11/30/20 1.82 calendar
NIH

RAGE/mDia1, Macrophage Trafficking and Inflammation in Regression of Diabetic Atherosclerosis
The major goal of this grant is to probe the mechanisms by which macrophage (MΦ) RAGE impairs regression of atherosclerosis in diabetic or IR mice.
Role: Multi-PIs (Schmidt & Ramasamy-(Contact-PI))

1P01 HL131481-A1 (Fisher, PI) 05/01/17-04/30/22 2.40 calendar
NIH
Macrophage Dysfunction in Obesity, Diabetes and Atherosclerosis
Major goal of this application is to determine mechanisms of macrophage trafficking, metabolism and inflammation in the context of RAGE/DIAPH1 in obesity
Role: Co-I

1 R01 HL135987-01A1 05/01/17 – 04/30/21 0.91 calendar
NIH
Fatty Acids: Ischemic Protection and Repair
The goal of this project is to assess how lipid metabolism in cardiomyocytes and white blood cells affects heart injury and repair after ischemia/reperfusion.
Role: Multi-PIs (Goldberg (contact PI) & Ramasamy)

<u>THIS AWARD</u>
USAMRAA Dept. of the Army 09/30/17-09/29/21 (NCE)
W81XWH-17-1-0201/0202
RAGE/Diaph1, Diabetes, and Kidney Disease: Mechanisms and Novel Therapeutic Strategies
Major goals for this grant involves (a) testing the hypothesis that RAGE and DIAPH1 mediate podocyte dysfunction in DN through disengagement of homeostatic actin cytoskeleton dynamics and upregulation of pro-inflammatory and pro-fibrotic molecules (b) testing the hypothesis that RAGE and DIAPH1-expressing macrophages contribute to structural and functional derangements in DN through upregulation of tissue-destructive and profibrotic mediators and (c) determining if administration of novel small molecule antagonists of RAGE-DIAPH1 interaction in diabetic mice protects against DN.
Role: Partnering PI (Schmidt, Contact PI)

2R01HL073029-12A1 (Goldberg) 08/10/18-07/31/22 0.91 calendar

Mechanisms of Fatty Acid Uptake by Cardiac Muscle
The goal of this project is to understand how lipids are obtained by the heart and their roles in normal physiology and in pathological conditions.
Role: Co-I

1P01HL146367-01 08/01/19- 07/31/24 2.86 calendar
NHLBI
Macrophages, Cell-Cell Communication, Ischemic Injury in Diabetes and the RAGE/DIAPH1 Signaling Axis
The major goal of this grant application is to probe the mechanisms and identify new therapies for untoward monocyte and macrophage responses in ischemia, which, together with cellular

perturbation in the microenvironment, amplify damage in myocardial infarction and peripheral arterial disease, especially in diabetes.
Role: Project 1 and Scientific Core Leader

(NEW)
1R01DK122456-01A1 07/01/20-03/31/25 0.68 calendar
NIDDK
Targeting RAGE/DIAPH1: Novel Therapeutic Strategy for Diabetic Complications
The major goal of this grant is to develop small molecule antagonists to treat diabetic complications.
Role: MPI (Schmidt (contact PI)/Ramasamy)

INACTIVE
(ENDED)
1R24DK103032 (Schmidt) 08/01/14-07/31/20 0.60 calendar
NIH (NCE)
Targeting RAGE-mDia1 in Diabetic Complications: Mechanisms & Therapeutics
Major goal of this application is to develop small molecule inhibitors of the interaction of the RAGE cytoplasmic domain with DIAPH1.
Role: Co-I

(ENDED)
P01HL60901 (Schmidt) 07/15/11-11/30/18 0.12 calendar
NIH No Cost Extension
RAGE and Mechanisms of Vascular Dysfunction
This grant focuses on the mechanisms by which diabetes accelerates atherosclerosis via RAGE.
Role: Project 2, 3, Core B Leader, Core C Co-Leader

(ENDED)
1R25DK092170-01A1 07/2012-06/2017 0.2 calendar
NIH
A New Era of Targeted Drug Discovery and the Path of Development from Molecular
This grant funds a graduate level course focused on challenges in drug discovery for the treatment of diabetic complications and obesity.
Role: Multiple PIs (Ramasamy & Gold-von Simson)

8. SPECIAL REPORTING REQUIREMENTS:

- **COLLABORATIVE AWARDS:**
  A copy of this report will be submitted to *https://ers.amedd.army.mil.*

9. APPENDICES:

Nothing to report