Award Number: W81XWH-21-1-0550

TITLE: Genetic Determinants of Focal Segmental Glomerulosclerosis in Mouse and Humans

PRINCIPAL INVESTIGATOR: Ali G. Gharavi

CONTRACTING ORGANIZATION: Columbia University Medical Center

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Fort Detrick, Maryland 21702-5012

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Ali G. Gharavi

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U.S. Army Medical Research and Development Command

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12. DISTRIBUTION / AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT. The goals of this project are to identify genetic determinants of focal segmental glomerulosclerosis (FSGS). We have shown that the development nephropathy in the HIV-1 transgenic mice (TgFVB) is highly strain dependent. Linkage mapping in murine crosses have shown that there are at least 4 FSGS susceptibility loci among inbred strains. Furthermore, F1 hybrids between TgFVB and other inbred strains show highly variable penetrance of nephropathy, indicating the feasibility of mapping genes using F1 hybrids for association mapping. Our previous work, we identified a Candidate gene Ssbp2, (PMID: 34893534) associated with the susceptibility to HIV associated nephropathy. We now have *Ssbp2* null mice generated and using these mice we are able to knock out *Sspb2* either globally or we can specifically target specific cells including the podocyte where Ssbp2 is highly expresses. We are in the process of generating Ssbp2 null mice on two HIVAN resistant strains (C57BL/10J and C57BL6/NJ) and two susceptible strains (A/J and FVB/NJ). We are immunophenotyping and transcriptionally profiling the resident lymphocyte populations in the HIV-1 transgenic mice (TgFVB) and demonstrate a population of CD107a+ activated lymphocytes residing in the kidney of the TgFVB mice. We have demonstrated these CD107a+ lymphocytes are involved in kidney damage leading to elevated levels of proteinuria. Our interim analysis of genetic modifiers of *APOL1* high risk genotypes, demonstrated the *APOL1* high risk genotype was associated with an increased with an increased risk of C1q nephropathy, FSGS, and hypertension-attributed chronic kidney disease (CKD). We observed a significant enrichment of rare missence variants in the inflammasome gene-set was identified in individuals with high risk *APOL1* genotypes and kidney disease.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The glomerular filtration barrier amongst mammals is highly conserved, and mouse models are highly relevant to understanding the human pathogenesis of FSGS. Frequently candidate genes identified in mouse models have been implicated in the cause of human disease, demonstrating the importance of genes identified in the mouse FSGS model are highly relevant to human disease. The identification of novel candidate genes, including *Ssbp2*, will allow us to evaluate these novel candidate genes in the pathogenesis of FSGS.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

FSGS, *APOL1*, Nephropathy, Mouse Kidney Disease,

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Generation of *Ssbp2* null mice by CRISPR mutagenesis in resistant and susceptible genetic backgrounds to demonstrate the causality for collapsing glomerulopathy on our HIVAN mouse line

Identify the earliest molecular drivers of FSGS via multi-parameter flow cytometry and single cell transcriptomic analysis of mouse kidneys from strains with contrasting susceptibility to HIVAN.

Replicate the *AHDC1* association and identify new genetic modifiers for *APOL1*- nephropathy in 500 CKD cases with *APOL1* high risk genotypes vs ~10,000 ethnically matched controls.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

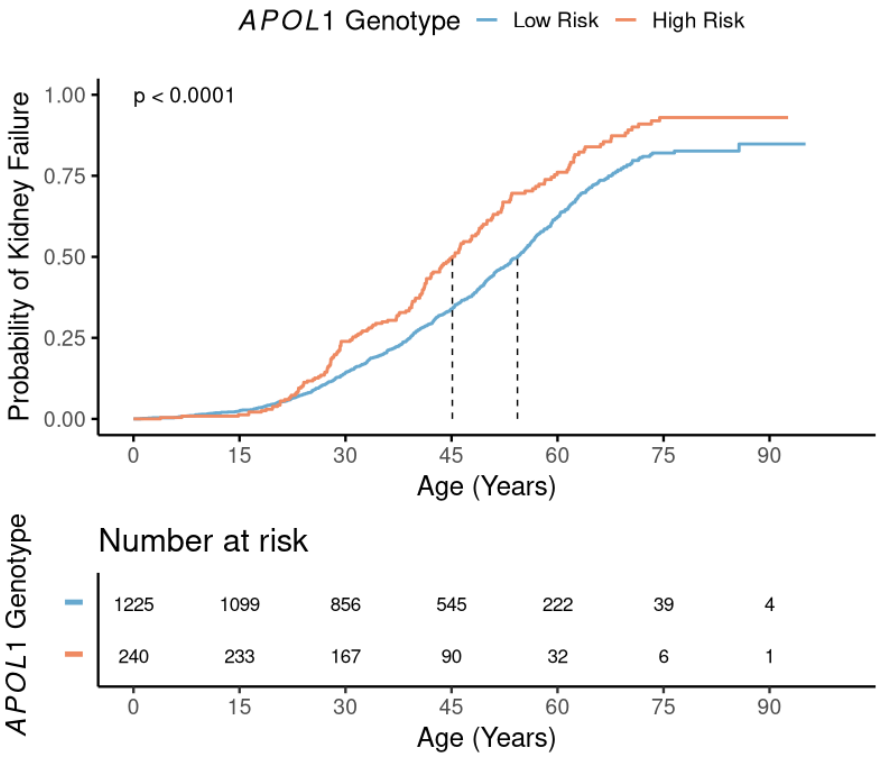
We have received the Ssbp2 null mice, and these mice were backcrossed on the C57BL/6 background. With these mice we are able generate global Ssbp2 null mice by crossing these mice with EIIA-Cre and we have generated conditional podocyte Ssbp2 null mice determining using the Nphs2-Cre mice.
We are in the process of crossing these mice onto the C57BL10/J, C57BL6/NJ. FVN/NJ, and A/J backgrounds.

Replicate the *AHDC1* association and identify new genetic modifiers for *APOL1*- nephropathy in 500 CKD cases with *APOL1* high risk genotypes vs ~10,000 ethnically matched controls.

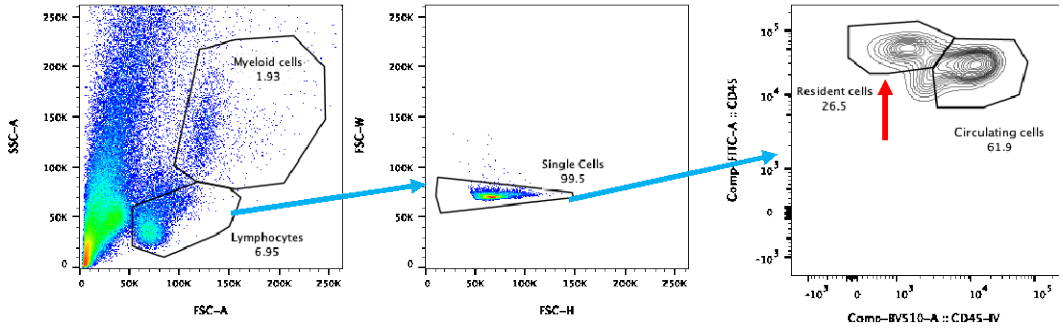
The clinical outcomes of individuals with high risk *APOL1* genotypes have not been assessed across disease categories, and are highly variable, suggesting the presence of effect modifiers. Using linked biobank, health record, and exome sequencing data, we assessed the risk of kidney failure and eGFR decline rate in chronic kidney disease (CKD) patients with high-risk (N=240) and low-risk (N= 1225) *APOL1* genotypes. Mendelian genetic kidney disease were identified in patients with high risk *APOL1* genotypes. Genetic modifiers of the effect of *APOL1* genotype on CKD were evaluated using an Exome-wide association study (ExWAS), and gene-based and gene-set based collapsing analyses.

APOL1 risk genotype was associated with an increased risk of C1q nephropathy, FSGS, and hypertension-attributed CKD. Compared to individuals with low-risk genotypes, individuals with high-risk *APOL1* genotypes had a higher risk of kidney failure (HR= 1.59, P = 1.1 x 10^{-6}), higher decline in eGFR (6.55 vs 3.63 mL/min/1.73m^2/year, P = 0.0007) and reached kidney failure at a younger age (45.1 vs 54.3 years), with the G1/G1 genotype demonstrating the highest risk (HR = 1.87, P = 2.1 x 10^{-6}). There was no interaction for *APOL1* risk genotype and specific primary causes of CKD on risk of kidney failure. Six individuals had high risk *APOL1* genotypes and a variant diagnostic of a monogenic nephropathy. Significant enrichment of rare missense variants in the inflammasome gene-set was identified in individuals with high risk *APOL1* genotypes and kidney disease (OR = 1.90, 2.03; Q_{FDR} = 0.038).

We have concluded that high risk *APOL1* genotypes are associated with an increased risk of kidney failure and eGFR decline rate. Rare missense variants in the inflammasome pathway may act as genetic modifiers of *APOL1* effect on kidney disease.

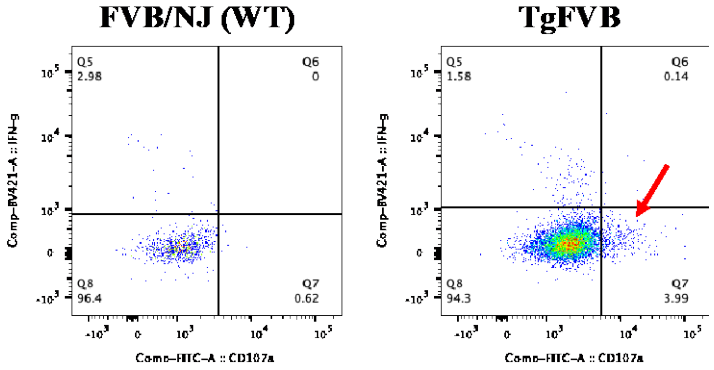


Our previous research demonstrated interstitial inflammation in HIV-1 transgenic mice, and we have are currently immunophenotyping the resident kidney lymphocytes in HIV-1 transgenic mice compared to WT mice.
To ensure we are examining the cells resident in the kidney, anti-CD45 antibodies are injected into the tail-veins of mice to label the circulating lymphocytes. This is an essential step, as the kidney is highly vascularized, we want to ensure we are accurately analyzing tissue resident lymphocytes for both phenotypic and single cell transcriptomic analysis.



Identification of the kidney resident lymphocyte populations denoted by the red arrow.

Analysis of the lymphocyte population demonstrated a population of activated lymphocytes in the Tg-FVB (HIV-1 transgenic mice, compared to the FVB/NJ (WT) mice. There was an increase in the number of lymphocytes isolated from the kidneys of the TgFVB mice compared to the WT mice. There was a significant increase in the percentage of cells expression CD107a, a marker of immune cell activation, inflammation, and cytotoxic degranulation. We are currently investigating mechanisms of induced CD107a expression on the cell surface of lymphocytes and this is demonstrated cytotoxic activity in an antigen-specific manner.



Identification of the CD107a+ lymphocytes residing in the kidneys of HIV-1 transgenic mice denoted by the red arrow.

We are currently defining the lymphocyte population to determine the activation status and the exhaustion station of the lymphocyte populations. These cells have been sorted for single cell sequencing experiments.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This award provides additional training to Dr. Steers, an Assistant Professor in Medical Sciences (promoted July 1st 2023) in my lab to utilize and learn additional skill sets.

Results generated in association with this award will be presented at conferences, seminars, and research meetings by Dr. Steers.

This award provides training for post-bac students and summer students who are currently working in the laboratory who intend to go on the graduate education.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Data was presented the data at the American Society of Nephrology 2021 meeting.
(Poster Title: GWAS in mice maps susceptibility to NIV Associated Nephropathy to the *Ssbp2* locus)

Data published in the Journal of the American Association of Nephrology
Clinical and Genetic Characteristics of CKD Patients with High-Risk APOL1 Genotypes
J Am Soc Nephrol. 2023 May 1;34(5):909-919

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Continue the generation of F1 HIV-1 Transgenic *Ssbp2* null and WT mice. Urine will be collected and analyzed for proteinuria, hematuria and NGAL for the phenotypic analysis of the newly generated transgenic F1 hybrids. Serum will be analyzed for blood urea nitrogen, albumin, cholesterol levels, immunological and inflammatory markers for the phenotypic analysis.

Continue the analysis of the infiltrating immune cells in the HIV-1 transgenic mice with high proteinuria and low proteinuria and the WT mice. Further define the inflammatory lymphocytes, using flow cytometry and single cell RNA-sequencing analysis. We are currently analyzing single cell sequencing data derived from the kidney resident lymphocytes population from HIV-1 transgenic mice and WT mice.

Continue collection and processing of DNA samples for exome sequencing and continue with the variant calling and annotation.

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

This proposal will combine human genetics, mouse genetics and transcriptomics to study focal segmental glomerulosclerosis (FSGS). Using a comprehensive approach, we will aim to identify novel susceptibility genes and loci, and genetic modifiers for FSGS.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

This proposal will combine human genetics, mouse genetics and transcriptomics to study focal segmental glomerulosclerosis (FSGS). Using a comprehensive approach, we will aim to identify novel susceptibility genes and loci, and genetic modifiers for FSGS.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Steers NJ, Gupta Y, D'Agati VD, Lim TY, DeMaria N, Mo A, Liang J, Stevens KO, Ahram DF, Lam WY, Gagea M, Nagarajan L, Sanna-Cherchi S, Gharavi AG. GWAS in Mice Maps Susceptibility to HIV-Associated Nephropathy to the Ssbp2 Locus. J Am Soc Nephrol. 2022 Jan;33(1):108-120. doi: 10.1681/ASN.2021040543. Epub 2021 Dec 10. **PMID: 34893534**

Elliott MD, Marasa M, Cocchi E, Vena N, Zhang JY, Khan A, Krishna Murthy S, Bheda S, Milo Rasouly H, Povysil G, Kiryluk K, Gharavi AG. Clinical and Genetic Characteristics of CKD Patients with High-Risk APOL1 Genotypes
J Am Soc Nephrol. 2023 May 1;34(5):909-919

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Title: Effect of ApoL1 Genotype on Clinical Outcomes in a Population of Patients with Chronic Kidney Disease. *American Society of Nephrology, 2021*

Title: GWAS in mice maps susceptibility to NIV Associated Nephropathy to the *Ssbp2* locus. *American Society of Nephrology, 2021*

Data pertaining to this project is periodically presented in Laboratory meetings.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*

- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Ali Gharavi
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.2

Contribution to Project: Dr. Gharavi was responsible for achieving the overall goals of the study. He supervises mouse and human genetic studies at Columbia University.

Funding Support: Dr. Gharavi's funding portfolio currently includes NIH Grants:1OT2OD026556-01, 5RC2DK116690-03, 1RM1-HG011123-01A1, 5U01DK100876-08, 2R01DK080099-10, 2R01DK082753-10A1, 2U54DK104309-08 and DOD grants W81XWH2010762 and PR201425. AstraZeneca UK limited.

Name: Iulina Ionita-Laza
Project Role: Co- Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.6

Contribution to Project: sequencing data, analysis of datasets GWAS

Funding Support: Dr. Ionita-Laza's funding portfolio currently includes NIH Grants: 5R21HG012345-02, 5R01MH095797-08, 1RF1AG072272-01, 5R01DK080099-12, 1RF1AG066107-01A1, 5R01DK082753-11, 5RC2DK122397-03, 5R01AG072474-02, 5R25GM143298-02, R01HL169766, and 2R01DK105124-06, and DOD grant PR201425 and PR212415

Name: Vivette D'Agati
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.6

Contribution to Project: Dr. D'Agati perform standardized review and scoring of kidney biopsies for enrolled patients and mouse samples.

Funding Support: Dr. Agati's funding portfolio currently includes NIH Grants: NIH Grants: U01DK100876-10, 2R01DK109683-07, R01DK121846-04, R01DK129252-02, 1R01DK131525-02, R01DK129735-02, R01DK133912-01, and DOD grants: W81XWH2110550 and PR212415

Name: Nicholas Steers
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3.0

Contribution to Project: Dr. Steers is involved in the mouse breeding to generate the Ssbp2 null mice and the generation of the HIVAN mice. He is responsible for the cell isolation and processing of cells for the single cell RNA sequencing and will be involved in the single cell RNA sequencing analysis. He will be involved in the genetic analysis under the supervision of Dr. Ionita-Laza and Dr. Gharavi.
Funding Support: Dr. Steers is supported by the following NIH grant: 2R01DK082753-10A1 and DOD grant W81XWH2110550

Name: Sharvari Pathak
Project Role: Tech
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 6.0

Contribution to Project: Wet lab experiments, DNA preparation and plating. Processed mouse husbandry and genotyping and processing samples for histopathology.
Funding Support: N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**
   COLLABORATIVE AWARDS: N/A
   QUAD CHARTS: N/A

9. **APPENDICES:** N/A