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, New York, NY

REPORT DATE: July 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
July 2022	Annual	15Jun2021-14Jun2022

4. TITLE AND SUBTITLE

Genetic Determinants of Focal Segmental Glomerulosclerosis in Mouse and Humans

5a. CONTRACT NUMBER
W81XWH-21-1-0550

5b. GRANT NUMBER
W81XWH-21-1-0550, PR201425

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Ali G. Gharavi

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research And Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

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). Out 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** USAMRDC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	15	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. **INTRODUCTION:**

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:**

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

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

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?

We have now received the Ssbp2 null mice on the C57BL/6 background. With these mice we are able to either generate global Ssbp2 null mice or we are able to generate conditional Ssbp2 null mice determining the specific cell Ssbp2 can be knocked out.

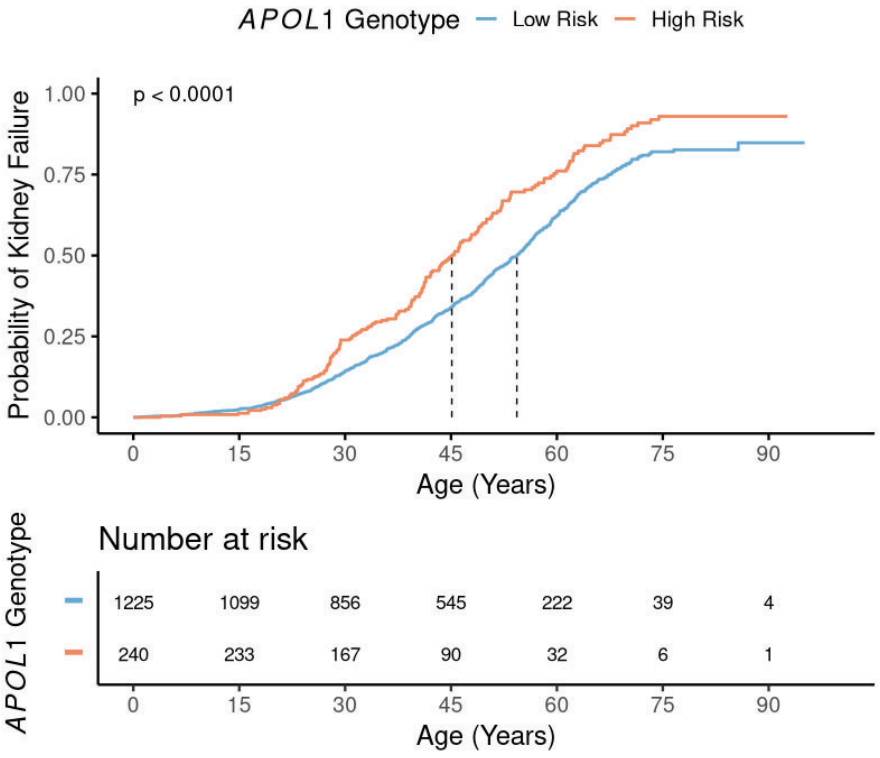
We are in the process of generating the *Ssbp2* null mice on the C57BL10/J, C57BL6/NJ. FVN/NJ and A/J background

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, $P = 1.1 \times 10^{-6}$), 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, $P = 2.1 \times 10^{-6}$). 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.



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.

This award provides additional training to Dr. Steers, an Instructor in Medical Sciences 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?

Data was presented the data at the American Society of Nephrology 2021 meeting

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

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.

Single cell RNA sequencing of mouse kidney cells from strains with contrasting susceptibility to HIVAN
The resident kidney cell and the infiltrating immune cell populations will be isolated and prepared for single cell RNA sequencing.

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

4. IMPACT:

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

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?

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?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

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

Nothing to report

Changes that had a significant impact on expenditures

Nothing to Report

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

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:

- **Publications, conference papers, and presentations**

Journal publications.

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**

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

Nothing to Report

Other publications, conference papers and presentations.

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

Data pertaining to this project is periodically presented in Laboratory meetings

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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.

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: 5R01MH095797-08, 1RF1AG072272-01, 1R21HG012345-01A1, 1R25GM143298-01, 5RC2DK116690-04, 5RC2DK122397-02, 5R01DK080099-11, 2R01DK082753-10A1, 1RF1AG066107-01A1, 1R01AG072474-01, 1K25DK128563-01 and DOD grant PR201425

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: 2P01DK056492, 2U01 DK100876-07, R01 DK115694-05, 1UG3DK114926-04, 2R01DK109683-06, R01DK121846-02, R01DK129252-01, 1R01DK131525-01, and DOD grant W81XWH2110550

Name: Nicholas Steers
Project Role: Instructor in Medical Sciences
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?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: