AWARD NUMBER: W81XWH-16-1-0450

TITLE: Multispecies, Integrative GWAS for Focal Segmental Glomerulosclerosis

PRINCIPAL INVESTIGATOR: ALI GHARAVI

CONTRACTING ORGANIZATION:
Columbia University
New York, NY 10032

REPORT DATE: December 2020

TYPE OF REPORT: FINAL

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
December 2020	Final	8-15-2016 to 8-14-2020

4. TITLE AND SUBTITLE
Multispecies, Integrative GWAS for Focal Segmental Glomerulosclerosis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0450

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Ali Gharavi, MD

E-Mail: ag2239@columbia.edu

5d. PROJECT NUMBER
0010856529

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Trustees of Columbia University
630 West 168th street
New York, NY 10032

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

Focal Segmental Glomerulosclerosis (FSGS) is one of the most common causes of nephrotic syndrome and several studies reported an increase of FSGS diagnosis with up to 18.7% and 47% of cases in adults and children receiving a kidney biopsy, respectively[1] . From a clinical perspective, idiopathic FSGS is characterized by high morbidity, poor response to medical therapy, and high rate of progression to end-stage renal disease requiring dialysis or transplantation. We approached this phenotype in a comprehensive fashion using both human genetics and mouse studies. In a human GWAS of FSGS, we detected genome-wide significant signals in the HLA locus and in the *APOL1* locus In a mouse model of collapsing FSGS, we detected a signal on Chr13.. Fine mapping studies and bioinformatics analyses suggested *Ssbp2*, as a lead candidate. Analysis of Ssbp2 null mice demonstrated that they develop spontaneous FSGS with aging, These data identify multiple risk loci and candidate genes for FSGS in mice and humans and implicate new pathways in disease pathogenesis.

15. SUBJECT TERMS

None Listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	7	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. Introduction

Focal Segmental Glomerulosclerosis (FSGS) is one of the most common causes of nephrotic syndrome and several studies reported an increase of FSGS diagnosis with up to 18.7% and 47% of cases in adults and children receiving a kidney biopsy, respectively[1] . From a clinical perspective, idiopathic FSGS is characterized by high morbidity, poor response to medical therapy, and high rate of progression to end-stage renal disease requiring dialysis or transplantation. We approached this phenotype in a comprehensive fashion using both human genetics and mouse studies. In the original grant, we pursued a multispecies GWAS with the following aims:

Specific aim 1: A Genome-wide association study (GWAS) 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 model of collapsing FSGS

Specific aim 3. Cross annotation between human and mouse GWAS and identification of downstream dysregulated pathways and networks

2. Keywords

DO Diversity Outcross strains
ESRD End stage renal disease
FSGS focal segmental glomerulosclerosis
GWAS Genome wide association study
HIVAN HIV- associated nephropathy
QTL quantitative trait loci
SNV Single nucleotide variant
TgFVB
Transgenic mouse with the proviral HIV genome with deletion of the gag and pol sequences in the FVB background
WES Whole exome sequencing

3. Accomplishments

GWAS identifies known and novel associations for adult and pediatric FSGS (manuscript in preparation)

We conducted genome-wide SNP genotyping in 4,136 FSGS cases in a multiethnic cohort of European, African American, and Asian adults and children. We are currently genotyping additional 1,564 cases. We conducted an interim GWAS in 2,639 FSGS cases and 16,765 controls (76% EUR, 14% AFR, 8% AMR, and 2% ASN). This cohort was composed of 11 subpopulations of cases and strictly genetically-matched controls. Meta-analysis was then performed using an inverse variance-weighted method (METAL software) (**Figure 1**). **The first association was at the HLA-DQA1 locus with the top signal at the marker rs9273349 *HLA-DQA1*** (*P* = 2.76 x 10^{-19}; OR 1.35). **After conditioning for this variant, a second genome-wide significant association was identified at the marker rs9275245 (*P* = 4.55 x 10^{-11}; OR 1.26) situated in the next haploblock at the *HLA-DQA1* locus**. Recent findings in three GWAS of pediatric steroid sensitive nephrotic syndrome identified significant association at this locus[2-4], suggesting that disease-associated variation at the *HLA-DQA1* is significant among major categories of nephrotic syndrome.

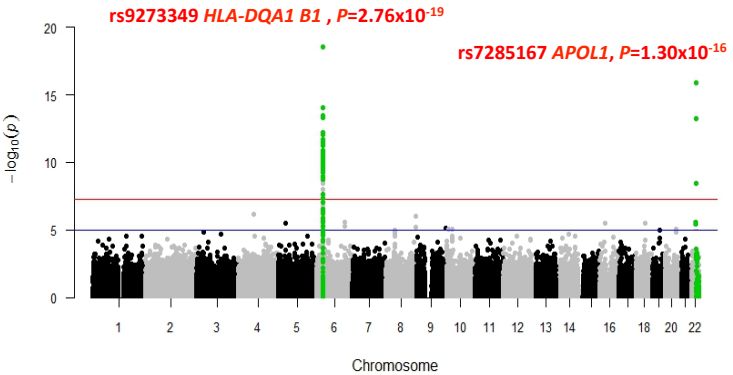

Figure 1. Manhattan plot of GWAS in the entire cohort of 2,639 FSGS cases and 16,765 controls showing highly significant association at the HLA and APOL1 loci.

The second top association was for the marker rs7285167 (P = 1.30 x 10^{-16}; OR 1.46) at the known *APOL1* locus for increased risk for kidney disease in African Americans[5]. This signal was entirely driven by 4/11 subpopulations with African ancestry in both pediatric (P = 7.20 x 10^{-9}; OR 2.28) and adult (P = 1.17 x 10^{-13}; OR 2.64) cohorts, demonstrating again striking similarities in the genetic architecture of common variants between adult and pediatric FSGS. We anticipate that analysis of the full cohort will provide many additional loci that will clarify pathogenesis and genetic architecture of this trait. These results will be followed up by Dr. Sanna-Cherchi as proposed by a separate expansion award application.

Mapping genetic susceptibility to collapsing FSGS in mouse model. Although mice do not possess an *APOL1* ortholog, HIV-1 transgenic mice on the FVBN/J background (TgFVB) display virtually all the clinical and molecular features of collapsing variant of FSGS[6-8] , indicating that alternative genetic lesions, in the absence of *APOL1*, can predispose to this disease[7,9] (**Figure 2**). To map genes for glomerulosclerosis, we generated F1 hybrids between TgFVB and 20 inbred strains (over 750 F1 hybrids generated). F1 hybrids between 129S1/SvImJ, Balb/CJ, C57BL/6J, C57BL/6NJ, C57BL/10J, C57BL/J, C58/J and CAST/EiJ are completely protected from disease, while the other F1 hybrids have evidence of disease (**Figure 2**). To map loci predisposing to collapsing FSGS, we performed a GWAS using a mixed linear model method with 160,000 SNPs, searching for haplotype distribution patterns that matched the high/low strain susceptibility pattern. This search identified a major locus on Chr 13 which is genome-wide significant (p=1x10-6, rs48893293, **Figure 3**). Remarkably, the top signal falls within a QTL, *HIVAN2*, which we had previously mapped using segregating crosses[7]. Notably, the Chr 13 interval contains the *Ssbp2* gene, encoding a DNA binding protein that stabilizes transcriptional complexes by prevent proteosomal degradation. *Ssbp2* is highly expressed in podocytes based on recent single cell transcriptomic data published by the Susztak and McMahon labs[10,11] (**Figure 4**). In addition, it has been shown to interact *LMX1B* a known FSGS genes, in yeast two hybrid assay[12]. Finally, *Ssbp2* null mice have been previously described and have been shown to develop cancer at multiple sites, but also develop chronic glomerulopathy. Finally,[13,14] there is a cis-eQTL for *Sspb2* in this interval in our F2 cross. There are two other genes in the vicinity, *Agt10* and *Acot12*, but they are not highly expressed in podocytes and have no known involvement in kidney disease. Taken together, these data strongly suggest that *Ssbp2* is the culprit gene producing susceptibility to HIVAN in the mouse. We now propose to pursue these studies by generating *Ssbp2* null mice in susceptible and resistant strains to demonstrate involvement in HIVAN and collapsing glomerulopathy.

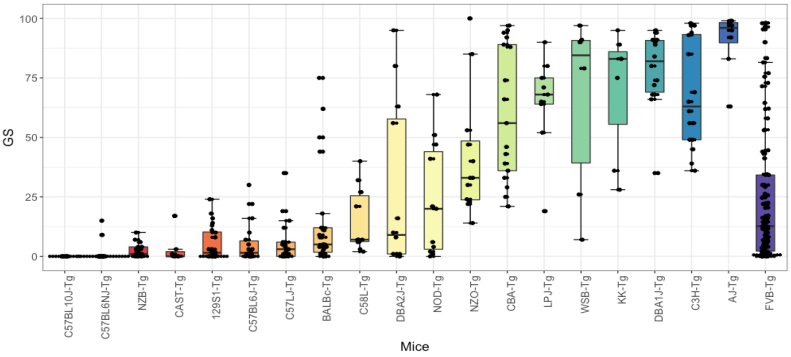

Figure 2: Significant interstrain variation in glomerulosclerosis in 20 inbred strains (N=10-20 per strain). There were no differences by sex.

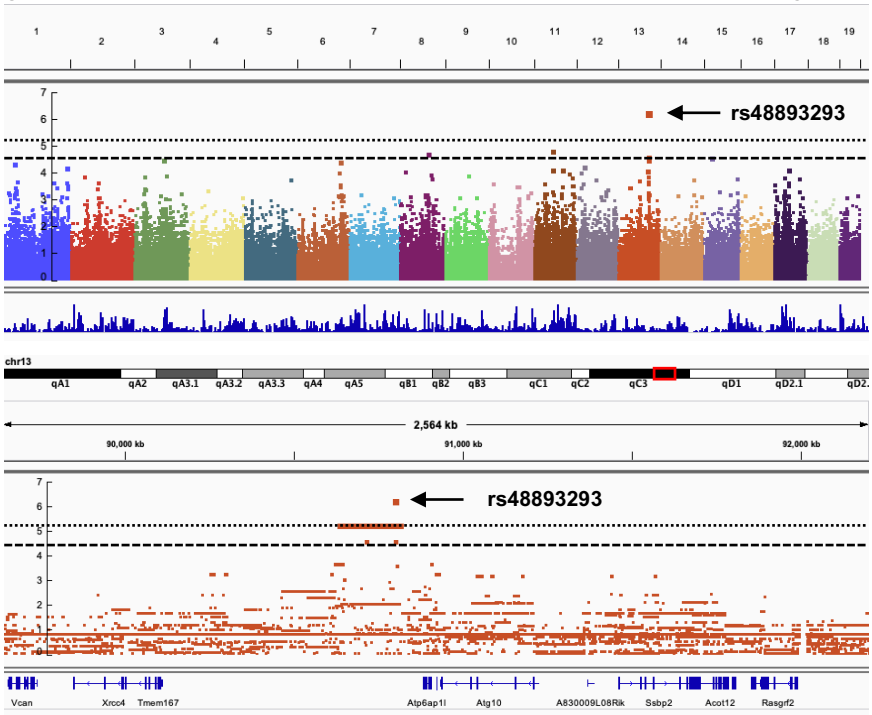

Figure 3. A GWAS of F1 hybrids identifies a signal on Chr13 with coincides with the HIVAN2 locus. **B.** The region contains 3 candidate genes, *Agt10*, *Ssbp2* and *Acot12*

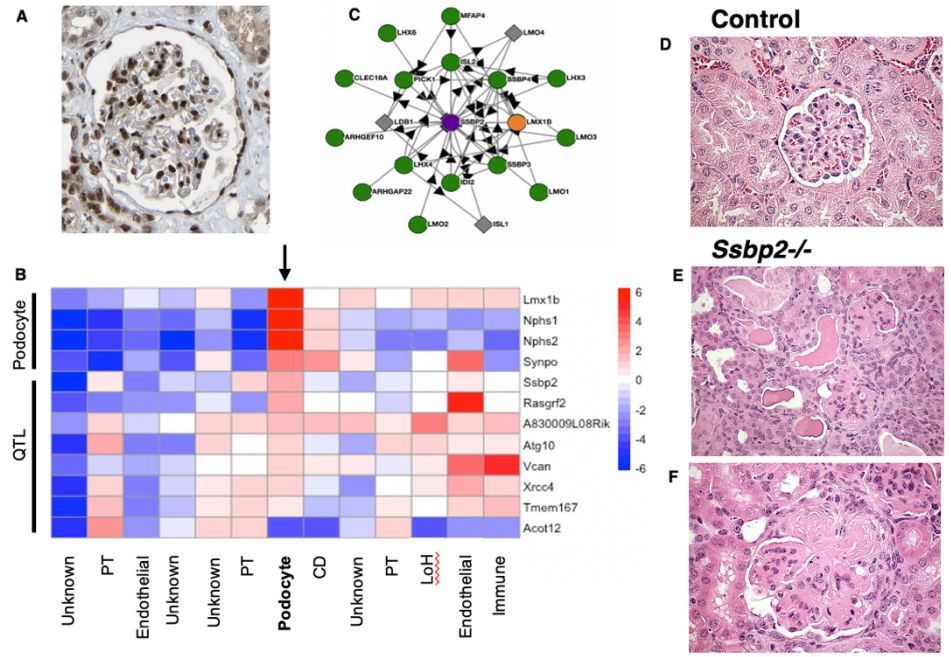

Figure 6. A SSBP2 is expressed in human podocytes (Protein Atlas). B. Single cell sequencing of kidney revealed 13 populations, and *Sspb2* is expressed in the population containing podocyte genes *Nphs1*, *Nphs2* and *Lmx1b,* whose orthologs are known genes for FSGS**. C**. Interrogation of Bioplex data identifies an interaction between SSBP2 and *LMX1B.* D. Histology slide of WT control mice. E. and F. histology slides of *Sspb2* null mice showing FSGS lesions with tubular casts, focal tubular atrophy and interstitial fibrosis. PT – proximal tubule, LoH – Loup of Henle, CD – collecting duct

How do studies of animal models viral collapsing glomerulopathy inform about FSGS. FSGS can arise due to developmental, environmental, as well as genetic defects. The HIVAN mouse model of FSGS is informative not only about HIV induced kidney injury but also about APOL1-assocaited glomerulopathies which contribute to a substantial proportion of kidney failure among African Americans. In addition, we have recently reported multiple cases of FSGS-collapsing glomerulopathy in the setting of SARS-CoV-2 in patients with high risk *APOL1* genotypes[58-61]. Therefore, these studies can also illuminate the pathogenesis kidney disease due to SARS-CoV-2 infection, which occurs in 30% of ICU cases. Elucidating novel candidate genes and dissecting mechanisms of glomerular damage will provide critical information for the design of novel therapy, and patient treatment and management.

Rare variant gene burden analysis identifies a modifier signal for *APOL1* nephropathy

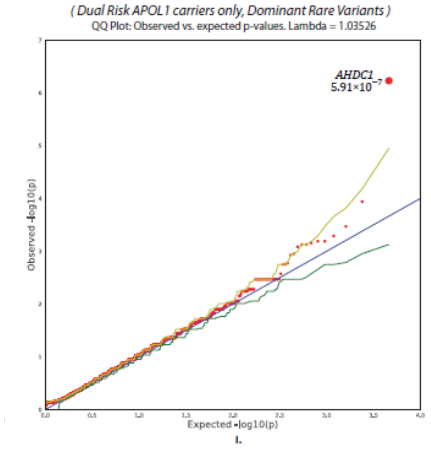

Figure 5. Quantile-Quantile for the modifier analyses for *APOL1*. AHDC1 achieved a suggestive threshold for *APOL1,* identifying a potential modifier (p = 5.9x10-7)

Through this award, we studied a large number of patients with FSGS and contributed samples to a collaborative study aiming to discover rare genetic variants modifying the development to CKD[15]. In this study, we identified 137 CKD cases with 2 *APOL1* risk variants. Comparing the 137 CKD cases with the *APOL1* risk genotypes to 2,209 ancestry-matched controls, we identified a suggestive signal in the *AT-Hook DNA Binding Motif Containing 1* gene (*AHDC1*; MIM:615790) in the rare non-benign model (p = 5.9x10-7; OR 14.0 [95% CI 5.0 – 37.9], Figure 5). This association was driven by nonsynonymous and indel variants. *AHDC1* encodes a nuclear protein with a predicted DNA binding domain that is ubiquitously expressed. *De novo* mutations cause Xia-Gibbs syndrome, an intellectual disability syndrome that also features sleep apnea and various dysmorphologies (OMIM 615829). The function of *AHDC1* is not completely understood, but this suggestive signal encourages further investigation to confirm this association, and, if replicated, to examine the potential links to *APOL1* biology. We will thus study larger cohorts in the expansion proposal and also determine whether *AHDC1* initiate podocyte injury in our single cell analysis of mouse kidneys.

4. Impact

At the time of writing, we are 3 years into the funding cycle. To date, we have presented 2 abstracts at the American Society of Nephrology and have one publication derived from this award. The work we have undertaken is comprehensive in scope and we have a preference to publish complete stories rather than incremental gains. Therefore, we anticipate publications reporting this analysis in the coming year.

5. **Changes/Problems**

The major challenges were essentially all logistical. For the human genetic studies, samples are now all in hand, but due to the budgeting structure, we had to perform the genotyping in batches across the 3 years, and are still waiting for the final batch to complete the human GWAS. Nonetheless the interim analysis is quite promising as it reveals the association of human FSGS with HLA locus for the first time, indicating a strong immune mediated component to disease. A second challenge arose for the procurement of mice. Our original plans for the mouse GWAS involved analysis of diversity outcross mice but because these mice were still under development at the initiation of the award, this created major delays. We finally had to switch plans and perform analysis of classic inbred strains (after consultation and approval from DOD program officials). This turned to our advantage as the mouse GWAS was highly informative. We now can turn our attention to validation of GWAS signals by generating *Sspb2* null mice.

6. Products

1-Cameron-Christie S, Wolock C, Groopman E, Petrovski S, Kamalakaran S, Povysil G, Vitsios D, Zhang M, Fleckner J, March RE, Gelfman S, Marasa M, Li Y, Sanna-Cherchi S, Kiryluk K, Allen AS, Fellström B, Haefliger C, Platt A, Goldstein DB, Ali Gharavi AG. Exome-based rare-variant analyses in chronic kidney disease. JASN 2019

2- NJ. Steers, YJ Na, ND DeMaria, WY Lam, VD D'Agati AG. Gharavi "Interstrain variation in severity of nephropathy and immunoglobulin levels in HIV-1 transgenic mice". American Society of Nephrology 2018

3- HLA Alleles Confer Risk to Primary Idiopathic Nephrotic Syndrome in Individuals of Caucasian Ancestry. Ahram D, Gilles C, Mitrotti A, …. Gharavi AG, Hildebrandt F, Sampson MG, Sanna-Cherchi S. ASN SA-PO593. American Society of Nephrology Kidney Week, 2017

4- Steers N, Gupta Y, DeMaria ND, Lim TY, Lam WY, Mo A, Liang J. Stevens K., D'Agati V, Sanna-Cherchi, Gharavi AG. GWAS in the mouse identifies loci for collapsing glomerulopathy (submitted)

7. Participants & Other Collaborating Organizations

None

8. Special Reporting Requirements

None

9. Appendices

None