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TITLE: Multiethnic Integrated GWAS to Illuminate the Pathobiology of FSGS

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14. ABSTRACT

Overarching Challenge: This project directly addresses the FY21 PRMP area "Focal Segmental Glomerulosclerosis". FSGS is a common form of kidney injury and a major cause of end-stage kidney disease (ESKD). It is particularly common among people of recent African ancestry. FSGS is not one disease, but rather a collection of entities with a similar histologic phenotype. There has been considerable progress in understanding the genetic basis of FSGS over the past two decades. Still, the underlying pathobiology of disease is largely obscure, and therapeutic options are limited. Until now there has not been a coordinated effort to pull together investigators, resources, and patient populations to tackle this problem using a genome-wide association (GWAS) approach. No studies also have combined large-scale genetics with mouse genetics and transcriptomic analyses. Thus, we are proposing a comprehensive approach to dissecting the complex molecular and genetic basis of focal segmental glomerulosclerosis (FSGS)

Background: FSGS is a common pattern of kidney injury in both adults and children. Over the past years, there have been large advances in understanding the genetic basis of FSGS. However, in the majority of cases, even when a positive family history is present, mutations in a known FSGS gene cannot be identified. The role of copy number variations (CNVs) and common variants with small to moderate effect size is poorly understood. In fact, no GWAS have been conducted for this trait so far. An improved understanding of the genetic architecture of FSGS is expected to lead to marked improvements in its diagnosis and ultimately its therapy.

Research Plan: This research program will consist of three independent but highly interrelated Aims. The principal goal here is to assess the contribution of common genetic variants to FSGS and how they relate to rare point mutations and CNVs. To do so, we will conduct the largest genome-wide study for common variants and CNV to date by analyzing over 12,000 FSGS multiethnic cases across the age of onset and therapy response spectrum. We will then conduct a series of post-GWAS analyses using multi-omics approaches to fine map, dissect and functionally interpret the loci identified in Aim 1, and then explore complex modes of genetic determination, including gene-gene interactions and polygenic risk scores (PRS). We will finally integrate genomic results with bulk and single-cell RNA sequencing from kidney subcompartments in human and mice to help resolve the molecular pathogenesis of FSGS. **Aim 1** will work to discover new alleles that confer risk to FSGS. A few loci have been implicated in steroid sensitive nephrotic syndrome, but, with the exclusion of *APOL1*, no causal common alleles for FSGS have been identified as yet. Based on our preliminary work we are certain that there are many moderately penetrant FSGS alleles and genes to be identified. We anticipate that we will identify and validate multiple new FSGS susceptibility genes and help identify critical biological pathways in the development of FSGS. Such alleles are also likely to play a role in disease course modification and outcome in both sporadic and genetic forms of disease. **Aim 2** will work to conduct a series of post-GWAS analyses. First, this aim will be directed at fine mapping and characterizing the loci discovered in Aim 1 using genome sequencing, eQTL and mQTL analyses. We will then explore complex modes of genetic determination, including screens for modifiers of highly penetrant allele and genotypes (*APOL1*, Mendelian FSGS genes), and the contribution of polygenic risk scores (PRS) to relevant FSGS outcomes such as progression to ESKD and recurrence after transplantation. Based on our preliminary data, we are certain that this approach will be successful in validating susceptibility genes for glomerulosclerosis, in identifying modifiers of *APOL1*, and in helping to optimize PRSs for FSGS. **Aim 3** will work to generate and analyze bulk and scRNA-seq from different mouse models of FSGS that are exemplars of known adult-onset (Actn4), pediatric (Nphs2), and immune-mediate (HIVAN) disease, that represent new mechanisms of disease causation (Trim8), as well as kidney biopsies from FSGS patients, in order to translate genetic findings into an understanding of their biological consequences.

Short-term impact: Improved understanding of the genetic basis FSGS with immediate improvement in diagnosis and rationally application of existing therapies.

Long-term impact: Better methods of treating and/or preventing FSGS and other related forms of chronic kidney injury and end-stage kidney disease. Reduction in the burden of chronic kidney disease and ESRD.

Military relevance: FSGS is a common cause of progressive kidney disease and eventual end-stage renal failure (ESRD). FSGS is particularly common among certain minority populations (African Americans, Latinos), groups overrepresented in the military. Discoveries emerging from our studies have the potential to improve the health of actively enlisted members, and those whose disease manifests later in life.

15. SUBJECT TERMS
FSGS, GWAS

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Focal segmental glomerulosclerosis (FSGS) is one of the major causes of end-stage kidney disease (ESKD) across populations and ages worldwide. Despite its impact on public health, pathobiology of disease is still obscure in most part, and therapeutic options are limited. Until now there has not been a coordinated effort to pull together investigators, resources, and patient populations to overcome these hurdles. Here we propose to overcome these challenges by putting together the largest worldwide cohorts of FSGS cases, conduct GWAS and gene-gene interaction studies, andintegrate the human association with transcriptomic data from podocytes and kidney tissue from mouse models of adult and pediatric onset FSGS.
- **Specific Aim 1:** A refined trans-ethnic genome-wide association study for common variants in over 12,000 FSGS cases and 30,000 matched controls
- **Specific Aim 2:** A post GWAS study to dissect new loci and test complex models of genetic determination including polygenic risk scores and gene-gene interactions
- **Specific Aim 3:** Integration of GWAS data with mouse genomic and transcriptomic studies conducted in multiple experimental models of pediatric and adult FSGS

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

APOL1:	Apolipoprotein L1
CKD:	Chronic Kidney Disease
CNV:	Copy number variant
CureGN:	Cure Glomerulonephropathy
eQTL:	Expression quantitative trait locus
ESKD:	End-stage kidney disease
FSGS:	Focal segmental glomerulosclerosis
GATK:	Genome Analysis Toolkit
GWAS:	Genome-wide association study
MCD:	Minimal Change Disease
NEPTUNE	Nephrotic Syndrome Study Network
NS:	Nephrotic syndrome
PCA:	Principal Component Analysis
SNP:	Single nucleotide polymorphism
SNV:	Single nucleotide variant
SRNS:	Steroid resistant Nephrotic syndrome
SSNS:	Steroid sensitive Nephrotic syndrome
TOPMed:	Trans-Omics for Precision Medicine Program
WES:	Whole exome sequencing
WGS:	Whole genome sequencing

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?

Major Task 1: To complete the genotyping and analyses for a FSGS GWAS for common variants

- Genotyping and GWAS analysis completed for Phase 1 of the Study: abstract accepted for presentation at American Society of Nephrology Kidney Week 2023; Manuscript in preparation, expected submission December 2023/January 2024
- Extended CNV analysis in >3,150 FSGS cases: abstract accepted for presentation at American Society of Nephrology Kidney Week 2023; Manuscript in preparation, expected submission November 2023

Major Task 2: To conduct a series of integrated multiomics approaches and analyses in order to identify the causal variants underlying the GWAS loci and test for complex models of genetic determination, including polygenic determination of risk and gene-gene-interactions

- Post-GWAS fine mapping and eQTL analyses ongoing and to be completed and integrated in the first GWAS manuscript in preparation (see above)
- First screen for *APOL1* genetic modifiers completed with a manuscript accepted for publication on the protective role of the p.N264K variants against FSGS

Major Task 3: Cross annotation GWASs and transcriptomics mouse models of FSGS

- Generation and analysis of transcriptomic data from mouse models is in process and will occupy mostly the second half of the second year and the third year of funding

What was accomplished under these goals?

1. Multi-ethnic genome-wide association study for idiopathic nephrotic syndrome identifies susceptibility loci across the life span, response to therapy and genetic ancestry (Yask Gupta, et al, Am Soc Nephrol Kidney Week 2023, Poster; *manuscript in preparation*)

Large-scale genetic association studies for idiopathic nephrotic syndrome (INS) cause by focal segmental glomerulosclerosis and minimal change disease are lacking, especially for forms of disease that are unresponsive to immunosuppressive treatment.
To address this knowledge gap, we conducted a large-scale genome-wide association study (GWAS) for common variants with paired exome sequencing in order to assess the complex polygenic architecture of non-Mendelian INS.
This study consists of 5,600 INS cases across the life span, genetic ancestry, and response to therapy, compared to over 50,000 genetically matched controls. More than 80% of cases had paired exome or genome sequencing. GWAS was conducted first on the entire cohort and then in subgroups based on age of onset, ancestry, and response to therapy. Analyses were conducted again after removal of cases harboring diagnostic/pathogenic Mendelian mutations in known FSGS genes and APOL1 high-risk genotypes. Ancestry specific GWAS were conducted using Regenie and Saige, metanalyses were conducted using Metal and TransMeta.

Trans-ethnic metanalysis of all cases irrespective of age of onset and response to therapy showed the known association of APOL1, driven by individuals of recent African ancestry (OR= 2.67, P= 4.24 x 10^{-61}), association with the HLA-DQA1 locus (OR=1.49, P= 5.91 x 10^{-24}) and with chromosome 4 (LDB2; OR=1.34, P= 4.48 x 10^{-9}). After removal of cases harboring pathogenic mutations causing Mendelian forms of FSGS and APOL1 HR genotypes, the strength for association to chromosome 4 increased and a novel locus on chromosome 15 reached genome-wide significance (ADAMTSL3; OR=1.53, P=2.73 x 10^{-8}). Ancestry-specific and sub-phenotype analyses according to the age of onset and response to glucocorticoid treatment resulted in validation and replication of loci for SSNS (e.g. CALHM6, CLEC16A), and several novel HLA loci (for adult SSNS and non-Mendelian SRNS), as well as novel non-HLA significant associations specific for clinical or ancestry sub-groups.
Interim results demonstrate multiple novel loci for INS, including pleiotropic risk alleles that predispose to NS across different sub-phenotypes, and loci specific to ancestry, age of onset, and response to therapy.

2. Large-scale case-control exome-wide association study identifies known and novel susceptibility genes for idiopathic nephrotic syndrome (Juntao Ke et al, Am Soc Nephrol Kidney Week 2023, Top Poster + Oral Presentation; *manuscript in preparation*)

The genetic causes of idiopathic nephrotic syndrome (INS) have traditionally been studied using family-based approaches. Comprehensive association studies across the age of onset, response to therapy, and ancestries are lacking.
We conducted an exome sequencing (ES) study on 5,271 children and adult cases with INS caused by focal segmental glomerulosclerosis or minimal change disease, including 1,853 steroid sensitive cases (SSNS) and 3,418 cases that were not known to be responsive to steroids (either steroid resistant or untreated/unknown). Per-gene burden of rare coding variants was assessed by exome-wide collapsing analysis comparing the above 5,271 cases and 28,637 population controls with ES data under dominant and recessive models. Analyses were conducted on the entire dataset and then again after removal of cases harboring diagnostic/pathogenic Mendelian mutations in known FSGS genes and APOL1 high-risk genotypes. Thresholds for significant and suggestive associations were generated by permutation analyses.
In the analysis on the entire cohort, we identified and retrieved association for many of the known FSGS genes, including *WT1* (best P= 1.09 × 10^{-23}; OR= 15.64), *COL4A5* (best P= 2.37 × 10^{-17}; OR= 9.83), *INF2* (best P= 1.87 × 10^{-11}; OR= 12.22), and several others, under dominant models, and *NPHS1* (best P= 1.56 × 10^{-21}; OR= 25.18), *NPHS2* (best P= 3.93 × 10^{-15}; OR= 30.58), *SMARCAL1* (9.91 x 10-9; OR= 31.97) and several others, under a recessive model. This analysis re-classified *CD2AP* as an autosomal recessive cause of INS, and points to mutations in *CLN5* and *OCRL* as common causes of FSGS phenocopies. Removal of solved cases and re-analysis prioritized 5 novel genes that exceeded the 5% false discovery rate (FDR), one gene that represented a phenotypic expansion of a known Mendelian neurodevelopmental disease, and three genes for which mouse models of the orthologues display glomerulopathy. Seven of them are novel candidates for steroid resistant NS and two for SSNS.
These findings expand our understanding of the genetic underpinning of INS/FSGS, identify novel candidate genes, and highlight the high genetic heterogeneity of disease.

3. Small but clinically-relevant Contribution of CNVs to Idiopathic Nephrotic Syndrome (Tze Y Lim, et al, Am Soc Nephrol Kidney Week 2023, Poster; *manuscript in preparation*)

Genetic studies for FSGS and nephrotic syndrome have overwhelmingly assessed the contribution of single nucleotide variants while the contribution of rare Copy Number Variations (CNV) to INS remains poorly understood.
We conducted chromosomal DNA microarray (CMA) genotyping on a multi-ethnic cohort 3,600 INS patients across the age of onset and response to immunosuppressive therapy. Interim analysis on 2,230 INS cases was conducted. To extract CNVs we used PennCNV using default parameters, and variants were annotated against the CNV start and stop boundaries corresponding to 221 known Genomic Disorders (GD) and a curated list of 126 genes that, when mutated, are known to cause INS/FSGS or a phenocopy of it. Subgroup analyses were conducted after removal of solved cases via exome sequencing, partitioning by age of onset, response to therapy, and genetic ancestry.
18 of 2,230 INS individuals (0.8%) carried a GD-CNV without significant enrichment for a particular subcategory. Notably, we identified 6 (0.3%) individuals with smaller deletions that encompassed INS-associated genes for which a loss-of-function mechanism of disease determination is known: *NPHS1* (N=2), *FRAS1*, *HNF1B*, *LMX1B*, and *WDR73*. These variants were observed almost exclusively in individuals with FSGS or steroid resistant NS.
This analysis showed that the overall CNV diagnostic rate for INS is relatively low, (< 2%), but should not be overlooked since these variants add to the overall genetic diagnostic workup and have direct implication in clinical management for NS (ex steroid avoidance) and risk stratification for extra-urinary complications associated to these variants.

4. **Strong protective effect of the *APOL1* p.N264K variant against G2-associated focal segmental glomerulosclerosis and kidney disease** (Yask Gupta, et al, *accepted for publication in Nature Communications*; https://www.medrxiv.org/content/10.1101/2023.08.02.23293554v1)

African Americans develop kidney disease at a rate five times higher than European Americans1. Two African ancestry-associated variants (G1 and G2) in the apolipoprotein L1 (APOL1) gene constitute major contributors to this disparity. These predispose to progressive kidney disease, with odds ratios for hypertension-associated end stage kidney disease (ESKD), focal segmental glomerulosclerosis (FSGS), and HIV-associated nephropathy exceeding 7, 17, and 30, respectively, when comparing APOL1 high-risk (APOL1-HR, i.e. individuals carrying either the G1/G1, G1/G2, or G2/G2 genotypes) to low-risk (APOL1-LR) genotypes.
Approximately 15% of individuals with an APOL1-HR genotype will develop ESKD, and a smaller fraction, estimated at 5%-8%, will develop FSGS8. The incomplete penetrance of APOL1-HR genotypes is thought to reflect the requirement for disease modifiers that potentiate APOL1 cytotoxicity. Genetic modifiers have been suggested but, to date, the identification of modifier genetic variants for APOL1-mediated kidney disease and, particularly, FSGS, remains elusive. In 2019, we studied the cytotoxic effect of multiple naturally and non-naturally occurring APOL1 haplotypes in experimental cell-based systems. We found that the toxicity of G1 and G2 alleles was substantially reduced when expressed on the haplotype defined by the APOL1 missense variant p.N264K (chr22:36265628 C>A; rs73885316), also associated with a partial loss of trypanolytic function. These data suggested, at a functional level, a protective effect for this variant against the deleterious cellular effects of the G1 and G2 APOL1 risk variants. The p.N264K defines one of the

common G0 (non-risk) APOL1 haplotypes, which is more frequent in individuals of European ancestry, but it is also present on a small fraction of G2 haplotypes in absence of G0, indicating two independent mutational events during evolution only on these two haplotypes. The p.N264K is therefore expected to be mutually exclusive with the APOL1 G1 allele.

To test the hypothesis that the G2-p.N264K haplotype differs in its genetic impact from the more common G2 risk allele without the p.N264K variant, we sought to compare its frequency in APOL1-HR subjects with FSGS to APOL1-HR controls without kidney disease. First, to eliminate potential confounding by the p.N264K haplotype defined by the more common APOL1 non-risk G0 allele, we excluded all individuals with non-risk, G0-containing genotypes, i.e., G0/G0, G0/G1, and G0/G2. We studied two case-control FSGS discovery cohorts: the first consisted of 434 APOL1-HR FSGS cases and 2,398 genetically matched APOL1-HR population controls subjected to Illumina DNA microarray genotyping and imputation; the second included 94 APOL1-HR FSGS cases and 208 genetically matched APOL1-HR controls with whole genome sequencing data, for a total of 528 FSGS cases and 2,606 population controls with no known kidney disease. Next, in order to investigate the impact of the p.N264K variant, we conducted a comprehensive analysis only on APOL1 high-risk individuals, employing categorical approaches (based on allelic frequency) and, as sensitivity analysis, regression-based (based on genotypes) statistical tests. In our APOL1- HR FSGS cohorts, we observed a strong protective effect for the p.N264K minor allele 'A' (MAF cases = 0.19 % and MAF controls = 2.7%, OR=0.07, 95%CI = 0.01-0.25, CMH test P=3.4x10-9) as compared to APOL1-HR controls. Stratifying the cohort for the three APOL1 high-risk genotypes showed that this variant was only observed within APOL1-HR individuals carrying the G2 allele (i.e., G1/G2 and G2/G2) and, as expected, never in G1/G1. These findings support a protective effect of the p.N264K variant only in the context of G2-containing APOL1-HR genotypes. In fact, the p.N264K variant seemed to confer complete protection against FSGS as it was never observed in cases in the presence of the G2/G2 genotype: OR=0, 95%CI 0-0.41; CMH test $P=4.4x10^{-4}$. A strong and significant protective effect was also observed for the G1/G2 genotype with a p.N264K MAF of 3.57% in controls as compared to 0.49% in cases (OR=0.14, 95%CI:0.16-0.52; CMH test $P=4.0x10^{-4}$). Consistent with these findings, analyzing individuals with G1/G2 or G2/G2 genotypes combined increased the level of statistical significance for the p.N264K protective effect (OR=0.08, 95%CI 0.01-0.3, CMH test $P=2x10^{-7}$). Sensitivity analyses that additionally adjust for population structure confirmed the results obtained by CMH, as Firth's regression tests supported the strong protective effect of the p.N264K variant against FSGS with comparable effect sizes. As expected from population distribution of haplotypes, in the context of APOL1-HR genotypes, the p.N264K is limited to G2-containing genotypes (i.e., G1/G2 or G2/G2). Nevertheless, a recombination event between the p.N264K and the G1 or G2 alleles (although very unlikely given the proximity of these APOL1 alleles), could result in contamination from the European G0-p.N264K haplotype due to local ancestry admixture. To evaluate this scenario, in our final sensitivity analysis we conducted haplotype-of-origin analysis in the discovery cohort using Tractor, a statistical framework that deconvolutes the local haplotypes into ancestral (in this case European and African) haplotypes. This confirmatory analysis showed a significant protective effect of the p.N264K variant exclusively originating from the African haplotype (OR=0.10, 95%CI=0.02-0.29, $P=1.3x10^{-7}$), while the European haplotype was non-significant (OR(ADJ)=0.74, 95%CI= 0.00-11.37, P=0.85) despite larger sample size. Again, stratifying for G1/G2 or G2/G2 further validated the G2-specific protective effect of the p.N264K variant for the African haplotype (OR=0.12, 95%CI=0.02-0.35, $P=3.53x10^{-6}$) but not for the European haplotype (OR(ADJ)=0.76, CI=0.00-12.75, P=0.86).

Overall, these results support a strong protective effect of the APOL1 p.N264K missense variant against APOL1-associated FSGS, but this effect occurs exclusively on G2-containing APOL1 high-risk genotypes of African origin. In practical terms, based on these analyses, APOL1-HR individuals are at least 8.3 times less likely to develop FSGS if they carry one copy of the p.N264K missense variant.

Finally, to test the generalizability of these findings to milder forms of APOL1-associated kidney disease, we investigated the protective effect of the APOL1 p.N264K in individuals from the REasons for Geographic and Racial Differences in Stroke (REGARDS) and Electronic Medical Records and Genomics Phase III (eMERGE-III) studies. In aggregate, these cohorts included 1,573 APOL1-HR individuals with available kidney function data. Of these, 276 had CKD stage 3 (REGARDS, N=150; eMERGE-III, N=126) or worse (considered as cases), and 1,297 genetically-matched APOL1-HR controls (REGARDS, N=893; eMERGE-III, N=404) with estimated glomerular filtration rate (eGFR) >60ml/min/1.73m2. Despite the smaller sample size, milder form of APOL1-associated kidney disease, and incomplete clinical data to classify and exclude unrelated causes for CKD in these cohorts, the findings revealed a direction-consistent protective effect for the p.N264K variant among individuals with the G2-APOL1-HR genotypes, by which p.N264K carriers were 3.3 times less likely to have CKD3 or worse (OR=0.30, 95%CI: 0.11-0.83, CMH P=0.023), with this likely representing an underestimation due to confounders as mentioned above.

These results have immediate and broad implications for translational research and clinical practice. First, from the genetic standpoint, it is important to note that we observed a very large effect of p.N264K on mitigating the consequences of the G2 risk allele but saw no evidence of this variant on the more common G1 risk allele. As consequence, because p.N264K and G1 alleles are mutually exclusive, this finding raises the possibility of additional genetic modifiers specific to G1 and, in general, identifiable by considering genotype-specific APOL1 studies. In addition to studies of the APOL1 high-risk genotype as a single genetic driver, analyses conducted by partitioning cohorts into the three specific APOL1 high-risk genotypes, although might require larger sample sizes, are likely to provide significant additional insight into the genetics and underlying biology of APOL1-associated FSGS and kidney disease. Second, our genetic observations are in agreement with our previous functional studies showing that the p.N264K variant is able to reverse the cytotoxic effect of both G1 and G2 risk variants in cell-based assays14. Therefore, conceptually, it may be best to regard the p.N264-G2 and p.K264-G2 simply as different alleles that encode different proteins. As such, they likely adopt different conformations and/or have different activities at the protein level. This will become clearer as we learn more about the APOL1 protein structure(s) in the future.

Taken together, these data support the hypothesis that the p.N264K missense variant negates the toxic effect of the G2 allele, and will allow the reclassification of a fraction of APOL1 G1/G2 or G2/G2 high-risk individuals as having a non-high-risk genotype if p.N264K is also present. This discovery has substantial, immediate, and clinically-relevant implications. First, individuals affected by CKD or ESKD with APOL1 G1/G2 or G2/2 high-risk genotypes but with the p.N264K missense variant are unlikely to have APOL1-associated FSGS, and therefore an additional cause (immune, toxic, structural, or others) should be investigated because this will likely result in a different therapeutic approach. Second, importantly, in kidney transplant settings, these results can significantly affect donor selection and both donor kidney, and recipient graft, outcome. In fact, APOL1 G2-HR donors who are p.N264K positive will likely have kidney outcomes similar to any of the G1G0, G2G0, and G0G0 low-risk donors, thus expanding donors' pool; kidney transplant recipients of a APOL1-HR-p.N264K kidney will likely have low risk for developing de novo FSGS on the graft or graft failure from APOL1-associated kidney disease. Third, incorporation of this

knowledge will allow more accurate study design for new intervention trials by which individuals with APOL1-HR-p.N264K genotypes should not be included in the intervention arm as cases since this genotype is genetically and functionally a low-risk genotype. Finally, the knowledge presented here will affect family risk stratification and planning, and, in general, CKD risk ascertainment at the population level.

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

Tze Y. Lim. Ms. Lim, MS, joined my lab in 2017 as lead bioinformatician. Ms. Lim has been working and helping postdoc and scientists in the lab with the computational biology component of projects related to this grant: analysis of exome sequencing, DNA microarrays for GWAS, RNA sequencing etc. Ms. Lim has also been heavily involved in training with the ultimate goal of proceeding to graduate school in order to obtain a PhD degree in computational biology/genetics applied to FSGS. During the tenure of this award she attended several courses, including: The Advanced Sequencing Technology & Applications, Cold Spring Harbor Laboratory; The Medical Genetics for Internal Medicine "How to Integrate Genetics into Internal Medicine Practice" by The Center for Precision Medicine and Genomics course; and the Getting started with statistical software, Columbia University Department of Biostatistic. Since she joined the lab she coauthored 14 publications, including 8 published during this first year of funding.

Yask Gupta, PhD, joined my lab in December 2019 as postdoctoral research scientist / computational biology. Dr. Gupta has been leading the GWAS effort for FSGS that are at the core of this DoD award. Similarly to Ms Lim, he attended several courses, including: The 2023 Podocyte Meeting in Philadelphia; The Medical Genetics for Internal Medicine "How to Integrate Genetics into Internal Medicine Practice" by The Center for Precision Medicine and Genomics course; and the Getting started with statistical software, Columbia University Department of Biostatistic. Since he joined the lab, he coauthored 6 publications, including 5 published during this first year of funding. Importantly, he is the first author of the *APOL1* modifier paper recently accepted for publication in *Nature Communications* (see above).

Juntao Ke, PhD, joined my lab in 2020 as postdoctoral research scientist. Dr. Ke has been leading the FSGS rare variant studies using exome and genome sequencing that are complementary to the common variant studies at the core of this DoD award. He also attended several courses, including: The 2023 Podocyte Meeting in Philadelphia; The Medical Genetics for Internal Medicine "How to Integrate Genetics into Internal Medicine Practice" by The Center for Precision Medicine and Genomics course; and the Getting started with statistical software, Columbia University Department of Biostatistic. Since he joined the lab, he coauthored 3 publications, all during this first year of funding.

All trainees participated and presented original work at national and international meetings, including the American Society of Nephrology Kidney, the American Society of Human Genetics; the Podocyte Meeting; the CAIRIBU meeting; and others.

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?

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the first year of funding we nearly completed the generation of human genetics data and made very significant progress towards the completion of the aims of this grant. Hence, in the second year of funding we will dedicate our efforts mostly toward two goals: 1) computational work to analyze and bring to publication the bulk of human genetics data generated thus far. We anticipate at least three manuscripts from this effort (see preliminary data above); 2) generation of the mouse transcriptomic data as described in aim 3 of the proposal. This work will be completed then in year 3 of the proposal for the final integration with human genetics data.

4. **IMPACT:** D*escribe 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?

To date in less than one year of funding, we accomplished important goals with significant impact: 1) we completed the first GWAS for FSGS and identified multiple novel associations; this work is accepted for poster presentation at the American Society of Nephrology Kidney week 2023 and will result in a high-impact publication describing, for the first time, the complex genetic determination of FSGS. 2) We have completed a first, large-scale exome-wide case-control association analysis for rare variant and identified known and novel associations. This work beyond representing a significant advancement in the field, it also allows to identify FSGS cases with known Mendelian disease, which in turn can obscure associations for common variants with small-to-moderate effect size thus helping refine our GWAS. 3) We have shown a small, but clinically relevant contribution of structural variants to the etiology of FSGS, thus improving our diagnostic toolkiti. 4) Finally, we have identified and reported the first results of our APOL1 genetic modifier analysis, with the discovery of the p.N264K protective missense variant, resulting in important and direct implications for clinical management of FSGS in underrepresented minorities (URM)s.

What was the impact on other disciplines?
"Nothing to Report."

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:** *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:*

While there are no changes in the SOW nor in our timeline, and hence we can assure that the milestones of the proposed program are being met, given our rapid and relevant advancement in data generation and preliminary analysis, we respectfully ask to rebudget some of the funding dedicated to the DNA microarrays genotyping (complete for the phase 1 studies) to support computational, analytical and manuscript preparation.
As described in our preliminary results, we are rapidly moving towards delivery of very important and clinically actionable results, with analyses that will populate at least 3 additional high-impact publications. Therefore, in order to accomplish these milestones we request to transfer dollars from the human se quencing effort (currently budgeted for year 2, split in for exome and low pass sequencing) toward the effort of trainees.

Remember that significant changes in objectives and scope require prior approval by the agency.

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.

No problems nor delays: we are on track to deliver the first group of manuscripts based on the first phase of data aggregation studies

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.

We had no changes not delays.
As described above, we have generated all necessary data and we are on track to deliver the first round of manuscripts.
Since we have generated a significant amount of data (sequencing and microarray) we now face the hurdle of data analysis and publication. In order to stay on track and allow prompt completion of the first set of milestones, we ask to rebudget, only for year 2, from the requested funding for exome and low pass sequencing to salary effort for trainees to face this increase analytical demand.

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

No changes

Significant changes in use of biohazards and/or select agents

No changes

6. PRODUCTS:

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

  Journal publications.

1) Gupta Y, Friedman DJ, McNulty MT, Khan A, Lane B, Wang C, Ke J, Jin G, Wooden B, Knob A L, Lim TY, Appel GB, Huggins K, Liu L, Mitrotti A, Stangl MC, Bomback A, Westland R, Bodria M, Marasa M, Shang N, Cohen DJ, Crew RJ, Morello W, Canetta P, Radhakrishnan J, Martino J, Liu Q, Chung WK, Espinoza A, Luo Y, Wei WQ, Feng Q, Weng C, Fang Y, Kullo IJ, Naderian M, Limdi N, Irvin MR, Tiwari H, Mohan S, Rao M, Dube GK, Chaudhary NS, Gutiérrez OM, Judd SE, Cushman M, Lange LA, Lange EM, Bivona DL, Verbitsky M, Winkler CA, Kopp JB, Santoriello D, Batal I, Pinheiro SVB, Oliveira E , Simoes e Silva AC, Pisani I, Fiaccadori E, Lin F, Gesualdo L, Amoroso A, Ghiggeri GM, D'Agati VD, Magistroni R, Kenny EE, Loos RJF, Montini G, Hildebrandt F, Paul D S, Petrovski S, Goldstein DB, Kretzler M, Gbadegesin R, Gharavi AG, Kiryluk K,

Sampson M G, Pollak MR, and **Sanna-Cherchi S[$]**. Strong protective effect of the APOL1 p.N264K variant against G2-associated focal segmental glomerulosclerosis and kidney disease. medRxiv. 2023. PMID: 37577628. **Nature Communications**, accepted for publication.

2) Marasa M*,Ahram DF*, Rehman AU*, Mitrotti A, Abhyankar A, Jain NG, Weng PL, Piva SE, Fernandez HE, Uy NS, Chatterjee D, Kil BH, Nestor JG, Felice V, Robinson D, Whyte D, Gharavi AG, Appel GB, Radhakrishnan J, Santoriello D, Bomback A, Lin F, D'Agati VD[$], Jobanputra V[$], and **Sanna-Cherchi S[$]**. Implementation and feasibility of clinical genome sequencing embedded into the outpatient nephrology care for patients with proteinuric kidney disease. **Kidney International Reports**. 2023 May 26;8(8):1638-1647. doi: 10.1016/j.ekir.2023.05.021. PMID: 37547535
3) Barry A*, McNulty MT*, Jia X*, Gupta Y*, Debiec H*, Luo Y, Nagano C, Horinouchi T, Jung S, Colucci M, Ahram DF, Mitrotti A, Sinha A, Teeninga N, Jin G, Shril S, Caridi G, Bodria M, Lim TY, Westland R, Zanoni F, Marasa M, Turudic D, Giordano M, Gesualdo L, Magistroni R, Pisani I, Fiaccadori E, Reiterova J, Maringhini S, Morello W, Montini G, Weng PL, Scolari F, Saraga M, Tasic V, Santoro D, van Wijk JAE, Milošević D, Kawai Y, Kiryluk K, Pollak MR, Gharavi A, Lin F, Simœs E Silva AC, Loos RJF, Kenny EE, Schreuder MF, Zurowska A, Dossier C, Ariceta G, Drozynska-Duklas M, Hogan J, Jankauskiene A, Hildebrandt F, Prikhodina L, Song K, Bagga A, Cheong H 2nd, Ghiggeri GM, Vachvanichsanong P, Nozu K, Lee D, Vivarelli M, Raychaudhuri S, Tokunaga K[$], **Sanna-Cherchi S[$]**, Ronco P[$], Iijima K[$], Sampson MG[$]. Multi-population genome-wide association study implicates immune and non-immune factors in pediatric steroid-sensitive nephrotic syndrome. **Nature Communications**. 2023 Apr 29;14(1):2481. doi: 10.1038/s41467-023-37985-w. PMID: 37120605

Books or other non-periodical, one-time publications.
Nothing to report

Other publications, conference papers and presentations.

The following abstracts have been accepted for presentation at the upcoming American Society of Nephrology Kidney Week, Philadelphia, Nov 2-6

1) Multi-ethnic genome-wide association study for idiopathic nephrotic syndrome identifies susceptibility loci across the life span, response to therapy and genetic ancestry (Yask Gupta, et al, Am Soc Nephrol Kidney Week 2023, Poster; *manuscript in preparation*)

2) Large-scale case-control exome-wide association study identifies known and novel susceptibility genes for idiopathic nephrotic syndrome (Juntao Ke et al, Am Soc Nephrol Kidney Week 2023, Poster; *manuscript in preparation*)

3) Small but clinically-relevant Contribution of CNVs to Idiopathic Nephrotic Syndrome (Tze Y Lim, et al, Am Soc Nephrol Kidney Week 2023, Poster; *manuscript in preparation*)

- **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**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
No change

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: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*