Population Architecture using Genomics and Epidemiology (PAGE)
Ver. 09/30/16

PAGE Manuscript Proposal Template
Submit proposals by email to Kari North or Steve Buyske

All sections must be completed; incomplete applications will be returned.
Do not exceed 3 pages in length (not including references).

PAGE Ms. Number: __3639___ Submission Date: _03/1/2020___ [Approval Date: 03/23/2020]

Title of Proposed MS: Metabolomics of APOL1 carriers with kidney disease

Abbreviated Title of Proposed MS: APOL1 metabolomics and kidney disease

I. INVESTIGATOR INFORMATION:

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Authorship model: Typical PAGE genetic epidemiology paper with coauthors from all participating studies.

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Study	Contacted? Y/N	Declined? / Other?

Names, affiliations, email address of non-PAGE investigators proposed as co-authors:

II. SCIENTIFIC RATIONALE (Please be specific and concise)

Background

Chronic kidney disease (CKD) is prevalent in 14% of the US population[1] and is a major risk factor for cardiovascular morbidity and mortality. Adverse outcomes increase with progressively lower estimated glomerular filtration rate (eGFR) and higher urine albumin creatinine ratio (UACR)[2; 3]. African Americans carry a disproportionate burden of CKD and end-stage kidney disease (ESKD)[4; 5]. Two variants (G1 and G2) of the apolipoprotein L1 gene (*APOL1*) located on chromosome 22 are common in populations of West African ancestry and increase the risk for CKD and ESKD[6]. Among African Americans, the allele frequencies of G1 (rs73885319A>G, S342G) and G2 (rs71785313 TTATAA/− N388Y389/−) are 22.5% and 14.6%, respectively[7]. G1 and G2 occur on distinct haplotypes and 15% of African Americans carry the two risk alleles necessary to confer increased CKD risk.

Initial case-control studies of APOL1 focused on the rare diseases focal segmental glomerulosclerosis (FSGS) and Human immunodeficiency Virus (HIV)-nephropathy. Results were dramatic, with the presence of two risk alleles conferring a 17-fold risk for FSGS and 29-fold increased risk of HIV-nephropathy[8]. Subsequent cohort studies examined whether APOL1 risk alleles modified the risk of more common causes of CKD such as hypertension and diabetes and found significant associations only in nondiabetic CKD, with adjusted odds ratios of 3.5 (95%CI 2.1-5.7) for incident albuminuria[9], 1.5 (95%CI 1.0-2.2) for incident CKD and 1.9 (95%CI 1.2-2.9) for ESKD[10]. While APOL1 risk alleles have been found to consistently increase the risk only for incident nondiabetic CKD, the presence of two risk variants do appear to hasten the deterioration of eGFR, regardless of diabetes status[11].

The mechanisms by which APOL1 risk variants increase the risk for CKD and its progression have yet to be fully elucidated. In vitro and mouse model studies have revealed that mRNA (but not protein) from APOL1 risk alleles, activate protein kinase R, resulting in podocyte injury and proteinuria[12]. APOL1 risk variants have also been linked to inflammation[13], mitochondrial dysfunction[14] and cytotoxicity[13; 15; 16]. In humans, APOL1 protein concentrations do not vary by genotype, but proteins from high risk alleles may coalesce differently with protein complexes[17]. We propose to study associations between APOL1 G1 and G2 risk alleles and metabolomic, proteomic and epigenetic data in PAGE to investigate potentially overlapping mechanistic pathways through which they increase CKD risk.

III. OBJECTIVES AND PLAN (Please be specific and concise)

Are APOL1 alleles associated with metabolomic and methylomic dysregulation?
Is there modification by CKD?
Is there modification by type of CKD?
Is there interaction between metabolites/methylation and APOL1 genotypes on both prevalent or incident CKD?

See hypotheses below.

g. **Ancestry information used? No __ Yes_X_ How is it used in the analyses?**

Control for population stratification.

h. **Anticipated date of draft manuscript to P&P: _2021_____**

i. What manuscript proposals listed on *www.pagestudy.org/index.php/manuscripts/* **are most related to the work proposed here? Approved PAGE ms. numbers: ____NA ____ ____**

- **If any: Have the lead authors of these proposals been contacted for comments and/or collaboration? Yes__ No__**

IV. METHODS

a. Data. We will restrict analyses to African American and Hispanic/Latino datasets with metabolomic and/or methylation typing due to the absence of the G1 and G2 alleles in European Ancestry data [18]. The datasets and samples sizes are in **Table 1**. A recessive genetic model will be used that allows for compound heterozygotes to test our hypotheses that known APOL1 recessive variants are associated with changes in metabolites and/or methylation. We will assess the impact of potential phasing errors and we will conduct sensitivity analysis excluding potential compound heterozygotes. Due to the heterogenous nature of the omics data (e.g. platform, batch effects), we will conduct analyses within each study and then meta-analyze or use a discovery/replication design.

Table 1: Omics Data Included

Study	Metabolomics	Methylation
HCHS/SOL	3924 H/L on metabolon	NA
MESA	4220 untargeted	759 H/L and 525 AA
ARIC	2436 AA on Metabolon	2821 AA
WHI		806 H/L and 1432 AA
CARDIA	~1600 AA untargeted	NA

MethylOMICs. DNA methylation data is available from MESA, ARIC, and WHI measured in individuals from whole blood assays using Illumina arrays (typically the 450k array). Beta scores, which estimate the methylation level using a ratio of intensities between methylated and unmethylated alleles, will be used along with detection *p*-values representing the likelihood of detection relative to background. Based on prior work, we will eliminate CpGs where the probe sequence maps to a location that does not match the annotation file or to more than one locus. Data will be normalized with SWAN contained in the R package minfi[139]. For the small subset with the new Illumina array, most CpG sites from the 450K chip are present on the new EPIC array.

MetabolOMICs. MetabolOMICs data are more abundant, with a sample drawn from fasting serum or plasma. We have untargeted data in MESA and CARDIA. For each targeted metabolite, there is a detection threshold. Generally, detection is quite good (with few below limits) and in most cases imputation to the detection limit is performed. For HCHS/SOL and ARIC, all targeted metabolomic data are from Metabolon. We will primarily focus on ~300 metabolites that overlap across panels. Metabolites will be standardized (centered at the mean and scaled by the standard deviation) prior to analysis. Importantly, TOPMed and CHARGE investigators are working to harmonize metabolomic data across multiple platforms—e.g., MESA data are being harmonized with the Metabolon platform used in ARIC and HCHS/SOL. Several open-source data repositories will be used to access chemical, biological and molecular information about metabolites, including the

Kyoto Encyclopedia of Genes and Genomes (KEGG)[140], the Metabolic Pathway Database[141], and the MetabolOMICs GWAS Server[19; 20].

b. Hypotheses: to be tested

1) Are APOL1 risk alleles (G1 and G2) associated with certain metabolites/methylation sites?

- Recessive model with sensitivity analyses for compound heterozygotes

1a) Is this modified by the presence of CKD?

- Same as above in the subset of people with CKD at exam omics were measured.

1b) is this modified by the presence of nondiabetic CKD?

- Same as above in the subset of people with CKD, but not diabetes, at exam omics were measured.

2) Is there interaction between metabolites/methylation and APOL1 genotypes on CKD?

a) For prevalent disease
b) For incident disease in the future;

c. Statistical analyses. A recessive genetic model will be used that allows for compound heterozygotes to test our hypotheses that known *APOL1* recessive variants are associated with metabolites and/or methylation. We will assess the impact of potential phasing errors and we will conduct sensitivity analysis excluding potential compound heterozygotes. Due to the heterogenous nature of the omics data (e.g. platform, batch effects), we will conduct analyses within each study and then meta-analyze or use a discovery/replication design. For each study, we will use linear mixed effects (LME) models with each OMIC as the outcome variable and the APOL1 defining case/control status as a fixed effect predictor and random effects to account for sample relatedness if needed. For metabolOMICs, we will use log or inverse-normal transformations to achieve approximate normality. For methylOMICs we will use the inverse normal transformation of the probe Beta-values (ratios of methylated probe intensity to overall intensity) as the response. Effect modification will be pursued only if power allows.

]**V. SOURCE OF DATA TO BE USED** (Provide rationale for any data whose relevance to this manuscript is not obvious): **Check all that apply:**

Aggregate/summary data to be generated by investigators of the study(ies) mentioned:

] ISMMS; **[X]** CALiCO; **[ ]** MEC; **[X]** WHI; **[ ]** CC; **[ ]** Other:__MESA_____
If CALiCo please specify:
Included on MEGA Array: **[X]** SOL

Studies not on MEGA: **[X]** ARIC; **[X]** CARDIA; **[ ]** SHS-Fam; **[ ]** SHS-Cohort
I, AM_(initials)_, affirm that this proposal has been reviewed and approved by all listed investigators.

V1. REFERENCES

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VI. IF USING SOL DATA (Please provide the information below)

a. Keywords: APOL1, kideney disease, metabolomics

b. Using biomarker data? Yes _X__ No ___

c. Where will the SOL data analyses be performed?

UNC

VII. IF USING CHS DATA (Please provide the information below)

a. Do you propose use of data from a participant's DNA?