National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

ApoL1 and Kidney Disease Conference

College Park Marriott Hotel & Conference Center
Hyattsville, MD
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Experiments and Areas of Research Suggested by Participants

Clinical/Human subjects research
Franceschini
Characterization of ApoL1-related CKD in African American populations without and with CKD, such as to identify factors (environmental and genetic) that contribute to disease - Or even better, that are associated with no transition to disease. Who are these healthy individuals who are carriers of ApoL1 2 risk alleles, and what was their lifetime exposure experience? It would be of interest to know their trajectory in birth cohorts, studies of pregnancy and in children. Ideally, we should be using our best approaches in epidemiology/clinical research for that including longitudinal studies, both in established cohorts with standardized measures and rich environmental factors (including accounting for SES, co-morbidities, toxins), but also using EMR samples so to capture treatment, and medical resource use.

Franceschini
Apply findings from basic research in clinical/epidemiological research - for example, if there is evidence for a mechanism related to lipids, we could look at this in our studies. If the protein is harmful to podocytes, should we be checking UACR in African Americans?

Le
Assess genetic factors which modify the influence of ApoL1 high risk variants on the course of kidney disease progression and on plasma levels of markers of oxidative stress and apoptosis - Genetic modifiers may provide meaningful information regarding pathways regulated by the gene of interest, as well as modifiable factors for therapeutic target. In this regard, our preliminary data show that the GSTM1(0) and the ApoL1 high risk alleles confer additive deleterious effects in AASK participants. Interestingly, those with ApoL1 high risk alleles but homozygous for the GSTM1 active allele (GSTM1 1/1) appear to be protected from CKD progression. In addition, we find that Gstm1 knockout mice have significantly increased levels of superoxide in the kidney, and worse kidney injury in angiotensin II-induced hypertension than wild-type mice. The deleterious additive effect of GSTM1(0) allele to worsen clinical outcomes in AASK participants carrying the ApoL1 high risk alleles may suggest that ApoL1 influences oxidative stress. Oxidative stress and apoptosis are closely linked biological processes. Moreover both oxidative stress and apoptosis have been linked to chronic kidney disease. The joint effects of GSTM1 and ApoL1 on clinical outcomes and markers of oxidative stress and apoptosis should be examined in CKD patients.

Ashley-Koch
Expand metabolomics work both in SCD-CKD [Sickle Cell Disease-Chronic Kidney Disease] cases and in non-SCD CKD cohorts – this will allow investigators to sort out what signals may be specific to the ApoL1 risk variants and what may be distinct in SCD vs. non-SCD.

Reidy
Effect of ApoL1 on birth outcomes and nephron number – Is this a direct effect or is prematurity/low nephron number a second hit resulting in ApoL1 disease? If it affects perinatal/birth outcomes there is a potential for tighter screening and intervention. If prematurity is a risk factor it provides a new tool to understand which premies need to be followed for renal issues (there are no current recommendations of how premies should be followed for renal issues beyond checking blood pressures in the first 2 years of life). The way to study this is to examine a perinatal/neonatal cohort, collect placentas, maternal and baby DNA and pregnancy outcomes (preeclampsia/eclampsia)/ neonatal outcomes

(prematurity). The goal would be to enroll all early in pregnancy and there will be a subset with fetal demise and neonatal deaths and one could do nephron (and podocyte) counts on those kidneys. There could then be longitudinal followup to assess who develops disease. There is as an active effort nationally to develop neonatal cohorts and this would fit in well.

Freedman
Identification of non-HIV viruses that may serve as second hits in ApoL1-nephropathy - these might be amenable to treatment to slow nephropathy progression. This project involves looking at urine and kidney tissue (possibly blood also).

Sampson
GWAS using 'super-controls' - Identify a cohort of older African-American individuals with 2 risk alleles who do not have any sign of kidney disease (normal eGFR, no proteinuria) and perform a GWAS of this group of "super-controls" versus African-Americans with FSGS and 2 risk alleles. Collect detailed histories from these cases and controls in terms of environmental and infectious exposures as well as characteristics such as birth history and their BMI. The hypothesis would be that we could identify genetic or environmental differences between these two groups that teach us about factors that either potentiate or protect against the development of ApoL1-associated proteinuric disease.

Transplant

Franceschini
ApoL1 genotyping for prognosis - Evaluate the benefits/costs of genotyping African American (and Hispanic) donors for prognosis, considering the ethical and cost issues. It would be interesting to have some cost analysis done for this purpose.

Freedman
Prospective study of African American deceased kidney donor ApoL1 genotypes and their recipient ApoL1 genotypes - with testing for interactions between them. Donor kidney tissue ApoL1 protein and mRNA should also be assessed at the time of initial transplantation for effects on allograft survival.

Doshi
Investigate African American live kidney donors - are they at an increased risk of developing hypertension and ESRD than age-, gender-, race- and time to follow-up matched healthy controls (non-donors)? Can this increased risk can be explained by familial/genetic factors i.e. ApoL1 risk alleles that are shared between the donor and the recipient (absent in the non-donor, control group)? Use historical and prospective living kidney donors to:

- Compare the post-donation incidence of hypertension and trajectory of blood pressure between donors with two versus zero/one APOL1 risk alleles.
- Compare the trajectory of post-donation renal function between donors with two versus zero/one APOL1 risk alleles.
- Collect samples from donated organ for study of podocyte injury

Basic

Pollak
ApoL1 toxicity - conduct ApoL1-mediated cell toxicity assay for small molecules which rescue the G1 and G2 associated lethality. Rationale: ApoL1 risk variants never would have been discovered without non-hypothesis based experiments. Now everyone is pursuing the same one or two pathways for mechanism. A non-hypothesis based mechanism screen is needed to identify new pathways.

Weisz
What is the basis for ApoL1 toxicity? - For example, do podocytes or other possible affected cell types with one or two variant alleles have altered lysosomal pH? Are there altered lysosomal or ER stress responses in these cells? What modifies expression of ApoL1?

Le

Use healthy kidney tissues obtained from donor kidney immediately prior to implantation to determine the differences in gene expression networks and epigenetics influenced by ApoL1 G1/G2 risk variants versus the G0 variant - Gene expression networks and epigenetics may provide mechanistic role of a gene of interest when appropriate or ideal animal models are not available. However, gene expression and epigenetics are influenced by disease states, and therefore alterations in their profiles may reflect more of a response to disease rather than direct effect of the gene of interest. Use of healthy kidney tissues from donor kidney carrying the ApoL1 high risk variants to determine changes in gene expression networks and epigenetics would be more advantageous than using "normal kidney area" from kidneys removed from patients with to renal neoplasms to avoid the possibility of gene expression changes in "normal tissue" that could result from paracrine effects of or response to the nearby tumor.

Edwards

Establish the sub cellular membrane fraction in which ApoL1 resides in human podocytes - isolate that membrane fraction from cells expressing the various forms, and assess transport/permeability/channel properties of the protein.

Fornoni

Cholesterol and ApoL1 - Data suggest that accumulation of cholesterol in podocytes in CKD causes podocyte injury and that cells expressing G1 or G2 variants accumulate intracellular cholesterol. How do ApoL1 risk alleles influence cholesterol efflux from cells? As we do not know if cellular specific or circulating ApoL1 is important, then there are two key experiments. One is to test cholesterol efflux from WT macrophages exposed to the sera collected from patients with different risk alleles and the other is to test cholesterol efflux in macrophages isolated from patients with different risk alleles.

Fornoni

Use of iPS [induced pluripotent stem cells] - Develop iPS-derived human podocytes modified by genomic editing to express the different ApoL1 risk variants and use for testing cholesterol efflux.

Weisz

Site of action of ApoL1 - What is the primary affected cell type relevant to ApoL1-associated CKD? Endothelial cells, podocytes, proximal tubule cells?

Weisz

Cell biology of ApoL1 - Are there structural changes in G1 and G2 that affect folding/stability/membrane insertion? Given that the protein has no disulfides or glycans it would appear that it can more easily escape ER quality control mechanisms than other secreted proteins. What is the trafficking route/stability of ApoL1 in cells? Can endogenous ApoL1 cause cellular changes? What is the cellular site of action? If it's lysosomes, how does newly synthesized ApoL1 get to this compartment? Cells typically work hard to shunt their secreted proteins away from lysosomal routes, although it's possible that the protein is associated with membranes and percolates passively into lysosomes where it is activated where it may be activated. Do the variants cause trouble because they don't insert as well into lipoprotein particles and/or are more likely than G0 to be "free" in the bloodstream? Do they associate better or worse than G0 with lipids?

Weisz

Second hits – It is unlikely that second hits affect the ApoL1 target cells directly, so how do they synergize systemically with G1 and G2 to propel disease progression?

Ashley-Koch

Zebrafish models of ApoL1 - Create a zebrafish ApoL1 CRISPR mutant on a fli1-eGFP reporter line background so that we can investigate the potential effects on vascular endothelial cells and determine what that relation is to the kidney phenotypes. In addition, create a zebrafish APOL1 CRISPR mutant on the pod-NTR-mCherry background so that we can flow sort the podocytes and further investigate gene*gene interactions, through RNAseq.

Susztak

Determine the critical cell type(s) for APOL1-mediated disease development - characterize the phenotype of transgenic

mice with podocyte, tubule epithelial cell, liver, endothelial and vascular smooth muscle specific inducible reference (G0) and risk allele (G1 and G2) ApoL1 expression. Our preliminary experiments indicate that podocyte specific, but not tubule specific, risk allele ApoL1 induces nephrotic syndrome and glomerulosclerosis in mice. While most human cells can express ApoL1, we propose that the phenotype heterogeneity might be related to cell type specific expression and regulation of ApoL1. For example while podocyte specific risk allele ApoL1 is associated with FSGS development, hypertensive renal disease could be induced by vascular ApoL1 expression. Create and study different mouse lines with cell type specific temporal control of ApoL1 expression, and study expression levels and phenotypes including albuminuria, kidney function, blood pressure, renal histology, podocyte number, hyalinosis, and atherosclerosis.