# Online Proceedings

## SESSION 1E

### FROM VIRAL PATHOGENESIS TO GENETIC DISEASES TO BUILDING A BETTER KIDNEY

*Session Moderator: Michael Lagunoff, Microbiology*
**MGH 231**
*12:30 PM to 2:15 PM*

* Note: Titles in order of presentation.

**Generation of Human Pluripotent Stem Cells without Cilia Establishes a Novel Model of Polycystic Kidney Disease**
*Christine Vu Tran, ,*
*Mentor: Benjamin Freedman, Medicine/Nephrology*
*Mentor: Nelly Cruz, Medicine*

Primary cilia are antenna-like structures on the surface of diverse cell types. Cilia are important for signal transduction and processes such as cell division and development, but their precise role in the cell remains poorly understood. Defects in cilia result in a wide spectrum of genetic disorders called ciliopathies, which commonly involve polycystic kidney disease (PKD), the formation of fluid-filled sacs in the kidney which eventually result in kidney failure. To understand the role of cilia in these diseases, we generated human pluripotent stem cells (hPSCs) with defects in cilia formation for the first time. We used the CRISPR-Cas9 genome editing system to introduce mutations in two different genes that are required for cilia formation, kinesin family member 3A (KIF3A) and kinesin family member 3B (KIF3B). We isolated four independent cells lines with indel mutations in exon 3 of the KIF3A gene and two in exon 2 of KIF3B. The introduced mutations led to frameshifts and protein truncations. Immunoblot analysis confirmed that no full-length protein was produced in these mutant cells. The cells were stained with acetylated alpha-tubulin, a marker of cilia. No cilia were detected in the mutant hPSCs, while an average of 48% cilia were detected in controls of identical genetic background. Surprisingly, the absence of cilia did not alter hPSC pluripotency, self-renewal, amniotic cavity formation, or growth. Further, we differentiated these cell lines into kidney organoids. The cilia deficient kidney organoids formed cysts reminiscent of PKD, while the isogenic controls did not. In conclusion, we have generated human cells and organoids lacking an entire organelle, the primary cilium, and used these to conclusively link cilia to PKD. These cilia deficient hPSC lines can be used to study cilia function, to model various ciliopathies, and to screen for drugs that can ameliorate the disease phenotypes associated with them.

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**Using Induced Pluripotent Stem Cells to Change Urine into Kidney Tissue**
*Kezia Caroline Philip, ,*
*Mary Gates Scholar*
*Mentor: Benjamin Freedman, Medicine/Nephrology*
*Mentor: Nelly Cruz, Medicine*
*Mentor: Ramila Gulieva, Nephrology*

Approximately 14% of the United States population is currently living with chronic kidney disease (CKD), demonstrating the importance of developing effective treatments. Presently, the mechanisms and driving forces of the many diseases that comprise the spectrum of CKD are not well understood. Current methods for acquiring kidney cells for research purposes require an invasive biopsy taken directly from the kidney. We theorized that patient kidney cells can be acquired from a significantly less invasive urine sample, be expanded, and subsequently differentiated into new kidney tissues. We developed a method for accomplishing this, which enabled the collection of cells from the urine samples of over 40 different patients located across the globe with various subtypes of CKD. Reverse transcription polymerase chain reactions (RT-PCRs) probing for the expression of XIST, a RNA gene exclusively expressed in females, were conducted on urinary cells from patients who have had kidney transplants with donors of the opposite gender. Using this assay, we show that these urinary cells originated in the

donor kidney. By introducing a set of transcription factors expressed in embryonic stem cells, urinary cells were further reprogrammed to induced pluripotent stem cells (iPSCs), an undifferentiated, stem cell-like state. Subsequently, the iPSCs were differentiated into patient-specific kidney organoids, marking the first time that new kidney-like structures have been generated from a urine sample. Hollow tubules, which better mimic the architecture of the kidney, were created by incorporating these urinary cells or iPSC-derived kidney organoid cells into three-dimensional microfluidic devices wherein normal cell viability and morphology was maintained up to twenty days. Immunohistological stainings indicate similar protein expression between the cell types, which will be further investigated in the upcoming months. These studies improve our ability to regenerate kidney tissue and disease processes from patients' own bodies, starting with a simple urine sample.

## POSTER SESSION 2

**Commons East, Easel 49**

*1:00 PM to 2:30 PM*

**Investigating the Cyst-Inducing/-Inhibiting Effects of Drugs on Kidney Organoids Differentiated from PKD Mutant Human Pluripotent Stem Cells**

*Kosuke Winston, ,*
*Mary Gates Scholar*
*Mentor: Benjamin Freedman, Medicine/Nephrology*
*Mentor: Nelly Cruz, Medicine*

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder in which fluid-filled cysts form in the kidneys and other organs. Mutations in the PKD1 and PKD2 genes, encoding the polycystin-1 (PC1) and polycystin-2 (PC2) proteins respectively, result in ADPKD. The underlying mechanisms that connect these mutations to ADPKD pathogenesis are poorly understood. Therefore, there is a great need to understand the mechanism of this disease and find an effective treatment. Our laboratory has developed an ADPKD cellular model by differentiating human pluripotent stem cells (hPSC) with mutations in PKD1 or PKD2 into kidney organoids. We are using this model to screen drugs that potentially induce or inhibit cyst formation in a dish. The cyclic AMP signaling pathway is hypothesized to contribute to cystogenesis in PKD. To test this, PKD mutant hPSC lines and isogenic controls were differentiated into kidney organoids and treated with forskolin, a strong cAMP agonist. Images of live organoids in adherent cultures were acquired in intervals and quantified for cyst formation using the cell counter feature of ImageJ. Organoids were subsequently fixed, stained, and imaged with fluorescent microscopy. Forskolin, induced a rapid and dose-dependent swelling in kidney organoids derived from both PKD hPSCs and isogenic controls. After removal of the drug, the tubules returned to its original form. These studies show that chemical modifiers of cystogenesis can be successfully tested using this system. Surprisingly, cyclic AMP does not appear to have effects that are specific to PKD. We are currently testing other drugs and culture conditions for cyst-inducing or –inhibiting effects. Interestingly, PKD kidney organoids form cysts in suspension cultures at significant higher rates than adherent cultures, revealing a role for the microenvironment in cystogenesis. These findings help us gain insight into the disease pathophysiology, which will ultimately guide us to discoveries of better treatments for PKD.

## POSTER SESSION 2

**MGH 241, Easel 143**

*1:00 PM to 2:30 PM*

**Malaria: The Switching Signals from Asexual Merozoites Infected Blood Cells to the Sexual Gametocyte Stage in *Plasmodium***

*Brenda Janet Robles, ,*
*Mary Gates Scholar*
*Mentor: Nelly Camargo, Kappe Laboratory, Center for Infectious Disease Research*

According to the World Health Organization, Malaria is a disease that affected nearly 216 million people worldwide and caused 445,000 deaths in 2016. The disease is a Global Health concern that mainly affects young children and pregnant women in the subtropical and tropical poor regions, such as Africa and South America. My internship at the Center for Infectious Disease Research gave me an opportunity to learn more about the *Plasmodium* parasites that cause malaria and the research behind developing a cure. I learned to evaluate *Plasmodium* blood stages with light microscopy and to evaluate the morphological difference between the sexual and asexual stages of the parasite life cycle as well as to put this in context with the disease transmission and symptoms. I also evaluated morphological differences between rodent and human parasites. The female Anopheles mosquito is the vector that transmits *Plasmodium* Sporozoites into the host's bloodstream, where the parasite starts a journey to the liver. After reaching the liver, the Sporozoite starts a process of transformation into liver stage forms which eventually matures into a Schizont and releases Merozoites that will return to the host's bloodstream. In the bloodstream, the parasite invades red blood cells through asexual replication, the Merozoites amplify and continue to invade erythrocytes which result in the illness symptoms of malaria. Some of these parasites get a switching signal and become gametocytes, the transmission stages of the parasite. The mosquito is able to acquire the gametocytes during a blood meal. On this project, we will look into the transcript levels of a few genes at the stage of gametocyte by qPCR analysis.