ATS 2024 Highlights

Respiratory Structure and Function Early Career Professionals

Get to know members of the RSF Assembly

Daniel J. Wallman, MD

(He, Him, His)
Assistant Professor
Pulmonary, Allergy, Sleep and Critical Care
Center for Regenerative Medicine
Boston University and Boston Medical Center
dwallman@bu.edu

Is your research clinical, basic science or translational?

Basic Science and Translational

Tell us about your research?

At the Center for Regenerative Medicine (CReM) I use the induced pluripotent stem cell (iPSC) and induced basal cell (iBC) platforms, coupled with gene editing techniques, to map genotype-to-phenotype of a variants of uncertain significance (VUS) in genes implicated in primary ciliary dyskinesia (PCD) and study ciliogenesis. Through this I aim to develop cell-based gene therapy for PCD.

Where do you see yourself in 5 years?

As a physician scientist at Boston University and Boston Medical Center I have the unique opportunity to translate my research to clinical care. In 5-10 years, I hope to be treating patients with PCD and other monogenic lung disease in a specialized pulmonary clinic with cutting edge gene and cell-based therapy.

What do you find is the major benefit of RSF Assembly Membership?

The opportunity to engage and network with other individuals/groups who are engaged in similar or near-similar research.

ATS

Please follow us on LinkedIn and X @ATS_RSF

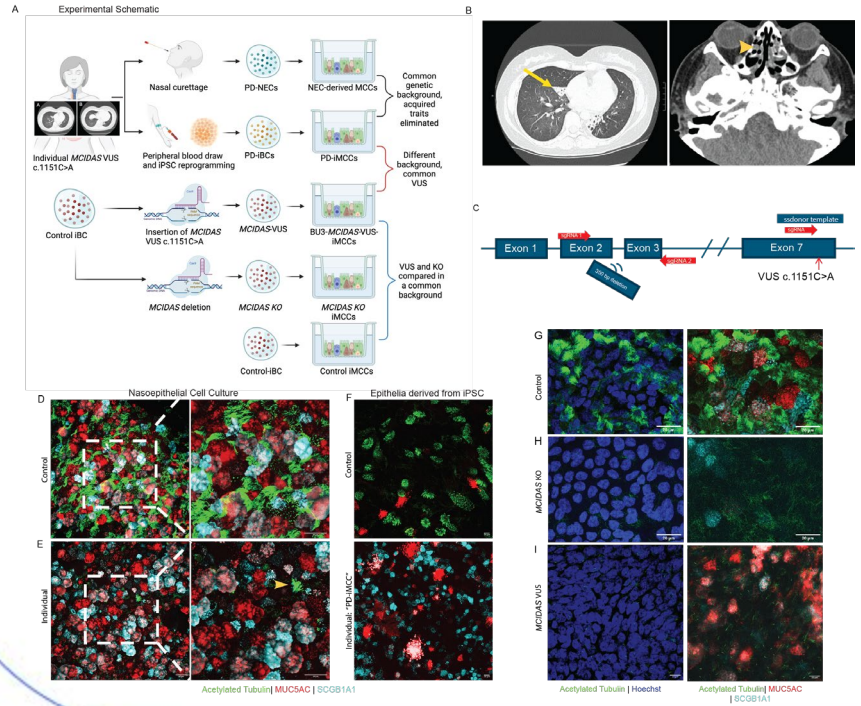
If you or someone you know would like to be featured as an ATS RSF ECP please email Carolyn Wang (carolyn.wang@hli.ubc.ca)

ATS 2024 Highlights

Respiratory Structure and Function Early Career Professionals

Daniel J. Wallman, MD

(He, Him, His)
Assistant Professor
Pulmonary, Allergy, Sleep and Critical Care
Boston University/CReM



Genotype-phenotype assessment of a variant of unknown significance in Multicillin using CRISPR/Cas9 precision mutagenesis in induced basal cells for rapid in vitro assessment of Primary Ciliary Dyskinesia

Objective: Thousands of variants of unknown significance (VUS) have been identified in genes known to cause primary ciliary dyskinesia (PCD) and confirmatory testing is currently a bottleneck. We aim to develop a high-throughput pipeline using the human induced basal cell (iBC) system to determine the pathogenicity of VUS implicated in PCD. Here, we exemplify our progress in using CRISPR-Cas9 to map the genotype-to-phenotype of a VUS in *Multicillin* (*MCIDAS*) in iBCs.

Methods: An individual with chronic cough, bronchiectasis, sinusitis and testing suggestive of PCD harbors a *MCIDAS* VUS c.1151C>A(p.Pro384His). We use CRISPR-Cas9 to perform knock out and precision mutagenesis in *MCIDAS* to insert the VUS c.1151C>A in iBCs, differentiate multiciliated cells (iMCCs) from edited iBCs using standard ALI culture techniques and perform phenotypic assessments. We validate our process against MCCs generated from a) induced pluripotent stem cells (iPSC) derived from the individual and b) their nasoepithelial cells.

Results: Sanger sequencing demonstrates successful VUS insertion. RT-qPCR was performed to assess for expression of markers of ciliogenesis (i.e. *MCIDAS*, *FOXJI*, *DNAH5*) and mucociliary differentiation (*MUC5AC*, *SCGB1A1*). Immunostaining of MCC markers demonstrate an attenuated MCC program (see figure).

Conclusion: VUS c.1151C>A was successfully inserted into human iBCs and functional pathogenicity of a VUS is determined. Gene editing of iBCs provides a rapid genotype-to-phenotype assessment that can provide insight to missense mutations in cases of suspected ciliopathies while eliminating laborious and time intensive iPSC reprogramming and differentiation processes.

Legend - A: Schematic of methods. **B:** CT Chest and Sinus demonstrating bronchiectasis (Arrow) and sinusitis (arrow head). **C:** Gene editing schematic. **D&E:** Human nasoepithelial cell culture depicting normal ciliogenesis from a control sample (**D**) and decreased multiciliated cells from the individual's cells (**E**). **F: E**pithelia derived from "control" iPSCs demonstrating multiciliated cells vs iPSCs derived from the individual "PD-iPSCs" devoid of multiciliated cells. (**G-I**) Epithelia derived from control iBCs (**G**), *MCIDAS* knockout iBCs **(H),** and iBCs with the VUS inserted (**I**) demonstrating an attenuated multiciliated cell program.

ATS

Please follow us on LinkedIn and X @ATS_RSF

If you or someone you know would like to be featured as an ATS RSF ECP please email Carolyn Wang (carolyn.wang@hli.ubc.ca)