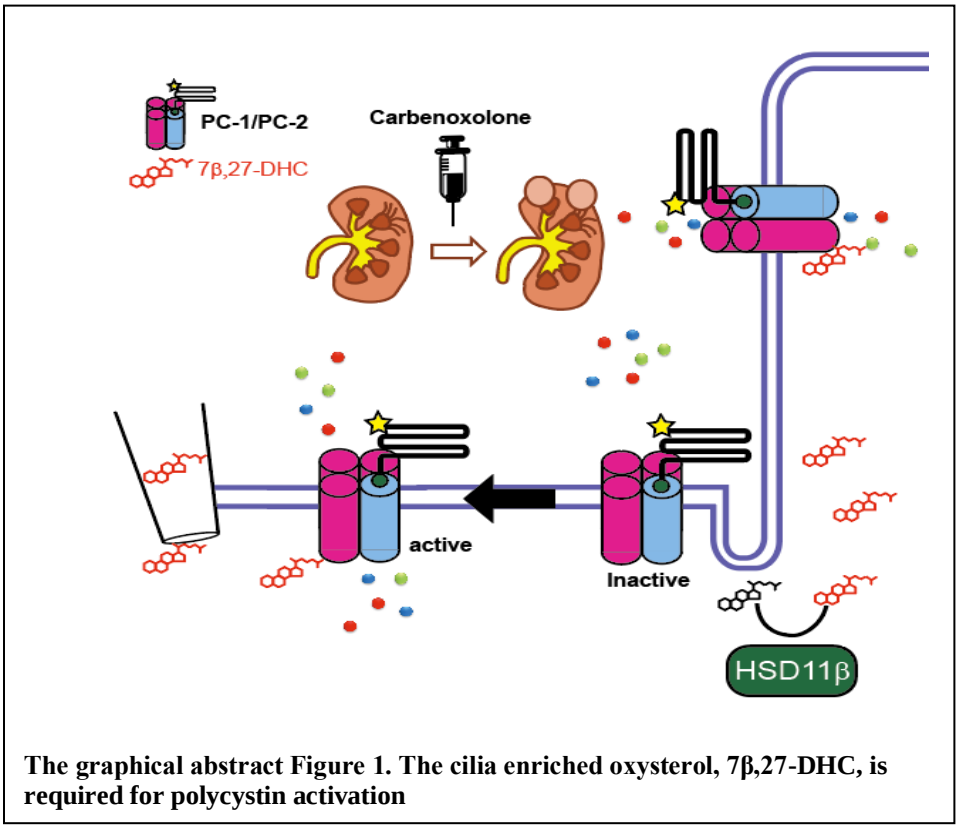
The cilia enriched oxysterol, 7β,27-DHC, is required for polycystin activation

Kotdaji Ha, Ph.D;[1] Nadine Mundt, Ph.D[1], Paola Bisignano, Ph.D[2], Aide Pinedo, B.S[1], Erhu Cao, Ph.D[3], Jeremy F. Reiter, M.D,Ph.D[4,5], David Raleigh, M.D.,Ph.D[6], and Markus Delling, Ph.D[1]

[1]Department of Physiology, University of California San Francisco, San Francisco CA, USA; [2]Lawrence Livermore National Laboratory, Bioscience and Biotechnology Division, CA, USA;[3]Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT, USA;[4]Department of Biochemistry and Biophysics, Cardiovascular Research Institute, University of California San Francisco, San Francisco CA, USA;[5]Chen Zuckerberg Biohub, San Francisco CA, USA;[6]Department of Radiation Oncology, University of California San Francisco, San Francisco CA, USA



The graphical abstract Figure 1. The cilia enriched oxysterol, 7β,27-DHC, is required for polycystin activation

PC-1 and PC-2 form a heteromeric ion channel complex (hereafter called the Polycystin complex) that is abundantly expressed on primary cilia of renal epithelial cells. Mutations within the polycystin complex cause Autosomal Dominant Polycystic Kidney Disease (ADPKD). The Polycystin complex forms a non-selective cation channel, yet the spatial and temporal regulation of the polycystin complex within the ciliary membrane remains poorly understood, partially due to technical limitations posed by the tiny ciliary compartment. Here, we employ our novel assays to functionally reconstitute the polycystin complex in the plasma membrane. Using whole-cell and ciliary patch-clamp recordings we identified a ciliary enriched oxysterol, 7β,27-DHC, as a critical component required for activation of the polycystin complex. We identified a novel oxysterol binding pocket in PC-2 using molecular docking simulation. We also identified two amino acids within the PC-2 oxysterol binding pocket, E208 and R581, to be critical for 7β,27-DHC dependent polycystin activation in both the plasma membrane and ciliary compartment. Further, we can show that the pharmacological and genetic inhibition of oxysterol synthesis by carbenoxolone (CNX) reduces channel activity in primary cilia. Our findings identified a unique second messenger that regulates the polycystin complex. We hypothesize that cilia-enriched lipids license the polycystin complex to be functional only in the ciliary organelle, thus providing novel insights into the spatial regulation of the polycystin complex. Our results also establish a framework to target the same allosteric regulatory site in the polycystin complex to identify activators of the polycystin channels as novel therapeutic strategies for ADPKD.

This work was supported by National Institute of Health Grant R01DK127277 (MD and EC), National Research Foundation of Korea (NTF) grant funded by the Korean government (MSIT) (No.2019R1A6A3A03033302) (KH) and PKD Foundation (A137178) (KH). A portion of this work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 (PB).