Genetic blueprint revealed for kidney design and formation

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Researchers have generated the first comprehensive genetic blueprint of a forming mammalian organ, shedding light on the genetic and molecular dynamics of kidney development.

Part of an international consortium sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), a research team led by Cincinnati Children's Hospital Medical Center reports the creation of a detailed genome-based atlas for understanding healthy and abnormal kidney development and disease. Published in the Nov. 11 *Developmental Cell* and featured on the journal's cover, the research provides a molecular genetic map detailing "gene expression analysis of all the major elements of kidney formation," according to the investigators.

The study, involving embryonic mice, shows how the entire genome is regulated to produce thousands of specific genes that are mixed and re-mixed to form genetic teams. The teams work together to direct formation of 15 embryonic compartments in the developing kidney – from the earliest phases when stem cells are told how to differentiate into specific kidney cells to the development of nephrons, the kidney's primary functioning unit.

"This study establishes a baseline for what changing gene expression looks like in a normal developing kidney in a very global way," said Steven Potter, Ph.D., a researcher in the division of Developmental Biology at Cincinnati Children's and the study's senior author. "Now we have molecular insights that will allow us to understand specific interactions throughout all stages of kidney development."

Dr. Potter explained this will let researchers analyze kidney abnormalities in mutant mice "in a much more complete and profound way than ever before. Given the mouse's genetic similarities with people, this should help us understand the underpinnings of human disease," he said.

The researchers conducted their multi-step analysis of mouse embryonic kidneys that were aged 15.5 days. This developmental time point in a mouse's normal 19- to 21-day gestation allows multiple stages of kidney formation to be studied at once because of how the organ develops. The organ's outer layers contain early stem cells that are still differentiating to become specific cell types, while inside the organ structures are forming at intermediate and more mature stages. This enabled measurement of varied gene expression stage-by-stage, compartment by compartment, the researchers said.

One of the study's more unexpected discoveries is overlapping gene expression between the kidney's different structures, according to Eric Brunskill, Ph.D., the study's lead author. Most of the thousands of genes involved in making a mammalian kidney are expressed at some level in every compartment. Previously it had been thought each kidney compartment would have unique genes driving its development, and those genes would not be expressed in the cells of other structures. This is not the case, as the research team found only a small number of genes expressed exclusively in specific kidney structures.

"Instead of it being a digital on-off pattern, where you might have many unique genes expressed in one part of the kidney but not in the other structures, we instead see a more analog picture, where almost all of the genes are expressed in the different parts but at varied levels," Dr. Brunskill said.

Helping make this discovery possible is the study's use of microarray technology to measure relative expression levels of every gene in each unique structure and developmental stage. Combined with two other technologies to precisely isolate specific types of cell populations (laser capture micro-

dissection and florescent activated cell sorting), microarray analysis allowed a more quantitative and sensitive measure of varied gene expression than ever observed in a developing organ system.

Computational biology analysis (bioinformatics) then let the researchers see how different sets of genes cooperate through circuits or pathways, some already defined and others defined in this study for the first time. The genes cooperate by signaling each other, telling cells when to grow, when to make tubes, when to turn on pumps or perform other critical functions, said Bruce Aronow, Ph.D., study co-author and scientific director of the Center for Computational Medicine at Cincinnati Children's. The study also makes new headway into identifying different transcription factors – "boss" genes that regulate the activity of other genes – and the target genes they may activate or repress.

Given that about one in every 500 births results in a kidney development abnormality, this provides insight into genetic programs that are critical to deciding how kidney stem cells form structures in the adult kidney. The researchers identified genes that regulate DNA transcription, establish functioning developmental processes, and are involved in pattern specification, cell differentiation and organ compartment shaping.

Source: Cincinnati Children's Hospital Medical Center