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GENETICS

# Genetic and functional studies reveal genetic drivers of renal disease in DiGeorge syndrome

DiGeorge syndrome is a multisystemic disease that can affect the heart, endocrine, nervous and immune systems; congenital anomalies of the kidney and urinary tract (CAKUT) are found in about one-third of patients. The syndrome is caused by deletions on chromosome 22q11.2 but the underlying genetic drivers of kidney defects are unknown. New research has identified three genes — *SNAP29*, *AIFM3* and *CRKL* — within a 370 kb region of 22q11.2 as being crucial to the renal phenotype of DiGeorge syndrome. "Our study provides strong evidence for a critical role of the distal portion of the 22q11.2 DiGeorge locus as the main variant conferring risk of CAKUT and, within this region, of *CRKL* as the main single driver," says Simone Sanna-Cherchi.

Sanna-Cherchi explains that his research has traditionally focused on the identification of genetic variants with large effect size that predispose children to kidney disease, especially CAKUT. "Genetic determination of CAKUT is complex, involving copy number variations (CNVs) as well as point mutations, and ultimate proof of causality requires functional modelling in relevant animal systems," he says. "Nicholas Katsanis's work has been at the forefront of developing and validating assays to enable the functional interpretation of genetic variants in zebrafish in a systematic fashion. It was clear that combining our expertise would lead to advancement in the field of paediatric genetic research and nephrology."

The researchers conducted a genome-wide search for CNVs in 2,080 affected children and 22,094 controls and identified deletions at the chromosome 22q11.2 locus in 1.1% of patients and 0.01% of controls. "This study identified a minimal deleted region of just 370 kb containing nine genes, suggesting that the kidney defects observed in patients with DiGeorge syndrome were attributable to at least one of these genes," says Sanna-Cherchi. "Identification of genetic drivers of CNV phenotypes has been traditionally very challenging. Here we conducted systematic knockdown and knockout experiments on all tractable candidate genes in zebrafish and used convolution of the proximal pronephric tubule as a readout of kidney malformations." Suppression of *crkl*, *aifm3* and *snap29* expression led to severe convolution defects; although these defects could be induced by inactivation of *crkl* alone, co-suppression of *snap20* and *aifm3* was required to induce the same phenotype. "Consistent with these data, resequencing of *CRKL* in over 500 patients with kidney agenesis or hypodysplasia identified rare point mutations, implicating this gene as the main driver of the kidney defects observed in patients with DiGeorge syndrome and as a cause of CAKUT in the general population," says Sanna-Cherchi. Finally, the researchers showed that inactivation of *Crkl* in mice led to developmental defects similar to those observed in patients with CAKUT.

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Sanna-Cherchi notes that their findings open several avenues for further research. "First, the identification of these novel variants augments our diagnostic repertoire, and their diagnostic and prognostic value can now be tested in extended populations. Second, the identification of these variants represents a critical step in understanding the genetic basis of congenital kidney defects associated with DiGeorge syndrome, which will facilitate the development of diagnostic and therapeutic tools. Third, as DiGeorge syndrome is characterized by a variety of neurocognitive problems, we plan to determine whether *CRKL* gene function is critical for brain development as well as kidney and urinary tract development." The researchers also emphasize that their study represents an example of how the use of large-scale genomics, coupled with functional modelling in vertebrates, can aid study of the genetic architecture of complex genetic diseases. "As we know, CNVs are more frequent mutagens than are point mutations," notes Katsanis. "As such, learning to dissect causality in CNVs and to start probing genetic interactions of genes inside CNVs will be of exceptional importance, especially as we transition from whole exome sequencing — which often fails to detect CNVs — to whole genome sequencing.

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