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GLOMERULAR DISEASE

Novel candidate genes implicated in FSGS

Genetic mutations in podocyte genes can increase susceptibility to focal segmental glomerulosclerosis (FSGS), but only a small proportion of patients have been identified who have a clear genetic basis underlying their disease. A new study from Haiyang Yu and colleagues describes the development of a pipeline to detect FSGS susceptibility genes. Nine FSGS candidate genes were identified by next-generation sequencing and three were validated *in vivo* using a novel high-throughput method.

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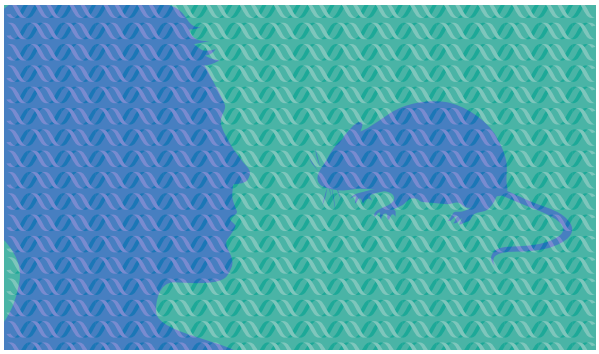

Lara Crow/NPG

The researchers performed DNA sequencing of 2,500 genes that are highly and/or specifically expressed in podocytes, in 214 unrelated individuals of Northern European ancestry with biopsy-confirmed sporadic or familial FSGS. They found missense variants in *WNK4, KANK1,* and *ARHGEF17* by single variant analyses. *XYLT1, KAT2B, BPTF, DLG5, WNK4,* and *GC1* were identified as potential FSGS susceptibility genes by rare variant analyses. Enrichment was also found in two known FSGS susceptibility genes: *APOL1* and *COL4A4*.

Next, a high-throughput method was designed to validate the candidate genes. An embryonic stem (ES) cell line was generated from FSGS-susceptible *Cd2ap*$^{+/-}$*; Synpo*$^{+/-}$ mice, which was engineered to express a podocyte-specific doxycycline (DOX)-inducible transactivator. *WNK4, KANK1, ARHGEF17, DLG5,* and *KAT2B* were knocked down in the ES cells using shRNAs targeted specifically to the *Hprt* locus. *KANK2* was also targeted by shRNA, as the precise mouse orthologue for *KANK1* is unknown. Upon verification of shRNA targeting *in vitro*, mice were generated by laser-assisted microinjection of the ES cells into eight-cell embryos. Consequently, the mice were almost 100% derived from the shRNA-targeted ES cells without the need for further breeding.

Proteinuria was assessed in the animals 4–8 weeks after treatment with DOX. Substantial proteinuria was identified in the *Wnk4, Arhgef17,* and *Kank2* mice by 8 weeks, but not in the *Kank1* mice until 12 weeks; podocyte foot process effacement was also observed. No effect on renal function was detected in the *Dlg5* animals.

The researchers conclude that their data support "a broader role for genetic susceptibility of both sporadic and familial cases of FSGS". They propose that their methodology allows for rapid evaluation of candidate disease genes, and that this pipeline could be applied to other common or rare diseases.

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