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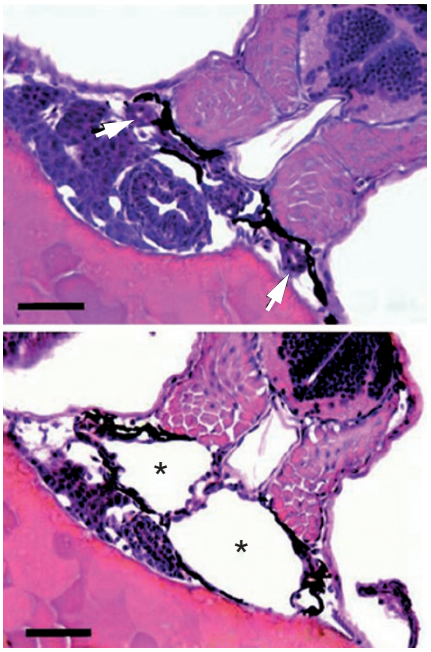
GENETICS

FAN1 mutations result in defective DNA damage repair and karyomegalic interstitial nephritis

Mutations in *FAN1* lead to a defective DNA damage response (DDR) and karyomegalic interstitial nephritis (KIN), according to new research. "Our findings implicate susceptibility to environmental genotoxins and inadequate DNA repair as novel mechanisms contributing to renal fibrosis and [chronic kidney disease]", state the researchers.

To identify causative genes for nephronophthisis (NPHP)-like ciliopathies, Weibin Zhou, Edgar Otto *et al.* examined two affected siblings of New Zealand Maori descent. Renal histology demonstrated the presence of enlarged nuclei (karyomegaly), leading to a diagnosis of KIN—a rare NPHP-like fibrotic kidney disease. The researchers performed homozygosity mapping and exome sequencing, revealing a homozygous nonsense mutation in *FAN1*, which encodes Fanconi anemia-associated nuclease 1. Zhou *et al.* then sequenced *FAN1* exons in DNA samples obtained from 10 families with KIN. They identified 12 different *FAN1* mutations in nine of the 10 families; these mutations were absent in 96 healthy controls, thereby identifying recessive mutations in *FAN1* as an important cause of KIN.

The FAN1 protein acts in a DNA repair pathway that is involved in the repair of interstrand cross-link (ICL) damage. To investigate the effect of the *FAN1* mutation on genome maintenance and DDR, the researchers in collaboration with Agata Smogorzewska, studied the response of *FAN1*-mutant cells to the ICL-inducing agents mitomycin C and diepoxybutane. Upon exposure of primary fibroblasts and lymphoblastoid cell lines from individuals with *FAN1* mutations to mitomycin C, chromatid breaks and radial chromosomes on metaphase spreads were observed, consistent with a role of FAN1 in DDR. Both mitomycin C and diepoxybutane reduced the survival of *FAN1*-mutant cells, demonstrating that *FAN1* deficiency causes ICL sensitivity.



Zebrafish embryos with knockdown of *p53* alone at 72 h postfertilization show normal pronephric tubules (arrowheads, upper panel) whereas knockdown of both *fan1* and *p53* at 72 h postfertilization results in pronephric kidney cysts (asterisks, lower panel). Nature Publishing Group © Zhou, W. *et al. Nat. Genet.* doi:10.1038/ng.2347.

To repair the ICL sensitivity defect, the researchers transduced fibroblasts from one of the affected siblings with either wild-type *FAN1* cDNA or with *FAN1* cDNA harbouring KIN-associated mutations. Wild-type *FAN1* rescued the mitomycin C sensitivity defect; however, none of the cDNAs carrying mutations from individuals with KIN complemented the defect, with the exception of one variant that partially rescued cell survival with exposure to mitomycin C, suggesting that this particular variant represents a hypomorphic allele of *FAN1*. Transduction with a cDNA carrying a mutation known to inhibit interaction of FAN1 with FANCD2, necessary for recruitment of FAN1 to sites of ICL damage via the Fanconi anaemia pathway, fully rescued the mitomycin C sensitivity defect. The researchers believe that this finding suggests that FAN1 has the ability to act independently of the Fanconi anaemia pathway. In support of this theory, the researchers found that depletion of *FANCD2* in *FAN1*-mutant cells resulted in a worsening of the mitomycin C sensitivity defect, suggesting additive pathways.

Most DDR pathways and NPHP-like ciliopathies are conserved in zebrafish; therefore, the researchers performed morpholino oligonucleotide knockdown of *fan1* in zebrafish embryos. Knockdown of *fan1* resulted in NPHP-like and DDR phenotypes, characterized by a shortened body axis, microcephaly, microphthalmia and massive apoptotic cell death. Zhou *et al.* found increased levels of γH2AX, indicative of activated DDR signalling. Suppressing apoptosis by knocking down p53 function revealed phenotypes characteristic of NPHP-like ciliopathies, including pronephric renal cysts. "Loss of *fan1* function results in ciliopathy-related phenotypes, which are revealed when p53 function is inhibited", the authors state.

The researchers also examined γH2AX levels in a small sample of human biopsy samples from kidney transplant recipients. Levels of γH2AX were increased in samples with histological evidence of kidney damage, suggesting that DDR might contribute to renal damage in adults with chronic kidney disease as well as in individuals with NPHP-like ciliopathies.

Zhou *et al.* say that their study raises the question of whether individuals who carry hypomorphic alleles of *FAN1* rather than two null alleles might develop renal failure later in life. "It will be important to examine a potential role for ICL-causing genotoxins in the absolute and relative increase in end-stage kidney disease", they explain.

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Original article Zhou, W. *et al. FAN1* mutations cause karyomegalic interstitial nephritis, linking chronic kidney failure to defective DNA damage repair. *Nat. Genet.* doi:10.1038/ng.2347