Unraveling disease mechanisms in ciliopathies

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Ciliopathies represent a group of rare and severe genetic disorders caused by serious ciliary dysfunction. These diseases are individually rare, but they occur frequently in a collective sense, affecting 1 in 1000 births. Cilia are sensory antennae that play a key role in signaling mechanosensation and chemosensation at the surface of most mammalian cells. Impairment of ciliary function can lead to various clinical manifestations, one of the main ones is kidney damage, generally leading to end-stage kidney disease (ESKD) in patients. The spectrum of renal ciliopathies includes ADPKD (autosomal dominant polycystic kidney disease) in adults or ARPKD (autosomal recessive polycystic kidney disease), renal cystic dysplasia, and nephronophthisis (NPHP) in pediatric patients. NPHP is a chronic tubulointerstitial nephropathy, autosomal recessive by nature, and is generally characterized by polyuria and polydipsia, smaller kidneys, and massive interstitial fibrosis. It is associated with corticomedullary cysts in the later stage of the disease, leading to end-stage renal disease (ESRD) at a median age of 13 years old. To date, more than 23 genes have been linked with NPHP, the most frequent being *NPHP1* with nearly 25% of affected individuals harboring a homozygous deletion of this gene. Researchers have gathered a large cohort of patients with NPHP, with genetic analysis identifying the variants responsible for the disease in 600 affected individuals, amounting to 70% of overall ESKD subjects. The main gene involved in this disorder is NPHP1, which encodes a protein that is localized to the cilia, and all compartments of the cilia are affected.

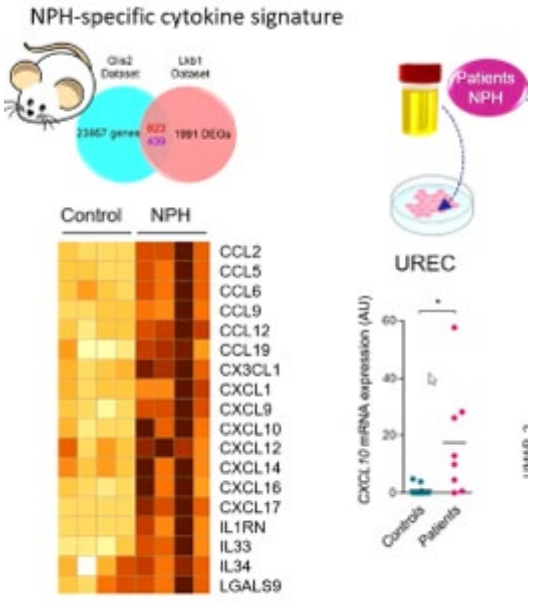

Figure 1. Exploring renal inflammation in nephronophthisis

The objective of the laboratory research was to try to decipher the key signaling pathways that occur through cilia to find a common pathological mechanism that can be targeted in these patients. To investigate this matter further, researchers analyzed two mouse models of nephronophthisis and

identified a specific cytokine signature that was abnormally up-regulated at the transcriptomic level. This signature was also present in urinary-derived renal epithelial cells (UREC) from patients with nephronophthisis. Clinicians and researchers believe that this abnormal cytokine expression would recruit immune cells in the kidney, leading to inflammation and fibrosis. Finally, the kidney biopsies from patients with nephronophthisis were compared to controls and CKD biopsies with the same lesion, and recruitment of macrophage T-cells and neutrophils was observed, which is specific to nephronophthisis.

Research is focused on compounds that could rescue phenotypes in renal cell lines deficient for NPHP1, such as increased cell migration and defects in ciliogenesis.

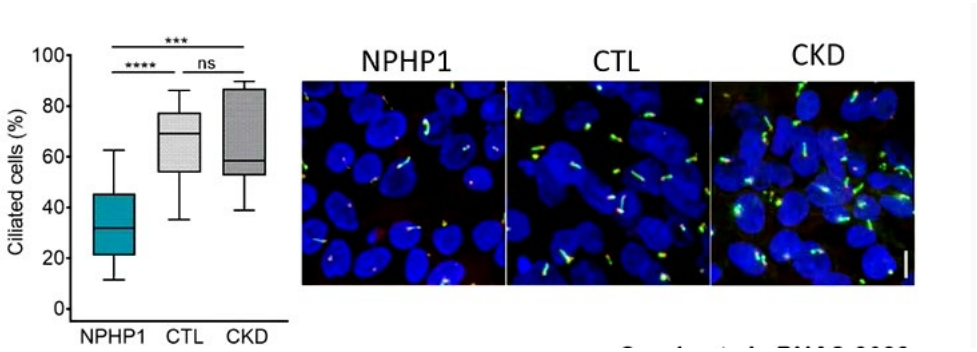

Figure 2. Defect in ciliogenesis in NPHP1 URECs

Through a model of immortalized UREC renal cells, it was found that one compound in particular, Alprostadil or PGE1, an analog of prostaglandin E2, was able to rescue ciliogenesis in URECs from patients with *NPHP1* variants to levels similar to those observed in control cells. Interestingly, a specific agonist of the EP2 PGE2 receptor showed a similar rescue of ciliogenesis in URECs from patients with *NPHP1*. Research also found that PGE1 was able to partially rescue defects in ciliary composition, such as the presence of adenylyl cyclase 3, in URECs from patients with *NPHP1*. It was investigated whether the positive effect of PGE1 on ciliogenesis was mediated via the cAMP and protein kinase A (PKA) pathway. It was found that cAMP/ protein kinase A (PKA) activation by the adenylyl cyclase activator forskolin mimics PGE1 treatment. To further understand the role of this pathway in ciliogenesis, the effect of PGE1 treatment on p27kip1, a key positive regulator of cellular quiescence and ciliogenesis was examined. Results showed that p27kip1 expression was reduced in NPHP1 cells compared to controls, and treatment with PGE1 restored p27kip1 expression. Another pathway important for cilia elongation and ciliogenesis is the regulation of RhoA which increased activity inhibiting ciliogenesis. Interestingly, p27kip1 is known to inhibit RhoA activation, and it was confirmed that this pathway was downregulated in *NPHP1* cells treated with PGE1. This suggests that prostaglandins may work to rescue ciliogenesis in *NPHP1* cells by downregulation the RhoA pathway partly through the stimulation of p27kip1 which also induces cellular quiescence and ciliogenesis by other means. A large-scale RNA transcriptomic analysis was conducted to further investigate the effect of prostaglandins in *NPHP1* conditions. Comparative transcriptomic analysis of *NPHP1* and control URECs was achieved to identify dysregulated gene expression. *NPHP1* URECs with and without treatment were also analyzed, resulting in the identification of 128 dysregulated genes. Enrichment analysis was performed to identify key processes and signaling pathways modulated by prostaglandins. These included actin cytoskeleton

organization, extracellular matrix-receptor interaction, and focal adhesion. A downregulation of fatty acid oxidation was also observed.

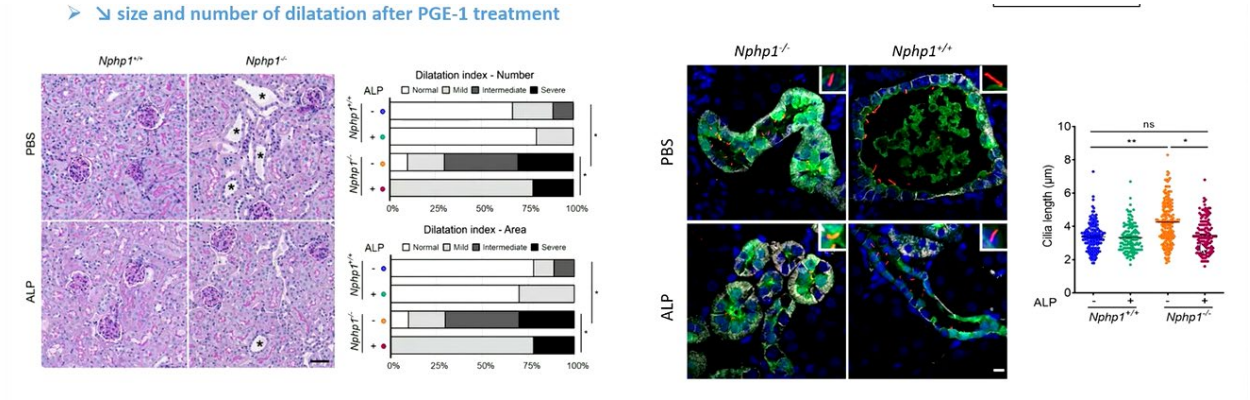

Figure 3. Impact of PGE-1 on kidney phenotypes in NPHP1 knockout mice

To validate the findings in vivo, *Nphp1* knockout mice were generated using a CRISPR/Cas9 system that resulted in a homozygous ATG deletion in exon 1. This resulted in the complete absence of the *Nphp1* transcripts and loss of the protein in the transition zone from cilia in the kidney tubular cells. There was evidence of renal lesions, including tubular dilatation in the distal part of the nephron, abnormal cilia length, and an abnormal signature of fibrosis in RNA profiling analyses. These lesions were consistent in two and five months old mice, and located in the distal part of the nephron, similar to what has been observed in humans. However, there were no observed alterations in kidney function. Another model for ciliopathy, the zebrafish larva with a primitive kidney, was also used. This model exhibited kidney lesions and ciliary defects. It was previously shown that the *nphp4* morpholino model (RNA interference) develop cystic lesions and that treatment with prostaglandins ameliorated these lesions. In the proximal tubules, the treatment also prevented cystic lesions. These results suggest that Prostaglandins may also prevent renal and ciliopathy-related phenotypes in other NPHP models.

Key points

1. From a cell-based screening of small molecules, a signaling pathway was identified, as well as a series of promising molecules for the treatment of nephronophthisis.
2. Prostaglandin E2 analogues are capable of restoring ciliogenesis and ciliary composition in renal tubular cells of *NPHP1* patients. They also have a beneficial effect on fibrotic gene expression and renal lesions in in vitro and in vivo models.
3. PGE1 and EP2 agonist may have an impact on UREC cells from patients with mutations in other NPH genes and other NPH mouse models

Further reading

(1) Garcia H, Serafin AS, Silbermann F, et al. Agonists of prostaglandin E2 receptors as potential first in class treatment for nephronophthisis and related ciliopathies. Proc Natl Acad Sci U S A. 2022;119(18):e2115960119. doi: 10.1073/pnas.2115960119

(2) Quatredeniers M, Bienaimé F, Ferri G, et al. The renal inflammatory network of nephronophthisis. *Hum Mol Genet*. 2022;31(13):2121-2136. doi:10.1093/hmg/ddac014

(3) Goetz SC, Anderson KV. The primary cilium: a signaling centre during vertebrate development. Nat Rev Genet. 2010;11(5):331-44. doi: 10.1038/nrg2774.

(4) Konya V, Marsche G, Schuligoi R, Heinemann A. E-type prostanoid receptor 4 (EP4) in disease and therapy. Pharmacol Ther. 2013;138(3):485-502. doi: 10.1016/j.pharmthera.2013.03.006.

(5) Yin H, Xue W, Chen S, et al. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol. 2014;32(6):551-3. doi: 10.1038/nbt.2884. Erratum in: Nat Biotechnol. 2014;32(9):952.