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P165 -Ankyrin Repeat and Single KH domain 1 (ANKHD1) is a novel driver of polycystic kidney disease in vivo.

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BACKGROUND: Autosomal Dominant polycystic kidney disease is a devastating disease, characterised by the progressive enlargement of bilateral renal cysts. Mutations in the Polycystic kidney disease 1 (Pkd1) gene are responsible for over 80% of ADPKD cases. How Pkd1 mutations lead to ADPKD is largely unknown, however several signalling abnormalities and increased proliferation are observed in ADPKD kidneys. The development of new therapies heavily relies on gaining a better understanding the mechanisms leading to cystic growth. Our group has recently shown that the JAK2/STAT5 signalling pathway is enhanced in ADPKD and its inhibition leads to reduced proliferation in murine and human models of ADPKD (Fragiadaki et al, 2017, Patera et al, 2019). To understand how the JAK/STAT pathway is regulated we performed a genome-wide RNA interference screen, identifying ankyrin repeat and single KH domain protein 1 (ANKHD1) as a central regulator of multiple components of the JAK/STAT pathway (Fisher, Fragiadaki et al, 2018). ANKHD1 has not been studied in the context of ADPKD previously. Given that ANKHD1 controls JAK/STAT activity, which is abnormally activated in ADPKD, we studied ANKHD1 in mouse and human models of ADPKD.

HYPOTHESIS & METHODS: We hypothesise that genetically reducing the expression of ANKHD1 will limit JAK/STAT activity, which will in turn improve renal function in mouse models of ADPKD in vivo. To test our hypothesis, we used mice with hypomorphic deletion of the Pkd1 gene (the Pkd1nl/nl model). We crossed Pkd1nl/nl mice with the ANKHD1 knockout mice to generate mice with and without deletion of the ANKHD1 locus in the Pkd1 background (Ankhd1KO;Pkd1nl/nl). To assess whether knocking out ANKHD1 affects ADPKD development, we estimated blood urea nitrogen, normalised renal size and cystic index. Expression and localisation of ANKHD1 was immunohistochemically studied. Proliferation was assessed by staining cells with ser10 histone H3, followed by flow cytometry or by cyclin D1 staining in mouse kidney tissues, followed by microscopy.

RESULTS: Firstly, widespread ANKHD1 expression was observed in both wild-type and polycystic kidneys, with stronger expression found in cystic tubules. To determine whether ANKHD1 contributes to cystogenesis in vivo, we deleted ANKHD1 in mice with polycystic kidney disease. Deletion of ANKHD1 led to a robust and significant improvement in renal function, assessed by renal volume, cystic index and blood urea nitrogen. These data strongly indicate that ANKHD1 is a driver of polycystic kidney disease. To understand how ANKHD1 drives cystic disease, we studied JAK/STAT activation and proliferation, since ANKHD1 regulates JAK/STAT activity in non-renal cells (Fisher, Fragiadaki et al, 2018). We found that mouse kidneys of animals lacking ANKHD1 had reduced JAK/STAT activity and lower proliferation rate in vivo. Critically, ANKHD1 siRNA led to markedly reduced proliferation in human ADPKD-derived cells, suggesting it is relevant to human disease.

CONCLUSION: We have provided conclusive evidence that ANKHD1 is a strong regulator of polycystic kidney disease by modulating JAK/STAT activity and proliferation of renal cells, these findings provide a platform for the discovery of novel therapeutics for patients with ADPKD.