Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease

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Autosomal dominant polycystic kidney disease (ADPKD) is a leading cause of end-stage renal disease. The vasopressin V2 receptor (VPV2R) antagonist OPC31260 has been effective in two animal models of PKD with pathologies that are probably related. Here we show, in a mouse model of ADPKD (*Pkd2*$^{-/tm1Som}$), a similar cellular phenotype and response to OPC31260 treatment, with reduction of renal cyclic AMP (cAMP) levels, prevention of renal enlargement, marked inhibition of cystogenesis and protection of renal function.

ADPKD is genetically heterogeneous, with two genes (*PKD1* and *PKD2*) expressing interacting polycystin proteins. The polycystin complex has a role in the regulation of intracellular calcium[1–3]. Increased cAMP levels, possibly related to altered intracellular calcium homeostasis[4], may underlie the proliferative and secretory phenotype of cystic epithelium[5–7]. OPC31260, an antagonist of VPV2R, the major cAMP agonist in the collecting duct, has recently been shown to be an effective therapy in two models orthologous to human autosomal recessive PKD (ARPKD; PCK rat) and nephronophthisis (*pcy* mouse)[8].

In this study, we tested the efficacy of OPC31260 in an animal model of ADPKD. The *Pkd*$^{-/tm1Som}$ mouse (orthologous to human *PKD2*) was selected because, unlike *Pkd1*$^{+/-}$ or *Pkd2*$^{+/-}$ models, it reliably develops renal cysts within 3 months and is most amenable to study[9]. Renal cAMP levels and expression of aquaporin-2 and VPV2R were higher in *Pkd2*$^{-/tm1Som}$ than in wild-type mice (**Fig. 1a**,**b**). As in ARPKD, the cysts in ADPKD patients are predominantly derived from the collecting duct[10], and a defect in urine concentration is one of its earliest manifestations[11]. *Pkd2*$^{-/tm1Som}$ renal cysts originate predominantly from the collecting duct and distal nephron, as reported previously[9]. We found that 52% of renal cysts stained positively for collecting duct markers[12], whereas only 3% derived from the thick ascending limb and none derived from the proximal tubule (**Fig. 1c–f**). The remaining cysts were negative for all markers, suggesting a degree of dedifferentiation.

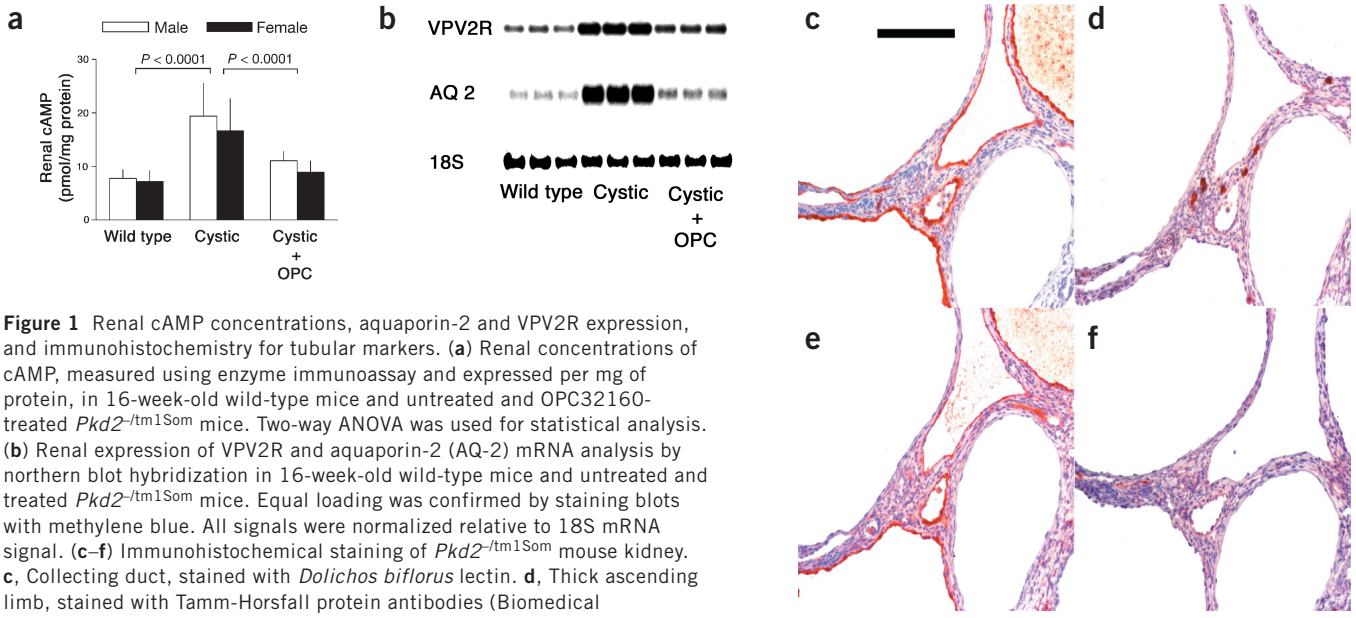

Figure 1 Renal cAMP concentrations, aquaporin-2 and VPV2R expression, and immunohistochemistry for tubular markers. (**a**) Renal concentrations of cAMP, measured using enzyme immunoassay and expressed per mg of protein, in 16-week-old wild-type mice and untreated and OPC32160-treated *Pkd2*$^{-/tm1Som}$ mice. Two-way ANOVA was used for statistical analysis. (**b**) Renal expression of VPV2R and aquaporin-2 (AQ-2) mRNA analysis by northern blot hybridization in 16-week-old wild-type mice and untreated and treated *Pkd2*$^{-/tm1Som}$ mice. Equal loading was confirmed by staining blots with methylene blue. All signals were normalized relative to 18S mRNA signal. (**c**–**f**) Immunohistochemical staining of *Pkd2*$^{-/tm1Som}$ mouse kidney. **c**, Collecting duct, stained with *Dolichos biflorus* lectin. **d**, Thick ascending limb, stained with Tamm-Horsfall protein antibodies (Biomedical Technologies). **e**, Collecting duct, stained with F13 antibodies (provided by E. Avner, Rainbow Babies and Children's Hospital & Case Western Reserve University). **f**, Proximal tubule, stained with antibody to lysozyme (BioGenex). Diameter cutoff of 200 μm was used to differentiate cysts from dilated tubules. Scale bar, 200 μm.

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Published online 29 February 2004; doi:10.1038/nm1004

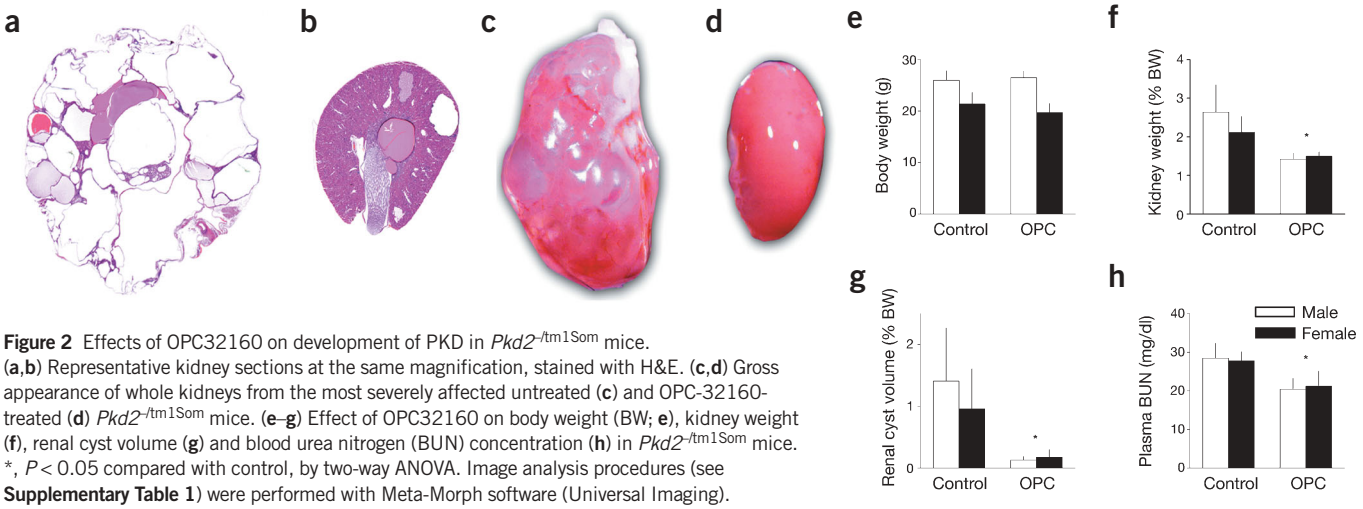

Figure 2 Effects of OPC32160 on development of PKD in *Pkd2*$^{-/tm1Som}$ mice. (**a**,**b**) Representative kidney sections at the same magnification, stained with H&E. (**c**,**d**) Gross appearance of whole kidneys from the most severely affected untreated (**c**) and OPC-32160-treated (**d**) *Pkd2*$^{-/tm1Som}$ mice. (**e**–**g**) Effect of OPC32160 on body weight (BW; **e**), kidney weight (**f**), renal cyst volume (**g**) and blood urea nitrogen (BUN) concentration (**h**) in *Pkd2*$^{-/tm1Som}$ mice. *, $P < 0.05$ compared with control, by two-way ANOVA. Image analysis procedures (see **Supplementary Table 1**) were performed with Meta-Morph software (Universal Imaging).

OPC31260 (0.05%), administered in the diet to *Pkd2*$^{-/tm1Som}$ mice between 3 and 16 weeks of age, markedly reduced the renal accumulation of cAMP and corrected the overexpression of aquaporin-2 and VPV2R (**Fig. 1a**,**b**). OPC31260 also markedly inhibited disease development, as reflected by lower kidney weights, plasma blood urea nitrogen concentrations, numbers of renal cysts and fibrosis volumes, and by mitotic and apoptotic indices (**Fig. 2** and **Supplementary Table 1** online). The kidney weights of treated *Pkd2*$^{-/tm1Som}$ mice were similar to those of wild-type mice ($1.4 \pm 0.2\%$ of body weight), indicating that renal enlargement was prevented. OPC31260 did not have a significant effect on polycystic liver disease or tail-cuff blood pressures. It was well tolerated and did not cause electrolyte abnormalities. Urine outputs and osmolalities in treated and untreated mice were similar, possibly because OPC31260's aquaretic effect was compensated for by its beneficial effect on the disease.

ADPKD is one of the most common life-threatening monogenic disorders, and causes 5% of end-stage renal disease in the United States. Recent studies have shown that cAMP has a central role in cystogenesis, stimulating fluid secretion in normal collecting ducts[5] and isolated ADPKD cysts[6]. Its effect on epithelial cell proliferation is more complex. Adenylyl cyclase agonists and 8Br-cAMP activate the ERK cascade and increase proliferation of ADPKD cells, while inhibiting proliferation of normal kidney cortex cells[7]. The mechanisms responsible for this phenotypic switch are unknown, but a similar change can be induced when collecting duct epithelial cells are treated with Ca^{2+} channel blockers, suggesting the importance of cross-talk between the Ca^{2+} and cAMP signaling pathways[13].

The ability of VPV2R antagonists to markedly slow disease progression in an animal model has shown their potential to delay the requirement for transplantation or dialysis in ADPKD patients, although the usefulness of such antagonists in PKD is yet to be proven. These drugs are attractive because of their apparent safety in preclinical and clinical studies, which can probably be explained by their renoselectivity[14]. Several VPV2R antagonists are in phase 3 efficacy and safety trials for hyponatremia and disorders of water retention, such as congestive heart failure and cirrhosis. Given the present lack of effective therapies and the apparent safety of VPV2R antagonists, clinical trials of these compounds in ADPKD seem appropriate.

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant DK44863 (V.E.T.) and by a grant from the Polycystic Kidney Disease Foundation (V.H.G.). OPC31260 was a gift from Otsuka Pharmaceutical. Technical assistance was provided by Ming Li.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Medicine* website for details).

Received 19 November 2003; accepted 30 January 2004
Published online at http://www.nature.com/naturemedicine/

1. Koulen, P. *et al. Nat. Cell Biol.* **4**, 191–197 (2002).
2. Nauli, S.M. *et al. Nat. Genet.* **33**, 129–137 (2003).
3. Qian, Q. *et al. Hum. Mol. Genet.* **12**, 1875–1880 (2003).
4. Chabardes, D., Imbert-Teboul, M. & Elalouf, J.M. *Cell. Signal.* **11**, 651–663 (1999).
5. Wallace, D.P., Rome, L.A., Sullivan, L.P. & Grantham, J.J. *Am. J. Physiol. Renal Physiol.* **280**, F1019–F1029 (2001).
6. Ye, M. & Grantham, J. *N. Engl. J. Med.* **329**, 310–313 (1993).
7. Yamaguchi, T. *et al. Kidney Int.* **57**, 1460–1471 (2000).
8. Gattone, V.H., Wang, X., Harris, P.C. & Torres, V.E. *Nat. Med.* **9**, 1323–1326 (2003).
9. Wu, G. *et al. Cell* **93**, 177–188 (1998).
10. Verani, R.R. & Silva, F.G. *Mod. Pathol.* **1**, 457–463 (1988).
11. Gabow, P. *et al. Kidney Int.* **35**, 675–680 (1989).
12. Sweeney, W.E., Jr. *et al. Am. J. Physiol. Cell Physiol.* **281**, C1695–C1705 (2001).
13. Yamaguchi, T., Wallace, D.P., Grantham, J.J. & Calvet, J.P. *J. Am. Soc. Nephrol.* **13**, 105A (2002).
14. Thibonnier, M., Coles, P., Thibonnier, A. & Shoham, M. *Annu. Rev. Pharmacol. Toxicol.* **41**, 175–202 (2001).