nature medicine

Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist

Vincent H Gattone II[1], Xiaofang Wang[2], Peter C Harris[2] & Vicente E Torres[2]

The polycystic kidney diseases (PKDs) are a group of genetic disorders causing significant renal failure and death in children and adults. There are no effective treatments. Two childhood forms, autosomal recessive PKD (ARPKD) and nephronophthisis (NPH), are characterized by collecting-duct cysts[1,2]. We used animal models orthologous to the human disorders to test whether a vasopressin V2 receptor (VPV2R) antagonist, OPC31260, would be effective against early or established disease. Adenosine-3′,5′-cyclic monophosphate (cAMP) has a major role in cystogenesis[3,4], and the VPV2R is the major cAMP agonist in the collecting duct[5,6]. OPC31260 administration lowered renal cAMP, inhibited disease development and either halted progression or caused regression of established disease. These results indicate that OPC31260 may be an effective treatment for these disorders and that clinical trials should be considered.

PKD is accompanied by abnormalities in two major intracellular messengers, Ca^{2+} and cAMP (**Fig. 1**). Polycystin-2 may control intracellular Ca^{2+} ($[Ca^{2+}]_i$) through its proposed function as an inositol-1,4,5-triphosphate (IP_3)-sensitive channel that mediates Ca^{2+} release from the endoplasmic reticulum[7]. Alternatively, in primary cilia it may act as a channel that translates an extracellular signal, sensed by polycystin-1, into a Ca^{2+} influx that in turn induces Ca^{2+} release by intracellular ryanodine receptors[8]. Disruption of the polycystin pathway can result in reduced $[Ca^{2+}]_i$, as has recently been shown in freshly dissociated aortic vascular smooth muscle cells from *Pkd2*$^{+/-}$ mice[9]. Stimulation of Ca^{2+}-inhibitable adenylyl cyclase VI and inhibition of Ca^{2+}-dependent cAMP phosphodiesterases by reduction of $[Ca^{2+}]_i$ in the collecting ducts[10] may explain the increased levels of cellular cAMP[11] and expression of cAMP-dependent genes[12] in various animal models of PKD. Polycystin-1 might also act as a G_i-protein-coupled receptor and directly inhibit adenylyl cyclase when activated by its unspecified ligand[13].

To study the renal accumulation of cAMP and the expression of cAMP-regulated genes in PKD, we chose two models closely related to important human diseases that cause end-stage renal failure in childhood. The PCK rat, a model of human ARPKD, has a splicing mutation (IVS35-2A→T) that skips exon 36 and leads to a frameshift in *Pkhd1*, the rat ortholog of *PKHD1* (ref. 14). The *pcy* mouse is a model of the human autosomal recessive cystic kidney disease that is caused by a missense mutation in *NPHP3*, the gene mutated in adolescent NPH[15]. Both models have a defect in urine concentration, and eventually progress to renal failure. Renal cAMP expression was significantly ($P < 0.001$) higher in PCK rats and CD1/*pcy* mice than in the wild-type animals (**Fig. 2a**,**b**). Expression of aquaporin-2, a gene positively regulated by cAMP[16], and VPV2R was also increased (**Fig. 2c–f**). The mechanism responsible for the activation of the VPV2R pathway is not known, although fibrocystin, like the polycystins and other proteins mutated in animal models of PKD, has recently been located in the primary cilia of collecting-duct principal cells[17]. Disruption of this pathway, as described above, might be directly responsible for the increased cAMP levels and expression of cAMP-dependent genes. Alternatively, disruption of the medullary architecture and urinary concentration ability by the cysts could lead to compensatory activation of the VPV2R pathway. This seems less likely, as impairment of urinary concentration capacity occurs early in these animal models, before the development of major structural changes.

The increased renal accumulation of cAMP may affect secretion from and proliferation of the cystic epithelium. The renal collecting-duct epithelium is capable of very active chloride ion–driven fluid secretion that is stimulated by cAMP (ref. 6). The effect on epithelial cell proliferation is more complex, as cAMP can exert opposite effects in different cell types[18]. Thus, adenylyl cyclase agonists and 8-Br-cAMP activate the extracellular signal-regulated kinase cascade and increase proliferation in cells derived from polycystic kidneys, while having an inhibitory effect on cells derived from normal kidney cortex[3,4]. The mechanisms responsible for this phenotypic switch from cAMP-induced inhibition to cAMP-induced stimulation of cell proliferation are not known. Recent observations of a similar phenotypic switch when collecting-duct epithelial cells were treated with the nonspecific cation channel blocker gadolinium, or with L-type calcium channel blockers, suggest the importance of cross-talk between Ca^{2+} and cAMP signaling pathways in the pathogenesis of PKD[19].

Because vasopressin, the major adenylyl cyclase agonist in the principal cells of the renal collecting duct, acts through VPV2R (ref. 5), studies were designed to determine whether OPC31260, an antagonist of this receptor, could prevent the renal accumulation of cAMP and influence the course of PKD. The initiation and duration of treatment were chosen to ascertain effects on both disease development and progression.

[1]Anatomy and Cell Biology, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, Indiana 46202, USA. [2]Division of Nephrology, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, USA. Correspondence should be addressed to V.E.T. (torres.vicente@mayo.edu) or V.H.G. (gattone@anatomy.iupui.edu).

Published online 21 September 2003; doi:10.1038/nm935

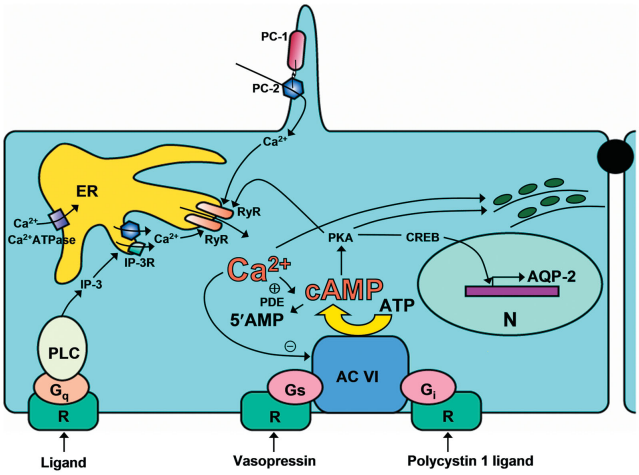

Figure 1 Polycystin pathway can influence intracellular regulation of Ca^{2+} and cAMP in collecting-duct epithelial cells from polycystic kidneys. Polycystin-1 (PC-1) acts as a sensor for extracellular signals. Polycystin-2 (PC-2) is regulated by polycystin-1 and acts as a Ca^{2+} channel that can induce the release of Ca^{2+} from intracellular stores. Disruption of the polycystin pathway results in reduced $[Ca^{2+}]_i$, which in turn stimulates adenylyl cyclase VI (AC VI), inhibits cAMP-dependent phosphodiesterases (PDE) and results in increased levels of intracellular cAMP. ER, endoplasmic reticulum; RyR, ryanodine receptor; R, G-protein-coupled receptor; PLC, phospholipase C; AQP-2, aquaporin-2; N, nucleus; PKA, protein kinase A; CREB, cAMP-responsive element binding protein.

Administration of 0.1% or 0.05% OPC31260 to PCK rats between 3 and 10 weeks of age markedly reduced the renal accumulation of cAMP and inhibited disease development, as reflected by lower kidney weights, plasma creatinine and blood urea nitrogen (BUN) concentrations, renal cyst volumes and mitotic and apoptotic indices (**Figs. 3** and **4a** and **Supplementary Table 1** online). Fibrosis at this stage of disease is very mild; a beneficial effect of borderline statistical significance ($P = 0.057$) was detected only in the rats receiving 0.1% OPC31260. By comparing the kidney weights of the treated and untreated PCK rats to those of 10-week-old wild-type Sprague-Dawley rats (0.80 ± 0.05% of body weight), we estimated the degree of protection by OPC31260 to be 60% for the 0.05% group and 75% for the 0.1% group. Administration of 0.05% OPC31260 to PCK rats from 10 to 18 weeks of age inhibited renal accumulation of cAMP and disease progression, as reflected by a decrease in the above criteria, as well as in renal fibrosis and systolic blood pressure (**Figs. 3** and **4a** and **Supplementary Table 2** online). The weights of the kidneys of the treated rats at 18 weeks of age were identical to those of the control PCK rats at 10 weeks of age, indicating that OPC31260 treatment completely halted disease progression. OPC31260 did not have a significant effect on fibropolycystic liver disease (results not shown), consistent with the absence of VPV2R in the liver.

Administration of 0.1% OPC31260 to CD1/*pcy* mice between 4 and 30 weeks of age markedly inhibited the renal accumulation of cAMP and disease development, as reflected by lower kidney weights, plasma BUN concentrations, renal cyst and fibrosis volumes and mitotic and apoptotic indices (**Figs. 3** and **4b** and **Supplementary Table 3** online). Administration of 0.1% OPC31260 to CD1/*pcy* mice between 15 and 30 weeks of age markedly inhibited the renal accumulation of cAMP levels and disease progression, as reflected by a decrease in the above criteria (**Figs. 3** and **4b** and **Supplementary Table 3** online). Kidney weights of mice that started treatment at 15 weeks and were killed at 30 weeks of age were significantly ($P = 0.004$) lower than those of untreated mice at 15 weeks of age, indicating that OPC31260 not only halted disease progression but also induced disease regression. OPC31260 administration to wild-type mice lowered urine osmolarity from 2,449 ± 223 to 1,106 ± 368 mosm/l ($P < 0.001$) but had no significant effect on blood biochemistry or renal histology (data not shown). This is the first interventional study in PKD that shows regression of cystic disease in a rodent model. The lack of detectable disease regression in the PCK study might have resulted from the shorter duration of treatment and lower concentration of the drug.

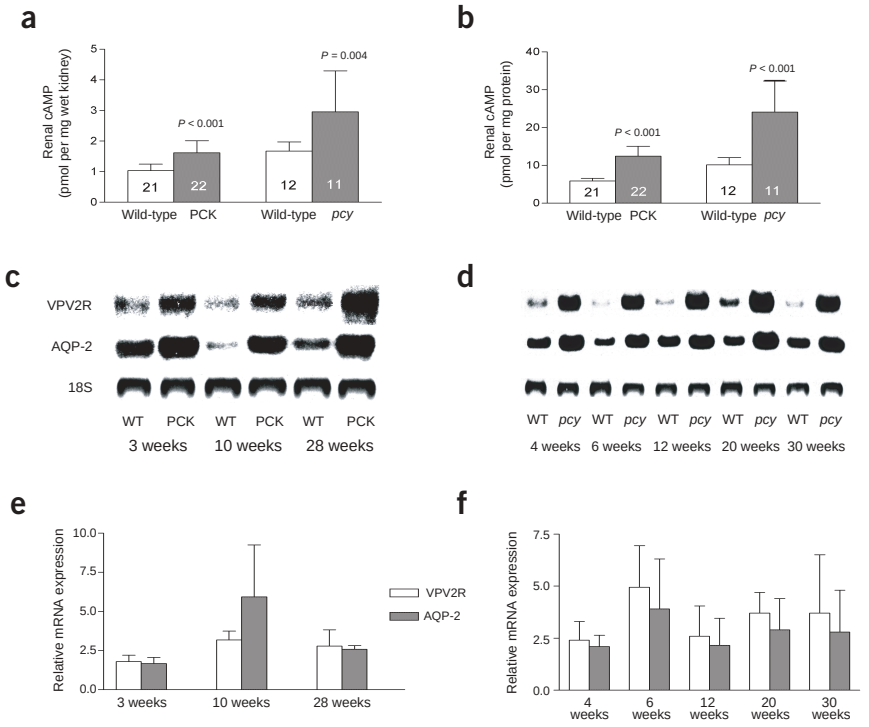

Figure 2 Renal levels of cAMP, VPV2R and aquaporin-2 mRNA. (**a**,**b**) cAMP expression per mg of wet tissue (**a**) or protein (**b**) in the kidneys of 10-week-old wild-type and PCK rats and 30-week-old wild-type and CD1/*pcy* mice. Numbers indicate mice per group. (**c**,**d**) Renal expression of VPV2R and aquaporin-2 (AQP-2) mRNA in wild-type (WT) and PCK rats (**c**) or CD1/*pcy* mice (**d**) by northern blot hybridization. Equal loading was confirmed by staining the blots with methylene blue. (**e**) Relative abundance of VPV2R and aquaporin-2 mRNA in cystic kidneys of PCK rats (**e**) or *pcy* mice (**f**), compared with wild-type kidneys at the indicated ages. All signals were normalized to the 18S mRNA signal. Each bar represents mean ± s.d. of three experiments. Cystic animals had significantly higher expression of VPV2R ($P < 0.001$) and aquaporin-2 ($P = 0.023$ for rats; $P < 0.001$ for mice) mRNA compared with wild-type animals.

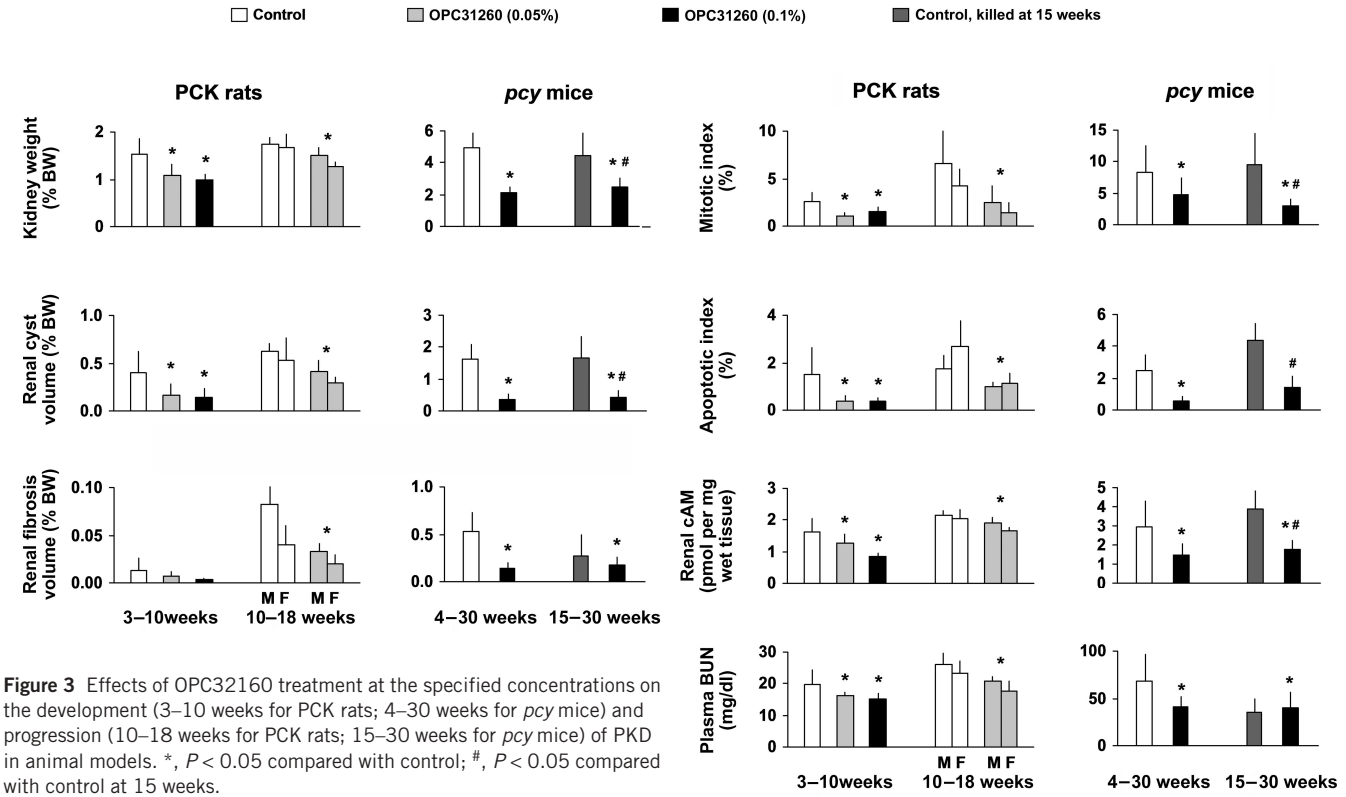

Figure 3 Effects of OPC32160 treatment at the specified concentrations on the development (3–10 weeks for PCK rats; 4–30 weeks for *pcy* mice) and progression (10–18 weeks for PCK rats; 15–30 weeks for *pcy* mice) of PKD in animal models. *, $P < 0.05$ compared with control; #, $P < 0.05$ compared with control at 15 weeks.

As previously reported in wild-type rats with free access to water[20], OPC31260 was well tolerated and did not cause significant electrolyte abnormalities. In the present study, a significant ($P = 0.045$) difference in urine output between the treated and untreated PCK rats was detected only during the first week of treatment, possibly indicating that the aquaretic effect of the drug was compensated by its beneficial effect on the disease (**Supplementary Tables 1** and **2** online). No significant difference in urine osmolality was detected between treated and untreated animals (**Supplementary Tables 1** and **3** online).

The VPV2R antagonists are attractive because of their renoselectivity (a result of the restricted expression of VPV2R on collecting-duct principal cells and endothelial cells[21,22]) and their apparent safety in preclinical and clinical studies. VPV2R antagonist administration to humans in phase 1 or 2 clinical trials has not resulted in adverse effects, except for the expected mild to moderate thirst that was well tolerated by all of the subjects[23]. No adverse effects related to possible vascular effects have been described[21]. Several VPV2R antagonists are currently in phase 3 efficacy and safety trials for hyponatremia caused by inappropriate antidiuretic hormone secretion, and for disorders of water retention such as congestive heart failure and cirrhosis. Given the paucity of effective therapies and the lack of adverse effects associated with the administration of VPV2R antagonists, clinical trials in patients with ARPKD and NPH may be warranted.

The results of this study may also be relevant to human ADPKD. As in ARPKD, a urine concentration defect is one of the earliest manifestations of ADPKD[24], and most of the cysts in ADPKD patients[25] and *Pkd2*$^{-/tm1Som}$ mice[26] derive from the collecting duct. As in the PCK rat and the *pcy* mouse, the renal concentration of cAMP is higher in *Pkd2*$^{-/tm1Som}$ mice than in wild-type mice (unpublished observations). Expression of the polycystin-1 C-terminal tail in mouse collecting-duct cells converts their proliferative phenotype, probably by a dominant-negative effect, from one that is inhibited by

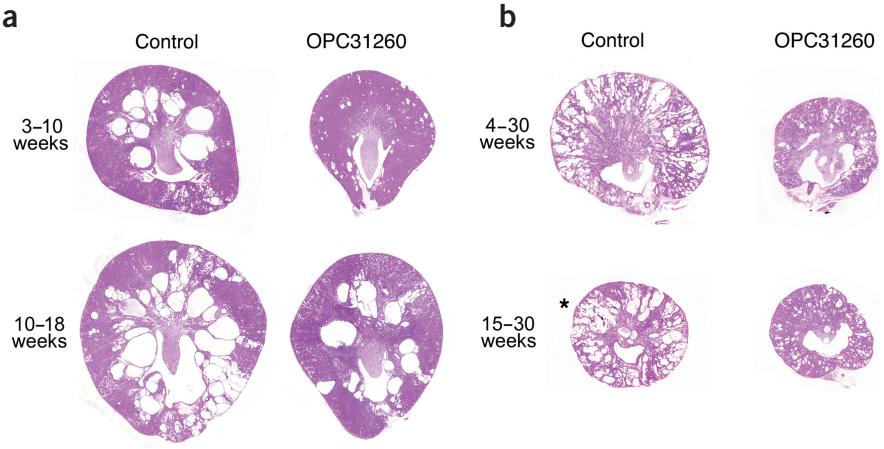

Figure 4 Representative kidney sections from PCK rats and CD1/*pcy* mice treated with OPC32160 and from untreated controls. (**a**) Kidney sections from PCK rats treated with OPC32160 between 3–10 or 10–18 weeks of age, compared with untreated controls. (**b**) Kidney sections from CD1/*pcy* mice treated with OPC32160 between 4–30 weeks or 15–30 weeks of age, compared with untreated controls. *, untreated control rat killed at 15 weeks of age.

cAMP to one that is stimulated by cAMP[27]. Finally, cAMP and agonists of adenylyl cyclase stimulate the proliferation of epithelial cells derived from ADPKD kidneys, but not of those derived from normal human kidneys[3,4].

In summary, the present study provides a strong rationale for an effective and safe potential treatment for PKD using VPV2R antagonists.

METHODS

Experimental animals and study design. Institutional Animal Care and Use Committees approved the use of PCK rats on a Sprague-Dawley strain (maintained at the animal facilities of the Mayo Clinic), *pcy* mice on a CD1 strain (maintained at Indiana University School of Medicine) and wild-type Sprague-Dawley rats and *pcy* mice, as well as the rat and mouse experimental protocols used here. The rats and mice were divided into control and treated groups at the specified ages. OPC31260 was added to ground rodent chow (Teklad 7017) at the specified concentrations. OPC31260 is an orally bioavailable nonpeptide VPV2R antagonist, without VPV2R agonist effects, that blocks the vasopressin-induced production of cAMP and its antidiuretic action in a dose-dependent fashion[20].

Experimental protocol. Twenty-four-hour urine outputs in metabolic cages (PCK rats only) were obtained weekly and tail-cuff blood pressures were obtained before the animals were weighed and killed. Rats were anesthetized with intraperitoneal ketamine (60 mg/kg) and xylazine (10 mg/kg), and mice with intraperitoneal sodium pentobarbital (50 mg/kg). Blood was obtained by cardiac puncture for determination of serum creatinine and BUN levels. Urine from *pcy* mice was obtained by bladder puncture. The right kidney and liver (PCK rats only) were placed into preweighed vials containing 10% formaldehyde in phosphate buffer (pH 7.4), and the tissues were embedded in paraffin for histological studies. The left kidneys were immediately frozen in liquid nitrogen for determination of cAMP.

Histomorphometric analysis and immunohistology. Transverse tissue sections (4 μm), including cortex, medulla and papilla, were stained with H&E to measure cyst volumes and with picrosirius red collagen stain to measure fibrosis[28]. Image analysis procedures were done with Meta-Morph software (Universal Imaging). We used immunostaining for proliferating cell nuclear antigen with monoclonal IgG2a antibody (Santa Cruz Biotechnology) to measure proliferation. We used the TUNEL assay with the ApopTag Peroxidase In Situ Apoptosis Detection Kit (Chemicon International) to measure apoptosis. Fields (×400) of renal medulla were randomly selected, and 500–1,000 tubular epithelial cell nuclei per tissue section were counted. The mitotic and apoptotic indices were calculated as the percentage of cells positive for proliferating cell nuclear antigen or TUNEL.

Content of cAMP in whole kidneys. Kidneys were ground to fine powder under liquid nitrogen in a stainless steel mortar and homogenized in ten volumes of cold 5% trichloroacetic acid in a glass-Teflon tissue grinder. After centrifugation at 600 *g* for 10 min, supernatants were extracted with three volumes of water-saturated ether. After drying the aqueous extracts, reconstituted samples were processed without acetylation using an enzyme immunoassay kit (Sigma-Aldrich). Results were expressed in pmol per mg of wet tissue or pmol per mg of protein[11].

Renal expression of VPV2R and aquaporin-2 mRNA. Total RNA was extracted from wild-type and cystic kidney pairs using TRIzol reagent (Invitrogen), and analyzed by northern blot hybridization using formaldehyde-agarose gel electrophoresis with transfer of RNA to nylon membranes. ^{32}P-labeled probes for VPV2R and aquaporin-2 were applied and the hybridized blots were exposed to X-ray film to obtain autoradiographs. Autoradiographs of hybridized blots were scanned, background was subtracted and corrected for RNA loading, and the pixel density of the cystic kidney was divided by that of the normal kidney to calculate the fold increase in mRNA expression in the cystic kidney.

Statistical analysis. Comparisons between groups were made using one-way or two-way ANOVA, with least significant difference comparisons of the means or Student *t*-test as appropriate. Data are expressed as mean ± s.d.

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

ACKNOWLEDGMENTS

This work was supported by grants from the PKD Foundation and the Indiana University School of Medicine (V.H.G.) and by National Institutes of Health grant DK44863 (V.E.T.). OPC31260 was a gift from Otsuka Pharmaceutical. C. Trambaugh, A. Yu and M. Branden provided technical assistance.

COMPETING INTERESTS STATEMENT

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

Received 12 May; accepted 2 September 2003
Published online at http://www.nature.com/naturemedicine/

1. Zerres, K. & Mucher, G. Autosomal recessive polycystic kidney disease. *J. Mol. Med.* **76**, 303–309 (1998).
2. Hildebrandt, F. & Otto, E. Molecular genetics of nephronophthisis and medullary cystic kidney disease. *J. Am. Soc. Nephrol.* **11**, 1753–1761 (2000).
3. Yamaguchi, T. *et al.* cAMP stimulates the *in vitro* proliferation of renal cyst epithelial cells by activating the extracellular signal-regulated kinase pathway. *Kidney Int.* **57**, 1460–1471 (2000).
4. Hanaoka, K. & Guggino, W. cAMP regulates cell proliferation and cyst formation in autosomal polycystic kidney disease cells. *J. Am. Soc. Nephrol.* **11**, 1179–1187 (2000).
5. Yasuda, G. & Jeffries, W.B. Regulation of cAMP production in initial and terminal inner medullary collecting ducts. *Kidney Int.* **54**, 80–86 (1998).
6. Wallace, D.P., Rome, L.A., Sullivan, L.P. & Grantham, J.J. cAMP-dependent fluid secretion in rat inner medullary collecting ducts. *Am. J. Physiol. Renal Physiol.* **280**, F1019–F1029 (2001).
7. Koulen, P. *et al.* Polycystin-2 is an intracellular calcium release channel. *Nat. Cell Biol.* **4**, 191–197 (2002).
8. Nauli, S.M. *et al.* Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* **33**, 129–137 (2003).
9. Qian, Q. *et al. Pkd2* haploinsufficiency alters intracellular calcium regulation in vascular smooth muscle cells. *Hum. Mol. Genet.* **12**, 1875–1880 (2003).
10. Chabardes, D., Imbert-Teboul, M. & Elalouf, J.M. Functional properties of Ca^{2+}-inhibitable type 5 and type 6 adenylyl cyclases and role of Ca2+ increase in the inhibition of intracellular cAMP content. *Cell Signal.* **11**, 651–663 (1999).
11. Yamaguchi, T., Nagao, S., Kasahara, M., Takahashi, H. & Grantham, J. Renal accumulation and excretion of cyclic adenosine monophosphate in a murine model of slowly progressive polycystic kidney disease. *Am. J. Kidney Dis.* **30**, 703–709 (1997).
12. Gattone, V.H., Maser, R.L., Tian, C., Rosenberg, J.M. & Branden, M.G. Developmental expression of urine concentration-associated genes and their altered expression in murine infantile-type polycystic kidney disease. *Develop. Gen.* **24**, 309–318 (1999).
13. Parnell, S.C. *et al.* The polycystic kidney disease-1 protein, polycystin-1, binds and activates heterotrimeric G-proteins *in vitro*. *Biochem. Biophys. Res. Commun.* **251**, 625–631 (1998).
14. Ward, C.J. *et al.* The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat. Genet.* **30**, 259–269 (2002).
15. Olbrich, H. *et al.* Mutations in a novel gene, *NPHP3*, cause adolescent nephronophthisis, tapeto-retinal degeneration and hepatic fibrosis. *Nat. Genet.* **34**, 455–459 (2003).
16. Promeneur, D., Kwon, T.-H., Frokler, J., Knepper, M.A. & Nielsen, S. Vasopressin V_2-receptor-dependent regulation of AQP2 expression in Brattleboro rats. *Am. J. Physiol. Renal Physiol.* **279**, F370–F382 (2000).
17. Ward, C.J. *et al.* Cellular and subcellular localization of the ARPKD protein; fibrocystin is expressed on primary cilia. *Hum. Mol. Genet.*; published online 12 August 2003 (doi:10.1093/hmg/ddg274).
18. Cass, L.A. *et al.* Protein kinase A-dependent and -independent signaling pathways contribute to cyclic AMP-stimulated proliferation. *Mol. Cell Biol.* **19**, 5882–5891 (1999).
19. Yamaguchi, T., Wallace, D.P., Grantham, J.J. & Calvet, J.P. Decreased intracellular Ca^{2+}, which causes an ADPKD-like switch in the proliferative response of M-1 cells to cAMP, is associated with increased activation of Src, B-Raf, and ERK, and increased B-Raf protein. *J. Am. Soc. Nephrol.* **13**, 105A (2002).
20. Yamamura, Y. *et al.* Characterization of a novel aquaretic agent, OPC–31260, as an orally effective, nonpeptide vasopressin V2 receptor antagonist. *Br. J. Pharmacol.* **105**, 787–791 (1992).
21. Wong, L.L. & Verbalis, J.G. Vasopressin V2 receptor antagonists. *Cardiovasc. Res.* **51**, 391–402 (2001).
22. Thibonnier, M., Coles, P., Thibonnier, A. & Shoham, M. The basic and clinical pharmacology of nonpeptide vasopressin receptor antagonists. *Annu. Rev. Pharmacol. Toxicol.* **41**, 175–202 (2001).
23. Ohnishi, A. *et al.* Aquaretic effect of a potent, orally active, nonpeptide V2 antagonist in men. *J. Pharmacol. Exp. Ther.* **272**, 546–551 (1995).
24. Gabow, P. *et al.* The clinical utility of renal concentrating capacity in polycystic kidney disease. *Kidney Int.* **35**, 675–680 (1989).
25. Verani, R.R. & Silva, F.G. Histogenesis of the renal cysts in adult (autosomal dominant) polycystic kidney disease: a histochemical study. *Mod. Pathol.* **1**, 457–463 (1988).
26. Wu, G. *et al.* Somatic inactivation of PKD2 results in polycystic kidney disease. *Cell* **93**, 177–188 (1998).
27. Sutters, M. *et al.* Polycystin-1 transforms the cAMP growth-responsive phenotype of M-1 cells. *Kidney Int.* **60**, 484–494 (2001).
28. Junqueira, L.C.U., Bignolas, G. & Brentani, R.R. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem. J.* **11**, 447–455 (1979).