**Table 1 • Comparison between strains of the portion of the Il-2 protein sequence encoded by exon 1 of *Il2***

| Strain | Exon 1 predicted amino acid sequence |
|---|---|
| | x xxxx xxxx |
| C57BL/6 | MYSMQLASCVTLTLVLLVNSAPTSSST——SSSTAEAQQQQQQQQQQQHLEQLLMDLQELLSRME |
| B10.S | MYSMQLASCVTLTLVLLVNSAPTSSST——SSSTAEAQQQQQQQQQQQHLEQLLMDLQELLSRME |
| NOD | MYSMQLASCVTLTLVLLVNSAPTSSPTSSPTSSSTAEAQQQQQQQQ———HLEQLLMDLQELLSRME |
| SJL/J | MYSMQLASCVTLTLVLLVNSAPTSSPTSSPTSSSTAEAQQQQQQQQ———HLEQLLMDLQELLSRME |

Exon 1 of *Il2* was amplified by PCR from genomic DNA using oligonucleotide primers CMG72 (5′–TTGCCTATAGATGGGATGGC–3′) and CMG73 (5′–CAGTGACCT-CAAGTCCTGCA–3′; ref. 9) and sequenced with an ABI Prism 377 sequencing system. Differences between the strains are indicated by an x above differing residues.

that SJL/J mice have an *Il2* sequence identical to that of NOD mice; similarly, B10.S mice were found to have a sequence identical to that of B6 (Table 1). As in the NOD allele, the SJL/J allele differs from the B6 allele by a single base change (T to C) that results in a serine-to-proline substitution in the sixth amino acid residue of the mature protein. The SJL/J allele also has a duplication of a 12-bp segment of DNA that results in a 4-aa insertion, and a compensatory 12-bp deletion that results in a deletion of 4 glutamines from a stretch of 12 consecutive glutamines.

Compared with the Il-2 protein produced by B6 mice, NOD-produced Il-2 shows differences in glycosylation that may affect its functional half-life (L.S.W., manuscript in preparation). If the NOD/SJL allele of *Il2* influences EAE and diabetes susceptibility, a possible mechanism may lie in its role in T-cell selection in the thymus or in its function in the peripheral immune compartment. Insufficient levels of Il-2 may affect negative selection in the thymus, allowing the escape of self-reactive T cells[12]. Il-2 is also important in an autocrine feedback loop that regulates the expansion of antigen-specific, T-cell clones by inducing apoptotic cell death[13], and is essential for the maintenance of self-tolerance as evidenced by the development of severe autoimmunity in *Il2*$^{-/-}$ mice[14]. The identification of a narrowly defined locus that has effects in both EAE and diabetes may indicate that there are individual genes that contribute to the susceptibility to several autoimmune diseases. By using congenic mice to precisely define each of the loci contributing to EAE and diabetes susceptibility, we can begin to put together the pieces that determine the development of the autoimmune phenotype.

**Acknowledgements**

*We thank A. Abbas and L. Nicholson for review of the manuscript. These studies were carried out with the support of grants from the National Institutes of Health (RO1 NS 30843, RO1 NS 35685 and PO1 AI 39671) and National Multiple Sclerosis Society (RG 2571 and 2320) to V.K.K.*

**Jeffrey A. Encinas[1], Linda S. Wicker[2], Laurence B. Peterson[3], Akiko Mukasa[1], Cory Teuscher[4], Raymond Sobel[5], Howard L. Weiner[1], Christine E. Seidman[6], J.G. Seidman[6] & Vijay K. Kuchroo[1]**

*[1]Center for Neurologic Diseases, Department of Medicine, Brigham & Women's Hospital, Boston, Massachusetts 02115, USA. [2]Departments of Autoimmune Diseases Research and [3]Cellular and Molecular Pharmacology, Merck Research Laboratories, Rahway, New Jersey 07065, USA. [4]Department of Veterinary Pathobiology, The University of Illinois at Urbana-Champaign, Urbana, Illinois 61802, USA. [5]Department of Pathology, Stanford University School of Medicine and Laboratory Service, Veterans Affairs Medical Center, Palo Alto, California 94304, USA. [6]Department of Genetics and Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02115, USA. C.E.S. and J.G.S. are supported by Howard Hughes Medical Institute. Correspondence should be addressed to V.K.K. (e-mail: kuchroo@cnd.bwh.harvard.edu).*

1. Encinas, J.A. *et al. J. Immunol.* **157**, 2186–2192 (1996).
2. Sundvall, M. *et al. Nature Genet.* **10**, 313–317 (1995).
3. Todd, J.A. *et al. Nature* **351**, 542–547 (1991).
4. Ghosh, S. *et al. Nature Genet.* **4**, 404–409 (1993).
5. Vyse, T.J. & Todd, J.A. *Cell* **85**, 311–318 (1996).
6. Podolin, P.L. *et al. Mamm. Genome* **9**, 283–286 (1998).
7. Denny, P. *et al. Diabetes* **46**, 695–700 (1997).
8. Bernard, C.C. *et al. J. Mol. Med.* **75**, 77–88 (1997).
9. Chesnut, K., She, J.X., Cheng, I., Muralidharan, K. & Wakeland, E.K. *Mamm. Genome* **4**, 549–554 (1993).
10. Teuscher, C., Wardell, B.B., Lunceford, J.K., Michael, S.D. & Tung, K.S. *J. Exp. Med.* **183**, 631–637 (1996).
11. Matesanz, F., Alcina, A. & Pellicer, A. *Immunogenetics* **38**, 300–303 (1993).
12. Classen, J.B. & Shevach, E.M. *Autoimmunity* **15**, 55–59 (1993).
13. Lenardo, M.J. *et al. Int. Rev. Immunol.* **13**, 115–134 (1995).
14. Sadlack, B. *et al. Eur. J. Immunol.* **25**, 3053–3059 (1995).
15. Zamvil, S. *et al. Nature* **317**, 355–358 (1985).

# Late onset of renal and hepatic cysts in *Pkd1*-targeted heterozygotes

Autosomal dominant polycystic kidney disease (ADPKD) due to *PKD1* mutations is characterized by the progressive appearance of renal, hepatic and pancreatic cysts in adults[1]. We previously reported that targeted deletion of exon 34 in *Pkd1*, the mouse homologue of *PKD1*, results in renal cysts and perinatal death in homozygotes[2]. Here we report that *Pkd1*$^{+/-}$ mice progressively develop scattered renal and hepatic cysts.

Serial sections of 15 *Pkd1*$^{+/-}$ mice of 9–14 months of age revealed 1–7 renal cysts (of more than 5 times the normal tubule diameter) per animal in 12 mice (80%), of which 5 mice had bilateral cysts. After 16 months, 2–50 cysts were found in all 8 mice examined (100%, Table 1) and 6 of 8 mice had bilateral cysts. Renal excretory function (serum creatinine) was normal in all but one mouse with extensive disease. There were no cysts in five, age-matched, control littermates.

Cysts were seen from the cortex (Fig. 1*a*) to the inner medulla. Most cysts

**Table 1 • Number of renal cysts and liver function in *Pkd1*$^{+/-}$ mice**

| *Pkd1*$^{+/-}$ ID | 851 | 285 | 728 | 744 | 702 | 720 | 706 | 735 |
|---|---|---|---|---|---|---|---|---|
| Age (month) | 16 | 16 | 18 | 18 | 18.5 | 19 | 19 | 20 |
| Renal cysts | 25 | 2 | 2 | 3 | 4 | 3 | >50 | >50 |
| | Wild-type (n=3) | | *Pkd1*$^{+/-}$ 720 | | *Pkd1*$^{+/-}$ 753 | | *Pkd1*$^{+/-}$ 754 | |
| Liver cyst/parenchyma | 0% | | 25% | | 50% | | 75% | |
| Total protein (mg/dl) | 5,267±173 | | 5,400 | | 6,400 | | 3,800 | |
| ALT | 45±11 | | 56 | | 82 | | 152 | |
| AST | 176±40 | | 216 | | 258 | | 701 | |
| LDH | 438±110 | | 527 | | 928 | | 2,521 | |

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase.

had lost the characteristics of their tubule segment origins; only 1 of 10 cysts stained with a collecting duct marker and 2 of 10 with proximal tubule-specific lectin (Fig. 1*b*,*c*). Glomerular cysts were common (Fig. 1*a*). Cyst-lining epithelia ranging from columnar to cuboidal to squamous were found in large cysts (Fig. 1*d*), which were often surrounded by atrophic parenchyma with interstitial fibrosis and inflammation (Fig. 1*e*). Although basement membrane was normal as observed by light microscopy, epidermal growth factor receptor (EGFR) was mislocalized to apical membranes in cysts and some slightly dilated tubules (Fig. 1*f*,*g*), suggesting that EGFR mislocalization[3] may also be an early marker of cystic transformation in *Pkd1* deficiency. Polycystin-1 expression was found to be absent in some cysts (Fig. 1*h*), similar to what has been found in humans[5].

Liver cysts, which are common in ADPKD (ref. 6), were observable in 4 of 15 (27%) heterozygotes of 9–14 months of age and in 7 of 8 (87%) older mice. Cysts were filled with clear or dark-brown fluid (Fig. 1*i*,*j*) of up to 10 ml in volume occupying one- to two-thirds of the liver. Liver function impairment, occasionally seen in ADPKD, correlated with cyst volume and cholangitis (Table 1). Cyst fluid represents the bile salt-independent fraction of bile (data not shown), indicating that cyst epithelia, although originating from biliary ductule epithelia, have altered secretory function. This is similar to the human condition and supports the hypothesis that increased fluid in the cysts is due to increased secretion from the cystic epithelia[7,8].

Most liver cysts were lined by cuboidal or squamous biliary-like epithelium positive for cytokeratin 19, a biliary-specific epithelial marker (data not shown), and displayed regions of focal hyperplasia (Fig. 1*k*). Serial sections revealed ductal plate malformation with occasional biliary microhamartomas (BMH; Fig. 1*l*) that are thought to represent an early stage of liver cyst formation[9]. The prominent liver changes (Fig. 1*i*,*j*,*m*) combined with the absence of liver cysts in perinatal homozygotes suggest that *Pkd1* is required in the maintenance but not formation of biliary ducts.

The phenotype of *Pkd1*$^{+/-}$ mice recapitulates the renal and hepatic phenotypes of human ADPKD (refs 6,10,11). The gradual recruitment of cysts and the absence of polycystin-1 in some renal cysts are consistent with clinical progression in man and with the 'two hit' hypothesis of cyst development[12–15]. Therefore *Pkd1*$^{+/-}$ mice provide a relevant model of ADPKD pathophysiology.

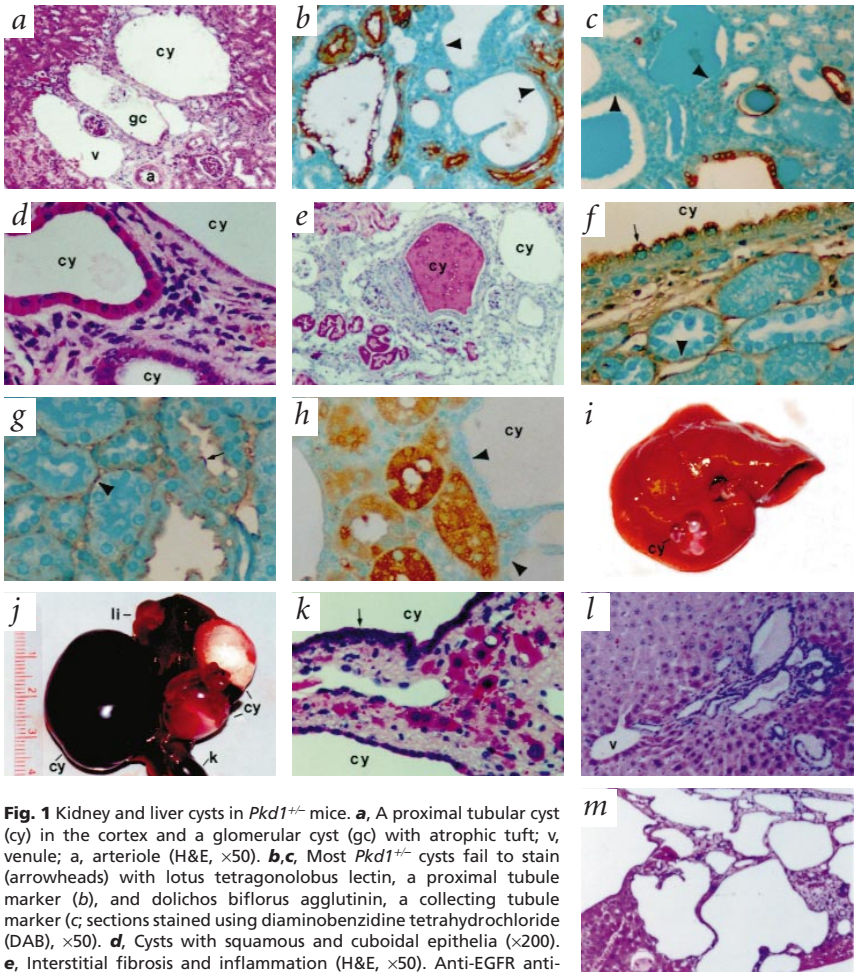


**Fig. 1** Kidney and liver cysts in *Pkd1*$^{+/-}$ mice. ***a***, A proximal tubular cyst (cy) in the cortex and a glomerular cyst (gc) with atrophic tuft; v, venule; a, arteriole (H&E, ×50). ***b***,***c***, Most *Pkd1*$^{+/-}$ cysts fail to stain (arrowheads) with lotus tetragonolobus lectin, a proximal tubule marker (*b*), and dolichos biflorus agglutinin, a collecting tubule marker (*c*; sections stained using diaminobenzidine tetrahydrochloride (DAB), ×50). ***d***, Cysts with squamous and cuboidal epithelia (×200). ***e***, Interstitial fibrosis and inflammation (H&E, ×50). Anti-EGFR antibody recognizes basolateral membranes of normal tubules (arrowheads), apical membranes of cysts (***f***, arrow) and slightly dilated tubules (***g***, arrow; DAB, ×200). ***h***, The monoclonal anti-polycystin-1 antibody PKS-A (ref. 4) fails to stain some renal cysts (arrowheads). ***i***, Liver cysts (cy) in an 11-month-old *Pkd1*$^{+/-}$ mouse are multilocular. ***j***, Cysts occupy 75% of the liver (li) with severe functional impairment (20 months) but kidneys (k) are normal in size. ***k***, Monolayer of cuboidal cyst epithelium with focal hyperplasia (arrow, ×200). ***l***, Ductal plate malformation with BMHs. v, terminal hepatic venule (×50). ***m***, Multiple liver cysts with little residual parenchyma (×50).

**Acknowledgements**

*We thank H. You and J. Edwards for technical assistance and S.T. Reeders and Y. Segal for discussions. This work was supported by the National Institutes of Health grants DK 40703 to S.T.R. and DK 51050 to J.Z., and the Polycystic Kidney Research Foundation.*

**Weining Lu[1], Xiaohong Fan[1], Nuria Basora[1], Hermik Babakhanlou[1], Terry Law[2], Nader Rifai[2], Peter C. Harris[3], Antonio R. Perez-Atayde[4], Helmut G. Rennke[5] & Jing Zhou[1]**

*[1]Renal Division, Department of Medicine, and [5]Department of Pathology, Brigham and Women's Hospital; [2]Department of Laboratory Medicine, and [4]Department of Pathology, Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115. [3]MRC Molecular Haematology Unit, John Radcliffe Hospital, Oxford, Headington, UK. Correspondence should be addressed to J.Z. (e-mail: zhou@rics.bwh.harvard.edu).*

1. Gabow, P.A. *N. Engl. J. Med.* **329**, 332–342 (1993).
2. Lu, W. *et al. Nature Genet.* **17**, 179–181 (1997).
3. Orellana, S.A., Sweeney, W.E., Neff, C.D. & Avner, E.D. *Kidney Int.* **47**, 490–499 (1995).
4. Ward, C.J. *et al. Proc. Natl Acad. Sci. USA* **93**, 1524–1528 (1996).
5. Geng, L. *et al. J. Clin. Invest.* **98**, 2674–2682 (1996).
6. Everson, G.T. *Am. J. Kidney. Dis.* **22**, 520–525 (1993).
7. Patterson, M., Gonzalez-Vitale, J.C. & Fagan, C.J. *Hepatology* **2**, 475–478 (1982).
8. Everson, G.T., Emmett, M., Brown, W.R., Redmond, P. & Thickman, D. *Hepatology* **11**, 557–565 (1990).
9. Ramos, A. *et al. Arch. Pathol. Lab. Med.* **114**, 180–184 (1990).
10. Fick, G.M. *et al. J. Am. Soc. Nephrol.* **4**, 1654–1660 (1994).
11. Gabow, P.A. & Grantham, J.J. in *Disease of Kidney* (eds Schrier, R.W. & Gottschalk, C.W.) 521–560 (Little Brown, Boston, 1997).
12. Reeders, S.T. *Nature Genet.* **1**, 235–237 (1992).
13. Qian, F., Watnick, T.J., Onuchic, L.F. & Germino, G.G. *Cell* **87**, 979–987 (1996).
14. Brasier, J.L. & Henske, E.P. *J. Clin. Invest.* **99**, 194–199 (1997).
15. Wu, G. *et al. Cell* **93**, 177–188 (1998).