

Somatic loss of polycystic disease genes contributes to the formation of isolated and polycystic liver cysts

We read with interest the paper by Urribarri *et al*¹ which describes that metalloprotease hyperactivity plays an important role in cyst expansion and that metalloprotease inhibition reduces cyst proliferation. As such, these results help to identify potential drug targets.² We hypothesise that the expansion and maintenance of the cyst is preceded by mutational events that trigger cytogenesis, and

we used genetic analysis to provide additional insight into this process. The majority of polycystic diseases are autosomal dominant disorders where every patient cell possesses one germ line mutation (first hit).³ As somatic second-hit mutations play an important role in liver and renal cyst formation,^{4–6} it was hypothesised that patients with polycystic disease have a DNA repair defect and accumulate somatic mutations.⁷ This was supported by a comparative genomic hybridisation study where renal cysts harboured multiple chromosomal aberrations, similar to cancers.^{8,9} In addition, patients without a germ line mutation can still develop sporadic cysts, which begs the question if and how somatic mutations contribute here.¹⁰ Therefore, we used a genome-wide approach to determine the type and extent of somatic mutations in solitary cysts and polycystic livers.

We collected cyst epithelium of 23 cysts (22 liver cysts, 1 kidney cyst) from 7 patients with different underlying diseases (table 1). All patients were initially screened for germ line mutations in *PRKCSH*, *SEC63* and *PKD2*. Cyst epithelial cells were collected from fresh tissue samples using EDTA and a CK19 staining was used to analyse the purity of each sample. We assessed copy-number variations and loss of heterozygosity (LOH)

regions through genome-wide high resolution cytogenetic array analysis (CytoScan HD, Affymetrix) and searched for intragenic mutations by sequencing.⁶

In the patients with autosomal dominant polycystic liver disease and autosomal dominant polycystic kidney disease (Patient#1, #2 and #3), we found that 13/18 cysts had acquired a somatic second-hit mutation (table 1). Using genome-wide data we could map the regions affected by LOH and determine the overall genome stability in the cysts (see online supplementary figure S1). LOH was caused by either terminal copy-number neutral (CNN) LOH (5–17 Mb) or interstitial deletions (16–45 Mb), depending on the patient. In Patient#2 we did not detect a germ line mutation in *PRKCSH*, *SEC63* or *PKD2*, but in 2/6 liver cysts we detected telomeric CNN LOH of respectively 5 Mb and 17 Mb on chromosome 16p suggesting that the affected gene may be located here (see online supplementary figure S1C). This region includes *PKD1*, the main gene for autosomal dominant polycystic kidney disease, however, sequencing of *PKD1* did not reveal any germ line mutation in genomic DNA of this patient. In Patient#3 we identified a novel *PKD2* c.1536_1538delTGT germ line mutation. In addition to the liver cysts, we also

analysed one kidney cyst of this patient and found loss of function of *PKD2* through telomeric CNN LOH (104 Mb).

As it is still unknown what drives cyst formation in sporadic liver cysts we also included 5 sporadic cysts in our analysis (table 1). Single (sporadic) hepatic cysts are found in 10% of the normal population with a clear age dependency. In one cyst we identified a complex biallelic deletion on chromosome 4q resulting in a homozygous loss of a 2.6 Mb region containing *PKD2* (see online supplementary figure S1E). We did not detect any genomic aberrations in other sporadic cysts.

In conclusion, loss of both wild type alleles seems sufficient to drive cyst formation, and consistent with the benign growth of the cysts we detected absence of genomic instability or additional large genomic aberrations. Therefore, the genetic changes that we see in these cysts fit with a somatic single-step model of cystogenesis. Finally, we found that a homozygous deletion of the *PKD2* genomic region in a solitary cyst provides the long-awaited support for the hypothesis that somatic loss of a polycystic disease gene contributes to the formation of sporadic cysts.

Manoe J Janssen,¹ Jody Salomon,¹
Wybrich R Cnossen,¹ Carsten Bergmann,^{2,3}
Rolph Pfundt,⁴ Joost P H Drenth¹

¹Department of Gastroenterology & Hepatology, Radboudumc, Institute for Genetic and Metabolic Diseases, Nijmegen, The Netherlands

²Department of Bioscientia, Center for Human Genetics, Ingelheim, Germany

³Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg, Germany

⁴Department of Human Genetics, Radboudumc, Institute for Genetic and Metabolic Diseases, Nijmegen, The Netherlands

Correspondence to Professor Joost P H Drenth, Department of Gastroenterology & Hepatology, Radboudumc, Institute for Genetic and Metabolic Diseases, P.O. Box 9101, code 455, Nijmegen 6500hb, The Netherlands; joostphdrenth@cs.com

Contributors MJJ: Study concept and design, acquisition of data, analysis and interpretation of data, statistical analysis and drafting and revising of the manuscript. JS: Acquisition of data, analysis and interpretation of data, technical support and revising the draft paper. CB: Acquisition of data, technical support and revising the draft paper. RP: Acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, technical and material support. JPHD: Study concept and design, critical revision of the manuscript for important intellectual content, obtained funding and supervised the study.

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Patient consent Obtained.

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Table 1 Mutation analysis in blood and cyst DNA

Patient information	Cyst nr	Somatic mutation
Patient#1—PCLD, female, age 60 years Heterozygous germ line mutation <i>PRKCSH</i> c.292+1G>C (Chr 19)	1	CNN LOH Chr 19p (18 Mb)
	2	CNN LOH Chr 19p (17 Mb)
	3	CNN LOH Chr 19p (13 Mb)
	4	<i>PRKCSH</i> c.374_375delAG
	5	<i>PRKCSH</i> c.102delC
	6	<i>PRKCSH</i> c.293-1G>A
Patient#2—PCLD, male, age 70 years Unknown germ line mutation	1	CNN LOH Chr 16p (17 Mb)
	2	CNN LOH Chr 16p (5 Mb)
	3	Not detected
	4	Not detected
	5	Not detected
	6	Not detected
Patient#3—ADPKD, female, age 56 years Heterozygous germ line mutation <i>PKD2</i> c.1536_1538delTGT (Chr 4)	1	Del Chr 4 (45 Mb)
	2	Del Chr 4 (34 Mb)
	3	Del Chr 4 (16 Mb)
	4*	CNN LOH Chr 4 (104 Mb)
	5	<i>PKD2</i> c.1002_1003delCC, Del Chr 5 (1.5 Mb)
	6	Not detected
Patient#4—Sporadic cyst, female, age 46 years	1	Biallelic deletion Chr 4q (2.6 Mb)
Patient#5—Sporadic cyst, female, age 58 years	1	Not detected
	2	Not detected
Patient#6—Sporadic cyst, female, age 56 years	1	Not detected
Patient#7—Sporadic cyst, male, age 70 years	1	Not detected

*Kidney cyst.

ADPKD, autosomal dominant polycystic kidney disease; Chr, Chromosome; CNN, Copy number neutral; LOH, loss of heterozygosity; PCLD, polycystic liver disease.

PostScript

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