The American Journal of Pathology, Vol. 193, No. 2, February 2023

The American Journal of PATHOLOGY
ajp.amjpathol.org

See related article on page 191

COMMENTARY

The Electrogenic Chloride Exchanger ClC5 as a Novel Player in Renal Cysts in Tuberous Sclerosis

Grazia Tamma

From the Department of Biosciences, Biotechnology, and Environment, University of Bari Aldo Moro, Bari, Italy

Renal cystic diseases are complex and multifaceted disorders that can have genetic or nongenetic bases. The etiology of these disorders is not unique but may be associated with other systemic diseases, acquired or inherited. With significant progress in genetics, several mutated genes have been associated with renal cyst development. Different abnormal protein functions, causing different renal cystic diseases, imply that several molecular mechanisms may lead to the formation of cysts. Cysts can be generated from any tract of the nephron; they usually generate in the distal nephron and in the collecting ducts.[1] Autosomal dominant polycystic kidney disease (ADPKD) is the most common cystic disorder, caused by mutations in the genes coding for polycystin-1 and polycystin-2. Renal cysts are also found in 50% of patients with tuberous sclerosis complex (TSC).[2] Patients with TSC can develop several disturbances, affecting many organs. Neurologic symptoms are often described, although renal defects can be considered the second most common features in TSC-affected individuals. In this issue of *The American Journal of Pathology*, Barone et al[3] proposed the electrogenic exchanger ClC5 as a possible candidate for mediating chloride secretion into the renal cyst lumen in TSC.

TSC is an autosomal dominant disease, displaying a birth incidence of 1:6000. TSC is due to loss-of-function mutations in the genes coding for TSC1 (hamartin) or TSC2 (tuberin). Physiologically, TSC2 down-regulates mammalian target of rapamycin (mTOR), which is a key player controlling cell proliferation, by modulating Ras homolog enriched in brain (RHEB) activity.[4,5] Cyst formation is a complex mechanism that is accompanied by altered tubulogenesis, increased apoptosis, and cellular proliferation.

In ADPKD and TSC, abnormal mTOR functioning has been observed. Several hypotheses have been raised to explain the molecular process leading to fluid secretion into the cysts. Fluid transport across the cyst-lining cells is sustained by transepithelial secretion of chloride. In ADPKD, several studies proposed the cAMP-regulated cystic fibrosis transmembrane regulator (CFTR) as the apical chloride channel involved in chloride accumulation into the lumen of the cysts. Accordingly, a significant reduction in cyst expansion has been demonstrated by using CFTR inhibitors or tolvaptan, which reduces the intracellular level of cAMP by inhibiting the vasopressin V2 receptor–dependent signaling. Tolvaptan, a selective V2 receptor antagonist, has been approved in the United States, Europe, and many other countries for slowing the progression of cyst development and retarding renal insufficiency in patients with ADPKD, although several adverse effects have also been reported.[6,7] More important, not all patients with ADPKD respond to tolvaptan treatment in terms of total kidney growth, suggesting possible alternative cAMP/V2 receptor–independent pathways promoting renal cysts.[8] On the other hand, compared with polycystin-1 knockout mice, mice carrying the codeletion of CFTR and polycystin-1 do not display a significant reduction in cystogenesis and proliferation, likely proposing that CFTR is not essential to promote the growth of renal cysts.[9] In this scenario, the findings reported by Barone et al[3] in this issue provide novel insights into the mechanism modulating chloride secretion into the lumen of TSC renal cysts. Using different transgenic mouse models of TSC,[3,10] the authors clearly and elegantly described the different and temporal phases of cystogenesis and identified ClC5 as a channel possibly

Accepted for publication November 30, 2022.
Disclosure: None declared.

Address correspondence to Grazia Tamma, Ph.D., Department of Bioscience, Biotechnology and Environment, University of Bari Aldo Moro, Via Orabona 4, 70125 Bari, Italy. E-mail: grazia.tamma@uniba.it

https://doi.org/10.1016/j.ajpath.2022.11.005

involved in chloride secretion in TSC renal cysts. Specifically, small cysts appeared in $Tsc2^{+/-}$ mice 300 days old, whereas larger cysts were observed secondary to a robust expression of forkhead box I1 (Foxi1) at 450 days of age. Forkhead genes code for a large family of transcription factors involved in cell differentiation and organogenesis. In the kidney, Foxi1 is a crucial factor modulating the differentiation and the expression of intercalated cells. Mice deficient for Foxi1 did not have functional intercalated cells and displayed a significant inability to acidify urine.[11] Conversely, Foxi1 induction promoted renal tubular differentiation of α-intercalated cells. Interestingly, a robust expression of Foxi1 has been found under treatment with lithium chloride, a known mood stabilizer, which causes an acquired form of nephrogenic diabetes insipidus. Indeed, some of the molecular signals associated with lithium chloride treatment share similarities with TSC cystogenesis in terms of Foxi1 induction, hyperproliferation, of α-intercalated cells, paralleled with a reduction of the renal principal collecting duct cells expressing the vasopressin-dependent water channel aquaporin 2. In line, in $Tsc2^{+/-}$ mice, a time-dependent decrease of aquaporin 2 expression, correlated with loss of renal principal cells, was found and was more pronounced at 450 days of age. At this stage, a remarkable costaining of ClC5 and H^+-ATPase was found on the apical membrane of cyst epithelia that do not express CFTR, which is mainly expressed in renal principal cells. More importantly, the costaining of ClC5 and H^+-ATPase was also found in human patients with TSC. By contrast, these proteins were expressed at a lower level in the renal cysts of patients with ADPKD.[3] Together, these findings indicate that different renal cystic disorders might share some molecular events, such as mTOR deregulation. Nevertheless, the mechanism sustaining and expanding renal cysts may have specific peculiarities that depend on cyst-lining cell types.

Results presented by Barone et al[3] leave, however, several open questions. i) What is the role of Foxi1 in the whole kidney? In this respect, the authors showed that in Tsc1/aquaporin 2 knockout mice, cyst formation, which precedes Foxi1 induction, occurs in the kidney cortex, whereas enhanced Foxi1 mRNA expression was found in both the cortex and medulla. ii) Can short- and long-loop nephrons, having a different expression of transporters and channels,[12,13] display differential sensitivity to cyst formation in TSC? iii) Considering the remarkable loss of principal cells expressing aquaporin 2, what about renal water balance in TSC animal models?

Although future investigations are still needed to depict a global picture leading to renal cyst formation, the findings published by Barone et al[3] shed more light on a selective mechanism underlying cystic expansion in patients with TSC that involves ClC5. The development of new drugs that inhibit ClC5 specifically to slow the increase of cyst's size is, therefore, an unmet need.

References

1. Sudarikova AV, Vasileva VY, Sultanova RF, Ilatovskaya DV: Recent advances in understanding ion transport mechanisms in polycystic kidney disease. Clin Sci (Lond) 2021, 135:2521−2540
2. Dixon BP, Hulbert JC, Bissler JJ: Tuberous sclerosis complex renal disease. Nephron Exp Nephrol 2011, 118:e15−e20
3. Barone S, Brooks M, Zahedi K, Holliday LS, Bissler J, Yu JJ, Soleimani M: Identification of an electrogenic $2Cl^-/H^+$ exchanger, ClC5, as a chloride-secreting transporter candidate in kidney cyst epithelium in tuberous sclerosis. Am J Pathol 2023, 193:191−200
4. Bissler JJ, Zadjali F, Bridges D, Astrinidis A, Barone S, Yao Y, Redd JR, Siroky BJ, Wang Y, Finley JT, Rusiniak ME, Baumann H, Zahedi K, Gross KW, Soleimani M: Tuberous sclerosis complex exhibits a new renal cystogenic mechanism. Physiol Rep 2019, 7:e13983
5. Neuman NA, Henske EP: Non-canonical functions of the tuberous sclerosis complex-Rheb signalling axis. EMBO Mol Med 2011, 3:189−200
6. Chebib FT, Perrone RD, Chapman AB, Dahl NK, Harris PC, Mrug M, Mustafa RA, Rastogi A, Watnick T, Yu ASL, Torres VE: A practical guide for treatment of rapidly progressive ADPKD with tolvaptan. J Am Soc Nephrol 2018, 29:2458−2470
7. De Rechter S, Breysem L, Mekahli D: Is autosomal dominant polycystic kidney disease becoming a pediatric disorder? Front Pediatr 2017, 5:272
8. Horie S, Muto S, Kawano H, Okada T, Shibasaki Y, Nakajima K, Ibuki T: Preservation of kidney function irrelevant of total kidney volume growth rate with tolvaptan treatment in patients with autosomal dominant polycystic kidney disease. Clin Exp Nephrol 2021, 25:467−478
9. Talbi K, Cabrita I, Kraus A, Hofmann S, Skoczynski K, Kunzelmann K, Buchholz B, Schreiber R: The chloride channel CFTR is not required for cyst growth in an ADPKD mouse model. FASEB J 2021, 35:e21897
10. Barone S, Zahedi K, Brooks M, Henske EP, Yang Y, Zhang E, Bissler JJ, Yu JJ, Soleimani M: Kidney intercalated cells and the transcription factor FOXi1 drive cystogenesis in tuberous sclerosis complex. Proc Natl Acad Sci U S A 2021, 118. e2020190118
11. Blomqvist SR, Vidarsson H, Fitzgerald S, Johansson BR, Ollerstam A, Brown R, Persson AE, Bergström GG, Enerbäck S: Distal renal tubular acidosis in mice that lack the forkhead transcription factor Foxi1. J Clin Invest 2004, 113:1560−1570
12. Pannabecker TL: Comparative physiology and architecture associated with the mammalian urine concentrating mechanism: role of inner medullary water and urea transport pathways in the rodent medulla. Am J Physiol Regul Integr Comp Physiol 2013, 304:R488−R503
13. Zhai XY, Fenton RA, Andreasen A, Thomsen JS, Christensen EI: Aquaporin-1 is not expressed in descending thin limbs of short-loop nephrons. J Am Soc Nephrol 2007, 18:2937−2944