# Decorating Histones in Polycystic Kidney Disease

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*JASN* 33: 1–2, 2022.
doi: https://doi.org/10.1681/ASN.2022070750

Autosomal dominant polycystic kidney disease (ADPKD) is perhaps the most common monogenetic condition known to humankind. The pathologic hallmark is the presence of innumerable fluid-filled tissue sacs called cysts that inundate the renal parenchyma. The cysts are derived from renal tubules, primarily the collecting duct, and are lined by a single layer of epithelial cells that exhibit high proliferation and excessive fluid secretion. Over years and decades, the relentless growth in cyst volume eventually produces massive bilateral kidneys and results in kidney failure in nearly 50% of individuals with ADPKD.

The inciting molecular event in virtually all clinically relevant cases is a monoallelic loss-of-function mutation of the *PKD1* gene or the *PKD2* gene. The precise sequence of events that unfold next is not entirely clear, but we know that multiple signaling cascades get activated. The cAMP pathway is the best studied, and its deregulation is the basis for using tolvaptan, the only Food and Drug Administration–approved treatment for ADPKD. However, the list of deregulated pathways is long and ever expanding. It includes many notable oncogenic and proproliferative pathways, such as c-Myc, STAT, mammalian target of rapamycin, YAP/TAZ, microRNAs, and many other signaling pathways.[1,2] Not surprisingly, this widespread dysregulation is facilitated by and leads to genome-wide transcriptomic rewiring. How does polycystic kidney disease (PKD) gene loss unleash this broad aberrant signaling? Is there a method behind this madness? There cannot be a single simple answer, but emerging evidence suggests that one clue may be to look at the epigenome.[3(preprint)]

Histones are structural nuclear proteins; the DNA wraps around them to form the nucleosome, the fundamental subunit of chromatin. Histones undergo dynamic post-translational chemical modifications, such as acetylation or methylation of lysine residues. These epigenetic modifications affect gene activity but without changing the DNA nucleotide sequence. The positively charged histone lysine binds to the negatively charged DNA. Acetylation neutralizes the lysine and loosens these DNA-histone interactions, making DNA more accessible to the transcription activation machinery. We and others have found that thousands of gene regulatory enhancers and super-enhancers exhibit increased histone lysine acetylation in cystic tissues, which may explain the widespread mRNA upregulation in PKD.[3(preprint)] Conversely, certain histone methylations enhance DNA compaction and contribute to gene silencing. However, acetylation and methylation are hardly the only histone modifications. In fact, with the advent of newer mass spectrometry–based approaches, the epigenetics field is evolving rapidly, and the repertoire of histone modifications has expanded dramatically in recent years.[4,5] The list now includes changes, such as histone lysine propionylation, succinylation, malonylation, glutarylation, $\beta$-hydroxybutyrylation, and crotonylation. These modifications have been studied in the context of metabolic stress, tissue injury, and carcinogenesis, but their role in kidney diseases remains unexplored.

In this issue of *JASN*, Dang *et al.*[6] examined the role of lysine crotonylation (Kcr), one of the newer histone modifications. Compared with the well-studied histone lysine acetylation, Kcr differs in hydrocarbon chain length, which may allow Kcr to exert enhanced *cis*-effects on gene activation. The metabolite crotonyl-CoA, an important component of fatty acid and amino acid metabolism, serves as the acyl donor for the Kcr reaction. The authors report that Kcr levels are elevated, whereas the enzyme CDYL, which hydrolyzes crotonyl-CoA and thereby, depletes the raw material needed for Kcr, is downregulated in ADPKD models. Crotonylation can be found on 28 lysine residues on various histones, each with its own unique effect on gene activity. Impressively, using HPLC-MS/MS–based proteomics, the authors pinpoint H3K18cr as the primary modification in a human *PKD1*-mutant cell line. Next, they discovered a set of PKD-relevant gene targets whose activation appears to rely on the Kcr modification. Importantly, they find that transgenic expression of CDYL lowers Kcr levels and attenuates cystic disease in a *Pkd1*-mutant mouse model. Finally, they demonstrate that the CDYL enzyme must form phase-separated condensates to execute crotonyl-CoA hydrolysis. A phase separation–incompetent CDYL is unable to lower Kcr levels or attenuate cyst size in a *pkd2* knockout zebrafish model.

This elegant biochemical work by Dang *et al.*[6] is the first to link Kcr to ADPKD progression, and it opens a new area

Published online ahead of print. Publication date available at www.jasn.org.

See related editorial, "Nuclear Condensation of CDYL Links Histone Crotonylation and Cystogenesis in Autosomal Dominant Polycystic Kidney Disease," on pages XXX–XXX.

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of research in kidney diseases. Like any other important study, this one too raises as many interesting questions as it answers. The authors provide a balanced discussion and highlight the need to confirm the cyst-modulating effect of CDYL/Kcr in additional ADPKD models and determine why CDYL is downregulated.

More exciting are the broader conceptual questions and implications. In the last 10 years, it has become clear that reprogrammed cellular metabolism is a pathologic hallmark of ADPKD. Altered metabolism is primarily thought to fulfill the anabolic needs of the proliferating cyst cells. However, reprogrammed metabolism is probably just as important in facilitating the epigenome rewiring, considering that it generates abundant raw materials (acetyl-CoA, acyl-CoA, and others) for the various histone modifications. Mapping out how altered nutrient utilization facilitates a cystogenesis-favorable epigenetic output will uncover metabolic vulnerabilities and provide ideas for targeted dietary therapeutic interventions. As an example, we recently showed that *Pkd1*-mutant cells are dependent on the methylation of cyst-pathogenic mRNAs for their growth. Restricting dietary methionine reduced mRNA methylation and attenuated cyst growth.[7] Microbiota-derived short-chain fatty acids may be the metabolic source for histone crotonylation.[4,8] Could these be the bottleneck for histone crotonylation? Additionally, how proximal are these epigenetic events to PKD gene loss? Interestingly, recent work suggests that Polycystin-1, the protein product of the *PKD1* gene, is directly involved in cellular energetics and mitochondrial function.[9,10] Thus, epigenome changes are unlikely to be a distal epiphenomenon, and the "Polycystin-1–mitochondria-epigenetic" axis is worth future exploration. More broadly, what is the physiologic function of crotonylation or any of the other new histone modifications? They do not exist to solely cause disease. What aspect of normal kidney development or function do they regulate? Understanding the physiologic function is the key to devising a safe therapeutic targeting strategy. Finally, are there other histone "decorations" awaiting discovery in ADPKD or for that matter, the larger kidney disease field?

## DISCLOSURES

Vishal Patel has patents involving anti–microRNA-17 for the treatment of ADPKD (16/466,752 and 15/753,865). V. Patel serves as a scientific consultant for Maze Therapeutics, Otsuka Pharmaceuticals, and Regulus Therapeutics. The laboratory of V. Patel has sponsored research agreements with Myonid Therapeutics, Regulus Therapeutics, Sanofi SA, and Vifor Pharma. V. Patel reports patents or royalties with Regulus Therapeutics and an advisory or leadership role with the Polycystic Kidney Disease Foundation and *Scientific Reports*. The remaining author has nothing to disclose.

## FUNDING

The work in our laboratory is supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases grant R01DK102572, the PKD Foundation, and the US Department of Defense grant D01W81XWH1810673.

## ACKNOWLEDGMENTS

The content of this article reflects the personal experience and views of the author(s) and should not be considered medical advice or recommendation. The content does not reflect the views or opinions of the American Society of Nephrology (ASN) or *JASN*. Responsibility for the information and views expressed herein lies entirely with the author(s).

## AUTHOR CONTRIBUTIONS

V. Patel wrote the original draft; and V. Patel and H. Ramalingam reviewed and edited the manuscript.

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