Innovation through Validation
The Case for Cross-Species Approaches

Kelly A. Hyndman

Kidney360 7: 695–697, 2026. doi: https://doi.org/10.34067/KID.0000001101

During 1929, in the American Journal of Physiology, Dr. August Krogh wrote, "For such a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied."[1] This statement was termed as the Krogh Principle in 1975 by Hans Krebs,[2] the Nobel Laurate who discovered the Krebs (tricarboxylic acid) cycle, and has been embraced by researchers who use a comparative approach. For centuries, scientists have used model organisms to test their hypotheses and relate the findings to human health. With the genome and molecular biology tool explosion in the early 2000s, it has now become easier to use a variety of species to study physiologic and pathologic processes. A great example is with the development of engineering genetic knockout/knockin rodent models predominantly in mice, which have dominated basic science research over the past decade and seem to be a favorite of a reviewer to ask, "have you considered a knockout mouse model?." But reflecting on the Krogh Principle, the mouse is likely not the answer to every question, yet modern scientific tools may not be available for every species. In this issue of *Kidney360*, Isnard *et al.*[3] reported transcriptional changes in the rat kidney using bulk RNA sequencing and spatial transcriptomics. Spatial transcriptomics combines imaging with RNA sequencing, thus allowing the integration of cellular structure with gene expression for a deeper understanding of the biology of the system. There are many commercial platforms available, and each has its own strengths and limitations.[4]

In their study, Isnard *et al.*[3] modeled three types of trauma on the basis of reports from the French TraumaBase registry, which included intensive care-admitted patients, 22% of whom had hemorrhagic shock (HS) and 18% had severe rhabdomyolysis (RM). Of these patients with RM, half also had HS.[3] These clinical conditions were modeled in Sprague Dawley rats by (*1*) inducing HS by controlled bleeding that causes a drop in mean arterial pressure to 35±2.5 mm Hg for 90 minutes, followed by a recusation protocol; (*2*) inducing RM with glycerol intramuscular injections; and (*3*) combining them to form the RM-HS group.

Mice, rats, and humans shared a common ancestor about 75 million years ago, and the mouse, rat, and human genomes encode a similar number of genes.[5] Moreover, almost all of the human disease-associated genes have orthologues in the rat.[5] However, one notable omission in the rodent genome is *APOL1*, whose variants are risk factors for kidney disease.[6] Nevertheless, most genes are conserved among these species. Given the larger body size of the rat compared with the mouse and other physiologic measures that closely align with human physiology, it was the preferred species for this study. However, there are a few limitations of using rats in certain molecular studies. First is the rat reference genome had errors and incomplete gene annotations in early versions (Rnor_6 or earlier). Thankfully, a new, improved annotated rat genome was released in 2024 (mRatBN7.2 also known as GRCr8).[7] A second concern is that for some technologies rat-specific probes are not yet available or may be cost prohibitive from generating custom genome wide species-specific probes. Isnard *et al.*[3] did not see this as a limitation and instead used the NanoString GeoMx digital spatial profiler platform with mouse probes and validated these for use in rats. To validate their approach, the authors mapped the sequence of the mouse probes to the rat genome and then compared the genes sequenced from the GeoMx with their bulk RNA-sequencing dataset. By setting a threshold of >90% nucleotide identity, they predicted that of the

Section of Cardio-Renal Physiology and Medicine, Division of Nephrology, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama

Correspondence: Dr. Kelly A. Hyndman, email: kellyhyndman@uabmc.edu

See related article, "The Spatially Resolved Kidney Transcriptome Signatures in Rat Models of Trauma-Induced Acute Kidney Injury," on pages 711–727.

19,965 mouse gene-specific probes 12,636 would bind to genes in the rat representing 63% gene coverage. Next, they correlated these predicted rat genes captured by GeoMx with those identified through their bulk kidney RNA sequencing. The GeoMX genes represented about 75% of the genes detected in their bulk kidney sequencing, and approximately 71% of the genes reported from the kidney of a male Wistar Han rat.[8] Given this high predicted overlap in potentially detectable genes, what did they report?

In their regions of interest, which included glomeruli, cortex, the cortical-outer medullary junction, and the medulla (132 regions analyzed), 9272 genes were sequenced with the GeoMx platform, compared with 10,391 from the bulk kidney RNA sequencing. Of these, 42% were conserved across both datasets. Although it seems plausible that bulk RNA sequencing would detect more genes that may not be represented in the selected regions of interest used in GeoMx, it is noteworthy that 25% of the GeoMX-identified genes were not captured in the bulk dataset. The specific genes and regions were not reported by the authors, but this finding may suggest enrichment in particular cell types (potentially rare cell types) that were too dilute to be detected in the whole-kidney transcriptome. These are exciting findings that would have been missed if not for their spatial transcriptomic approach.

The glomerular regions of interest had few differentially expressed genes, consistent with RM and HS being more injurious to the tubules.[9] As you might expect in AKI, differentially expressed genes in the cortex, cortical-outer medullary junction, and medulla were enriched in pathways associated with hypoxia, metabolic derangements, mitochondrial dysfunction, and inflammation. When comparing the transcriptomes from the RM and HS groups, RM resulted in changes in all three regions of interest, whereas HS predominantly drove transcriptional changes in the cortical-outer medullary junction. RM caused a robust activation of inflammation and oxidative responses, where HS caused a down regulation of genes involved in metabolism and mitochondrial respiration. RM led to medulla specific upregulation of the transcription factors *Stat5a* and *Stat5b*, whereas genes like *PLIN2*, a gene that encodes a protein involved in regulation of lipid metabolism, had cell and region-specific increases in RM and RM+HS kidneys. This gene was validated at the protein level by immunolocalization of it to the tubules in the RM rat and in tubules from a kidney biopsy from a person with early ischemic AKI. The authors concluded that RM dominates in driving transcriptional changes in response to trauma-induced AKI with RM+HS triggering synergistic but catastrophic transcriptional changes that associate with increased mortality. In addition, they proposed the working hypothesis that *PLIN2* may be central in promoting tubular injury during trauma.

By using spatial transcriptomics and validating this technology in rats, the authors identify new target genes and pathways for their clinically relevant models. As scientists, we should encourage—not discourage—the use of diverse models and approaches to better understand physiology and the initiation and progression of disease. Although human-derived organoids and other technologies are excellent tools for testing specific hypotheses, they cannot fully replicate the concerted interactions among different cell types, circulating factors, biophysical properties, chemical gradients (particularly in the medulla), cross-organ communication, and more. With advances in artificial intelligence and machine learning, we may begin to reduce the number of animals required to answer complex questions, but *in vivo* validation should remain an essential cog in the wheel of science. There are numerous examples of how discoveries in animals have led to important medical advances for humankind. One of my favorite examples is the discovery of a protein exendin-4 that was highly induced after the Gila monster (the only venomous lizard in the United States) ate a meal.[10] This protein is a glucagon-like peptide-1 (GLP-1) receptor agonist with longer action than GLP-1 and this research led to the development of the GLP-1 receptor agonists that are revolutionizing human health. As technology continues to develop at a rapid pace, the integration of diverse experimental models with cutting-edge computational tools will be critical for bridging fundamental discoveries to clinical breakthroughs. By embracing both traditional *in vivo* approaches and emerging platforms, we can ensure that scientific progress remains rigorous, translational, and ultimately transformative for human health.

Disclosures

Disclosure forms, as provided by each author, are available with the online version of the article at http://links.lww.com/KN9/B415.

Author Contributions

Conceptualization: Kelly A. Hyndman.
Funding acquisition: Kelly A. Hyndman.
Writing – original draft: Kelly A. Hyndman.
Writing – review & editing: Kelly A. Hyndman.

Funding

K.A. Hyndman: School of Medicine, University of Alabama at Birmingham (intramural support).

Acknowledgments

The content of this article reflects the personal experience and views of the author 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 *Kidney360*. Responsibility for the information and views expressed herein lies entirely with the author.

References

1. Krogh A. The progress of physiology. *Science.* 1929;70(1809):200–204. doi:10.1126/science.70.1809.200
2. Krebs HA. The August Krogh principle: "for many problems there is an animal on which it can be most conveniently studied". *J Exp Zool.* 1975;194(1):221–226. doi:10.1002/jez.1401940115
3. Isnard P, Laemmel E, Martinez T, et al. The spatially resolved kidney transcriptome signatures in rat models of trauma-induced acute kidney injury. *Kidney360.* 2026;7(4):711–727. doi:10.34067/KID.0000001045
4. Lim HJ, Wang Y, Buzdin A, Li X. A practical guide for choosing an optimal spatial transcriptomics technology from seven major commercially available options. *BMC Genomics.* 2025;26(1):47. doi:10.1186/s12864-025-11235-3

5. Gibbs RA, Weinstock GM, Metzker ML, et al.; Rat Genome Sequencing Project Consortium. Rat genome sequencing project C: genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature*. 2004;428(6982):493–521. doi:10.1038/nature02426
6. Pollak MR, Friedman DJ. APOL1 and APOL1-Associated kidney disease: a common disease, an unusual disease gene - proceedings of the Henry Shavelle professorship. *Glomerular Dis.* 2023;3(1):75–87. doi:10.1159/000529227
7. de Jong TV, Pan Y, Rastas P, et al. A revamped rat reference genome improves the discovery of genetic diversity in laboratory rats. *Cell Genom.* 2024;4(4):100527. doi:10.1016/j.xgen.2024.100527
8. Söllner JF, Leparc G, Hildebrandt T, et al. An RNA-seq atlas of gene expression in mouse and rat normal tissues. *Sci Data.* 2017;4:170185. doi:10.1038/sdata.2017.185
9. Hebert JF, Burfeind KG, Malinoski D, Hutchens MP. Molecular mechanisms of rhabdomyolysis-induced kidney injury: from bench to bedside. *Kidney Int Rep.* 2023;8(1):17–29. doi:10.1016/j.ekir.2022.09.026
10. Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem.* 1992;267(11):7402–7405. PMID: 1313797