

Supplementary Information

Title Longitudinal Multi-Modality MRI–Ultrasound Cyst-Level Analysis of Polycystic Kidney Disease in a Feline Model: A Proof-of-Concept Study

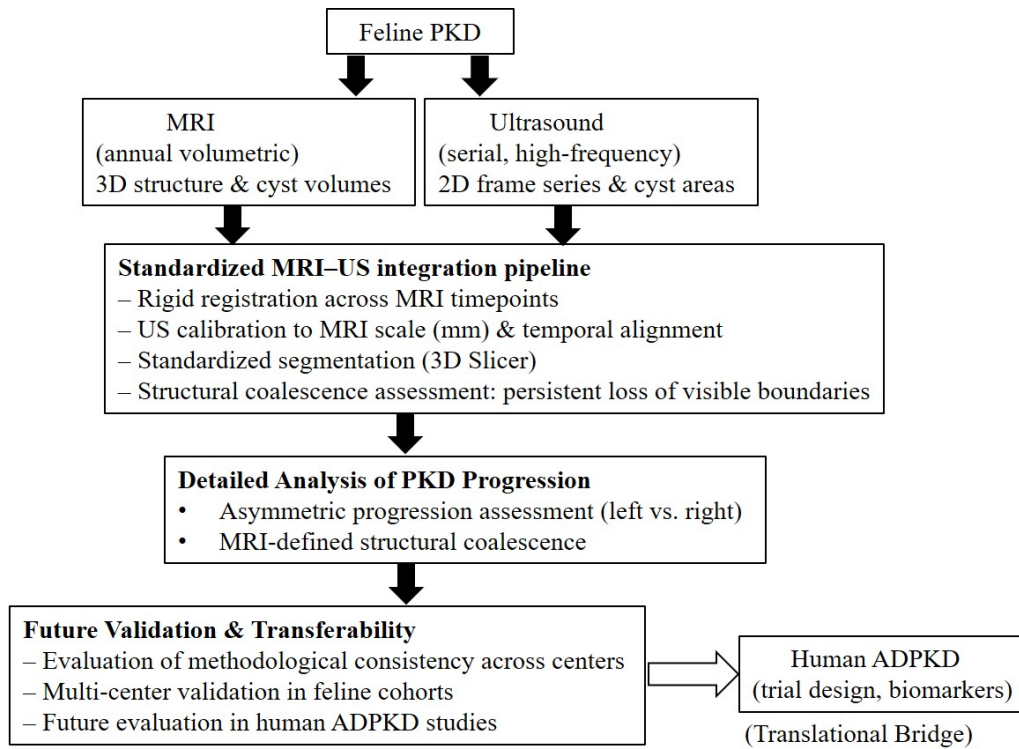
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Note. Supplementary materials are provided in a single file, as detailed in the following sections.

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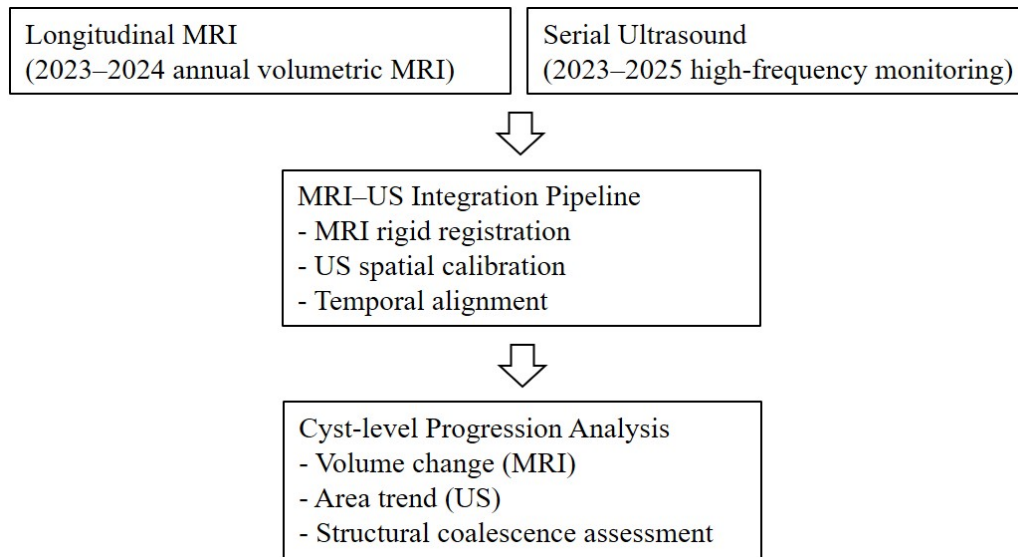
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Graphical Abstract

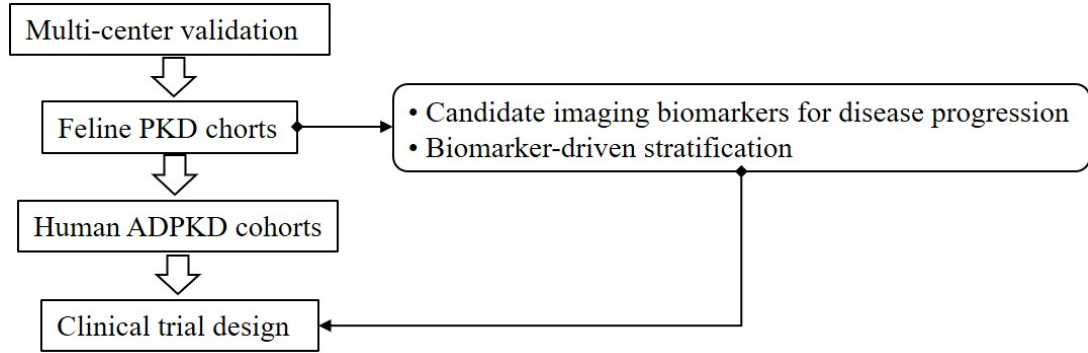


Graphical Abstract. **Summary of the MRI-US integrated framework for cyst progression analysis in feline PKD.** The approach combines high-resolution MRI with high-frequency ultrasound to capture volumetric progression and structural changes. This dual-modality workflow offers complementary spatial and temporal insights, enabling early detection of asymmetric disease progression. The framework demonstrates methodological feasibility and provides a basis for future multi-center validation.

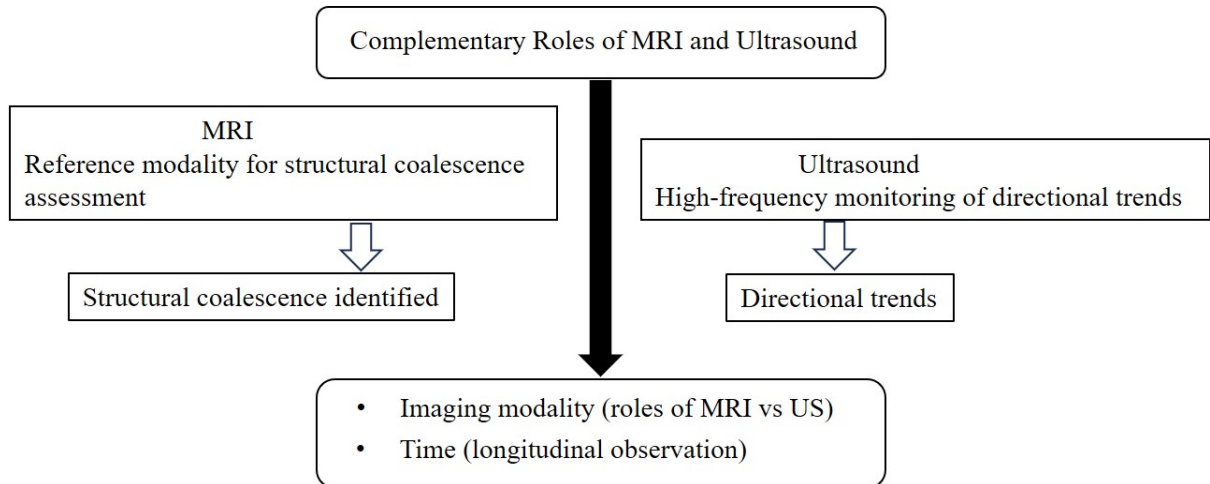
Supplementary Figures



Supplementary Fig. S1. **Workflow of a standardized longitudinal MRI–US integration framework for cyst progression analysis.** The diagram illustrates the steps for MRI–US registration, calibration, and segmentation used to derive longitudinal metrics for cyst progression analysis. Although based on a single case ($n=1$), the workflow demonstrates methodological feasibility and provides a foundation for future multi-center validation.



Supplementary Fig. S2. **Future directions of the framework.** A roadmap diagram illustrates the planned progression of this research framework. The workflow begins with multi-center validation in feline PKD cohorts, proceeds through human ADPKD cohorts, and culminates in clinical trial design. Future steps include the evaluation of MRI-defined structural coalescence as a candidate imaging biomarker for disease progression and its integration with biomarker-driven stratification (e.g., vasopressin dynamics, plasma copeptin levels). This conceptual roadmap highlights the translational trajectory from veterinary applications toward human clinical implementation, emphasizing the potential for cross-species validation and future clinical trial planning.



Supplementary Fig. S3. **Complementary functions of MRI and ultrasound in the current study.** MRI served as the reference modality for identifying structural coalescence, whereas ultrasound provided high-frequency monitoring of directional trends. Together, this figure demonstrates how the dual-modality workflow balances methodological rigor with practical applicability, bridging proof-of-concept results to future translational pathways.

Supplementary Methods

Imaging Protocols and Preprocessing. MRI volumes were acquired annually, and ultrasound images were obtained serially across multiple time points. MRI volumes were rigidly registered, and ultrasound images aligned to the MRI timeline. Segmentation was performed using 3D Slicer with fixed thresholds and smoothing parameters.

Data Sharing Protocol and Ethics. The data used in this study will be shared upon reasonable request after publication. In accordance with data-use agreements (DUA), appropriate privacy protection and compliance measures will be implemented to ensure the proper management of the data. The data will exclude personal identifiers and will be provided solely for academic and non-commercial research purposes. This protocol aims to support methodological transparency and facilitate future independent validation through controlled data access. All data sharing will be conducted with explicit written consent from the owner, ensuring transparency and compliance while respecting the welfare of the animals and the rights of the owners.

Supplementary Table

Supplementary Table S1. **Segmentation Parameter Settings and Standardization Checklist (3D Slicer).** This table outlines the segmentation parameter settings used in 3D Slicer, including the MRI and ultrasound modalities, segmentation methods, and data export protocols. Although based on a single case (n=1), standardized procedures were applied consistently across imaging timepoints, providing a methodological basis for future multi-center validation.

Item	Description	Standardization applied
Software platform	3D Slicer (v5.8.1), open-source	Version-controlled
Imaging modalities	MRI (T2-weighted), Ultrasound (2D)	Acquisition parameters fixed across timepoints
Segmentation method	Manual and semi-automated (thresholding, smoothing)	Same settings across sessions
Observer	Single trained operator	Same operator across all timepoints
ROI selection	Three largest cysts (MRI), largest cyst (US)	Defined <i>a priori</i>
Alignment	Rigid registration to anatomical landmarks	Standardized pipeline
Data export	Segment Statistics module outputs	CSV outputs archived