Comparative *PKD1* and *PKD2* Missense Variant Profiling Aids Molecular Diagnoses Across the ADPKD Spectrum and Reveals Common Pathomechanisms

Cynthia J. Sieben, Ph.D.;[1] Vladimir G. Gainullin, Ph.D.;[1] Christina M. Heyer;[1] Michael R. Hinten, Ph.D.;[1] Elizabeth K. Dillinger;[2] Sarah R. Senum;[1] Xiaobo Sun;[2] Ka Thao;[1] Ines Sturmlechner, Ph.D.;[3] and Peter C. Harris, Ph.D.[1,2]

[1]Department of Medicine, Division of Nephrology & Hypertension Research; [2]Department of Biochemistry & Molecular Biology; and [3]Department of Immunology, Mayo Clinic, Rochester, MN, USA.

Autosomal dominant polycystic kidney disease (ADPKD), characterized by fluid-filled renal cysts, often leading to kidney failure, is typically caused by monoallelic *PKD1* or *PKD2* variants. The advent of genome-wide variant screening has emphasized the importance of ADPKD molecular diagnostic methods to reliably determine the pathogenicity of variants of unknown significance (VUS).

Here, we aimed to develop improved methods for determining *PKD1* and *PKD2* missense variant pathogenicity. Utilizing 48 *PKD1* and 44 *PKD2* ADPKD variants, we assessed variant pathogenicity using: 1) current standards (American College of Medical Genetics and Genomics [ACMG] guidelines), 2) recently developed bioinformatic tools (Rare Exome Variant Ensemble Learner [REVEL] and Combined Annotation-Dependent Depletion [CADD]), 3) our in-house bioinformatic/manual prediction method (Variant Strength Group [VSG] Categorization), and 4) a novel cell-based flow cytometry assay (utilizes localization of polycystin 1 [PC1; *PKD1*] to the apical plasma membrane as a readout; requires PC2 [*PKD2*]).

According to ACMG guidelines, the majority of tested monoallelic *PKD1* and *PKD2* missense variants are VUS (only 22% *PKD1* and 18% *PKD2* pathogenic). However, using REVEL, CADD, our VSG categorization, and *in vitro* assay, we find that a larger number of these variants are predicted to be pathogenic (REVEL: 52% and 94%, CADD: 93% and 97%, VSG: 89% and 94%, and *in vitro*: 89% and 76%, *PKD1* and *PKD2*, respectively). Correlation analyses reveals the most consistency between our VSG categorization and *in vitro* analyses, and the *in vitro* assay provides information on allele penetrance and underlying pathomechanisms.

Together, these studies highlight the need for improved methods for determining variant pathogenicity, beyond the use of ACMG guidelines alone, and indicate that our VSG categorization and *in vitro* analyses are most informative. This suggests that developing an improved model, incorporating some of these additional tools, would provide a more efficient and reliable method for determining non-truncating variant pathogenicity.

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