Polycystic kidney disease caused by bilineal inheritance of truncating PKD1 as well as PKD2 mutations

Losekoot, M.; Meijer, E.; Hagen, E.C.; Belostotsky, V.; Borst, M. de; Tholens, A.; ... ; Peters, D.J.M.

Polycystic Kidney Disease Caused by Bilineal Inheritance of Truncating *PKD1* as Well as *PKD2* Mutations

Monique Losekoot[1], Esther Meijer[2], E. Christiaan Hagen[3], Vladimir Belostotsky[4], Martin de Borst[2], Aart Tholens[1], Marion Phylipsen[1], York Pei[5], Ron T. Gansevoort[2] and Dorien J.M. Peters[6]

[1]Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; [2]Department of Nephrology, University Medical Center Groningen, University Hospital Groningen, Groningen, Netherlands; [3]Department of Internal Medicine, Meander Medisch Centrum, Amersfoort, Netherlands; [4]Department of Pediatrics, Faculty of Health Sciences, McMaster Children's Hospital, Hamilton, Ontario, Canada; [5]Division of Nephrology, University Health Network, University of Toronto, Toronto, Ontario, Canada; and [6]Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands

Correspondence: Dorien J.M. Peters, Department of Human Genetics, Postzone S-04-P, Leiden University Medical Center (LUMC), P.O. Box 9600, 2300 RC Leiden, Netherlands. E-mail: d.j.m.peters@lumc.nl

Received 27 December 2019; revised 3 July 2020; accepted 7 July 2020; published online 17 July 2020

Kidney Int Rep (2020) **5,** 1828–1832; https://doi.org/10.1016/j.ekir.2020.07.006

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disorder. The disease is characterized by progressive cyst formation in both kidneys and loss of renal function, which typically leads to end-stage renal disease (ESRD) in the fourth to sixth decade of life.[1] In the majority of patients the disease is caused by a mutation in either the *PKD1*-gene or the *PKD2*-gene.[S1,S2]

The type of mutation, *PKD1* truncating, *PKD1* nontruncating or *PKD2* mutation, is a strong predictor for the age of ESRD.[2,3] In general, patients carrying a *PKD1* truncating mutation show a more rapidly progressing disease, whereas patients carrying a *PKD2*-mutation, show the mildest phenotype.[2] Nevertheless, the wide range in age at which ESRD is reached within families with ADPKD makes clear that additional modifying genes and/or modifying factors play a role.[S3–S5]

Co-inheritance of a *PKD1* as well as a *PKD2* pathogenic variant is extremely rare. The first case was described by Pei *et al.,* in a large family with segregation of a *PKD1* nontruncating mutation p.(Tyr528-Cys), initially identified by linkage, and a truncating *PKD2* mutation p.(Leu736*).[4] Two affected individuals were trans-heterozygous for these mutations and their renal disease was more severe than in individuals who had either mutation alone. Similarly, more progressive disease was described in a patient with bilineal inheritance of a truncating *PKD1* p.(Gln2196*) and a *PKD2* missense mutation p.(Arg420Gly).[5] Compound heterozygotes for 2 *PKD1* mutations have been described more frequently, including either one truncating with 1 missense mutation or 2 missense mutations in *trans*. Overall the phenotypes vary from more or less typical to early onset *in utero*.[S6–S10] In addition, a patient homozygous for a *PKD2* missense mutation with neonatal onset has been reported.[S11]

Here we describe for the first time 2 patients trans-heterozygote for truncating mutations in both *PKD1* and *PKD2*, and their families. The patient described in case 1 presented with a classical phenotype. However, disease progression accelerated much faster compared to the typical patients with a truncating mutation in either gene.[S12] The patient described in case 2, an offspring of 1 family with 3 generations ADPKD, was diagnosed soon after birth with cysts in both kidneys. Written informed consent was obtained for these studies.

CASE PRESENTATION

Patient From Family 1

A patient diagnosed with ADPKD at 18 years of age was referred to the University Medical Center in Groningen, the Netherlands. He participated in a clinical study (lanreotide vs. standard care), and at time of referral he was 36 years old and had an estimated glomerular filtration rate (eGFR) of 34 ml/min per 1.73 m^2

Table 1. Clinical and genetic characteristics of family members

Individual	Gender	*PKD1* mutation	*PKD2* mutation	Age at diagnosis, yr	eGFR (CKD-EPI), ml/min per 1.73 m^2	eGFR decline, ml/min per 1.73 m^2	ESRD age, yr	Renal volume (hTKV), ml/m	Additional features
Case 1									
Index	Male	c.439delG p.(Val147Trpfs*143)	c. 169C>T p.(Gln57*)	18	Age 36 yr: 34 Age 38 yr: 15	−7.6	38	Age 36 yr: 1315 Age 38 yr: 2296	Liver volume (ml): Age 36 yr: 9827 Age 38 yr: 11,758
Father	Male	c.439delG p.(Val147Trpfs*143)	—	45			60		
Mother	Female	—	c. 169C>T p.(Gln57*)	66	Age 66 yr: 46				
Brother	Male	—	c. 169C>T p.(Gln57*)	45	Age 45 yr: >90				CT scan: multiple kidney and liver cysts
Case 2									
Index	Male	c.7288C>T p.(Arg2430*)	c.124_125ins52bp p.(Ala42fs66*)	Postnatal	Age 4 yr: 132[a]				Ultrasound: multiple small cortical cysts
Father	Male	c.7288C>T p.(Arg2430*)	—	18	Age 31 yr: 56			Age 31 yr: 1246	Mild liver cysts
Mother	Female	—	c.124_125ins52bp p.(Ala42fs66*)	19	Age 30 yr: 56				Age 28 yr: mildly enlarged kidneys with small cysts bilaterally by ultrasound; mild liver cysts

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CT, computed tomography; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; hTKV, height-adjusted total kidney volume.
[a]Based on Schwartz formula from Schwartz GJ, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20:629–637.[S13]

(Table 1[S13]).[6] His height-adjusted total kidney volume (htTKV) was 1315 ml/m (Mayo class 1D). He showed a rapid decline in renal function with an eGFR of −7.6 ml/min per 1.73 m^2 per year to an eGFR of 15 ml/min per 1.73 m^2 and htTKV of 2296 ml/m. At 38 years of age, he started hemodialysis, and received a living unrelated kidney transplant after a right-sided nephrectomy at 41 years. In addition, his liver volume was exceptionally large: at referral this was 9.8 L, which increased to 14.2 L at 42 years of age. The patient was referred for exploration of liver transplantation in the future. At age 42 years, magnetic resonance angiography did not show intracranial aneurysms.

Mutation analysis of *PKD1* (NM_00100994.2) and *PKD2* (NM_000297.3) involving direct Sanger sequencing revealed the unique combination of a pathogenic truncating mutation in *PKD1* exon 4 c.439delG p.(Val147Trpfs*143), as well as a pathogenic truncating mutation in *PKD2* exon 1 c.169C>T p.(Gln57*) (Figure 1).[3,6,S14,S15] The *PKD1* mutation was also detected in his affected father, who presented with a classical phenotype, reaching renal failure at 60 years of age. His mother was presumed not to be affected, but DNA analysis showed that the *PKD2* mutation was present in her DNA as well. Recently, at the age of 66 years, she was diagnosed with mild ADPKD, having mild cystic disease in the kidneys and liver as shown on magnetic resonance imaging. Her eGFR (Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI]) was 46 ml/min per 1.73 m^2. Her mother was diagnosed with polycystic kidney disease and started dialysis at 66 years of age.

One of the patient's brothers is carrying only the *PKD2* mutation, presenting with mild PKD at 45 years of age.

Patient From Family 2

A 4-year-old boy who showed echogenic kidneys and enlarged kidneys at 35 weeks prenatally and who presented with renal cysts soon after birth (Table 1) was referred to the McMaster Children's Hospital (Hamilton, ON, Canada). His renal function was normal, with an eGFR of 132 (based on the Schwartz formula[S13]). On ultrasound, multiple small cortical cysts were seen in both kidneys. The largest cyst measured 13 × 9 × 9 mm in the upper pole of the right kidney and 14 × 13 × 10 mm in the lower pole of the left kidney. The right kidney measured 7.2 × 4.4 cm and the left kidney 7.7 × 3.5 cm, which is within the normal range at this age. There was a mild increase in size compared to 6 months earlier (right kidney: 6.5 × 3.3 cm; left kidney: 7.0 × 3.0 cm), but no hydronephrosis or hydroureter. Corticomedullary differentiation was preserved, and there were no signs of hydronephrosis or hydroureter. In the last 2 years, his ultrasound findings were stable, with mild interval enlargement of the renal cysts and mild interval growth of both kidneys. He continued to grow well. No albuminuria or high blood pressure were reported.

The families of both of his parents had been diagnosed with ADPKD (Figure 1). Sanger sequencing of both *PKD1* and *PKD2* showed that his mother is a carrier of a pathogenic truncating *PKD2* mutation in exon 1 c.124_125ins52bp p.(Ala42fs*66) and his father

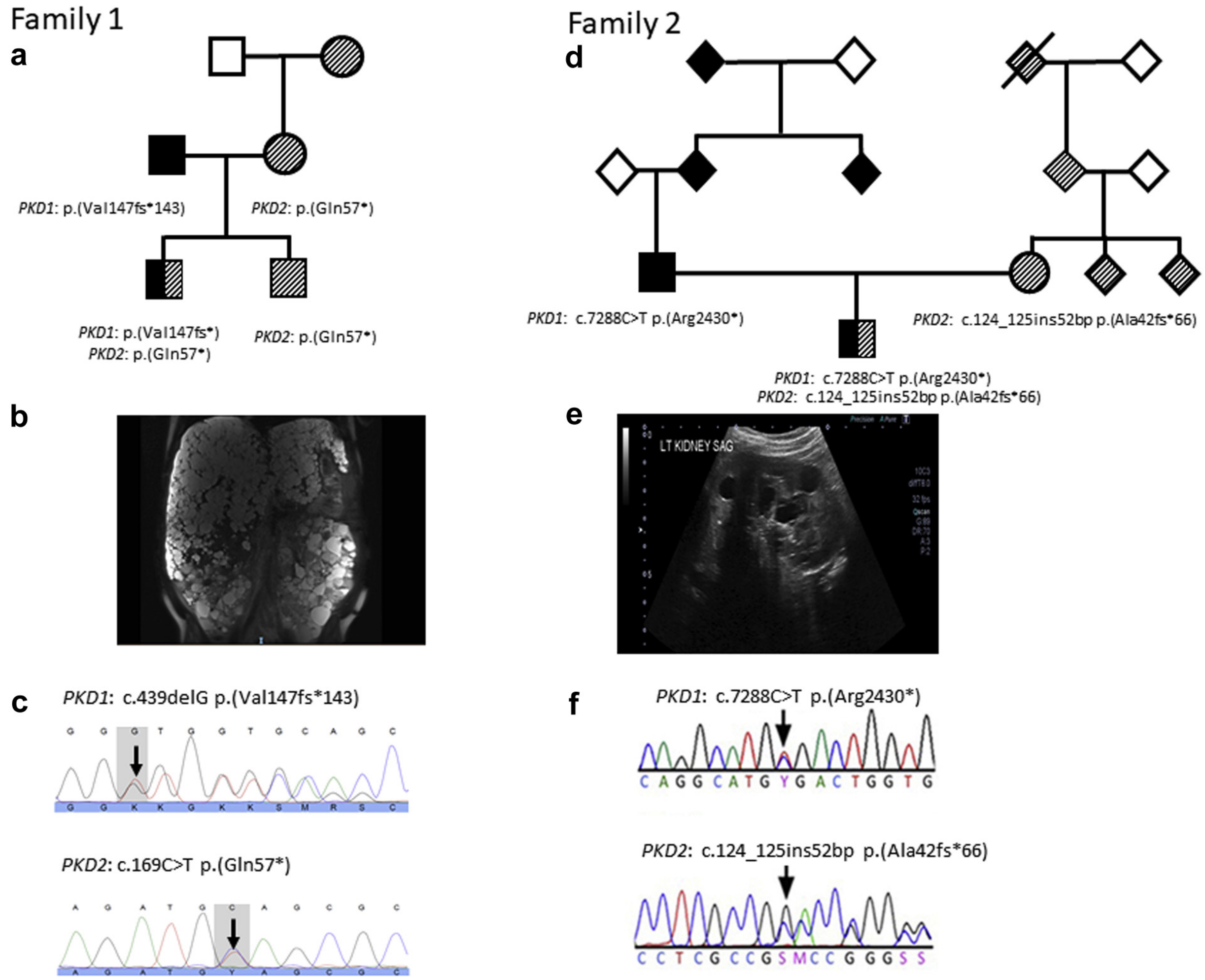

Figure 1. Case 1 (family 1): inheritance of a *PKD1* truncating mutation PKD1 (NM_001009944.2) exon 4: c.439delG p.(Val147Trpfs*143), and *PKD2* truncating mutation PKD2 (NM_000297.3) exon 1: c.169C>T p.(Gln57*). (a) Pedigree of the family with genotypes. Open symbols indicate healthy individuals; filled symbols indicate affected individuals carrying the *PKD1* mutation; shaded symbols indicate mildly affected individuals carrying the *PKD2* mutation. The patient carrying both mutations is indicated with a symbol with both a filled and striped pattern. (b) Magnetic resonance image of the patient at age 38 years. (c) Results of Sanger sequencing analysis of the forward strand of exon 4 of the *PKD1* gene (upper panel) and exon 1 of the *PKD2* gene (lower panel) in the index patient. The position of the mutation is indicated with a gray bar. Mutation analysis was done by direct Sanger sequencing (ABI3730 DNA Analyzer, Applied Biosystems) of all coding exons of *PKD1* and *PKD2*, including flanking intron sequences, as described previously.[6,S14] Case 2 (family 2): inheritance of a *PKD1* truncating mutation (NM_001009944.2) exon 18 c.7288C>T p.(Arg2430*) and *PKD2* truncating mutation (NM_000297.3) exon 1 c.124_125ins52bp p.(Ala42fs66*). (d) Pedigree (anonymized) of the family with genotypes; symbols as described for family 1. (e) Ultrasound image of the patient at 8 months of age. (f) Results of Sanger sequencing analysis of the forward strand of exon 18 of the *PKD1* gene (upper panel) and exon 1 of the *PKD2* gene (lower panel) in the index patient. The position of the mutation is indicated with arrows. Mutation analysis was done by direct Sanger sequencing (ABI3730 DNA Analyzer, Applied Biosystems, Thermo Fisher Scientific, Bleiswijk, the Netherlands) Sanger sequencing to confirm both parental mutations, as described previously.[3,S15]

has a truncating *PKD1* mutation in exon 18 c.7288C>T p.(Arg2430*). The young boy was affected with bilineal inherited disease, that is, carrying the paternal *PKD1* truncating mutation and the maternal *PKD2* truncating mutation.

The father was tested presymptomatically at age 18 years because of a positive family history for ADPKD and turned out to carry the familial *PKD1* mutation. At age 31, he showed moderate disease progression, with eGFR 56 ml/min per 1.73 m^2 and Ht-TKV of 1246 ml/m (Mayo class 1D). Other affected older relatives of the father reached renal failure between 46 and 50 years of age.

His mother was also tested presymptomatically at age 19 years because of a positive family history. She carries the *PKD2* mutation. At age 28, ultrasound imaging showed mildly enlarged kidneys with small cysts bilaterally. At age 30, her eGFR was 56 ml/min per 1.73 m^2. Both parents showed mild liver cysts. There are multiple older affected relatives of the mother, all having the typical mild disease.

DISCUSSION

Here we describe, for the first time, 2 patients carrying a truncating mutation both in *PKD1* and in *PKD2*. The patient described in case 1 developed ESRD at a young age, and the patient described in case 2 was diagnosed postnatally with cysts in both kidneys. Both patients had rapidly progressive disease that was more severe than that of their family members, who had only 1 mutation. The patient described in case 1 required kidney transplantation at the age of 38 years, which was much earlier compared to his father, who received a kidney transplant at the age of 60 years, as well as when compared to the median age for ESRD in patients with truncating *PKD1* mutations of 58 years.[2] Of interest, the patient described in case 1 also showed an extreme liver phenotype, suggesting that the combination of mutations also affects the growth of liver cysts.

The patient described in case 2 showed early presentation of a few renal cysts, detected postnatally by ultrasound, and at age 4 years, multiple cortical cysts could be seen. It is likely that this patient has a more progressive disease than the parents but data at young age from the parents or other affected relatives are not available. An alternative explanation could be the involvement of yet another gene. Therefore, a gene panel−based Next Generation Sequencing approach was used. Both index cases were analyzed for the presence of variants in a gene panel of 76 genes, postulated to be involved in cystic kidney disease (Supplementary Methods). In case 1, no additional variants were identified; however, in case 2, a heterozygous variant in the *VHL* gene (NM_000551.3 (c.556G>A p.[Glu186Lys]) was identified. There are conflicting interpretations of this variant, either as class 3 or class 4 (Supplementary Methods), and it is unlikely that this variant will have an effect on the phenotype of the patient. The parents have not been screened for this variant.

Thus far, only the combination of 1 truncating and 1 missense mutation or 2 missense mutations has been described.[4,5,7,S6−S11] These reports confirmed that in ADPKD patients, the level of functional polycystin-1 or polycystin-2 affects the age of presentation and the disease progression. In addition, it became clear that bilineal inheritance of a *PKD1* and a *PKD2* mutation caused more severe disease than either one of them, pin-pointing them as important disease modifiers.

The very severe disease manifestation seen in the patient described in case 1 is in agreement with a study in mice that found that biallelic heterozygous knock-out mice (*Pkd1*$^{+/-}$;*Pkd2*$^{+/-}$) showed more cysts than the single knock-outs, although renal cystic lesions were mild and variable in single as well as biallelic heterozygous knock-out mice. Importantly, although *Pkd1*$^{+/-}$;*Pkd2*$^{+/-}$ mice showed more cysts than the single knock-outs, they did not show increased mortality or a massive cystic phenotype,[8] in contrast to homozygous knock-out mice (*Pkd1*$^{-/-}$ *or Pkd2*$^{-/-}$), which are embryonic lethal.[S16−S19] The larger number of cysts in the *Pkd1*$^{+/-}$;*Pkd2*$^{+/-}$ mice seemed more than an additive effect, reflecting an increased number of cysts or increased cyst growth, or a combination of both. It is conceivable that triggers such as somatic mutations that have (virtually) no effect in the single heterozygous kidneys do have an effect on cyst formation or growth in the *Pkd1*$^{+/-}$;*Pkd2*$^{+/-}$ kidneys. Furthermore, it cannot be excluded that reduced levels of *Pkd1* or *Pkd2* increase the chance that somatic mutations will occur. Overall, the data confirm that the functional dosage of Polycystin-1 and Polycystin-2 affects disease severity.[8] Moreover, the combination of a truncating *PKD1* and *PKD2* mutation in patients is not lethal (Table 2).

Inter- and intrafamilial phenotypic variability in ADPKD is probably the result of a combination of environmental factors and modifying genetic factors, likely influencing different steps of the disease.[S20,S21] Obviously, the ADPKD genes themselves can function as important modifiers. Even more, a variety of studies have revealed a complex network of genetic and functional interactions between different cystic disease genes.[S22−S30] In agreement with this, a few patients carrying the combination of a *PKD1* mutation with genes involved in other cystic diseases such as *PKHD1* (autosomal recessive PKD) and *HNF1B* (renal cyst and diabetes syndrome) have been described. Overall, the phenotypes of these cases vary from more or less typical to early onset *in utero*.[S9]

Reports on bilineal inheritance of *PKD1* and *PKD2* mutations are rare. This is largely because of the prevalence of the disease and the lower frequency of *PKD2* mutations, which are associated with a milder phenotype.[S31] In routine DNA diagnostics, the *PKD2*-gene is frequently not analyzed after the identification of a *PKD1* mutation. Although rare, this might be critical in the case of a close relative as a living donor who might have a *PKD2* mutation. Furthermore, in recent years, algorithms have been developed that predict

Table 2. Teaching points

The combination of truncating mutations in both *PKD1* and *PKD2* is not embryonic lethal but results in severe, more rapidly progressing disease.
When genetic testing is performed, it is advisable to analyze both genes or a gene panel containing multiple genes, especially in patients with a severe phenotype or when considering a close relative as a living donor.
To correctly interpret the results of a clinical trial, genotyping of both *PKD1* and *PKD2*, or even a gene panel, is essential.
Prediction scores that integrate genetic and clinical data to predict renal survival in patients with autosomal dominant polycystic kidney disease (ADPKD) will become more accurate when genetic data for both genes are included.

individual disease progression and that are used for the optimal selection of patients in clinical trials or to select patients who will benefit from treatments when these become available.[S32,S33] The patient described in case 1 also illustrates that it is advisable to use genotype as part of the inclusion criteria for future clinical trials. This patient's yearly increase in total kidney volume was higher compared to that in the rest of the lanreotide treatment group, affecting the difference between the treated and control group.[6] In addition, the PROPKD score is a prognostic score that integrates genetic and clinical data to predict renal survival in patients with ADPKD.[9,S34] For these specific cases, predictions will become more accurate when genetic data for both genes *PKD1* and *PKD2* are included (Table 2).

With the expected decrease in costs, comprehensive genetic testing using next-generation sequencing methods will become more readily available. When genetic testing is performed, it is advisable to analyze both genes or a gene panel containing multiple genes, especially in patients with a severe phenotype.

In conclusion, our data show that the combination of truncating mutations in both genes is not embryonic lethal but results in a severe, more rapidly progressing disease, and support the role of the *PKD2* gene as a modifier of the more severe disease causing *PKD1* gene.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

This study is supported in part by grants from the Dutch Kidney Foundation (CP10.12 and CP15.01), the Dutch Ministry of Economic Affairs (LHSM15018), and the Canadian Institutes of Health Research (CIHR) Strategy for Patient Oriented Research (SPOR) program for the Canadians Seeking Solutions and Innovations to Overcome Chronic Kidney Disease (Can-SOLVE CKD) Network.

AUTHOR CONTRIBUTIONS

ML, YP, RTG, and DJMP, designed the study; EM, CH, VB, and MB performed clinical analyses; AT and MP performed genetic analyses; ML and DP produced the figures and drafted the paper; all the authors read, edited, and approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Additional Sequencing Methods and Interpretation of VHL Variant.

Supplementary References.

REFERENCES

1. Grantham JJ. Clinical practice. Autosomal dominant polycystic kidney disease. *N Engl J Med*. 2008;359:1477–1485.
2. Cornec-Le GE, Audrezet MP, Chen JM, et al. Type of PKD1 mutation influences renal outcome in ADPKD. *J Am Soc Nephrol*. 2013;24:1006–1013.
3. Hwang YH, Conklin J, Chan W, et al. Refining genotype-phenotype correlation in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2016;27:1861–1868.
4. Pei Y, Lan Z, Wang K, et al. A missense mutation in PKD1 attenuates the severity of renal disease. *Kidney Int*. 2012;81:412–417.
5. Elisakova V, Merta M, Reiterova J, et al. Bilineal inheritance of pathogenic PKD1 and PKD2 variants in a Czech family with autosomal dominant polycystic kidney disease—a case report. *BMC Nephrol*. 2018;19:163.
6. Meijer E, Visser FW, van Aerts RMM, et al. Effect of lanreotide on kidney function in patients with autosomal dominant polycystic kidney disease: the DIPAK 1 randomized clinical trial. *JAMA*. 2018;320:2010–2019.
7. Rossetti S, Kubly VJ, Consugar MB, et al. Incompletely penetrant PKD1 alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease. *Kidney Int*. 2009;75:848–855.
8. Wu G, Tian X, Nishimura S, et al. Trans-heterozygous Pkd1 and Pkd2 mutations modify expression of polycystic kidney disease. *Hum Mol Genet*. 2002;11:1845–1854.
9. Cornec-Le Gall E, Audrezet MP, Rousseau A, et al. The PROPKD Score: a new algorithm to predict renal survival in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2016;27:942–951.