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. In the majority of patients the disease is caused by a mutation in either the \( \text{PKD1} \)-gene or the \( \text{PKD2} \)-gene.\(^{1,2} \) The type of mutation, \( \text{PKD1} \)-truncating, \( \text{PKD1} \)-nontruncating or \( \text{PKD2} \)-mutation, is a strong predictor for the age of ESRD.\(^{2,3} \) In general, patients carrying a \( \text{PKD1} \)-truncating mutation show a more rapidly progressing disease, whereas patients carrying a \( \text{PKD2} \)-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.\(^{5,6,7} \)

Co-inheritance of a \( \text{PKD1} \) as well as a \( \text{PKD2} \) pathogenic variant is extremely rare. The first case was described by Pei et al., in a large family with segregation of a \( \text{PKD1} \) nontruncating mutation p.(Tyr528-Cys), initially identified by linkage, and a truncating \( \text{PKD2} \) 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 \( \text{PKD1} \) p.(Gln2196*) and a \( \text{PKD2} \) missense mutation p.(Arg420Gly).\(^{5} \) Compound heterozygotes for 2 \( \text{PKD1} \) mutations have been described more frequently, including either one truncating with 1 missense mutation or 2 missense mutations in \textit{trans}. Overall the phenotypes vary from more or less typical to early onset \textit{in utero}.\(^{6,8-10} \) In addition, a patient homozygous for a \( \text{PKD2} \) missense mutation with neonatal onset has been reported.\(^{11} \)

Here we describe for the first time 2 patients trans-heterozygote for truncating mutations in both \( \text{PKD1} \) and \( \text{PKD2} \), 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.\(^{12} \) 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\(^2\).
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 m² per year to an eGFR of 15 ml/min per 1.73 m² 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.

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 formula6). On ultrasound, multiple small cortical cysts were seen in both kidneys. The largest cyst measured $13 \times 9 \times 9$ mm in the upper pole of the right kidney and $14 \times 13 \times 10$ mm in the lower pole of the left kidney. The right kidney measured $7.2 \times 4.4$ cm and the left kidney $7.7 \times 3.5$ cm, which is within the normal range at this age. There was a mild increase in size compared to 6 months earlier (right kidney: $6.5 \times 3.3$ cm; left kidney: $7.0 \times 3.0$ cm), but no hydronephrosis or hydroureret. Corticomedullary differentiation was preserved, and there were no signs of hydronephrosis or hydroureret. 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...
has a truncating \textit{PKD1} mutation in exon 18 \textit{c.7288C>T} \textit{p.(Arg2430*)}. The young boy was affected with bilineal inherited disease, that is, carrying the paternal \textit{PKD1} truncating mutation and the maternal \textit{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 \textit{PKD1} mutation. At age 31, he showed moderate disease progression, with eGFR 56 ml/min per 1.73 m² 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 \textit{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 m². Both parents showed mild liver cysts. There are multiple older affected relatives of the mother, all having the typical mild disease.

\textbf{DISCUSSION}

Here we describe, for the first time, 2 patients carrying a truncating mutation both in \textit{PKD1} and in \textit{PKD2}. The
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.4,5,7,56–51 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.16–19 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.20,21 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.22–30 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.27

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.31 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. | S20, S21 |
| 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. | S30 |
| 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.\textsuperscript{5,12,33} 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.\textsuperscript{6} In addition, the PROPKD score is a prognostic score that integrates genetic and clinical data to predict renal survival in patients with ADPKD.\textsuperscript{9,34} For these specific cases, predictions will become more accurate when genetic data for both genes \textit{PKD1} and \textit{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 \textit{PKD2} gene as a modifier of the more severe disease causing \textit{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.

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