Lessons From the Clinic: ADPKD Genetic Test Unraveling Severe Phenotype, Intrafamilial Variability, and New, Rare Causing Genotype

Claudia Izzi1,2, Chiara Dordoni1,2, Elisa Delbarba1, Cinzia Mazza3, Gianfranco Savoldi3, Laura Econimo1, Roberta Cortinovis1, Letizia Zeni1,4, Eva Martin5, Federico Alberici1 and Francesco Scolari1

1Division of Nephrology and Dialysis, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia and ASST-Spedali Civili, Brescia, Italy; 2Medical Genetics Clinic, Department of Obstetrics and Gynecology, ASST-Spedali Civili, Brescia, Italy; 3Medical Genetics Laboratory, ASST-Spedali Civili, Brescia, Italy; 4Università degli Studi della Campania Luigi Vanvitelli, Napoli, Italy; and 5Radiology Unit, Montichiari Hospital, ASST-Spedali Civili, Brescia, Italy

Correspondence: Claudia Izzi, Division of Nephrology and Dialysis, Medical Genetics Clinic, Department of Obstetrics and Gynecology, University of Brescia and ASST-Spedali Civili, Brescia, Italy. E-mail: izziclaudia@yahoo.it

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Autosomal dominant polycystic kidney disease (ADPKD) is the prevalent inherited renal disease worldwide. Cystogenesis originates focally in the tubule and usually starts in utero although ADPKD symptoms usually develop in the fourth decade. The progressive cystic enlargement leads to end-stage renal disease in approximately 70% of patients with a median age of 58 years. Family history is present in >85% of cases; in 10% to 15% of cases, a family history may be absent because of de novo mutation, mosaicism, or mild disease.1,2,81,82

Pathogenic variants in PKD1 and PKD2 genes are responsible for 60% to 78% and 15% to 26% of ADPKD, respectively; approximately 10% to 15% of patients have no recognizable pathogenic variants.1,2,81,82 Recently, whole-exome sequencing studies identified mutations in other cystogenes (e.g., GANAB, DNAJB11, ALG8, ALG9) in a small proportion of patients with ADPKD.1,5,81 Somatic mosaicism can be an alternative explanation of the unresolved cases; finally, some patients may harbor missed rare pathogenic variants in noncoding region of PKD1 and PKD2.1

Renal phenotype variability is well-recognized in ADPKD, because of locus (PKD1 vs. PKD2) and allelic effect (protein-truncating vs. protein-nontruncating variant), gene modifiers, and stochastic and environmental factors.4,5,4,5 Nevertheless, in 1% to 2% of patients with ADPKD, intrafamilial variability may be extreme, characterized by onset in perinatal period in very early onset ADPKD (ADPKD-VEO) or before age of 15 years in early onset ADPKD.4 ADPKD-VEO/early onset ADPKD may carry unusual complex genotypes, characterized by biallelic PKD1 or PKD2 in transinheritance, digenic PKD1/PKD2 variants, or transheterozygosity for PKD1 and PKHD1 or HNF1B.5,7,56-58

Here, we describe a molecular study performed in 7 ADPKD pedigrees of our single-center ADPKD cohort (186 index patients) revealing a complex PKD genotype, including the first adult patient with a digenic inheritance because of PKD1 and PKHD1 variants. Detailed methods and clinical and molecular data are available in Supplementary Material (S1 and S2).

The key findings of our study were threefold. First, we confirm the association between the most severe renal phenotypes and complex genotype, including biallelic inheritance and digenic inheritance, with mutations in different cystogenes (PKD1/PKD2 and PKD1/PKHD1). Second, our data suggest the importance of genotyping in the presence of discordant renal disease severity among affected family members. In
this clinical setting, genotyping has a crucial role in terms of diagnosis (explaining the genetic background of the intrafamilial variability) and major implications for reproductive counseling (see Supplementary Material S3 for detailed genetic counseling information). Third, we first report the causative role of variants located in the untranslated region of *PKD1* gene, suggesting some genetically unexplained cases could harbor mutation in noncoding regions of the *PKD* genes.

Clinical and genetic data of families are detailed in Table 1 and Supplementary Material S2.

Family trees are detailed in Supplementary Figure S1.

In families 1 (Figure 1a and b) and 2, index cases (ICs) presented ADPKD-VEO mimicking autosomal recessive polycystic kidney disease (ARPKD) giving prenatal onset. ADPKD-VEO and ARPKD are difficult to differentiate in perinatal/neonatal period; however, to reach a conclusive diagnosis is relevant for renal prognosis, which is poorer in ARPKD, usually aggravated by hepatic fibrosis (Table 1). In the youngest twin daughters, the discordant early onset ADPKD phenotype was explained by the occurrence of a *de novo* pathogenic

### Table 1. Complex genotypes and clinical phenotypes of 7 ADPKD families revealing a marked intrafamilial phenotypic variability owing to complex genotypes

| P | Genotype: index variant alleles | Phenotype of the index case | Phenotype of the family members |
|---|--------------------------------|----------------------------|--------------------------------|
| 1 | - **PKD1** pat p.Gln4231<sup>a</sup> - **PKD1** mat p.Asp1332Asn | ADPKD-VEO: enlarged hyperechogenic kidneys in utero; enlarged palpable kidneys at birth; HTN, 7 yr; ESRD, 35 yr | Father: ADPKD; ESRD (after right traumatic nephrectomy), 46 yr;<sup>b</sup> Mother: no renal/liver cysts; normal eGFR, 64 yr |
| 2<sup>ab</sup> | - **PKD2** mat p.Ser349Pro - **PKD2** pat p.Gly1944Arg - **PKD2** pat p.Thr2003Ile | ADPKD-VEO: enlarged hyperechogenic kidneys in utero; oligohydramnios. Termination of pregnancy | Mother: ADPKD; HTN, 35 yr; lithiasis; normal eGFR, 38 yr Father: no renal/liver cysts; normal eGFR, 35 yr |
| 3 | - **PKD1** pat p.Arg1951Gln - **PKD1** dn p.Arg2402<sup>c</sup> | Twin 1<sup>d</sup>-ADPKD-EO; HTN, 12 yr; CKD II and TKV 6866cc, 19 yr Twin 2<sup>d</sup>-ADPKD-EO; HTN, 14 yr; CKD II and TKV 5866cc, 19 yr | Father: ADPKD; HTN, 30 yr; normal TKV/eGFR, 50 yr Sibling 1 (only paternal *PKD1* allele); ADPKD; normal TKV/eGFR 30 yr |
| 4 | - **PKD1** p.Cys259Tyr - **PKD2** p.Ala365fs | Sibling 1: ADPKD; HTN, 36 yr; CKD IIIb and TKV 3429cc, 50 yr Sibling 2: ADPKD; HTN, 32 yr; CKD IV and TKV 3315cc, 54 yr | Sibling 3 (maternal *PKD2* allele); ADPKD; CKD II, TKV 2780, 48 yr Mother: CKD,<sup>e</sup> 74 yr |
| 5 | - **PKD1** pat p.Gly1712Arg - **PKD1** mat p.Asp3088Asn | ADPKD, 24 yr, sCr<sub>1</sub> 1.4 CKD III, bilateral massive kidney enlargement left kidney 22 cm, right kidney 23 cm, 34 yr; ESRD, 38 yr. Massive liver enlargement. | Mother: no renal/liver cysts; normal eGFR Father: no renal/liver cysts; normal eGFR |
| 6 | - **PKD1** pat p.Arg1545Cys; - **PKD1** mat p.Arg1545Cys | ADPKD; HTN, 40 yr; CKD IV, 65 yr | Parents: bilateral cystic kidneys; never reached ESRD, 84–95 yr Daughter (PKD1 c.12460C>T–); no liver cysts; normal eGFR, 39 yr |
| 7 | - **PKD1** dn<sup>f</sup> 5' UTR deletion c.-1926–64del | ADPKD; CKD IIIb and TKV 8333cc, 38 yr | Mother: no renal/liver cysts; normal eGFR Father: few renal/liver cysts; normal eGFR Sibling: no renal/liver cysts; normal eGFR |

5' UTR, 5' untranslated region; ADPKD, autosomal dominant polycystic kidney disease; CKD, chronic kidney disease; dn, de novo; eGFR, estimated glomerular filtration rate; EO, early onset; EO, very early onset; ESRD, end-stage renal disease; HTN, hypertension; mat, maternal; P, pedigree; pat, paternal; TKV, total kidney volume; VEO, very early onset; yr, year old.

<sup>a</sup>Deceased. 
<sup>b</sup>Index DNA not available. 
<sup>c</sup>Monzygous twins. 
<sup>d</sup>De novo pathogenic mutation.

Moreover, recently, in the largest series of ADPKD-VEO,<sup>7</sup> a high prevalence (70%) of biallelic *PKD1* variants (hypomorphic variants in trans with a pathogenic variant) was reported; biallelic *PKD2* variants or transheterozygous *PKD1* and *PKD2* variants were found in few additional patients with ADPKD-VEO. The described complex genotypes lead to ADPKD-VEO genesis likely for a mechanism related to reduced gene dosage, according to the threshold model of cystogenesis.<sup>8–10</sup>

Family 3 is of interest because it underlines the need to go beyond the “simple” segregation of the germline PKD familiar variant in pedigree with relevant clinical variability. Indeed, in the youngest twin daughters, the discordant early onset ADPKD phenotype was explained by the occurrence of a *de novo* pathogenic
variant in cis with the paternal PKD1 mutation, thus contributing to more severe phenotype (Figure 1c and d).

Families 4, 5, and 6 exemplify digenic inheritance. In family 4, coinheritance of PKD1 and PKD2 variants is likely the major contributor to intrafamilial variability in adult patients. The most severely affected brothers (Figure 1e) carried both a pathogenic PKD1 missense variant and a truncating PKD2 variant, whereas the youngest sibling with a milder phenotype presented only the PKD2 variant.

In family 5, the clinical diagnosis of severe ADPKD with early manifestations was guided by the severely enlarged polycystic kidneys, typical of dominant form of the disease, and the absence of signs of liver fibrosis. The IC harbored both a de novo, likely pathogenic PKD1 variant and 2 in trans PKHD1 missense variants classified as likely pathogenic and variant of unknown significance which probably contributed to worsen the phenotype (Figure 1f and g). Mutations in multiple PKD genes exerting an aggravating effect have already been reported, that is, Bergmann et al. described 2 clinically discordant ARPKD fetuses born from a mother with PKD2. The authors suggested that the worsening of ARPKD disease in a fetus was due to the coinheritance of biallelic PKHD1 pathogenic variants with the maternal PKD2 variant. In a recent series of early ADPKD, heterozygous PKHD1 changes were detected in addition to the familial mutation in 4 patients. To explain the PKHD1 aggravating effect, Olson et al. described synergistic interactions between PKHD1 and PKD1 in murine models; indeed, digenic murine models for PKHD1 and PKD1 genes were found to develop a more severe renal cystic disease when compared with single PKHD1 homozygous murine model.

To the best of our knowledge, this is the first case of adult ADPKD aggravated by the presence of PKHD1 changes, supporting the hypothesis that PKHD1 and PKD1 gene products cooperate in a common pathway to maintain tubular integrity.

In pedigree 6, the IC presented a de novo atypical ADPKD characterized by renal cysts in slightly increased kidneys with advanced kidney failure (Figure 1a–h). Molecular analysis revealed a homozygous PKD1 hypomorphic variant; the healthy parents were found to be heterozygous. Of note, this variant has already been identified (in addition to another

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Figure 1. Imaging of relevant cases from ADPKD pedigrees revealing a marked phenotypic variability owing to complex genotypes. Pedigree 1: (a) abdomen MRI: index case with enlarged kidneys with multiple cysts; (b) abdomen CT scan: mother, normal kidney. Pedigree 3: abdomen CT scans: (c) index case with enlarged cystic kidney (left kidney length 151 mm) and (d) her father with cystic kidneys moderately enlarged (left kidney length 102 mm). Pedigree 4: (e) abdomen MRI: index case with severe enlargement of kidneys and multiple cysts. Pedigree 5: (f, g) abdomen MRI: index case with massive liver enlargement with multiple cysts. Pedigree 6: (h) abdomen MRI: index case with bilateral cortical renal cysts and small liver cysts. CT, computed tomography; MRI, magnetic resonance imaging.
PKD pathogenic variant) in 3 patients with VEO disease. Supplementary data indicate a hypomorphic variable effect in heterozygous state for this variant, whereas in homozygous state, it may cause mild cystic phenotype resembling late-onset ADPKD.

In family 7, the IC presented a de novo ADPKD phenotype. Next-generation sequencing analysis failed to identify the pathogenic variant, and we first describe a pathogenic *PKD1* deletion in noncoding 5′-untranslated region without involving exon 1 detected by multiplex-ligation–dependent probe amplification analysis (Supplementary Figure S2). The de novo occurrence and cDNA study supported the causative role of the deletion. This finding prompts genomic analysis beyond the coding region to enhance mutation detection in ADPKD when coding variants are not found.

In conclusion, our study supports an evolving role of genetic testing for ADPKD for diagnosis, prognosis, and familial counseling. Indeed, genotyping and elucidation of molecular mechanism underlying atypical but not rare ADPKD scenarios in the patients is important for the understanding of polycystic kidney disease, linking dosage effect and variability of phenotype severity, and for genetic counseling. We finally suggest that genetically unresolved cases could harbor pathogenic variants located in noncoding regions of *PKD* genes.

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**SUPPLEMENTARY MATERIAL**

Supplementary File (PDF)

**S1.** Supplementary Patients and Methods.

**S2.** Clinical and Genetic Patients’ Data.

**S3.** Implications for Genetic Counseling.

**S4.** Supplementary References.

**Figure S1.** ADPKD families’ pedigrees revealing a marked intrafamilial phenotypic variability owing to complex genotypes. The genotypes at *PKD1* and/or *PKD2* genes and *PKHD1* gene for pedigree 5 are indicated below with each subject symbol.

**Figure S2.** Potential transcription factor binding sites present in the deleted region upstream of the translation start site (ATG) are indicated. The transcription start site is indicated with an arrow. B. MLPA analysis of *PKD1* gene suggesting the presence of the heterozygous deletion in the 5′UTR (probe *PKD1* up 257) C. Zygosity of the SNP rs34197769 G/A (exon 35) on genomic DNA (IGV visualization) and cDNA (Sanger sequencing). Hemizigosity on cDNA indicates the absence of the transcript from one allele (see text).

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