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

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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,S1,S2]

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,S1,S2] Recently, whole-exome sequencing studies identified mutations in other cystogenes (e.g., *GANAB*, *DNAJB11*, *ALG8*, *ALG9*) in a small proportion of patients with ADPKD.[3,S3] 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,S4,S5] 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,S6–S8]

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, with digenic or biallelic inheritance, 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

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
	Pathogenic inherited allele	Hypomorphic inherited allele	*De novo* pathogenic allele		
1	- PKD1 pat p.Gln4231[b]	- 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;[a] Mother: no renal/liver cysts; normal eGFR, 64 yr Paternal aunt: ADPKD; CKD IV, 60 y
2[b]	- PKD2 mat p.Ser349Pro	- PKD1 pat p.Gly1944Arg - PKD2 pat p.Thr203Ile		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 Parents: wild-type PKHD1 sequence
3	- PKD1 pat p.Arg1951Gln		- PKD1 dn p.Arg2402[b]	Twin 1[a]-ADPKD-EO; HTN, 12 yr; CKD II and TKV 695cc, 19 yr Twin 2[c]-ADPKD-EO; HTN, 14 yr; CKD II and TKV 586cc, 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, [a] 74 yr
5		- PKHD1 pat p.Gly1712Arg - PKHD1 mat p.Asp3088Asn	- PKD1 dn c.2097+5_+6insT	ADPKD; 24 yr, sCr 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.Arg4154Cys; - PKD1 mat p.Arg4154Cys			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 *5′* UTR deletion c.-1926_- 64del	ADPKD; CKD IIIa and TKV 833cc, 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.
[a]Deceased.
[b]Index DNA not available.
[c]Monozygous twins.

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.[3]

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).[3,5,S9] Both ICs were negative for PKHD1 variants and revealed coinheritance of a PKD pathogenic variant with a hypomorphic PKD variant already identified in ADPKD-VEO cases.[4–6,S4–S8]

Moreover, recently, in the largest series of ADPKD-VEO,[7] a high prevalence (70%) of biallelic *PKD1* variants (hypomorphic variants *in trans* with a pathogenetic 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.[8,S9,S10]

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

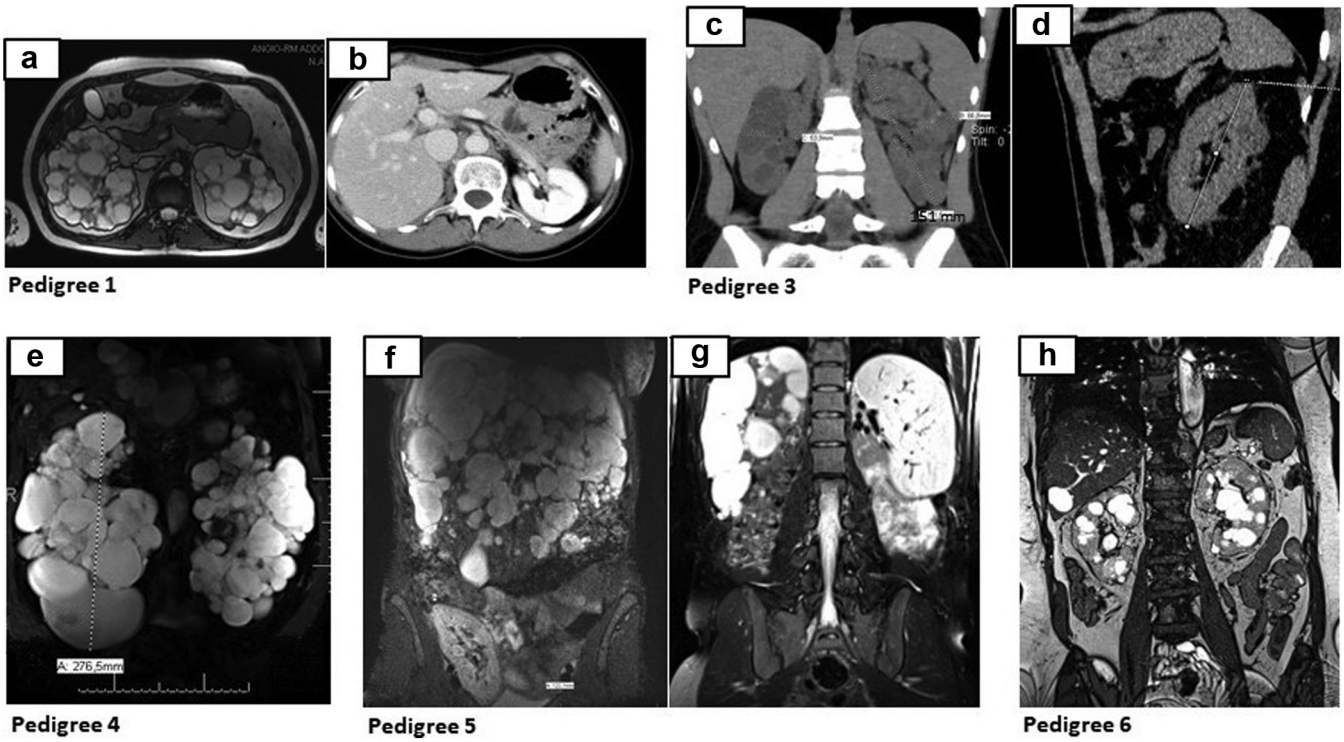

Figure 1. Imaging of relevant cases from ADPKD pedigrees revealing a marked phenotypic variability owing to complex genotypes. Pedigree 1: (a) abdomen MRI: index case with enlarged kidneys with multiple cysts; (b) abdomen CT scan: mother, normal kidney. Pedigree 3: abdomen CT scans: (c) index case with 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.

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.*[6] 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,[S8] heterozygous *PKHD1* changes were detected in addition to the familial mutation in 4 patients. To explain the *PKHD1* aggravating effect, Olson *et al.*[9] 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.[S11,S12]

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

PKD pathogenic variant) in 3 patients with VEO disease.[S8] The previous and present 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. Zigosity 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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