Clinical significance of genetic tests in ADPKD

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- The identification of PKD1 in 1995 and PKD2 in 1996 facilitated the development of DNA sequence–based molecular diagnostics.

ADPKD is genetically heterogeneous

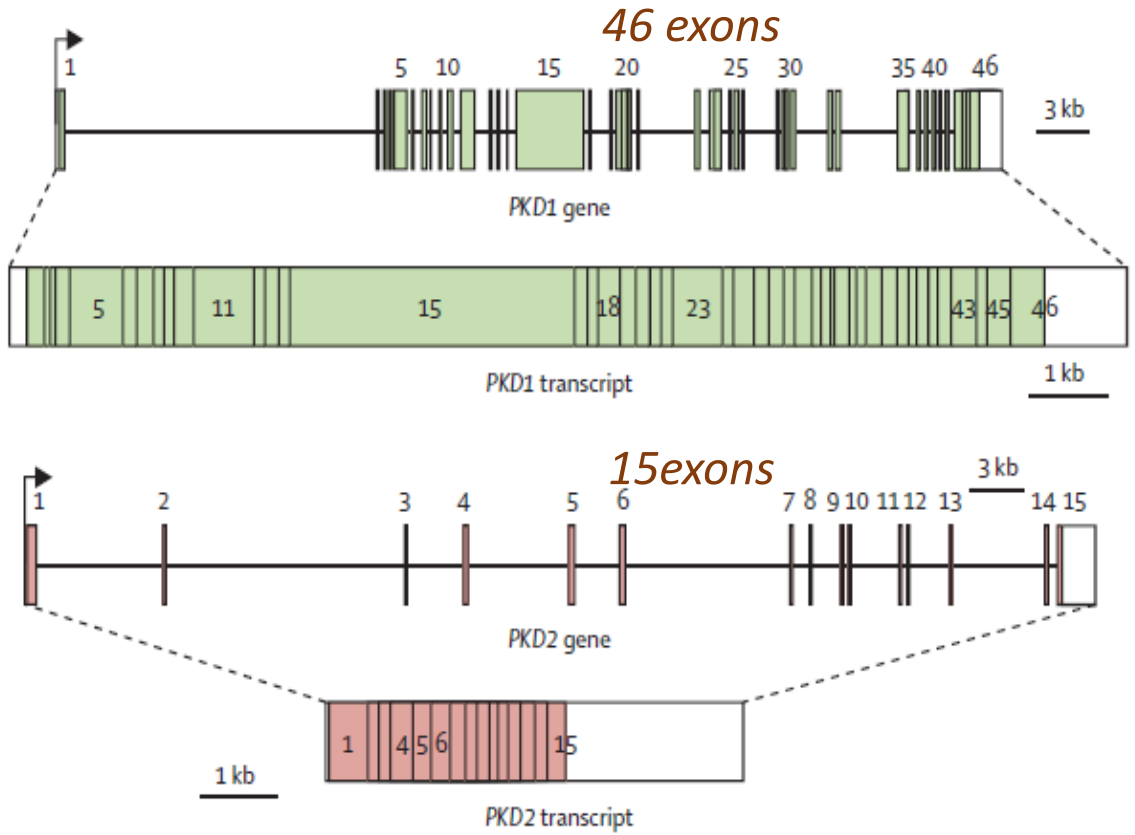
PKD1

- Chr. 16.p13.3; approximately 78% of families
- *50 kb genomic region*
- *producing a 12 909 bp mRNA transcript*

PKD2

- 4q21; approximately 15% of families
- *producing a 2907 bp mRNA transcript*

PKD1 and PKD2 genes and transcripts



Hughes et al, Nat Genet 1995
Mochizuki et al, Science 1996
Torres et al, Lancet. 2007

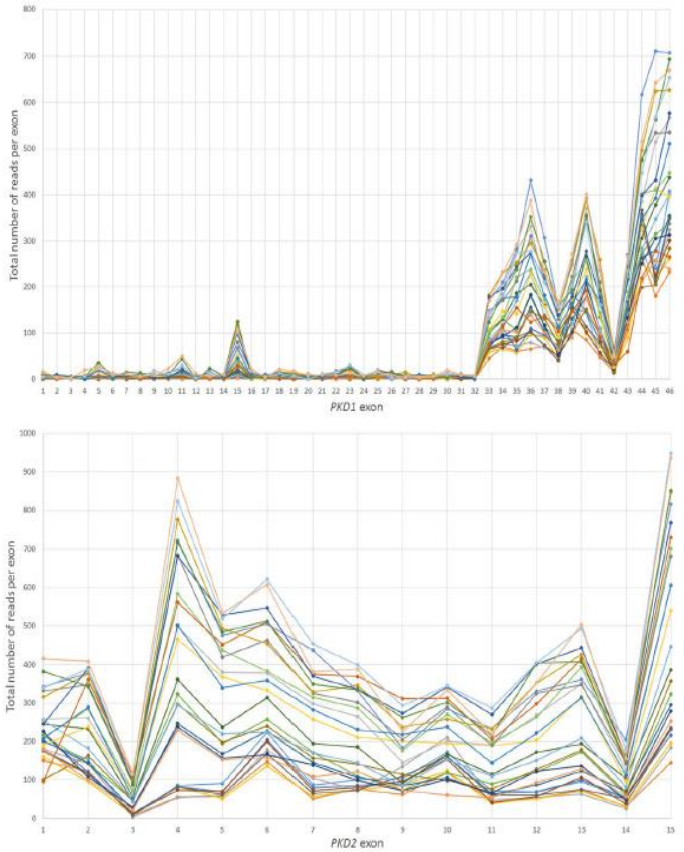
PKD1 mutation screening has been challenging

- ✓ **Large size**
- ✓ **High guanine–cytosine (GC) content**
- ✓ **Complexity**

  first 33 exons duplicated in 6 pseudogenes

  with ≈ 98% DNA sequence identity

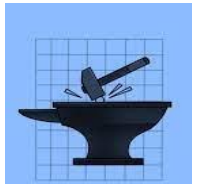
Genetic analysis of PKD2 is relatively easy



Song et al, Expert Rev Mol Diagn 2017
Ali et al, Sci Rep 2019

Gold standard

- Long range PCR
- Sanger sequencing

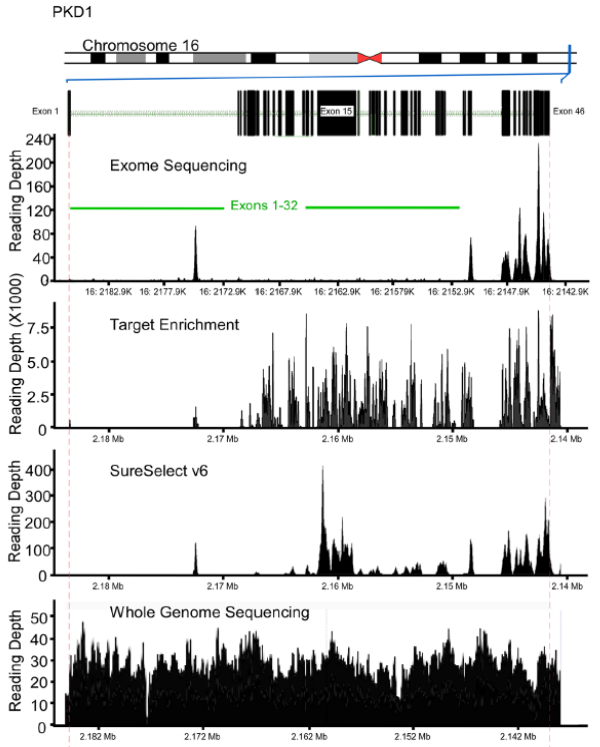


labor-intensive + costly

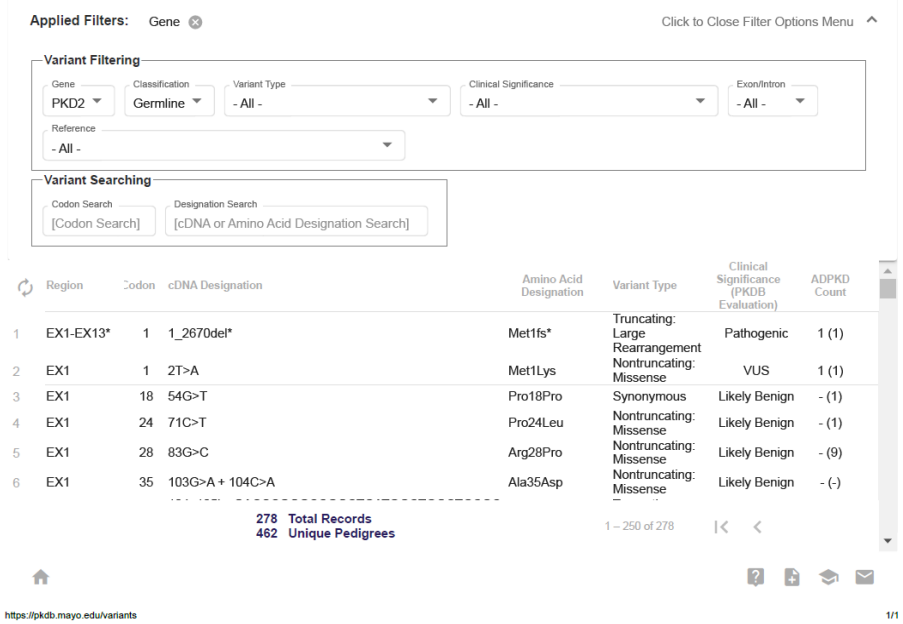
Large gene rearrangements account for 4% of mutations and are missed by Sanger sequencing

Next Generation Sequencing (NGS) technology

- whole exome sequencing (WES)
- whole genome sequencing (WGS)
- targeted enrichment methodologies



Ali et al, Sci Rep 2019



https://pkdb.mayo.edu/variants

- **NO single mutation accounts for >2% of cases**
- **HIGH degree of allelic heterogeneity**

GENE	GERMLINE	SOMATIC
PKD1	2322 Total Records 2077 Unique Pedigrees	9 Total Records 33 Unique Pedigrees
PKD2	278 Total Records 462 Unique Pedigrees	27 Total Records 43 Unique Pedigrees

Variant type
- Any Truncating -
- Any Nontruncating -
3'-UTR
5'-UTR
Nontruncating: Deletion/Insertion
Truncating: Deletion/Insertion
IVS Silent
IVS Unknown
Truncating: Large Rearrangement
Nontruncating: Nonstop
Truncating: Nonsense
Truncating: Splice
Nontruncating: Splice
Nontruncating: Missense
Synonymous

Clinical significance
Pathogenic
Likely Pathogenic
Benign
Likely Benign
Variant of Uncertain Significance (VUS)

Genetic studies in ADPK

- ✓**Epidemiologic information**
- ✓**Disease mechanisms**
- ✓**Providing prognostic value**
- ✓**Broadening of the phenotypic spectrum of cystic kidney diseases**
  - *Genetic Causes of PKD beyond PKD1 and PKD2*
- ✓**Improving diagnostics**

Estimating the prevalence of ADPKD has been challenging

- variable age-dependent penetrance
- incomplete clinical ascertainment in the general population

Prevalence estimates can have important implications for drug development, with orphan diseases defined by a prevalence of <1 per 2000

Epidemiologic studies of clinically ascertained cases of ADPKD reported a point prevalence of **2.4–9.0 per 10,000.**

Joly et al, Kidney Int 2015
Cornec-Le Gall Am J Kidney Dis 2017
Willey, Nephrol Dial Transplant 2017

Prevalence Estimates of Polycystic Kidney and Liver Disease by Population Sequencing

METHODS

Public databases of cardiovascular, diabetes, psychiatric, & control populations (gnomAD & BRAVO)

Whole Exome Sequencing
N = 123,136
Whole Genome Sequencing
N = 78,280

OUTCOME

~1 in 1000 carry a pathogenic mutation in an autosomal dominant polycystic kidney disease (ADPKD) gene and ~1 in 500 in an autosomal dominant polycystic liver disease (ADPLD) gene. Bioinformatic- and clinical database-predicted mutations led to inflated prevalence estimates.

CONCLUSION Individually rare mutations in cystogenic genes are cumulatively more common than expected. Unravelling their contribution to each individual's cystic phenotype remains challenging.

Lanktree et al, J Am Soc Nephrol. 2018

Observational Study JAMA. 2022 Dec 27;328(24):2412-2421. doi: 10.1001/jama.2022.22847.

Exome Sequencing of a Clinical Population for Autosomal Dominant Polycystic Kidney Disease

Alexander R Chang [1 2], Bryn S Moore [3], Jonathan Z Luo [3], Gino Sartori [4], Brian Fang [1], Steven Jacobs [2], Yoosif Abdalla [2], Mohammed Taher [1], David J Carey [3], William J Triffo [4], Gurmukteshwar Singh [1 2], Tooraj Mirshahi [3]

- **Retrospective observational study using an unselected health system–based cohort with exome sequencing enrolled from 2004 to 2020 and electronic health record data**
- **Of 174 172 patients 303 patients had ADPKD diagnosis codes**

1.74:1000

Insights into pathobiology of ADPKD from human and animal genetic studies

Germline mutation

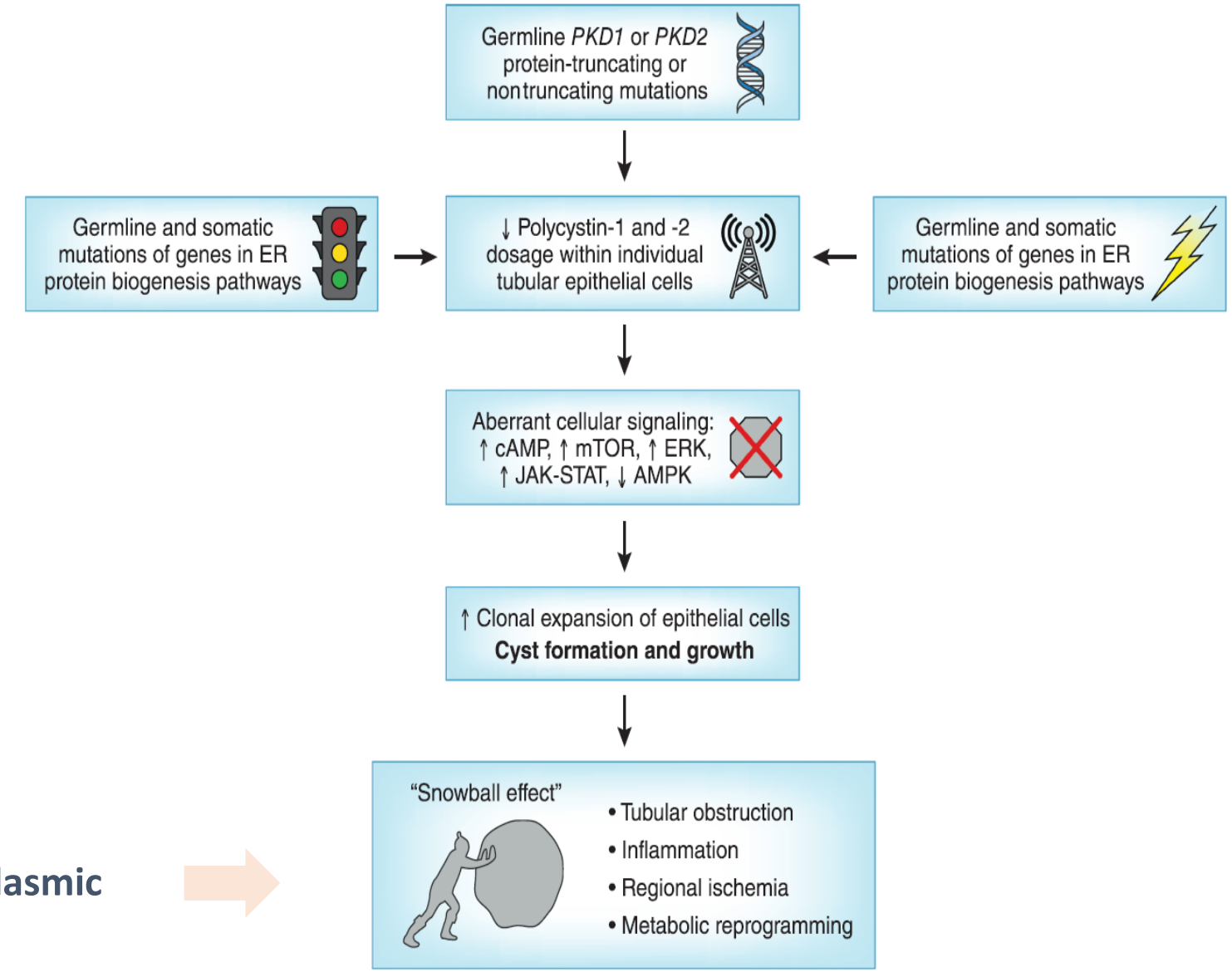
Second-hit

Somatic mutation

Threshold effect

Third-hit model

Gene mutations in endoplasmic reticulum proteins



Lanktree et al, CJASN 2021

Mutation class predicts disease variability

Cornec-Le Gall, J Am Soc Nephrol 2013
Heyer et al, J Am Soc Nephrol 2016
Hwang et al, J Am Soc Nephrol 2016
Cornec-Le Gall et al, J Am Soc Nephrol 2018

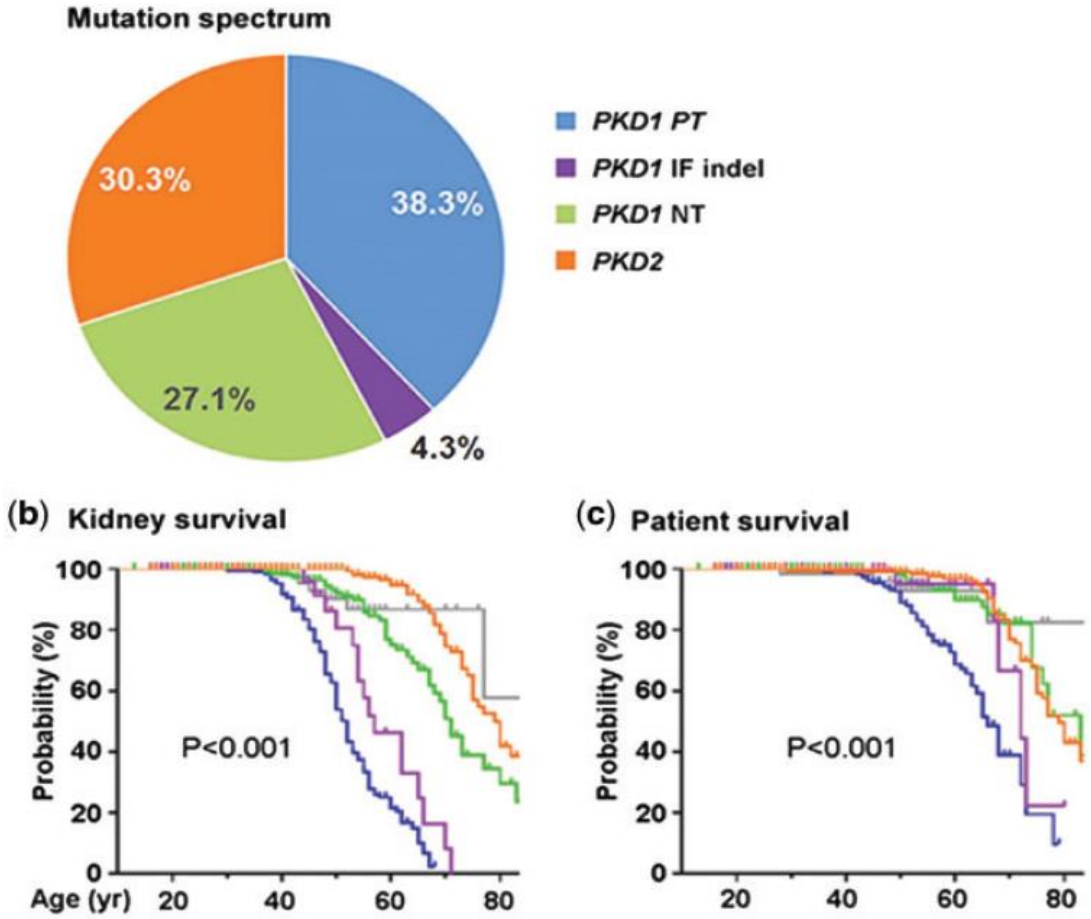
- **Protein-truncating PKD1 mutations**

➢ frameshift, nonsense, canonical splice site and large deletions

- **Nontruncating PKD1mutations**

➢Missense, in-frame insertions/deletions

- **PKD2 mutations**



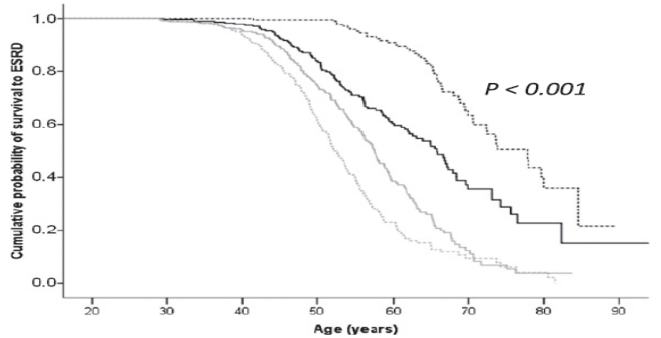
Incorporation of clinical risk factors with genetic results may further improve risk stratification in ADPKD as exemplified by the use of PROPKD (predicting renal outcome in ADPKD) scores.

- Cross sectional study
- 1341 patients from the Genkyst cohort
- Influence of clinical and genetic factors on renal survival

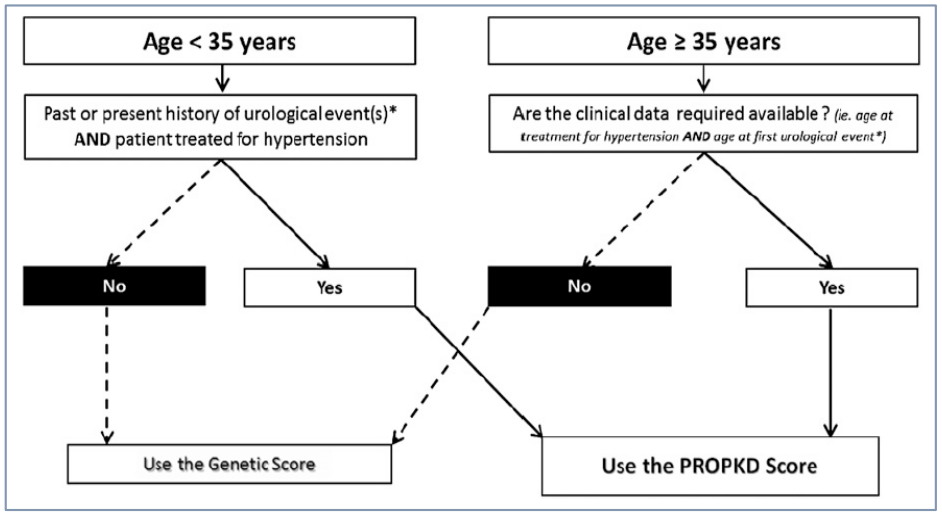
PRO-PKD SCORE

Variable	Patients (*n*)	HR (95% CI)	95% CI from Bootstrap Analysis	*P* Value	Points for PROPKD Score
Sex					
Female	541				0
Male	432	1.55 (1.29 to 1.88)	1.27 to 1.89	<0.001	1
Hypertension before age 35 yr					
No	679				0
Yes	294	2.11 (1.71 to 2.61)	1.71 to 2.62	<0.001	2
≥1 urologic event before age 35 yr					
No	734				0
Yes	239	1.73 (1.38 to 2.18)	1.35 to 2.24	<0.001	2
Mutation					
PKD2	186				0
PKD1 nontruncating	239	2.27 (1.57 to 3.28)	1.61 to 3.18	0.002	2
PKD1 truncating	548	4.75 (3.41 to 6.60)	3.63 to 6.60	<0.001	4

GENETIC SCORE



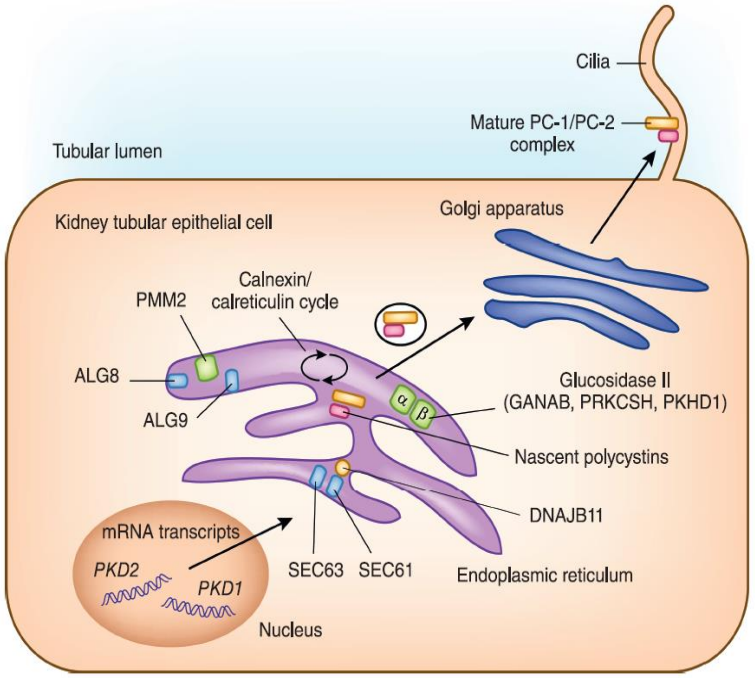
PKD2 (black dotted curve n=248)	235	216	181	116	38	9
PKD1 non truncating mutations (black curve n=323)	302	249	161	83	23	5
Women with *PKD1* truncating mutation (grey curve n=380)	346	295	185	59	10	1
Men with *PKD1* truncating mutations (grey dotted curve n=321)	298	243	128	33	7	2



Cornec Le-Gall J Am Soc Nephrol 2016

Genetic Causes of PKD beyond PKD1 and PKD2

Whole-exome sequencing identified mutations in rare cystic disease genes in patients who were originally labeled as "no mutation detected"



Autosomal dominant inheritance				
PKD1	Polycystin-1	1 in 1477	Moves from ER to cilia as complex with polycystin-2. Exact function remains unknown	ADPKD
PKD2	Polycystin-2	1 in 3914	Calcium permeable nonselective cation channel. Forms complex with polycystin-1	ADPKD
GANAB	α-Subunit of glucosidase II	1 in 4379	ER enzyme catalyzes hydrolysis of peptide-bound oligosaccharides	ADPKD
DNAJB11	DNAJ heat shock protein 40 subfamily B, member 11	1 in 12,312	ER glycoprotein cofactor for GRP78, required for protein trafficking	ADPKD
ALG9	α-1,2-mannosyltransferase	1 in 6156	Enzyme for protein *N*-glycosylation	ADPKD

Poroth et al, Am J Hum Genet 2016
Besse et al, J Am Soc Nephrol 2019
Cornec Le-Gall et al, Am J Hum Genet 2018

Scenarios where genetic testing is clinically indicated

✓Suspected ADPKD with no apparent family history

✓Suspected ADPKD with equivocal kidney imaging findings

✓ADPKD exclusion in young (e.g., ,25 yr old) at-risk subjects

- *Living related kidney donation evaluation*
- *Obtaining life or disability insurance*
- *Prenatal and preimplantation genetic diagnosis*

ADPKD DIAGNOSIS

- ✓ **positive family history**

plus

- ✓ **Ultrasonography**

or

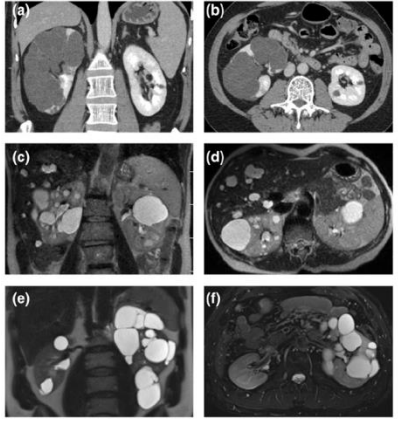
- ✓ **MRI in equivocal cases or young at-risk subjects**

} *validated age-dependent diagnostic criteria*

No apparent family history in up to 28% of patients suspected to have ADPKD

- unavailability of parental medical records
- mild PKD unrecognized in an affected parent
- genetic and nongenetic causes of renal cystic diseases
- de novo disease
- somatic and germline mosaicism

Iliuta et al, J Am Soc Nephrol 2017



Atypical Kidney Imaging

Present in up to 16% of patients suspected to have ADPKD

- ***Nontruncating PKD1 or PKD2*** mutations if positive family history and mild cystic disease
- ***Second kidney disease*** in patients with moderate to advanced CKD but mild cystic disease without kidney enlargement
- ***Somatic mosaicism*** if no family history of ADPKD and unilateral, asymmetric, segmental, or lopsided cystic disease

Class, Subclass, and Term	Description
1: Typical ADPKD	Bilateral and diffuse distribution, with mild, moderate, or severe replacement of kidney tissue by cysts, where all cysts contribute similarly to TKV. (Supplemental Figure 1, A and B)
2: Atypical ADPKD	
A	
Unilateral	Diffuse cystic involvement of one kidney causing marked renal enlargement with a normal contralateral kidney defined by a normal kidney volume (<275 ml in men; <244 ml in women) and having no or only 1–2 cysts (Supplemental Figure 1C)
Segmental	Cystic disease involving only one pole of one or both kidneys and sparing the remaining renal tissue (Supplemental Figure 1D)
Asymmetric	Diffuse cystic involvement of one kidney causing marked renal enlargement with mild segmental or minimal diffuse involvement of the contralateral kidney defined by a small number of cysts (>2 but <10) and volume accounting for <30% of TKV (Supplemental Figure 1E)
Lopsided	Bilateral distribution of renal cysts with mild replacement of kidney tissue with atypical cysts where ≤5 cysts account for ≥50% TKV (the largest cyst diameter is used to estimate individual cyst volume) (Supplemental Figure 1F)
B	
Bilateral presentation with acquired unilateral atrophy	Diffuse cystic involvement of one kidney causing moderate to severe renal enlargement with contralateral acquired atrophy (Supplemental Figure 1G)
Bilateral presentation with bilateral kidney atrophy	Impaired renal function (serum creatinine≥1.5 mg/dl) without significant enlargement of the kidneys, defined by an average length <14.5 cm, and replacement of kidney tissue by cysts with atrophy of the parenchyma (Supplemental Figure 1H)

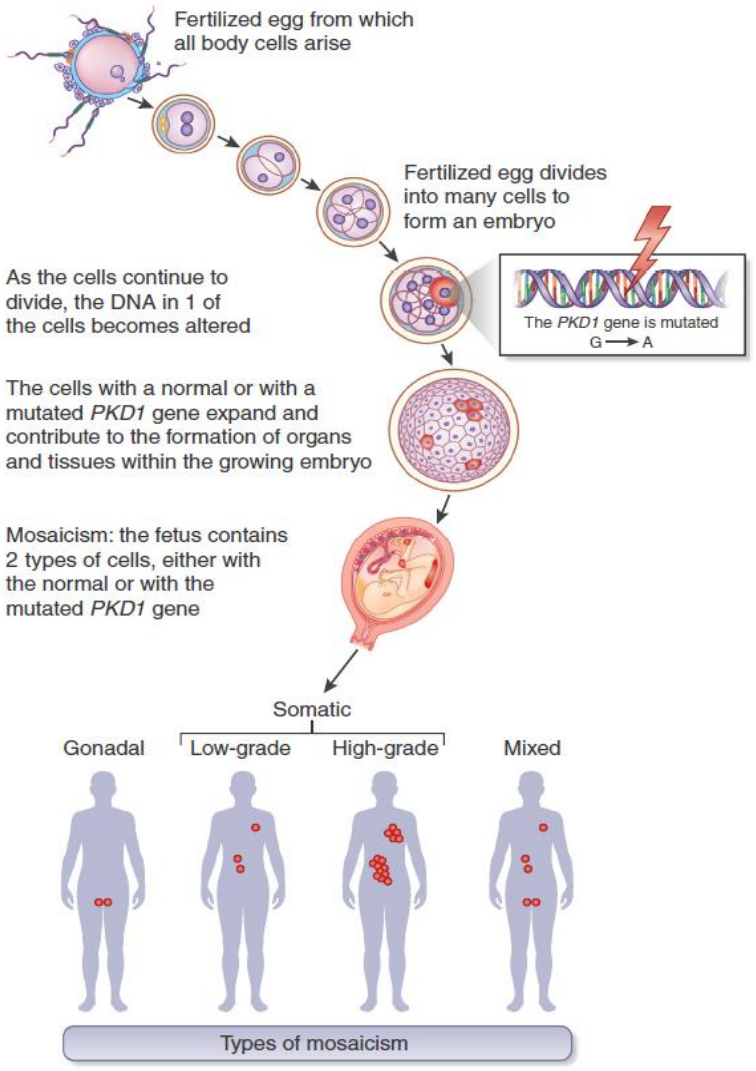
Irazabal et al, *J Am Soc Nephrol 2015*
Iliutal et al, J Am Soc Nephrol 2017

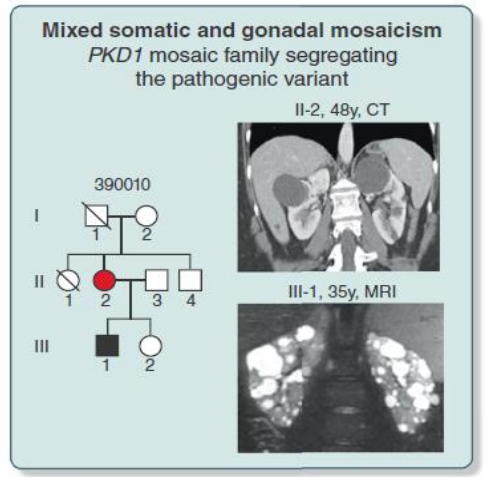
Mosaicism

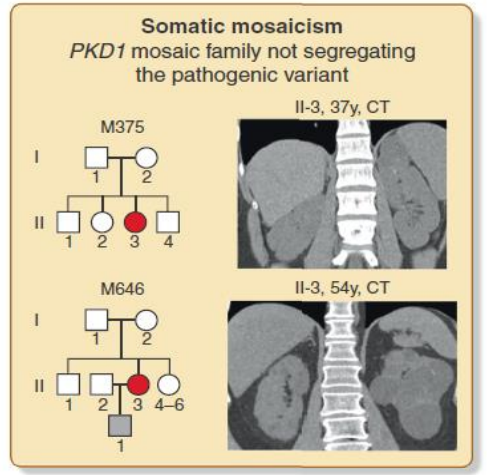
- *presence of 2 genetically distinct cell populations within 1 individual resulting from a somatic mutation during embryogenesis*

Mosaicism in ADPKD

- **Readily detectable mosaicism by NGS**
- ✓ *1% of typical ADPKD population*
- ✓ *10% of genetically unresolved PKD cases*



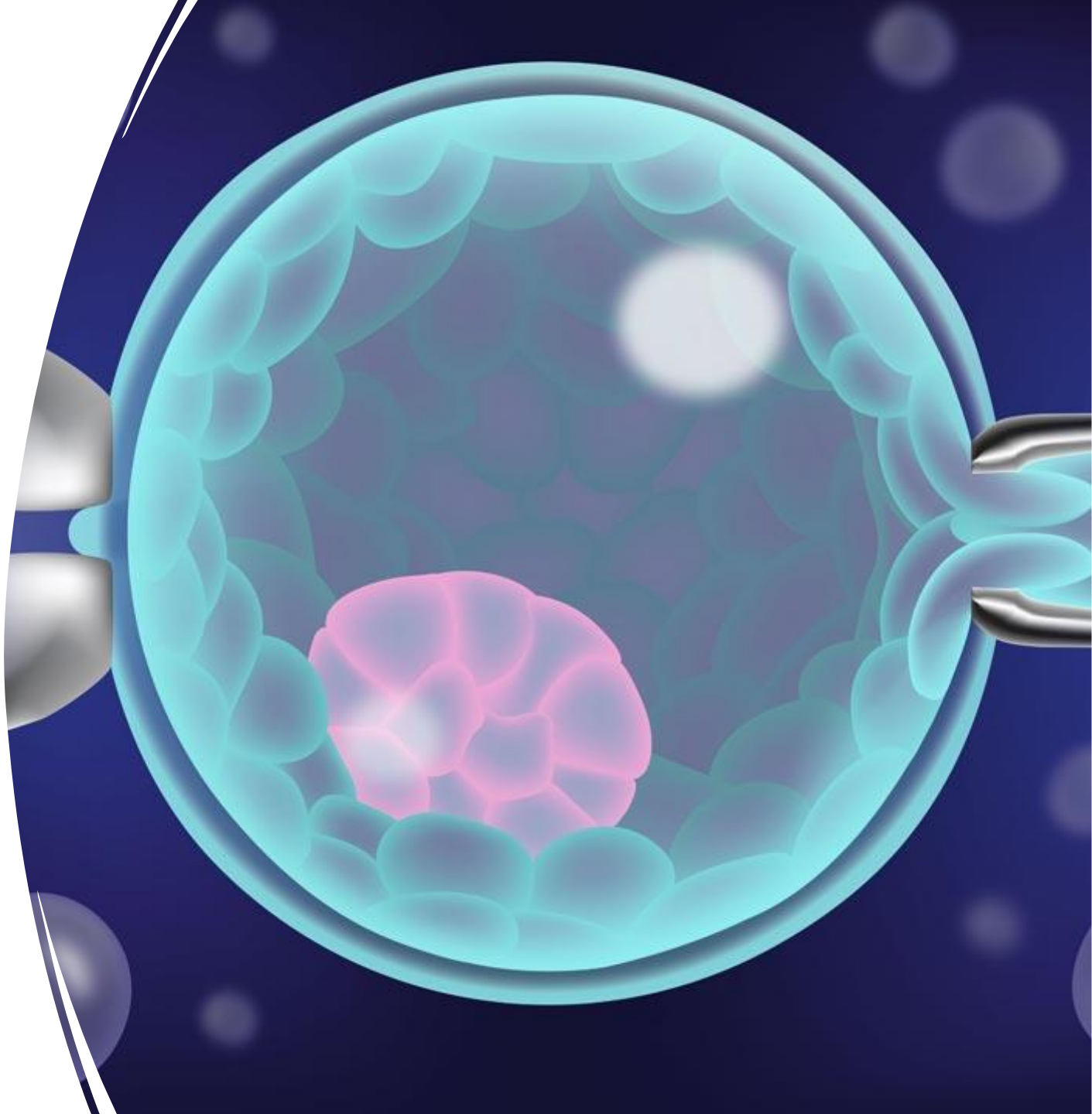




Hopp et al, Kidney Int 2020
Devuyst et al, Kidney Int 2020

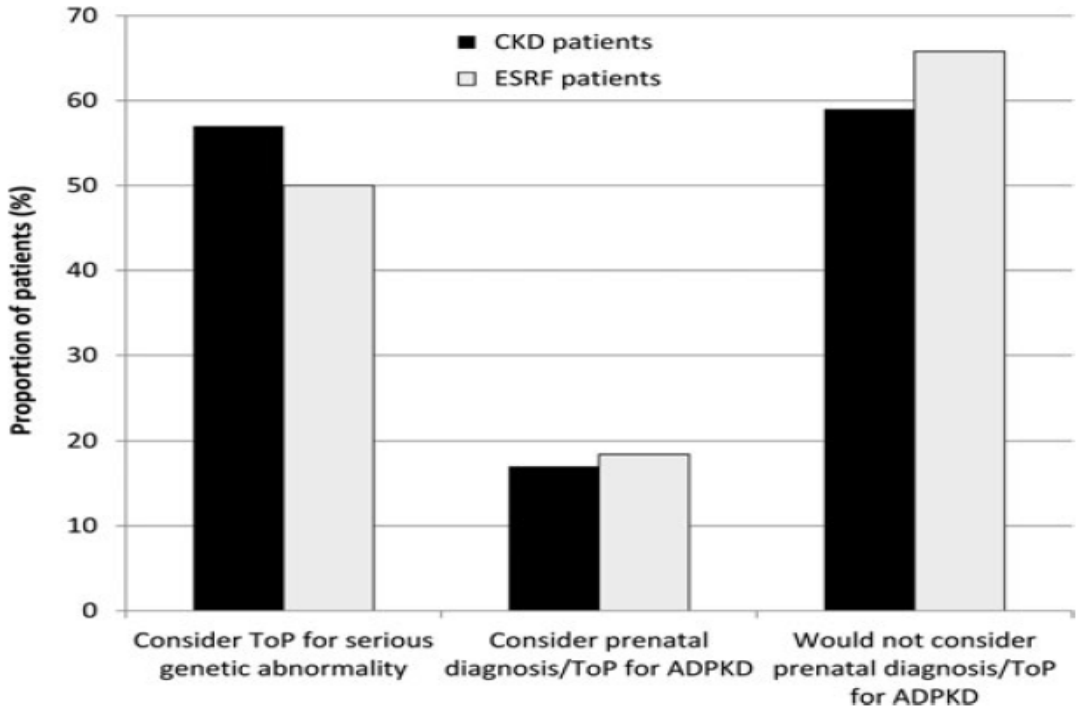
PGD involves extraction, amplification, and analysis of DNA removed from the blastomere or trophectoderm of embryos created using IVF

- Affected individuals have a 50% chance of passing the mutation to each of their offspring.
- Assisted reproductive technology using preimplantation genetic diagnosis (PGD) allows individuals with PKD to reduce risk to 1% - 2%.

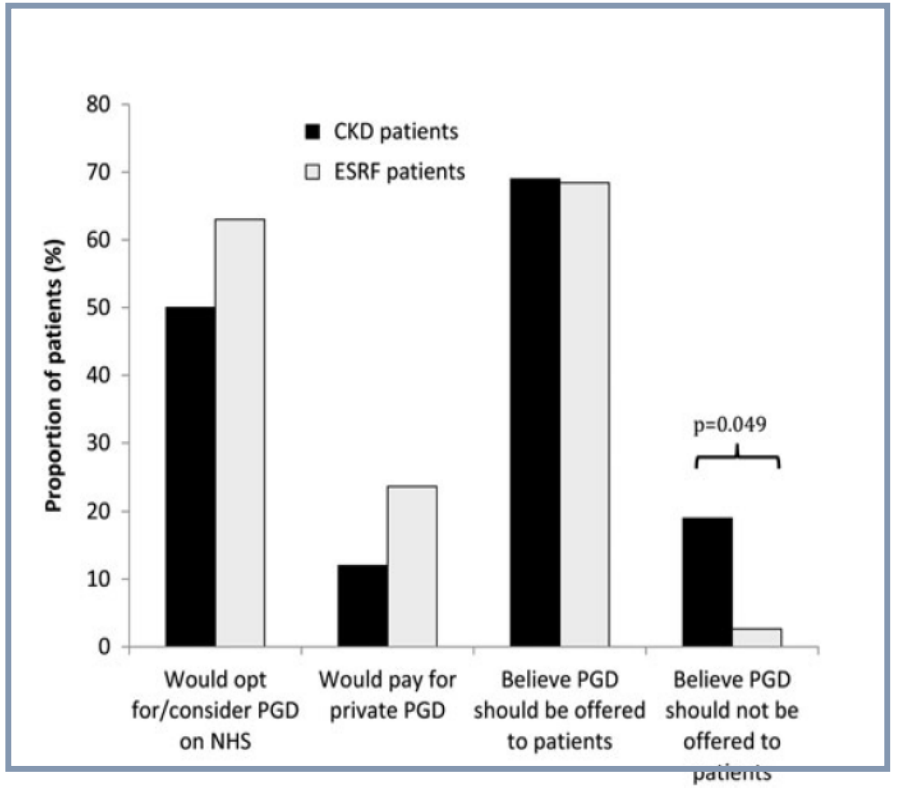


Greater acceptability for diagnostic methods that occur before embryo implantation

Attitudes to prenatal diagnosis and termination of pregnancy in ADPKD patients



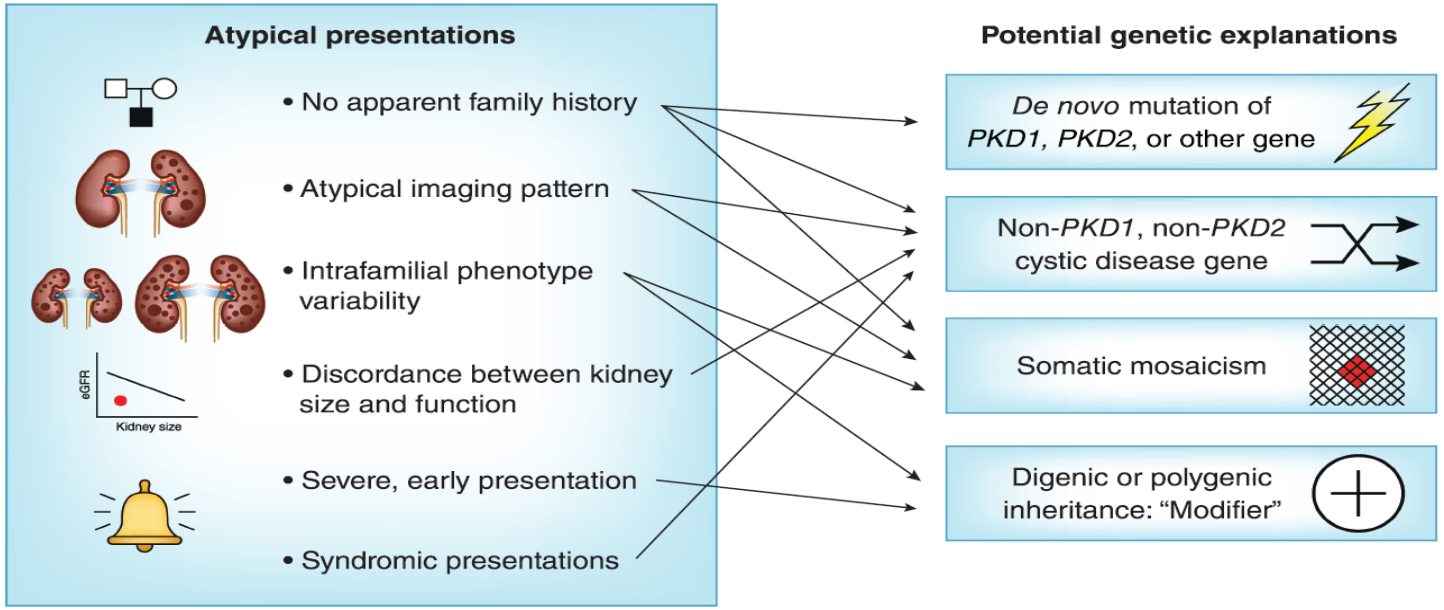
Attitudes to PGD in ADPKD patients



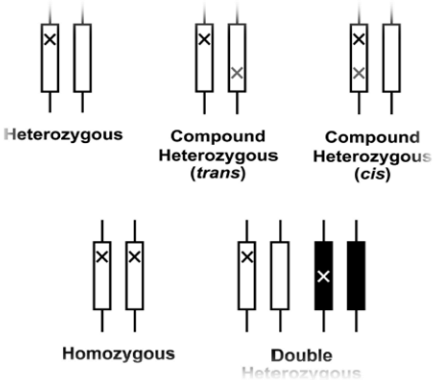
Swift et al, Genet Test Mol Biomarkers. 2016

Scenarios with evolving indication for genetic testing

- ✓ **Delineating the cause of atypical clinical presentation**
  - *Early and severe disease*
  - *Marked intrafamilial disease discordance*
  - *Syndromic forms of polycystic kidney disease*
- ✓ **Identifying "high-risk" patients for novel disease modifier therapy or clinical trial**



Severe PKD with bilateral kidney enlargement presenting in utero or early childhood can result in ESRD by teenage years or young adulthood

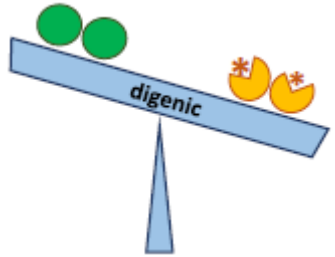


COMPOUND HETEROZYGOSITY

- *one protein-truncating PKD1 mutation in trans with a second nontruncating PKD1 mutation*

DIGENIC DISEASE

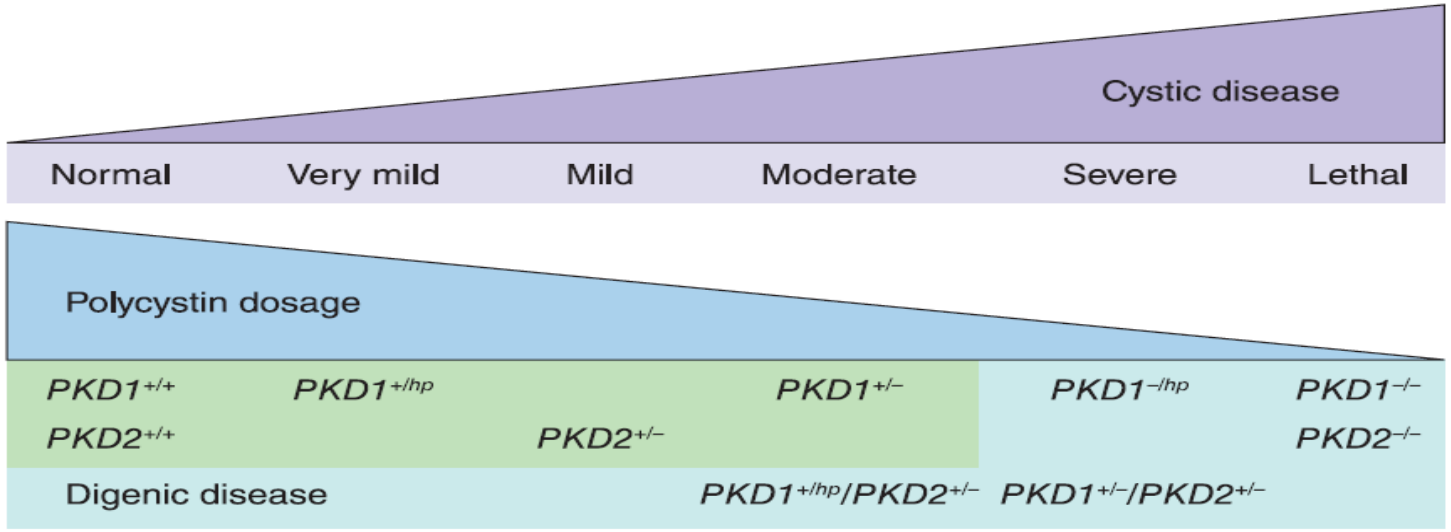
- *one PKD1 mutation and a second mutation in another cystic disease gene, such as PKD2, COL4A1, or HNF1B*



Biallelism of fully inactivating PKD1 or PKD2 mutations is not compatible with life

Paterson et al, Am J Hum Genet. 2001
Lanktree et al, CJASN 2021

Identification of families with bilineal ADPKD has important implications for genetic counseling

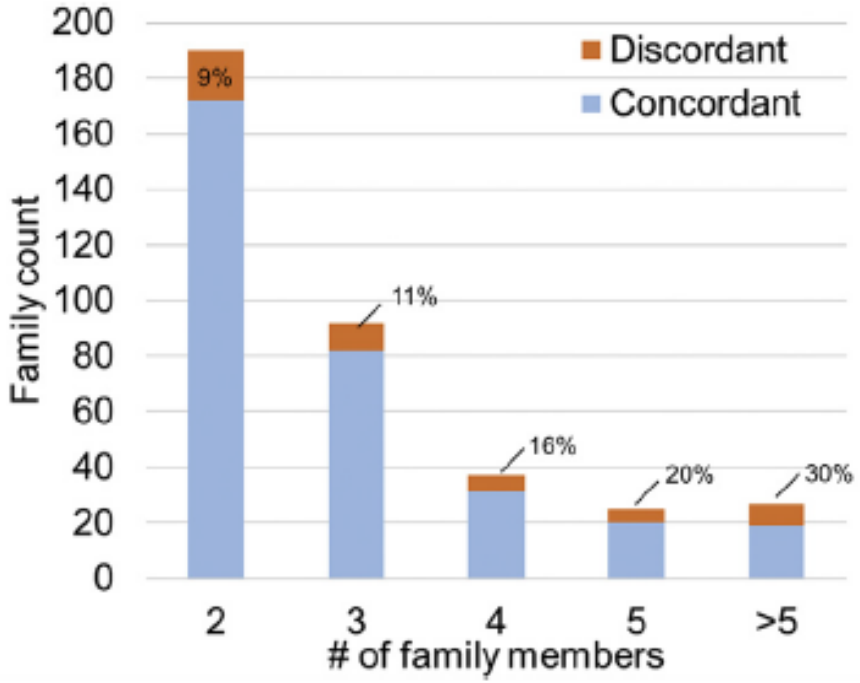


Bilineal disease is predicted to occur in ≈ 1 in 250,000–1,000,000 marriages in the general population

- one affected parent may have a mild form of ADPKD due to a nontruncating PKD1 or PKD2 mutation
- marked kidney disease discordance between family members
- high disease segregation ratio affecting ≈ 75% of children in large pedigrees

Extreme kidney disease discordance is present in at least 12% of families with ADPKD, regardless of the underlying mutated gene or mutation class.

- ***the extended Toronto Genetic Epidemiology Study of Polycystic Kidney Disease (eTGESP) cohort***
- ***1390 patients and 612 unrelated families with ADPKD***



Families were defined as discordant if they had at least 1 affected member with mild kidney disease and 1 member with severe kidney disease

Lanktree et al, Kidney Int Rep 2019

Marked intrafamilial disease variability by total kidney volume or eGFR adjusted for age

- **the presence of an unusual genetic underpinning**

  somatic mosaicism or digenic disease

- **coincidence of a second kidney disease**

  diabetes or glomerulonephritis

- **comorbidities**

  hypertension and obesity

- **environmental factors**

  smoking and water intake

Syndromic forms of PKD

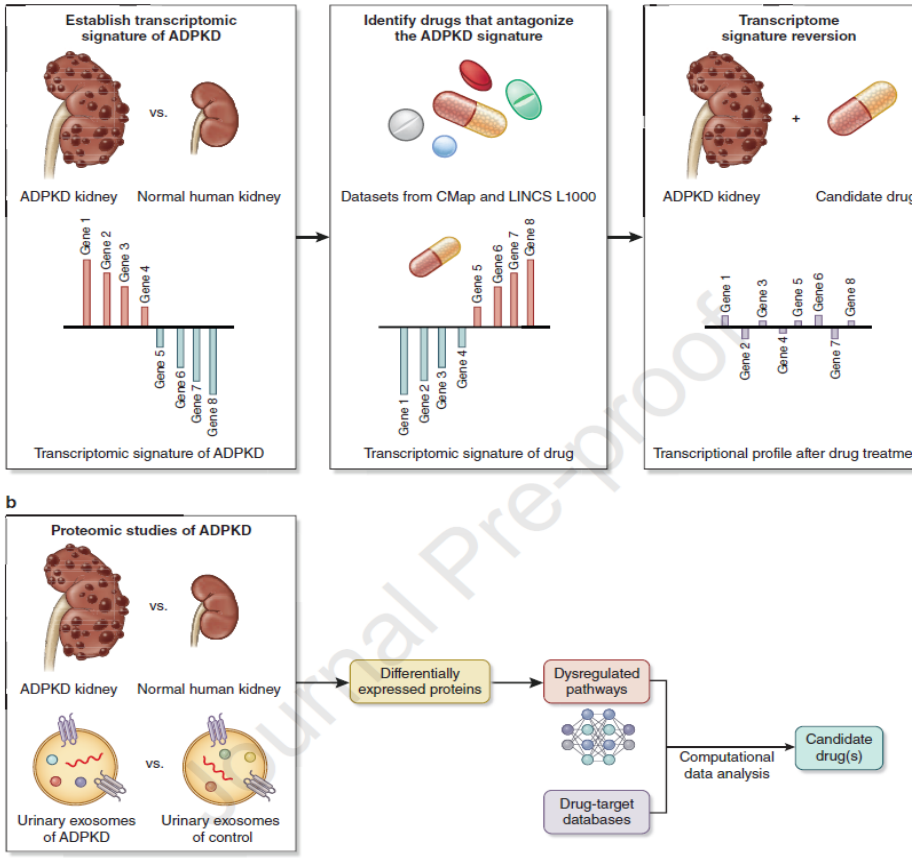
Tuberous sclerosis	Multiple and bilateral angiomyolipomas and renal cysts; kidney function usually preserved; possible evolution to ESKD, either by destruction of the renal parenchyma by multiple angiomyolipomas or following nephrectomies for haemorrhagic angiomyolipomas; if there is contiguous gene deletion of *TSC2* and *PKD1*, severe PKD with evolution to ESKD occurs before age 30 years	CNS (cortical tubers, astrocytomas, epilepsy, and mental retardation); skin lesions (facial angiofibromas and hypopigmented spots); pulmonary lymphangioleiomyomatosis; cardiac rhabdomyoma and retinal hamartoma; polycystic liver disease if contiguous deletion of both *PKD1* and *TSC2*
Von Hippel-Lindau disease	Bilateral renal cysts, renal cell carcinoma	Haemangioblastomas of the retina, spine, or brain; pheochromocytoma; neuroendocrine tumour of the pancreas
HANAC syndrome or *COL4A1*-related disease	Bilateral renal cysts occasionally reported; patients can develop renal insufficiency after about age 50–60 years	Microscopic haematuria, aneurysms, muscle cramps, elevated creatine phosphokinase, tortuosity of the retinal arteries
Oro-facial-digital syndrome type 1	X-linked, embryonically lethal in boys, PKD in women	Cleft palate, facial dysmorphy; syndactyly, clinodactyly, or polydactyly; mental retardation; polycystic liver disease

ADTKD-associated genes

ADTKD-*HNF1B*	Bilateral renal cysts in about 45% of affected individuals, occasionally mimics ADPKD imaging presentation; evolution to ESKD is highly variable, from childhood-onset ESKD to preserved kidney function throughout life	Diabetes, gout, hyperuricaemia, hypomagnesaemia, elevated liver enzymes, bicornate uterus, solitary kidney
ADTKD-*MUC1*	Normal or small-sized kidneys, few small renal cysts in half of patients; evolution to ESKD highly variable, age 20–70 years	Gout
ADTKD-*SEC61A1*	Normal or small-sized kidneys, bilateral small renal cysts in about 50% of individuals	Congenital anaemia, intrauterine growth retardation, neutropenia
ADTKD-*UMOD*	Normal or small-sized kidneys, few small renal cysts in a third of patients, unilateral or bilateral; evolution to ESKD highly variable, age 20–70 years	Gout

Drug repurposing in ADPKD

- GWAS
- Transcriptional gene profiling
- Investigation of the genomic data and integrating GWAS with other “omics” datasets might assist to identify compounds for repurposing in ADPKD



Xou et al, Kidney Int 2023

Patient Voice

- *What will I do with the information once I have it?*
- *Will it prevent me from obtaining medical or life insurance?*
- *Am I better off knowing or not knowing?*
- *Can I financially afford the test?*

- ✓ Lifestyle and Medication Intervention
- ✓ Family Planning
- ✓ Living donation
- ✓ Increased Knowledge About ADPKD

- ❖ Legal Protection
- ❖ Insurance
- ❖ Emotional support

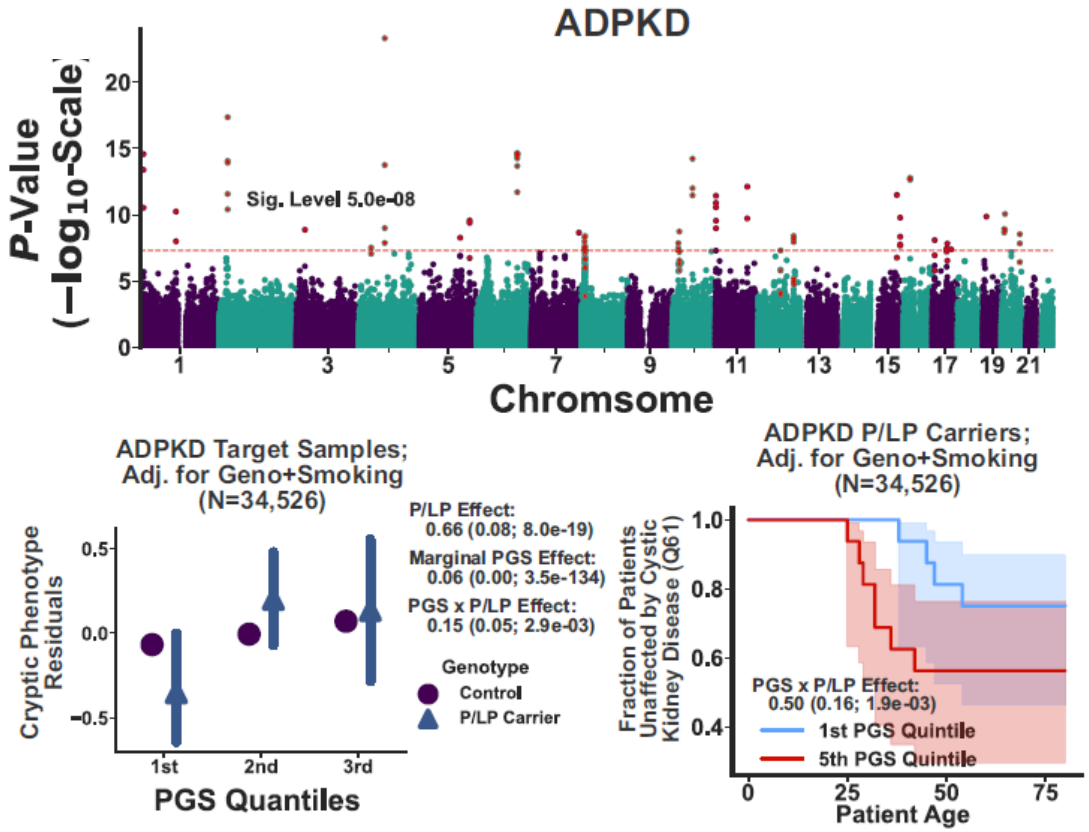

Excellent try, my dear Watson, but . . .
could you bring me a mirror, old boy?

- All patients with ADPKD who might consider having children in the future should be counseled about family planning and reproductive options.
- The decision to pursue or decline assisted reproductive technology or prenatal diagnosis of ADPKD is a personal choice based on patient beliefs, experience, and preferences.
- High risk disease factors may lead to patient interest in PGD.

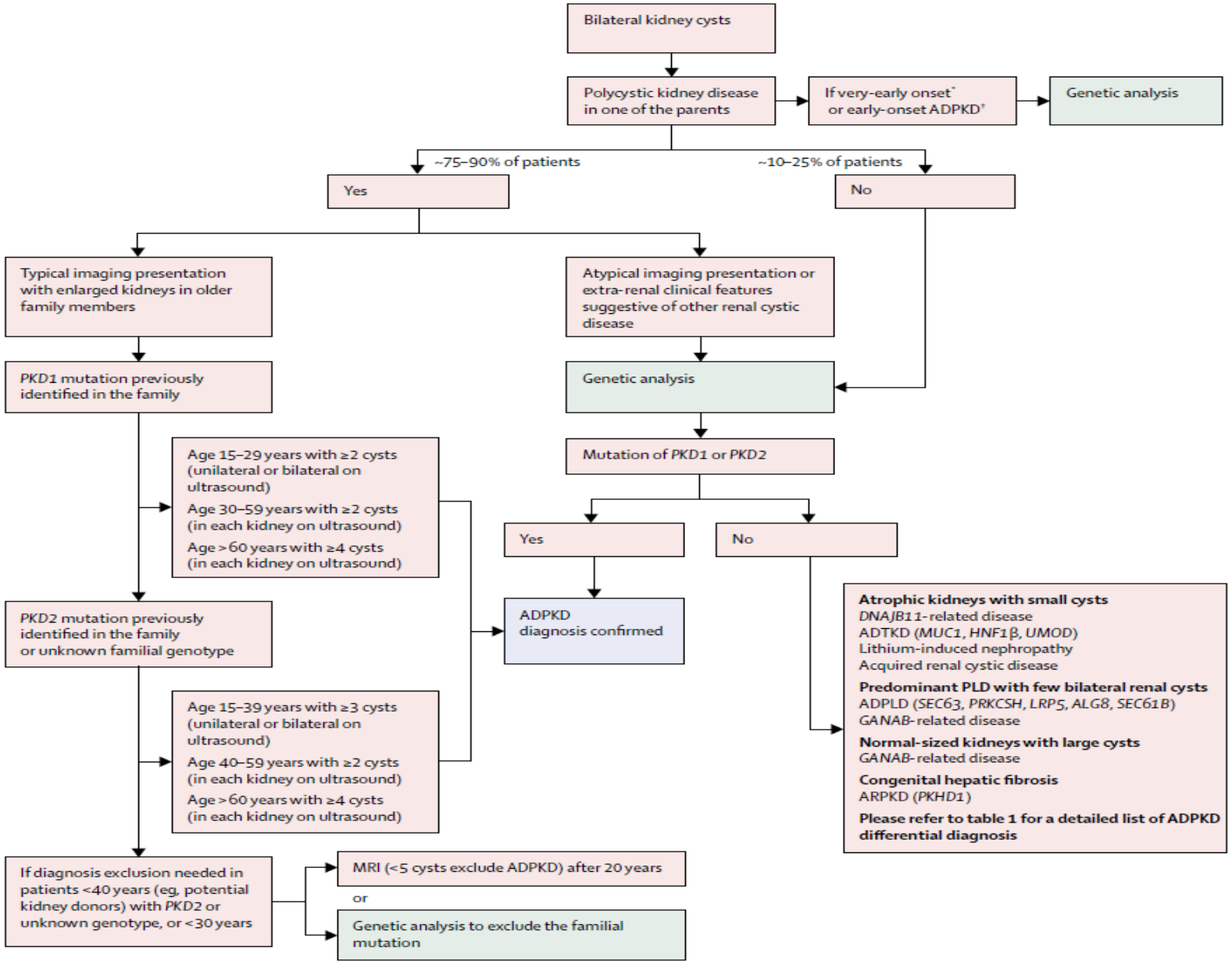
Murphy et al, Am J Kidney Dis 2018

- Cryptic phenotype analysis uses statistical modeling to infer quantitative traits that summarize disease-related phenotypic variability.
- These traits are estimated using symptoms documented in the electronic medical record.
- GWAS analyses for cryptic phenotypes, uncover common variation that is predictive of Mendelian disease-related diagnoses and outcomes.

- *GWAS for ADPKD uncovered 30 loci, most associated with blood pressure regulation.*
- *The cryptic phenotype polygenic scores (PGS) based on the associated loci was associated with the progression but not the diagnosis of ADPKD*



Blair et al, Nat Commun 2022



Cornec-Le Gall et al, Lancet 2019

- disease-modifying treatments in the pediatric population are
- lacking and obtaining a diagnosis in a presymptomatic state can
- be associated with emotional and psychological stress, we do
- not encourage screening beyond blood pressure measurement
- in pediatric cases. All subjects interested in undergoing genetic
- testing for ADPKD should be informed of the potential for genetic
- discrimination or protection from genetic discrimination,
- which varies fromcountry to country

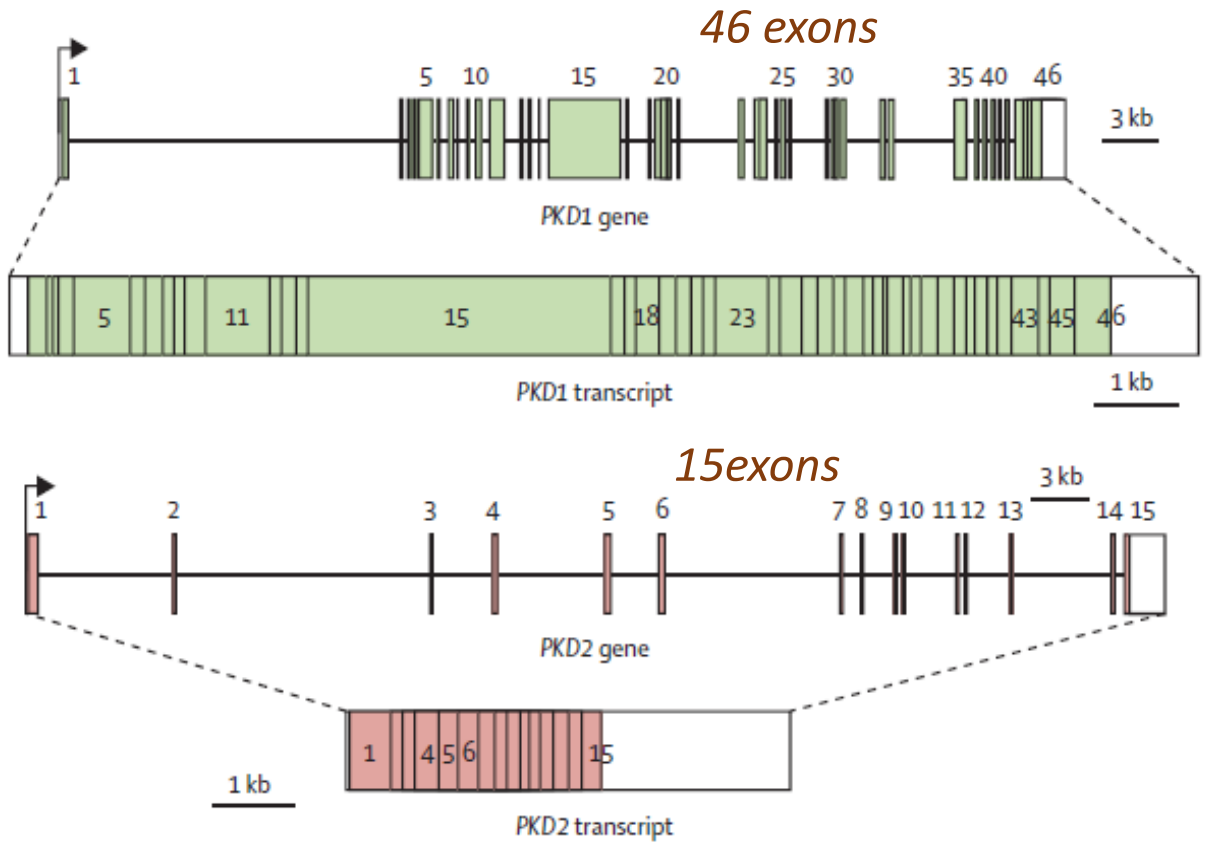
Differential diagnosis in ADPKD genotypes

	Associated disease	Renal phenotype	Extra-renal phenotype
Classical ADPKD*			
PKD1[1,20]	ADPKD-*PKD1* with truncating mutation	Innumerable bilateral kidney cysts causing progressive kidney enlargement and reduction in eGFR; median age at ESKD about 55 years	Polycystic liver disease, mild to severe
PKD1[1,20]	ADPKD-*PKD1* with non-truncating mutation	Innumerable bilateral kidney cysts causing progressive kidney enlargement and reduction in eGFR; median age at ESKD about 67 years	Polycystic liver disease, mild to severe
PKD2[1,20]	ADPKD-*PKD2*	Innumerable bilateral kidney cysts causing progressive kidney enlargement and reduction in eGFR; median age at ESKD about 79 years	Polycystic liver disease, mild to severe
ADPKD-like phenotype			
GANAB[14,15]	ADPKD-*GANAB*	Bilateral renal cysts, preserved kidney function	Polycystic liver disease, mild to severe
DNAJB11[18]	ADPKD-*DNAJB11*	Normal or small-sized kidneys with multiple small renal cysts; possible evolution to ESKD after 60 years	Polycystic liver disease, absent to moderate
ADTKD-associated genes			
HNF1B[21]	ADTKD-*HNF1B*	Bilateral renal cysts in about 45% of affected individuals, occasionally mimics ADPKD imaging presentation; evolution to ESKD is highly variable, from childhood-onset ESKD to preserved kidney function throughout life	Diabetes, gout, hyperuricaemia, hypomagnesaemia, elevated liver enzymes, bicornate uterus, solitary kidney
MUC1[22]	ADTKD-*MUC1*	Normal or small-sized kidneys, few small renal cysts in half of patients; evolution to ESKD highly variable, age 20–70 years	Gout
SEC61A1[16]	ADTKD-*SEC61A1*	Normal or small-sized kidneys, bilateral small renal cysts in about 50% of individuals	Congenital anaemia, intrauterine growth retardation, neutropenia
UMOD[23]	ADTKD-*UMOD*	Normal or small-sized kidneys, few small renal cysts in a third of patients, unilateral or bilateral; evolution to ESKD highly variable, age 20–70 years	Gout
ADPLD-associated genes			
PRKCSH[24]	ADPLD	Few renal cysts occasionally reported	Polycystic liver disease, mild to severe
SEC63[24]	ADPLD	Few renal cysts occasionally reported	Polycystic liver disease, mild to severe
ALG8[24]	ADPLD	Few renal cysts occasionally reported	Polycystic liver disease, mild to moderate
SEC61B[24]	ADPLD	No renal cysts observed to date in the two families reported with a pathogenic mutation in this gene	Polycystic liver disease, mild to moderate
LRP5[25,26]	ADPLD	Few renal cysts occasionally reported	Polycystic liver disease, mild to moderate
Recessive inheritance			
PKHD1[27]	ARPKD	Antenatally enlarged hyperechogenic kidneys; multiple bilateral millimetre-sized cysts; ESKD in the first decade of life in about 50% of individuals but milder renal presentation with diagnosis in adulthood possible	Congenital hepatic fibrosis, Caroli syndrome, small liver cysts in heterozygous patients
DZIP1L[28]	ARPKD	Antenatal enlarged hyperechogenic kidneys; multiple bilateral millimetre-sized cysts; progression to ESKD variable (second and third decade of life)	No obvious extra-renal manifestations reported in the seven patients identified to date
PMM2[17]	Hyperinsulinaemic hypoglycaemia with PKD	Antenatal enlarged hyperechogenic kidneys, enlarged kidneys with multiple cysts; progression to ESKD variable, from infancy to early adulthood	Hyperinsulinaemic hypoglycaemia; small liver cysts in some patients
Syndromic forms of PKD			
TSC1 or *TSC2*[29,30]	Tuberous sclerosis	Multiple and bilateral angiomyolipomas and renal cysts; kidney function usually preserved; possible evolution to ESKD, either by destruction of the renal parenchyma by multiple angiomyolipomas or following nephrectomies for haemorrhagic angiomyolipomas; if there is contiguous gene deletion of *TSC2* and *PKD1*, severe PKD with evolution to ESKD occurs before age 30 years	CNS (cortical tubers, astrocytomas, epilepsy, and mental retardation); skin lesions (facial angiofibromas and hypopigmented spots); pulmonary lymphangioleiomyomatosis; cardiac rhabdomyoma and retinal hamartoma; polycystic liver disease if contiguous deletion of both *PKD1* and *TSC2*
VHL[31]	Von Hippel-Lindau disease	Bilateral renal cysts, renal cell carcinoma	Haemangioblastomas of the retina, spine, or brain; pheochromocytoma; neuroendocrine tumour of the pancreas
COL4A1[32,33]	HANAC syndrome or *COL4A1*-related disease	Bilateral renal cysts occasionally reported; patients can develop renal insufficiency after about age 50–60 years	Microscopic haematuria, aneurysms, muscle cramps, elevated creatine phosphokinase, tortuosity of the retinal arteries
OFD1[34,35]	Oro-facial-digital syndrome type 1	X-linked, embryonically lethal in boys, PKD in women	Cleft palate, facial dysmorphy; syndactyly, clinodactyly, or polydactyly; mental retardation; polycystic liver disease

- The identification of PKD1 in 1995 and PKD2 in 1996 facilitated the development of DNA sequence–based molecular diagnostics.
- Driven by advancements in sequencing technology, our understanding of the complexities of the genetic basis of cystic kidney disease has evolved.

	PKD1-Associated ADPKD	*PKD2*-Associated ADPKD
Gene location	16p13.3	4q21
Protein product	Polycystin 1	Polycystin 2
Year gene discovered	1994	1996
No. of known pathogenic mutations in gene	>1,270	>200
Function of protein product	Receptor, adhesion molecule (not well understood)	Calcium-permeable nonselective cation channel
Proportion of ADPKD cases	64%-85%	15%-36%
No. of cysts	More numerous	Less numerous
Mean age at ESRD incidence, y	58.1	79.7

PKD1 and PKD2 genes and transcripts



Hughes et al, Nat Genet 1995
Mochizuki et al, Science 1996
Torres et al, Lancet. 2007

Evolution of Mutation Screening Technologies in ADPKD

- Future improvement of NGS-based molecular diagnostic for ADPKD will need to address several key issues including adequacy of target sequence coverage and read depth (especially in GC-rich regions), accuracy of mapping of raw sequence reads and variant calls to the duplicated region of PKD1 versus its pseudogene(s), and better identification of indels and CNVs.
- The use of WGS for molecular genetic diagnosis in ADPKD is promising but needs to be vigorously tested and validated

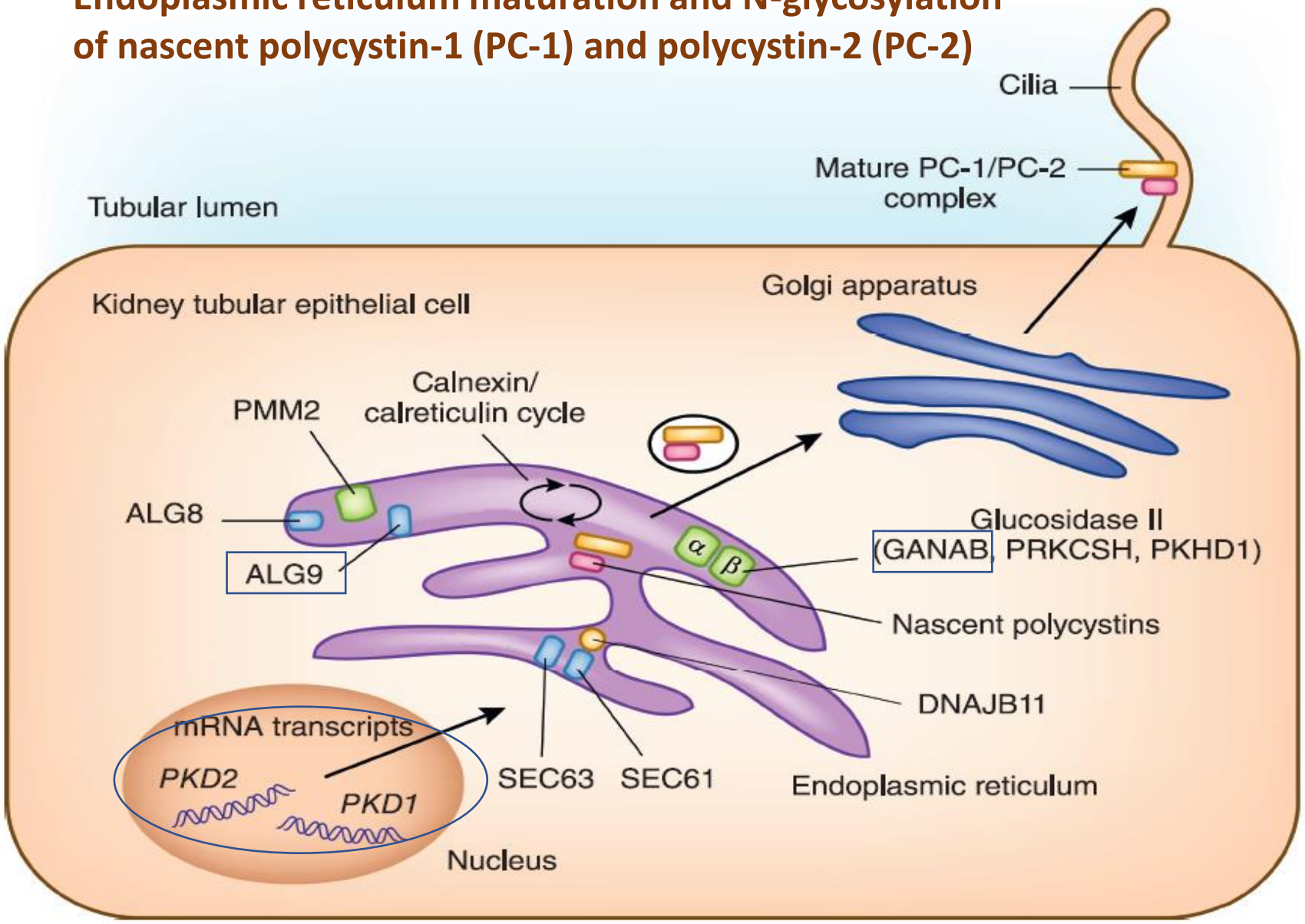
Song et al, Expert Rev Mol Diagn 2017

Genetic studies in ADPK

- Epidemiologic information
- Pathobiology mechanisms information
- Not all patients with multiple kidney cysts have ADPKD

- improving diagnostics
- providing prognostic value
- broadening the phenotypic spectrum of many kidney diseases

Endoplasmic reticulum maturation and N-glycosylation of nascent polycystin-1 (PC-1) and polycystin-2 (PC-2)



Lanktree et al, CJASN 2021

ADPKD

- 78% are due to mutations in PKD1
- 15% of cases are due to mutations in PKD2
- 0.3% are due to mutations in GANAB
- no mutation detected in the remaining cases

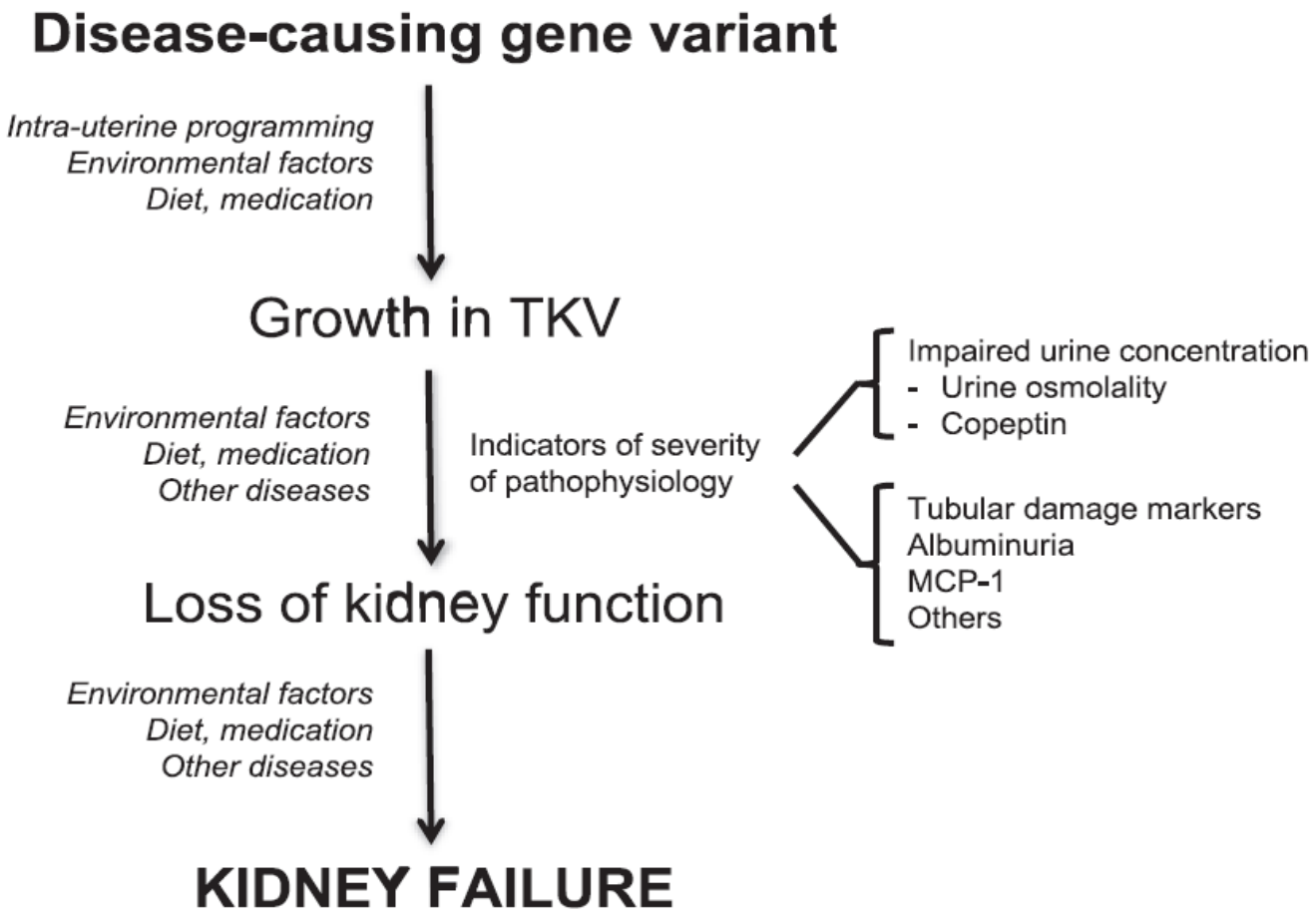
- **Gene linkage testing** and **direct mutation analysis/DNA sequencing**
- can determine if you have PKD with a 99 percent probability in those with family history. Linkage testing is not a direct analysis of the DNA sequence of the *PKD1* and *PKD2* genes. Instead, it relies on the identification of certain “markers” in the DNA of several members of a family in which PKD has been diagnosed. For linkage analysis, blood samples must be obtained from the person being tested as well as several (typically three or more) family members including those affected and unaffected by PKD. A detailed family history is also required. The results are typically reported to all family members that provided blood samples for the analysis

- **direct DNA sequencing** requires only a single sample from you (the person being tested). This method is a direct analysis of the DNA sequences of the *PKD1* and *PKD2* genes. It is private, and the results are only reported to you and your doctor.
- Using very specialized scientific equipment, each of the nearly 17,000 “bases” of DNA are analyzed and the entire sequence is thus determined.
- This method is capable of identifying those changes in the sequence that cause PKD.

. Minimal prevalence estimates of cystic kidney and liver diseases by population sequencing

Gene	Name	Minimal Prevalence[a]	Putative Function in Cystogenesis	Associated Disease
Autosomal dominant inheritance				
PKD1	Polycystin-1	1 in 1477	Moves from ER to cilia as complex with polycystin-2. Exact function remains unknown	ADPKD
PKD2	Polycystin-2	1 in 3914	Calcium permeable nonselective cation channel. Forms complex with polycystin-1	ADPKD
GANAB	α-Subunit of glucosidase II	1 in 4379	ER enzyme catalyzes hydrolysis of peptide-bound oligosaccharides	ADPKD
DNAJB11	DNAJ heat shock protein 40 subfamily B, member 11	1 in 12,312	ER glycoprotein cofactor for GRP78, required for protein trafficking	ADPKD
ALG9	α-1,2-mannosyltransferase	1 in 6156	Enzyme for protein *N*-glycosylation	ADPKD
PRKCSH	Protein Kinase C substrate, 80-kD heavy chain; β-subunit of glucosidase II	1 in 2077	ER enzyme catalyzes hydrolysis of peptide-bound oligosaccharides	ADPLD
SEC63	*Saccharomyces cerevisiae* homolog 63	1 in 4684	With SEC61 and GRP78, assists ER trafficking of membrane-inserted proteins	ADPLD
SEC61B	*S. cerevisiae* homolog 61, β-subunit	1 in 14,385	Core of translocon, a transmembrane channel for ER protein translocation	ADPLD
ALG8	α-3-glucosyltransferase	1 in 1429	Enzyme for protein *N*-glycosylation	ADPLD
LRP5	LDL receptor–related protein 5	1 in 3099	Coreceptor required for canonical Wnt signaling	ADPLD
TSC1	Hamartin	1 in 11,188	Facilitates HSP90 as chaperone for protein production including Tuberin; negative regulator of mTORC1	TSC
TSC2	Tuberin	1 in 2919	Activating GTPase of mTORC1 downregulators	TSC
VHL	VHL tumor suppressor	1 in 3301	Oxygen sensing, microtubule orientation, tumor suppression	VHL
COL4A1	Collagen type 4, α1	1 in 5594	Member of mesh-like type 4 basement membrane collagen	HANAC
Autosomal recessive inheritance				
PKHD1	Polycystic kidney and hepatic disease 1; Fibrocystin	1 in 201,993	Noncatalytic β-subunit of glucosidase II	ARPKD
DZIP1L	DAZ-interacting zinc finger protein 2	Approximately 1 in 3 million	Localizes to ciliary transition zone	ARPKD
PMM2	Phosphomannomutase 2	Approximately 1 in 3 million	Promoter mutation associated with reduced *N*-glycosylation	HIPKD

Markers of disease progression and factors contributing to the information contained in these markers



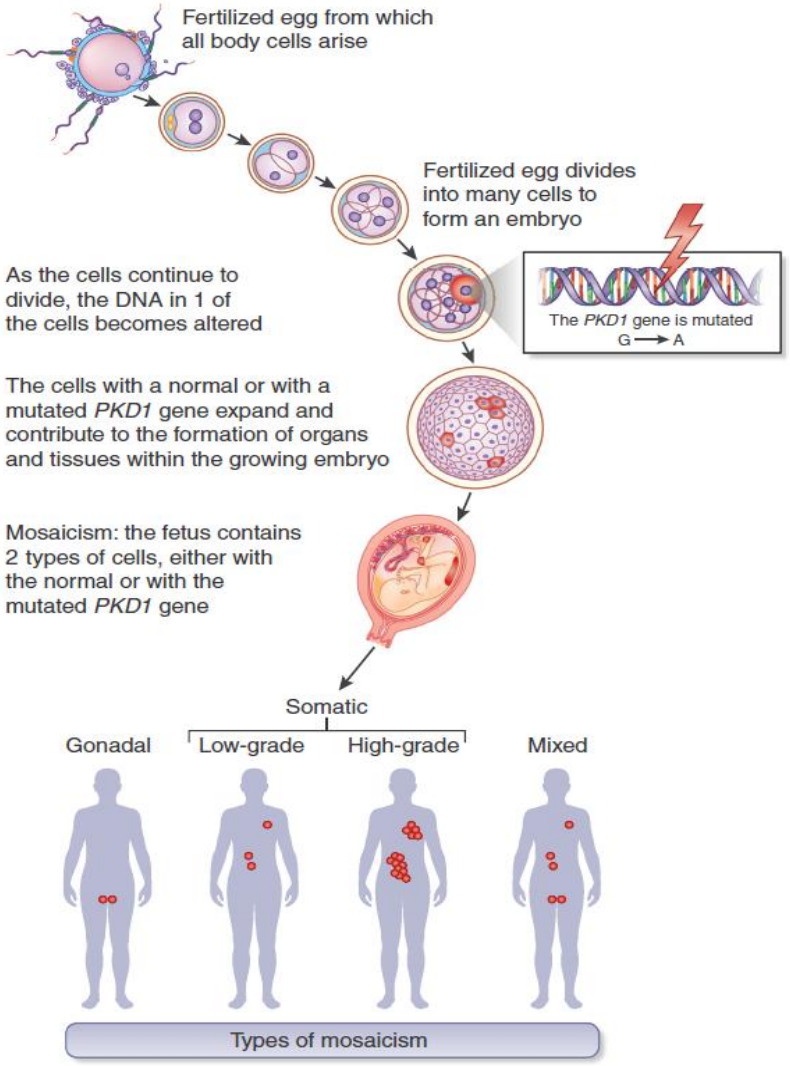
Müller et al, Nephrol Dial Transplant 2022

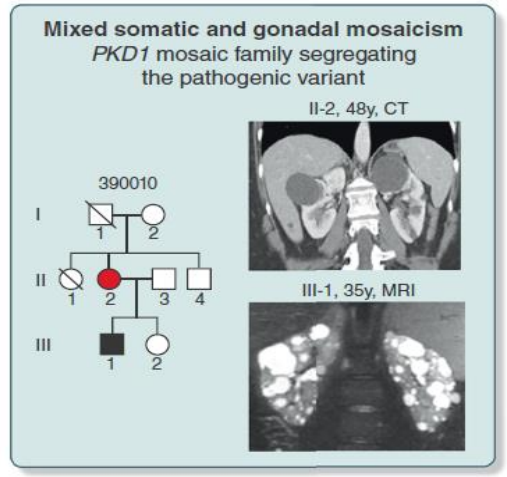
Mosaicism in ADPKD

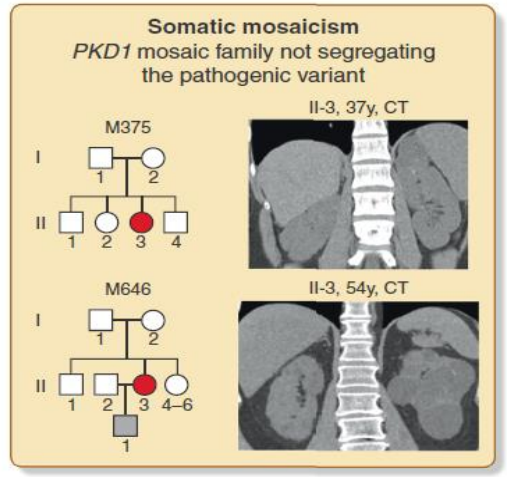
- **Readily detectable mosaicism by NGS**
- *1% of typical ADPKD population*
- *10% of genetically unresolved PKD cases*

Clinical findings highly suspicious of somatic mosaicism

- ✓ de novo PKD
- ✓ Mild PKD
- ✓ Asymmetric PKD
- ✓ unilateral PKD







Hopp et al, Kidney Int 2020
Devuyst et al, Kidney Int 2020

Syndromic Presentation

Differential diagnosis of cystic kidney diseases

Cystic disease	Gene	Renal features	Extrarenal features
ADPKD	*PKD1, PKD2*	Numerous bilateral cysts and kidney growth, progressive CKD	Liver cysts, intracranial and aortic aneurysms, heart valve defects, polycystic liver disease, colonic diverticulosis, hernias
Atypical ADPKD	*DNAJB11*	Few to many bilateral cysts interstitial fibrosis	Liver cysts without fibrosis
ADPLD	*PRKCSH, SEC63, GANAB, SEC61B, ALG8, LRP5*	No to a few renal cysts, no progression to ESRD	Liver cysts (usually mild)
Tuberous sclerosis complex	*TSC1, TSC2*	Angiomyolipomas, cysts	Cutaneous angiofibromas, retinal hamartomas, cardiac rhabdomyomas, LAM, cerebral tubers
ADTKD-UMOD, ADTKD-MUC1	*UMOD, MUC1*	CKD, occasional cysts	Hyperuricemia and gout
ADTKD-REN	*REN*	CKD, mild hypotension, cysts	Hyperuricemia, anemia, hyperkalemia
ADTKD-HNF1B	*HNF1B*	CKD, cysts, genitourinary tract malformations	MODY, hypomagnesemia, hypocalciuria, hyperuricemia with gout, mental retardation
Von Hippel–Lindau disease	*VHL*	Cysts, multifocal and bilateral clear cell carcinomas of the kidney	Retinal hemangiomas, cerebellar and spinal hemangioblastomas, pheochromocytoma
ARPKD	*PKHD1, DZIP1L*	Echogenic kidneys with medullary cysts diagnosed *in utero* and childhood	Congenital hepatic fibrosis, Caroli syndrome, hepatosplenomegaly, ascites, portal hypertension
Nephronophthisis	*NPHP1–13*	Corticomedullary cysts with normal or small kidneys, urinary concentrating and sodium reabsorption defect, progressive CKD	Retinitis pigmentosa, cerebellar vermis aplasia, hepatic fibrosis, skeletal dysplasia
Orofaciodigital syndrome	*OFD1*	Polycystic kidneys	Oral, facial, dental, digital and central nervous system anomalies
HANAC	*COL4A1*	Cysts, hematuria, decreased glomerular filtration rate	Muscle cramps, mild cerebral small vessel disease, retinal arteriolar tortuosity, intracranial aneurysms
HIPKD	*PMM2*	Antenatal or childhood onset polycystic kidney disease, CKD	Liver cysts, infantile hyperinsulinemic hypoglycemia

Lanktree et al, Nephrol Dial Transplant 2019

The genotype-phenotype relationship in ADPKD is not completely understood

- identity of the affected locus (PKD1 vs PKD2 mutation)
- allelic variant (truncating, nontruncating, or hypomorphic)
- timing of gene inactivation
- mosaicism
- genetic background

Syndromic Presentation

Marked intrafamilial disease discordance

Early and severe disease

- PGD involves extraction, amplification, and analysis of DNA removed from the blastomere or trophectoderm of embryos created using IVF
- The accuracy of PGD is highest when DNA analysis includes both direc mutation testing and linkage analysis of polymorphic markers closely linked to the gene of interest to ensure that there is no allelic drop out
- Identification of the pathogenic mutation is a prerequisite for PGD. If direct mutation analysis fails to identify a single pathogenic mutation, either because multiple potential pathogenic variants are identified or no mutation is detected, the use of family-based linkage analysis may be required to determine the locus of the pathogenic PKD mutation.
- The patient must have a large enough family to support linkage to PKD1 or PKD2.

Murphy et al, Am J Kidney Dis 2018

No Apparent Family History

Scenarios with evolving indication for genetic testing

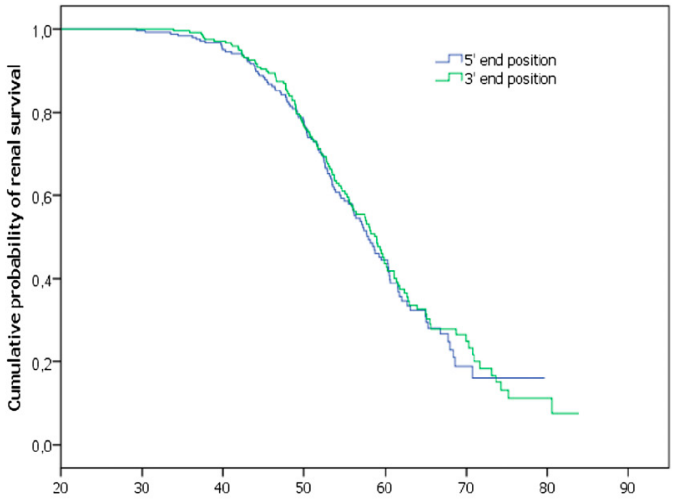
- ✓ **Identifying "high-risk" patients for novel disease modifier therapy or clinical trial**
- ✓ **Delineating the cause of atypical clinical presentation**
  - *Early and severe disease*
  - *Discrepancy between imaging findings and decrease in kidney function*
  - *Asymmetric, unilateral, segmental, or lopsided cystic kidneys*
  - *Marked intrafamilial disease discordance*
  - *Suspected somatic mosaicism*
  - *Syndromic forms of polycystic kidney disease*

Atypical kidney imaging

- All patients with ADPKD who might consider having children in the future should be counseled about family planning and reproductive options, including PGD and available genetic sservices.
- • The decision to pursue or decline assisted reproductive technology or prenatal diagnosis of ADPKD is a personal choice based on patient beliefs, experience, and preferences; either decision should be fully supported.
- • Genotype, early-onset hypertension, and large htTKV predict high risk for clinical progression to ESRD.
- • Other factors that may lead to patient interest in PGD include family history of aggressive disease manifestations such as intracranial aneurysm rupture or complications of hepatic cysts.
- • Initial genetic testing consists of identifying the disease causing mutation. In a minority of patients, no mutation may be identified.
- • DNA from additional affected family members may be required to determine the pathogenicity of a PKD gene variant and to increase the accuracy of PGD.

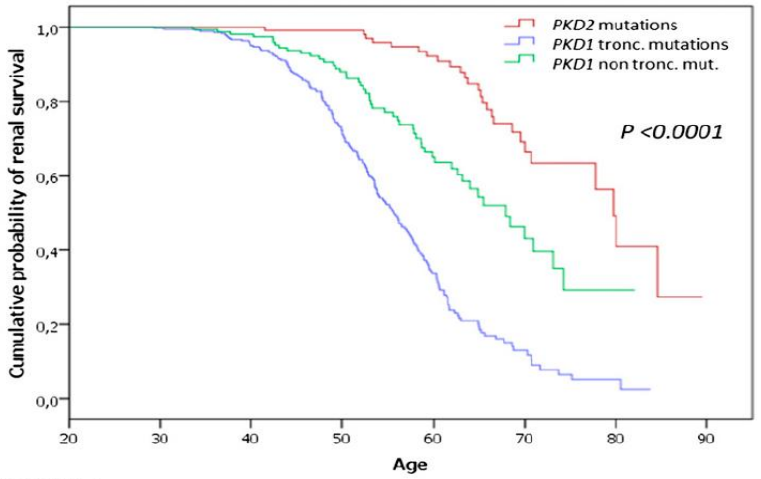
Murphy et al, Am J Kidney Dis 2018

PKD1 mutation type, but not its location, influences renal survival



Patients at risk :						
5' end mutations (n=282)	261	209	132	48	8	0
3' end mutations (n=289)	266	229	143	52	17	3

Patients carrying a truncating PKD1 mutation were 2.74 times more likely to develop ESRD than those carrying a nontruncating PKD1 mutation



Patients at risk :						
PKD1 truncating mutations (n=387)	356	296	175	53	11	2
PKD1 non truncating mutations (n=184)	172	144	134	48	15	1
PKD2 Mutations (n=133)	127	116	99	63	23	5

Cornec-Le Gall, J Am Soc Nephrol 2013

Comparison of NGS methods for PKD mutation screening

	Long-range PCR-based screening	Capture-based screening	Whole-genome sequencing
Target gene selection	At the time of PCR primer design	At the time of capture probe design	Computationally after sequencing
Capture efficiency at GC-rich region	High	Lower	Lower
False-positive rate	Low	Higher	Higher
Allelic dropout or bias	Yes	No	No
Detection of copy number variation	Difficult	Possible	Yes
Off-target capturing	Minimal	Yes	Yes
Ambiguous mapping	No	Yes	Yes
Sequencing of promoter and introns	If included in primer design	If included in probe design	Yes
Lab labor intensity	High	Low	Low
Computational intensity	Low	High	Highest
Costs of research sequencing	High	Low	Highest

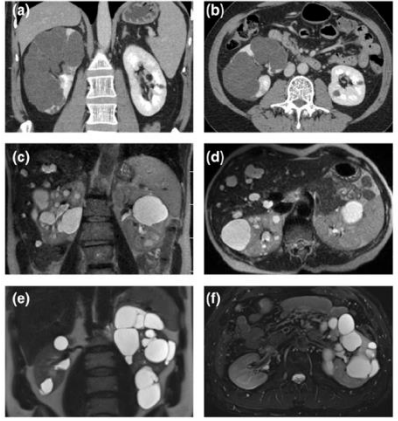
Song et al, Expert Rev Mol Diagn 2017
Lanktree et al, Nephrol Dial Transplant 2017

- Incomplete penetrance of many nontruncating PKD1 alleles has shown potential prognostic value.

✓not fully inactivating the protein

✓associated with milder kidney disease

- 30%–35% of PKD1 pathogenic alleles are nontruncating, it is a challenge to differentiate pathogenic from neutral changes, and their degree of penetrance.



Atypical Kidney Imaging

Present in up to 16% of patients suspected to have ADPKD

- ***Nontruncating PKD1 or PKD2*** mutations if positive family history and mild cystic disease
- ***Second kidney disease*** in patients with moderate to advanced CKD but mild cystic disease without kidney enlargement
- ***Somatic mosaicism*** if no family history of ADPKD and unilateral, asymmetric, segmental, or lopsided cystic disease

Class, Subclass, and Term	Description
1: Typical ADPKD	Bilateral and diffuse distribution, with mild, moderate, or severe replacement of kidney tissue by cysts, where all cysts contribute similarly to TKV. (Supplemental Figure 1, A and B)
2: Atypical ADPKD	
A	
Unilateral	Diffuse cystic involvement of one kidney causing marked renal enlargement with a normal contralateral kidney defined by a normal kidney volume (<275 ml in men; <244 ml in women) and having no or only 1–2 cysts (Supplemental Figure 1C)
Segmental	Cystic disease involving only one pole of one or both kidneys and sparing the remaining renal tissue (Supplemental Figure 1D)
Asymmetric	Diffuse cystic involvement of one kidney causing marked renal enlargement with mild segmental or minimal diffuse involvement of the contralateral kidney defined by a small number of cysts (>2 but <10) and volume accounting for <30% of TKV (Supplemental Figure 1E)
Lopsided	Bilateral distribution of renal cysts with mild replacement of kidney tissue with atypical cysts where ≤5 cysts account for ≥50% TKV (the largest cyst diameter is used to estimate individual cyst volume) (Supplemental Figure 1F)
B	
Bilateral presentation with acquired unilateral atrophy	Diffuse cystic involvement of one kidney causing moderate to severe renal enlargement with contralateral acquired atrophy (Supplemental Figure 1G)
Bilateral presentation with bilateral kidney atrophy	Impaired renal function (serum creatinine≥1.5 mg/dl) without significant enlargement of the kidneys, defined by an average length <14.5 cm, and replacement of kidney tissue by cysts with atrophy of the parenchyma (Supplemental Figure 1H)

Irazabal et al, *J Am Soc Nephrol 2015*
Iliutal et al, J Am Soc Nephrol 2017

de novo PKD with atypical kidney imaging patterns

suspect somatic mosaicism

- The diagnosis of mosaicism is challenging due to variable involvement of the affected cells resulting in a low mutation signal-to-noise ratio, and it is frequently missed by Sanger sequencing.
- Future studies using “molecular bar coding” of template DNA from different tissues (e.g., buccal mucosa and urinary epithelia) may improve the detection rate of mosaic cases with even lower variant allele fractions (<2% of reads).

Procedures and Potential Pitfalls of IVF With PGD

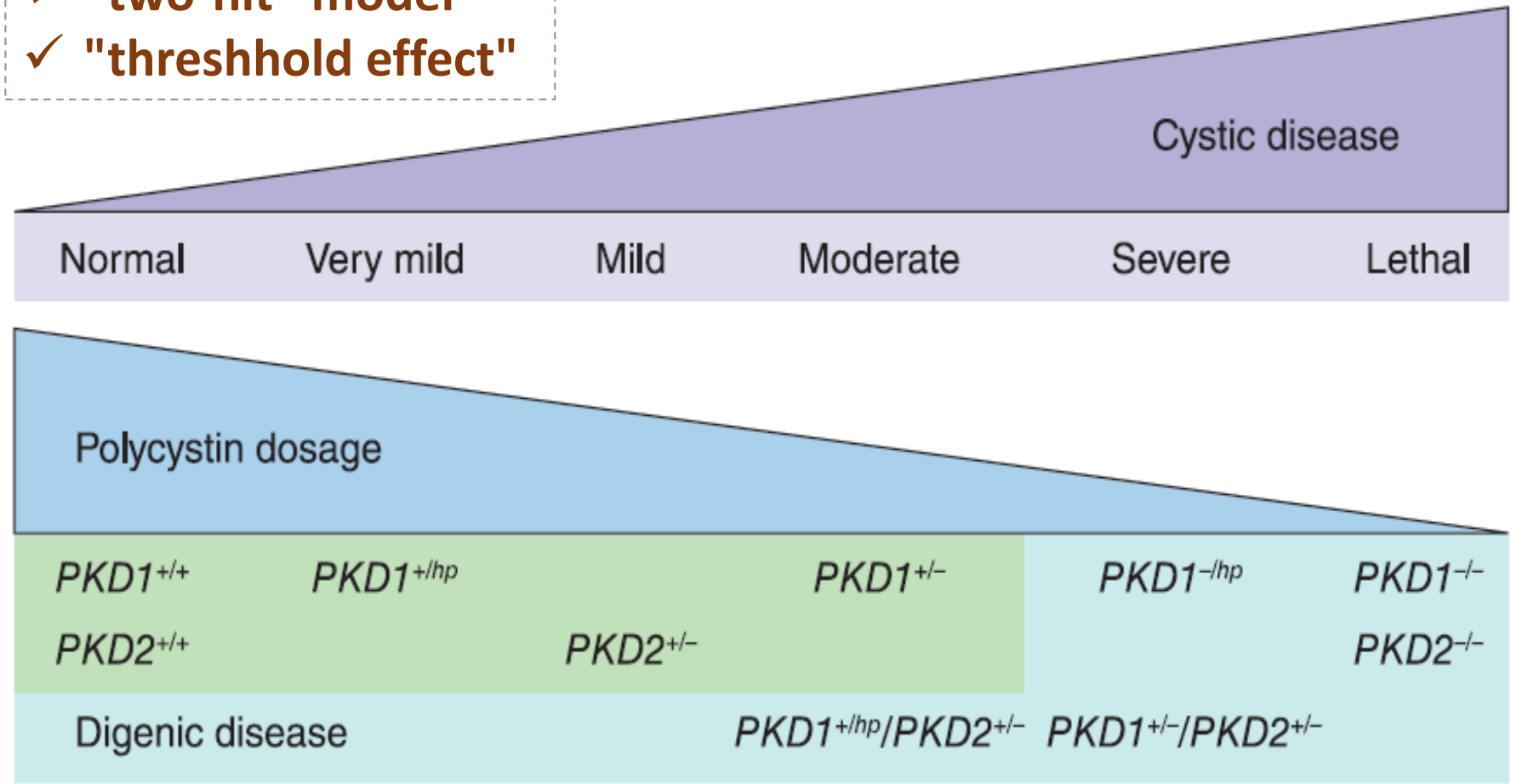
Procedure	Description	Potential Pitfall
1	Mutation identification for ADPKD using direct mutation analysis (common) or linkage analysis (rare)	Failure to identify a pathogenic mutation
2	Family linkage analysis may be needed to determine variant pathogenicity	Patient may have a de novo mutation or may not have enough affected family members to perform a linkage-based study
3	Egg harvest and sperm collection with subsequent IVF	Small risks to mother associated with IVF-related hormonal stimulation and egg harvesting; small increased risk for birth defects
4	DNA removal by biopsy from blastomere or trophectoderm	Damage to the embryo rendering it unusable
5	Genetic analysis of embryonic tissue by direct mutation analysis and/or linkage-based analysis	DNA degradation; low risk for misdiagnosis due to biological phenomenon or technical problems
6	Transfer to womb or freezing of embryos predicted to be without the pathogenic mutation	Pregnancy may not be achieved
7	Confirmatory prenatal or postnatal diagnosis	Low risks for miscarriage associated with confirmatory prenatal diagnosis procedure; risk that the fetus may have been misdiagnosed as unaffected

Murphy et al, Am J Kidney Dis 2018

- Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic hereditary kidney disease worldwide affecting all racial groups.
- Compex genetic mechanisms underlie the variable clinical presentation.

Effects of digenic disease on functional polycystin dosage and cystic disease severity

- ✓ “two-hit” model
- ✓ "threshhold effect"



Lanktree et al, CJASN 2021

Patients carrying a truncating PKD1 mutation were 2.74 times more likely to develop ESRD than those carrying a nontruncating PKD1 mutation

Disease	Number of Patients (Number of Pedigrees)	Median Age at ESRD, yr
ADPKD-*PKD1*^{T}	219 (54)	54
	701 (450)	55.1
	249 (72)	52.5
ADPKD-*PKD1*^{NT}	323 (228)	65.8
	152 (51)	70.8[a]
ADPKD-*PKD2*	291 (31)	74
	395 (71)	72
	117 (23)	70
	248 (172)	77.8
	213 (57)	80
	293 (203)	77.8
ADPKD-*GANAB*	22 (11)	No cases of ESRD described

Cornec-Le Gall, J Am Soc Nephrol 2013
Heyer et al, J Am Soc Nephrol 2016
Hwang et al, J Am Soc Nephrol 2016
Cornec-Le Gall et al, J Am Soc Nephrol 2018

Mutation class predicts disease variability

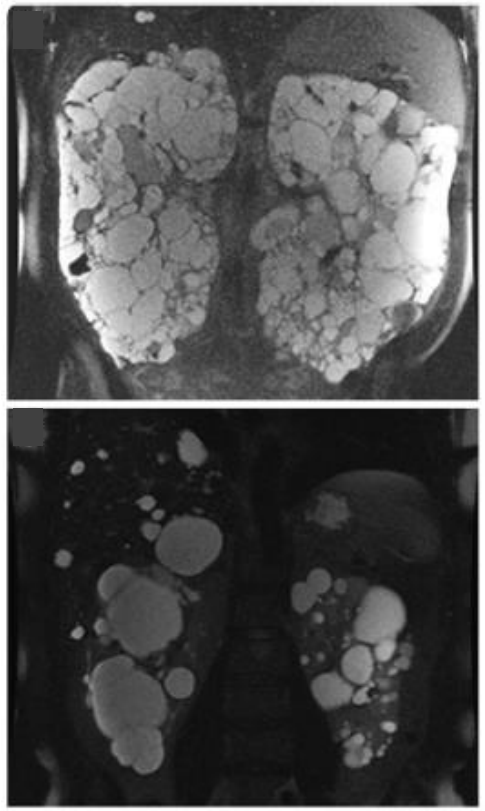
- **Protein-truncating PKD1 mutations**

➢ frameshift, nonsense, and canonical splice site mutations and large deletions

- **Nontruncating PKD1mutations**

➢missense and in-frame insertions/deletions

- **PKD2 mutations**



- **The high level of DNA sequence identity with the pseudogenes creates the possibility or both false positive and negative genotype calls**
  - a pseudogene mutation can be incorrectly called as present in PKD1
  - a PKD1 mutation can be missed if the signal is overwhelmed by the normal sequence in the pseudogenes when DNA capture assay is used

Mutation screening of patients with ADPKD with high-risk clinical features for progression

- 75% carried PKD1 mutations
- 15% carried PKD2 mutations
- at least 10% had no mutation detected

❑ *CRISP COHORT*
❑ *GENKYST COHORT*

Rossetti et al, J Am Soc Nephrol 2013

Cornec Le-Gall J Am Soc Nephrol 2016

No identifiable PKD1 or PKD2 mutation 6–11% of patients with PKD despite comprehensive screening.

Mutation screening of patients with normal or near-normal kidney function

- 60% carried PKD1 mutations
- 25% carried PKD2 mutations
- 15% had no mutation detected

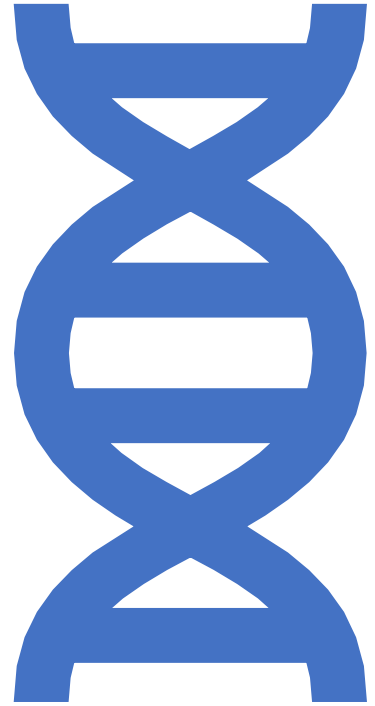
❑ *TGESP COHORT*

Hwang et al, J Am Soc Nephrol 2016

- Integrated results of NGS-based PKD genetic screen has the potential

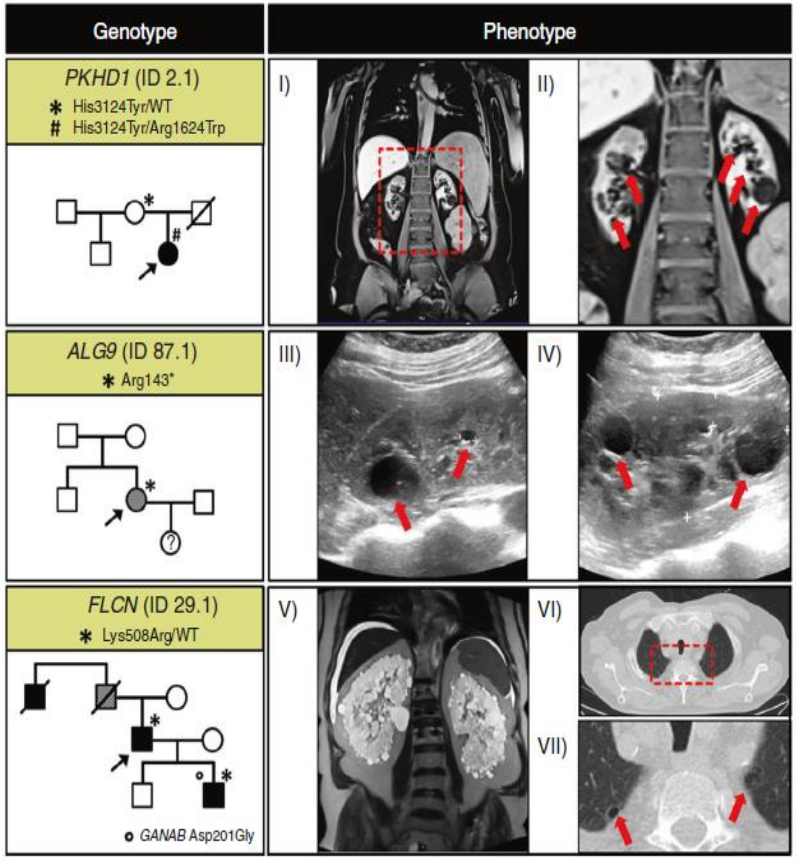
✓ to provide highly accurate risk stratification

✓to advance personalized precision medicine for ADPKD in the era of mechanism-based therapeutics



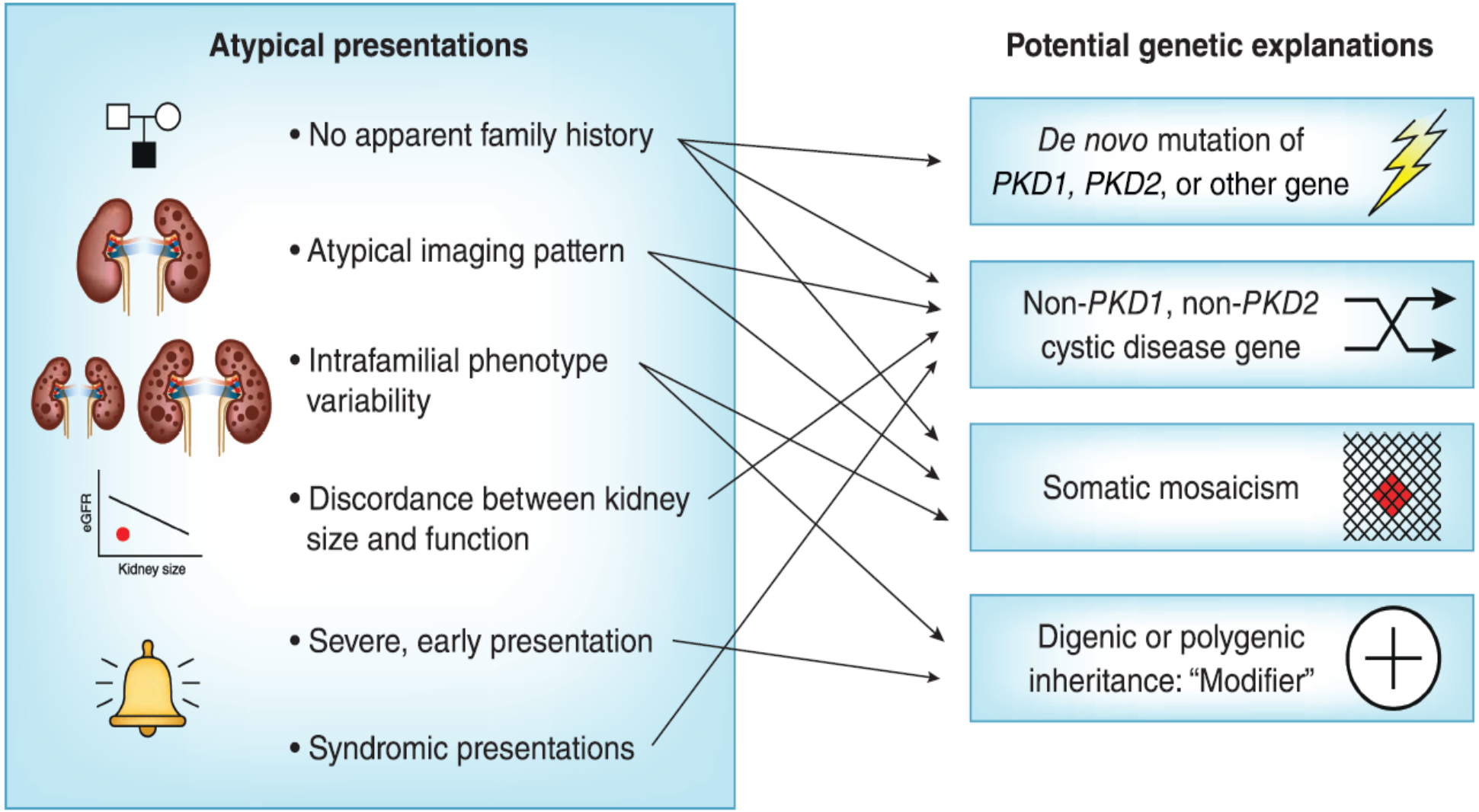
Matching clinical and genetic diagnoses in autosomal dominant polycystic kidney disease reveals novel phenocopies and potential candidate genes

- Genetic analysis enables detection of ADPKD phenocopies and excludes *PKD1/2*-negative patients from potentially harmful treatment.
- Patients suffering from conditions such as TSC may need mTOR inhibitor treatment which were not shown to be beneficial in ADPKD.
- As TSC and BHDS represent tumor syndromes, disease recognition is crucial for affected families, prompting intrafamilial screening for malignancies.



Schönauer et al, Genetics in Medicine 2020

Clinical scenarios of atypical ADPKD presentations and potential genetic explanations



Lanktree et al, CJASN 2021