ERKNet
The European Rare Kidney Disease Reference Network

Nephrology and genetics

Genetic diagnosis and application

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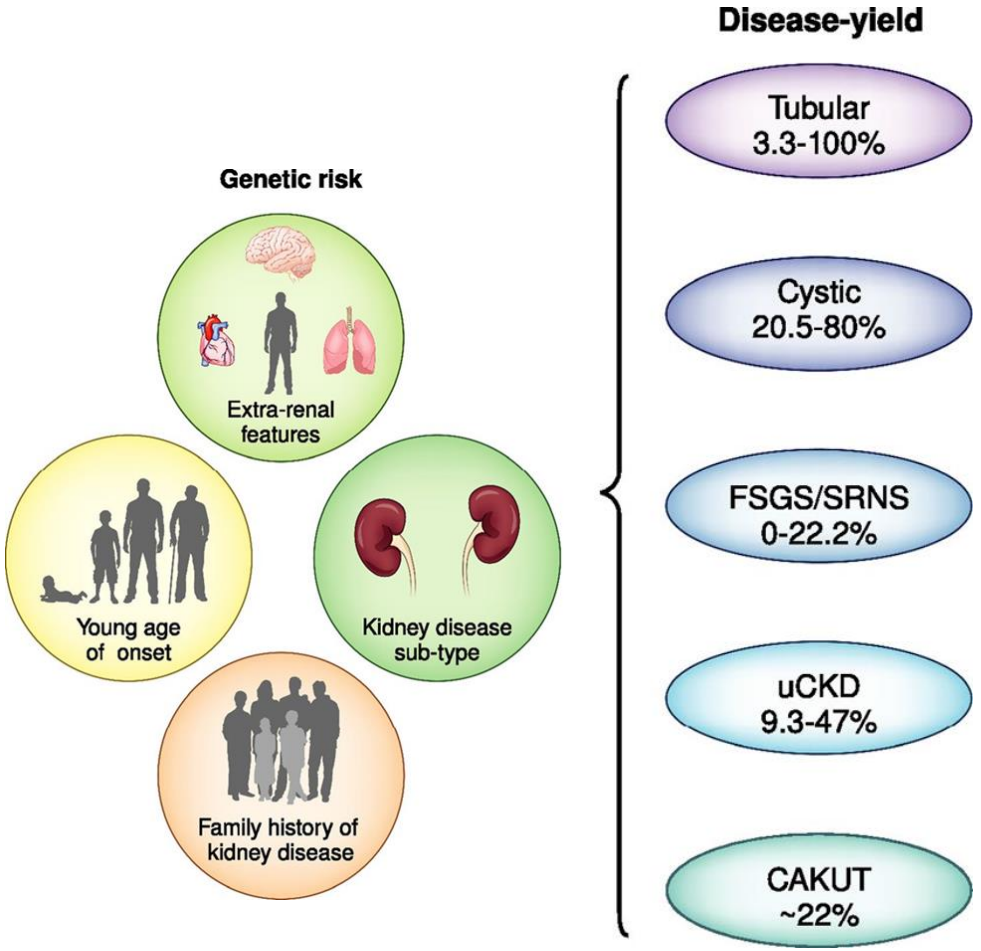
Rare kidney diseases

- 150 different disorders
- < 600 genes
- Overall prevalence:
  - 60-80 cases / 100,000 total population in Europe
- **30% of adults** with non-diabetic chronic kidney disease
- **and >50% of children** with CKD



Enrico Cocchi .CJASN July 2020, CJN.15141219

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ESKD

Table 1. Frequency of clinical diagnostic subgroups in patients with childhood-onset ESKD versus adult-onset ESKD

Diagnostic group	Childhood-onset ESKD[a]		Adult-onset ESKD[b]	
	N	%	*N*	%
CAKUT		(39)	NR	
Aplasia/hypoplasia/dysplasia	1769	16		
Obstructive uropathy	1713	15		
Reflux nephropathy	576	5		
Prune belly	279	2		
Glomerulonephritis	1845	16	8802	8
FSGS	1308	12	–	
Congenital nephrotic syndrome	289	3	–	
Membranous nephropathy	51	<1	–	
Cystic kidney disease			2482	2
PKD	339	3		
Medullary cystic kidney disease	305	3		
Cystinosis	225	2	NR	
Oxalosis	58	1	NR	
Renal infarct	144	1	NR	
Diabetic nephropathy	11	<1	51 339	44
Hypertension	0	0	33 585	29
Other/unknown	2270	20	20 782	18
Total	11 182	100	116 990	100

Note that where there is a much higher occurrence of CAKUT (39%) in childhood-onset disease, diabetic nephropathy (44%) and hypertensive nephropathy (29%) predominate in adult-onset disease.

[a]Adapted from Contributions of the Transplant Registry: The 2014 Annual Report of the North American Pediatric Renal Trials and Collaborative Studies, https://web.emmes.com/study/ped/annlrept/annualrept2014.pdf (8 February 2019, date last accessed).

[b]Adapted from the 2015 United States Renal Data System Annual Data Report detailing the primary cause of ESRD in incident cases of hemodialysis, peritoneal dialysis and transplantation in the US population, https://www.usrds.org/2015/download/vol2_USRDS_ESRD_15.pdf (8 February 2019, date last accessed).

NR, not reported; PKD, polycystic kidney disease.

Genetic nephrology workflow

Summarize clinical and phenotypic data

Consider hereditary disorders that are compatible with the clinical findings

Suspect single or several discrete genes

Targeted NGS panel

Genetically heterogeneous or multisystemic disease

WES/WGS

Clinical sequence interpretation: genes and variant classes that are broadly relevant to the phenotype

Patient-level interpretation : how concordant are the genetic findings with the patient's phenotype

Genetic diagnosis and clinical application

Adapted from Nat Rev Nephrol.10.1038

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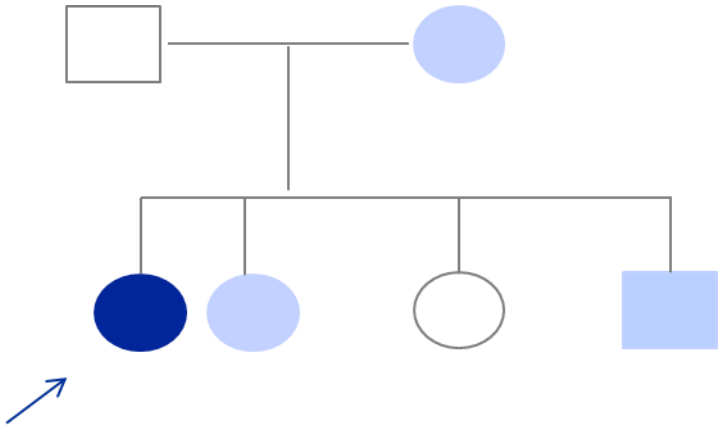
1. Case-based discussion



« **Blood in urine** » since the age of 5

Age 9 : first kidney biopsy

→ « endocapillary GN »

Age 23 : « hematuric cystitis »

Age 25 : proteinuria 2,5 g/24 h

→ 2nd kidney biopsy : **« FSGS »**

Age 26 : nephrotic syndrome (7g/24 h)

ser creat 0,8 mg/dl

R/ angiotensin blockers

Age 28 : proteinuria 2 g/24 h

→ second opinion about diagnosis before planning pregnancy

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ClinGen
Clinical Genome Resource

Glomerulopathy Gene Curation Expert Panel

Affiliated to **Kidney Disease CDWG**

Membership

The broad scope of the Glomerulopathy Gene Curation Expert Panel (GCEP) will be to curate genes associated with glomerular disorders and the altered function of podocytes, endothelial cells, mesangial cells, and the glomerular basement membrane (GBM). Defects in >150 genes have been asserted to cause disorders of the glomerular filtration barrier and this list will continue to expand. The initial focus of the Glomerulopathy GCEP will be on Mendelian disorders arising from the altered expression or function of glomerular-expressed genes, presenting with idiopathic haematuria and/or proteinuria. The GCEP will then go on to address monogenic disorders arising from genes expressed elsewhere that primarily impact the glomerular filtration barrier. Where significant overlap exists with the purview of other GCEPs, curations will be jointly reviewed. The gene list for curation was selected based on expert consensus, combined with review of literature and of multi-gene sequencing panels from the Genetic Testing Registry, and will be organized in order of priority, as described above.

Gene list: ACTN4, ACVRL1, ADA2, ADAMTS13, ALG1, ALMS1, AMN, ANKFY1, ANLN, APOA1, APOE, APOL1, ARHGAP24, ARHGDIA, B2M, C1QA, C1QB, ~~C1QC, C2, C3, C4A, CASP10~~, CBLIF, CD151, CD19, CD2AP, CD81, CDK20, CFH, CFHR5, CFI, CLCN5, COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, COL4A6, COPA, COQ2, COQ6, COQ7, COQ8A, COQ8B, COQ9, CRB2, CUBN, CYP11B2, DAAM2, DGKE, DHFR, DHTKD1, DKC1, DLC1, DLG3, DNASE1L3, E2F3, EMP2, ENG, ERCC6, ERCC8, FAT1, FGA, FN1, FOXC2, GAPVD1, GATA6, GDF2, GLA, GLB1, GON7, GSN, IL1RAP, INF2, ITGA3, ITGB4, ITSN1, ITSN2, KANK1, KANK2, KANK4, KIRREL1, LAGE3, LAMA5, LAMB2, LAMC2, LCAT, LMNA, LMX1B, LYZ, MAFB, MAGI2, MED28, MEFV, MTR, MTRR, MYH9, MYO1E, NARS2, NEIL1, NEU1, NLRP3, NOP10, NOS1AP, NPHP4, NPHS1, NPHS2, NUP107, NUP133, NUP160, NUP205, NUP37, NUP85, NUP93, NXF5, OCRL, OSGEP, PAX2, PDSS1, PDSS2, PGK1, PGM3, PLCE1, PLCG2, PMM2, PODXL, PRKCD, PTPRO, SCARB2, SGPL1, SLC17A5, SLC19A2, SLC19A3, SMARCAL1, SOX18, SYNPO, SYNPO2, TBC1D8B, TNFRSF1A, TNS2, TP53RK, TPRKB, TREX1, TRIM8, TRPC6, TTC21B, TTR, VIPAS39, VPS33B, WAS, WDR73, WT1, XPO5, YAP1, YRDC, ZAP70, ZMPSTE24

Expert Panel Status - *Approved Expert Panel*

Step 1	Step 2
Define Group	Expert Panel Approval
Complete Mar. 2021	*Completed Apr. 2021*

Chairs

Rachel Lennon, BMBS, PhD

Martin Pollak, MD

Cathy Quinlan, MD

Coordinators

Please contact a coordinator if you have questions.

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Hannah Dziadzio
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Two mutations in the gene *COL4A3* were identified

1. *p.Gly421Ser* on the maternal allele
2. *p.Arg1661Cys* on the paternal allele

→ **Alport, autosomal recessive**

***COL4A3/COL4A4* Mutations Producing Focal Segmental Glomerulosclerosis and Renal Failure in Thin Basement Membr**

Konstantinos Voskarides,* Loukas Da
Stalo Christodoulidou,† Valsamakis H
Yiannis Athanasiou,‖ Charalampos Pat
Kyriacos Kyriacou,‡ and Constantinos

J Am Soc Nephrol 18: 3004–301

Rare hereditary COL4A3/COL4A4 variants may be mistaken for familial focal segmental glomerulosclerosis

Andrew F. Malone[1,2], Paul J. Phelan[1,2], Gentzon Hall[1,2], Umran Cetincelik[3], Alison Homstad[1,4], Andrea S. Alonso[1,4], Ruiji Jiang[1,4], Thomas B. Lindsey[1], Guanghong Wu[1], Matthew A. Sparks[2], Stephen R. Smith[2], Nicholas J.A. Webb[5], Philip A. Kalra[6], Adebowale A. Adeyemo[7], Andrey S. Shaw[8], Peter J. Conlon[9], J. Charles Jennette[10], David N. Howell[11], Michelle P. Winn[1,2] and Rasheed A. Gbadegesin[1,4]

Kidney International (2014) **86,** 1253–1259

AS: clinical features

Renal disease

	Hemi-zygote	XL hetero-zygote	AR homo-zygote	AR hetero-zygote
Microscopic haematuria	all	90%	all	60%
Gross haematuria	60%	30%	70%	10%
Proteinuria	all	65%	all	15%
Probability ESRF by the age 40	90%	10%	90%	0

Mild renal disease if compound heterozygosity with 2 missense changes

JASN October 2003, 14:2603-2610
JASN December 2013, 24:1945-1954

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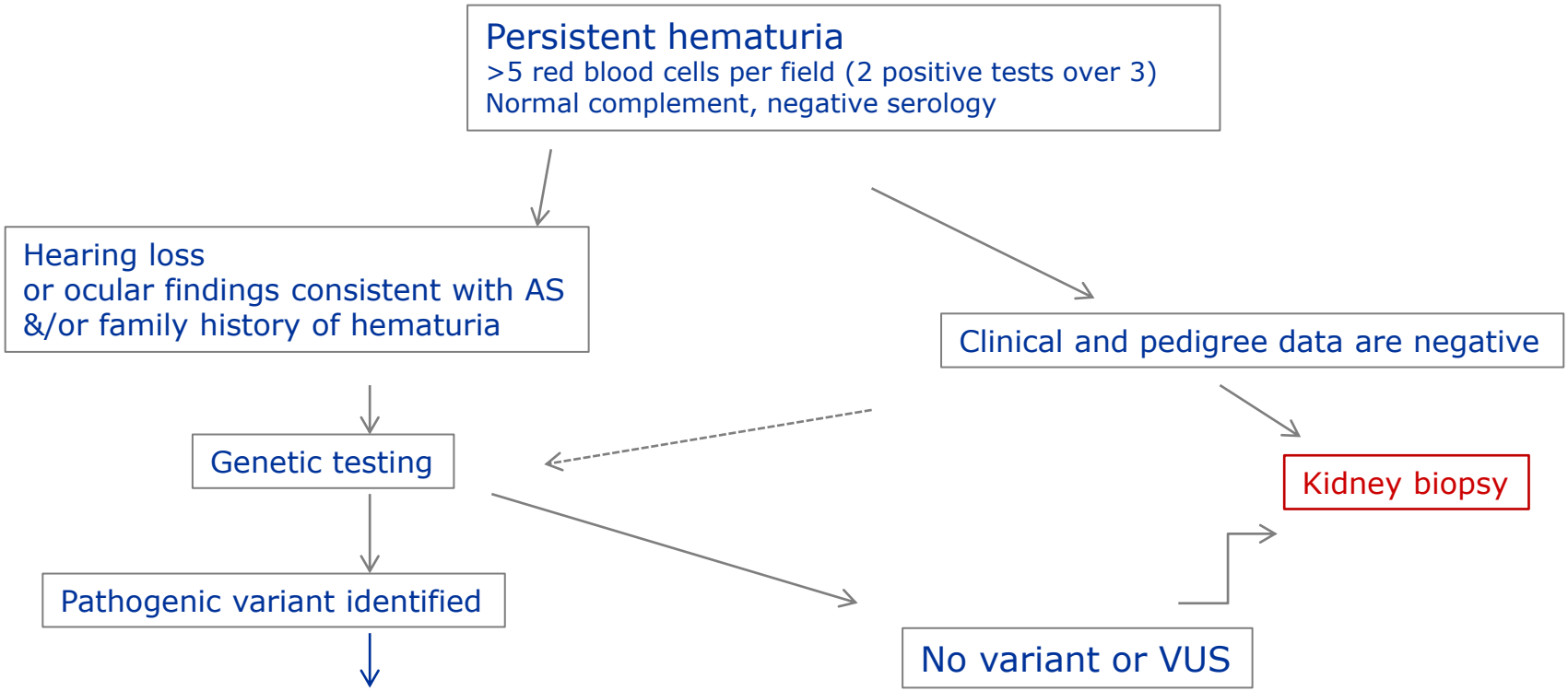
ACKD Advances in Chronic Kidney Disease
NATIONAL KIDNEY FOUNDATION
REVIEW ARTICLE | VOLUME 29, ISSUE 3, P225-230, MAY 01, 2022

What the Adult Nephrologist Should Know About Alport Syndrome

Clifford E. Kashtan



Manage as Alport syndrome

- regular follow up
- manage RAASi according recommendations
- screen at-risk relatives
- provide information about advocacy groups & registries

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Prevalence Estimates of Putative Pathogenic *COL4A3* – *COL4A5* Variants in Population Sequencing Databases and Their Implications for Alport Syndrome

JASN
JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY

How common is Alport syndrome?

gnomAD database of *COL4A3* – *COL4A5* variants in > 200,000 normal people were examined for predicted pathogenic variants using ACMG criteria

Gly -Xaa-Yaa-Gly-Xaa-Yaa-Gly

Gly -Xaa-Yaa-Asp-Xaa-Yaa-Gly

Truncating variants and Position 1 Gly substitutions were filtered (computational data, conservation in vertebrates, previous reports of pathogenicity) for predicted pathogenic variants

Prevalence of variants differed in different racial groups. Variants often had features that mitigated their clinical effects and explained why they were undetected.

Conclusion: Predicted pathogenic *COL4A5* variants were found in one in 2320 and heterozygous *COL4A3* or *COL4A4* variants in one in 106 individuals. However, these prevalence estimates do not include large deletions, intronic changes, non-Gly substitutions and known diagnoses.

doi: 10.1681/ASN.2020071065

COL4A5 variant:	1/2320 (recurrent change (p.Gly624Asp ~48%))
COL4A3/COL4A4 heterozygosity :	1/106
Compound heterozygous variants :	1/88.866
Digenic variants :	1/44.793

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Issues

- Diagnosis of progressive nephropathy made very early
- Carrier testing among relatives at risk
- Genetic counseling
  - The offspring of an individual with **autosomal recessive AS** are obligate heterozygotes (carriers) for a pathogenic variant
  - However, the risk to have a child affected with autosomal recessive AS is increased
  - Testing of reproductive partners is recommended
  - carrier frequency :~ 1%

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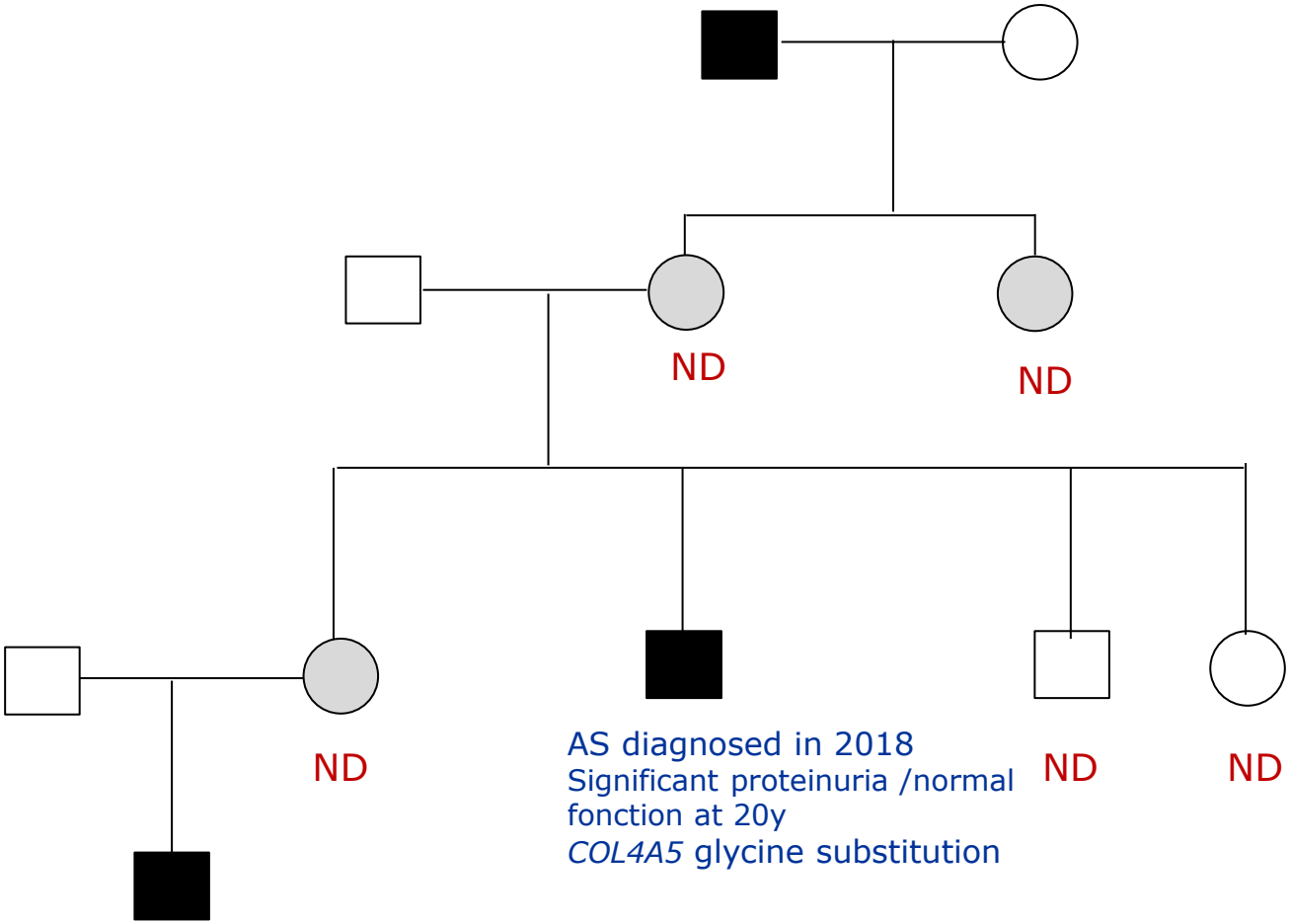
2. Case-based discussion



Prenatal testing at 32w in January 2022
Genetic counseling in October 2022

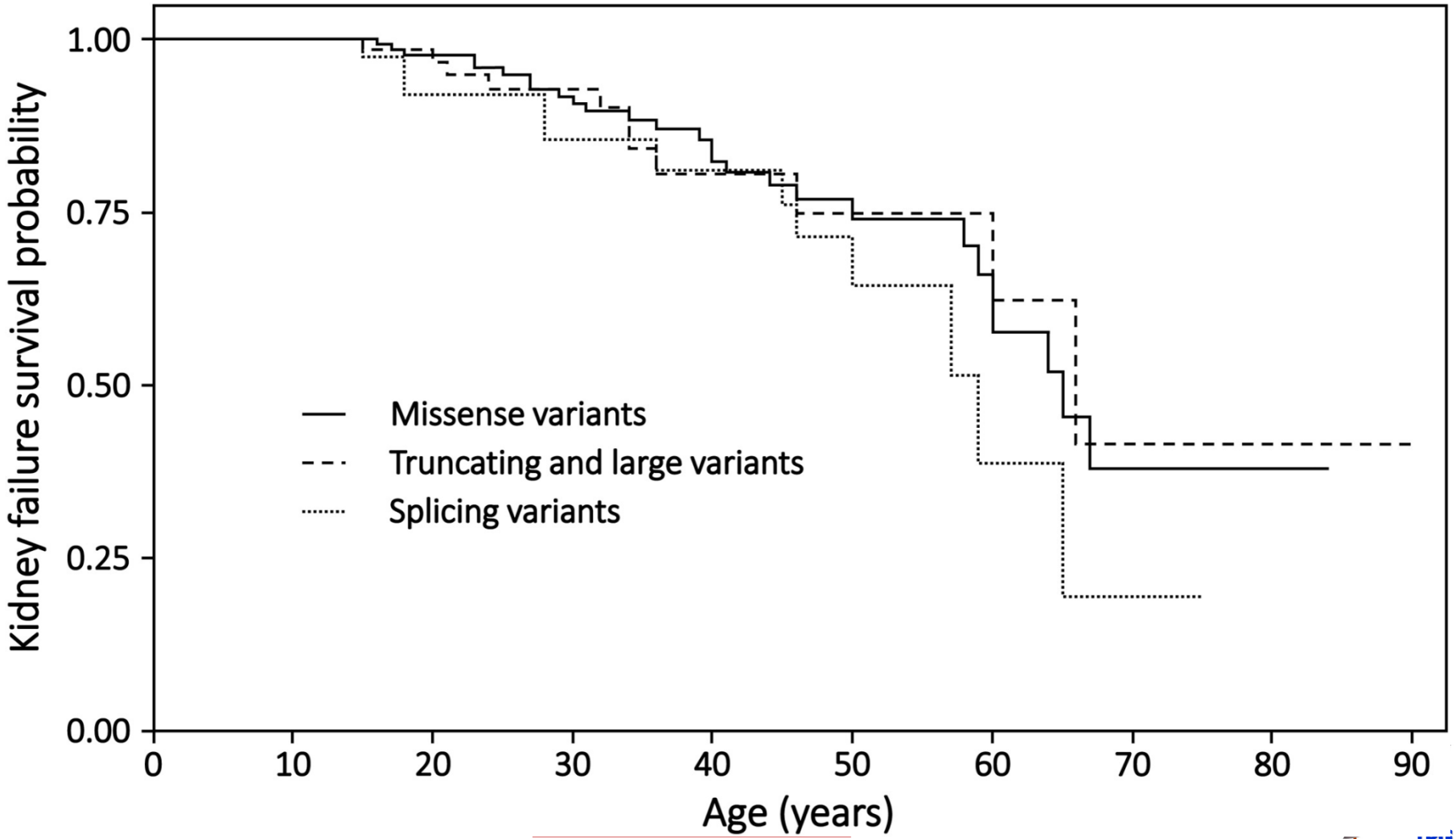
KI REPORTS
Kidney International Reports
ISN

CLINICAL RESEARCH | VOLUME 7, ISSUE 11, P2454-2461, NOVEMBER 01, 2022

A Systematic Review of Pathogenic *COL4A5* Variants and Proteinuria in Women and Girls With X-linked Alport Syndrome

Joel T. Gibson • Mikayla de Gooyer • Mary Huang • Judy Savige

Open Access • Published: August 29, 2022 • DOI: https://doi.org/10.1016/j.ekir.2022.08.021 •



X-linked kidney disorders in women

Table 1. Common X-linked Kidney Disorders

Gene	OMIM	Disease	Male Phenotype (Untreated)	Female Phenotype (Untreated)
COL4A5	301050	Alport syndrome	Kidney failure, 100% Hearing loss, 90%	Kidney failure, 10%-40% Hearing loss, 30%
GLA	301500	Fabry disease	Kidney failure, 14% Neuropathic pain, 76% Cardiac disease, >50% Hypohidrosis, 54%	Kidney failure, 10% Neuropathic pain, 64% Cardiac disease, 59% Hypohidrosis, 25%
AVPR2	304800	Nephrogenic diabetes insipidus	Concentrating defect, 100%	Concentrating defect, 25%
PHEX	307800	X-linked hypophosphatemic rickets	Bone disease, 100%	Bone disease, 85%
CLCN5	300009	Dent disease type 1	Nephrocalcinosis/kidney stones, 100%	Nephrocalcinosis/kidney stones, 42%
OCRL	309000	Lowe syndrome	Congenital cataracts Intellectual disability Fanconi syndrome	Rarely reported

Quinlan C, Rheault MN., 2022

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Genetic testing does not always involve DNA

- Detailed fetal ultrasound
- Renal ultrasound with PKD
  - May provide presymptomatic diagnosis (modified Ravine criteria)

Age	Number of cysts
15–39 years	At least 3 unilateral or bilateral kidney cysts
40–59 years	At least 2 cysts in each kidney
Above 60 years	At least 4 cysts in each kidney

JASN January 2009, 20 (1) 205-212
JASN March 2015, 26 (3) 746-753

  - Cannot be used to exclude ADPKD in childhood
    - NPV : 83.5-99%, according the causative gene for USS done <30 years

JASN March 2015, 26 (3) 746-753

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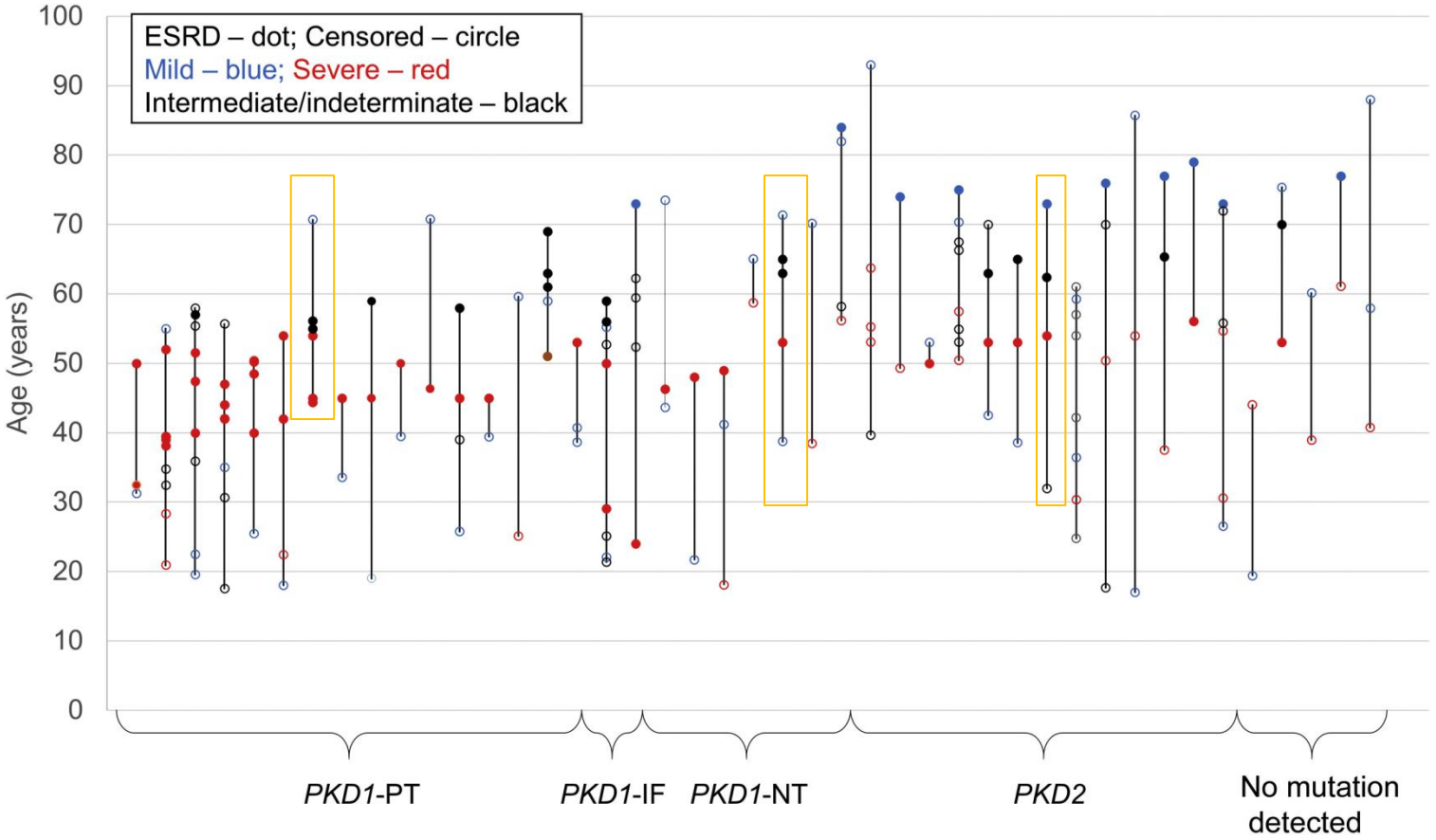
Adulhood condition with intrafamilial variability



Cohort including 1390 patients from 612 unrelated families with ADPKD
Discordance with at least 1 mild and 1 severe case was observed in 43 of 371
Mayo MRI-based classification

Kidney International Reports, July 2019, 7;995-1003

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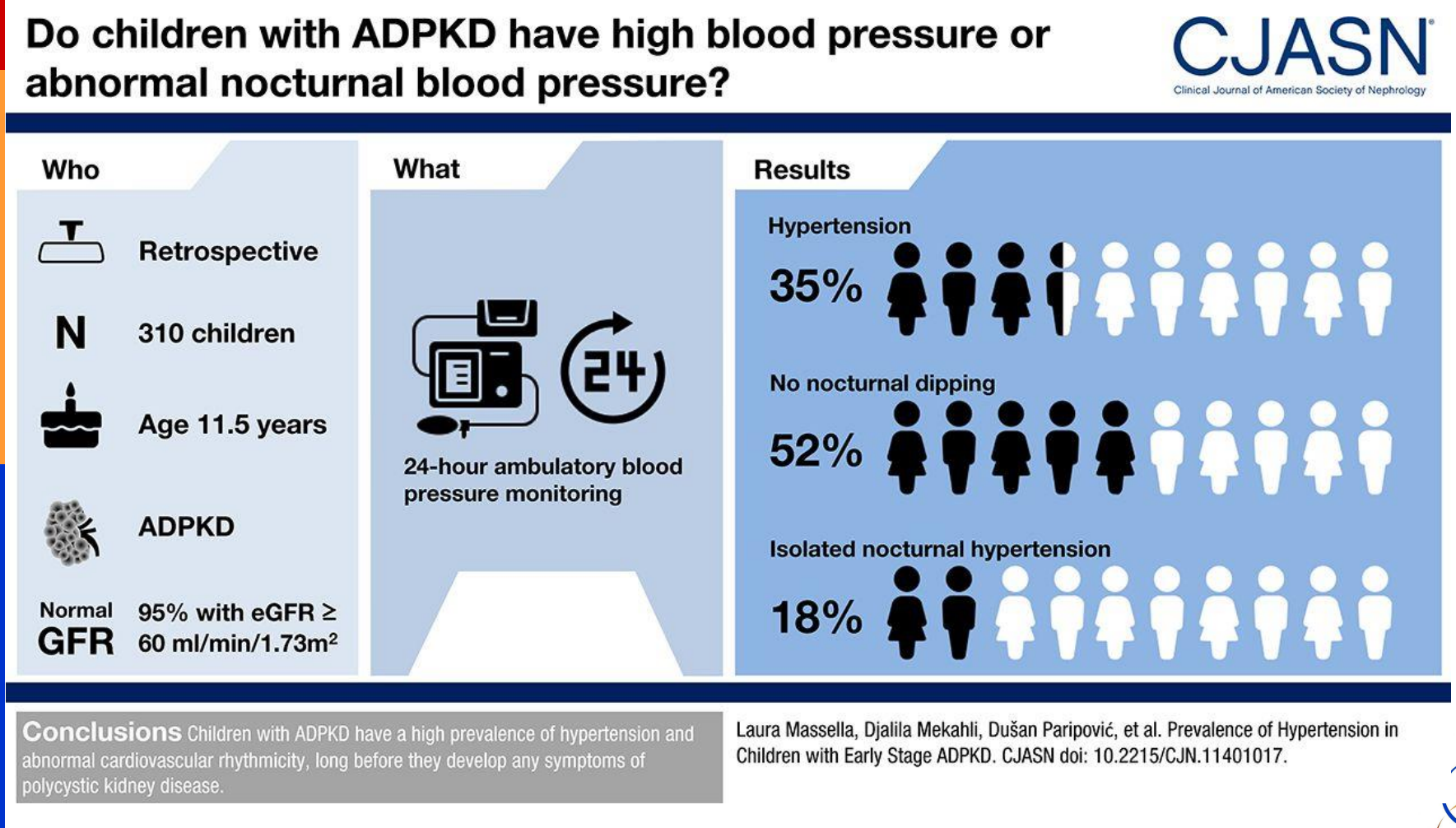
In adolescence



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OPEN

EVIDENCE-BASED GUIDELINE

International consensus statement on the diagnosis and management of autosomal dominant polycystic kidney disease in children and young people

Radiological diagnosis

Recommendation 2.1
Ultrasonography is the current radiological method of choice to screen for autosomal dominant polycystic kidney disease (ADPKD) in children (evidence level B; recommendation level moderate).

Recommendation 2.2
In a child under 15 years with a positive family history of ADPKD, sonographic detection of one or more kidney cysts is highly suggestive of ADPKD (evidence level B, recommendation level moderate). In a fetus or neonate with a positive family history of ADPKD, hyperechogenic and/or enlarged kidneys (>2 s.d.) on ultrasonography are suggestive of ADPKD (evidence level C; recommendation level moderate).

Recommendation 2.3
If kidney ultrasonography is normal in an at-risk child, this finding does not exclude ADPKD. However, if making a diagnosis based on ultrasonography is requested, it is not necessary to rescreen at intervals shorter than 3 years (evidence level C; recommendation level moderate).

Molecular diagnosis in children

Recommendation 3.1
We recommend offering genetic testing for cystic kidney disease genes to infants and children with very-early-onset (VEO) symptomatic disease independent of family history and to those with progressive disease (increasing cyst number or kidney volume) and a negative family history (evidence level B; recommendation level moderate).

Recommendation 3.2
In patients with a positive family history and unusually severe clinical course, genetic testing may be beneficial (evidence level D; recommendation level weak).

Recommendation 3.3
We do not recommend genetic testing in patients with a single cyst, no extrarenal findings and a negative family history of autosomal dominant polycystic kidney disease (ADPKD) (evidence level B–C; recommendation level moderate).

Recommendation 3.4
For genetic testing in children with VEO polycystic kidney disease or unusually progressive disease with a negative family history, we suggest using a multigene panel, including cystic kidney disease genes with a protocol adequately covering *PKD1* rather than testing single ADPKD genes (evidence level C; recommendation level weak).

Nature Reviews Nephrology, 2019, 15;713–726

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Comprehensive analysis of PKD1 and other ciliopathy genes

Ultra-deep sequencing of PKD1 (and other genes) with an average coverage of 1500 reads

Bioinformatic analysis
variants calling with GATK HaplotypeCaller, UnifiedGenotyper, varscan, platypus, freebayes and bcftools for SNPs and small indels

Pathogenic variant identified

Validation by targeted LR-PCR Sanger, MLPA analysis

In negative cases

Bioinformatic re-analysis
- insufficient coverage for known critical sites
- low variant allele fraction

Resequencing by LR-PCR Sanger

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Renal ciliopathies and associated genotypes

Kidney Cystic and Ciliopathy Disorders Variant Curation Expert Panel

Affiliated to **Kidney Disease CDWG**

Membership Documents

Kidney Cystic and Ciliopathy Disorders VCEP will focus on cystic kidney disease, with an initial focus on Autosomal Dominant Polycystic Kidney Disease (ADPKD). This group plans to begin with the most common and highly penetrant genes within our area of focus for variant curation and steadily enlarge the scope of the project overtime to encompass a wider range of cystic kidney disease genes, as outlined below.

The Kidney Cystic and Ciliopathy Disorders VCEP aims to specify the existing ACMG guidelines for variant classification in PKD genes and review variants for expert classification including resolving discrepancies in existing variant classification in ClinVar prioritizing the most common genes and variants associated to PKD.

Phase 1: Focus on *PKD1*

We expect that initial efforts will focus on the key player in autosomal dominant PKD (*PKD1*) which poses considerable issues surrounding the implementation of the ACMG/AMP guidelines and is responsible for approximately 78% of PKD cases. The establishment of guidelines surrounding pathogenicity assessment of previously reported plus unreported missense variants in *PKD1* has been identified as a key goal of the VCEP.

Phase 2: Widen scope to include all autosomal dominant PKD genes

Once guidelines for *PKD1* have been established, the expert panel's expertise will be applied to the wider range of autosomal dominant PKD genes (*PKD2, GANAB, ALG9* and *DNAJB11*) as well as the autosomal recessive PKD (ARPKD) gene, *PKHD1*.

Expert Panel Status

Step 1	Step 2	Step 3	Step 4
Define Group	Develop Classification Rules	Pilot Rules	Expert Panel Approval
Completed Jul. 2020			

Chairs

Katherine Benson, PhD

Peter Harris, PhD

Coordinators

Please contact a coordinator if you have questions.

Alicia Byrne, PhD
abyrne@broadinstitute.org

Hannah Dziadzio
hdziadzi@broadinstitute.org

Dominique J. McConnachie, Jennifer L. Stow, Andrew J. Mallett, AJKD, 2021

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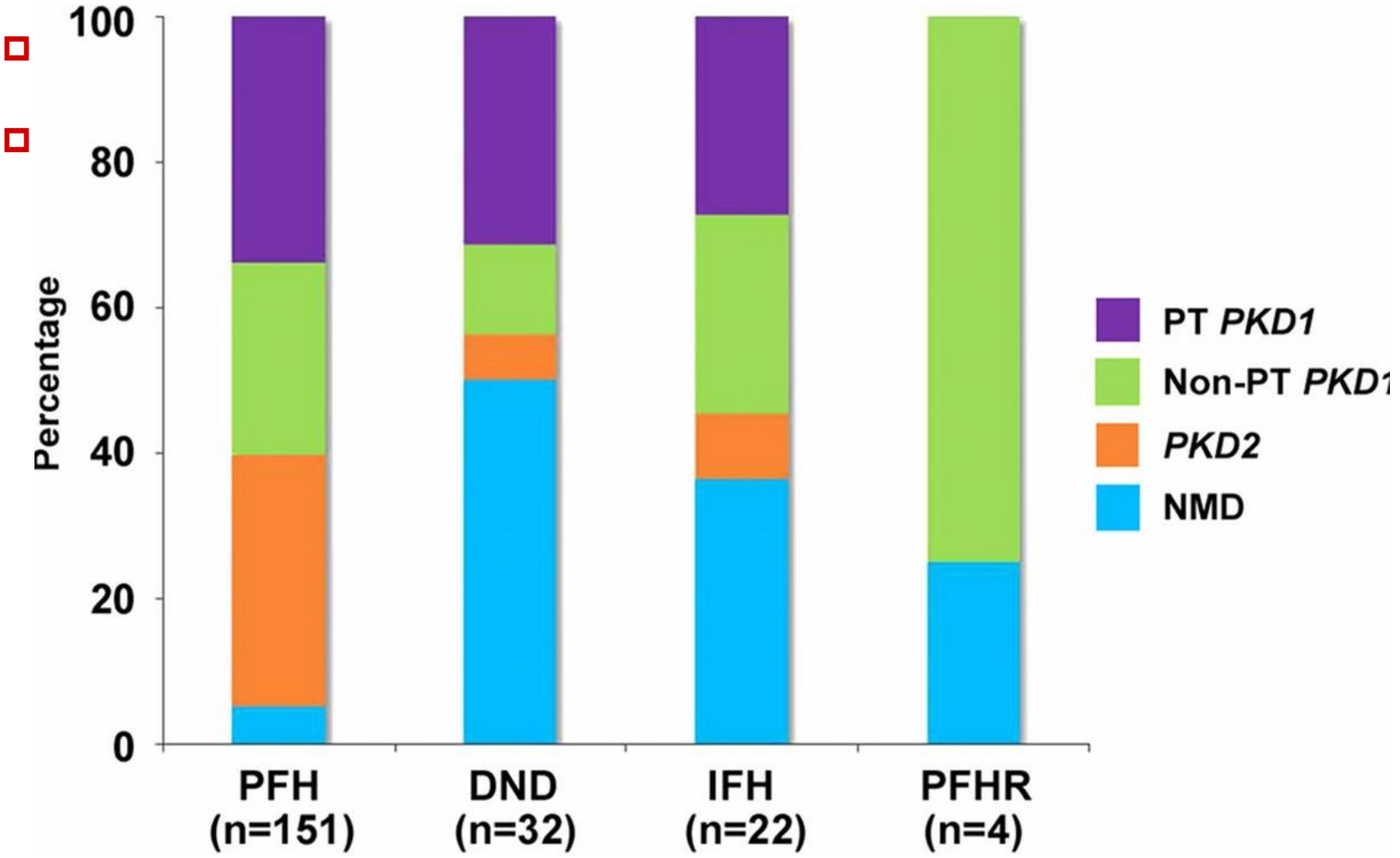
PKD with ou without positive family history (FH)



JASN September 2017, 28 (9) 2768-2776

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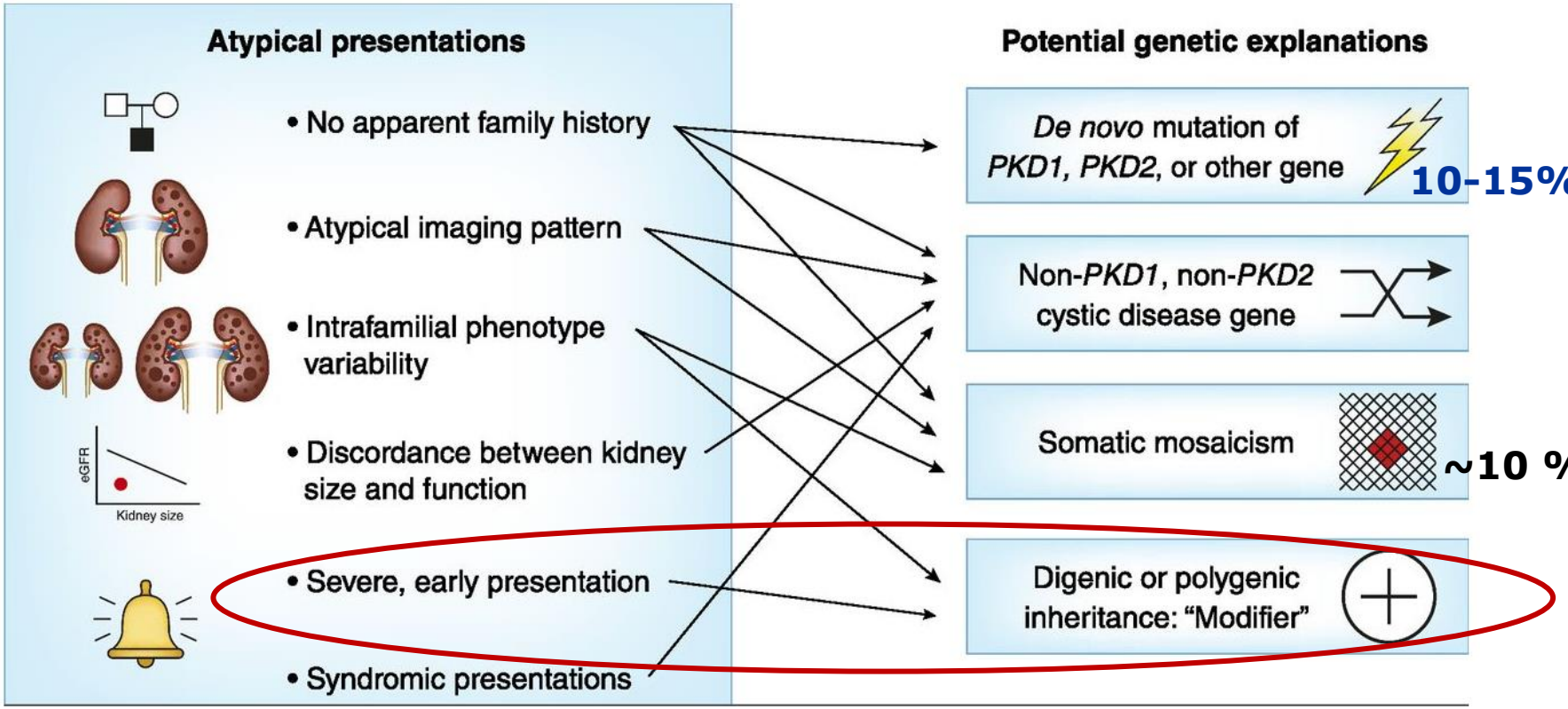
Insights into Autosomal Dominant Polycystic Kidney Disease from Genetic Studies

Matthew B. Lanktree,[1] Amirreza Haghighi,[2] Ighli di Bari,[2] Xuewen Song,[2] and York Pei[2]



Adapted from:
CJASN, 2021 May;16: 790–799
Kidney Int, 2020 Feb;97(2):370-382

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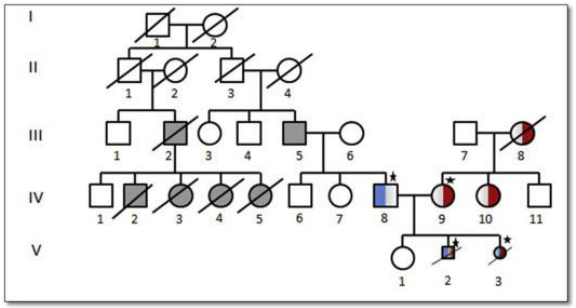
Very-early-onset PKD



- Diagnosis *in utero* or in infancy up to the age of 18m
- Positive FH **in ~50%**
- Rate of de novo pathogenic variant **up to 24%**
- Molecular diagnosis
  - Contiguous *PKD1* and *TSC2* deletion
  - HNF1*B* gene deletion
  - Biallelic *PKHD1* pathogenic variants
  - Compound *PKD1* heterozygosity
    - One protein-truncating *PKD1* pathogenic variant in trans with a second nontruncating *PKD1* variant (positive FH)
    - One nontruncating *PKD1* pathogenic variant and a second mutation in another cystic disease gene, such as *PKD2*, *COL4A1*, or *HNF1B* (positive FH)
    - Two hypomorphic *PKD1* variants in trans (no FH)

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Genetics inMedicine

www.nature.com/gim

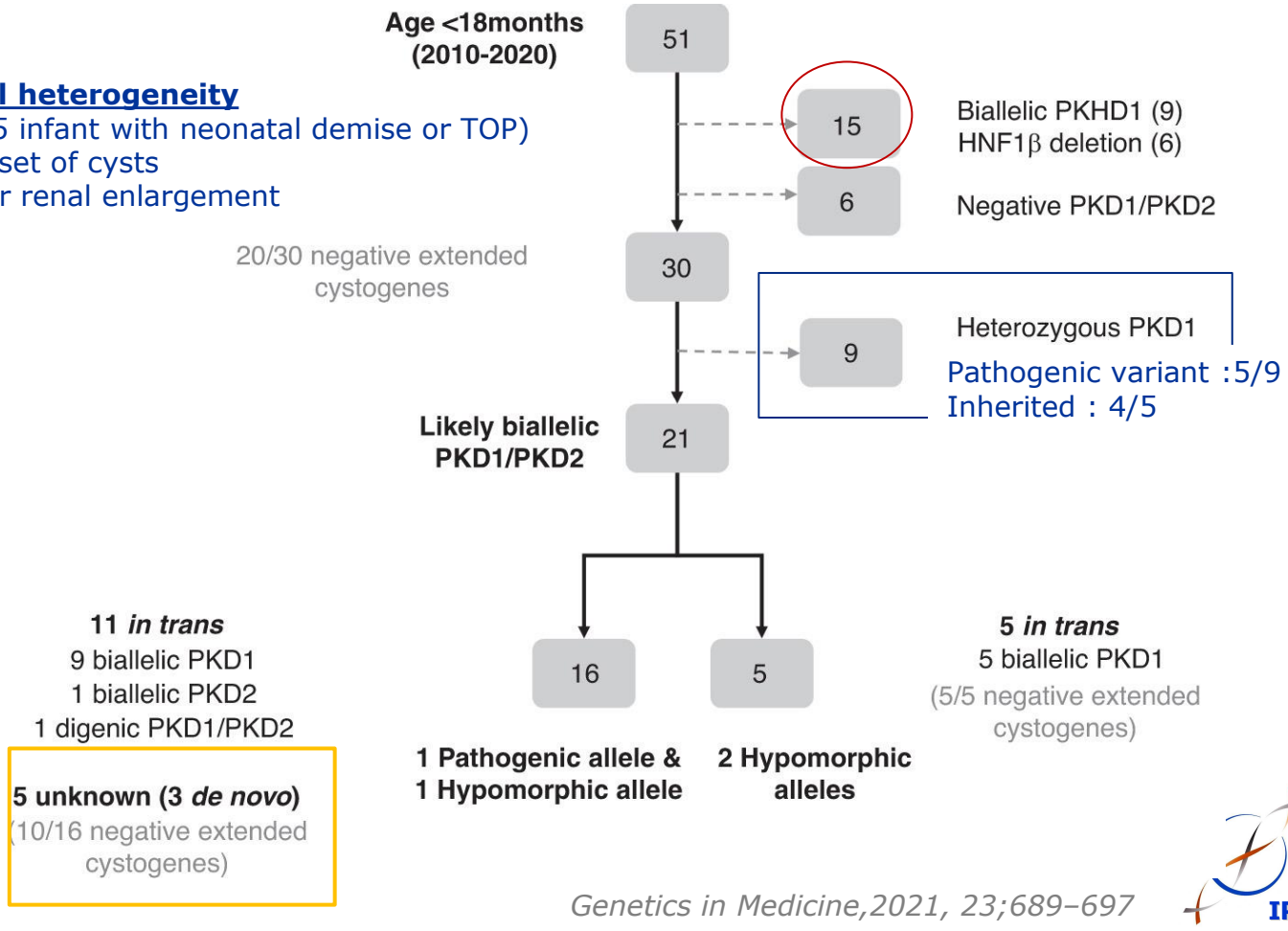
Check for updates

ARTICLE

Biallelic inheritance of hypomorphic *PKD1* variants is highly prevalent in very early onset polycystic kidney disease

Miranda Durkie, FRCPath[1], Jiehan Chong, MRCP[2], Manoj K. Valluru, PhD[2], Peter C. Harris, PhD[3] and Albert C. M. Ong, DM, FRCP[2]✉

Large clinical heterogeneity
from severe (5 infant with neonatal demise or TOP)
to prenatal onset of cysts
without HTA or renal enlargement



Genetics in Medicine,2021, 23;689–697

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Genet Med. 2019 June ; 21(6): 1425–1434. doi:10.1038/s41436-018-0324-x.

Population data improves variant interpretation in autosomal dominant polycystic kidney disease

Amali C. Mallawaarachchi, MBBS, FRACP[1], Timothy J. Furlong, PhD, FRACP[1], John Shine, PhD[1], Peter C. Harris, PhD[2], Mark J. Cowley, PhD[3,4]

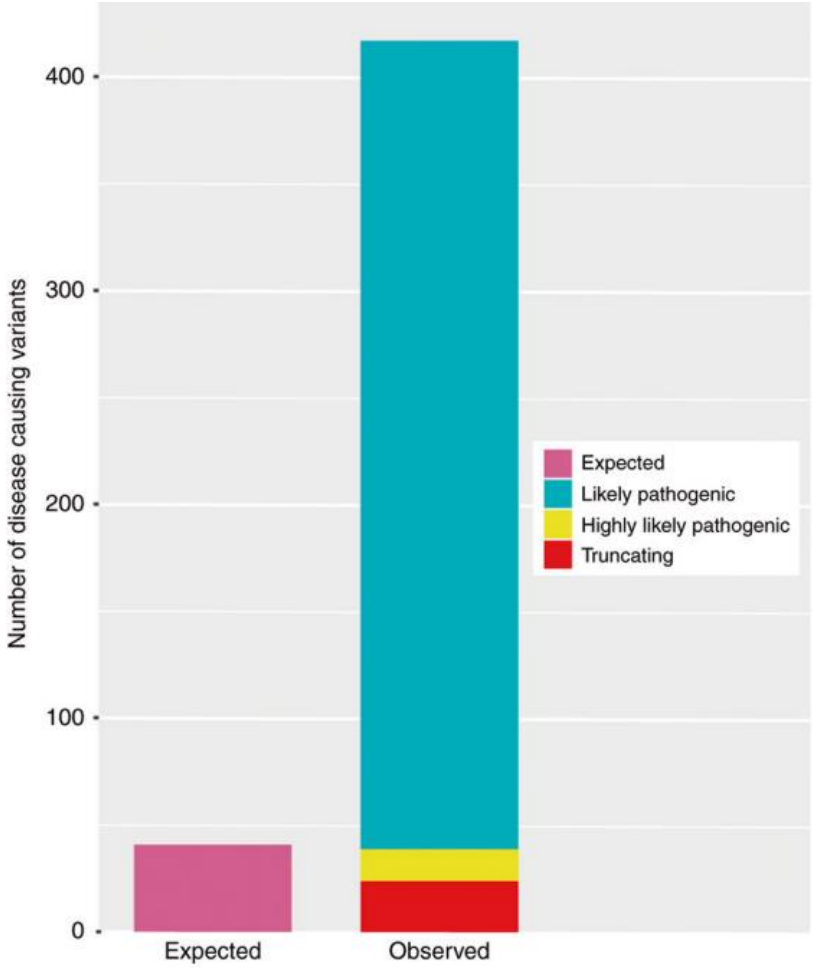

Fig. 3. Expected and observed disease-causing variants in ExAC.

1. A total of 418 records in ExAC
2. **A disease prevalence of up to 6.9 per 1000**
3. Number of variants currently classified as likely pathogenic in the PKDB may be hypomorphic, or weakly penetrant

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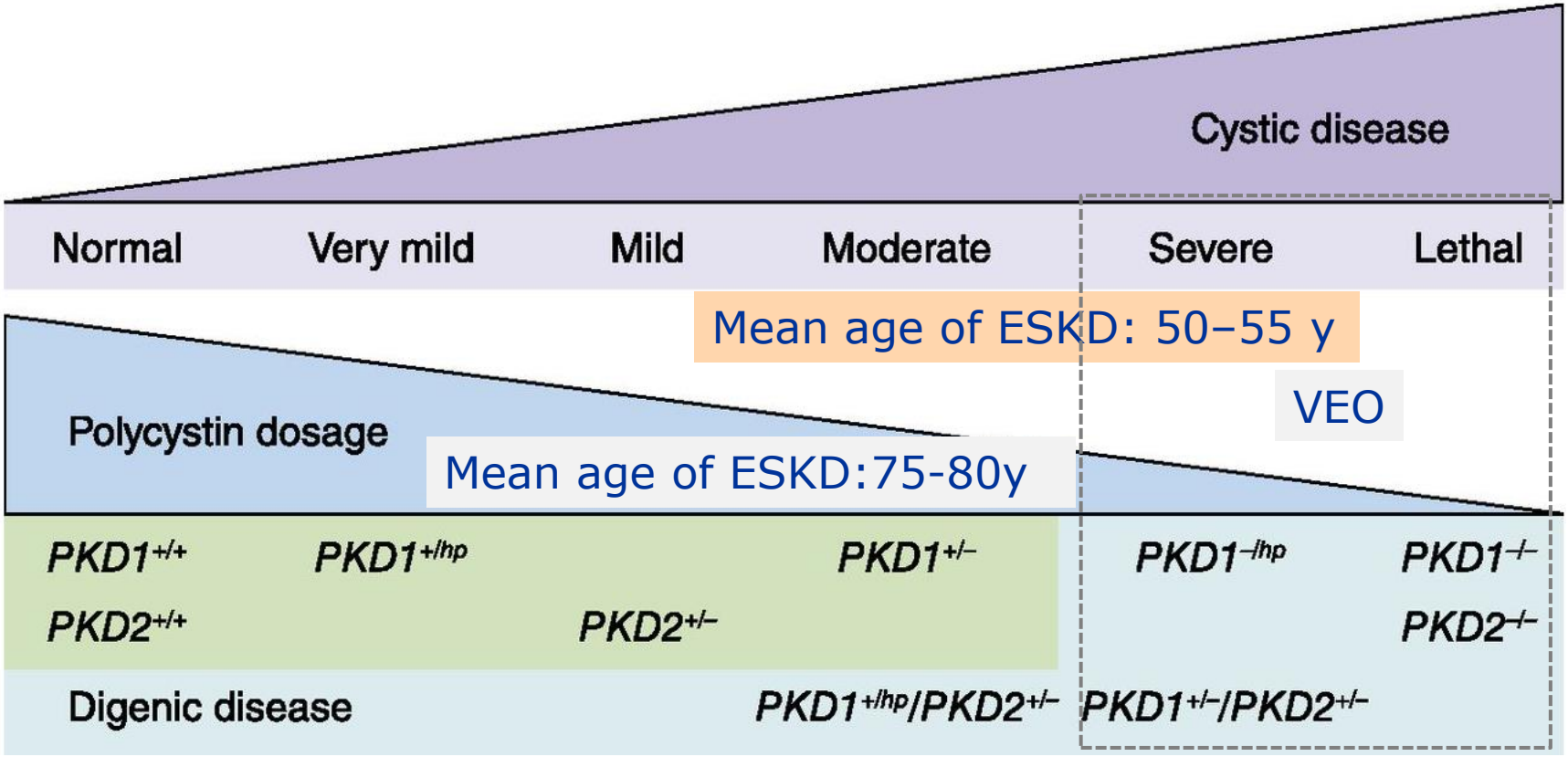
Summary



http://pkdb.mayo.edu

CJASN, May 2021,16: 790–799