Brigham and Women's Hospital
Founding Member, Mass General Brigham

GENETIC TESTING: HOW TO INTEGRATE IT INTO PRACTICE

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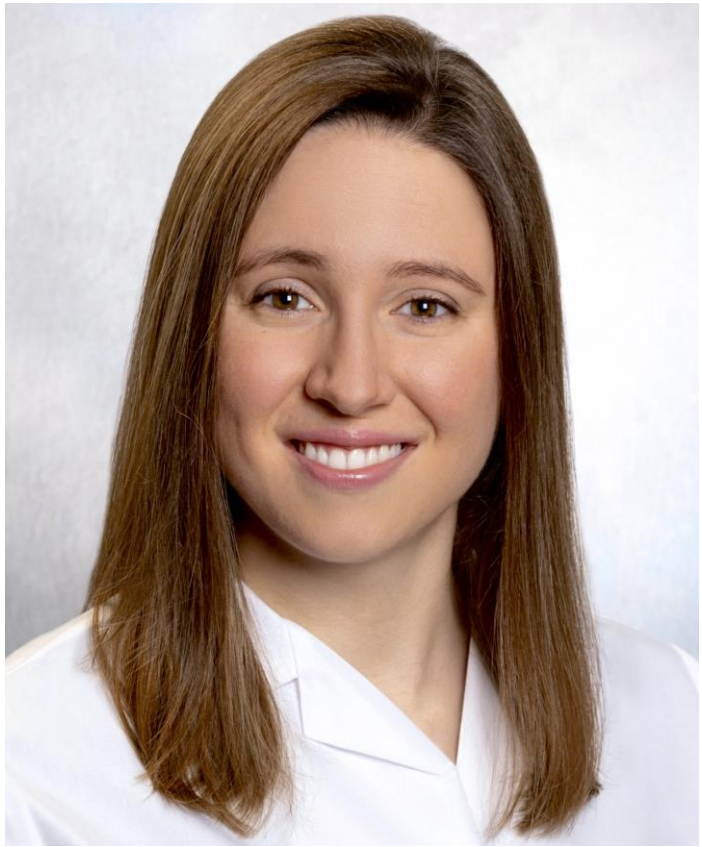
Harvard Medical School
August 11, 2026

Ana Claudia Onuchic-Whitford, MD



University of São Paulo School of Medicine, Brazil

Internal Medicine Residency @ University of São Paulo, Brazil

Internal Medicine Residency @ University of Connecticut

Nephrology Fellowship @ Brigham and Women's Hospital & Massachusetts General Hospital Joint Program

Postdoctoral Research Fellowship @ Boston Children's Hospital

Kidney Disease Initiative, Broad Institute of MIT and Harvard

Affiliated Research Faculty @ Boston Children's Hospital

Associate Physician @ Division of Renal Medicine, BWH

Instructor in Medicine @ Harvard Medical School

Co-Director, MGB Kidney Genetics & PKD Clinic

- Clinical focus: Genetic Kidney Disease
- Research focus: Genetic Kidney Disease, Nephrotic Syndrome, Computational Genomics

Disclosures

None.

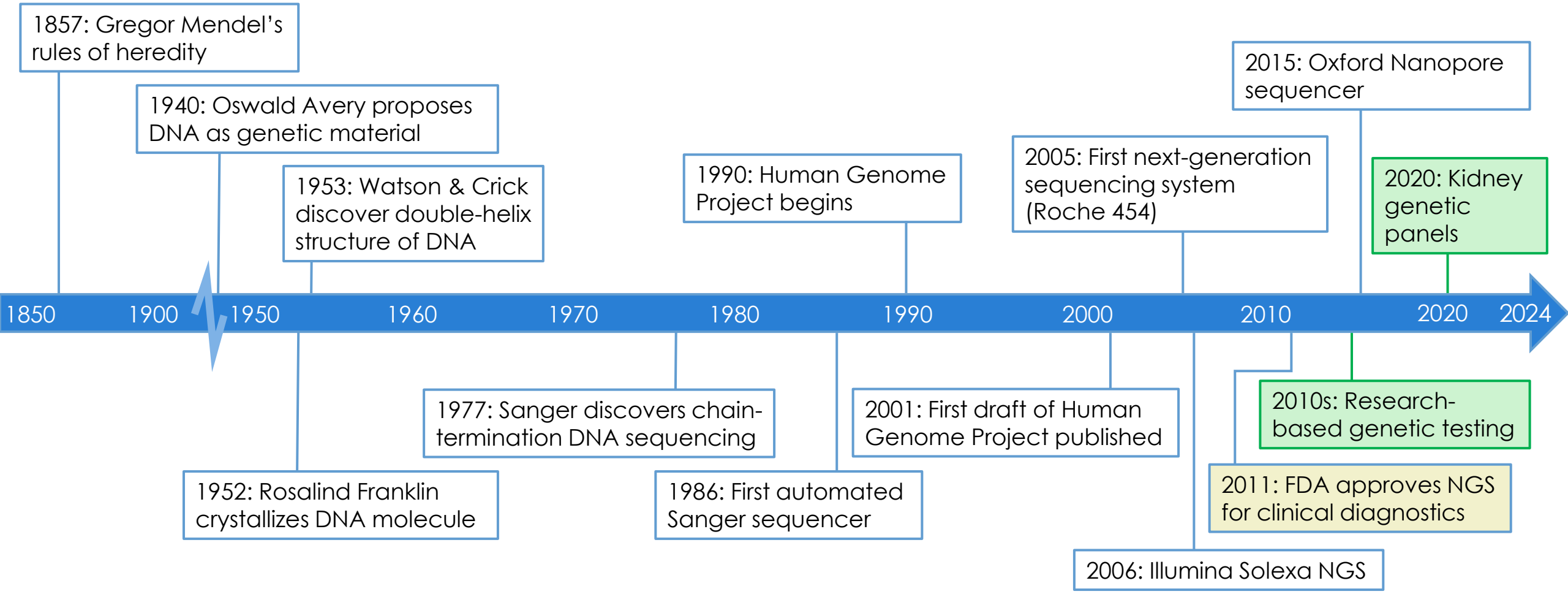
Two key learning objectives

1) Understand the indications, benefits, risks, challenges and strategies associated with genetic testing in patients with kidney disease.
2) Increase diagnostic awareness of genetic kidney disorders, with a focus in adult nephrology.

Note: Genetic aspects of the following disorders will be covered in separate lectures during this course, and thus will **not** be the focus of this session.

- Polycystic kidney disease (Dr. Fouad Chebib)
- APOL1-mediated kidney disease (Dr. Opeyemi Olabisi)
- Thrombotic microangiopathies (complement-mediated kidney disease) (Dr. Jean Francis)

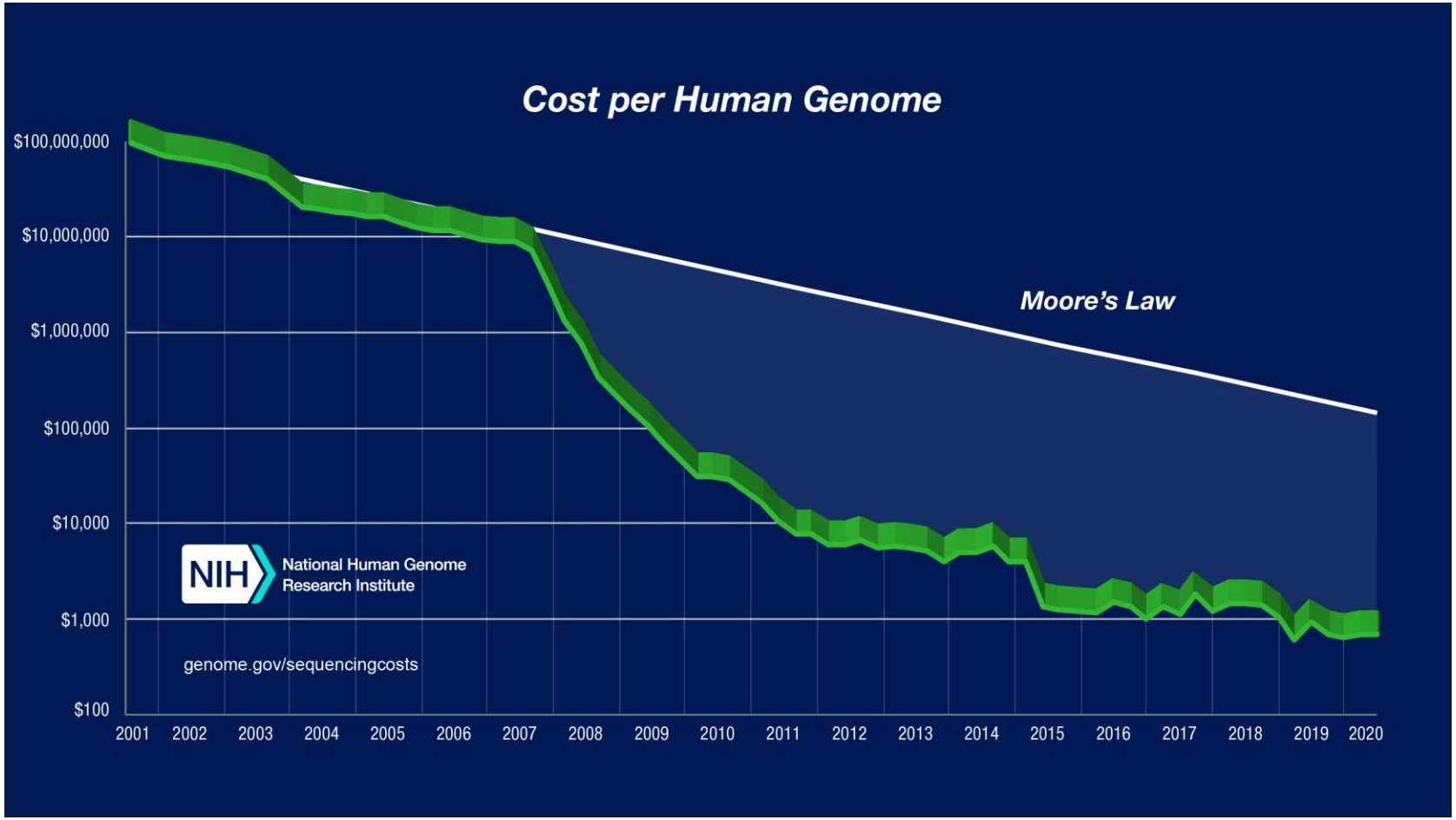
Timeline of genetic sequencing



Efthymiou et al, Curr Opin Neurol 2016; Chaitankar et al, Prog Retin Eye Res; Pereira et al, J Clin Med 2020

Sequencing costs

Y axis: logarithmic scale



Moore's law: long-term trend in computer hardware industry that involves doubling of 'compute power' every two years

January 2008: sudden, dramatic outpacing of Moore's law

Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP) Available at: www.genome.gov/sequencingcostsdata. Accessed 9/28/2021.

Audience Response Question #1

What is the main reason for the sudden and significant drop in genetic sequencing costs in early 2008?

a) Sequencing centers transitioned from Sanger-based to next-generation sequencing (NGS)
b) The Genetic Information Nondiscrimination Act of 2008 (GINA) was passed in the United States
c) The NIH initiated funding for the "All of Us" Research Program
d) The Food and Drug Administration (FDA) approved the use of next-generation sequencing for clinical diagnostics
e) The first next-generation sequencing system (Illumina Solexa) was developed in 2007

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Sequencing costs

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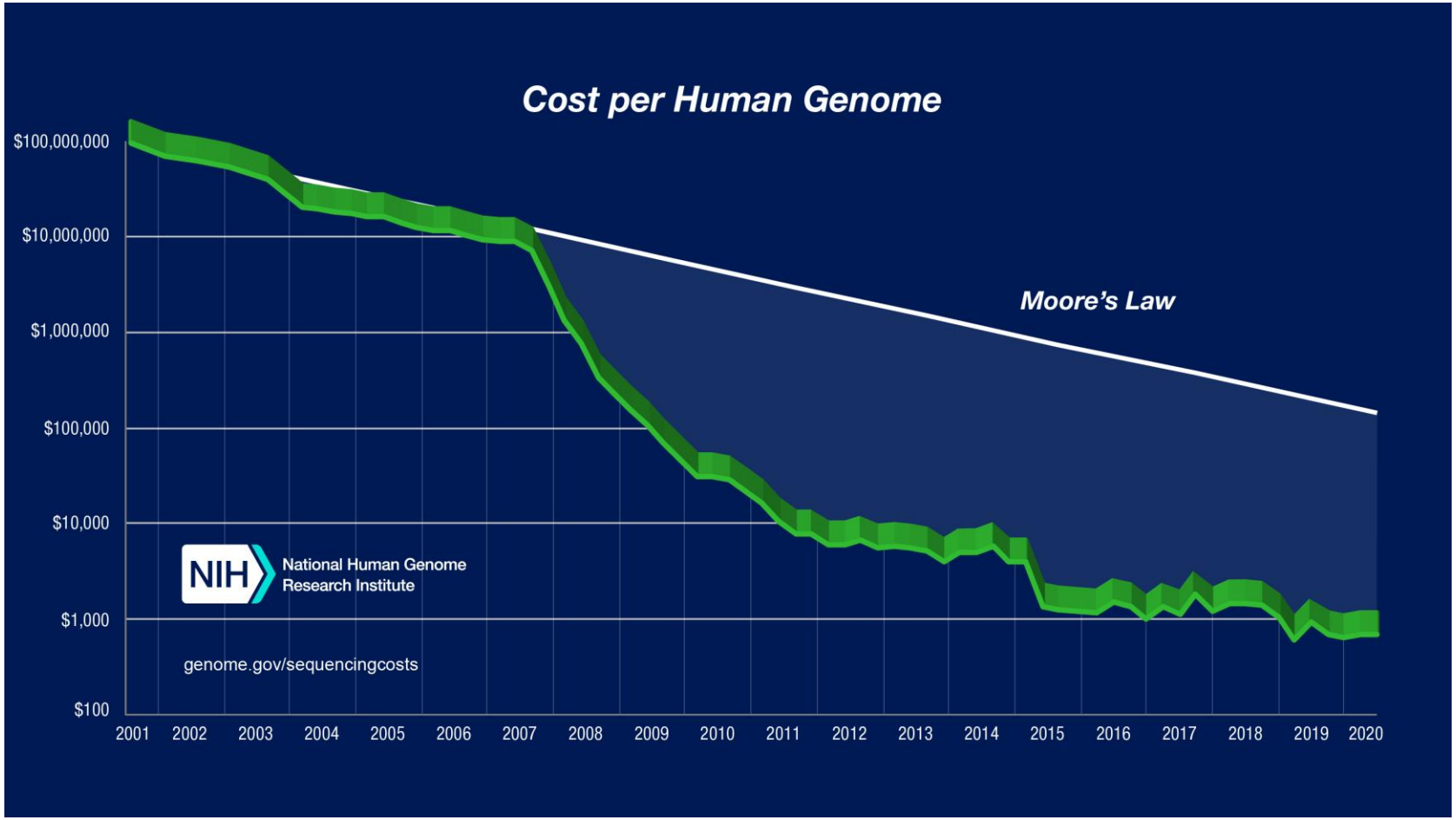


Moore's law: long-term trend in computer hardware industry that involves doubling of 'compute power' every two years

Does not include cost of:

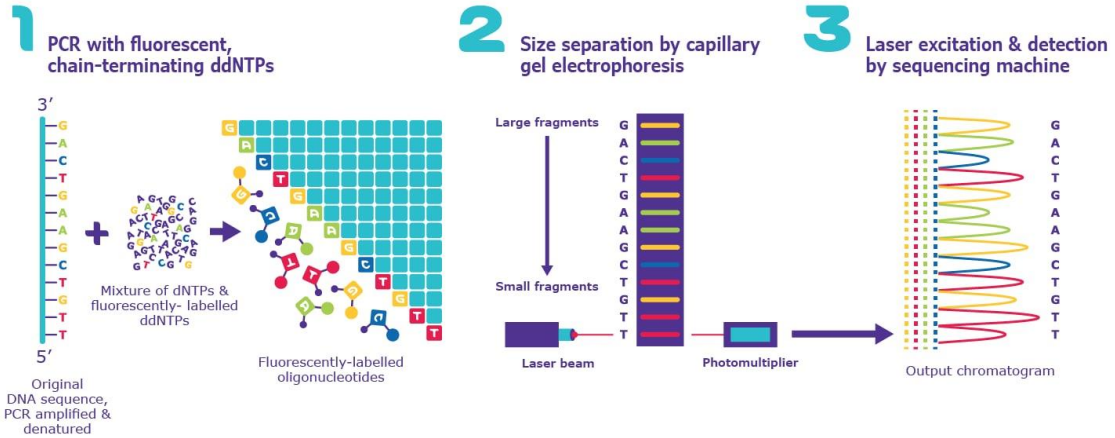
- **Development** of technology and bioinformatics tools
- **Data analysis:**
  - DNA sequence assembly/alignment
  - Variant identification
  - Interpretation of genetic findings

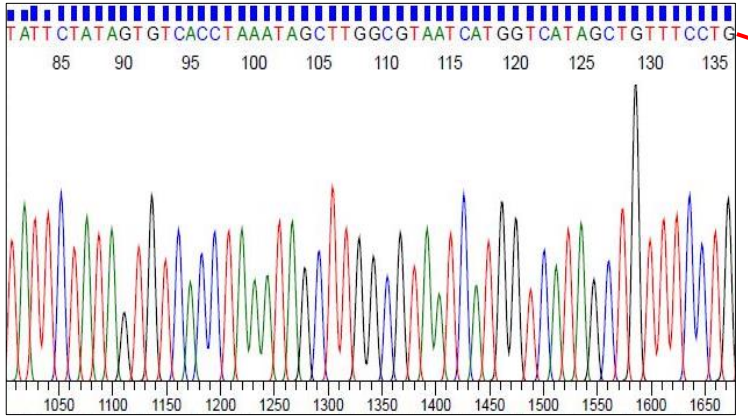
January 2008: sudden, dramatic outpacing of Moore's law as sequencing centers transition from Sanger-based to next-generation sequencing (NGS)

Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP) Available at: www.genome.gov/sequencingcostsdata. Accessed 9/28/2021.

Sanger sequencing

(dideoxy chain <u>termination</u>)

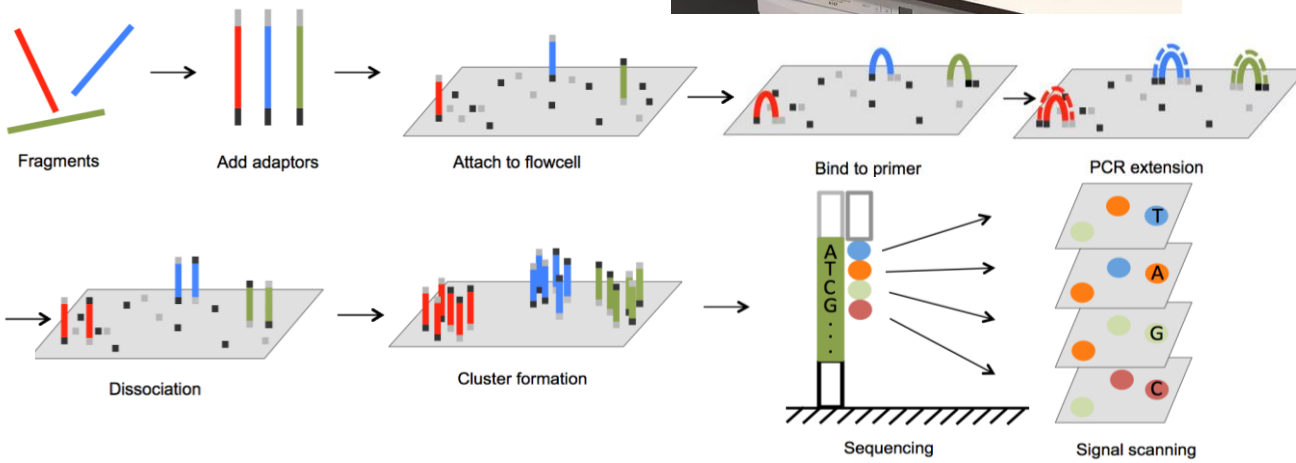




Cost per 1 million base pairs = **~$500.00**

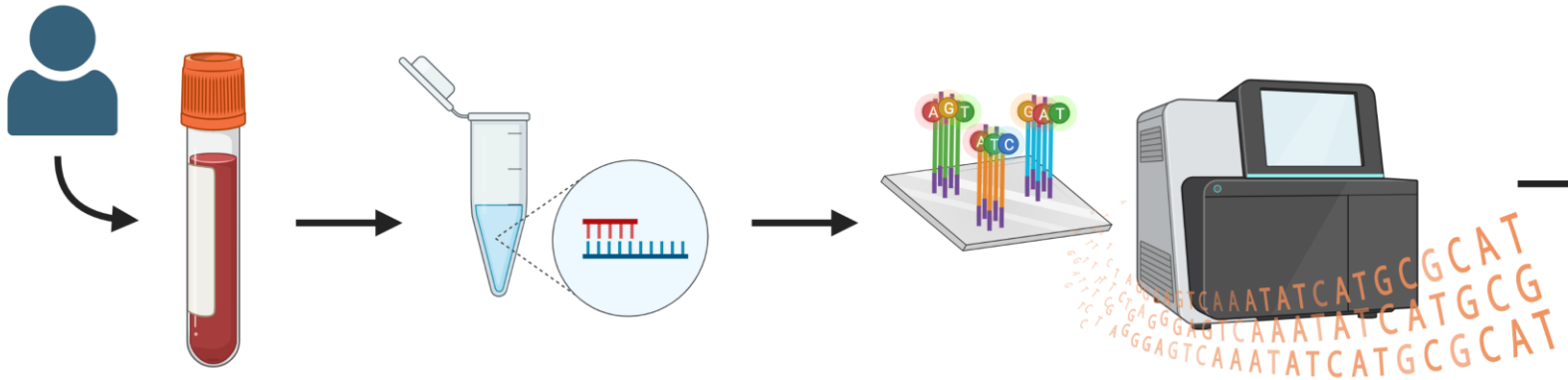
Next-generation sequencing (NGS)

(massively <u>parallel</u> sequencing)



Cost per 1 million base pairs = **< $0.50**

International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature 2001; www.cecerelab.com/news; www.sigmaaldrich.com/US/en/technical-documents/protocol/genomics/sequencing/sanger-sequencing; www.intechopen.com/chapters/49419; dnacore.missouri.edu/dataevaluation.html; esources.qiagenbioinformatics.com/manuals/clcgenomicsworkbench/700/index.php?manual=Extract_parts_mapping.html; www.ncbi.nlm.nih.gov/books/NBK274079/



DNA extraction

DNA sequencing

Alignment of reads

DNA sequence variants:

- *PKD1* c.1349T>A
- *UMOD* c.845G>T
- *NPHS2* c.278A>C
- …

Variant calling & annotation

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

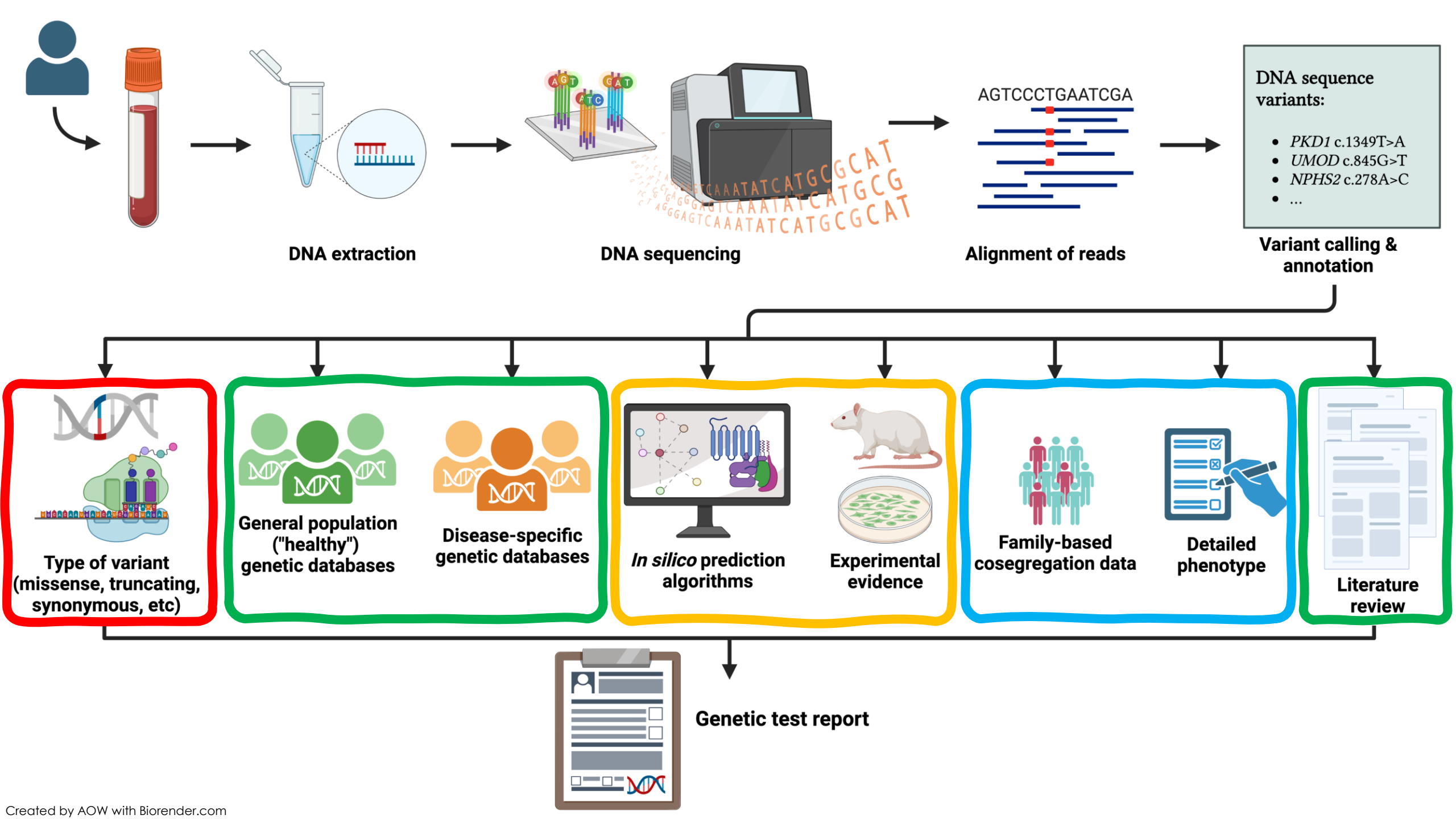
Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

Variants classified into 5 categories:

- Pathogenic
- Likely Pathogenic (>90% certainty)
- Variant of Uncertain Significance
- Likely Benign (>90% certainty)
- Benign

Genetic test report

Created by AOW with Biorender.com


DNA extraction
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AGTCCCTGAATCGA
Alignment of reads
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...
Variant calling & annotation
Type of variant (missense, truncating, synonymous, etc)
General population ("healthy") genetic databases
Disease-specific genetic databases
In silico prediction algorithms
Experimental evidence
Family-based cosegregation data
Detailed phenotype
Literature review
Genetic test report
Created by AOW with Biorender.com

FINAL RESULTS SUMMARY

Positive

A heterozygous pathogenic variant in the *PKD1* gene was detected.

FINDINGS: POSITIVE VARIANT

Gene	Condition(s)	Inheritance	Variant(s)	Zygosity	Classification
PKD1	Polycystic Kidney Disease 1	Autosomal Dominant	c.6406C>T (p.Gln2136*)	Heterozygous	**Pathogenic**

ADDITIONAL FINDINGS: CARRIER VARIANTS

The following heterozygous variants were detected in this individual. For any gene that is associated with an autosomal recessive condition, the variant is not sufficient to cause the condition by itself. For any gene that is associated with both autosomal recessive and autosomal dominant conditions, the variant has been interpreted as a carrier variant based on the current evidence and/or clinical information provided. Clinical correlation is recommended on an individual basis.

Gene	Condition(s)	Inheritance	Variant(s)	Zygosity	Classification
SLC37A4	Glycogen Storage Disease, Type 1B/1C	Autosomal Recessive	c.1108_1109del (p.Leu370Valfs*53)	Heterozygous	**Pathogenic**
NPHS2	Congenital Nephrotic Syndrome, Type 2	Autosomal Recessive	c.686G>A (p.Arg229Gln)	Heterozygous	**Likely Pathogenic**

ADDITIONAL FINDINGS: VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS)

Variants of uncertain significance (VUS) are common and the American College of Medical Genetics and Genomics (ACMG) does NOT recommend that a VUS be used in clinical decision making. A VUS means that a change in the DNA was detected, but there is not enough information to determine whether or not it results in disease. Medical management should be based on the patient's personal and/or family history.

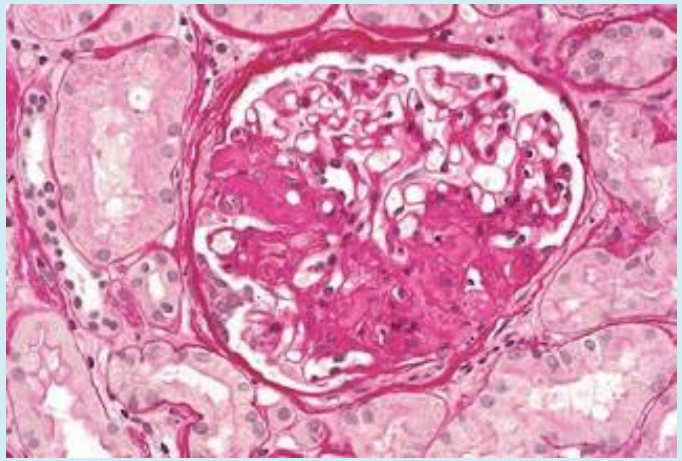
Gene	Condition(s)	Inheritance	Variant(s)	Zygosity	Classification
NR3C2	Pseudohypoaldosteronism Type I, Autosomal Dominant Hypertension, Early-Onset	Autosomal Dominant	c.2311C>T (p.Arg771Cys)	Heterozygous	Unknown Significance
NEK8	Nephronophthisis 9; Renal-Hepatic-Pancreatic Dysplasia 2	Autosomal Recessive	c.1966C>T (p.Arg656Trp)	Heterozygous	Unknown Significance
SDCCAG8	Senior Loken Syndrome, Type 7; Bardet-Biedl Syndrome 16	Autosomal Recessive	c.278C>T (p.Pro93Leu)	Heterozygous	Unknown Significance
ATP7B	Wilson Disease	Autosomal Recessive	c.814G>A (p.Val272Ile)	Heterozygous	Unknown Significance

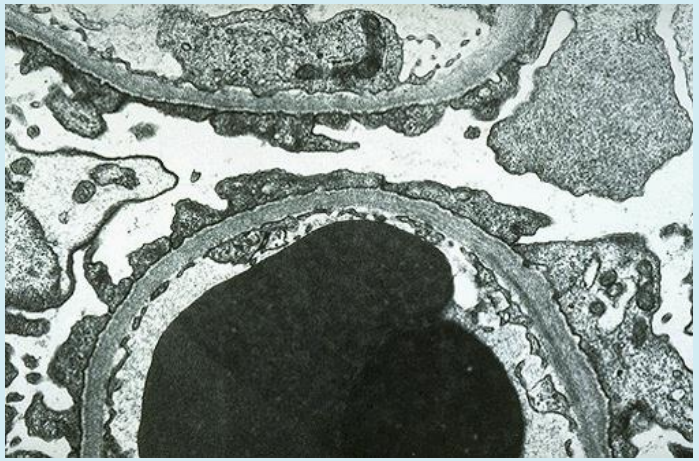
Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

Audience Response Question #2

26 yo pregnant woman with no PMH p/w pre-eclampsia and LE edema.
Labs: Cr 0.8 mg/dl, eGFR 86 ml/min, UA 3+ protein, UPCR 3.3g/g.
Family history: father with proteinuria + ESKD at 50yo, attributed to HTN/DM.
Renal biopsy is performed.
Is clinical genetic testing indicated?
If so, what is the usually recommended initial strategy?





a) No; there are currently no FDA-approved options for genetic forms of this disease and there would be no change in management
b) Not at this time; may inform future related kidney transplant donation but would not change immediate management strategy
c) Yes; sequencing of the *NPHS1*, *NPHS2*, *PLCE1*, *WT1* and *TRPC6* genes
d) Yes; targeted gene panel testing of >50 genes
e) Yes; clinical whole exome sequencing

Images: NephSap, July 2011; webpath.med.utah.edu/RENAHTML/RENAL102.html

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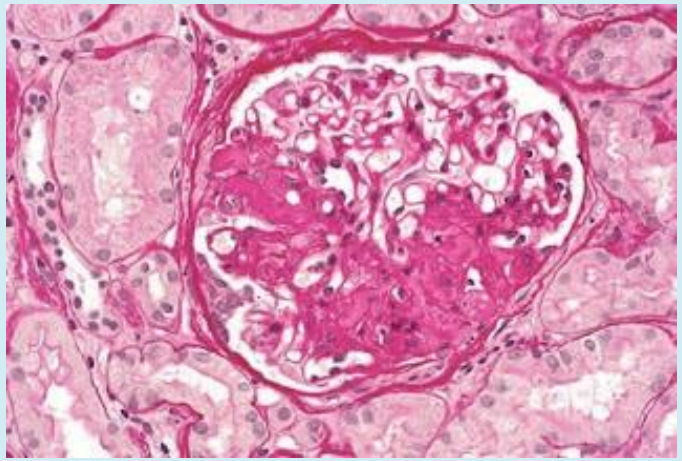

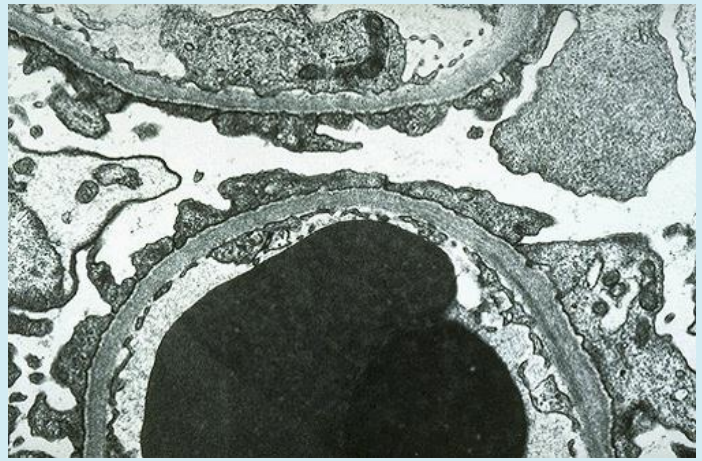

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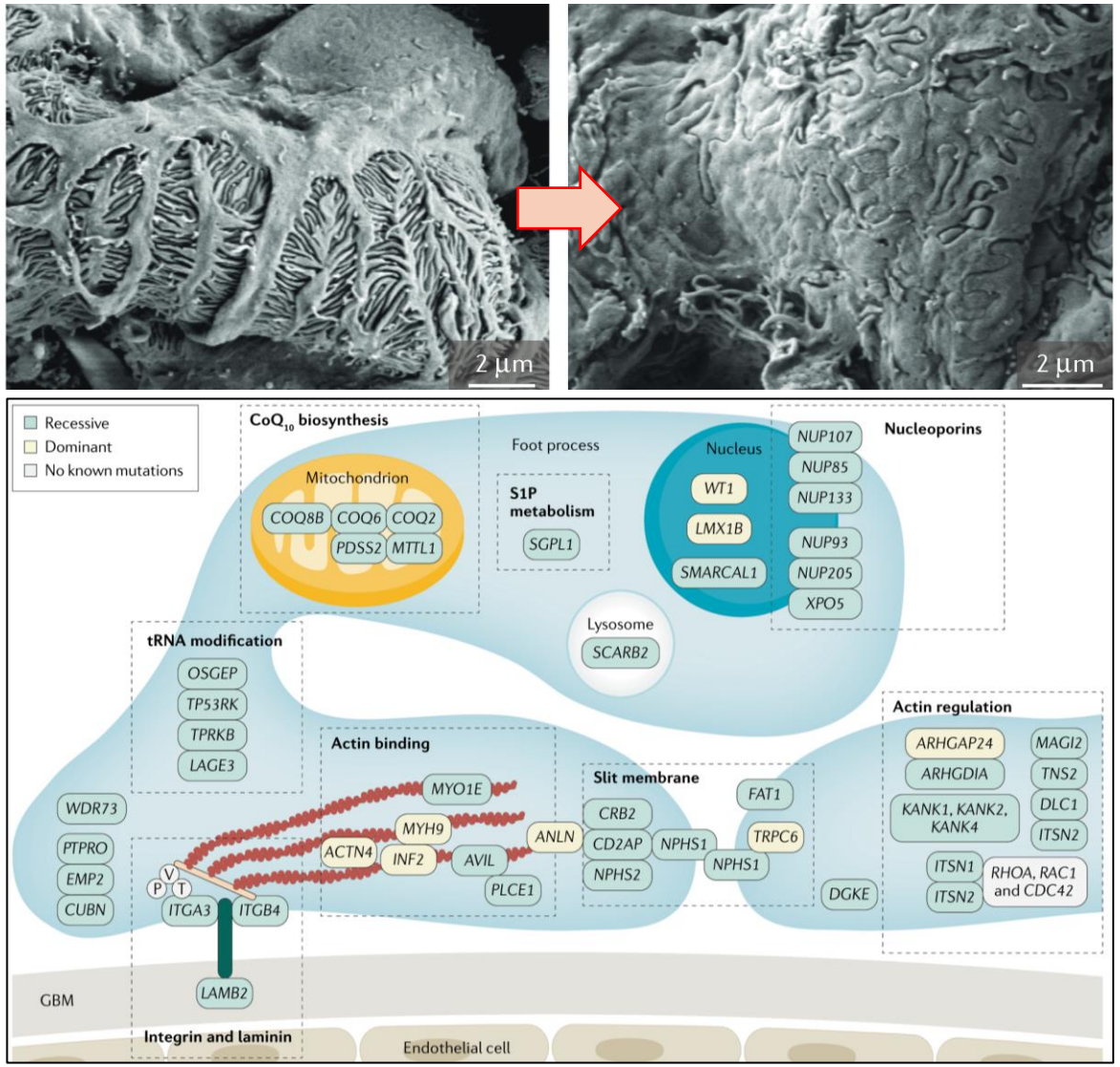
Genetic Podocytopathies
("Genetic FSGS", Steroid-resistant nephrotic syndrome)

- Caused by mutations in genes encoding proteins that maintain integrity of slit diaphragm, podocyte structure, glomerular filtration barrier
- This leads to glomerular injury, podocyte foot process effacement, proteinuria, glomerulosclerosis
- <u>Histologic spectrum:</u>
  - Diffuse mesangial sclerosis (pediatric)
  - Minimal change disease
  - FSGS
  - Collapsing glomerulopathy
- Nonspecific lesions represent patterns of podocyte injury = "Podocytopathies"
- Most often do not respond to steroids or immunosuppression



Kopp et al, Nat Rev Nephrol 2020; Perico et al, Nat Rev Nephrol 2016

Positive

Test Interpretation: This result is consistent with a molecular diagnosis of the condition(s) listed. Clinical correlation of these results is recommended.

Gene	Condition(s)	Inheritance	Variant(s)	Zygosity	Classification
TRPC6	Focal Segmental Glomerulosclerosis 2	Autosomal Dominant	c.2683C>T (p.Arg895Cys)	Heterozygous	**Pathogenic**

TRPC6

Single heterozygous pathogenic/likely pathogenic variants in TRPC6 are associated with Focal Segmental Glomerulosclerosis, type 2 (FSGS2). This condition is characterized by scarring of the glomeruli that can result in hypoalbuminemia, proteinuria, hyperlipidemia, and edema. Over time this can lead to kidney failure. **FSGS2 has reduced penetrance** been associated with a gain-of-function mechanism, though there are 19458060, 26892346). MedGen UID: 349053.

Chr11(GRCh37):g.101323799G>A NM_004621.6:c.2683C>T (p.Arg8

- This variant is predicted to result in a single amino acid substitu
- This missense variant has been reported in the heterozygous s glomerulosclerosis (FSGS) and steroid-resistant nephrotic synd as de novo in two individuals with proteinuria or nephrotic syndr the gene (p.Arg895Leu) has been associated with collapsing gl 21734084, 30655312, 26892346).
- Computational predictions for p.Arg895Cys (2P/0B AGVGD, SI (granthamDist = 180). For missense variants, if applicable, a RE pathogenicity.
- This variant is absent from the Broad gnomAD dataset.

FN1

Single heterozygous pathogenic/likely pathogenic variants in FN1 are associated with glomerulopathy with fibronectin deposits 2. Glomerulopathy with fibronectin deposits 2 is a condition characterized by buildup of abnormal fibronectin-1 protein in the glomeruli leading to proteinuria and hematuria, which lead to kidney failure. This condition typically occurs again after transplantation. MedGen UID: 356149 Single heterozygous pathogenic/likely pathogenic variants in FN1 are associated with corner fracture type spondylometaphyseal dysplasia. Corner fracture type spondylometaphyseal dysplasia is a condition characterized by short stature, hip abnormalities, corner-fracture like bone abnormalities, and vertebral anomalies. Kidney abnormalities are not known to be a feature of corner fracture type spondylometaphyseal dysplasia. MedGen UID: 98146.

Chr2(GRCh37):g.216236756T>C NM_212482.4:c.6590A>G (p.Asn2197Ser), Heterozygous, Classification: Unknown Significance

- This variant is predicted to result in a single amino acid substitution (missense) of Asn to Ser at codon 2197 in exon 40 of the *FN1* gene.
- Computational predictions for p.Asn2197Ser (0P/2B /AGVGD, SIFT) (REVEL = 0.11) (gnomAD: Z = 1.65 [Exp: 1367.3, Obs: 1196]) (granthamDist = 46). For missense variants, if applicable, a REVEL score ≥0.75 is considered as supporting computational evidence of pathogenicity.
- This variant is absent from the Broad gnomAD dataset.

KMT2D

Single heterozygous pathogenic/likely pathogenic variants in KMT2D are associated with Kabuki syndrome 1. This is a multisystemic condition characterized by postnatal growth deficiency, minor skeletal anomalies, persistence of fetal fingertip pads, mild-to-moderate intellectual disability, distinct facial features. Other features can include structural and functional abnormalities of the heart, endocrine system, intestinal tract, hearing, vision, joints, teeth, and immune system. Kidney abnormalities include Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) and commonly involve single fused kidneys, crossed fused renal ectopia, renal dysplasia, hydronephrosis, duplication of the collecting system, and ureteropelvic junction obstruction. MedGen UID: 893727.

Variant(s) of Uncertain Significance

A Variant of Uncertain Significance (VUS) means that a change determine whether or not it results in disease.

Gene	Condition(s)	Inheritance			
FN1	Glomerulopathy With Fibronectin Deposits 2 (AD); Corner Fracture Type Spondylometaphyseal Dysplasia (AD)	Autosomal Dominant	c.6590A>G (p.Asn2197Ser)	Heterozygous	Unknown Significance
KMT2D	Kabuki Syndrome 1	Autosomal Dominant	c.15716C>T (p.Pro5239Leu)	Heterozygous	Unknown Significance

THE LANCET February 7, 2026 Articles

TRPC6 inhibition for the treatment of focal segmental glomerulosclerosis: a randomised, placebo-controlled, phase 2 trial of BI 764198

Howard Trachtman, Matthias Kretzler, Loreto Gesualdo, Nicholas Cross, Biruh Workeneh, Jessica Kaufeld, Björn Meijers, Zhiming Ye, Qinkai Chen, Vimal K Derebail, Monica Suet Ying Ng, Bo Ji, Maximilian T Lobmeyer, Silke Retlich, Fabia T Licarião Rocha, Srinivasa Prasad, Nima Soleymanlou

Funding Boehringer Ingelheim.

*BI 764198 (Apecotrep) is a novel, potent, selective small-molecule TRPC6 inhibitor

"BI 764198 lowered proteinuria and was well tolerated by participants in this trial. This is the first evidence of efficacy with a podocyte-targeted therapy in FSGS.

Larger randomised controlled trials over longer treatment durations, enabling meaningful subgroup analyses, are planned to evaluate the safety and efficacy of BI 764198 treatment in FSGS"

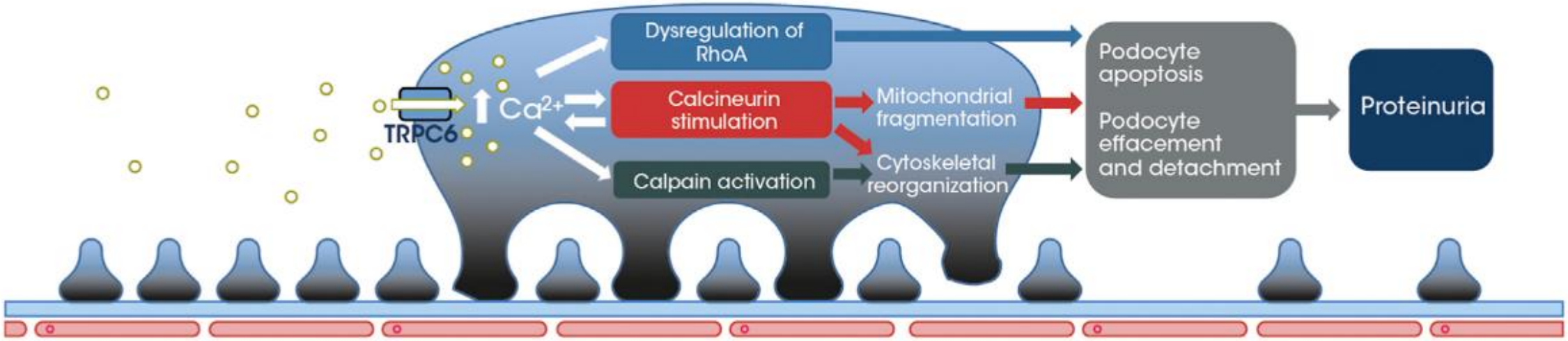
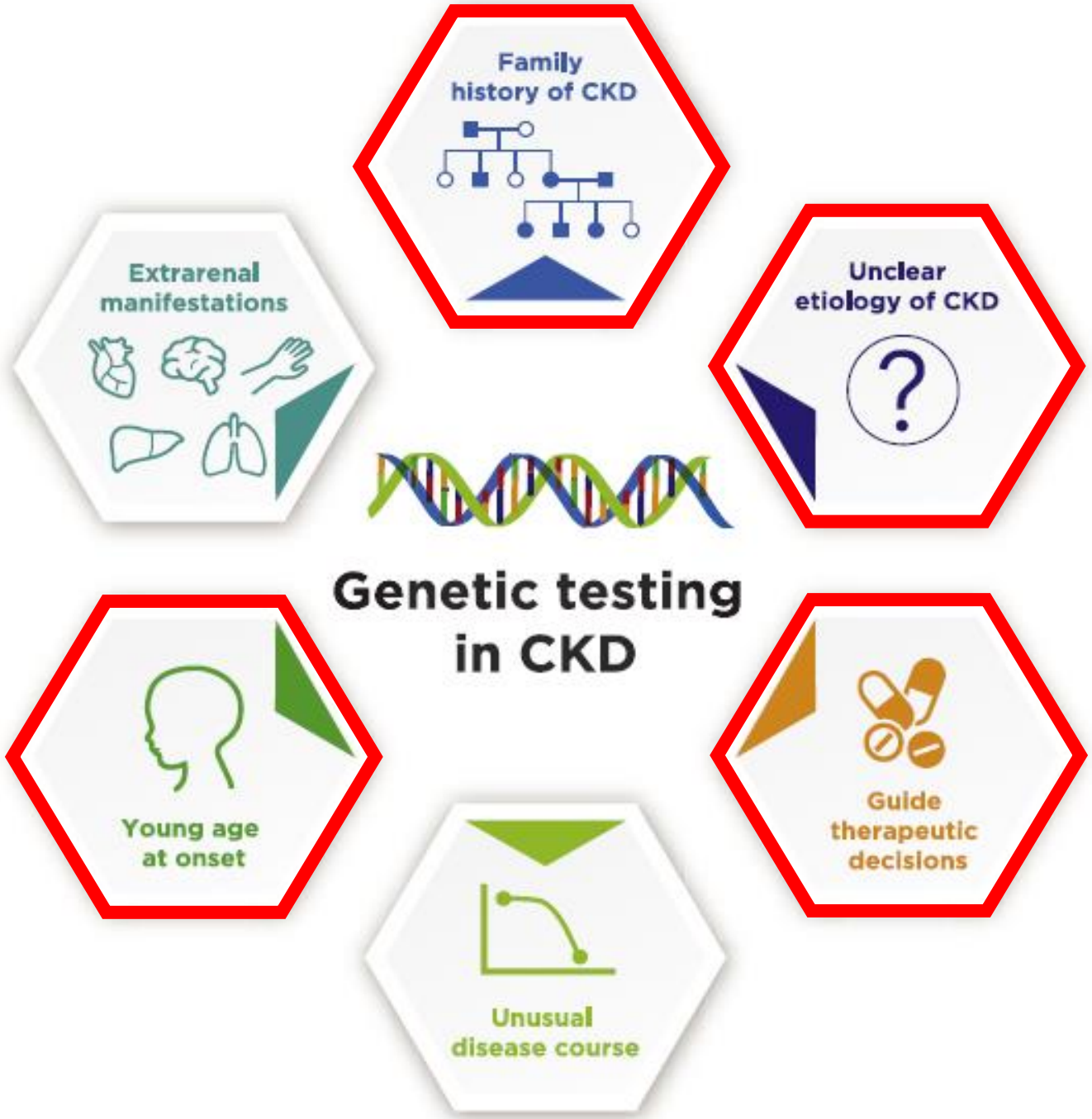


Figure 1. Mechanism of action of TRPC6 overexpression/activity in podocytes. TRPC6 overexpression or increase in function can lead to uncontrolled Ca^{2+} influx into podocytes. This induces cytoskeletal reorganization, detachment of podocyte foot processes from the glomerular basement membrane, and podocyte depletion, resulting in proteinuria.

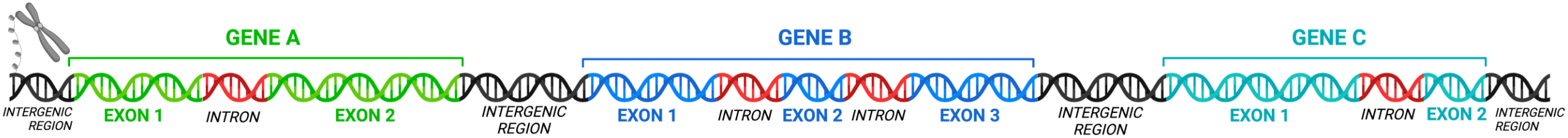
Trachtman et al, *KI Reports* 2023

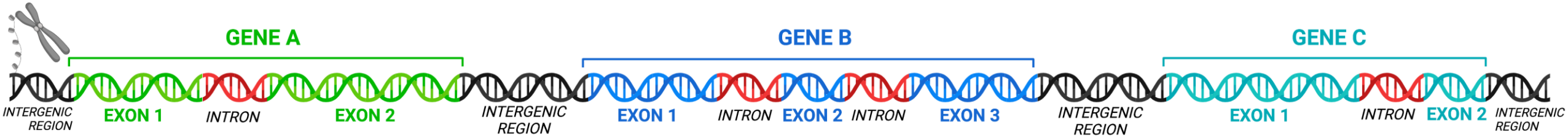
Indications for genetic testing



But what genetic test should be ordered?

Devarajan et al, *Kidney Med*, 2022





TARGETED VARIANT ANALYSIS

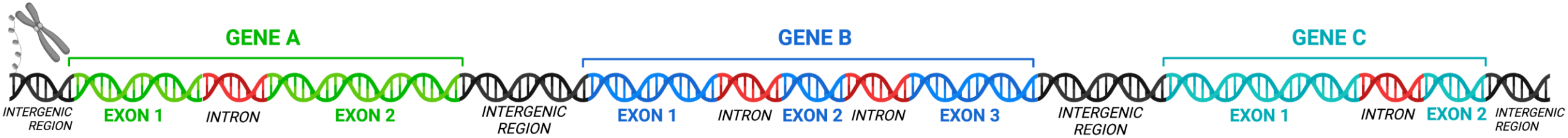
Use: Assess known family variant. **Advantages:** straightforward, lowest cost (sometimes free), no incidental findings. **Disadvantages:** very limited scope; some companies may not perform test for VUSs.

SANGER SEQUENCING (e.g. single gene analysis, or specific DNA region)

Use: Evaluate gene/region not well captured by NGS. **Advantages:** highest quality sequencing, usually no incidental findings. **Disadvantages:** often customized request, variable cost.

TARGETED GENE PANEL (next-generation sequencing; coding regions (exons) of select, disease-specific genes)

Use: Evaluate multiple genes (~20-400) implicated in a certain disease or group of diseases. Cost: ~$150-500 (copay), but up to ~$1000+ if not covered by insurance.
Advantages: Targeted search -- most cost-effective, less incidental findings, logistically easier to order (several disorder-specific panel options), sometimes customizable, higher quality sequencing (smaller #genes).
Disadvantages: Gene selection often predetermined and fixed (most commercial panels). Genetic analysis cannot be expanded later. Newly discovered genes need to be added. Low sensitivity for structural variants.



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WHOLE EXOME SEQUENCING (next-generation sequencing; all exons of ~ALL genes, ~2% of genome)

Use: Evaluate almost all coding genes. Useful in syndromic, complex, atypical cases (not a straightforward phenotype), or if panel negative but high suspicion for genetic cause. Cost: >$1000 (clinical).
Advantages: Very comprehensive genetic evaluation. If preferred, can perform in silico selection of genes initially, and can expand analysis to other genes later. Can identify novel genes.
Disadvantages: Broad search: less cost-effective, more VUS, more incidental findings, more genetic data to interpret. Low sensitivity for structural variants.

Audience Response Question #3

Currently, what is the approximate diagnostic yield of monogenic (single-gene) kidney disease among adult and pediatric CKD patients, respectively?

a) <1%, 10%
b) 5%, 15%
c) 10%, 30%
d) 30%, 50%
e) 40%, 70%

Groopman et al, NEJM 2019; Mallett et al, Orphanet J Rare Dis 2014; Connaughton & Hildebrandt, Nephrol Dial Transplant 2020

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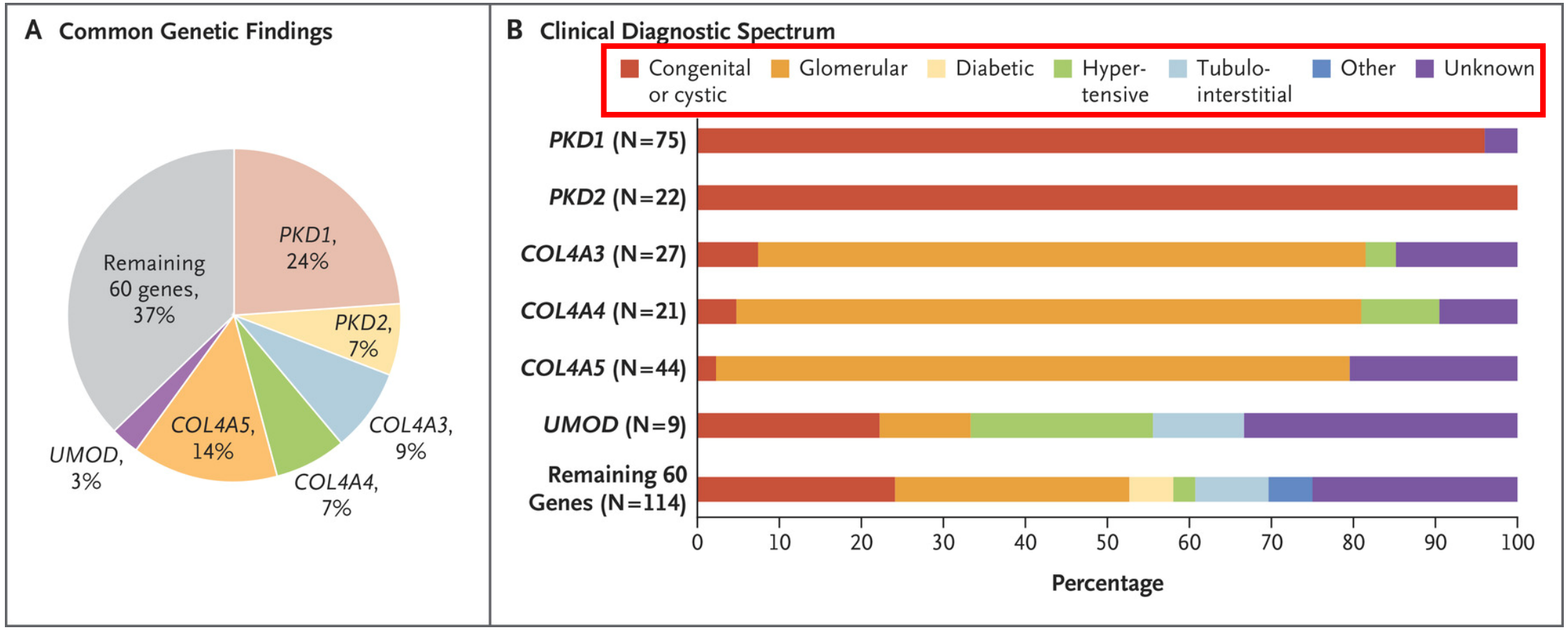
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Diagnostic Utility of Exome Sequencing for Kidney Disease

Exome sequencing in a cohort of 3,315 patients with CKD (64.7% ESKD) yielded a genetic diagnosis in 9.3% of cases.

Family history of kidney disease was associated with an increased chance of genetic diagnostic yield (OR=3.4, p-value=2.7×10^{-13}).



Groopman et al, NEJM 2019

Table 2. Diagnostic Yield and Heterogeneity of Genetic Diagnoses across Clinical Diagnostic Categories.

Clinical Diagnosis	Sequencing Performed	Diagnostic Variants Present	Diagnostic Yield	Distinct Monogenic Disorders Detected	Singleton Genetic Diagnoses
	number of patients		*percent*	*number*	
Congenital or cystic renal disease	531	127	23.9	27	20
Glomerulopathy	1411	101	7.2	23	14
Diabetic nephropathy	370	6	1.6	3	2
Hypertensive nephropathy	319	8	2.5	6	4
Tubulointerstitial disease	244	11	4.5	10	9
Other	159	6	3.8	4	2
Nephropathy of unknown origin	281	48	17.1	28	17
Total	3315	307	9.3	66*	39*

* A total of 27 genetic diagnoses were found multiple times, 21 of which were found among patients in different clinical diagnostic subgroups.

Groopman et al, NEJM 2019

Audience Response Question #4

42yo F referred for **microalbuminuria** during uncomplicated pregnancy, persistent 6 months post-partum.

Labs: Cr 1.1 mg/dl, eGFR 80 ml/min. UACR 150 mg/g. UAs (last 3y): 2+ blood, 10-50 RBCs. Serologic workup negative.

Family history: father heavy smoker with ESKD at 45yo, US with few kidney cysts; biopsy thin basement membranes and FSGS. Sister blood in urine, 2 left cysts.

What diagnosis is most likely, and what is/are the gene(s) usually involved?

a) X-Linked Alport Syndrome, *COL4A5*
b) Autosomal Dominant Polycystic Kidney Disease, *IFT140*
c) Autosomal Recessive Alport Syndrome, *COL4A3* or *COL4A4*
d) HANAC (hereditary angiopathy with nephropathy, aneurysms & muscle cramps), *COL4A1*
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f) APOL1-mediated kidney disease, *APOL1*

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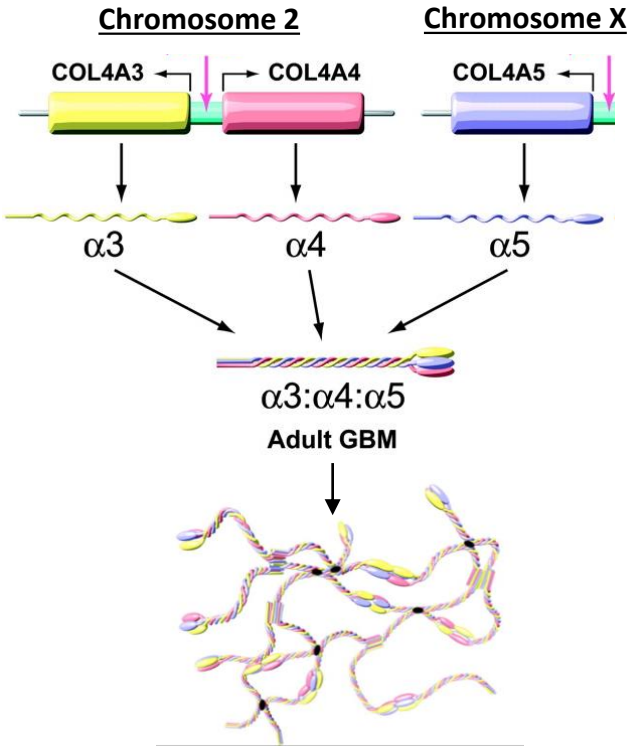
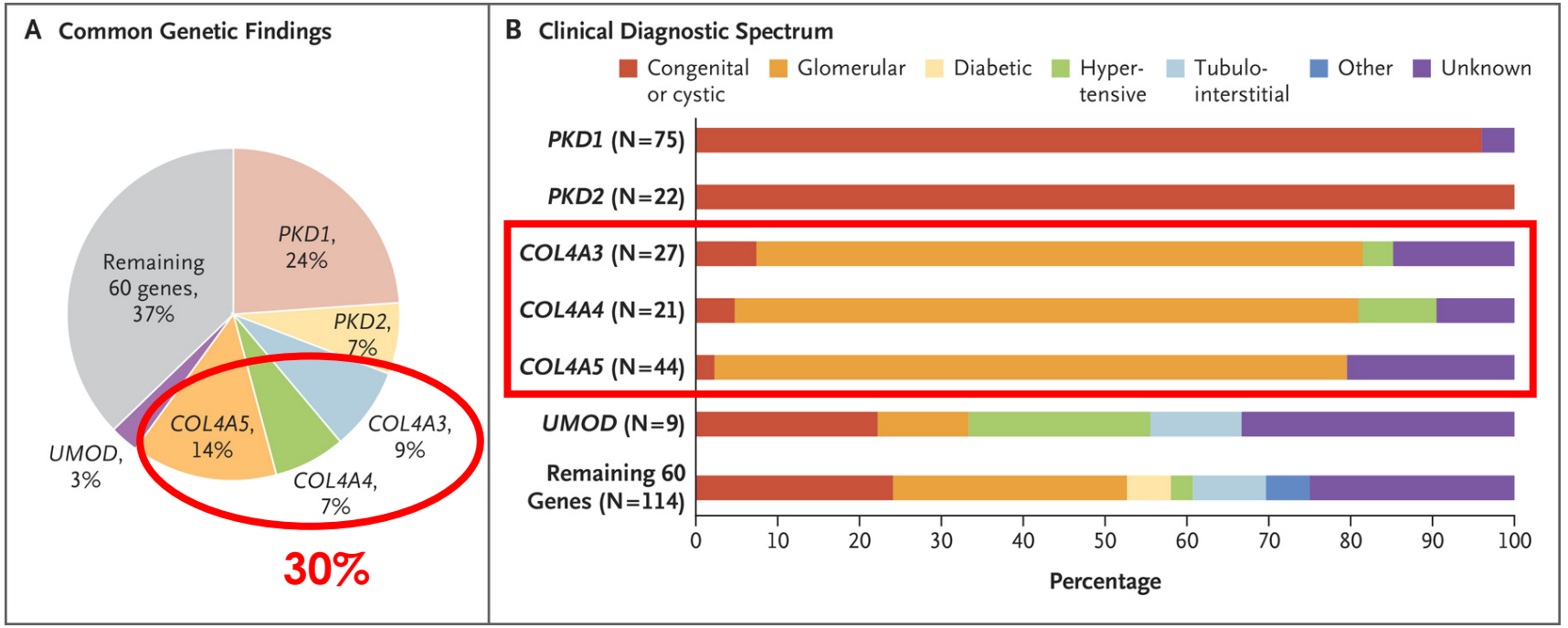
Alport Syndrome

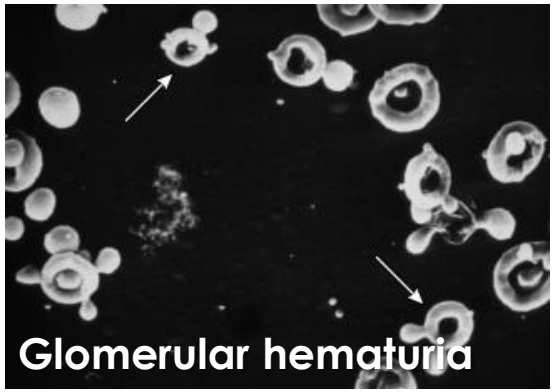
- Inherited primary basement membrane disorder (GBM + eye + cochlea) due to pathogenic variants in genes encoding type IV collagen alpha-3 (***COL4A3***), alpha-4 (***COL4A4***) and alpha-5 (***COL4A5***) chains
- Genetically heterogenous: inheritance can be X-linked, autosomal recessive, or autosomal dominant
- Clinically heterogenous: ranging from isolated hematuria to progressive renal disease (significant lifetime risk for kidney failure), sensorineural deafness (cochlea) and ocular abnormalities



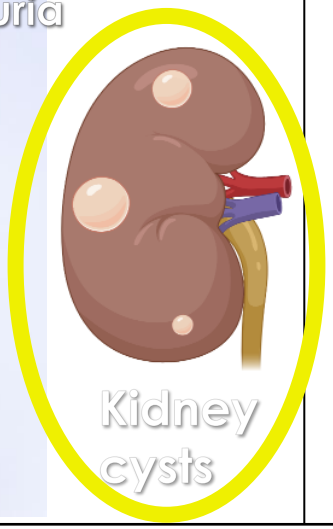


Tryggvason and Patrakka, JASN 2006; Groopman et al, NEJM 2019

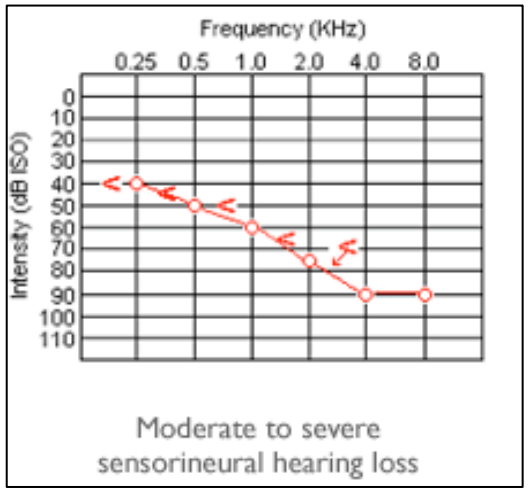
Alport Syndrome: Clinical Presentation

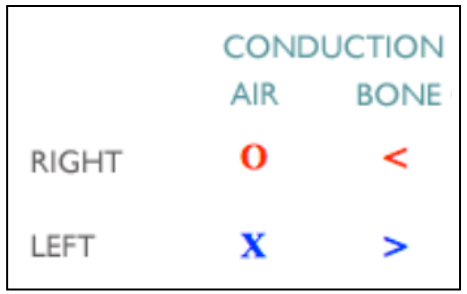


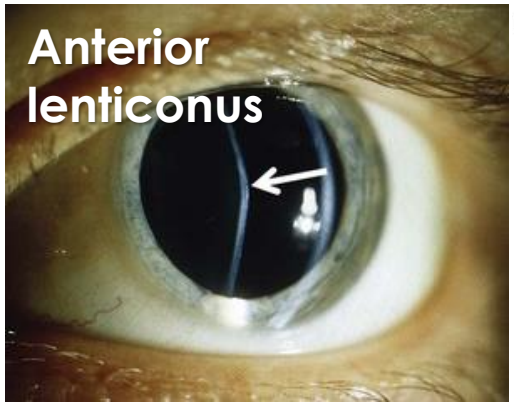
Gross hematuria
Albuminuria
Renal failure

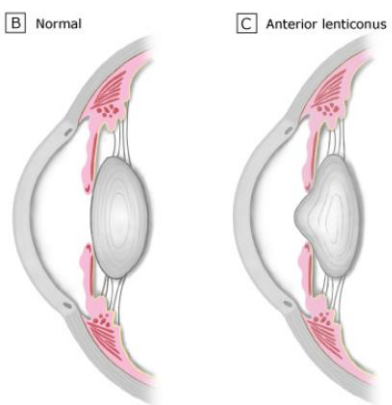


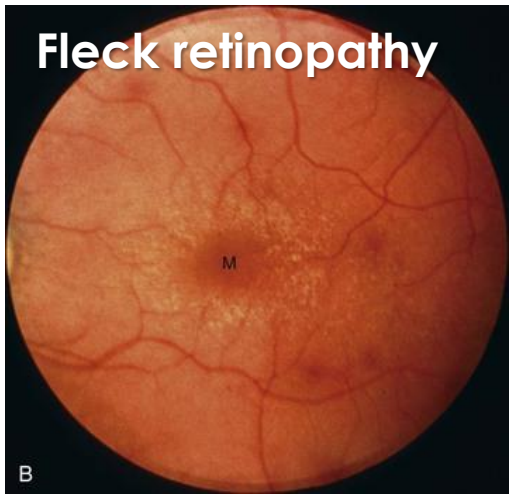
Sensorineural hearing loss

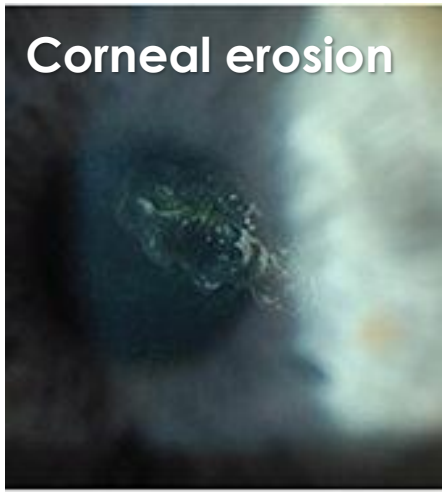




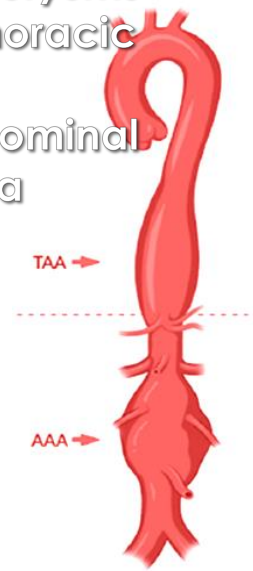








Aneurysms of thoracic & abdominal aorta



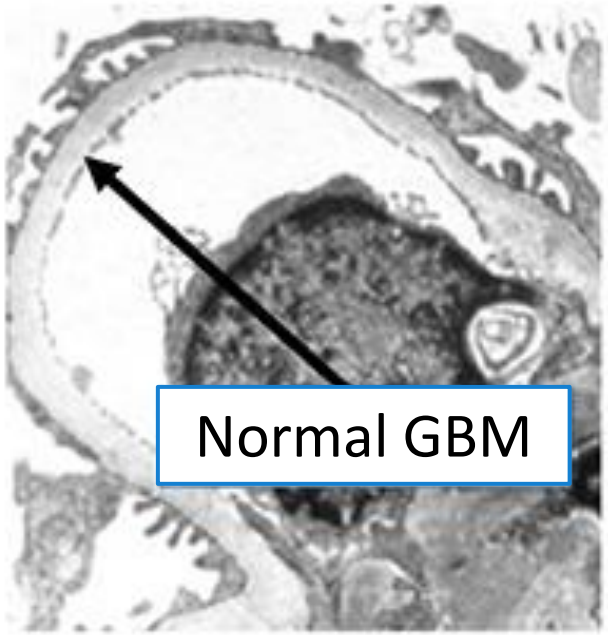
Images adapted from: Kashtan CE, abdominalkey.com/alport-and-other-familial-glomerular-syndromes, uptodate.com, paediatricentservices.com.au/hearing-assessments/audiogram, scarysymptoms.com/2015/08/blood-in-urine-caused-by-cancer-other, moyeseye.com/learn/recurrent-corneal-erosions; stepwards.com/?page_id=2886

Spectrum of disease in Alport Syndrome

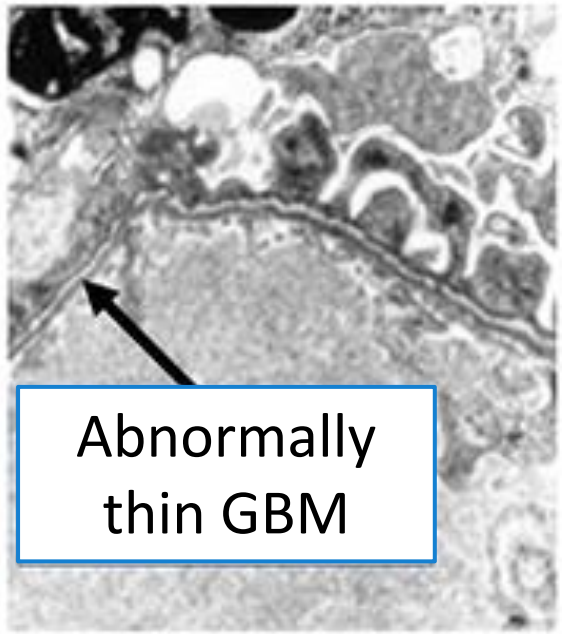
1 mutation in *COL4A3* or *COL4A4*	TBMN ADAS
1 mutation in *COL4A5* (X chrom, female)	XLAS "carrier"

2 mutations in *COL4A3* or *COL4A4*	ARAS
1 mutation in *COL4A5* (X chr, male)	XLAS

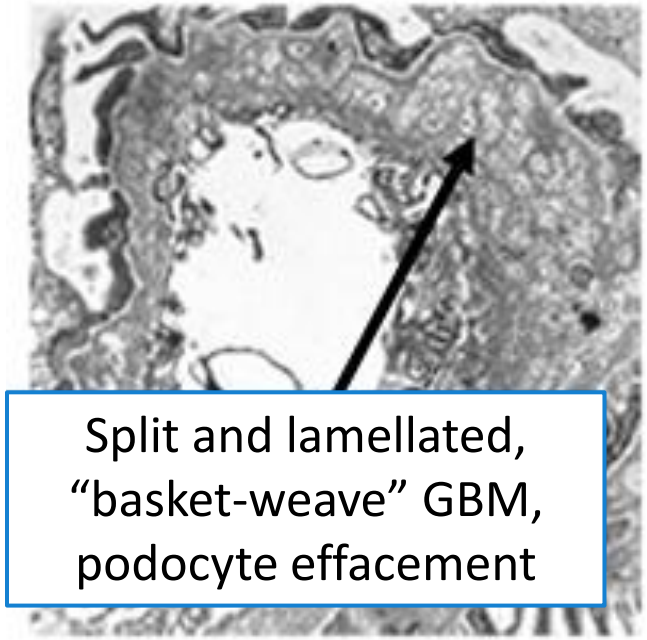
Any type of AS may progress to FSGS



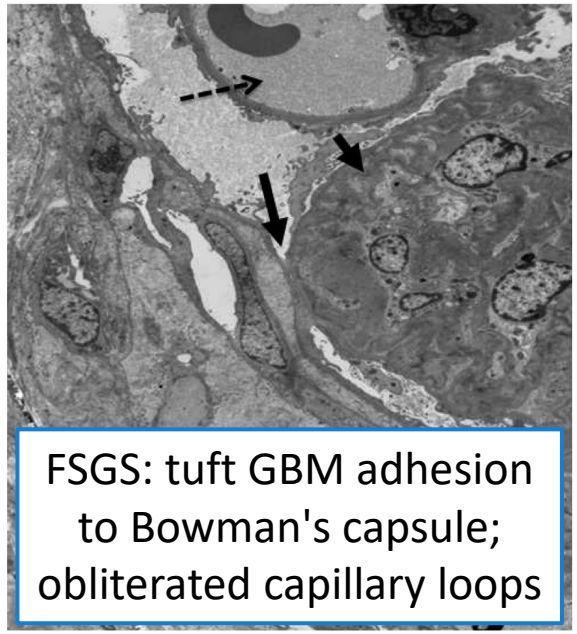
Normal GBM



Abnormally thin GBM



Split and lamellated, "basket-weave" GBM, podocyte effacement



FSGS: tuft GBM adhesion to Bowman's capsule; obliterated capillary loops

Thin Basement Membrane Nephropathy (TBMN)
Autosomal Dominant Alport Syndrome (ADAS)
X-Linked Alport Syndrome (female) (XLAS)

Digenic Alport Syndrome
Autosomal Recessive Alport Syndrome (ARAS)
X-Linked Alport Syndrome (male) (XLAS)

Adapted from unckidneycenter.org/kidneyhealthlibrary/glomerular-disease/alport-syndrome; Jefferson & Shankland, Adv Chronic Kidney Dis 2014

TYPE OF ALPORT SYNDROME		GENE(S)	VARIANTS (MUTATIONS)	RISK OF ESRD	HEARING LOSS	OCULAR LESIONS	COMMENTS
X-LINKED		*COL4A5*	Hemizygous (Males)	100%	100%	90%	Onset of ESRD and extrarenal manifestations a/w genotype
			Heterozygous (Females) *Lyonization!	Up to 25%	30%	15%	Risk factors for progression: gross hematuria, sensorineural hearing loss, proteinuria, GBM thickening/lamellation
AUTOSOMAL RECESSIVE		*COL4A3* or *COL4A4*	Biallelic (Homozygous or Compound Heterozygous)	100%	Frequent	Frequent	Onset of ESRD and extrarenal manifestations a/w genotype
AUTOSOMAL DOMINANT	**"THIN BASEMENT MEMBRANE NEPHROPATHY"**	*COL4A3* or *COL4A4*	Heterozygous	Zero	Rare	Rare	"Benign familial hematuria" Term sometimes used, but less as vague/controversial (actually ADAS? gene involved? biopsy?) *__Not all__ TBMN cases have mutations in *COL4A3-5* genes!
*leading to progressive kidney dz →	**AUTOSOMAL DOMINANT ALPORT SYNDROME***	*COL4A3* or *COL4A4*	Heterozygous	≥ 20%	Infrequent	Infrequent	Risk factors for progression: proteinuria, FSGS, GBM thickening/ lamellation, sensorineural hearing loss, or evidence of progression in family
DIGENIC (~"additive" allelic effect)		*COL4A3* +/- *COL4A4* +/- *COL4A5*	2 of 3 genes: each Heterozygous or Hemizygous	Higher than single variant	Variable	Variable	Inheritance pattern can simulate AD (variants in cis), AR (variants in trans), non-Mendelian (COL4A5+)

Adapted from: "Alport syndrome: a unified classification of genetic disorders of collagen IV a345: a position paper of the Alport Syndrome Classification Working Group," Kashtan et al, Kidney International 2018

Alport Syndrome: Management

- **Angiotensin blockade (ACEi, e.g. ramipril/lisinopril; ARB):** ↓ proteinuria, slows glomerulosclerosis/CKD progression
  - Initiate when there is **microalbuminuria (UACR ≥ 30 mg/g)**, or at time of diagnosis (table)
  - If UPCR > 1mg/mg despite maximal RAASi, additional measures to suppress proteinuria are reasonable, if tolerated (e.g. add **mineralocorticoide antagonist** – finerenone (initial promising evidence in AS, but in our experience not yet approved by insurance for this diagnosis alone); spironolactone (discuss risks using together with ACEi/ARB – eGFR decline, hyperK, hypoTN)
  - **SGLT2i** currently not in AS guidelines and being studied, but can be given in setting of other risk factors (DM), and many AS experts suggest considering in cases with persistent proteinuria
- **Renal transplantation** is the preferred option for ESKD
  - Carefully evaluate **living related donors**
  - Low risk of **anti-GBM** antibody-mediated glomerulonephritis in allograft
- **Audiologic evaluation** for children starting age 5-6yo (annual follow up) and adults
- **Ophthalmologic examination** – regular, comprehensive eye exam
- Currently no formal guidelines regarding **screening for aortic abnormalities** – consider in **males with XLAS**, +FH
- Patients with pathogenic variants in *COL4A3-5* and **FSGS** without other apparent cause, **avoid immunosuppression**

	Indication for treatment (ACEi)
XLAS males	At time of diagnosis, if age > 12 to 24 months
XLAS females	Microalbuminuria
ARAS	At time of diagnosis, if age > 12 to 24 months
ADAS (heterozygous variant in *COL4A3* or *COL4A4*)	Microalbuminuria

XLAS, X-linked Alport syndrome; *ARAS*, autosomal recessive Alport syndrome; *ADAS*, autosomal dominant Alport syndrome

Kashtan and Gross, Pediatr Nephrol 2021

Audience Response Question #5

This patient's kidney gene panel reveals a heterozygous, pathogenic variant in *COL4A4*, c/w Autosomal Dominant Alport Syndrome.

The genetic test also reveals a heterozygous, likely pathogenic variant in *NPHS2*. **Biallelic** (homozygous or compound heterozygous) mutations in *NPHS2* (encoding podocin) cause **autosomal recessive** nephrotic syndrome.

How do we interpret her *NPHS2* variant from a clinical standpoint?

a) Risk allele

b) Carrier status

c) Disease-associated

d) Variant of uncertain significance

e) Disease-causing

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Variant of Uncertain Significance (VUS)

- Variant that is unable to be classified into one of the other 4 categories at present time
  - Current scientific/clinical evidence cannot determine (based on ACMG guidelines) whether the variant is (likely) pathogenic or benign
- In principle, VUSs should not be used for clinical decision-making
  - However, expert review and additional data (e.g. variant characteristics, clinical features and family history) can help re-classify
- If the VUS is in a gene consistent with patient's disease, family testing may help re-classify the variant
  - E.g., if there is strong correlation between disease and VUS in different family members

NOTE:
PKD2 is a gene for ADPKD (autosomal dominant PKD). A PKD patient had genetic testing with a VUS in *PKD2*. 5y later, repeat test reclassified variant as pathogenic.

Carrier

- Individual who carries one pathogenic variant (heterozygous) for an autosomal recessive disorder
  - For autosomal recessive diseases, only one abnormal gene copy does not cause disease (caused by 2 abnormal gene copies)
  - Therefore, the patient "carries" the one variant, which does not affect them, but can be "carried" / passed along to their children
  - If a child inherits another mutation in the same gene from the other parent, they may develop the recessive disease
- For a "carrier" patient, variant should not be considered disease-causing
  - Unless scientific evidence of clinical consequences of variant in heterozygous state

NOTE:
PKHD1 is a gene for ARPKD (autosomal recessive PKD). Parents of affected children were labeled as "carriers." Later studies found some carriers with kidney/liver cysts.

www.cancer.gov/publications/dictionaries/genetics-dictionary/def/carrier; Gunay-Aygun, Mol Genet Metab 2011; Besse, J Clin Invest 2017

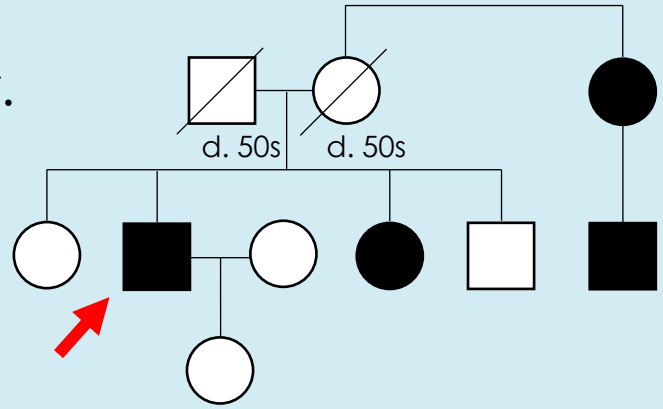
Audience Response Question #6

55 yo M with HTN, HLD, gout and slowly progressive CKD4.

US: 9cm kidneys, 3 medullary cysts and increased echogenicity. UA bland. Biopsy: 70% IFTA. Serologic evaluation negative.

Family history: sister with CKD in 40s, maternal aunt with ESKD in 60s, maternal cousin with kidney issues in 50s. Patient's parents died in their 50s.

His 30yo daughter is healthy, eager to donate a kidney to her father.



What is the most likely clinical diagnosis?

a) ADPKD (Autosomal Dominant Polycystic Kidney Disease)
b) CAKUT (Congenital Anomalies of Kidney and Urinary Tract)
c) HANAC (Hereditary Angiopathy with Nephropathy, Aneurysms and muscle Cramps)
d) MSK (Medullary Sponge Kidney)
e) ADTKD (Autosomal Dominant Tubulointerstitial Kidney Disease)

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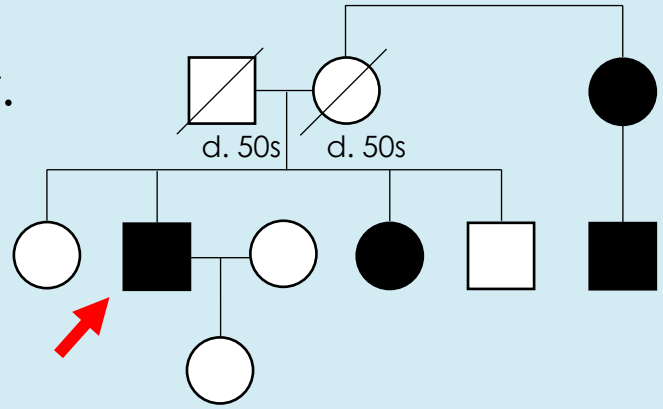

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Autosomal Dominant Tubulointerstitial Kidney Disease (ADTKD)

- Autosomal dominant inheritance
- **Slowly progressive CKD;** impaired renal function typically appears in teenage years
- Variable **adult ESKD onset** (~20 to 80 yo)
- **Bland urine sediment**, with no/minimal proteinuria
- Occasional kidney cysts
- **Biopsy: diffuse tubulointerstitial disease**
- Can be associated with **hyperuricemia**
- Few non-renal manifestations
- 3rd most common monogenic kidney disease, ~5% (after ADPKD and Alport)

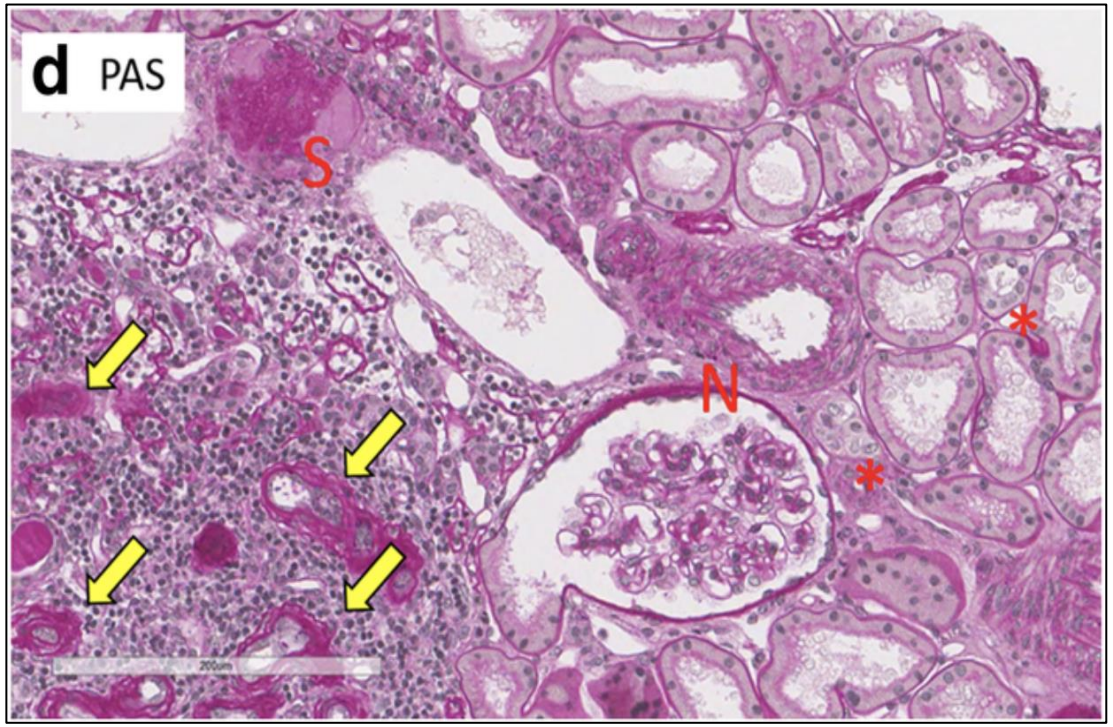


Table 3 | Usual findings on renal histology in patients with ADTKD

- Interstitial fibrosis
- Tubular atrophy
- Thickening and lamellation of tubular basement membranes
- Possibly tubular dilatation (microcysts)
- Negative immunofluorescence for complement and immunoglobulins

Devuyst et al, Nat Rev Dis Primers 2019; Onoe et al, BMC Nephrol 2021; Eckardt et al, Kidney Int 2015

Table 4 | Possible but not obligatory findings according to the underlying genetic defect (patient or family)

	UMOD	*MUC1*	*REN*	*HNF1B*
Clinical/imaging	Early gout (for age), occasional renal cysts (usually not medullary)[26–28]	Gout (less frequent), occasional renal cysts (usually not medullary)[26–28]	Mild hypotension, increased risk for AKI, anemia during childhood	MODY5, few bilateral renal cysts, genital abnormalities, pancreatic atrophy
Presentation during childhood	Rare (occasionally with gout)	None	Frequent	Frequent (prenatal ultrasound findings)
Laboratory	Hyperuricemia, low fractional excretion of urate (<5%), low urinary excretion of uromodulin	Hyperuricemia (less frequent)	Hyperuricemia and hyperkalemia, low urinary excretion of uromodulin Low-normal plasma renin	Hypomagnesemia, hypokalemia, liver function test abnormalities Hyperuricemia
Histology	Intracellular uromodulin deposits in TAL profiles	Intracellular accumulation of MUC1-fs in distal tubules[a]	Reduced renin staining in cells of the juxtaglomerular apparatus	

Abbreviations: AKI, acute kidney injury; HNF1B, hepatocyte nuclear factor 1β; MODY5, maturity onset diabetes mellitus of the young type 5; MUC1, mucin-1; MUC1-fs, mucin-1 frameshift protein; REN, renin; TAL, thick ascending limb of Henle's loop; UMOD, uromodulin.

[a]This test is currently available only in selected research laboratories. (MUC1 – e.g. Broad Institute)

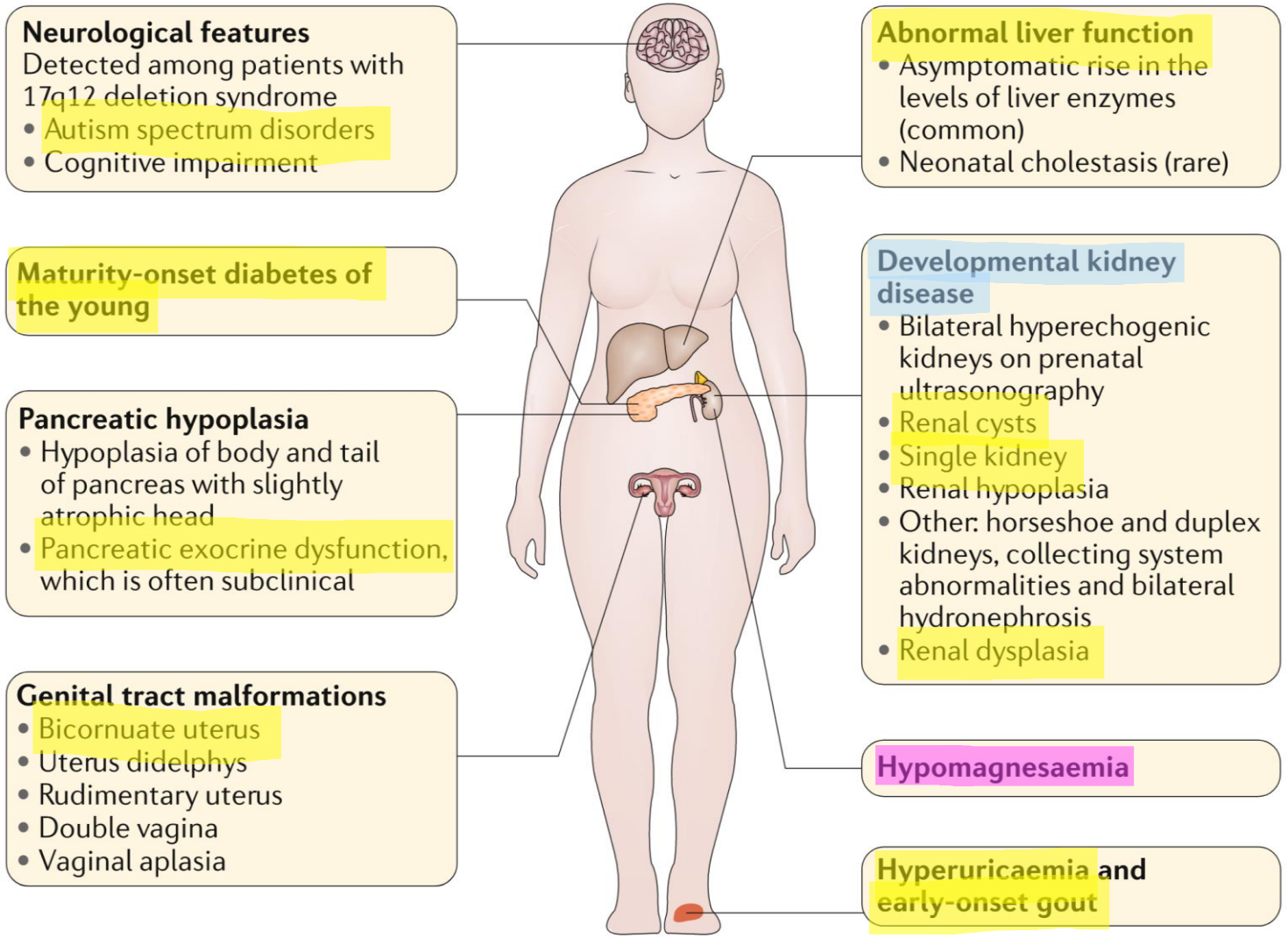
*ADTKD previously named: 'Medullary Cystic Kidney Disease (MCKD) type 2', 'Familial Juvenile Hyperuricemic Nephropathy (FJHN)', or 'Uromodulin-Associated Kidney Disease (UAKD)' for UMOD-related diseases and 'MCKD type 1' for the disease caused by MUC1 mutations.

Adapted from Eckardt et al, Kidney Int 2015 and Devuyst et al, Nat Rev Dis Primers 2019

HNF1B-related disease

CHECK MAGNESIUM!

- Encodes **transcription factor** hepatocyte nuclear factor 1β
- Important role in **kidney development**
- **Autosomal dominant** inheritance
- **Significant variability** in clinical presentation, even among family members
- **"Renal cyst and diabetes syndrome"**
- **Can mimic several different disorders**: differential diagnosis for ADPKD, ADTKD, CAKUT and Gitelman syndrome



Devyust et al, Nature Rev Neph 2019; Clissold et al, Nature Rev Neph 2015

Audience Response Question #7

The prior patient has a high-quality, extensive (>400 genes) kidney panel, which is negative for any pathogenic variants.

All below are potential benefits of genetic testing for kidney disease, EXCEPT FOR:
*(*Note: excluding instances of targeted familial variant testing)*

a) Ending "diagnostic odysseys," which often have significant burden for patients, their families, and the healthcare system
b) Enabling referral to clinical trials targeting certain genetic disorders
c) Ruling out a genetic cause of kidney disease
d) Guiding/changing clinical management
e) Informing long-term clinical prognosis
f) Prompting screening for associated extrarenal manifestations
g) Informing family counseling and planning

"Genetics in CKD: conclusions from a KDIGO Controversies Conference," Kidney Int 2022; Groopman et al, Nat Rev Nephrol 2018

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f) Prompting screening for associated extrarenal manifestations
g) Informing family counseling and planning

"Genetics in CKD: conclusions from a KDIGO Controversies Conference," Kidney Int 2022; Groopman et al, Nat Rev Nephrol 2018

A negative genetic test report does not rule out a genetic cause of disease

**excluding targeted familial variant testing*

- **Gene may not be included in a commercial panel**
  - Example: cystic kidney disease panel **without *COL4A3-5* or *HNF1B***
- **Gene, mutation or mode of inheritance may not have been discovered**
  - Example: **heterozygous *IFT140* mutations** were only discovered to cause autosomal dominant polycystic kidney disease in **2022**
- **Variant may not be captured by next-generation sequencing**
  - Non-coding (intronic or regulatory) variants
    - Example: deep **intronic mutations in *DGKE*** (aHUS)
  - Structural variants (large deletions or insertions)
    - Example: **deletion of entire *HNF1B* gene** (part of chromosomal 17q12 deletion)
  - Very repetitive DNA regions
    - Example: ***MUC1*** (variable number tandem repeat sequences)

MUC1 testing sent separately and shows a cytosine insertion. Patient's daughter is tested for this familial variant and does not have it. She is able to safely donate a kidney to her father!

Knoers et al, Neprol Dial Transplant 2022

Audience Response Question #8

50yo M referred by Ophthalmology – reason for referral unclear.

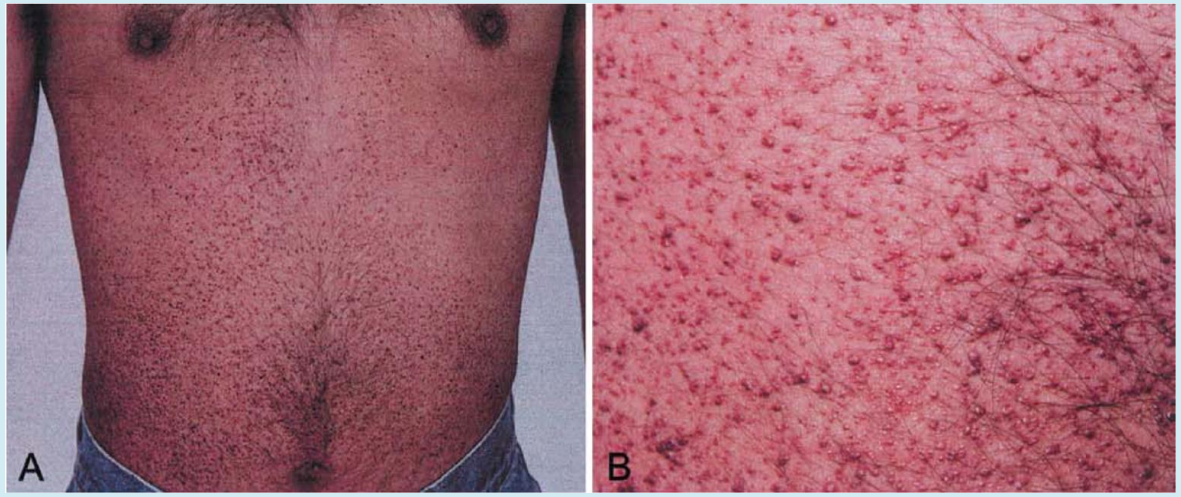
ROS: Polyuria and polydipsia. Intermittent pain in hands/legs since childhood, on B12 and gabapentin. Recurring abdominal pain, nausea, diarrhea and cold intolerance since teenage years.

FH: mother died in 40s of heart attack; daughter with protein in urine during pregnancy.

Labs: Cr 1.4, eGFR 57, lytes/TSH WNL, UPCR 1.3 g/g. US: renal sinus cysts.

What is the most likely diagnosis and gene involved?

a) Atypical Hemolytic-Uremic Syndrome (*CFH*)
b) Neurofibromatosis (*NF1*)
c) Tuberous Sclerosis Complex (*TSC1* or *TSC2*)
d) Fabry disease (*GLA*)
e) Polycystic liver disease (*GANAB*)



Cho & Kopp, Pediatr Nephrol 2004

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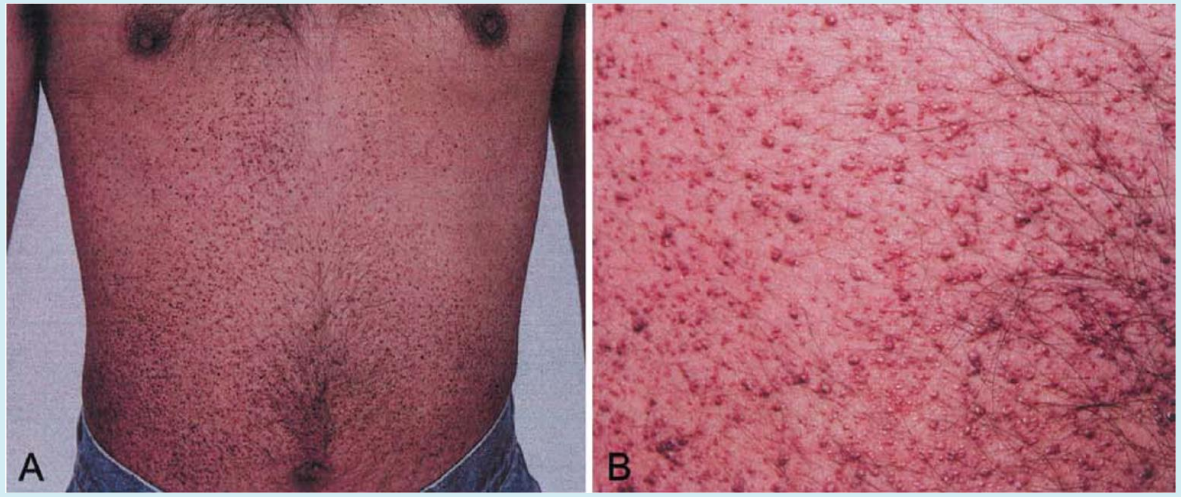
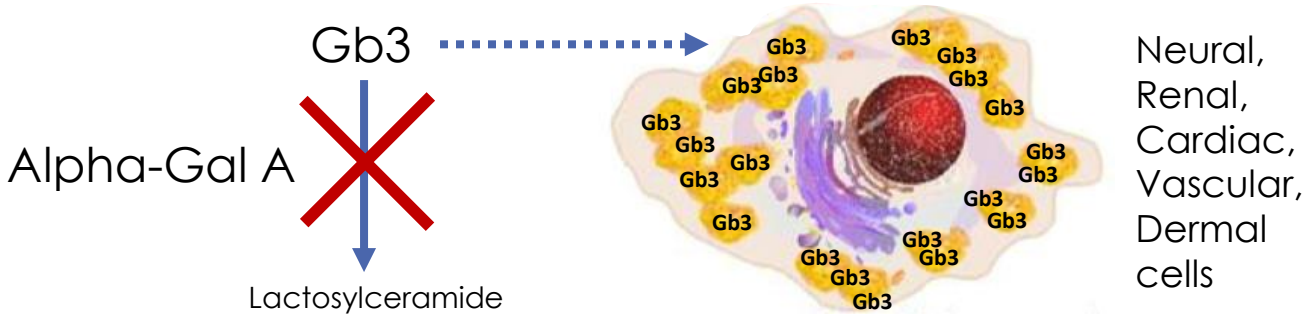


Cho & Kopp, Pediatr Nephrol 2004

Fabry Disease

- Most prevalent **lysosomal storage disorder**
- **Deficiency of alpha-galactosidase A** (alpha-Gal A) → cleaves globotriaosylceramide (**Gb3**)
  - Gb3 accumulates in multiple cells, tissues
- Disease is caused by mutations in ***GLA*** (encodes alpha-Gal A)
  - *GLA* gene is located on the X chromosome → **X-linked inheritance**
  - Phenotypic variation in women due to X chromosome inactivation
- Clinical course is correlated with magnitude of **alpha-Gal A activity**
  - **None/minimal (<3%):** Classic Fabry disease
  - **Residual (3-35%):** Atypical, later onset (3rd – 7th decade); cardiac/renal variants



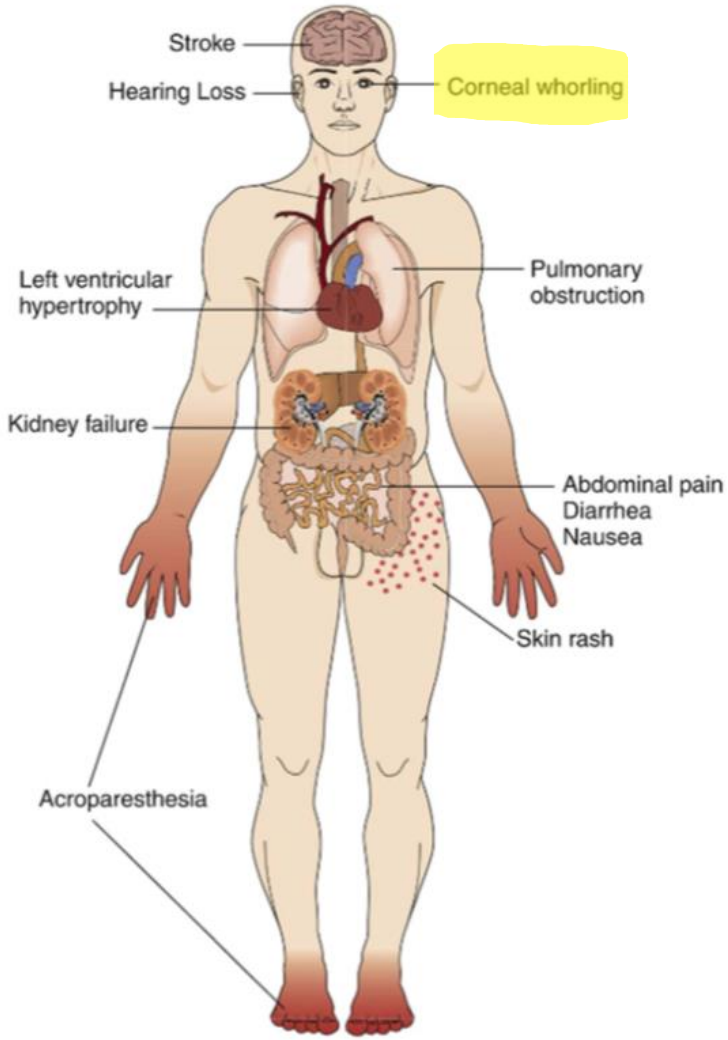
Neural, Renal, Cardiac, Vascular, Dermal cells

TREATMENT

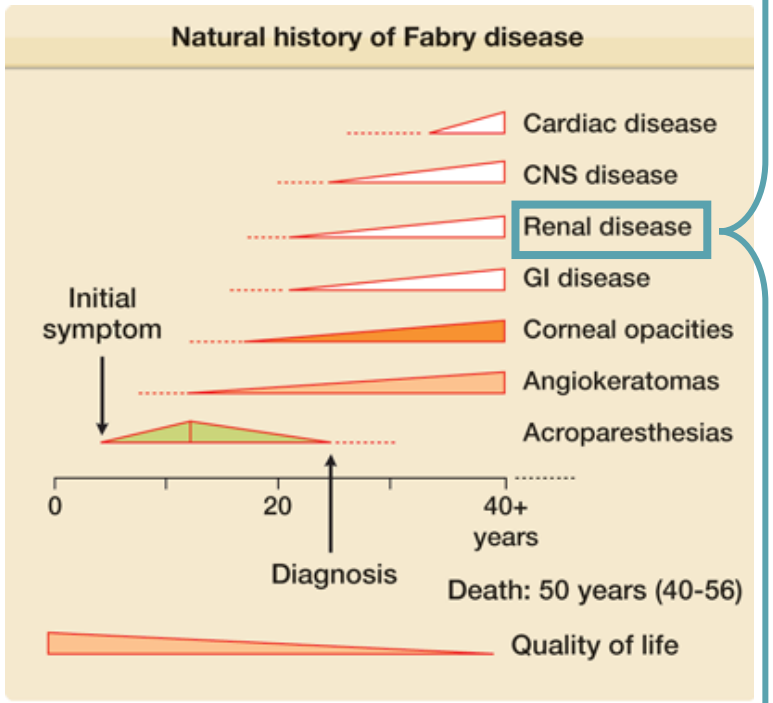
- **Enzyme replacement therapy:** infusion every 2 weeks
- **Pharmacological chaperone:** stabilizes mutant forms of alpha-Gal A → **increases enzyme activity**
  - **Migalastat (oral)**
  - Only for **amenable genetic variants (list)**
- Kidney disease:
  - **ACEi/ARB**
  - **Transplantation:** treatment of choice, usually no recurrence
  - Survival on dialysis is lower than other pts

Zarate & Hopkin, Lancet 2008; www.incanursing.ie/wp-content/uploads/2016/01/Fabry%E2%80%99-s-Disease-ICS-2016.pdf; www.lysosomalstorageresearch.ca

Fabry Disease: Clinical Presentation



(CLASSIC FORM)



Source: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K: *Fitzpatrick's Dermatology in General Medicine, 8th Edition*: www.accessmedicine.com
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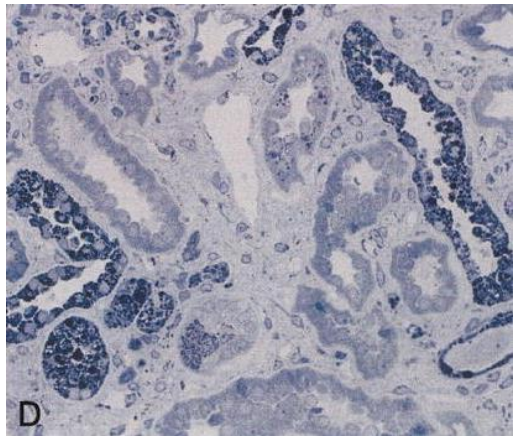
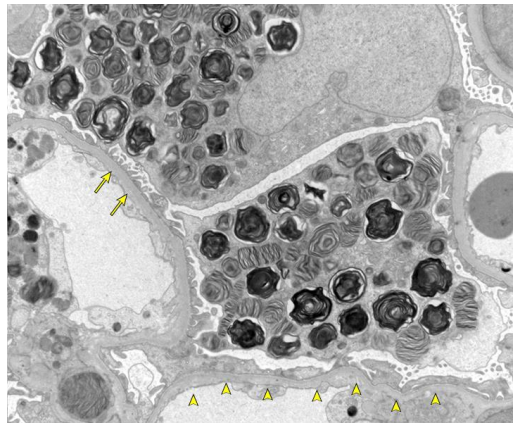
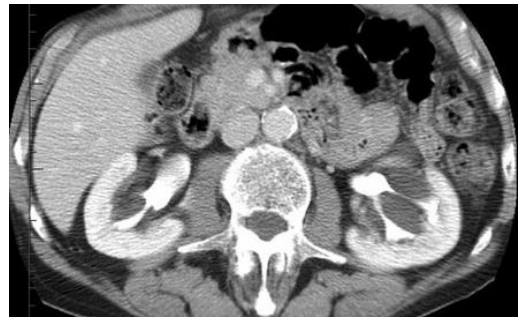
Distal tubules: impaired urinary concentrating ability → polyuria and polydipsia



Podocytes: zebra bodies (enlarged lysosomes with lamellated structures [Gb3 deposits]), foot process effacement → proteinuria → CKD → ESKD



Renal sinus and parapelvic **cysts**



Fitzpatrick's Dermatology in General Medicine, 8th ed., Chapter 136; Cho & Kopp, Ped Nephrol 2004; radiopaedia.org/articles/renal-sinus-cyst; med.nyu.edu/medicine/nephrology/sites/default/files/nephrology/Clinical%20Case%20Conference%2012.22.15.pdf; Branton MH et al, Medicine 2002 © Lippincott Williams & Wilkins; uptodate.com, courtesy of Michael Mauer.

Audience Response Question #9

In addition to a hemizygous pathogenic variant in *GLA*, the patient's genetic test report includes this finding:

Gene	Condition	Inheritance	Variant	Zygosity	Classification
RET	MEN2A, Familial Medullary Thyroid Carcinoma	Autosomal Dominant	c.2671T>G (p.Ser891Ala)	Heterozygous	Pathogenic

What is the next best step regarding this *RET* variant?

a) Refer the patient to Cancer Genetics and Endocrinology for time-sensitive consultation.

b) Call patient and provide reassurance, as they are carriers for this variant and thus not expected to have disease, but may pass the variant to a child.

c) Contact genetic testing company and notify them of the inclusion of a cancer gene in a kidney disease panel (patient does not have cancer, and may not want to know about this risk).

d) Call patient and explain that this is not clinically relevant, as it is an incidental finding.

e) Contact patient's adult children and offer genetic cascade testing through the clinical laboratory.

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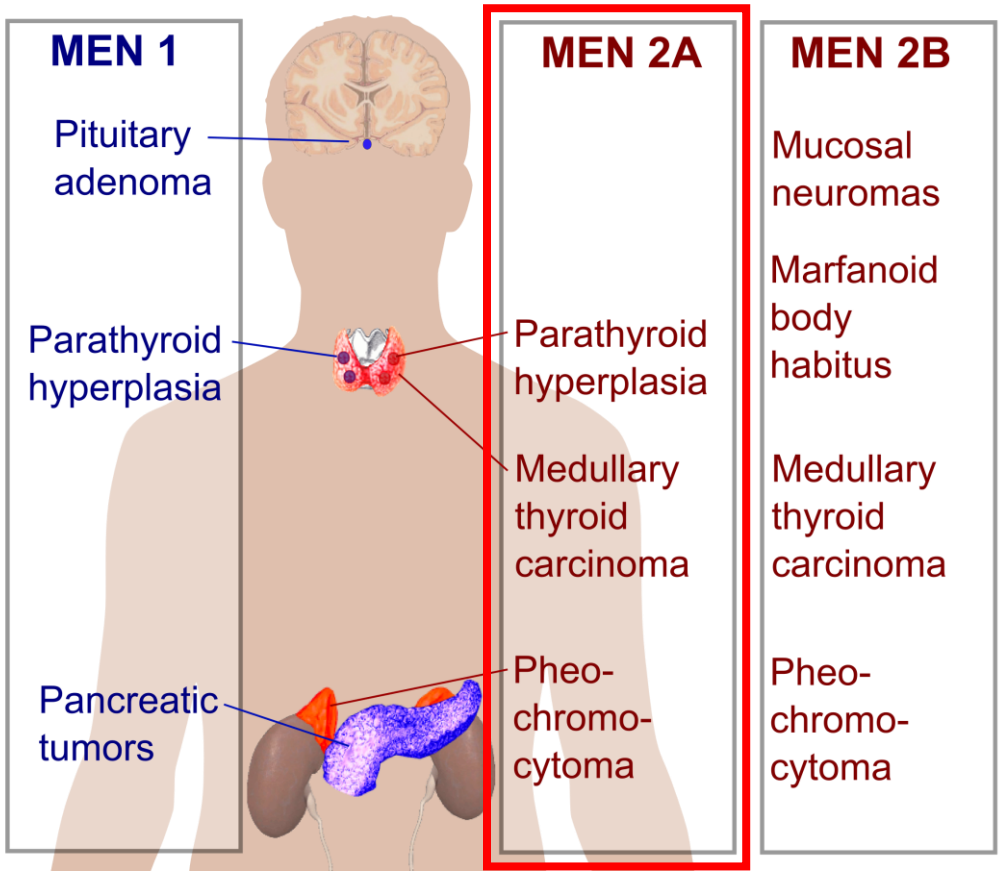
RET

- **Tyrosine kinase receptor:** transduction of growth & differentiation signals in developing tissues, **e.g. kidney**
- Mutations can cause:
  - **Congenital anomalies of kidney & urinary tract (CAKUT)**
  - Hirschsprung disease (intestinal aganglionosis)
  - **Multiple endocrine neoplasia type 2 (MEN2) hereditary cancer syndrome**
- p.Ser891Ala variant reported in many families with MEN2A
- Patients with MEN2A:
  - **Almost all: medullary thyroid cancer (MTC), often young**
  - Often multicentric → total (prophylactic) thyroidectomy **(timing based on specific RET variant)**
  - (First check/resect possible pheochromocytoma)



Timely referral to Cancer Genetics, Endocrinology, Surgical Oncology
PTH >1000, Ca 11. Calcitonin high. Thyroid US: nodule. PET-CT: no metastases
Thyroidectomy: pathology showed MTC. Genetic screening for his family members

Short & Smyth, Nat Rev Nephrol 2016; Wells et al, Thyroid 2015; www.uptodate.com/contents/approach-to-therapy-in-multiple-endocrine-neoplasia-type-2; en.wikipedia.org/wiki/Multiple_endocrine_neoplasia

Pre-test genetic counseling

GINA
GENETIC INFORMATION NONDISCRIMINATION ACT

POSITIVE RESULTS	• **PATHOGENIC and LIKELY PATHOGENIC variants, compatible with mode of inheritance of disorder**
	  • "Actionable" findings: can be used for clinical decision-making, as per evidence-based recommendations
	• **GINA (Genetic Information Nondiscrimination Act of 2008): federal law that protects individuals from genetic discrimination in most health insurance and employment**
	  • Health insurers and employers can't use genetic data to make eligibility/coverage and employment decisions
	• GINA does not cover life insurance, disability insurance or long-term care insurance – patients can be denied coverage or charged higher fees
	  • However, individuals may also have similar issues with positive family history of genetic disease
	• ACA (Affordable Care Act): health insurance can't refuse c… "pre-existing condition" (this includes clinical genetic disease

NEGATIVE RESULTS	• **No (likely) pathogenic variants to explain clinical disease** • **Does not rule out genetic cause** (aside from targeted familia… • If very typical presentation of genetic disease or strong family history, consider further genetic testing • Genetic test reports include a list of all tested genes and potential test limitations (usually at the end)

UNCERTAIN RESULTS	• **Variants of uncertain significance (VUS)**
	  • New clinical/scientific evidence may lead to possible reclassification in the future
	• Pathogenic variants for non-clinically manifest and/or non fully-penetrant disease
	  • E.g. asymptomatic young adult with variant causal of late-onset disease of variable severity

INCIDENTAL RESULTS	• Unexpected genetic findings • Often unrelated to reason for testing (e.g. unexpected renal disease; or cancer, heart disease, etc) • **Remember that this may be an actionable finding! (refer to the appropriate specialist)**

"Genetics in CKD: conclusions from a KDIGO Controversies Conference," Kidney Int 2022; www.GINAHelp.org; www.hhs.gov/answers/health-insurance-reform/can-i-get-coverage-if-i-have-a-pre-existing-condition/index.html#:~:text=Yes.,that%20new%20health%20coverage%20starts

Summary: indications for genetic testing

Table 4 | Potential indications for genetic testing for monogenic forms of CKD

- The clinical work indicates the possibility of a genetic disease, such as—
  - high prevalence of monogenic subtypes within the clinical category (e.g., congenital/cystic nephropathies or steroid-resistant nephrotic syndrome)
  - positive family history of kidney disease
  - early age of onset (pediatric CKD)
  - syndromic/multisystem features
  - consanguinity
  - possibility of identifying a condition amenable to targeted treatment (e.g., enzyme replacement therapy for Fabry disease)
- The individual is an at-risk relative of a patient with a known monogenic disease, especially when the individual is a potential kidney donor
- As an alternative to kidney biopsy in patients at high risk of biopsy-related complications, especially when there is a high pre-test probability of finding a genetic variant based on family or clinical history
- CKD or kidney failure of unknown etiology when kidney biopsy would not be informative due to advanced disease, and other features suggestive of hereditary disease are present
- Information to guide continuation of immunosuppressive therapy (e.g., in steroid-resistant or partially responsive nephrotic syndrome)
- Genetic testing can provide prognostic information (e.g., ADPKD or Alport Syndrome, age at kidney failure)
- Diagnosis of diseases with risk of recurrence in kidney allografts (e.g., aHUS/TMA, primary hyperoxaluria)

ADPKD, autosomal dominant polycystic kidney disease; aHUS, atypical hemolytic uremic syndrome; CKD, chronic kidney disease; TMA, thrombotic microangiopathy.

Take Home Points

- **Genetic testing is increasingly being used in clinical nephrology.** Expanding genomic literacy among nephrologists is critical for its successful implementation.
- Next-generation DNA sequencing generates large amounts of genetic data, thus **adequate interpretation of variants is crucial to diagnosis and medical decision-making.**
- Targeted gene panels provide relatively fast (3-6 weeks), sensitive and cost-effective sequencing. **Be sure to know which genes are included (or not) in each panel.**
- **Prevalence of genetic diagnoses in adult CKD is substantial and increasing**, as more patients have access to genetic testing and additional disease-causing genes and variants are discovered.
- Genetic diagnostic yield is higher in patients with **congenital or cystic disease, CKD of unknown etiology, family history of kidney disease, and young age of onset** (although there is no upper-age limit for monogenic CKD).
- **A negative result of a genetic test does not rule out a genetic etiology of disease** (aside from excluding the presence of familial variants in relatives).
- Patients should receive **comprehensive genetic counseling before and after** genetic testing is performed.
- Partnering with **genetic counselors and creating multidisciplinary genetics-focused teams** can increase access to genetic testing, facilitate cascade family testing, improve interpretation of genetic results, and optimize next steps regarding actionable findings.

Brief References (=highly recommended reading)

1. **Genomic medicine for kidney disease.** Groopman EE, Rasouly HM, Gharavi AG. Nat Rev Nephrol, 2018. PMID: 29307893.
2. **Genetics in chronic kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference.** KDIGO Conference Participants. Kidney Int, 2022. PMID: 35460632.
3. **Personalized medicine in chronic kidney disease by detection of monogenic mutations.** Connaughton DM, Hildebrandt F. Nephrol Dial Transplant, 2020. PMID: 30809662.
4. **Genetic testing in the diagnosis of chronic kidney disease: recommendations for clinical practice.** Knoers N, Antignac C, Bergmann C, et al. Nephrol Dial Transplant, 2022. PMID: 34264297.
5. **Clinical Interpretation of Sequence Variants.** Zhang J, Yao Y, He H, Shen J. Curr Protoc Hum Genet, 2020. PMID: 32176464. [This manuscript is more technical and takes the reader through the variant interpretation process.]

Thank you and good luck!