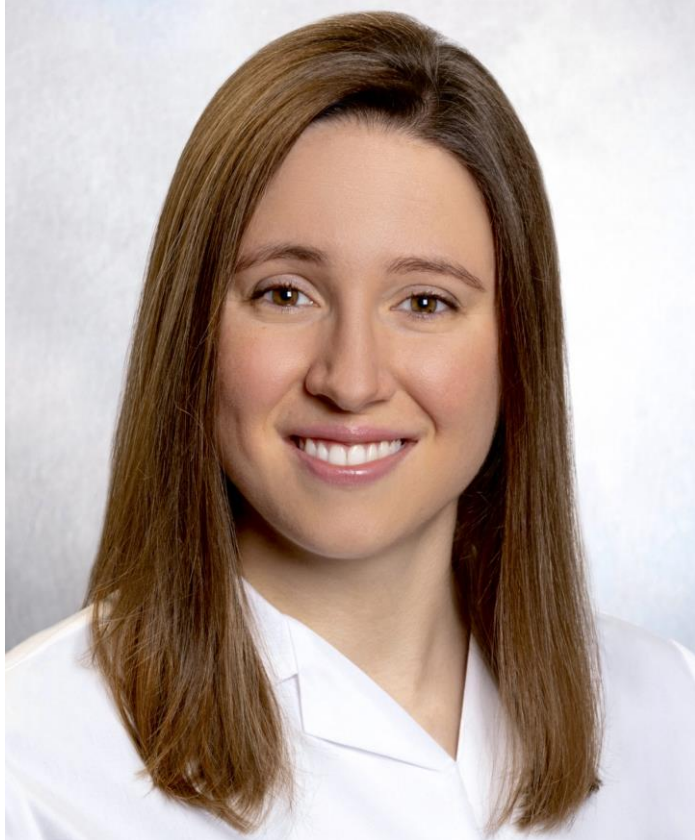


GENETIC TESTING: HOW TO INTEGRATE IT INTO PRACTICE

Ana C. Onuchic-Whitford, MD
Director, Kidney Genetics & PKD Clinic
Division of Renal Medicine, Department of Medicine
Brigham and Women's Hospital
Instructor in Medicine
Harvard Medical School
August 12, 2025

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

Director, Kidney Genetics & PKD Clinic @ BWH

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



Disclosures

Current institutional/foundation research funding from
- ASN KidneyCure Sharon Anderson Research Fellowship

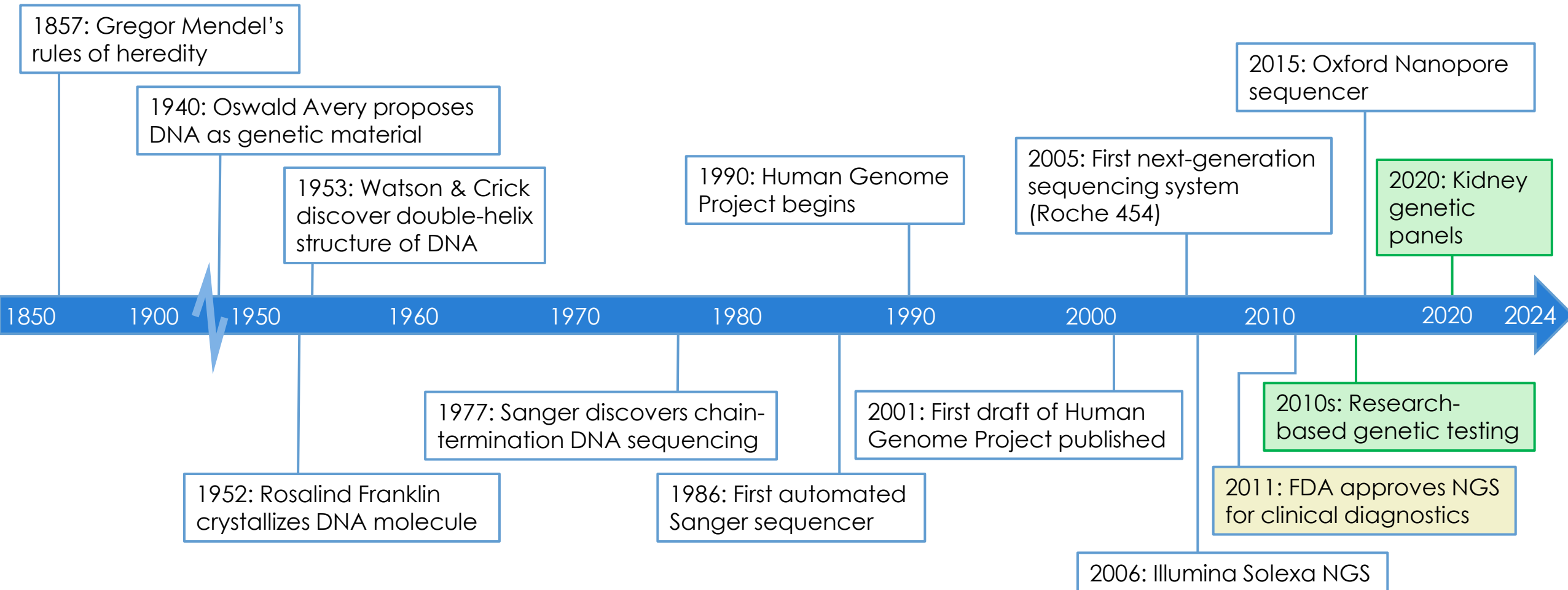
Two key learning objectives

- 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.

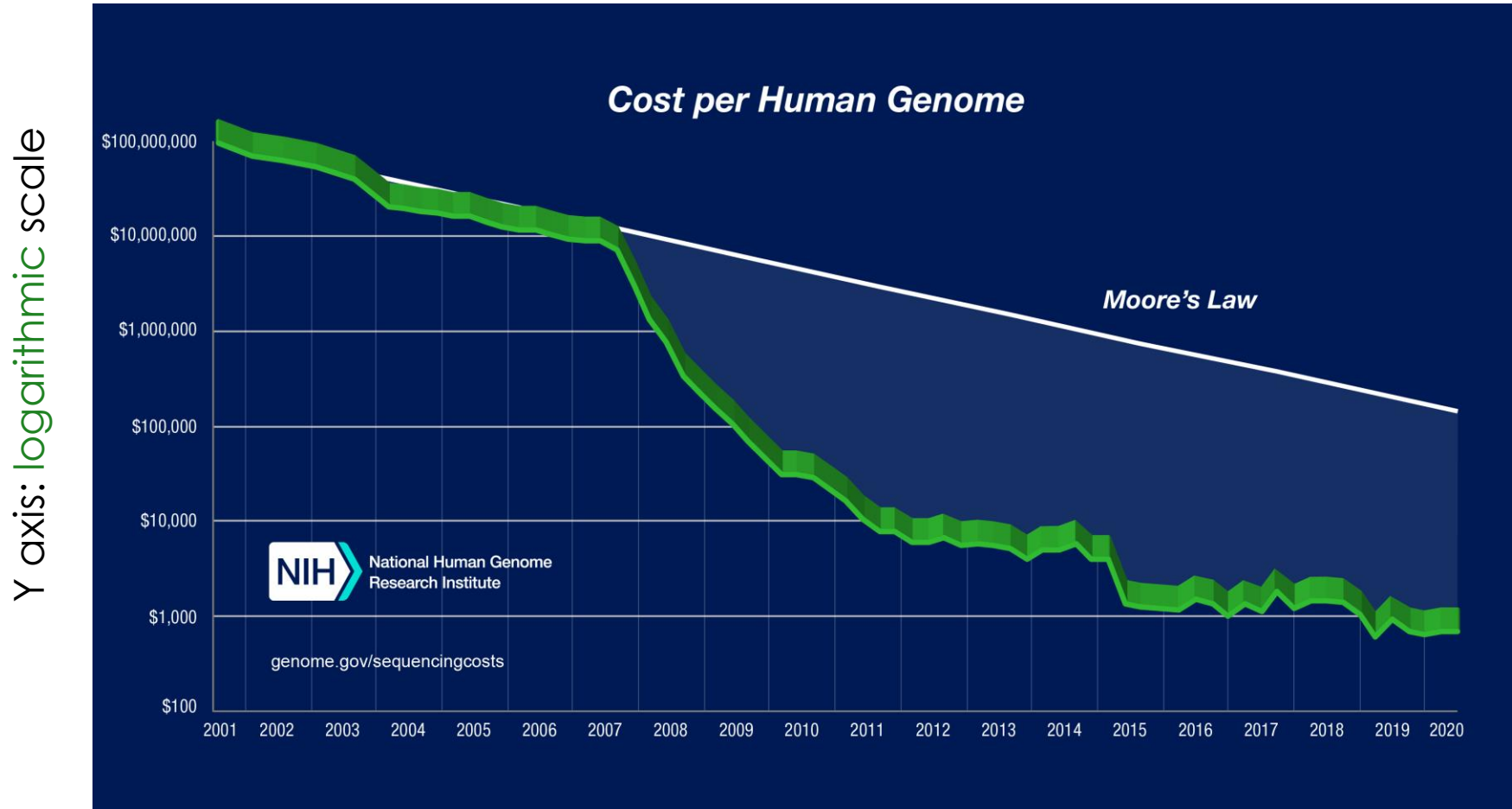
Note: Genetic aspects of the following disorders will be covered in separate lectures during this course, and thus will **not** be the focus of this session.

- 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



Sequencing costs



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

Audience Response Question #1

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

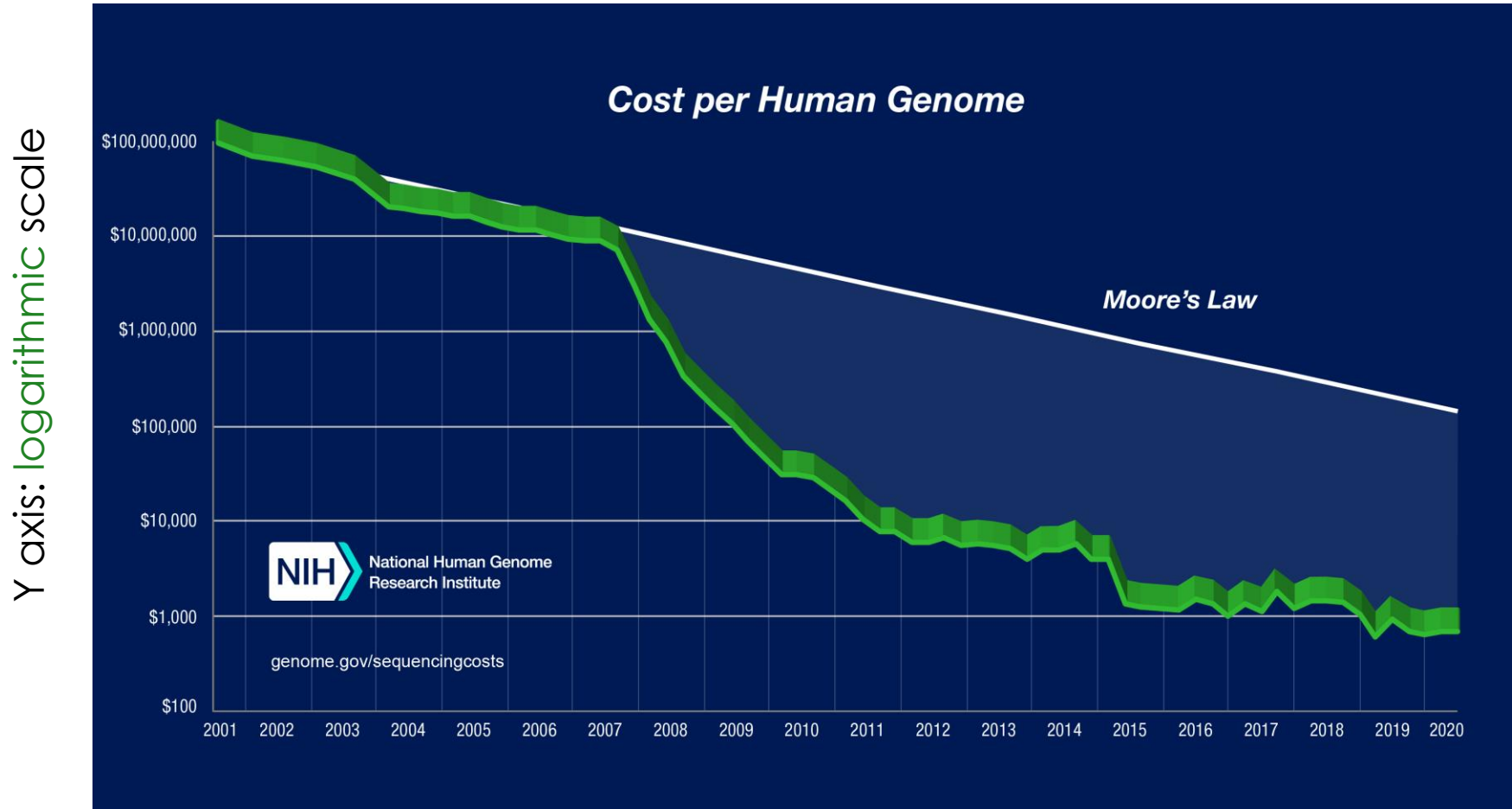
- a) Sequencing centers transitioned from Sanger-based to next-generation sequencing (NGS)
- b) The Genetic Information Nondiscrimination Act of 2008 (GINA) was passed in the United States
- c) The NIH initiated funding for the "All of Us" Research Program
- d) The Food and Drug Administration (FDA)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

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

Sequencing costs



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

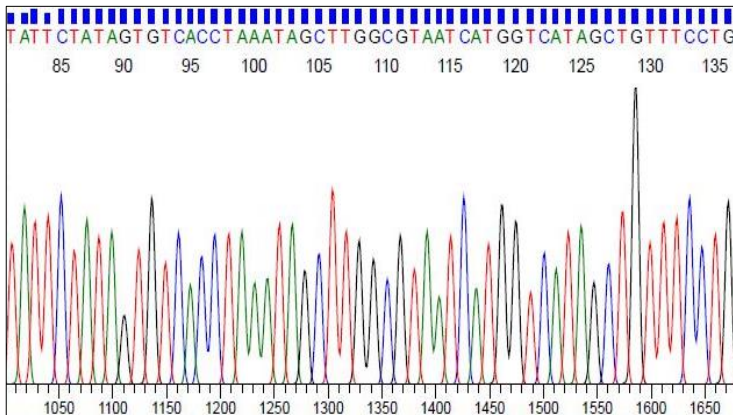
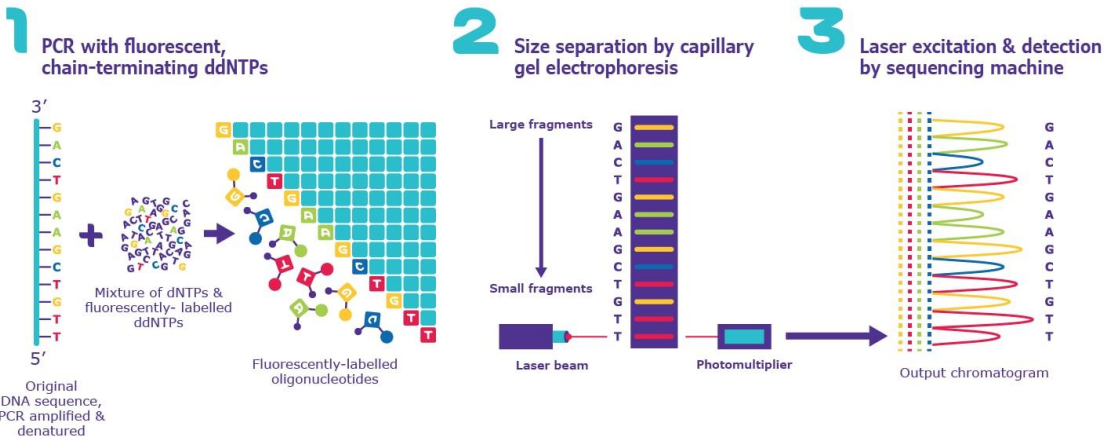
Does not include cost of:

- **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)

Sanger sequencing

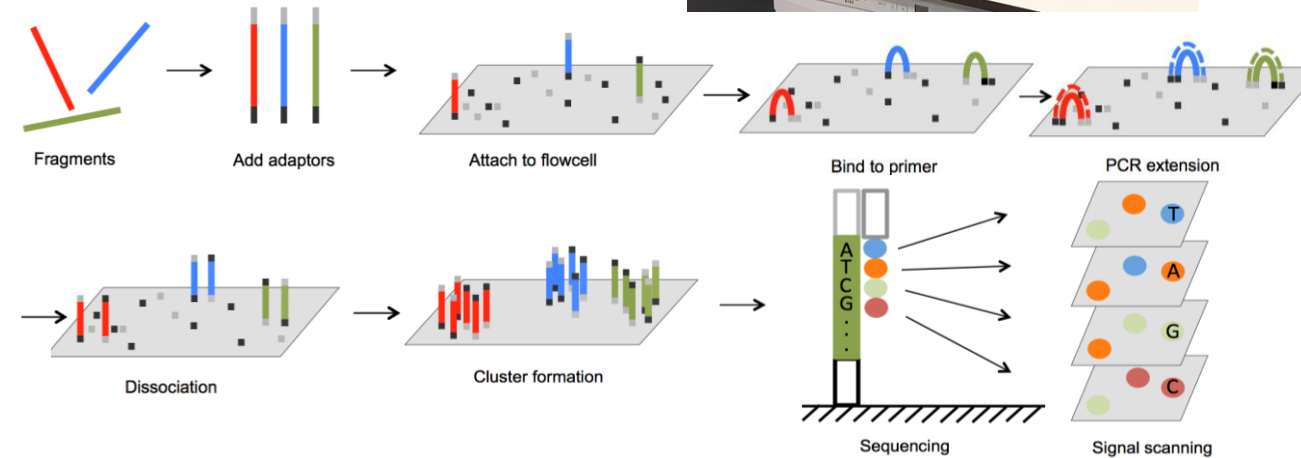
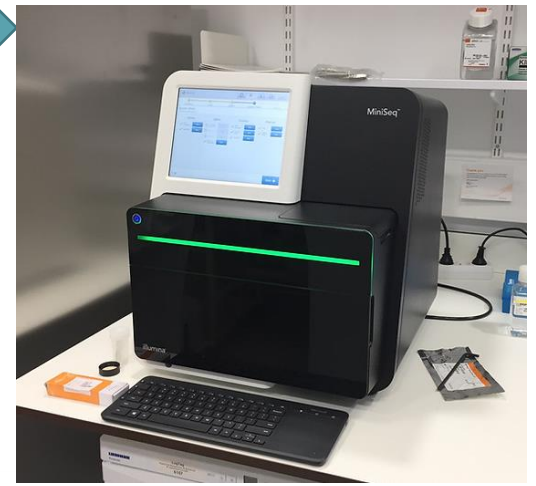
(dideoxy chain termination)



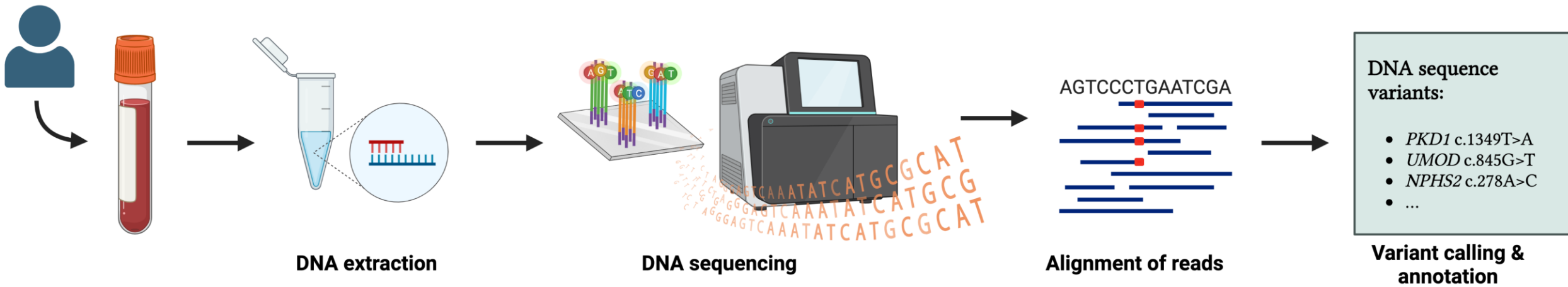
Cost per 1 million base pairs =
~\$500.00

Next-generation sequencing

(massively parallel sequencing)



Cost per 1 million base pairs =
< \$0.50

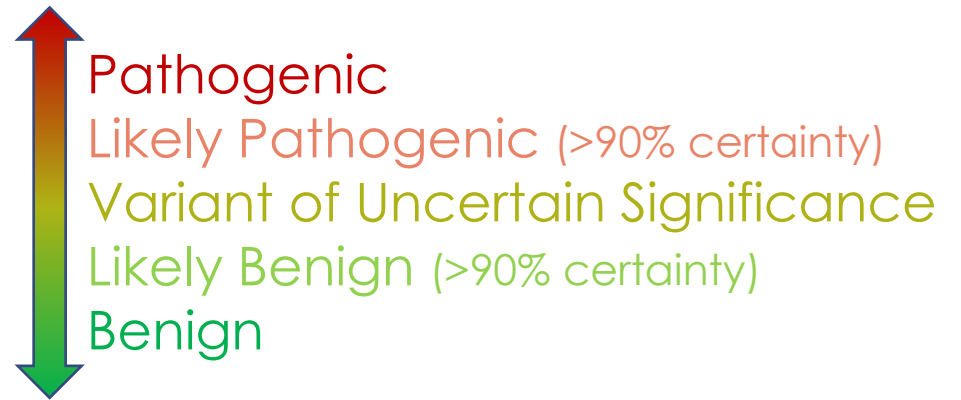


© American College of Medical Genetics and Genomics **ACMG STANDARDS AND GUIDELINES** | **Genetics in Medicine**

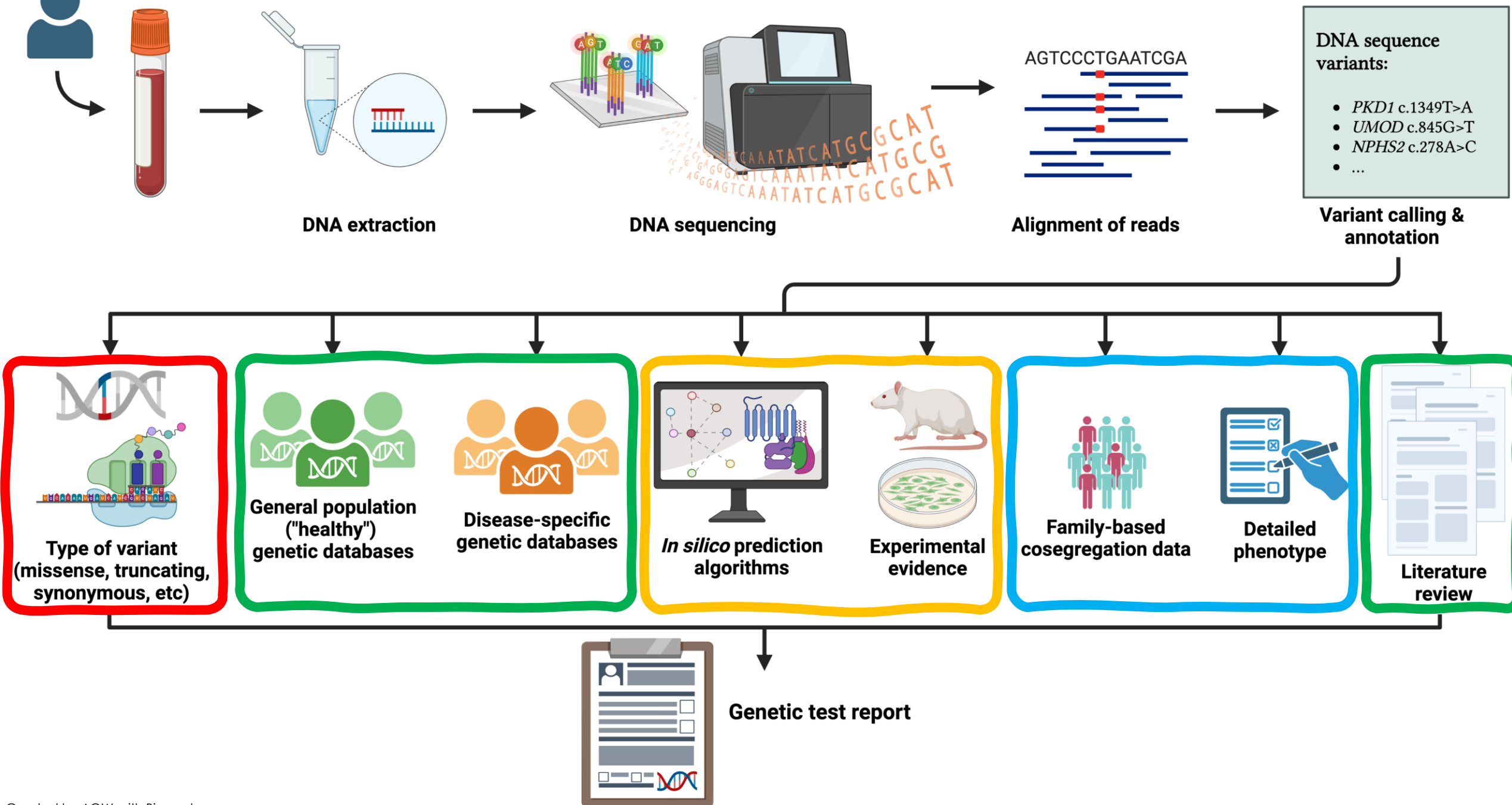
Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD¹, Nazneen Aziz, PhD^{2,16}, Sherri Bale, PhD³, David Bick, MD⁴, Soma Das, PhD⁵, Julie Gastier-Foster, PhD^{6,7,8}, Wayne W. Grody, MD, PhD^{9,10,11}, Madhuri Hegde, PhD¹², Elaine Lyon, PhD¹³, Elaine Spector, PhD¹⁴, Karl Voelkerding, MD¹³ and Heidi L. Rehm, PhD¹⁵; on behalf of the ACMG Laboratory Quality Assurance Committee

Variants classified into 5 categories:



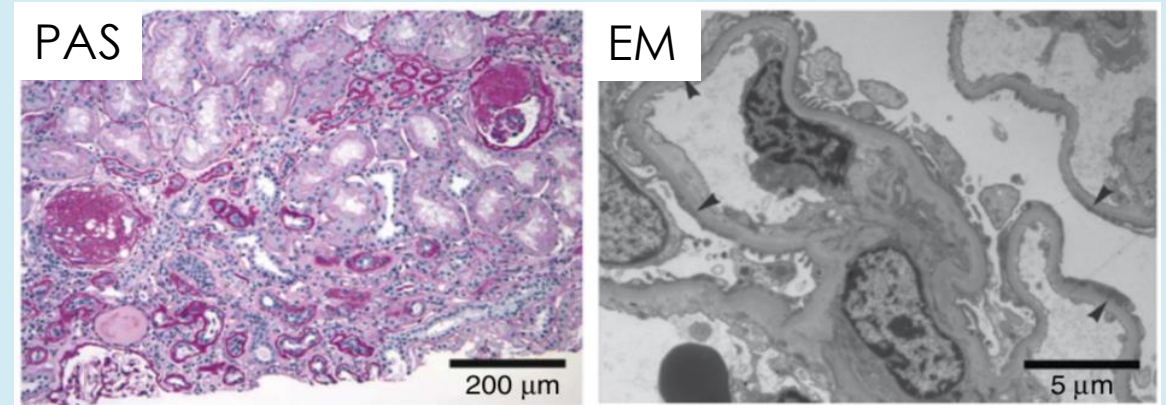
Genetic test report



Audience Response Question #2

26 yo woman with no PMH presents with progressive **lower extremity edema**.
Labs: Cr 1.7mg/dl, eGFR 46ml/min, UA 3+ protein and trace blood, **UPCR 3.7g/g**.
Family history: **mother with ESKD** at age 50, attributed to longstanding HTN.
Renal biopsy is performed.

Is clinical genetic testing indicated?
If so, what is the usually recommended initial strategy?

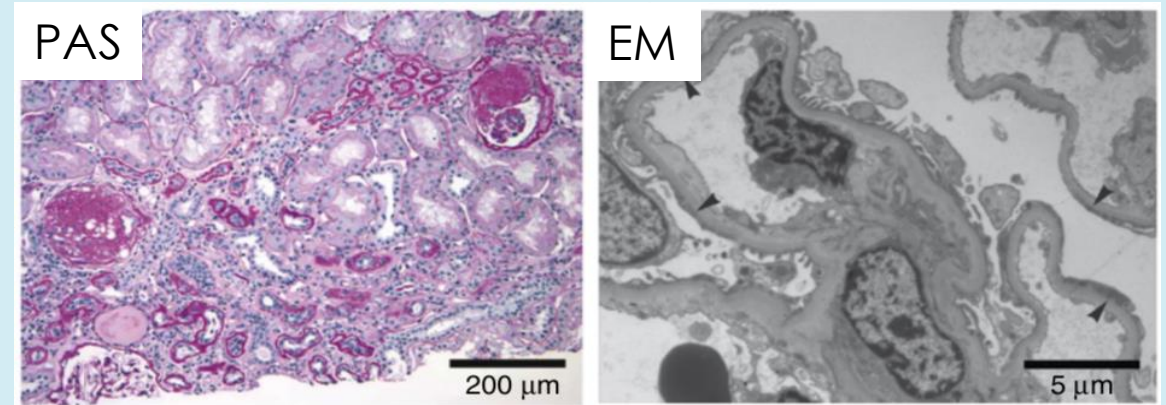


- a) No; there are currently no FDA-approved options for genetic forms of this disease and there would be no change in management
- b) Not at this time; may inform future related kidney transplant donation but would not change immediate management strategy
- c) Yes; sequencing of the *NPHS1*, *NPHS2*,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

Audience Response Question #2

26 yo woman with no PMH presents with progressive **lower extremity edema**.
Labs: Cr 1.7mg/dl, eGFR 46ml/min, UA 3+ protein and trace blood, **UPCR 3.7g/g**.
Family history: **mother with ESKD** at age 50, attributed to longstanding HTN.
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

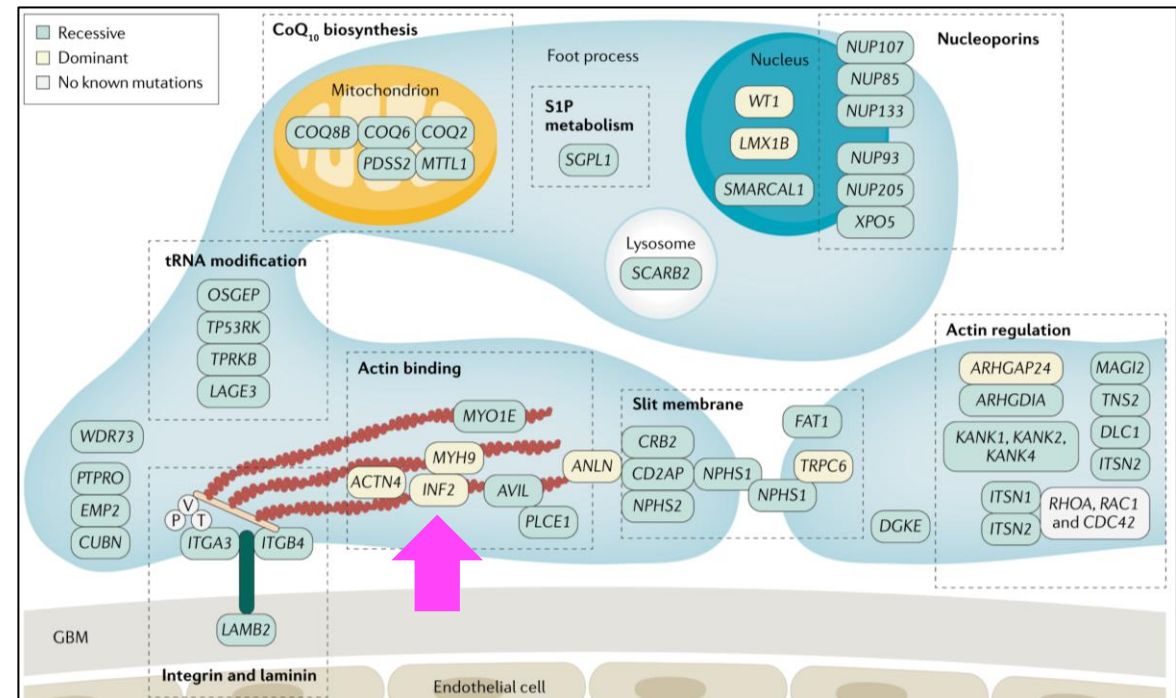
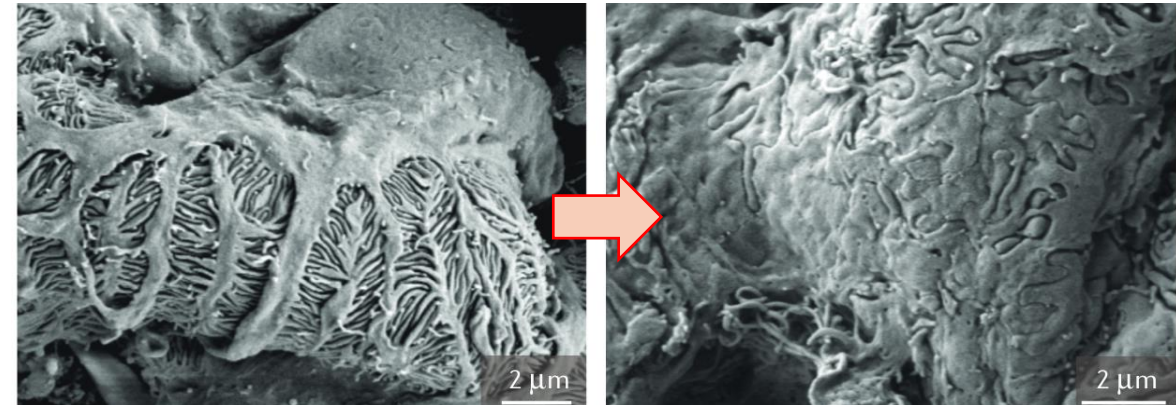
Genetic Nephrotic Syndrome

("Genetic FSGS," Podocytopathies, Steroid-resistant NS)

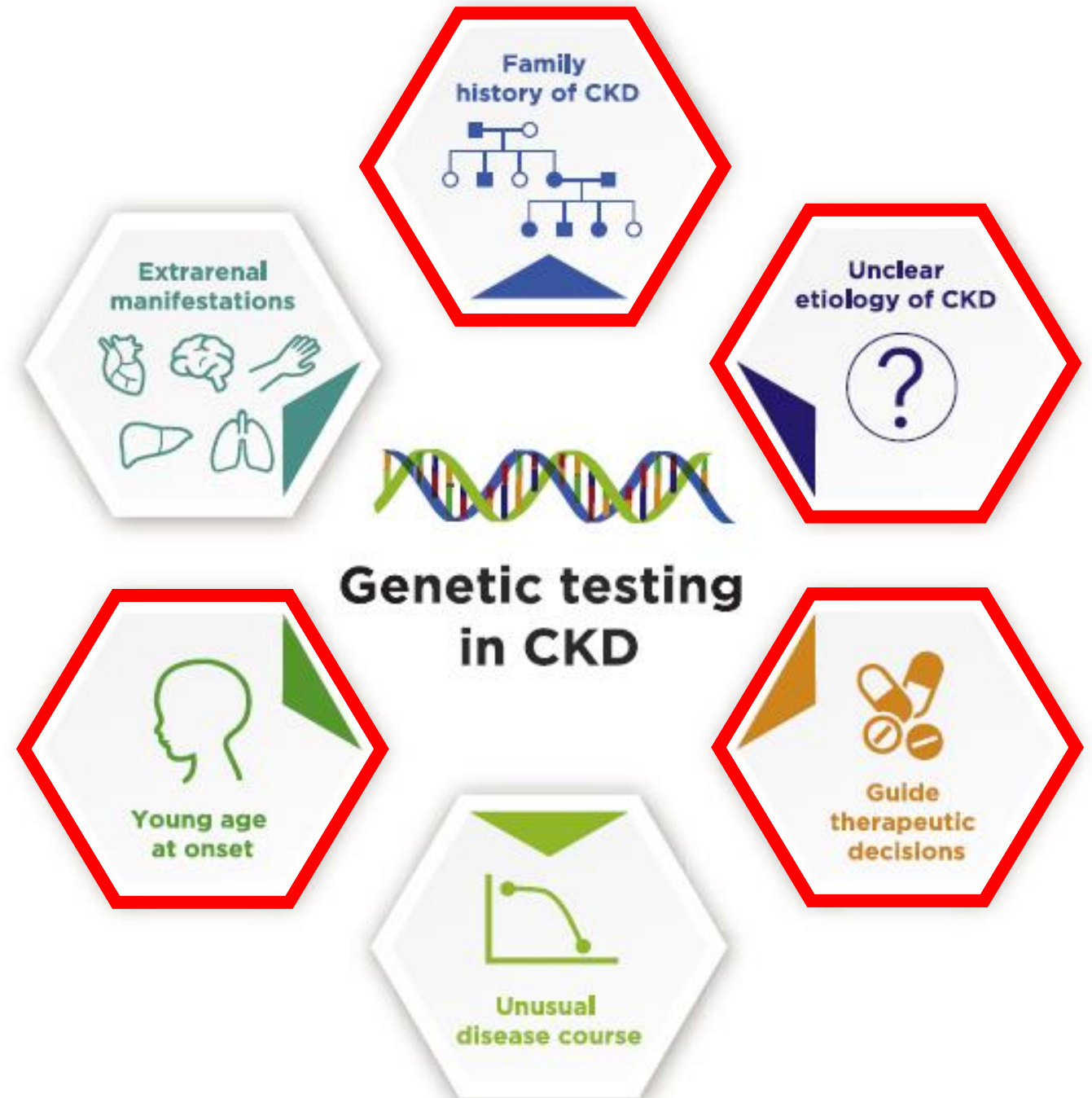
- Mutations in genes encoding proteins that maintain integrity of slit diaphragm, podocyte structure, glomerular filtration barrier
- This leads to glomerular injury, podocyte foot process effacement, proteinuria, sclerosis
- Histologic spectrum:
 - Diffuse mesangial sclerosis
 - Minimal change disease
 - FSGS
 - Collapsing glomerulopathy
- Nonspecific lesions represent patterns of podocyte injury = "Podocytopathies"

Case 1:

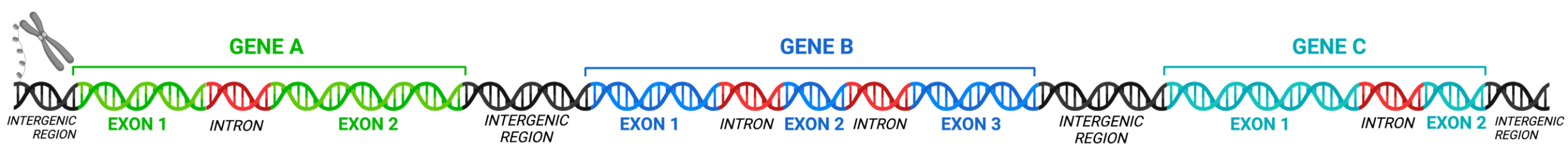
- Gene panel testing: **pathogenic variant in *INF2***
- While awaiting genetic results, treated with immunosuppression, with **no response & many side effects**
- Upon genetic diagnosis, immunosuppression stopped, and **sibling tested for variant prior to kidney donation**



Indications for genetic testing



But what genetic test should be ordered?



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%

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%

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, $p\text{-value}=2.7\times 10^{-13}$).

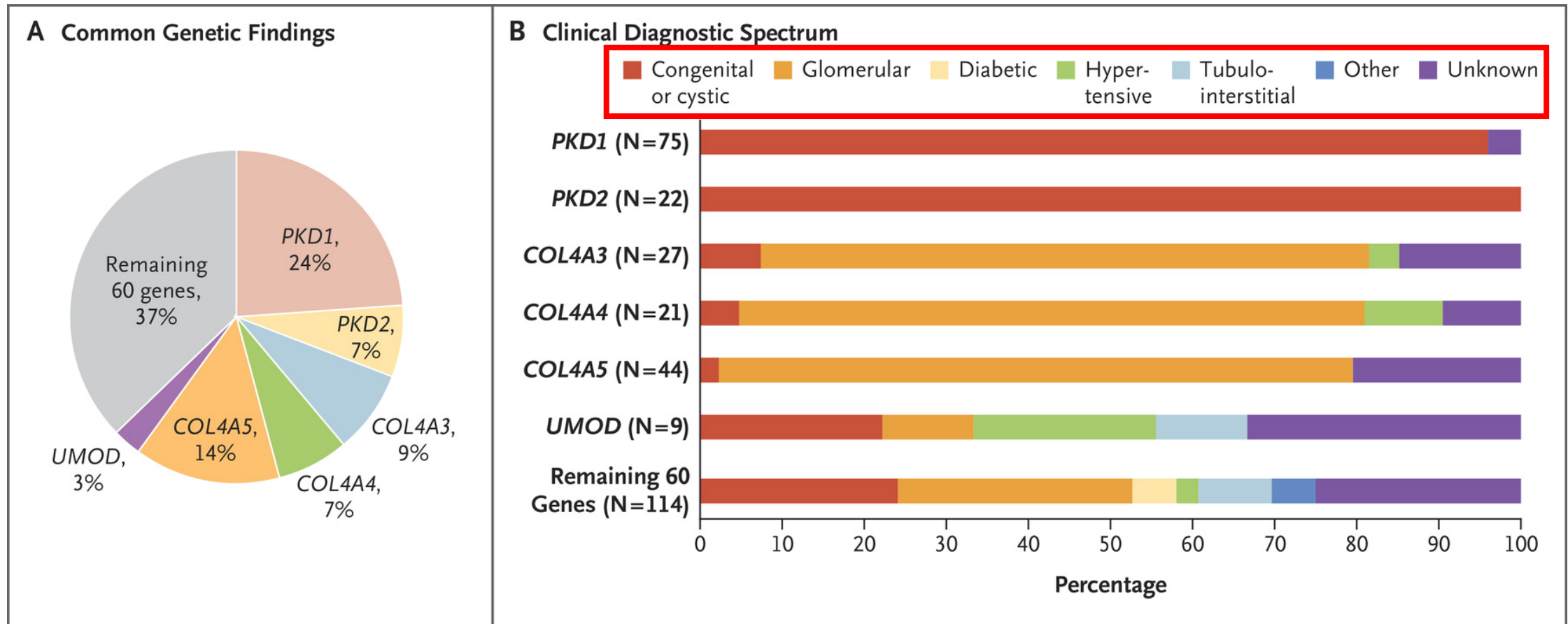


Table 2. Diagnostic Yield and Heterogeneity of Genetic Diagnoses across Clinical Diagnostic Categories.

| Clinical Diagnosis | Sequencing Performed | Diagnostic Variants Present | Diagnostic Yield | Distinct Monogenic Disorders Detected | Singleton Genetic Diagnoses |
|------------------------------------|---------------------------|-----------------------------------|---------------------|--|-----------------------------------|
| | <i>number of patients</i> | | <i>percent</i> | <i>number</i> | |
| 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.

Audience Response Question #4

40yo woman referred for **mild proteinuria** first seen during uncomplicated pregnancy. Six months post-partum, OBGYN noted persistent 1+ protein on urine dipstick.

Family history: **father** heavy smoker with **ESKD at 45yo**, US with few bilateral kidney cysts; his biopsy showed **thin basement membranes and diffuse glomerular sclerosis**. Older **sister had blood in urine** attributed to 2 left kidney cysts. Mother and brother are healthy.

Labs: Cr 1.5 mg/dl, eGFR 55 ml/min. UACR 0.3 g/g. UAs over last 3 years: 2-3+ blood, trace-1+ protein, 10-50 RBCs. Ultrasound normal. Serologic workup is non-diagnostic.

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 and muscle cramps), *COL4A1*
- e) Autosomal Dominant Alport Syndrome, *COL4A3* or *COL4A4*
- f) APOL1-mediated kidney disease, *APOL1*

Audience Response Question #4

40yo woman referred for **mild proteinuria** first seen during uncomplicated pregnancy. Six months post-partum, OBGYN noted persistent 1+ protein on urine dipstick.

Family history: **father** heavy smoker with **ESKD at 45yo**, US with few bilateral kidney cysts; his biopsy showed **thin basement membranes and diffuse glomerular sclerosis**. Older **sister had blood in urine** attributed to 2 left kidney cysts. Mother and brother are healthy.

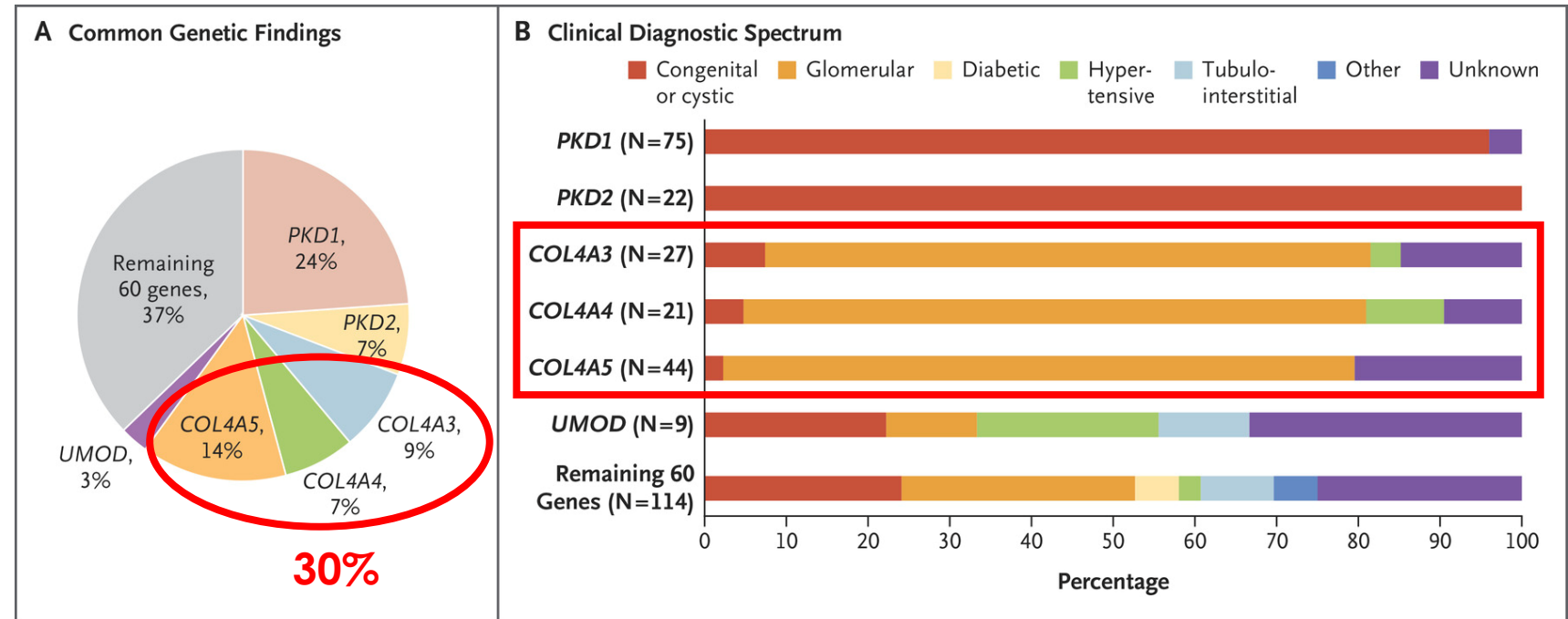
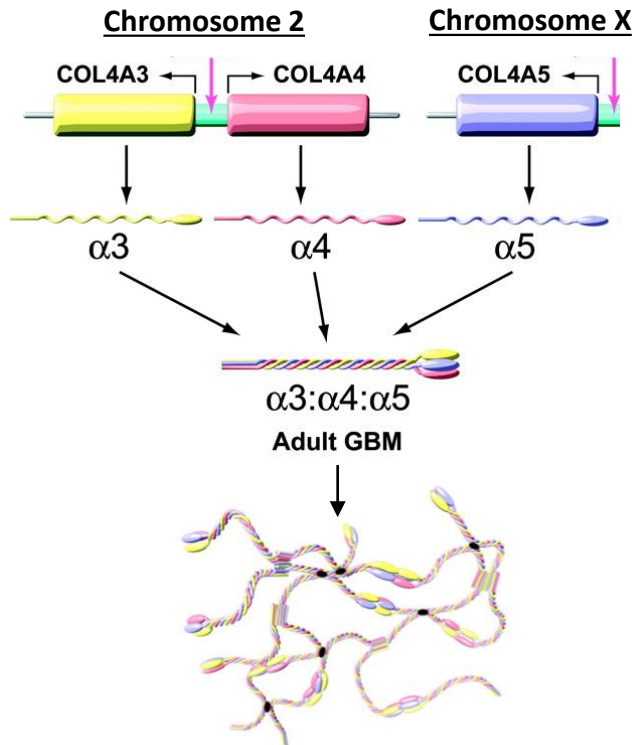
Labs: Cr 1.5 mg/dl, eGFR 55 ml/min. UACR 0.3 g/g. UAs over last 3 years: 2-3+ blood, trace-1+ protein, 10-50 RBCs. Ultrasound normal. Serologic workup is non-diagnostic.

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

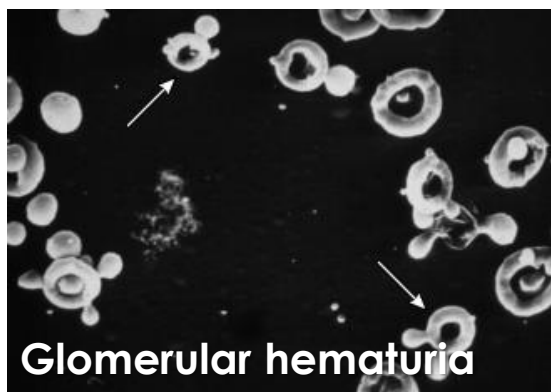
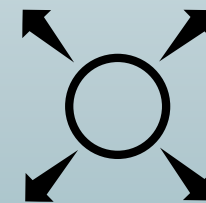
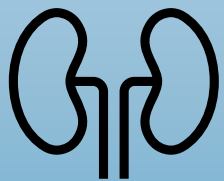
- a) X-Linked Alport Syndrome, COL4A5
- b) Autosomal Dominant Polycystic Kidney Disease, IFT140
- c) Autosomal Recessive Alport Syndrome, COL4A3 or COL4A4
- d) HANAC (hereditary angiopathy with nephropathy, aneurysms and muscle cramps), COL4A1
- e) Autosomal Dominant Alport Syndrome, COL4A3 or COL4A4**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

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

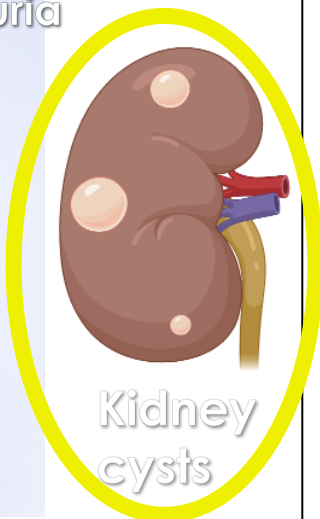


Alport Syndrome: Clinical Presentation



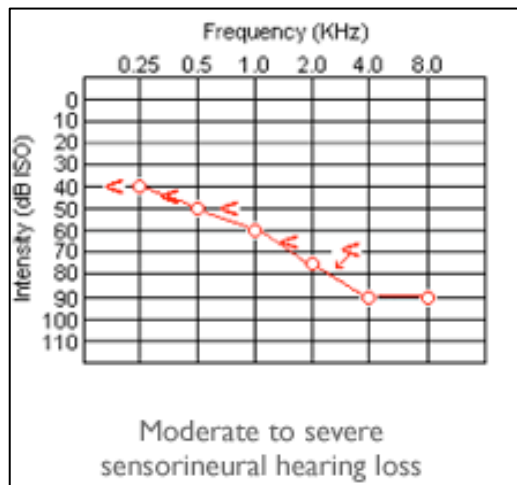
Glomerular hematuria

Gross hematuria
Albuminuria
Renal failure



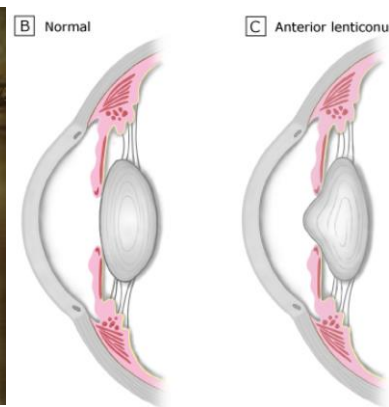
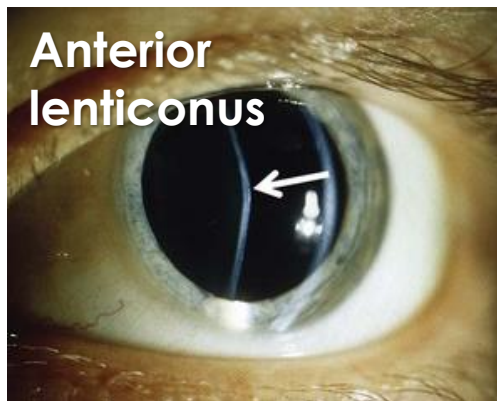
Kidney cysts

Sensorineural hearing loss

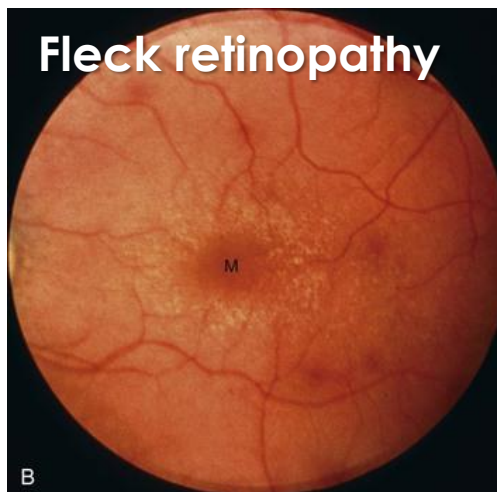


| | CONDUCTION | |
|-------|------------|------|
| | AIR | BONE |
| RIGHT | O | < |
| LEFT | X | > |

Anterior lenticonus



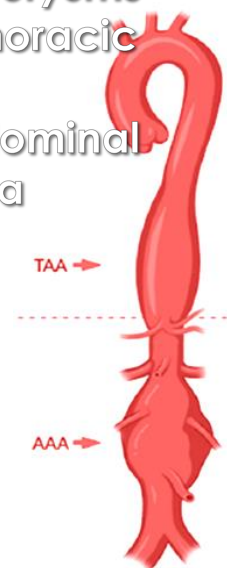
Fleck retinopathy



Corneal erosion



Aneurysms of thoracic & abdominal aorta



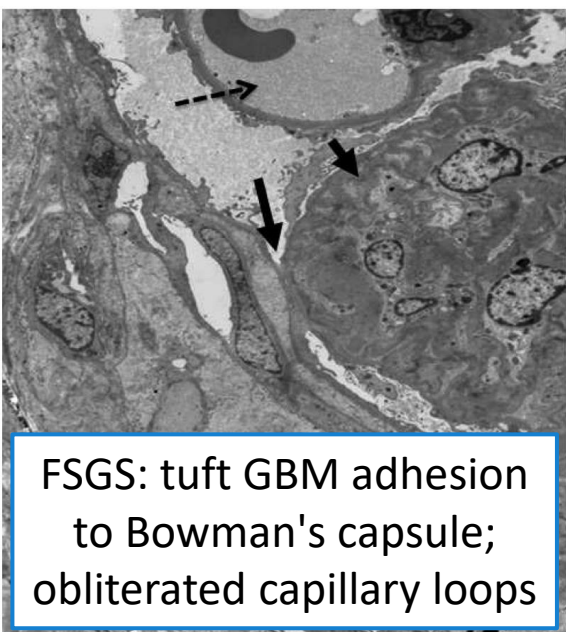
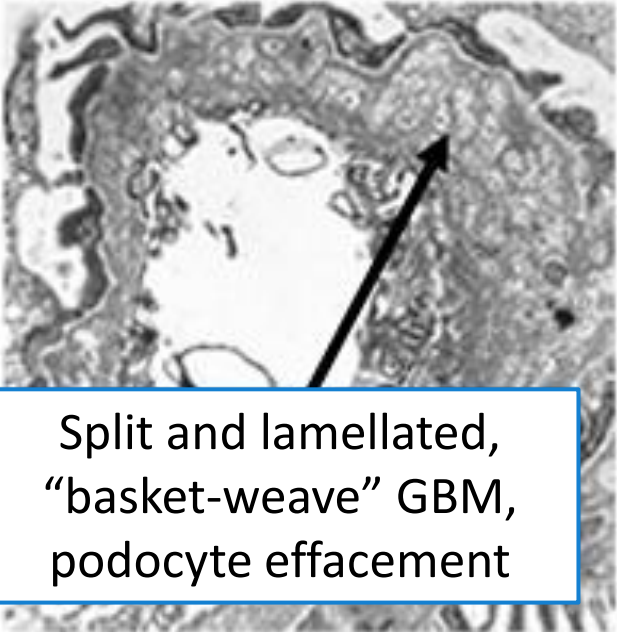
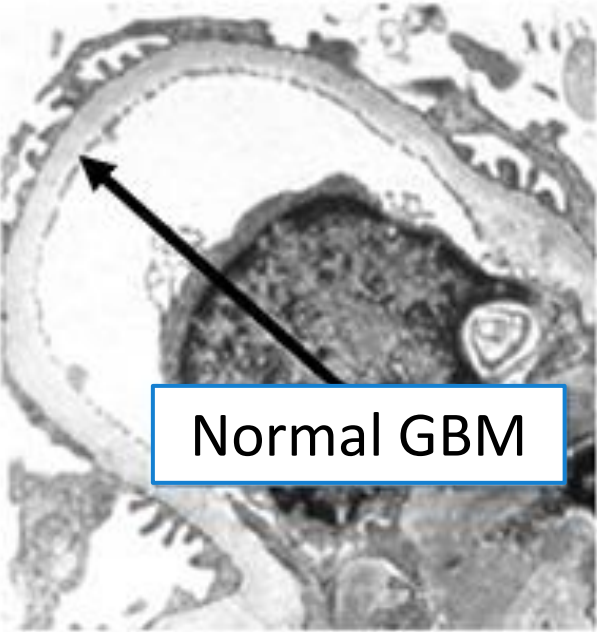
(rare) Leiomyomatosis

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



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)

| 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 | <u>2 of 3 genes:</u> 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+) |

Audience Response Question #5

The prior patient has kidney genetic panel testing, which reveals a heterozygous, pathogenic variant in *COL4A4*. She is diagnosed with Autosomal Dominant Alport Syndrome.

This genetic panel 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

Audience Response Question #5

The prior patient has kidney genetic panel testing, which reveals a heterozygous, pathogenic variant in *COL4A4*. She is diagnosed with Autosomal Dominant Alport Syndrome.

This genetic panel 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

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, potential clinical relevance can be assessed through expert review, in the context of variant characteristics, clinical features and family history
- If the VUS is in a gene consistent with patient's disease, family testing can sometimes help re-classify the variant as pathogenic
 - 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 thorough expert review and/or scientific evidence determine there are clinical consequences of this 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.

Alport Syndrome: Management

- **Angiotensin blockade (ACEi, e.g. ramipril/lisinopril; ARB)**
 - Reduces proteinuria and slows glomerulosclerosis and CKD progression
 - Initiate when there is **microalbuminuria (UACR ≥ 30 mg/g)**, or at time of diagnosis (table)
 - If UPCR persistently high (e.g. > 1 g/g despite maximal RAASi), additional measures to suppress proteinuria are reasonable, if tolerated (e.g. add **aldosterone antagonist** – discuss risks)
 - SGLT2i currently not in AS guidelines and being studied, but some experts suggest consideration in persistent proteinuria
- **Renal transplantation** is the preferred option for ESKD
 - Carefully evaluate **living related donors**
 - Risk (low ~3%) of **anti-GBM** antibody-mediated glomerulonephritis in allograft (in ARAS and in males with XLAS)
- **Audiologic evaluation:** 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 mutations 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 <i>COL4A3</i> or <i>COL4A4</i>) | Microalbuminuria |

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

Audience Response Question #6

65 yo man with PMH of HTN, HLD, gout and slowly progressive CKD4.

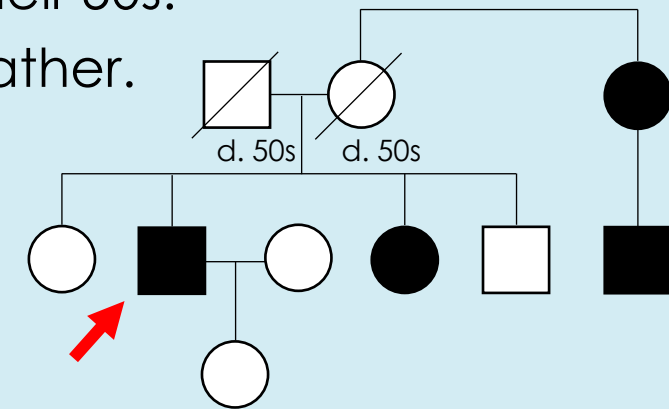
Ultrasound: 9cm kidneys, 3 medullary cysts and increased echogenicity. UA no blood, protein, cells or casts. Biopsy: 70% interstitial fibrosis and tubular atrophy. Serologic evaluation negative.

Family history: sister just diagnosed with CKD, maternal aunt with ESKD in 70s, maternal male cousin with kidney problems in 60s. Patient's parents died in their 50s.

His 35yo daughter is healthy and eager to donate a kidney to her father.

What is the most likely clinical diagnosis?

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



Audience Response Question #6

65 yo man with PMH of HTN, HLD, gout and slowly progressive CKD4.

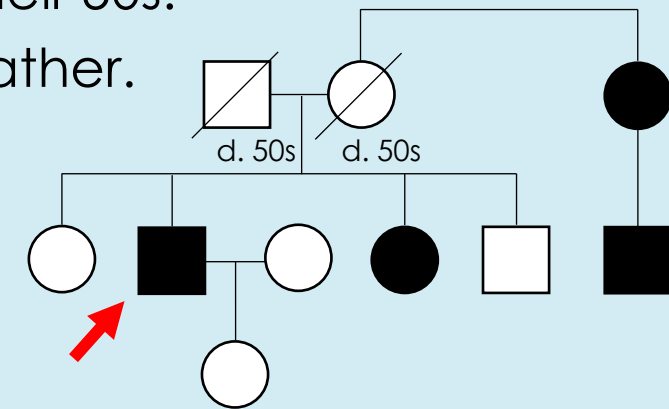
Ultrasound: 9cm kidneys, 3 medullary cysts and increased echogenicity. UA no blood, protein, cells or casts. Biopsy: 70% interstitial fibrosis and tubular atrophy. Serologic evaluation negative.

Family history: sister just diagnosed with CKD, maternal aunt with ESKD in 70s, maternal male cousin with kidney problems in 60s. Patient's parents died in their 50s.

His 35yo daughter is healthy and eager to donate a kidney to her father.

What is the most likely clinical diagnosis?

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



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.

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)

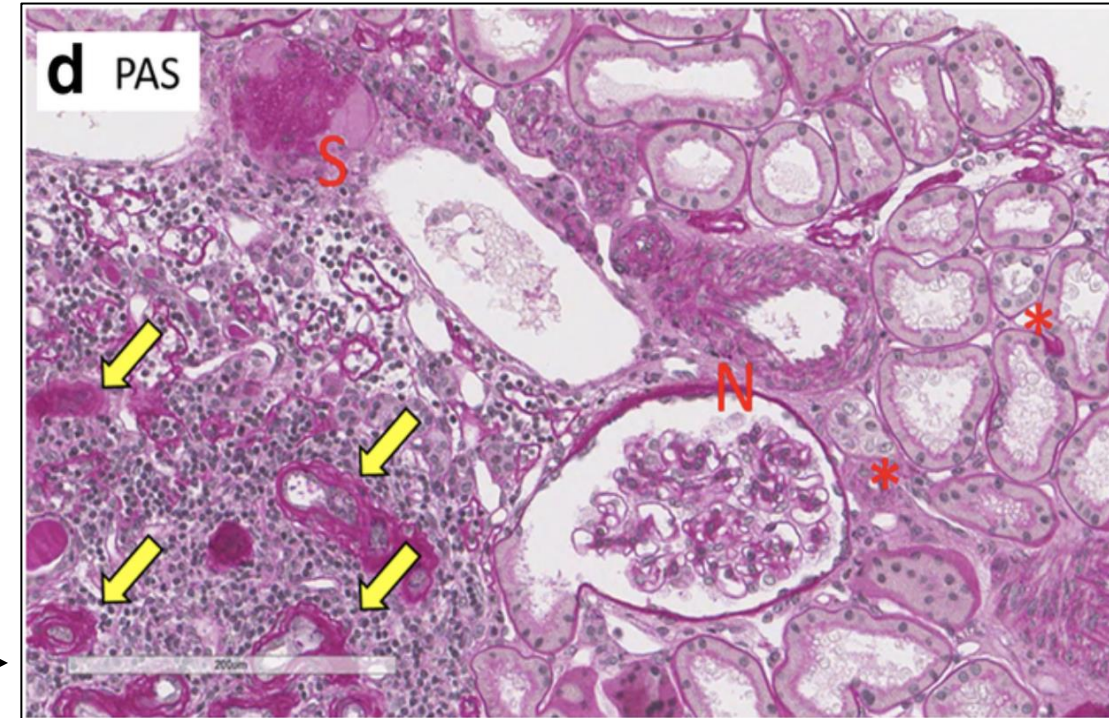


Table 3 | Usual findings on renal histology in patients with ADTKD

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

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 | |

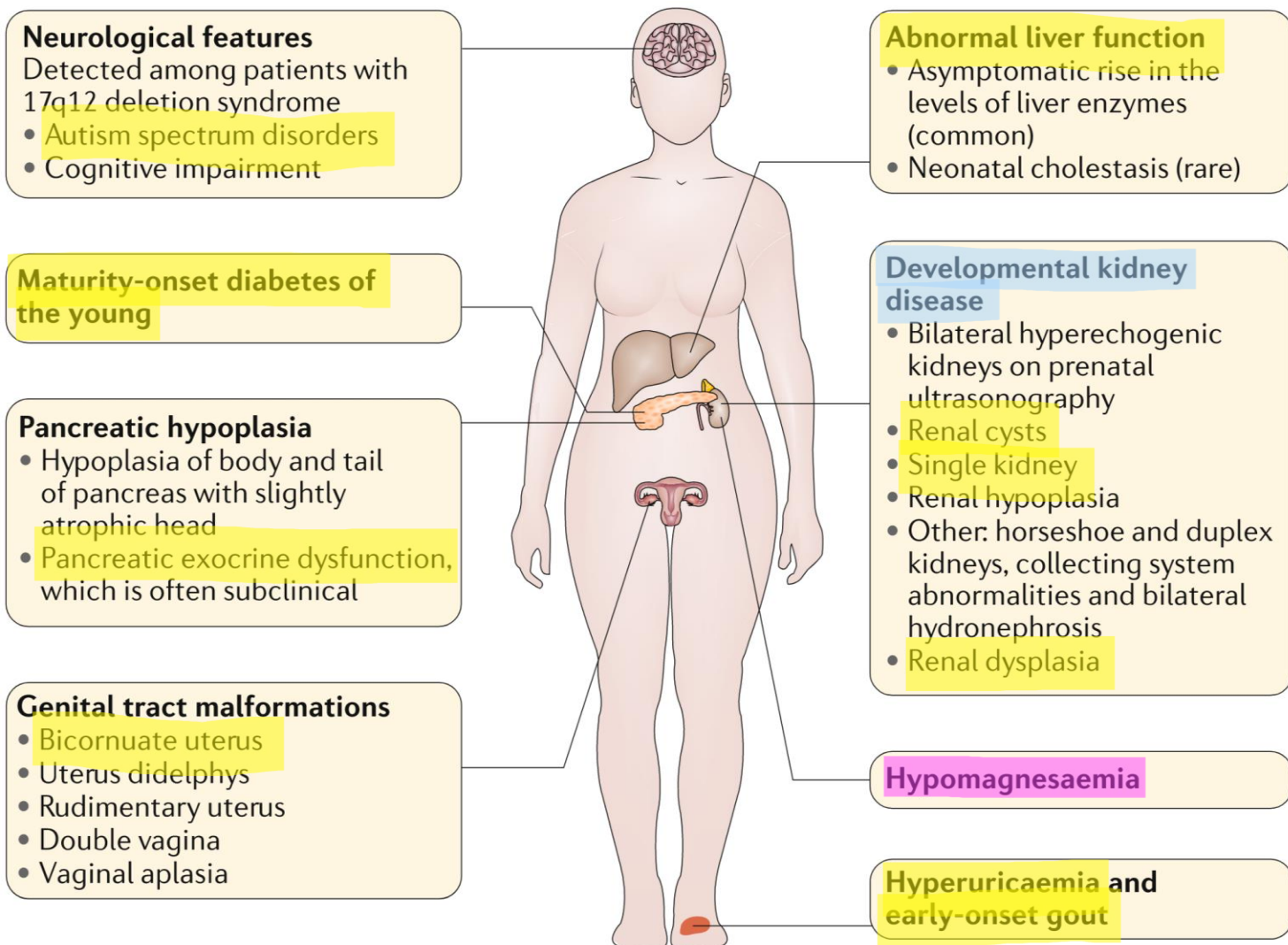
Abbreviations: AKI, acute kidney injury; HNF1B, hepatocyte nuclear factor 1β; MODY5, maturity onset diabetes mellitus of the young type 5; MUC1, mucin-1; MUC1-fs, mucin-1 frameshift protein; REN, renin; TAL, thick ascending limb of Henle's loop; UMOD, uromodulin.

^aThis 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.

HNF1B-related disease

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



Audience Response Question #7

The prior patient has genetic panel testing which reveals a heterozygous pathogenic variant in *UMOD*.

His daughter is tested and does not have this variant. She proceeds with kidney donation to her father, and both are doing well several years later.

All the below are potential benefits of genetic testing for kidney disease, EXCEPT FOR:

- 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

Audience Response Question #7

The prior patient has genetic panel testing which reveals a heterozygous pathogenic variant in *UMOD*.

His daughter is tested and does not have this variant. She proceeds with kidney donation to her father, and both are doing well several years later.

All the below are potential benefits of genetic testing for kidney disease, EXCEPT FOR:

- 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

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

- **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 the entire HNF1B gene** (part of chromosomal 17q12 deletion)
 - Very repetitive DNA regions
 - Example: **MUC1** (variable number tandem repeat sequences)

Audience Response Question #8

50yo man is referred to Nephrology from his out-of-state Ophthalmologist. ROS:

- Frequent urination, drinks a lot of water
- Intermittent pain in hands and legs since childhood, on B12 and gabapentin
- Recurring abdominal pain, nausea, diarrhea and cold intolerance since teenager
- Was in a car accident and couldn't move his right arm for an hour

Family history: mother died in her 40s after heart attack; daughter had protein in urine during pregnancy.

Physical exam: below. Labs: Cr 1.4, eGFR 57, electrolytes/TSH normal, UPCR 1.3g/g.

Ultrasound: renal sinus cysts.

What is the most likely diagnosis and gene involved?

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



Audience Response Question #8

50yo man is referred to Nephrology from his out-of-state Ophthalmologist. ROS:

- Frequent urination, drinks a lot of water
- Intermittent pain in hands and legs since childhood, on B12 and gabapentin
- Recurring abdominal pain, nausea, diarrhea and cold intolerance since teenager
- Was in a car accident and couldn't move his right arm for an hour

Family history: mother died in her 40s after heart attack; daughter had protein in urine during pregnancy.

Physical exam: below. Labs: Cr 1.4, eGFR 57, electrolytes/TSH normal, UPCR 1.3g/g.

Ultrasound: renal sinus cysts.

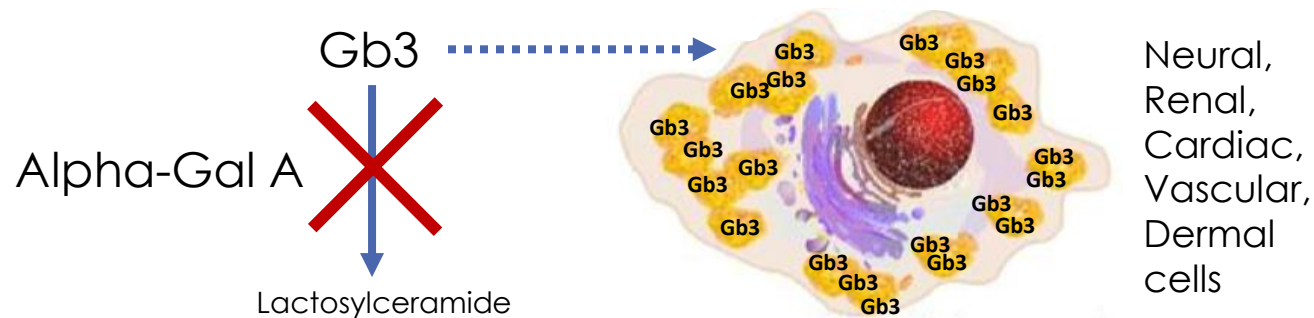
What is the most likely diagnosis and gene involved?

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



Fabry Disease

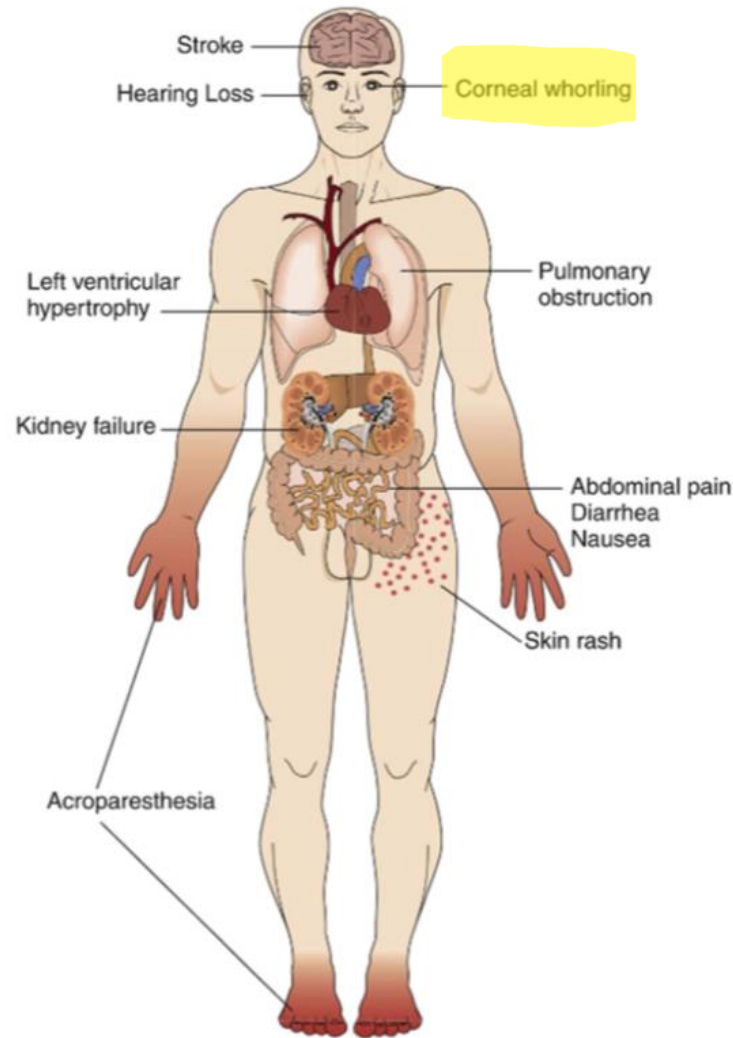
- Most prevalent **lysosomal storage disorder**
- **Deficiency of alpha-galactosidase A** (alpha-Gal A) → cleaves globotriaosylceramide (**Gb3**)
 - Gb3 accumulates in multiple cells, tissues
- Disease is caused by mutations in **GLA** (encodes alpha-Gal A)
 - GLA gene is located on the X chromosome → **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



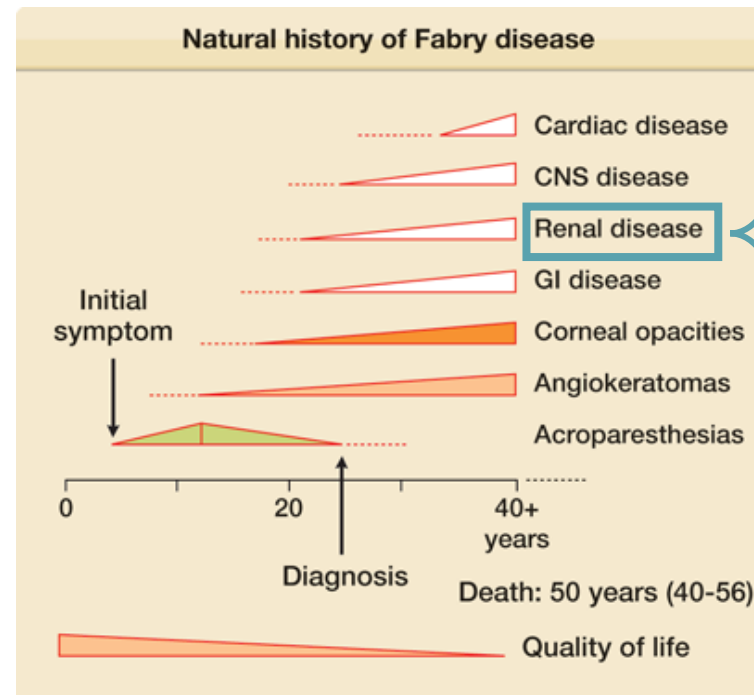
TREATMENT

- **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

Fabry Disease: Clinical Presentation



(CLASSIC FORM)



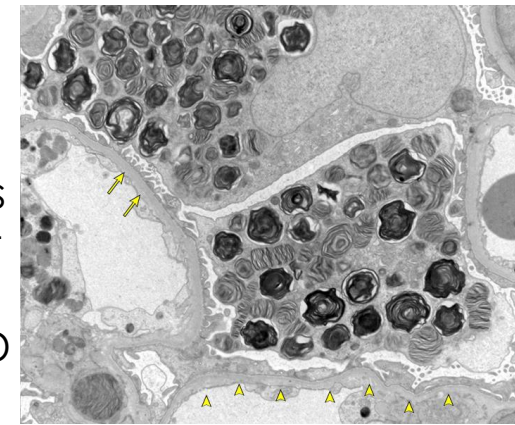
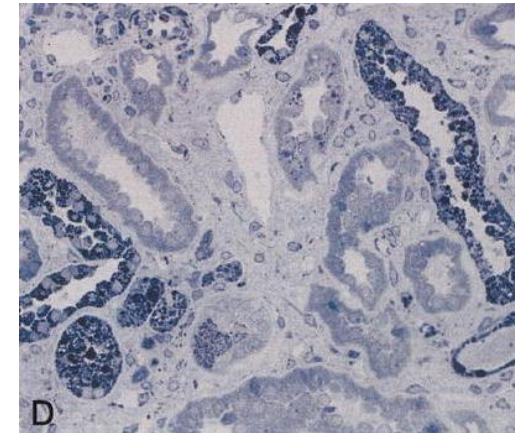
Source: Goldsmith LA, Katz SI, Gilchrist BA, Paller AS, Leffell DJ, Wolff K: *Fitzpatrick's Dermatology in General Medicine, 8th Edition*: www.accessmedicine.com
Copyright © The McGraw-Hill Companies, Inc. All rights reserved.

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**



Audience Response Question #9

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

| Gene | Condition | Inheritance | Variant | Zygosity | Classification |
|------------|---|--------------------|-------------------------|--------------|----------------|
| <i>RET</i> | 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) Contact the genetic testing company to notify them of inadvertent inclusion of a cancer gene in a kidney disease panel.
- b) Call patient and explain that this is not clinically actionable, as it is an incidental finding.
- c) Call patient and provide reassurance, as they are carriers for this variant and thus are not expected to have clinical disease, but could pass the variant to a child.
- d) Refer the patient to Cancer Genetics and Endocrinology for time-sensitive consultation.
- e) Call patient's children and grandchildren and offer genetic cascade testing through the clinical laboratory.

Audience Response Question #9

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

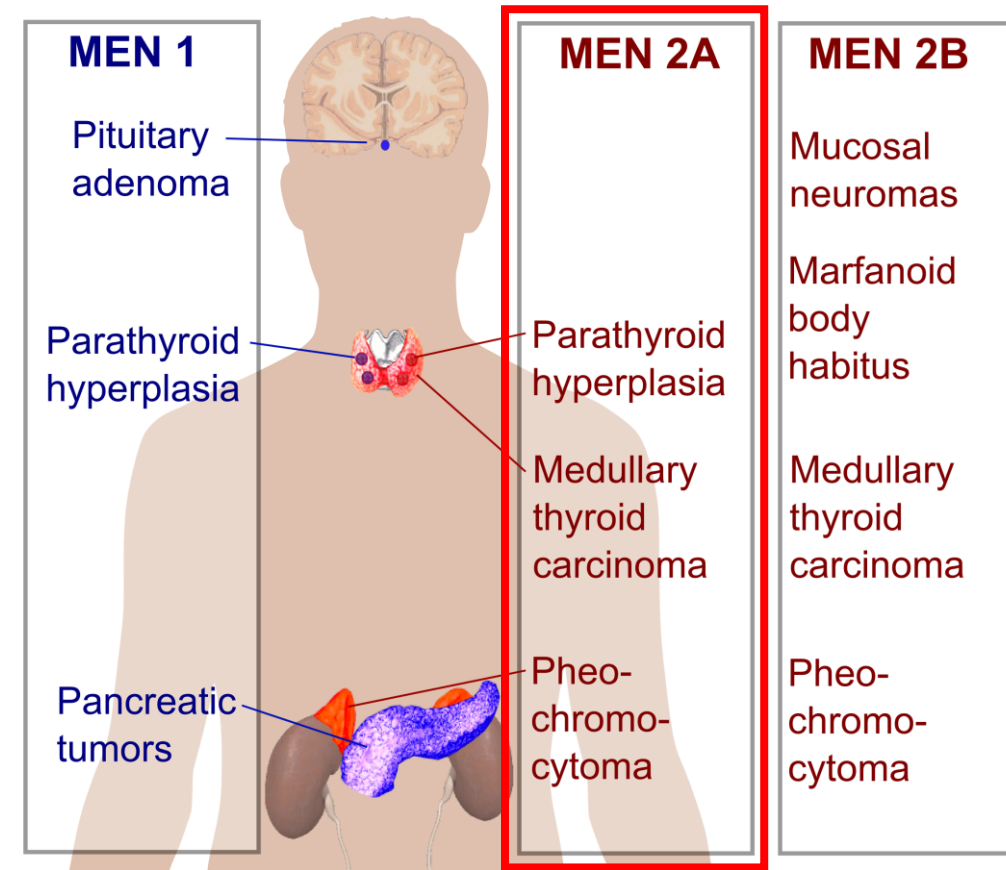
| Gene | Condition | Inheritance | Variant | Zygosity | Classification |
|------------|---|--------------------|-------------------------|--------------|----------------|
| <i>RET</i> | MEN2A, Familial Medullary Thyroid Carcinoma | Autosomal Dominant | c.2671T>G (p.Ser891Ala) | Heterozygous | Pathogenic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

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

- a) Contact the genetic testing company to notify them of inadvertent inclusion of a cancer gene in a kidney disease panel.
- b) Call patient and explain that this is not clinically actionable, as it is an incidental finding.
- c) Call patient and provide reassurance, as they are carriers for this variant and thus are not expected to have clinical disease, but could pass the variant to a child.
- d) Refer the patient to Cancer Genetics and Endocrinology for time-sensitive consultation.**
- e) Call patient's children and grandchildren and offer genetic cascade testing through the clinical laboratory.

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
Calcitonin high, Thyroid US: nodule, PTH >1000, Ca 11, PET-CT: no metastases
Thyroidectomy: pathology showed MTC. Genetic screening for family members

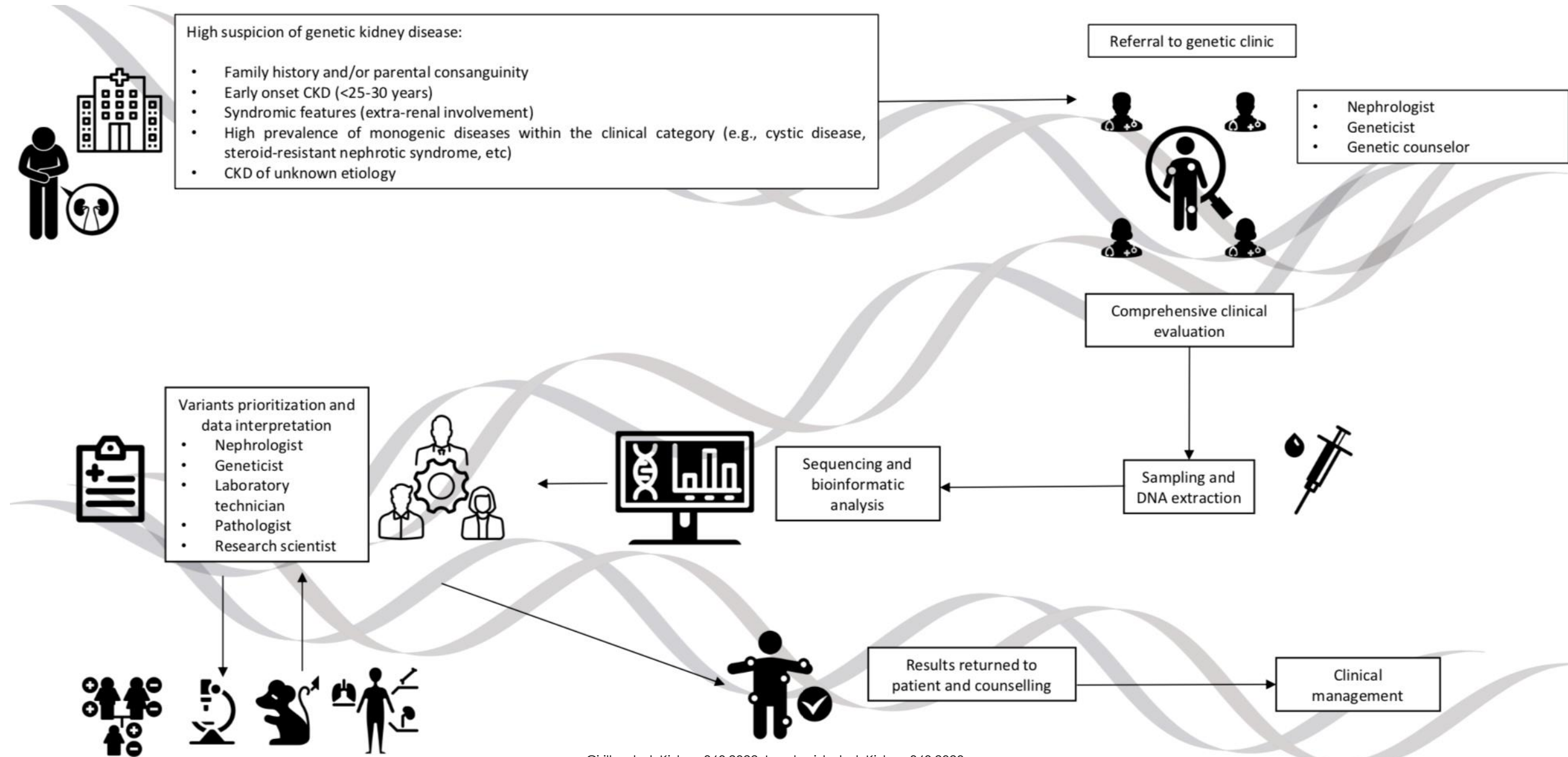
Pre-test genetic counseling

| | |
|---------------------------|--|
| POSITIVE RESULTS | <ul style="list-style-type: none"> • PATHOGENIC and LIKELY PATHOGENIC variants, compatible with mode of inheritance of disorder <ul style="list-style-type: none"> • “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 <ul style="list-style-type: none"> • 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 <ul style="list-style-type: none"> • However, individuals may also have similar issues with positive family history of genetic disease • ACA (Affordable Care Act): health insurance can't refuse coverage or increase cost because of a “pre-existing condition” (this includes clinical genetic disease) |
| NEGATIVE RESULTS | <ul style="list-style-type: none"> • No (likely) pathogenic variants to explain clinical disease • Does not rule out genetic cause • 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 | <ul style="list-style-type: none"> • Variants of uncertain significance (VUS) <ul style="list-style-type: none"> • New clinical/scientific evidence may lead to possible reclassification in the future • Pathogenic variants for non-clinically manifest and/or non fully-penetrant disease <ul style="list-style-type: none"> • e.g. high-risk APOL1 genotype • e.g. asymptomatic young adult with variant causal of late-onset disease of variable severity |
| INCIDENTAL RESULTS | <ul style="list-style-type: none"> • Unexpected genetic findings <ul style="list-style-type: none"> • 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) |



**Written, signed
informed consent**

Incorporating genetic testing into the renal clinic



Take Home Points

- With decreasing cost, increasing availability, and scientific discoveries, genetic testing is increasingly being used in clinical nephrology. Expanding genomic literacy among nephrologists is critical for its successful implementation.
- The most commonly used DNA sequencing method, next-generation 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, and have been recommended as a first-line test for molecular diagnosis of genetic kidney disease.
- Prevalence of genetic diagnoses in adult CKD is significant and increasing, as more patients have access to genetic testing and additional disease-causing genes and variants are discovered.
- Diagnostic yield of genetic testing is higher in patients with congenital or cystic disease, CKD of unknown etiology, family history of kidney disease, and young age of onset. However, 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.
- Patients should receive comprehensive genetic counseling before and after genetic testing is performed. Some states/countries require written informed consent to be obtained.
- Partnering with genetic counselors and creating multidisciplinary genetics-focused teams can increase access to genetic testing, facilitate cascade family testing if indicated, 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!