HELLENIC SOCIETY OF NEPHROLOGY
MEETING & SEMINAR
Combined with:
18th BANTAO CONGRESS
October 19-22, 2023
Makedonia Palace Hotel THESSALONIKI, GREECE

Genetic testing in CKD
when, how and why

Kostas Stylianou
Assistant Professor of Nephrology
University of Crete
Head of the Nephrology Department
University Hospital of Heraklion

Kidney Genes

- The human genome contains 3.2 billion base pairs and the average genome differs from the reference genome at 4 million sites (variation 0.1%)
- There are 22000 known genes in the human genome, of which **4000 are known to cause disease**, susceptibility to disease or benign changes in laboratory values
- Of these genes, **625 (15%)** are known to cause monogenic kidney disease

Susan Murray. Nephrol Dial Transplant (2020) 35: 1113–1132

Genetic or Familial CKD

- Around 25-37% of patients with CKD self-report a positive family history of CKD . Despite this high percentage, Mendelian causes are estimated to account for approximately 10% of cases of adult ESRD. This proportion is much higher in children (70%)
- Around 15% of all incident patients who reach ESRD lack a primary diagnosis (CKD or ESRD of unknown etiology). It is very likely that a significant proportion of them carry a genetic defect, responsible for the pathophysiology of their CKD.

N Engl J Med 2019;380:142-51.

Inherited kidney disease

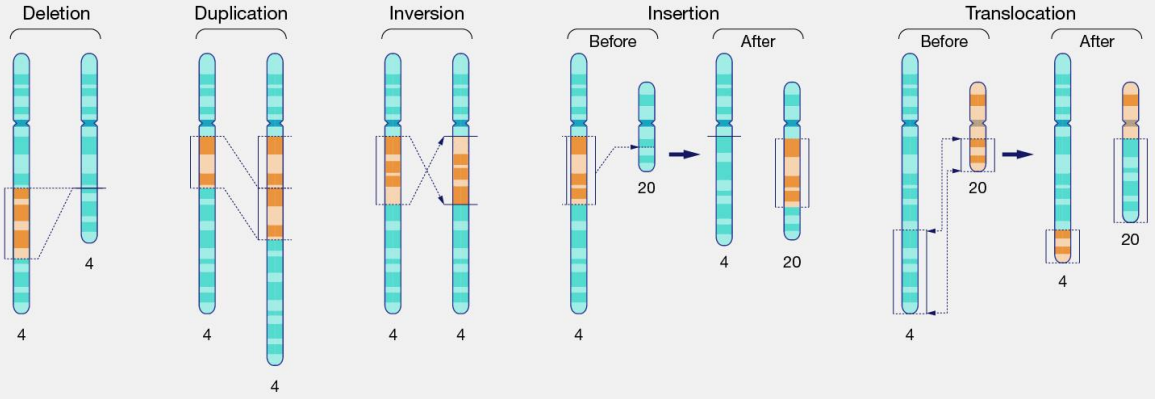
- There are many kidney diseases that are inherited in a **monogenic** fashion due to a variant in a single gene, but there are equally as many kidney diseases that are influenced in **a polygenic** fashion (DM, HTN)
- Most Mendelian inherited kidney diseases are rare. However**, as a group they represent a significant burden of disease**.
- They are the main cause of CKD and ESRD in the pediatric population.

doi:10.1038/nrneph.2017.167.

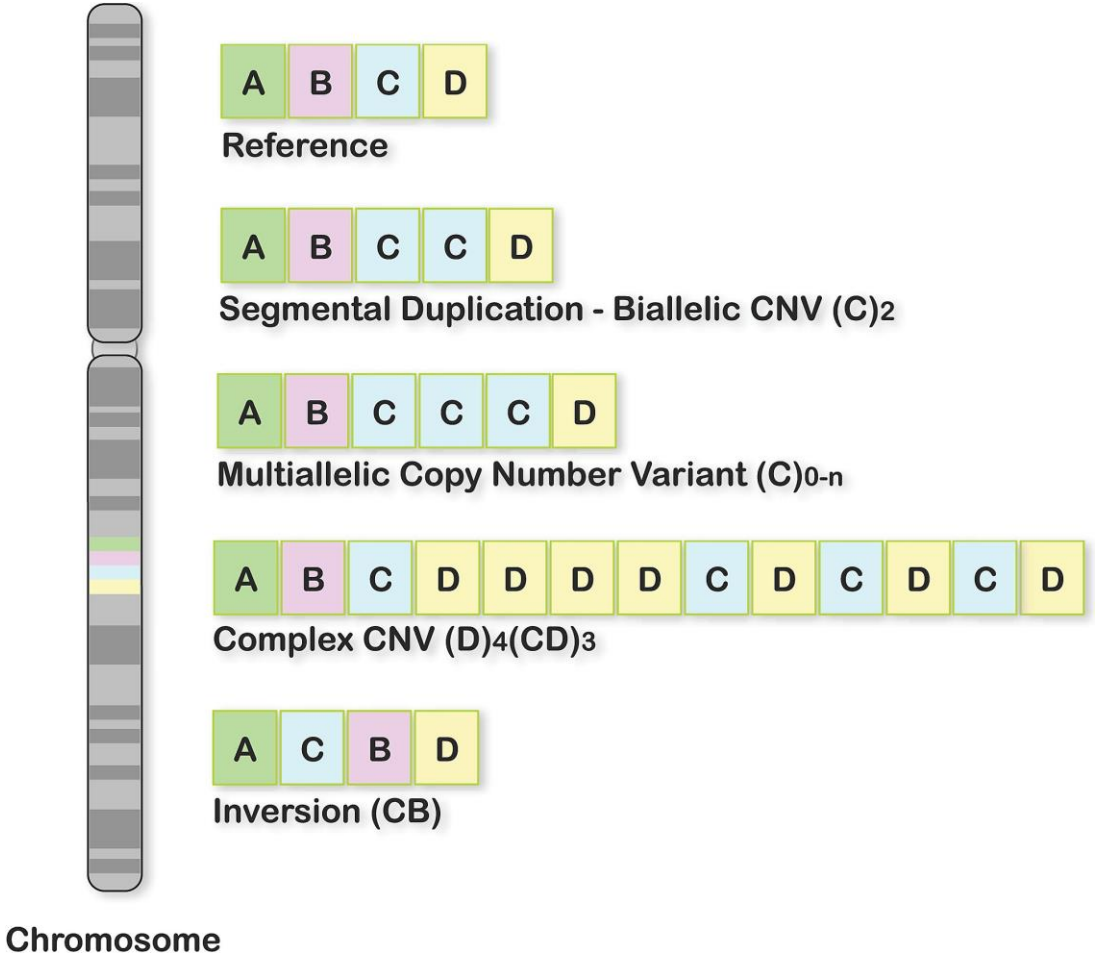
Mutations

Single nucleotide variations

Large structural variants (large DNA segments defects)



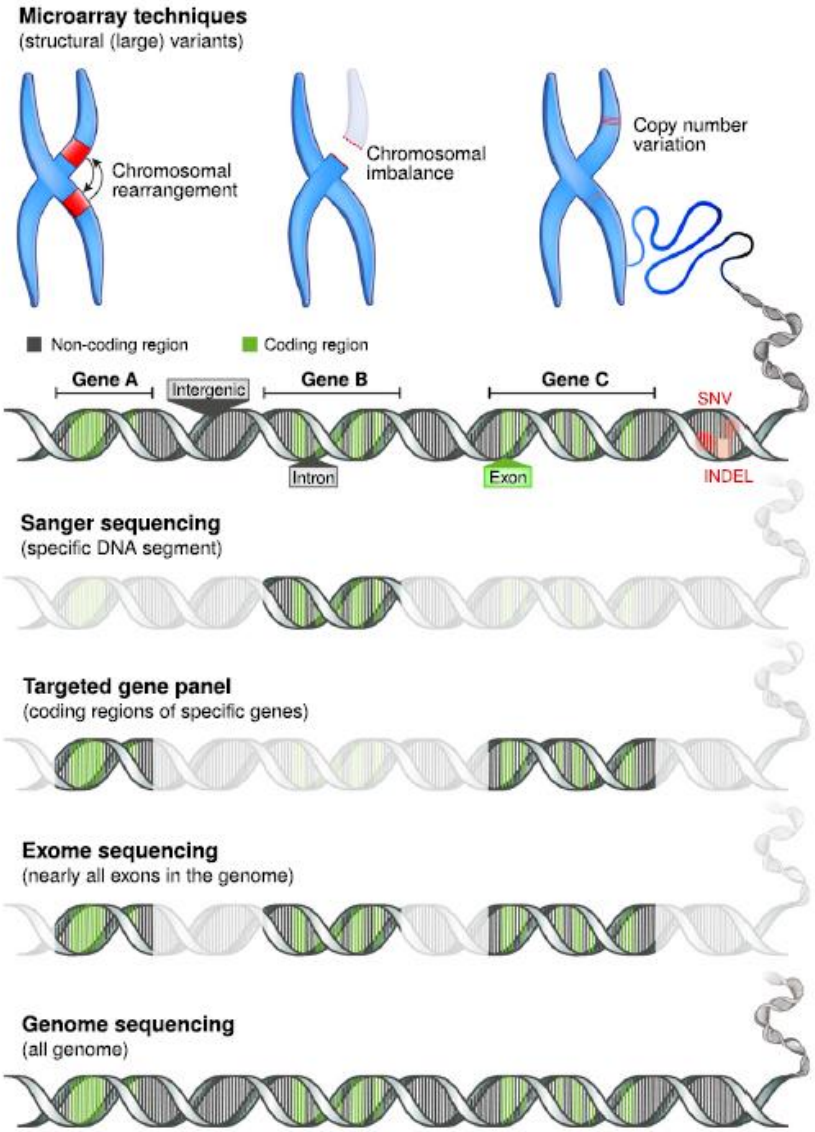
Types of Genomic Structural Changes Affecting Segments of DNA, Leading to Deletions, Duplications, Inversions, and CNV Changes (Biallelic, Multiallelic, and Complex)



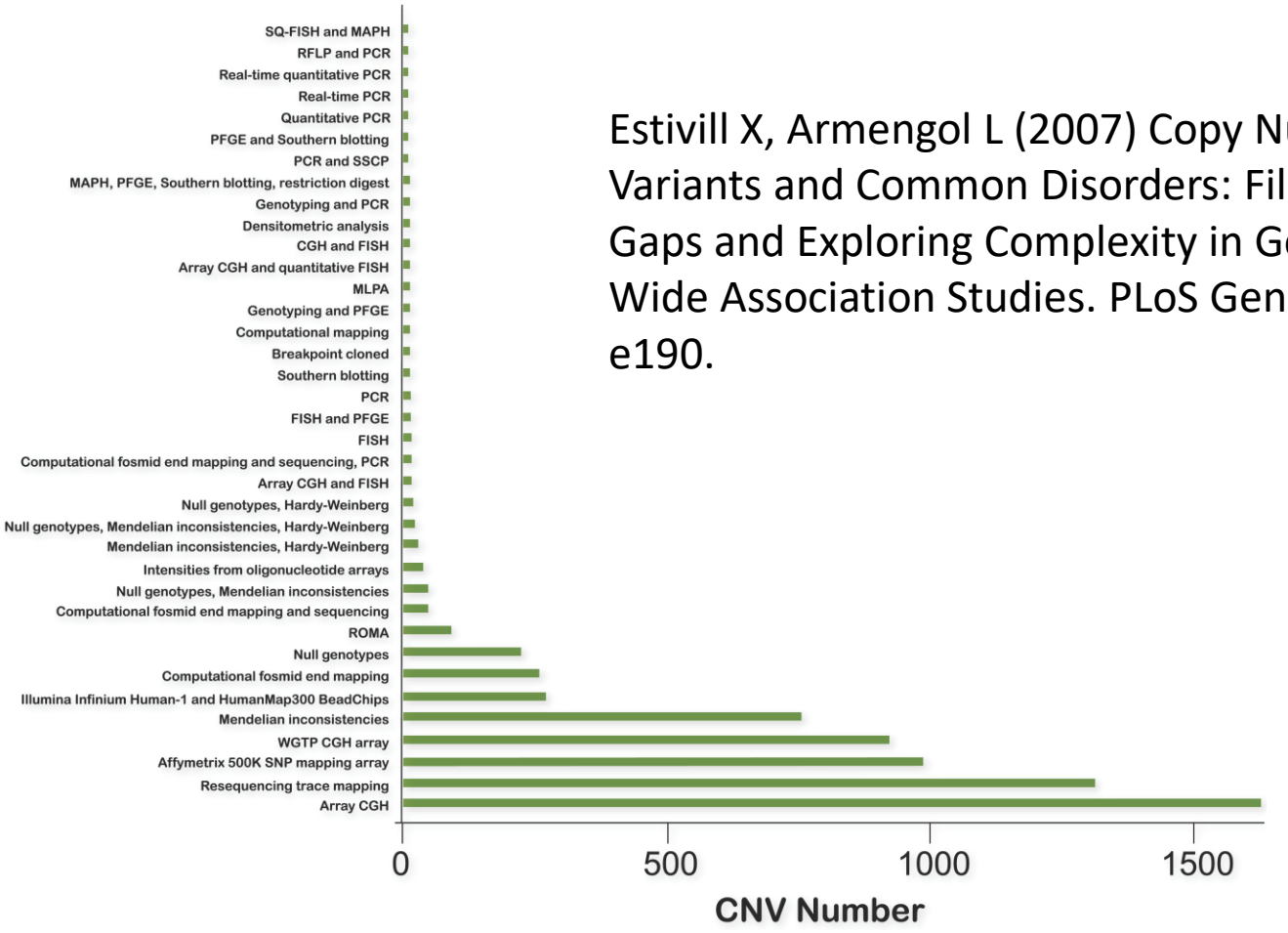
Methods of genetic testing

large DNA defects

small DNA defects



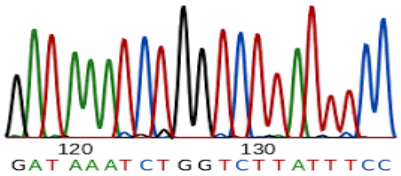
Myriad methods have been employed to identify structural variants in the human genome.



Estivill X, Armengol L (2007) Copy Number Variants and Common Disorders: Filling the Gaps and Exploring Complexity in Genome-Wide Association Studies. PLoS Genet 3(10): e190.

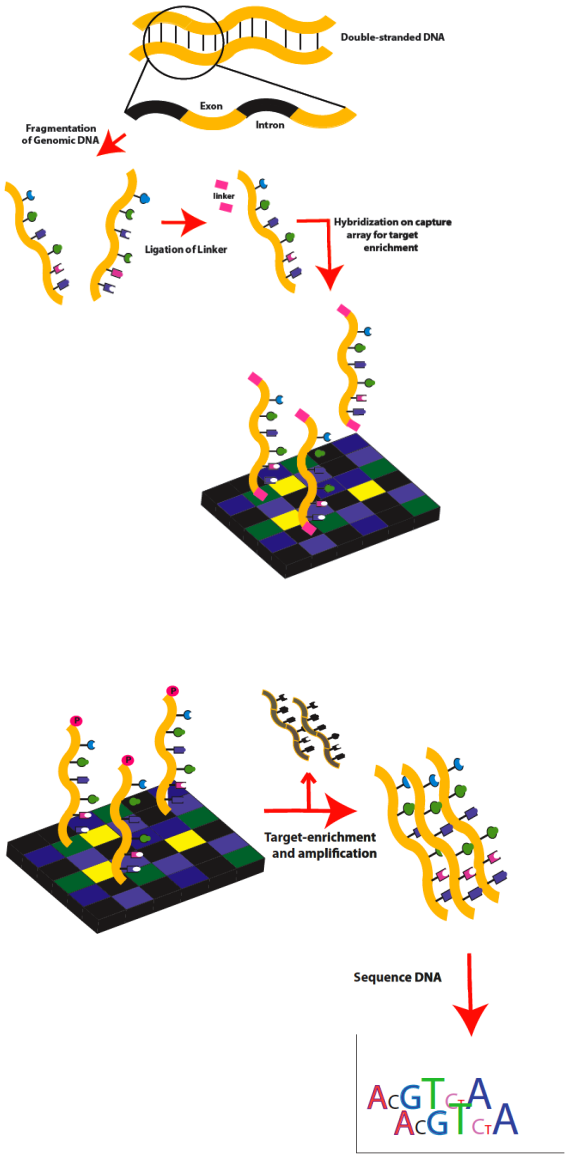
Genetic testing techniques

- We use Sanger analysis when the phenotype is highly specific for a particular genetic disease and to verify results from other tests
- Sanger sequencing is expensive and time consuming.
  - Large genes (COL4A5, PKD1)
  - Many Genes - Genetic heterogeneity (FSGS, nephrocalcinosis)
- In these instances massively parallel sequencing (MPS) is the preferred option: a) Targeted gene panels (NGS panel), b) whole exome sequencing (WES), and c) whole genome sequencing (WGS).



WHOLE EXOME & WHOLE GENOME SEQUENCING (WES, WGS)

- WES: Genomic technique for sequencing all <u>protein-coding</u> regions of genes (all exons = exome) constituting 1-2% of the genome.
- WES can detect 75% of the pathogenic mutations in the genome
- WGS analyzes whole genome (exons, introns, intergenic areas) and can detect the rest 25% of pathogenic mutations



Susan Murray. Nephrol Dial Transplant (2020) 35: 1113–1132

WHOLE EXOME SEQUENCING (WES)

- The diagnostic yield of WES outperforms targeted gene panels in both adults and children
- WES allows for the discovery of **new mutations**, **multiple mutations**, **incidental mutations** in actionable genes, new phenotype-genotype correlations and for **future re-analysis**
- CNV detection is also possible but not always optimal.
- Difficult to analyze genes relevant to nephrology: a duplicated region of PKD1 and the tandem repeats of MUC1 (blind spots)

WHOLE GENOME SEQUENCING (WGS)

- WGS can characterize noncoding regions, enabling detection of splicing or regulatory variants.
- However, currently, there is limited benefit due to our incomplete understanding of the function of most of the noncoding regions
- It is more efficient than WES for rich GC content areas, and for CNV detection, (duplicated region of PKD1 or the MUC1 tandem repeats)

Genetic testing in Nephrology

Method	Detects	Example	Diagnostic Yield
CMA (chromosomal micro arrays)	CNVs & large DNA rearrangements (100kb)	CAKUT	10-17% cJASN 2020
NGS PANELS	SNV, small Indels in selected genes (<250 bp)	FSGS, ALPORT, OXALURIA	Up to 80% selected populations
WES	SNV Indels in coding DNA	CKD unknown etiology NPHP-Ciliopathies (>90genes)	Up to 70% για NPHP
WGS	SNV Indels in coding and non coding DNA	CKD unknown etiology, aHUS (DGKE), Alport, Gitelman, PKD1	Detects 20-40% of mutations that are lost by CMA & WES
MLPA (multiplex ligation dependent probe amplification)	large deletion-duplications (<50kb)	PKD1, αGLA , HNF1b	
Long-range PCR	GC-rich repeats	ADTKD-MUC1	

Groopman, Nat Rev Nephrol. 2018

Diagnostic yield from several cohorts

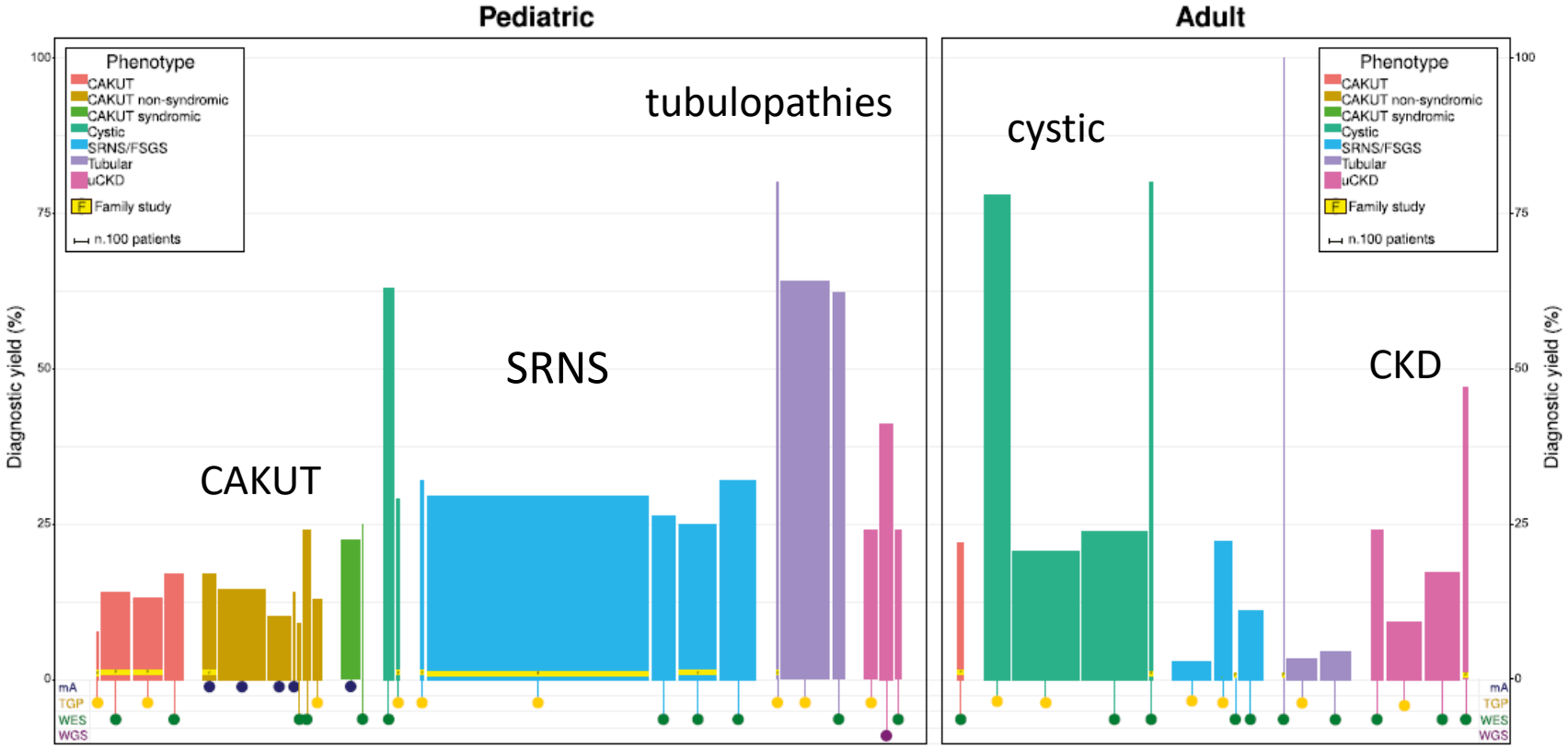


Figure 3. | Diagnostic yield per phenotype and genetic test type. The figure represents the diagnostic yield in different phenotype cohorts obtained through different genetic test type in pediatric and adult genetic studies on kidney disease. The *y* axis represents the percentage of diagnostic rate for the cohort (specified above each bar with the relative citation). The *x* axis represents the study under consideration; the width of the bar is dependent on the sample size for each study. The familial yellow flag indicates whether the study considered families (not individuals). The colored legend below the plot indicates the genetic test utilized in the study cohort. mA, chromosomal microarray techniques (especially in CAKUT); TGP, targeted gene panel; WES, whole exome sequencing; WGS, whole genome sequencing. The studies depicted here are presented in the Supplemental Material.

The diagnostic yield depends on the presence of family history, age of presentation, and underlying pathology

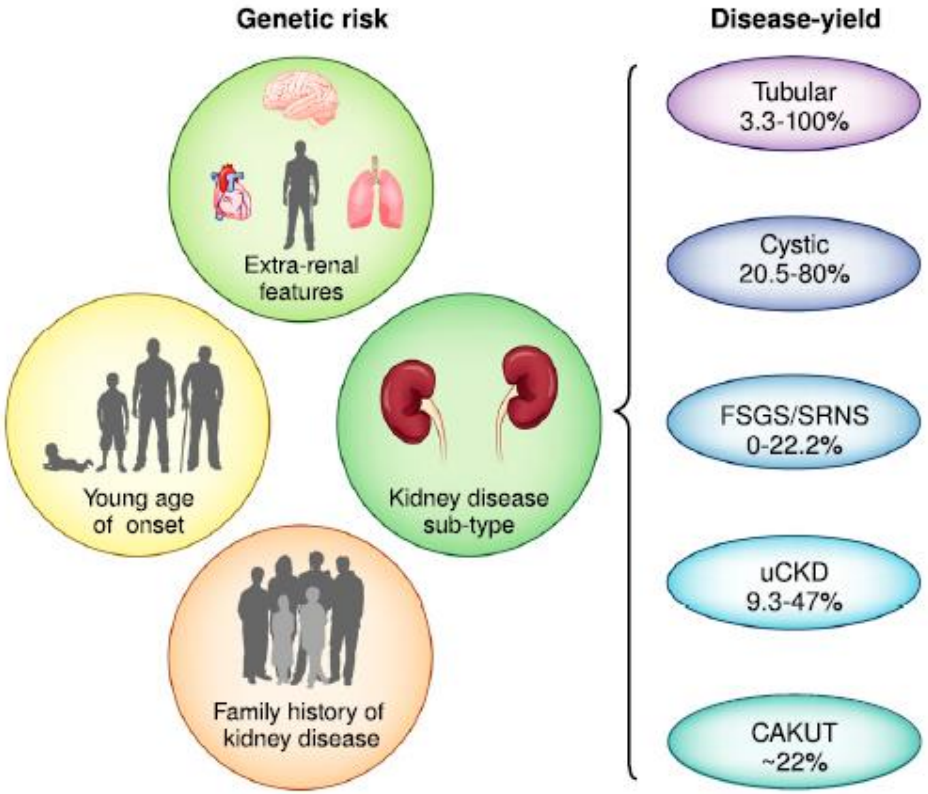
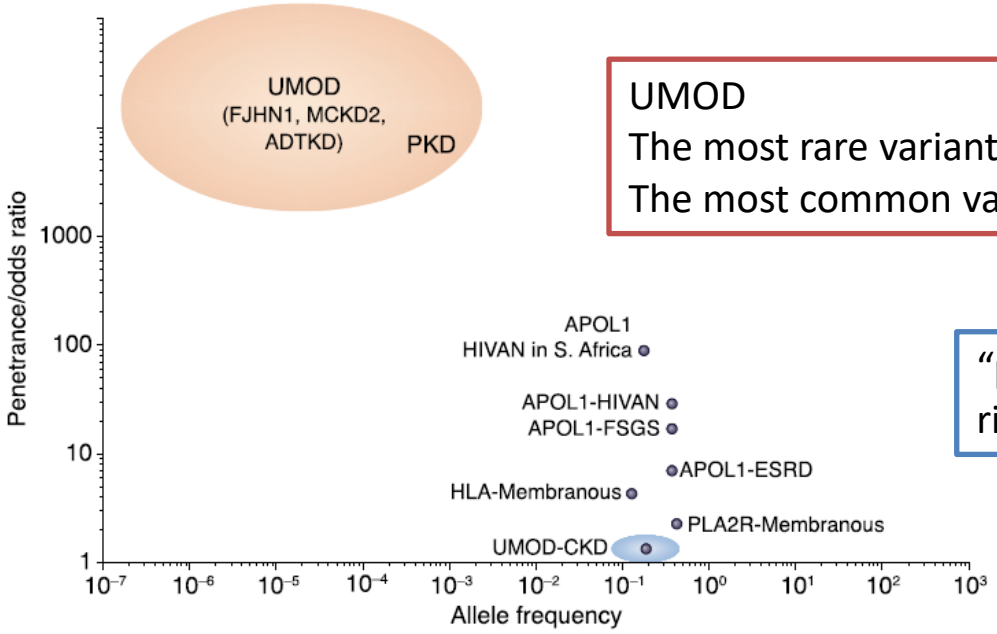


Figure 2. | Clinical determinants of genetic risk and their influence on genetic test type. The figure summarizes the clinical characteristics shaping the pretest risk of a genetic disease in a nephrology patient. Young age at onset, family history of kidney disease, and presence of extrarenal features are all predictive of genetic disease. Moreover, depending on the clinical diagnosis, the diagnostic yield of genetic testing varies. The yields of different modalities is shown in Figure 3. CAKUT, congenital anomalies of kidney and urinary tract; SRNS, steroid-resistant nephrotic syndrome; uCKD, CKD of unknown etiology.

CJASN 15: 1497–1510, October, 2020

Inverse correlation between frequency of a variant with the severity of phenotype (careful interpretation of the genetic results)



UMOD
The most rare variants: ADTKD
The most common variant: CKD

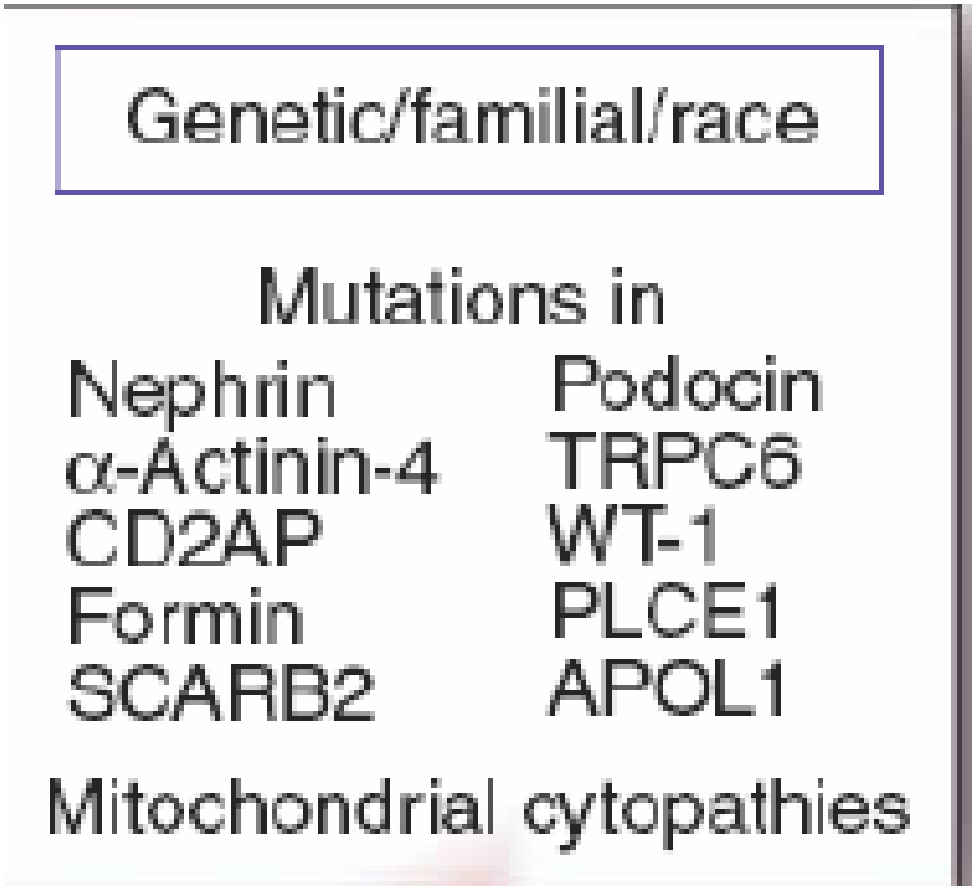
"polygenic risk scores"

CJASN 15: 268–275, February, 2020

Figure 2. | Different kidney disease relevant gene variants have a large range of allele frequencies and effect sizes. Genetic variants that cause or predispose to kidney disease can be characterized according to their frequency (*x* axis) and penetrance (*y* axis). Mendelian disease gene variants are very rare but very powerful (red ellipse). It is hard to precisely ascertain their frequency or effect size because detection typically depends on the variant being highly penetrant and the phenotype being unambiguous. The variants that cause susceptibility to common complex kidney phenotypes, such as low GFR (CKD), have small effect sizes, and therefore, they need to be common to be detected (blue ellipse). Some variants have unusual combinations of moderately strong effect size and high frequency (APOL1 and PLA2R). Most of these variants identified to date are immunity genes where the allele that promotes kidney dysfunction may also confer some benefit to the immune system. Some genes fall into more than one category. For example, common variants in UMOD are among the strongest common contributors in studies of CKD or eGFR, whereas rare variants can cause severe Mendelian phenotypes. HIVAN, HIV-associated nephropathy.

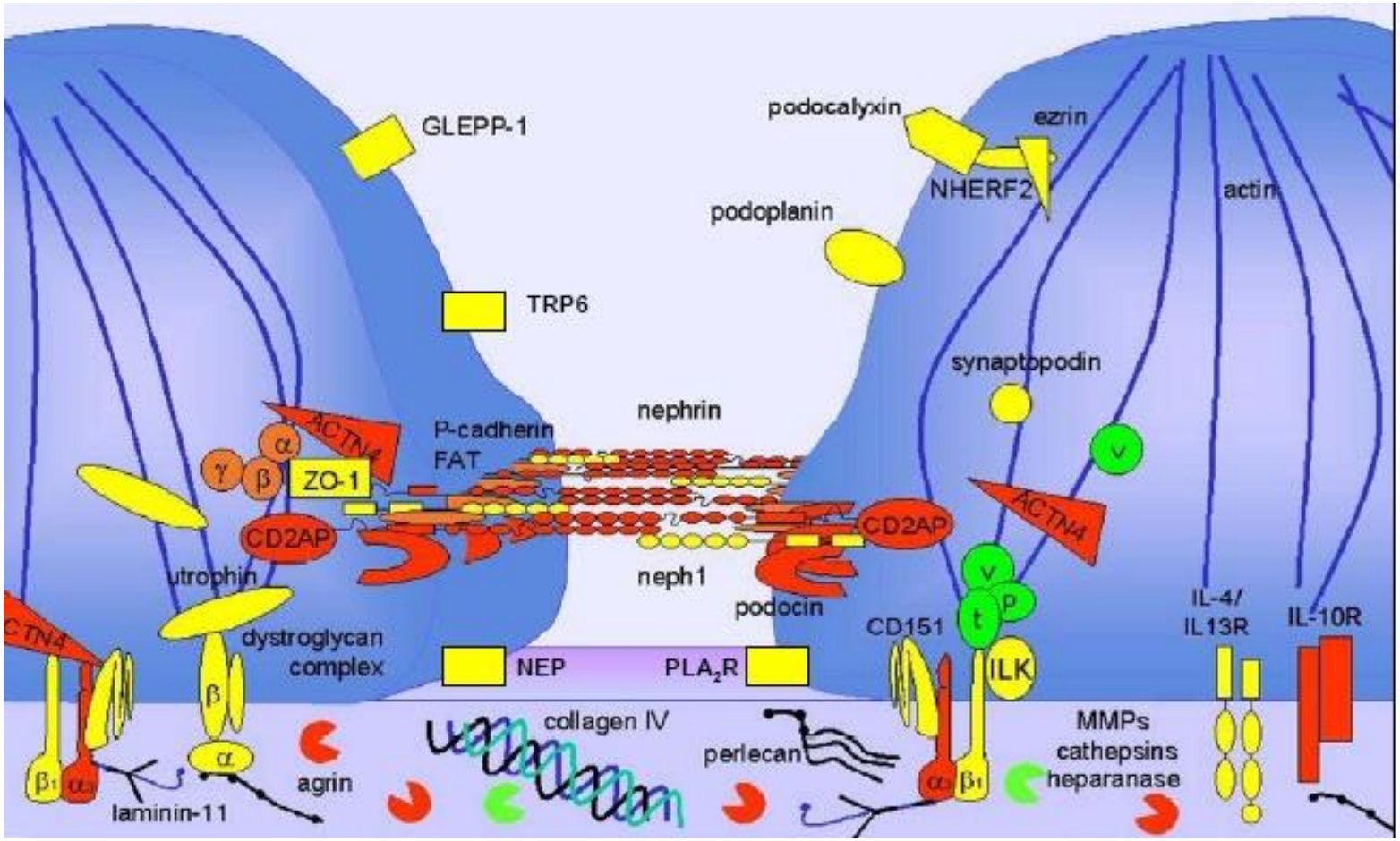
medullary cystic kidney disease-2 (MCKD2), autosomal dominant tubulointerstitial kidney disease (ADTKD)
familial juvenile hyperuricemic nephropathy (FJHN)

Hereditary podocytopathies (first studied, early age)



80 genes so far

Podocyte proteins



FSGS

Table 4. Genes causing syndromic and non-syndrome FSGS

Gene	Protein	Inheritance	Syndrome	Renal phenotype
			Non-syndromic forms of FSGS	
Cell signalling				
PLCE1	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase epsilon-1	AR	No	Steroid-resistant nephrotic syndrome
KANK2	KN motif and ankyrin repeat domain-containing protein 2	AR	No	Early onset, steroid-resistant nephrotic syndrome, haematuria
KANK4	KN motif and ankyrin repeat domains 4	AD	No	VUS, may contribute to FSGS
TRPC6	Transient receptor potential cation channel, subfamily C, member 6	AD	No	FSGS
Slit diaphragm-associated proteins				
NPHS1	Nephrin	AR	No	Congenital nephrotic syndrome or FSGS
NPHS2	Podocin	AR	No	Early-onset FSGS
CD2AP	CD2-associated protein	AD/AR	No	Early-onset FSGS
MYO1E	Myosin 1E	AR	No	Early-onset FSGS
MAGI2	Membrane-associated guanylate kinase, WW and PDZ domain-containing 2	AR	No	Steroid-resistant congenital nephrotic syndrome
Nuclear pore complex proteins				
XPO5	Exportin 5	AR	No	Childhood-onset steroid nephrotic syndrome
NUP85	Nucleoporin 85 kDa	AR	No	Childhood-onset FSGS
NUP93	Nucleoporin 93 kDa	AR	No	Childhood-onset FSGS
NUP205	Nucleoporin 205 kDa	AR		Childhood-onset FSGS
NUP160	Nucleoporin 160 kDa	AR	No	Steroid-resistant nephrotic syndrome in the second decade
Cell membrane-associated proteins				
PTPRO	Protein tyrosine phosphatase receptor-type, O	AR	No	Steroid resistant nephrotic syndrome in childhood
MP2	Epithelial membrane protein 2	AR	No	Childhood-onset steroid-resistant nephrotic syndrome
Mitochondrial function				
COQ8B	Coenzyme Q8B	AR	No	Steroid-resistant nephrotic syndrome
Cilia				
TTC21B	Tetratricopeptide repeat domain-containing protein 21B	AR	No	Nephronophthisis, adolescent-onset FSGS with tubulointerstitial lesions
AVIL	Advillin	AR	No	Childhood-onset FSGS
Cytoskeleton, cell polarity adhesion				
INF2	Inverted foramin 2	AD	No	FSGS, other, severe histological appearances
ACTN4	Actinin-α-4	AD	No	Congenital steroid-resistant nephrotic syndrome
ARHGAP24	Rho GTPase activating protein 24	AD	No	Adolescent-onset FSGS
ANLN	Actin binding protein anillin	AD	No	FSGS with variable age of onset
CRB2	Crumbs cell polarity complex component 2	AR	No	Childhood-onset FSGS, congenital steroid-resistant nephrotic syndrome
ARHGDIA	Rho GDP dissociation inhibitor α	AR	No	Congenital or early-onset steroid-resistant nephrotic syndrome
FAT1	Fat atypical cadherin	AR	No	VUS, may contribute to steroid-resistant nephrotic syndrome

FSGS

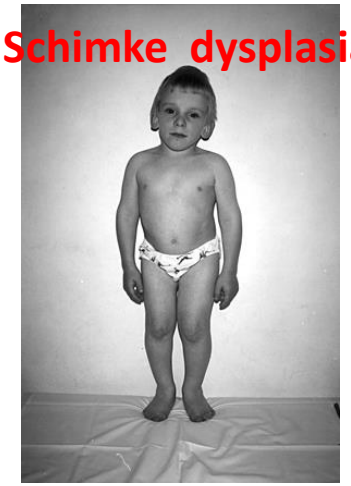
Table 4. Genes causing syndromic and non-syndrome FSGS

Gene	Protein	Inheritance	Syndrome	Renal phenotype
DNA repair, transcription				
NXF5	Nuclear RNA export factor 5	X-linked	No	Adult-onset nephrotic syndrome
Cell vesicles				
TBC1D8B	TBC1 domain family protein 8	X-linked	No	Early-onset steroid-resistant nephrotic syndrome Syndromic forms of FSGS
Lysosome				
SCARB2	Lysosomal integral membrane protein 2	AR	Action myoclonus-renal failure syndrome (ataxia, myoclonus, collapsing FSGS)	FSGS
DNA repair, transcription				
WT1	Wilm's tumour 1	AD	Denys–Drash syndrome (Wilms' tumour, male pseudohermaphroditism, FSGS), Frasier syndrome (FSGS, gonadoblastoma, male pseudohermaphroditism isolated congenital or childhood-onset diffuse mesangial sclerosis)	Childhood-onset FSGS
PAX2	Paired box 2	AD	Renal coloboma syndrome (renal hypoplasia, childhood-onset FSGS, optic nerve colobomas)	Adult-onset FSGS
EYA1	Eyes absent homolog 1	AD	Branchio-oto-renal syndrome (abnormalities in branchial, ear and renal development)	Adult-onset FSGS
LMX1B	LIM homoeobox transcription factor 1β	AD	Nail–Patella syndrome (hypoplastic or absent patella, dysplasia of elbows, short stature dystrophic nails, frequently glaucoma)	FSGS
SMARCAL1	SMARCA-like protein 1	AR	Schimke immuno-osseous dysplasia (immunodeficiency, skeletal dysplasia, childhood-onset FSGS)	Childhood-onset FSGS
LMNA	Lamin A	AD	Familial partial lipodystrophy with adult-onset FSGS, other syndromes	Adult-onset FSGS
WDR73	WD repeat-containing protein 73	AR	Galloway–Mowat syndrome (microcephaly joint contractures and developmental delay)	Childhood-onset steroid-resistant nephrotic syndrome
Cell matrix				
COL4A3	α3 Type 4 collagen	AR, AD	ATS (deafness, renal failure, ocular abnormalities)	FSGS, ATS
COL4A4	α4 Type 4 collagen	AR, AD	ATS (deafness, renal failure, ocular abnormalities)	FSGS, ATS
COL4A5	α5 Type 4 collagen	X-linked	ATS (deafness, renal failure, ocular abnormalities)	FSGS, ATS
ITGB4	Integrin-β4	AR	Epidermolysis bullosa	Steroid-resistant nephrotic syndrome
LAMB2	Laminin-β2	AR	Pierson syndrome (microcoria, neuromuscular junction defects, early-onset FSGS or diffuse mesangial sclerosis)	Childhood-onset steroid-resistant nephrotic syndrome
Cytoskeleton, cell polarity adhesion				
MYH9	Myosin heavy chain 9	AD	Epstein–Fechtner syndrome (FSGS, cataracts, sensorineural deafness macrothrombocytopaenia leucocyte inclusions)	Childhood-onset FSGS
Mitochondrial function				
MT-TL1	mitochondrial tRNA for leucine 1	Mitochondrial	mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes (MELAS)	Secondary FSGS
MT-TL2	Mitochondrially encoded tRNA leucine 2	Mitochondrial	MELAS	Secondary FSGS
MT-TY	Mitochondrially encoded tRNA tyrosine	Mitochondrial	Ophthalmoplaegia, dilated cardiomyopathy, FSGS	Secondary FSGS

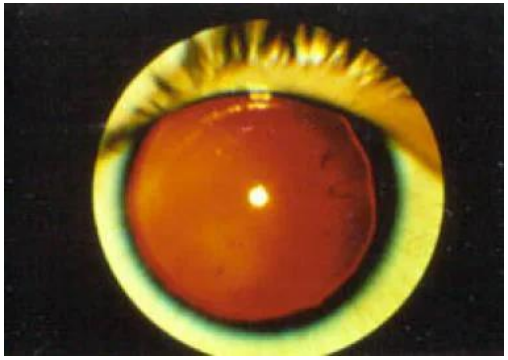
TABLE 5.1 Genetic causes of idiopathic nephrotic syndrome

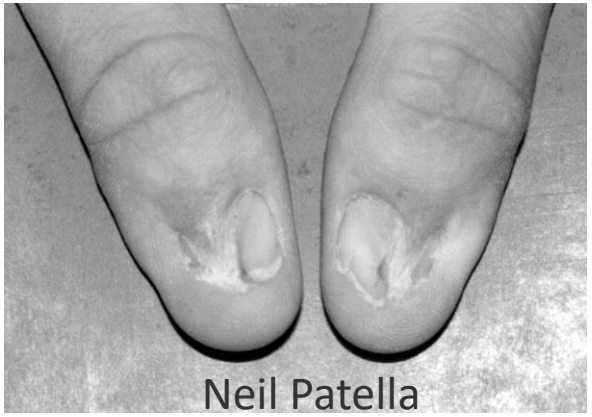
Gene	Protein	Disease	Locus
NPHS1	Nephrin	Congenital nephrotic syndrome of the Finnish type	19q13.1
NPHS2	Podocin	Autosomal recessive steroid-resistant nephrotic syndrome	1q25-31
WT1	Wilms' tumor 1	Denys-Drash syndrome, Frasier syndrome, isolated FSGS and diffuse mesangial sclerosis, WAGR syndrome	11p13
LAMB2	Laminin-β2	Pierson syndrome	3p21
PLCE1	Phospholipase C epsilon 1	Diffuse mesangial sclerosis and FSGS	10q23
SMARCAL1	SW1/SNF2-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1	Schimke immuno-osseous dysplasia	2q34-q36
SCARB2	Scavenger receptor 2	Action myoclonus renal failure	4q13-21
LMX1B	LIM-homeodomain transcription factor 1, beta	Nail-patella syndrome	9q34.1
COQ2	Parahydroxygenzoate-polyprenyltransferase enzyme	COQ2 deficiency	4q21-q22
PDSS1	Decaprenyl diphosphate synthase-1	COQ10 deficiency	10p12.1
PDSS2	Decaprenyl diphosphate synthase-2	COQ10 deficiency	6q21
MTTL1	Mitochondrial tRNA for leucine (UUR)	FSGS, with or without nephrotic syndrome	Mitochondrial

Schimke dysplasia



WAGR





Neil Patella

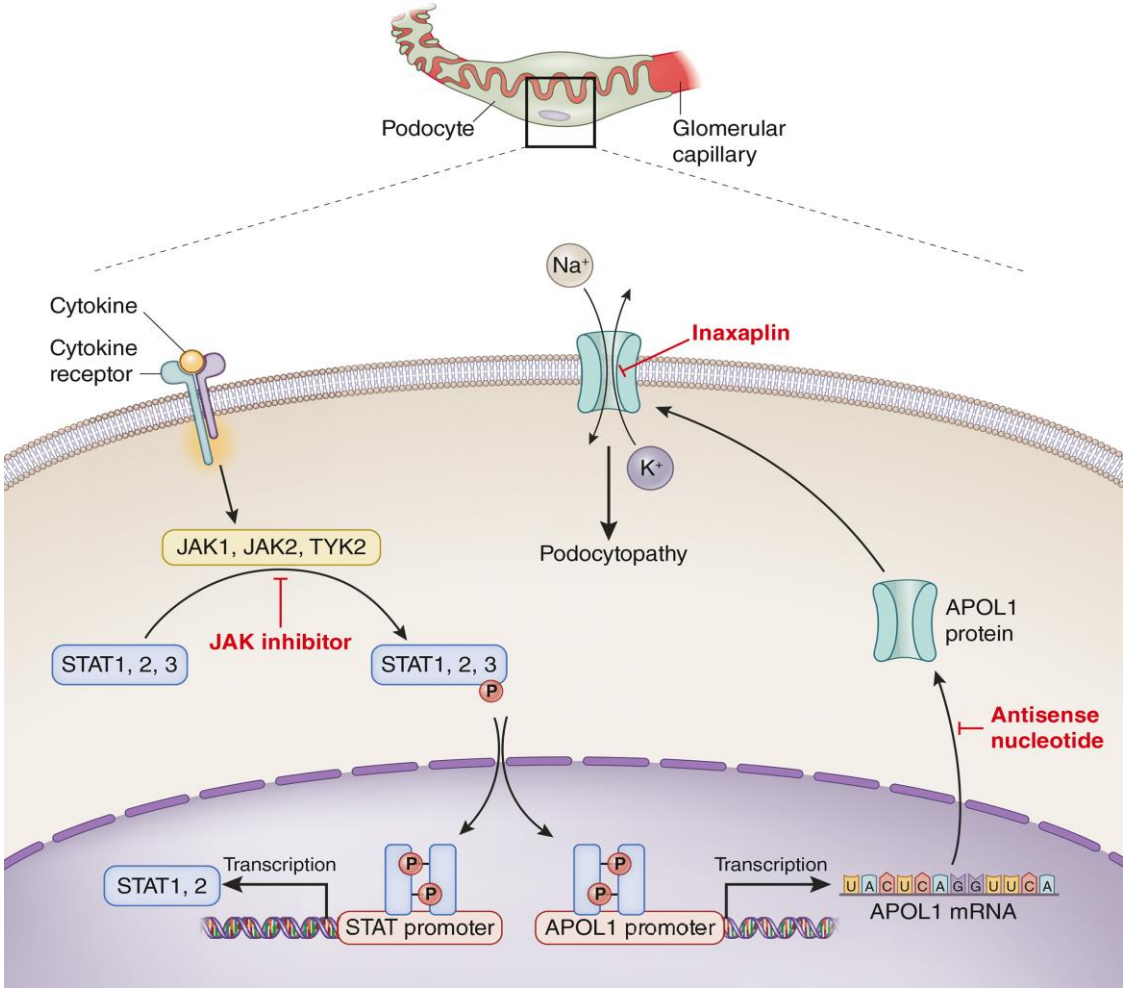
Identification of the cause of FSGS may have a significant impact on treatment.

- The majority of monogenic forms of FSGS do not respond to corticosteroids and have a very low risk of recurrence after transplantation.
- Identifying the underlying genetic cause may avoid unnecessary exposure to toxic steroid and other immunosuppressive regimens.
- Conversely, the absence of genetic mutations in structural components of the filtration barrier, would indicate an acquired, immunologic cause of the condition and would support the use of immunosuppression

Identification of the cause of FSGS may have a significant impact on treatment

- Genetic testing can identify cases that may respond to therapy with glucocorticoids (PLCE1) or cases that may benefit from other therapies:
  - coenzyme Q10 supplementation when there is a coenzyme Q10 biosynthesis-associated mutations (ADCK4, COQ2, COQ6, PDSS2)
  - vitamin B12 in the context of a cubilin mutation.

Kidney International 2023 DOI: (10.1016/j.kint.2023.04.022)



Genetics, pave the way for the discovery of new treatments.

Kidney disease–associated APOL1 variants (G1 and G2) proteins form cation pores at the plasma membrane (PM) that transport Na+ and K+ down their concentration gradients across the PM, thereby causing podocyte injury. Inaxaplin specifically blocks the aberrant cation channel function of G1 and G2 and thereby prevents podocyte injury. Egbuna *et al.*[8] reported that Inaxaplin reduced proteinuria in APOL1-associated FSGS. Inhibition of APOL1 production either by blocking JAK-STAT signaling or by APOL1 antisense oligonucleotide is an alternative therapeutic strategy that is under investigation

Whole exome sequencing identifies causative mutations in the majority of consanguineous or familial cases with childhood-onset increased renal echogenicity

Daniela A. Braun[1,19], Markus Schueler[1,19], Jan Halbritter[1,2], Heon Yung Gee[1], Jonathan D. Porath[1],

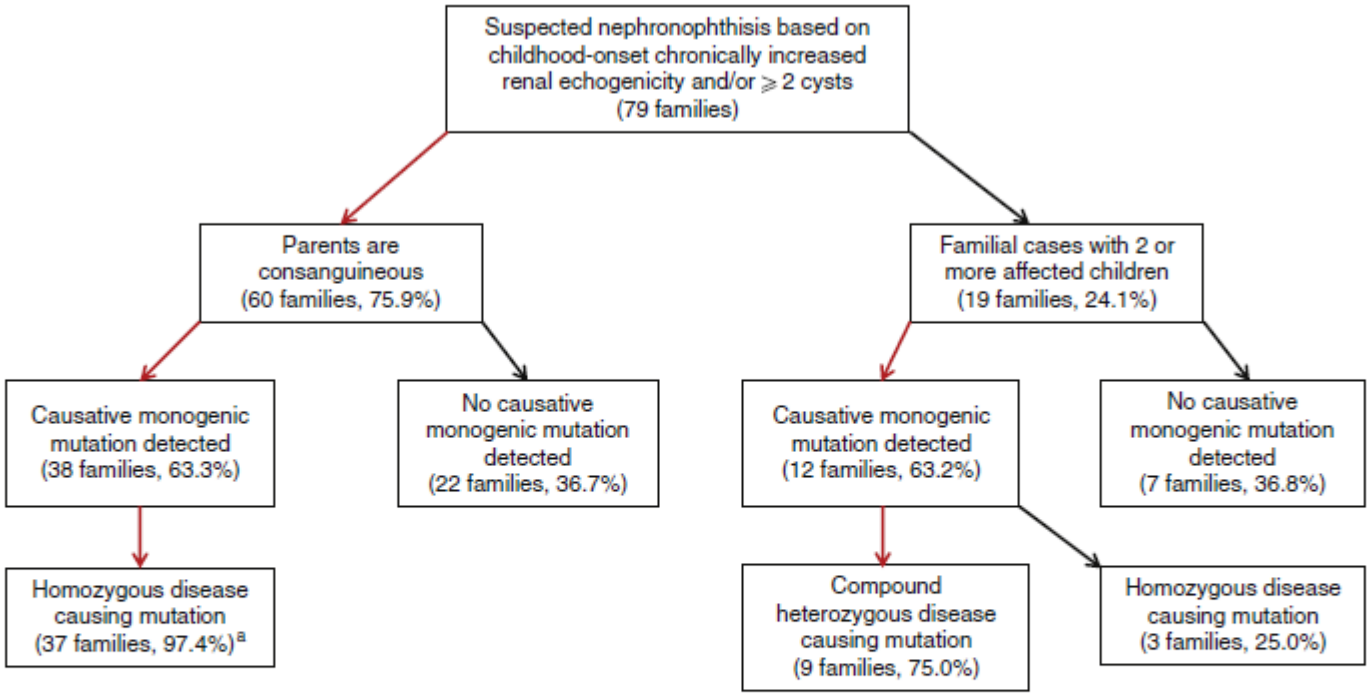
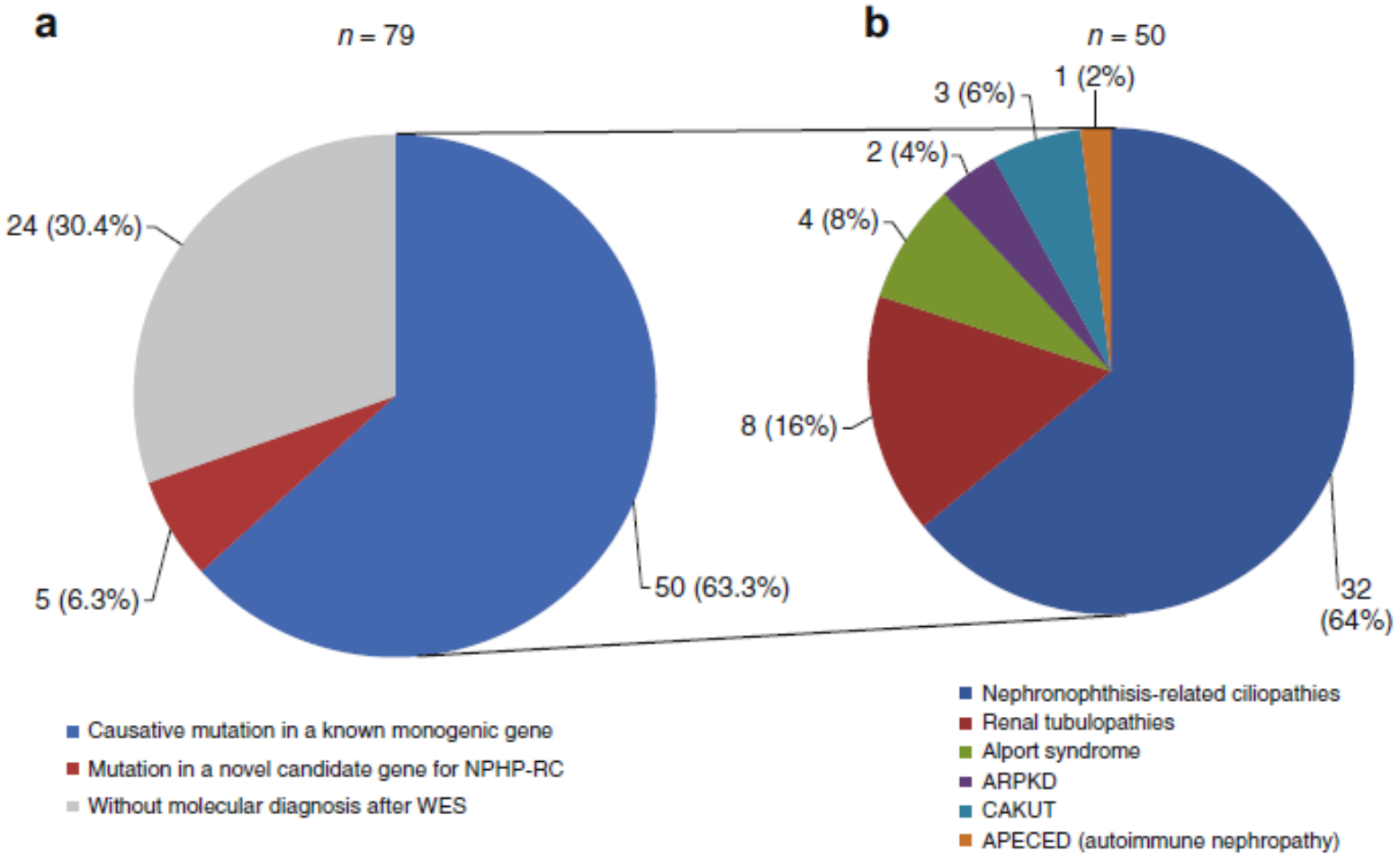


Figure 2| Algorithm for molecular diagnostics in consanguineous or familial cases of suspected nephronophthisis based on renal ultrasound presentation. Of the 79 families with childhood-onset increased renal echogenicity and/or ≥2 cysts on renal ultrasound imaging, 60 individuals were born of consanguineous unions and 19 families were nonconsanguineous with 2 or more affected children. In 63.3% of consanguineous families, we identified a mutation in a known monogenic disease gene as causative. The majority of these mutations were, as postulated, present in the homozygous state. In 63.2% of familial cases, we identified a causative mutation in a recessive, monogenic disease gene. Three-fourths of these mutations were compound heterozygous. [a]In one consanguineous family, a single heterozygous mutation in the dominant gene *HNF1B* was identified as the molecular disease cause.

79 families with childhood-onset chronically increased echogenicity or ≥2 cysts on renal ultrasound.



Identifying the specific mutation can help determine the prognosis regarding age of onset, kidney prognosis and dysfunction of other organs

The genetic landscape and clinical spectrum of nephronophthisis and related ciliopathies.

kidney INTERNATIONAL

ISN INTERNATIONAL SOCIETY OF NEPHROLOGY

Cohort

600 patients

1990 - 2020
INSERM,
Imagine Institute &
Necker Hospital
(Paris, France)

nephronophthisis /
renal ciliopathy

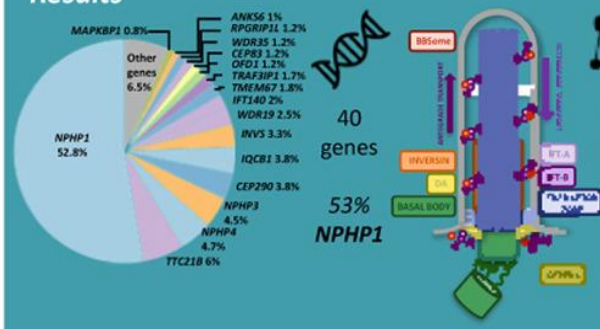
+/-

Methods

biallelic variants
in ciliopathy genes

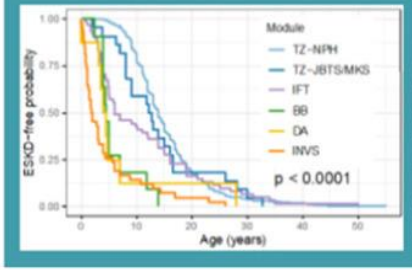
- Sanger sequencing
- QMPSF
- NGS Gene panel
- WES

Results



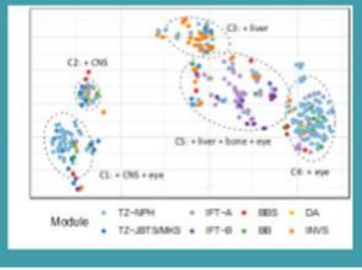
18% infantile
48% juvenile
34% late-onset

Kidney



Kidney prognosis associated with mutation types, genes and ciliary modules

Extrarenal



Genes belonging to the same ciliary module share phenotypic similarities

Petzold et al, 2023

CONCLUSION Nephronophthisis and related renal ciliopathies are clinically and genetically complex disorders. Phenotypic groups associated with genes and variant types improving precise diagnosis and prognosis of kidney disease onset as well as pattern of extrarenal defects.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Diagnostic Utility of Exome Sequencing for Kidney Disease

- WES in 2 large cohorts combined:
- Assessment of Survival and Cardiovascular Events (AURORA), a clinical trial involving 2773 patients with **ESRD** who were 50-80 years of age. 280 medical centers, in 25 nations (Greece included)
- 2187 patients from the Columbia University Medical Center (CUMC) Genetic Studies **CKD** project, a genetic research and biobanking study recruiting patients who are seen by the CUMC Nephrology Division for the evaluation and management of nephropathy

Emily E. Groopman. N Engl J Med 2019; 380:142-151DOI: 10.1056/NEJMoa1806891

Table 1. Clinical Characteristics of the Patients.*

Characteristic	AURORA Cohort (N=1128)	CUMC Cohort (N=2187)	Overall Study Population (N=3315)
	number of patients (percent)		
Age at time of study entry			
0–21 yr	0	278 (12.7)	278 (8.4)
22–44 yr	0	713 (32.6)	713 (21.5)
45–64 yr	560 (49.6)	800 (36.6)	1360 (41.0)
≥65 yr	568 (50.4)	396 (18.1)	964 (29.1)
Sex			
Female	427 (37.9)	945 (43.2)	1372 (41.4)
Male	701 (62.1)	1242 (56.8)	1943 (58.6)
Race or ethnic group†			
White	1023 (90.7)	1113 (50.9)	2136 (64.4)
Hispanic	50 (4.4)	435 (19.9)	485 (14.6)
Black	18 (1.6)	330 (15.1)	348 (10.5)
Asian	20 (1.8)	224 (10.2)	244 (7.4)
Other or unspecified	17 (1.5)	85 (3.9)	102 (3.1)
Clinical diagnosis			
Congenital or cystic renal disease	159 (14.1)	372 (17.0)	531 (16.0)
Glomerulopathy	231 (20.5)	1180 (54.0)	1411 (42.6)
Diabetic nephropathy	184 (16.3)	186 (8.5)	370 (11.2)
Hypertensive nephropathy	193 (17.1)	126 (5.8)	319 (9.6)
Tubulointerstitial disease	212 (18.8)	32 (1.5)	244 (7.4)
Other	50 (4.4)	109 (5.0)	159 (4.8)
Nephropathy of unknown origin	99 (8.8)	182 (8.3)	281 (8.5)
End-stage renal disease‡	1128 (100.0)	1016 (46.5)	2144 (64.7)
Family history of kidney disease§	—	619 (28.3)	—

* CUMC denotes Columbia University Medical Center.
† Race and ethnic group were reported by the patients.
‡ In the AURORA trial design, all patients had end-stage renal disease at the time of trial entry.
§ Family history data were available only for patients in the CUMC cohort.

Results

- 625 nephropathy-associated genes examined
- 59 genes are recommended by the ACMG for reporting as medically actionable
- diagnostic variants were detected in 307 of the 3315 patients (9.3%), encompassing 66 distinct monogenic disorders
  - 206 (67%) had an autosomal dominant disease,
  - 42 (14%) an autosomal recessive disease, and
  - 54 (18%) an X-linked disease
- This yield is similar to that observed for cancer, for which genomic diagnostics are routinely used.

Results

- 202 variants (59%) had been previously reported as pathogenic
- 141 variants (41%) new.
- The majority of diagnostic variants (228 of 343 [66%]) were absent from population control databases (new or extremely rare?)

Results

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.

652 renal genes, 39 diagnoses =6% have a clinical presence,
94% of known renal genes were not detected (extremely rare)

Variable clinical diagnostic spectrum before genetic testing

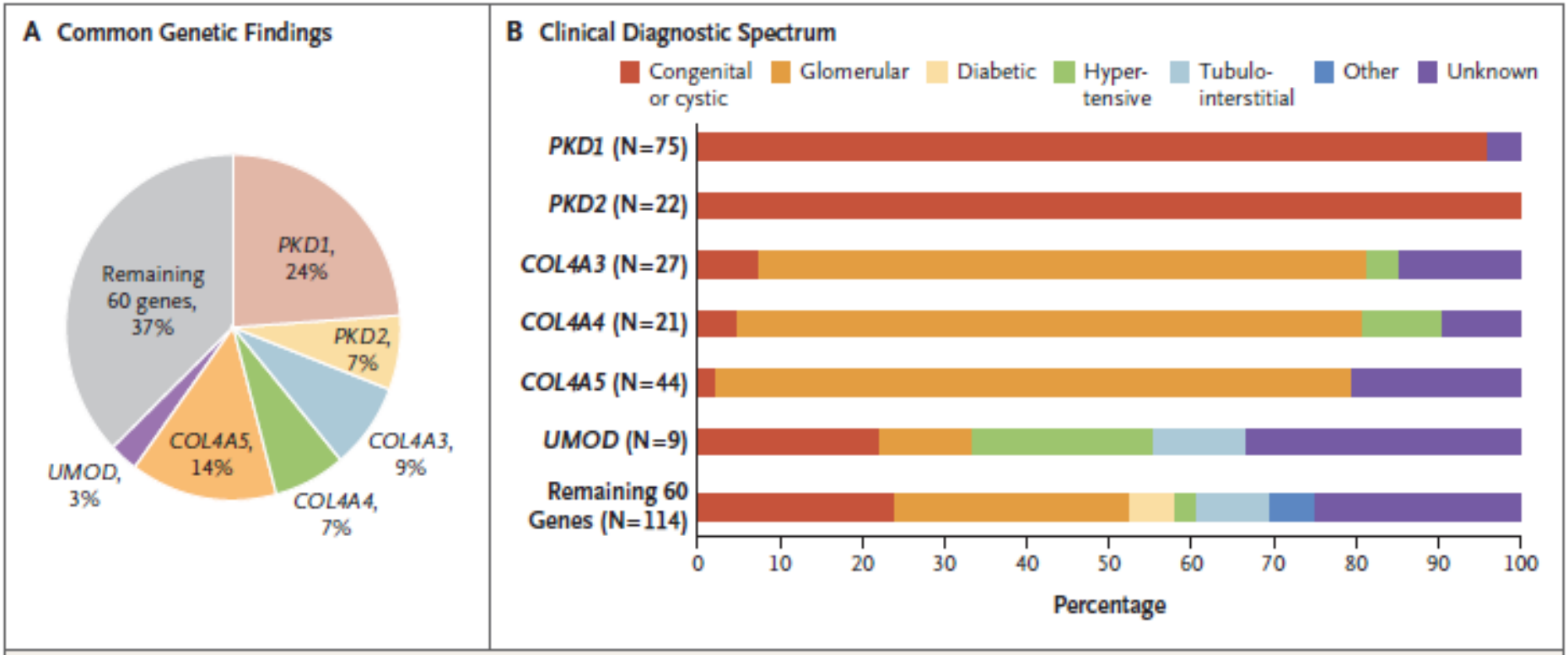


Figure 1. Common Genetic Findings and the Clinical Diagnostic Spectrum.

Panel A shows the most common diagnostic genetic findings. In total, 312 genetic diagnoses, representing 66 distinct monogenic disorders, were detected in 307 patients, with 5 patients (2%) harboring dual molecular diagnoses (Tables S8 through S10 in Supplementary Appendix 1 and Table S7 in Supplementary Appendix 2). Of the 66 distinct monogenic disorders observed, 6 collectively accounted for 63% of the genetic diagnoses: autosomal dominant polycystic disease due to mutations in *PKD1* (75 patients) or *PKD2* (22); glomerulopathy due to mutations in *COL4A3* (27), *COL4A4* (21), or *COL4A5* (44); and *UMOD*-associated tubulointerstitial disease (9). Percentages do not total 100 because of rounding. Panel B shows the clinical diagnostic spectrum of patients with diagnostic variants in these genes; the percentage of patients belonging to a given diagnostic category among all the patients found to have diagnostic variants in the gene is shown. Patients who had diagnostic findings for nephropathy associated with *COL4A3*, *COL4A4*, or *COL4A5* or for *UMOD*-associated tubulointerstitial disease had a broad spectrum of clinical diagnoses. The clinical diagnostic spectrum that was observed for the other 60 genes, which accounted for the remaining 37% of genetic diagnoses, is shown alongside for comparison. The categories of clinical diagnoses are congenital or cystic renal disease, glomerulopathy, diabetic nephropathy, hypertensive nephropathy, tubulointerstitial disease, and nephropathy of unknown origin.

Results on Alport: in 62% we had no idea of the correct diagnosis

- Only 35 of the 91 patients (38%) with diagnostic variants in *COL4A3, A4, A5 had a correct clinical* diagnosis (Alport syndrome or TBMD)
- The remaining 56 patients (62%) had other clinical diagnoses
  - FSGS (16%),
  - unspecified glomerulopathy (22%)
  - congenital renal disease (4%),
  - hypertensive nephropathy (3%),
  - nephropathy of unknown origin (15%)

In the majority of these patients (89%), the genetic diagnosis gave a new clinical insight

Table 4. Diagnostic Utility and Clinical Implications of Genetic Findings in the 167 Patients in the CUMC Cohort with Genetic Diagnoses.

Diagnostic Utility of Genetic Findings	Patients	Distinct Monogenic Disorders Detected	Singleton Genetic Diagnoses	Genetic Diagnosis with Implications for Clinical Management*
		number		*number (percent)*
Confirmed suspected hereditary cause	45	12	5	34 (76)
Discerned specific subcategory of condition within broader clinical disease category	65	36	24	58 (89)
Reclassified disease	18	11	7	18 (100)
Identified molecular cause for undiagnosed condition	39	22	11	39 (100)
Total	167	55†	35†	149 (89)

* Implications for clinical management included informing prognosis (e.g., regarding disease severity or transplantation), initiating referral for subspecialty care, and influencing the choice of therapy — for example, the use or avoidance of agents or referral of patients to clinical trials of therapies targeted to the underlying genetic disease.

† A total of 20 genetic diagnoses were found multiple times, 16 of which were found among patients in different diagnostic utility categories.

Diagnostic implications

- For 88 of the 167 patients (53%), the genetic diagnosis could initiate referral and evaluation for previously unrecognized extrarenal features of the associated diseases, spanning 15 different medical specialties (ENT, eye, etc)

Diagnostic implications

- In a significant proportion of Pts (34%), the genetic findings reclassified their disease or provided a cause for undiagnosed nephropathy, emphasizing the usefulness of the "***agnostic***" approach of exome sequencing
- This approach assesses genes that otherwise may have gone unevaluated with the use of single-gene or phenotype-driven panel testing.
- Applying a phenotype-specific Gene panel in this study would have resolved, at most, 136 cases (44.3%) in the overall population.

Genetic diagnosis can help the physician to diagnose extrarenal pathologies

60th ERA CONGRESS MILAN & VIRTUAL JUNE 15-18, 2023

Nephrocalcinosis In 3 Generations Of Female Patients Due To a Pathogenic MEN1 Mutation.

K. DERMITZAKI [1], I. PETRAKIS [1], CH. PLEROS [1], M. KONIDAKI [1], D. LYGEROU [1], A. ANTONAKIS [1], S. MARAGKOU [1], K. STYLIANOU [1]
1. Nephrology Department, University General Hospital, Heraklion, Crete, Greece

INTRODUCTION

Multiple Endocrine Neoplasia type 1 (**MEN1**) syndrome, has an autosomal dominant transmission pattern, characterized by hyperparathyroidism, benign or malignant tumours in pancreas islets, pituitary tumours, thymic carcinomas, adrenal cortex adenomas and angio-fibromas of the skin. We are describing 3 cases of MEN1 with first and cardinal manifestations within the kidney.

AIM

Underline the role of genetic counselling and genetic screening in cases of familial nephrocalcinosis.

METHOD

Whole exome sequencing (WES) was performed. Bioinformatics analysis was performed with Ingenuity Clinical Insights software (Qiagen Inc.) utilizing Human Gene Mutation Database (HGMD). Patient electronic record was utilized after receiving informed consent.

RESULTS

Female : 28 years old

- Horseshoe kidney
- **Primary hyperparathyroidism**
- **Hypercalcemia**
- **Magnesiuria**
- **Nephrocalcinosis.**

Her Mother : **48 years old**

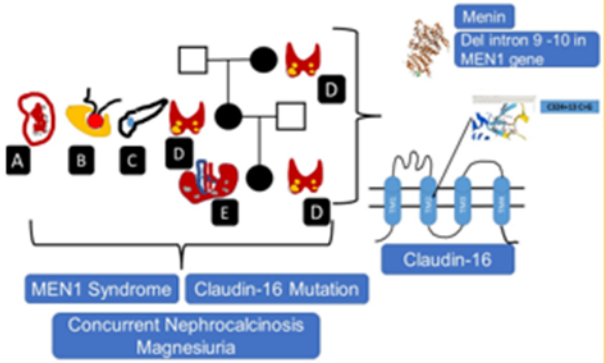
- **Hyperparathyroidism**
- **Hypercalcemia**
- **Magnesiuria**
- **Nephrocalcinosis.**
- **Endocrine neoplasia : Increased serum gastrin and chromogranin from Pancreas Mass, Pituitary adenoma (increased Prolactin)**

Her Grandmother: 84 years old

- **Hyperparathyroidism**

WES Analysis:

- 12 base deletion between intron 9 and exon 10 in MEN1 gene (c.1351-3_1359delCAGGTGCGGCAG) (1).
- CLDN16 gene accompanying alteration (c.324+13C>G, rs369250510)



Schematic representation of family tree and associated clinical phenotypes. Grandmother presenting only with parathyroid adenoma (D). Mother presenting with parathyroid adenoma (D), pancreas tumours (C), Pituitary adenoma (B) and nephrocalcinosis (A). Patient presenting with parathyroid adenoma (D) and Horseshoe kidney nephrocalcinosis (E). All patients bear the same mutations in MEN-1 and Claudin-16 gene.

CONCLUSIONS

In familial nephrocalcinosis / lithiasis / hyperparathyroidism genetic counselling and screening defines individualized treatment and may prevent extra renal disease manifestations.

REFERENCES

Klein RD et al, Clinical testing for multiple endocrine neoplasia type 1 in a DNA diagnostic laboratory. Genet Med. 2005 Feb;7(2):131-8

CONTACT INFORMATION

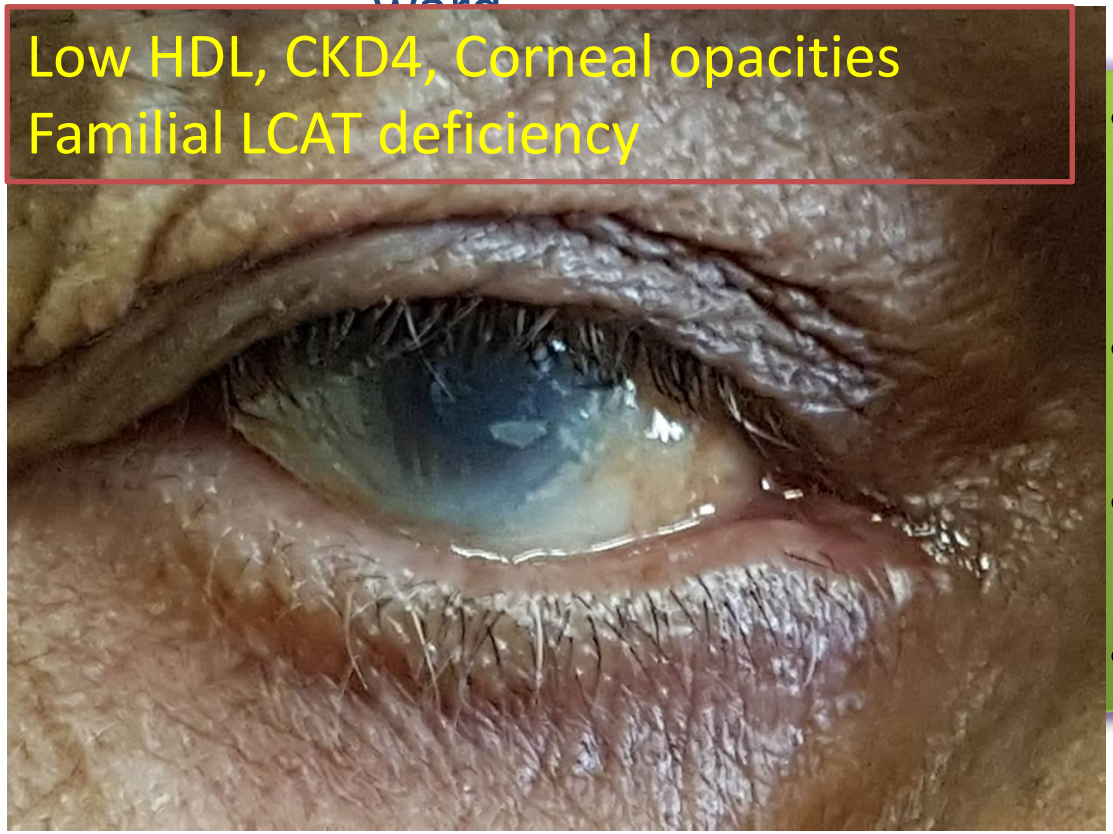
I. Petrakis MD, Ph.D, petrakgia@gmail.com

Therapeutic implications (CKD cohort)

- For 84 patients (50%), the genetic diagnosis could inform therapy:
  - by disfavoring immunosuppression among patients who were found to have monogenic forms of FSGS,
  - By initiating multidisciplinary care (e.g BRCA2),
  - By leading to the initiation of tailored therapies (e.g Dent disease),
  - By prompting referral to clinical trials that were targeted to the genetic disorder identified.

It always starts with a patient…

63 yo Male in Hematology ward



- Chronic anemia- unknown cause
- Proteinuria- 40yo
- DMT2- 46yo
- CKD3- 56yo

And then came a second one…

43 yo M

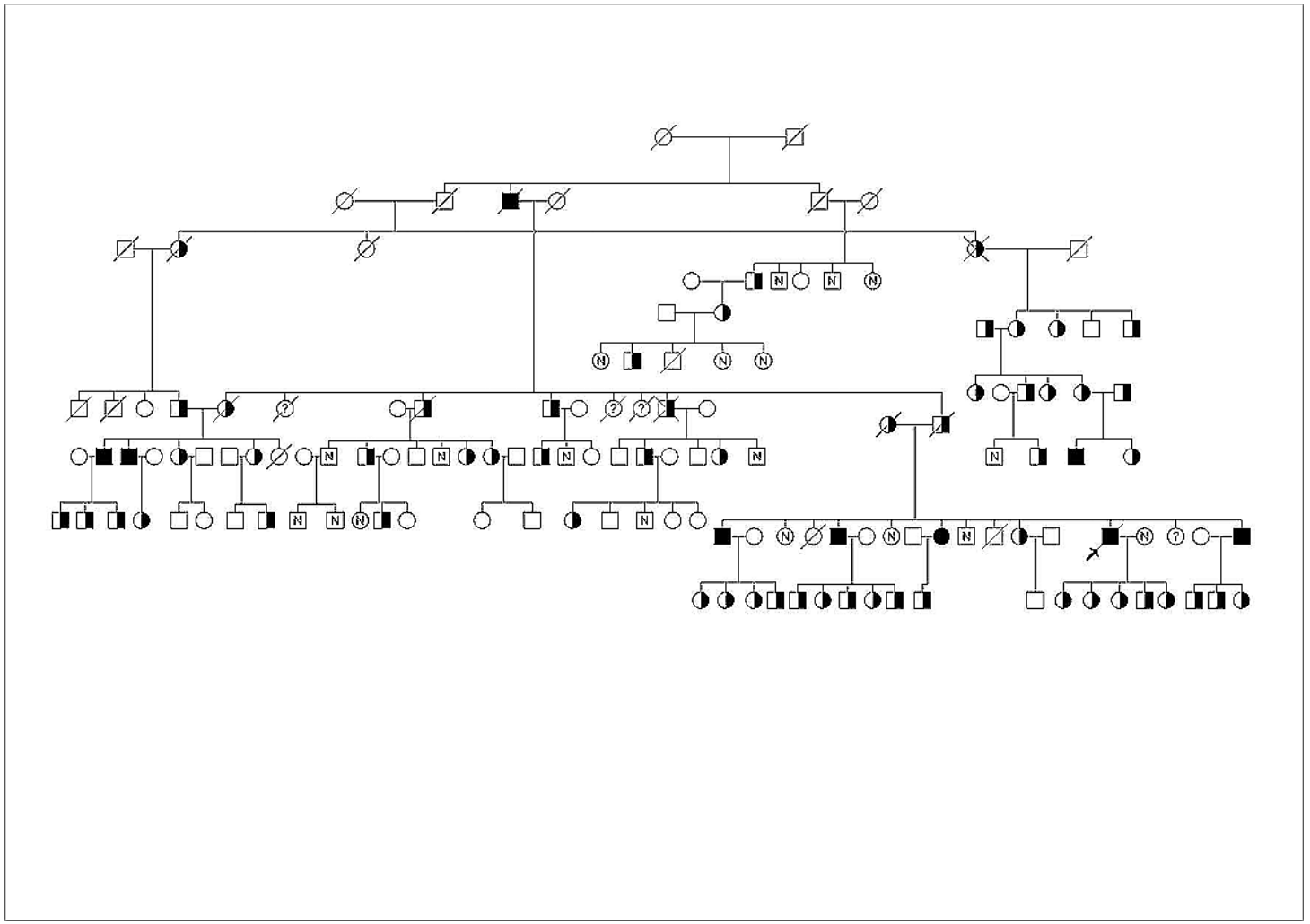
- Prote
- New

c

ince

n2

And ends up with hundreds of pts



Our early experience with WES

Renal diseases: 71% (10/14) diagnostic rate with WES

Pt.	Age (years)	Sex (M/F)	Phenotype	Gene	Disease
1ΧαΜα	28	F	Hematuria, proteinuria and hypertension	*COL4A5*	**Alport Syndrome**
2ΒαΚα	45	M	Hypokalaemia, hypercalcaemia, and nephrocalcinosis	*OCRL*	**Dent disease 2**
3Καπ	35	M	Nephrotic syndrome, renal failure	*MAGI2*	**Nephrotic syndrome 15**
4Ζακ	40	F	Hypokalemic alkalosis, hypocalciuria	*SLC12A3*	**Gitelman syndrome**
5Ζοι	22	F	Focal segmental glomerulosclerosis	*PLCE1*	**Nephrotic syndrome 3**
6	48	M	Hypertrophic Cardiomyopathy & Keratosis palmoplantaris	*MYH7* *DSG1*	Cardiomyopathy, hyper-Keratosis palmoplantaris striata I
7Ιωα	33	F	retinitis pigmentosa proteinuria	ADCK4	**Nephrotic syndrome 9**
8	50	F	Cystic kidney disease, proteinuria ESRD	*COL4A5*	**Alport Syndrome**
9Νιω	30	M	C3GN	*CFH*	**C3 glomerulopathy**
10Τσι	32	F	Severe Hypomagnesemia, Headaches	*Cyclin M2* *CNNM2*	**Renal Hypomagnesemia 6**

THE FIRST CASE DNAJB11 ASSOCIATED NEPHROPATHY IN GREECE (AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE-6/ ADPKD-6). WCN23-0585

K. Dermitzaki, I.Petrakis, E. Drosataki, M. Papapanagiotou, C. Pleros, D. Lygerou, I. Stavrakaki, M. Konidaki, M. Mitrakos, N. Papadakis, S. Maragou, A. Androvitsanea, N. Kroustalakis, K. Stylianou.

Department of Nephrology, Heraklion University Hospital, Medical School, University of Crete, Greece

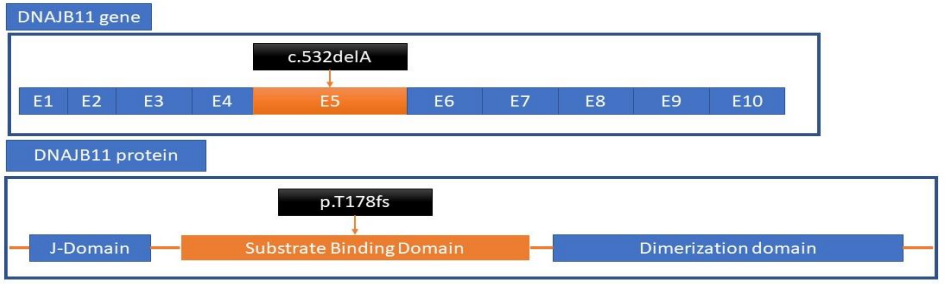
Introduction: Many patients with a family history of chronic kidney disease (CKD) present multiple cystic kidney lesions without suffering from classical adult polycystic kidney disease (ADPKD). DNAJB11 associated nephropathy was first described in 2018 in 7 kindreds with monoallelic mutations in DNAJB11 gene (1). DNJAB11 shortage disrupts PKD1 maturation and transport in cellular membrane and causes an aberrant hold of uromodulin and MUC1 in the thick ascending limb of loop of Henle. These changes result in mixed polycystic and tubulointerstitial kidney disease phenotype with a late onset (60-90 years). DNAJB11 mutations are associated with the clinical phenotype in patients with ADPKD-6 (OMIM: 618061). The disease is transmitted with an autosomal dominant mode, renal cysts are usually small (0, 3-3 cm) and the kidneys are not enlarged. Some patients present with interstitial fibrosis and about half of them present liver cysts.

Results: Index patient is a 59-year-old Caucasian female with CKD stage IIIa, bearing multiple cortical cysts in both kidneys (maximum diameter 2 cm), without kidney size enlargement and a positive family history for CKD: Her father and her grandfather developed CKD-III and IV respectively in advanced age. Clinically, the patient showed a large amount of angiokeratomas in her periumbilical region. Routine blood biochemistry other than renal function was normal. After obtaining informed consent we performed WES which showed a c.532delA (p.T178fs*10) in DNAJB11 gene (Figure 1). Fabry disease was excluded.

Figure 1: Abundant renal cysts of variable dimensions within the renal parenchyma in our index patient (MRI) baring the mutation c.532delA (black box) resulting in an altered protein product (p.T178fs).

Methods: Whole exome sequencing (WES) was performed within 3 generations of a kindred with microcystic kidney disease and CKD progression in advanced age (over 50 years). Bioinformatics analysis was performed with Ingenuity Clinical Insights software (Qiagen Inc.) utilizing Human Gene Mutation Database (HGMD).

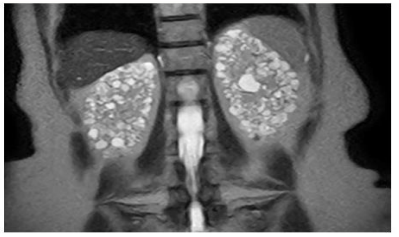
Conclusions: We are describing the first patient with ADPKD6 in Greece. The monoallelic mutation p.T178fd*10 in DNAJB11 causes polycystic kidney disease type 6 with late onset CKD and a full penetrance in each generation (2). Ongoing genetic analysis will show the exact prevalence in the island of Crete and will allow a better description of the clinical phenotype.



DNAJB11 associated clinical phenotype

CKD IIIA

+



ADTKD

Table 3. Characteristics of the four major causes of ADTKD

Condition	ADTKD-*MUC1*	ADTKD-*UMOD*	ADTKD-*REN*	ADTKD-*HNF1B*	ADTKD-*SEC61A1*
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant
Age of onset of CKD (years)	18–50	18–50	First year of life	Variable, may be antenatal	10–50
Onset in childhood	No	Gout rarely	Frequent	May have prenatal findings	Variable
Renal features	Progressive renal disease with bland urinary sediment	Progressive renal disease with bland urinary sediment	Progressive renal disease with bland urinary sediment, Prone to acute kidney injury	CAKUT Progressive renal disease with bland urinary sediment	Progressive renal disease, small dysplastic kidneys without cysts
Gout	In advanced CKD	Early onset, frequent	Early onset, frequent	Early onset, frequent	In second decade of life
Other electrolyte abnormalities	None identified	Low fractional excretion of urate, low urinary excretion of uromodulin	Hyperkalaemia,low urinary excretion of uromodulin	Hypomagnesaemia, hypokalaemia	None identified
Other clinical features	None	None	Anaemia, Hyporeninaemia Mild hypotension	Diabetes, deranged liver function tests, genital malformation, Pancreatic atrophy Variable penetrance mong family members	Congenital anaemia, intrauterine and postnatal growth retardation, polydactyly, mild mental retardation
Histopathology	Non-specific tublointerstitial atrophy	Non-specific tubulointerstitial atrophy	Non-specific tubulointerstitial atrophy	Non-specific tubulointerstitial atrophy	Small foci of tubulointerstitial lesions

- **Renal biopsy will not provide a precise diagnosis** in ADTKD,
- **Autosomal dominant pattern** means that multiple family members may be affected, and the **variable age of onset** and **bland radiological and urinary sediment** findings mean it may be difficult to distinguish the affected from the unaffected through clinical screening alone. **Thus, genetic testing is imperative**.
- Urinary staining may become a useful non-invasive test for ADTKD-MUC1 (MUC1-fs).
- A small molecule **P24 trafficking protein 9**, has been shown to promote lysosomal degradation of the toxic MUC1-fs from cells and reverse proteinopathy.
- The same therapy may have potential treatment implications for ADTKD-UMOD and other proteinopathies.

ADTKD example. WES or MLPA ?

- Male 24 yo, progressive decline of eGFR
- Left kidney agenesis, pancreatic body and tail agenesis
- NID-DM since age 22, currently on GLP1 agonist
- Normal liver function,
- Magnesiuria, Hypomagnesemia, Hyperparathyroidism
- Hypospadias repair surgery some years ago.
- Brother with similar phenotype plus liver dysfunction
- Grandmother and mother with DM

WES or MLPA ?

- WES
  - SLC12A3 c.791C>G HOMO, common variant
  - COL4A3 C1721C>T (p.Pro574Leu) HOMO, benign
  - **HNF1A** c.1460 G>A ,HOMO, DOM, CADD 18, benign
  - **HNF1A** c.79A>C, HOMO DOM, CADD=22, benign
  - PKD2 c.-82 G>C, benign
  - PHEX c1482+31_1482+32delTT GnomAD=0%, VUS
- **MLPA:**
  - **HETEROZYGOUS, DELETION of HNF1B gene, AUTOSOMAL DOMINANT DISEASE (MODY5)**

Study of complement proteins in aHUS & C3G (KDIGO)

Supplementary Table 3. Complement studies for aHUS and C3G

Test	aHUS	C3G
Complement protein levels	C3, C4, FB*, C5*	C3, C4, FB*, C5*
Complement regulatory protein levels	FH, FI, Properdin*, CD46#	FH, FI, Properdin*
Complement split products	C3c*, C3d*, Bb*, sC5b-9*	C3c*, C3d*, Bb*, sC5b-9*
Complement functional assays	CH50, AH50, hemolytic assays, FH assays*	CH50, AH50, hemolytic assays, FH assays*
Autoantibodies	Anti-FH	Anti-FH, anti-FB*, C3Nef*, C4Nef*
Tests to detect plasma cell dyscrasia	-	Serum free light chains, SEP
Genetic screening	*CFH, CFI, C3, CD46, CFB* Genomic rearrangements across the *FH-FHR* locus (e.g., by MLPA) Sequencing of coding regions and assessment of CNV Non-complement genetic screening includes *THBD* and *DGKE*	*CFH, CFI, C3, CFB* Genomic rearrangements across the *FH-FHR* locus (e.g. by MLPA) Sequencing of coding regions and assessment of CNV Non-complement genetic screening includes *DGKE*

*Currently available only at specific laboratories; they are research and not clinically validated assays
#CD46 is also known as MCP
Modified from Angioi *et al.*[14] Investigation for plasma cell dyscrasias is warranted in individuals with C3G as monoclonal gammopathy has been reported in these patients.[15, 16]

Abbreviations: AH50, alternative pathway hemolytic assay; C3, complement component 3; C3Nef, C3 nephritic factor; C4, complement component 4; C4Nef, C4 nephritic factor; C5, complement component 5; *CFB*, complement factor B gene; *CFH*, complement factor H gene; *CFHR*, complement factor H related genes; *CFI*, complement factor I gene; CH50, classical pathway hemolytic assay; CNV, copy number variation; *DGKE* gene, diacylglycerol kinase epsilon gene; FB, complement factor B; FH, complement factor H; FI, complement factor I; MLPA, multiplex ligation-dependent probe amplification; sC5b-9, soluble C5b-9; SEP, serum protein electrophoresis; THBD, thrombomodulin.

Goodship: aHUS and C3 glomerulopathy: a KDIGO conference report Kidney International (2017) 91, 539–551

Diagnostic yield of genetic testing in aHUS

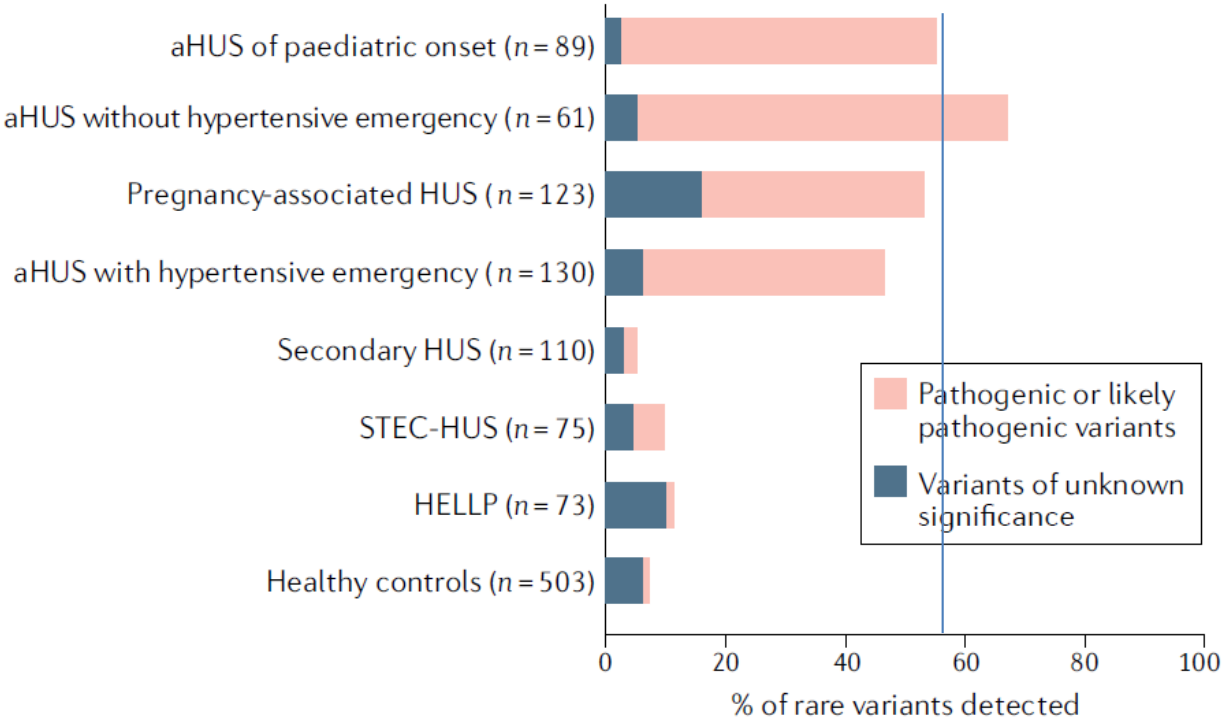


Fig. 2 | **Frequencies of rare variants in *CFH*, *CFI*, *MCP*, *C3*, *CFB* and *THMD* genes identified in TMA.** Pathogenic and likely pathogenic variants in complement genes are more frequent in atypical haemolytic uraemic syndrome (aHUS) of paediatric onset (data from the French HUS cohort[12]), aHUS of adult onset with or without hypertensive emergencies (data from the French HUS cohort[12,18]) and pregnancy-associated HUS

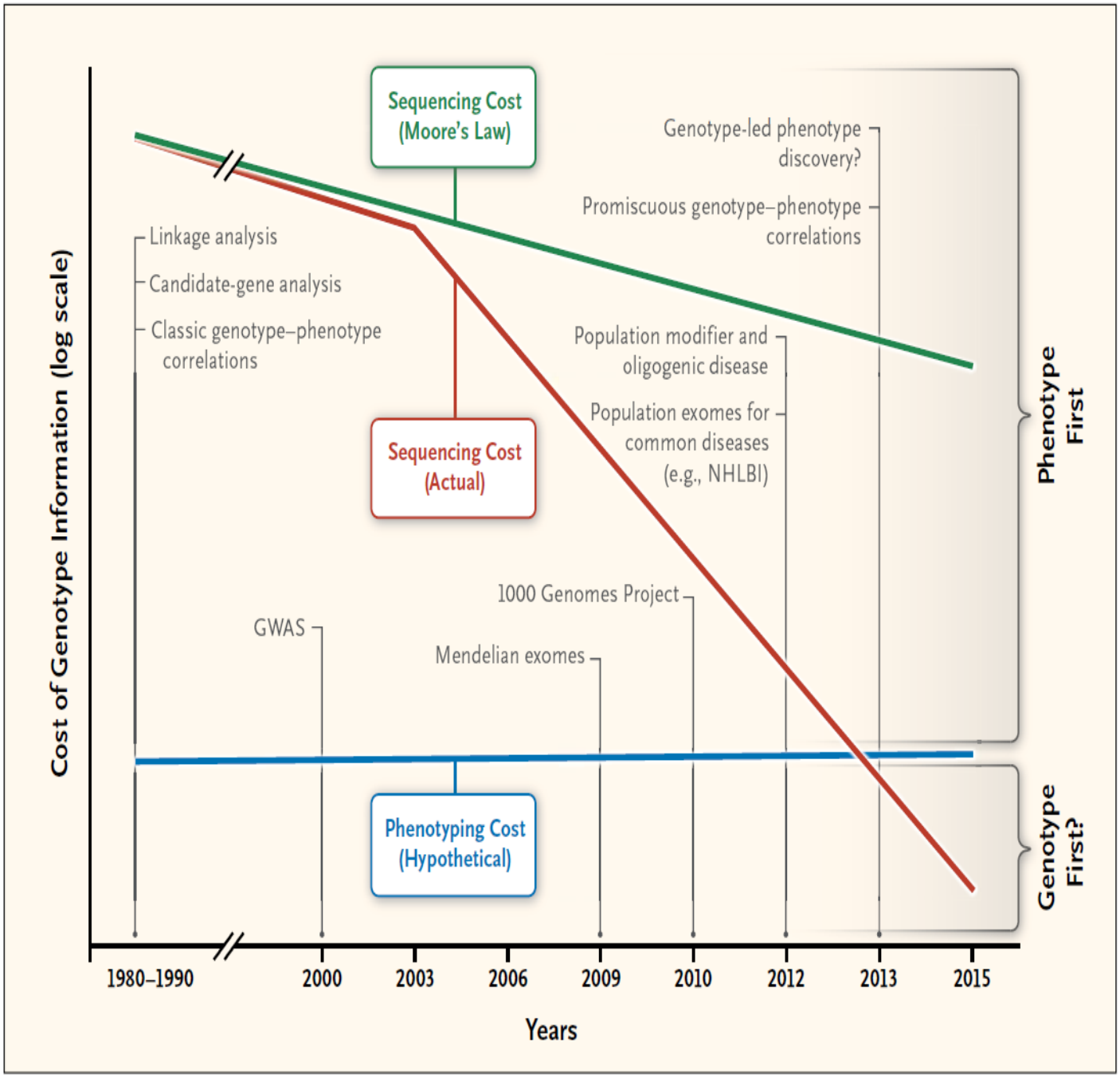
https://doi.org/10.1038/s41581-021-00424-4

Complement genetics in aHUS is a valuable tool

- To provide proof of a link between complement dysregulation and the disease,
- to assess disease severity
- to predict the risk of recurrence after kidney transplantation and enable prophylactic complement blockade
- To predict the risk of disease relapse after discontinuation of eculizumab treatment (60% vs 5%).

Conclusions: genetic testing can help

- Set the correct diagnosis
- Estimate the risk of nephropathy progression,
- Identify other affected organs and to initiate multidisciplinary care
- Guide family counseling
- Allow donor selection for transplantation
- Offer correct treatment, avoidance of unnecessary or dangerous treatments.
- Discovery of new treatments



The Decreasing Cost of Genotype Information.

Lu et al, N Engl J Med, 2014