Genetic testing in the diagnosis of chronic kidney disease

Course on Genetics and Kidney diseases
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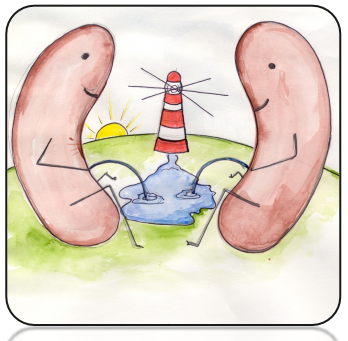

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Working Group on Inherited Kidney Disorders

ERKNet
The European Rare Kidney Disease Reference Network

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Genetic testing in the diagnosis of chronic kidney disease: recommendations for clinical practice

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REVIEW ARTICLE

Review of genetic testing in kidney disease patients: Diagnostic yield of single nucleotide variants and copy number variations evaluated across and within kidney phenotype groups

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KDIGO executive conclusions

www.kidney-international.org

Genetics in chronic kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference

Check for updates

OPEN

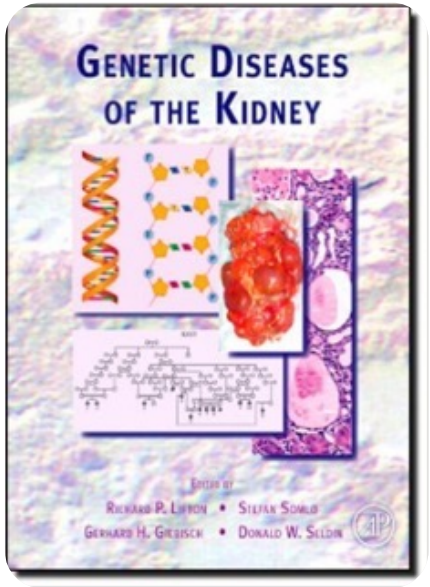
KDIGO Conference Participants[1]

Monogenic diseases underestimated, but important cause chronic kidney disease (CKD)

Monogenic kidney disease:

> 70 % of children progressing to renal-replacement therapy

> 10-15% of adults progressing to renal-replacement therapy



Mutations in > 600 genes associated with inherited kidney diseases
Genetic testing increasingly being used in diagnostic process

ERKNet
The European
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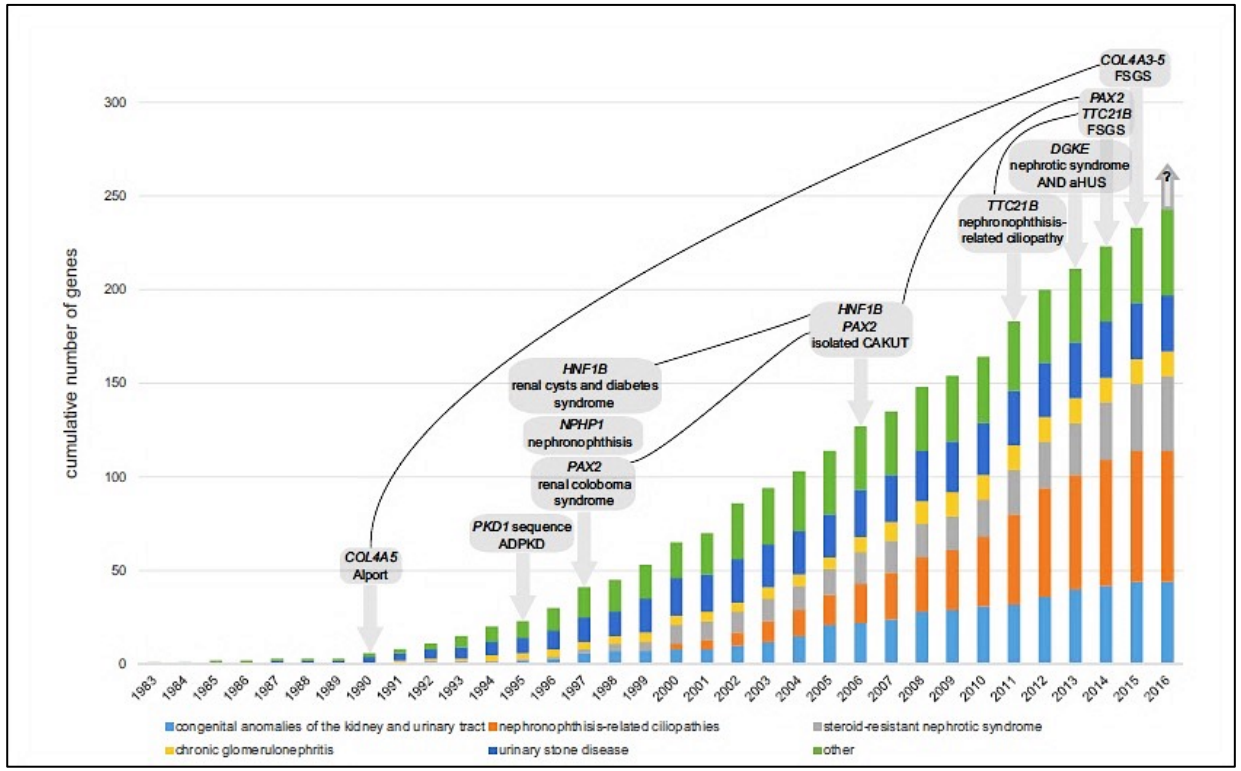
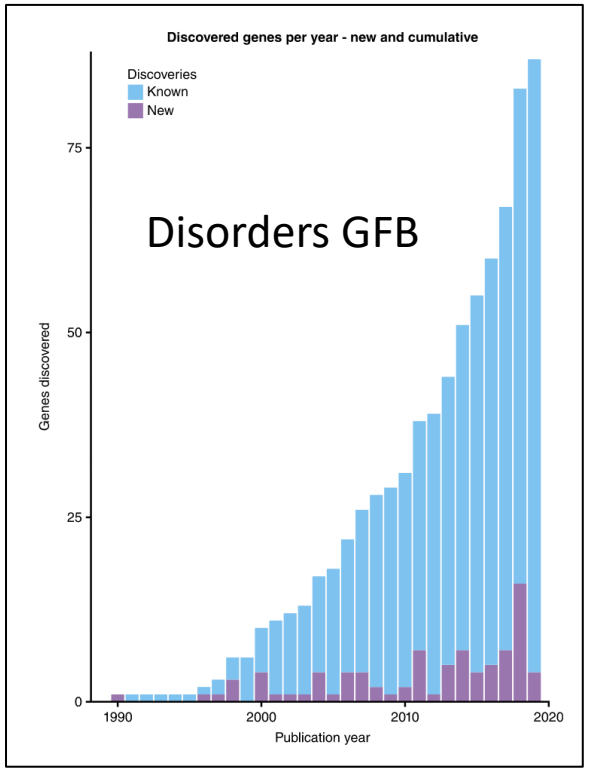
Reference Network

Scientific impact: discovery novel disease genes

Acceleration molecular insights into aetiology of genetic kidney disorders, many of them rare disorders



van Eerde et al., KI 2016



Li et al., CJASN 2020

Different testing modalities and their current indications in nephrology

Test	Indications	Examples
Sanger sequencing	Minimal locus heterogeneity	Fabry disease (*GLA*) Cystinosis (*CTNS*)
CGH/SNP array MLPA	Large copy number variants (CNVs) suspected	CAKUT Nephronophthisis (*NPHP1*)
Targeted phenotype-associated gene panel Targeted ES (virtual gene panel)	Disorders with locus heterogeneity Disorders with overlapping phenotypes Disorders with common pathways	Steroid resistant nephrotic syndrome (SRNS) Hereditary tubulopathies Complement-related disorders
ES	Phenotype indistinct & underlying cause unknown Second tier test after gene panel testing (open up exome)	Unexplained kidney failure
GS	Research for unsolved cases after WES Emerging clinical use	ADPKD (*PKD1*)

Limitations gene testing using MPS gene panels or ES

Reason	Examples
Phenocopies may be missed	Mutations *CTNS* (cystinosis), *AGXT* (primary hyperoxaluria), *GLA* (Fabry) can mimic SRNS
Detection large copy number variants (CNVs) from gene panels/ES data challenging; specific CNV detection algorithms not automatically performed in diagnostic setting	*HNF1B* and *NPHP1* full gene deletions (CAKUT and nephronophthisis, respectively)
Variants in some genomic regions poorly discovered with MPS gene panels or ES	High GC-content in first exon *COL4A3* (Alport syndrome) *PKD1* (ADPKD) has high GC-content and sequence homology with six pseudogenes located nearby
Some pathogenic variants not discovered by any of the MPS-based techniques	Cytosine insertion in variable-number tandem repeat (VNTR) sequences *MUC1* (MUC1-ADTKD)
Variants in non-coding (intronic or regulatory) regions or imprinting defects not detected	Deep intronic mutations in *DGKE* (aHUS) Imprinting defect Beckwith Wiedemann syndrome

Copy number variant (CNV) detection in diagnostics

CNVs: Structural variations in genome of individual in form of gains (duplications) or losses (deletions) of DNA fragments

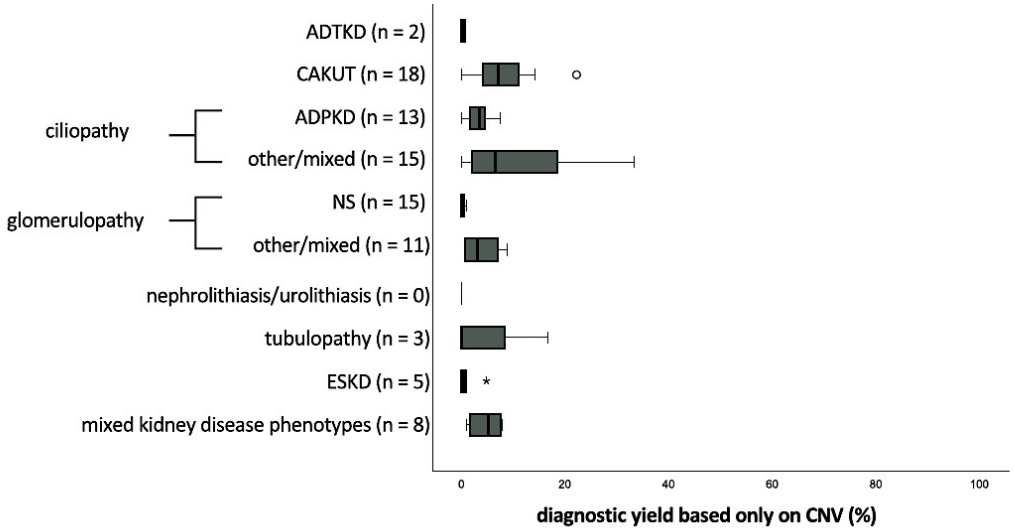
CNVs not easily picked up by MPS-based gene panels or ES

- Sophisticated bioinformatic tools (i.e. ExomeCopy, ExomeDepth) necessary to detect those large CNVs from gene panel or ES data
- Not yet routinely used in all diagnostic laboratories

Preferred methodologies for routine diagnostics of large CNVs in many labs:

- Microarray-based technique (CGH- or SNP-arrays)
- Multiplex ligation-dependent probe amplification (MLPA)

Systematic literature search (115 articles): CNVs are important cause for genetic renal diseases

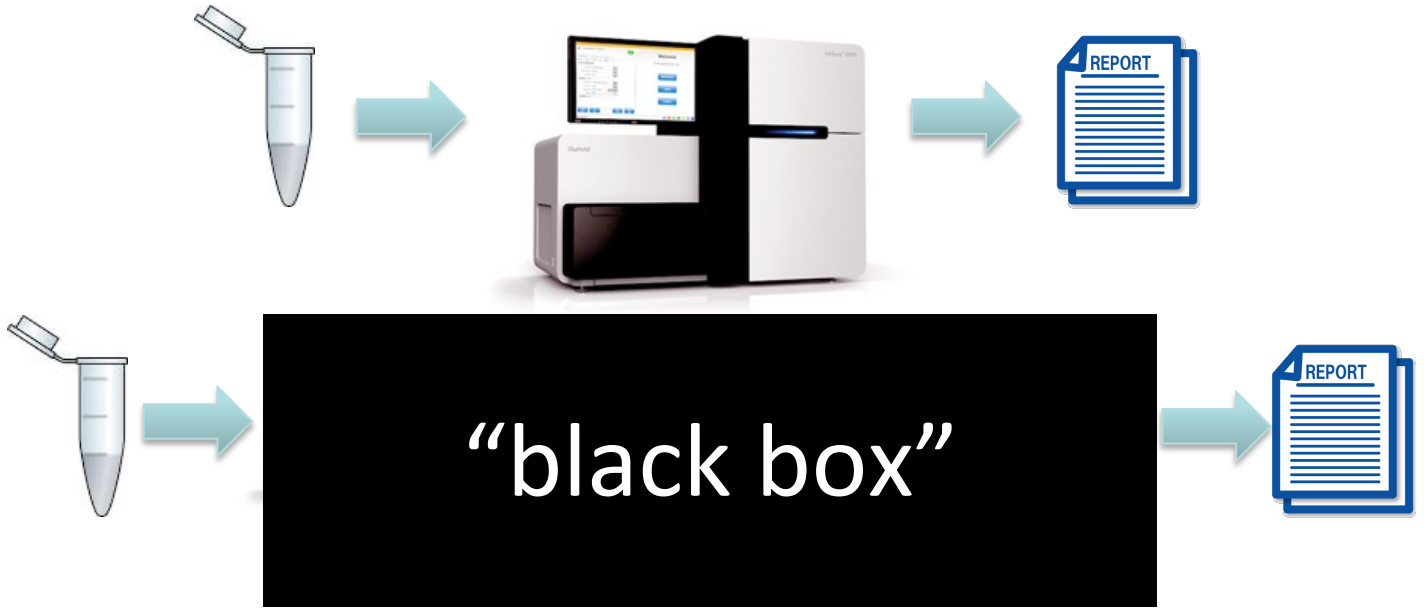


Claus et al., AJMG 2022

Challenge: interpretation identified variants

Genetic testing in MPS era

Sample in → Diagnosis out



Pathogenicity identified variants?

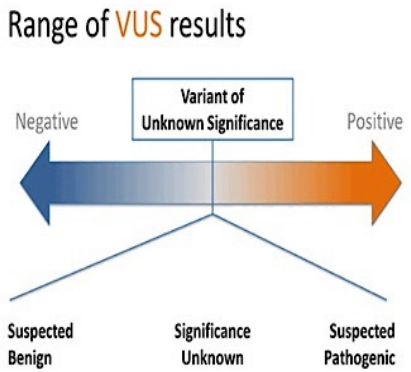
All testing and molecular classification in accredited molecular genetics laboratories
In silico tools for predicting pathogenicity (i.e. SIFT, Polyphen) < 80% accuracy

Interdisciplinary expert boards (including nephrologists, clinical geneticists, molecular biologists) assembled to discuss potential genetic diagnostic findings

American College of Medical Genetics and Genomics (ACMG) variant classification

Class	Description
1	Clearly not pathogenic
2	Unlikely to be pathogenic
3	Unknown significance (VUS)
4	Likely to be pathogenic
5	Clearly pathogenic

Most difficult outcome: class 3, variants of unknown significant (VUS)
Local hospital policies differ whether or not to disclose VUS to patients



ACMG recommendations:

- VUS should not be used in clinical decision-making
- Efforts to resolve classification (i.e. segregation analysis, functional studies, data sharing)

KDIGO participants, KI 2022;Umlai et al., Briefing Bioinform 2022

Pathogenicity identified variants?

Pathogenicity of some variants previously reported as pathogenic mutations questionable with available knowledge of large databases (i.e. gnomAD)

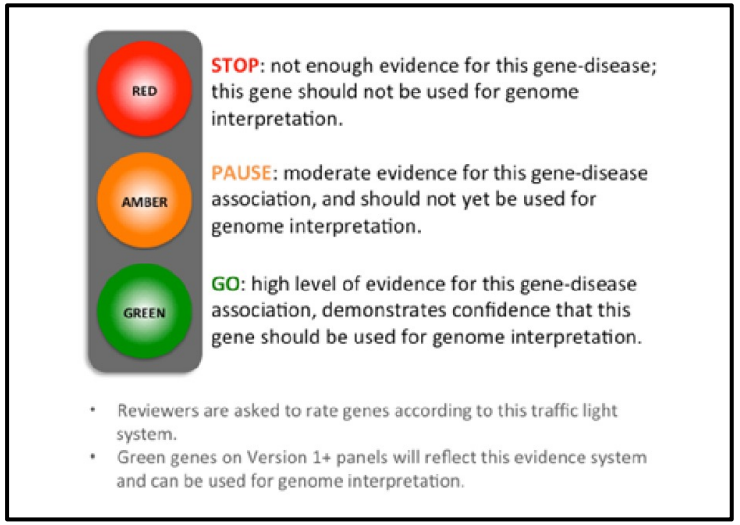
Consult updated clinical variant databases:

- **ClinVar** https://www.ncbi.nlm.nih.gov/clinvar/
- **LOVD** https://www.lovd.nl

Curation of data:

- **ClinGen** (https://clinicalgenome.org): specific clinical domain groups/expert panels helpful in defining clinical relevance identified genes

- **Genomics England PanelApp/PanelApp Australia:** Crowd-sourcing tools to allow gene panels to be shared, downloaded, viewed, and evaluated by scientific Community



https://panelapp.genomicsengland.co.uk/

Increasing clinical impact of MPS-based genetic testing

Establish diagnosis: end diagnostic odyssey

- Bringing peace of mind to family
- Avoiding further expensive and fruitless testing (may obviate need kidney biopsy)
- Reclassification clinical/histological diagnosis

Diagnostic yield significantly increased with MPS-based techniques

Overall **diagnostic yield** in patients with presumably known and unknown causes of CKD:

- 30% in paediatric cohorts
- 6-30% in adult cohorts

Knoers et al., NDT 2022; KDIGO participants, KI 2022

Diagnostic yield targeted ES in adult CKD

- **3000 adult** CKD patients, >21 years of age (two cohorts)
- ES with prioritization variants in **625 nephropathy-associated genes**
- **Diagnostic yield 9.3%**, encompassing 66 monogenic disorders

Clinical Diagnosis	Sequencing Performed	Diagnostic Variants Present	Diagnostic Yield
	number of patients		*percent*
Congenital or cystic renal disease	531	127	23.9
Glomerulopathy	1411	101	7.2
Diabetic nephropathy	370	6	1.6
Hypertensive nephropathy	319	8	2.5
Tubulointerstitial disease	244	11	4.5
Other	159	6	3.8
Nephropathy of unknown origin	281	48	17.1
Total	3315	307	9.3

Groopman et al., NEJM 2019

Diagnostic yield targeted ES in adult CKD

Study (n= total adult CKD/ESRD patients)	ES-based MPS panel n=nephropathy-associated genes	Total diagnostic yield %	Diagnostic yield within tested group CKD unknown origin (%)
1 (92) 58% familial	287	23	56
2 (3000)	625	9.3	17.1
3 (114) 68% familial 14% extrarenal	478	37	47

Diagnostic yield influenced by: sample size, inclusion criteria, MPS approach, selection genes

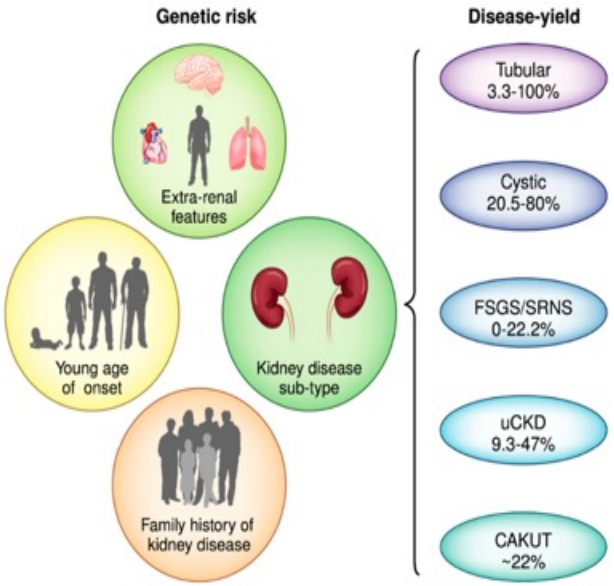
All research cohorts !

1. Lata et al., 2018; 2. Groopman et al., NEJM 2019; 3. Connaughton et al., KI 2019

Important points

- In study 3 diagnostic yield not different between cases with childhood-onset and adult-onset CKD
- 10-22% **reclassification** clinical/pathological diagnosis

Predictors high yield

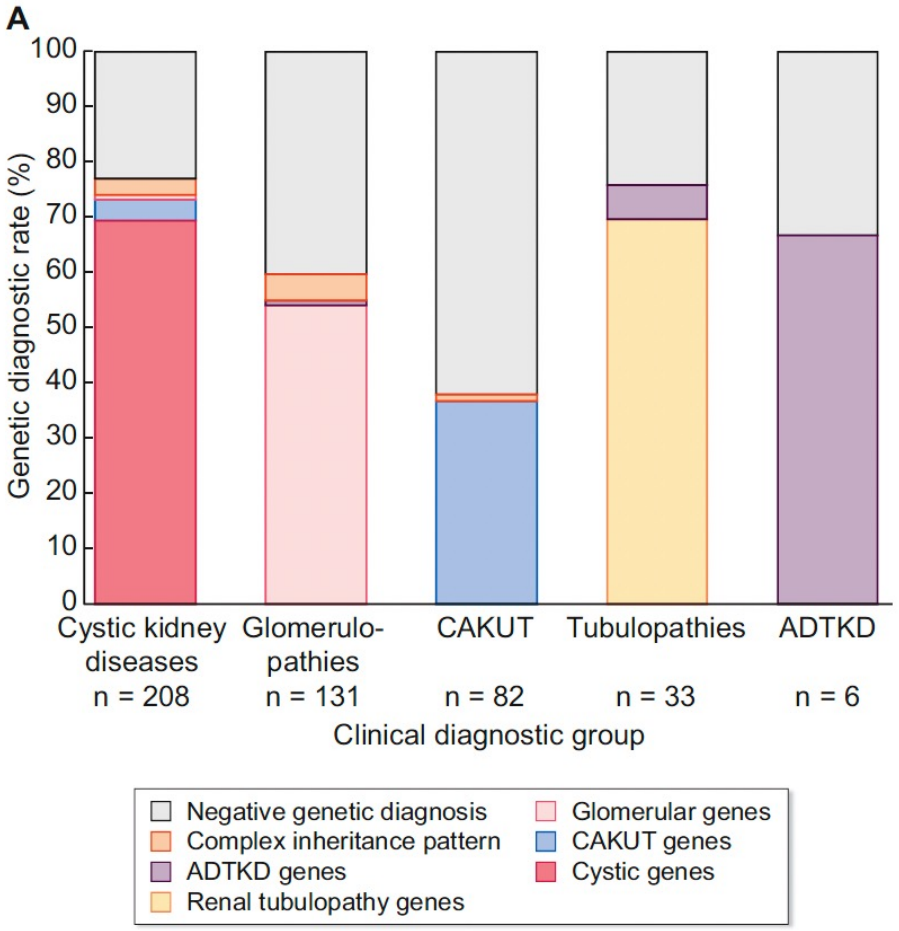


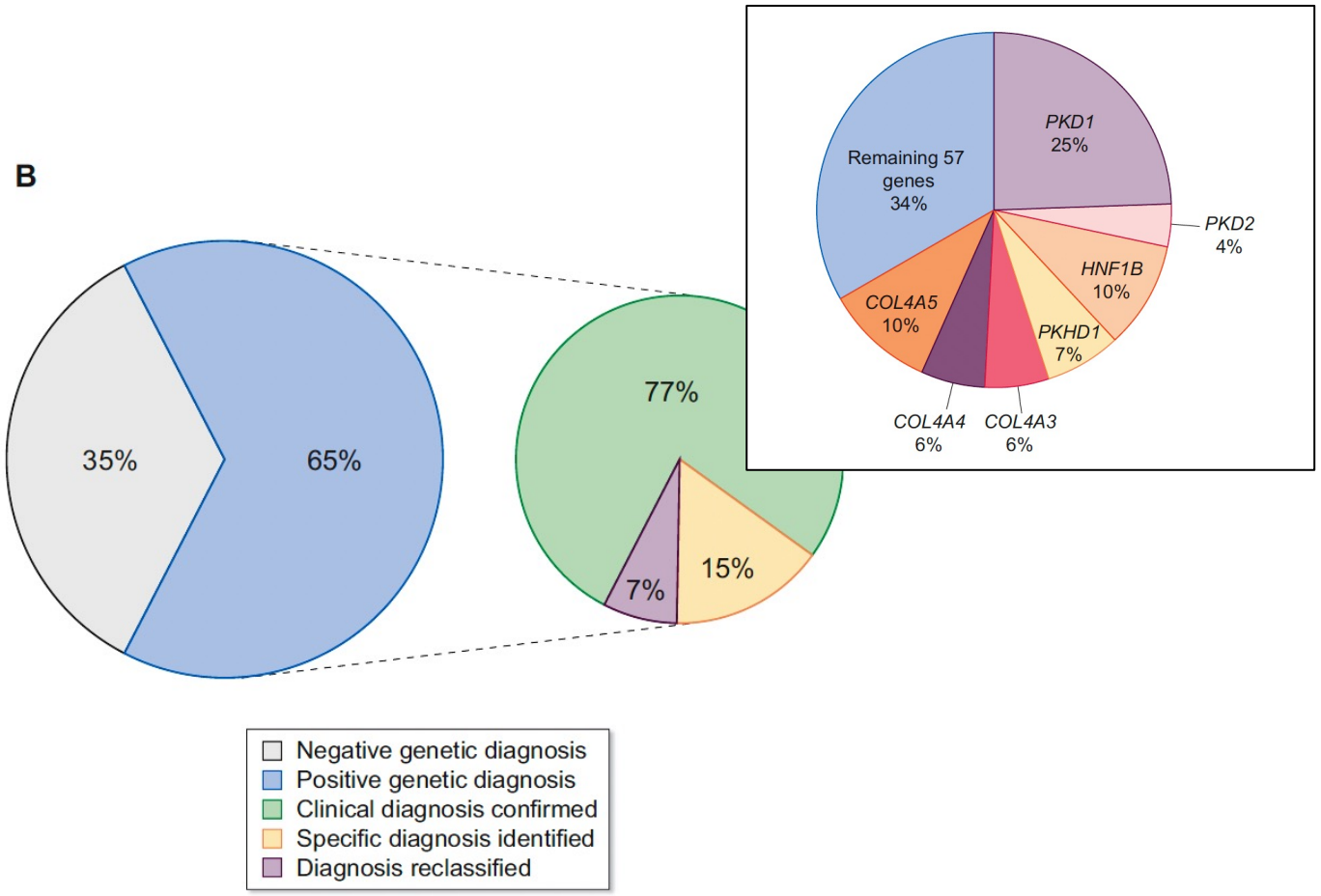
Cocchi et al., CJASN 2020

Diagnostic yield genetic testing clinical study

460 patients from different Spanish hospitals suspected of monogenic disease

- Early onset CKD (0 month-30 years)
- Family history 49%
- Extra-renal manifestations 36%



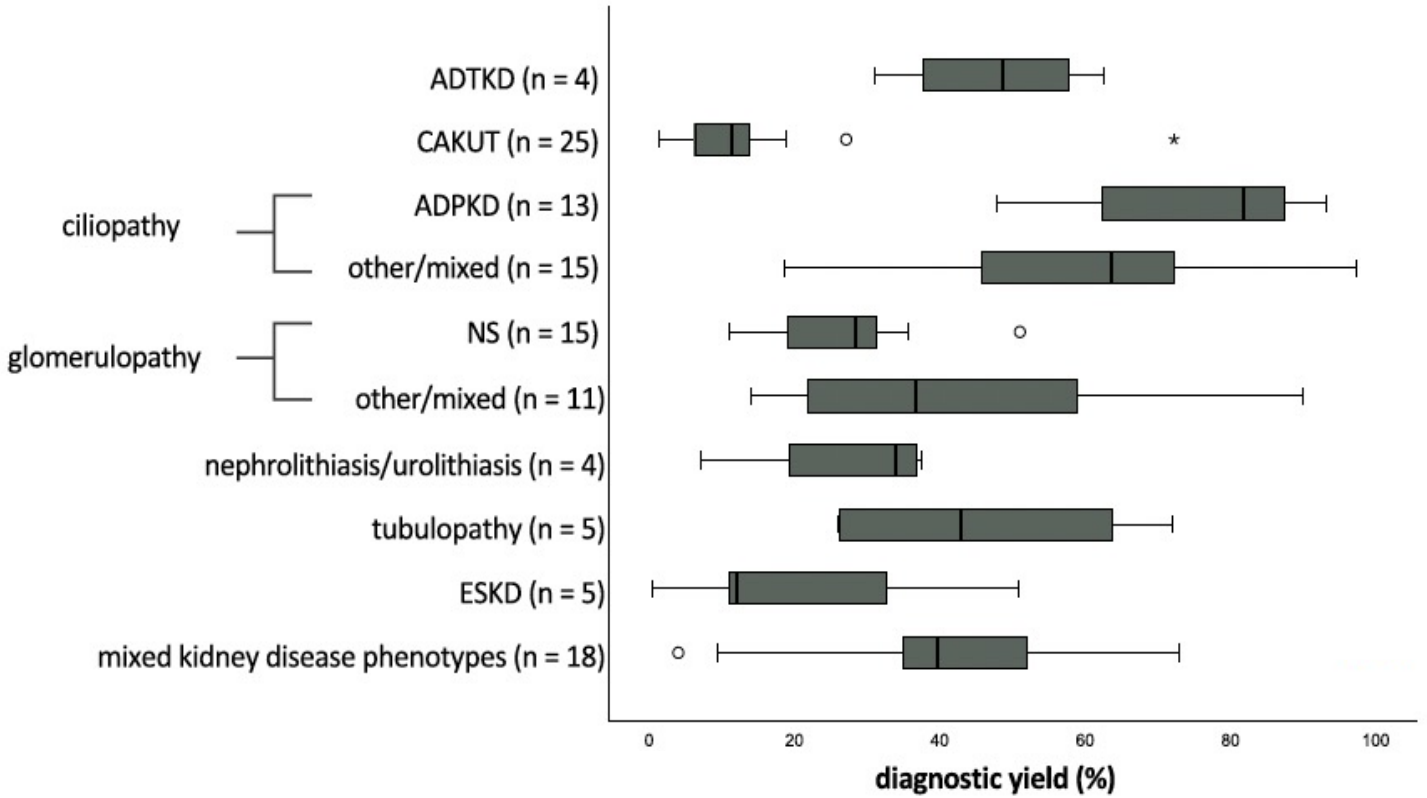


Custom-capture based kidney disease panel , 316 genes

Domingo-Gallego et al., NDT 2022

Overall diagnostic yield genetic testing 115 studies

Systematic literature search (115 articles)

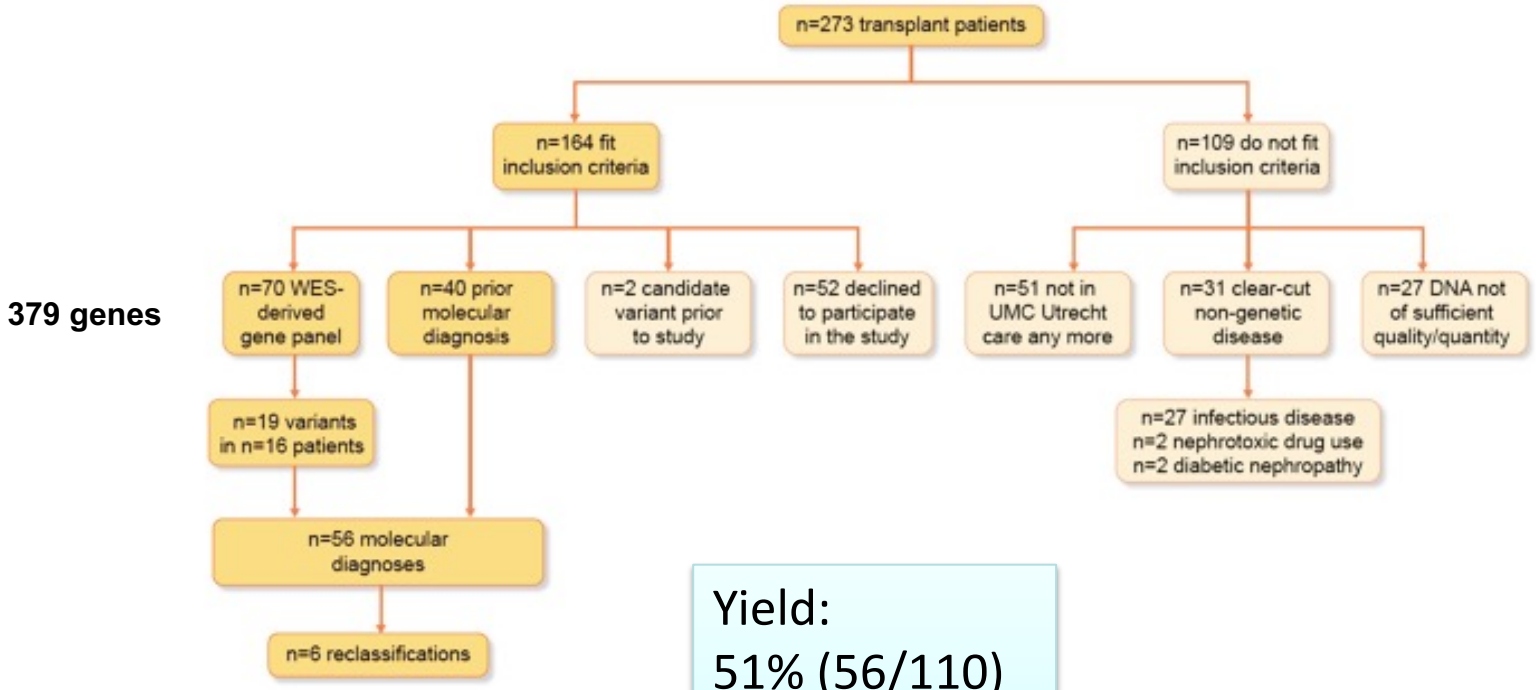


Claus et al., AJMG 2022

Test viability “genetics-first” approach for CKD in daily practice

Inclusion: kidney transplant recipients with first kidney transplant at age <50 and without clear-cut non-genetic disease

No selection for: familial cases, cases with very early onset, phenotypes with high likelihood of underlying genetic cause



Yield:
51% (56/110)
34% (56/164)
21% (56/273)

Snoek et al., NDT 2022

Added value genetic testing to renal biopsy

Molecular diagnosis	Original histological diagnosis	Reexamined histological diagnosis	Likelihood of MGKD based on reexamined biopsy
NPHS2	FSGS	FSGS	Not determinable due to lack of IF and EM
COL4A3	Primary FSGS	FSGS	Not determinable due to lack of IF and EM
INF2	Secondary FSGS	Secondary FSGS	Not likely
NPHS2	FSGS, not all classifications for Finnish type	Diffuse mesangial sclerosis	Very likely
PAX2	Secondary FSGS	Secondary FSGS	Not likely
COL4A3	Possibly Alport syndrome	Alport syndrome	Very likely
COL4A4	IgA nephropathy	IgA nefropathy. Oxford-score: M1, E1, S1, T1	Not likely
CFTR	Nodular glomerulosclerosis	Diabetic nephropathy	Not likely

Snoek et al., NDT 2022

Dutch nationwide prospective VARIETY cohort study

Determine diagnostic yield of MPS-based gene panel testing in patients with unexplained CKD in routine healthcare setting

Nationwide cohort study

- Patients can be included from every hospital in the Netherlands
- Active screening in 7 hospitals

Patients

- eGFR <60 mL/min/1.73 m^2 before age of 50 years
- CKD due to unknown/unclear cause

Clinical and demographic data collection

- Electronic health record
- Questionnaire

VARIETY

umcg

Patients	N
Included	348
Results from genetic testing	229

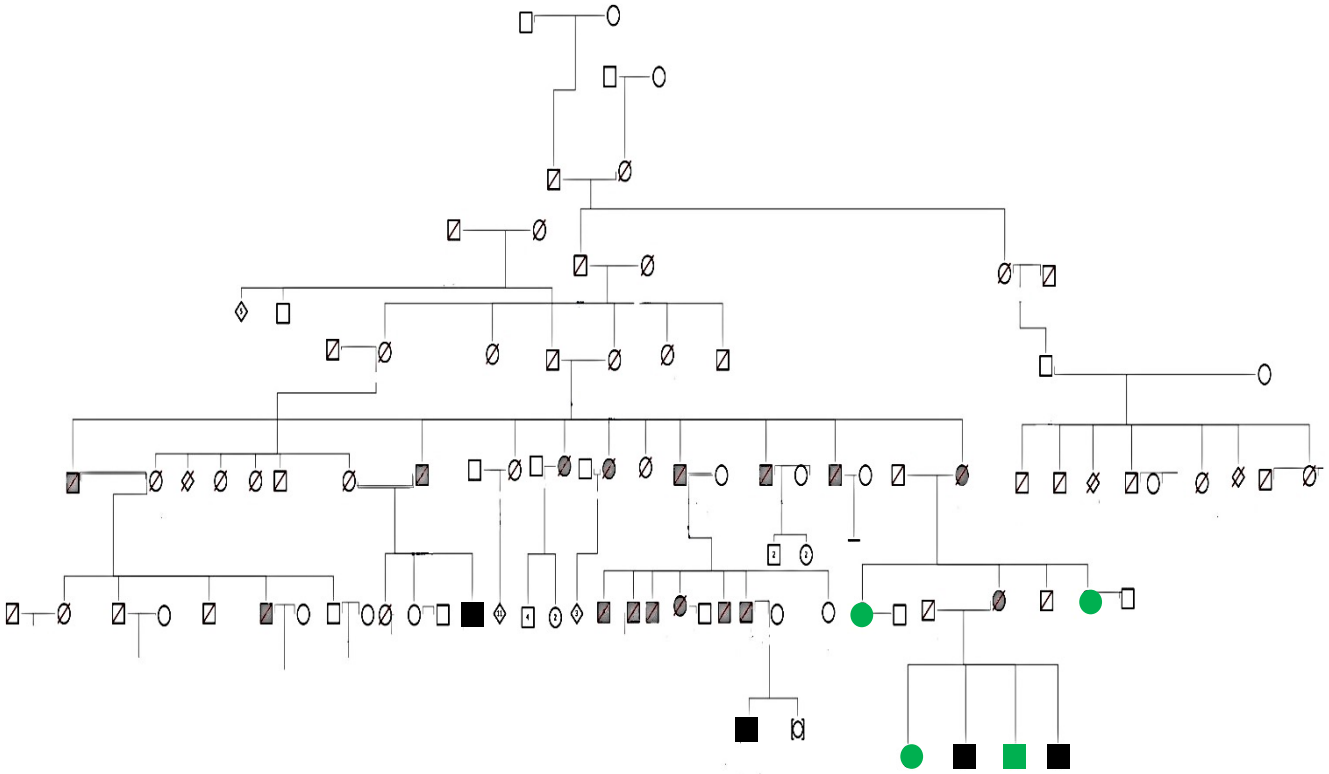
Interim results:

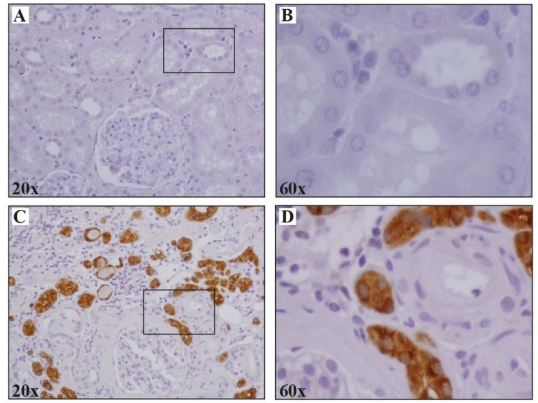
- Diagnostic yield 19%
- VUS 12%
- Carrier 11%

Most involved genes: *COL4A3-4-5, NPHP1*

De Haan, BMC open 2022

Unexplained early-onset kidney failure in 60 years known large Dutch family finally explained





Immunohistochemical detection MUC1-fs in kidney tissue from control and patient

ES and filtering for 141 CKD genes → ***MUC1*** frameshift variant p.(Ser119Profs*119)
Classical *MUC1* cytosine insertion analysis: negative

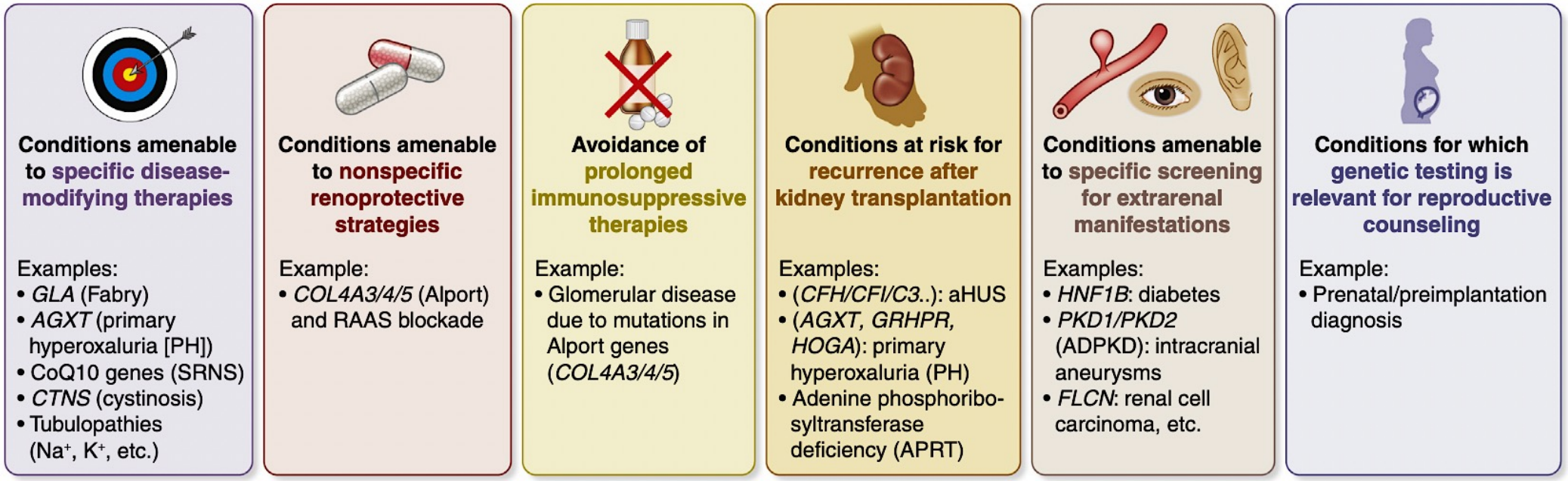
Autosomal Dominant Tubulo-Interstitial Kidney Disease (ADTKD)- *MUC1*

de Haan et al., submitted

Increasing clinical impact of MPS-based genetic testing in CKD

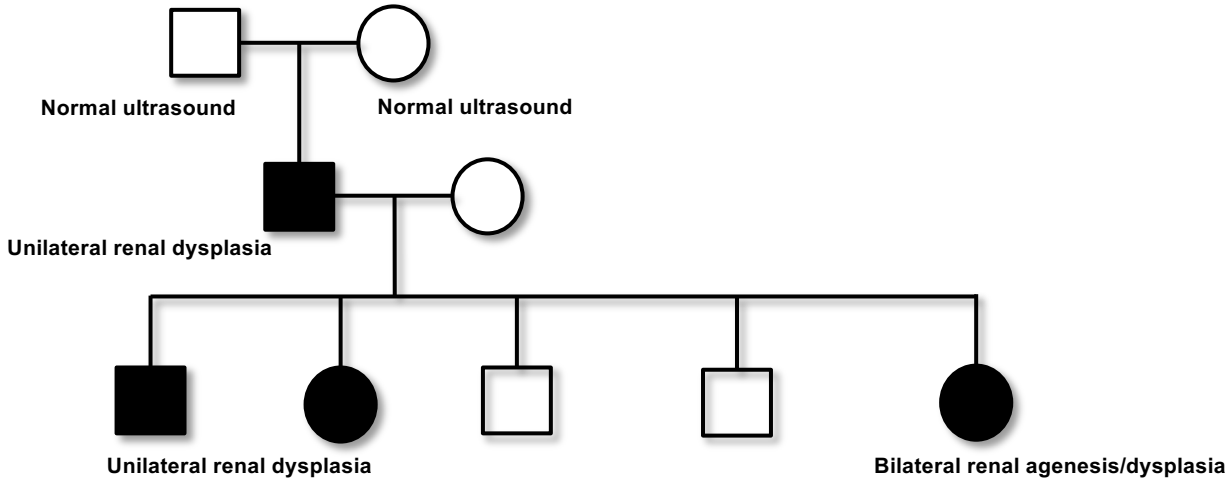
Establish diagnosis: **end diagnostic odyssey**

Clues for **management**

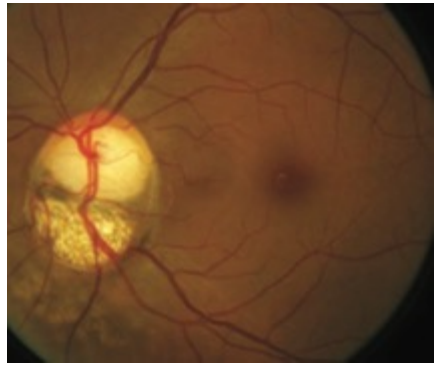


Knoers et al., NDT 2022; KDIGO participants, KI 2022

Clinical impact genetic diagnosis in CKD: screening for extrarenal manifestations



***ES: de novo pathogenic PAX2* mutation**



Ophthalmological examinations: optic nerve coloboma's in *PAX2* mutation carriers

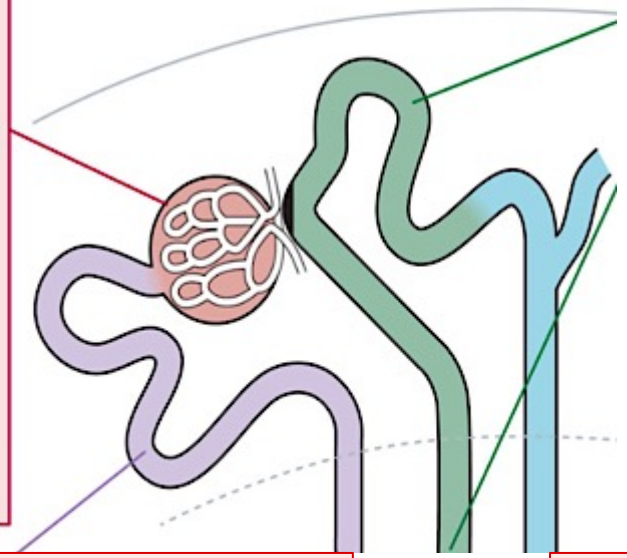
➔ **Renal coloboma syndrome**

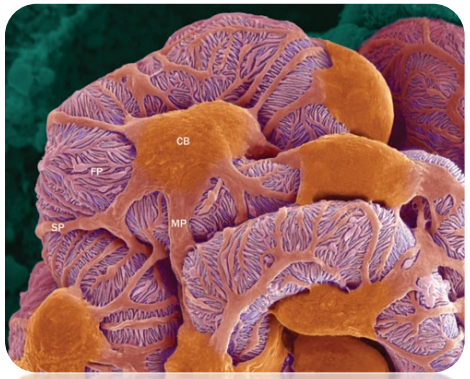
Clinical impact genetic diagnosis in CKD: prognosis and management for glomerulopathies

Glomerular diseases

- Congenital steroid-resistant nephrotic syndrome
- Denys-Drash syndrome, Frasier's syndrome
- Wilms' tumour, aniridia, genitourinary abnormalities, and mental retardation (WAGR) syndrome
- Pierson's syndrome
- Nail-patella syndrome
- Schimke immuno-osseous dystrophy
- Mitochondrial disorders with steroid-resistant nephrotic syndrome
- Fabry's disease
- Alport's syndrome
- Benign familial haematuria (thin basement membrane)
- Fechtner syndrome (Alport's syndrome with macrothrombocytopenia)
- Alport's syndrome with leiomyomatosis
- Familial amyloidosis

Adapted from Devuyst et al., Lancet 2014





Welsh & Saleem 2011

Steroid-resistant nephrotic syndrome

- **Autosomal recessive**
- **Autosomal dominant**
- **X-linked**
- **> 50genes**

Alport syndrome

- **X-linked**
- **Autosomal dominant**
- **Autosomal recessive**
- **3 genes**

Clinical impact genetic diagnosis in CKD: prognosis and management for glomerulopathies (1)

Prognosis

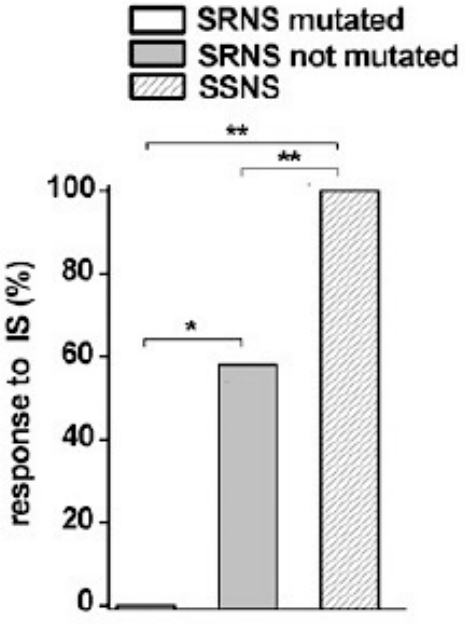
Type *COL4A* mutation in patients with Alport syndrome: information regarding renal and extrarenal (e.g. hearing loss) phenotypes

Prevent prescription of ineffective therapy

Immunosuppressive drugs should not be given to patients with genetic glomerulopathies.
Instead RAAS-inhibition for nephroprotection

Recommendation regarding (change) in therapy

Identification pathogenic variants in genes co-enzyme Q10 biosynthesis pathway (i.e. *COQ6, ADCK4*) in patients with rare form of nephrotic syndrome → treatment with co-enzyme Q10 beneficial



Giglio et al., JASN 2015

Clinical impact genetic diagnosis in CKD: prognosis and management for glomerulopathies (2)

Palliative care:

Case own practice: Severely ill female neonate with congenital nephrotic syndrome, severe brain abnormalities, hypothyroidism, facial dysmorphism

ES: Galloway-Mowat syndrome (nephrosis-microcephaly syndrome) → Palliative care introduced

Predicting post-transplant recurrence kidney disease

Very low in genetic glomerulopathies

In setting of kidney transplantation/eligibility of living related donors

Donor screening of diseases with dominant transmission/ intra-familiar variability and incomplete and age-dependent penetrance (i.e. *NPHS2, COL4A3/A4/A5*)

Indications genetic testing CKD

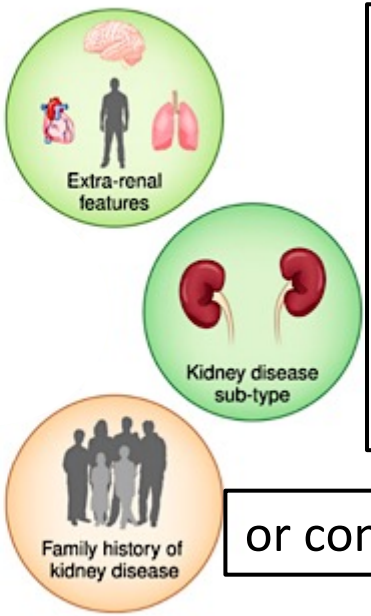
Conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference

- Clinical work indicates possibility genetic disease:
  - High prevalence of monogenic subtypes in clinical category (cystic disease/SRNS)
  - Positive family history
  - Early age of onset
  - Multisystem features
  - Consanguinity
  - Possibility of identifying disease amenable to targeted treatment (Fabry)
- At-risk family member (potential kidney donor)
- Alternative to kidney biopsy (patients with high-risk biopsy-related complications)
- CKD of unknown/unclear aetiology
- Information to guide continuation of immunosuppressive therapy
- Genetic testing can provide prognostic information (Alport)
- Diagnosis diseases with high risk of recurrence in kidney allografts (aHUS/TMA)

KDIGO participants, KI 2022

Genetic testing in CAKUT ?

Predictors high diagnostic yield



Higher in severe CAKUT affecting both renal parenchymas, with or without urinary tract defects

Posterior urethral valves/isolated ureteral phenotypes rarely associated with known monogenic cause: no standard genetic testing

or consanguinity

Adapted from Cocchi et al., CJASN 2020

No one-size-fits all approach:

Consider:

- Potential diagnostic yield/clinical clues for monogenic CAKUT
- Test's costs
- Payer's situation
- Ethical issues (unsolicited findings)

Westland et al., CJASN 2021, Knoers et al., NDT 2021

Genetic testing CKD of unknown/unclear aetiology

Who?

Patients with severe CKD/ESKD

- onset before the age of 50
- clear-cut non-genetic diagnosis (e.g. acute nephrotoxicity, diabetec nephropathy, infectious nephropathy) has been excluded

What?

Tiered exome-based diagnostic approach

- large targeted multi-gene panel, involving all known nephropathy genes
- open up exome backbone in case no causative variant(s) is (are) identified

Why?

- Management
- Family planning/Reproductive options
- Decisions about transplantation
- Testing family members at risk
- Eligibility living-related kidney donor

Importance of pre- and post-test counselling

Recommendations pre-test genetic counselling

- Inform patients on possible outcomes of genetic test, including the possibility test may not give any positive results
- Emphasize that a genetic diagnosis may, but not always will lead to change in management and/or to prognostic information
- Mention possible psychosocial consequences of receiving a definite diagnosis, including consequences for prognosis, and the chances of developing extrarenal symptoms in some disorders
- Mention possible implications for insurability: i.e. Alport syndrome genetic testing in patient with mild hematuria
- Discuss possibility of VUS and unsolicited findings (UFs), and their potential implications, including those for family members, explain the hospital's policy with regard to these findings and emphasize the patient's right to not receive these results

Unsolicited findings (UFs) from MPS-based genetic testing

- Unanticipated findings not related to initial reason for genetic testing
  - Overall frequency UFs: 0,58%
  - 0,03% when restricted disease gene panels were used
  - 1,03% when exome was analysed

- Could be predictive of risk for other diseases, which may or may not be medically actionable

- May also have implications for family members (i.e. *BRCA1/2* mutations)

- Reporting UFs subject of ongoing debate:
  - **Recommendation ACMG**: identification and return of IFs from a minimum of 59 actionable genes, unless patients opt out

  - **Recommendations European Society of Human Genetics & Canadian College Medical Geneticists**: restrict testing to regions of genome linked to patient's phenotype in order to avoid detection of UFs

  - None of these policies have been accepted as general standard

Van der Schoot et al., EJHG 2022

Importance of pre- and post-test counselling

Recommendations post-test genetic counselling

- In case of (likely) pathogenic mutations, provide more information on the associated disease and if possible, on prognosis and possibilities for (change of) management. Also mention the patient advocacy groups for the specific disease(s)

- Discuss recurrence risks and possibilities for family planning, including reproduction options, such as prenatal diagnosis and pre-implantation genetic testing

- Discuss the potential implications of the genetic results for family members and, if applicable, mention the possibility of presymptomatic testing

- In case of returning VUS, discuss the need for additional investigations

Take home messages

- MPS-based gene panels and ES have increasingly found their place in routine clinical diagnosis of monogenic kidney diseases with important implications for diagnostic yield (end diagnostic odyssey) and management
- Predictors of high diagnostic yield are: family history, extrarenal features, young age of onset, kidney disease type (high in cystic kidney disease, ADTKD, tubulopathies, steroid-resistant nephrotic syndrome, low in CAKUT)
- Genetic testing: reclassification clinical/pathological diagnosis in 10-22% of cases
- Genetic testing may also be useful in CKD of unknown origin, with onset before age of 50 and when clear-cut non-genetic diagnosis has been excluded
- Management: disease specific treatment, renal protection, recurrence risk after transplantation, screening extrarenal manifestations, carrier screening, counseling and reproductive options
- Challenges in establishing pathogenicity of identified variants; data sharing and consulting updated variant databases and variant curation expert panels are essential
- Important ethical challenges; especially how to deal with VUSses and with unsolicited findings

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