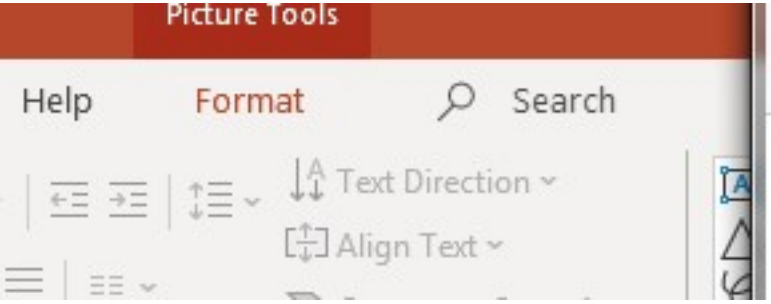
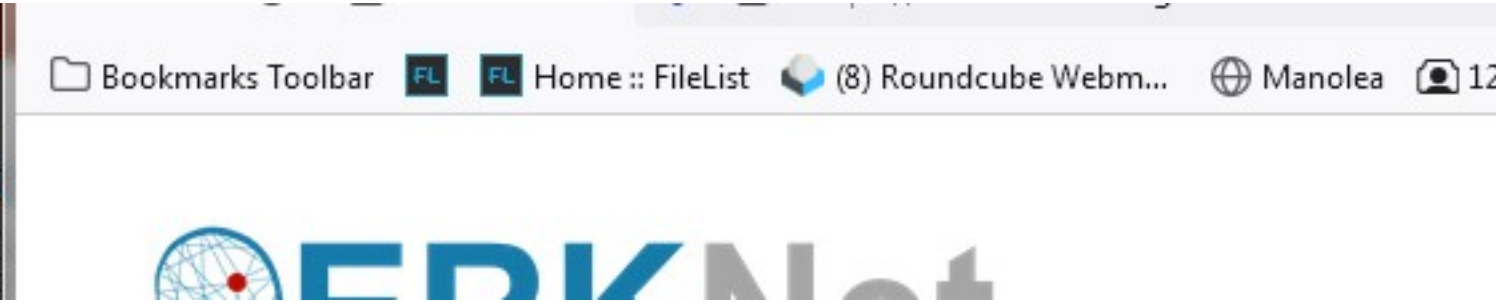




Complement role in kidney pathology

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

Adrian Lur

04 Apr 20

Sko

Conflict of interests

…some

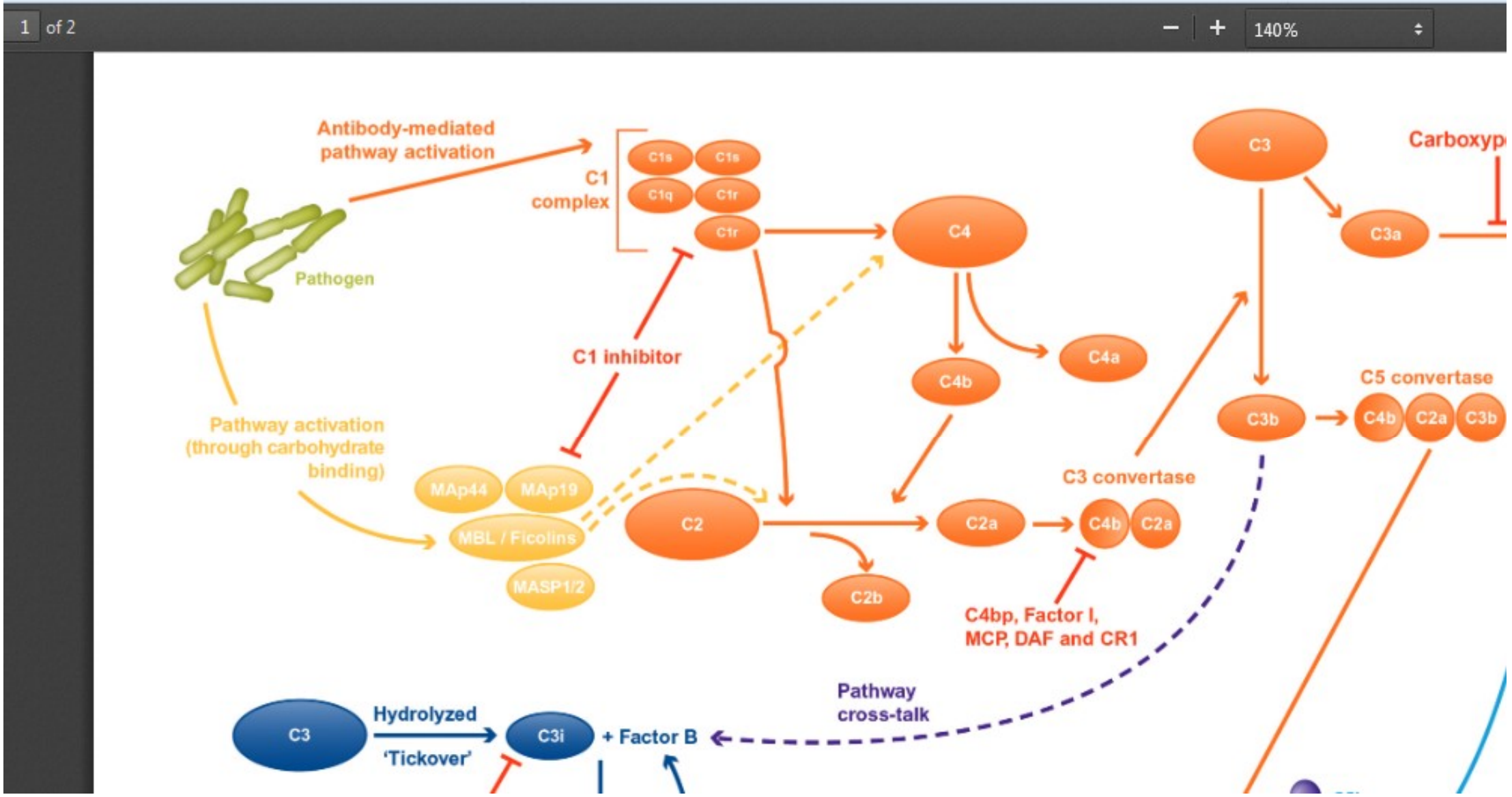
Alexion, Alnylam, Akari, Baxter, Orphan Europe, Abbvie

- General Aspects about complement
- Complement testing – driving the patient management (Examples)
- New therapies

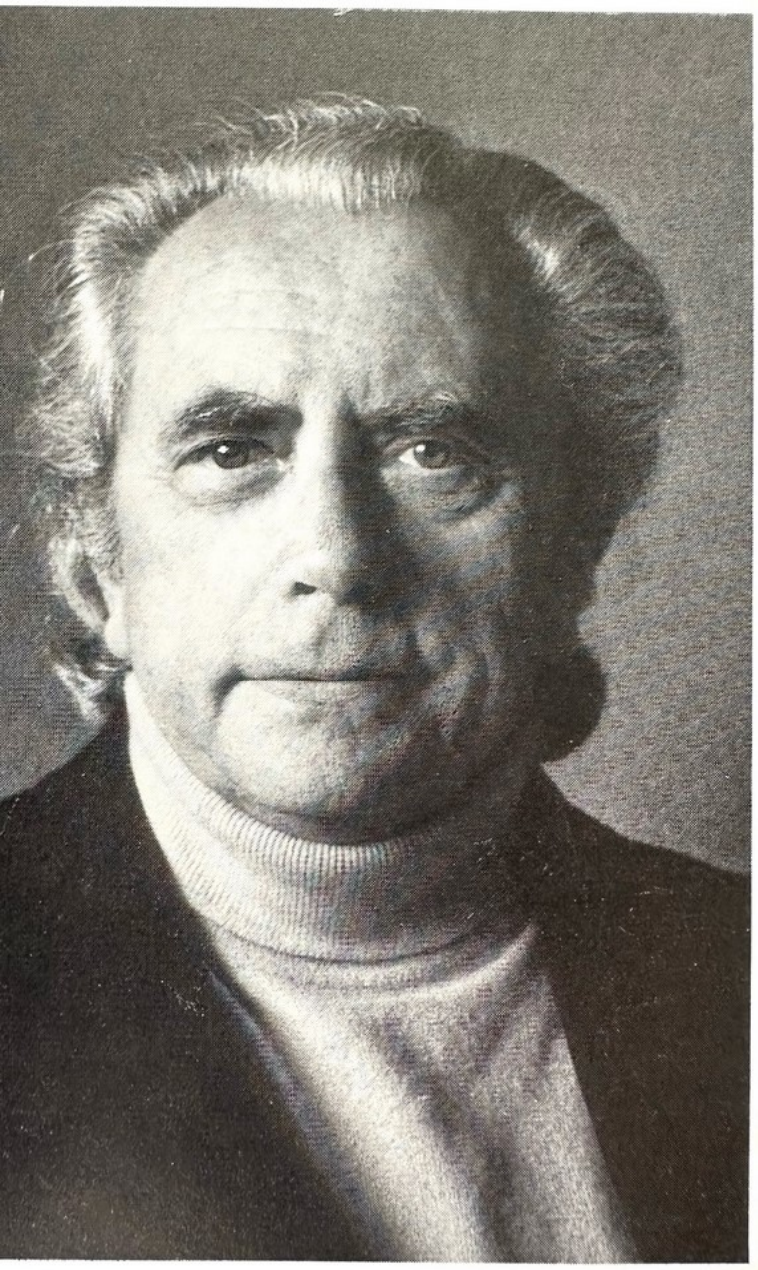
Complement system – 500 million years ago

Complement system – 100 years ago

- Serum from guinea pigs that had recovered from cholera killed the cholera bacterium in vitro
- Heating the serum destroyed its killing activity
- The heat-inactivated serum maintained its ability to protect the animals from illness
- 1899 - Paul Ehrlich renamed the heat-sensitive component "complement”….. because it is something in the blood that "complements" the cells of the immune system

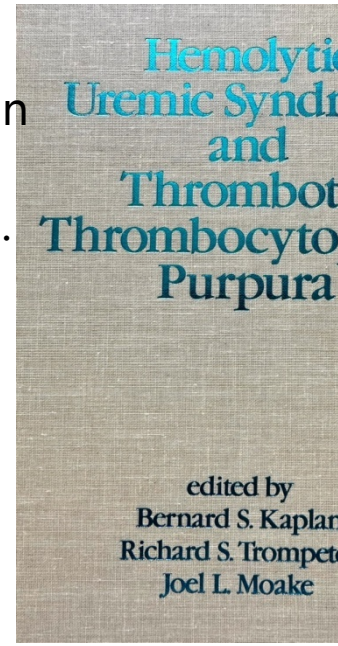


http://www.abcam.com/index.html?pageconfig=resource&rid=15159&source=pagetrap&viapagetrap=



e 1 Professor Conrad Gasser, 1912–1982.

Gasser C, Gautier E, Steck A, Siebenmann RE, Oechslin R: [Hemolytic-uremic syndrome: Bilateral necrosis of the renal cortex in acute acquired hemolytic anemia]. Schweiz Med Wochenschr 85: 905–909, 1955



Hämolytisch-urämische Syndrome:
Bilaterale Nierenrindennekrosen bei akuten erworbenen hämolytischen Anämien

Von C. Gasser, E. Gautier und Annemarie Steck (klinischer Teil) und R. E. Siebenmann und R. Oechslin (pathologisch-anatomischer Teil)

Hiezu 4 Abbildungen Seite 929

Aus einer Gruppe von 10 letal verlaufenen Krankheitsbildern, die mit Urämie und hämolytischer Anämie einhergingen, werden 5 akute Fälle beschrieben, die charakterisiert sind durch eine aus unbekannter Ursache plötzlich einsetzende akute intravasale Hämo-

Figure 3 Gasser, C., Gautier, C., Steck, A., Siebenmann, R. E., and Oechslin, R., Hamolytisch-uramische syndrome: Bilaterale nierenindennekrosen bei akuten erworbenen hamolytischen anamien. *Schweiz. Med. Woschenschr.*, 85:905–909 (1955).

plan BS, Thomson PD, MacNab GM: Letter: Serum- complement levels in haemolytic-uraemic syndrome. ncet 2: 1505–1506, 1973

plan BS: Haemolytic uremic syndrome with recurrent episodes: An important subset. Clin Nephrol 8: 495–3, 1977

ha MR, van Es LA: Further evidence for the antibody nature of C3 nephritic factor (C3NeF). J Immunol 123: 5–758, 1979

rry PL, McEnery PT, McAdams AJ, West CD: Membrano- proliferative glomerulonephritis in two sibships. Cli phrol 16: 101–106, 1981

leman TH, Forristal J, Kosaka T, West CD: Inherited complement component deficiencies in mbranoproliferative glomerulonephritis. Kidney Int 24: 681–690, 1983

Dysregulation of Complement

- Atypical hemolytic uremic syndrome,
- C3 glomerulopathies

Overactivation of Complement

- Lupus nephritis
- Anti-glomerular basement membrane glomerulonephritis
- Antineutrophil cytoplasmic antibody-associated vasculitis
- Membranous nephropathy
- C1Q nephropathy
- IgA nephropathy
- Immune complexes-associated membranoproliferative glomerulonephrit

Complement In Renal Transplantation

- Ischemia-reperfusion injury,
- Cell-mediated rejection,
- Antibody-mediated rejection

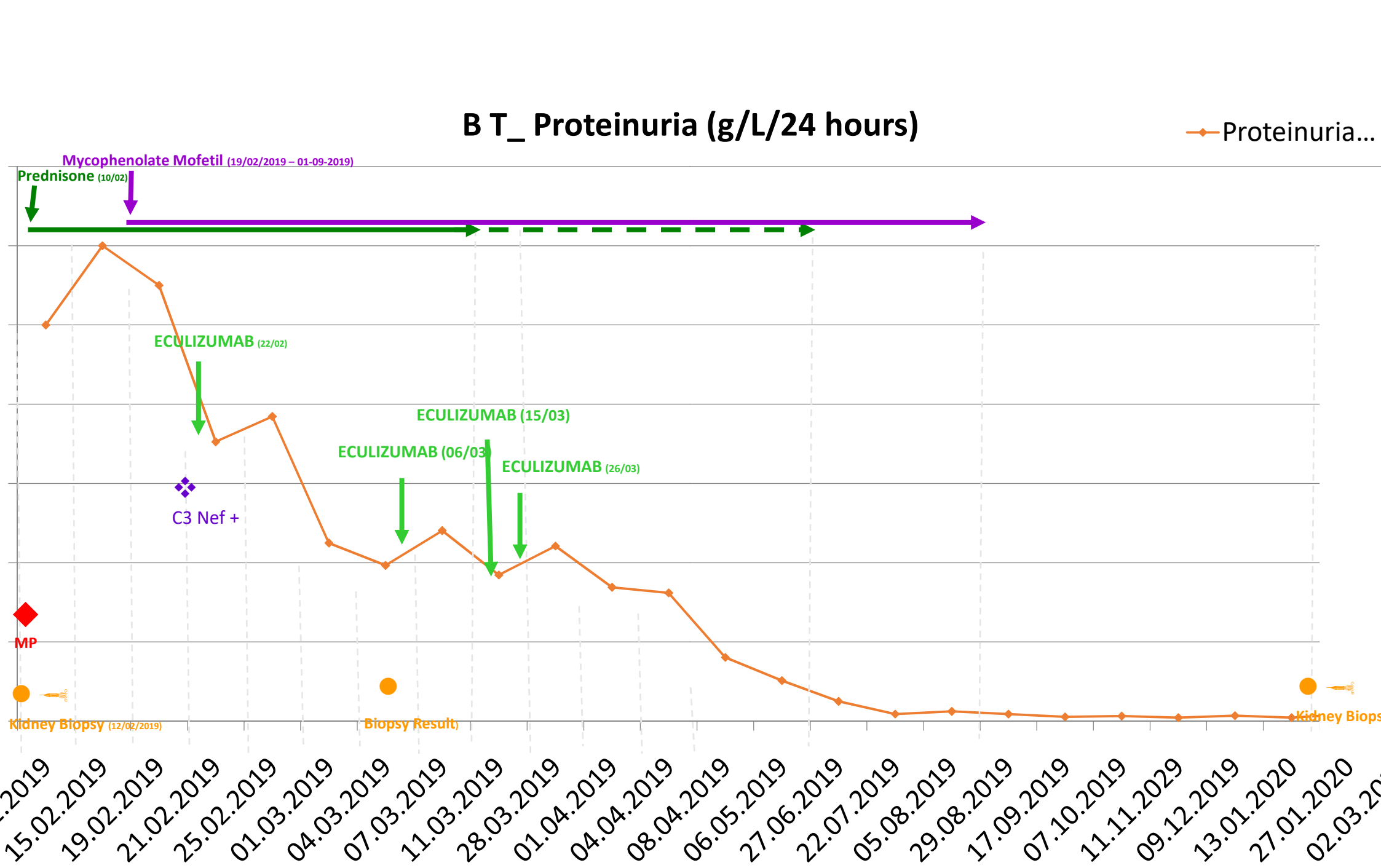
Back to our patients

Case 1 – C3 GN – C3 Nef Positive

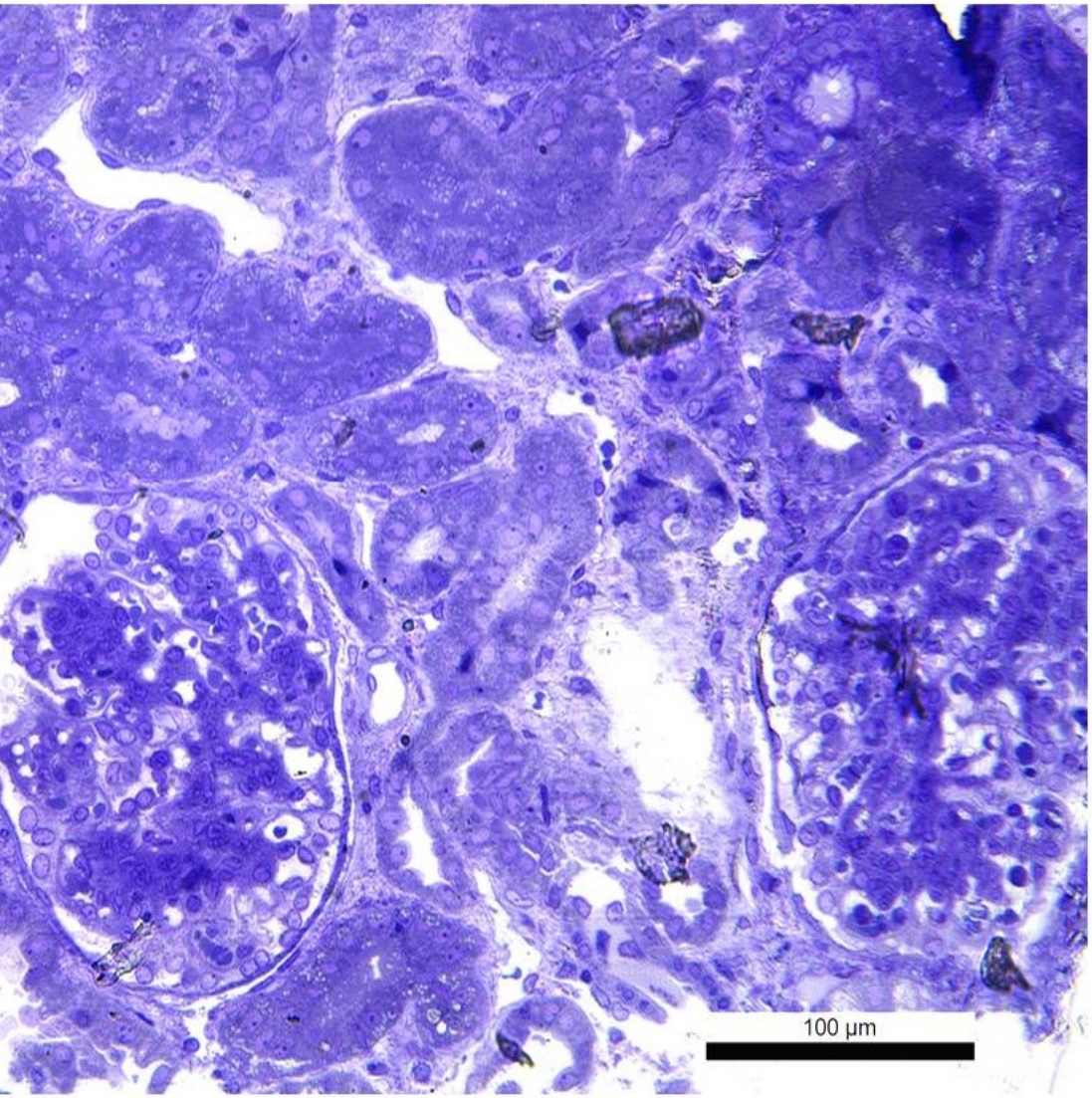
- TEO – 6 years old
  - Nephrotic Proteinuria
  - Hematuria
  - HTA
  - Edema
  - Low urine output

– Hematuria - micro
– LOW C3, Normal-C4

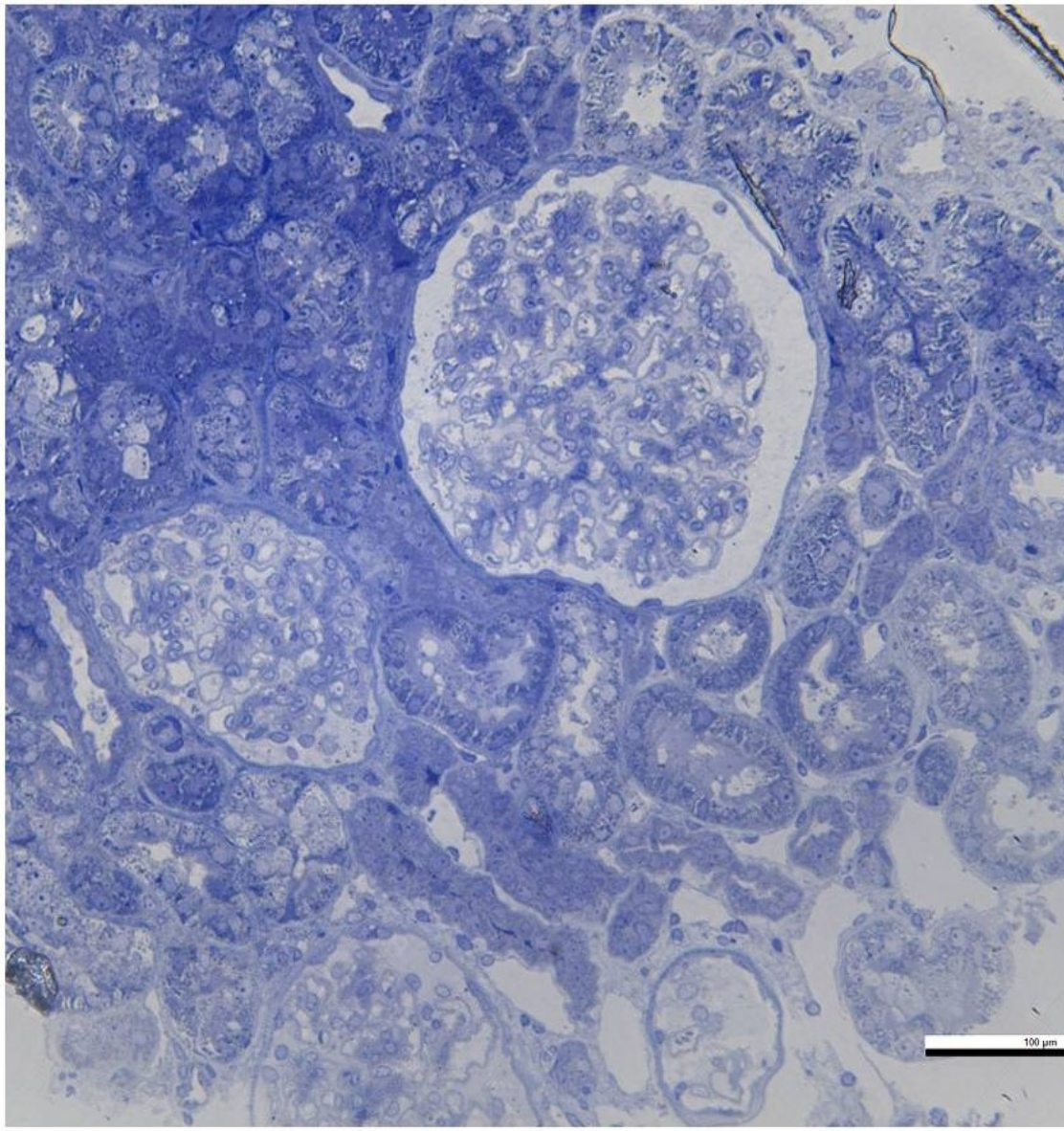
- Hight Chol
- Low Alb


B T_ Proteinuria (g/L/24 hours)
Proteinuria...
Mycophenolate Mofetil (19/02/2019 – 01-09-2019)
Prednisone (10/02)
ECULIZUMAB (22/02)
ECULIZUMAB (15/03)
ECULIZUMAB (06/03)
ECULIZUMAB (26/03)
C3 Nef +
MP
Kidney Biopsy (12/02/2019)
Biopsy Result)
Kidney Biops
15.02.2019
19.02.2019
21.02.2019
25.02.2019
01.03.2019
04.03.2019
07.03.2019
11.03.2019
28.03.2019
01.04.2019
04.04.2019
08.04.2019
06.05.2019
27.06.2019
22.07.2019
05.08.2019
29.08.2019
17.09.2019
07.10.2019
11.11.2029
09.12.2019
13.01.2020
27.01.2020
02.03.20

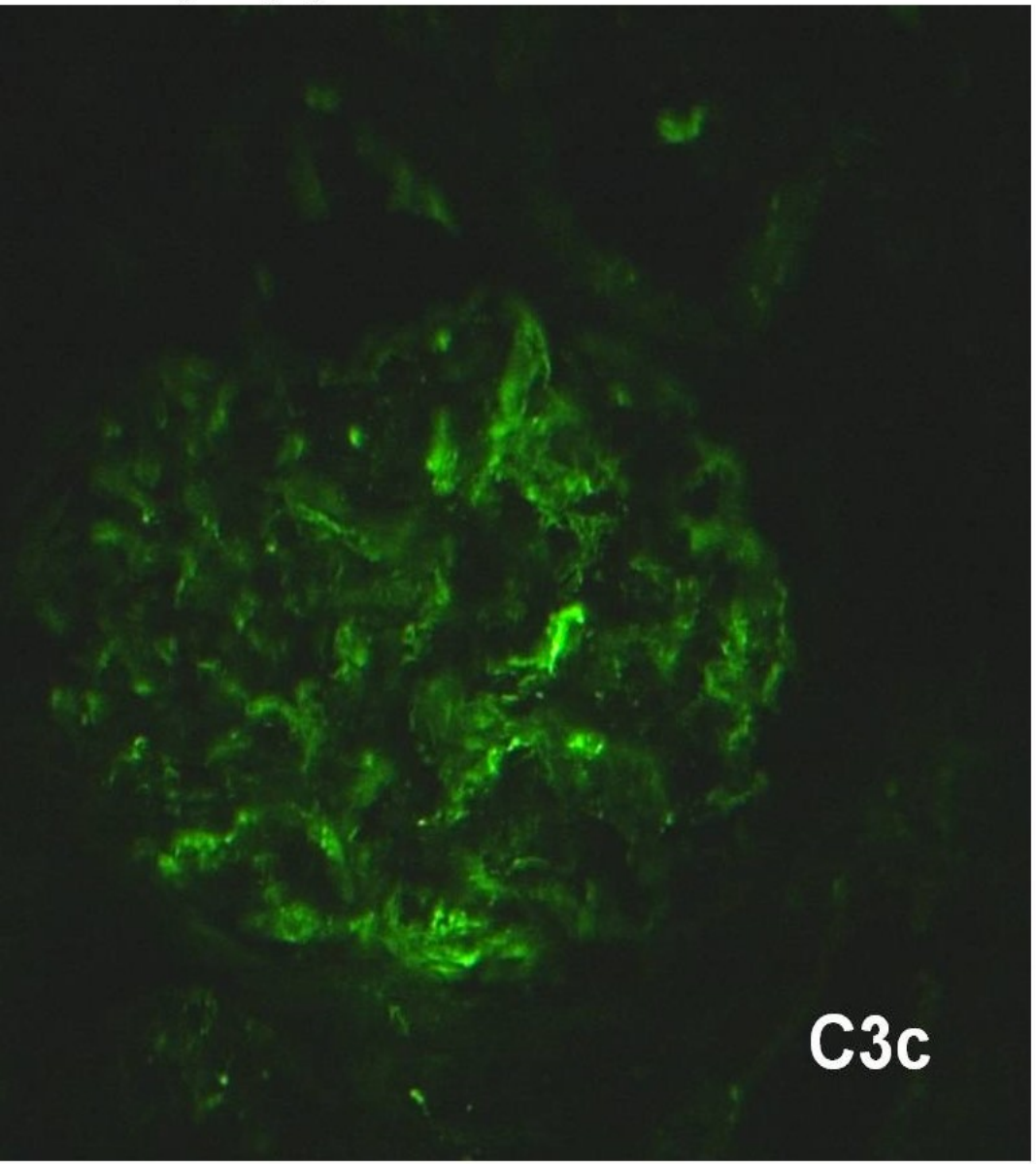
Kidney Biopsy 12.02.2019



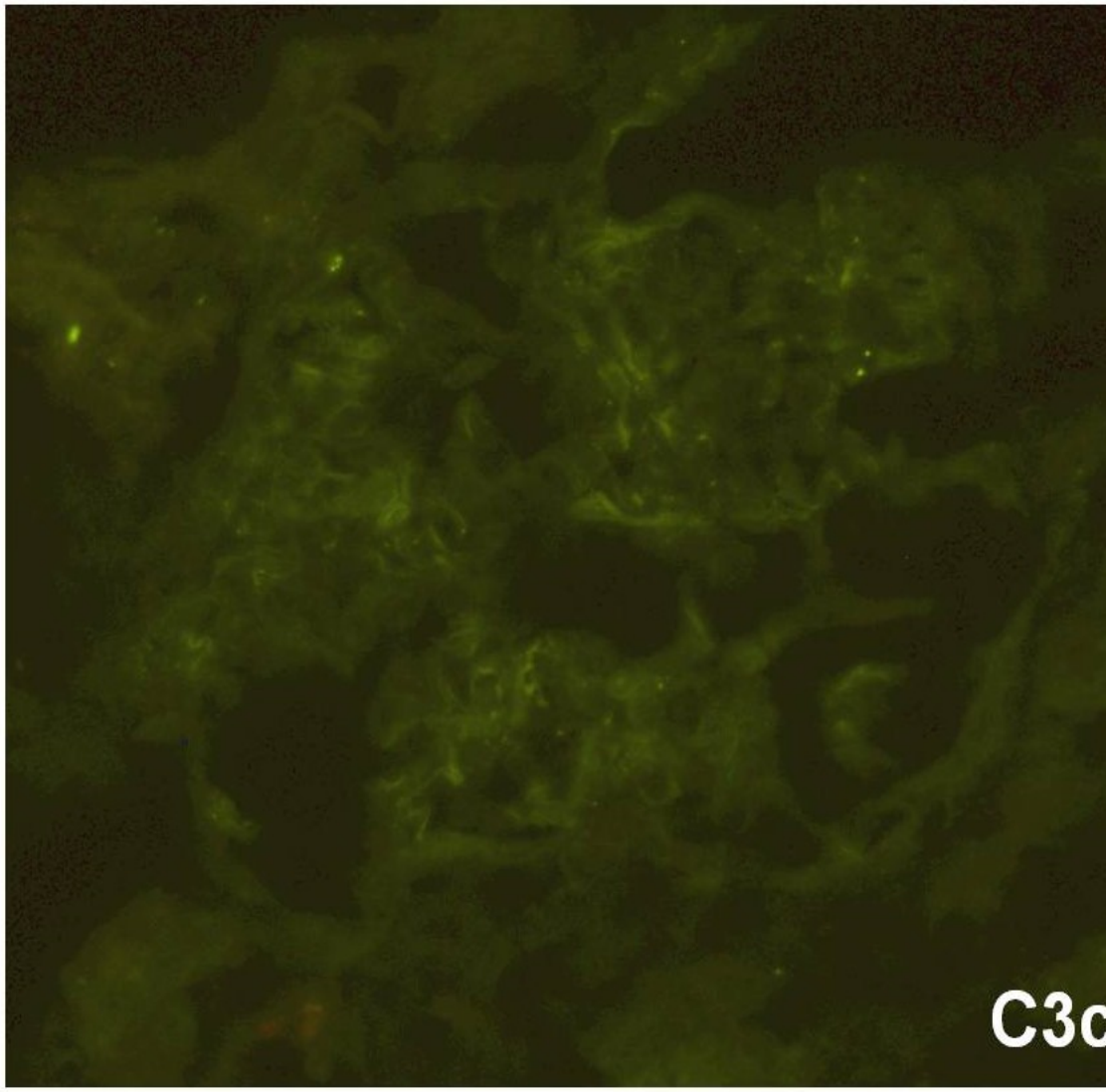
Kidney Biopsy 28.01.2020



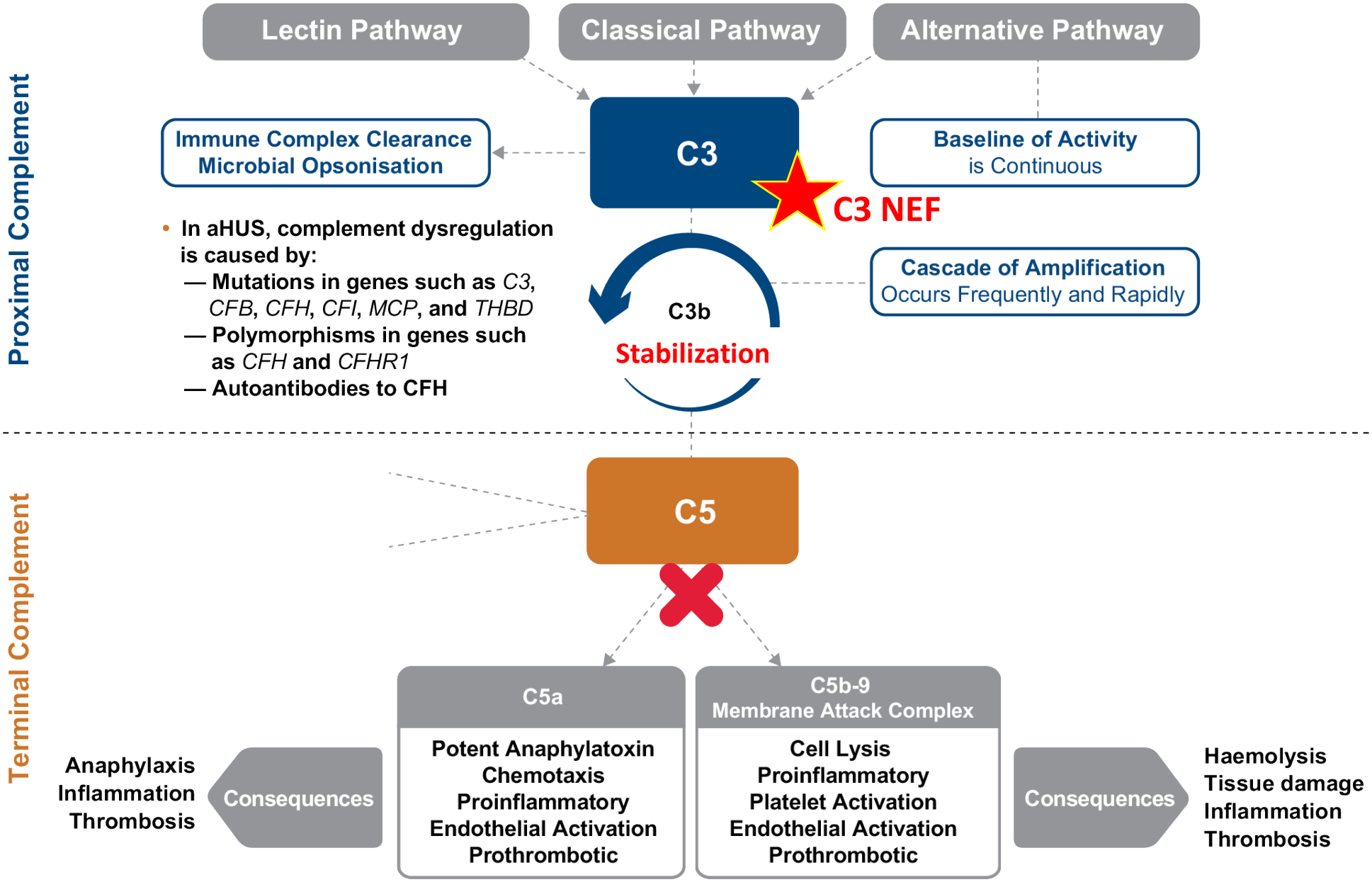
Kidney Biopsy 12.02.2019



Kidney Biopsy 28.01.2020



Case
C3 G
C3 N
Posit



ase 2 – aHUS – anti CFH atb

age 5, female

pale, fatigue, red urine

Vomiting, diarrheic stools

The family remembers that all of them ate some kind of beef meet and all of them got sick.

CNP: [illegible]
Varsta: 4 ani 11 luni
Sex: F
MRN:
Adresa:

DATE	HB	PLT	Crea	LDH	HPT	C3	C4	Proteinuria	Hematuria	Treatment	Blood
15.01.2018	8.4	14000	0.85	1653				nefritic	macro		
16.01.2018	6.2	13000	0.98	1659	0.07	54	23	nefritic	macro		RBC trans
17.01.2018	9.1	18000	1.44					nefritic	macro		
18.01.2018	12	25000						nefritic	macro		
19.01.2018	10.4	30000	2.42			79	20.3	nefritic	macro	PEX	
20.01.2018	7	49000	2.1	1674				nefritic	macro	PEX	RBC trans
21.01.2018								nefritic	macro		
22.01.2018	6.7	53000	1.85		0.07	49.6	11.1	nefritic	macro	PEX	RBC trans
23.01.2018	8.7	65000	1.62	915				nefritic	macro	**ECULIZUMAB**	
23.01.2018											
24.01.2018	7.8	104000	1.54	895				nefritic	macro		RBC trans
25.01.2018	9.8	119000						nefritic	macro		
26.01.2018	9.3	135000	1.3	1032	0.07	60.1	16.1	nefritic	macro		
27.01.2018											
28.01.2018	7.8	131000	0.83		0.07	71.2	26.1				
29.01.2018											
30.01.2018	7.8	163000	0.7	694		71.2	25.1			**ECULIZUMAB**	
31.01.2018	7.7	149000									RBC trans

COMPLEMENT

Complement C3: **0,5 g/L (**reference range 0,9-1,8 g/L)
Complement C4: **0,36 g/L** (reference range: 0,15-0,55 g/L)
Factor H antigen: **49 mg/L** (reference range 250-880 mg/L)
Anti- factor H IgG autoantibody: **positive (10900 AU/mL, ref <110)**

GENETICS

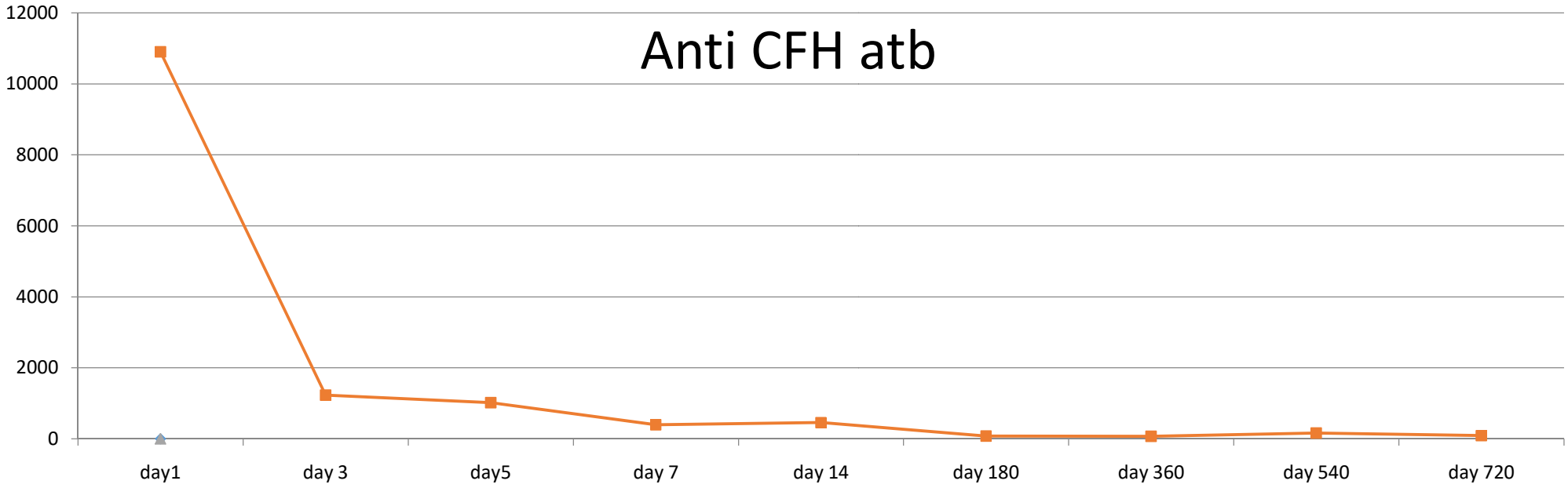
The patient was found to be homozygous for a common deletion of CFHR1 and CFHR3 genes, no other copy number alterations were identified in the studied genes.
The patient was found to be **heterozygous** for the **MCPggaac** haplotype of the *CD46* gene reported as a risk factor of developing aHUS.

The patient was found to be **heterozygous** for the **R102G** and **P314L** polymorphisms of the *C3* gene. The rare alleles of these common variations as well as their haplotype were shown to be susceptibility factors for dense deposit disease.

The patient was found to be **heterozygous** for the ***CFH* V62I** missense variation that was reported as a protective variant against the development of aHUS.

Theraphy

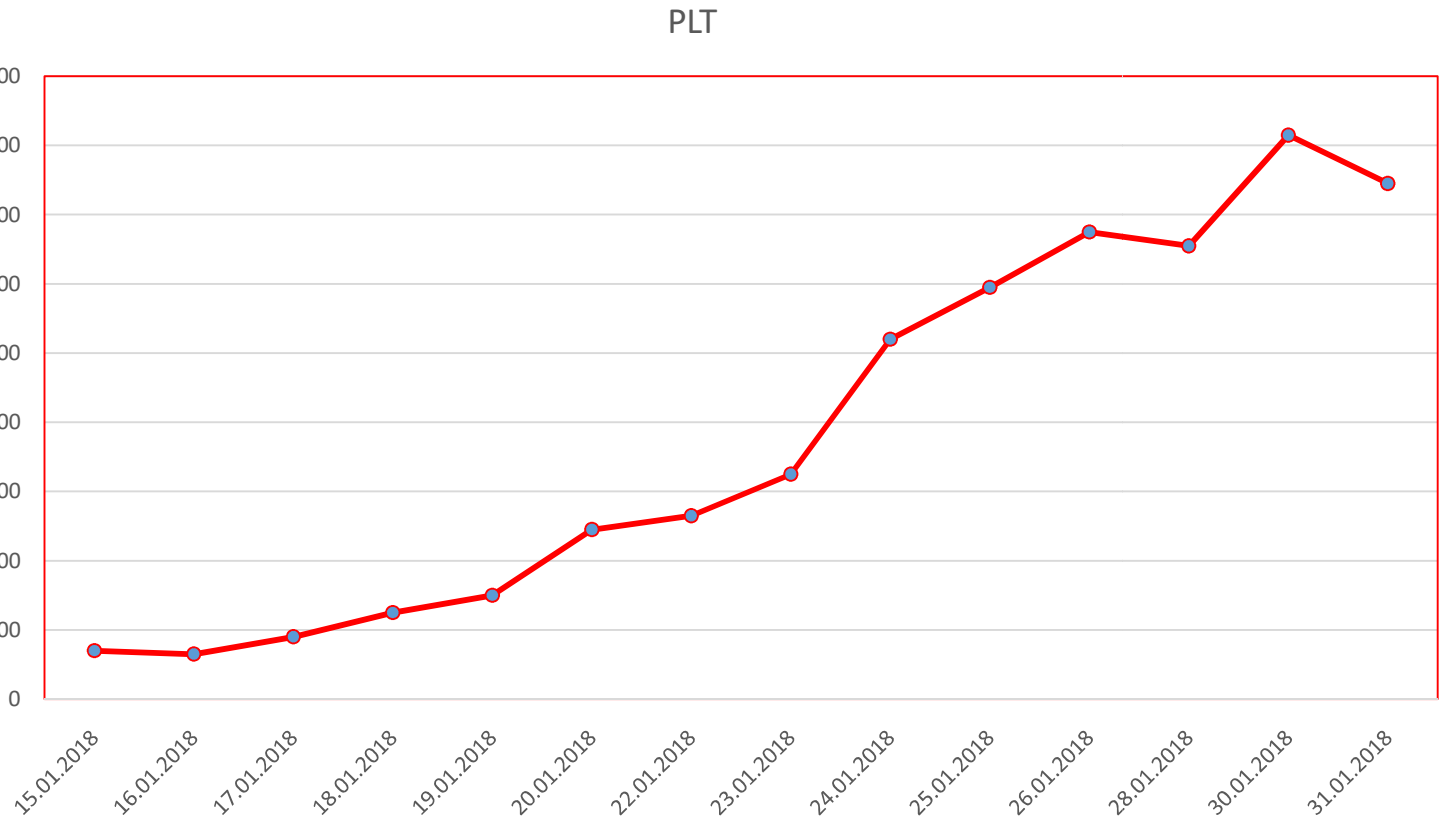
Plasma Exchange – 3 session – dramatic decline in the anti Complement factor H Antibodies, but modest effect on the clinical condition of the patient

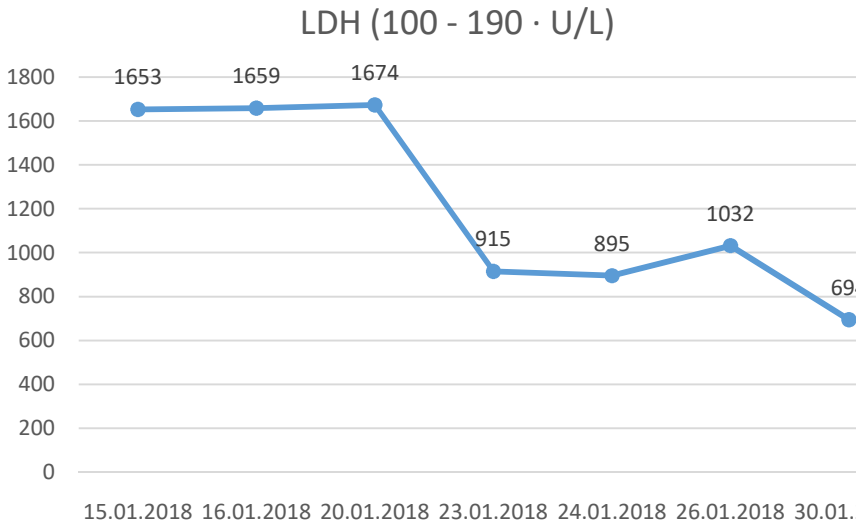


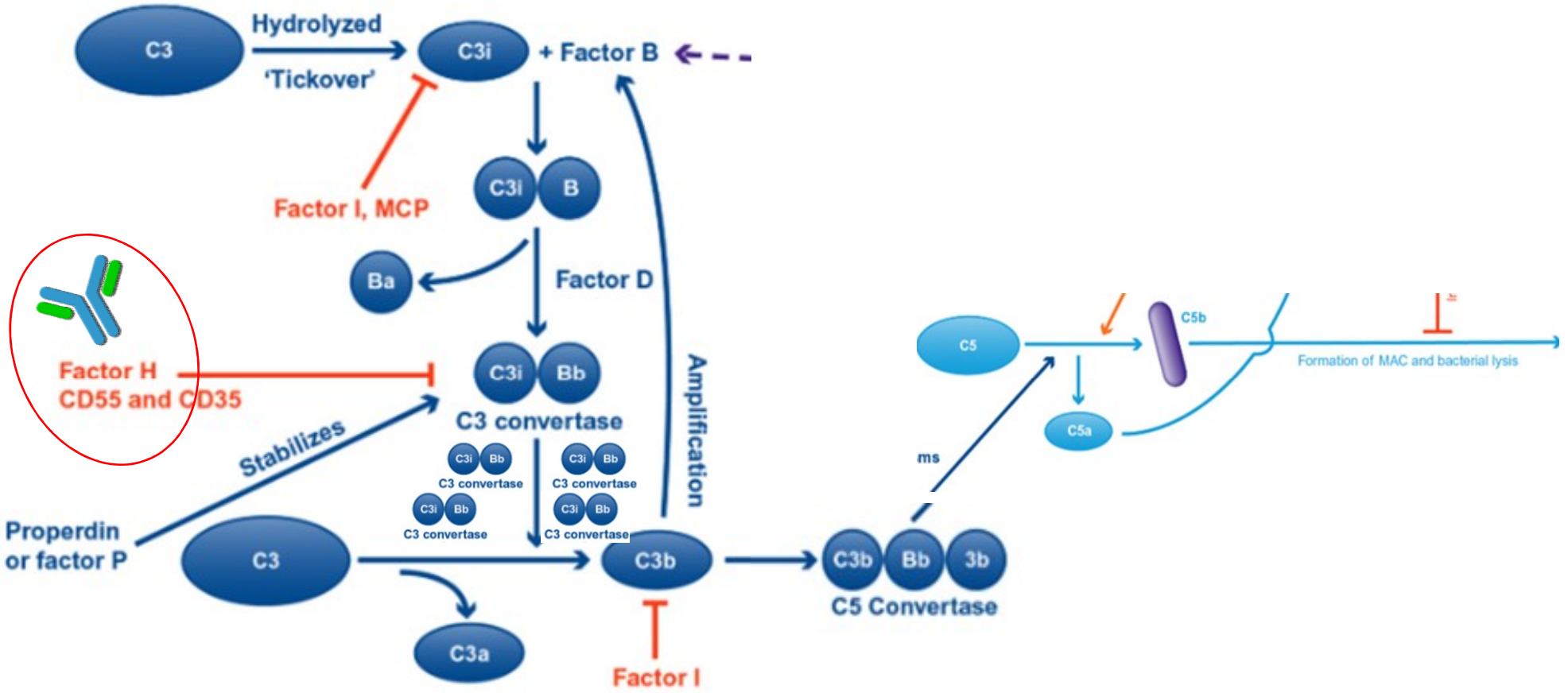
Theraphy

- No immunosuppressant therapy
- Hemodialysis was performed for 2 sessions
- 5 blood transfusion
- **Eculizumab start at day 8**

Case 4 – aHUS – anti CFH atb

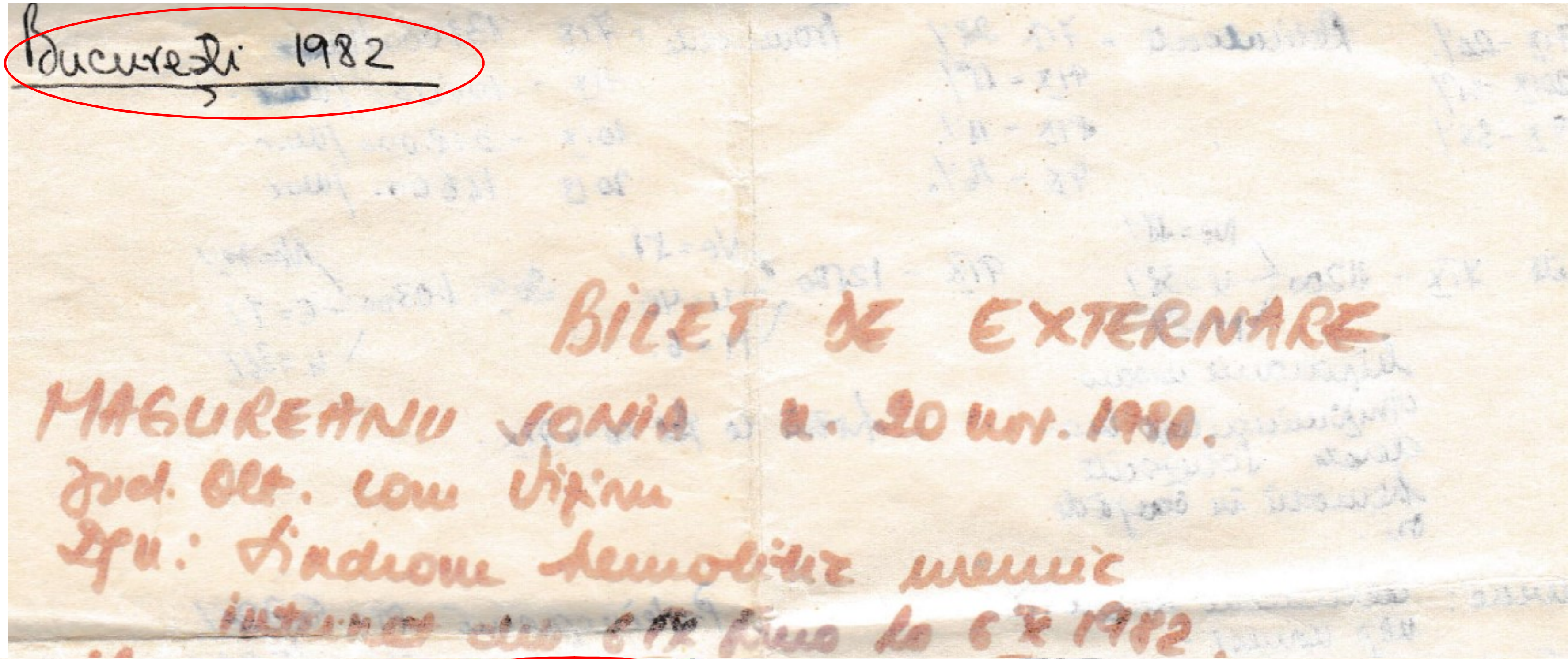





C3
Hydrolyzed
'Tickover'
C3i
+ Factor B
Factor I, MCP
C3i
B
Ba
Factor D
Factor H
CD55 and CD35
C3i
Bb
C3 convertase
Stabilizes
Properdin
or factor P
C3
C3a
C3b
Factor I
Amplification
C3 convertase
C3 convertase
C3 convertase
C3 convertase
C5
C5b
C5a
Formation of MAC and bacterial lysis
ms
C3b
Bb
3b
C5 Convertase

ase 3 –
HUS -
D46
nut

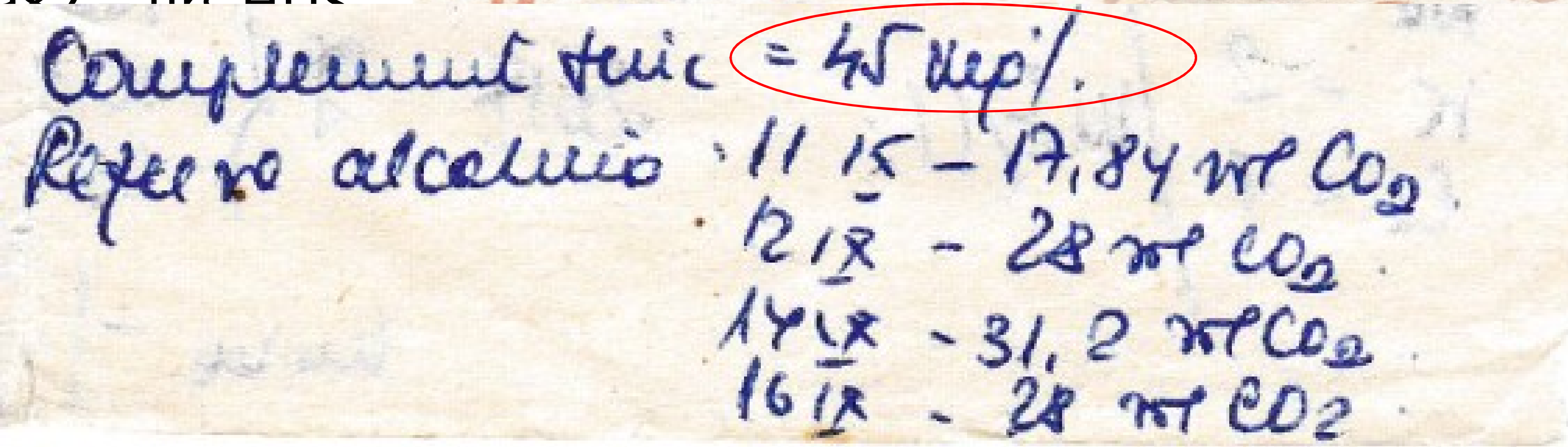
onia –
ucharest
982 – firs HUS



Bucureşti 1982

BILET DE EXTERNARE

MAGUREANU SONIA n. 20 ian. 1980.
Jud. Olt. com. Vişina
Dg.: Sindrom hemolitic uremic
internat din 6 IX până la 6 X 1982



Complement seric = 45 hep%.
Rezerva alcalina: 11 IX – 17,84 ml CO_2
12 IX – 28 ml CO_2
14 IX – 31,2 ml CO_2
16 IX – 28 ml CO_2

Trombocite = 7 IX 133.000/mm

Sonia – USA – Florida, 2012 – second HUS episode. 30 years apart

- PEX
- HD

CONSULTATION: 1/30/12

ou, Dr. Friedlander for this consultation.

FOR CONSULTATION: This consultation was requested for evaluation and tic management.

OF PRESENT ILLNESS: Ms. Sonia Magureanu is a 31-year-old Romanian with no significant past medical history, who stated she was doing well he started her birth control pills about 2 months ago. She became sively fatigued and weak, and over the past week, had no appetite with s and she has not taken anything by mouth. She noted her ankles are g to swell, so she presented to the Emergency Room. The patient was o be in acute renal failure with anemia and thrombocytopenia. The subsequently underwent transfusion of 2 units of packed red blood nd consultation was requested for management. She has been started on n 1 g IV q. 12 h. The patient denies any history of preceding upper tory tract symptomatology or sore throat. She has had no episodes of ng diarrhea. Although, she does state she had 1 episode 2 days ago. not notice any change in the color of her urine or change in her habits. She has had no history of rash or joint symptomatology. as been no history of recent travel. She denies any history of HIV risk factors. She does take a multivitamin from a GNC. She was also onsteroidals approximately twice a day, anytime she had a headache. re no other members of her household who have been ill and there has history of recent travel. She has not seen a physician in several

IMPRESSION:
1. Acute renal failure with anemia and thrombocytopenia - history
initiation of birth control pills about 2 months ago. She states wh
started feeling fatigued, had weakness and over the past week with
cannot eat anything and stopped with her p.o. intake. She was admit
above; however, of note, her temperature and white count were normal
denied any history of preceding upper respiratory tract symptomatol
gastrointestinal complaints. She did use nonsteroidals for intermi

headache, which may be contributing to the renal failure. There are
members of her household who have been ill and there has been no histo
recent travel. She denies any history of human immunodeficiency virus
risk factors. Of note, her complement levels are normal and her neuro
exam is also normal. Questionable hemolytic uremic syndrome/thromboti
thrombocytopenic purpura.
A. Rule out drug related, questionable birth control pill.
B. Rule out underlying human immunodeficiency virus.
C. Rule out autoimmune.

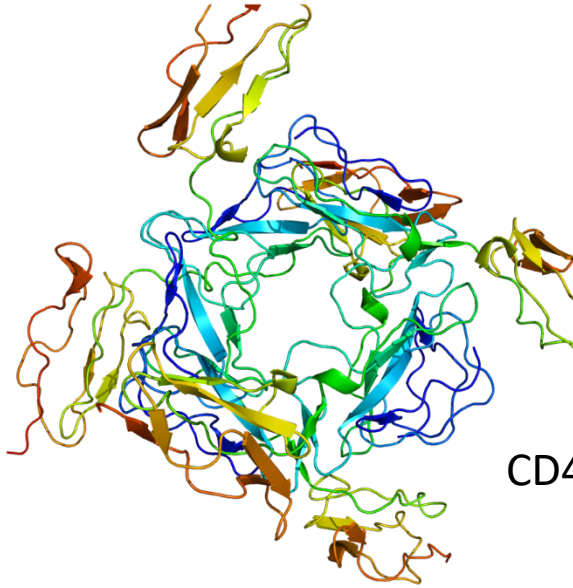
PLAN:
1. Cultures, HIV and hepatitis serologies have been ordered.
2. We will add stool cultures if diarrhea.
3. We would adjust Rocephin to 1 g IV daily and continue until cultu
negative.
4. Supportive care. Renal following, will likely require biopsy.

- Sonia – 2017 – Bucharest, Budapest.
- Genetic testing for high suspicion of alternative complement pathway activation TMA

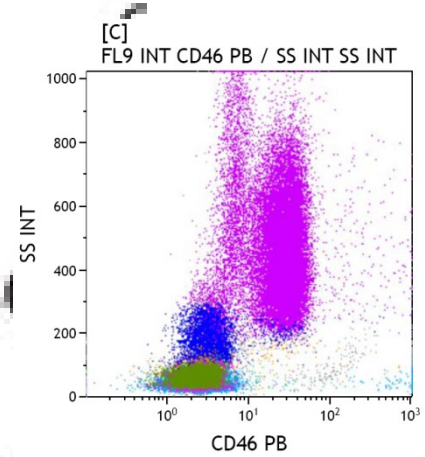
ADAMTS13 metalloprotease activity:	96 (reference range 67-150 %)
Total complement activity, classical pathway (hemolytic test):	49 CH50/ml (ref range 48-103 CH50/ml)
Total complement activity, alternative pathway (WIELISA-Alt):	73 % (reference range 70-105%)
Complement C3:	0,71 g/L (reference range 0,9-1,8
Complement C4:	0,18 g/L (reference range: 0,15-0, g/L)
Factor H antigen:	508 (reference range 250-880 mg
Complement factor I antigen:	105 % (reference range 70-130%)
Complement factor B antigen:	42 % (reference range 70-130%)
Anti- factor H IgG autoantibody:	negative (46 AU/mL, ref <110)
C1q antigen:	93 mg/L (ref: 60-180)
Anti-C1q IgG autoantibody	1 U/mL (ref <52)
sC5b-9 (terminal complement complex):	166 ng/mL (ref 110-252 ng/mL)
C3a anaphylatoxin:	150 ng/mL (ref 70-270 ng/mL)

- mutation causing the deletion of a thymine base (**c.280_280delT**) in the ***CD46*** gene that generates a reading frameshift at amino acid 94 and a premature stop codon at amino acid position 107
- mutation (**c.856G>T**) that affects the last nucleotide of exon 6 of the ***CD46*** gene, which is a highly conserved position in the splice site consensus sequence

- mutation causing the deletion of a thymine base (**c.280_280delT**) in the ***CD46*** gene that generates a reading frameshift at amino acid 94 and a premature stop codon at amino acid position 107.
- mutation (**c.856G>T**) that affects the last nucleotide of **exon 6** of the ***CD46*** gene, which is a **highly conserved** position in the splice site consensus sequence



(a)



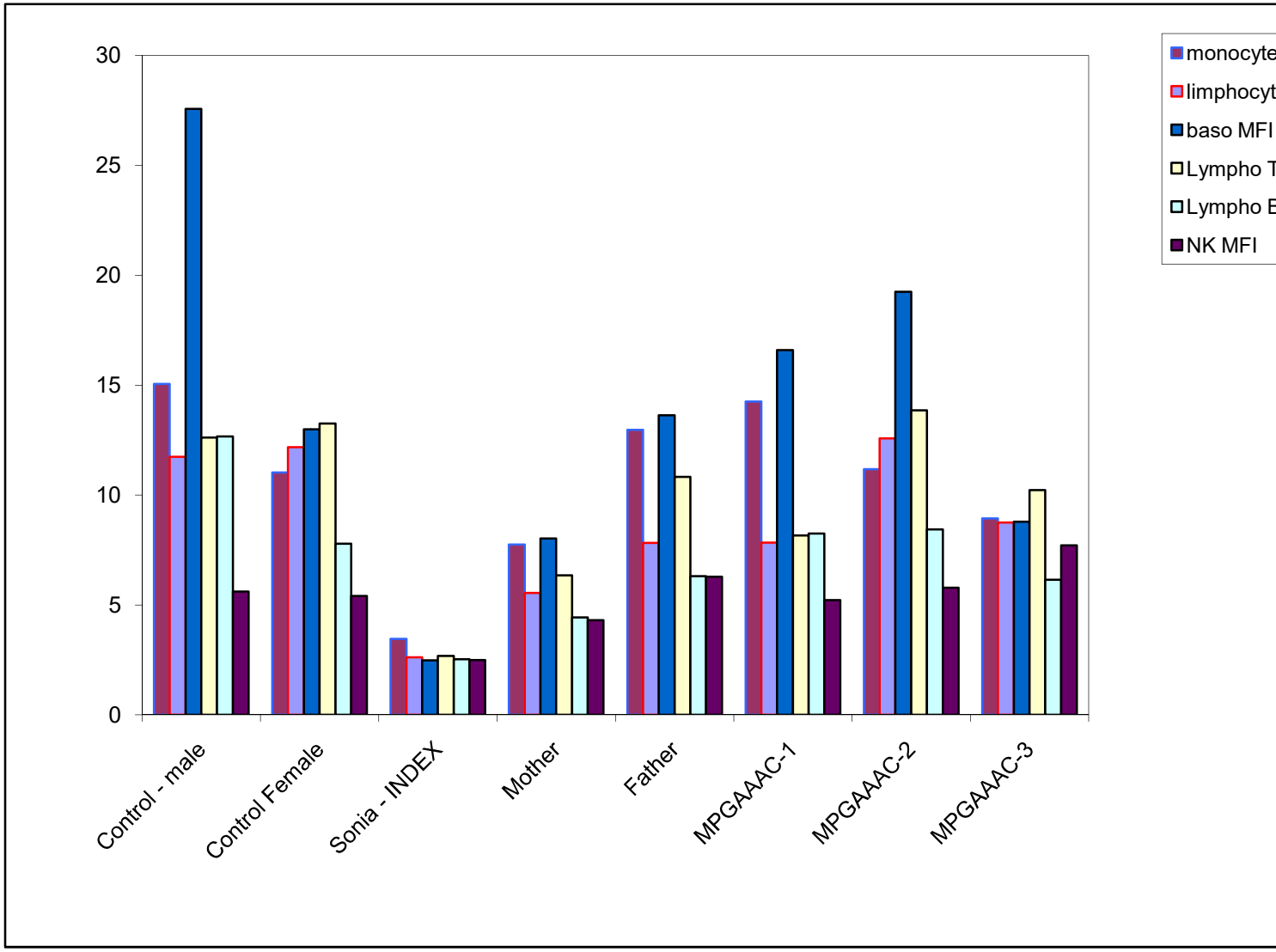
C3b

SCR

SCR

SCR

Exon 6



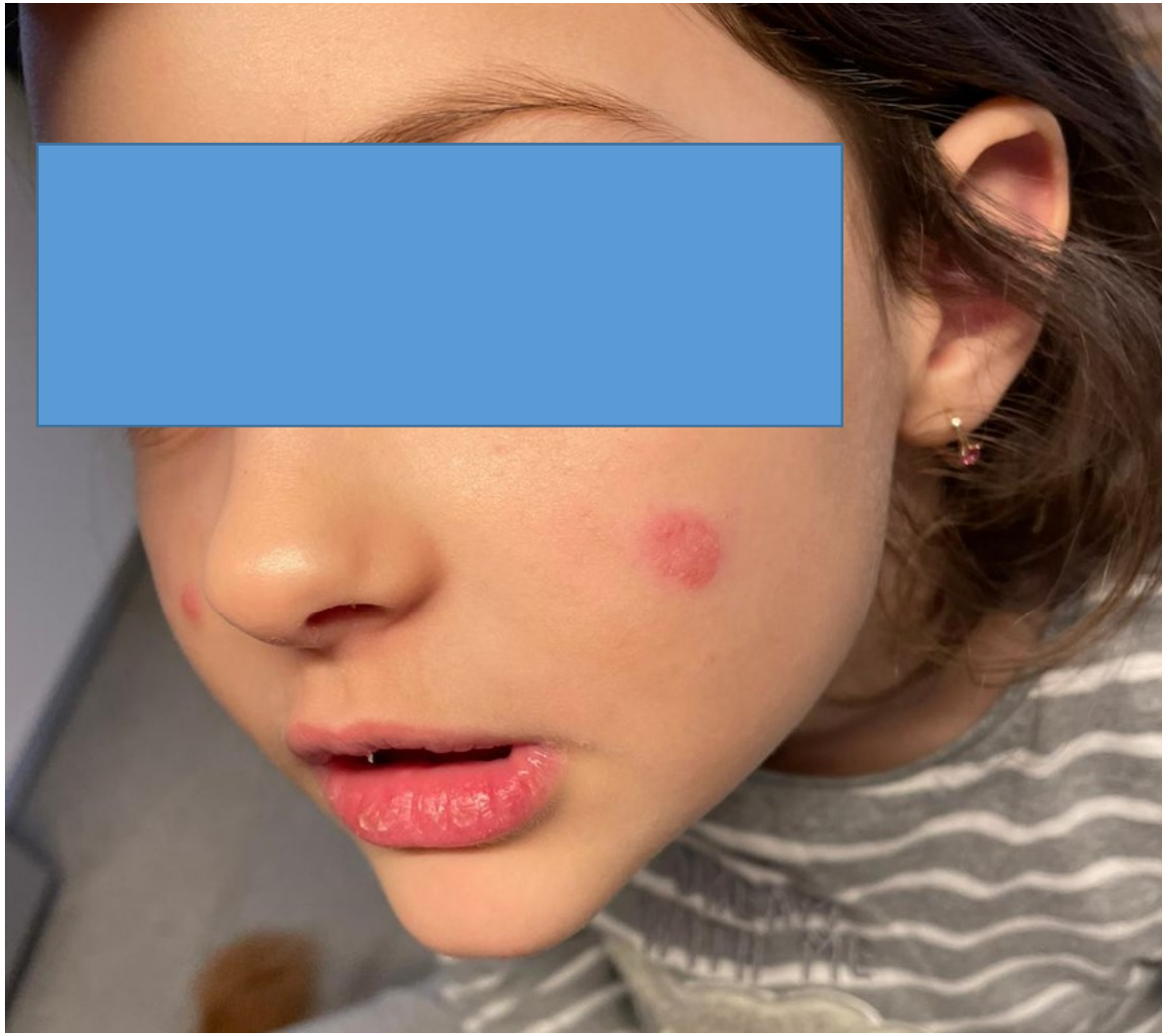
- Sonia – 2017
  - No benefit from immunosuppression
  - Disputable benefit from PEX
  - Very good candidate for Renal Tx with almost no risk of relapse

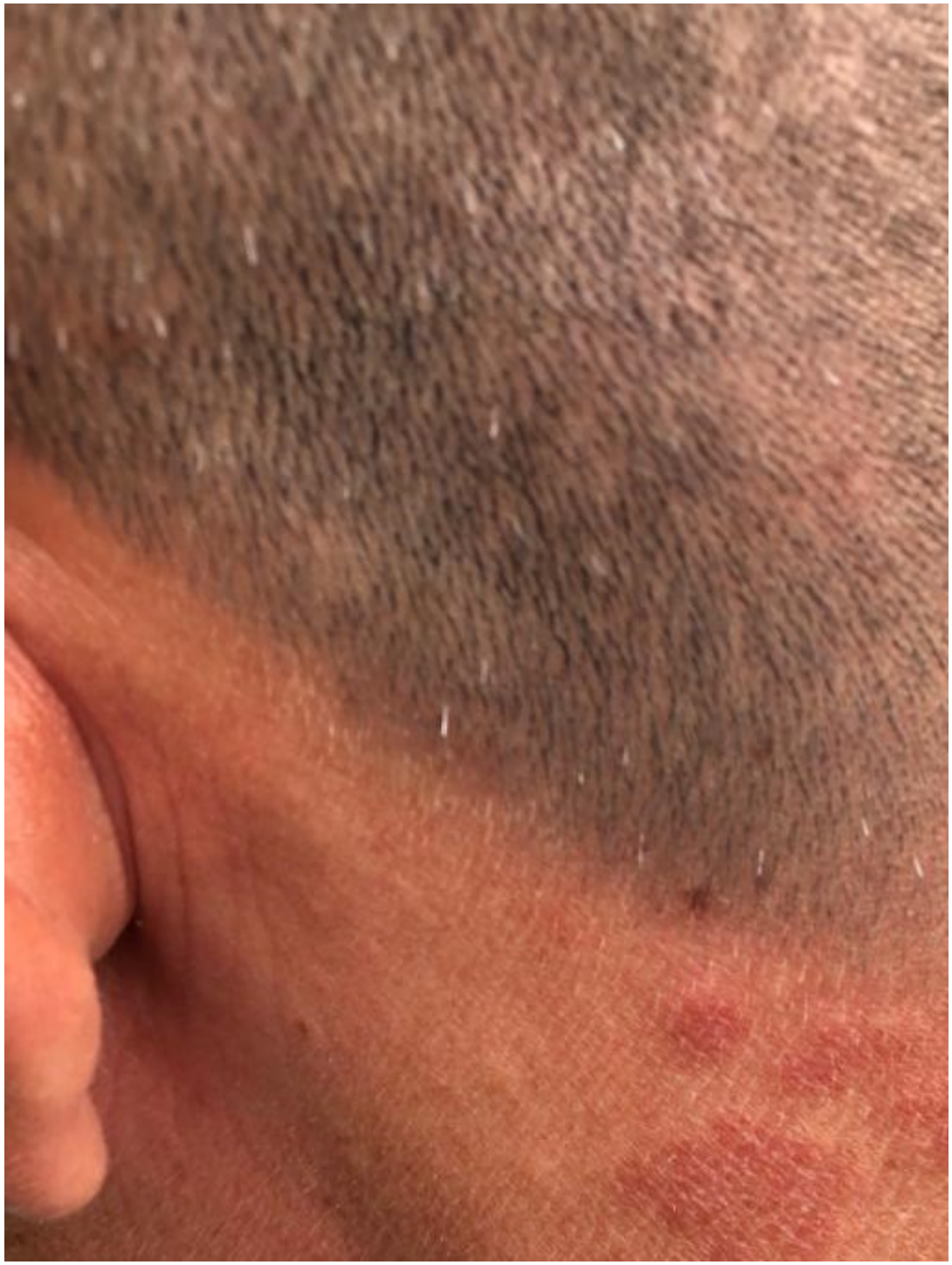
  - Functional test

CASE 4 - LUPUS

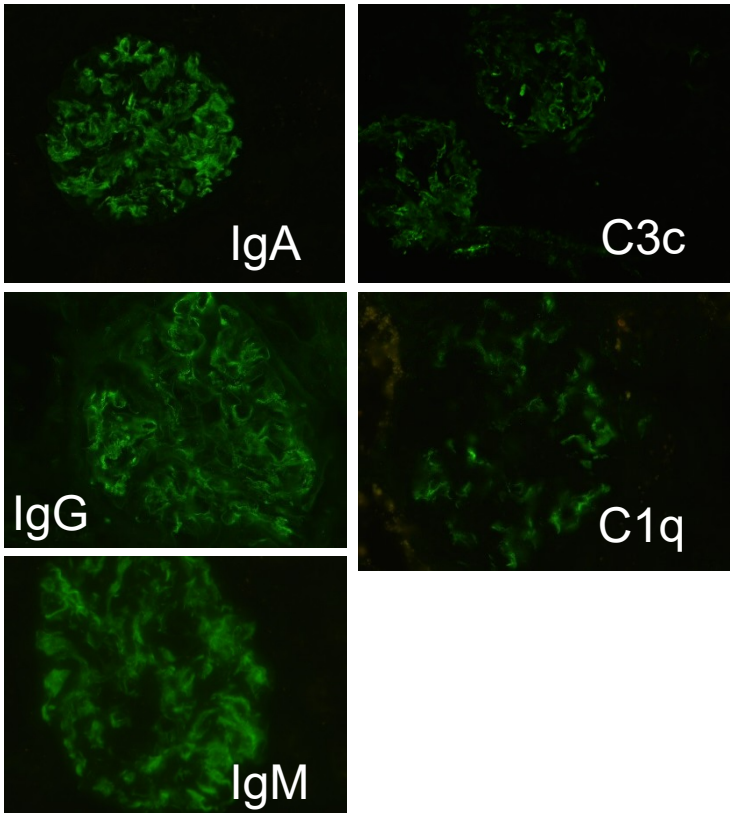
- ANASTASIA-6 years old
- Discoid rash
- joint pain

- **WBC 3120/mm3**
- Hb 13.9g/dl
- **PLT 144000/mm3**
- **Anti ds DNA >200u/ml (intens +)**
- **Intense positive ANA**
- **Intense Positive aniti C1Q**
- **C3 28.4 mg/dl**
- **C4 2.12 mg/dl**
- **NORMAL URINE SEDIMENT**



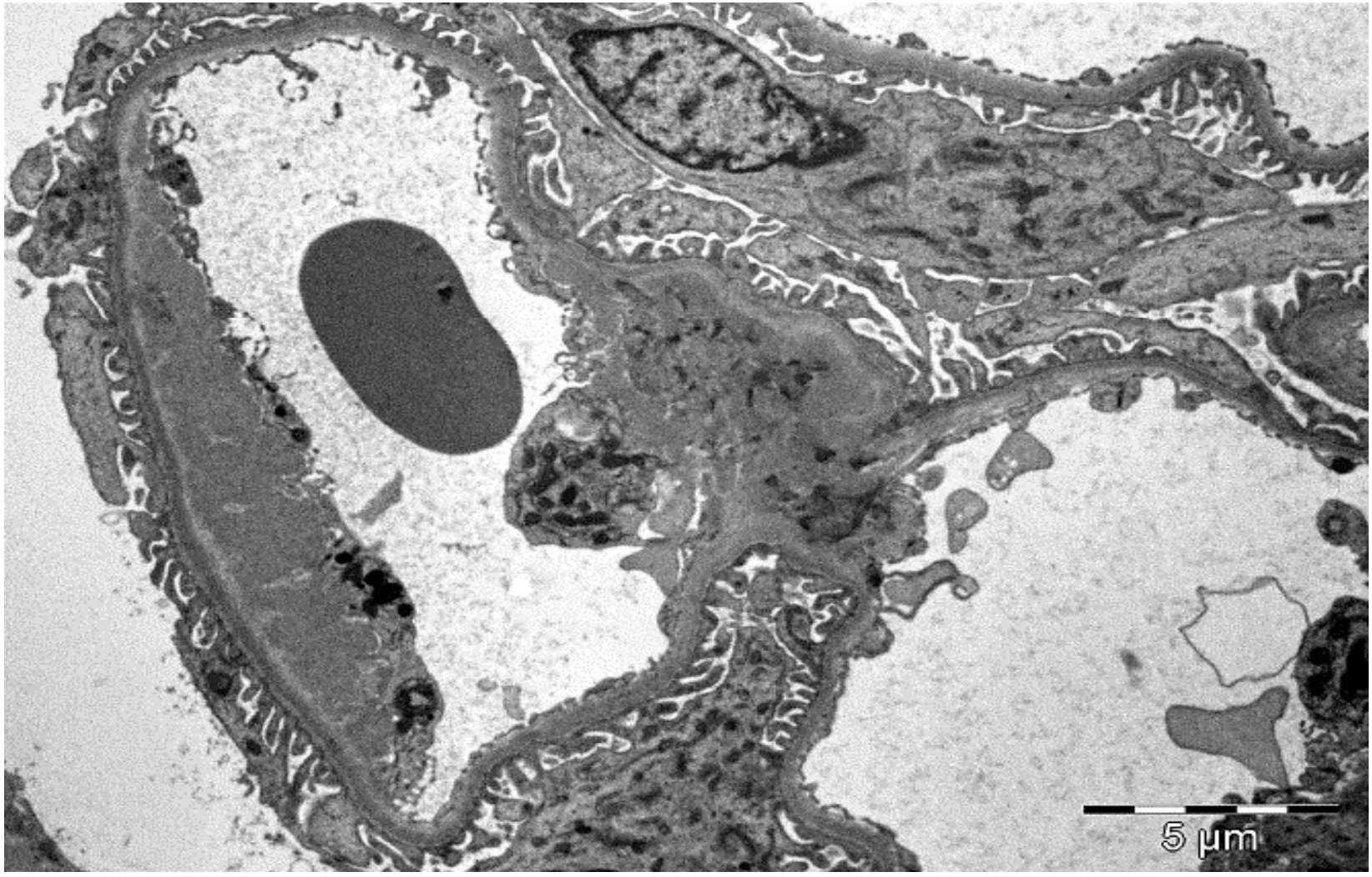


class III – LUPUS NEPHRITIS

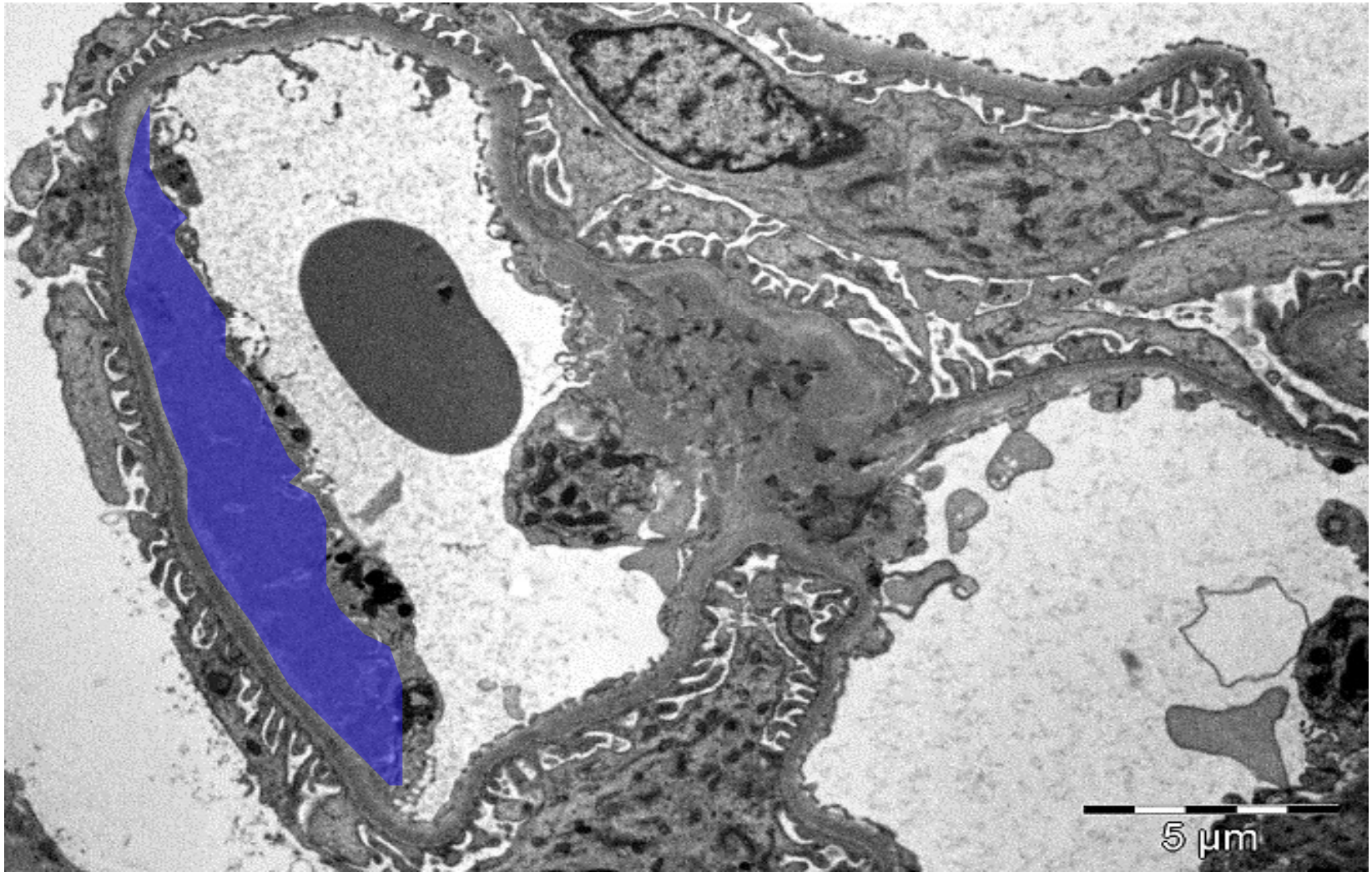


IgA – intense + granular in the mesangium and MBG
IgG – intense + granular in mesangium, MBG, focal in MBT and vessels
IgM – intense + granular in the mesangium C1q – intense + granular in the mesangium and MBG
C3c – intense + granular in mesangium and MBG, focal in MBT and in vessels

class III – LUPUS NEPHRITIS



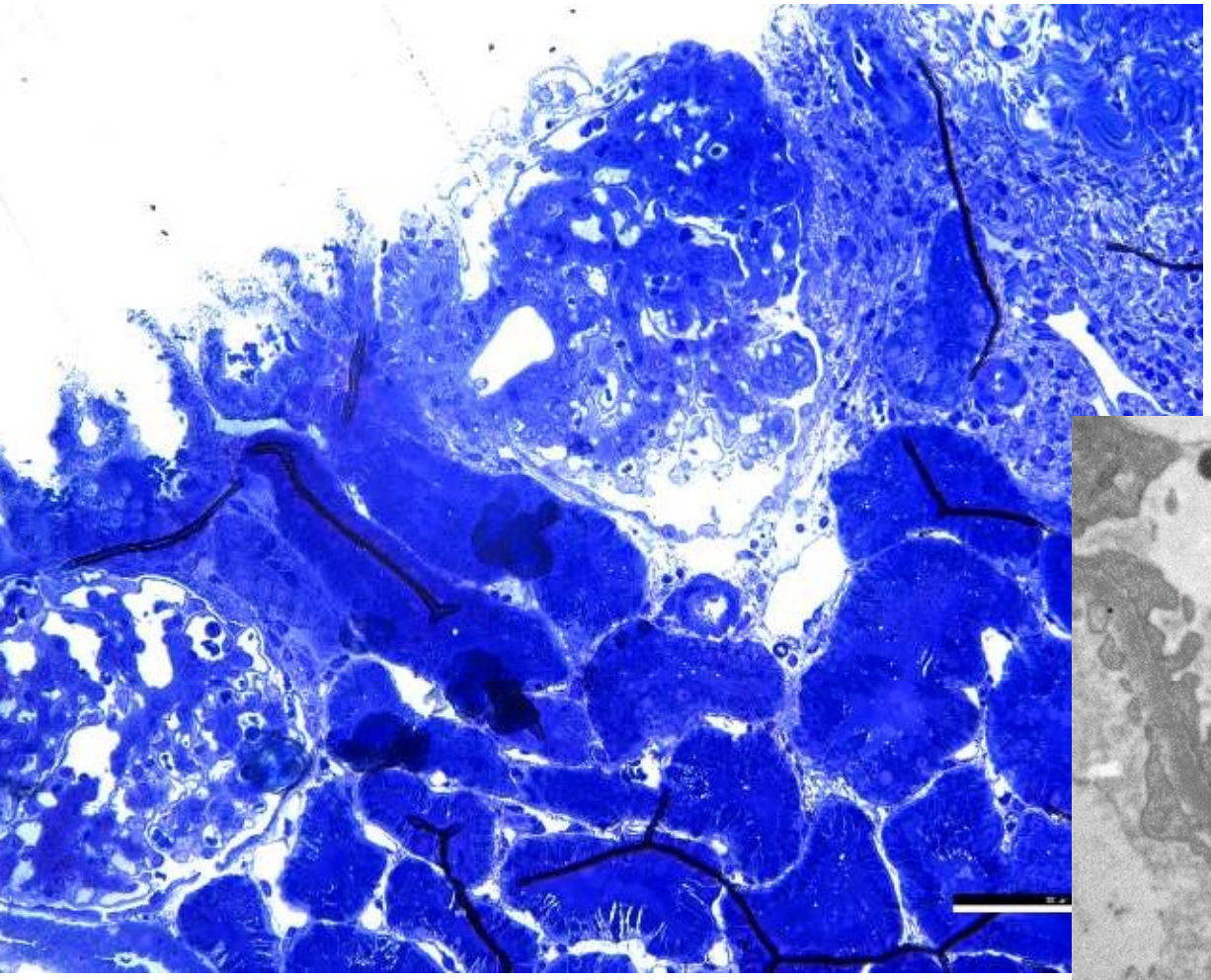
class III – LUPUS NEPHRITIS

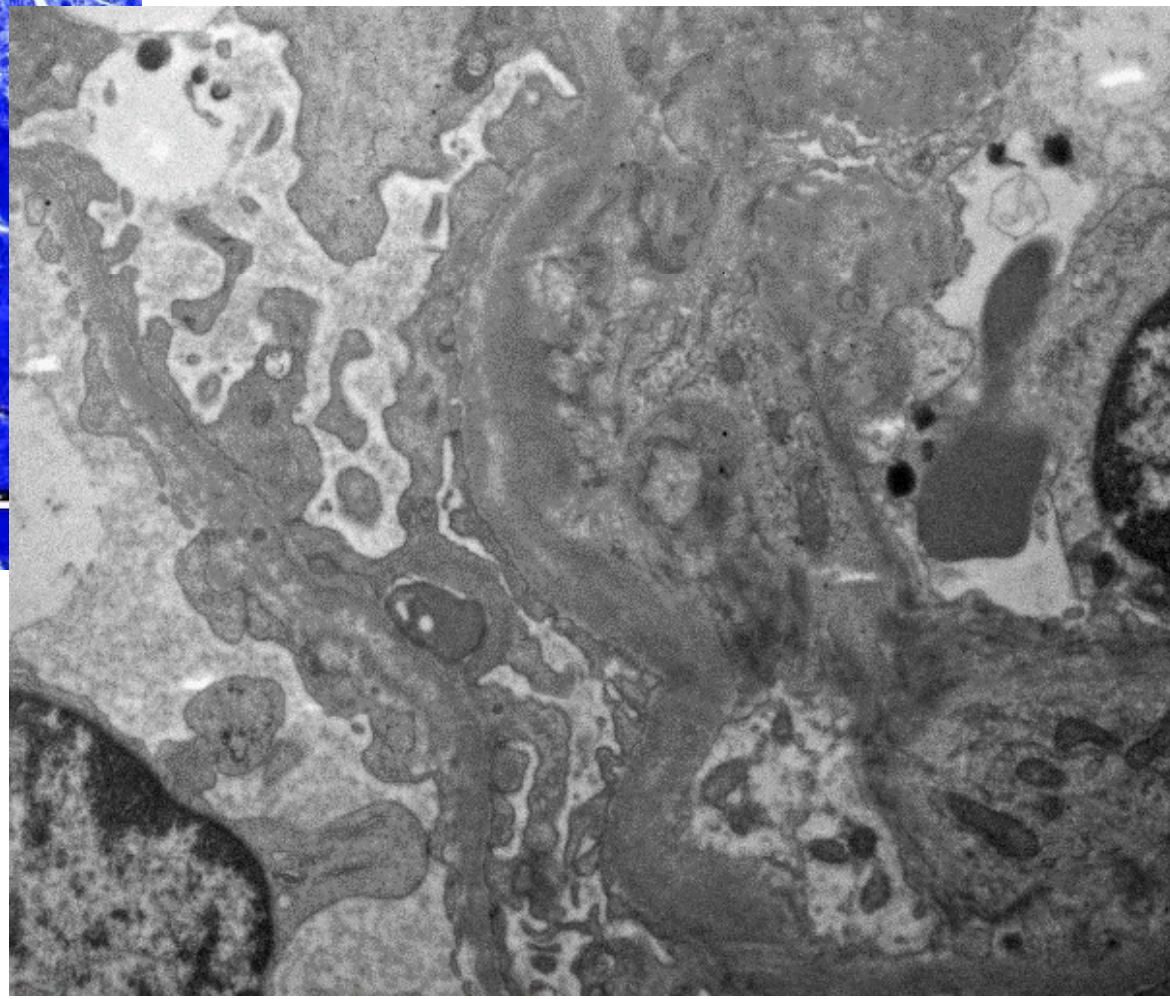


Gene (transcript)	Nucleotide (protein)	Zigosity	Described by:	Minor Allele Frequency (MAF)	Variant classificati
CFI (NM_001318057)	c.1666del p.(Glu556Lysfs*26)	Het	-	-	**Variant o unknow significan**

CASE 4 IgA nephropathy + thrombotic microangipathy

11 years old boy
2 recurrent episodes of macroscopic hematuria during upper espiratory tract infection
low C3
normal ASLO titer.
mild proteinuria,
persistent microscopic hematuria
normal renal function.
IgA nephropathy on Renal biopsy + thrombotic microangipathy





CLINICAL RESEARCH www.jasn.org

Variants in Complement F Factor H-Related Protein Affect Complement Activ

Li Zhu,*†‡§ Ya-Ling Zhai,*†‡§ Feng-Mei Wang,
Su-Fang Shi,*†‡§ Li-Jun Liu,*†‡§ Feng Yu,*†‡§
and Hong Zhang*†‡§

*Renal Division, Department of Medicine, Peking Unive
Peking University, Beijing, China; ‡Key Laboratory of R
§Key Laboratory of Chronic Kidney Disease Prevention ar
China; ‖Department of Microbiology, University of Alaba
of Medicine, Columbia University College of Physicians

ABSTRACT

Complement activation is common in patients w

Example Highlights

850+ clinical trials related to complement therapeutics have been registered till date; majority (53%) of these trials were / are being conducted across various clinical sites based in Europe

Clinical Trial Analysis

Cumulative Distribution by Trial Registration Year, (Pre-2015-2021)[1]

Popular Disease Indications by Number of Clinical Trials[2,3]

Note 1: Trials with unknown status, have not been included in this representation
Note 2: Clinical trials being conducted for more than one indication, have been counted multiple times
Note 3: Indications for which more than 25 clinical trials are being conducted, have been included in this analysis
Note 4: Clinical trials that are being conducted across more than one location (country-wise), have been counted multiple times

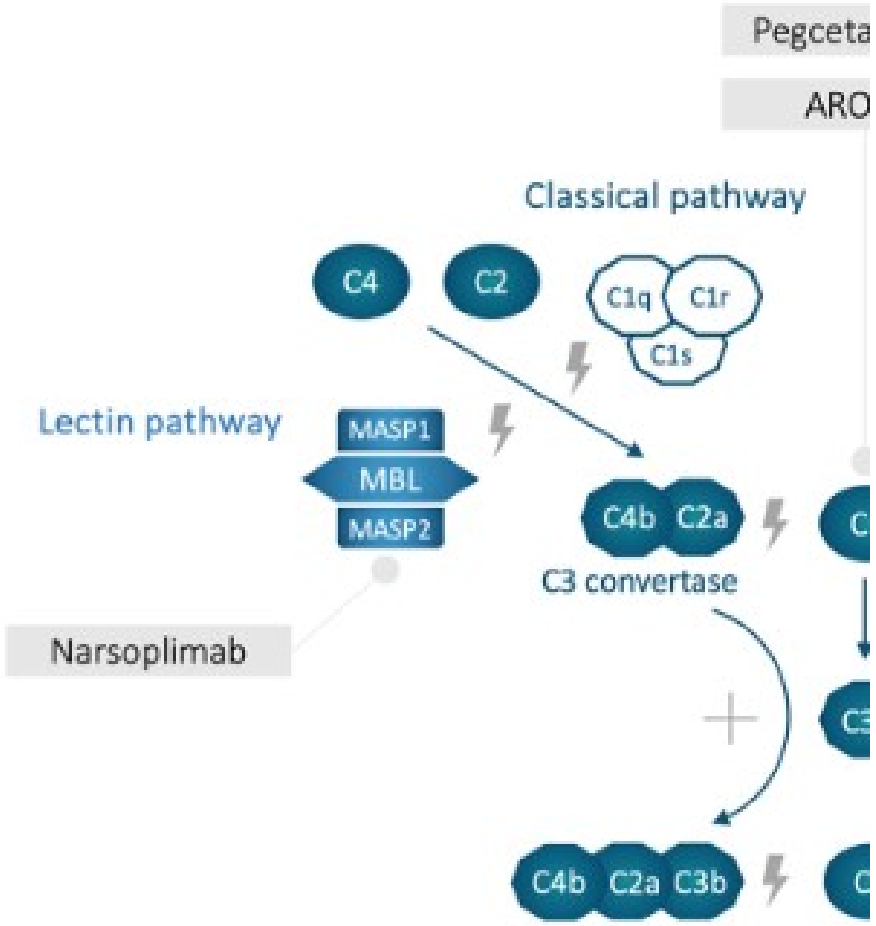
Clinical Trial Analysis

Distribution by Location of Trial[4]

- https://www.rootsanalysis.com/reports/next-generation-complement-therapeutics-market.html

Kidney International Reports (2024) 9, 563–573

REVIEW



Take home message

- we have witnessed tremendous advances in our understandig of complement
- Now we have several innovative therapies that have radically improved patients' outcomes
- When we speak about complement and complemen blocking therapies is "all or nothing"
- Hit fast, hit hard

IPNA

Teaching Course

SEPNWG XI

XI SEPNWG Meeting

Hereditary Kidney Disease: Bucharest international Symposium on Nephrology's Hidden Challenges

Topics:

aHUS
C3 GN
Primary Hyperoxaluria
Fabry disease

Bucharest, Romania
08-09 November 2024

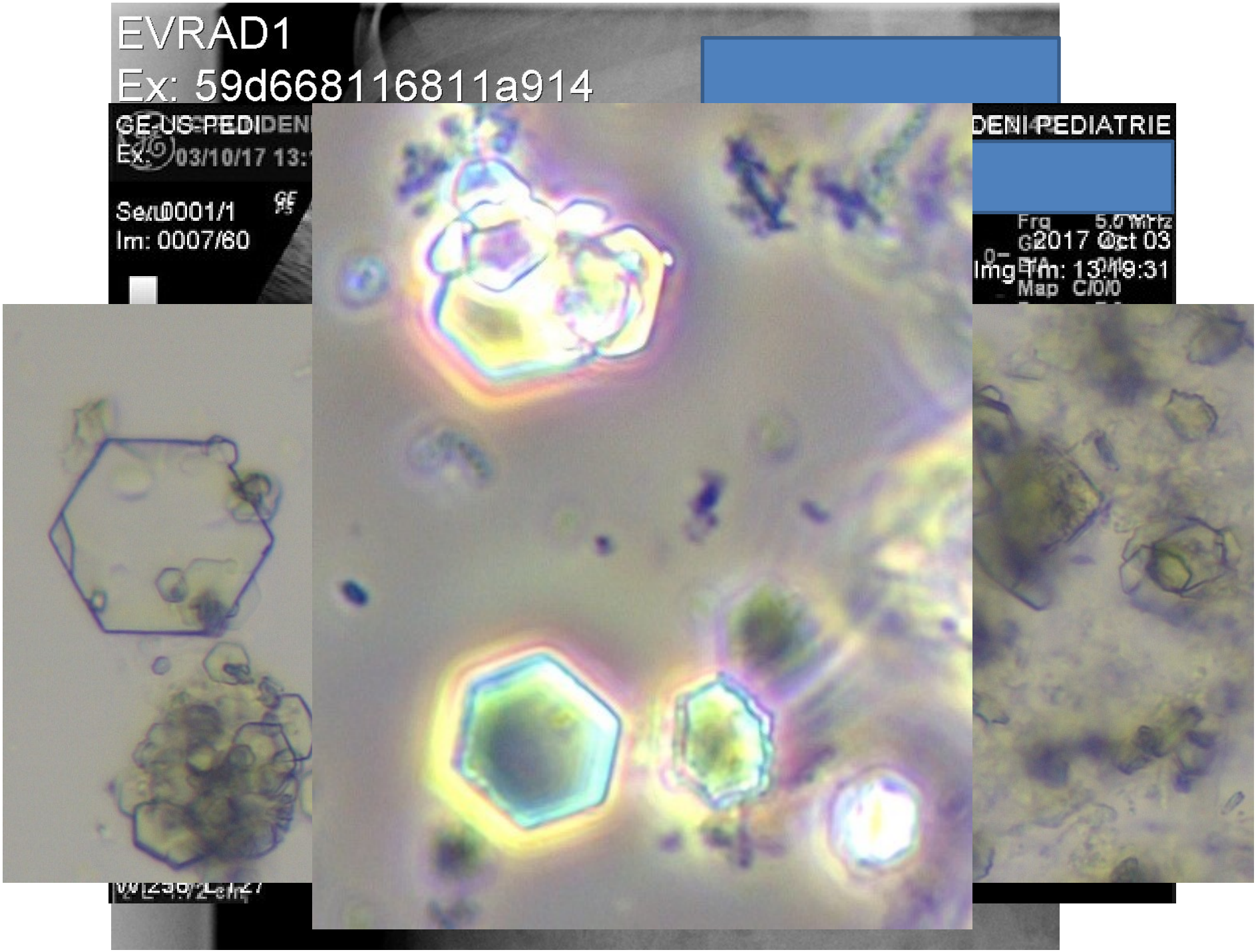
www.sepnwg.ro

Images in rare kidney diseases

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

Adrian Catalin Lungu
04 Apr 2024
Skopje

Stones


EVRAD1
Ex: 59d668116811a914
GE US PEDI
Ex:
03/10/17 13:
Ser 0001/1
Im: 0007/60
DENI PEDIATRIE
Frq 5.0MHz
2017 Oct 03
Map C/0/0

Aminoacizi in urina

Urina 24h / cromatografie de lichide cuplata cu spectrometrie de masa

	Alanina	3	'0.1g creatir	< 54
	Acid alfa-amino butiric	< 1	'0.1g creatir	< 2
A	Arginina	92	'0.1g creatir	< 3
	Asparagina	2	'0.1g creatir	< 55
	Acid aspartic	< 1	'0.1g creatir	< 6
	Carnozina	5	'0.1g creatir	< 11
	Citrulina	3	'0.1g creatir	< 35
C	Cistina	20	'0.1g creatir	< 9
	Glutamina	6	'0.1g creatir	< 51
	Acid glutamic	1	'0.1g creatir	< 61
	Glicina	8	'0.1g creatir	< 155
	Histidina	14	'0.1g creatir	< 110
	Hidroxiprolina	< 1	'0.1g creatir	< 54
	Izoleucina	< 1	'0.1g creatir	< 3
	Leucina	1	'0.1g creatir	< 2
L	Lizina	110	'0.1g creatir	< 65
	Metionina	< 1	'0.1g creatir	< 2
	3-Metilhistidina	4	'0.1g creatir	< 19
O	Ornitina	30	'0.1g creatir	< 6
	Fenilalanina	2	'0.1g creatir	< 11
	Fosfoetanolamina	< 1	'0.1g creatir	< 2
	Prolina	< 1	'0.1g creatir	< 47
	Sarcozina	< 1	'0.1g creatir	< 15

RESULTS

Sequence analysis identified the heterozygous sequence variant c.647C>T in exon 3 of the *SLC3A1* gene in INDEX

This mutation leads to an amino acid exchange from threonine to methionine at the amino acid position 216 (p.Thr216Met).

By quantitative MLPA analysis, also a heterozygous *SLC3A1* duplication of exons 5-9 was detected in INDEX

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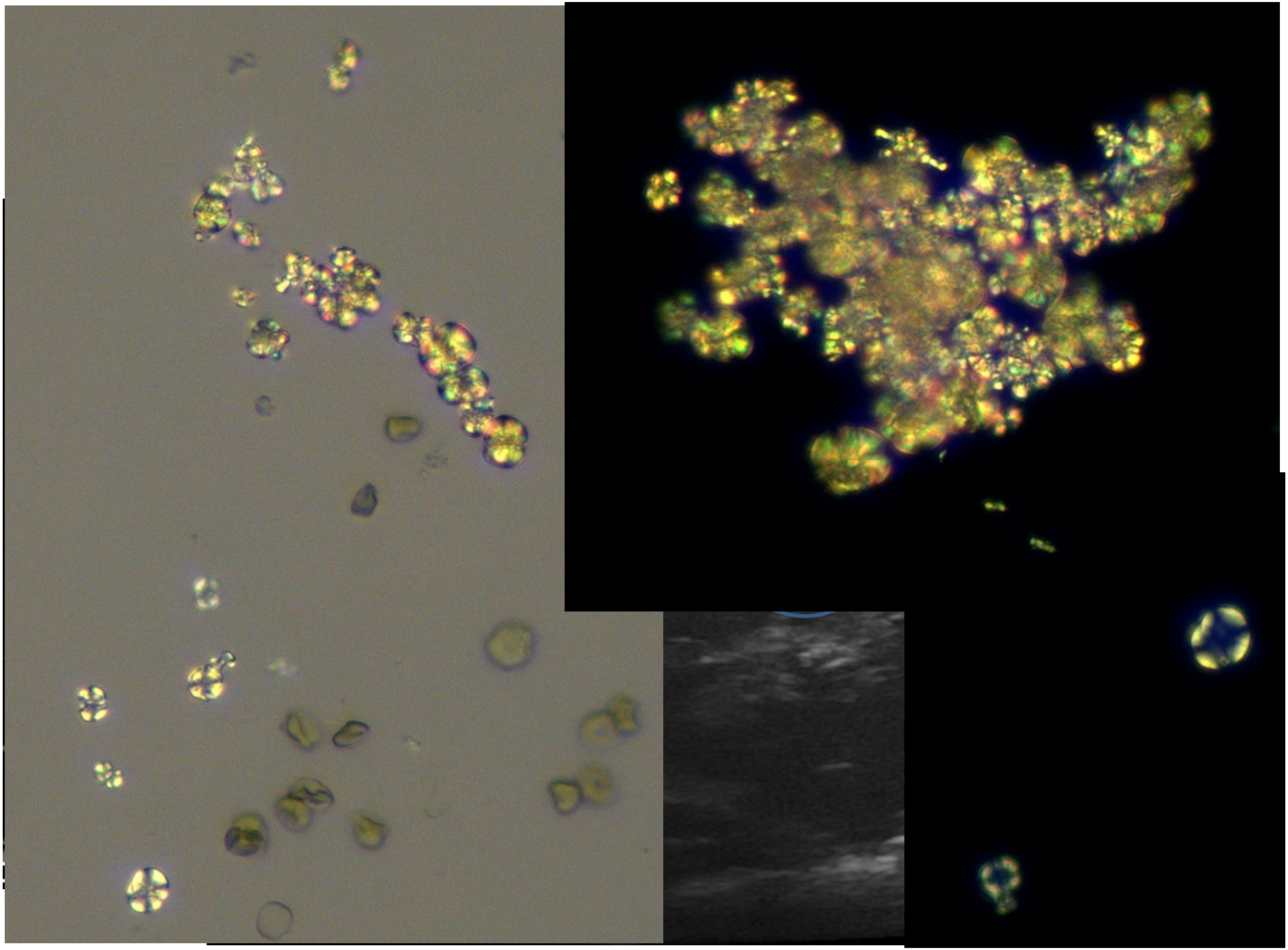
Vorstandsvorsitzender: Prof. Dr. Edgar Schömig
Steuernummer: 223/5911/1092 • Ust-IdNr.: DE 215 420 431

Anfahrt Institut für Humangenetik
KVB-Linie 9 bis „Lindenburg" oder Bus-Linie 146 bis „Geibelstraße"

Sequence analysis of exon 3 of the *SLC3A1* gene on DNA of MOTHER **identified the heterozygous sequence variant c.647C>T.**

Quantitative MLPA analysis on DNA of FATHER **identified the heterozygous *SLC3A1* duplication of exons 5-9.**

Sequence analysis of exon 3 of the *SLC3A1* gene was without pathological findings.



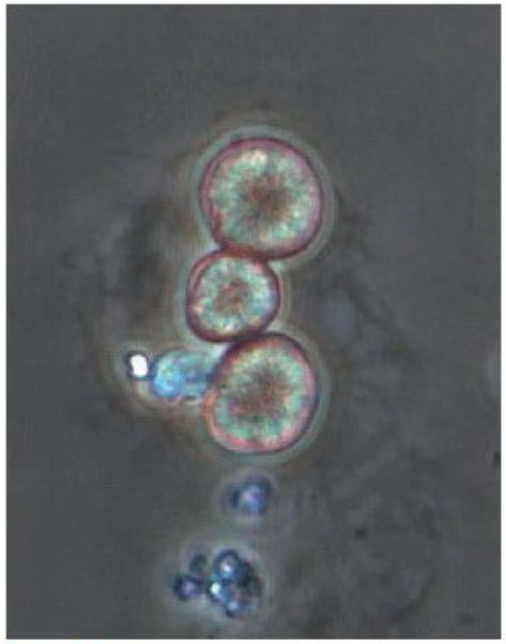


FIGURE 2.204 2.8-DHA crystals with atypical appearance (phase contrast, original magnification x 400).

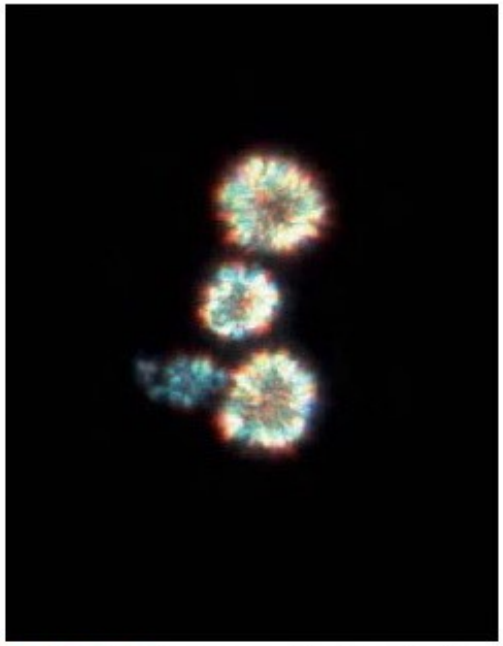
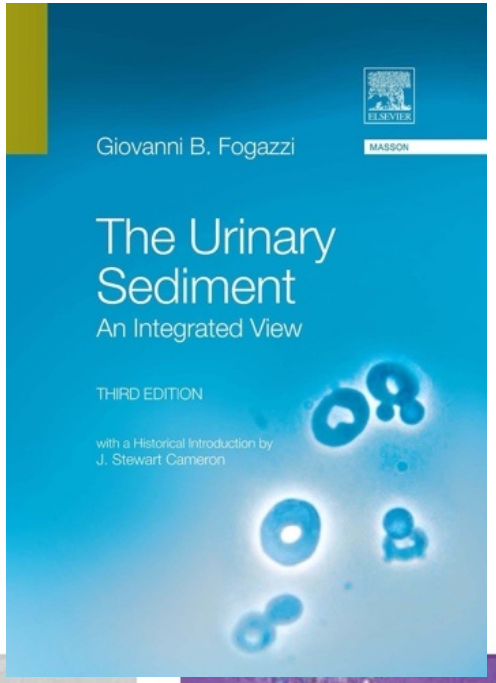


FIGURE 2.205 The same crystals shown in Figure 2.204 as seen by polarized light. Note that also the birefringence features are atypical. These crystals could correctly be identified only after infrared spectroscopy analysis (original magnification, x 400).



??????Adenine Phosphoribosyl-transferase Deficiency

2,8-Dihydroxyadeninuria; APRT Deficiency

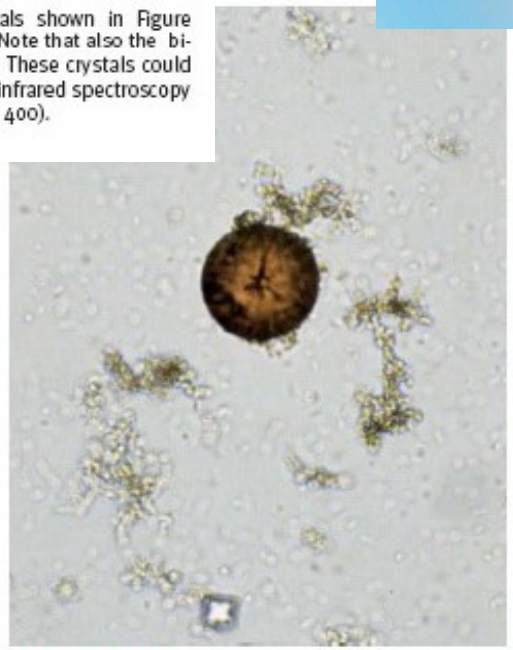


FIGURE 2.202 A crystal of 2.8-DHA (bright field, original magnification, x 400). Courtesy of Prof. Michel Daudon, Paris, France.

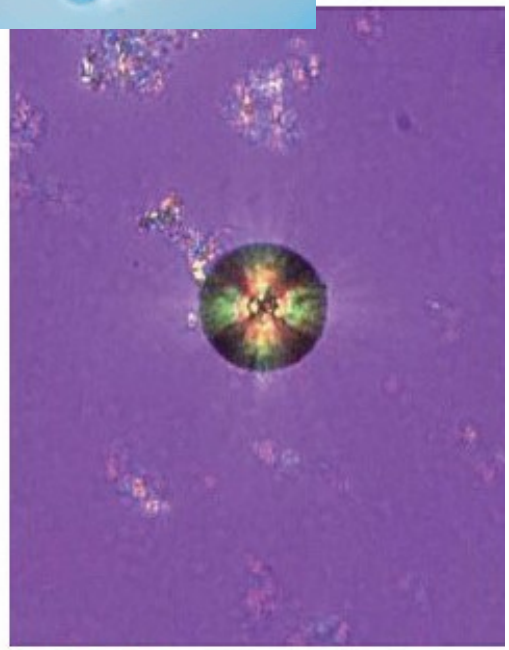


FIGURE 2.203 The same crystal shown in Figure 2.202 as seen by polarized light (original magnification, x 400). Courtesy of Prof. Michel Daudon, Paris, France.

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
GJB2	NM_004004.6	c.269T>C, p.(Leu90Pro)	HET	missense_variant	AD,AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs80338945	GRCh37/hg19	13:20763452	A/G		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	178/282666	probably damaging	deleterious	disease causing	Bart-Pumphrey syndrome, Deafness, autosomal recessive 1A, Hystrix-like ichthyosis with deafness, Keratitis-icthyosis-deafness syndrome, Keratoderma, palmoplantar, with deafness, Vohwinkel syndrome	

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
GJB2	NM_004004.6	c.551G>C, p.(Arg184Pro)	HET	missense_variant	AD,AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs80338950	GRCh37/hg19	13:20763170	C/G		

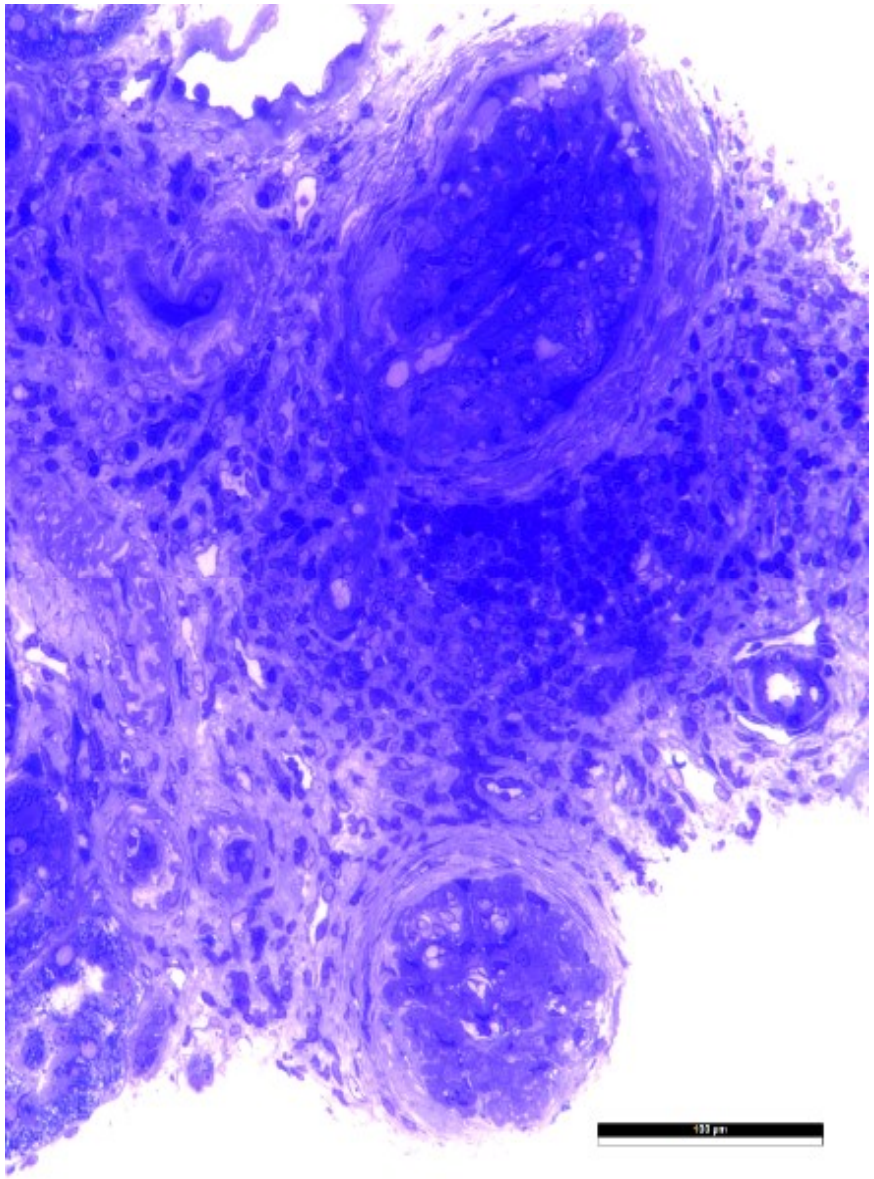
This variants explain the hearing problems

HPRT1	NM_000194.3	c.577C>T, p.(Leu193Phe)	HEM	missense_variant	X-linked

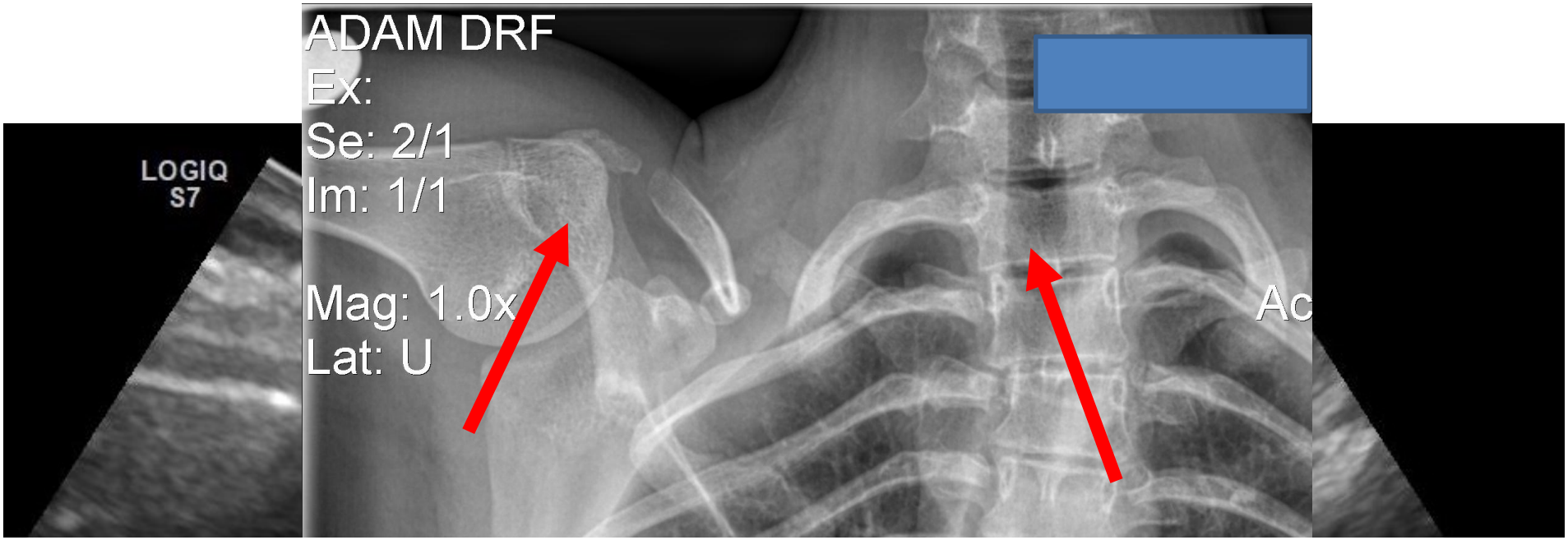
?? **Lesch-Nyhan syndrome**

Zero enzime activity - impaired kidney function, acute gouty arthritis, and self-mutilating behaviors

????? **Kelley-Seegmiller syndrome –** milder form of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency - hereditary disorder of purine metabolism, s associated with uric acid overproduction leading to urolithiasis, and early-onset gout.



FSGS
Ischemia
Tubular atrophy
Important inflamation

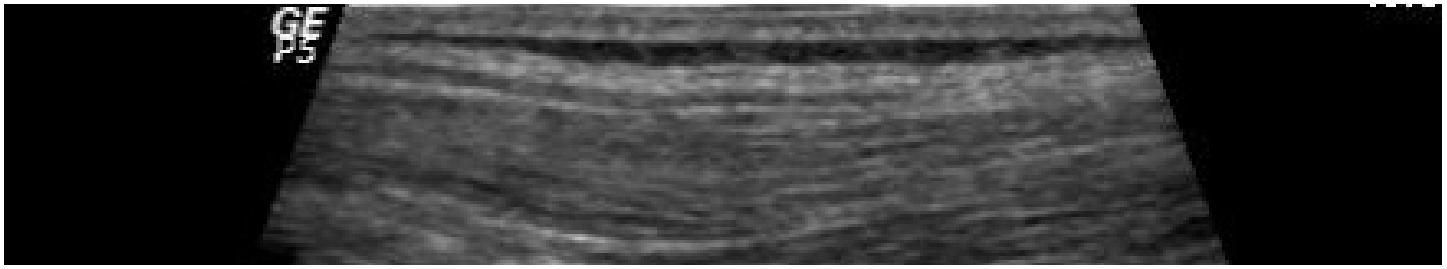


GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
SMARCA4	NM_001128849.3	c.2933G>A, p.(Arg978Gln)	HET	missense_variant	AD	**Likely pathogenic**
	ID	**ASSEMBLY** GRCh37/hg19	**POS** 19:11134267	**REF/ALT** G/A		
	gnomAD AC/AN 0/247936	**POLYPHEN** probably damaging	**SIFT** deleterious	**MUTTASTER** disease causing	**PHENOTYPE** Coffin-Siris syndrome, Rhabdoid tumor predisposition syndrome	

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
GNPTG	NM_032520.5	c.883C>G, p.(Leu295Val)	HET	missense_variant	AR	**Variant of uncertain significance**
	ID	**ASSEMBLY** GRCh37/hg19	**POS** 16:1413057	**REF/ALT** C/G		
	gnomAD AC/AN 4/282520	**POLYPHEN** probably damaging	**SIFT** deleterious low confidence	**MUTTASTER** polymorphism	**PHENOTYPE** Mucolipidosis	
GENE	**TRANSCRIPT**	**NOMENCLATURE**	**GENOTYPE**	**CONSEQUENCE**	**INHERITANCE**	**CLASSIFICATION**
CYP24A1	NM_000782.5	c.989C>T, p.(Thr330Met)	HET	missense_variant, splice_region_variant	AR	**Variant of uncertain significance**
	ID	**ASSEMBLY** GRCh37/hg19	**POS** 20:52779257	**REF/ALT** G/A		
	gnomAD AC/AN 21/282848	**POLYPHEN** probably damaging	**SIFT** deleterious	**MUTTASTER** disease causing	**PHENOTYPE** Hypercalcemia infantile 1	

Nephrocalcinosis cases

05



Result

Genotype: CYP24A1 (NM_000782.4): c.[428_430delAAG](;)[999_1006del] p.[(Glu143del)](;)[(Ser334fs)] (Chr20(GRCh37):g.[52789467_52789469del](;)[52775647_52775654del])

Conclusion

[redacted] shows compound heterozygosity for the c.428_430del p.(Glu143del) and c.999_1006del p.(Ser334fs) mutations in the CYP24A1 gene. Mutations in this gene are associated with autosomal recessively inherited infantile hypercalcemia 1 (OMIM#143880). The c.428_430del p.(Glu143del) mutation is a known pathogenic mutation, described before in patients. The c.999_1006del p.(Ser334fs) mutation has not been described in patients before, but leads to a frameshift in the CYP24A1 mRNA, shortly followed by a premature stopcodon. This mutation will either lead to the formation of a truncated protein product or no protein production (possible nonsense-mediated-decay of CYP24A1 mRNA). We consider this to be a pathogenic mutation.

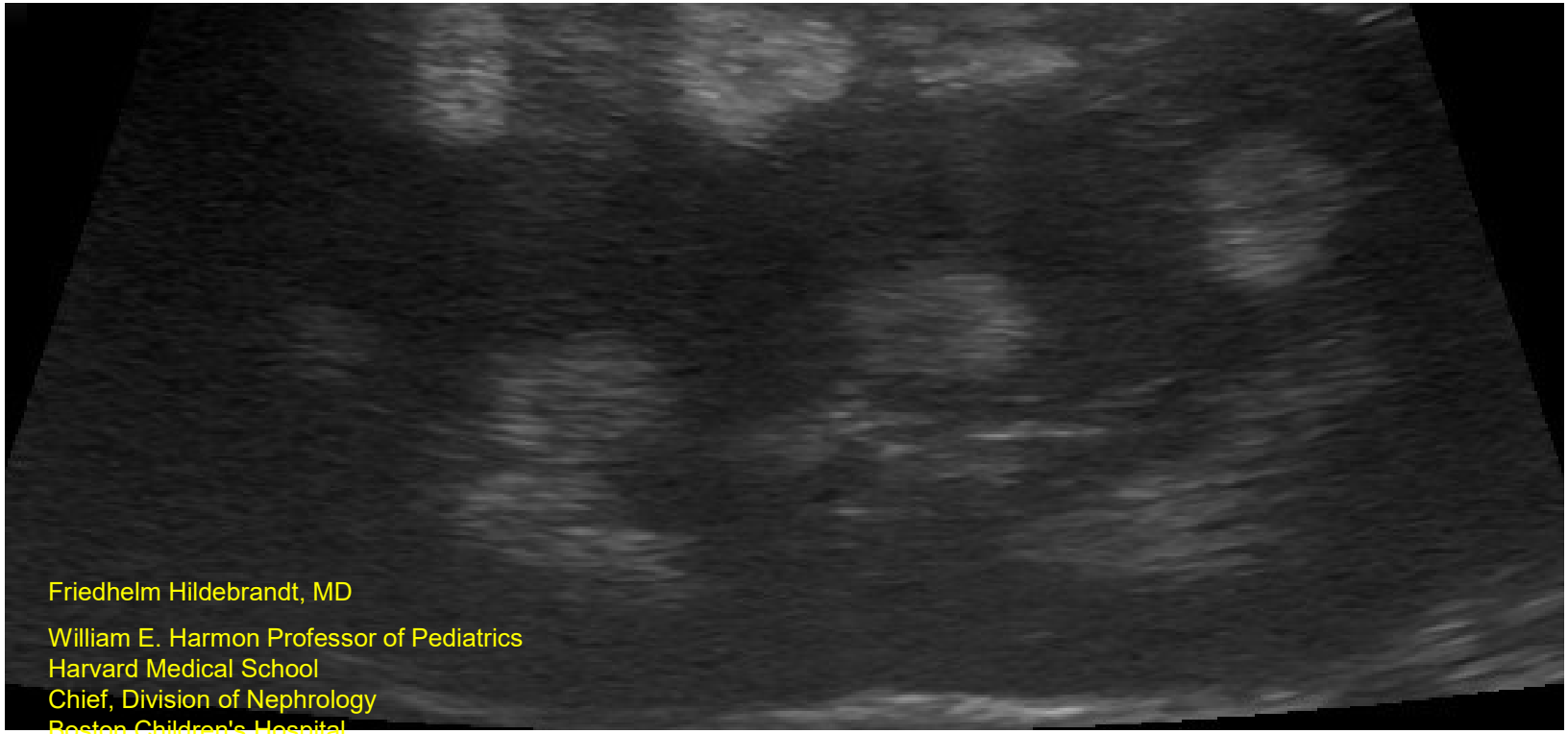
Compound heterozygosity for these two pathogenic mutations supports the assumption that mutations in the CYP24A1 gene underly the clinical phenotype in this patient. Carrier analysis in the parents of [redacted] has confirmed the compound heterozygosity of these mutations (see our refs. DN2016/13162 and DN2016/13163). Referral to a clinical geneticist for genetic counseling of the patient and his family is recommended.

06

Research-based mutation analysis of the genomic DNA of your patient by Sanger sequencing showed the following result in the gene ***SLC12A1*** (solute carrier family 12 member 1; NM_001184832).

Gene	Mutation	Zygosity
SLC12A1	Exon 7	homozygosity

Bartter Syndrome, Type 1



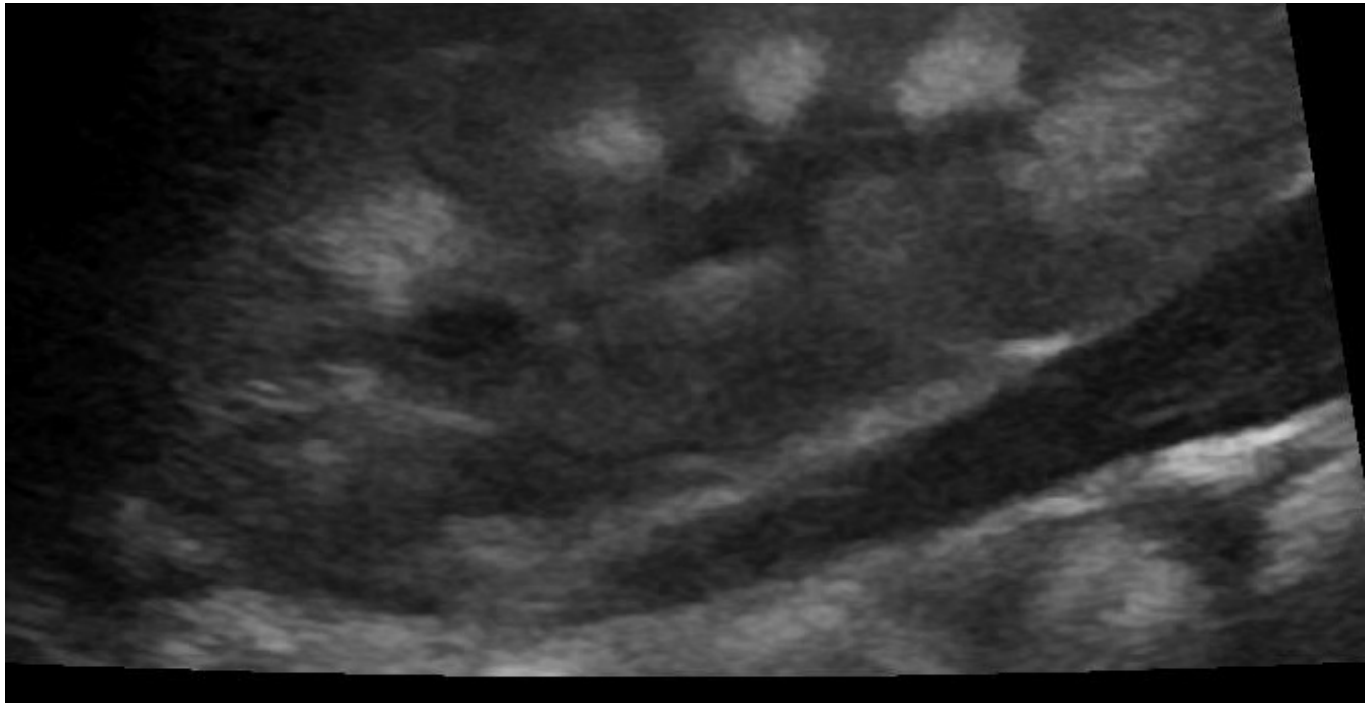
Friedhelm Hildebrandt, MD

William E. Harmon Professor of Pediatrics
Harvard Medical School
Chief, Division of Nephrology
Boston Children's Hospital

012

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
SLC12A1	NM_000338.3	c.1163del, p.(Phe388Serfs*40)	HET	frameshift_variant	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	15:48527142	AT/A		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	2/250966	N/A	N/A	N/A	Bartter syndrome; antenatal	

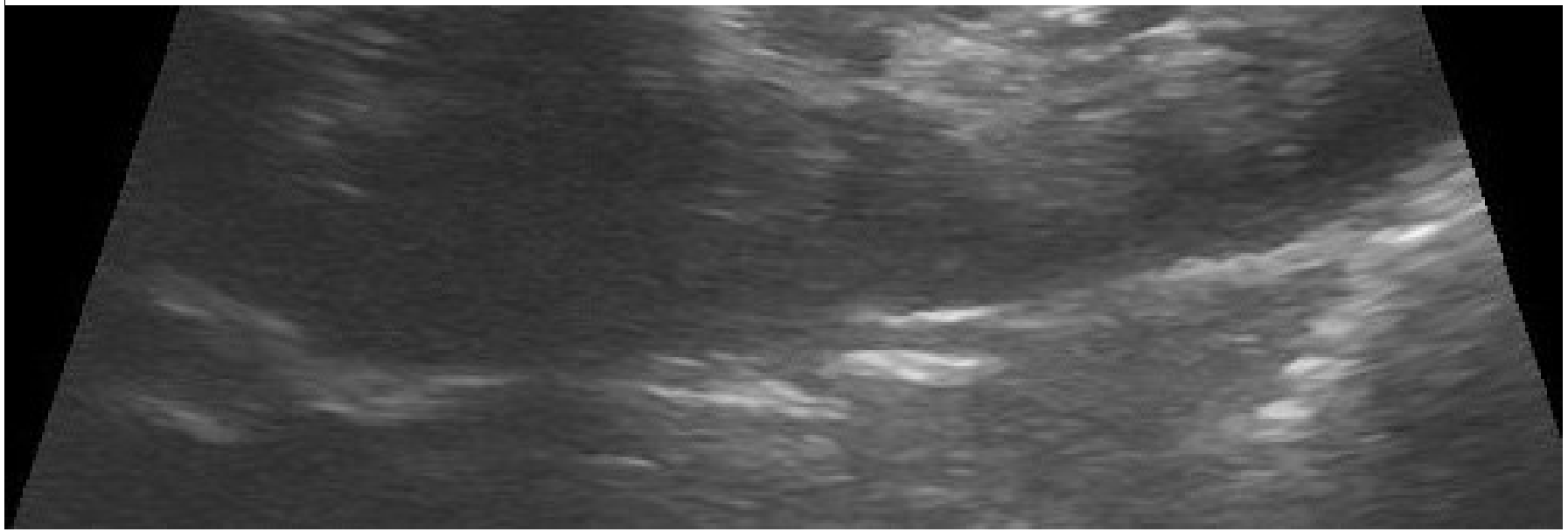
GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
SLC12A1	NM_000338.3	c.2755G>C, p.(Asp919His)	HET	missense_variant	AR	**Likely pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	15:48580365	G/C		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	1/241152	probably damaging	deleterious	disease causing	Bartter syndrome; antenatal	



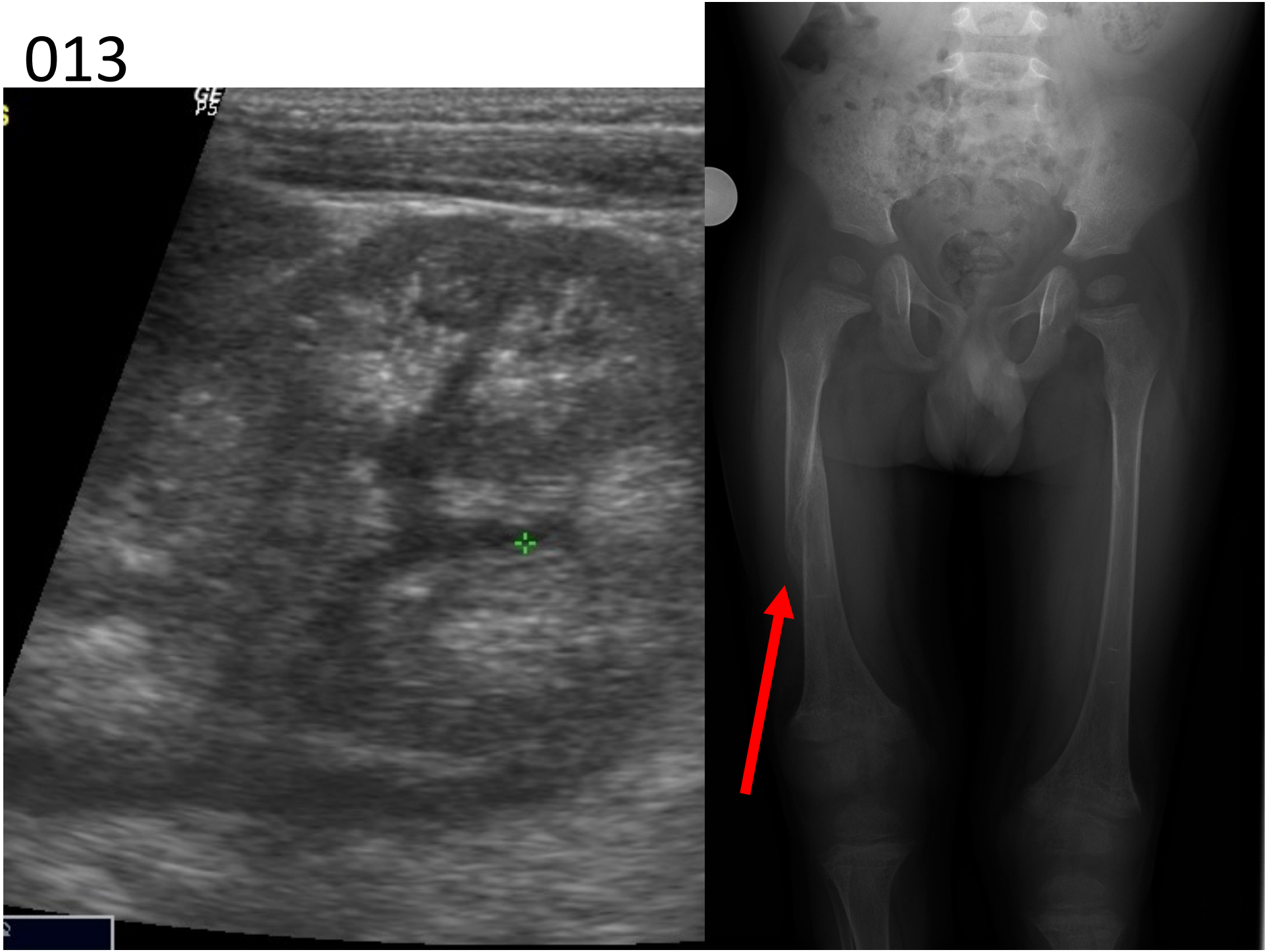
011

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
KCNJ1	NM_000220.5	c.996_999del, p.(Glu334Glyfs*35)	HET	frameshift_variant	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	11:128709196	CCTTT/C		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	26/282682	N/A	N/A	N/A	Bartter syndrome, antenatal, type 2	

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
KCNJ1	NM_000220.5	c.658C>T, p.(Leu220Phe)	HET	missense_variant	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs200320892	GRCh37/hg19	11:128709538	G/A		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	89/249254	probably damaging	tolerated	disease causing	Bartter syndrome, antenatal, type 2	



013



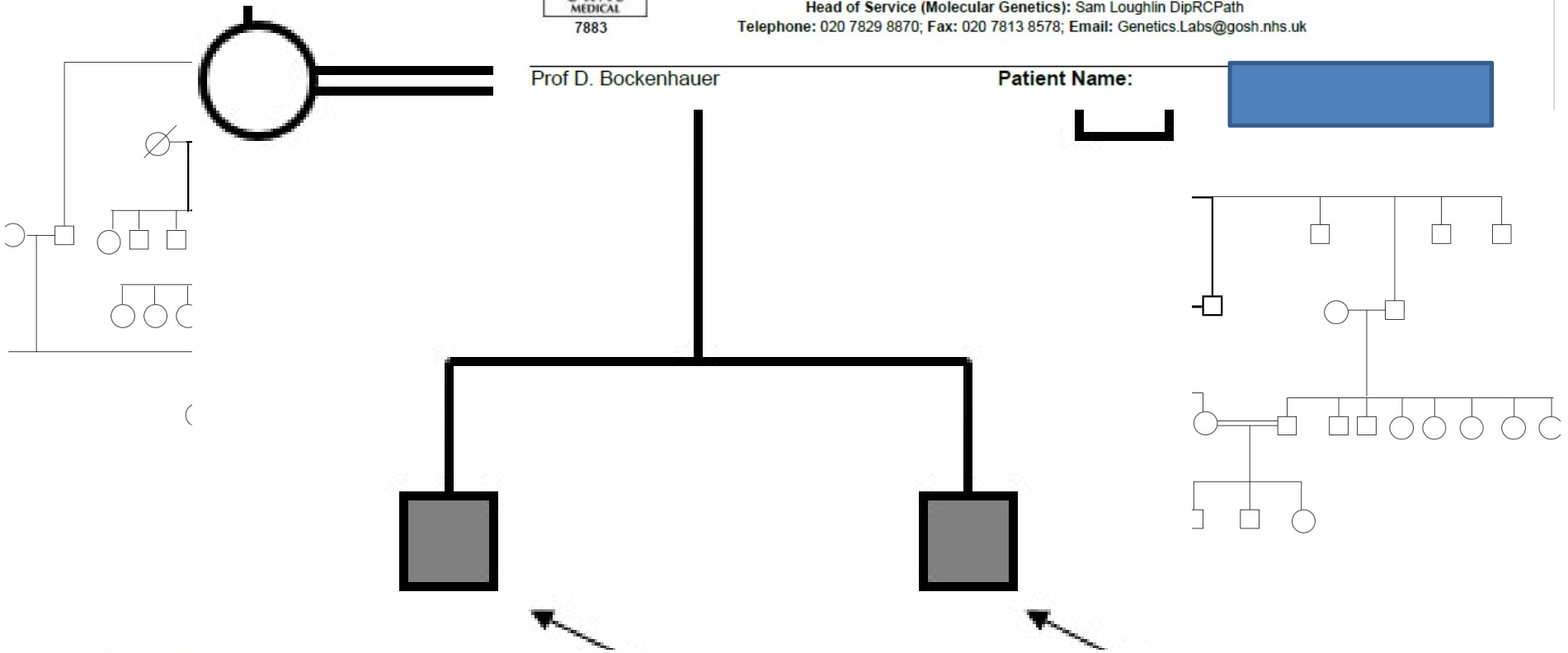
013

Rare & Inherited Disease Genomic Laboratory
Great Ormond Street Hospital for Children NHS Foundation Trust
Levels 4-6 Barclay House, 37 Queen Square, London WC1N 3BH

Director: Lucy Jenkins FRCPath
Head of Service (Cytogenetics): Deborah Morrogh FRCPath
Head of Service (Molecular Genetics): Sam Loughlin DipRCPath
Telephone: 020 7829 8870; **Fax:** 020 7813 8578; **Email:** Genetics.Labs@gosh.nhs.uk

UKAS MEDICAL 7883

NHS London North NHS Genomic Laboratory Hub



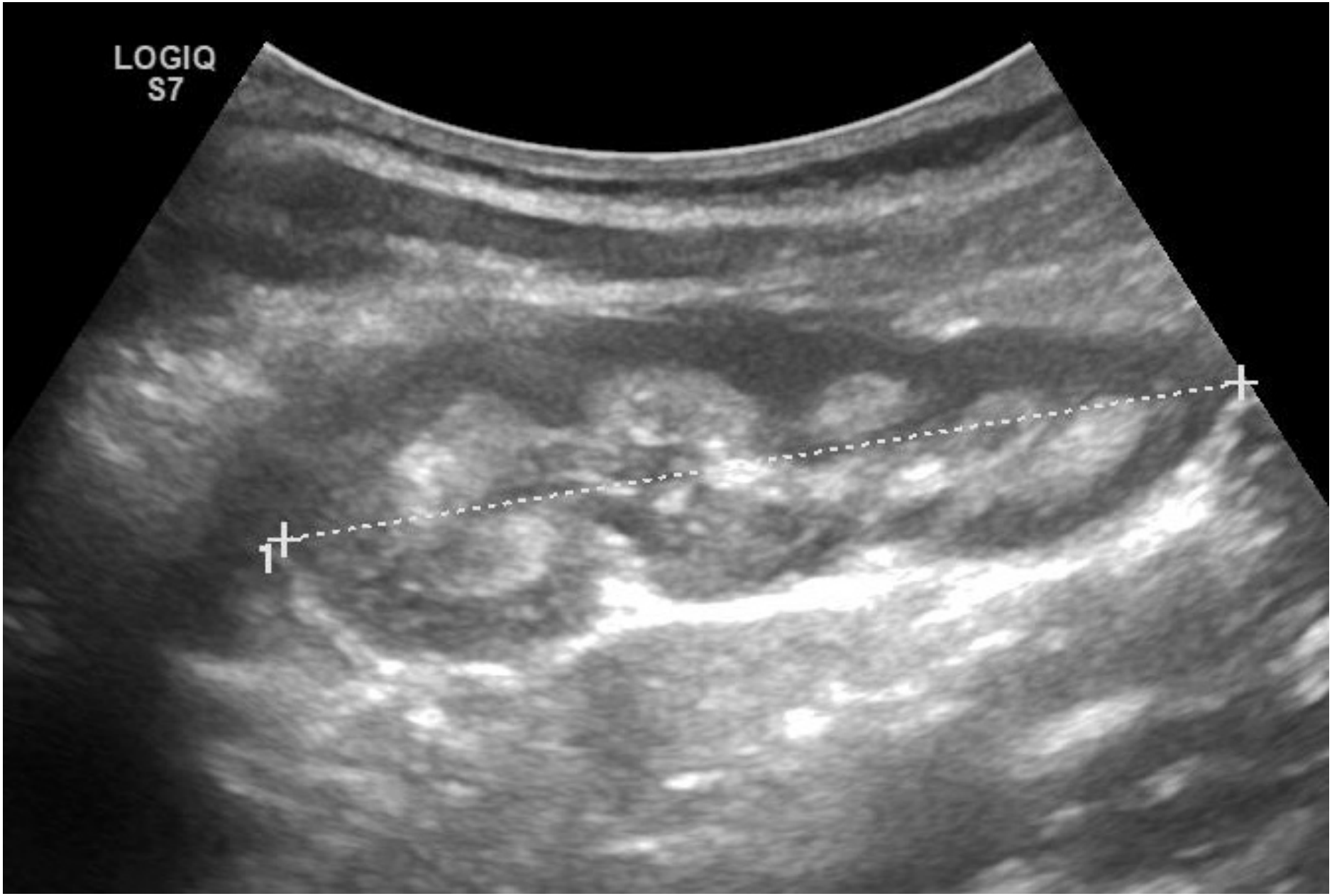
Prof D. Bockenhauer

Patient Name: [redacted]

RESULTS

Name (Date of Birth)	**Episode**	**PANEL RESULT**	**Conclusion**
[redacted] (11-11-2014)	19G10886	*ATP6V1B1* c.586-2A>G homozygote	**Consistent with a diagnosis of distal renal tubular acidosis**

04



04

Abnormal facial shape; Atrial septal defect; Decreased body weight; Dysphagia; Failure to thrive; Feeding difficulties; Gastrostomy tube feeding in infancy; Gingival overgrowth; Hypotonia; Language impairment; Leukoencephalopathy; Microcephaly; Narrow forehead; Nephrocalcinosis; Premature birth; Proptosis; Short stature; Syndactyly

INTERPRETATION

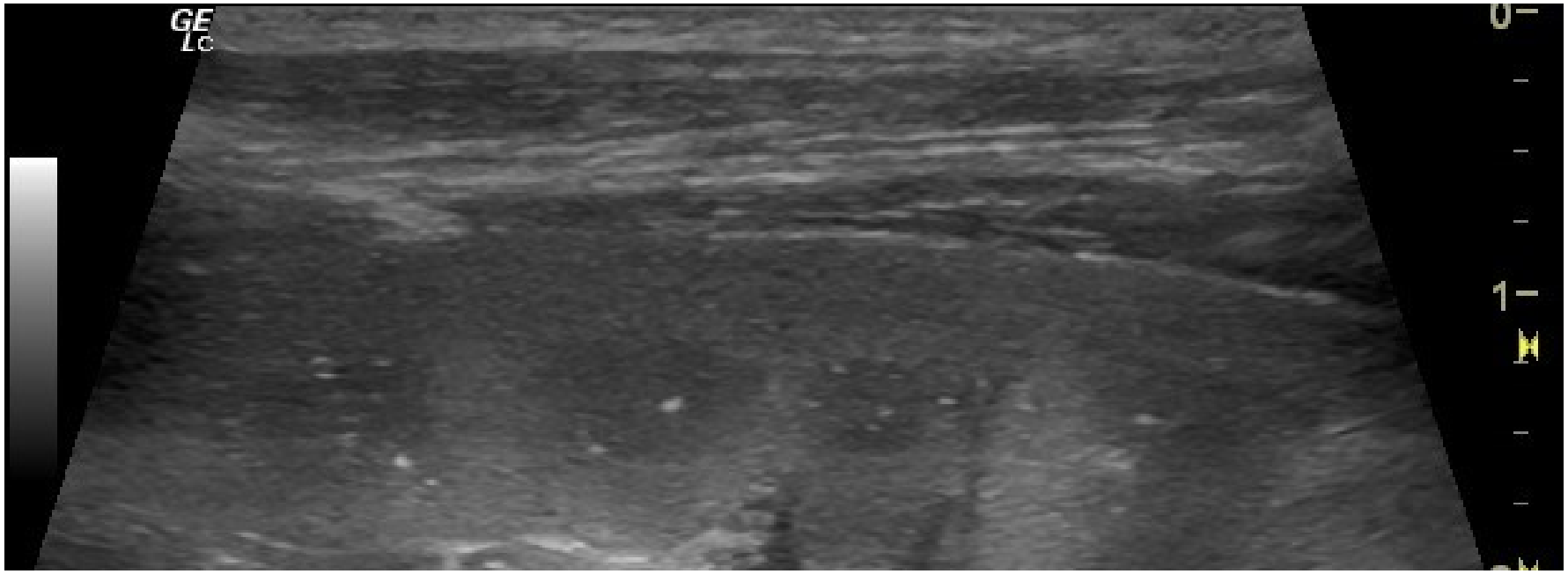
A heterozygous pathogenic and a heterozygous likely pathogenic variant was identified in the *DHCR7* gene. **This finding is consistent with the genetic diagnosis of an autosomal recessive Smith-Lemli-Opitz syndrome.** Parental carrier testing is needed to identify the phase of the detected variants.

A variant of uncertain significance was identified in the mitochondrial gene *MT-ND3* in heteroplasmic state. Pathogenic variants in this gene are associated with Mitochondrial Complex I Deficiency. Based on current evidence, the clinical relevance of this variant remains unclear.

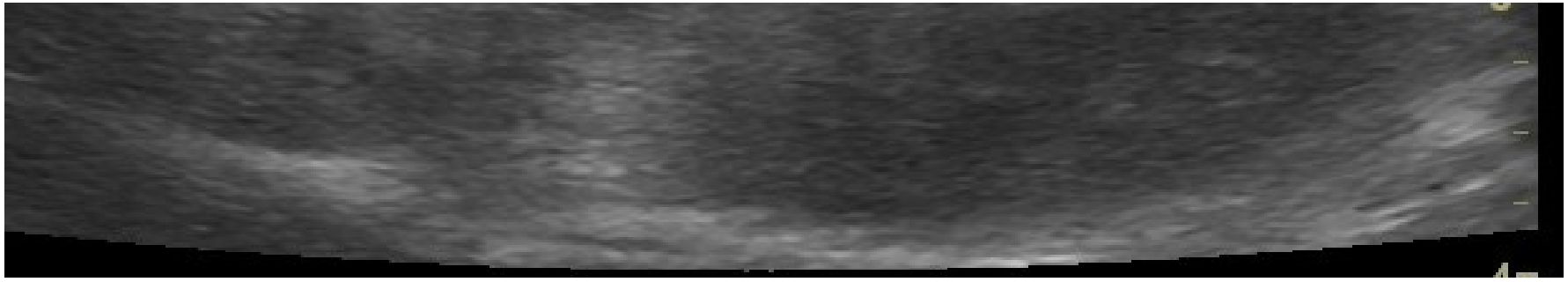
No further clinically relevant variants related to the described phenotype were detected.

DENT

09



GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
CLCN5	NM_000084.5	c.789_791del, p.(Leu263del)	HEM	inframe_deletion	X-linked	**Variant of uncertain significance**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	X:49850697	GTAT/G		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	0/0	N/A	N/A	N/A		



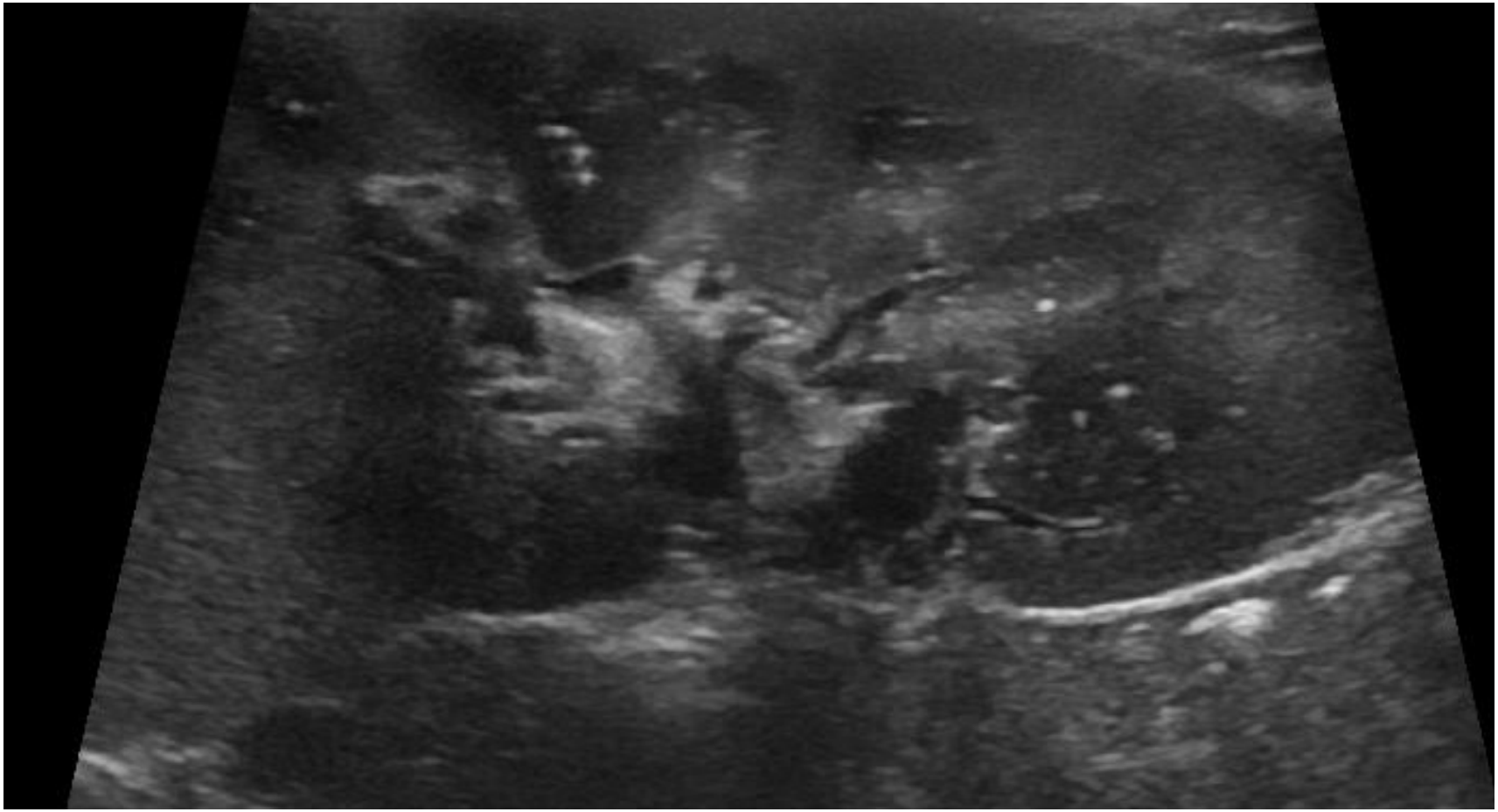
010

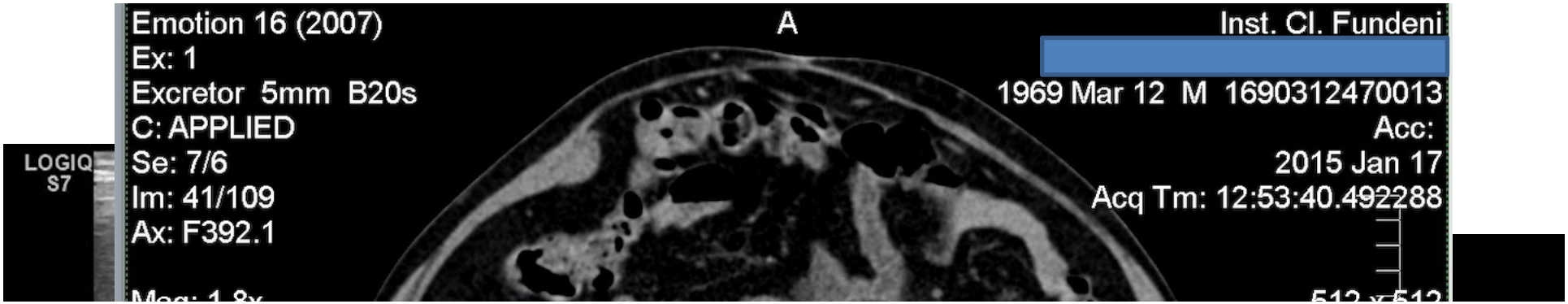
GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
CLCN5	NM_000084.4	c.794G>A, p.(Ser265Asn)	HEM	missense_variant	X-linked	**Likely pathogenic**

ID	ASSEMBLY	POS	REF/ALT
	GRCh37/hg19	X:49850707	G/A

gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER
0/0	probably damaging	deleterious	disease causing

PHENOTYPE
Dent disease,
Hypophosphatemic rickets,
Nephrolithiasis type I,
Proteinuria low molecular weight with hypercalciuric nephrocalcinosis



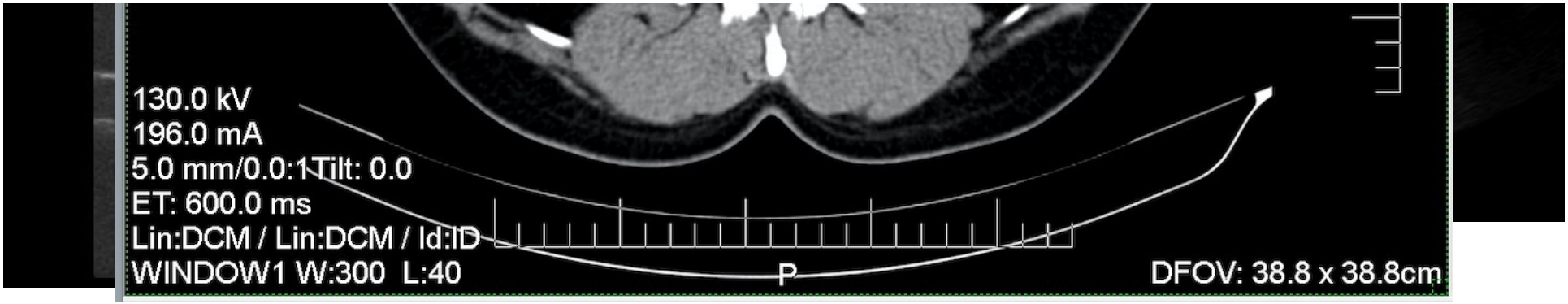


GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
CLCN5	NM_000084.5	c.1561C>T, p.(Leu521Phe)	HEM	missense_variant	X-linked	**Likely pathogenic**

ID	ASSEMBLY	POS	REF/ALT
	GRCh37/hg19	X:49854799	C/T

gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER
0/0	possibly damaging	deleterious	disease causing

PHENOTYPE
Dent disease,
Hypophosphatemic rickets,
Nephrolithiasis I,
Proteinuria,
low molecular weight,
with hypercalciuric nephrocalcinosis



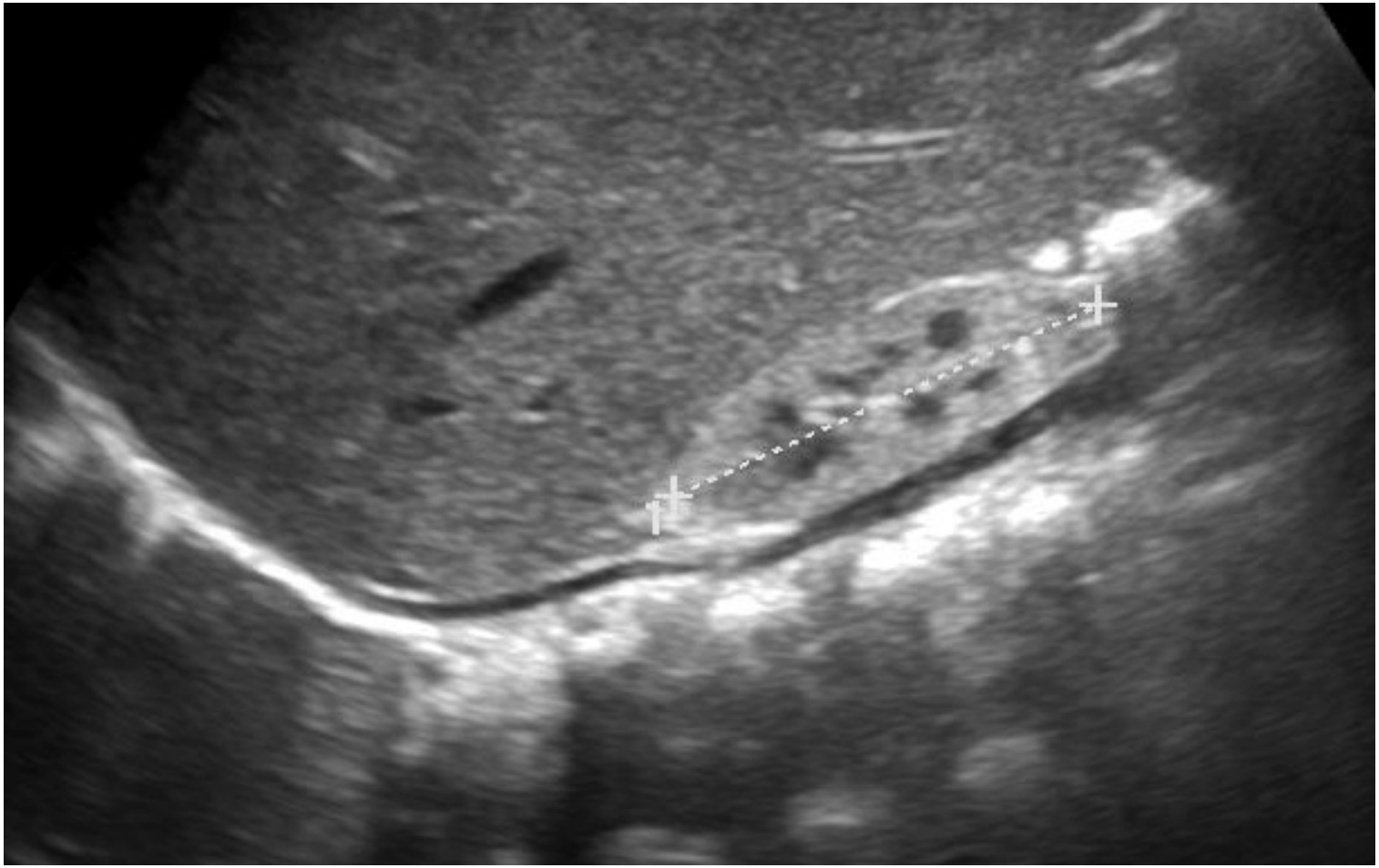
Ciliopaties

Cysts

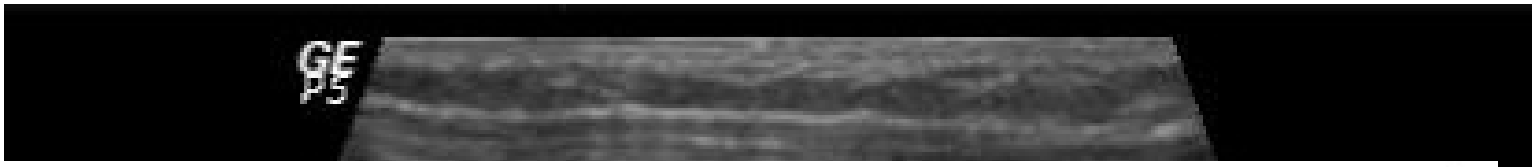
GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
NUP205	NM_015135.3	c.3128G>C, p.(Gly1043Ala)	HOM	missense_variant	AR	**Variant of uncertain significance**

ID	ASSEMBLY	POS	REF/ALT
	GRCh37/hg19	7:135292052	G/C

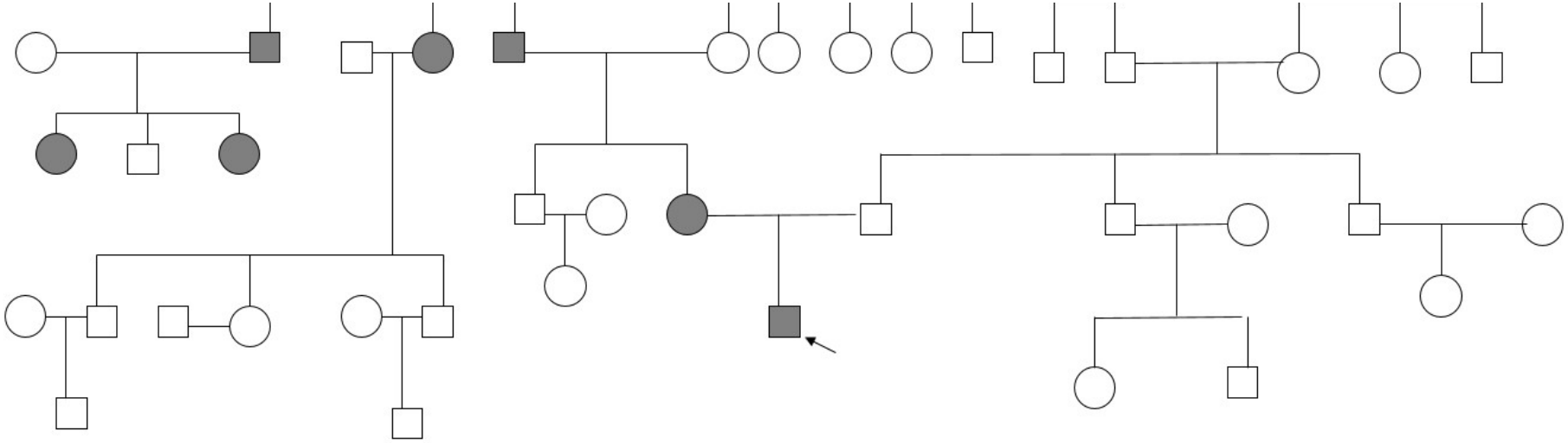
gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER	PHENOTYPE
7/282860	probably damaging	deleterious	disease causing	Nephrotic syndrome, type 13

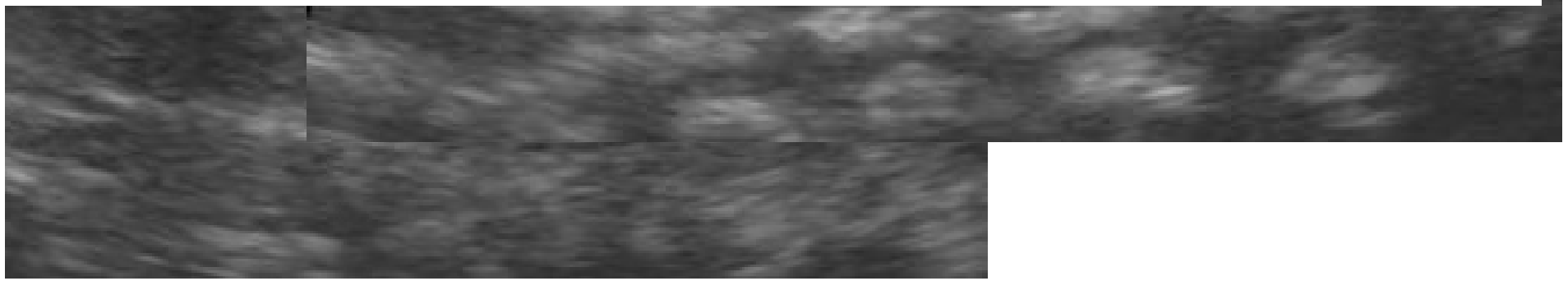


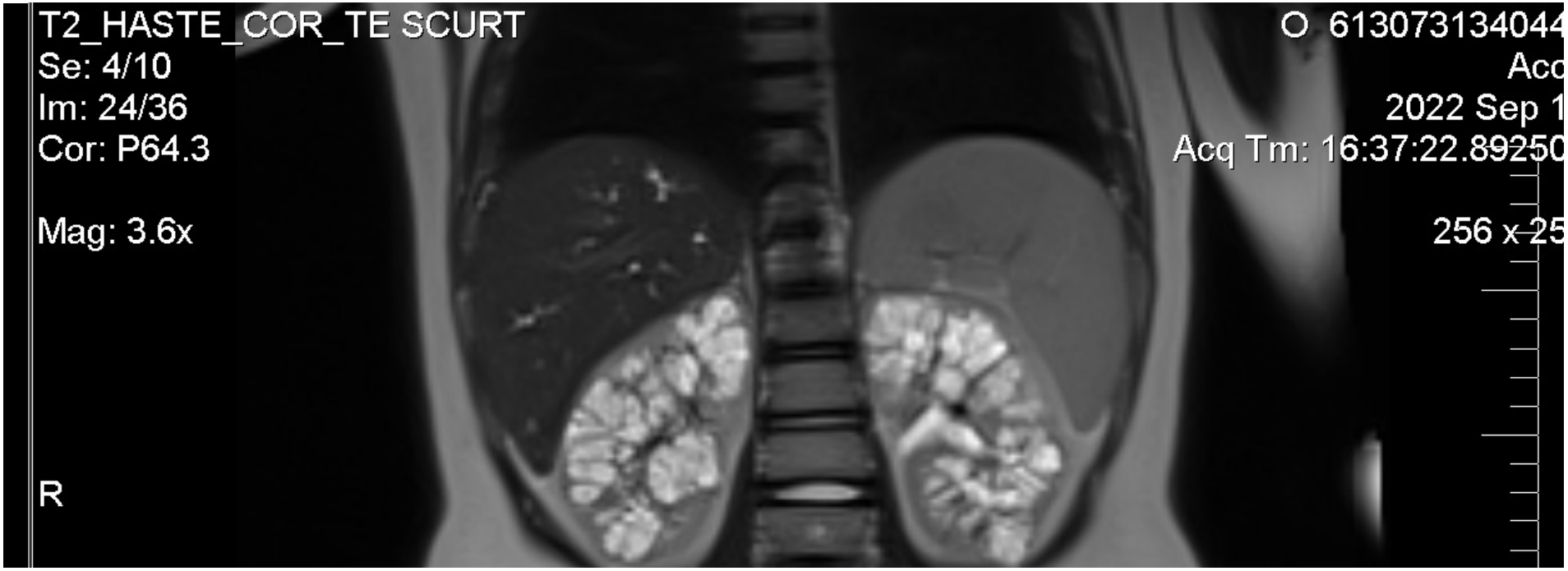

Pediatrie Fundeni
02/29/24 11:50:11AM
ADM
FERARU, NATALIA
6240216440021
MI 1.
Bardet–Biedl syndrome



GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
PKD1	NM_001009944.2	c.11537+1del	HET	splice_donor_variant	AD	**Likely pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	16:2141780	AC/A		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	0/0	N/A	N/A	N/A	Polycystic kidney disease	







GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
PKHD1	NM_138694.4	c.370C>T, p.(Arg124*)	HET	stop_gained	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
		GRCh37/hg19	6:51944718	G/A		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	3/282830	N/A	N/A	disease causing	Polycystic kidney disease	

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
PKHD1	NM_138694.4	c.10658T>C, p.(Ile3553Thr)	HET	missense_variant	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs137852948	GRCh37/hg19	6:51524266	A/G		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	1/31404	benign	deleterious	disease causing	Polycystic kidney disease	

Thank you

Never Delegate Understanding

Tim Kastelle

IPNA
Teaching Course

SEPNWG XI
XI SEPNWG Meeting

Hereditary Kidney Disease: Bucharest international Symposium on Nephrology's Hidden Challenges

Topics:

aHUS
C3 GN
Primary Hyperoxaluria
Fabry disease

Bucharest, Romania
08-09 November 2024

www.sepnwg.ro

RARE DISEASES IN PEDIATRIC NEPHROLOGY
(IPNA Sponsored Teaching Course)
Skopje, North Macedonia, April 2024.

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

Genetic counseling in rare kidney diseases

Adrijan Sarajlija

Mother and Child Health Care Institute of Serbia “Dr Vukan Čupić”

University of Belgrade, Faculty of Medicine, Serbia

Basics

- The concept of **genetic counselling** was introduced by **Sheldon Reed** in 1947

- Genetic counseling is a process in which a specialist helps interested individuals **understand and adapt** to the medical, psychological, and familial implications of hereditary diseases

- In many countries, there is a specialized educational profile for genetic counselors

Sheldon **Clark** ***Reed*** *(1910-2003)*

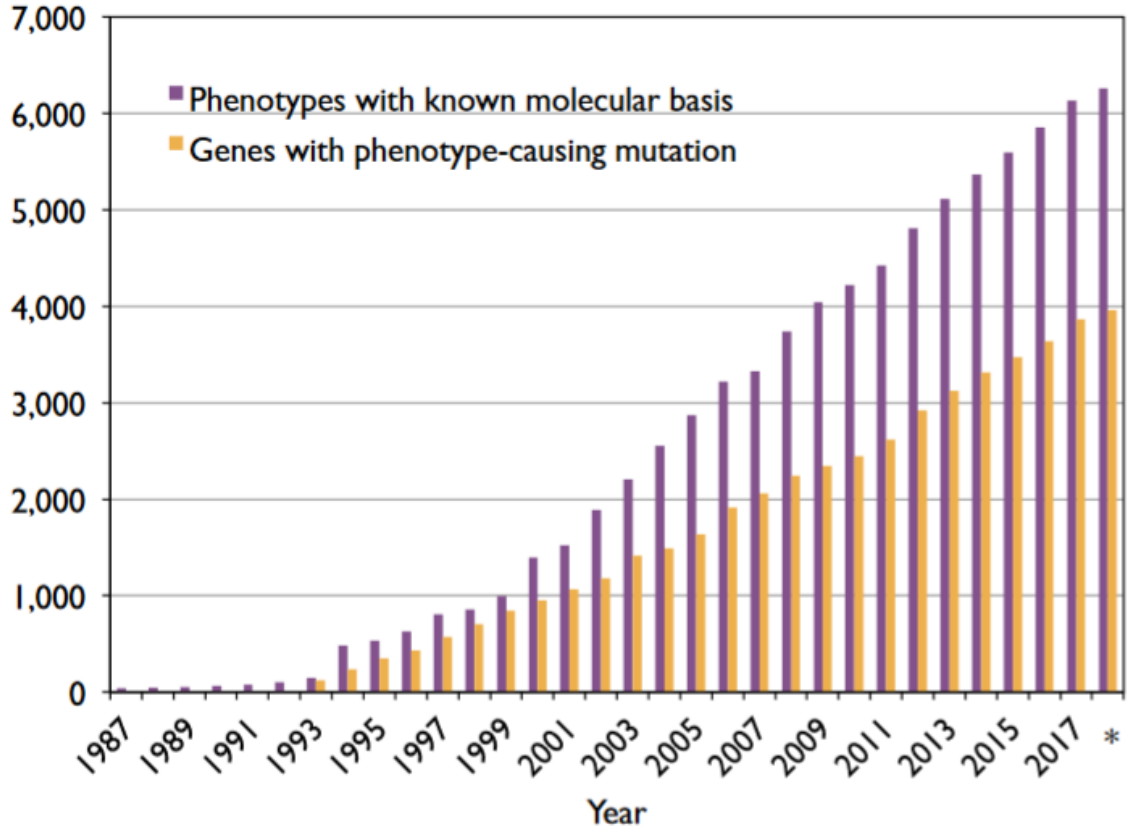
Roles of genetic counselor:
as proposed by *European Board of Medical Genetics* - EBMG)

- To **identify the needs** of individuals and families
- To **gather, select, and interpret** significant clinical and genetic information, results, findings, literature...
- To **assist individuals** in understanding the genetic basis of the disease and the risks of illness within the family
- To **provide information** to the family about the possibility of genetic testing, treatment, scientific research...
- To **assist in choosing the best option** and psychological adaptation to the current situation
- To **assist in accessing medical professionals** and services relevant to the inherited disease

Catapano F, El Hachmi M, Ketterer-Heng N, Renieri A, Mari F, Morris M, Cordier C. The role of the Genetic Counsellor in the multidisciplinary team: the perception of geneticists in Europe. Eur J Hum Genet. 2022 Dec;30(12):1432-1438.

Advance of molecular genetic diagnostics

- The advent of **gene sequencing** techniques has revolutionized the diagnosis of hereditary diseases
- The number of diseases with clarified genetic bases exceeds 7,000
- Dozens of hereditary nephrological diseases are now better understood due to gene sequencing



source: https://www.omim.org/statistics/geneMap

OMIM Gene Map Statistics

OMIM Morbid Map Scorecard (Updated April 2nd, 2024) :

Total number of phenotypes* for which the molecular basis is known	7,512
Total number of genes with phenotype-causing mutation	4,899

* Phenotypes include (1) single-gene mendelian disorders and traits; (2) susceptibilities to cancer and complex disease (e.g., BRCA1 and familial breast-ovarian cancer susceptibility, 113705.0001, and CFH and macular degeneration, 134370.0008); (3) variations that lead to abnormal but benign laboratory test values ("nondiseases") and blood groups (e.g., lactate dehydrogenase B deficiency, 150100.0001 and ABO blood group system, 110300.0001); and (4) select somatic cell genetic disease (e.g., GNAS and McCune-Albright syndrome, 139320.0008 and IDH1 and glioblastoma multiforme, 147700.0001.)

Distribution of Phenotypes across Genes (Updated April 2nd, 2024) :

Number of genes with 1 phenotype	3,432
Number of genes with 2 phenotypes	892
Number of genes with 3 phenotypes	322
Number of genes with 4+ phenotypes	253

Diagnostic value of genomic tests in pediatrics

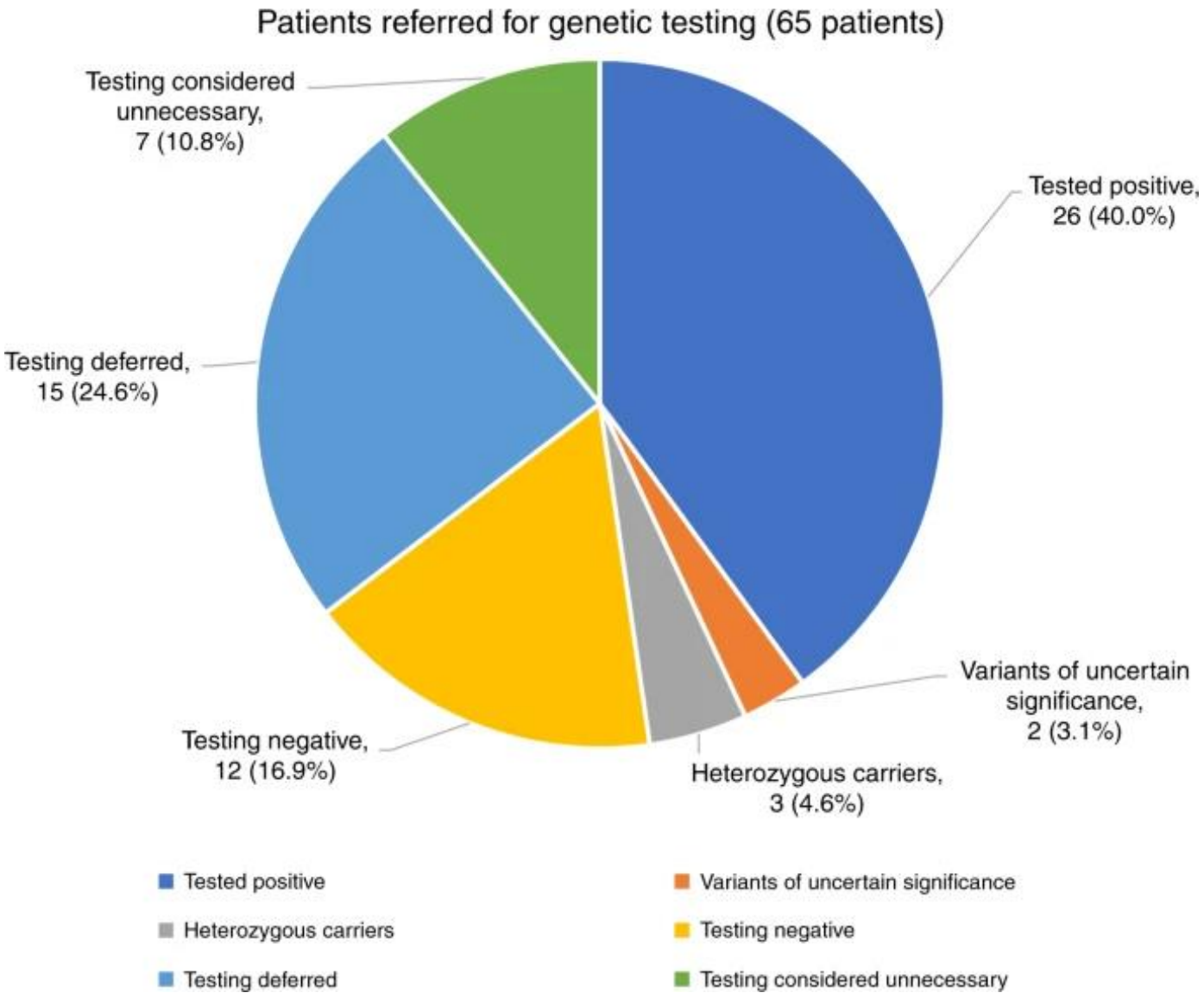
- Exome and whole genome sequencing have a role in **diagnosing a broad array of conditions**, both common and rare

- **Diagnosing rare diseases** should be exact and timely

- Reduction of emotional distress for families and high medical expenditures

- Recent studies have demonstrated that monogenic causes can be identified in up to **10% of adults** and **20%-50% of children with CKD**
- 20%–27% of patients with CKD/ESKD report a positive family history
- 10%–65% of patients with a family history may have a genetic cause identified
- up to 24% of a more general CKD cohort may have a genetic cause identified

Aron AW, Dahl NK, Besse W. A Practical Guide to Genetic Testing for Kidney Disorders of Unknown Etiology. Kidney360. 2022 Jul 8;3(9):1640-1651.



Mean age: 39.9 years

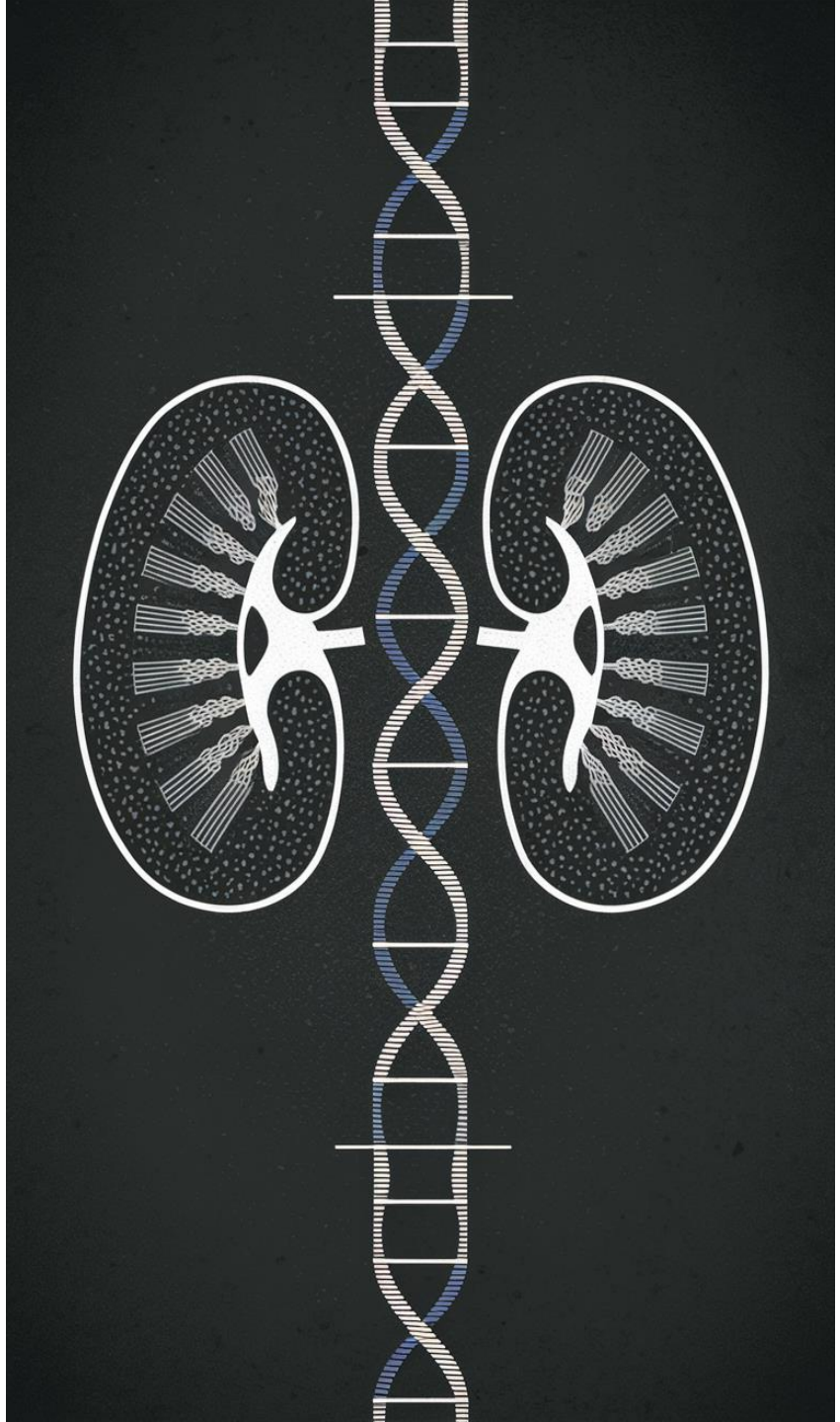
9 - Alport syndrome,
7 – ADPKD
2 - FSGS
2 - with PAX2-mediated CAKUT,
1 each with ARPKD, Dent, Frasier, Gordon, Gitelman, and Zellweger syndromes

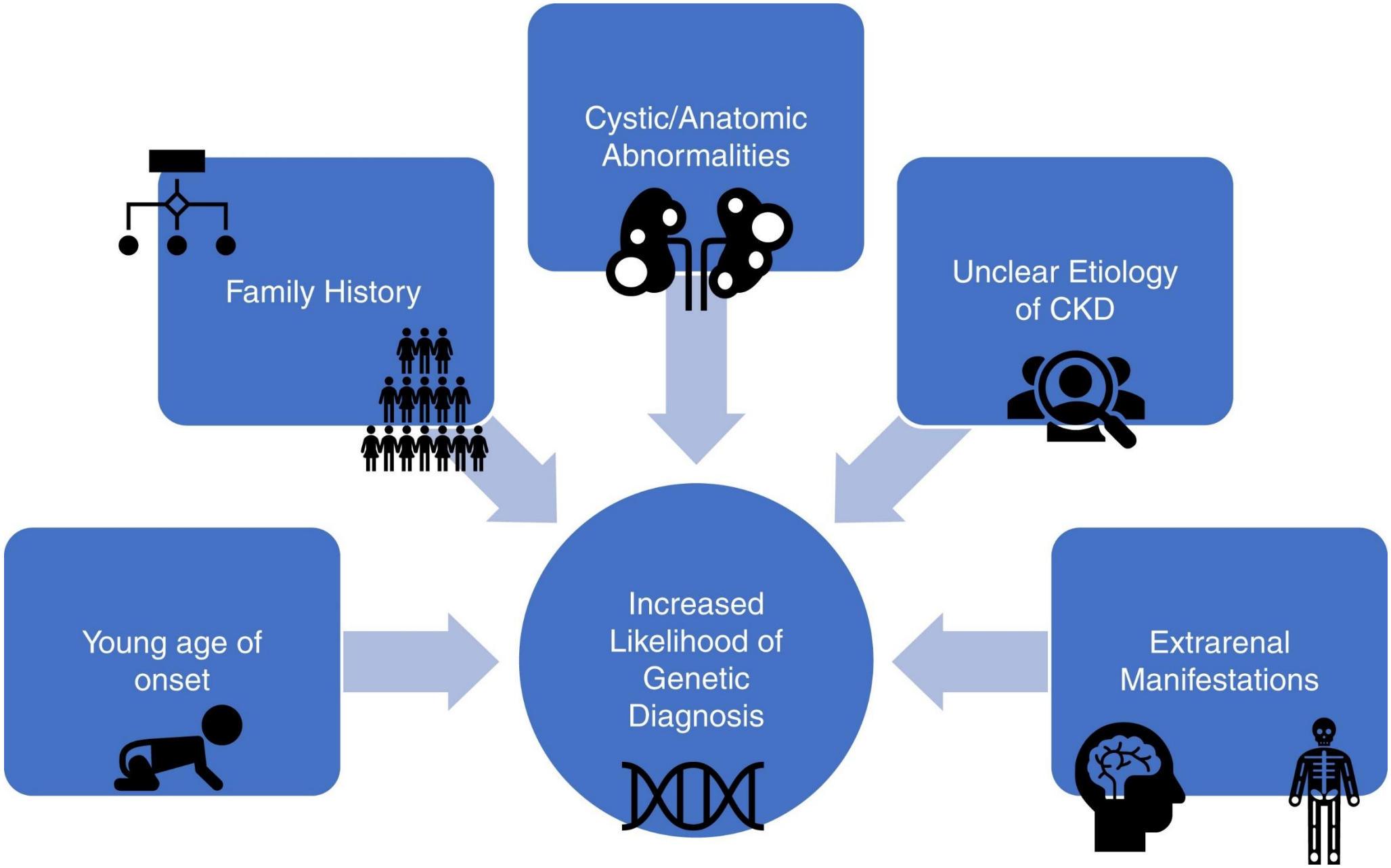
Thomas, C.P., Freese, M.E., Ounda, A. *et al.* **Initial experience from a renal genetics clinic demonstrates a distinct role in patient management.** *Genet Med* **22**, 1025–1035 (2020).

Nephrogenetics

Notable yield of genetic testing in patients with:

- persistent hematuria
- nephrotic syndrome
- focal segmental glomerulosclerosis
- nephrolithiasis
- congenital anomalies of the kidney and urinary tract
- cystic kidney disease
- CKD of unknown etiology




Cystic/Anatomic Abnormalities
Family History
Unclear Etiology of CKD
Young age of onset
Increased Likelihood of Genetic Diagnosis
Extrarenal Manifestations

Genetics in nephrology practice

- **72%** of nephrologist report using genetic test use in their practice
- On average, tests were ordered for **3.8%** of their patient population
- Both users and nonusers of genetic tests indicated high cost and poor availability or lack of ease of genetic testing as the **most significant perceived barriers to implementation**

Mrug M, Bloom MS, Seto C, Malhotra M, Tabriziani H, Gauthier P, Sidlow V, McKanna T, Billings PR. Genetic Testing for Chronic Kidney Diseases: Clinical Utility and Barriers Perceived by Nephrologists. Kidney Med. 2021 Oct 5;3(6):1050-1056.

Roles of clinical geneticist in nephrology

- **Assessing the need** for genetic testing for different kidney diseases
- Counseling and testing for patients and **presymptomatic** family members
- Overseeing the **management** of rare multisystem inherited diseases with a renal component (mitochondrial disease, Fabry disease, tuberous sclerosis…)
- Helping with the **transition** to an adult nephrology practice for patients with genetic renal disease referred by pediatric nephrology providers
- Evaluation of kidney **transplant candidates** and their asymptomatic living donors

Clinical genetic testing of symptomatic individuals

Predictive genetic investigations of asymptomatic individuals

Fabry disease (x-linked LSD)

- Fabry disease is **progressive** and often becomes symptomatic in childhood
- Age of symptom onset varies even within the same family
- Life threatening complications are rare in pediatric patients
- On average, there are **five family members** diagnosed with Fabry disease **for every proband**

- <u>Diagnostic limitations:</u>

- enzyme assay can be normal in heterozygous females
- the percentage of residual α-gal-A enzyme activity does not correlate with clinical severity
- mutations **frequently cannot predict** disease severity

Issues in genetic counselling for Fabry disease

- **testing "healthy" minors** and psychological and social implications
- reproductive options including prenatal diagnosis and preimplantation diagnosis
- teratogenic risk of frequently used medications in Fabry disease such as Dilantin, Carbamazepine (Tegretol), and ACE Inhibitors in pregnancy
- testing kidney donors, particularly family members, prior to transplant for Fabry disease

- Starting ERT early, before the age of 16, in male FD patients with a classical phenotype is associated with reduced risk and severity of albuminuria
- Starting ERT early, before the age of 16, in male FD patients with a classical phenotype slows down left ventricular hypertrophy

van der Veen SJ, Körver S, Hirsch A, Hollak CEM, Wijburg FA, Brands MM, Tøndel C, van Kuilenburg ABP, Langeveld M. Early start of enzyme replacement therapy in pediatric male patients with classical Fabry disease is associated with attenuated disease progression. Mol Genet Metab. 2022 Feb;135(2):163-169.

- Early treatment with ERT, before presumed symptom onset, can reduce lyso Gb3 levels below detectable limits
- Early treatment also has the potential to enhance QoL, including cognitive, social and developmental health

Kritzer A, Siddharth A, Leestma K, Bodamer O. Early initiation of enzyme replacement therapy in classical Fabry disease normalizes biomarkers in clinically asymptomatic pediatric patients. Mol Genet Metab Rep. 2019 Oct 19;21:100530.

"If medical or psychosocial benefits of genetic testing will not be realized until adulthood, testing should typically be postponed. Presymptomatic genetic testing of a child should be approached with great caution."

“Presymptomatic and predictive genetic testing of children for conditions that manifest in adulthood is acceptable only if preventive measures can be initiated before reaching adulthood.”

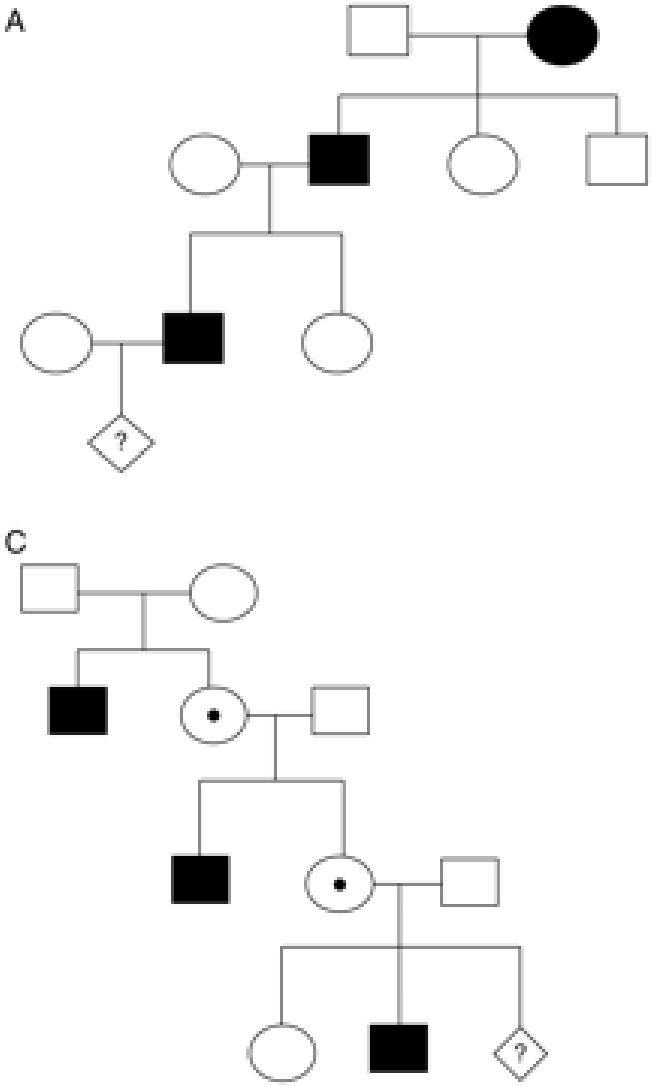
“If this condition is not met, presymptomatic and predictive genetic testing in minors for these disorders should be postponed until the individual reaches maturity and the ability to understand the nature of the decision and its consequences.”

National Society of Genetic Counselors

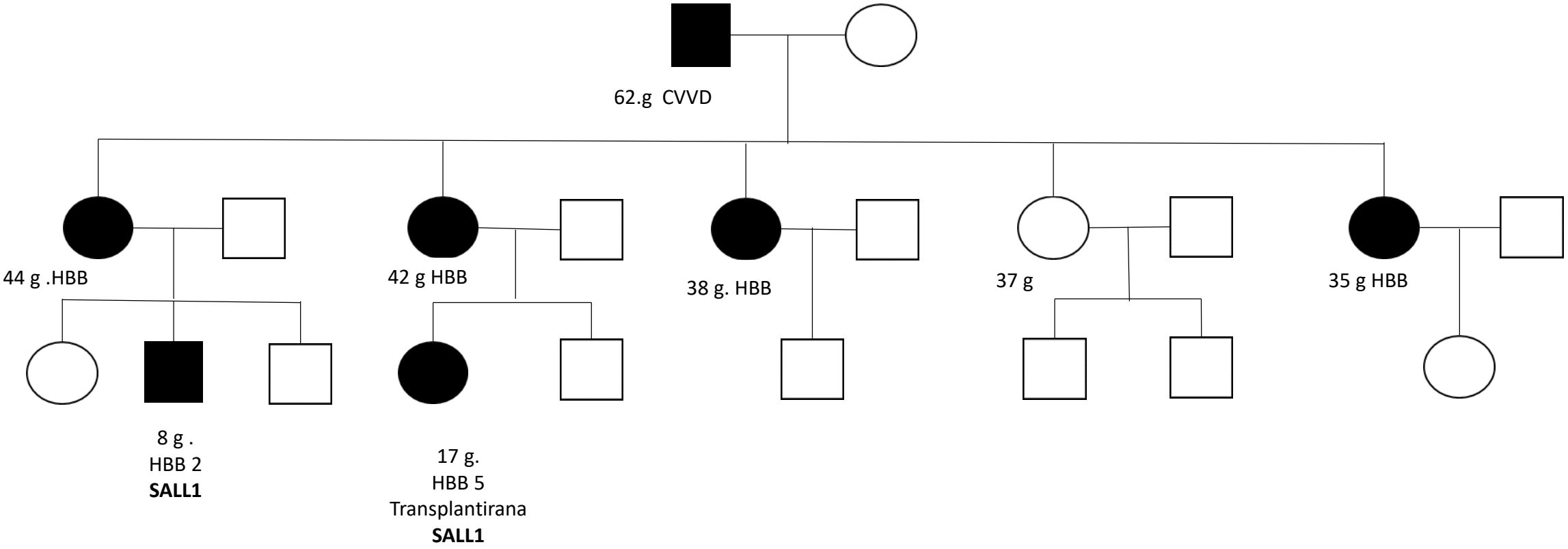
- The National Society of Genetic Counselors recommends postponing predictive genetic testing of children for conditions that arise in adulthood if the results will not significantly benefit the child...
- Predictive testing should ideally be delayed until the individual has the capacity to consider the risks, benefits, and limitations of this information... in order to preserve their autonomy and right to an open future.

Challenges in (nephro)genetics

- Difficulties in interpreting genetic variants of unknown significance
- Genetic variability and incomplete penetrance
- Availability of diagnosis and therapy
- Ethical and psychosocial constraints

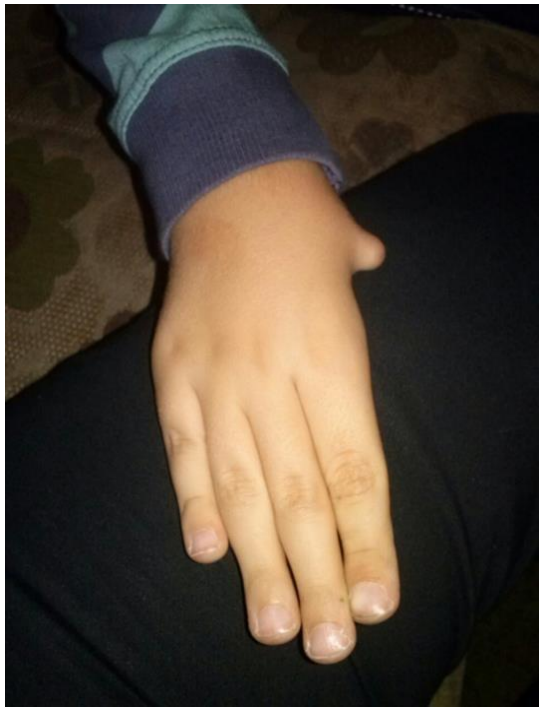
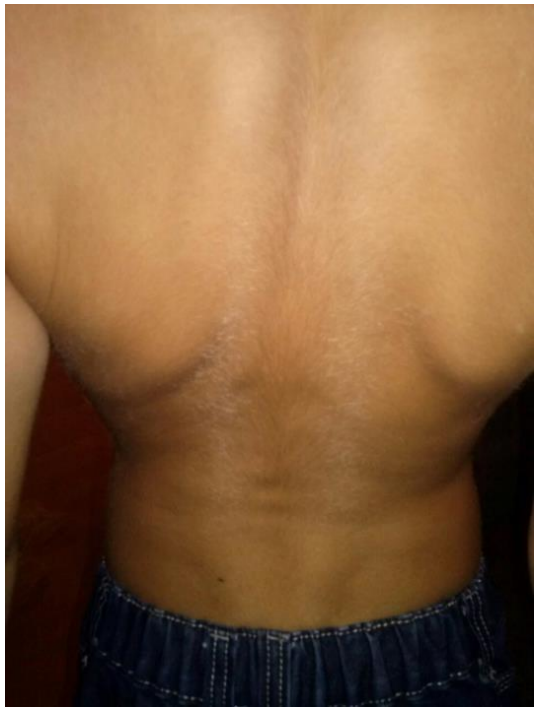


CKD in multiple family members across generations; autosomal dominant



CKD + imperforate anus, malformed ears, finger anomalies…



Townes-Brocks syndrome

- *SALL1* gene mutation, autosomal dominant, OMIM 602218
- VERY VARIABLE PHENOTYPE IN **ONE** FAMILY

Clinical phenotype

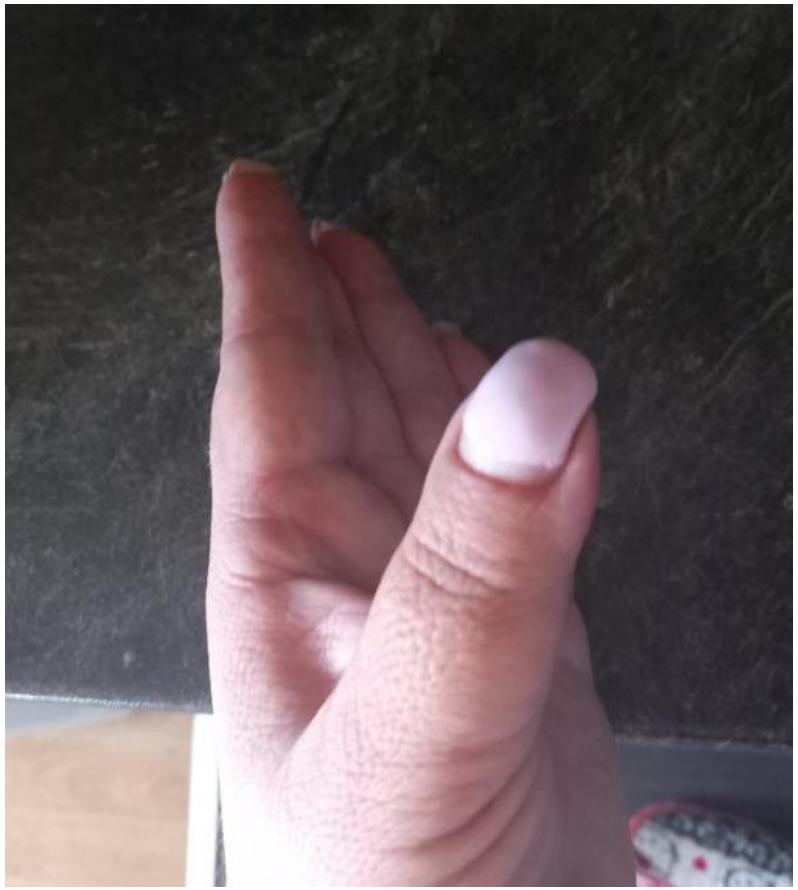
Anal atresia

Malformed thumbs

Malformed ears

CAKUT (ectopy, hipoplasia, agenesis)

Congenital heart defect



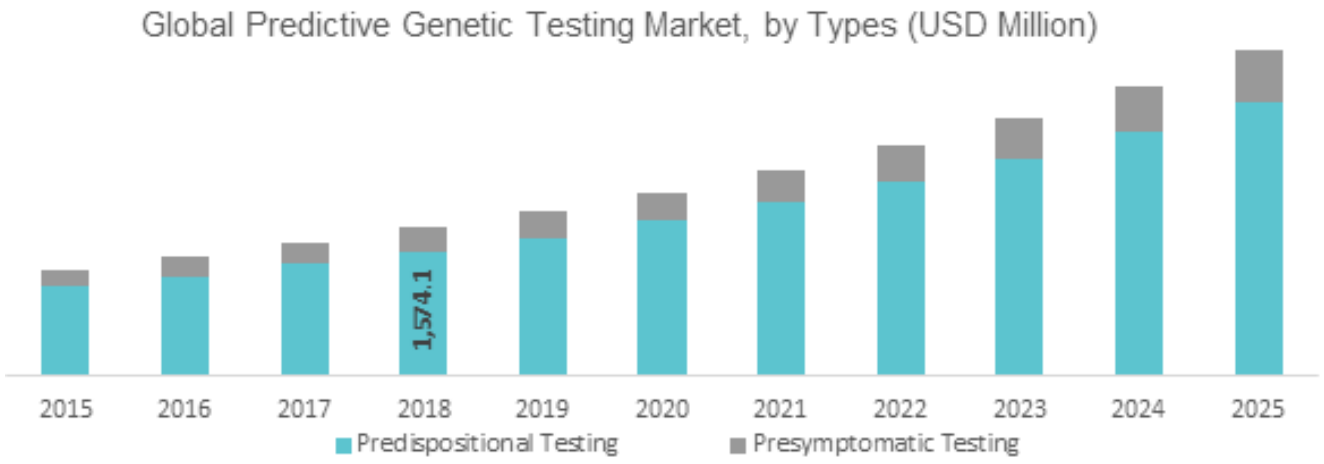
- The rapid growth of genetic testing over the last 3 decades has created a **demand for genetic counseling services**

- In the United States and Canada there are currently 50-60 accredited training programs typically granting master's degrees in medical genetics or genetic counselling

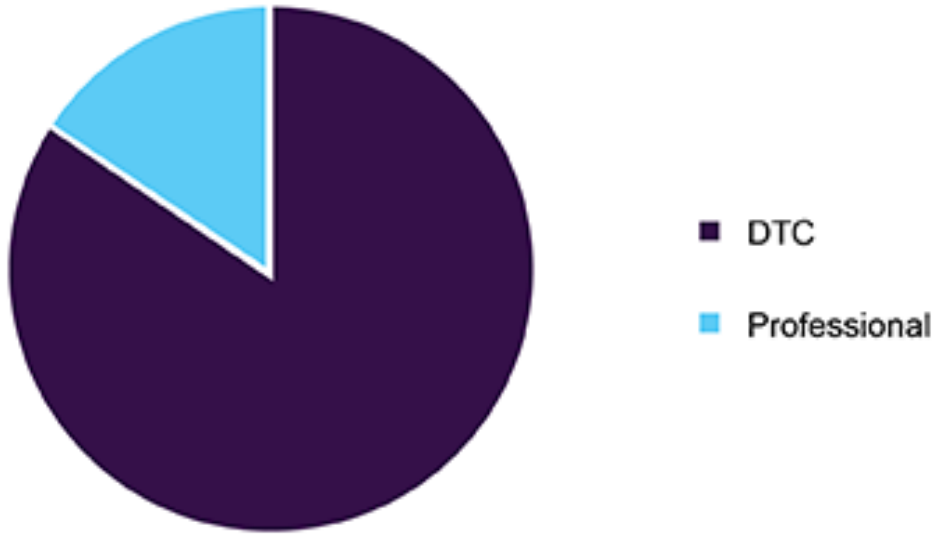
- Genetic counselors are certified by passing a board examination through the American Board of Genetic Counseling

- The number of certified genetic counselors has risen from 495 in 1993, to 5629 in April 2021, and is expected to grow to 10,000 by 2030.

Who makes the indication for predictive genetic testing today?



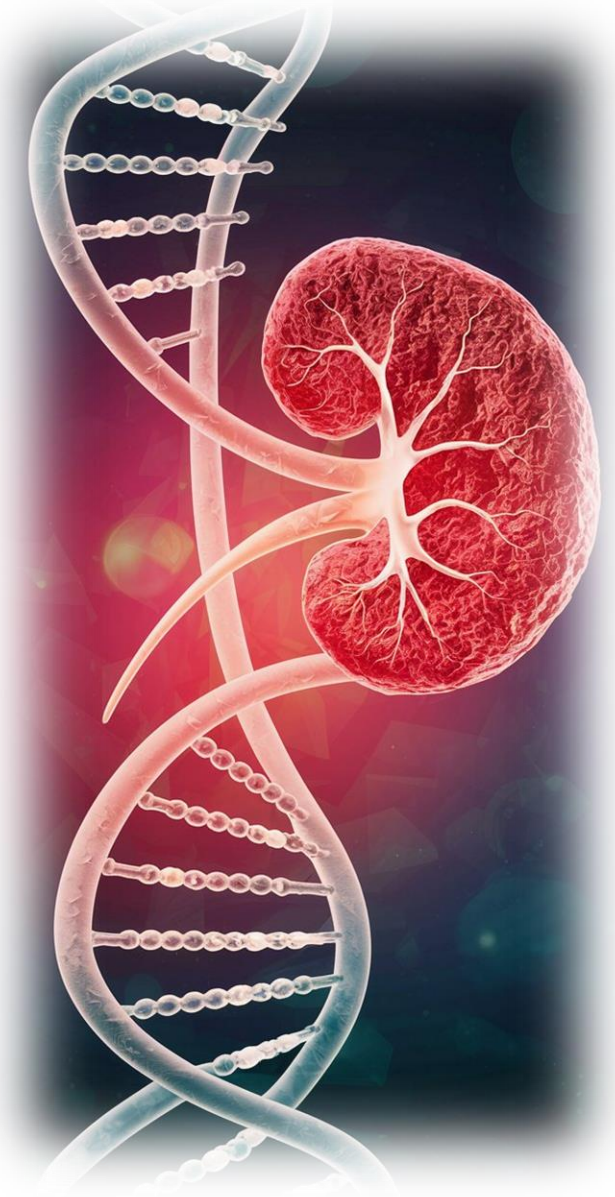
Source: Adroit Market Research © 2019

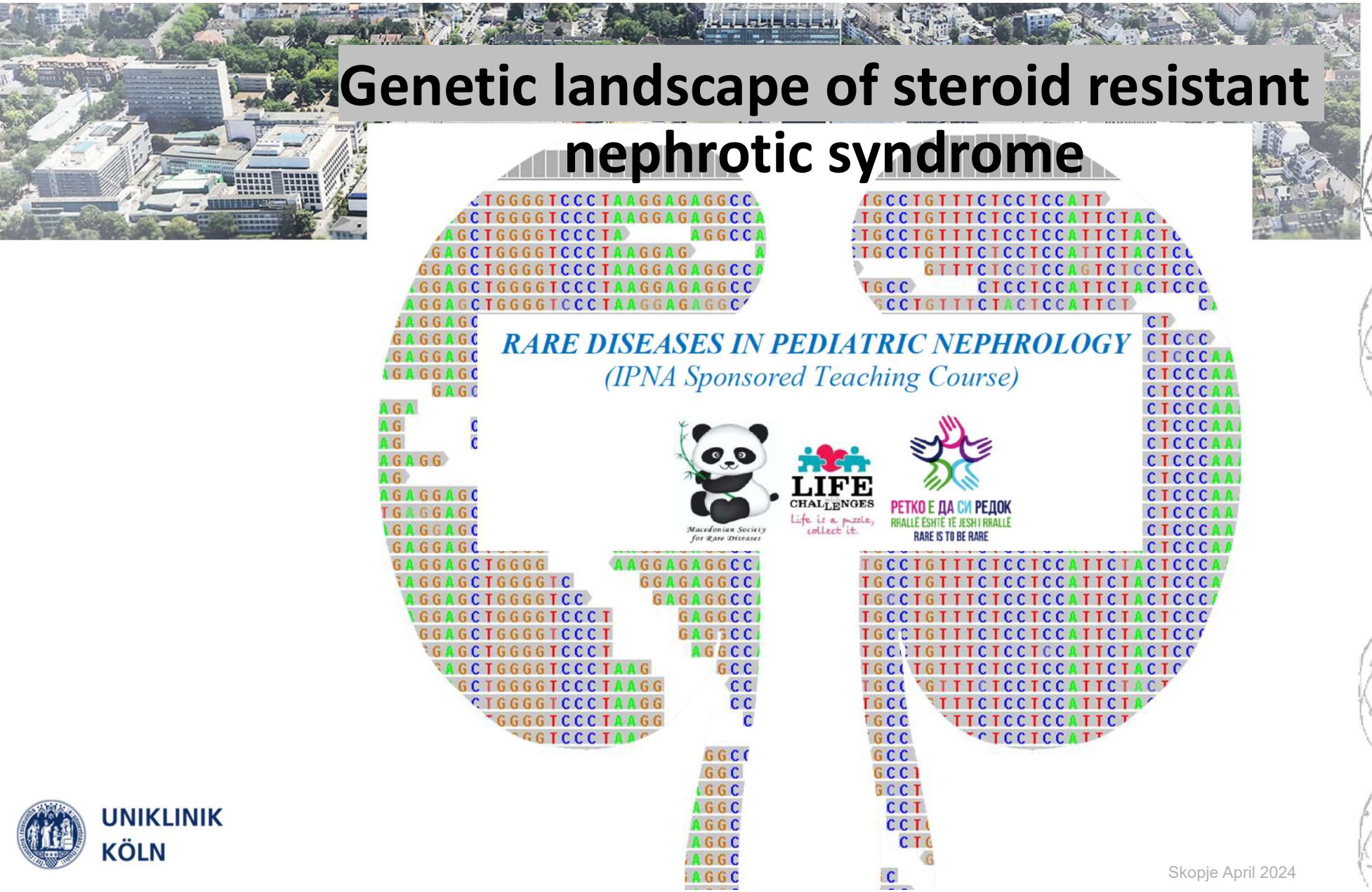


iewresearch.com

Perspectives of genetic testing/counseling in nephrology

- Growth in demand for genetic testing
- Improvement of genetic test interpretation
- Development of pharmacotherapy/precision medicine
- Increased possibilities for presymptomatic intervention
- Enhancement of recommendations in the context of ethical principles





Genetic landscape of steroid resistant nephrotic syndrome

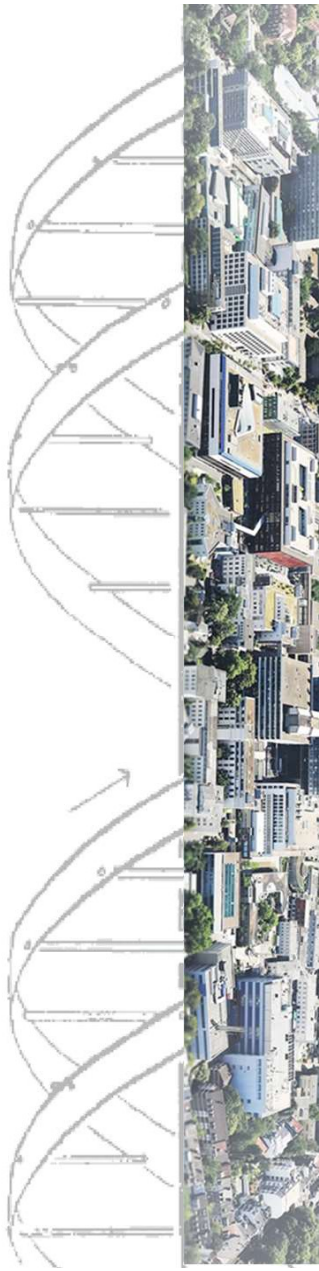
UNIKLINIK KÖLN

Skopje April 2024

conflict of interest

received consulting honoraria not related to this meeting from

Alnylam Pharmaceuticals
Novo Nordisk



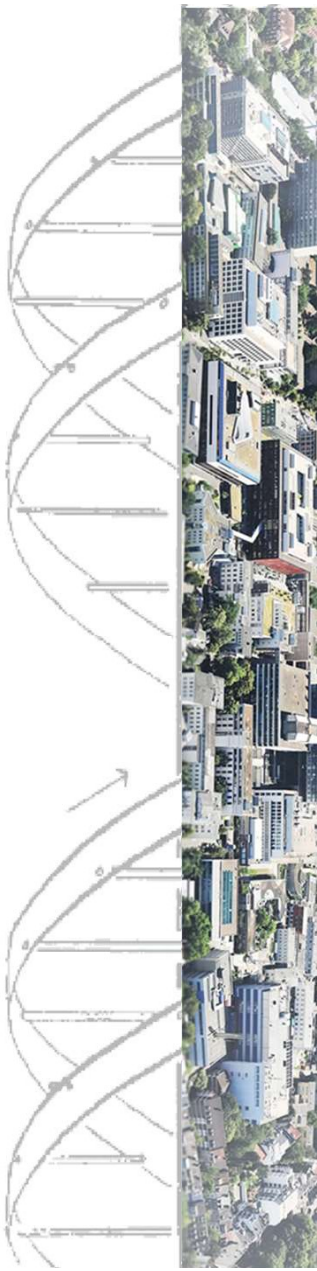
rare diseases

OMIM Entry Statistics

Number of Entries in OMIM (Updated August 24th, 2023) :

MIM Number Prefix	Autosomal	X Linked	Y Linked	Mitochondrial	Totals
Gene description *	16,212	767	51	37	17,067
Gene and phenotype, combined +	21	0	0	0	21
Phenotype description, molecular basis known #	6,281	379	5	34	6,699
Phenotype description or locus, molecular basis unknown %	1,393	112	4	0	1,509
Other, mainly phenotypes with suspected mendelian basis	1,640	102	3	0	1,745
Totals	25,547	1,360	63	71	27,041

OMIM® - Online Mendelian Inheritance in Man®



OMIM Gene Map Statistics

OMIM Morbid Map Scorecard (Updated March 26th, 2024) :

Total number of phenotypes* for which the molecular basis is known	7,509
Total number of genes with phenotype-causing mutation	4,897

* Phenotypes include (1) single-gene mendelian disorders and traits; (2) susceptibilities to cancer and complex disease (e.g., BRCA1 and familial breast-ovarian cancer susceptibility, 113705.0001, and CFH and macular degeneration, 134370.0008); (3) variations that lead to abnormal but benign laboratory test values ("nondiseases") and blood groups (e.g., lactate dehydrogenase B deficiency, 150100.0001 and ABO blood group system, 110300.0001); and (4) select somatic cell genetic disease (e.g., GNAS and McCune-Albright syndrome, 139320.0008 and IDH1 and glioblastoma multiforme, 147700.0001.)

Distribution of Phenotypes across Genes (Updated March 26th, 2024) :

Number of genes with 1 phenotype	3,431
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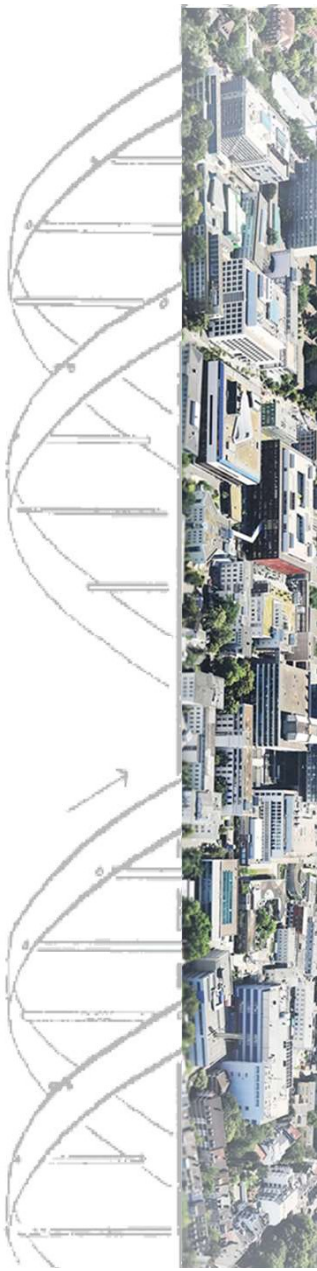
Number of genes with 3 phenotypes	321
Number of genes with 4+ phenotypes	253

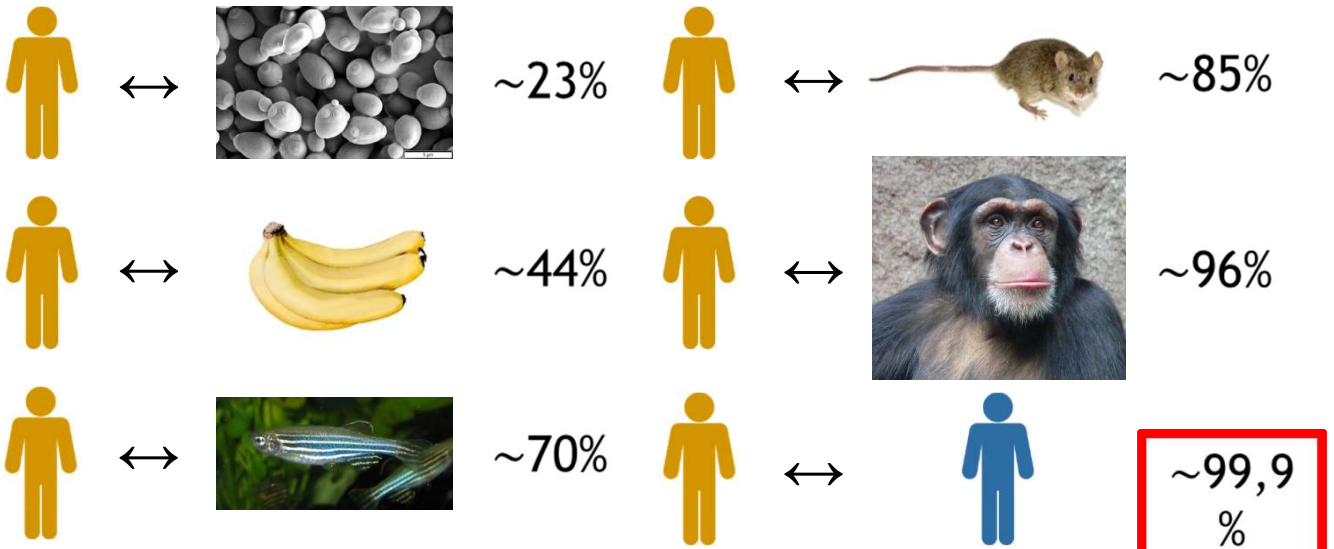
Dissected OMIM Morbid Map Scorecard (Updated March 26th, 2024) :

Class of phenotype	Phenotype	Gene *
Single gene disorders and traits	6,447	4,532
Susceptibility to complex disease or infection	680	503
"Nondiseases"	151	118
Somatic cell genetic disease	238	131

*Some genes may be counted more than once because mutations in a gene may cause more than one phenotype and the phenotypes may be of different classes (e.g., activating somatic BRAF mutation underlying cancer, 164757.0001. and germline BRAF mutation in Noonan syndrome, 164757.0022.)

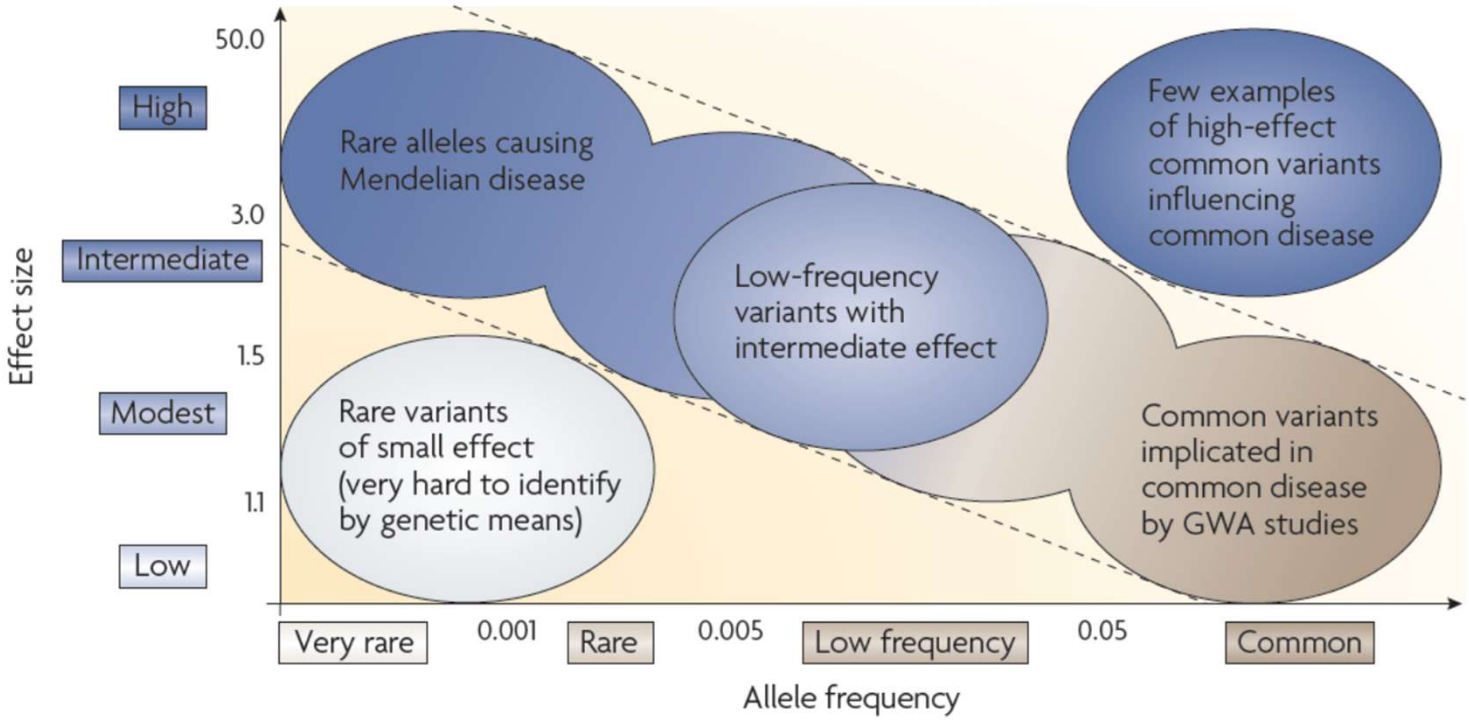


genetic variability and phenotype (1)

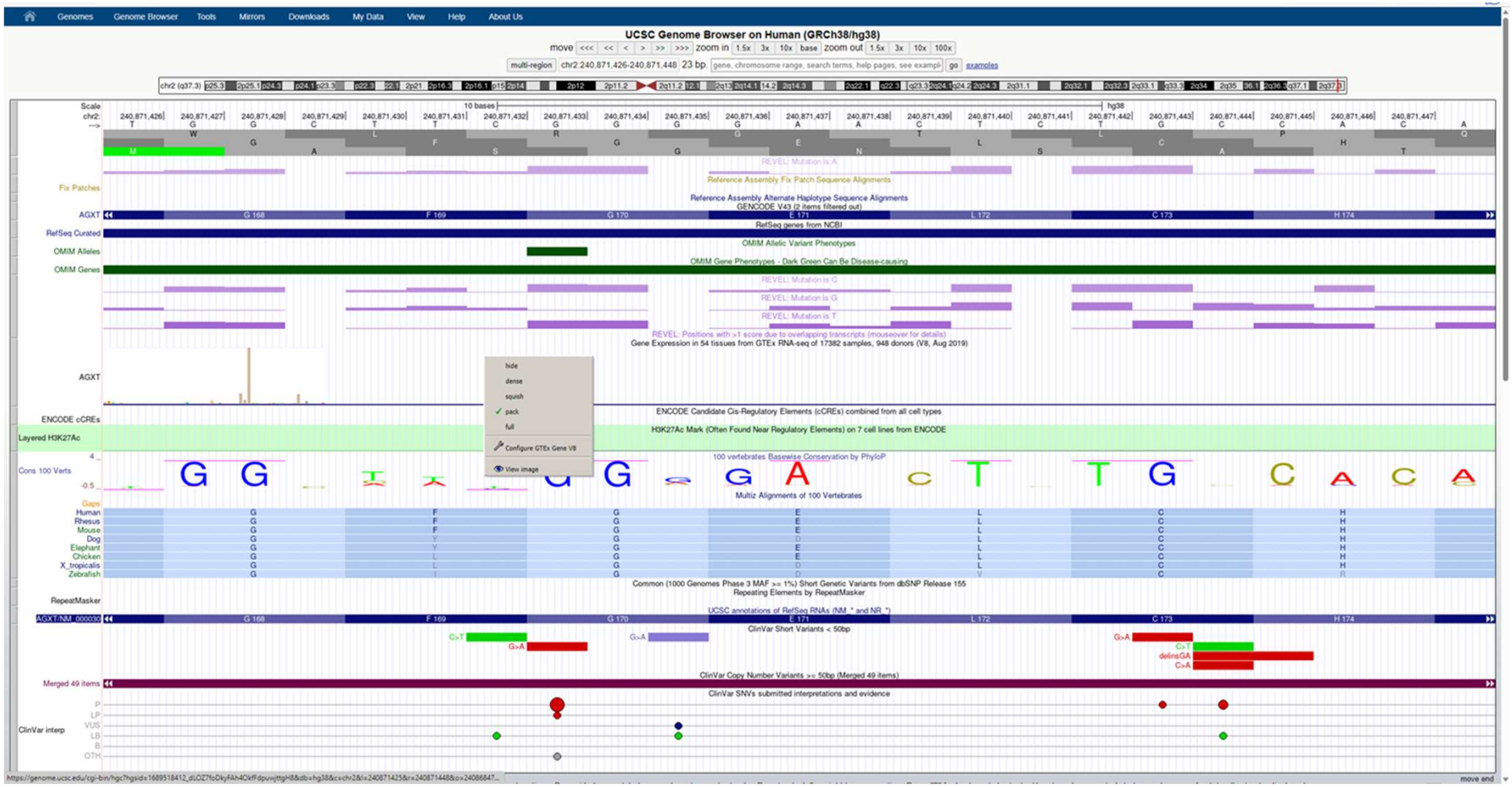


genetic variability (2)

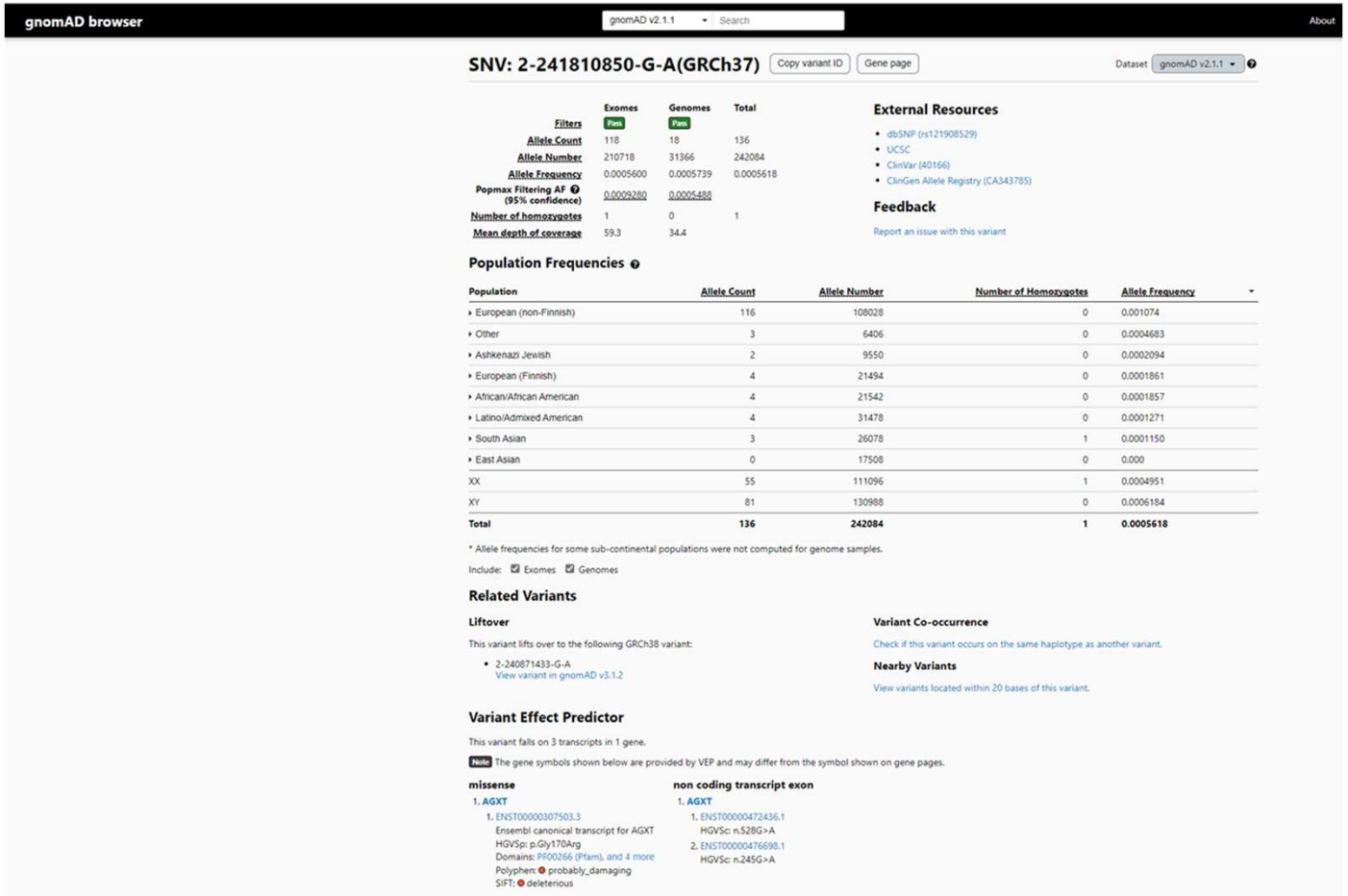
- **you differ from your neighbour at 1 in 1000 base positions = 3.3 million positions in total**



genetic variability – (UCSC) genome browser (3)



genetic variability – gnomAD browser (4)



gnomAD browser

gnomAD v2.1.1 ▾ Search

About

SNV: 2-241810850-G-A(GRCh37) Copy variant ID | Gene page

Dataset gnomAD v2.1.1 ▾

	Exomes	Genomes	Total
Filters	Pass	Pass	
Allele Count	118	18	136
Allele Number	210718	31366	242084
Allele Frequency	0.0005600	0.0005739	0.0005618
Popmax Filtering AF (95% confidence)	0.0009280	0.0005488	
Number of homozygotes	1	0	1
Mean depth of coverage	59.3	34.4	

External Resources

- dbSNP (rs121908529)
- UCSC
- ClinVar (40166)
- ClinGen Allele Registry (CA343785)

Feedback

Report an issue with this variant

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
European (non-Finnish)	116	108028	0	0.001074
Other	3	6406	0	0.0004683
Ashkenazi Jewish	2	9550	0	0.0002094
European (Finnish)	4	21494	0	0.0001861
African/African American	4	21542	0	0.0001857
Latino/Admixed American	4	31478	0	0.0001271
South Asian	3	26078	1	0.0001150
East Asian	0	17508	0	0.000
XX	55	111096	1	0.0004951
XY	81	130988	0	0.0006184
Total	**136**	**242084**	**1**	**0.0005618**

* Allele frequencies for some sub-continental populations were not computed for genome samples.

Include: ☑ Exomes ☑ Genomes

Related Variants

Liftover

This variant lifts over to the following GRCh38 variant:

- 2-240871433-G-A
  View variant in gnomAD v3.1.2

Variant Co-occurrence

Check if this variant occurs on the same haplotype as another variant.

Nearby Variants

View variants located within 20 bases of this variant.

Variant Effect Predictor

This variant falls on 3 transcripts in 1 gene.

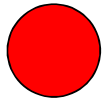
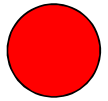
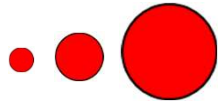
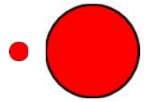
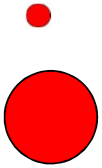
Note The gene symbols shown below are provided by VEP and may differ from the symbol shown on gene pages.

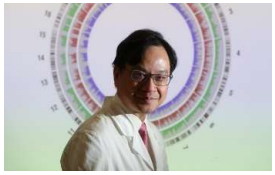
missense

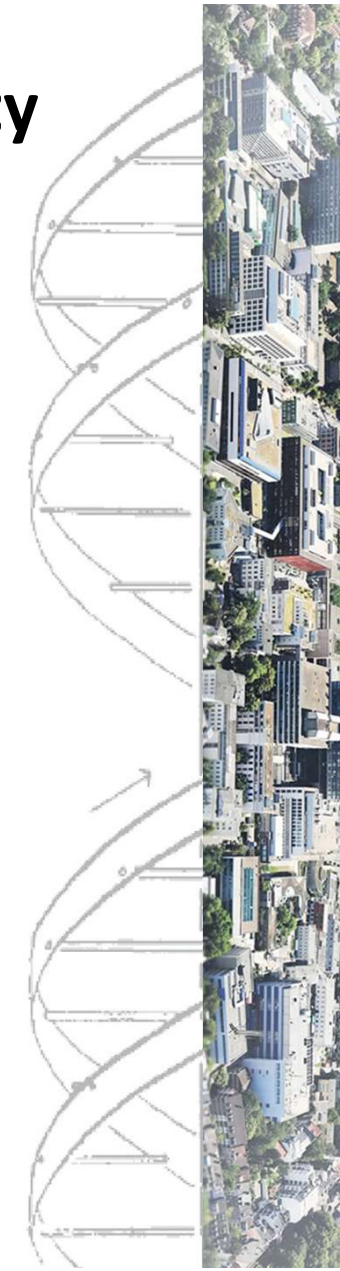
1. AGXT
   1. ENST00000307503.3
      Ensembl canonical transcript for AGXT
      HGVSp: p.Gly170Arg
      Domains: PF00266 (Pfam), and 4 more
      Polyphen: probably_damaging
      SIFT: deleterious

non coding transcript exon

1. AGXT
   1. ENST00000472436.1
      HGVSc: n.528G>A
   2. ENST00000476698.1
      HGVSc: n.245G>A

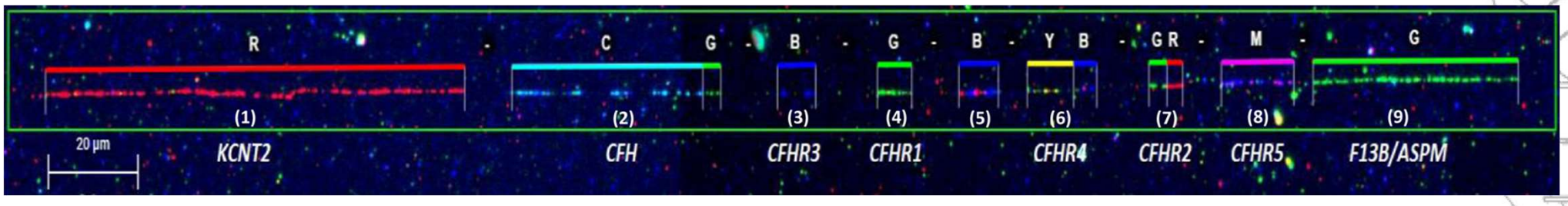
method	resolution	and	capacity
• karyotyping	3 to 5 mb		
• array-CGH	5 to 50 kb		
• targeted sequenzierung (Sanger sequencing)	1 bp		
• NGS-(sequencing by synthesis) gene panels, WES, WGS problem highly homolog regions /repeats …	1 bp		
• longread sequencing (SMRT/ONT)	1bp	 	
• cfDNA, noninvasive prenatal testing (NIPT)	chromosor 1 bp	 	

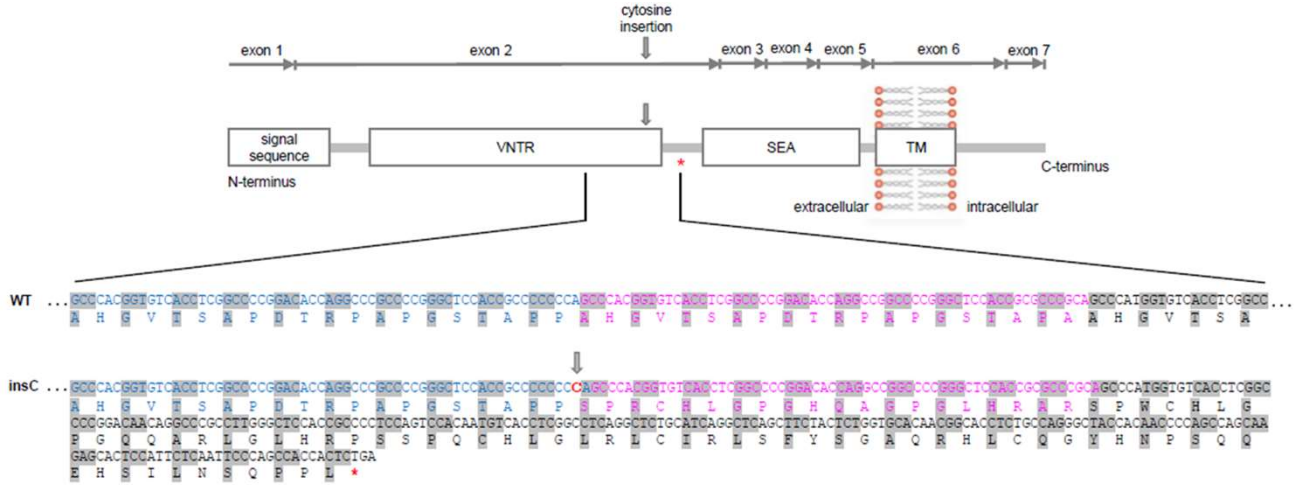




prototypic blind spots to NGS

RCA Gencluster molecular combing (genomic vision)





***MUC1* VNTR (ADTKD)**

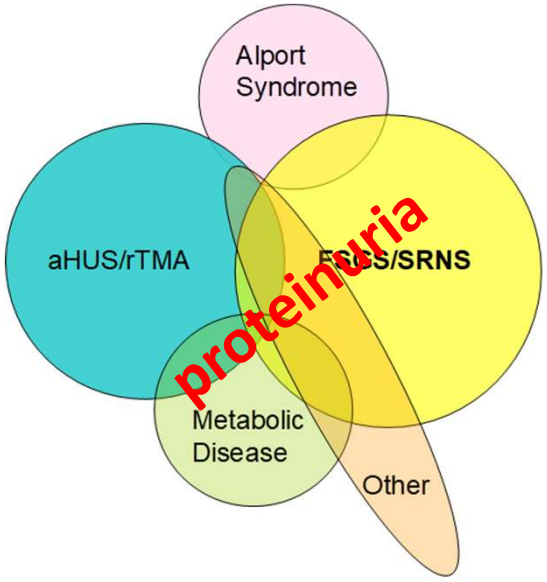
Tschernoster et al. J Mol Diagn 2022

monogenic nephrotic syndrome (SRNS/FSGS)

ACTN4, AMN, ANLN, APOE, APOL1, ARHGDIA, C1GALT1C1, C3, CD46, CD151, CD2AP, CFH, CFI, CLCN5, COL4A3, COL4A4, COL4A5, COQ2, COQ6, COQ8B, CRB2, CUBN, DAAM2, DGKE, DLC1, EHD1, EMP2, FAT1, FN1, GLA, GON7, INF2, ITGA3, ITSN1, ITSN2, KANK2, KANK1, KANK4, KIRREL1, LAGE3 LAMA5, LAMB2, LCAT, LMX1B, LRP2, MAGI2, MEFV, MYH9, MYO1E, NPHS1, NPHS2, NOS1AP, NUP85, NUP93, NUP107, NUP133, NUP160, NUP205, OCRL, OSGEP, P3H2, PAX2, PDSS2, PLCE1, PODXL, PRDM15, PTPRO, RCAN1, SCARB2, SGPL1, SMARCAL1, TBC1D8B, TNS2, TP53RK, TPRKB, TRIM8, TRPC6, TTC21B, WDR73, WT1, YRDC (n=82)

isolated
ACTN4, NPHS2
…

syndromal
WT1, SMARCAL1,
…

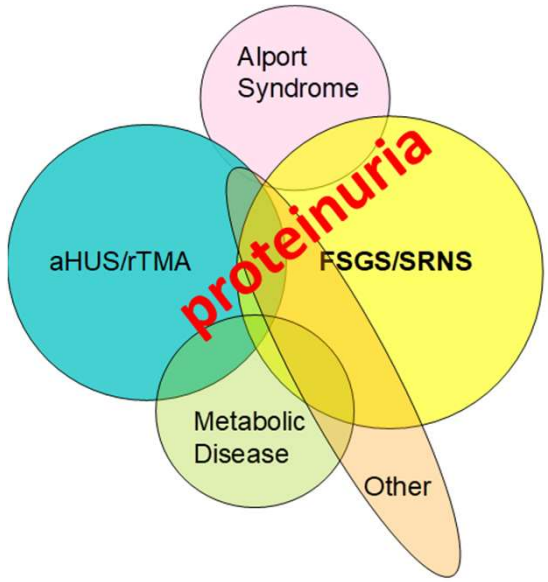


congenital
WT1, NPHS1
…

late onset
TRPC6, INF2,
…

monogenic proteinuria (SRNS/FSGS)
my ommissions today

 # Alport syndrome >>> Julia Hoefele
 # common forms of congenital nephrotic syndrome >>> Sandra Habbig
 # caHUS (forms and proteinuria) >>> Daniel Turudic
 # metabolic diseases and proteinuria



monogenic nephrotic syndrome(SRNS/FSGS) - semantics

„canonical FSGS genes“:

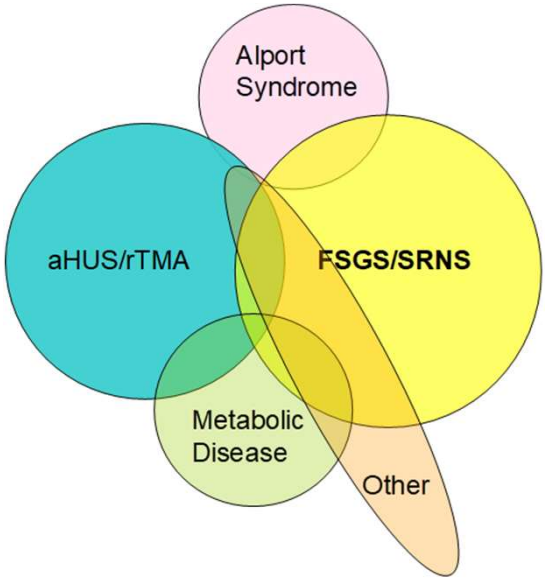
ACTN4
INF2
NPHS1
NPHS2
TRPC6
APOL1

genes important for glomerular development

PAX2
WT1
LMX1B
COL4A3
COL4A4
COL4A5

Other genes reported with the histological label FSGS

CLCN5
UMOD
Mitochondrial genes
ANLN
NPHP3
NPHP1
HNF1B
CLCNKB
…



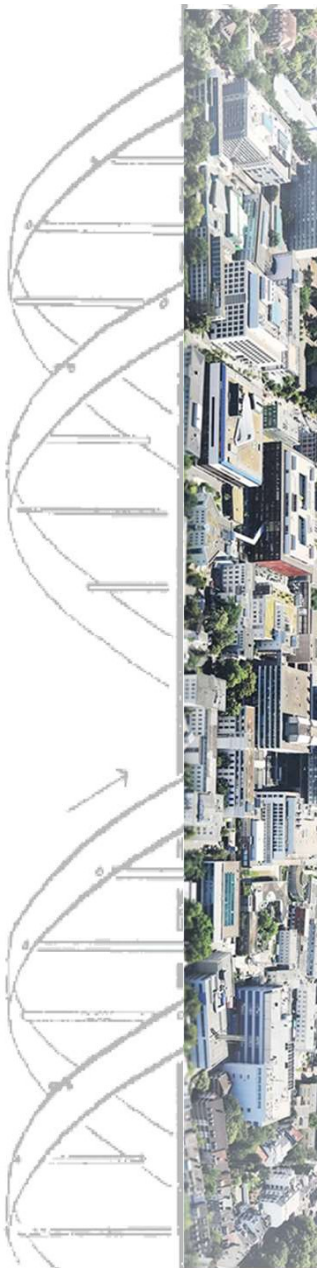
monogenic proteinuria gene panels (SRNS/FSGS)

ACTN4, AMN, ANLN, **APOE, APOL1 (risk polymorphisms), ARHGDIA,** C1GALT1C1, C3, CD46, CD151, CD2AP, CFH, CFI, **CLCN5, COL4A3, COL4A4, COL4A5, COQ2, COQ6, COQ8B,** CRB2, **CUBN,** DAAM2, **DGKE,** DLC1, EHD1, EMP2, **FAT1, FN1, GLA, GON7, INF2,** ITGA3, ITSN1, ITSN2, KANK2, KANK1, KANK4, KIRREL1, **LAGE3** LAMA5, **LAMB2, LCAT, LMX1B, LRP2, MAGI2, MEFV, MYH9, MYO1E, NPHS1, NPHS2,** NOS1AP, **NUP85, NUP93, NUP107, NUP133,** NUP160, NUP205, **OCRL, OSGEP,** P3H2, **PAX2,** PDSS2, **PLCE1,** PODXL, **PRDM15, PTPRO,** RCAN1, **SCARB2, SGPL1, SMARCAL1, TBC1D8B,** TNS2, **TP53RK, TPRKB, TRIM8, TRPC6,** TTC21B, **WDR73, WT1,** YRDC **(n=82)**

orange very rare / candidate genes n=16 (but respected authors & journals)

black „my consensus“ genes n=15

bold frequently reported consensus genes n=46



monogenic proteinuria gene panels (SRNS/FSGS) curation

ACTN4, AMN, ANLN, **APOE, APOL1 (risk polymorphisms), ARHGDIA,** C1GALT1C1, C3, CD46, CD151, CD2AP, CFH, CFI, **CLCN5, COL4A3, COL4A4, COL4A5, COQ2, COQ6, COQ8B,** CRB2, **CUBN,** DAAM2, **DGKE,** DLC1, EHD1, EMP2, **FAT1, FN1, GLA, GON7, INF2,** ITGA3, ITSN1, ITSN2, KANK2, KANK1, KANK4, KIRREL1, **LAGE3** LAMA5, **LAMB2, LCAT, LMX1B, LRP2, MAGI2, MEFV, MYH9, MYO1E, NPHS1, NPHS2,** NOS1AP, **NUP85, NUP93, NUP107, NUP133,** NUP160, NUP205, **OCRL, OSGEP,** P3H2, **PAX2,** PDSS2, **PLCE1,** PODXL, **PRDM15, PTPRO,** RCAN1, **SCARB2, SGPL1, SMARCAL1, TBC1D8B,** TNS2, **TP53RK, TPRKB, TRIM8, TRPC6,** TTC21B, **WDR73, WT1,** YRDC **(n=82)**

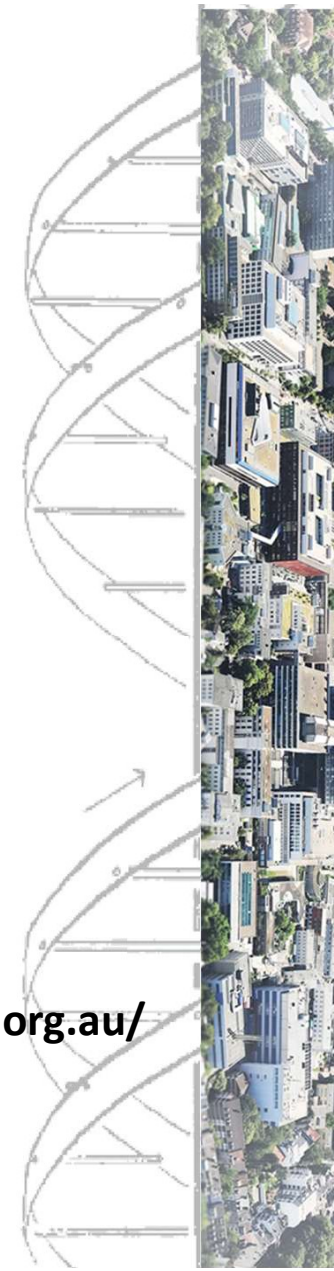
orange very rare / candidate genes n=16 (but respected authors & journals)
black my consensus genes n=15
bold frequently reported consensus genes n=46

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

https://www.australiangenomics.org.au/

Australian Genomics

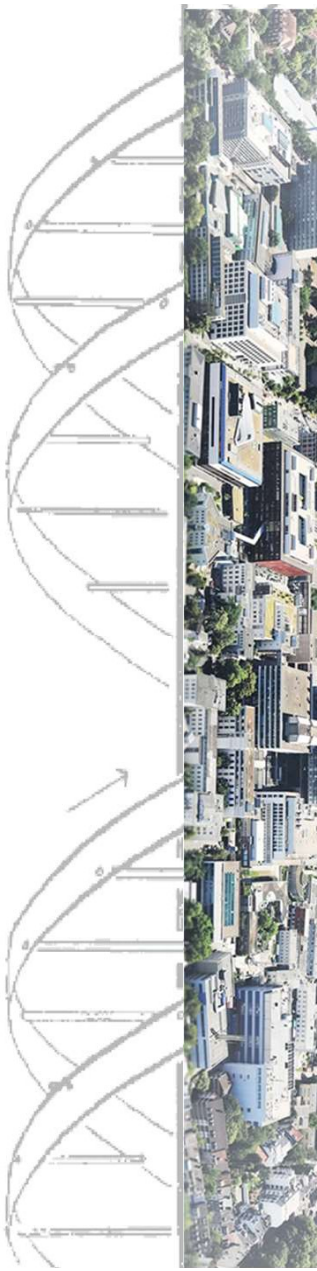
Skopje April 2024



monogenic proteinuria gene panels (SRNS/FSGS) – UCMC yield

ACTN4, AMN, ANLN, **APOE**, **APOL1** (risk polymorphisms), **ARHGDIA**, C1GALT1C1, C3, CD46, CD151, CD2AP, CFH, CFI, **CLCN5, COL4A3, COL4A4, COL4A5, COQ2, COQ6, COQ8B**, CRB2, **CUBN**, DAAM2, **DGKE**, DLC1, EHD1, EMP2, **FAT1**, **FN1**, **GLA**, **GON7**, **INF2**, ITGA3, ITSN1, ITSN2, KANK2, KANK1, KANK4, KIRREL1, LAGE3, LAMA5, **LAMB2**, **LCAT**, **LMX1B**, **LRP2**, **MAGI2**, **MEFV**, **MYH9**, **MYO1E**, **NPHS1**, **NPHS2**, NOS1AP, **NUP85**, **NUP93**, **NUP107**, **NUP133**, NUP160, NUP205, **OCRL**, **OSGEP**, P3H2, **PAX2**, PDSS2, **PLCE1**, PODXL, **PRDM15**, **PTPRO**, RCAN1, **SCARB2**, **SGPL1**, **SMARCAL1**, **TBC1D8B**, TNS2, **TP53RK**, **TPRKB**, **TRIM8**, **TRPC6**, TTC21B, **WDR73**, **WT1**, YRDC **(n=82)**

„recovery rate“ 50/82 41/46 (bold)



monogenic proteinuria (SRNS/FSGS) curated databases

Example 1: *KANK1* Gee et al. JCI 2015

Pro: authors/journal **Con: not reconfirmed since 2015**

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QUICK GENE: Search

All 44 mutations in KANK1

missense/nonsense | splicing | small insertions | gross deletions

Missense/nonsense : 37 mutations [back to top]

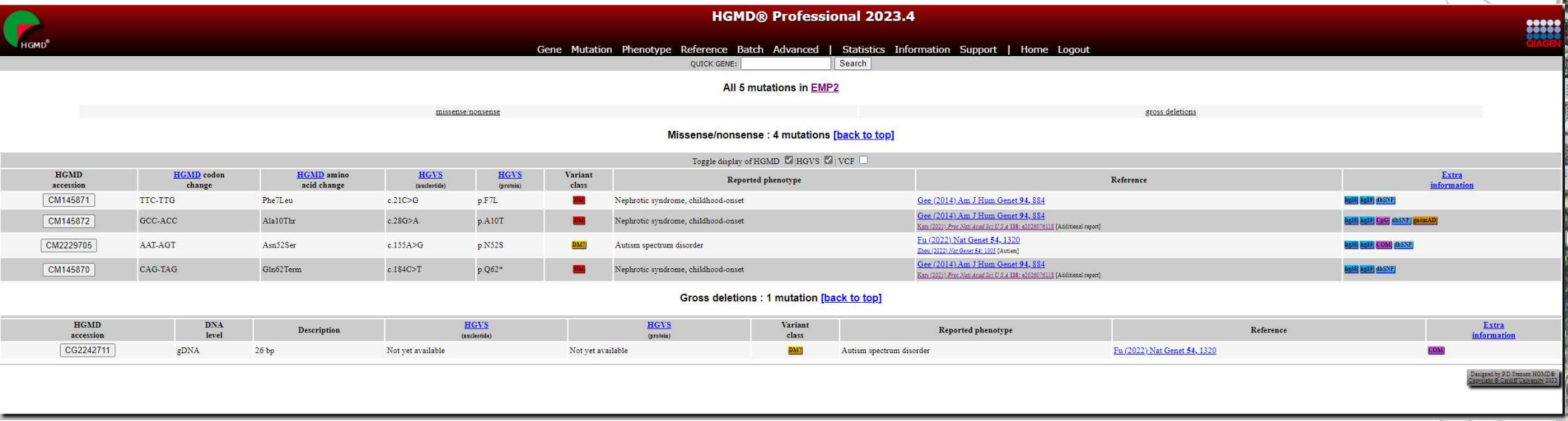
Toggle display of HGMD ☑ HGVS ☑ VCF ☐

HGMD accession	HGMD codon change	HGMD amino acid change	HGVS (nucleotide)	HGVS (protein)	Variant class	Reported phenotype	Reference	Extra information
CM2055648	TAT-TGT	Tyr37Cys	c.110A>G	p.Y37C	DM?	Developmental disorder	Kaplanis (2020) Nature 586, 757 Zhou (2022) Nat Genet 54, 1305 [Autism]	hg38 hg19 COM dbSNP
CM2315113	GAT-GTT	Asp50Val	c.149A>T	p.D50V	DM	Amyotrophic lateral sclerosis	Farrugia Wismayer (2023) Neurobiol Aging 123, 200	hg38 hg19 gnomAD
CM2037145	CGG-CAG	Arg67Gln	c.200G>A	p.R67Q	DM?	Cerebral palsy	Landini (2020) Clin J Am Soc Nephrol 15, 89	hg38 hg19 COM CpG dbSNP gnomAD
CM2077618	CCG-TCG	Pro69Ser	c.205C>T	p.P69S	DM?	Developmental disorder	Kaplanis (2020) Nature 586, 757 Zhou (2022) Nat Genet 54, 1305 [Autism]	hg38 hg19 COM
CM2124450	TTT-ATT	Phe131Ile	c.391T>A	p.F131I	DM?	Obsessive-compulsive disorder	Halvorsen (2021) Nat Neurosci 24, 1071	hg38 hg19 COM
CM1939821	GCT-GGT	Ala228Gly	c.683C>G	p.A228G	DM?	Focal segmental glomerulosclerosis	Wang (2019) J Am Soc Nephrol 30, 1625	hg38 hg19 COM gnomAD
CM2122299	CAG-TAG	Gln261Term	c.781C>T	p.Q261*	DM?	Neurodevelopmental disorder	Nassir (2021) Hum Genomics 15, 68	hg38 hg19 COM
CM2037144	TAT-TGT	Tyr324Cys	c.971A>G	p.Y324C	DM?	Cerebral palsy	Landini (2020) Clin J Am Soc Nephrol 15, 89	hg38 hg19 COM dbSNP
CM2258533	CGG-CAG	Arg368Gln	c.1103G>A	p.R368Q	DM?	Congenital heart defects	Meerschaut (2022) Genes (Basel) 13,	hg38 hg19 COM CpG gnomAD
CM2055130	GAG-GAA	Glu394Glu	c.1182G>A	p.E394=	DM?	Developmental disorder	Kaplanis (2020) Nature 586, 757 Zhou (2022) Nat Genet 54, 1305 [Autism]	hg38 hg19 COM
CM2047352	GCT-CCT	Ala402Pro	c.1204G>C	p.A402P	DM?	Developmental disorder	Kaplanis (2020) Nature 586, 757 Zhou (2022) Nat Genet 54, 1305 [Autism]	hg38 hg19 COM
CM2233620	GAG-GGG	Glu406Gly	c.1217A>G	p.E406G	DM?	Autism spectrum disorder	Fu (2022) Nat Genet 54, 1320	hg38 hg19 COM dbSNP
CM154807	GAA-AAA	Glu454Lys	c.1360G>A	p.E454K	DM	Nephrotic syndrome, steroid sensitive	Gee (2015) J Clin Invest 125, 2375	hg38 hg19
CM2033853	GCT-GTT	Ala495Val	c.1484C>T	p.A495V	DM	Developmental disorder with metabolic disorder	Dong (2020) J Med Genet 57, 558	hg38 hg19 COM dbSNP
CM2246699	TCG-TTG	Ser497Leu	c.1490C>T	p.S497L	DM?	Autism	Zhou (2022) Nat Genet 54, 1305	hg38 hg19 COM CpG dbSNP
CM2233621	GCC-GGC	Ala504Gly	c.1511C>G	p.A504G	DM?	Autism spectrum disorder	Fu (2022) Nat Genet 54, 1320	hg38 hg19 COM
CM2052937	CAG-GAG	Gln521Glu	c.1561C>G	p.Q521E	DM?	Developmental disorder	Kaplanis (2020) Nature 586, 757 Zhou (2022) Nat Genet 54, 1305 [Autism]	hg38 hg19 COM

monogenic proteinuria (SRNS/FSGS) curated databases

Example 2: *EMP2* Gee et al. AJHG 2014

Pro: authors/journal **Con: not reconfirmed since 2014**



HGMD® Professional 2023.4

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QUICK GENE: Search

All 5 mutations in EMP2

missense/nonsense gross deletions

Missense/nonsense : 4 mutations [back to top]

Toggle display of HGMD ☑ | HGVS ☑ | VCF ☐

HGMD accession	HGMD codon change	HGMD amino acid change	HGVS (nucleotide)	HGVS (protein)	Variant class	Reported phenotype	Reference	Extra information
CM145871	TTC-TTG	Phe7Leu	c.21C>G	p.F7L	DM	Nephrotic syndrome, childhood-onset	Gee (2014) Am J Hum Genet 94, 884	hg38 hg19 dbSNP
CM145872	GCC-ACC	Ala10Thr	c.28G>A	p.A10T	DM	Nephrotic syndrome, childhood-onset	Gee (2014) Am J Hum Genet 94, 884 Kars (2021) Proc Natl Acad Sci U S A 118: e2026076118 [Additional report]	hg38 hg19 CpG dbSNP gnomAD
CM2229705	AAT-AGT	Asn52Ser	c.155A>G	p.N52S	DM?	Autism spectrum disorder	Fu (2022) Nat Genet 54, 1320 Zhou (2022) Nat Genet 54: 1305 [Autism]	hg38 hg19 COM dbSNP
CM145870	CAG-TAG	Gln62Term	c.184C>T	p.Q62*	DM	Nephrotic syndrome, childhood-onset	Gee (2014) Am J Hum Genet 94, 884 Kars (2021) Proc Natl Acad Sci U S A 118: e2026076118 [Additional report]	hg38 hg19 dbSNP

Gross deletions : 1 mutation [back to top]

HGMD accession	DNA level	Description	HGVS (nucleotide)	HGVS (protein)	Variant class	Reported phenotype	Reference	Extra information
CG2242711	gDNA	26 bp	Not yet available	Not yet available	DM?	Autism spectrum disorder	Fu (2022) Nat Genet 54, 1320	COM

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monogenic proteinuria (SRNS/FSGS) other help curated databases

Example 3 *PTPRO* Ozaltin et al. AJHG 2011

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missense/nonsense | splicing | regulatory | small deletions

Missense/nonsense : 18 mutations [back to top]

Toggle display of HGMD ☑ HGVS ☑ VCF ☐

HGMD accession	HGMD codon change	HGMD amino acid change	HGVS (nucleotide)	HGVS (protein)	Variant class	Reported phenotype	Reference	Extra information
CM2263283	ACA-GCA	Thr176Ala	c.526A>G	p.T176A	DM?	Autism	Zhou (2022) Nat Genet 54, 1305	hg38 hg19 COM
CM1711527	ATT-GTT	Ile228Val	c.682A>G	p.I228V	DM?	Emphysema, susceptibility to	Radder (2017) Am J Respir Crit Care Med 196, 159	hg38 hg19 dbSNP gnomAD
CM1711528	TCC-TGC	Ser229Cys	c.686C>G	p.S229C	DM?	Emphysema, susceptibility to	Radder (2017) Am J Respir Crit Care Med 196, 159	hg38 hg19 dbSNP
CM1711529	CAT-CGT	His362Arg	c.1085A>G	p.H362R	DM?	Emphysema, susceptibility to	Radder (2017) Am J Respir Crit Care Med 196, 159	hg38 hg19 dbSNP gnomAD
CM1711526	AAC-AAG	Asn370Lys	c.1110C>G	p.N370K	DP	Emphysema, susceptibility to	Radder (2017) Am J Respir Crit Care Med 196, 159	hg38 hg19 dbSNP gnomAD
CM1718435	GAG-AAG	Glu434Lys	c.1300G>A	p.E434K	DM?	Nephrotic syndrome, steroid resistant	Sen (2017) J Med Genet 54, 795	hg38 hg19 COM CpG dbSNP gnomAD
CM188126	TTT-TTC	Phe526Phe	c.1578T>C	p.F526=	DM?	Nephrotic syndrome, steroid resistant	Bezdíčka (2018) Pediatr Nephrol 33, 1347	hg38 hg19 COM dbSNP gnomAD
CM1718451	ACG-ATG	Thr544Met	c.1631C>T	p.T544M	DM?	Nephrotic syndrome, steroid resistant	Sen (2017) J Med Genet 54, 795	hg38 hg19 COM CpG dbSNP gnomAD
CM2126402	GTG-GCG	Val593Ala	c.1778T>C	p.V593A	DM	Nephrotic syndrome, steroid sensitive	Thakor (2021) Mol Biol Rep 48, 7193	hg38 hg19 COM dbSNP
CM2063222	ACG-ATG	Thr612Met	c.1835C>T	p.T612M	DM?	Developmental disorder	Kaplanis (2020) Nature 586, 757 Zhou (2022) Nat Genet 54, 1305 [Autism]	hg38 hg19 COM CpG
CM2126430	TCT-TAT	Ser663Tyr	c.1988C>A	p.S663Y	DM?	Nephrotic syndrome, steroid sensitive	Thakor (2021) Mol Biol Rep 48, 7193	hg38 hg19 COM
CM1718414	TGT-TAT	Cys706Tyr	c.2117G>A	p.C706Y	DM?	Nephrotic syndrome, steroid resistant	Sen (2017) J Med Genet 54, 795	hg38 hg19 COM
CM2239303	CGC-CTC	Arg717Leu	c.2150G>T	p.R717L	DM?	Autism spectrum disorder	Fu (2022) Nat Genet 54, 1320 Zhou (2022) Nat Genet 54, 1305 [Autism]	hg38 hg19 COM
CM2247640	GTG-ATG	Val822Met	c.2464G>A	p.V822M	DM?	Autism	Zhou (2022) Nat Genet 54, 1305	hg38 hg19 COM
CM2126431	ACC-ATC	Thr885Ile	c.2654C>T	p.T885I	DM?	Nephrotic syndrome, steroid sensitive	Thakor (2021) Mol Biol Rep 48, 7193	hg38 hg19 COM
CM2060267	CGT-TGT	Arg967Cys	c.2899C>T	p.R967C	DM?	Developmental disorder	Kaplanis (2020) Nature 586, 757 Zhou (2022) Nat Genet 54, 1305 [Autism]	hg38 hg19 COM CpG gnomAD
CM188127	GGG-GCG	Gly1166Ala	c.3497G>C	p.G1166A	DM?	Nephrotic syndrome, steroid resistant	Bezdíčka (2018) Pediatr Nephrol 33, 1347 Thakor (2021) Mol Biol Rep 48, 7193 [Nephrotic syndrome, steroid sensitive]	hg38 hg19 COM dbSNP
CM2126429	AGC-TGC	Ser1214Cys	c.3640A>T	p.S1214C	DM?	Nephrotic syndrome, steroid resistant	Thakor (2021) Mol Biol Rep 48, 7193	hg38 hg19 COM

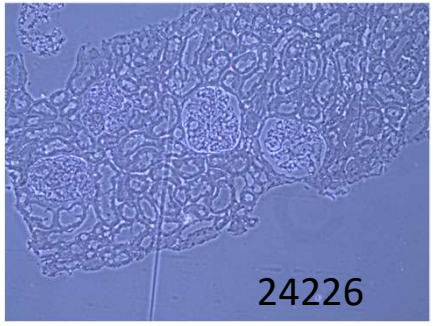
Splicing : 2 mutations [back to top]

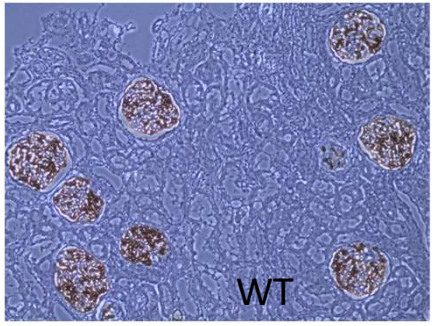
HGMD accession	HGMD splicing mutation	HGVS (nucleotide)	Variant class	Reported phenotype	Reference	Extra information
CS115583	IVS16 ds G-T +1	c.2627+1G>T	DM	Nephrotic syndrome, childhood-onset	Ozaltin (2011) Am J Hum Genet 89, 139 Xiong (2015) Science 347: 1254806 [Additional report]	hg38 hg19
CS115584	IVS19 ds G-A +1	c.2829+1G>A	DM	Nephrotic syndrome, childhood-onset	Ozaltin (2011) Am J Hum Genet 89, 139 Xiong (2015) Science 347: 1254806 [Additional report]	hg38 hg19 COM

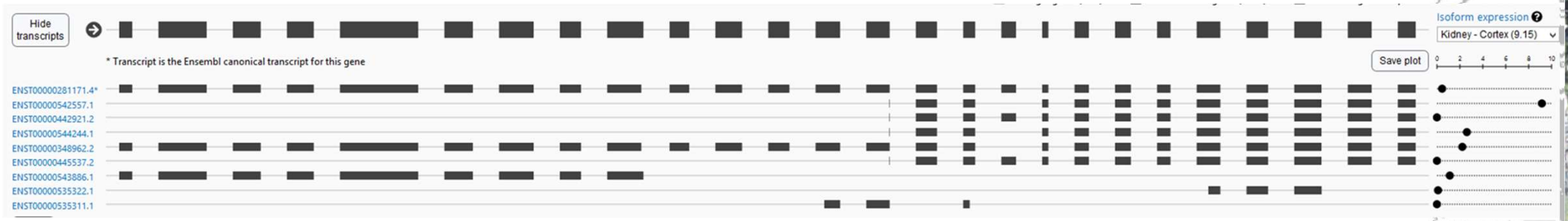
Regulatory : 1 mutation [back to top]

monogenic proteinuria (SRNS/FSGS)
PTPRO (GLEPP1)

- **Patient 24226: *PTPRO* c.2437+1G>A;2437+1G>A**
- **Ozaltin et al. c.2627+1G>T and c.2828+1G>A**
- **Hypothesis: Splicing mutation with skipping of exon 14 in the long transcript and exon 2 in the short transcript**







monogenic proteinuria (SRNS/FSGS) other help curated databases

Example 4 *FAT1* Gee et al. Nat Commun 2016
FAT1 mutations cause a glomerulotubular nephropathy

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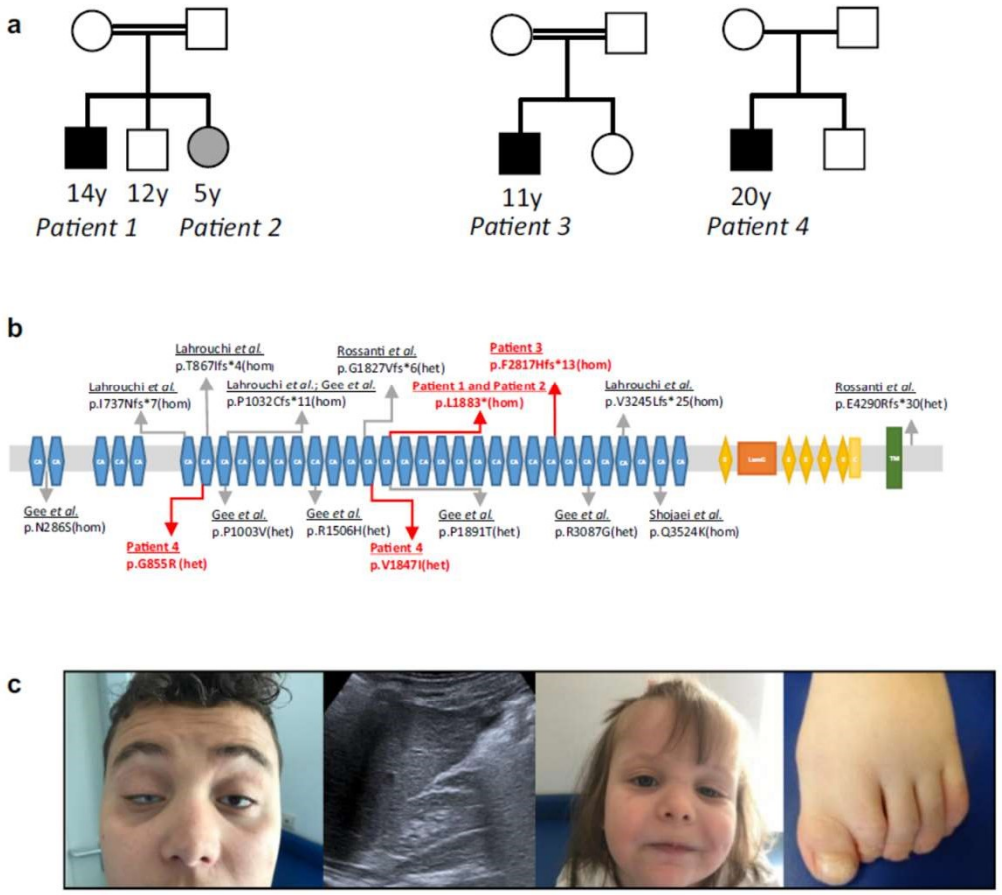
QUICK GENE: Search

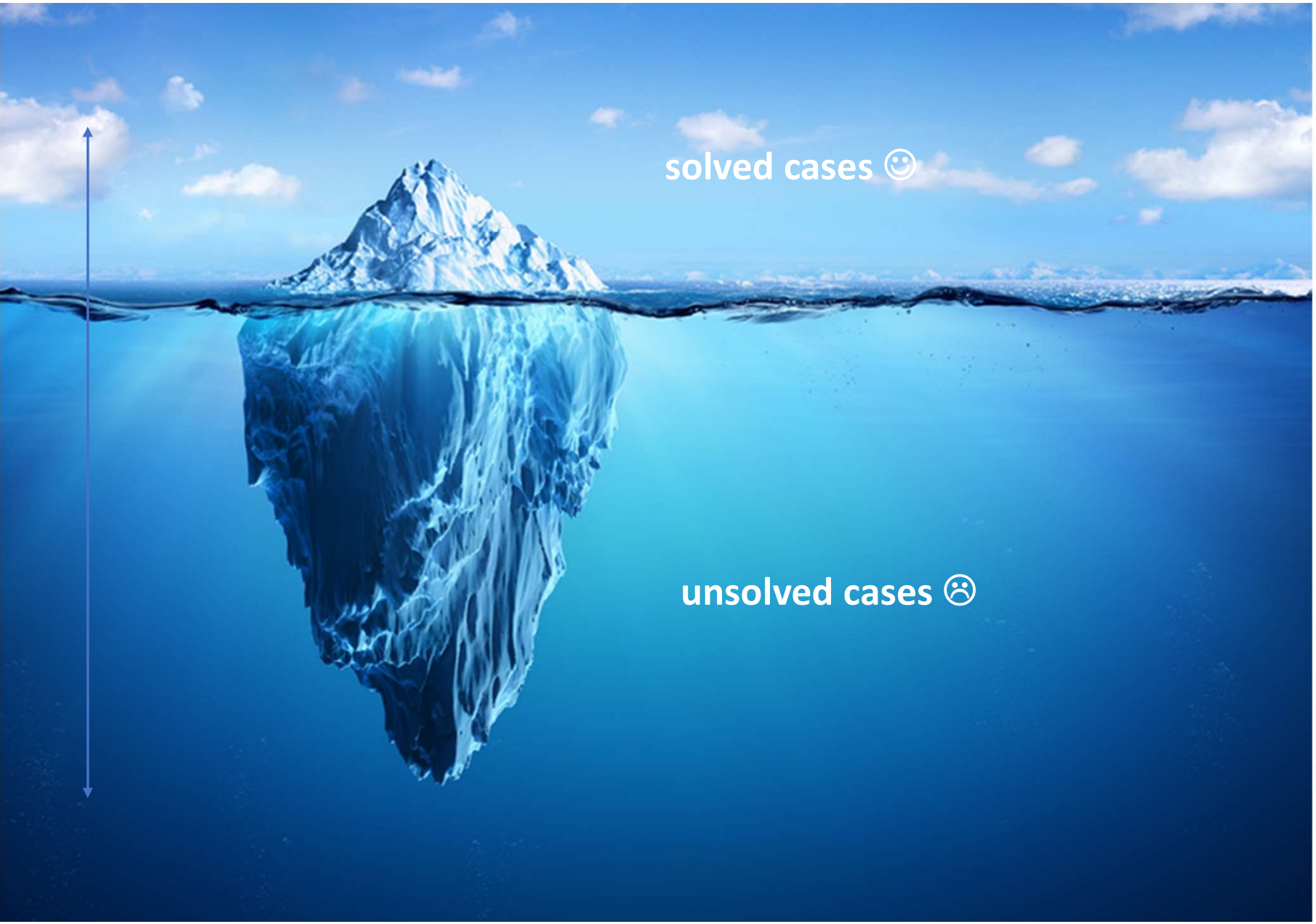
CM2069073	CCC-CCT	Pro1372Pro	c.4116C>T	p.P1372=	DM?	Developmental disorder	Kaplanis (2020) Nature 586, 757 Zhou (2022) Nat Genet 54: 1305 [Autism]	hg38 hg19 COM CpG dbSNP
CM2111950	TTC-TGC	Phe1402Cys	c.4205T>G	p.F1402C	DM?	Schizophrenia	Lencz (2021) Neuron 109, 1465	hg38 hg19 dbSNP
CM152617	CGT-CAT	Arg1453His	c.4358G>A	p.R1453H	DM	Facioscapulohumeral dystrophy-like phenotype	Puppo (2015) Hum Mutat 36, 443 Kaplanis (2020) Nature 586: 757 [Developmental disorder] Zhou (2022) Nat Genet 54: 1305 [Autism] Kars (2021) Proc Natl Acad Sci U S A 118: e2026076118 [Additional report]	hg38 hg19 CpG dbSNP gnomAD
CM1716401	ATC-ACC	Ile1478Thr	c.4433T>C	p.I1478T	DM?	Spinocerebellar ataxia	Nibbeling (2017) Brain 140, 2860 Wang (2019) J Am Soc Nephrol 30: 1625 [Focal segmental glomerulosclerosis]	hg38 hg19 dbSNP gnomAD
CM1939757	ATC-GTC	Ile1478Val	c.4432A>G	p.I1478V	DM?	Focal segmental glomerulosclerosis	Wang (2019) J Am Soc Nephrol 30, 1625	hg38 hg19 COM dbSNP gnomAD
CM2118446	CGT-TGT	Arg1506Cys	c.4516C>T	p.R1506C	DM?	Head and neck squamous cell carcinoma	Cury (2021) Oral Oncol 122,	hg38 hg19 COM CpG gnomAD
CM162554	CGT-CAT	Arg1506His	c.4517G>A	p.R1506H	DM	Nephrotic syndrome, tubular ectasia and haematuria	Gee (2016) Nat Commun 7, 10822	hg38 hg19 CpG dbSNP
CM152618	GCA-ACA	Ala1575Thr	c.4723G>A	p.A1575T	DM	Facioscapulohumeral dystrophy-like phenotype	Puppo (2015) Hum Mutat 36, 443	hg38 hg19 dbSNP
CM1939753	ACG-ATG	Thr1585Met	c.4754C>T	p.T1585M	DM?	Focal segmental glomerulosclerosis	Wang (2019) J Am Soc Nephrol 30, 1625	hg38 hg19 COM CpG dbSNP gnomAD
CM1416239	GTG-GAG	Val1597Glu	c.4790T>A	p.V1597E	DM?	Autism spectrum disorder	Iossifov (2014) Nature 515, 216 Lim (2017) Nat Neurosci 20: 1217 [Autism spectrum disorder, increased risk of] Turner (2019) Am J Hum Genet 104: 1274 [Autism] Zhou (2019) Nat Genet 51: 973 [Additional report] 2 more reference(s)...	hg38 hg19 COM
CM2118440	ATT-GTT	Ile1619Val	c.4855A>G	p.I1619V	DM?	Head and neck squamous cell carcinoma	Cury (2021) Oral Oncol 122,	hg38 hg19 COM dbSNP
CM2257599	CGA-CAA	Arg1627Gln	c.4880G>A	p.R1627Q	DM?	Hereditary diffuse gastric cancer	Liu (2022) JAMA Netw Open 5,	hg38 hg19 COM CpG dbSNP gnomAD
CM2118442	CAT-CGT	His1692Arg	c.5075A>G	p.H1692R	DM?	Head and neck squamous cell carcinoma	Cury (2021) Oral Oncol 122,	hg38 hg19 COM dbSNP
CM2257598	GCG-ACG	Ala1762Thr	c.5284G>A	p.A1762T	DM?	Hereditary diffuse gastric cancer	Liu (2022) JAMA Netw Open 5,	hg38 hg19 COM CpG dbSNP gnomAD
CM2111949	TTT-GTT	Phe1765Val	c.5293T>G	p.F1765V	DM?	Schizophrenia	Lencz (2021) Neuron 109, 1465	hg38 hg19
CM2245543	ACA-ATA	Thr1771Ile	c.5312C>T	p.T1771I	DM?	Autism	Zhou (2022) Nat Genet 54, 1305	hg38 hg19 COM
CM2223785	ATT-GTT	Ile1774Val	c.5320A>G	p.I1774V	DM?	Coloboma and nephropathy	Esmaeilzadeh (2022) CEN Case Rep 11, 404	hg38 hg19 dbSNP gnomAD
CM2055968	GTC-GTT	Val1790Val	c.5370C>T	p.V1790=	DM?	Developmental disorder	Kaplanis (2020) Nature 586, 757 Zhou (2022) Nat Genet 54: 1305 [Autism]	hg38 hg19 COM
CM1514839	GAT-GGT	Asp1800Gly	c.5399A>G	p.D1800G	DM?	Congenital anomalies of kidney and urinary tract	Nicolaou (2016) Kidney Int 89, 476	hg38 hg19 COM dbSNP
CM2313821	CAT-TAT	His1830Tyr	c.5488C>T	p.H1830Y	DM?	Renal rickets	Saha (2023) BMC Nephrol 24, 212	hg38 hg19 COM
CM2111898	GTC-ATC	Val1847Ile	c.5539G>A	p.V1847I	DM	Renal & ocular abnormalities	Fabretti (2021) Kidney Int Rep 6, 1368	hg38 hg19 CpG dbSNP gnomAD
CM1514838	CCA-TCA	Pro1855Ser	c.5563C>T	p.P1855S	DM?	Congenital anomalies of kidney and urinary tract	Nicolaou (2016) Kidney Int 89, 476 Chrestani (2022) Clin Genet 101: 494 [Peters' anomaly]	hg38 hg19 COM dbSNP
CM2111894	TTA-TAA	Leu1883Term	c.5648T>A	p.L1883*	DM	Ptosis & syndactyly	Fabretti (2021) Kidney Int Rep 6, 1368	hg38 hg19 COM
CM162555	CCA-ACA	Pro1891Thr	c.5671C>A	p.P1891T	DM	Nephrotic syndrome, tubular ectasia and haematuria	Gee (2016) Nat Commun 7, 10822	hg38 hg19 dbSNP gnomAD
CM1716377	GAC-CAC	Asp1930His	c.5788G>C	p.D1930H	DM	Spinocerebellar ataxia	Nibbeling (2017) Brain 140, 2860	hg38 hg19 dbSNP gnomAD
CM1514837	AAT-AGT	Asn2009Ser	c.6026A>G	p.N2009S	DM?	Congenital anomalies of kidney and urinary tract	Nicolaou (2016) Kidney Int 89, 476 Pehlivan (2019) Am J Hum Genet 105: 132 [Arthrogryposis]	hg38 hg19 COM dbSNP gnomAD

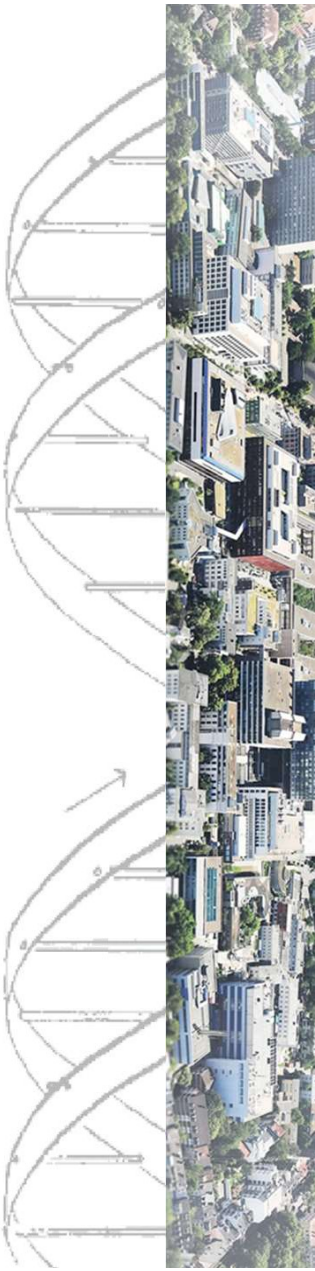
Example 4 *FAT1* Gee et al. Nat Commun 2016
Lahrouchi et al. Nat Commun 2019

Homozygous frameshift mutations in FAT1 cause a syndrome characterized by colobomatous-microphthalmia, ptosis, nephropathy and syndactyly

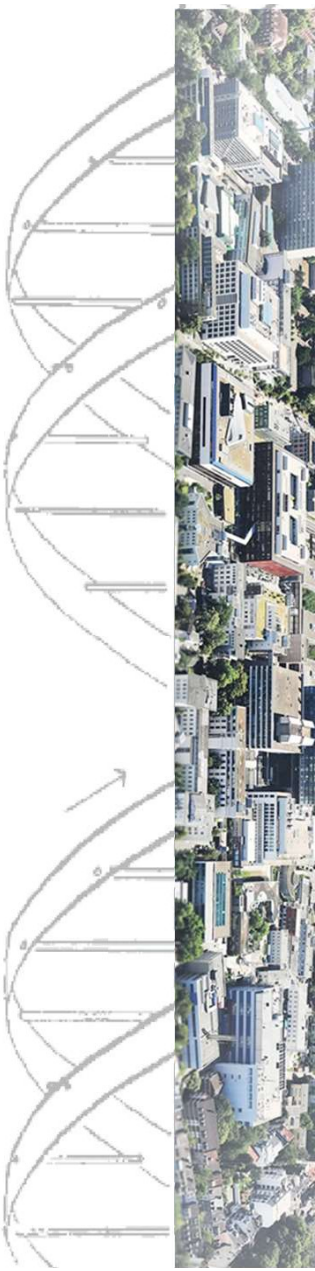


Fabretti et al. Kidney Int Rep 2022


solved cases ☺
unsolved cases ☹




Geklärte Fälle ☺
Nota bene
"The reward for good work is more work." – Francesca Elisia
+ prognosis?
+ centers?
+ therapeutic options and concepts?
+ family members at risk?
+ prenatal testing etc.

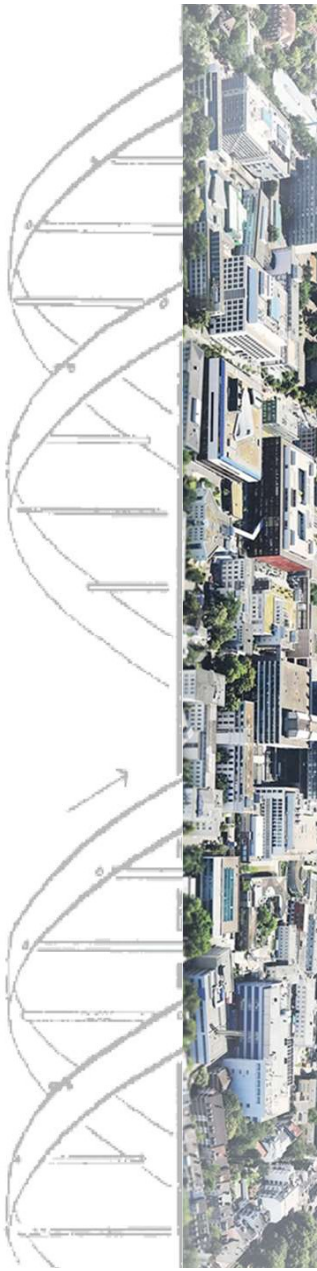


monogenic proteinuria (SRNS/FSGS) genotype and outcome

 # general outcome genetic forms of SRNS/FSGS poor
 # mostly not responsive to immunosupressive regimens
 # high risk of progressive CKD and renal failure
 # exceptions reported

Exceptions:

Alport-Syndrom (susceptible mutations (e.g. G624D))
COQ10 deficiency
Cobalamine deficiency (MMACHC)
TRPC6 assocaited FSGS – TRPC6 inhibitor
 # caHUS
 # APOL1 associated kidney disease/FSGS



monogenic proteinuria (SRNS/FSGS) genotype and outcome

Rapid Response to Cyclosporin A and Favorable Renal Outcome in Nongenetic Versus Genetic Steroid–Resistant Nephrotic Syndrome

Anja K. Büscher, Bodo B. Beck,† Anette Melk,‡ Julia Hoefele,§ Birgitta Kranz,‖ Daniel Bamborschke,† Sabr Bärbel Lange-Sperandio,¶ Theresa Jungraithmayr,** Lutz T. Weber,†† Markus J. Kemper,‡‡ Burkhard Tönsho Peter F. Hoyer,* Martin Konrad,‖ and Stefanie Weber* for the German Pediatric Nephrology Association (G*

clinical investigation www.kidney-int

A multicenter retrospective study of calcineurin inhibitors in nephrotic syndrome secondary to podocyte gene variants

see commentary on

Georgia Malakasioti[1], Daniela Iancu[2], Anastasiia Milovanova[3], Alexey Tsygin[3], Tomoko Horinou China Nagano[4], Kandai Nozu[4], Koichi Kamei[5], Shuichiro Fujinaga[6], Kazumoto Iijima[7], Hee Gyur Rajiv Sinha[9], Biswanath Basu[10], William Morello[11], Giovanni Montini[11,12], Aoife Waters[13], Olivia Zeynep Yürük Yıldırım[15], Sibel Yel[16], İsmail Dursun[16], Hugh J. McCarthy[17], Marina Vivarelli[18], Larisa Prikhodina[19], Martine T.P. Besouw[20], Eugene Yu-hin Chan[21], Wenyan Huang[22], Markus J. Sebastian Loos[23], Chanel Prestidge[24], William Wong[24], Galia Zlatanova[25], Rasmus Ehren[26], Lutz T Hassib Chehade[27], Nakysa Hooman[28], Marcin Tkaczyk[29], Małgorzata Stańczyk[29], Michael Miligk Kjell Tullus[13]; on behalf of the CNI in Monogenic SRNS Study Investigators[31]

Lay Summary

Calcineurin inhibitors (CNI) are immunosuppressive medications very efficacious in childhood steroid-resistant nephrotic syndrome (SRNS). However, there is a subgroup of children with genetic mutations responsible for the disease in whom CNI are considered non-efficacious and are contraindicated. Yet, to date, there are no studies that have specifically addressed the efficacy of CNI in genetic SRNS and how they could affect long-term kidney prognosis. We retrospectively assessed the records of 141 children with genetically confirmed SRNS from 37 international pediatric nephrology centers who had received CNI treatment. Approximately 1 in 4 children showed response to therapy, but more importantly, children responding to this treatment had a 75% lower risk for kidney failure compared with those who did not respond. Our study is the first to show that CNI can actually work in children with genetic SRNS and increase kidney survival, reducing the need for kidney replacement therapy.

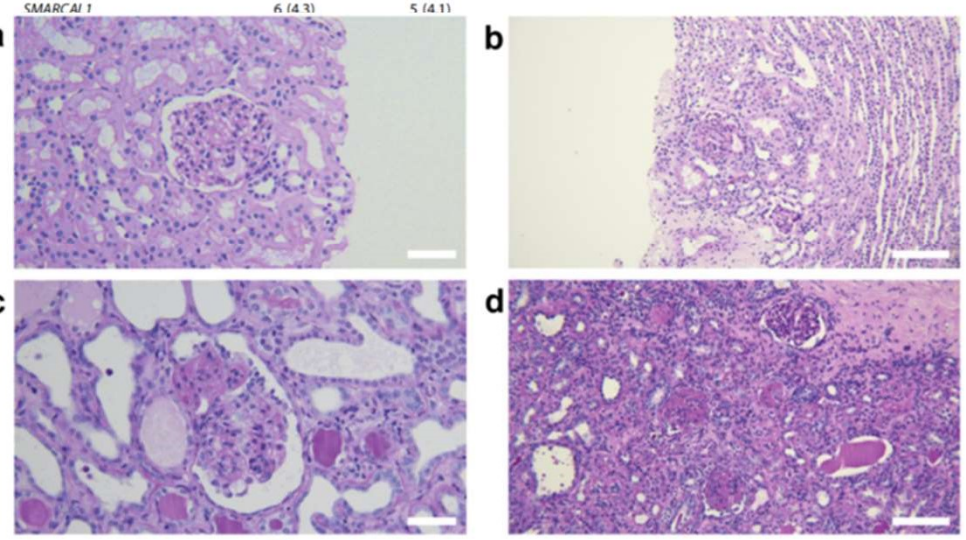
monogenic proteinuria (SRNS/FSGS) genotype and outcome

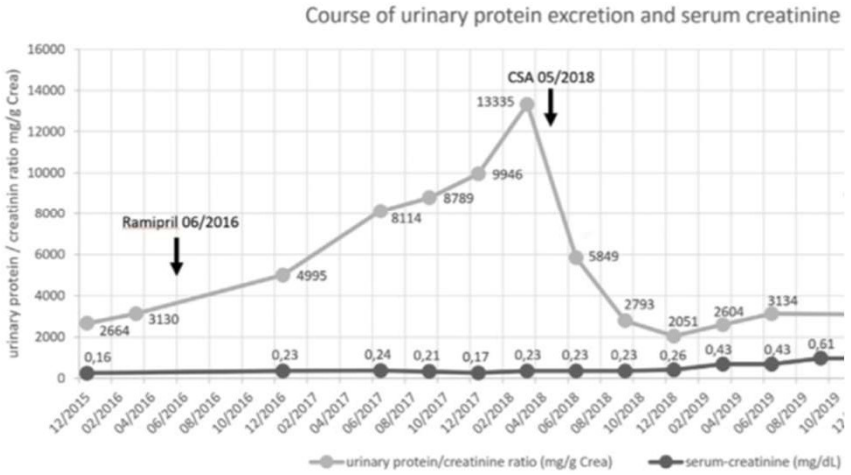
Characteristic	Possibly pathogenic and pathogenic genotype (N = 141)	Pathogenic genotype (N = 122)
Age at presentation, mo	34 (0–193)	31.5 (0–193)
Follow-up (from clinical presentation), mo	55 (5.5–243.6)	54.5 (7.6–243.6)
Female	81 (57.4)	72 (59)
Ethnicity		
Caucasian	78 (55.3)	71 (58.2)
African American	1 (0.7)	1 (0.8)
Asian	57 (40.4)	46 (37.7)
Hispanic	2 (1.4)	2 (1.6)
Other	3 (2.1)	2 (1.6)
Presentation		
CNS	15 (10.6)	15 (12.3)
INS	16 (11.3)	15 (12.3)
Overt NS	110 (78)	92 (75.4)
Family history of NS/CKD	30 (21.3)	28 (23)
Time between clinical and genetic diagnosis, mo	33.2 (0.8–245)	28.4 (0.8–229.3)
Gene mutated		
NPHS1	15 (10.6)	14 (11.5)
NPHS2	49 (34.8)	48 (39.3)
WT1	15 (10.6)	12 (9.8)
INF2	8 (5.7)	6 (4.9)
SMARCAL1	6 (4.3)	5 (4.1)
Normal kidney function	41 (29.3)	38 (31.4)
CKD stage 2–4	39 (27.9)	34 (28.1)
Kidney failure	60 (42.9)	49 (40.5)

Modeling of *ACTN4*-Based Podocytopathy Using *Drosophila* Nephrocytes

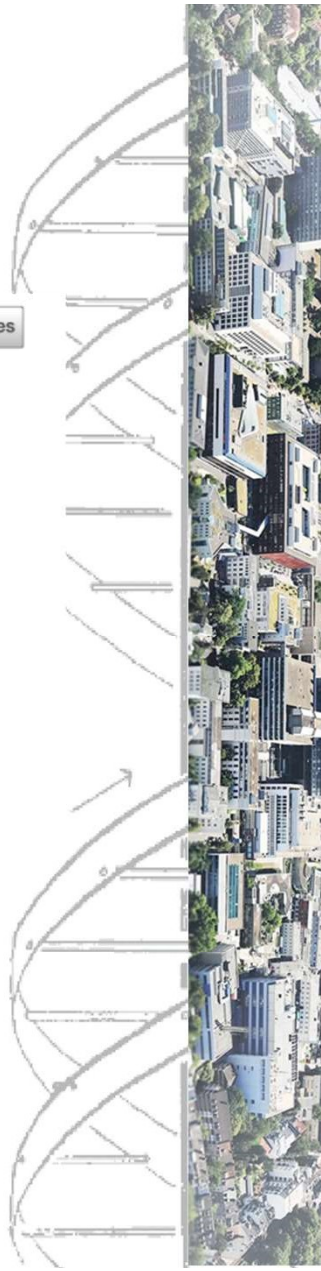
Check for updates

Johanna Odenthal[1], Sebastian Dittrich[1], Vivian Ludwig[1], Tim Merz[1], Katrin Reitmeier[1], Björn Reusch[3,4], Martin Höhne[1], Zülfü C. Cosgun[5], Maximilian Hohenadel[6], Jovana Putnik[7], Heike Göbel[8], Markus M. Rinschen[9,10,11], Janine Altmüller[12,13], Sybille Koehler[1], Bernhard Schermer[1,2], Thomas Benzing[1,2], Bodo B. Beck[3,4], Paul T. Brinkkötter[1], Sandra Habbig[5,14] and Malte P. Bartram[1,14]





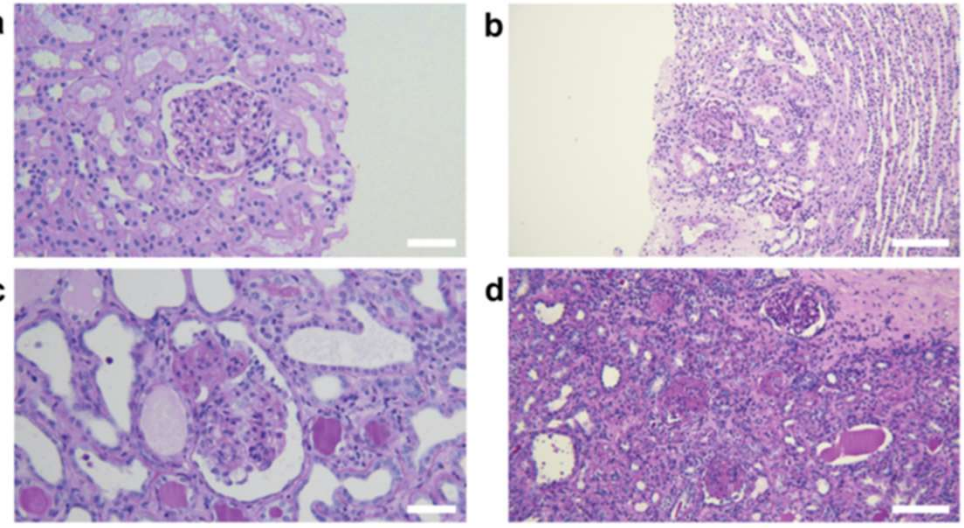
Kidney Int Rep 2022

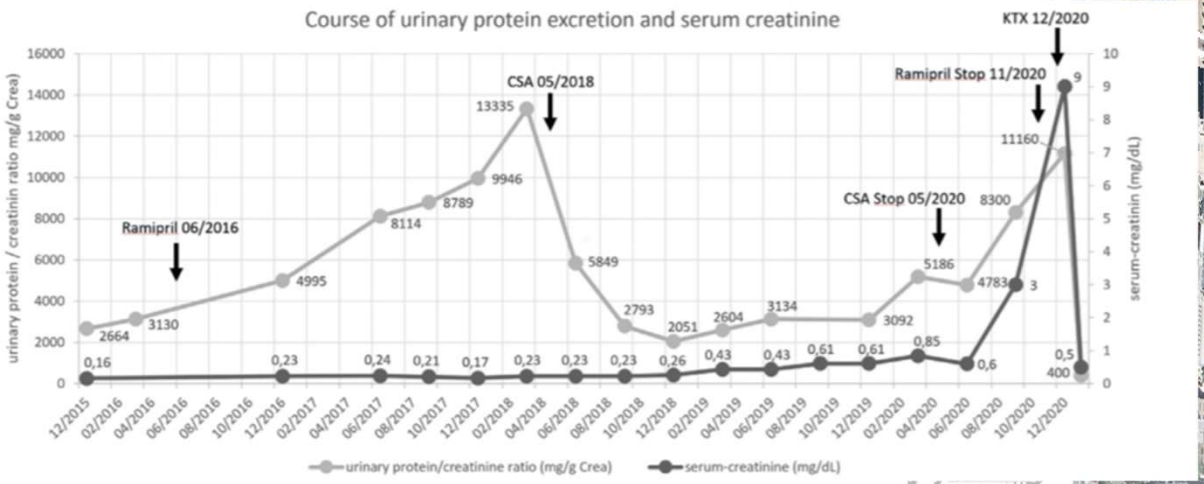


monogenic proteinuria (SRNS/FSGS) genotype and outcome

Modeling of *ACTN4*-Based Podocytopathy Using *Drosophila* Nephrocytes

Johanna Odenthal[1], Sebastian Dittrich[1], Vivian Ludwig[1], Tim Merz[1], Katrin Reitmeier[1], Björn Reusch[3,4], Martin Höhne[1], Zülfü C. Cosgun[5], Maximilian Hohenadel[6], Jovana Putnik[7], Heike Göbel[8], Markus M. Rinschen[9,10,11], Janine Altmüller[12,13], Sybille Koehler[1], Bernhard Schermer[1,2], Thomas Benzing[1,2], Bodo B. Beck[3,4], Paul T. Brinkkötter[1], Sandra Habbig[5,14] and Malte P. Bartram[1,14]

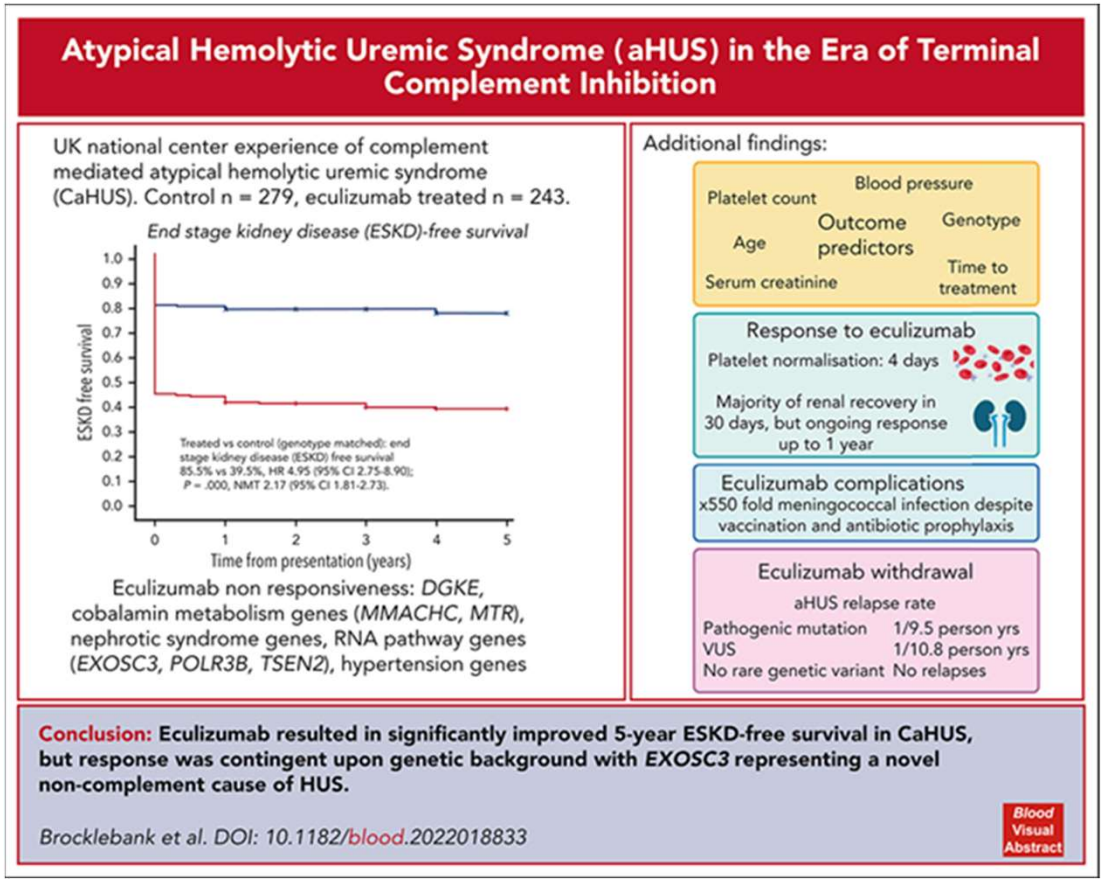




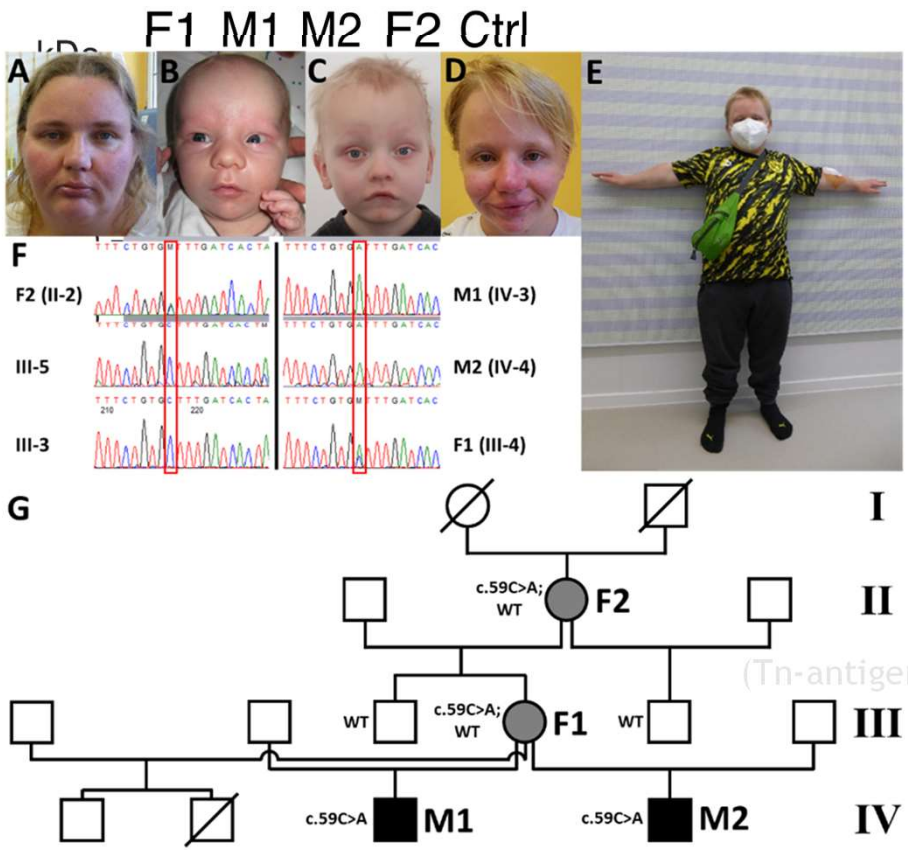
Kidney Int Rep 2022

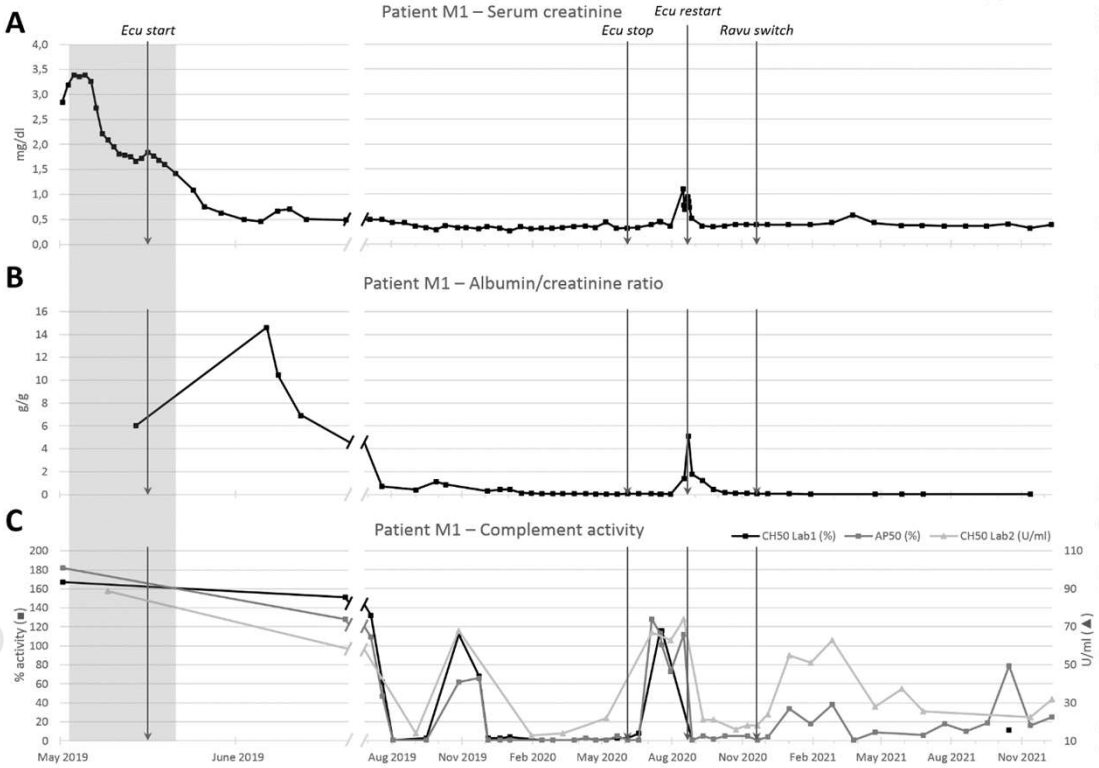
caHUS: C3, CD46, CFB, CFH, CFI, structural variants/hybrid genes

CFH/CFHR gene cluster



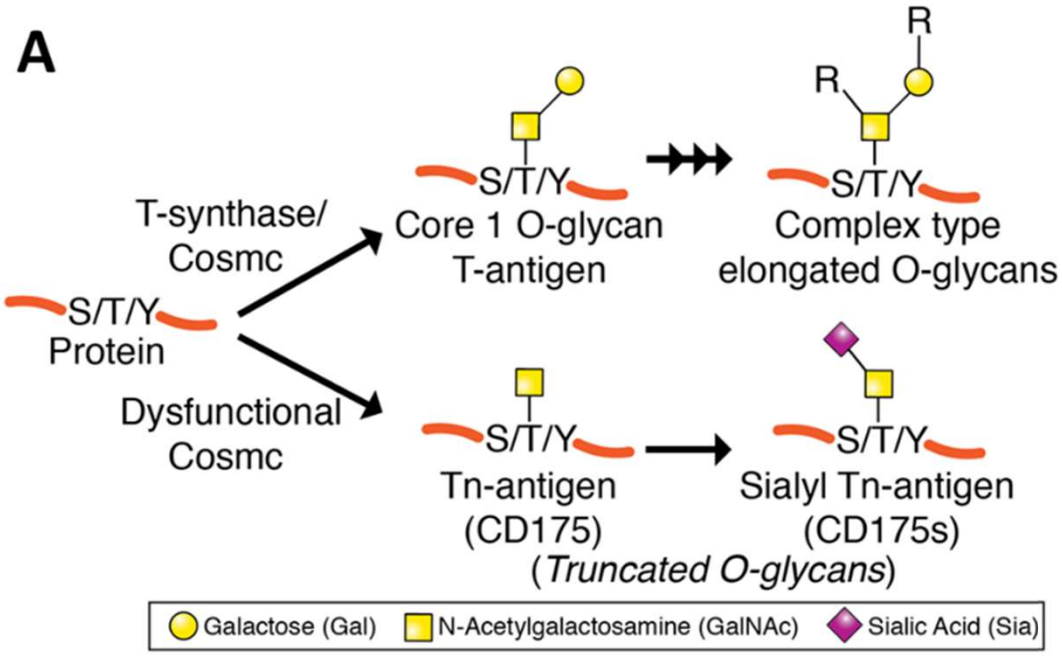
aHUS-*like, responsive to Eculizumab*

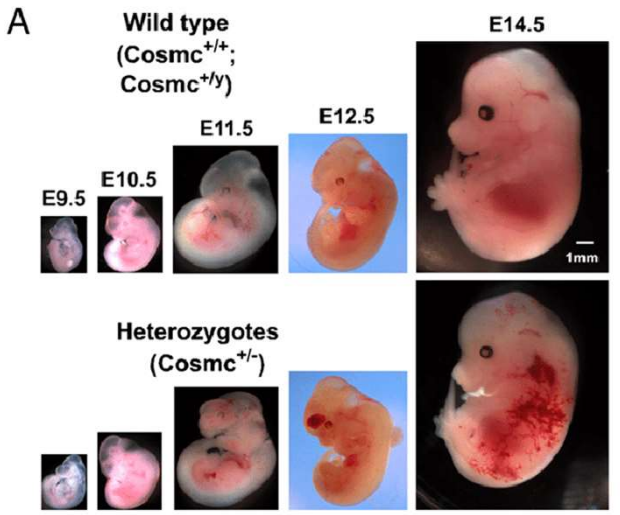




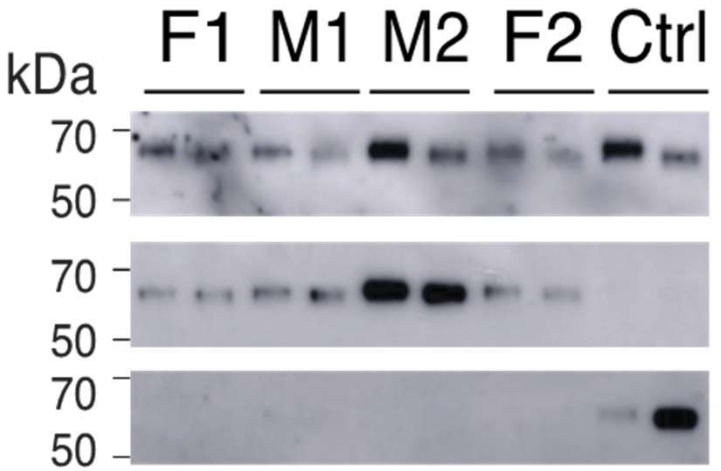
Erger&Aryal et al., *PNAS 2023*

C1GALT1C1 p.Ala20Asp





Wang et al., *PNAS*, 2010



Erger&Aryal et al., *PNAS 2023*

aHUS-like, responsive to Eculizumab

ESHG

www.nature.com/ejhg

ARTICLE

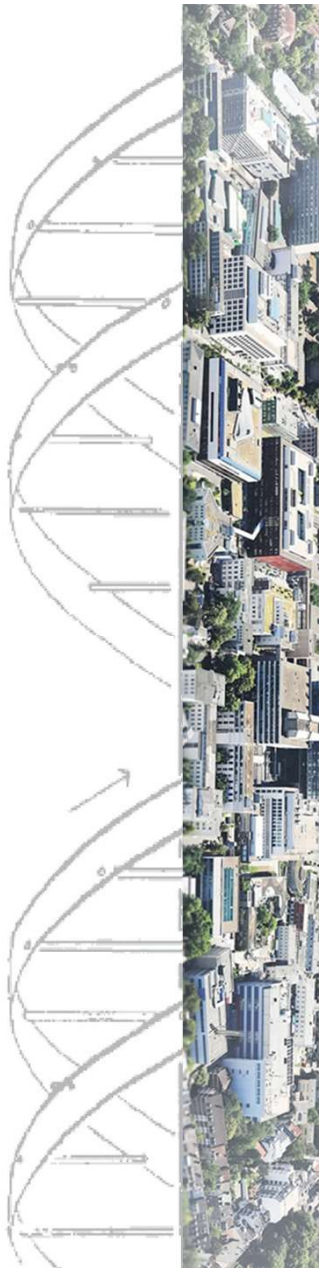
Check for updates

X-linked *C1GALT1C1* mutation causes atypical hemolytic uremic syndrome

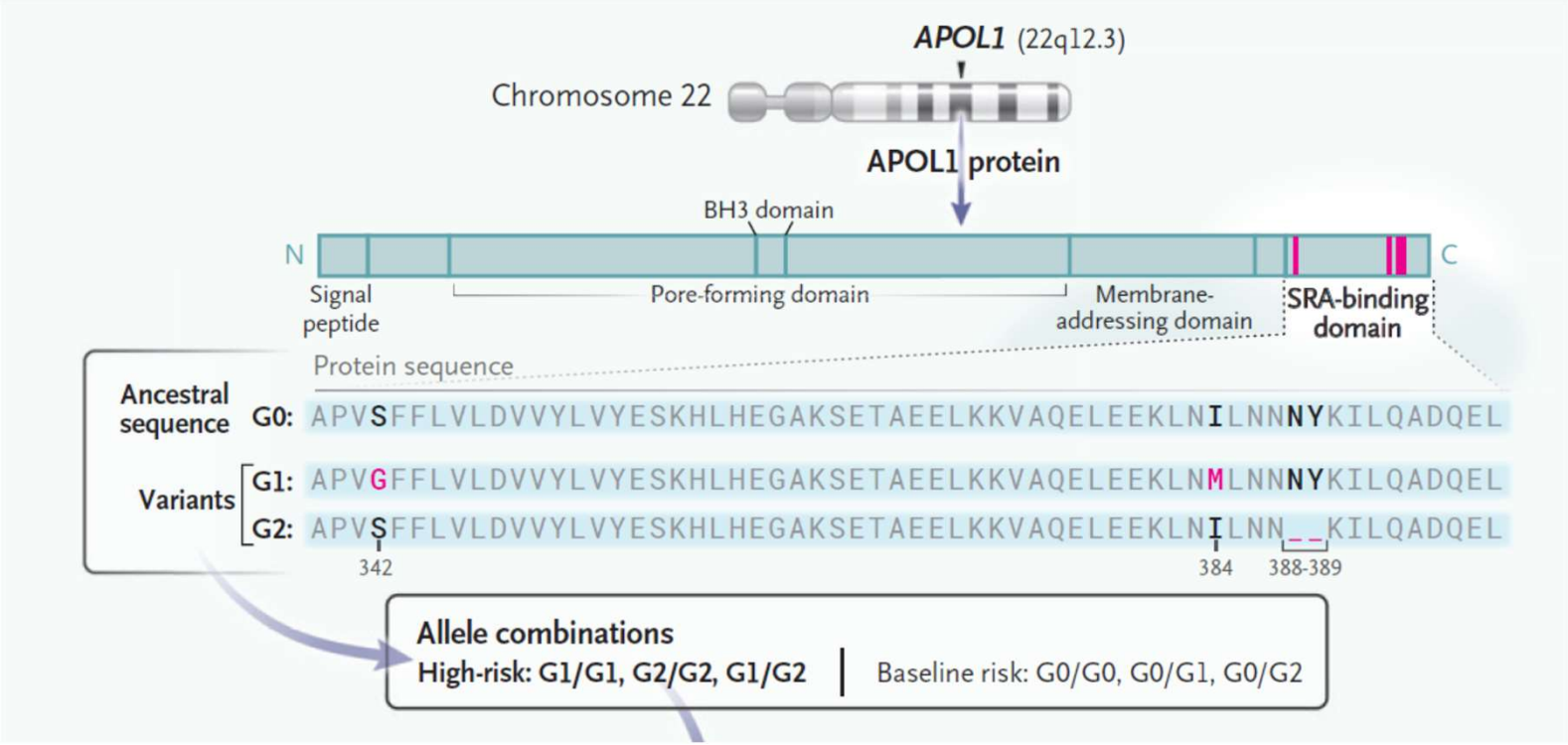
Noam Hadar[1], Ruth Schreiber[2], Marina Eskin-Schwartz[1,3], Eyal Kristal[4], George Shubinsky[5], Galina Ling[4], Idan Cohen[6], Michael Geylis[2], Amit Nahum[2,7], Yuval Yogev[1] and Ohad S. Birk[1,3,6]✉

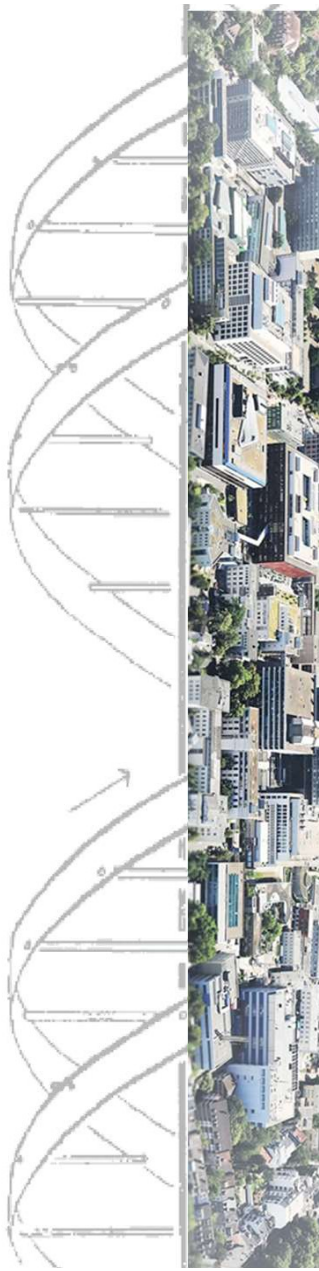
Gene-Phenotype Relationships

Location	Phenotype (View Clinical Synopses)	Phenotype MIM number	Inheritance	Phenotype mapping key
Xq24	Hemolytic uremic syndrome, atypical, 8, with rhizomelic short stature	301110	XLR	3
	Tn polyagglutination syndrome, somatic	300622		3

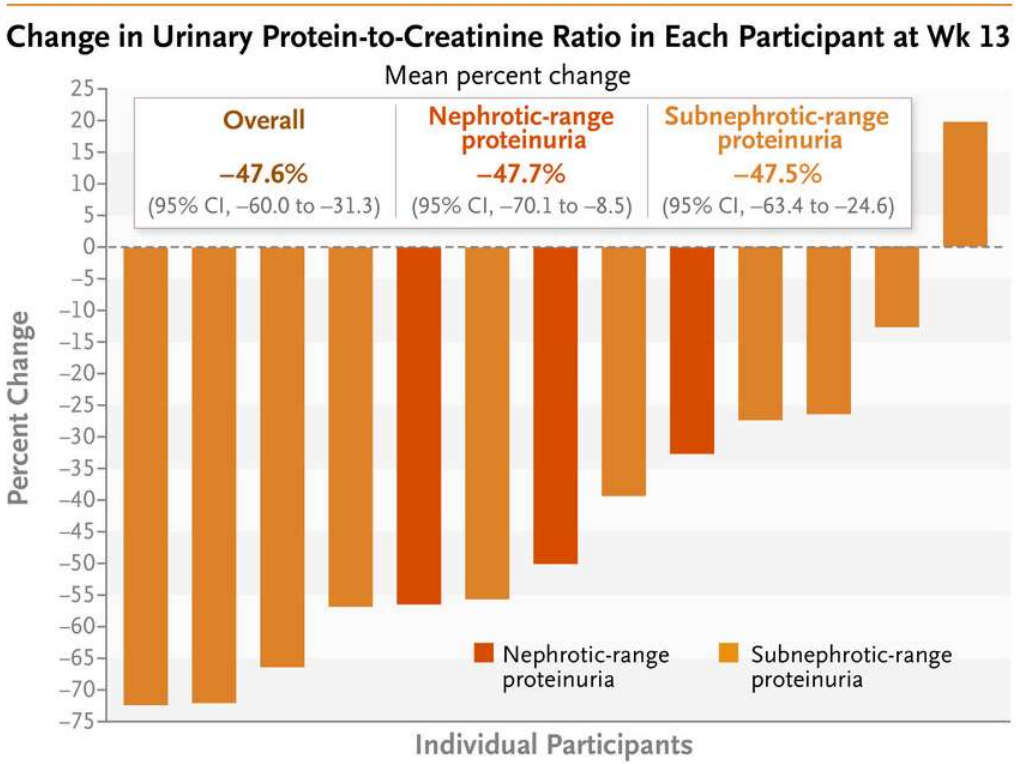


APOL1 risk alleles

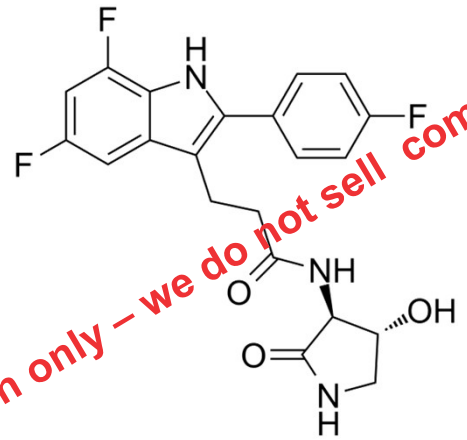




APOL1 risk alleles and treatment

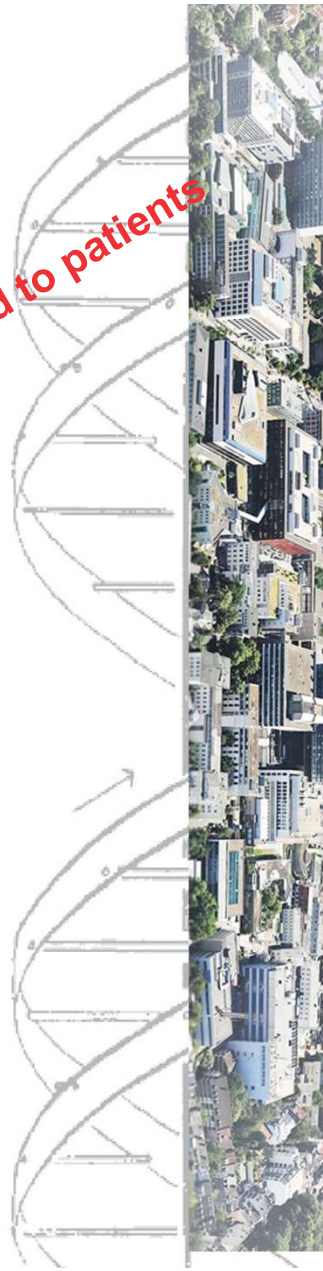


Egbuna et al., *NEJM 2023*

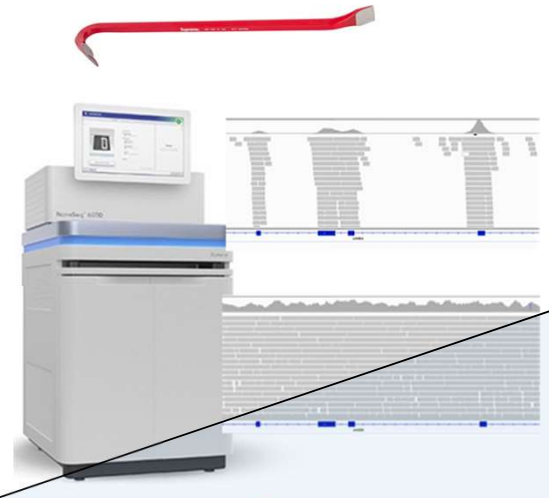


Research only – we do not sell compound to patients

Inaxaplin



phenotyping is frequently the bottleneck

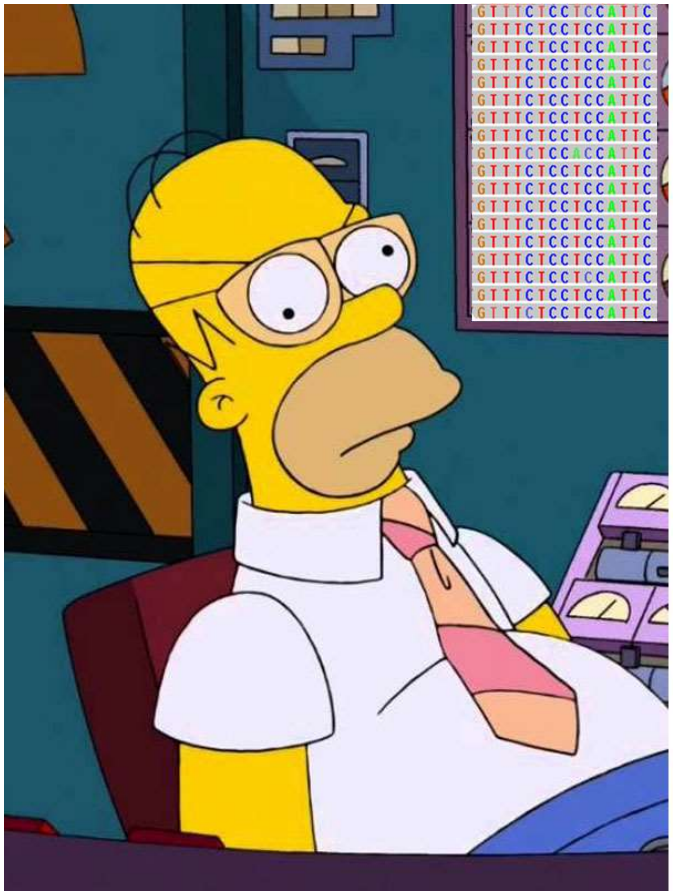


phenotyping
complex (interdisciplinary)
clinical findings
lab findings
Imaging findings
behaviour

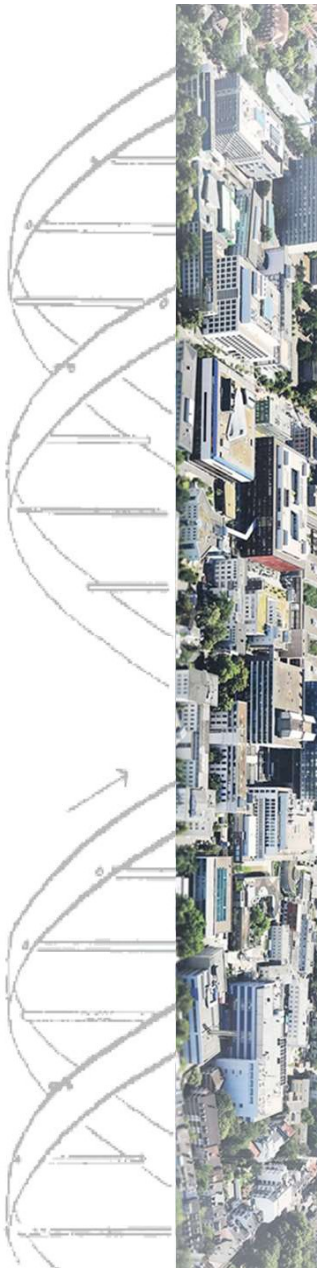
less standardized and
scalable

reverse genetics
requires correlation with phenptype

thank you for your attention



"I like work, it fascinates me. I can sit and look at it for hours"



How to investigate pediatric nephrolithiasis for a rare disease?

Constantinos J. Stefanidis, MD, PhD, FESPN

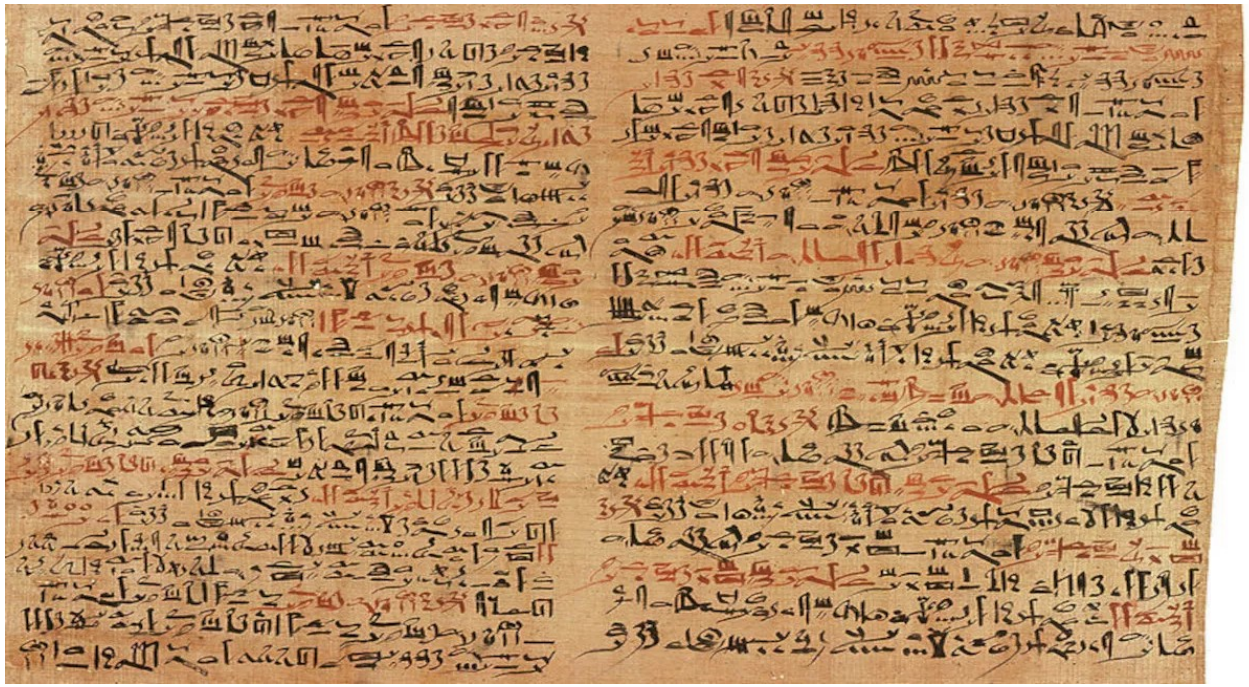
Head of Pediatric Nephrology
"MITERA" Children's Hospital, Athens, Greece

RARE DISEASES IN PEDIATRIC NEPHROLOGY
Skopje, Republic of North Macedonia 4th to 6th April 2024

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

What we learned all these years?

1550 BC: An Egyptian papyrus described kidney and bladder stones and their management



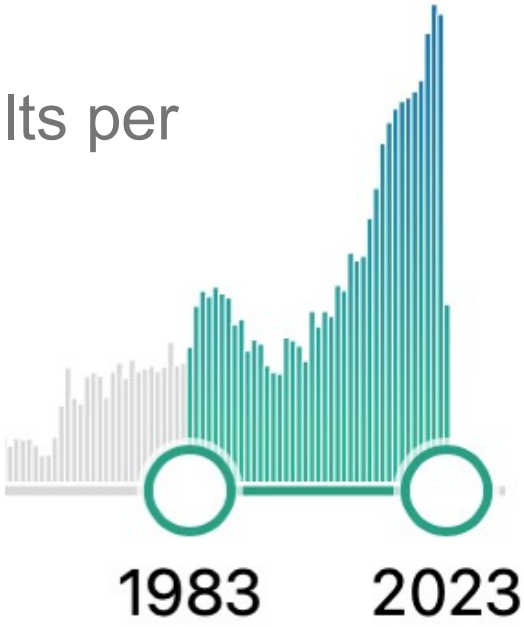
What we learned all these years?

PubMed®

nephrolithiasis

20,260 results

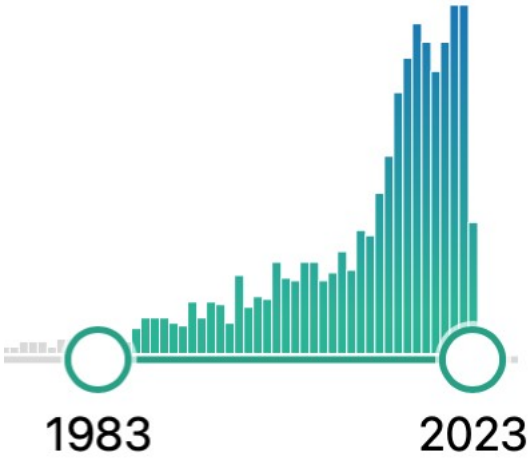
Results per year



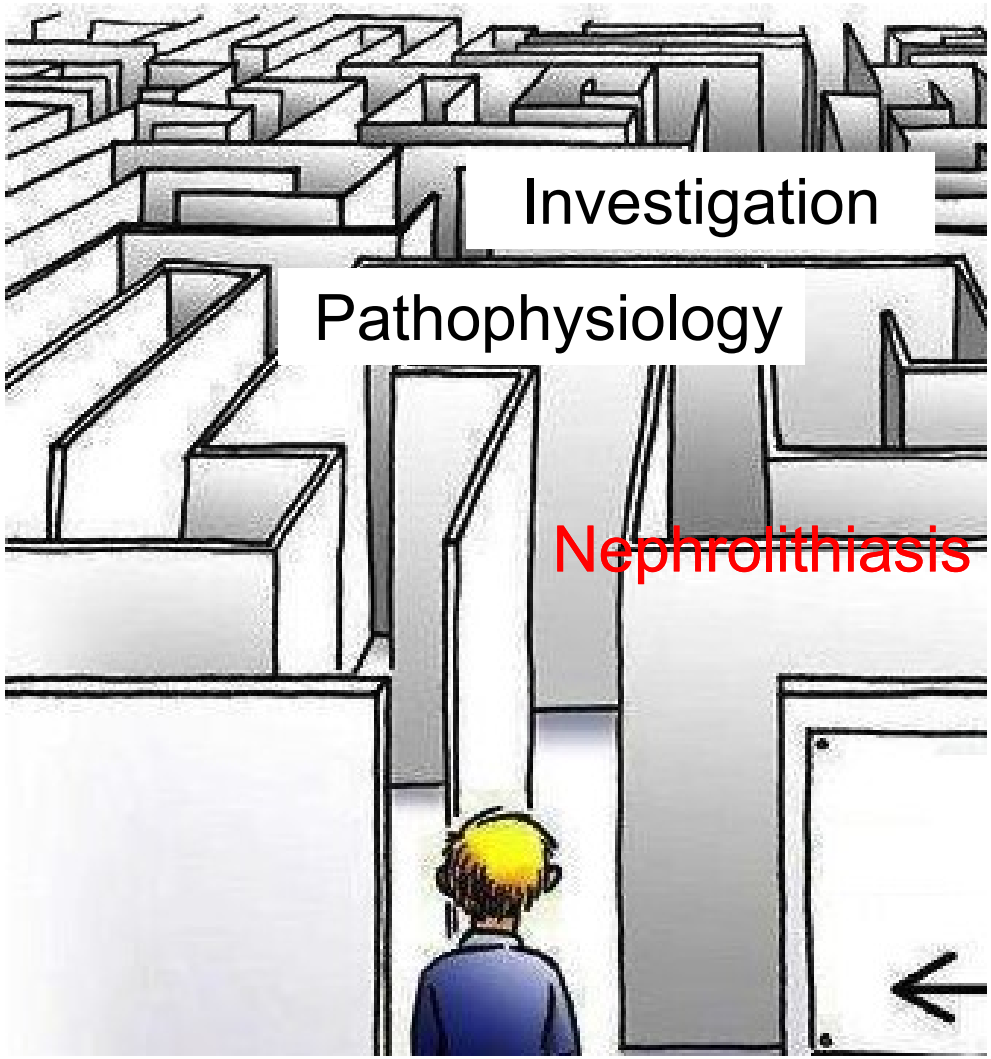
PubMed®

pediatric nephrolithiasis

1,446 results



Nephrolithiasis in children: a practical approach




Kidney stones with symptoms
Kidney stones with no symptoms
History of macroscopic hematuria
with pain and dialatation of pelvis
Hematuria, hypercalciuria with
family history of kidney stones

Incidence of nephrolithiasis in children

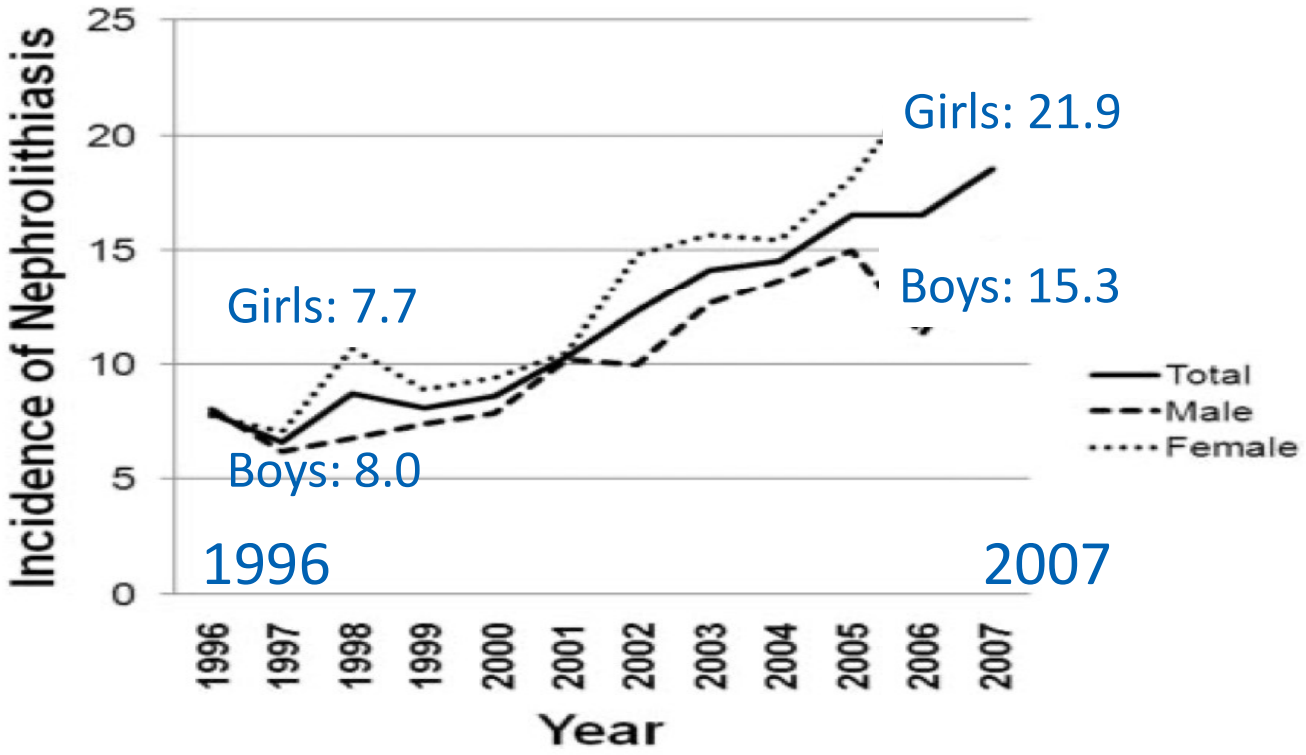
North America and Europe:

Yearly incidence in children:
<10 per 100.000 population

Pediatric nephrolithiasis is a rare disease
(<10 cases/ 100,000 population)

Incidence of pediatric nephrolithiasis (S. Carolina)

per 100,000 children

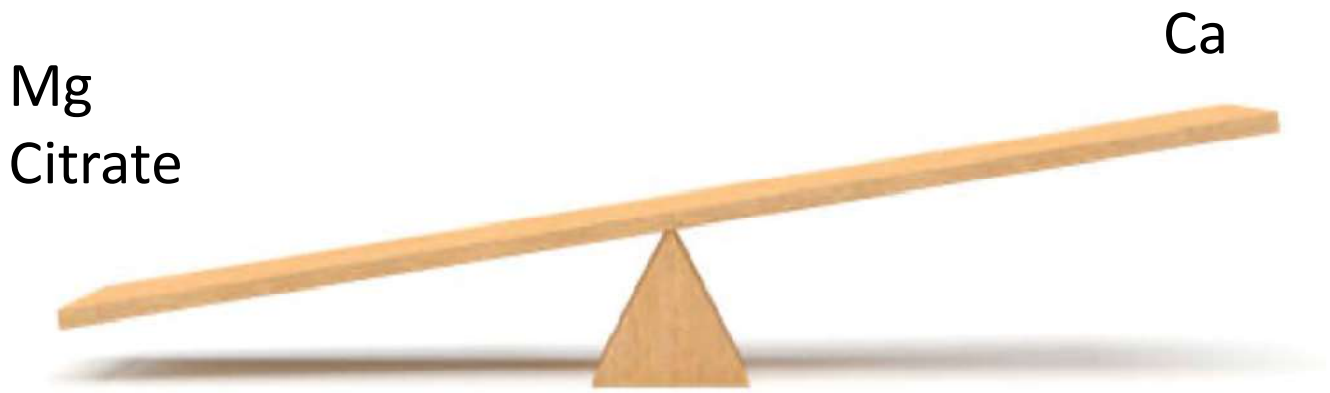


Sas DJ, et al. J Pediatr, 2010

Why children have lower incidence of KS?

Children have a higher urine concentration of calcium, but have also a higher concentration of inhibitors of crystallization (Mg, citrate)

Miyake OA et al. Urology 2001

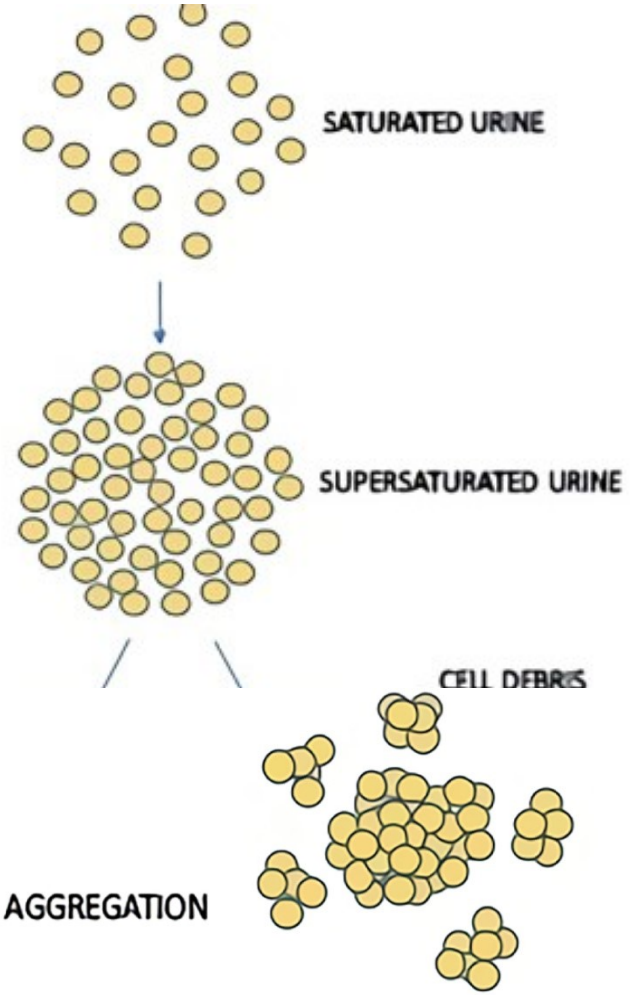


Supersaturation and crystallization

Urine: complex solution containing Ca, Ox, other ions and macromolecules that can interact and modulate:

Supersaturation

Crystallization

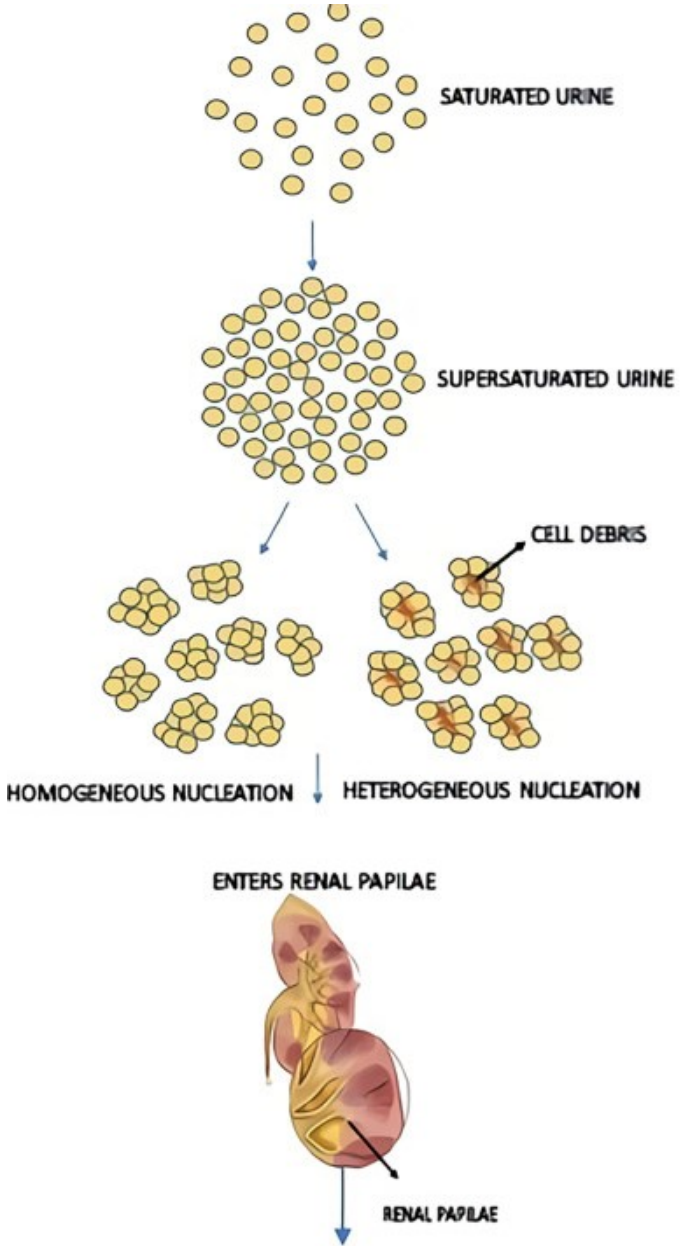


Khan SR. Urol Res 2006

Renal epithelial attachment

Crystal retention by attachment to renal epithelial cells

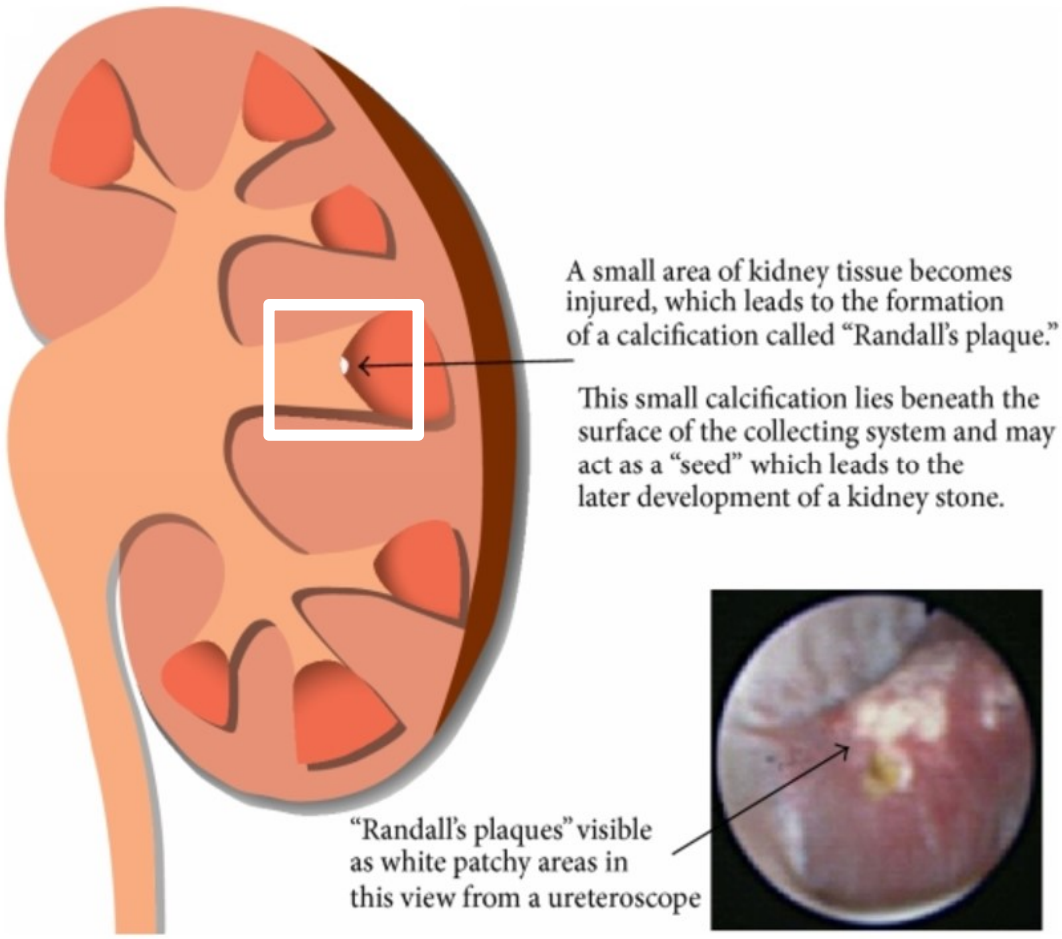
This modulates the retention of crystals in the interstitium of the kidneys



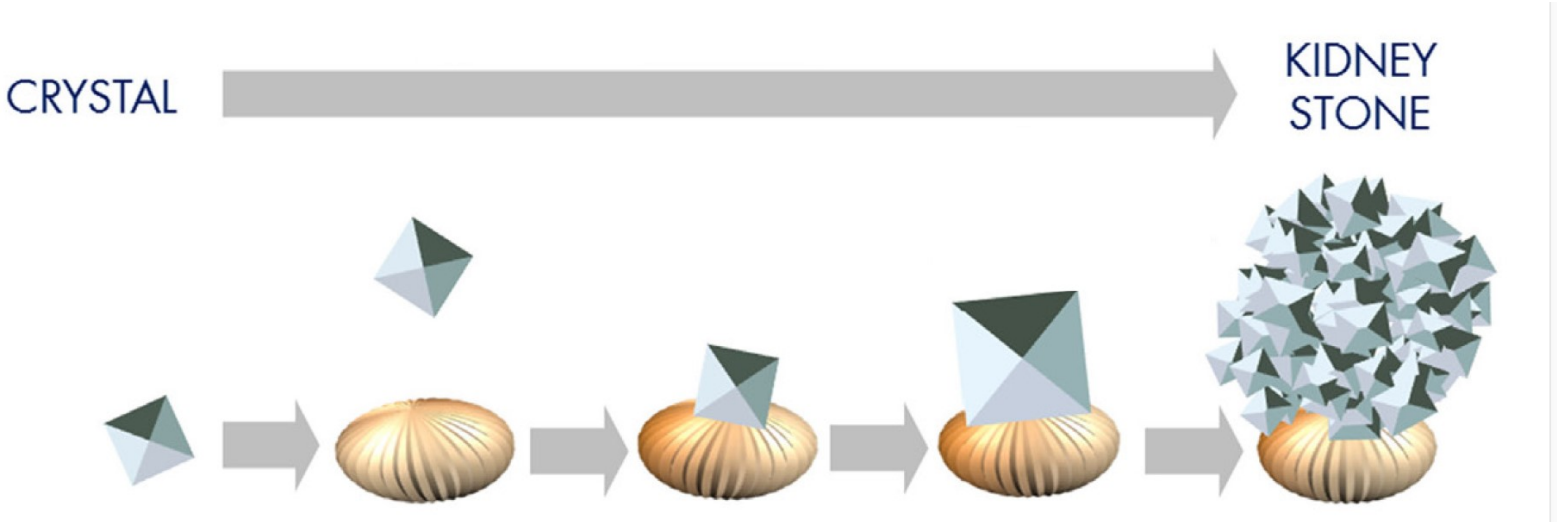
Khan SR. Urol Res 2006

Randall's plaques

Crystals are initially located to the surface of the papillae to form a stone nidus



Lithogenesis

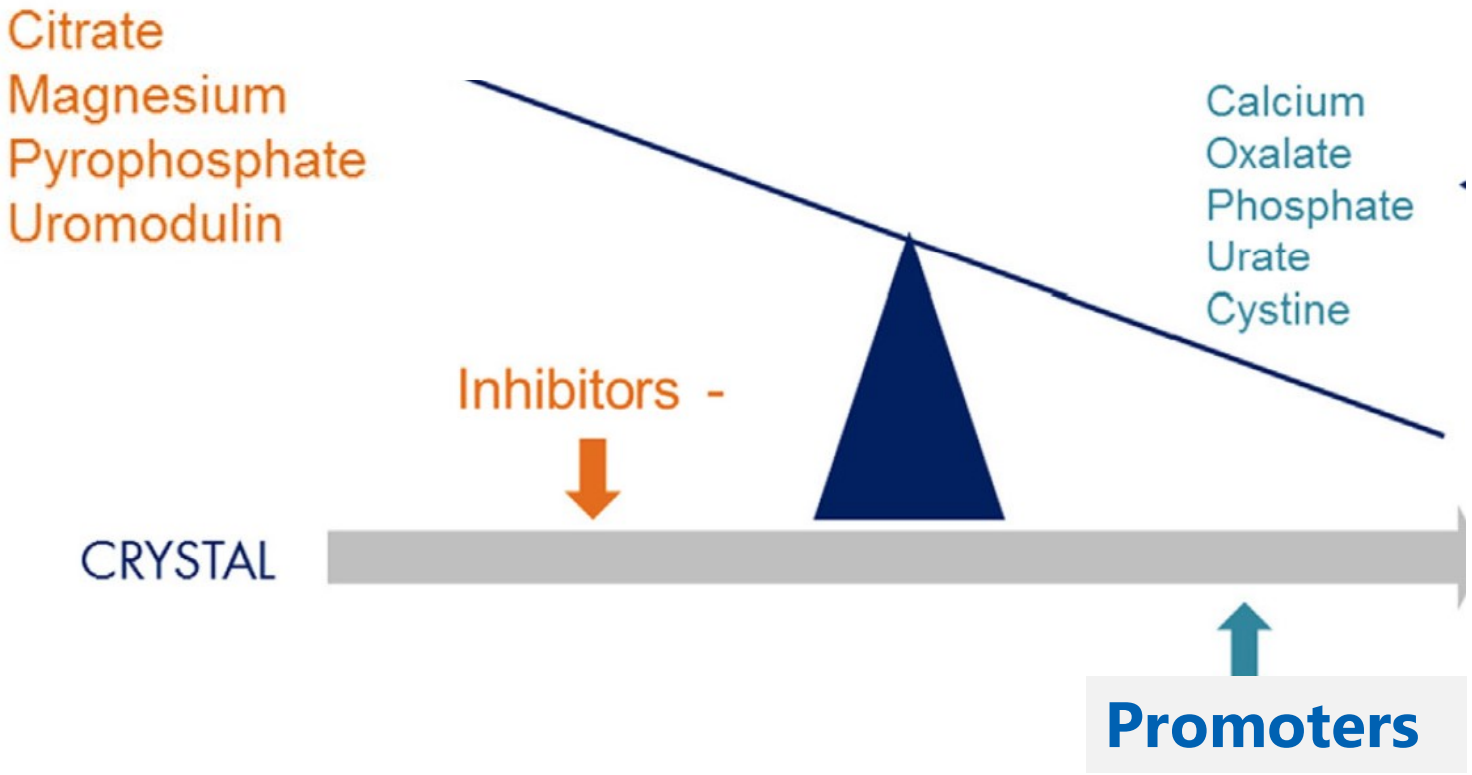


Randall's plaque

Halbritter J et al. Frontiers Ped 2018

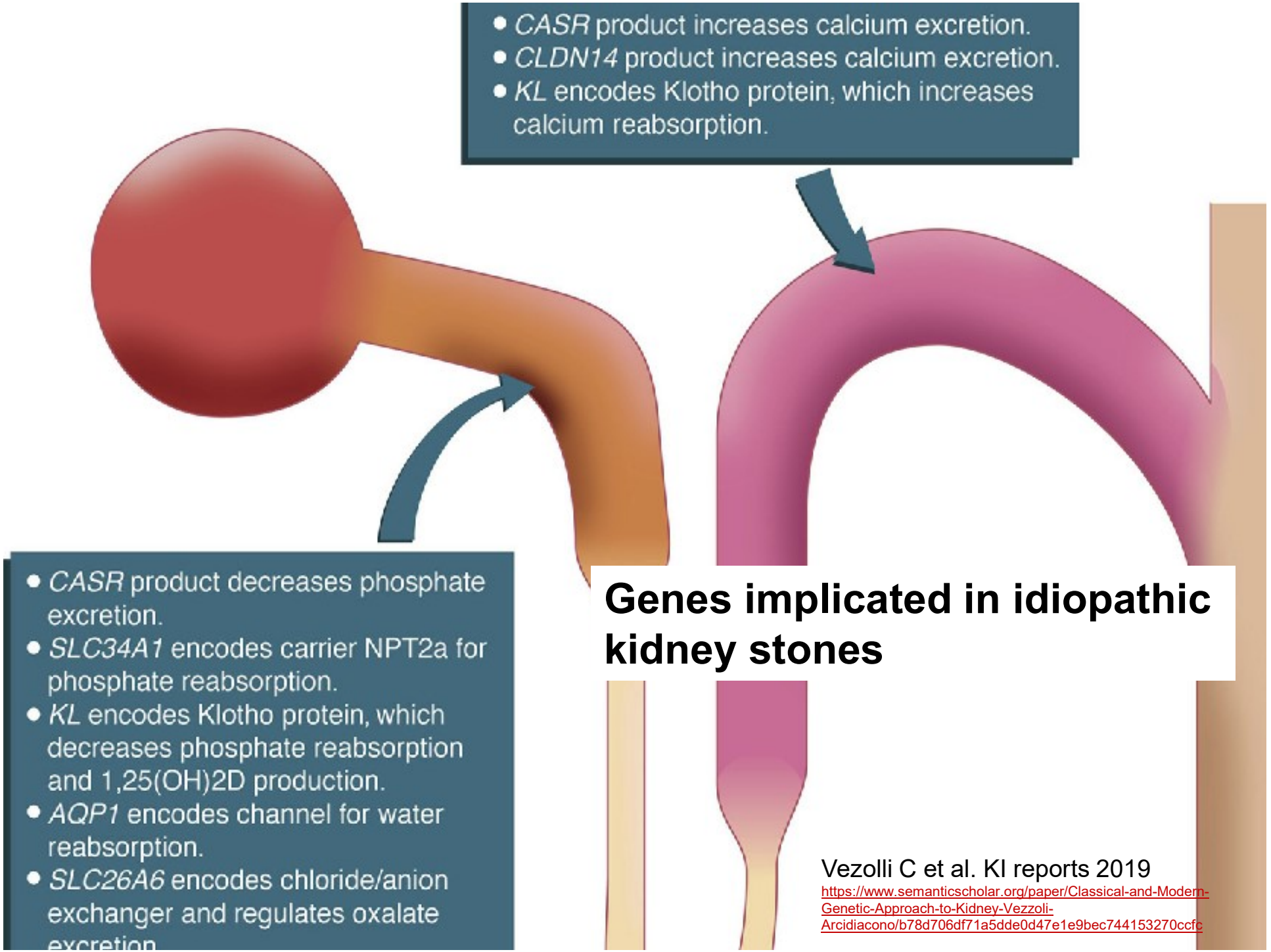
Imbalance of inhibitors and promoters of crystallization

Urinary **inhibitors** and **promoters** are controlled by renal and intestinal transporters

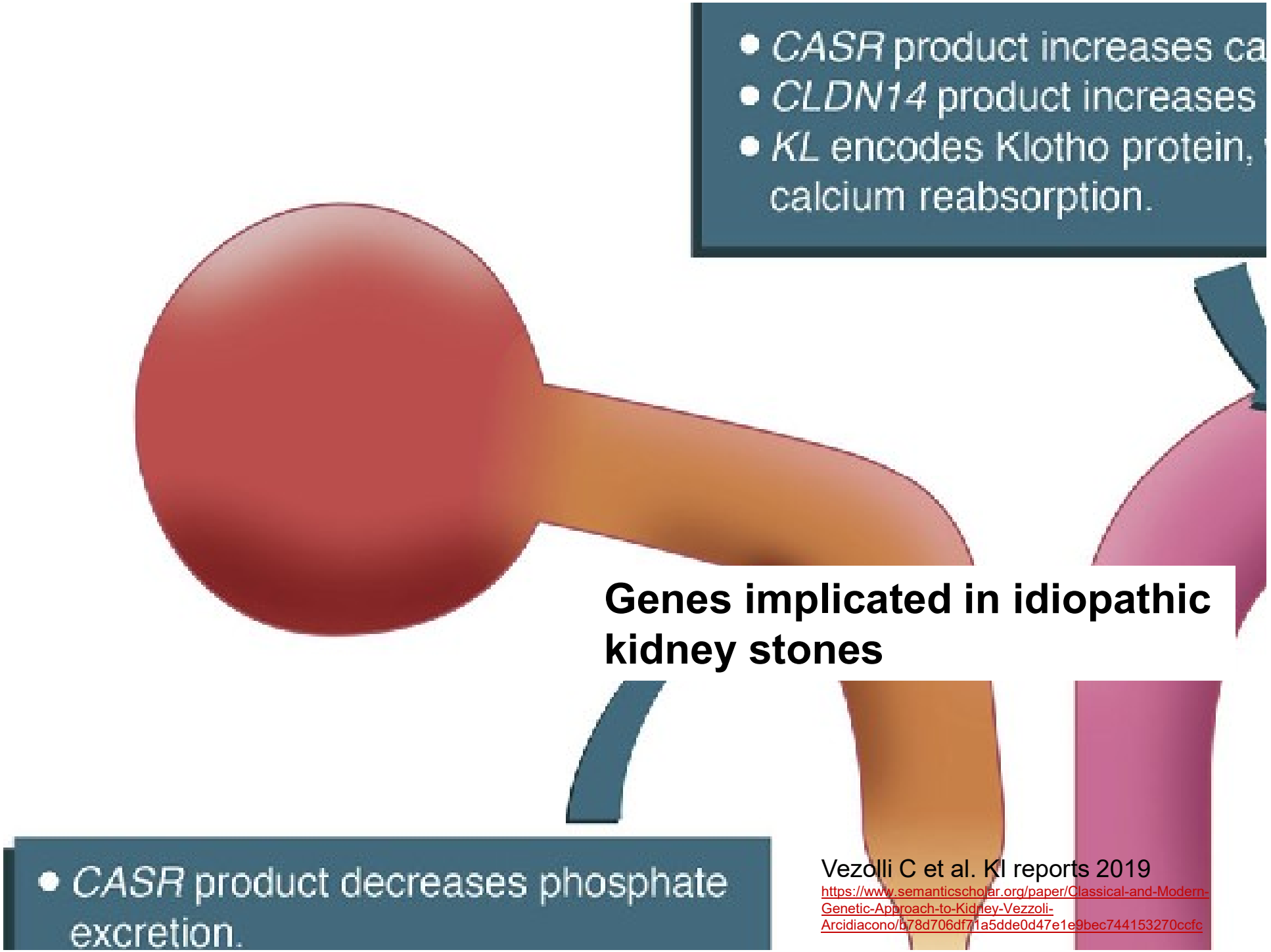


Halbritter J et al. Frontiers Ped 2018

Genes implicated in idiopathic kidney stones



Vezolli C et al. KI reports 2019
https://www.semanticscholar.org/paper/Classical-and-Modern-Genetic-Approach-to-Kidney-Vezzoli-Arcidiacono/b78d706df71a5dde0d47e1e9bec744153270ccfc



Vezolli C et al. KI reports 2019
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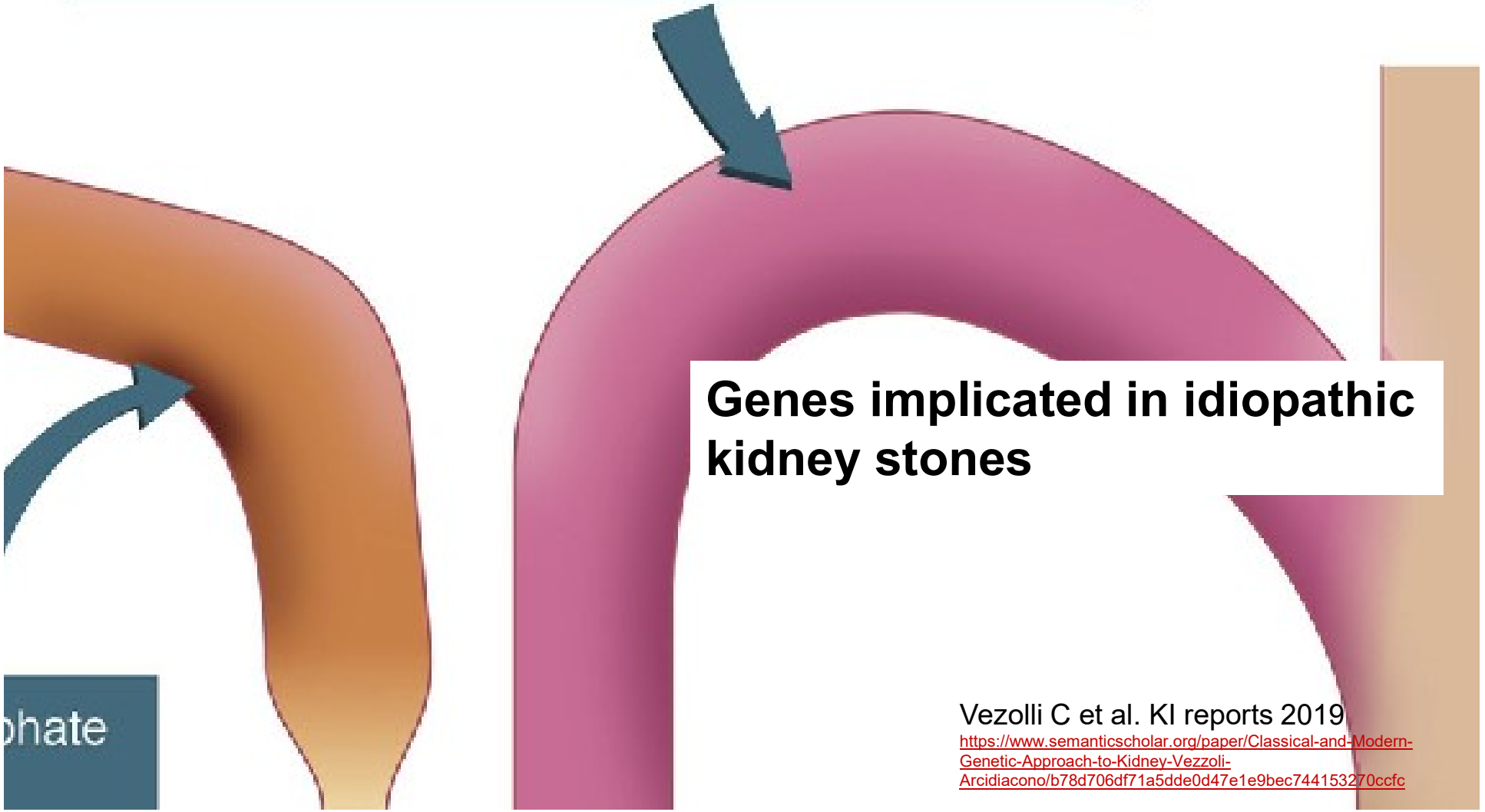
- *CASR* product increases calcium excretion.
- *CLDN14* product increases calcium excretion.
- *KL* encodes Klotho protein, which increases calcium reabsorption.



Genes implicated in idiopathic kidney stones

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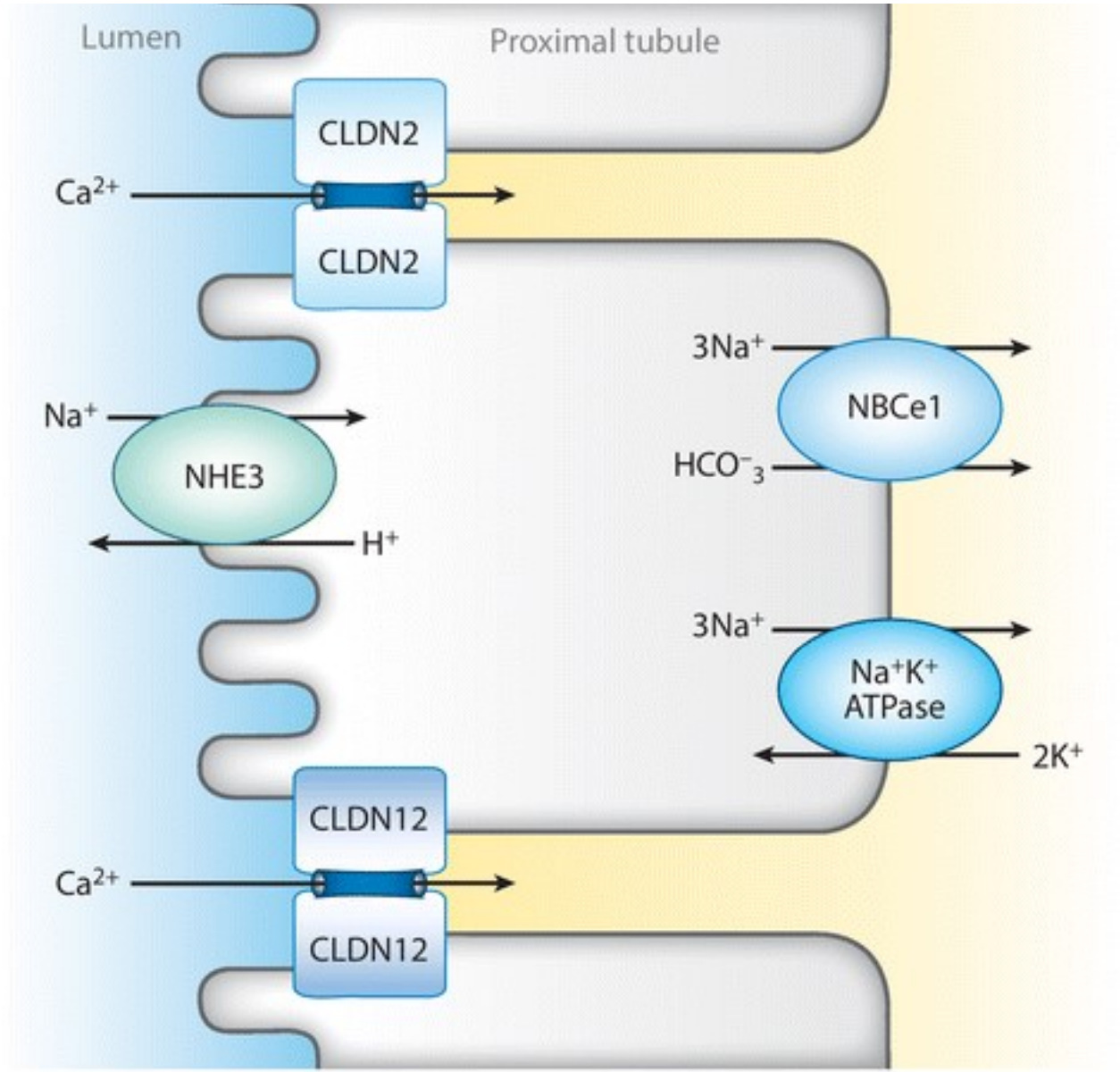
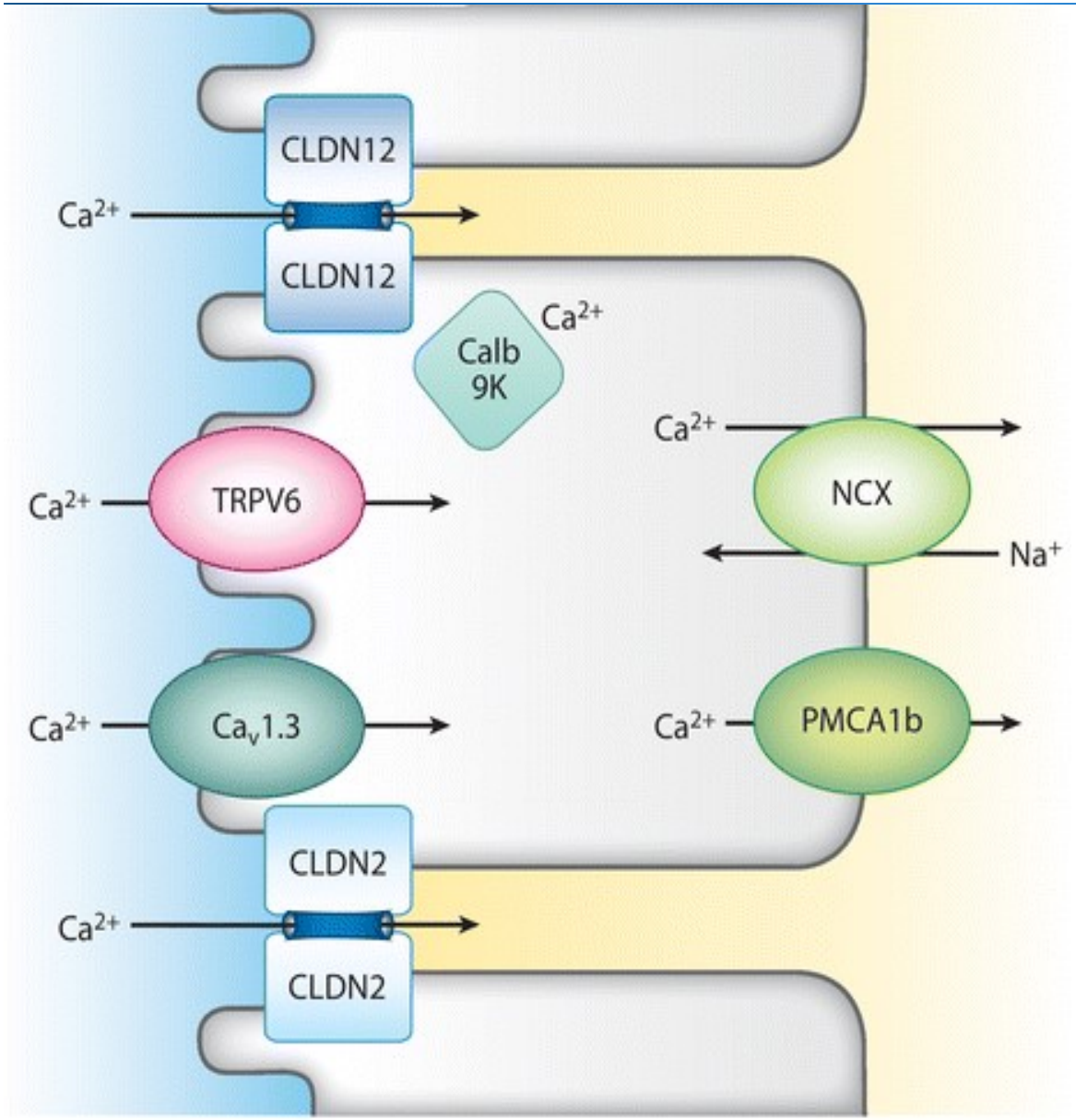


Alexander RT, et al. 2022
Annu. Rev. Physiol. 84:559–83

Molecular pathway of calcium transport in the proximal tubule.

Sodium reabsorption is mediated primarily by:
1. Apical influx via the epithelial sodium proton exchanger NHE3
2. Basolateral secretion by the sodium-potassium-ATPase (Na+K+ATPase)
3. The electrogenic sodium bicarbonate cotransporter (NBCe1).

This drives **paracellular calcium reabsorption through claudin-2** (CLDN2) or claudin-12 (CLDN12) pores.

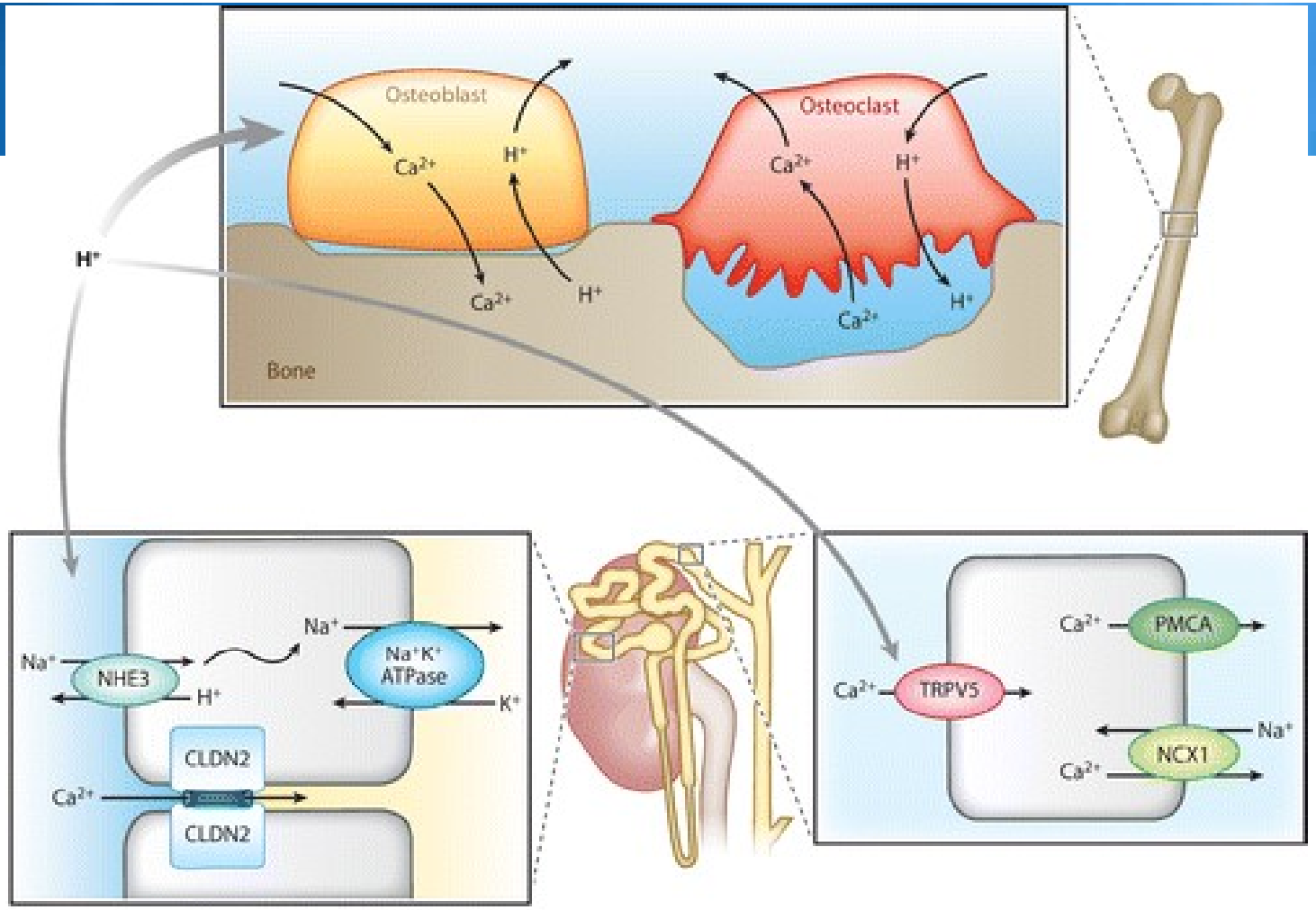


Alexander RT, et al. 2022
Annu. Rev. Physiol. 84:559–83

Molecular pathways of calcium absorption from the intestine.

Transcellular Ca absorption from the duodenum and the colon is mediated via apical entry by transient receptor potential vanilloid 6 (TRPV6), buffering and shuttling are mediated by calbindin-D9K (Calb9K), and basolateral efflux is mediated by Na2+/Ca2+ exchanger (NCX) and plasma membrane calcium-dependent ATPase 1b (PMCA1b).

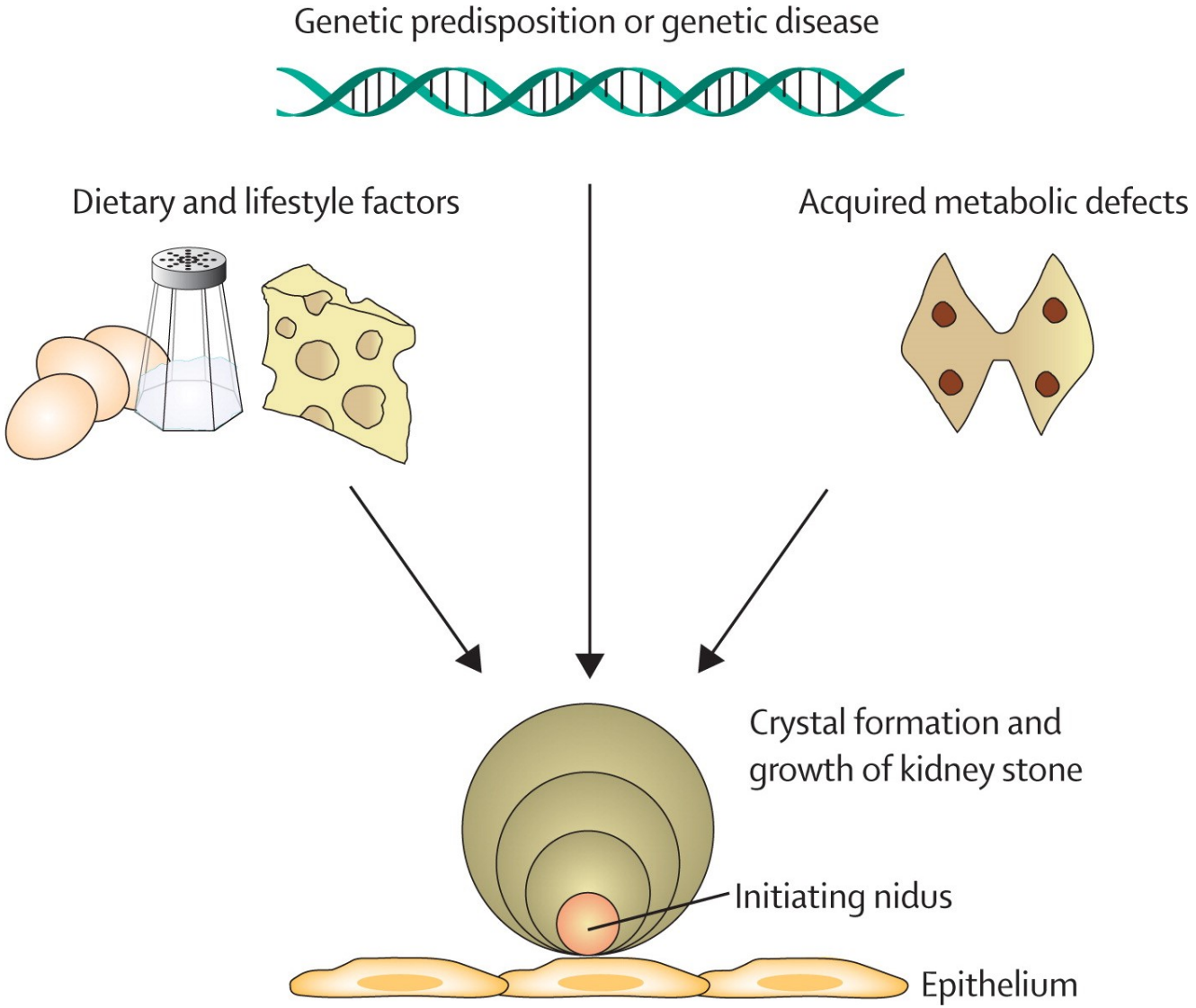
Paracellular calcium absorption is proposed to occur through claudin-2 (CLDN2) and claudin-12 (CLDN12). The apical voltage-gated calcium channel Cav1.3 has also been proposed to mediate apical calcium influx.



Acidosis stimulates Ca reabsorption from the proximal tubule

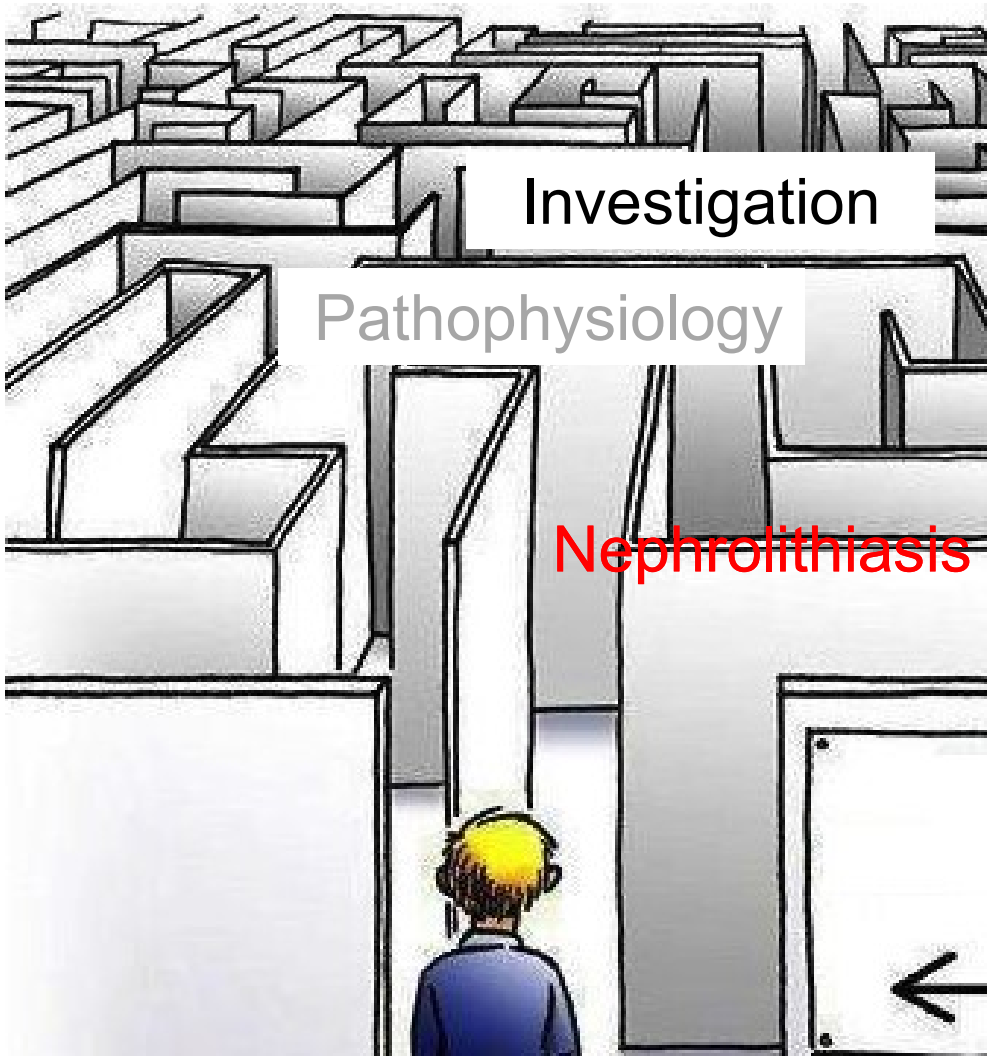
Acidosis promotes the release of calcium from the bones, thereby favoring mineral release

Etiology of calcium stones

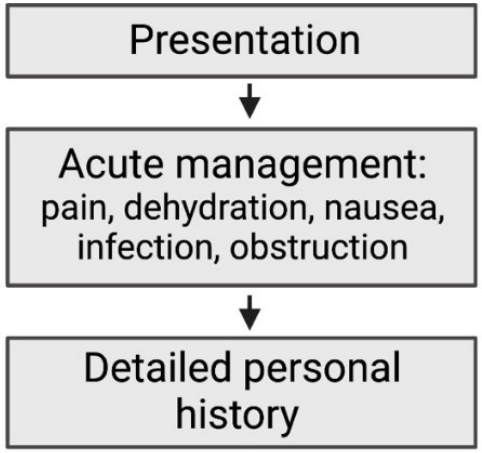


Moe OW. Lancet 2006

Nephrolithiasis in children: a practical approach



Investigation of nephrolithiasis



Risk factors for kidney stones in children

Inadequate hydration

Increase in dietary Na intake

produces higher urinary Ca excretion

Low intake of potassium

Marra G J Nephrol 2018

Risk factors for kidney stones in children

Inadequate hydration

Increase in dietary Na intake

produces higher urinary Ca excretion

Low intake of potassium

High intake of proteins

↑ Ca, UA, Ox excretion

↓ urinary pH, which ↓ precipitation of UA and CaOx.

↓ urinary citrate

Marra G J Nephrol 2018

Risk factors for kidney stones in children

UTIs caused by a urease-producing organism (Proteus or Klebsiella)

History of CAKUT

Perinatal medical history (prematurity, vitamin D supplementation)

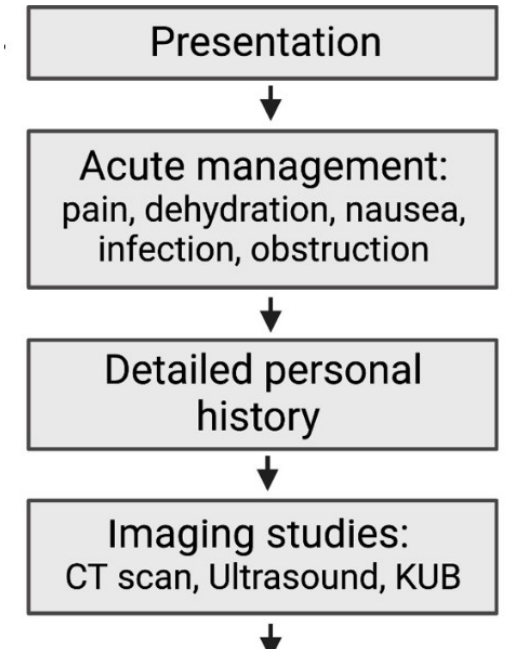
Conditions leading to immobilization

Medications associated with stone formation

Malabsorptive intestinal diseases and conditions

Marra G J Nephrol 2018

Management of nephrolithiasis

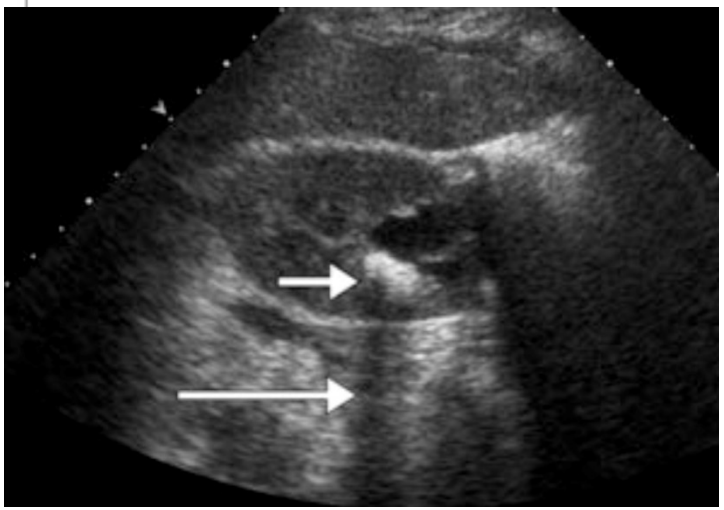


Imaging

KUB X-ray

Radio density	
Radio-opaque	Calcium oxalate Carbonate apatite $Ca_5(PO_4)_3\ 2H_20$ Brushite $CaHPO_4\ 2H_20$
Intermediate	Cystine Struvite $MgNH_4PO_4\ 6H20$
Radiolucent	Uric acid Xanthine 2,8 Dihydroxyadenine

Renal ultrasound



Edvardsson V. Ped Nephrol 2016

Imaging

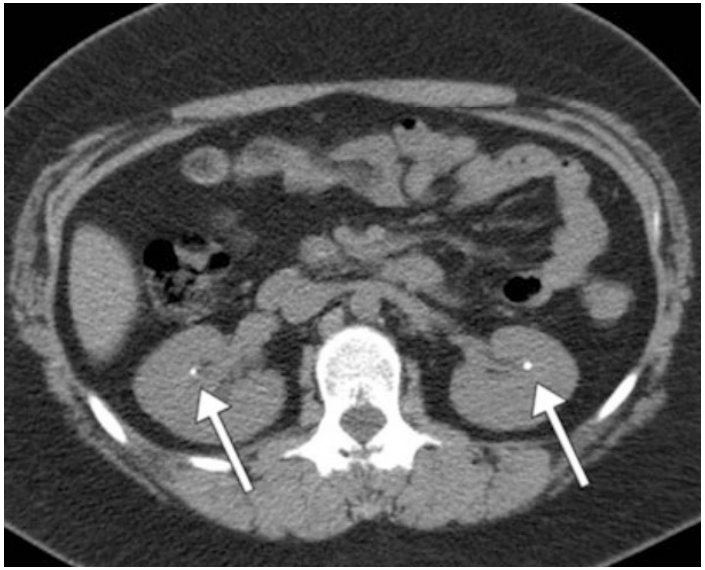
KUB X-ray

Renal ultrasound

CT scan

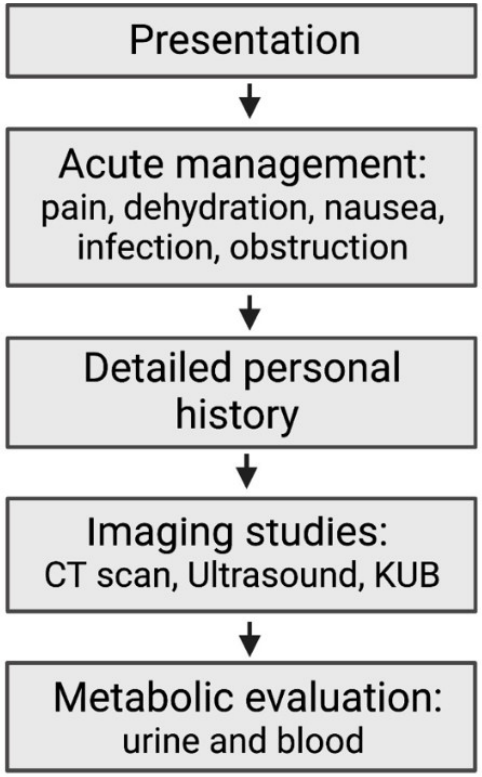
In 50 patients 13 KS were not diagnosed by US
12 stones < 5 mm
3 stones in ureter

Passerotti C et al. J Urol 2009

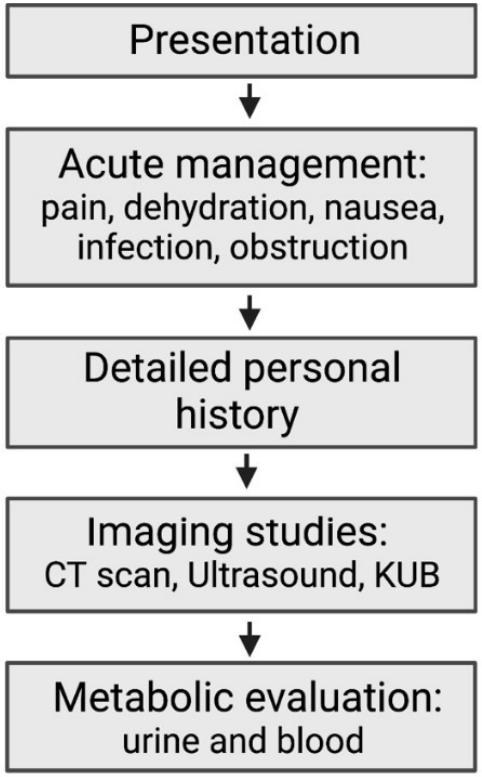


Edvardsson V. Ped Nephrol 2016

Management of nephrolithiasis



Management of nephrolithiasis



First-line tests in pediatric nephrolithiasis

Urine collection	Once	Oxalates and amino acids
	3 times	Creatinine, proteins, beta2 microglobulins, Na, K, Cl, Ca, P, Mg, uric acid, citrate
Urine microscopic examination	3 times	Crystals and urinary sediment
Blood test	Once	Urea, creatinine, uric acid, Na, K, Cl, Ca, P, Mg, EAB

Marra G et al. J Nephrol 2018

Stone risk in as many as one in four children may be misclassified if normative values of only a single 24-hour urine are used.

733 pediatric patients

Ellison JS et al. J Pediatr Urol 2017

Metabolic risk factors present in children with stones

	No. Totals (%)	No. Neurogenic Bladder (%)	No. Anatomical Abnormality (%)	No. Isolated (%)
None	14 (31.1)	2 (22.2)	1 (33.3)	11 (33.3)
Hypocitraturia	13 (28.9)	4 (44.4)	0	9 (27.3)
Hypercalciuria	6 (13.3)	1 (11.1)	1 (33.3)	4 (12.1)
Hyperoxaluria	2 (4.4)	0	0	2 (6.1)
Hyperuricosuria	1 (2.2)	0	0	1 (3.0)
Hypocitraturia + hypercalciuria	3 (6.7)	1 (11.1)	0	2 (6.1)
Hypercalciuria + hyperoxaluria	4 (8.9)	1 (11.1)	0	3 (9.1)
Hypocitraturia + hypercalciuria + hyperoxaluria	2 (4.4)	0	1 (33.3)	1 (3.0)
Totals	45	9	3	33

Kovacevic L, et al. J Urol 2012

Limited metabolic assessment

Hypercalciuria and low urine volume were considered the most common abnormalities in pediatric stone formers

Recent studies noted that hypocitraturia was more common

Hypocitraturia corresponds to a low consumption of potassium and magnesium

Chan KH et al. J Pediatr Urol 2018

Limited metabolic assessment

Retrospective study 2005-2015

380 patients <18 years

Low urine volume <1ml/kg/hour,

Elevated	24-hour calcium	>4.0 (1 mol) mg/kg
Decreased	24-hour citrate	<4.2 mg (27 mol) /kg
Elevated	24-hour oxalate	>52 mg (0.58 mmol)/1.73m2
Elevated	24-hour uric acid	>20 mg (0.12 mmol) /kg
Decreased	24-hour Mg	<4.5 mg (0.2 mmol) /kg

Chan KH et al. J Pediatr Urol 2018

Limited metabolic assessment

would have detected almost all clinically significant metabolic abnormalities

Potassium (low) 61 (76.3%)

Magnesium (low) 57 (71.3%)

Citrate (low) 55 (69.6%)

Low urine volume 42 (52.5%)

Calcium (high) 18 (22.5%)

Oxalate (high) 12 (15.0%)

Uric acid (high) 2 (2.5%)

pH (high) 27 (33.8%)

Sodium (high) 20 (25.0%)

Phosphorus (low) 14 (17.5%)

pH (low) 12 (15.0%)

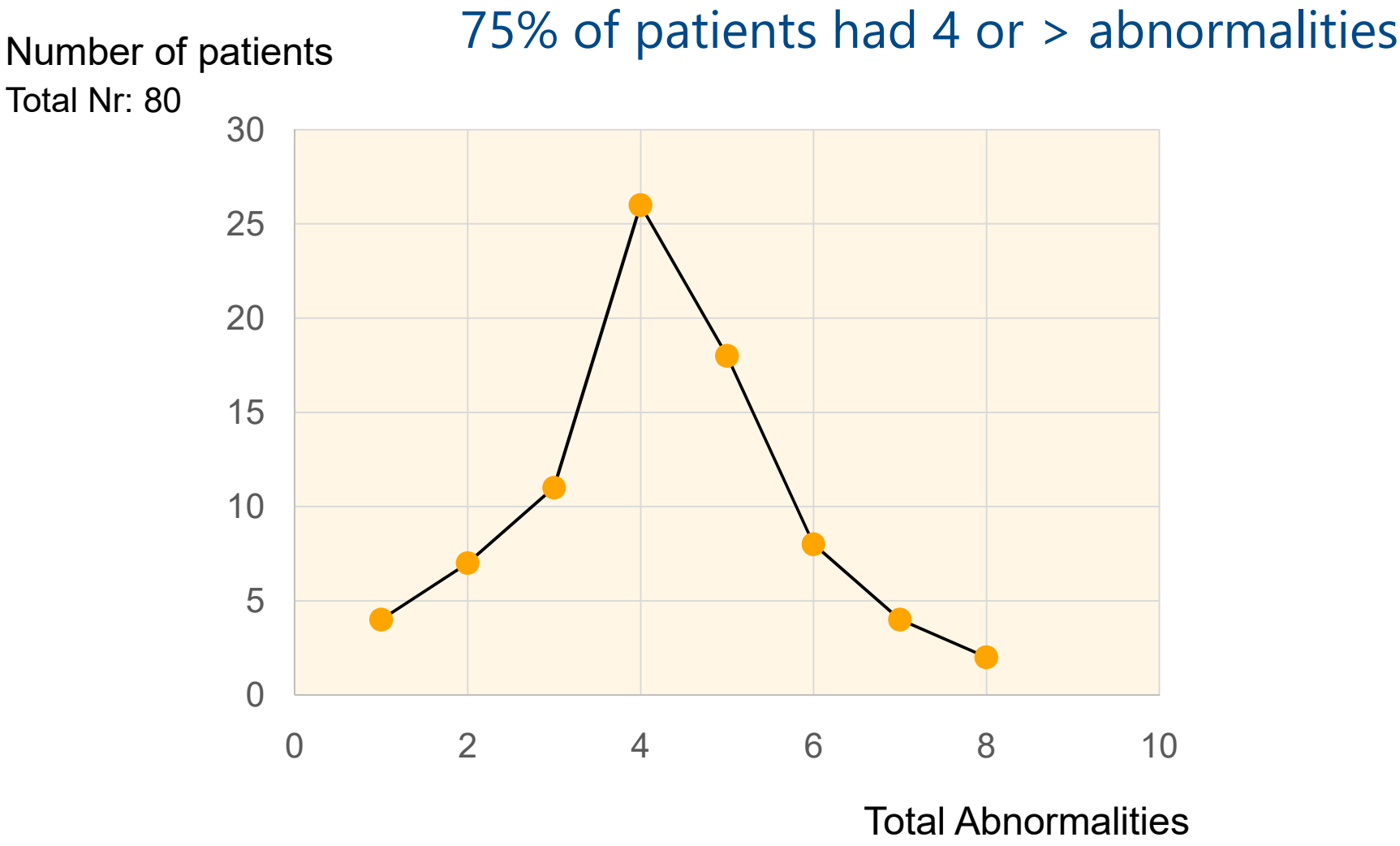
Phosphorus (high) 7 (8.8%)

Sodium (low) 2 (2.5%)

Chan KH et al. J Pediatr Urol 2018

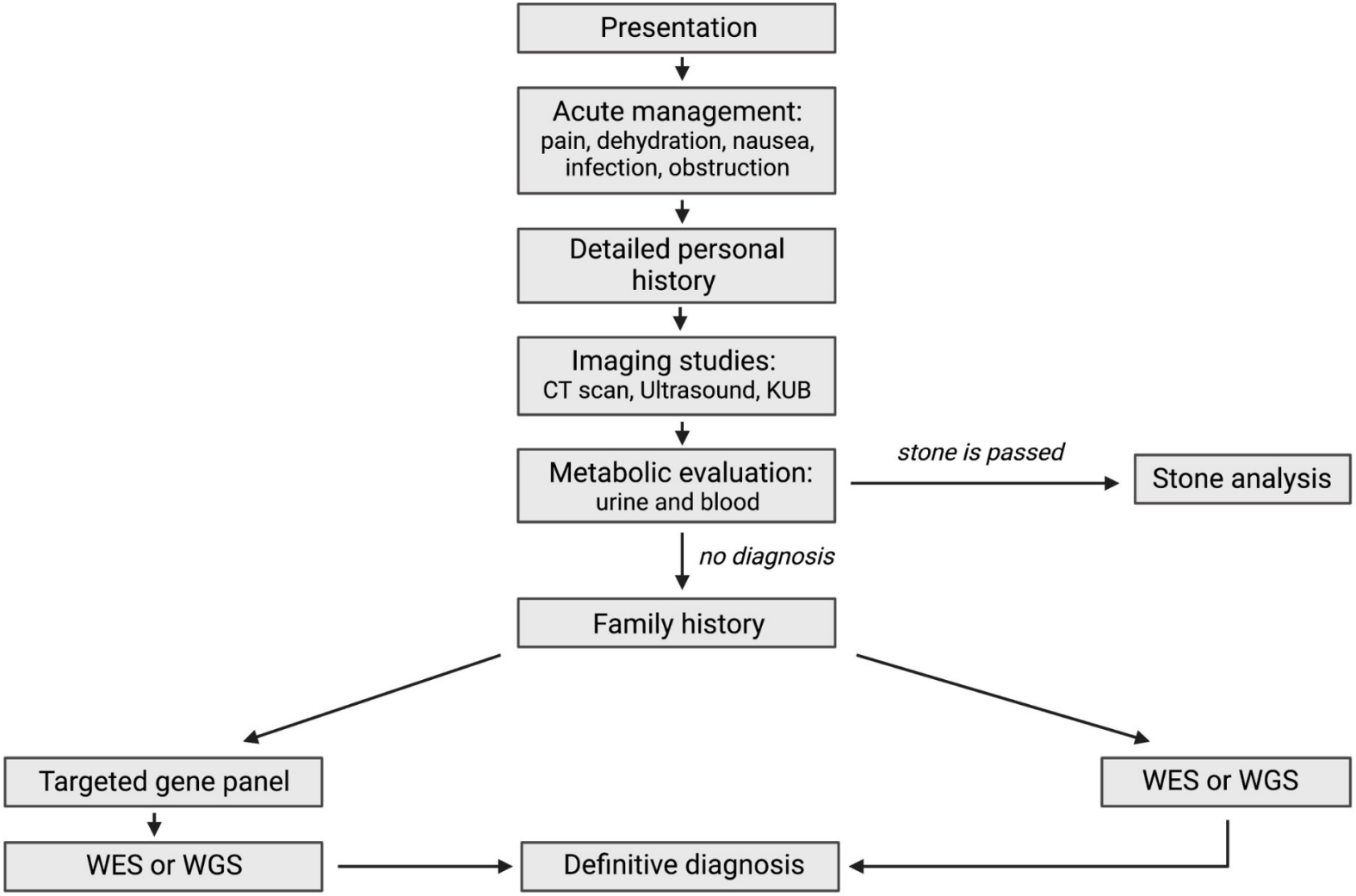
Limited metabolic assessment

75% of patients had 4 or > abnormalities



Chan KH et al. J Pediatr Urol 2018

Management of nephrolithiasis



Schott C et al. Frontiers Urol. 2022
https://www.frontiersin.org/articles/10.3389/fruro.2022.1075711/full

When to suspect rare causes of kidney stones?

Currently, there are 41 known genes with monogenic causation for nephrolithiasis

History	Family history of nephrocalcinosis
	Growth retardation, rickets, CKD
Imaging	Nephrocalcinosis
	Radiolucent kidney stones
	Multiple stones, bilateral stones
Lab results	Unexplained kidney failure
	Mild-moderate proteinuria
	Increased urine b2 microglobulin

Primary hyperoxaluria

PH1 accounts for 85% of patients,
PH2 8-10% and
PH3 5-7%.

Chan KH et al. J Pediatr Urol 2018

Challenges in the diagnosis of PH1

Kidney stone in a child

Recurrent lithiasis

Nephrocalcinosis

Diffuse nephrocalcinosis is noted in about one-half of patients with PH1 with nephrolithiasis

Milliner et al. GeneReviews 2017

Challenges in the diagnosis of PH1

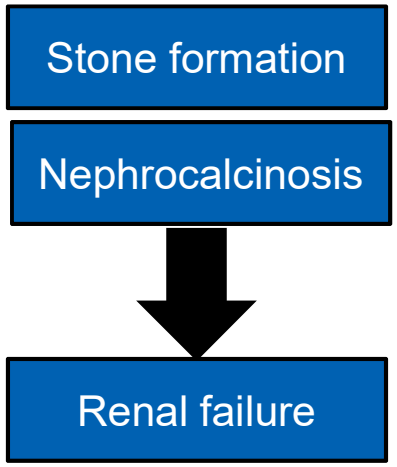
Family history of stones

Failure to thrive in infancy

Progressive kidney function decline that commonly progresses to ESRD

Milliner et al. GeneReviews 2017

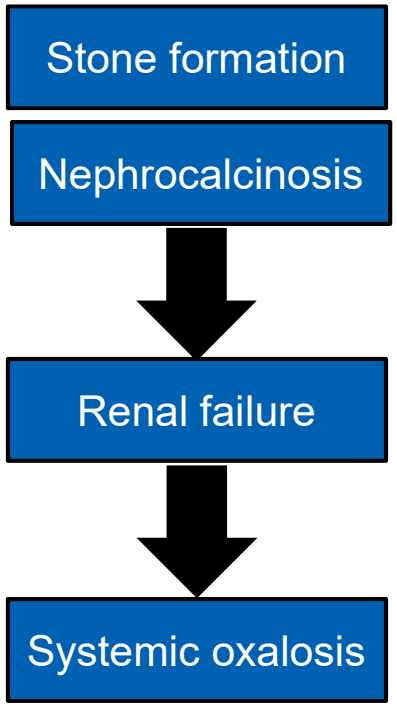
PH1 and oxalosis



Overproduction of oxalate results in insoluble calcium oxalate crystals, which may lead to recurrent kidney stones, diffuse nephrocalcinosis and progressive renal disease

Cochat & Rumsby. *N Engl J Med* 2013;369:649–58

PH1 and oxalosis



Overproduction of oxalate results in insoluble calcium oxalate crystals, which may lead to recurrent kidney stones, diffuse nephrocalcinosis and progressive renal disease

As renal function declines, POx levels increase, causing patients to develop systemic oxalosis

Cochat & Rumsby. *N Engl J Med* 2013;369:649–58

nature reviews nephrology

Consensus statement

Clinical practice recomm
for primary hyperoxaluri

Oxalate measurements SHOULD BE repeated on at least two times, but preferably three to confirm that levels are elevated, particularly if findings are equivocal

Exclusion of enteric causes of hyperoxaluria (for example, chronic pancreatitis, cystic fibrosis, inflammatory bowel syndrome or bariatric surgery)

Groothoff JW, et al. Nat Rev Nephrol 2023

Take home messages

A stepwise approach is recommended for the management of pediatric kidney stones

Early diagnosis of monogenic diseases is a priority

Management should be individualized

THANK YOU
FOR YOUR
ATTENTION

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

Methylmalonic acidemia and the kidney

Prof. dr. sc. Danko Milošević

DISORDERS OF COBALAMIN METABOLISM

Hyperammonemias belong to a considerable variety of diseases

- N-acetylglutamate syntethase deficiency (NAGS)
- methylmalonic acidemia (MMA)
- propionic acidemia (PA)
- isovaleric acidemia (IA)
- ornitine transcarbamylase deficiency (OTC)
- carbamyl phosphate synthetase deficiency (CPSID)

All hyperammonemias should be considered with utmost clinical attention.

Clinical signs and symptoms (acute presentation):

Nonspecific:

- neonatal sepsis-like image
- temperature instability (OTC, CPSID)
- respiratory distress (NAGS)
- hyperventilation

Nervous system:

- Altered level of consciousness (from lethargy and somnolence to coma) mimicking encephalitis or drug intoxication
- Acute encephalopathy
- Seizures (in general not isolated but in the context of altered level of consciousness)
- Movement disorders (PA, OTC, CPSID)
- Stroke-like episodes (MMA)

Gastrointestinal system:

- Vomiting and feeding difficulties

Hematologic findings:

- neutropenia, pancytopenia

Cardiac:

- acute cardiac failure (mostly on basis of cardiomyopathy)
- arrhythmias

Other:

- distinctive odor of sweaty feet (IA)

Chronic presentation (ofter triggered by a secondary infection such as a viral infection)

- Often episodic signs and symptoms of metabolic acidosis (odour IA in acute phase)
- failure to thrive (NAGS, MMA, PA, IA, OTC, CPSID)
- avoidance of protein

- **Nervous system:**
- hypotonia (CPSID, NAGS)
- developmental delay (learning disabilities, intellectual disability) (NAGS, MMA, PA, IA, OTC, CPSID)
- movement disorders / dystonia (NAGS, CPSID)
- seizures (IA)
- Cerebellar hemorrhage (IA)
- optic atrophy (MMA,PA)
- psychiatric symptoms (hallucinations, psychotic attacks) (OTC)
- headaches (OTC)

Gastrointestinal system:
- recurrent vomiting with ketoacidosis
- abnormal feeding behavior (anorexia)
- constipation
- pancreatitis (MMA; PA)
- progressive liver damage (OTC)

Hematologic findings:
- neutropenia, pancytopenia
- secondary hemophagocytosis (rare)

Cardiac (more frequent in PA):
- cardiomyopathy
- prolonged QTc interval in ECG

Kidney (more frequent in MMA):
- chronic renal failure in MMA

Other:
- Dermatitis (OTC)
- Hearing loss (rare)
- brittle hair (OTC)

Case report: Male siblings 2 months old:

First sibling: Mother noticed petechial spots on child's face.
Physical examination except petechial bleeding on the head, body and limbs was normal.
Laboratory analysis revealed thrombocytopenia (4 Tr x 10^9), neutrophils (5.0%)

CRP, procalcitonine, coagulation tests, GuK, ionogram, total proteins, plasma free hemoglobin, creatinine, amylase, fibrinogen, d-dimers, bilirubin, AST, ALT, lactate, urine, 24 h proteinuria were within normal values, LDH 403

Extensive search fo infection included parvo B19: all negative.
Neonatal immune thrombocitopenia (NAIT): negative
Specific trombocite antibiodies in all family members: negative
Immunohematologic tests for erythtocite antibiodies: negative

Renal doppler: negative
Cardiac US: normal
Brain US: normal
Abdominal US: negative

Therapy until receiving results of methymalonic aciduria:

Methyprednisolone pulses (3 pulses)
Immunoglobulins (IVIG)
Packed RBC 3x
Thrombocyte conc.
FFP (2x)

Methylmalonic acid: 1.01 (n.v.< 0.51) 9.2 H mmol/mol kreat (n.v.< 1)
Ammonemia 29,5; 43 [umol/L]
Homocistein 10.3; 9.8[umol/L]

Glutaminic acid 133 umol/L 10 – 133
Leucin 198 H umol/L 45 - 160
Phenilalanin 101 H umol/L 23 – 75
Methionin 50 H umol/L 15 - 35

Folic acid: normal
B12: 226 (n.v. 241)

DG. Methylmalonic acidemia

Th:Dietary regime
B12 0.5 mg i.m. 3x
Carbaglu®

2nd sibling: Accepted for evaluation because of his brother´s petechial bleeding.
Physical examination was normal.
Laboratory: Tr 275 x 10^9, neutrophils 7.5%

All clinical and immunological tests that were performed in the first sibling were done in the second sibling. Laboratory tests were normal, except for LDH 315.

Therapy until receiving results of methymalonic aciduria:

- Immunoglobulins (IVIG)
- Packed RBC 2x
- FFP (1x)

Methylmalonic acid: 15.4 H mmol/mol kreat; 1.15 H umol/L (n.v.< 1)
Ammonemia 29,5; 28; 45.2[umol/L]
Homocistein 10 [umol/L]

Serin 191 H umol/L 60 – 186
Glicin 403 H umol/L 60 – 380
Citrulin 41 H umol/L 3 – 40
Alanin 697 H umol/L 100 – 439
Glutaminic acid 191 H umol/L 10 – 133
Histidin 123 H umol/L 30 – 112
Arginin 140 H umol/L 10 – 130
Prolin 318 H umol/L 50 – 298
Lizin 221 H umol/L 45 – 196
Methionin 42 H umol/L 15 - 35
Valin 354 H umol/L 60 - 294
Tirozin 132 H umol/L 20 – 120
Izoleucin 95 umol/L 28 - 95
Leucin 235 H umol/L 45 - 160
Phenilalanin 130 H umol/L 23 - 75
Triptofan 74 H umol/L 23 - 71

Folic acid: normal
B12: 218 (n.v. 241)

DG. Methylmalonic acidemia

Th:Dietary regime
B12 0.5 mg i.m. 3x
Carbaglu®

Male child: 4 years of age treated in clinical hospital because of:

- Recurrent vomiting with periodic serum ammonia above normal level (samples taken considerable time after vomiting)
- Anorexia (body weight stagnant about 20 kg)
- Failure to thrive
- Developmental delay (learning disabilities, intellectual disability)

We found on several occasions methylmalonic acid (methylM uri=11.9[mmol/molcreatine]methylM plasma=0.32[umol/L], methylM urine=2.7[mmol/mol creatine]methylMplasma=0.13) with disturbance of acid-base balance in the sense of compensated metabolic acidosis with increased anion gap (current bicarbonate 16.0 mmol/L, BE -8.0,Lactate 3.8).

DG: **Methylmalonic acidemia**
Rasopathy

Signal pathway RAS/MAPK that appears to be disrupted is involved in a number of biological reactions of energy homeostasis including metabolic remodeling, mitochondria processing and production of energy.

Genetic analysis: heterozygous mutation c.1741C>T

Therapy: Carbaglu®
Dietary regime

Outcome: 8 years:

- Normal weight
- No vomiting
- Physical activity and condition slightly enhanced
- Excellent in school
- ADHD

- CKD is a common complication of the MMA.
- Usual equations overestimate GFR.
- Therefore, measured GFR should be performed to inform therapeutic decisions such as dialysis and/or transplantation.

Dao, M., Arnoux, JB., Bienaimé, F. *et al.* Long-term renal outcome in methylmalonic acidemia in adolescents and adults. *Orphanet J Rare Dis* **16**, 220 (2021).

- The majority of patients with MMA develop renal impairment at a young age.
- Liver transplantation is curative, and patients with advanced renal failure may benefit from combined liver and kidney transplantation.

Alkhunaizi AM, Al-Sannaa N. Renal Involvement in Methylmalonic Aciduria. Kidney Int Rep. 2017 Apr 28;2(5):956-960

- 1-^{13} C-propionate oxidation breath test (POBT) measure metabolic capacity and the changes in circulating proteins to assess mitochondrial dysfunction (fibroblast growth factor 21 [FGF21] and growth differentiation factor 15 [GDF15]) and kidney injury (lipocalin-2 [LCN2]).
- Biomarker concentrations are higher in patients with the severe mut^{0} -type and cblB-type MMA, correlate with a decreased POBT, and show a significant response postliver transplant.

Manoli I, Gebremariam A, McCoy S, Pass AR, Gagné J, Hall C, Ferry S, Van Ryzin C, Sloan JL, Sacchetti E, Catesini G, Rizzo C, Martinelli D, Spada M, Dionisi-Vici C, Venditti CP. Biomarkers to predict disease progression and therapeutic response in isolated methylmalonic acidemia. J Inherit Metab Dis. 2023 Jul;46(4):554-57

Liver / combined liver-kidney transplantation in MMA:

Solid-organ transplantation, such as single liver (LT) or kidney transplantation (KT), or combined liver/kidney transplantation, has become an effective alternative treatment option in recent decades. Isolated liver transplantation should be performed early in life to maintain normal renal function.

Low protein diet:

The basic principles of dietary management are similar for MMA and PA patients. The mainstay of nutrition therapy is a low protein intake, limiting but ensuring essential requirements of the propionic acid precursor amino acids, isoleucine, valine, methionine, and threonine to reduce elevated concentrations of metabolites

Patients with mild forms of MMA/PA may tolerate a natural protein intake that is equal to or exceeds the FAO/WHO/UNU (2007) safe levels of protein intake

Amino acid supplements*:* Although supplementary, precursor free amino acids are commonly used to contribute to the total protein intake; their efficacy has not been fully assessed

Energy requirements: Little is known about energy requirements in MMA/PA. Whilst this should be individually determined, there should be a balance between preventing catabolism and overfeeding, particularly if there is decreased physical activity. The FAO/WHO/UNU (2007) recommendations can be used to guide energy requirements

There are few published reports of successful demand **breast feeding** in MMA/PA and some do not advocate this in MMA/PA. Expressed breast milk should be encouraged if demand breast feeding is impracticable. For MMA/PA particular breast milk advantages include its low protein and amino acid content, protection against infection, and reduction in gut propionate.

Breast feeding or breast milk with or without MMA/PA precursor-free amino acids may be considered in the dietary treatment of newly diagnosed neonates/infants.

MMA patients are at increased risk for osteoporosis. Recommendations for bone health include optimizing nutrition, ensuring adequate calcium and vitamin D. Baseline DEXA is recommended at 10 years and follow-up according to bone-health status.
Extra attention should be paid to MMA patients with chronic kidney disease.

Carbaglu® is a structural analogue of the human N-Acetyl-glutamate, will replace NAG and reactivate urea cycle.
It either alone or combined with ammonia scavengers, produces greater reductions in plasma ammonia levels than ammonia scavengers alone (Chakrapani 2018)
Dietary recommendations can be mitigated with this medication.

Carbaglu long-term treatment

- Why Carbaglu?
- Behind the approval

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

FABRY DISEASE

DIMITAR ROUSSINOV

Sofia, Bulgaria

RARE DISEASES IN PEDIATRIC NEPHROLOGY

IPNA TEACHING COURSE

Hotel Holyday Inn, Skopje, Republic of North Macedonia, 4-6 April 2024

DISCLOSURE

Dimitar Roussinov is a member of the Paediatric Committee (PDCO) of the European Medicines Agency (EMA). Presented opinions are personal and do not engage both institutions with a positions or future decisions.

FABRY DISEASE

- Fabry disease(FD) is a rare, X-linked lysosomal storage disease
- It is caused by mutations in the α-galactosidase A gene (GLA), leading to partial or full deficiency of the enzyme α-galactosidase A (α-Gal A), resulting in accumulation into cellular lysosomes of globotriaocylceramide (Gb3) and its derivative globotriaosylsphingosine (Lyso-Gb3)
- There are more than 1000 mutations reported, around 60% without important clinical significance
- However, there are mutations with late, “nonclasical” onset and course and others with unclear genotype - phenotype

FABRY DISEASE

- The first descripton of FD (angiokeratoma) by Anderson dates back to 1898
- The long “journey” across 3 centuries leads to contemporary knowledge for a treatable, very complex and heterogeneous, multisystemic disease with high morbidity and mortality
- The incidence has been revised from initial estimations of 1:40 000 – 170 000 to 1:1250 in a neonatal screening
- Before introduction of the enzyme replacement trerapy (ERT) only 25% of heterozygous males survived 50 years and nobody 60
- They have reduced life expectancy by 25 years, while heterozygous females by 10

MULTISYSTEMIC DISEASE

Since almost all body cells accumulate Gb3 and Lyso-Gb3, numerous organs/organ systems are affected and patients show varying degrees of progressive functional deficits.

MANIFESTATIONS IN FD

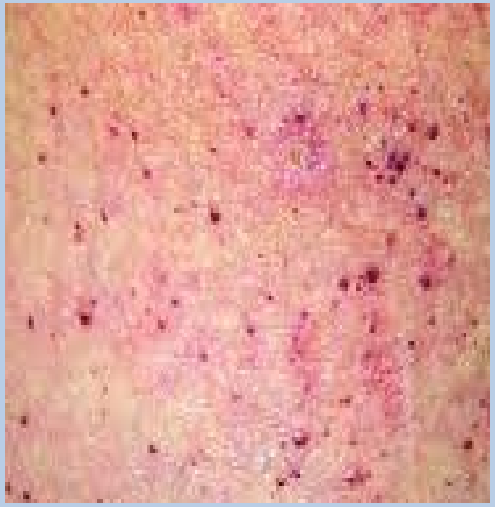
- Traditionally it has been believed FD is a disease of adulthood because progressive renal failure, cardiomyopathy with potentially malignant cardiac arrhythmias and strokes developed at that stage
- However, it is known currently the process and symptoms start already in infancy
- The earliest symptoms are:
  - > pain / dysesthesia
  - > reduced or absent sweating
  - > cornea verticillata
  - > angiokeratoma
  - > Gastrointestinal complaints

MANIFESTATIONS IN FD

Childhood, adolescence (≤ 16 years)

Peripheral/ autonomous nervous system:
Acroparesthesia and neuropathic burning pain of the hands and feet, "pain crises" triggered by cold, heat, physical or emotional stress, intercurrent diseases, or alcohol consumption (detectable small-fiber neuropathy)
Hypohidrosis, reduced saliva and tear production, impaired intestinal motility, orthostatic dysregulation, vertigo

Skin: Angiokeratoma, mostly in groups gluteal, periumbilical, scrotal and on the thighs, sometimes on the lips, fngertips, mucous membranes (oral mucosa and conjunctiva)



Gastrointestinal: Gastrointestinal complaints (postprandial abdominal pain, fatulence, diarrhea, gastric refux)

Lung: Obstructive (and restrictive) respiratory diseases

Ears: Progressive sensorineural hearing loss (particularly high frequencies), tinnitus

MANIFESTATIONS IN FD

Childhood, adolescence (≤ 16 years)

Eyes: Cornea verticillata, tortuositas vasorum (conspicuous tortuosity of the conjunctival and retinal vessels), Fabry cataract

Musculoskeletal system: Characteristic deformation of the interphalangeal joints of the fingers, in some cases drum fail fingers and toes. Ossified tendon insertions, degenerative joint changes, aseptic bone necrosis

Robustness: Physical exhaustion, fatigue

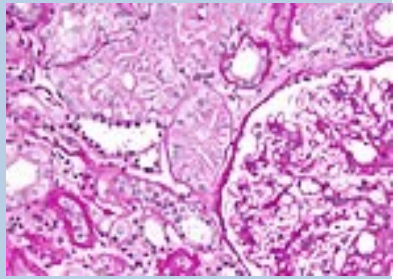
Additional manifestations: Reduced body growth, delayed puberty, fertility disorder, impotence, characteristic facial features, anomaly in the oral and dental area such as cysts and pseudocysts of the maxillary sinus

First renal and cardiac abnormalities: (including microalbuminuria, proteinuria, abnormal heart rate variability)

MANIFESTATIONS IN FD

Early adulthood (17−30 years)

In addition to the above-mentioned manifestations:



Fabry nephropathy: Proteinuria and progressive renal insufciency; often renal cysts (unclear cause), renal hypertension, focal segmental glomerolusclerosis

Fabry cardiomyopathy: Left ventricular hypertrophy (mostly concentric), conduction disorders (atrial fbrillation, supraventricular and ventricular tachycardia), valve dysfunction (mitral valve, aortic valve), angina pectoris, intramyocardial fibrosis ("late enhancement" in cardiac MRI)

Cerebral manifestation: Transient ischemic attack (TIA), ischemic insult, rare intracerebral hemorrhage, ectasia of the basilar artery and white matter lesions (lesions of the white matter in the cerebral MRI), disturbed cerebral blood fow, lymphedema of the lower extremity, depression, psychoses, limited quality of life

MANIFESTATIONS IN FD

Later adulthood (> 30 years)

Progression of the above-listed manifestations:

Renal insufficiency (dialysis, renal transplantation)

Heart failure, malignant arrhythmia, recurrent TIAs and insults, vascular dementia

TREATMENT

Treatment of patients with FD should be performed by a multidisciplinary team

The following therapeutic goals should be aimed for in the context of multimodal care:

- **Reduction of complaints (especially pain reduction)**
- **Delaying/preventing the progression of organ manifestations (especially in the kidney, heart, and central nervous system)**
- **Improvement of quality of life**
- **Normalization of life expectancy**

NONSPECIFIC TREATMENT

Neuropathic pain: Avoidance of pain triggers such as heat, cold, physical strain, stress, overtiredness medication: pregabalin, in case of resistance to therapy possibly in combination with a dual serotonin and noradrenalin reuptake inhibitor (e.g., duloxetine)

Stroke: Platelet-aggregation inhibition

Depression: serotonin reuptake inhibitors

CKD, albuminuria/proteinuria: RAS blocker (ACE inhibitor, ARB), anemia therapy

ESRD: Dialysis, kidney transplantation (first choice therapy)

Hypertension: Antihypertensives, e.g., ACE inhibitors or ARBs (no beta blockers in patients with sinus bradycardia)

Ventricular tachycardia: Antiarrhythmics, implantable cardioverter defibrillator (ICD)

NONSPECIFIC TREATMENT

Bradykardia: Pacemaker implantation

Coronary stenosis: PTCA, ACVB

Heart failure: Diuretics, ACE inhibitor (ARB for patients with ACE inhibitor intolerance), pacemaker or ICD implantation, heart transplantation

Dyslipidemia: Statins

Airway obstruction: Abstention from nicotine, possibly bronchodilators

Delayed gastric emptying, dyspepsia: Small and frequent meals; metoclopramide, H2 blocker

Pronounced hearing loss: Hearing aids, cochlear implant

SPECIFIC TREATMENT – WHEN?

- All clinical consensuses recommend specific treatment to start if symptoms or organ damage are present
- Opinions are divided when to start treatment in asymptomatic patients
- Accumulated clinical experience in treatment of FD shows as early the diagnosis is made and therapy
- started as successful is it
- It is believed the early start of treatment during childhood might prevent organ damage latter
- Some guidelines are treatment of asymptomatic patients to start from 7 years of age (Germain et al. 2019)

TREATMENT – ERT

- Since 2001 two products are registered in Europe for ERT in FD
- Agalsidase alfa (Replagal®)
  - > for children above 7 years of age
  - > dose 0.2 mg/kg i.v. every 2 weeks

- Agalsidase beta (Fabrazyme®)
  - > for children above 2 years of age
  - > dose 1.0 mg/kg i.v. every 2 weeks

TREATMENT – ERT

- ERT reduces plasma levels of Gb3, significantly decreases the rate of incidence of cardio-vascular and cerebro-vascular events, stops or slows down progression of CKD, improves quality of life in adult patients
- In children decreases accumulation of Gb3 in tissues, plasma and urine, improves pain, gastrointestinal symptoms, quality of life, energy and activity

TREATMENT – ERT

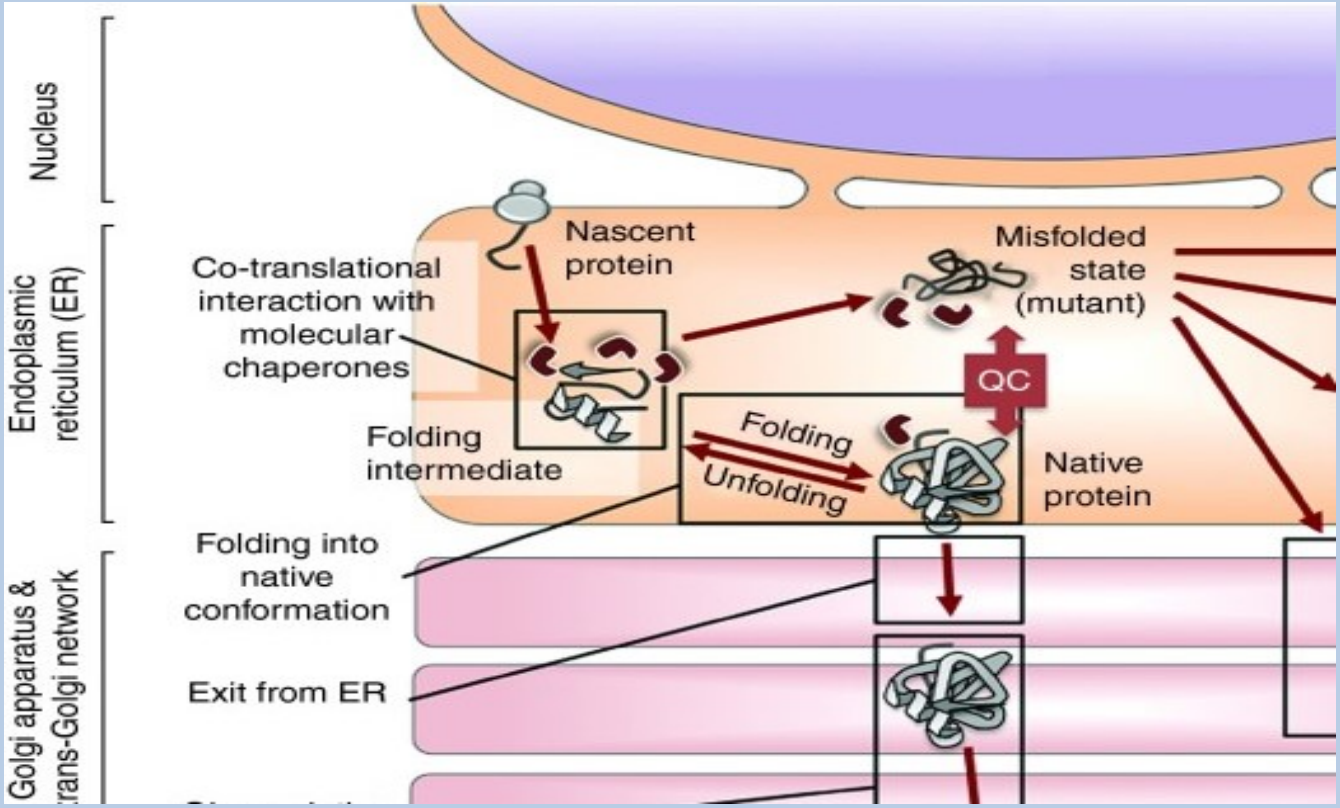
- Pegunigalsidase alfa (Elfabrio®) was approved for treatment of adults in 2023.
- It is as safe and effective as Fabrazyme and can prevent kidney decline in adults with FD over two years (BALANCE Ph 3 clinical trial)
- Infusion-related side effects occur significantly less often
- Dose - 1.0 mg/kg i.v. every 2 weeks
  (2.0 mg/kg) i.v. every 4 weeks
  under evaluation

ERT - PROBLEMS

- Intravenous infusion every 2 weeks
- Reactions during infusion – up to 59%
- Anaphylactoid reactions – up to 1%
- Anti drug antibodies (ADA) – block the product and decrease the effect or there is a need for dose increase

TREATMENT – CHAPERONES

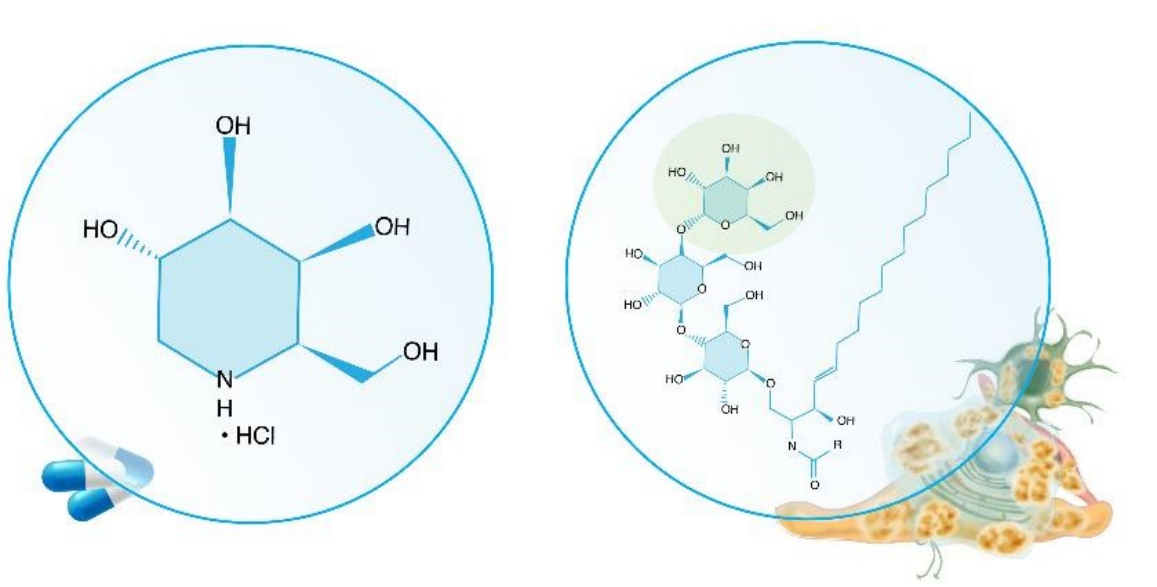
- **Pharmacologic chaperones are medicinal products, small molecules able to help proteins to achieve the right shape, to be stable and functional and to restore their normal traffic**



TREATMENT – MIGALASTAT

- In 2016 migalastat (Galafold ®) was registered for treatment of FD
- It is a structural analog of the terminal galactose group of Gb3

Migalastat[1] **Gb3[2]**



1. Johnson FK et al. *Clin Pharmacol Drug Dev*. 2013;2(2):120-132; **2.** ChemID*plus*: a TOXNET database. Bethesda, MD: US National Library of Medicine. At: chem.nlm.nih.gov/chemidplus/structure/viewer/71965-57-6. Accessed June 13, 2018.

TREATMENT – MIGALASTAT

- Migalastat is indicated for long term treatment of patients with diagnosed FD from 12 years of age and have amenable mutation (over 3% activity)
- There are new mutations identified constantly and their sensitivity to migalastat is tested
- There are about 367 amenable and 711 nonamenable mutations
- Data concerning new amenable mutations are updated in Migalastat Amenability Table, available online - https://www.galafoldamenabilitytable.com/hcp

TREATMENT – MIGALASTAT

- **Dosing:**
  - > 1 capsule (123 mg) - orally, once per day on the same time
  - > children ≥12 to <18 years of age and weight ≥ 45 kg
  - > not to be taken up to 2 hours before and after meal (up to 40 % reduction in absorbtion)
- **Contraindications**
  - > hypersensitivity to the active or additive substances
  - > not indicated in patients with GFR less than 30 ml/min/1.73 m^2
- **Follow up of renal function, echocardiography and biochemistry parameters is recommended every 6 months**
- **No decrease in proteinuria rate has been observed in patients treated with Galafold**

European Medicines Agency. Migalastat (Galafold) SmPC: Accessed July 2021

ATTRACT – CONCLUSIONS

- After 18 months treatment with Migalastat there is a statistically significant decrease in LVMi, while in patients on ERT it is not significant
- Migalastat and ERT have similar and comparable therapeutic effects on renal function as well as on the whole final clinical evaluation
- Migalastat is safe and well tolerated
- The most common adverse event is headache in around 10% of patients, while in adolescents – upper respiratory tract infections

TREATMENT – FUTURE

- Substrate reduction therapy (SRT) aims to reduce the substrate and, therefore, the subsequent inhibition of Gb3 accumulation in the cells. Lucerastat is a low molecular weight iminosugar under evaluation.
- Gene therapy is based on the introduction of DNA carrying the genetic code for the AGAL protein into patients' cells. At least 3 products are under investigation.

CONCLUSIONS

- FD is a multisystemic disease, starting during the childhood and progressing with age
- Males with the classical form develop early symptoms and have worsening of the quality of life
- Females usually have more benign course, but some may show a phenotype as the classical one
- It should be noted all currently available therapies do not reduce already accumulated in cells Gb3
- Early diagnosis and treatment are promising strategy to reduce organ damage, morbidity and accelerated mortality

THANK YOU FOR YOUR ATENTION

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Institut für Humangenetik

Type-IV-collagen-related nephropathies

Julia Hoefele

DFG Deutsche Forschungsgemeinschaft
GPN Gesellschaft für Pädiatrische Nephrologie
european society for paediatric nephrology
Federal Ministry of Education and Research

Conflict of interest

No conflicts of interest to declare.

- CKD causes
- Type-IV-collagen-related nephropathies
- Mosaicism
- Projects on Alport syndrome
- Summary

CKD causes in adult and pediatric nephrology?

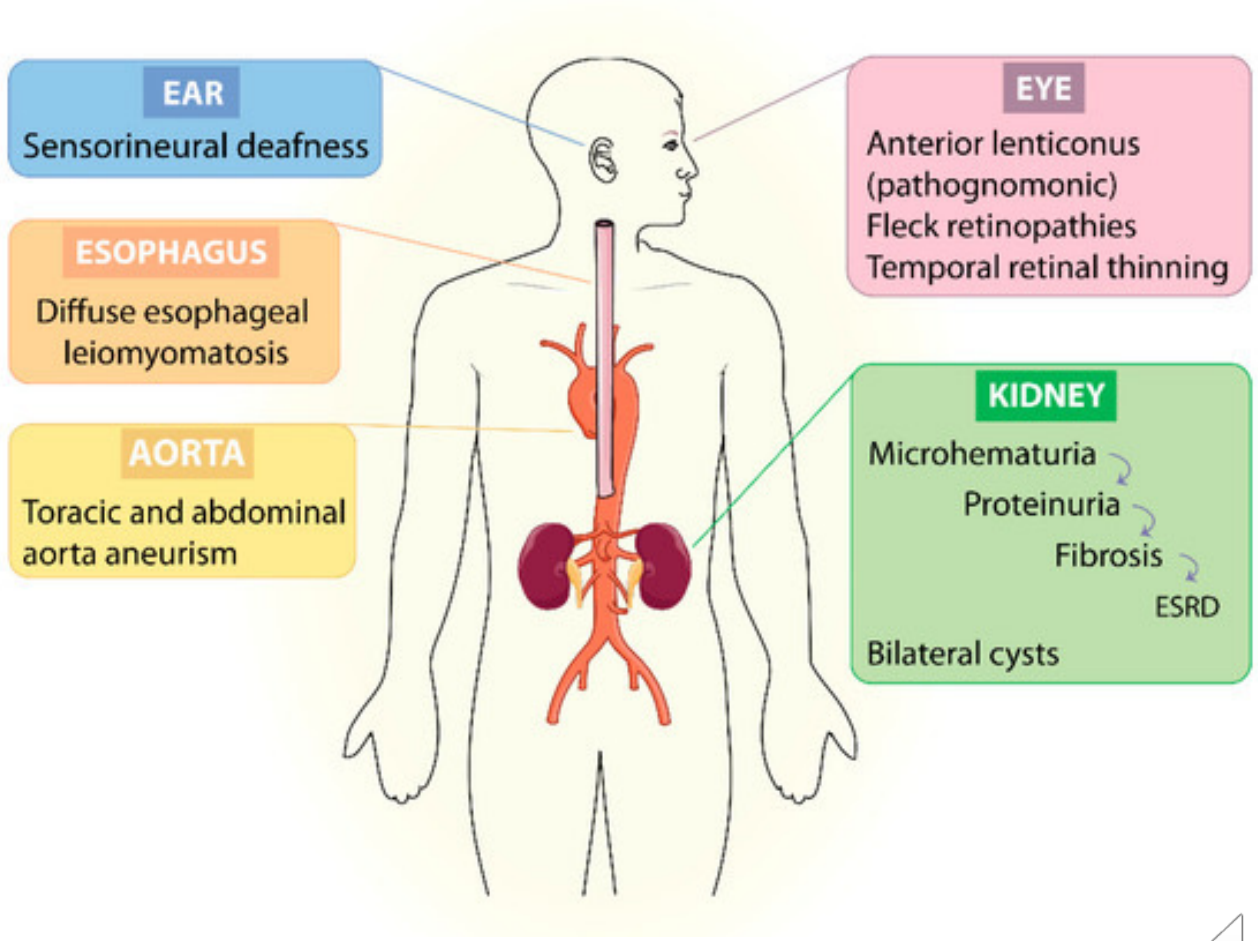
Causes of CKD in adults	Causes of CKD in children
1. Diabetes (38.0%)	1. CAKUT (up to 50%)
2. Renovascular disease (12.2%)	2. Cystic kidney disease (13%)
3. Glomerulonephritis (10.2%)	3. Infection/cortical necrosis (11%)
4. Polycystic kidney disease (3.3%)	4. Nephrotic syndrome (6%)
5. Pyelonephritis (2.5%)	5. Systemic diseases
6. Drug-induced (1.7%)	6. Trauma
7. Other/unknown (18.0%/14.2%)	7. Urinary blockage or reflux

Kitzler and Chun, *Canadian Journal of Kidney Health and Disease*, 2023

Clinical symptoms

Different Types of these Nephropathies

- Alport syndrome
  - Microscopic hematuria, proteinuria, ESKF until age 40 years
  - Sensorineural hearing impairment, characteristic ocular abnormalities, leiomyomatosis



Martínez-Pulleiro et al., *I J Mol Sci*, 2021

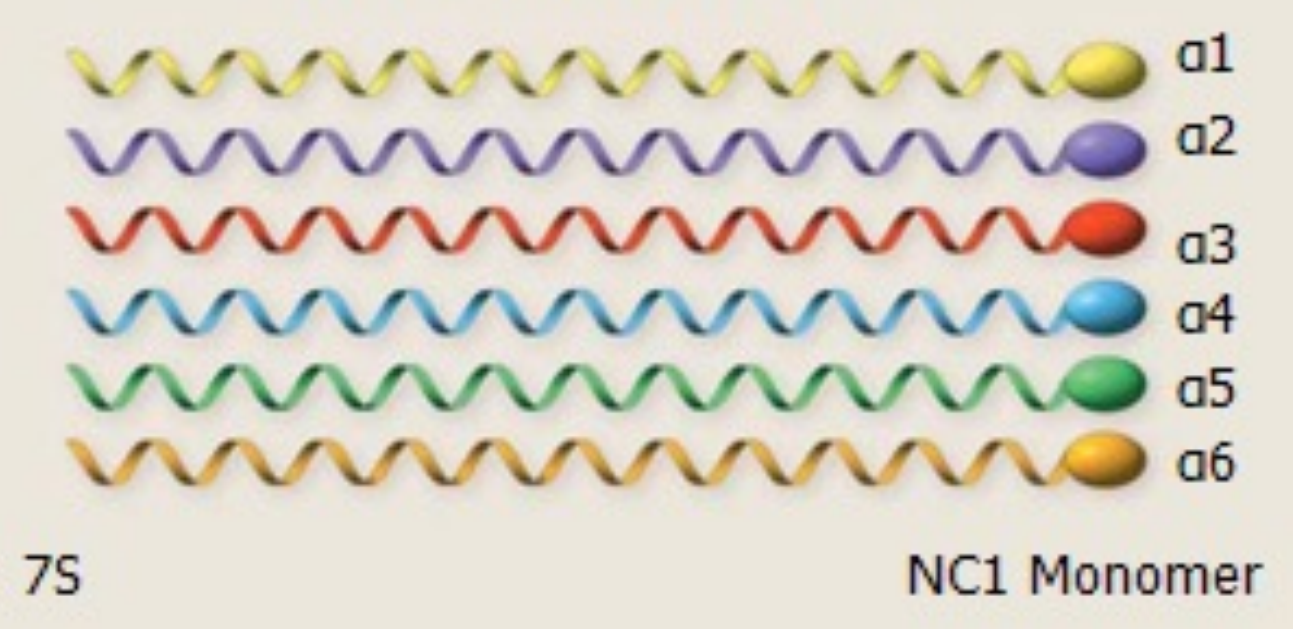
Type-IV-collagen-related Nephropathies

- Thin basement membrane nephropathy (TBMN)
  - Microscopic hematuria, sometimes small proteinuria
  - Development of CKD in 20% individuals at older age (> 60 years of age)

Pathogenesis

Type IV Collagen

- Six different type IV collagen chains:
  - α1, α2, α3, α4, α5, and α6



Hudson et al, *N Engl J Med*, 2003

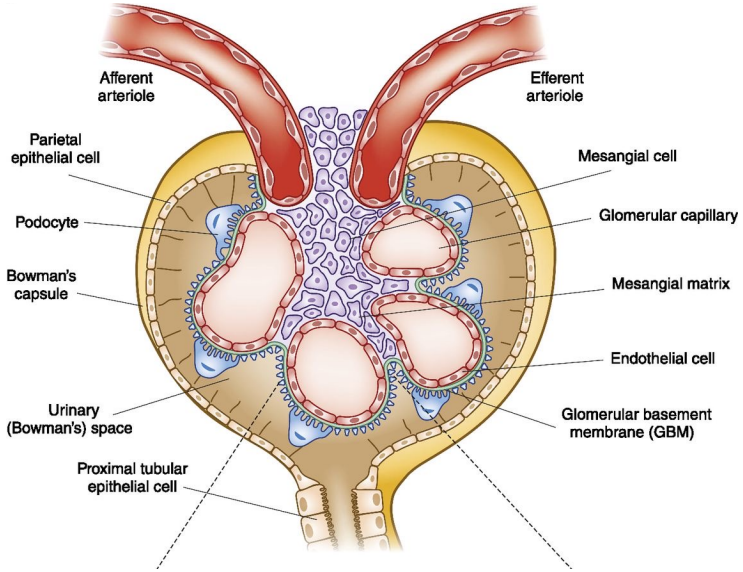
Type IV Collagen

- Three sets of triple helical moleculs (protomers):
  - α1.α1.α2, α3.α4.α5, α5.α5.α6

Hudson et al, *N Engl J Med*, 2003

Protomers

- α1.α1.α2:
  - Appearance in the embryo at the start of early capillary formation
  - Replacement by α3.α4.α5 (mature glomerular capillary) and α5.α5.α6 (Bowman‘s capsule)
- α3.α4.α5:
  - Kidney (glomerular basment membrane), lung, testis, cochlea, eye
- α5.α5.α6:
  - Kidney (Bowman‘s capsule), skin, smooth muscle, oesophagus



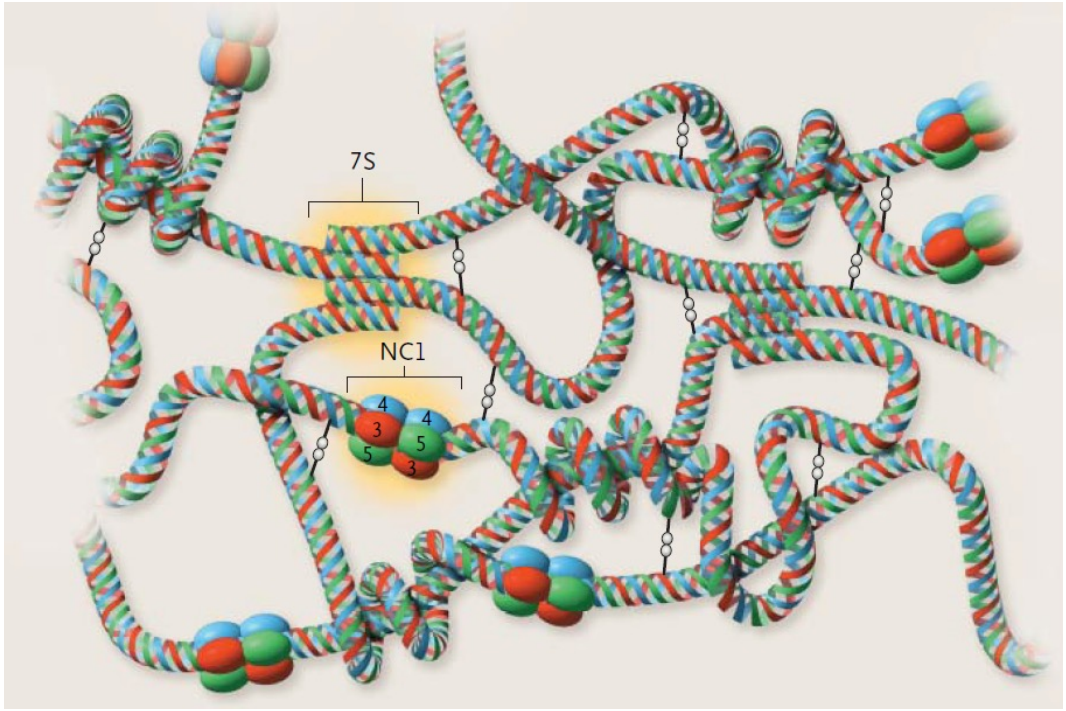
www.cjasn.asnjournals.org

Network Configuration

- Combining two NC1 trimers
  → formation of hexamers

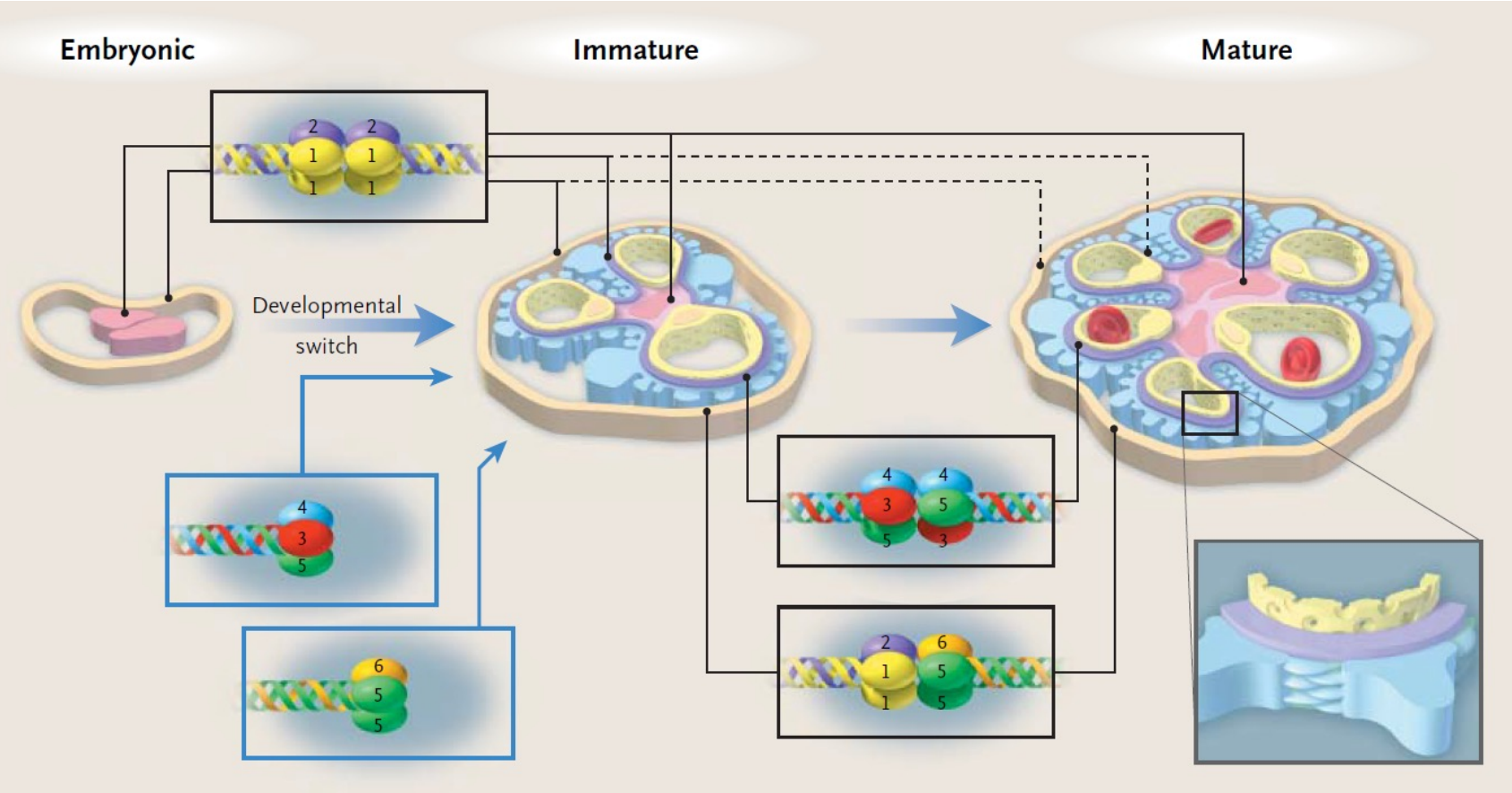
- Combining 7S domains
  → formation of tetramers

- Networks:
  - α1.α1.α2-α1.α1.α2
  - α3.α4.α5-α3.α4.α5
  - α1.α1.α2-α5.α5.α6



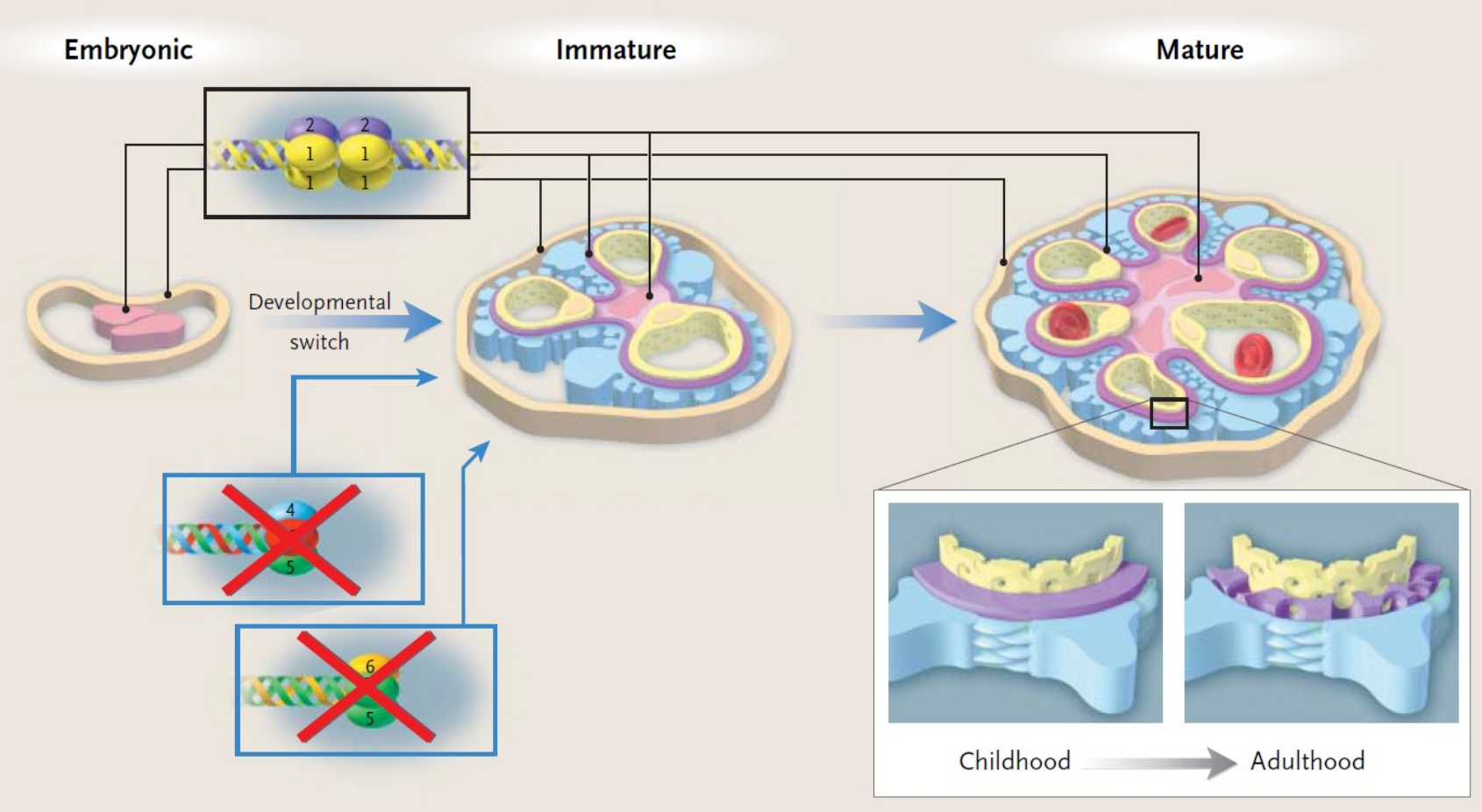
Hudson et al, *N Engl J Med*, 2003

Normal Glomerular Development



Hudson et al, *N Engl J Med*, 2003

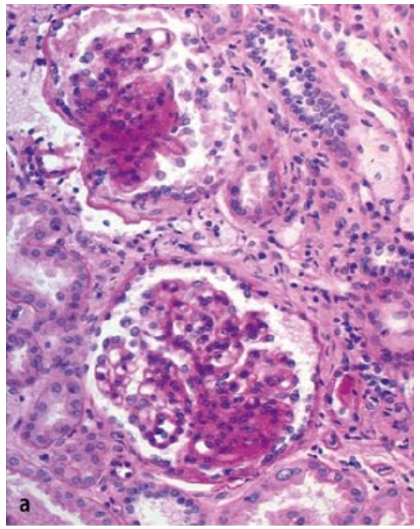
Glomerular Development in Alport syndrome

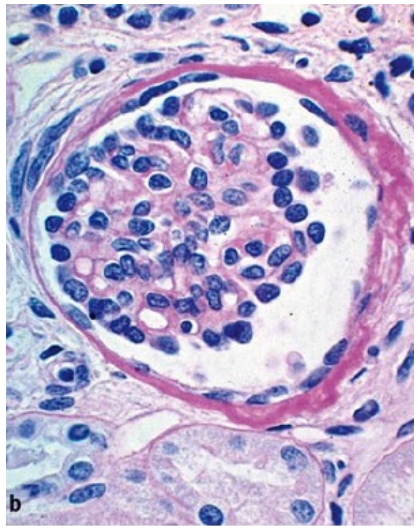


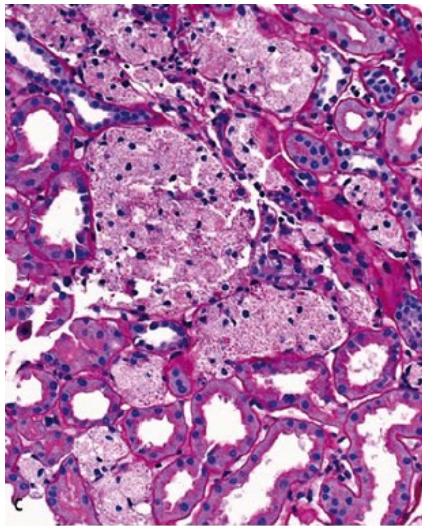
Hudson et al, *N Engl J Med*, 2003

Histological Features: Light Microscopy

- Mesangioproliferative changes
- Tubular atrophy
- Glomerular sclerosis
- Interstitial fibrosis



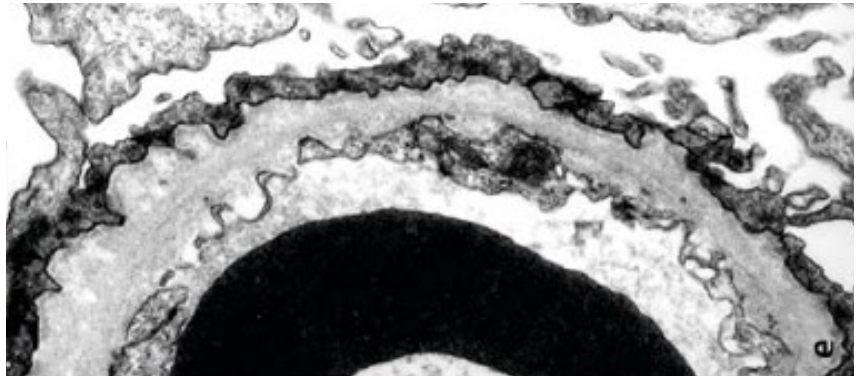


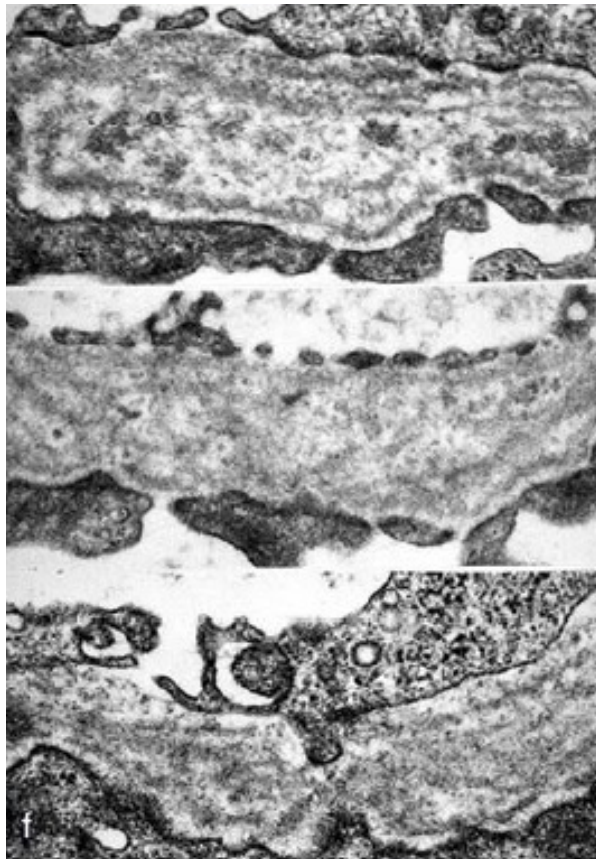


Hopfer & Mihatsch, *Der Nephrologe*, 2010

Histological Features: Electron Microscopy

- Areas of thinning and splitting of the glomerular basement membrane





Hopfer & Mihatsch, *Der Nephrologe*, 2010

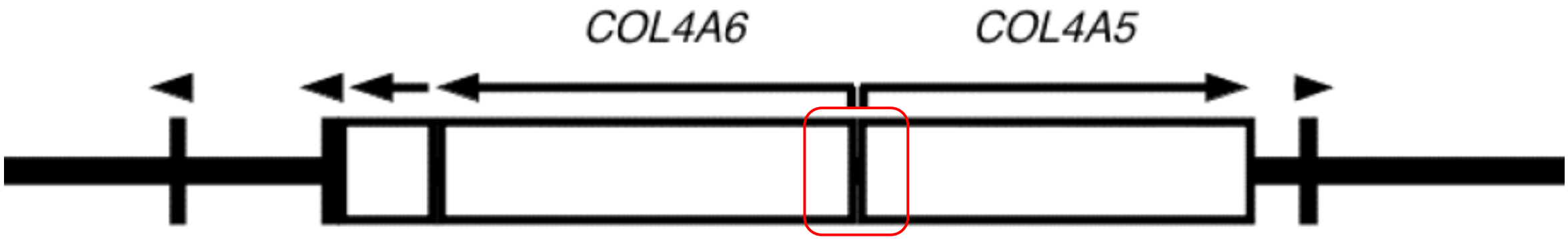
Genetic causes

Genetic causes: Alport Syndrome and TBMN

- Alport syndrome
  - X-linked: *COL4A5*
    - 60-85% of cases
    - Incidence: 1:5.000
  - Autosomal recessive: *COL4A3*/*COL4A4*
  - Autosomal dominant: *COL4A3*/*COL4A4*
    - Diverse frequencies described
  - Digenic inheritance
- Thin basement membrane nephropathy
  - Autosomal dominant: *COL4A3*/*COL4A4*

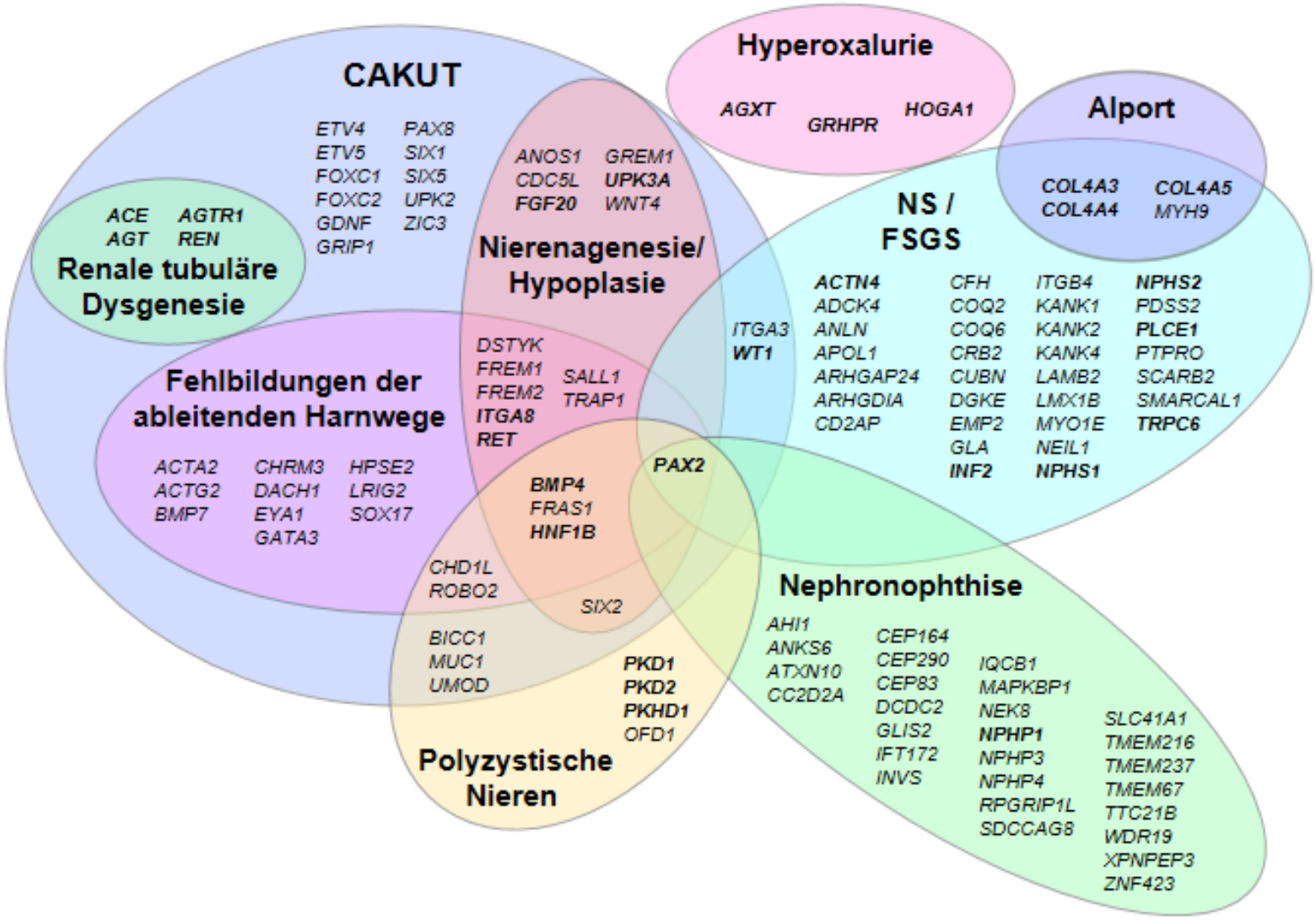
Genetic causes: Alport syndrome and Leiomyomatosis

- Partial deletion of *COL4A5* and *COL4A6* ranging from intron 2 of *COL4A6* to intron 1 of *COL4A5*



Thielen et al., *Hum Mutat*, 2003

Phenocopies



www.medizinische-genetik.de

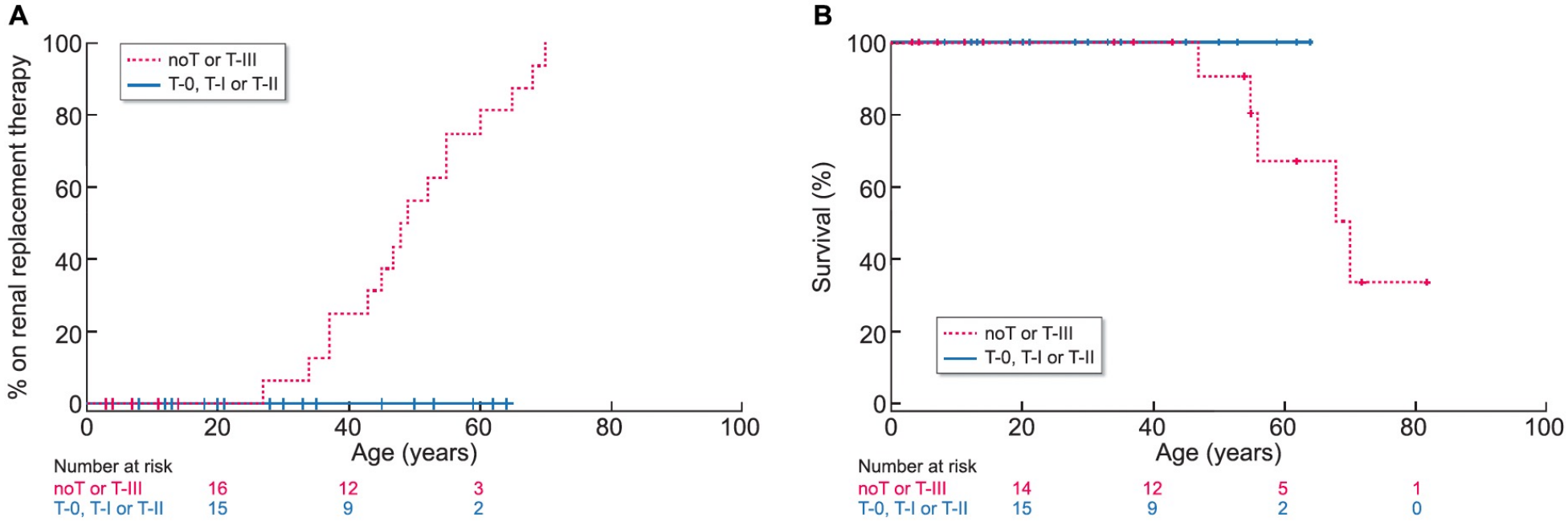
Own Phenocopy Data

- 19% of phenocopies in exome-sequencing solved cases
  - 5 clinical FSGS (genetically Alport syndrome)
  - 3 clinical Alport syndrome (genetically FSGS, Dent Disease)
  - 1 clinical ciliopathy (genetically 17q12 microdeletion)
  - 3 others

Riedhammer et al., Exome Sequencing and Identification of Phenocopies in Patients With Clinically Presumed Hereditary Nephropathies. *Am J Kidney Dis*, 2020

Therapy

Effect of Therapy and Renal Failure

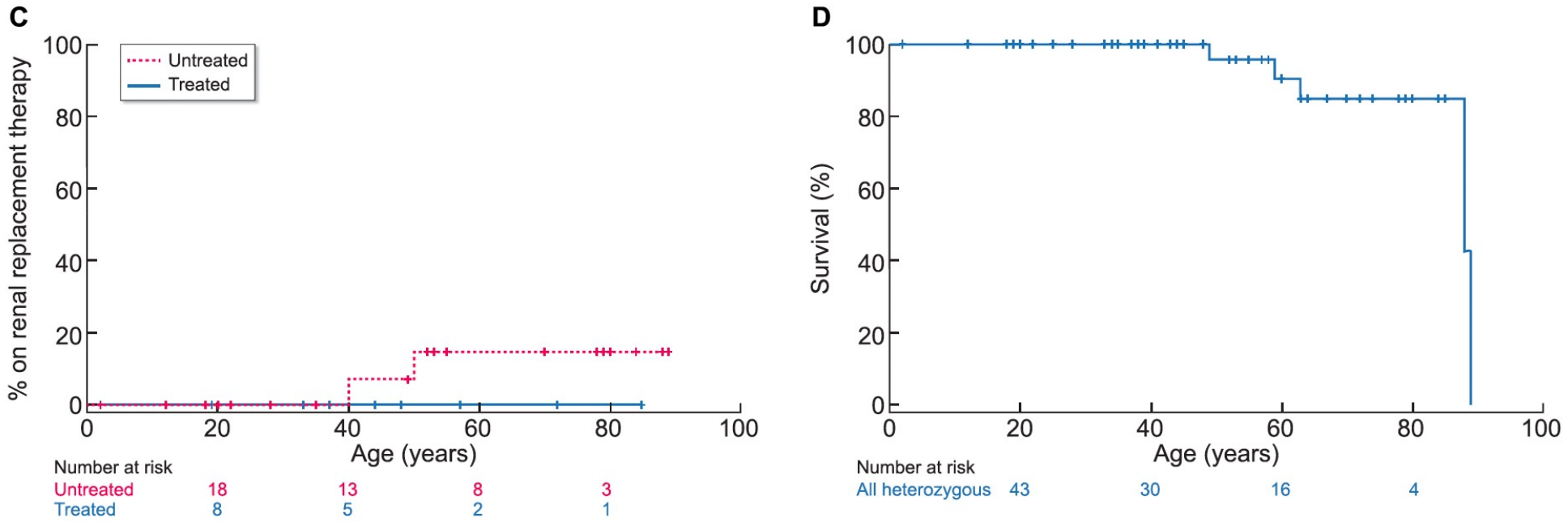


Boeckhaus et al., *Nephrol Dial Transplant*, 2022

A) Hemizygous individuals

B) Hemizygous individuals

Effect of Therapy and Renal Failure



Boeckhaus et al., *Nephrol Dial Transplant*, 2022

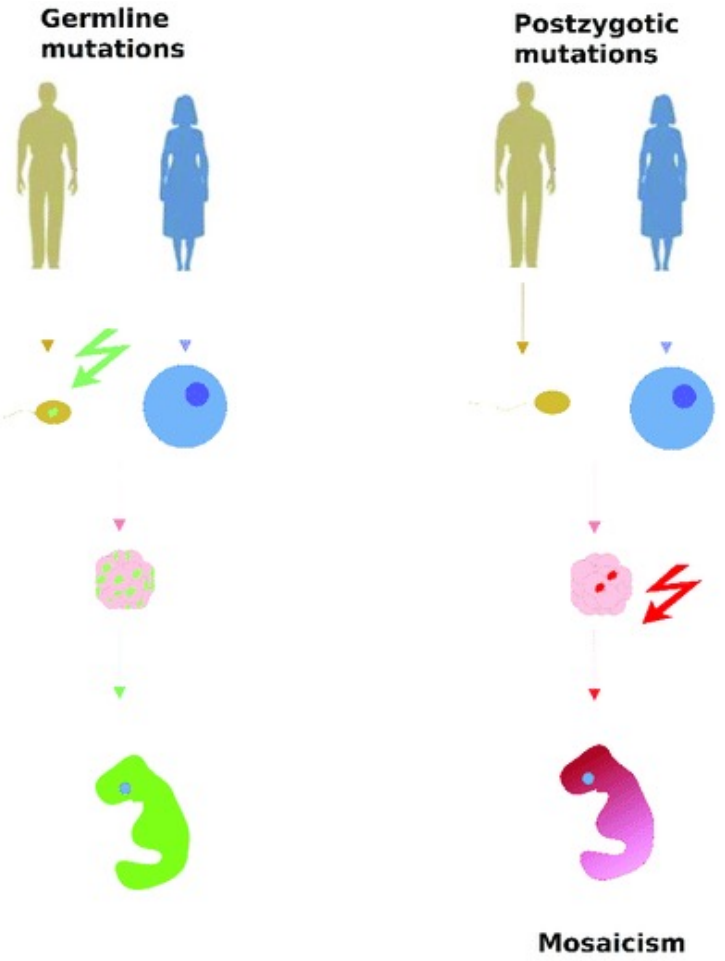
C) Heterozygous individuals

D) Heterozygous individuals

Mocaisism

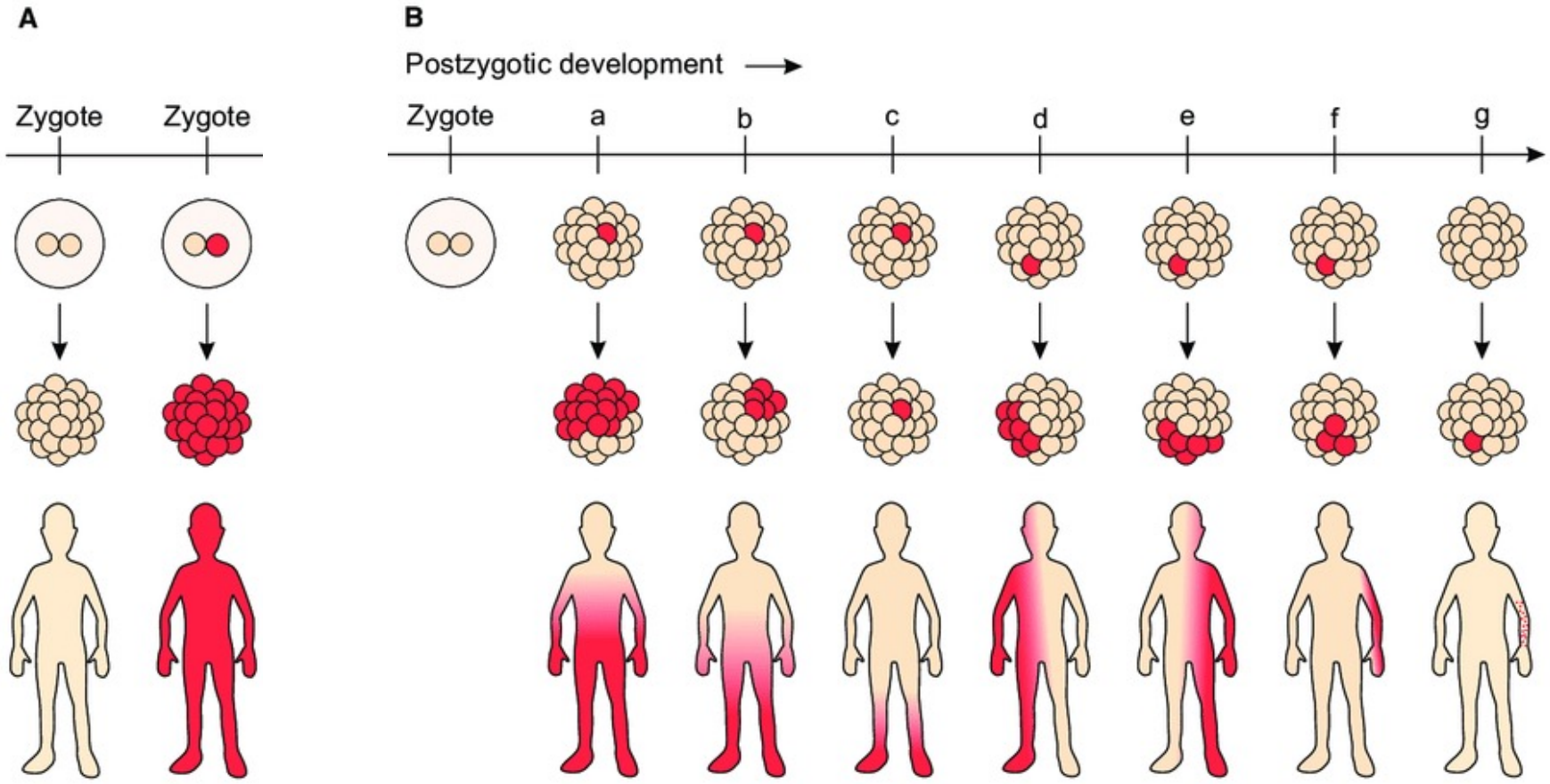
General information on mosaicism

- Mosaicism refers to the presence of two or more genetically distinct cell populations within one individual's body, resulting from a post-zygotic variant that occurs after conception



https://www.researchgate.net

Postzygotic Mosaicism



https://www.researchgate.net

Information on Mosaicism in Alport syndrome

- Limited data on mosaicism in individuals with Alport syndrome
- Clinical presentation depends on the grade and the location of the mosaic
  - Mild to severe phenotypes can be observed
- Depending on the grade of mosaicism and the used molecular technique, it might be difficult to detect

Project:

Is there a dominant-negative effect in individuals with heterozygous disease-causing variants in *COL4A3*/*COL4A4*?

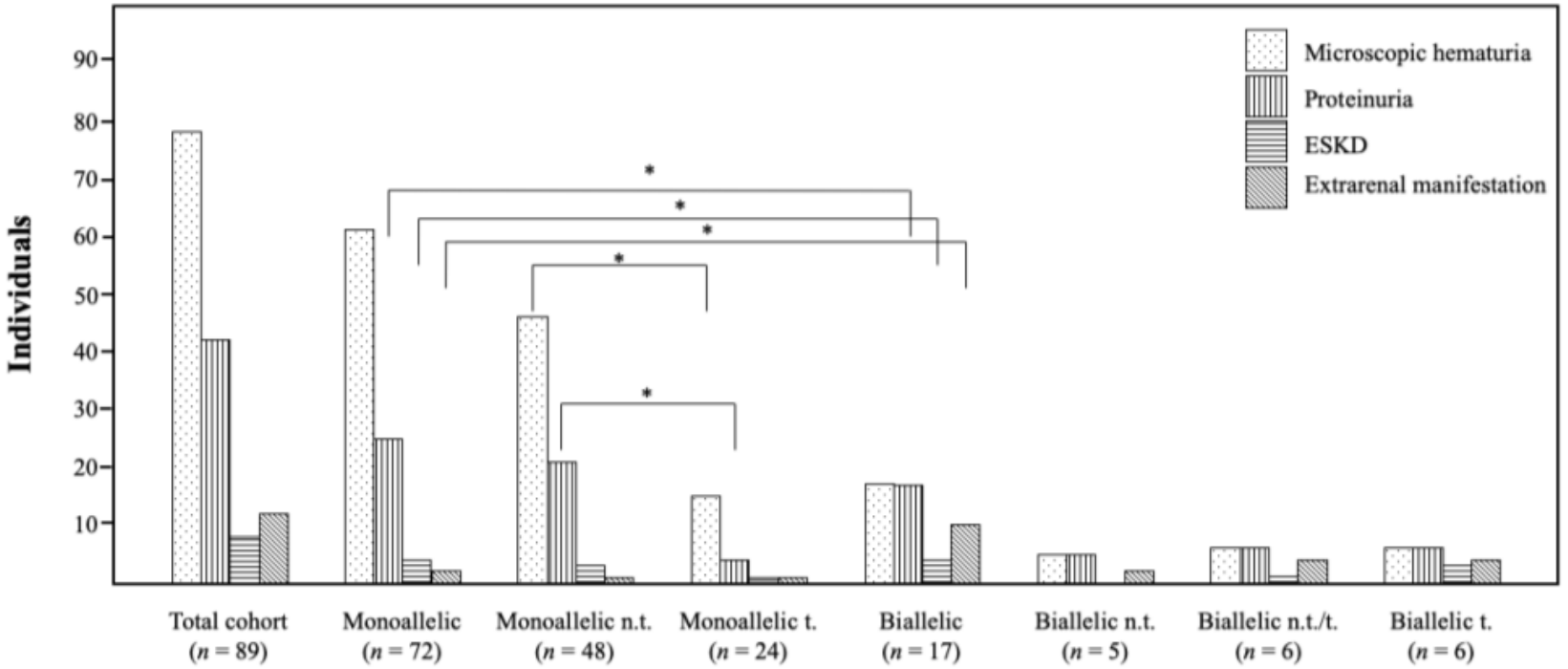
Riedhammer et al., *Clin Genet* 2024

Material & Methods

- Cohort of 89 individuals with autosomal Alport syndrome or TBMN
  - Monoallelic variants
  - Biallelic variants

Results

- Indivi
  micro
- Indivi
  repor
  variar



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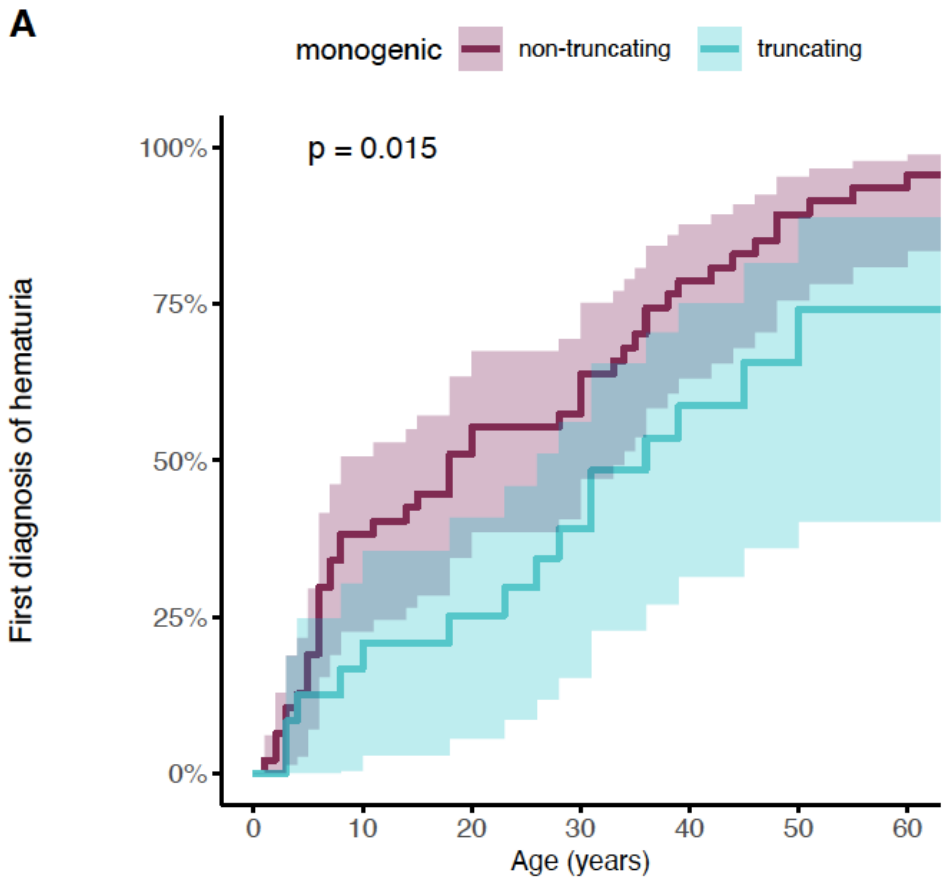
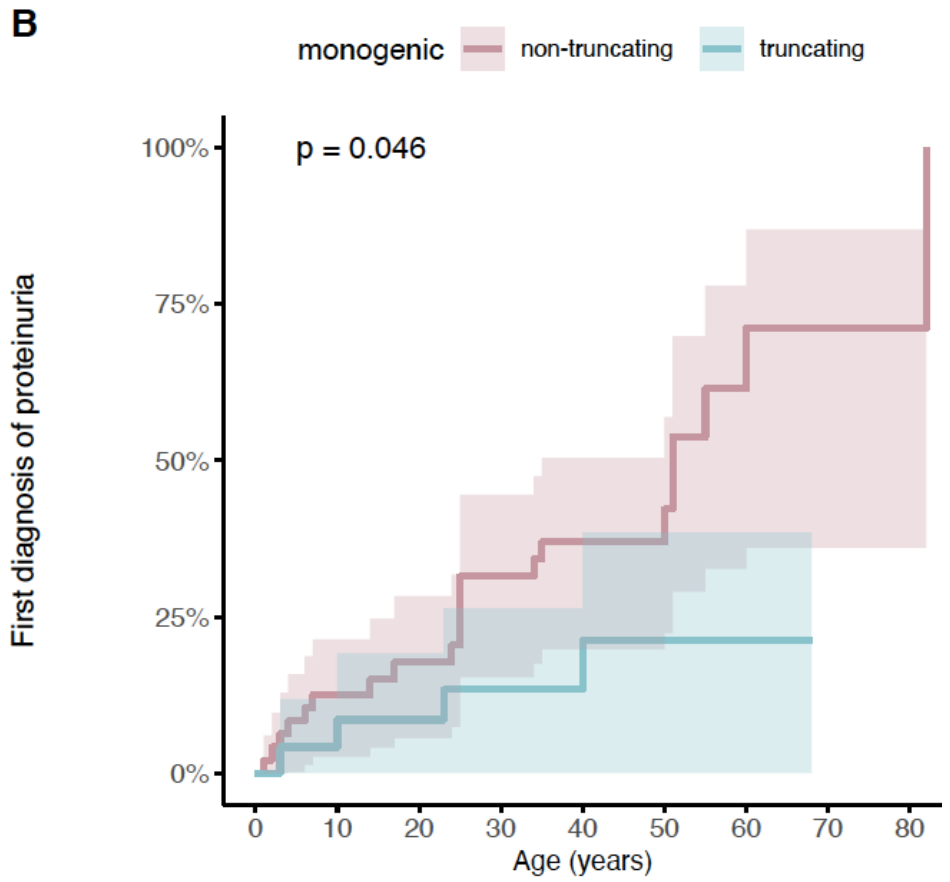
Results


A
monogenic
non-truncating
truncating
p = 0.015
First diagnosis of hematuria
100%
75%
50%
25%
0%
0
10
20
30
40
50
60
Age (years)


B
monogenic
non-truncating
truncating
p = 0.046
First diagnosis of proteinuria
100%
75%
50%
25%
0%
0
10
20
30
40
50
60
70
80
Age (years)

Conclusion

- The results of this study implicated a potential dominant-negative effect as an explanation for the heterozygous non-truncating variants in individuals with a more severe phenotype

→ Knowledge of genotype is important for the prognosis of the disease and the planned treatment

Project:

Are there other disease-causing mechanisms?

Ongoing project

Material & Methods

- Two affected individuals within a family from Iceland
  - Mother: mildly affected with Alport syndrome
  - Son: classical Alport syndrome

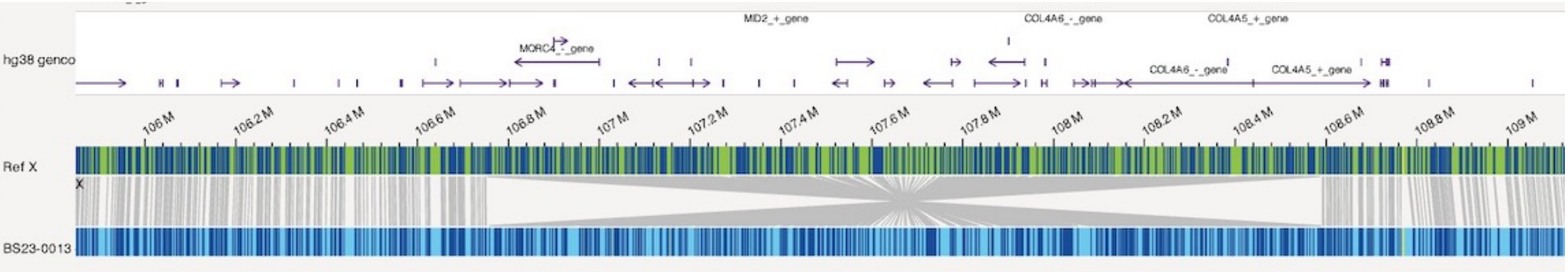
→ X-linked inheritance

Results & Conclusion

- Panel diagnostics *COL4A3-5* and genome sequencing
  - No disease-causing variants identified
- Optical Genome Mapping (Bionano)
  - Identification of a paracentric inversion on the X chromosome disrupting *COL4A5*

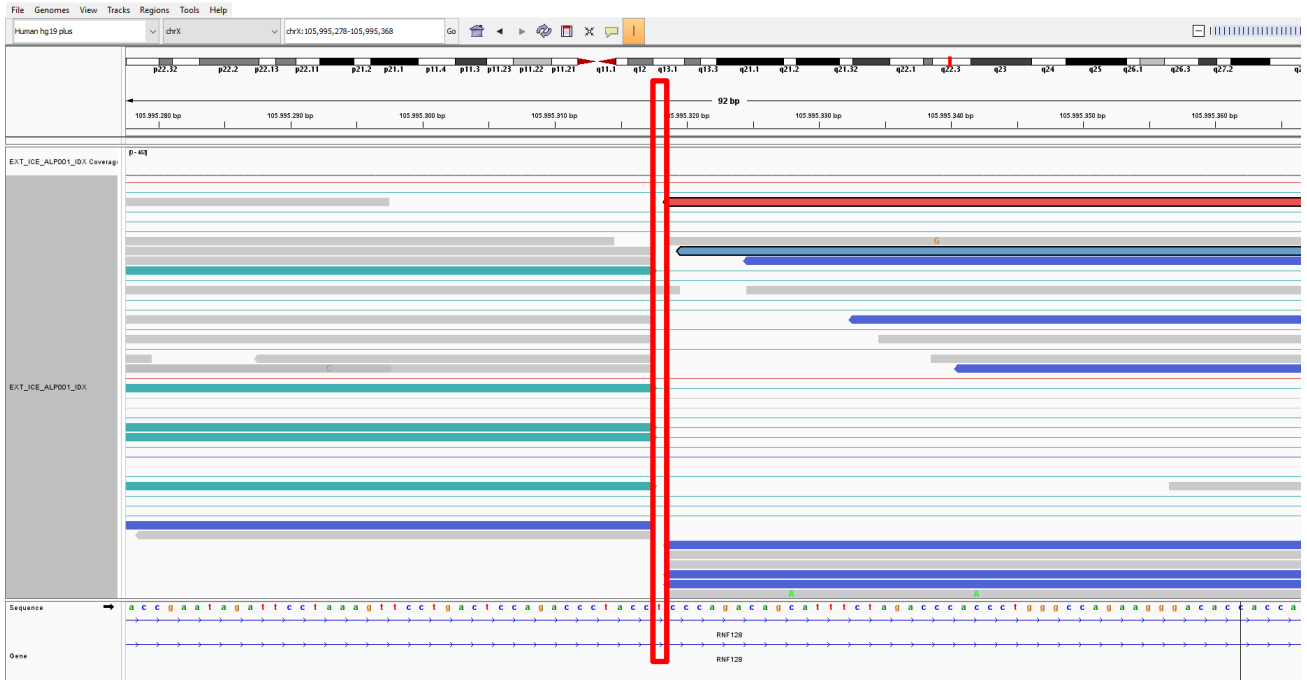
→ Identification of a new disease mechanisms

Results & Conclusion



- Re-analysis of the genome sequencing data
  → confirmation of the inversion

Genome sequencing

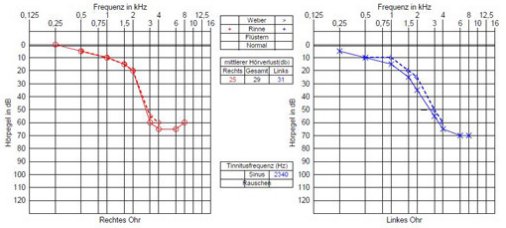


Conclusion

- Individuals with clear phenotypic symptoms should be genetically completely evaluated
- If no variant can be detected with the regular diagnostic tests, these individuals should be included into research projects

Total Summary

- Extrarenal manifestations like hearing impairment and occular involvement should be regularly monitored
- Early treatment essential to avoid disease progression
- Genetically broad spectrum of causes



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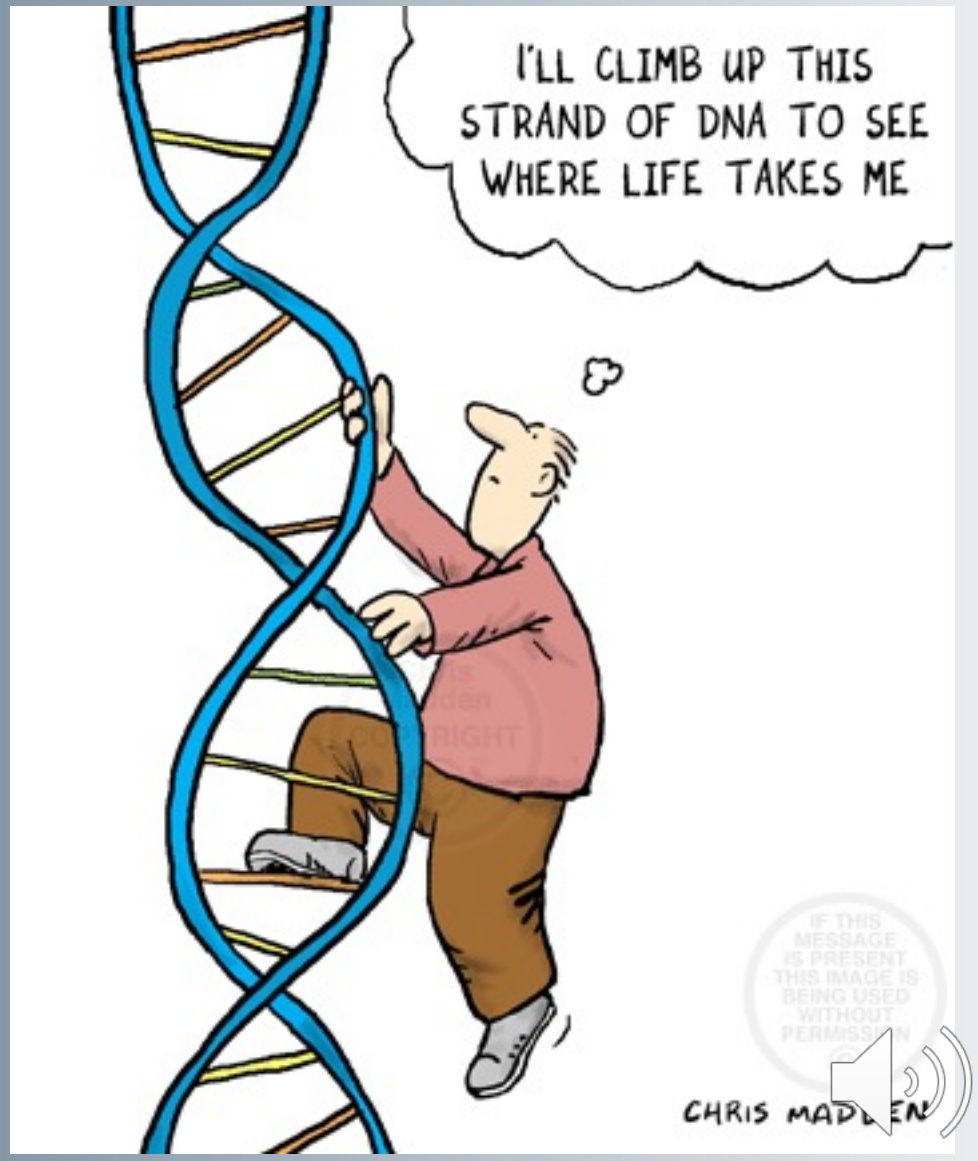
Thank you for your attention!



julia.hoefele@tum.de

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IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

Congenital Nephrotic Syndrome

Sandra Habbig

04/2024 IPNA Teaching Course, RARE DISEASES IN PEDIATRIC NEPHROLOGY, Skopje, Republic of North Macedoniaa

Outline

Definition of Congenital Nephrotic Syndrome (CNS)

Clinical Presentation and Diagnosis of CNS

Management of CNS

Definition of CNS

Nephrotic-range proteinuria and oedema that manifest *in utero* or during the first 3 months of life

Diagnosis:
- massive proteinuria, edema
- enlarged hyperechoic kidney
- variable kidney function at birth
- enlarged placenta (>25% birth weight)

Complications:
- hemodynamic instability
- recurrent infections
- thromboses

Most patients with CNS progess to ESKD within a few years.

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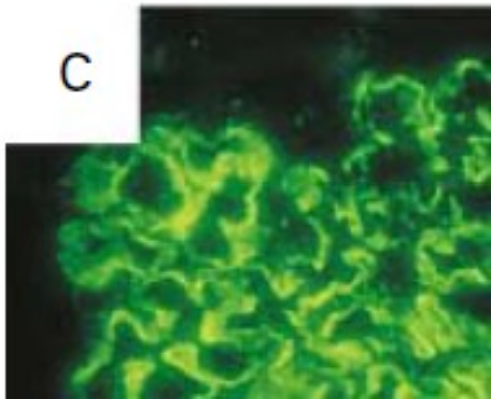
Definition of CNS

Nephrotic-range proteinuria and oedema that manifest *in utero* or during the first 3 months of life

Etiology:	Podocyte gene pathogenic variants	Genetic CNS
	Congenital infections	Infectious CNS
	Maternal allo-immune disease	Non-genetic non infectious CNS

Non-genetic, non infectious CNS:
Antenatal Membranous GN due to Anti-Neutral Endopeptidase Antibodies

Age	Serum Creatinine Concentration
	mg/dl
1 day	ND
2 days	1.9
4 days	2.7
5 days	2.2
6 days	1.6
22 days	1.4



Kidney biopsy specimen, 4 weeks of age, fluorescein-isothiocyanate–labeled antihuman IgG antibody

Debiec et al, N Engl J Med, Vol. 346, No. 26, June 27, 2002

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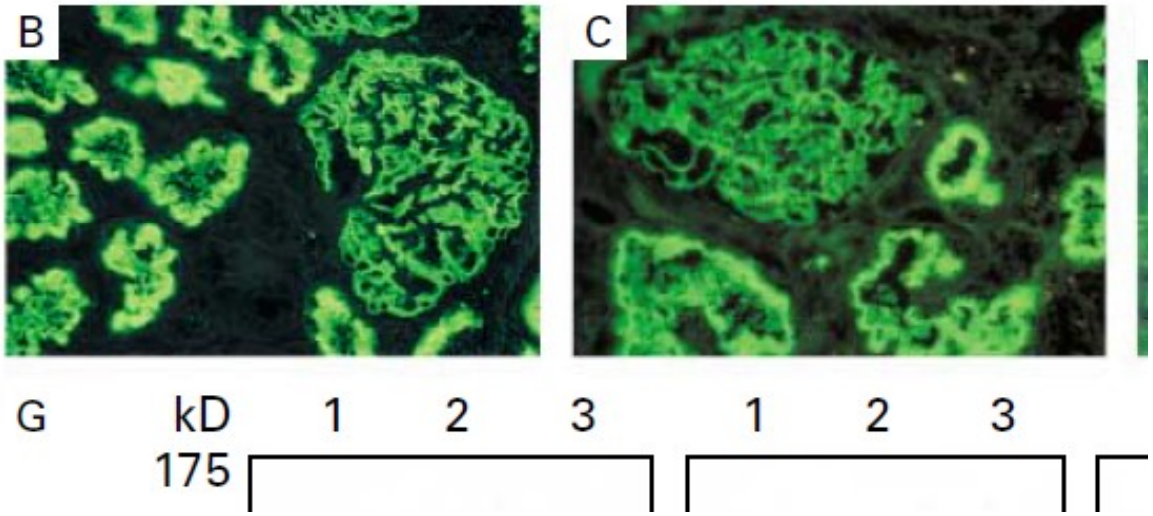
Non-genetic, non infectious CNS:
Antenatal Membranous GN due to Anti-Neutral Endopeptidase Antibodies



Debiec et al, N Engl J Med, Vol. 346, No. 26, June 27, 2002

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Infectious CNS

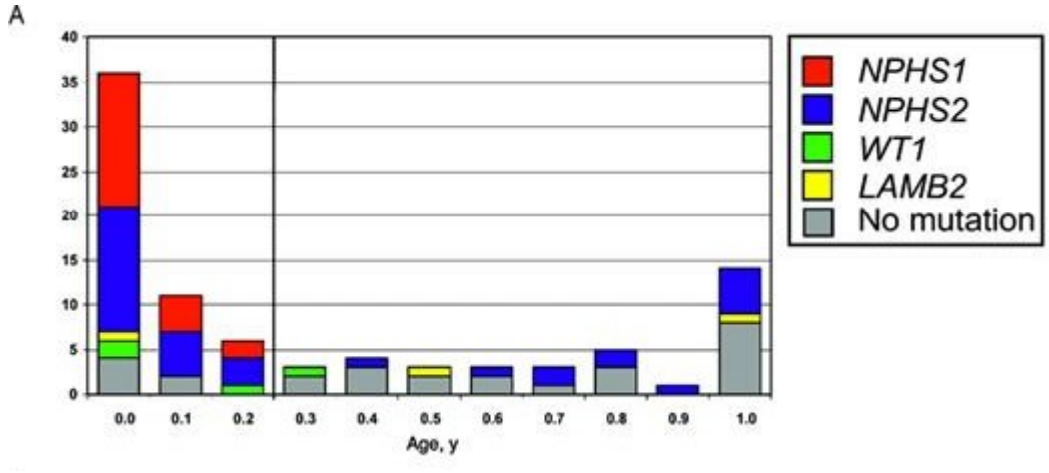
Infectious CNS:

- *Congenital syphilis*
- *Toxoplasmosis*
- *Rubella*
- *CMV*
- *Hepatitis B, Hepatitis C*
- *Herpes*
- *HIV*
- *Bordetella pertussis*

-> specific therapy e.g. penicilline in Syphilis + genetic testing

Genetic CNS

Mutations in NS during the first 12 months of life



89 central European and Turkish children: 60.7% (54 of 89) of individuals manifested within the first 3 months of life (CNS). Of these, 87.0% (47 of 54) carried disease-causing mutations.

Hinkes et al, Pediatrics (2007) 119 (4): e907–e919

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Genetic CNS

Gene/locus	Protein	Inherit-ance	Function	Renal pat
NPHS1 19q13.1	Nephrin	AR	Main component of the SD Crucial for the integrity of actin cytoskeleton	CNF
NPHS2 1q25-31	Podocin	AR	Scaffold protein linking plasma membrane to actin cytoskeleton	CNS, SRN
WT1 11p13	Wilms tumor 1	AD	Located in Podocyte Transcription factor Tumor suppressor Regulator for renal differentiation & gonadal development	DDS Frasier syr WAGR syr Isolated D Isolated F
LAMB2 3p21	Laminin- β2	AR	Located in GBM Links GBM with actin cytoskeleton	DMS Pierson syndrome
PLCE1	Phospho-lipase	AR	Located in podocyte	DMS

AbuMaziad et al, Journal of Perinatology (2021), 41:2704-2712

When to perform genetic testing?

- A genetic screening comprising all CNS-related genes ist recommend as first-line diagnostic measure in every patient with CNS
  - to identify the etiology of CNS
  - to plan multi-disciplinary management, esp. with regard to complications (risk of Wilms tumour)
  - to better provide data on the prognosis (neurodevelopmental involvement)
  - to enable genetic counselling of the family

- screening of *NPHS1, NPHS2, WT1, PLCE1* and *LAMB2* will identify > 80% of genetic forms

- mutations in less commonly mutated genes provide an additional of 5% diagnoses

European Journal of Human Genetics
https://doi.org/10.1038/s41431-020-0642-8

ARTICLE

Genetic aspects of congenital nephrotic syndrome: statement from the ERKNet–ESPN inherited glomer

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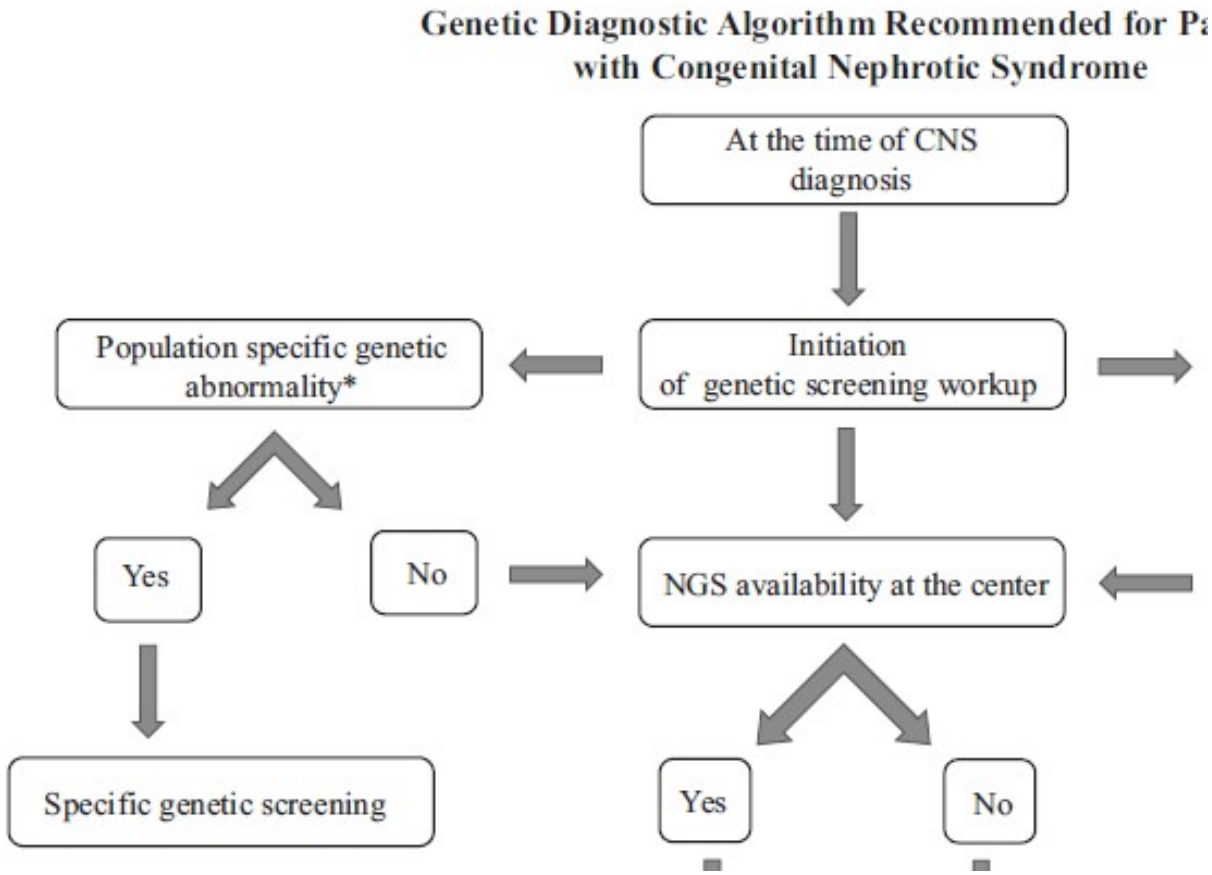
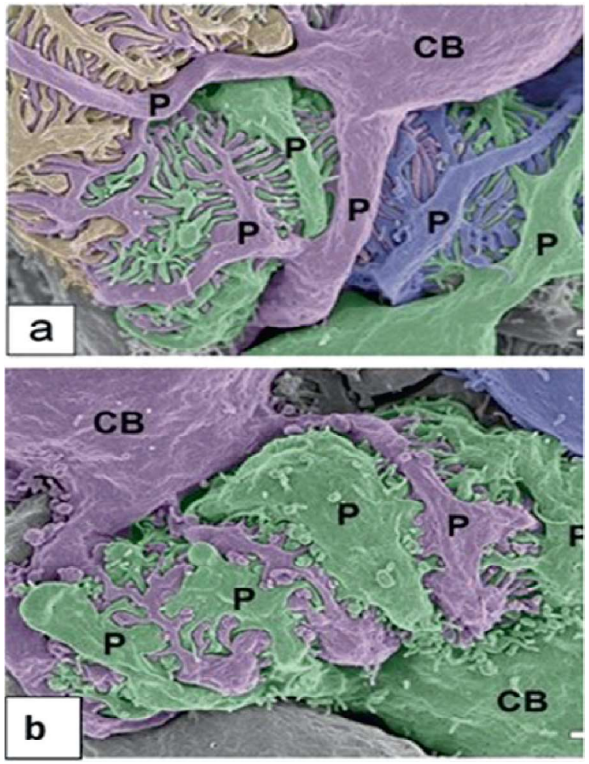


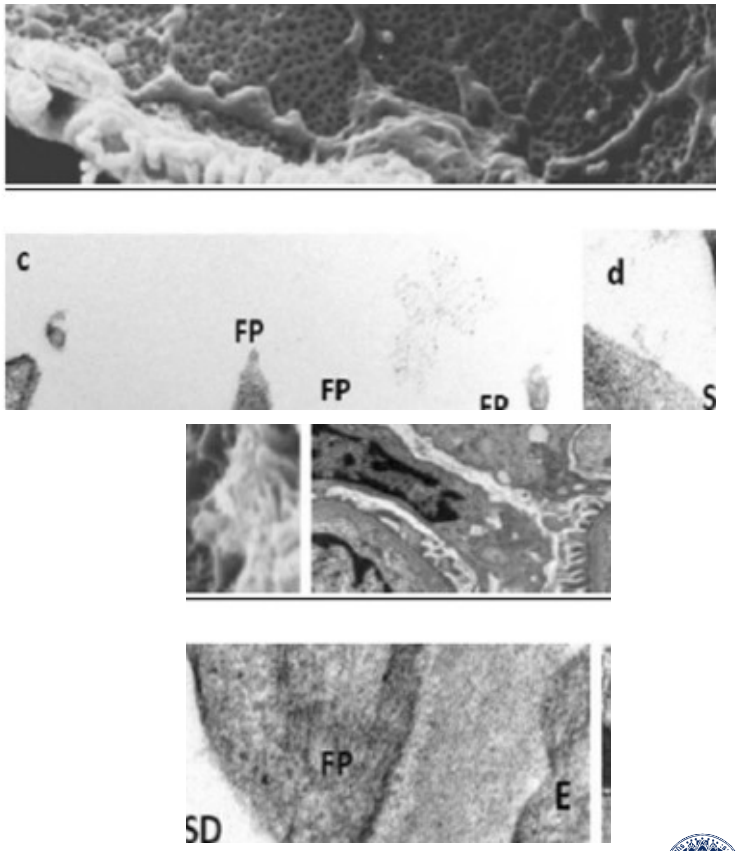
Lipska-Zietkiewicz et al, EJHG (2020), https://doi.org/10.1038/s41431-020-0642-8

When to perform a kidney biopsy?

- Non-invasive molecular diagnostic methods have largely replaced kidney biopsies
- routine kidney biopsy is not recommended in patients with CNS
- Kidney biopsy may be considered if a genetic diagnosis cannot be established and/ or in rare cases with suspected congenital membranous nephropathy due to maternal anti.neutral endopeptidase antibodies

The glomerular filtration barrier





AbuMaziad et al, Journal of Perinatology (2021), 41:2704-2712

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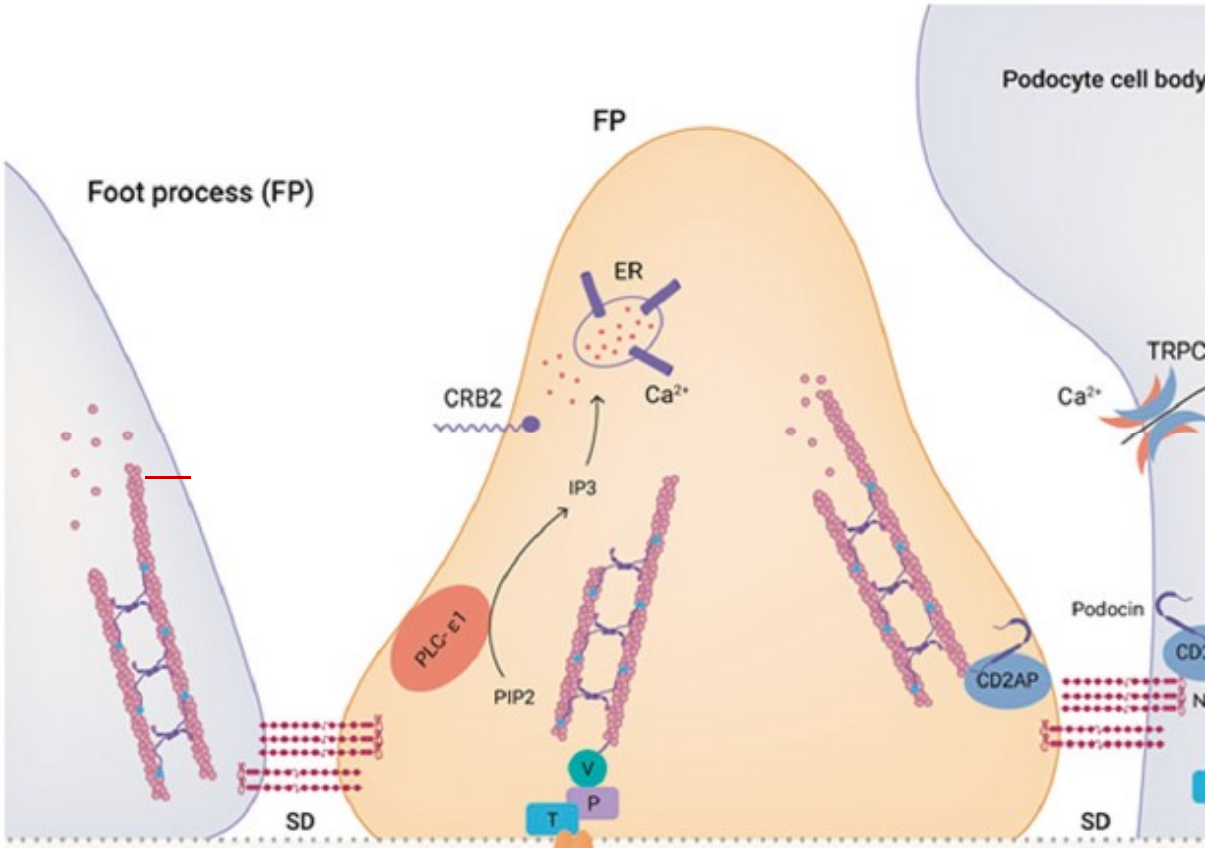
The glomerular filtration barrier



AbuMaziad et al, Journal of Perinatology (2021), 41:2704-2712

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Boyer et al, Nature Reviews Nephrology 2021 Apr;17(4):277-289

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Diagnosis of CNS

First-line evaluation

- Growth chart: height or length, weight, head circumferer calculation of BMI and annual height velocity.
- Blood pressure.
- Physical examination: volaemia, signs of oedema (e.g. asc effusions).
- Blood biochemistry: blood count, levels of sodium, chlori creatinine, urea, protein, albumin, cholesterol, fasting trig
- Levels of thyroid-stimulating hormone and free thyroxine
- Serum IgG level.

+ **evaluation of dysmorphic features** and skeletal abnormalities, genital examination, hearing test, ophthalmological examination

+ neurological examination and standardized evaluation of **cognitive status** (with or without brain MRI)

LAB

sonography

Boyer et al, Nature Reviews Nephrology (2021)

Management of CNS

Patients with CNS should be managed in a multidisciplinary team!

-> rapid referral of children with CNS to a specialized paediatric nephrology unit due to the complexity of the disease and fluid management.

Boyer et al, Nature Reviews Nephrology (2021)

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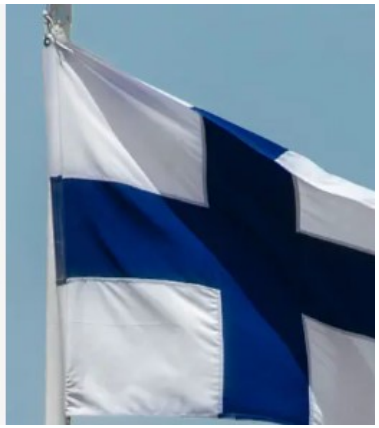
Learning from Finland…



Finnish-type nephrotic syndrome

1/8000 in Finland *versus* 0.5/100.000 in Europe/USA

> 120 cases in Cologne

https://www.guinnessworldrecords.com/news/2023/

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The Finnish approach

75 Finnish children followed 1965-1973: nutritional support, no albumin infusions, **mean survival 7.6 months** (non developed uremia before death, death due to infections, thrombotic complications and hemodynamic instability)

early 1970s, Minnesota: KTX as therapeutic option

since 1971 active treatment: high caloric, high protein, low sodium diet, diuretics, IV albumin, 25% died before KTX ->

CNS no longer considered a lethal condition but intensive , medical treatment + KTX were offered since then

Huttunen N-P (1976) Congenital nephrotic syndrome of Finnish type. Study of 75 patients. Arch Dis Child 51:344–348, Holmberg C, Antikainen M, Rönnholm K, Ala-Houhala M, Jalanko H (1995) Management of congenital nephrotic syndrome of the Finnish type. Pediatr Nephrol 9:87–93

The Finnish approach

Active Finnish treatment strategy published in 1995 (in every 0-3 months old infant with heavy proteinuria):

(1) Management of nephrosis from birth to the age of 6–10 months (weight 7 kg),

(2) Bilateral nephrectomy and peritoneal dialysis (PD) for 3–6 months,

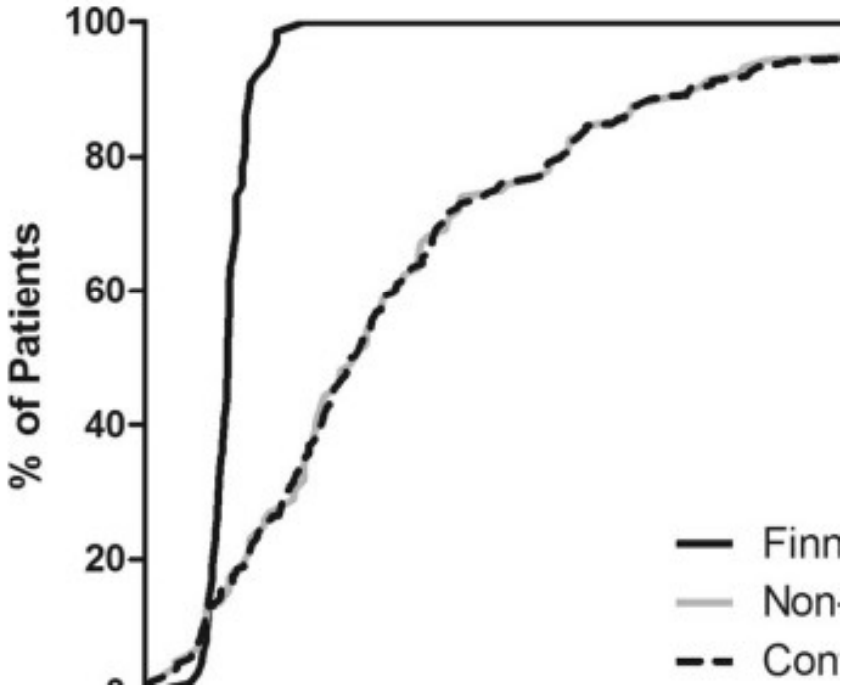
(3) KTx with extra peritoneal engraftment when the weight of 10 kg has been reached (usually 1–1.5 years of age).

Treatment applied to > 150 CNS children with CNS, nearly 90% survival, 16 deaths

(2 nephrotic phase, 7 on dialysis, 7 after KTx)

Huttunen N-P (1976) Congenital nephrotic syndrome of Finnish type. Study of 75 patietns. Arch Dis Child 51:344–348, Holmberg C, Antikainen M, Rönnholm K, Ala-Houhala M, Jalanko H (1995) Management of congenital nephrotic syndrome of the Finnish type. Pediatr Nephrol 9:87–93

Finnish *NPHS1* patients *versus* non-Finnish *NPHS1* patients (ESPN/ERA-EDTA registry)



Finish NPHS1 more frequently have a „severe“ genotype

Hölttä et al, Pediatr Nephrol (2016) 31:2317–2325

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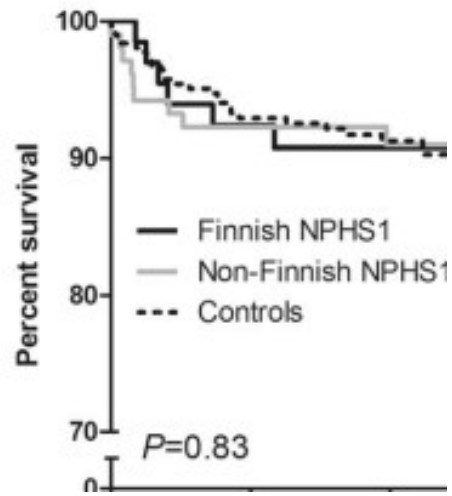
Finnish *NPHS1* patients versus non-Finnish *NPHS1* patients (ESPN(ERA-EDTA registry)

A



- 5-year patient and graft survival were independent of the timing of RRT initiation and RTX
- 5-year patient and graft survival were excellent in both, Finnish and non-Finnish NPHS1 patients on RRT
- and was comparable with CAKUT patients with equally early RRT onset

Hölttä et al, Pediatr Nephrol (2016) 31:2317–2325

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The „conservative“ approach

- 80 children from 17 tertiary nephrology units in Europe
- comparable outcome between 25 *NPHS1* patients with early nephrectomy (median age 9 months) *versus* 17 *NPHS1* patients on conservative management
- at final follow-up (34 months):
  - 80% KTX in nephrectomy group, 1 death
  - 24% KTX, 53% without RRT, 2 deaths

-> individualized, stepwise approach with prolonged conservative management as an promising alternative to early bilateral nephrectomies and dialysis in children with CNS and *NPHS1* mutations.

Dufek et al (2019) Management of children with congenital nephrotic syndrome: challenging treatment paradigms. Nephrol Dial Transplant 34: 1369–1377

Management of CNS – Fluids and albumin

- Recommendation to avoid intravenous fluids and saline. Oral fluid intake should be concentrated if necessary to avoid marked oedema
- Use if albumin infusions based on clinical signs of hypovolemia or upon failure to thrive
- No albumin infusions based on serum albumin levels
- avoid central venous lines due to the high risk of thrombosis
- if central venous line – provide prophylactic anticoagulation

Boyer et al, Nature Reviews Nephrology 2021 Apr;17(4):277-289

Management of CNS – antiproteinuric therapy

- RAAS-blocking therapy such as **ACE inhibitors or angiotensin receptor blockers** are recommended in children with CNS aged > 4 weeks

- Start with the short-acting ACE inhibitor captopril, escalating the dosage from 0.01 to 0.5 mg/kg per dose in children younger than younger than 3 months (maximum dosage of 2 mg/kg/day). Older infants should receive 0.15–3 mg/kg per dose (maximum dosage of 6 mg/kg/day).

- **No combined use** of ACE inhibitors and ARB (risk of AKI!)

- The use of **prostaglandin inhibitors** may be considered as add-on treatment (indomethacin dosed incrementally from 0.5 to 3 mg/kg/day) – but should bes stopped if no clinical benefit after 2-4 weeks

Boyer et al, Nature Reviews Nephrology 2021 Apr;17(4):277-289

Management of CNS – Nephrectomy

- no routine early nephrectomy

- Unilateral or bilateral nephrectomy in patients with severe complications, including failure to thrive, thrombosis and/or daysbalance in euvolemia despite optimized conservative treatment

- Bilateral nephrectomy before KTX in patients with persisting NS and/or a *WT1*-dominant pathogenic variant

Boyer et al, Nature Reviews Nephrology 2021 Apr;17(4):277-289

Individual approach - two patients from Cologne

- both carry the identical homozygous mutation

- 2 year old girl, stable with ACE inhibitor (Ramipril), serum-albumin 20 g/L, proteiuria: 19.000-33545 mg/g creatinine, no complications, stable (normal) kidney function

- 3 year old girl, CNS, severe pneumonia, thrombotic complications, sequential bilateral nephrectomy, peritoneal dialysis

Management of CNS – Thrombosis prophylaxis

- High risk of potentially life-threatening venous and arterial thromboembolic complications
- thrombophilic predisposition due to CNS ***and*** treatment-related risks (e.g. CVL as a strong pro-thrombotic risk factor)
- recent study reported no effect of anti-thrombotic prophylaxis with warfarin, heparin or aspirin on the incidence of thrombotic events (50% associated to CVL)
- preventive anticoagulation should be considered during states of increased thrombosis risk (owing to acute illness, risk of dehydration, inserted central lines and/or thrombocytosis >750,000/ml) and/or in patients with a previous thrombosis
- No recommendation on the therapeutic agent:
    - Low molecular weight heparins may be ineffective due to low antithrombin III levels
    - Warfarin is used in Finnish patients without bleeding comlications
    - Magnesium and calcium supplements to avoid low levels (which may promote thromboses)

Dufek et al, Nephrol Dial Transplant 2019, 34: 1369–1377

Boyer et al, Nature Reviews Nephrology 2021 Apr;17(4):277-289

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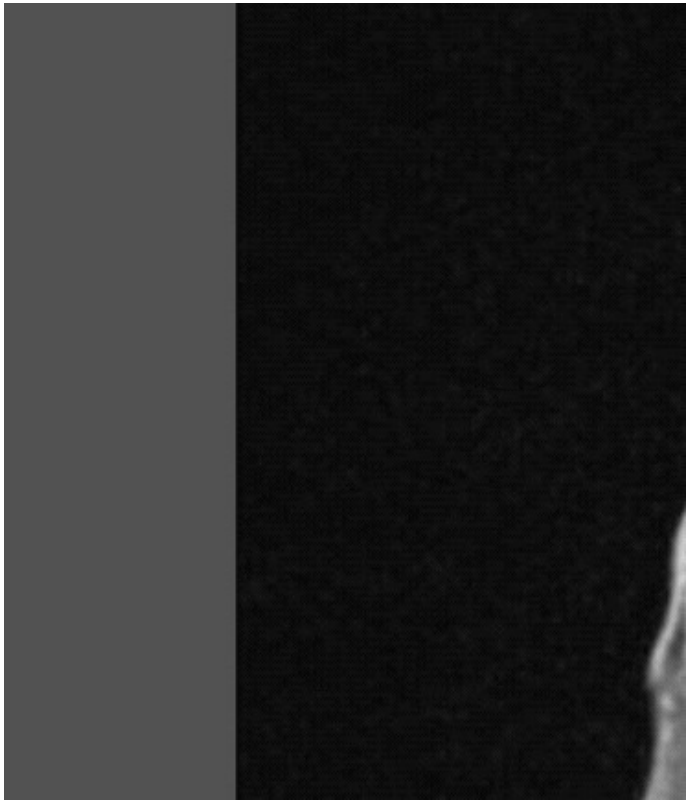
A case from Cologne

- Preterm birth (gestational age 33 weeks)
- heavy proteinuria
- *Severe superior sagittal sinus thrombosis + Transverse sinus thrombosis*
- *Intraventricular hemorrhage (IVH)*
- Transfer to Cologne at the age of 4 weeks



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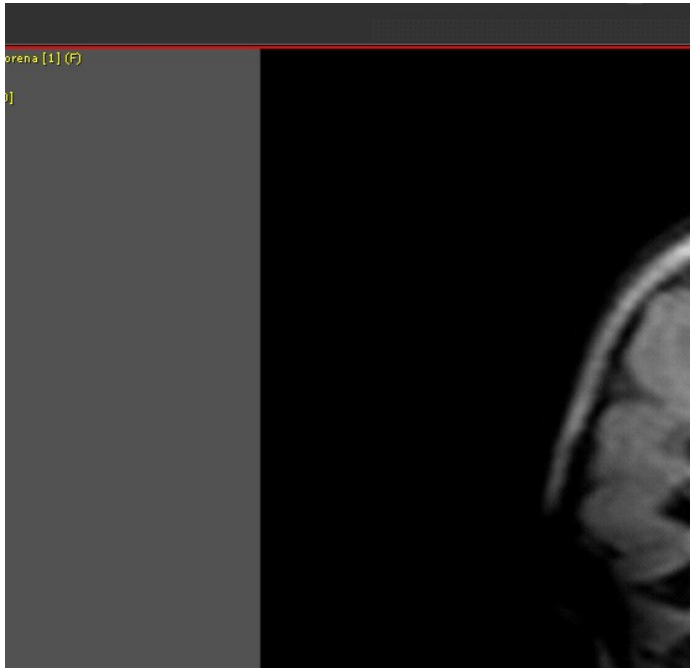
A case from Cologne

- continuation of heparin, later low.molecular-weight heparin
- ventriculoperitoneal (VP) shunt
- MRI shows cystic formation, **Periventricular Leukomalacia**



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A case from Cologne

- Identification of a homozygous mutation in NPHS1 (Nephrin)

Immunosuppression and Renal Outcome and Pediatric Steroid-Resistant Nephrotic

Anja K. Büscher,* Birgitta Kranz,† Rainer Büscher,* Friedheln
Bernd Dworniczak,§ Petra Pennekamp,§ Eberhard Kuwertz-B
Anne-Margret Wingen,* Ulrike John,‖ Markus Kemper,¶ Leo
Stefanie Weber,* and Martin Konrad†
*Pediatric Nephrology, Pediatrics II, University of Duisburg-Essen, Essen, Gern

CJASN 2010

E5	M	DelTCAinsCC2617 (H);2552C>T (H)	L904X and *A851V*	CNS	N	Finnish type	40	RTx
E6	F	DelTCAinsCC2617 (H);2552C>T (H)	L904X and *A851V*	CNS	0	ND	—	CRI
E8	F	DelTCAinsCC2617 (H);2552C>T (H)	L904X and *A851V*	CNS	0	ND	—	CRI
E12	F	DelTCAinsCC2617 (H);2552C>T (H)	L904X and *A851V*	CNS	N	ND	49	RTx
E14	F	DelTCAinsCC2617 (H);2552C>T (H)	L904X and *A851V*	CNS	0	MC	23	RTx
E15	M	DelTCAinsCC2617 (H);2552C>T (H)	L904X and *A851V*	CNS	0	ND	23	RTx

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Management of CNS – infections and immunoglobulins

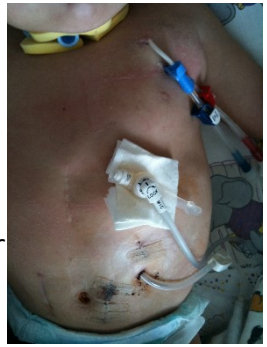
- Infections are one of the major causes of death in children with CNS
- high loss of IgG and complement proteins via urine
- Prophylactic antibiotics have not been shown to be effective to avoid sepsis -> no prophylactic antibiotic treatment but prompt initiation in cases of suspected bacterial infection

- 50% of infused IgG is lost within one day!
- However, preventive IgG infusions may be considered in patients with low circulating IgG levels and recurrent and/or severe infections

Boyer et al, Nature Reviews Nephrology 2021 Apr;17(4):277-289

Management of CNS – alimentation

- Calory intake is of utmost importance!
- ideally help of an expert dietician
- enteral tube feeding in those with insufficient oral nutrition
- diet with high energy (130 kcal/kg per day) and high protein content (4 g/kg per day) but low salt content (<0.5 g per day in babies aged <6 months, <1 g per day in infants aged 7–12 months, <2 g per day in children aged 1–3 year)



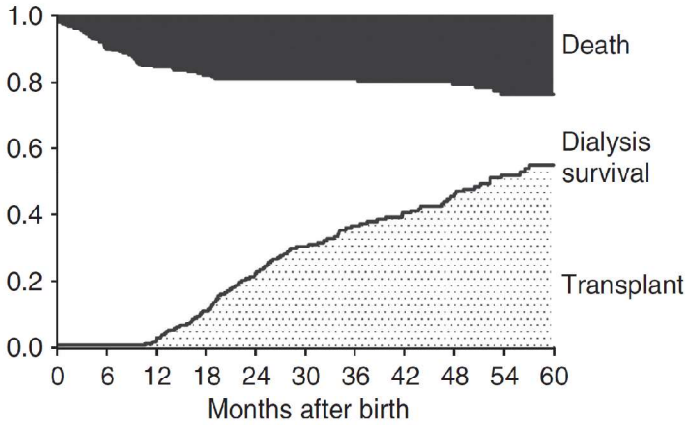
Boyer et al, Nature Reviews Nephrology 2021 Apr;17(4):277-289

Management – Kidney failure

- The use of dialysis in children with CNS should follow the general guidelines for kidney replacement therapy in infants and children.

- good outcome also with dialysis initiation during the first month of life

- Peritoneal dialysis is the mode of choice in CNS patients



Van Straalen et al, Pediatrics 2014

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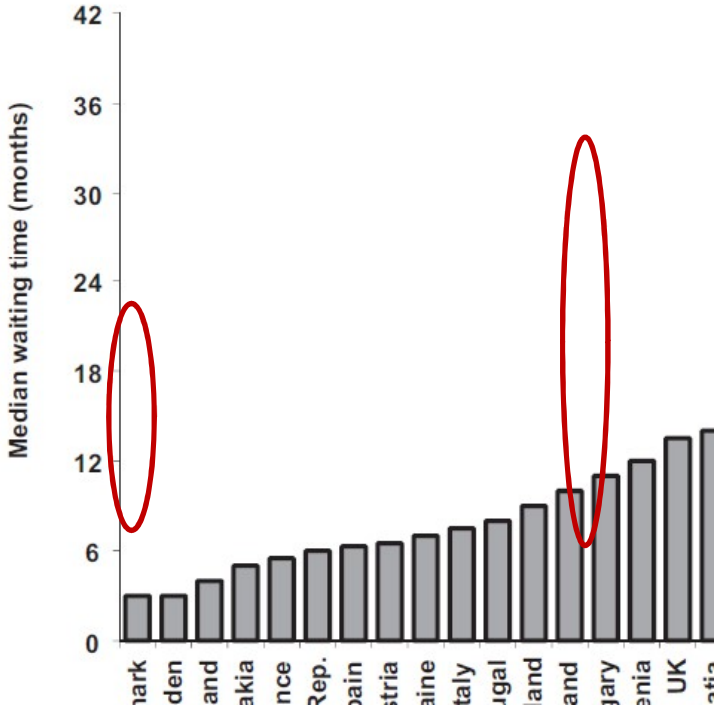
Management – Kidney transplantion



Harambat et al, Am J of Transpl 2013

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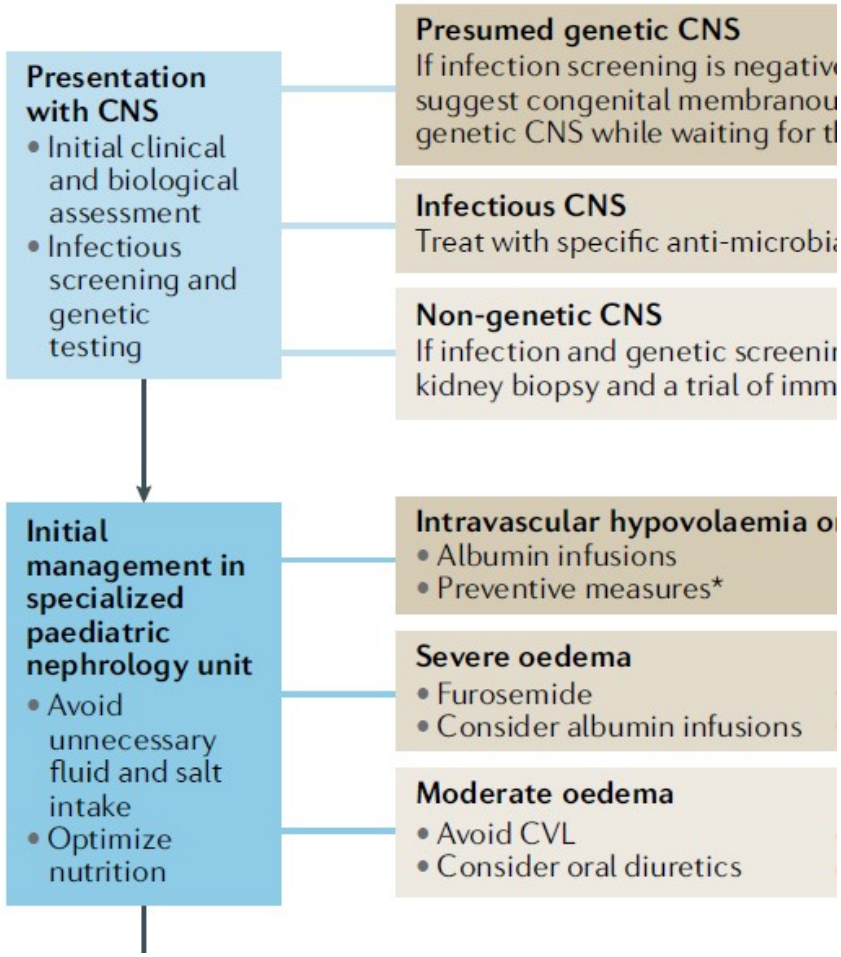

*Preventive measures: prophylaxis for thrombosis, infection and anaemia, adequate nutrition and growth hormone substitution.

Boyer et al, Nature Reviews Nephrology 2021 Apr;17(4):277-289

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Conclusion - CNS

- comprises a **wide spectrum** of clinical phenotypes

- **management should be based on the clinical severity**, the course of disease and the opportunities at your center (waiting time for KTX etc)
  - infants with no or minimal symptoms -> avoid aggressive and potentially dangerous treatments
  - critically ill infants -> rapid and intensive symptomatic treatments to avoid complications

Thank you!

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

RARE DISEASES IN PEDIATRIC NEPHROLOGY

(IPNA Sponsored Teaching Course), Skopje, April 5-6,2024

Renal glucosuria and beyond

Velibor Tasic

University Children's Hospital Skopje, N. Macedonia

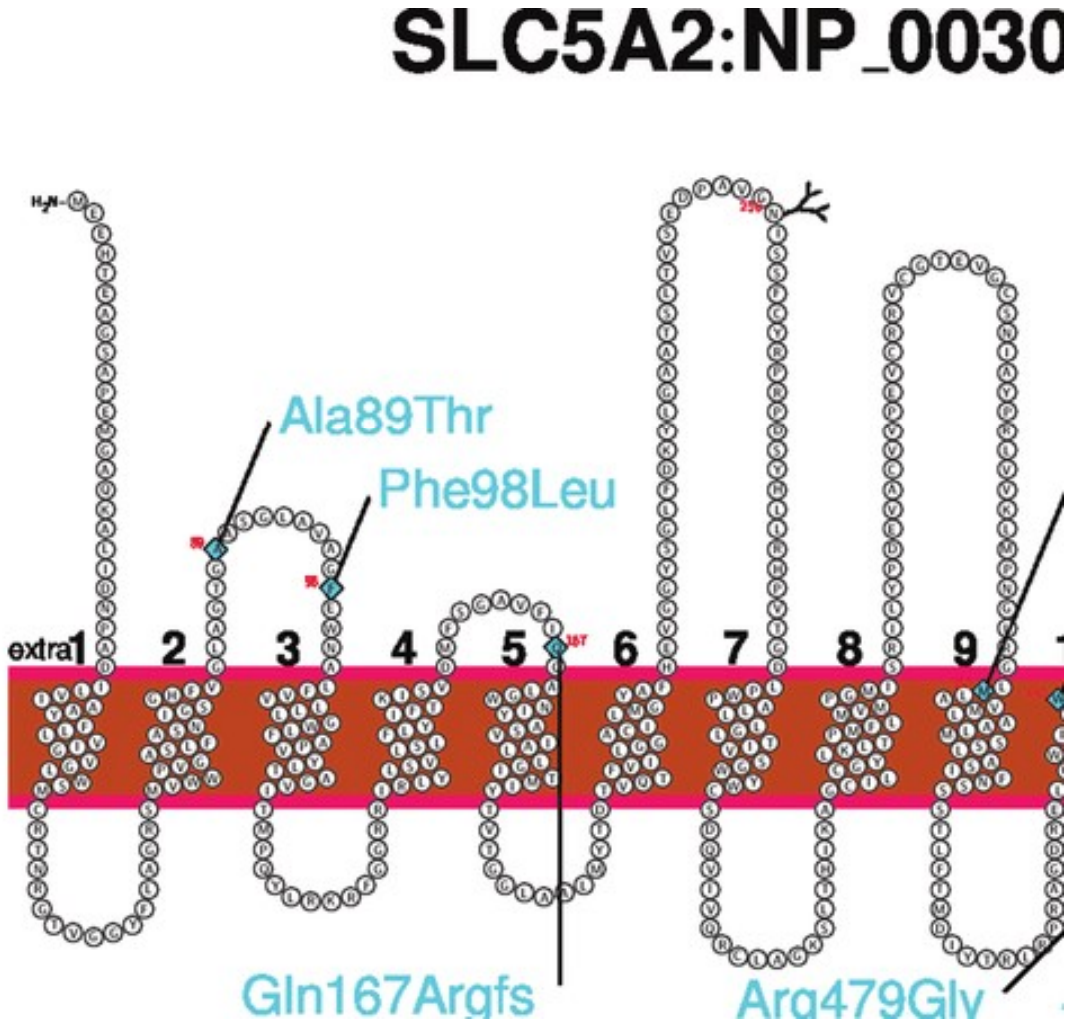
Renal glucosuria

- Renal glucosuria is glucose in the urine without hyperglycemia; it results from either an acquired or an inherited, isolated defect in glucose transport or occurs with other renal tubule disorders.
- In this lecture we will focus on isolated renal glucosuria (familial renal glucosuria-FRG)

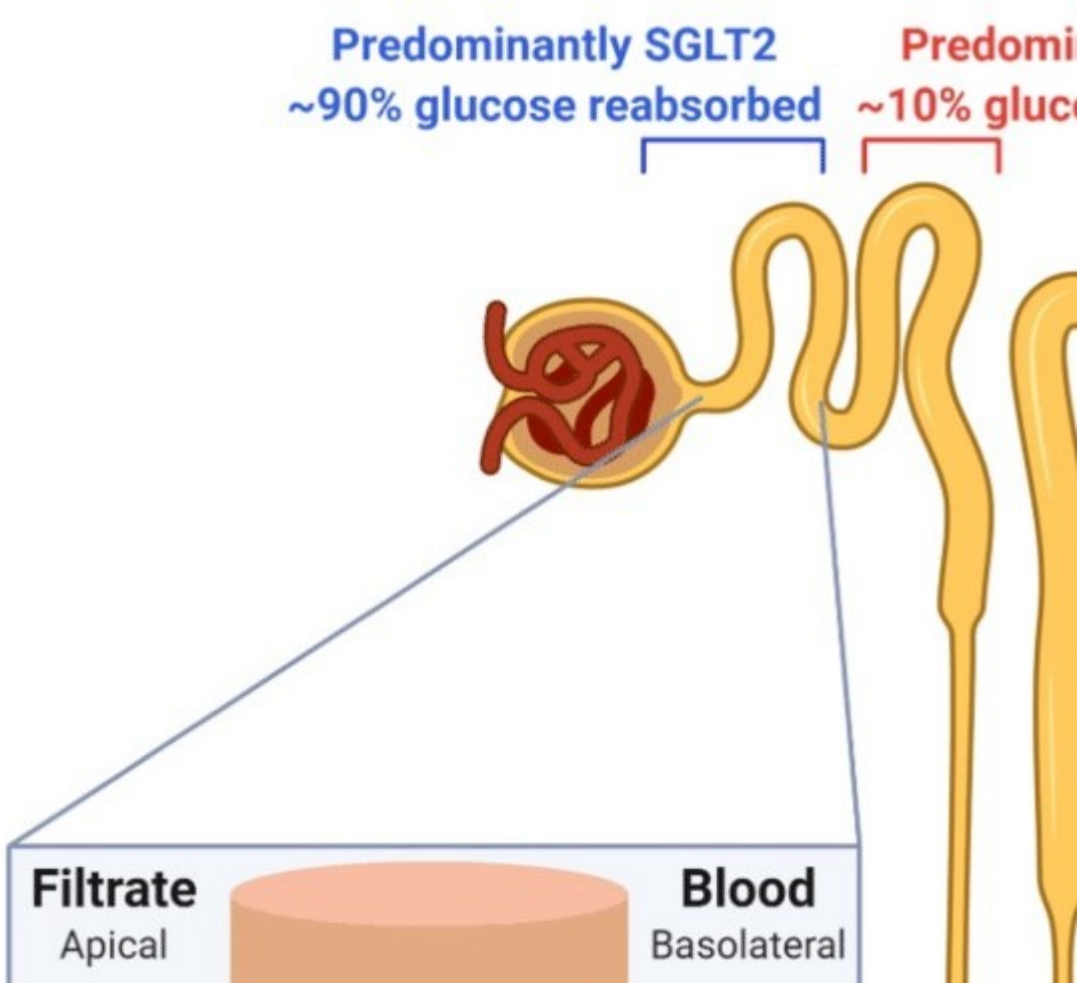
Familial renal glucosuria (FRG)

- It is most commonly due to mutations in the SLC5A2 gene coding for the glucose transporter SGLT2 in the proximal tubule.
- Familial renal glucosuria can be inherited in an autosomal recessive or autosomal dominant pattern.
- To date, over 86 mutations of the SLC5A2 have been identified, including missense mutations, nonsense mutations, small deletions, and splicing mutations.
- Individuals with 2 mutations usually have a more severe phenotype with greater glucose wasting compared to those with 1 mutation

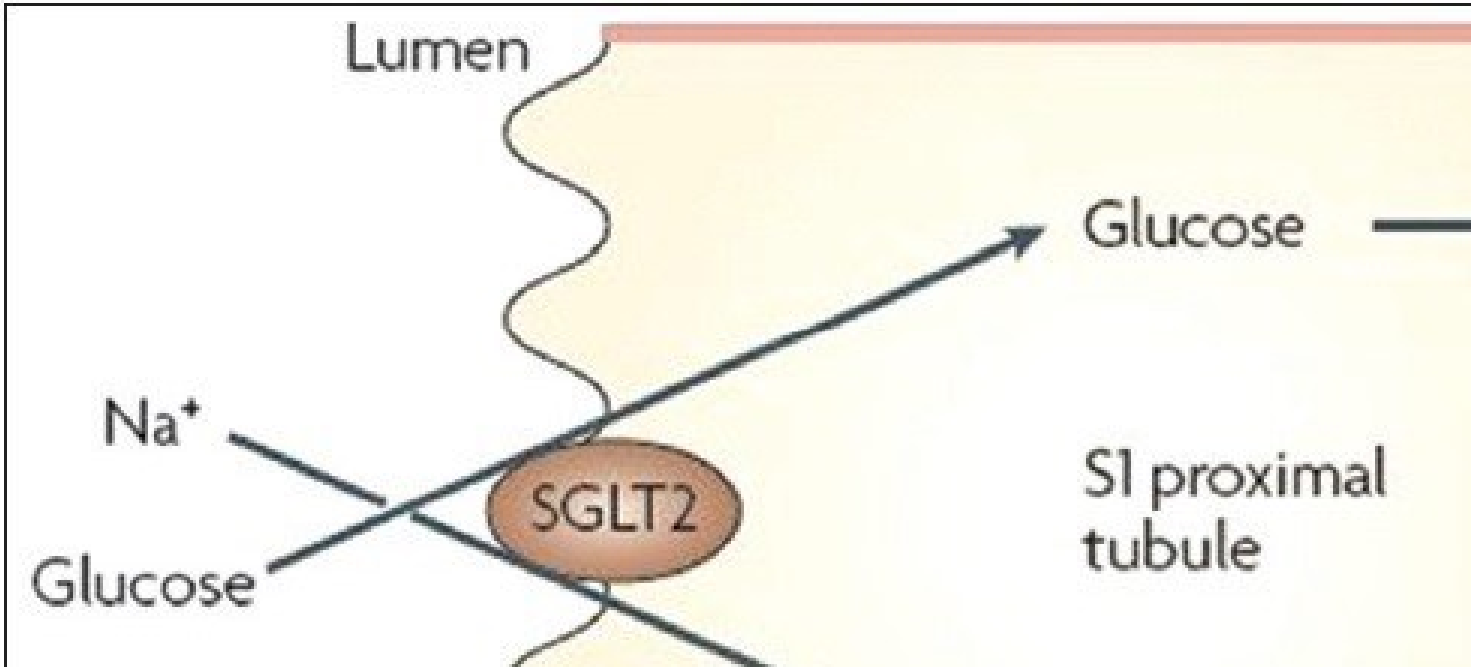
Structure of the SLC5A2 gene



Pathophysiology



Pathophysiology



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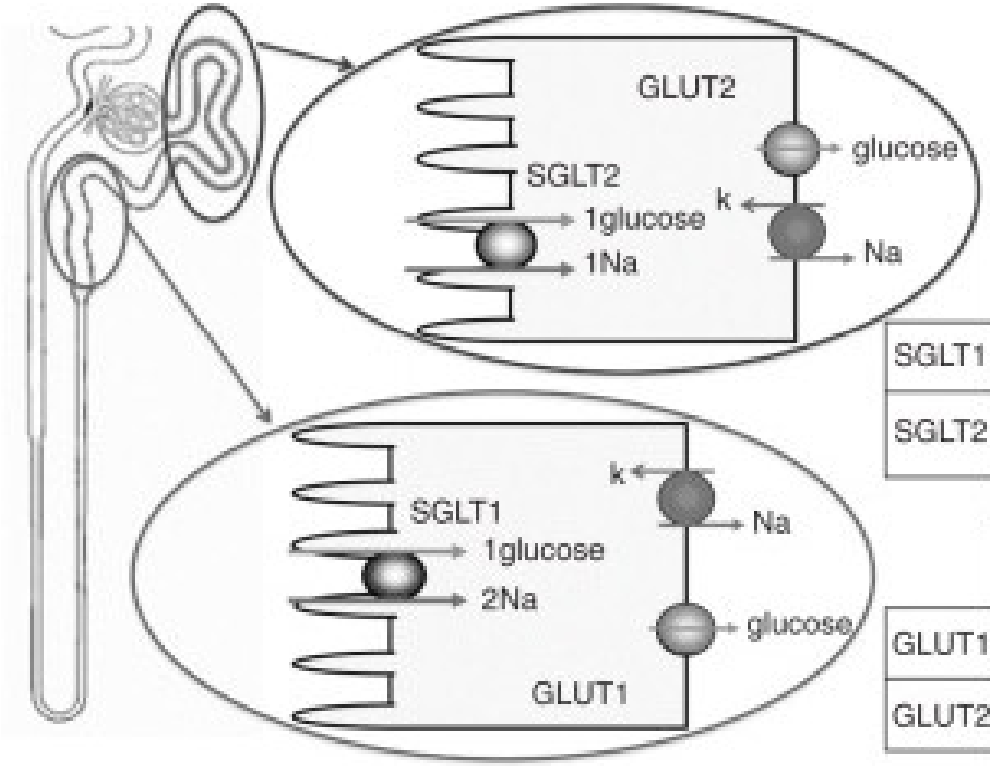
Pathophysiology



	Km glucose
SGLT1	0.35mM
SGLT2	1.6mM

	Km glucose
GLUT1	1-2μM
GLUT2	15-20μM

Clinical features of FRG

- Polyuria and enuresis and later a mild growth and pubertal maturation
- Episodic dehydration and ketosis during pregnancy and starvation
- Presence of several autoantibodies without clinical evidence of autoimmune disease
- An increased incidence of urinary tract infections
- Activation of the renin-angiotensin-aldosterone system, secondary to natriuresis and possible extracellular volume depletion
- Hypercalciuria
- Selective aminoaciduria
- Hypouricemia
- Personal comment – Apart from hyperaminoaciduria none were seen by the author of this presentation

Genetic and clinical characterization of familial renal glucosuria

KEY LEARNING POINTS

What was known:

- Familial renal glucosuria (FRG) is caused by variants in SLC5A2, the gene encoding
  The genotype–phenotype relationship in FRG patients has not been systematicall
- SGLT2 inhibitors, a novel class of antidiabetic agents with cardiac and renal protec
  2 diabetes. Detailed phenotyping of FRG patients might shed light on the long-ter

This study adds:

- We provide a visualized summary of the genotype–phenotype relationship in FRG
  by variant type and variant location. Homozygous missense variants, especially t
  lead to severe glucosuria, highlighting the role of key residues in the transport fu
- There is a potential functional link between SGLT2 and proximal tubule transpo

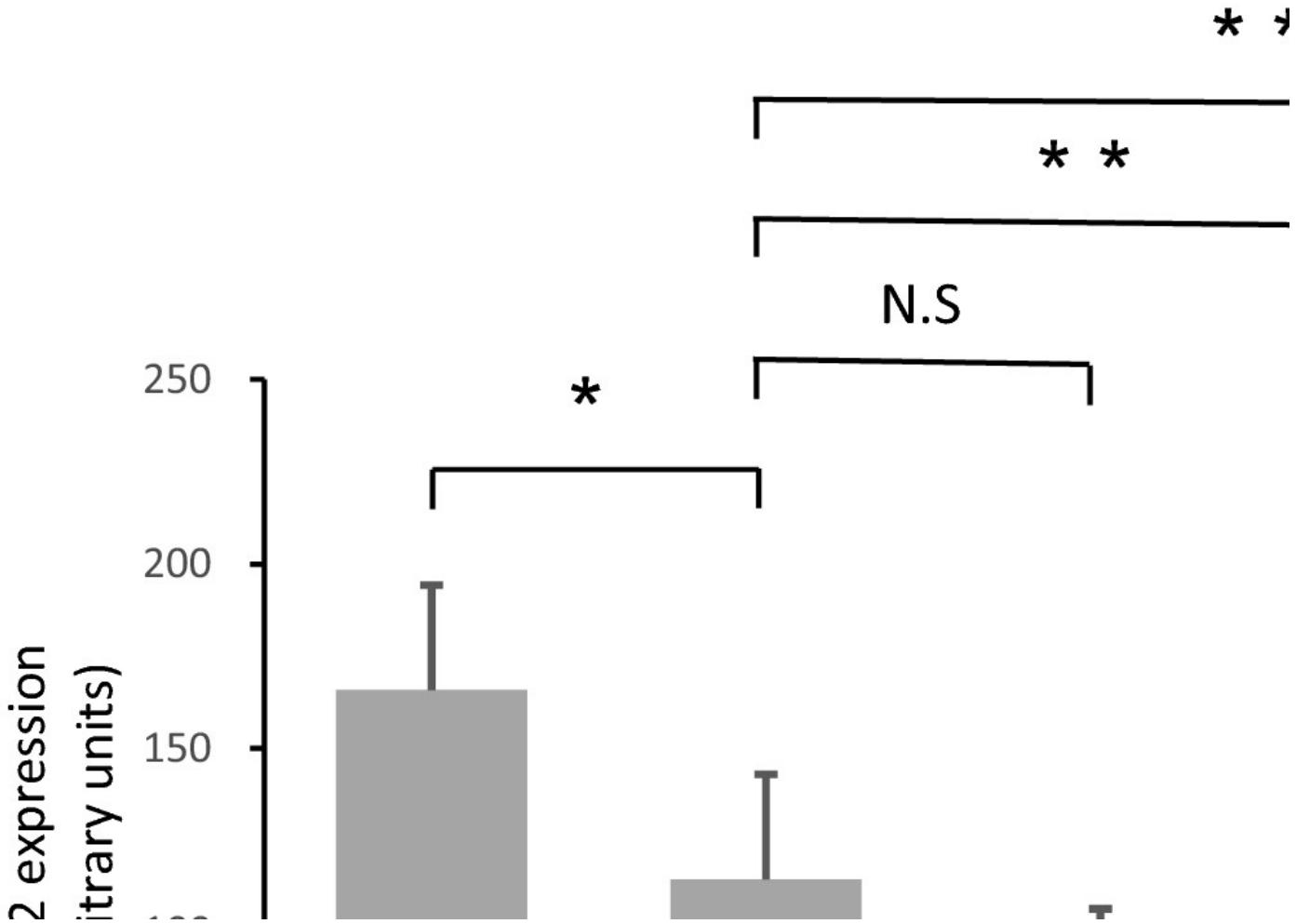
Xu L et a. Clin Kidney J. 2024

IPNA International Pediatric Nephrology Association
GREAT CARE FOR LITTLE KIDNEYS. EVERYWHERE

From rare to common diseases!

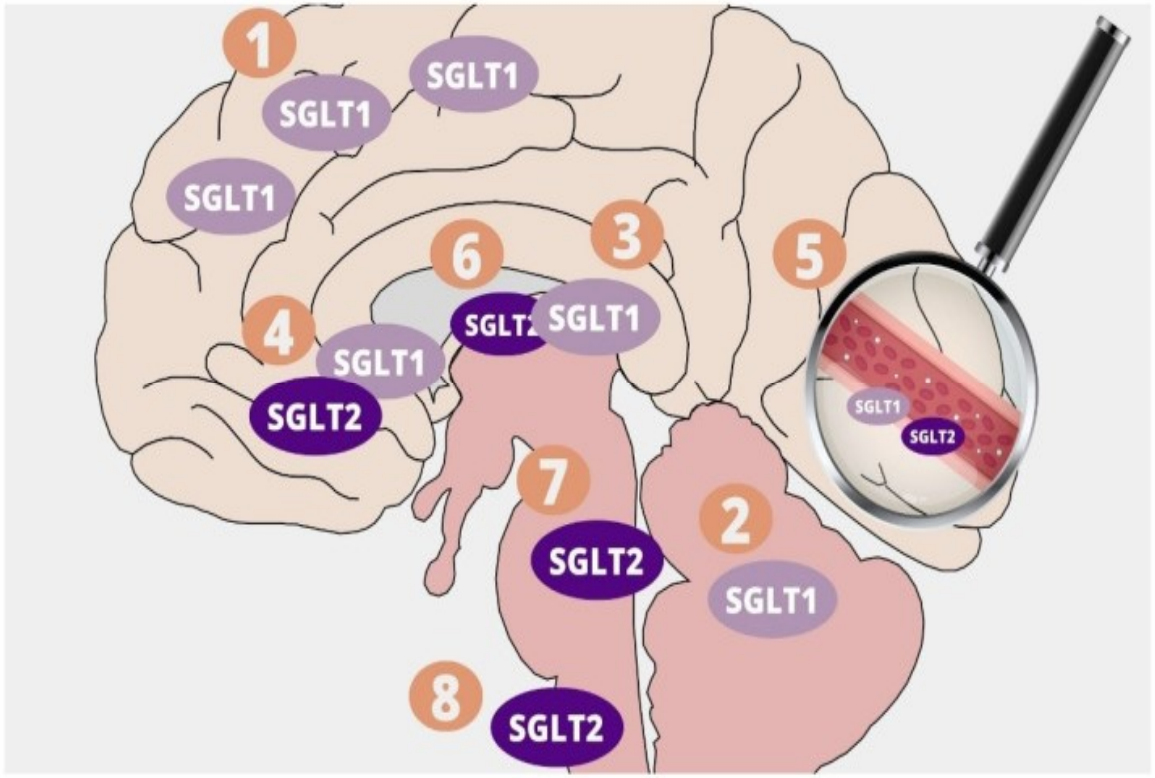
- FRG is a trivial disease with minimal clinical significance
- But the knowledge we have gained from studying the genetics and pathophysiology of FRG is of enormous importance to the community because it has led to the development of very important drugs called SGLT inhibitors.
- Today, these drugs are used to treat diabetes mellitus type 2, chronic heart failure and chronic kidney disease in adults.

SGLT2 expression in various tissues



Nakano et al. Medical Molecular Morphology2022

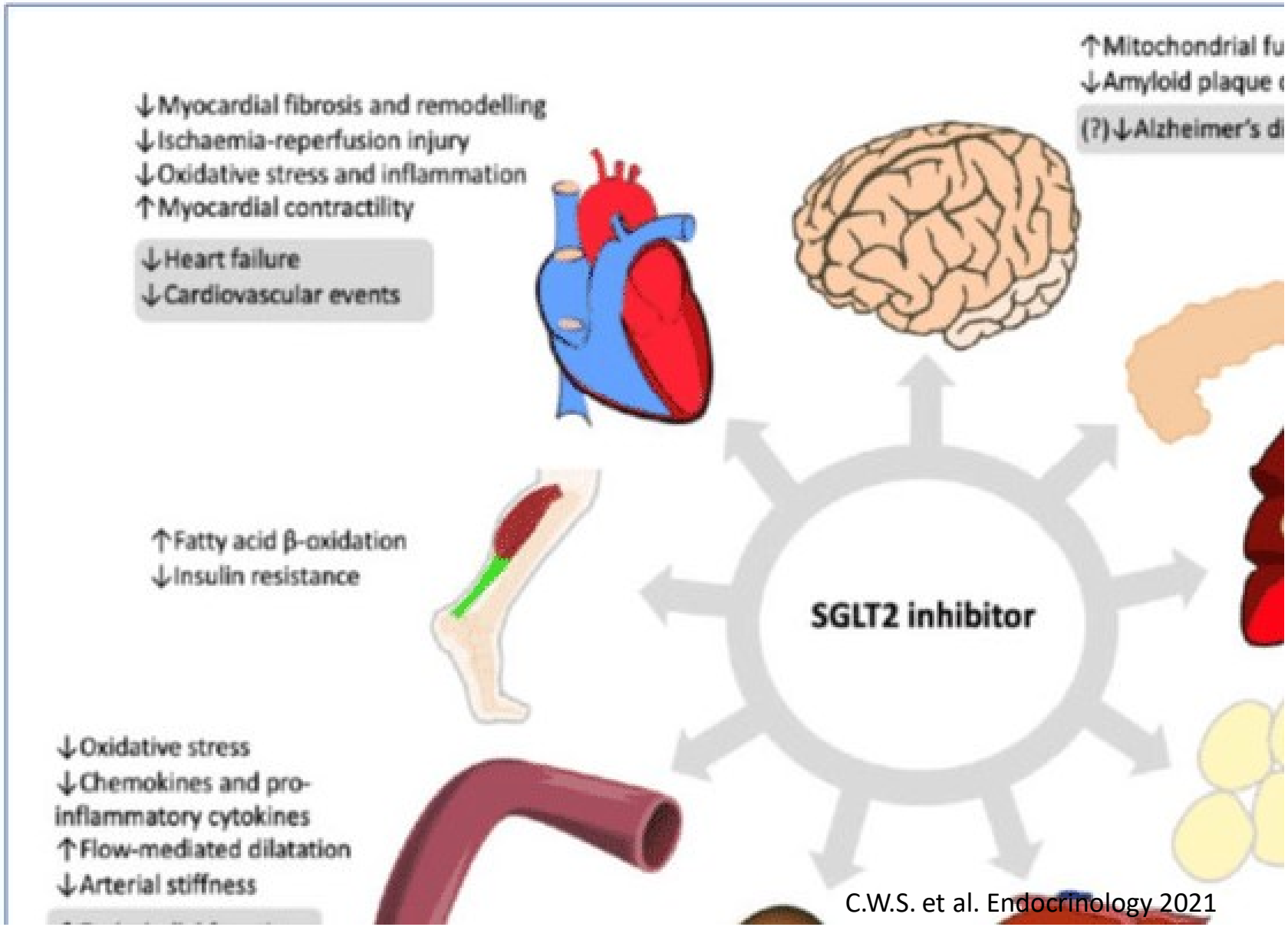
Neuroprotective effect of SGLT2 inhibitors



SGLT2i have also been shown to improve insulin sensitivity in the brain of obese rats by decreasing brain inflammation, brain apoptosis, and brain oxidative stress, with a final effect of improvement in mitochondrial brain function as well as a strong increase in hippocampus synaptic plasticity

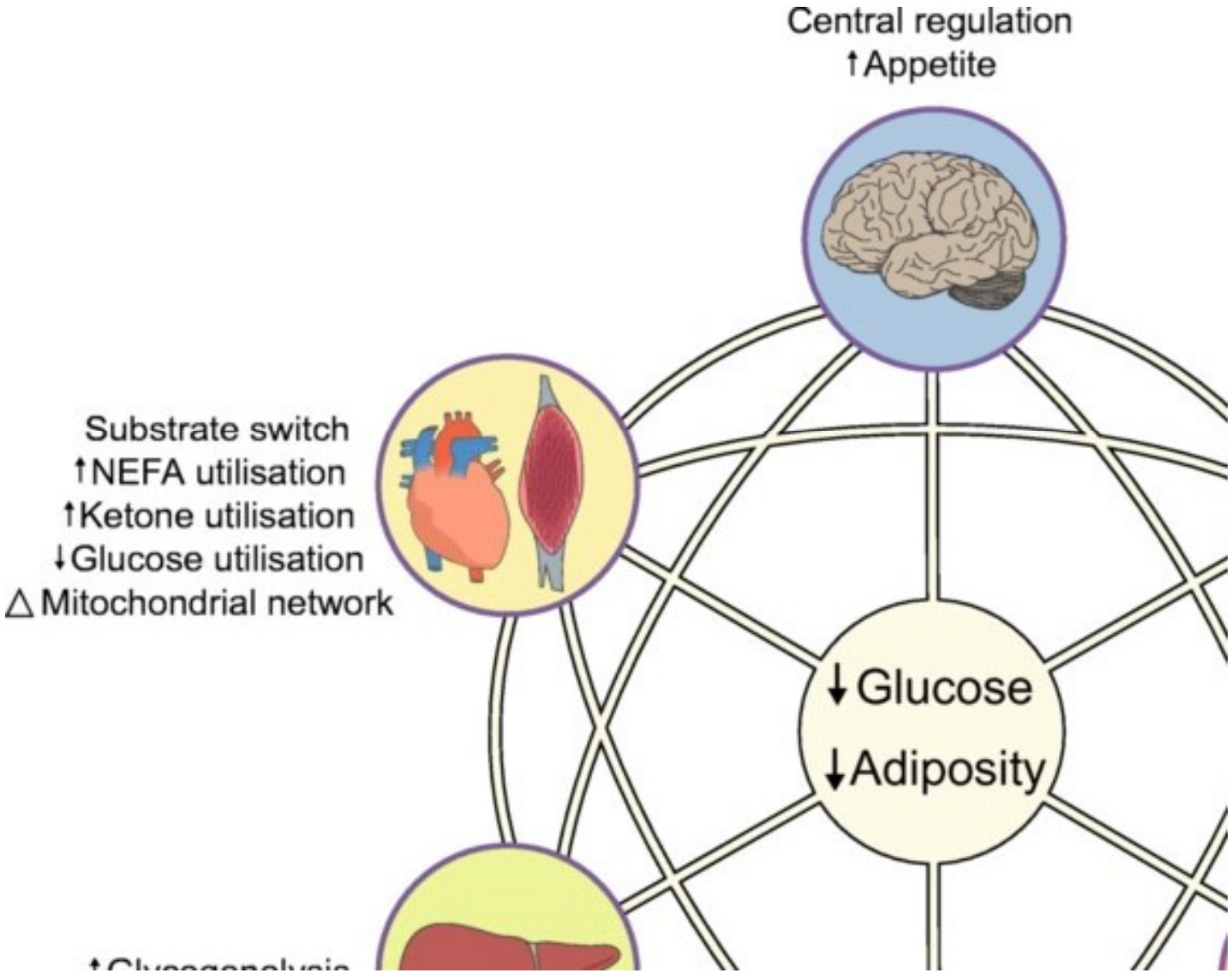
Pawlos et al, Molecules 2021

SGLT2 inhibition has pleiotropic effects on multiple organ systems



C.W.S. et al. Endocrinology 2021

Effect of SGLT2 inhibitors on metabolism, renal function and blood pressure



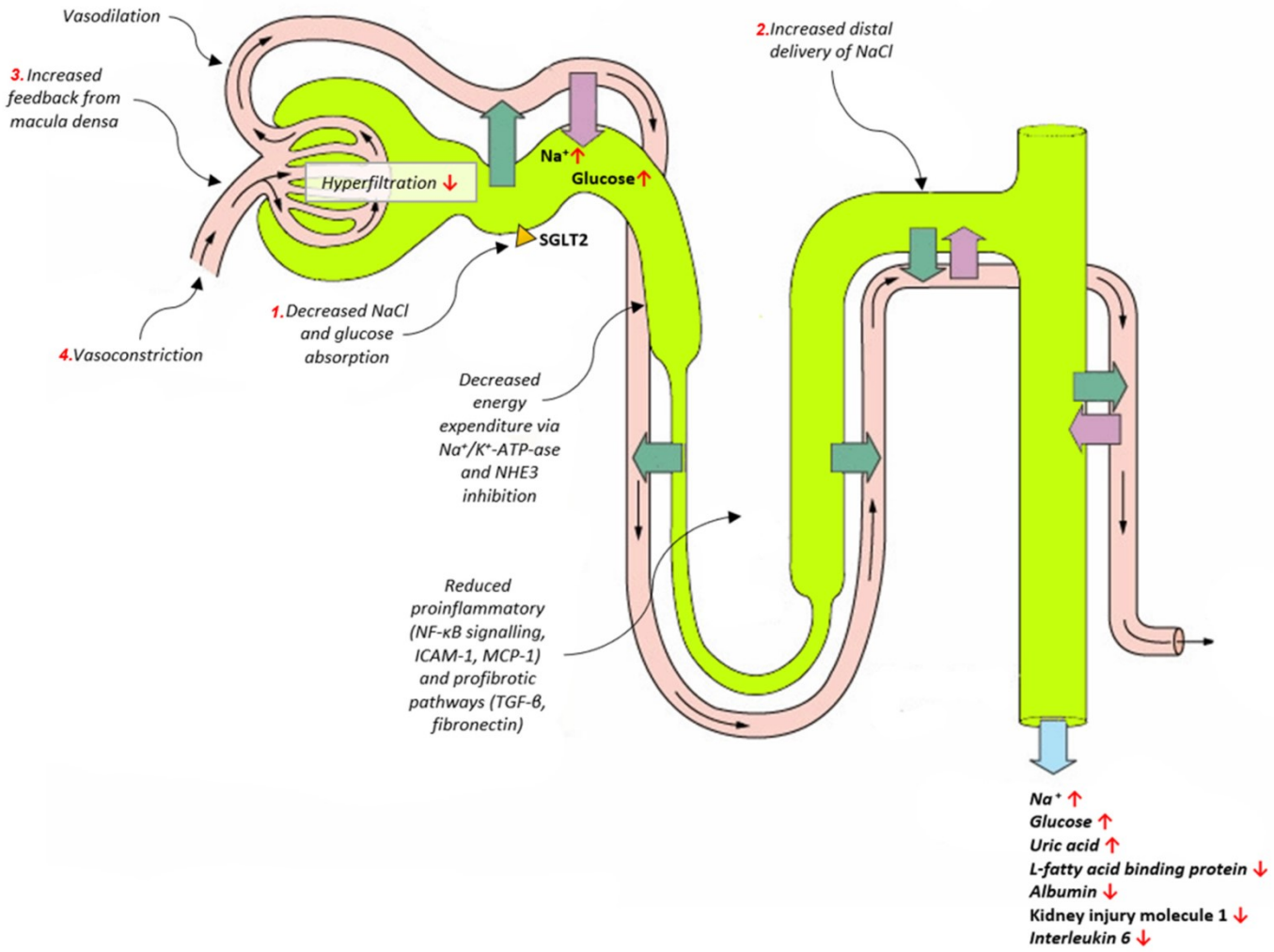
Thimas M and Cherney DZI. Diabetologia 2018

Renoprotective Mechanisms of Actions of SGLT2i

- Antihyperglycemic
- Anti-inflammatory → Decreasing inflammatory and reactive oxygen species.
- Antioxidant
- Promote tubule-glomerular feedback → Decrease glomerular hyperfiltration
- Activate adenosine mono-phosphate-activated protein kinase → Decrease glomerular and tubular injury
- Hemodynamic changes → Decrease albuminuria
- Improve lipid profile
- Reduce body weight
- Natriuresis → Mild decrease in systolic and diastolic blood pressure
- Attenuate renal ischemia-reperfusion injury
- Decrease serum uric acid

Abdelrahman et al. J Clin Med 2024

Mechanism of action of SGLT2i



Skrabic R et al. Biomedicines 2022

IPNA International Pediatric Nephrology Association
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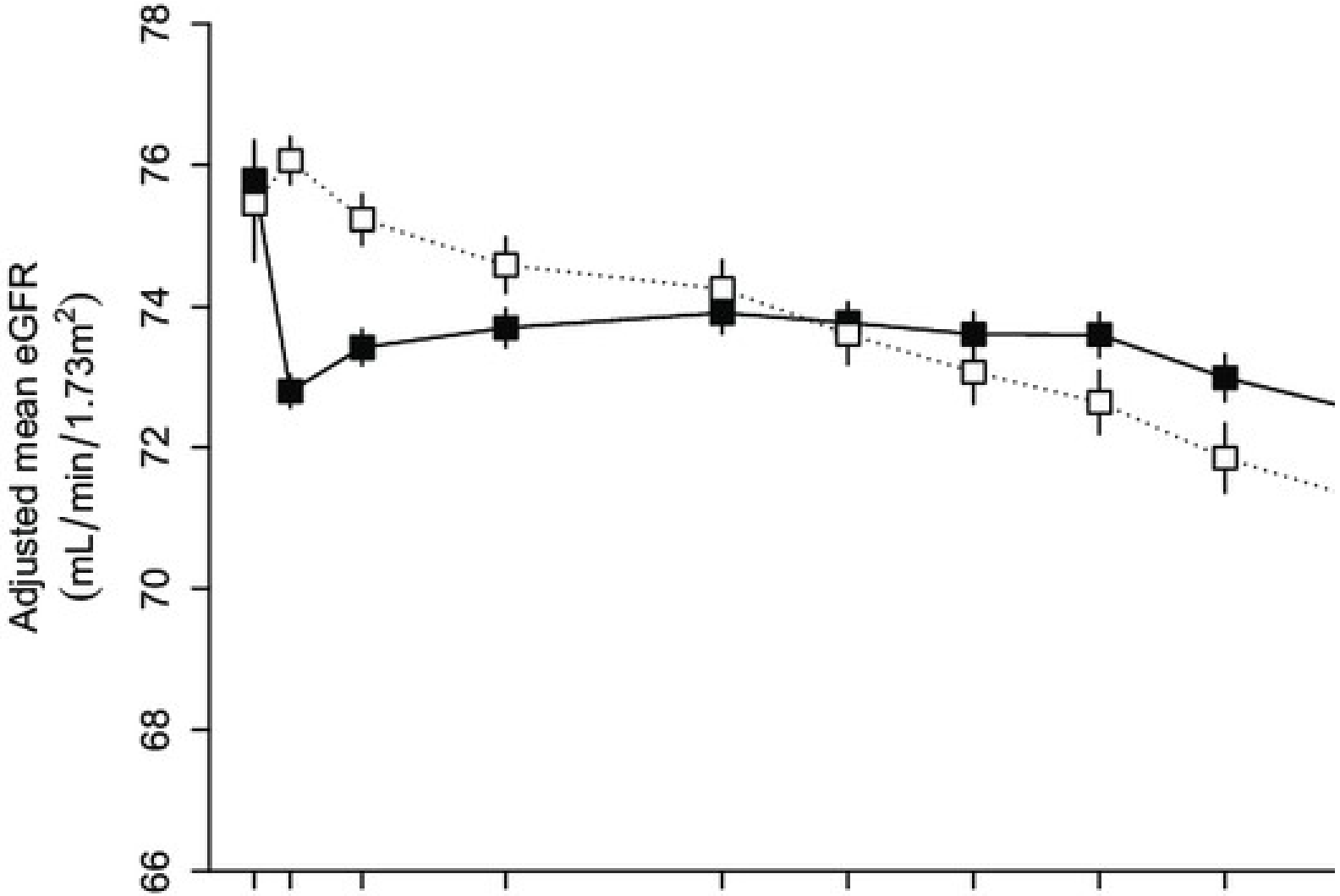
Efect of SGLTi in adult trials

- Empagliflozin-EMPA-REG-OUTCOME trial
- Canagliflozin-CANVAS (canagliflozin CV assesment study)

Both studies demonstrated:

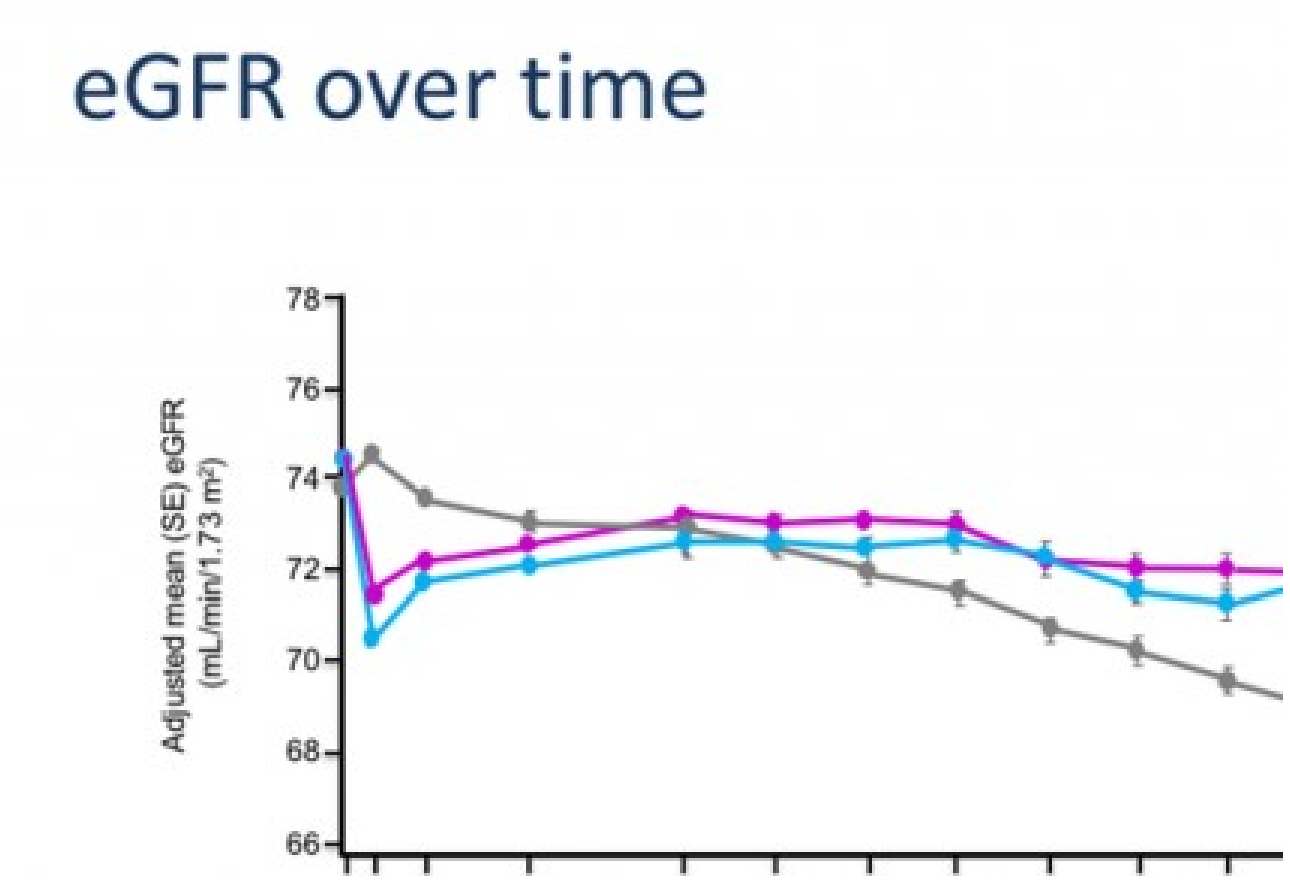
- Improved serum glucose/HbA1c
- Weight loss, better BP control
- Reduction in CHF prevalence
- Reduction in albuminuria (40-50%)
- After initial decline, better eGFR over time
- 20% better survival

SGLT2i and eGFR

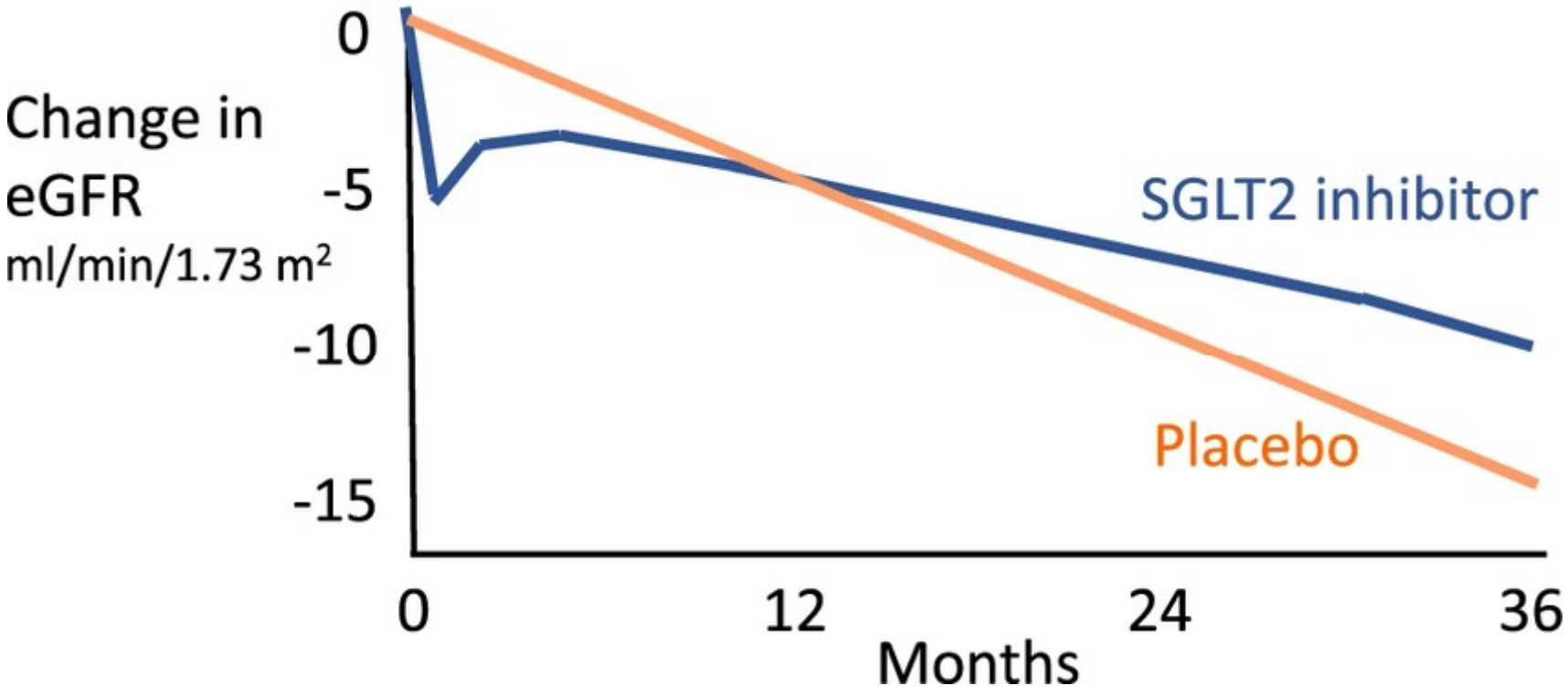


Zinman, N Engl J Med 2015

SGLT2i and eGFR



SGLT2i and eGFR



Cumulative data from EMPA-REG, CREDENCE and DAPA-CKD trials

Bailey CJ&Bellary S. Current Diabetes Report 2022

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Benefits and Side effects

Benefits

- Improved CV outcomes
- Decreased albuminuria
- Stabilization of eGFR

Side effects

- UTI’s
- Vaginal yeast infections
- Osmotic diuresis

SGLT2i in Kidney Disorders

- Underline mechanisms are not completely clarified
- These benefits are not due exclusively to glucose lowering effects
- Natriuresis and glucose induced osmotic diuresis lead to reduced intraglomerular pressure
- Improved hemodynamics preservs kidney function
- Additive benefits to RAAS inhibition
- High risks when administered at GFR <30ml/min/1,73m2.

SGLT2i in children with CKD

- FDA has not approved SGLT2i for children
- Reports in adolescents with T2DM show similar benefits to that reported in adults
- One should expect that children with CKD would have similar benefits as reported in adults
- Prospective controlled trials are warranted

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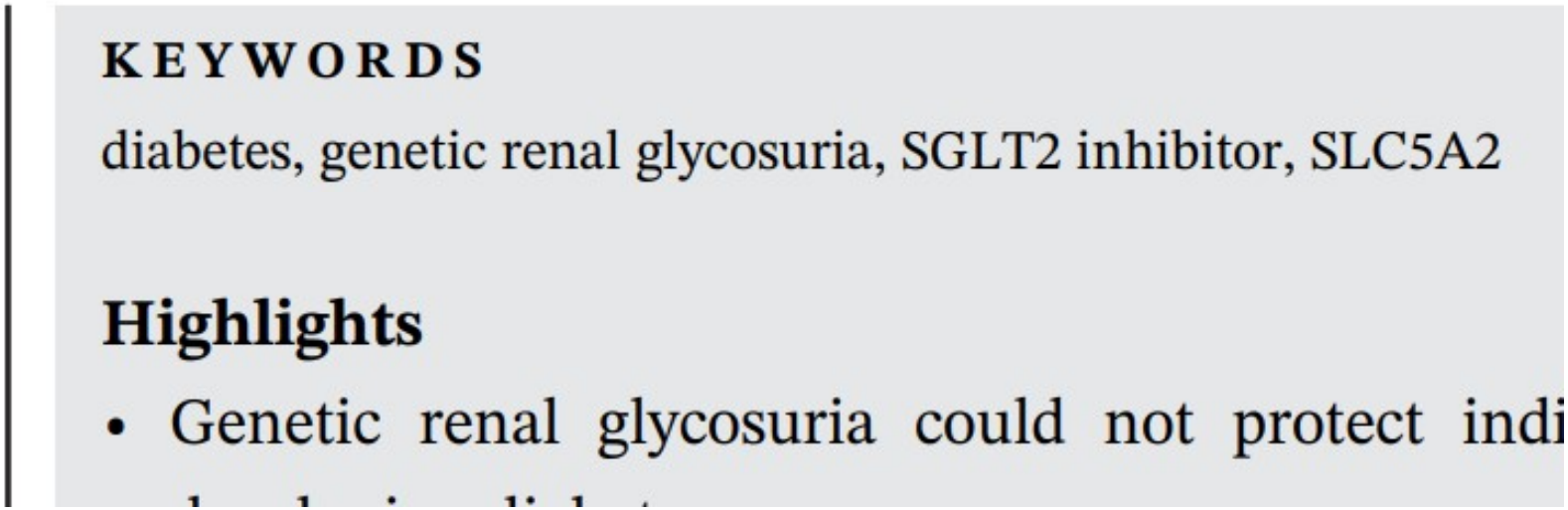
Appeal for pediatric trials

Side effects of SGLT2i (adult trials)

- Hypoglycemia
- Diabetic ketoacidosis
- Genital mycotic infections
- Urosepsis and pyelonephritis
- Acute kidney injury
- Fournier gangrene
- Hyperkalemia

Can SGLT2 gene mutation protect from diabetes?

Journal of Diabetes
Open Access



KEYWORDS

diabetes, genetic renal glycosuria, SGLT2 inhibitor, SLC5A2

Highlights

- Genetic renal glycosuria could not protect indi

Patient 1 –glucosuria from 2,2 g to 103 g/d after SGLT2i

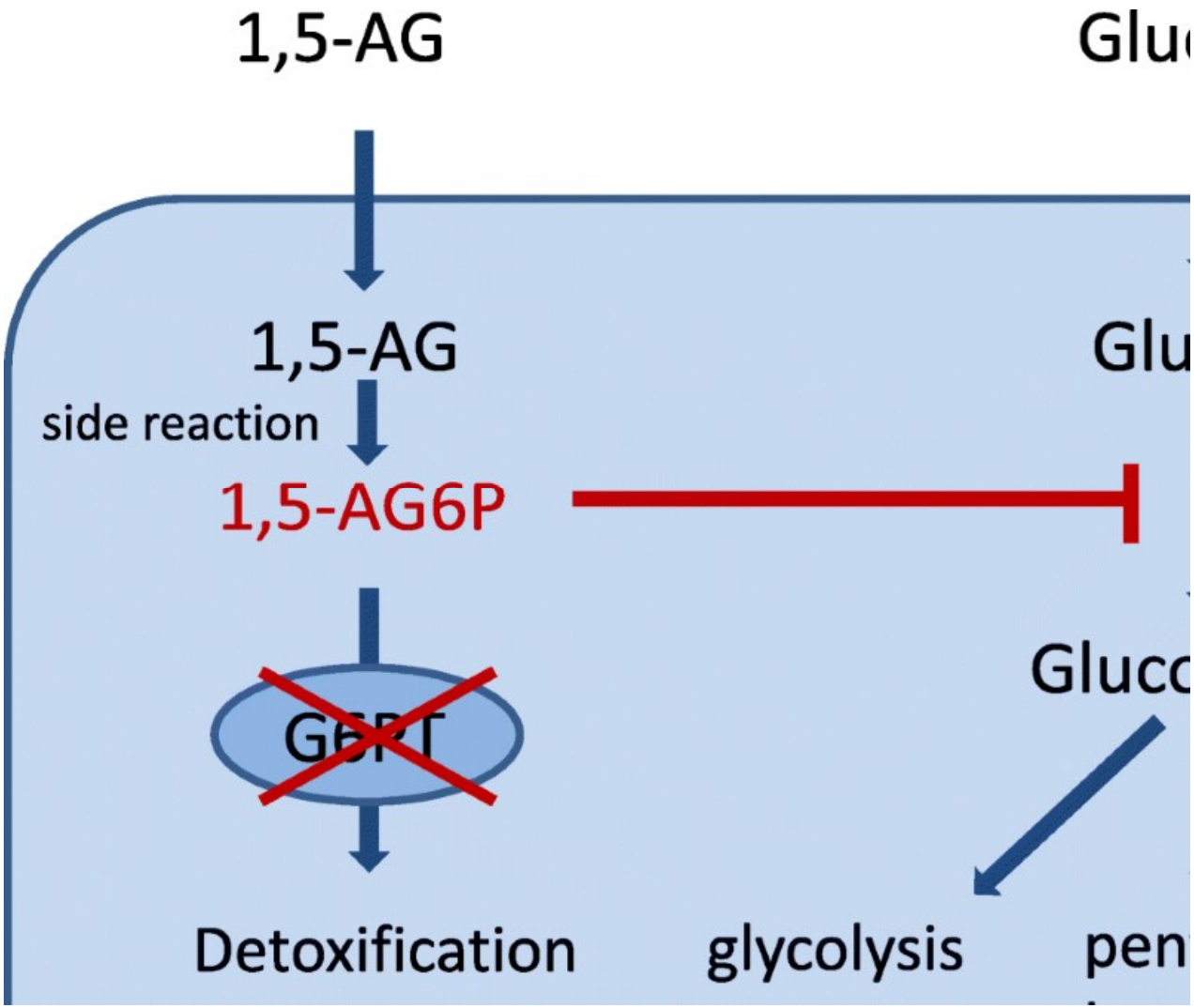
Patient 2 – glucosuria from 121.4 g to 185.8 g/d after SGLT2i

Ren Q et al. Journal of Diabetes 2022

SGLT2i and Glycogen Storage Disease Type Ib (What we learned)

- Glycogen storage disease 1b is caused by biallelic variants in SLC37A4
- SGLT2-inhibitor empagliflozin has provided a mechanism-based treatment option for the symptoms caused by neutropenia/neutrophil dysfunction (e.g. mucosal lesions, inflammatory bowel disease).
- SGLT2 is also responsible for the reabsorption of 1,5-anhydroglucitol (1,5AG). SGLT2 inhibitors were shown to decrease plasma 1,5AG and restore a normal neutrophil count and function

Patophysiology of Glycogen Storage Disease 1b



Sarah C. Grünert SC et al. Orphanet Journal of Rare Diseases 2020

Adverse events in children with GSD 1b treated with empaglifozin

Serious

- ❑ Hypoglycemia (18%) ???
- ❑ Lactic acidosis (4%) ???
- ❑ Ketoacidosis (0%)
- ❑ Anaphylactic reaction (1%)

Milder

- ❑ Fungal genital infections (1%)
- ❑ UTI (6%)
- ❑ Skin rashes (3%)
- ❑ Pruritus (1%)
- ❑ Dehydration (0%)

Grunert SC et al. Genetics in Medicine 2022

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Instead of conclusion

- We learned very much from a trivial disease FRG
- We should learn about the long term outcome of FGR; are these patients protected from T2DM, CHF and CKD?
- We should strongly advocate realization of controlled SGLT2i trials in children with CKD
- Our current research focus: is there difference in serum/urinary proteomics between FRG patients and those treated with SGLT2i

I was happy to collaborate with J Calado, who did a great research in FRG. After 15 years internet friendship we met first time in vivo in Bern last year.

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NEPHROPATHIC CYSTINOSIS – AN UPDATE

YAACOV FRISHBERG, MD

SHAARE ZEDEK MEDICAL CENTER

JERUSALEM, ISRAEL

SKOPJE APR052024

DISCLOSURES

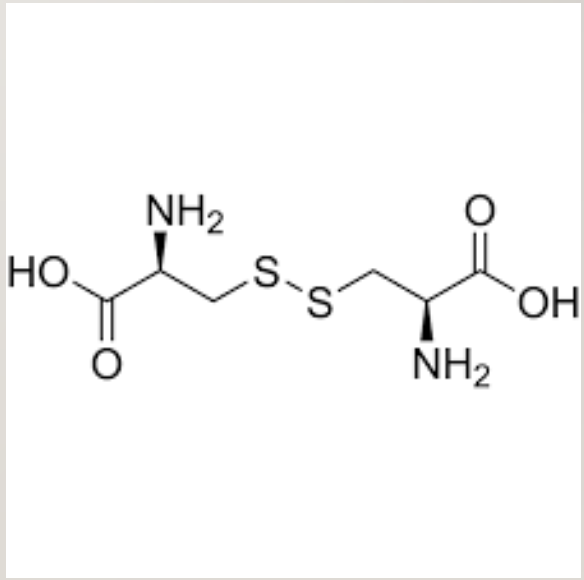
- None with respect to this talk

CYSTINOSIS

- Rare recessively inherited multi-system lysosomal storage disorder
- Incidence 0.5-1:100,000 live births
- Caused by mutations in *CTNS*, encoding cystinosin
- Cystinosin is a lysosomal cystine/H^+ symporter
- Progressive accumulation of lysosomal cystine in most tissues
- Nephropathic cystinosis (OMIM 219800) is the most common and severe form
- Other forms [continuum] : intermediate- and ocular-cystinosis

CYSTEINE –CYSTINE CONVERSION

- Cysteine is a non-essential amino acid containing a thiol group
- Cystine is a dimer of 2 cysteine molecules covalently linked by a di-sulfide bond
- Cystine is insoluble and precipitates if >2mM



THE *CTNS* GENE

- Mapped to 17p13
- 12 exons, 367 aa
- A 57,257-bp deletion removing the first 9 exons and part of exon 10 of CTNS
- Present in 50% of European cystinosis patients
- Carrier frequency
  - USA 1:200
  - Germany 1:370

PATHOLOGY

- Lysosomal cystine crystals rectangular/hexagonal
- Birefringent under polarized light
- Interstitial inflammatory cell infiltrates → fibrosis
- Narrowing of kidney tubules – swan-neck deformity → glomerlo-tubular disconnection
- Glomerular endothelial proliferation, necrosis, hyalinization
- Crystals within glomeruli (multi-nucleated podocytes) → FSGS

EARLY CLINICAL MANIFESTATIONS

- Fanconi syndrome diagnosed during the second half of the 1st year of life
  - Polyuria, polydipsia, dehydration
  - Poor appetite, nausea, vomiting
  - Phosphaturia, hypercalciuria → Rickets
  - Symptomatic hypocalcemia [after alkalinizing therapy]
- Growth impairment
  - Decreased weight and height percentiles between 6-12 months of age
  - Combination of CKD, acidosis, poor caloric intake, rickets
- Glomerular involvement → progressive CKD [kidney failure ~ 10yrs]

DIAGNOSIS

- Phenotype: Fanconi syndrome, polyuria, FTT, short stature, rickets, corneal crystals
- Family history
- Elevated WBC/neutrophils cystine levels [expressed as nmol half-cystine/mg protein]
- Trough levels
- Genetically-confirmed diagnosis

CYSTINOSIS IN ADULTS

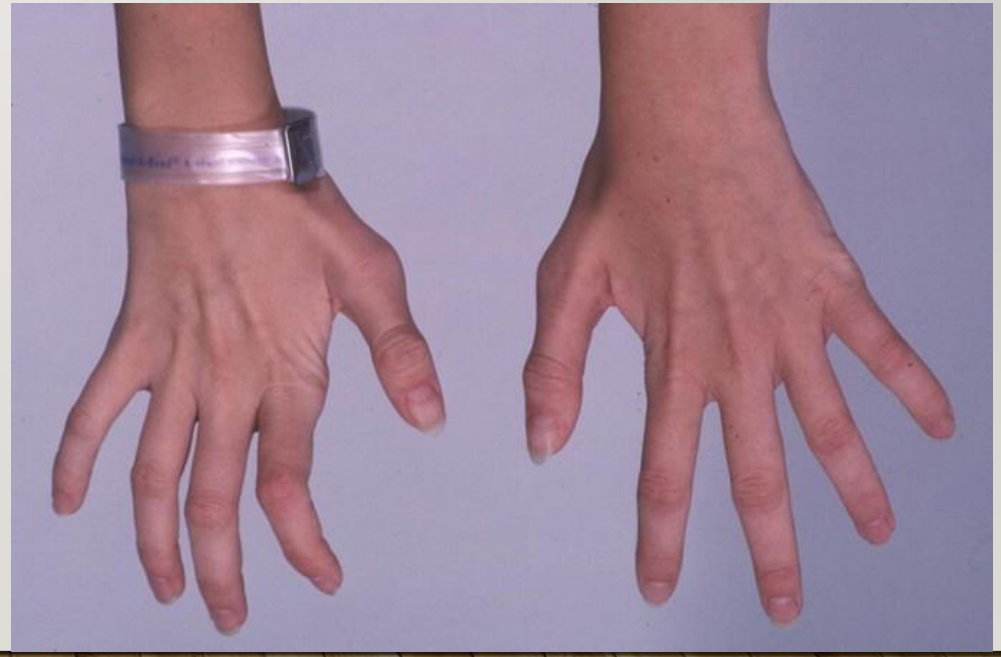
Table 2 Frequency of complications in 100 adults with cystinosis[a]

Finding frequency	(%)
Hypothyroidism	75
Male hypogonadism	74
Pulmonary dysfunction	69
Swallowing abnormality	60
Myopathy	50
Retinopathy	32
Vascular calcifications	31
Diabetes mellitus	24
Cerebral calcifications	22

[a]92% of patients had received a renal allograft; many had not benefited from early treatment with cysteamine

MYOPATHY

- Myopathy – starting in hands → arms, legs, shoulders, neck, chest
- Poor tongue and lip strength, hypophonic speech, swallowing difficulties
- Restrictive lung disease
- EMG – myopathic
- NCV - normal



FERTILITY

- Both males and females - delayed puberty
- Males - Absence of secondary sexual characteristics
- Primary hypogonadism - low testosterone; elevated LH/FSH
- Azoospermia

- Females – no impairment in ovulatory function and may give birth [high risk pregnancies]
- Cysteamine should be discontinued during pregnancy [based on rats' study]
- Kidney Int Rep 2024:9:214

CYSTINOSIN

- Cystine transporter outside the lysosome
- Driven by H^+ gradient
- Additional functions → not all symptoms are corrected by cystine depletion
  - Abnormal autophagy (autophagosomes, mitophagosomes, accumulation of perinuclear enlarged lysosomes)
  - Endo-lysosomal dynamics
  - Altered mTORC1 activity
  - Increased cell death (physical rupture of membranes, oxidative stress, mitochondrial damage, direct stimulation of apoptosis)
  - Altered glutathione metabolism → oxidation

AUTOPHAGY AND AUTOPHAGOSOMES

- Degradation of cytoplasmic organelles, proteins and macromolecules and recycling of the breakdown products
- Conserved eukaryotic cellular recycling process
- Plays major role in cell survival and maintenance

- Autophagosomes
  - Double membrane vesicles engulfing intracellular material
  - Transport the cargo to lysosomes for subsequent degradation

AUTOPHAGOSOME

- Cell Discovery 2020:6:33

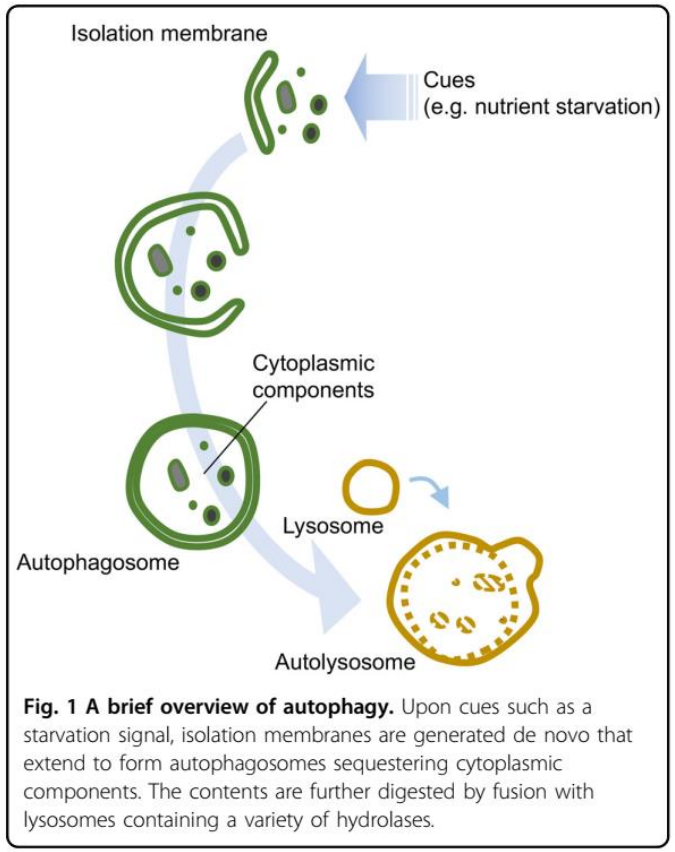
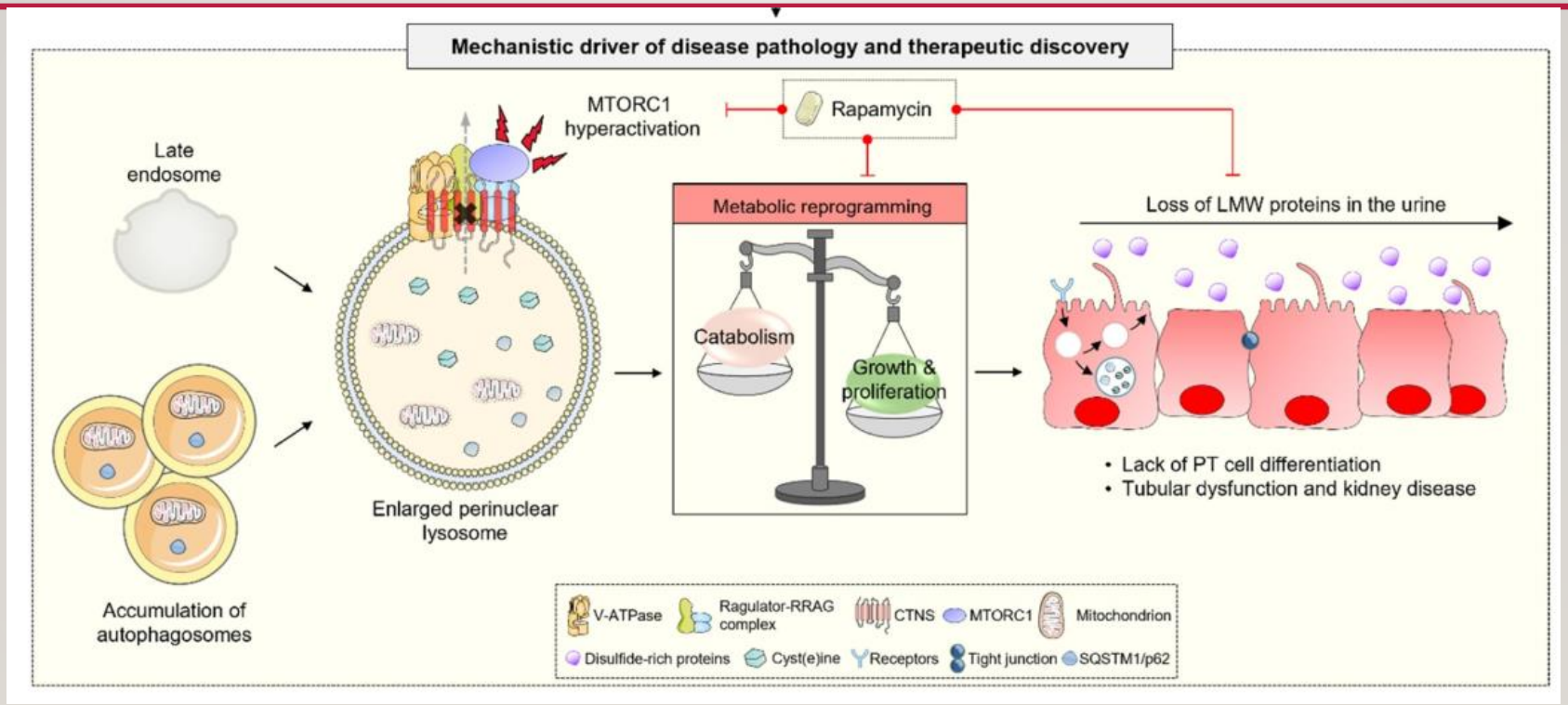


Fig. 1 A brief overview of autophagy. Upon cues such as a starvation signal, isolation membranes are generated de novo that extend to form autophagosomes sequestering cytoplasmic components. The contents are further digested by fusion with lysosomes containing a variety of hydrolases.

THE CTNS-MTORC1 AXIS COUPLES LYSOSOMAL CYSTINE TO EPITHELIAL CELL FATE

- Cystinosin interacts with the lysosomal membrane proteins that mediate fusion with autophagosomes
- Disturbing clearance of intracellular debris: misfolded proteins and damaged mitochondria
- Catabolic abnormalities are mirrored by anabolic programs for growth and proliferation
- In $Ctns^{-/-}$ rats, increased level in genes regulating cell cycle and driving proliferation
- Lack of PTC differentiation ⟶ LMW proteinuria
- MTORC1 inhibition as a potential therapeutic?

THE CTNS-MTORC1 AXIS COUPLES LYSOSOMAL CYSTINE TO EPITHELIAL CELL FATE

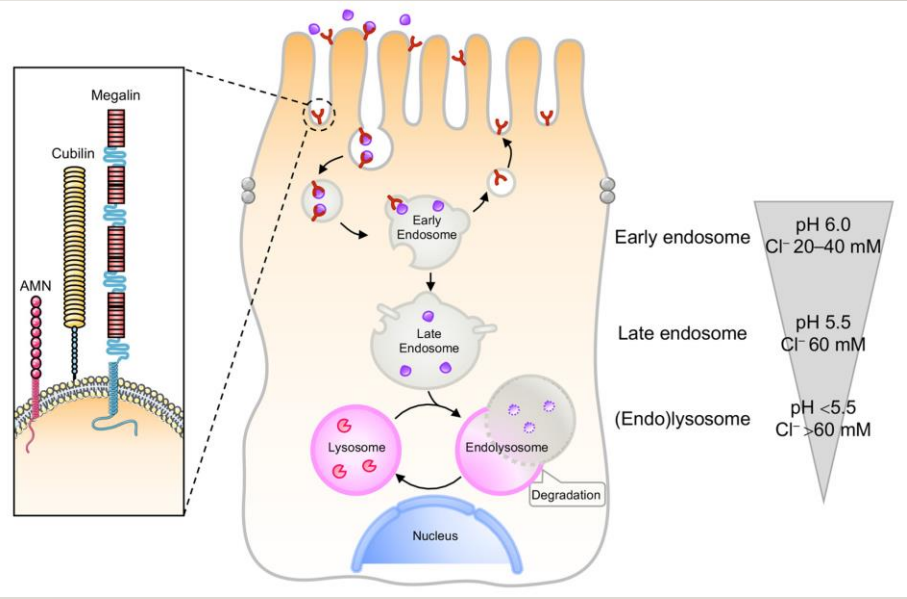


- Autophagy 2024:20:202

ABNORMAL ENDO-LYSOSOMAL DYNAMICS

- Defective receptor-mediated endocytosis in PTC
- Impaired trafficking of cubilin/megalin to the plasma membrane (In rat KO, also NaPi2a, SGLT2)*
- Impaired endocytosis
- Low molecular weight proteinuria, phospahturia, glycosuria

- *Hum Mol Genet 2022:31:2262



ER-ASSOCIATED DEGRADATION

- A variant referred to as cystinosin(7Δ)
- Retained in the ER and undergoes ER-associated degradation (ERAD)
- ERAD targets misfolded proteins for ubiquitination and degradation by the proteasome [protein-degrading complex]
- Chaperons known to treat CFTR F508Δ, facilitated folding and trafficking of cystinosin(7Δ) in patient fibroblasts
- 70% reduction in luminal cystine
- Re-purposing and precision medicine

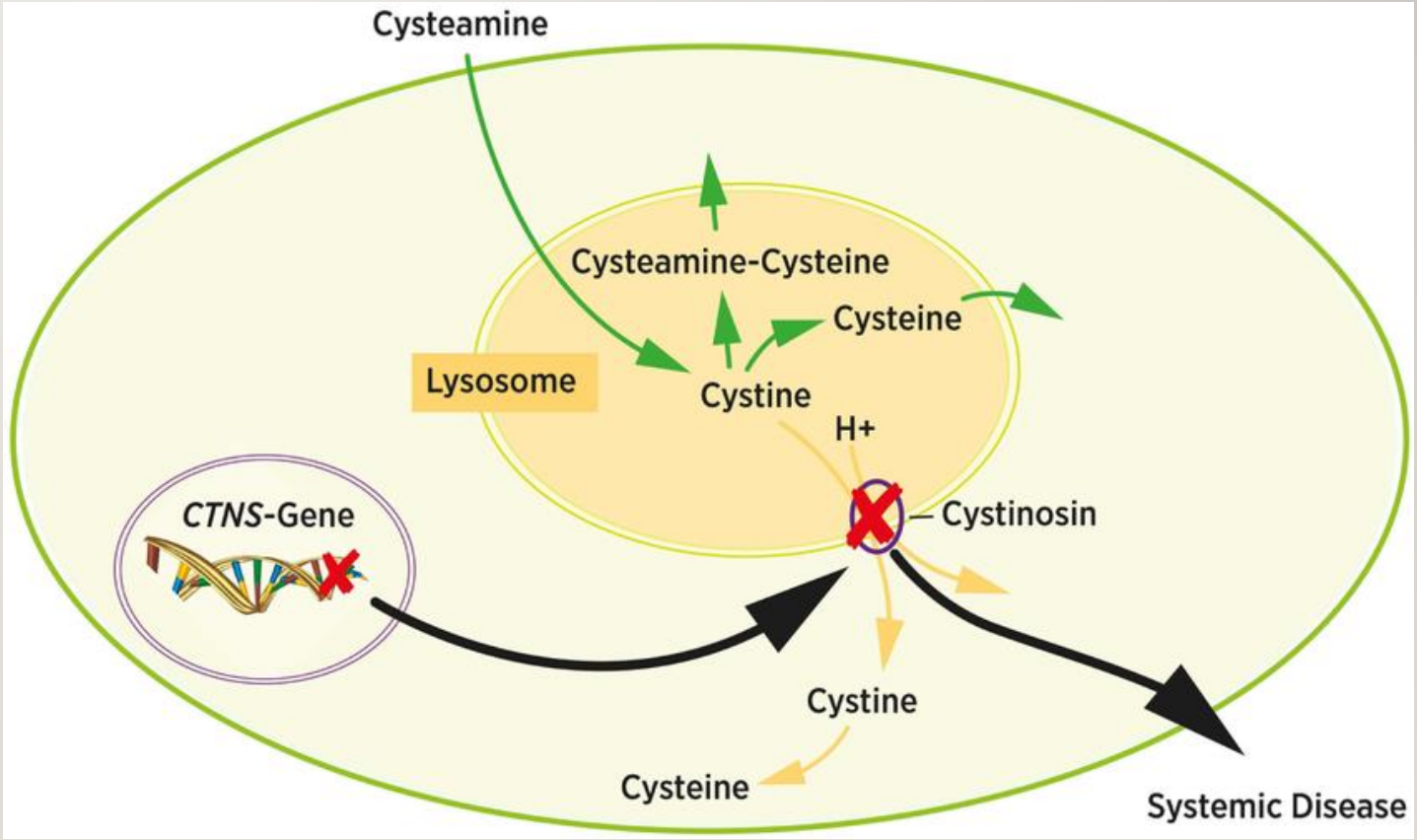
- JCI 2023:133(19): e169551

TREATMENT

- Replacement of renal losses [TID/QID]: potassium, phosphate and alkali supplementation
- Avoid dehydration episodes and maintain caloric intake
- Allow salt craving
- In infants/toddlers – consider PEG insertion
- Vitamin D supplements to increase calcium/phosphate GI absorption
- Consider thyroid hormone replacement, GH therapy or indomethacin
- Cysteamine eye drops
- KRT considerations similar to other etiologies with good outcome

LYSOSOMAL TRANSPORT DISORDER - CYSTINE DEPLETING DRUGS

- Cysteine + PQLC2 transporters



DIFFICULTIES

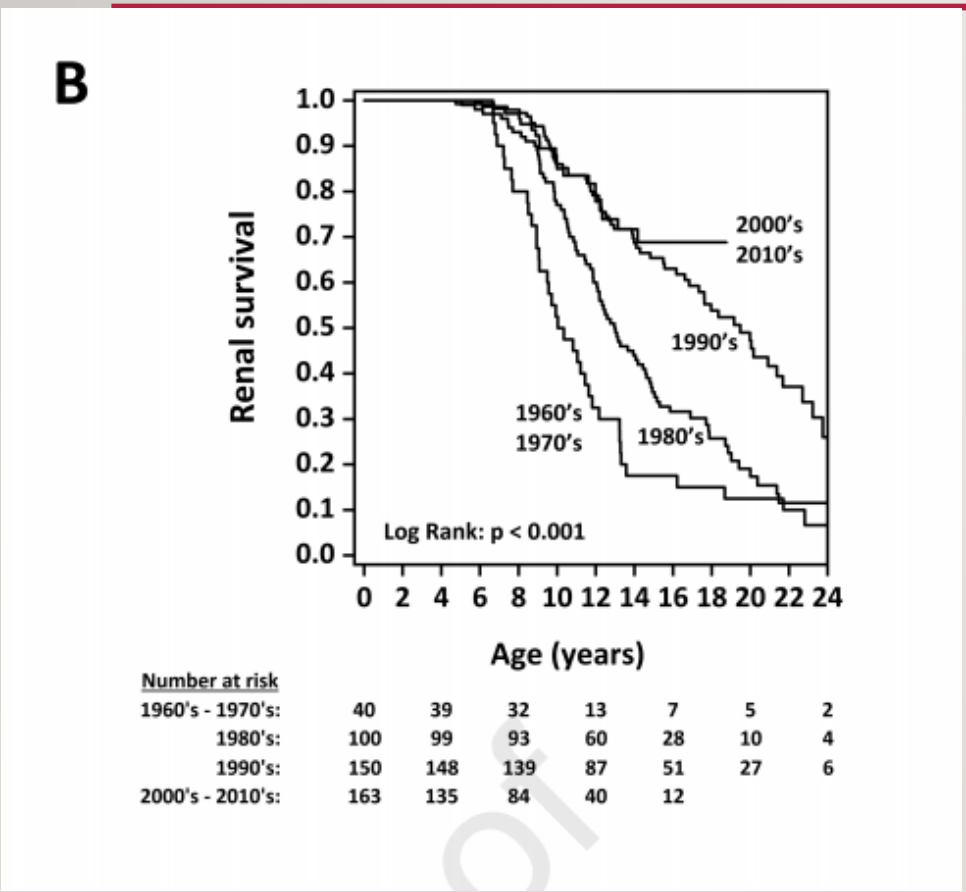
- Short term effect – q 6 hour dose
- Halitosis
- Body odor
- Gastric ulcers

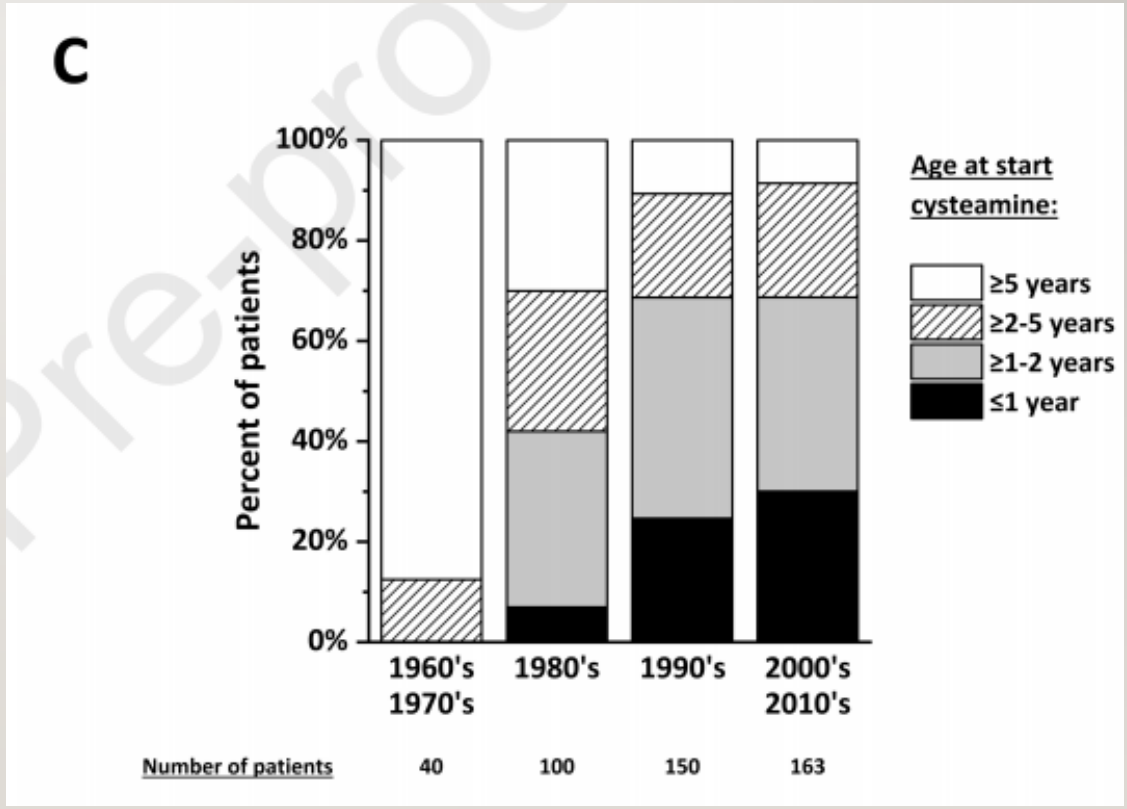
INTERNATIONAL STUDY – OUTCOMES OVER 5 DECADES

- International cohort of 453 patients born 1964-2016
- Cysteamine proposed for use in 1970's and introduced in 1980's
- Minimum F/U 3 years
- Risk factors for progression: late initiation of cysteamine and high leukocyte cystine levels
- No specific effect to: gender, specific genetic variant, the use of Indomethacin or ACEi
- Improved linear growth associated with early initiation of cysteamine

- Kidney Int 2021: DOI: 10.1016/j.kint.2021.06.019

RENAL SURVIVAL AND CYSTEAMINE TREATMENT

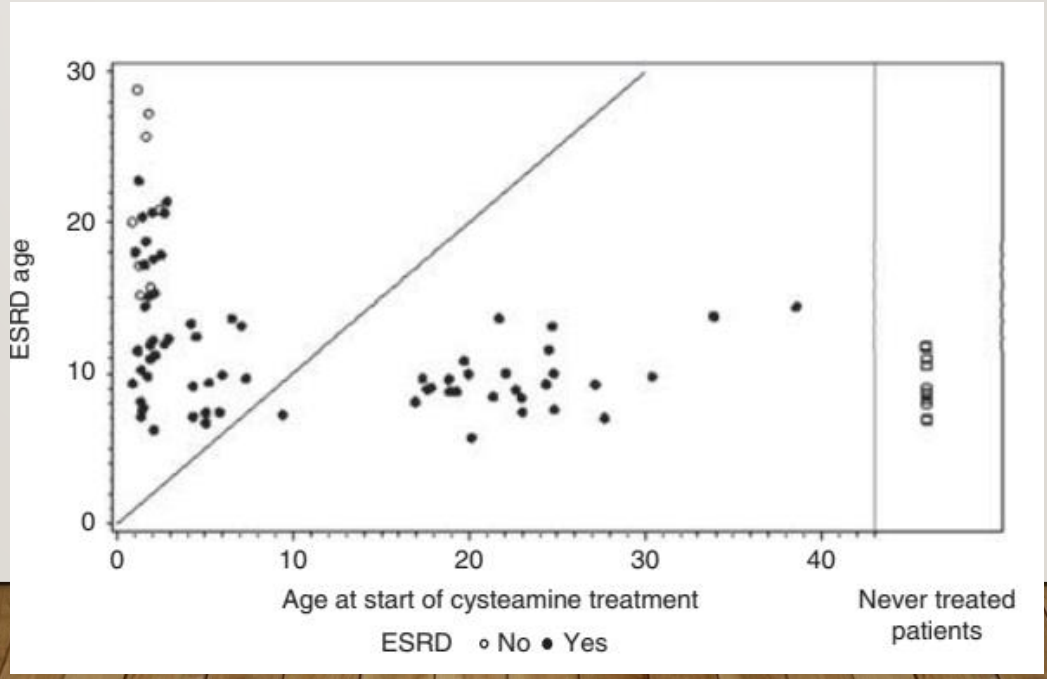




CYSTEAMINE THERAPY DELAYS PROGRESSION

- 86 adult patients (mean age 26.7 yrs)
- 75 treated with cysteamine: initiated at mean age of 9.9 yrs; mean duration 17.7 yrs
- Age of ESRD 13.6 and 9.6 yrs for cysteamine initiation before or after 5 years, respectively

- Kidney Int 2012:81:179



EXTRA-RENAL COMPLICATIONS

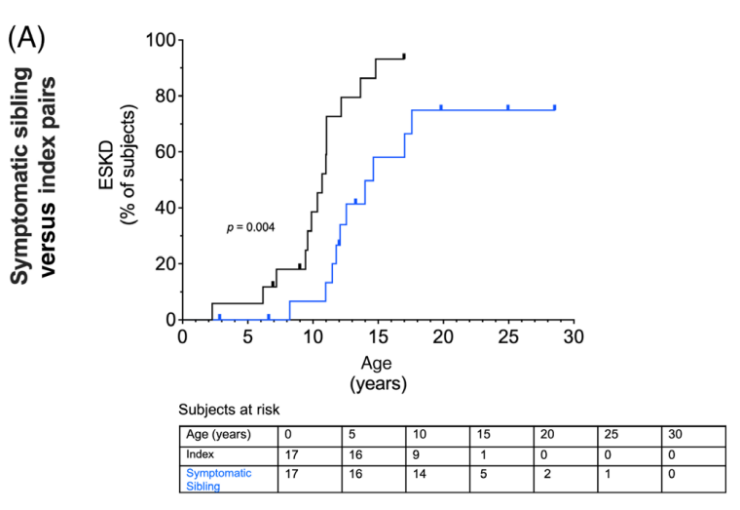
- Hypothyroidism 62 (mean age 13.4)
- Diabetes 48 (mean age 17.1)
- Neuromuscular 32 (mean age 23.3)
  - Myopathy (69%), swallowing impairment (53%), paresis (75%), stroke related (38%), mental deterioration (56%), seizures (31.3%)
- Cysteamine therapy delayed the development of complications
  - Particularly if initiated < 5 years
  - Even if initiated > 5 years
- Compliance?
- Leukocyte cystine level was optimal in **28%** of patients

CAN VERY EARLY CYSTEAMINE MAINTAIN TUBULAR AND GLOMERULAR FUNCTION?

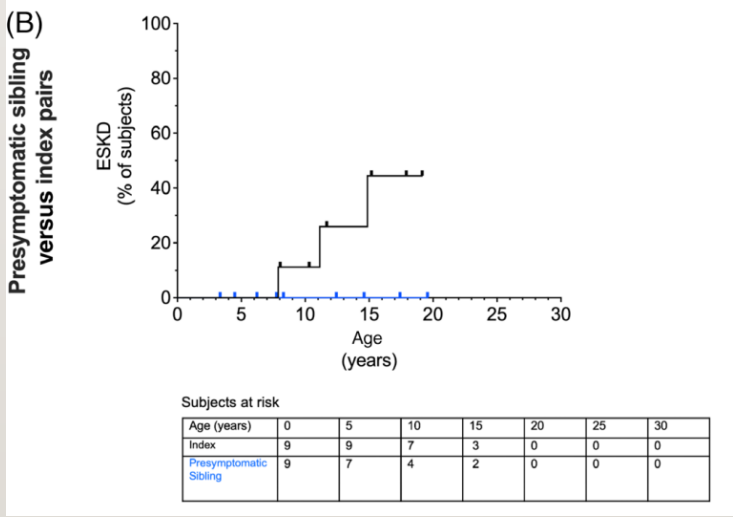
- 6 infants diagnosed with cystinosis before 2 mts of age (5 affected siblings; 1-NBS)
- Cysteamine initiated between 0.2-1.6 months
- Last F/U: 2-18 years
- 4/6 never required K, P, HCO3, citrate supplementation
- 5/6 never required Ca supplements
- Generalized amino-aciduria in all (x4)
- 1/6 non-compliant and had reduced eGFR
- Mol Genet Metab 2022:136:232

A MULTI-CENTER SIBLING STUDY

- Siblings diagnosed with Cystinosis start treatment at a younger age
- Siblings show slower progression to ESKD
- Siblings display similar incidence of ERC independent of age at cysteamine initiation



Age (years)	0	5	10	15	20	25	30
Index	17	16	9	1	0	0	0
Symptomatic Sibling	17	16	14	5	2	1	0



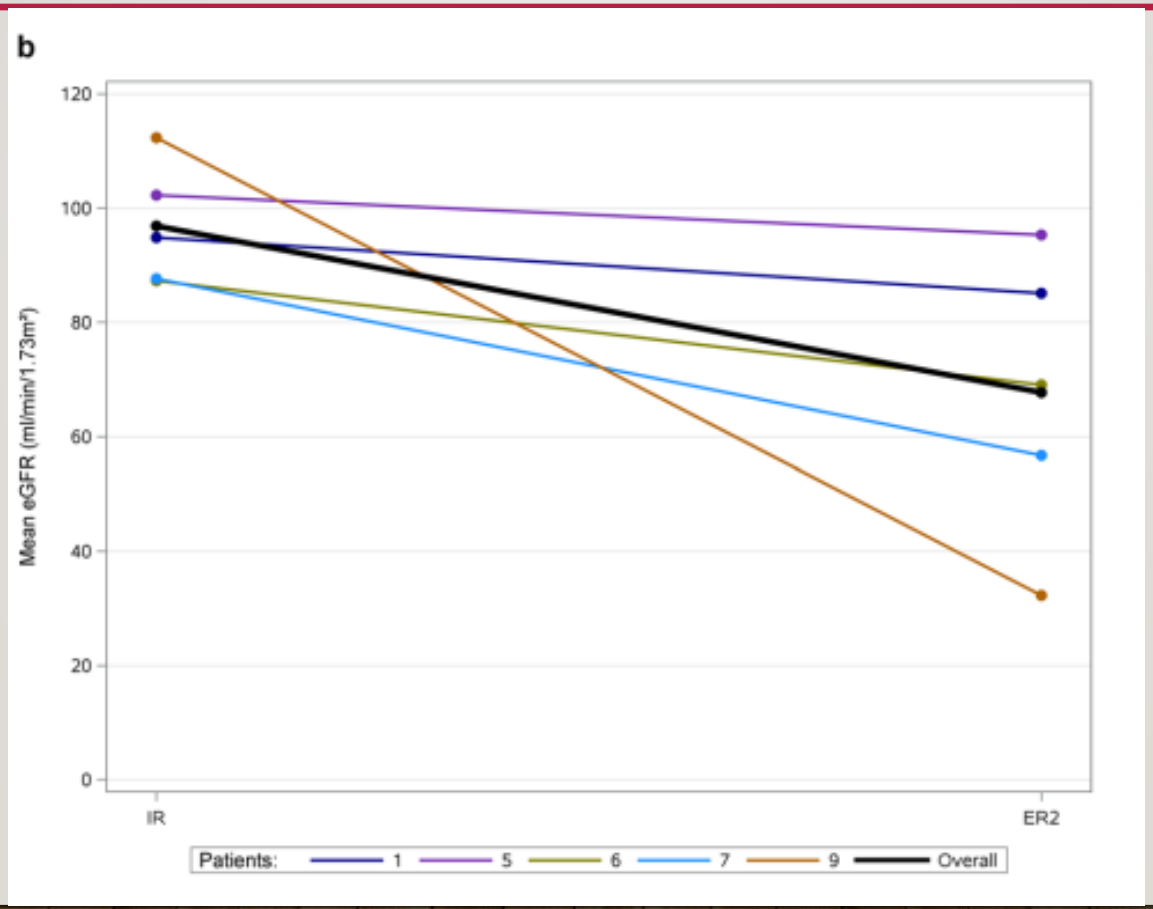
Age (years)	0	5	10	15	20	25	30
Index	9	9	7	3	0	0	0
Presymptomatic Sibling	9	7	4	2	0	0	0

- J Inherit Metab Dis 2023:46:43

TRANSITION FROM IR TO ER CYSTEAMINE

- 10 patients (pediatric and adults) from Norway
- Retrospective (transition ± 6 years)
- Comparable leukocyte cystine levels despite dose reduction
- Similar growth rate
- Halitosis: 4/7 improved, 1/7 unchanged, 2/7 worsened
- 1 patient switched back for ADR
- Pediatr Nephrol 2023:38:3671

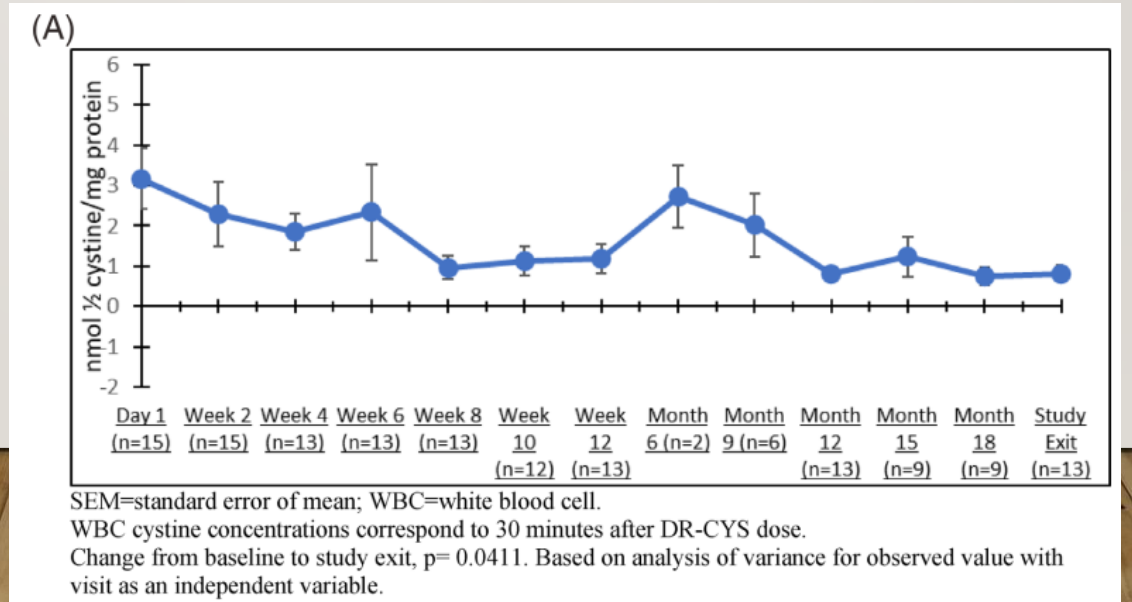
CHANGE IN EGFR/YEAR IN NON-TRANSPLANTED



DR CYSTEAMINE TO NAÏVE INFANTS AND TODDLERS

- Prospective open-label study
- 15 children naïve to treatment – mean age 2.2 years
- 14/15 completed 12 months and 10/15 – 18 months
- 10/13 reached WBC cystine levels < 1 nmol ½ cystine/mg protein

- JIMD Reports 2022:63:66



SEM=standard error of mean; WBC=white blood cell.
WBC cystine concentrations correspond to 30 minutes after DR-CYS dose.
Change from baseline to study exit, p= 0.0411. Based on analysis of variance for observed value with visit as an independent variable.

- Improved height and weight Z scores
- eGFR 55.9 → 63.8 ml/min/1.73m2
- No SAE related to the study drug
- No mention of GI symptoms/halitosis

DR CYSTEAMINE

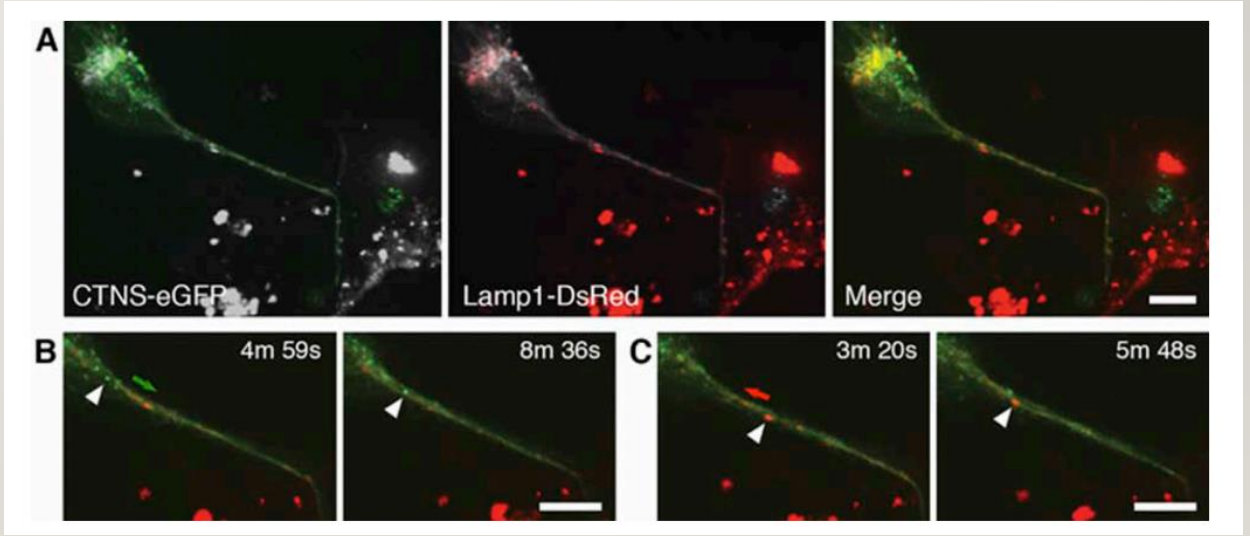
- Non-inferior
- Better compliance
- Long-term studies are needed
- GI complaints?
- Expensive
  - What are the indications for naïve patients/switch from IR cysteamine?
  - Improve access to medications around the globe

HEMATOPOIETIC STEM CELL GENE THERAPY

- Pre-clinical proof of concept - Cells 2021:10:3273
- $Ctns^{-/-}$ mice – a relevant mouse model for cystinosis (mild FS, CKD, thyroid, eye)
- Hematopoietic Stem and Progenitor Cells (analogous to human CD34+) of wild type mice
- Abundant tissue integration of BM-derived cells (matured into macrophages)
- Decreased cystine accumulation and preservation of kidney function
- Rescue corneal involvement
- Cystinosin is a trans-membrane lysosomal protein and not a secreted enzyme –
- How does it work?

TUNNELING NANOTUBES (TNT)

- Plasma membrane extensions which can facilitate inter-cellular transport of organelles
- Direct cell contact
- TNT-mediated lysosomal transfer is bidirectional



- Stem Cells 2015: 33:301

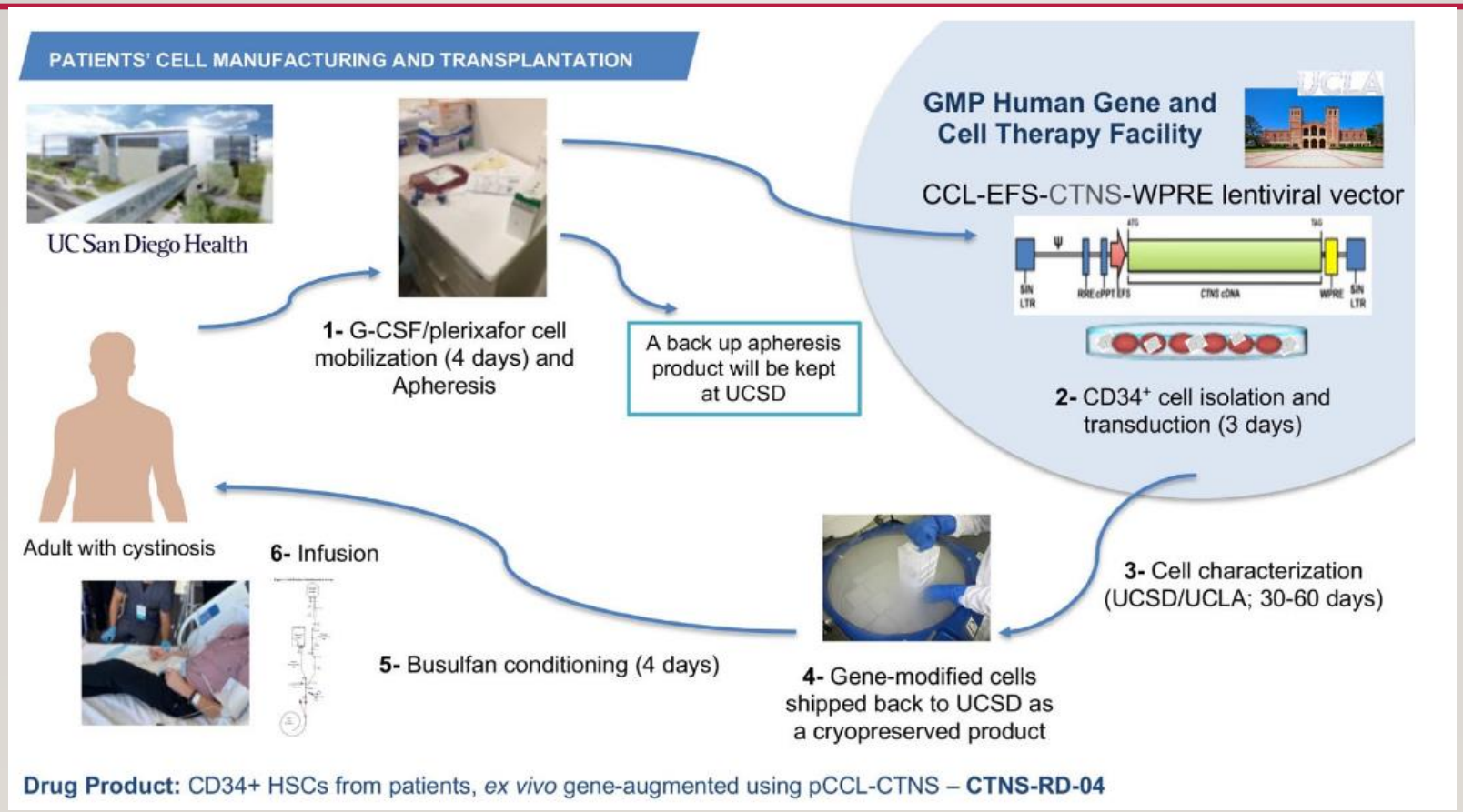
OF MICE AND MEN

- Allogeneic HSPC transplantation and the risk of GVHD
- Allogeneic HSPC transplantation to a 16 year old cystinotic patient [treated with cysteamine] from a full-HLA matched unrelated donor
- Second transplantation from the same donor due to partial graft failure
- Severe GVHD and death of multi-resistant Ps pneumonia
- Nevertheless, kidney function stabilized, improved polyuria, photophobia score improved, decreased cystine accumulation in stomach biopsy

EX-VIVO GENE-MODIFIED CELL THERAPY

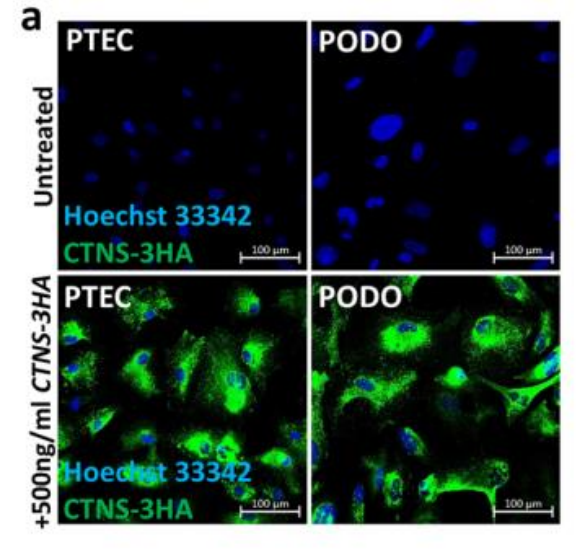
- Autologous HSPC gene therapy
- Myeloablation conditioning (reduced intensity) but no immunosuppressants post transplant
- A third-generation lentiviral vector has been shown to be safe
- Approval for recruitment of 6 patients

PHASE 1/2 FIRST IN HUMAN OPEN LABEL CLINICAL TRIAL CTNS-RD-04



CTNS MRNA DELIVERY IN CYSTINOSIS MODELS

- PTEC and podocytes derived from cystinosis patient
- Transfected with synthetic CTNS mRNA



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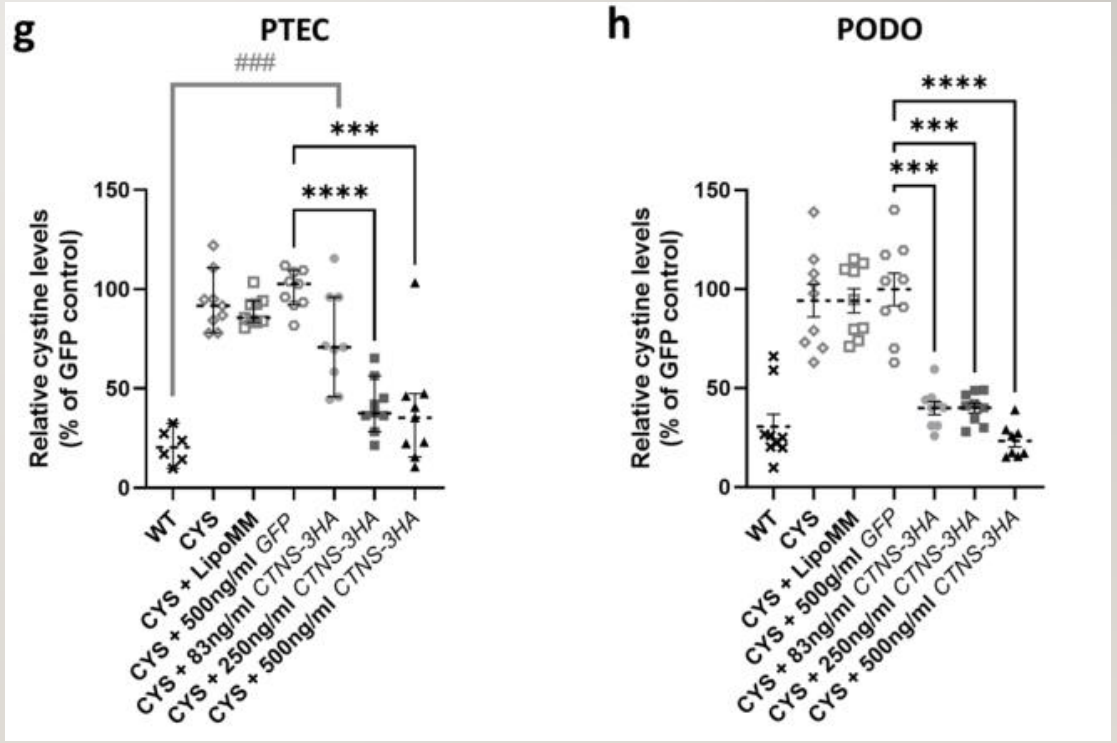

THANK YOU!

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