University of Cyprus
Department of Biological Sciences

MOLECULAR MEDICINE RESEARCH CENTER UNIVERSITY OF CYPRUS

CY-Biobank

Familial microscopic hematuria as a paradigm for a "multifactorial" Mendelian disease: A unique Cyprus experience

Croatian Society for Human Genetics

21 February 2017

Constantinos Deltas
Director, Molecular Medicine Research Center
University of Cyprus

Objectives of lecture

1. Present a glimpse of past research
2. Focus on one major project
   a) Alport and thin basement membrane nephropathy
   b) Familial microscopic hematuria as a paradigm for a “multifactorial” Mendelian disease:
      A unique Cyprus experience
   c) The role of genetic modifiers: A hypothesis

Who we are

- Established a diagnostics and research lab in the newly created Cyprus Institute of Neurology and Genetics, which served the medical community and the patients, 1991
- Established the newly created Department of Biological Sciences, hired new faculty, started new undergraduate and graduate programs of study, 2002
- Assisted in the development of the Medical School of UCY, hired new faculty, contributed in developing teaching curricula and currently in the process of designing a graduate program of studies, 2003
- Established the first Biobank in Cyprus through external funding, approved by the Cyprus National Bioethics Committee, 2011

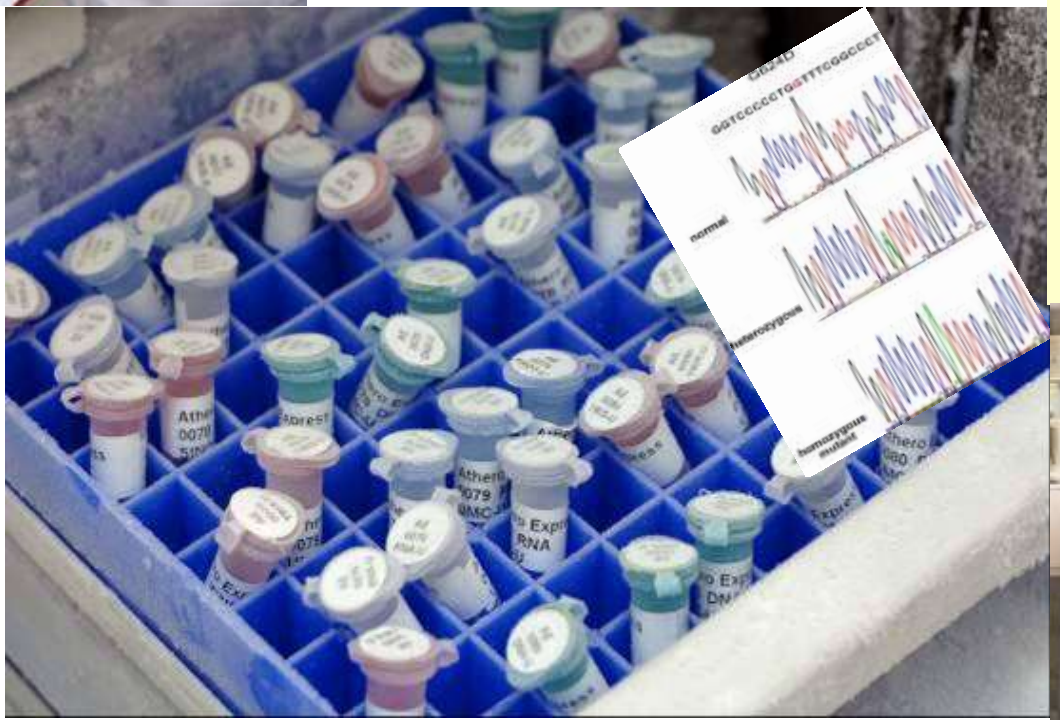
BIOBANK

>5,000 samples, not always with complete medical records

CY-Biobank

- ✔ Medical info

- ✔ DNA
- ✔ Plasma
- ✔ Serum
- ✔ Urine
- ✔ Biopsies



CY-Biobank

BBMRI.cy
Biobanking and
BioMolecular resources
Research Infrastructure
Cyprus

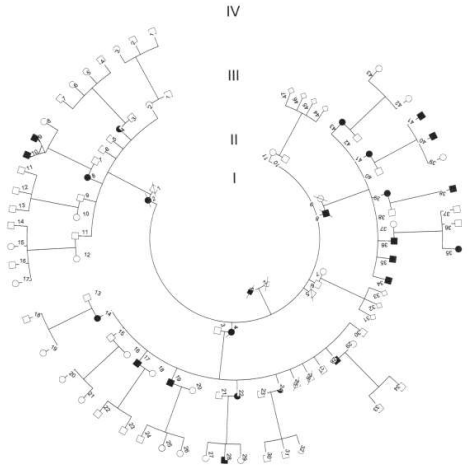
Past research and diagnostics

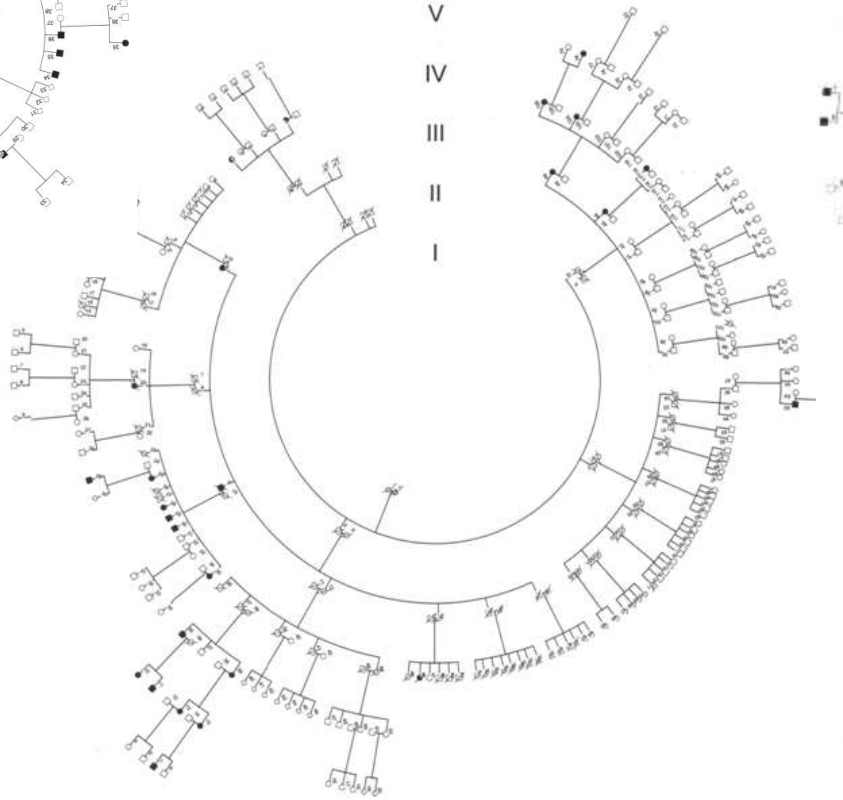
Kidney related projects

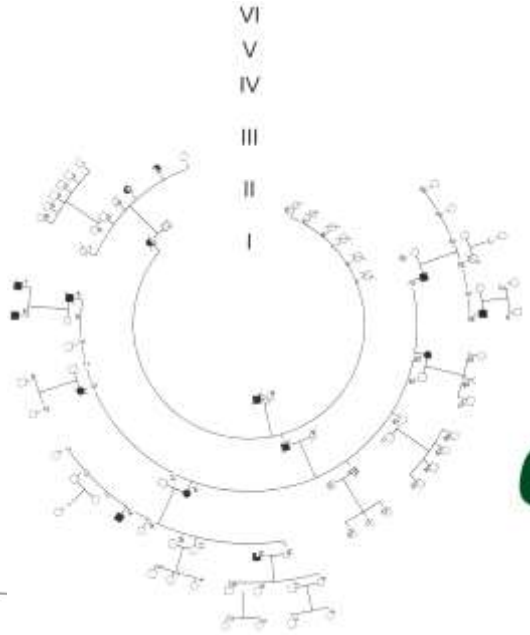
1. Polycystic kidney disease (*PKD1*, *PKD2*)
2. Medullary cystic kidney disease (*MCKD1/MUC1*)
3. Distal renal tubular acidosis (*ATP6V1B1*)
4. Branchio-oto-renal syndrome (*EYE*)
5. Cystinuria (*SLC3A1*, *SLC7A9*)
6. C3/CFHR5 glomerulonephritis (*CFHR5*)
7. Focal segmental glomerulosclerosis
8. Nephrotic syndrome (*NPHS2*, *PLCE1*)
9. Hypertensive nephrosclerosis (*MTHFR*)
10. Collagen IV nephropathies
    - ❖Alport syndrome (*COL4A3*, *COL4A4*, *COL4A5*)
    - ❖Thin basement membrane nephropathy (*COL4A3*, *COL4A4*)

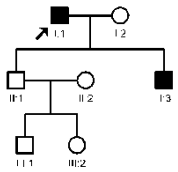
Other

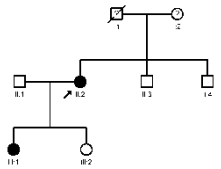
1. Cystic Fibrosis (*CFTR*)
2. Medullary thyroid carcinoma (*RET*)
3. Familial Mediterranean fever (*MEFV*)
4. Hereditary thrombophilia (Factor *V* Leiden, *MTHFR*, Prothrombin)

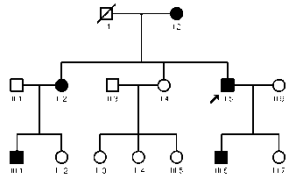


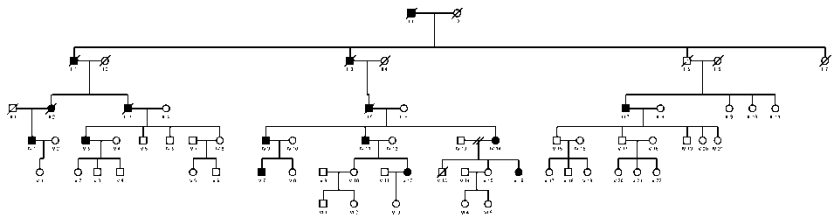


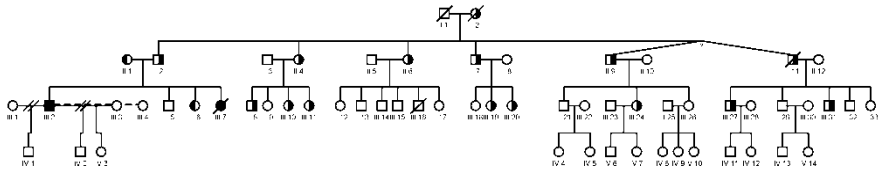


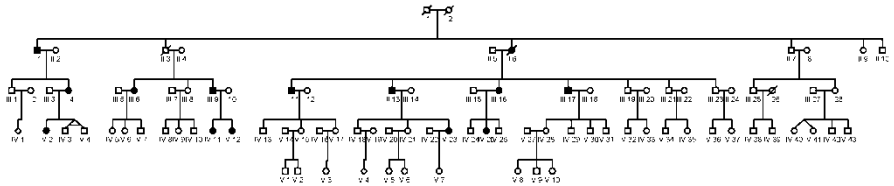












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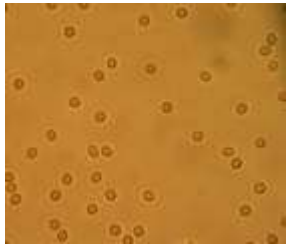
Microscopic hematuria

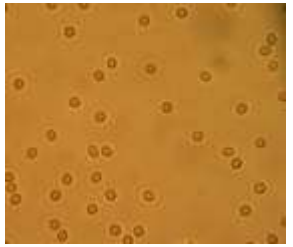
http://www.suite101.com/content/microscopic-hematuria-in-adults-a132528



- The presence of more than 3-5 red blood cells per high power field in light microscope of centrifuged urine
- It is a frequent finding in the general population, estimated to be 0.19-21%, depending on the study
- There is no consensus regarding the need for performing a biopsy when there is isolated microscopic hematuria
- There are well known inherited renal diseases that present with microscopic hematuria since childhood. They can be mild or severe and progressive

Familial Microscopic Hematuria

http://www.suite101.com/content/microscopic-hematuria-in-adults-a132528



It can be the presenting symptom of:

- IgA Nephropathy (mostly sporadic, rarely familial)

1. Young males with X-linked Alport Syndrome (Chr. X, *COL4A5*)

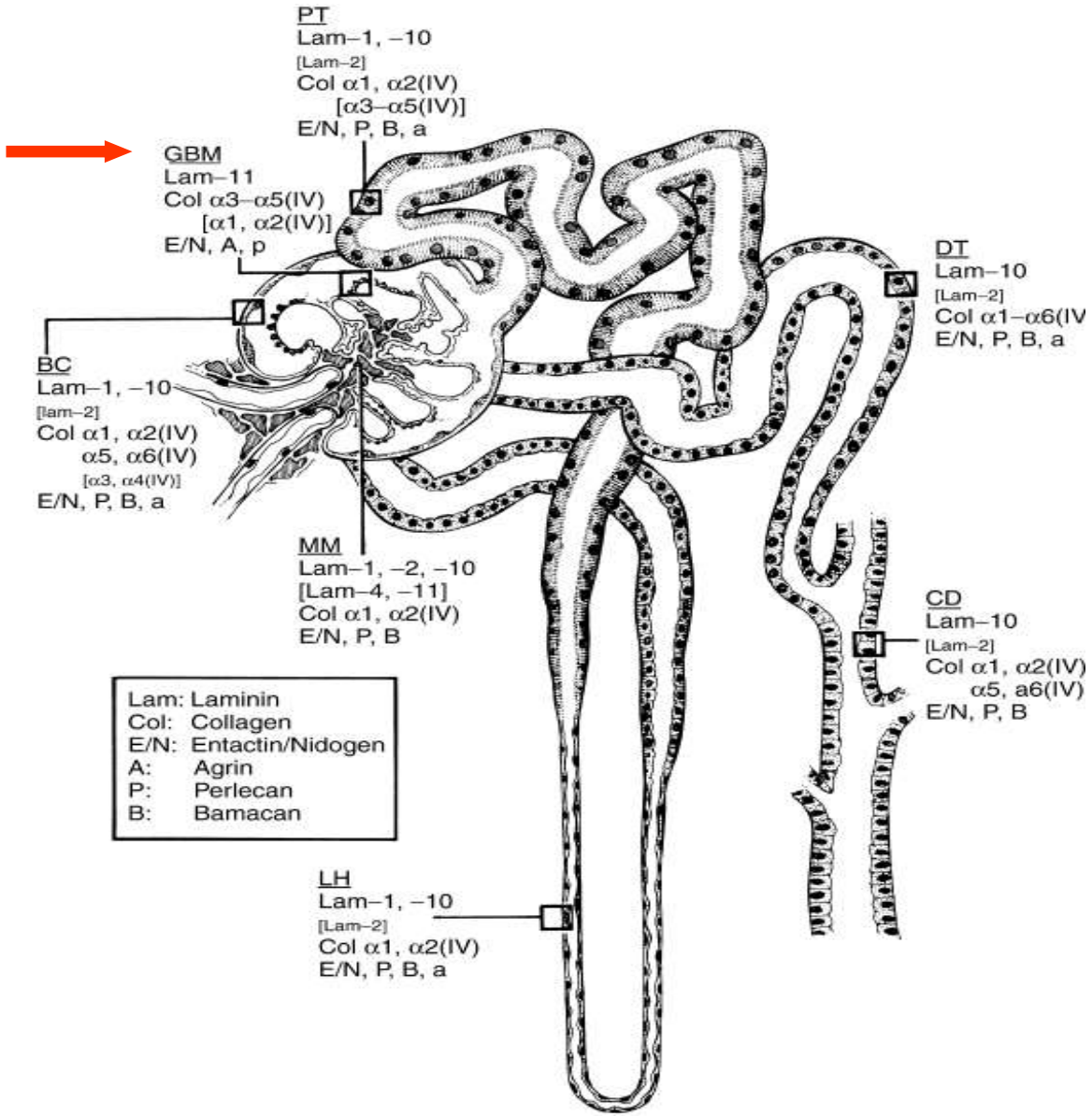
2. Female heterozygous carriers of the X-linked Alport Syndrome (Chr. X, *COL4A5*)

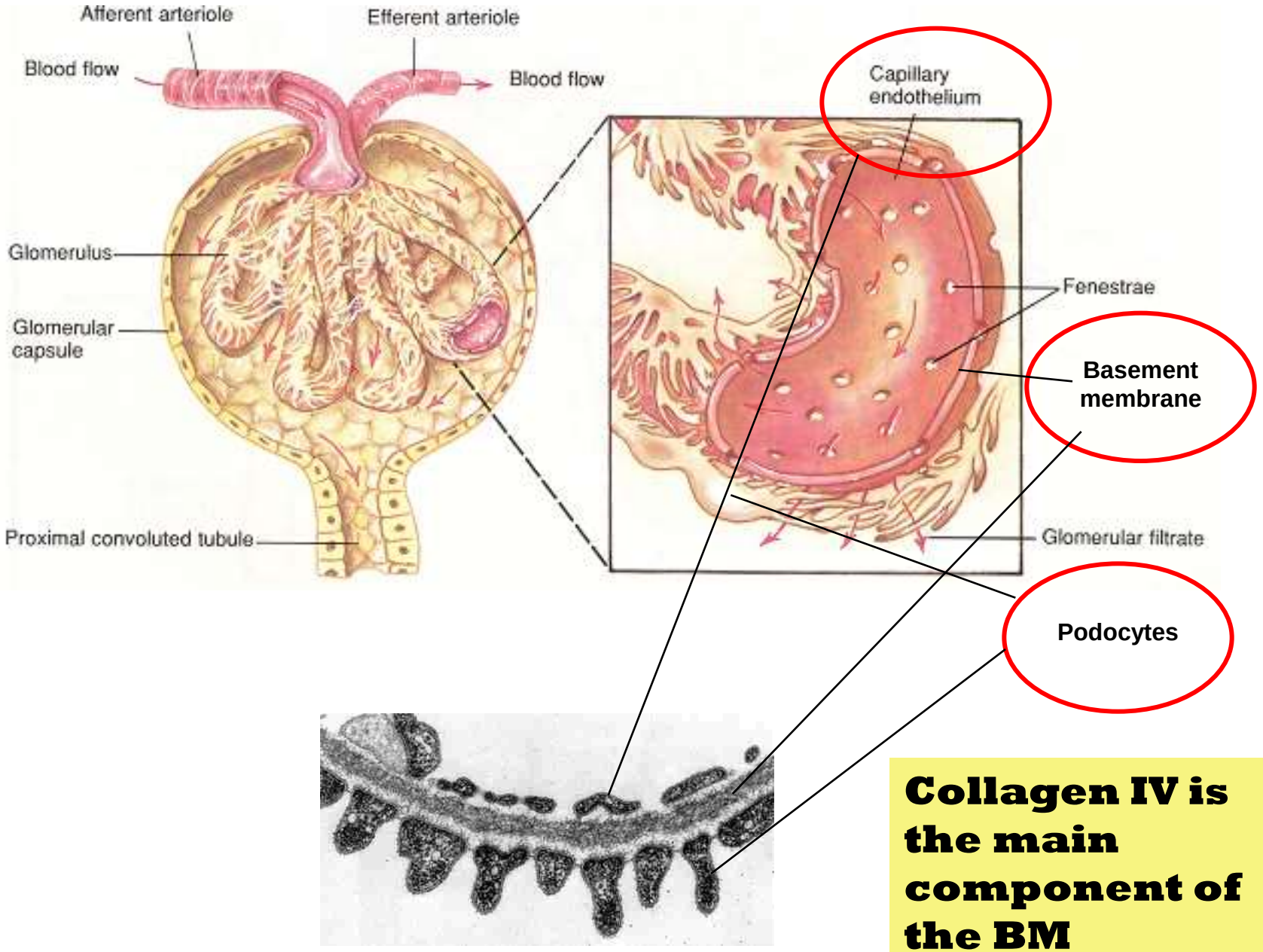
3. Male and female patients with the autosomal recessive Alport Syndrome (Chr. 2, *COL4A3/COL4A4*)

4. Male and female heterozygous carriers of *COL4A3/COL4A4* mutations (Thin Basement Membrane Nephropathy)

5. C3 glomerulopathy as a result of mutations in the *CFHR5* gene (isolated deposition of complement C3 in the glomerulus without immune complexes)
6. *MYH9* mutations (May-Hegglin anomaly, Fechtner, Sebastian, & Epstein syndromes)
7. Fibronectin depositions glomerulopathy (*FN1* gene)

Micro-anatomy of the nephron





Collagen IV is the main component of the BM

Collagen IV

All collagens are trimeric molecules, where a variable part of the protein sequence contains Glycine at every third position

Gly-X-Y

Positions X & Y are frequently occupied by prolines

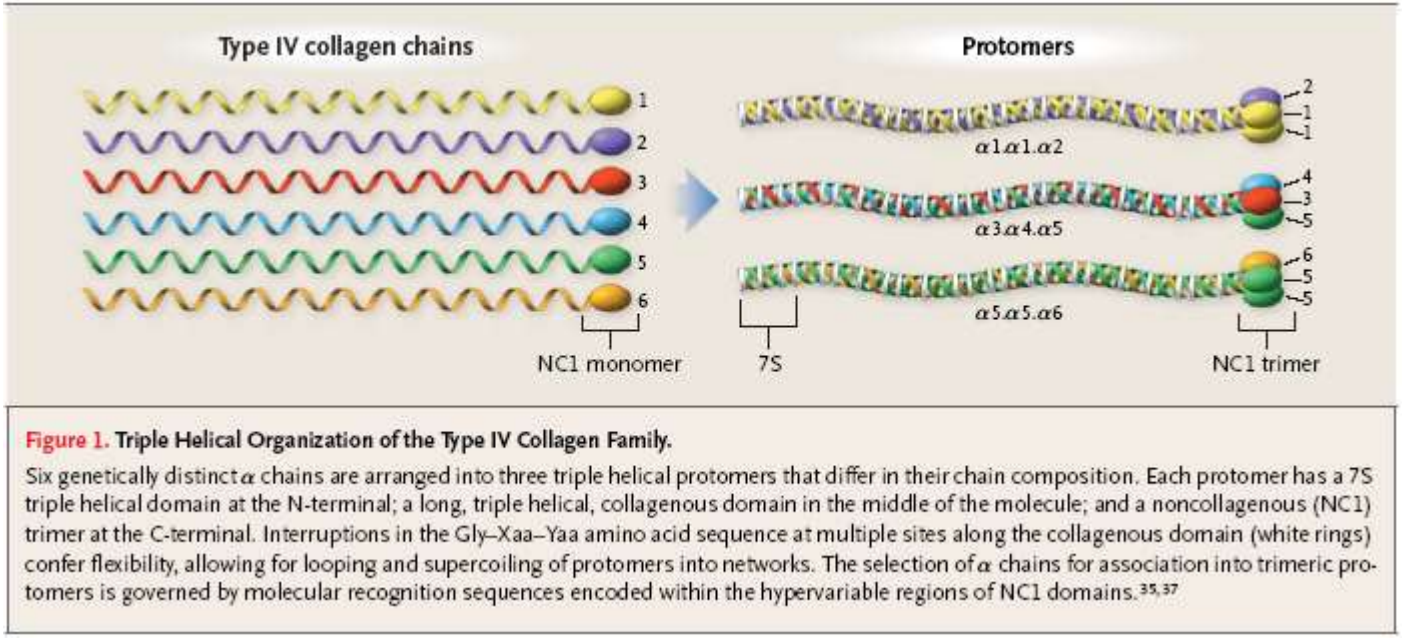


Figure 1. **Triple Helical Organization of the Type IV Collagen Family.**
Six genetically distinct α chains are arranged into three triple helical protomers that differ in their chain composition. Each protomer has a 7S triple helical domain at the N-terminal; a long, triple helical, collagenous domain in the middle of the molecule; and a noncollagenous (NC1) trimer at the C-terminal. Interruptions in the Gly–Xaa–Yaa amino acid sequence at multiple sites along the collagenous domain (white rings) confer flexibility, allowing for looping and supercoiling of protomers into networks. The selection of α chains for association into trimeric protomers is governed by molecular recognition sequences encoded within the hypervariable regions of NC1 domains.[35,37]

Hudson and Tryggvason 2003. N Eng J Med 348:2543-2556.

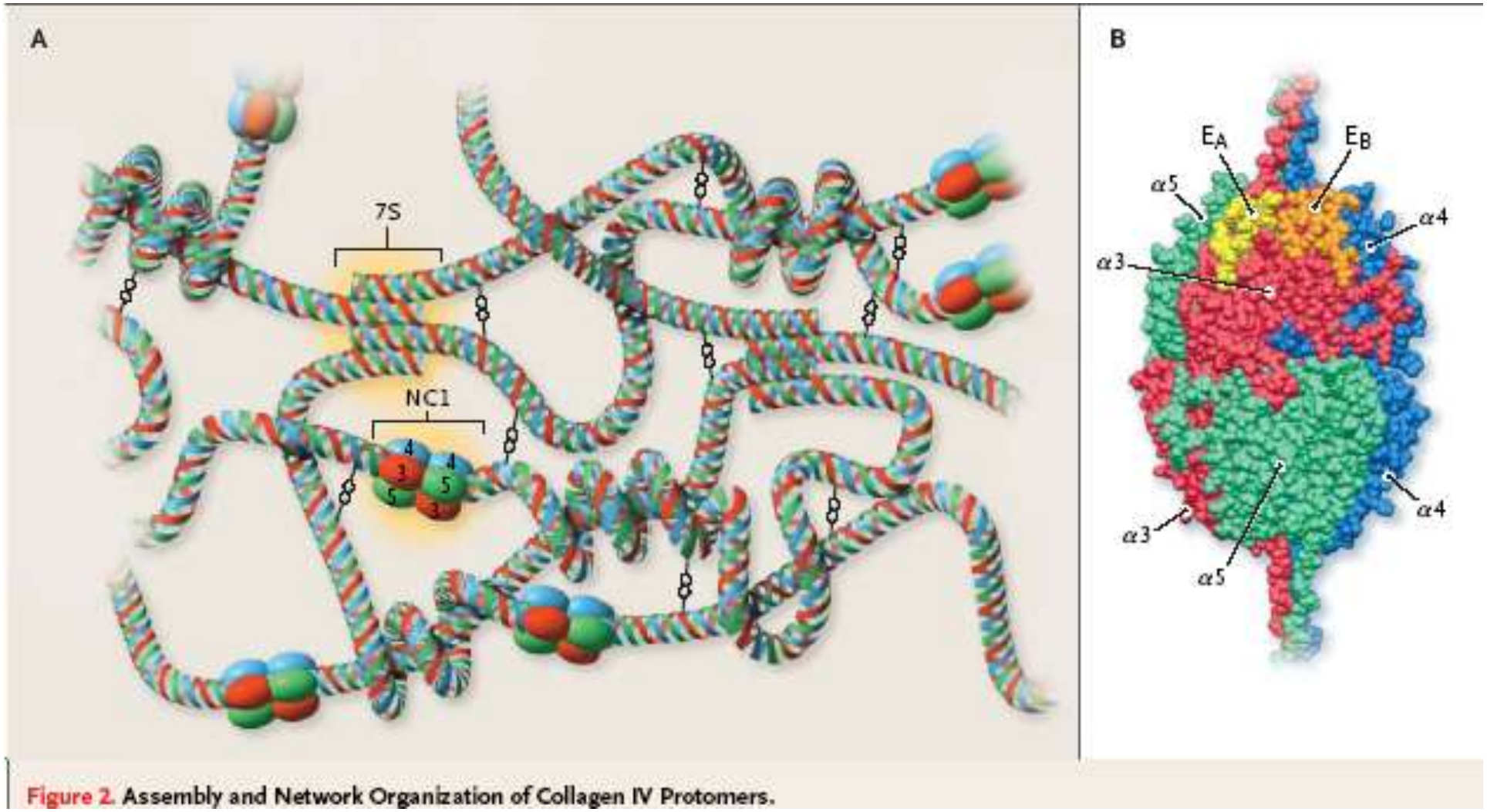


Figure 2. Assembly and Network Organization of Collagen IV Protomers.

Collagen IV network in the basement membrane

a3a4a5 OR a5a5a6

Hudson and Tryggvason 2003. N Eng J Med 348:2543-2556.

Collagen IV nephropathies

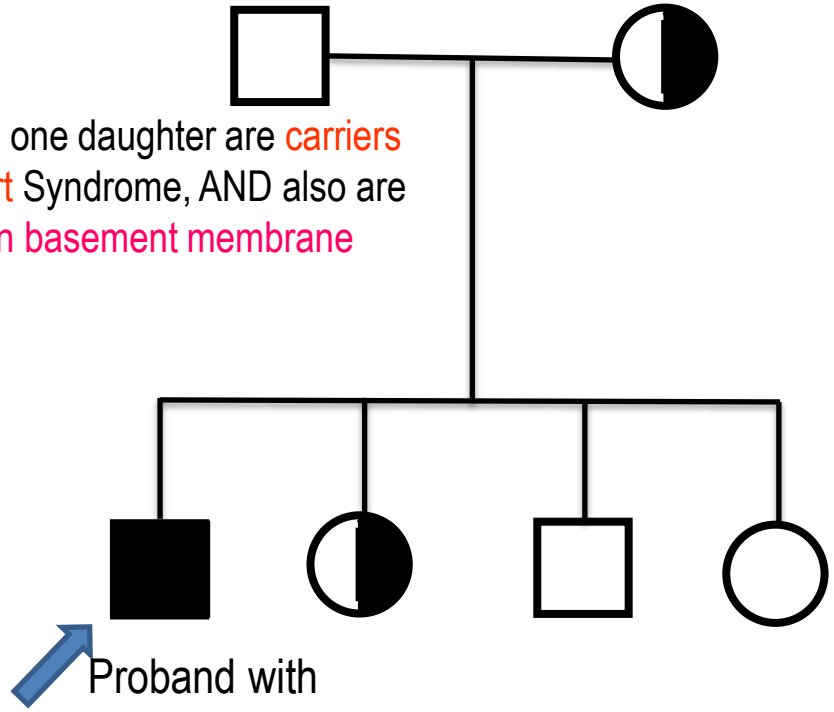
- Alport Syndrome
  - X-linked
  - ***Autosomal recessive***
  - Autosomal dominant

- Thin basement membrane nephropathy
  - Benign for life OR
  - Progressive

- Patients with thin basement membrane nephropathy are actually the heterozygous carriers of the ***autosomal recessive*** Alport Syndrome, who are not healthy!!!

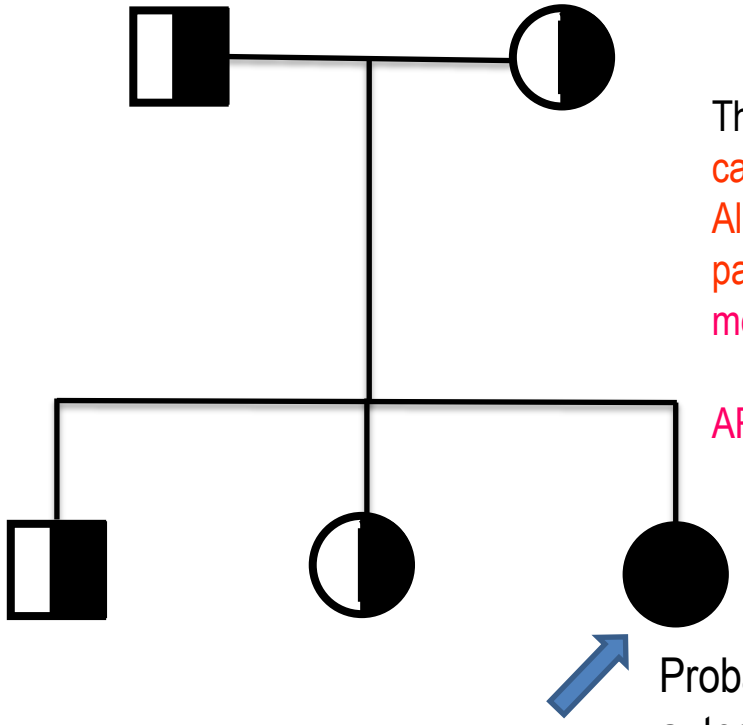
Carriers and patients with X-linked Alport Syndrome (*COL4A5*)

The mother and one daughter are carriers of x-linked Alport Syndrome, AND also are patients with thin basement membrane nephropathy



Proband with X-linked alport syndrome

Carriers and patients with Autosomal Recessive Alport Syndrome (*COL4A3* or *COL4A4*)



The parents and two children are carriers of autosomal recessive Alport Syndrome, AND also are patients with thin basement membrane nephropathy

ARE THEY HEALTHY?

Proband with autosomal recessive alport syndrome

Carriers of autosomal recessive Alport Syndrome (ARAS)
OR
Thin Basement Membrane Nephropathy (TBMN)
(a form of familial hematuria)

- TBMN has an estimated prevalence of about 0.3-1% in the general population (Gregory MC, Semin Nephrol 2005)
- TBMN is genetically heterogeneous, 40-50% caused by heterozygous mutations in *COL4A3/A4* (collagen IV nephropathy, ARAS)
- Presents with microscopic hematuria
- Formerly considered nearly always benign, also referred to as Benign Familial Hematuria, with excellent prognosis

- How Benign is it, really?
  - Experience varies between centers, perhaps because of differences in population gene pools and heterogeneity in genetic background and / or environment

- Careful study of the literature was revealing and informative…

Arch Intern Med/Vol 131, Feb 1973

Clinical Notes

Familial Benign Essential Hematuria

MAJ Philip W. Rogers, MC, USA; LTC Neil A. Kurtzman, MC, USA; MAJ Simon M. Bunn, Jr., MC, USA; and MAJ Martin G. White, MC, USA, Fort Sam Houston, Tex

'The abnormality causing the hematuria can be called "benign" only after prolonged observation over a period of years with neither further morbidity nor mortality'.

One of the explanations for this adverse development was the probable co-inheritance of another glomerulopathy, perhaps IgAN, focal segmental glomerulosclerosis, minimal change disease, mesangioproliferative glomerulonephritis or others, something that cannot be excluded entirely considering the fairly high estimated prevalence of TBMN.

Of course, there is room for other explanations! Genetic modifiers?

Deltas et al, NDT 2013

Am. J. Nephrol. 5: 103–109 (1985)

0250–8095/85/0052–0103$2.75/0

Abnormally Thin Glomerular Basement Membranes Associated with Hematuria, Proteinuria or Renal Failure in Adults

Frederick E. Dische[a], *Michael J. Weston*[b], *Victor Parsons*[b]

[a] Department of Pathology and [b] Renal Unit, Dulwich Hospital (King's College Hospital), London, UK

- Reported on 14 patients aged 11-51 yr, whose main abnormality was the thin glomerular basement membrane.
- Several of their patients had progressive disease including hypertension and renal impairment while one had reached ESRD.
- Three family members of this small cohort demonstrated similar renal symptoms

Kidney International, Vol. 51 (1997), pp. 1596–1601

Thin GBM nephropathy: Premature glomerular obsolescence is associated with hypertension and late onset renal failure

CHRISTINA M. G. NIEUWHOF, FRANS DE HEER, PETER DE LEEUW, and PETER J. C. VAN BREDA VRIESMAN

- Results of a prospective study with a 12-year follow up of 19 patients with TBMN and microscopic (18/19) or macroscopic hematuria (1/19).
- They were the first to note clearly the association between TBMN and late onset renal impairment on long follow up in elderly patients. In 13.5% of their patients focal global glomerulosclerosis was also detected.
- In six first degree relatives of these 19 patients ESKD was established, prompting the authors ***to conclude that TBMN predisposes*** to premature glomerular obsolescence, which with sufficient time leads to increased incidence of hypertension and late onset renal insufficiency.
- Interestingly the same authors mentioned that in a separate series of TBMN patients they noted an increased proteinuria associated with FSGS in the renal biopsy. Based on their admittedly small patient cohort they commented that the prognosis of TBMN may not be as benign as generally thought.

Thin basement membrane disease with heavy proteinuria or nephrotic syndrome at presentation.

Am J Kidney Dis. 2000 Apr;35(4):E15.

Nogueira M, Cartwright J Jr, Horn K, Doe N, Shappell S, Barrios R, Coroneos E, Truong LD.

- Eight patients, 32-66 yr, three of whom had pure TBMN and five had TBMN with heavy proteinuria or nephrotic syndrome at presentation. They referred to a ***dual diagnosis of TBMN associated with FSGS***.
- Four patients responded to steroids resulting in remission, while hematuria persisted, something that prompted them to hypothesize that the nephrotic syndrome was not related to TBMN but rather was the manifestation of another associated glomerular disease.
- The authors made special reference to the fact that TBMN is as frequent as 5% to 10% in the general population and it is reasonable to expect TBMN to be co-inherited with other glomerular diseases that are diagnosed by renal biopsy.
- They made a point regarding the significance of long follow up in detecting possible disease progression after the initial diagnosis.

Kidney International, Vol. 66 (2004), pp. 909–913

Signs and symptoms of thin basement membrane nephropathy: A prospective regional study on primary glomerular disease—The Limburg Renal Registry

PIETER VAN PAASSEN, PETER J.C. VAN BREDA VRIESMAN, HENK VAN RIE, and JAN WILLEM COHEN TERVAERT

- The Limburg Renal Registry: Of 22 patients who originally had been classified as primary FSGS with microscopic hematuria, 50% turned out to be secondary FSGS due to TBMN, ***thereby admitting that FSGS can be precipitated on the genetic background of TBMN***.
- They suggested that ***TBMN is not a benign condition*** particularly in patients of late middle age. In a series of 92 patients with TBMN in the same study, 11 middle-aged adults (12%) had FSGS who developed hypertension or renal insufficiency, the FSGS being secondary to the TBMN.
- Five patients with TBMN had developed nephrotic syndrome in the presence of erythrocyturia and proteinuria >5g/day. They hypothesised that a long follow up of patients with TBMN will identify increasing numbers of subjects who will succumb to renal function impairment.

All previous work was not accompanied by molecular testing.

More recently:

- Severe TBMN on long follow up

  Vs

- Autosomal dominant Alport Syndrome with later age at onset

LATE ONSET ALPORT NEPHROPATHY (LOAN)

Autosomal dominant Alport Syndrome/1

Previous publications
1. Jefferson JA, Lemmink HH, Hughes AE*, et al.* Autosomal dominant Alport syndrome linked to the type IV collagen alpha 3 and alpha 4 genes (COL4A3 and COL4A4). Nephrol Dial Transplant 1997;12(8):1595-9.
2. van der Loop FT, Heidet L, Timmer ED*, et al.* Autosomal dominant Alport syndrome caused by a COL4A3 splice site mutation. Kidney Int 2000;58(5):1870-5.
3. Ciccarese et al. Idetification of a new mutation in he alpha 4 (IV) collagen gene in a family with autosomal dominant Alport syndrome and hypersholesterolaemia. Nephrol Dial Transplant 2001;16(10):2008-12.
4. Longo I, Porcedda P, Mari F*, et al.* COL4A3/COL4A4 mutations: from familial hematuria to autosomal-dominant or recessive Alport syndrome. Kidney Int 2002;61(6):1947-56.
5. Pescucci C, Mari F, Longo I*, et al.* Autosomal-dominant Alport syndrome: natural history of a disease due to COL4A3 or COL4A4 gene. Kidney Int 2004;65(5):1598-603.
6. Kharrat, M., S. Makni, et al. Autosomal dominant Alport's syndrome: study of a large Tunisian family. Saudi J Kidney Dis Transpl 2006;17(3): 320-325.
7. Marcocci E, Uliana V, Bruttini M*, et al.* Autosomal dominant Alport syndrome: molecular analysis of the COL4A4 gene and clinical outcome. Nephrol Dial Transplant 2009;24(5):1464-71.
8. Fallerini, C., L. Dosa, et al. Unbiased next generation sequencing analysis confirms the existence of autosomal dominant Alport syndrome in a relevant fraction of cases. Clin Genet 2013

Autosomal dominant Alport Syndrome/2

- Symptoms, findings
  - Microscopic hematuria, some had macroscopic hematuria
  - Low or heavy proteinuria, elevated serum creatinine
  - Severe renal failure and ESRD usually after 40-60 yo
  - On biopsy, thinning/thickenning, splitting of GBM, no lamellation reported
  - Some had sensorineural hearing loss at various ages, since young age
  - Ocular signs only reported by Fallerini et al (Clin Genet 2013), 5/35 (14%)

CLINICAL RESEARCH www.jasn.org

A turning point…

COL4A3/COL4A4 Mutations Producing Focal Segmental Glomerulosclerosis and Renal Failure in Thin Basement Membrane Nephropathy

Konstantinos Voskarides,* Loukas Damianou,† Vassos Neocleous,‡ Ioanna Zouvani,§ Stalo Christodoulidou,† Valsamakis Hadjiconstantinou,† Kyriacos Ioannou,‖ Yiannis Athanasiou,‖ Charalampos Patsias,¶ Efstathios Alexopoulos,** Alkis Pierides,‖ Kyriacos Kyriacou,‡ and Constantinos Deltas*‡

*Department of Biological Sciences, University of Cyprus, ‡Cyprus Institute of Neurology and Genetics, and Departments of §Histopathology and ‖Nephrology, Nicosia General Hospital, Nicosia, Cyprus; †Department of Nephrology, Evangelismos Hospital, Athens, Greece; ¶Department of Nephrology, Larnaca General Hospital, Larnaca, Cyprus; and **Department of Nephrology, Aristotle University of Thessalloniki, Greece

families clinically affected with thin basement membrane nephropathy. These families first came to our attention because they segregated microscopic hematuria, mild proteinuria, and variable degrees of renal impairment, but a dual diagnosis of focal segmental glomerulosclerosis (FSGS) and thin basement membrane nephropathy was made in 20 biopsied cases. Molecular studies identified founder mutations in both *COL4A3* and *COL4A4* genes in 10 families. None of 82 heterozygous patients had any extrarenal manifestations, supporting the diagnosis of

Voskarides K et al, JASN 2007

Nephrol Dial Transplant (2009) 1: 1–9
doi: 10.1093/ndt/gfp158

NDT
Nephrology Dialysis Transplantation

Original Article

Clinico-pathological correlations in 127 patients in 11 large pedigrees, segregating one of three heterozygous mutations in the *COL4A3/COL4A4* genes associated with familial haematuria and significant late progression to proteinuria and chronic kidney disease from focal segmental glomerulosclerosis

Alkis Pierides[1], Konstantinos Voskarides[2], Yiannis Athanasiou[1], Kyriacos Ioannou[1], Loukas Damianou[3,4], Maria Arsali[1], Michalis Zavros[1], Michael Pierides[5], Vasilios Vargemezis[4], Charalambos Patsias[1], Ioanna Zouvani[6], Avraam Elia[7], Kyriacos Kyriacou[8] and Constantinos Deltas[2]

Conclusions. Our data confirm for the first time a definite association of heterozygous *COL4A3/COL4A4* mutations with familial microscopic haematuria, thin basement membrane nephropathy and the late development of familial proteinuria, CRF, and ESRD, due to FSGS, indicating that the term 'benign familial haematuria' is a misnomer, at least in this cohort. A strong hypothesis for a causal relationship between these mutations and FSGS is also made. Benign familial haematuria may not be so benign as commonly thought.

Pierides A et al, NDT 2009

The Cyprus Experience
Initial report on 82 patients

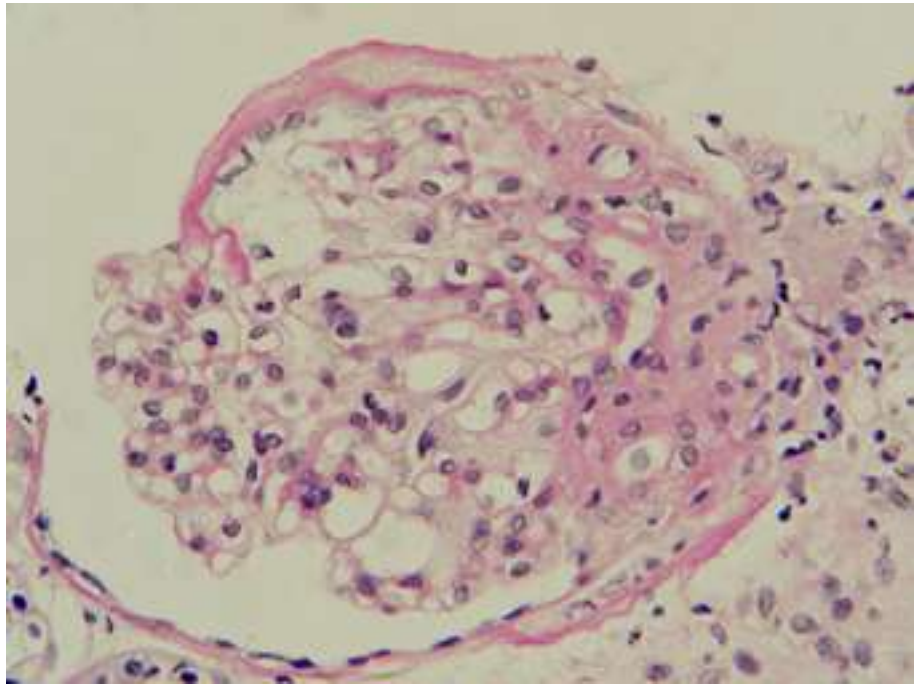
All 13 families were initially diagnosed with **familial Focal Segmental Glomerulosclerosis**

We excluded *ACTN4*, *CD2AP* and *TRPC6*

In 10 of 13 families we found heterozygous mutations in *COL4A3/COL4A4* genes, supporting Thin Basement Membrane Nephropathy

A significant percentage of these patients developed CKD or ESRD

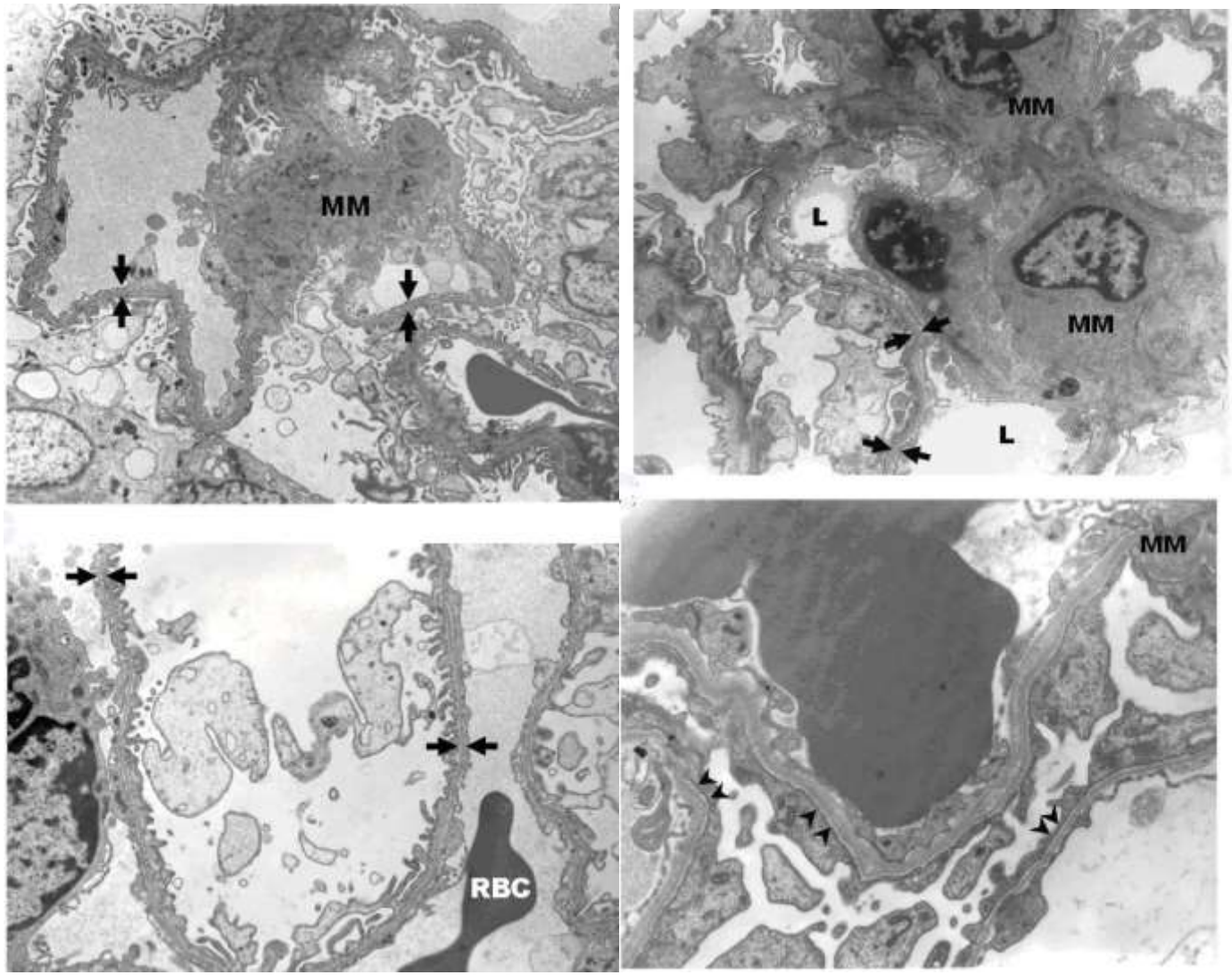
Dept of Histology, NGH/Dr Zouvani



CY-5303

-Voskarides et al, *J Am Soc Nephrol* 2007
-Pierides et al, *Nephrol Dial Transplant* 2009
-Deltas C, *Pediatr Nephrol* 2009

The Revelation - A dual diagnosis of
Familial FSGS in the presence of Thin Basement Membrane Nephropathy
Podocyte Foot Process Effacement

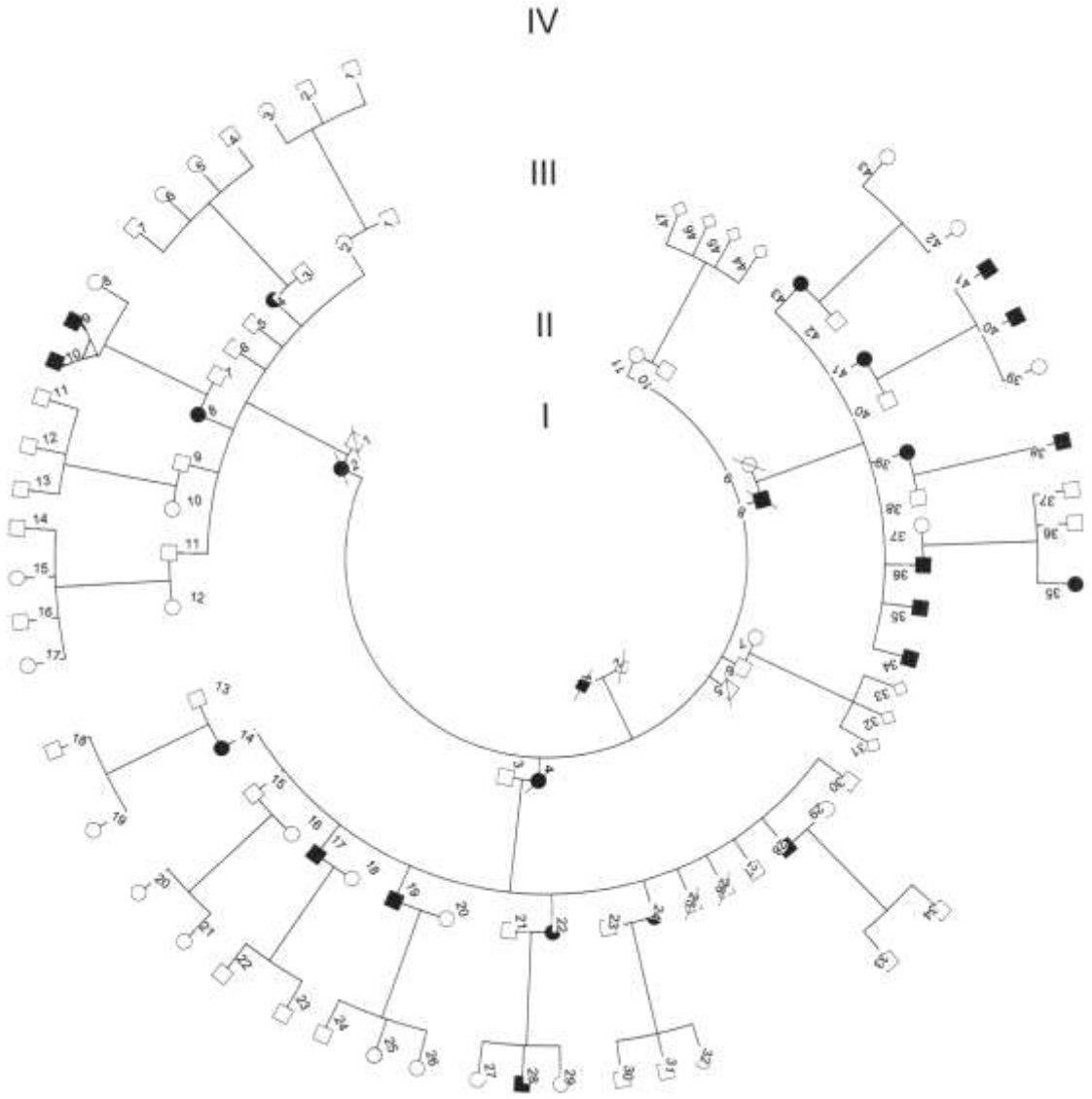


Electron Microscopy: Dept of EM/CING, Dr K. Kyriacou

«Στερνή μου γνώση
να σ' είχα πρώτη»

-Voskarides et al, *J Am Soc Nephrol* 2007
-Pierides et al, *Nephrol Dial Transplant* 2009
-Deltas C. *Pediatr Nephrol* 2009

Family 5301-Mutation *COL4A3* / G1334E

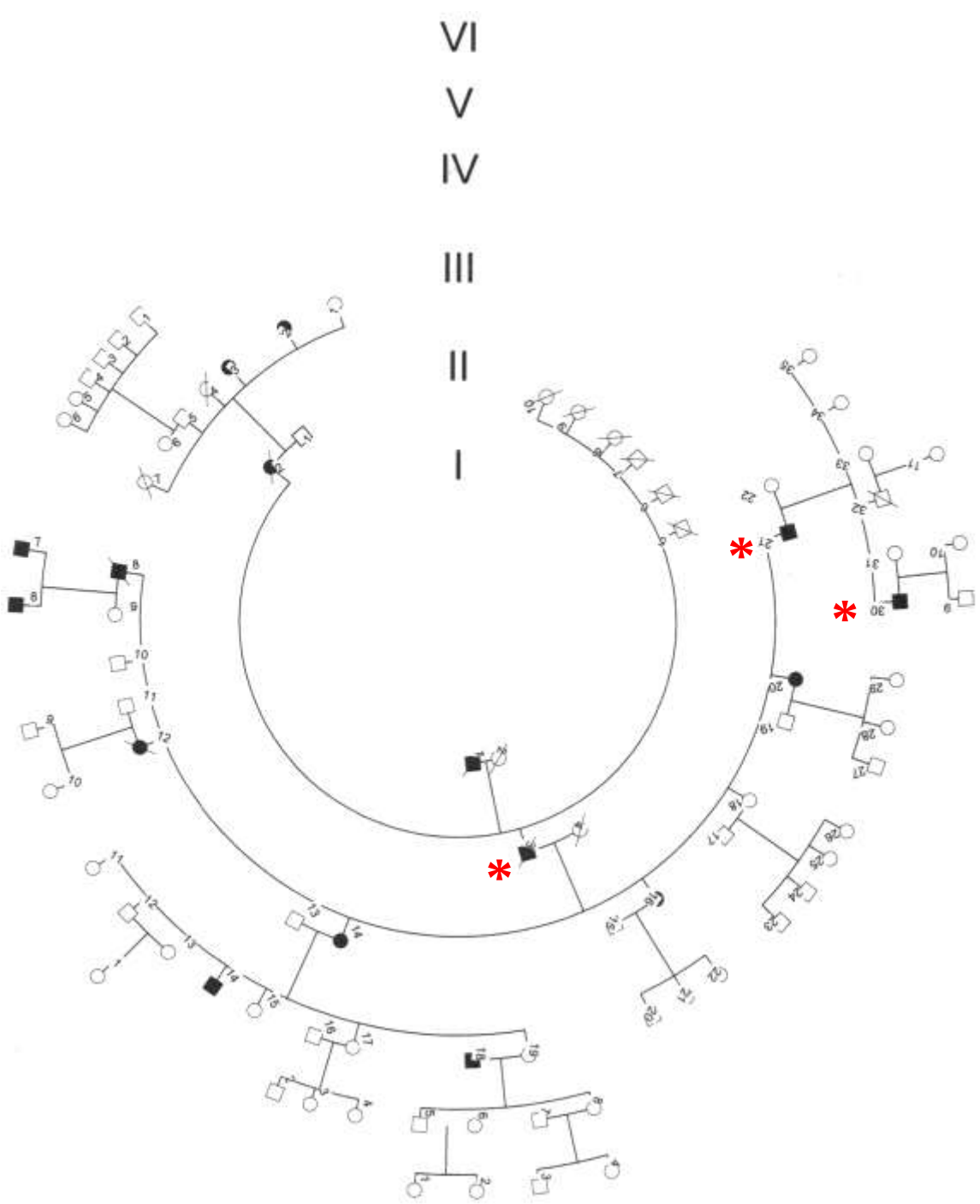


Patients start with microhematuria and progress over 20, 30 or 40 years of follow-up to proteinuria, CKD & ESRD, usually NO DEAFNESS and NO OCULAR problems.

Patients of generation II reached ESRD

Most patients in generation III have CRF or ESRD

GREAT Phenotypic Heterogeneity and age-dependent penetrance



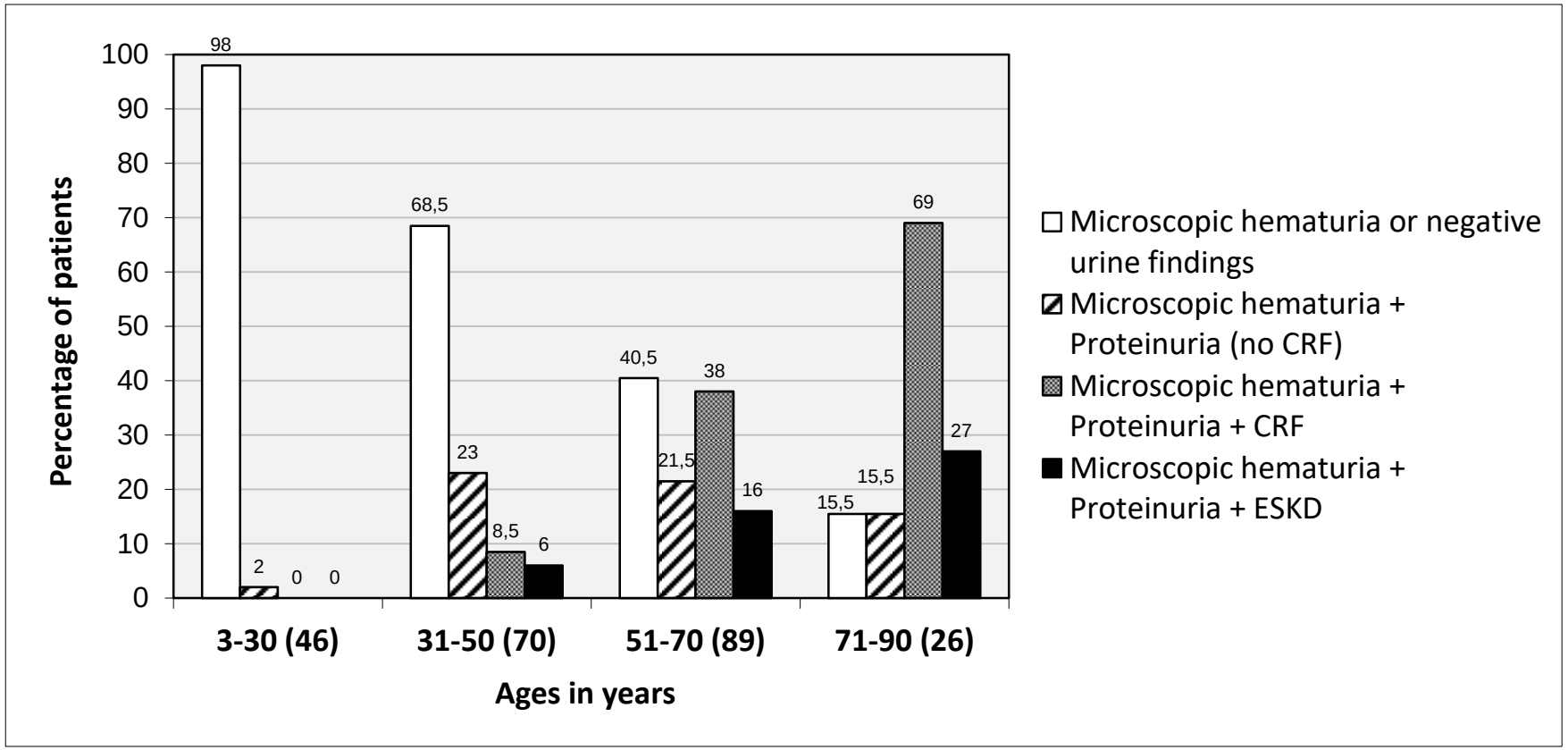
CY5307 family
COL4A3-G1334E mutation

There is a clear three generation CRF inheritance in individuals **II3, III21, IV30**

GREAT Phenotypic Heterogeneity
and age-dependent penetrance

Autosomal Recessive Alport -Thin Basement Membrane Nephropathy

Late Onset Alport Nephropathy



-231 live patients with TBMN, heterozygous for known *COL4A3/A4* mutations (18/08/2014)
(number of patients in parenthesis, on X-axis)

-Until 30 years there is only isolated microscopic hematuria

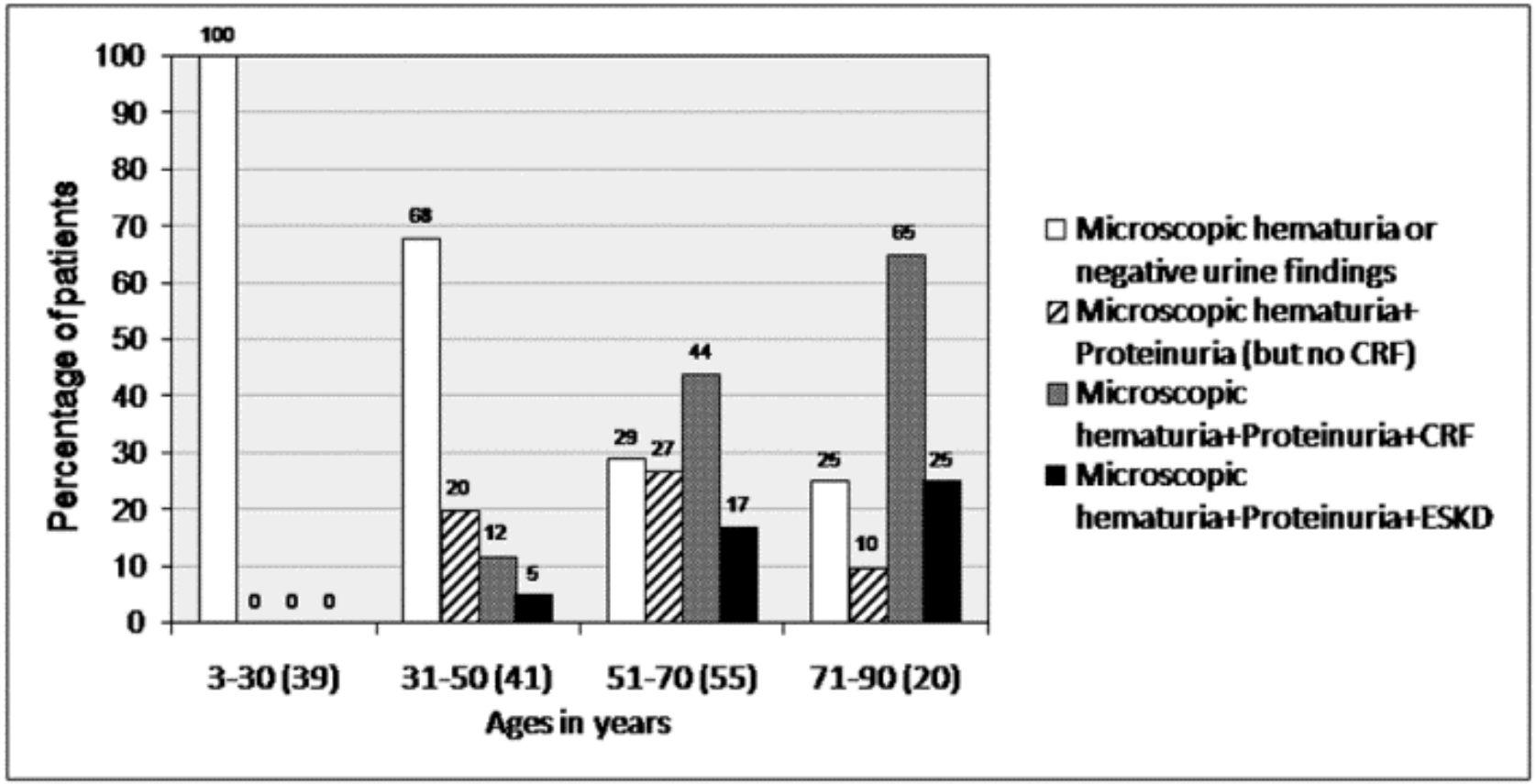
-Among patients aged 51-70 years, 38% developed chronic renal failure.

-"~~BENIGN~~" familial hematuria is not benign at all.

Deltas et al, *Nephrol Dial Transplant* 2013 and unpublished results

Autosomal Recessive Alport -Thin Basement Membrane Nephropathy

Late Onset Alport Nephropathy



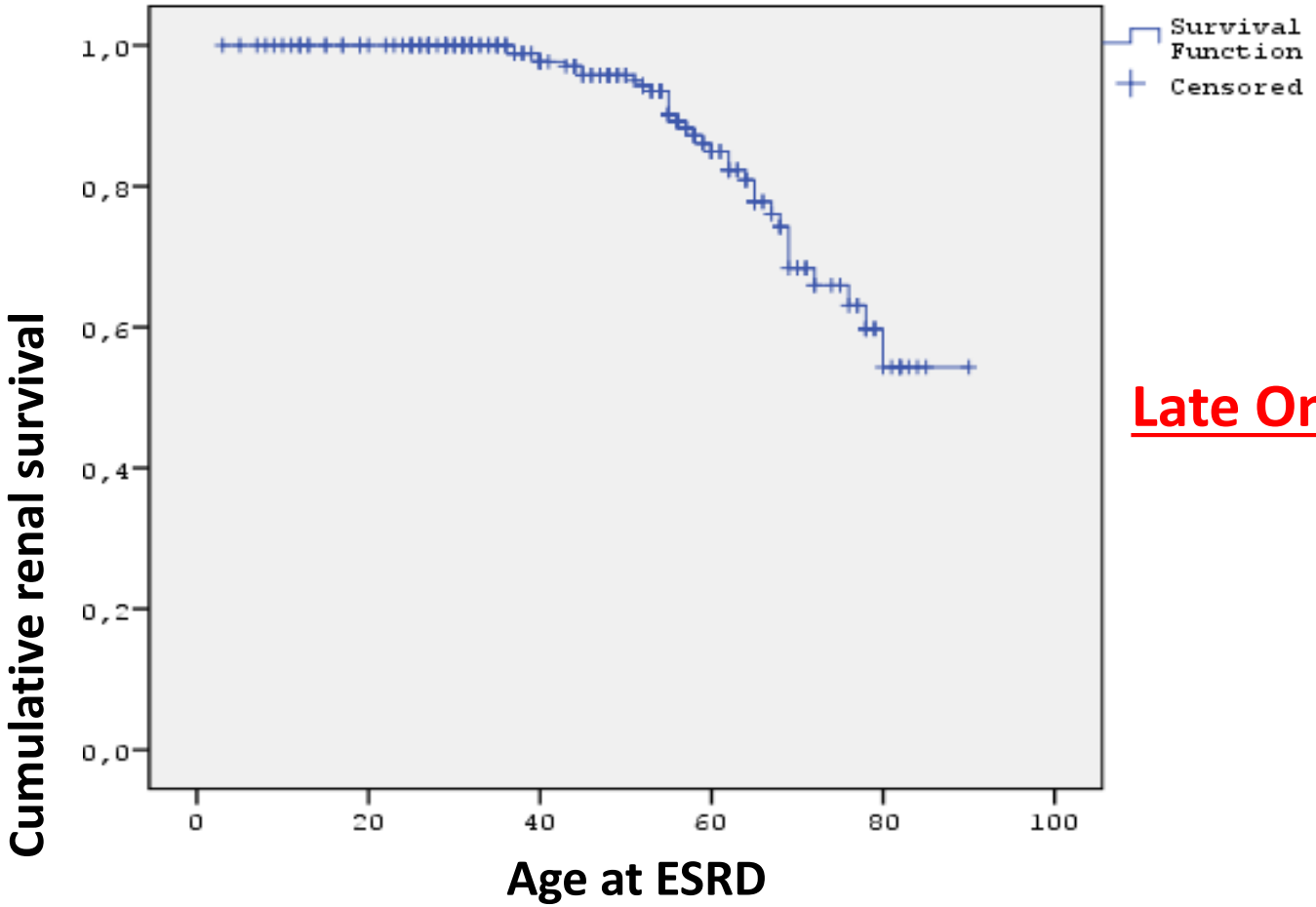
-155 live patients with TBMN, carrying a founder mutation, *COL4A3*-G1334E
(number of patients in parenthesis, on X-axis).

-Among patients aged 51-70 years 44% progress to chronic renal failure of variable degree, including ESRD

-"~~BENIGN~~" familial hematuria is not benign at all.

Deltas et al, *Nephrol Dial Transplant* 2013

Autosomal Recessive Alport -Thin Basement Membrane Nephropathy



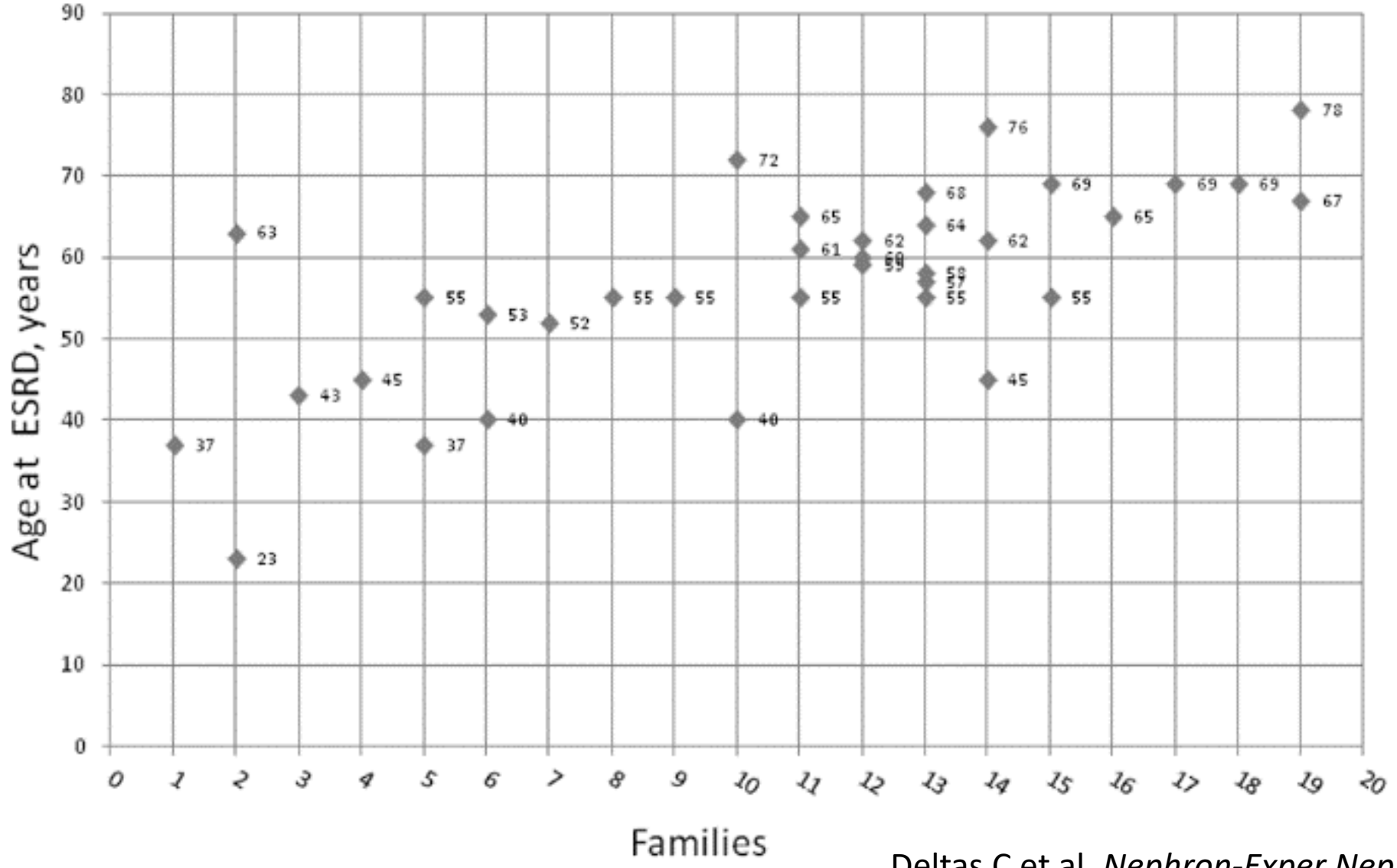
Late Onset Alport Nephropathy

-Kaplan-Meier analysis of renal survival in **248** TBMN patients
-No association of gender and disease progression.
-By the age of 70 years nearly 35-40% reach ESRD, a fact which clearly challenges the formerly thought benign nature of the disease, at least in this cohort.
-"~~BENIGN~~" familial hematuria is not benign at all.

Deltas et al, *Nephrol Dial Transplant* 2013 and unpublished results

Impressive phenotypic heterogeneity amongst patients with thin basement membrane nephropathy (heterozygous mutations in genes *COL4A3/A4*)

LATE ONSET ALPORT NEPHROPATHY



Deltas C et al, *Nephron-Exper Nephrol & Genet 2015*

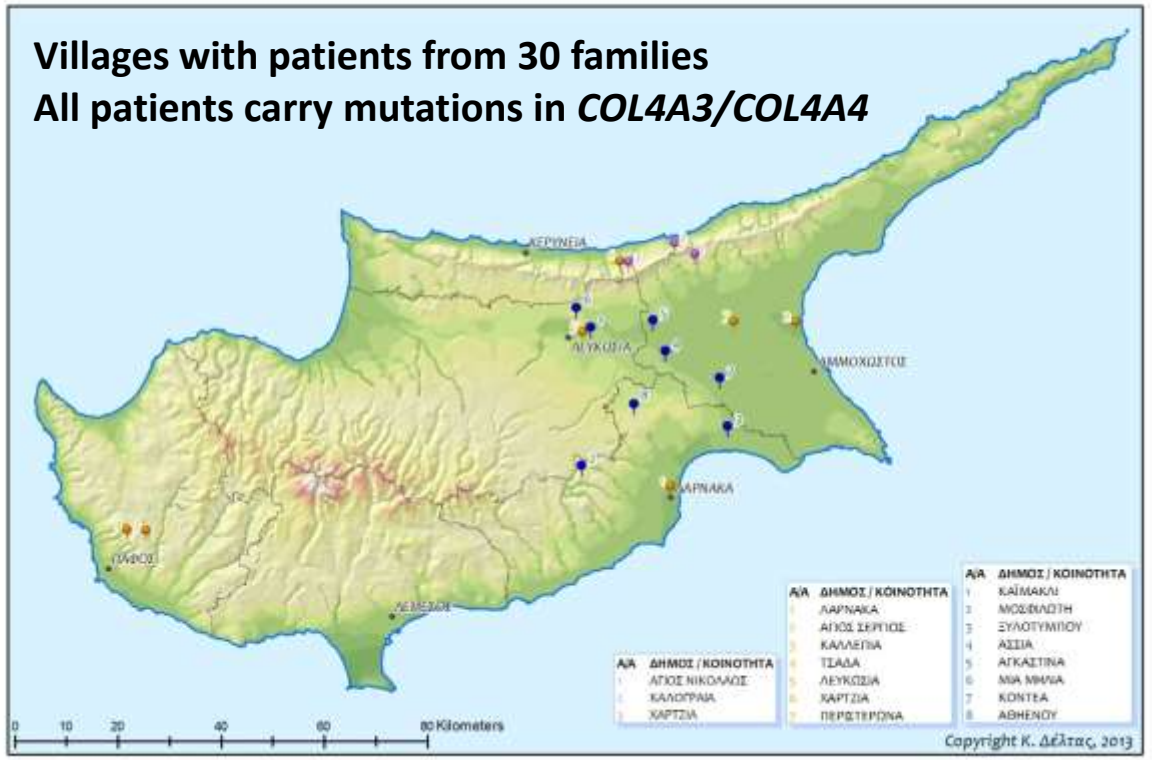
26 biopsies in carriers of 17 families of autosomal recessive Alport

Family	Biopsy result	Age at biopsy	Mutation
CY-5301	FSGS (3), TBMN-FSGS(1)	45, 53, 51, 47	COL4A3-G1334E
CY-5303	TBMN-FSGS(3)	48, 48, 40	COL4A4-c.3854del
CY-5304	TBMN-FSGS(1)	35	COL4A3-G1334E
CY-5306	FSGS (1)	32	COL4A3-G1334E
CY-5307	TBMN-FSGS(2)	60, 63	COL4A3-G1334E
CY-5313	TBMN-FSGS(2)	41, 52	COL4A3-G1334E
CY-5314	TBMN-FSGS(2)	53, 57	COL4A3-G1334E
CY-5323	FSGS (1)	37	COL4A3-G871C
CY-4201	FSGS (1)	58	COL4A3-G871C
CY-5467	TBMN & Alport signs (1)	51	COL4A3-G1334E
CY-5321	TBMN-FSGS(1)	?	COL4A4-c.3854delG
CY-5371	TBMN, FSGS (2)	??	COL4A3-G1334E
CY-5374	TBMN-FSGS(1)	60	COL4A3-G1334E
CY-5376	TBMN-FSGS(1)	?	COL4A3-G1334E
CY-5442	FSGS (1)	35	COL4A3-G1334E
CY-5346	TBMN-FSGS (1)	45	COL4A3-G871C
CY-5322/4204*	TBMN-FSGS(1)	40	COL4A3-G1077D

The genetic map of Cyprus

Thin Basement Membrane Nephropathy presenting as FSGS

Villages with patients from 30 families
All patients carry mutations in *COL4A3/COL4A4*



A peasant roaming in Mesaoria

The inheritance paradox

Putative explanations for the adverse course of disease in TBMN patients

- Considering that the heterogeneity is observed even within same families:
  1. Co-inheritance of a separate serious condition
  2. Co-occurrence of a separate not heritable condition, by pure chance (eg IgAN)
  3. Co-inheritance of ***genetic modifiers*** that on their own are totally benign
  4. Environmental factors
  5. Epigenetic factors

Pediatr Nephrol
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BRIEF REPORT

Evidence that *NPHS2*-R229Q predisposes to proteinuria and renal failure in familial hematuria

Konstantinos Voskarides · Maria Arsali · Yiannis Athanasiou · Avraam Elia · Alkis Pierides · Constantinos Deltas

Pediatr Nephrol

Table 1 Frequencies and statistics of *R229Q–NPHS2* by disease and by severity

Cohort	Number	Genotype counts			Genotype frequency			Allele counts		Allele frequencies		*P* values		
		RR	RQ	QQ	RR	RQ	QQ	R	Q	R	Q	Cases vs general population[a]	Mild vs severe[a]	Mild vs severe[b]
General population	150	144	6	0	0.960	0.040	0	294	6	0.980	0.020			
TBMN	44	44	0	0	1	0	0	88	0	1	0			
CFHR5	18	18	0	0	1	0	0	36	0	1	0			
Mild	62	62	0	0	1	0	0	124	0	1	0	0.184		
TBMN	58	55	3	0	0.948	0.052	0	113	3	0.974	0.026			
CFHR5	27	21	6	0	0.778	0.222	0	48	6	0.889	0.111			
Severe	85	76	9	0	0.894	0.106	0	161	9	0.947	0.053	0.056	0.010*	0.043*

TBMN thin basement membrane nephropathy, *CFHR5* complement factor H R5

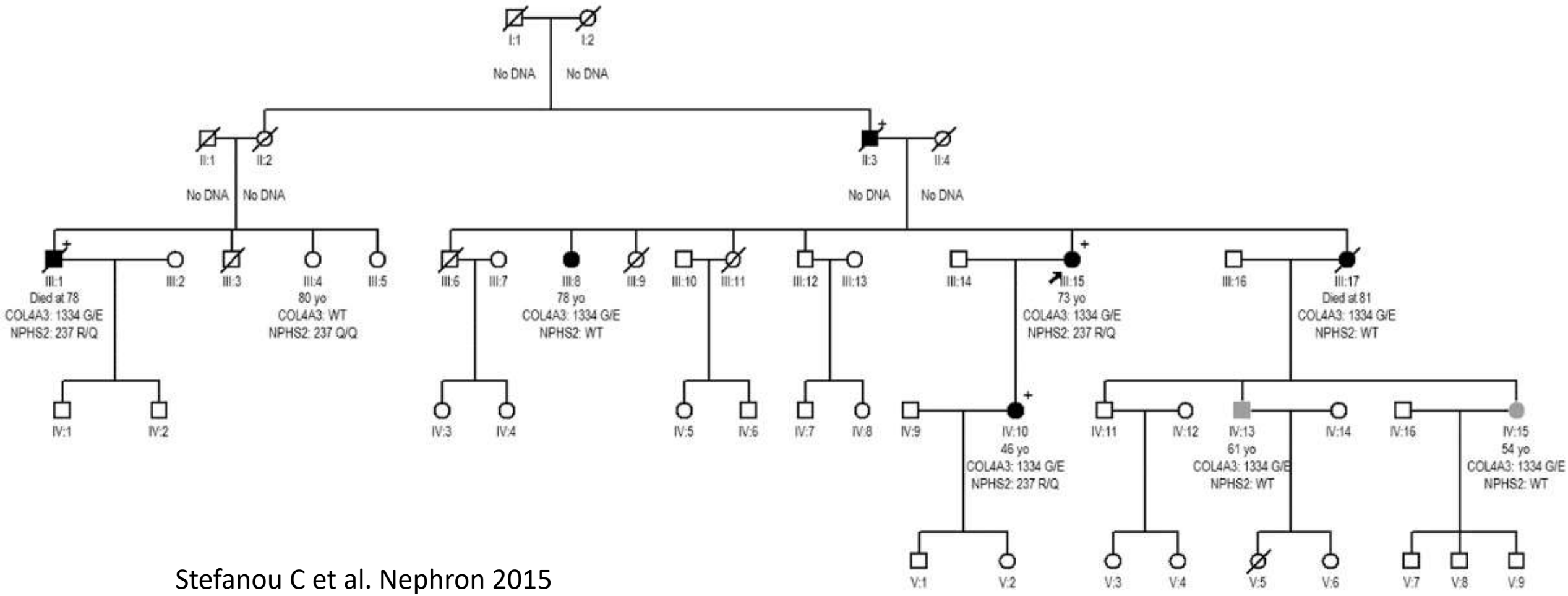
[a] Genotypic association using two-sited Fisher's exact test; [b] allelic association, correcting the *p* values using kinship coefficients (see text)

*Statistical significance ($p<0.05$)

-Tonna et al, *Pediatr Nephrol*, 2008
-Voskarides et al, *Pediatr Nephrol*, 2011

Family: CY5304 | *COl4A3*-p.Gly1334Glu
Thin basement membrane nephropathy

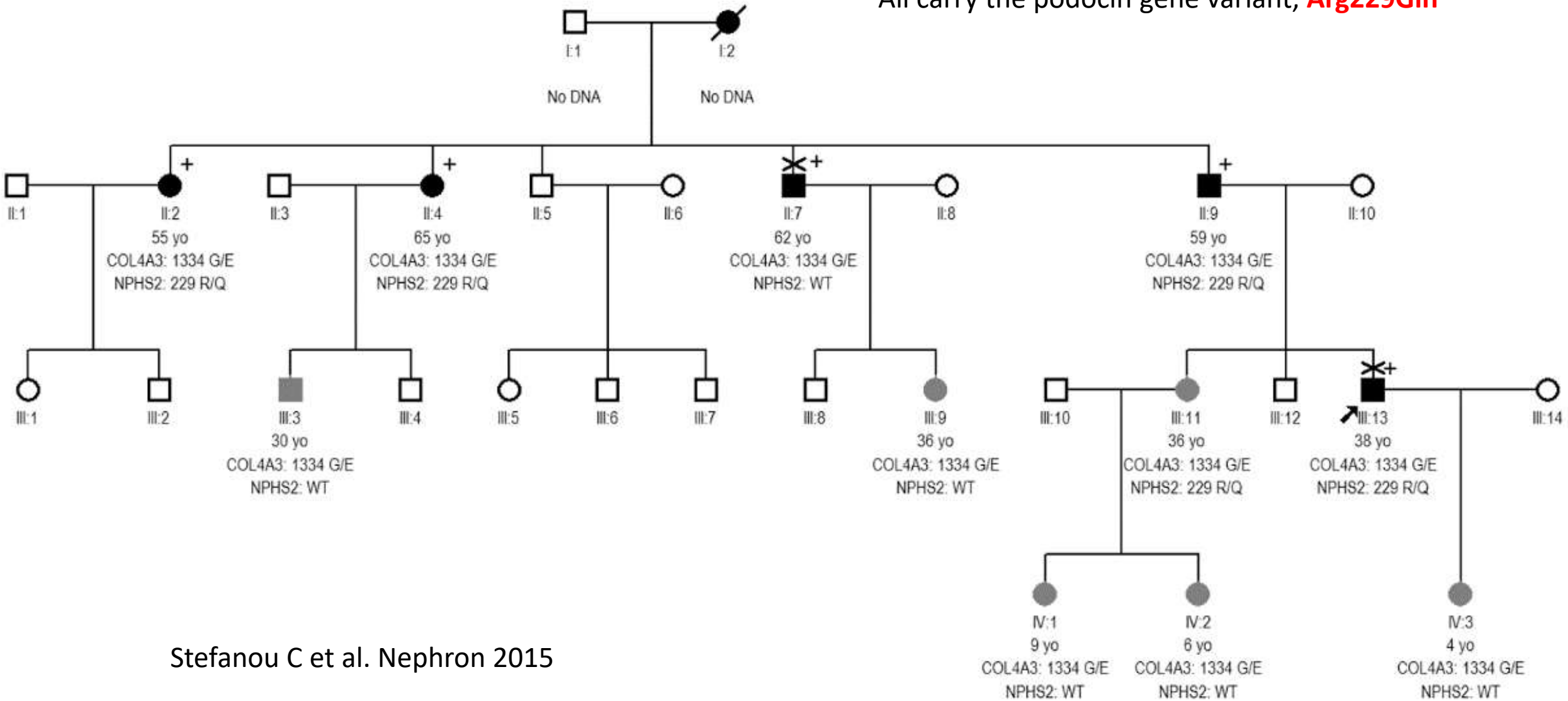
Patients with a cross symbol have a severe phenotype
All three carry the podocin gene variant, **Glu237Gln**



Stefanou C et al. Nephron 2015

Family: CY5376 | *COl4A3*-p.Gly1334Glu
Thin basement membrane nephropathy

Patients with a cross symbol (+) have a severe phenotype
All carry the podocin gene variant, **Arg229Gln**



Stefanou C et al. Nephron 2015

Table 3. Clinical information for the seven "severe" patients carrying a heterozygous mutation in *COL4A3* and a modifier in the *NPHS2* gene

Family/ Patient/Gender	Mutations	Age at ESRD	Biopsy	Other*	Age by 2013	Age of death
CY5304 / III:1 Male	COL4A3-p.Gly1334Glu / NPHS2-p.Glu237Gln	78	ND			78
CY5304 / III:15 Female	COL4A3-p.Gly1334Glu / NPHS2-p.Glu237Gln	67	ND		73	
CY5304 / IV:10 Female	COL4A3-p.Gly1334Glu / NPHS2-p.Glu237Gln		TBMN-FSGS	s.cr.: 0,93mg/dl proteinuria: 700mg /24hrs	46	
CY5376 / II:2 Female	COL4A3-p.Gly1334Glu / NPHS2-p.Arg229Gln		ND	s.cr.: 1,70mg/dl proteinuria: 1200mg /24hrs	55	
CY5376 / II:4 Female	COL4A3-p.Gly1334Glu / NPHS2-p.Arg229Gln		ND	s.cr.: 1,45mg/dl proteinuria: 600mg /24hrs	65	
CY5376 / II:9 Male	COL4A3-p.Gly1334Glu / NPHS2-p.Arg229Gln		ND	s.cr.: 1,40mg/dl proteinuria	59	
CY5376 / III:13 Male	COL4A3-p.Gly1334Glu / NPHS2-p.Arg229Gln	37	ND	vesicoureteral reflux since childhood	38	

Stefanou C et al. Nephron 2015

Whole Exome Sequencing of 260 patients with Thin Basement Membrane Nephropathy

- Average coverage of 80X
- The percentage of mapped reads was high (>95%), and we get less than 20% of duplicated reads
- Overall we identified **837,313 variants** (SNPs and INDELs) in the cohort of 260 samples. A large proportion of these variants was never reported in public databases
- Missense variants represent ~**10%** of the total number of variants identified, UTRs harbour ~25% of the variants, and ~46% are located in intronic regions which are included in our analysis because we extended the target regions of 200bp up- and down-stream.

Table 3. Genomic location of variants (from KGGSeq)

Feature	Number	Explanation
Frameshift	3,932 (0.47%)	Short insertion or deletion result in a completely different translation from the original.
Nonframeshift	3,432 (0.41%)	Short insertion or deletion results in loss of amino acids in the translated proteins.
Startloss	360 (0.043%)	Indels or nucleotide substitution result in the loss of start codon(ATG) (mutated into a non-start codon).
Stoploss	226 (0.027%)	Indels or nucleotide substitution result in the loss of stop codons (TAG, TAA, TGA).
Stopgain	2,226 (0.26%)	Indels or nucleotide substitution result in the new stop codons (TAG, TAA, TGA), which may truncate the protein.
Splicing	29,104 (3.47%)	Variant is within 13-bp of a splicing junction.
Missense	90,501 (10.8%)	Variants result in a codon coding for a different amino acid (missense)

Synonymous	56,551 (6.754%)	Nucleotide substitution does not change amino acid.
Exonic	25 (0.003%)	Due to loss of sequences in reference database, this variant can only be mapped into exonic region without more precise annotation.
5UTR	44,081 (5.265%)	Within a 5' untranslated region
3UTR	166,223 (19.85%)	Within a 3' untranslated region
Intronic	385,777 (46.07%)	Within an intron
Upstream	22,443 (2.68%)	Within 1-kb region upstream of transcription start site
Downstream	19,630 (2.34%)	Within 1-kb region downstream of transcription end site
ncRNA	12,663 (1.51%)	Within a transcript without protein-coding annotation in the gene definition
Intergenic	139 (0.017%)	Variant is in intergenic region

Whole Exome Sequencing

ERA-EDTA funded project

COL4Alport

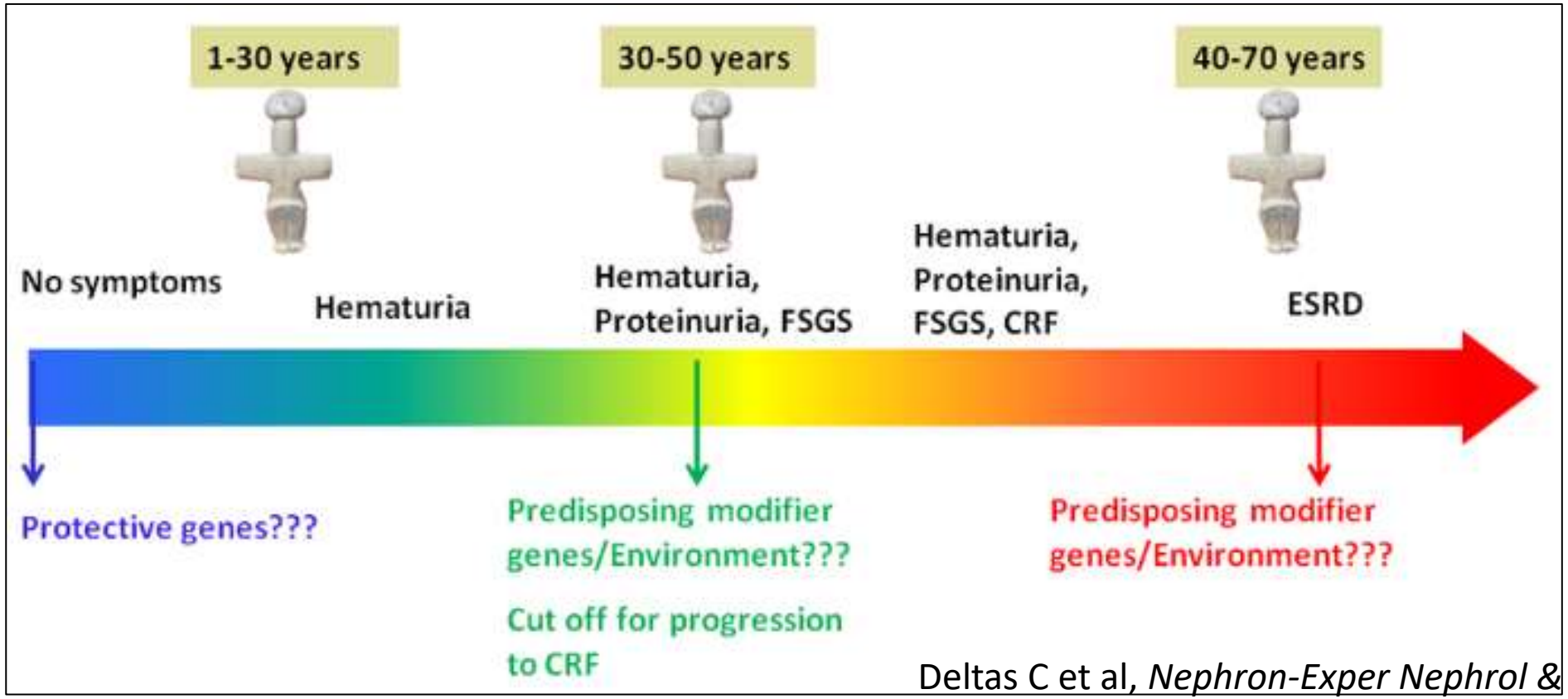
Why some patients do better than others?

- 260 patients with TBMN
- Classified as “Severely” or “Mildly” affected
- >800,000 DNA variants identified
- Most variants in intronic, non-coding regions
- About 10,000 DNA variants in exonic coding regions
- Identifying good candidates as genetic modifiers is a ***challenge***
- Detecting digenic inheritance is a rare event
  - Fallerini et al, Clin Genet 2016
  - Mencarelli et al, J Med Genet 2015

Carriers of autosomal recessive Alport Syndrome, Thin Basement Membrane Nephropathy, frequently presenting with FSGS

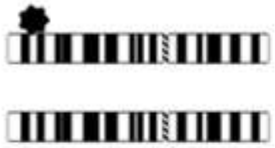
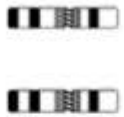
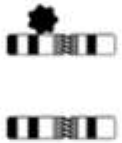
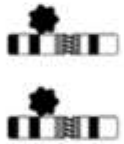
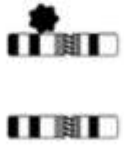
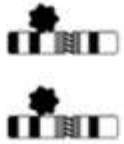
LATE ONSET ALPORT NEPHROPATHY

-Reduced penetrance accompanied by age-dependent penetrance
-Progressive impairment of kidney function
-The full spectrum of the phenotype behaves as a ***multifactorial*** condition, implicating ***primary*** genes, ***modifier*** genes and ***environmental*** factors



Deltas C et al, *Nephron-Exper Nephrol & Genet 2015*

A working hypothesis

COL4A3 or *COL4A4* mutation (chr. 2)	*NEPH3* variant (chr. 19)	
		Mostly familial benign hematuria
		TBMN and gradual loss of kidney function
		TBMN and gradual loss of kidney function Perhaps susceptible to fast progression
		Normal kidney function
		Susceptibility to microalbuminuria

FSGS treatment options

Question: Why is the correct diagnosis of significance?

Answer: Because it may dictate treatment

- Primary FSGS
  - Non specific therapy (ACE inhibitors, ARBs, optimal blood pressure control, statins, diet)
  - Specific therapy (immunosuppressive drugs: prednisone, MMF, cyclosporine, cyclophosphamide)

- Secondary FSGS
  - Non specific therapy (ACE inhibitors, ARBs, optimal blood pressure control, statins, diet)

Conclusions/1

1. It is not unusual for TBMN/COL4 mutations to present as FSGS and be mistaken for FSGS

2. "~~**BENIGN**~~" familial hematuria is a misnomer for a significant % of carriers of ARAS/TBMN, who develop FSGS and progress to chromic kidney function decline (CKD/ESRD)

3. During childhood TBMN ***is a Benign*** condition. However, ALL adults with TBMN who progress to FSGS and CRF/ESRD went through childhood☺

4. Consider preparing detailed pedigrees for identifying inheritance pattern. It is of paramount importance to have ***long follow-up*** into adulthood and maintain good archives

Conclusions/2

5. In familial MH consider at least a single biopsy in a family. It may assist DNA analysis and obviate the need for more biopsies.

6. DNA sequencing remains the gold-standard for the final diagnosis. ***Next generation sequencing*** is expected to boost the analysis and lead to robust characterization of more patients on the borderline of several distinct diagnoses.

7. In patients who are carriers of ARAS/TBMN, the expression of the full spectrum of the phenotype behaves as a ***multifactorial*** condition, implicating ***primary*** genes, ***modifier*** genes and ***environmental*** factors

Conclusions/3

- 8. Perhaps a better name for thin basement membrane nephropathy would be:

  Late Onset Alport Nephropathy (LOAN)

The genetic heritage of Cypriots through special topics of genetics

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Dr Alkis Pierides

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- Dr Konstant. Voskarides, Univ. of Cyprus
- Dr Pan. Demosthenous, Univ. of Cyprus
- Dr Greg. Papagregoriou, Univ. of Cyprus
- Dr Myrtani Pieri, University of Cyprus
- Dr Isavella Savva, Univ. of Cyprus
- Dr Kamil Erguler, Univ. of Cyprus/Cyprus Inst
- Charis Stephanou, Univ. of Cyprus
- Louiza Papazachariou. Univ. of Cyprus
- Dr Kyriacos Felekkis, University of Nicosia
- Natasa Anastasiadou, Nicosia General Hospital
- Dr Kyriacos Kyriacou, The Cyprus Inst. of Neurology and Genetics (EM)
- Dr Alkis Pierides, Hippokrateion Hospital
- Dr Michalis Zavros, Nicosia General Hospital
- Dr Yiannis Athanasiou, Nicosia General Hosp
- Dr Kyriacos Ioannou, Nicosia General Hospital
- Dr Akis Lazarou, Limassol General Hospital
- Dr Maria Arsali, Limassol General Hospital
- Dr Loukas Damianou, Limassol General Hosp
- Dr Ioanna Zouvani, Nicosia General Hospital
- Dr Michalis Hadjigavriel, Larnaca General Hosp
- Dr Maria Kkolou, Larnaca General Hospital
- Dr Androulla Pastelli, Larnaca General Hospital
- Dr Panag. Loukaidou, Larnaca General Hospital
- Dr Christoforos Stavrou, Evangelismos, Pafos

Thank you for your attention!

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