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

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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

- Medical info
- DNA
- Plasma
- Serum
- Urine
- Biopsies
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*)
Microscopic hematuria

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

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

\[\text{Gly-X-Y}\]

Positions X & Y are frequently occupied by prolines

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Collagen IV network in the basement membrane

\[ \alpha_3\alpha_4\alpha_5 \text{ OR } \alpha_5\alpha_5\alpha_6 \]

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

Frederick E. Dischea, Michael J. Westonb, Victor Parsonsb

aDepartment of Pathology and bRenal 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
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.
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.
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)**
### Previous publications


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%)
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,¶ Efthathios 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, Lamaca General Hospital, Lamaca, Cyprus; and **Department of Nephrology, Aristotle University of Thessaloniki, 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
Clinico-pathological correlations in 127 patients in 11 large pedigrees, segregating one of three heterozygous mutations in the \textit{COL4A3/COL4A4} genes associated with familial haematuria and significant late progression to proteinuria and chronic kidney disease from focal segmental glomerulosclerosis.

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

Conclusions. Our data confirm for the first time a definite association of heterozygous \textit{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.
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.

-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

-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
There is a clear three generation CRF inheritance in individuals II3, III21, IV30
GREAT Phenotypic Heterogeneity and age-dependent penetrance
- 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
- 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

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

<table>
<thead>
<tr>
<th>Family</th>
<th>Biopsy result</th>
<th>Age at biopsy</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY-5301</td>
<td>FSGS (3), TBMN-FSGS(1)</td>
<td>45, 53, 51, 47</td>
<td>COL4A3-G1334E</td>
</tr>
<tr>
<td>CY-5303</td>
<td>TBMN-FSGS(3)</td>
<td>48, 48, 40</td>
<td>COL4A4-c.3854del</td>
</tr>
<tr>
<td>CY-5304</td>
<td>TBMN-FSGS(1)</td>
<td>35</td>
<td>COL4A3-G1334E</td>
</tr>
<tr>
<td>CY-5306</td>
<td>FSGS (1)</td>
<td>32</td>
<td>COL4A3-G1334E</td>
</tr>
<tr>
<td>CY-5307</td>
<td>TBMN-FSGS(2)</td>
<td>60, 63</td>
<td>COL4A3-G1334E</td>
</tr>
<tr>
<td>CY-5313</td>
<td>TBMN-FSGS(2)</td>
<td>41, 52</td>
<td>COL4A3-G1334E</td>
</tr>
<tr>
<td>CY-5314</td>
<td>TBMN-FSGS(2)</td>
<td>53, 57</td>
<td>COL4A3-G1334E</td>
</tr>
<tr>
<td>CY-5323</td>
<td>FSGS (1)</td>
<td>37</td>
<td>COL4A3-G871C</td>
</tr>
<tr>
<td>CY-4201</td>
<td>FSGS (1)</td>
<td>58</td>
<td>COL4A3-G871C</td>
</tr>
<tr>
<td>CY-5467</td>
<td>TBMN &amp; Alport signs (1)</td>
<td>51</td>
<td>COL4A3-G1334E</td>
</tr>
<tr>
<td>CY-5321</td>
<td>TBMN-FSGS(1)</td>
<td>?</td>
<td>COL4A4-c.3854delG</td>
</tr>
<tr>
<td>CY-5371</td>
<td>TBMN, FSGS (2)</td>
<td>??</td>
<td>COL4A3-G1334E</td>
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<td>CY-5374</td>
<td>TBMN-FSGS(2)</td>
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<td>COL4A3-G1334E</td>
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<tr>
<td>CY-5376</td>
<td>TBMN-FSGS(1)</td>
<td>?</td>
<td>COL4A3-G1334E</td>
</tr>
<tr>
<td>CY-5442</td>
<td>FSGS (1)</td>
<td>35</td>
<td>COL4A3-G1334E</td>
</tr>
<tr>
<td>CY-5346</td>
<td>TBMN-FSGS (1)</td>
<td>45</td>
<td>COL4A3-G871C</td>
</tr>
<tr>
<td>CY-5322/4204*</td>
<td>TBMN-FSGS(1)</td>
<td>40</td>
<td>COL4A3-G1077D</td>
</tr>
</tbody>
</table>
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 (e.g., IgAN)
  3. Co-inheritance of *genetic modifiers* that on their own are totally benign
  4. Environmental factors
  5. Epigenetic factors
Evidence that \textit{NPHS2-R229Q} predisposes to proteinuria and renal failure in familial hematuria

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

\textit{Pediatr Nephrol}

Table 1: Frequencies and statistics of \textit{R229Q-NPHS2} by disease and by severity

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Number</th>
<th>Genotype counts</th>
<th>Genotype frequency</th>
<th>Allele counts</th>
<th>Allele frequencies</th>
<th>$P$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RR</td>
<td>RQ</td>
<td>QQ</td>
<td>RR</td>
<td>RQ</td>
</tr>
<tr>
<td>General population</td>
<td>150</td>
<td>144</td>
<td>6</td>
<td>0</td>
<td>0.960</td>
<td>0.040</td>
</tr>
<tr>
<td>TBMN</td>
<td>44</td>
<td>44</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CFHR5</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>62</td>
<td>62</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>TBMN</td>
<td>58</td>
<td>55</td>
<td>3</td>
<td>0</td>
<td>0.948</td>
<td>0.052</td>
</tr>
<tr>
<td>CFHR5</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>0</td>
<td>0.778</td>
<td>0.222</td>
</tr>
<tr>
<td>Severe</td>
<td>85</td>
<td>76</td>
<td>9</td>
<td>0</td>
<td>0.894</td>
<td>0.106</td>
</tr>
</tbody>
</table>

\textit{TBMN} thin basement membrane nephropathy, \textit{CFHR5} complement factor H R5

\textsuperscript{a} Genotypic association using two-sided Fisher’s exact test; \textsuperscript{b} allelic association, correcting the $p$ values using kinship coefficients (see text)

\textsuperscript{*} Statistical significance ($p<0.05$)
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
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 \textit{COL4A3} and a modifier in the \textit{NPHS2} gene

<table>
<thead>
<tr>
<th>Family/Patient/Gender</th>
<th>Mutations</th>
<th>Age at ESRD</th>
<th>Biopsy</th>
<th>Other*</th>
<th>Age by 2013</th>
<th>Age of death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>s.cr.: 0,93mg/dl proteinuria: 700mg /24hrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CY5376 / II:2 Female</td>
<td>COL4A3-p.Gly1334Glu/NPHS2-p.Arg229Gln</td>
<td>ND</td>
<td></td>
<td>s.cr.: 1,70mg/dl proteinuria: 1200mg /24hrs</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>CY5376 / II:4 Female</td>
<td>COL4A3-p.Gly1334Glu/NPHS2-p.Arg229Gln</td>
<td>ND</td>
<td></td>
<td>s.cr.: 1,45mg/dl proteinuria: 600mg /24hrs</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>CY5376 / II:9 Male</td>
<td>COL4A3-p.Gly1334Glu/NPHS2-p.Arg229Gln</td>
<td>ND</td>
<td></td>
<td>s.cr.: 1,40mg/dl proteinuria</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Feature</th>
<th>Number</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frameshift</td>
<td>3,932 (0.47%)</td>
<td>Short insertion or deletion result in a completely different translation from the original.</td>
</tr>
<tr>
<td>Nonframeshift</td>
<td>3,432 (0.41%)</td>
<td>Short insertion or deletion results in loss of amino acids in the translated proteins.</td>
</tr>
<tr>
<td>Startloss</td>
<td>360 (0.043%)</td>
<td>Indels or nucleotide substitution result in the loss of start codon (ATG) (mutated into a non-start codon).</td>
</tr>
<tr>
<td>Stoploss</td>
<td>226 (0.027%)</td>
<td>Indels or nucleotide substitution result in the loss of stop codons (TAG, TAA, TGA).</td>
</tr>
<tr>
<td>Stopgain</td>
<td>2,226 (0.26%)</td>
<td>Indels or nucleotide substitution result in the new stop codons (TAG, TAA, TGA), which may truncate the protein.</td>
</tr>
<tr>
<td>Splicing</td>
<td>29,104 (3.47%)</td>
<td>Variant is within 13-bp of a splicing junction.</td>
</tr>
<tr>
<td>Missense</td>
<td>90,501 (10.8%)</td>
<td>Variants result in a codon coding for a different amino acid (missense).</td>
</tr>
<tr>
<td>Category</td>
<td>Count</td>
<td>Percentage</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>Synonymous</td>
<td>56,551</td>
<td>6.754%</td>
</tr>
<tr>
<td>Exonic</td>
<td>25</td>
<td>0.003%</td>
</tr>
<tr>
<td>5'UTR</td>
<td>44,081</td>
<td>5.265%</td>
</tr>
<tr>
<td>3'UTR</td>
<td>166,223</td>
<td>19.85%</td>
</tr>
<tr>
<td>Intronic</td>
<td>385,777</td>
<td>46.07%</td>
</tr>
<tr>
<td>Upstream</td>
<td>22,443</td>
<td>2.68%</td>
</tr>
<tr>
<td>Downstream</td>
<td>19,630</td>
<td>2.34%</td>
</tr>
<tr>
<td>ncRNA</td>
<td>12,663</td>
<td>1.51%</td>
</tr>
<tr>
<td>Intergenic</td>
<td>139</td>
<td>0.017%</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>COL4A3 or COL4A4 mutation (chr. 2)</th>
<th>NEPH3 variant (chr. 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Diagram" /></td>
<td>Mostly familial benign hematuria</td>
</tr>
<tr>
<td><img src="image2" alt="Diagram" /></td>
<td>TBMN and gradual loss of kidney function</td>
</tr>
<tr>
<td><img src="image3" alt="Diagram" /></td>
<td>TBMN and gradual loss of kidney function</td>
</tr>
<tr>
<td><img src="image4" alt="Diagram" /></td>
<td>Perhaps susceptible to fast progression</td>
</tr>
<tr>
<td><img src="image5" alt="Diagram" /></td>
<td>Normal kidney function</td>
</tr>
<tr>
<td><img src="image6" alt="Diagram" /></td>
<td>Susceptibility to microalbuminuria</td>
</tr>
</tbody>
</table>
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
Dr Alkis Pierides
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Thank you for your attention!