Inherited Kidney Diseases: Familial Hematuria, a phenotypic CHAMELEON

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Objectives

1) What is familial hematuria? - Describe the problem

2) Describe voluminous findings of research in Cyprus

3) Discuss the broad phenotypic heterogeneity, a phenotypic Chameleon

4) Offer likely explanations for the broad phenotypic heterogeneity

• Key words: Alport Syndrome; Thin basement membrane nephropathy; Familial hematuria; Microscopic hematuria; Benign familial hematuria; Molecular diagnostics; Genetic and phenotypic heterogeneity; Broad spectrum of phenotypes; Genetic modifiers

• MOST IMPORTANT TERMS:
  ➢ Thin basement membrane nephropathy
  ➢ Microscopic hematuria
  ➢ Phenotypic heterogeneity
Microscopic hematuria

The presence of more than 3 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
Probable Medical Scenario

- A young 6-yo boy, in a check up for any reason (eg some infection) he presented with 30-40 erythrocytes in urine (microscopic hematuria)
- He has no micro-albuminuria nor proteinuria
- The serum creatinine is normal (creatinine is a kidney function marker)
- There is no known wide family history of microscopic hematuria or kidney disease
- The mother, when asked, mentions that occasionally her urine analysis demonstrates the presence of erythrocytes, beyond normal number. She was never investigated further.
Could that be an incidental finding without any further significance?

6-yo
Microscopic hematuria
Is mother’s finding a mere coincidence?

Microscopic hematuria

Microscopic hematuria (MH)
X-linked Alport Syndrome

MH

MH

MH

MH

1930-1958
He died at age 28-yo
Perhaps a kidney problem. Allegedly the urinated blood.

Molecular investigation reveals a causative mutation in the \textit{COL4A5} gene (X-linked). Other family members also are found mutation positive. A renal biopsy is not indicated as the diagnosis is now settled.

He is expected to be on dialysis before the age of 30 years. He will need a kidney transplant.
X-linked Alport Syndrome  
A rare disease

- Serious hereditary kidney disease-glomerulopathy, childhood onset
- Usually the affected boys reach end-stage renal disease by the age of 30 years. Some times later.
- Other accompanying symptoms can be hearing loss (85%) and ocular problems (44%)
- Histologically, the ultrastructural presentation on electron microscopy shows a characteristic pathognomonic picture
- Depending on the mutation, immunofluorescence microscopy against the collagen IV chains, demonstrates absence of staining or weak signal
- All daughters of affected males will have one copy of the mutant gene. They will have a much milder disease or late onset severe kidney failure
- The sons of affected males are healthy
- The children of affected women have a 50% chance of inheriting the mutant gene
Figure 1: Electropherograms showing the DNA mutations identified in **COL4A5**. Upper panel, normal sequence; medium panel, heterozygous female; lower panel, hemizygous mutant. Note that very near the single nucleotide deletion of 2946delT, there is a polymorphism with A or G in the heterozygous woman. The mutant allele of the male patient is coinherited with the G allele.

X-linked Alport

Milder phenotype: The overlap of symptoms between men and women prompted the initial diagnosis of TBMN

Family CY-4206: Mutation P628L

Demosthenous et al, *Clin Genet* 2012
X-linked Alport

Milder phenotype: Initial diagnosis was TBMN

Family CY-4212: Mutation P628L

Two Greek-Cypriot families
Fam. CY4206: ESKD at ages 30, 31, 34, 44 & 56 years
Fam. CY4212: ESKD at ages 45 & 52 years
Two patients at 51 & 57-yr have SCr 1.6 & 1.5 mg %

Demosthenous et al, Clin Genet 2012
Micro-anatomy of the nephron
Collagen IV is the main component of the BM
Collagen IV nephropathies
Rare disorders

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

- Thin basement membrane nephropathy (a "frequent" rare disease in Cyprus)
  - It can be Benign for life
  - It can be Progressive, leading to end-stage renal disease and the need for dialysis or kidney transplantation

- Patients with thin basement membrane nephropathy are actually the heterozygous carriers of the *autosomal recessive* Alport Syndrome, who are not healthy!!!
Alport Syndrome can also be an Autosomal Recessive Disorder, affecting equally frequently men and women.

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

Microscopic hematuria

Healthy

Microscopic hematuria

Microscopic hematuria

Proband with autosomal recessive Alport syndrome
- Microscopic hematuria
- Proteinuria
- Hearing loss
- Ocular problems

These are the patients presenting with a Phenotypic CHAMELEON.
Carriers of autosomal recessive Alport Syndrome (ARAS) OR Thin Basement Membrane Nephropathy (TBMN) (a frequent 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

- Please Note: Many rare serious hereditary disorders present late, not shortly after birth
- We should be equally sensitive!
A turning point...

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

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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
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 ESKD.


The Cyprus Experience
Initial report on 82 patients

Dept of Histology, NGH/Dr Zouvani

CY-5303
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

Deltas C. *Pediatr Nephrol* 2009
Patients start with microhematuria and progress over 20, 30 or 40 years of follow-up to proteinuria, CKD & ESRF, usually NO DEAFNESS and NO OCULAR problems.

Patients of generation II reached ESRF

Most patients in generation III have CRF or ESRF

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
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 \textit{COL4A3/A4})

Deltas C et al, \textit{Nephron-Exper Nephrol & Genet 2015}
<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>
</tr>
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<td>CY-5374</td>
<td>TBMN-FSGS(1)</td>
<td>60</td>
<td>COL4A3-G1334E</td>
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<td>CY-5376</td>
<td>TBMN-FSGS(1)</td>
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<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>
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
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}, 2011

Table 1  Frequencies and statistics of \textit{R229Q}–\textit{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>
<th>Cases vs general population(^a)</th>
<th>Mild vs severe(^b)</th>
<th>Mild vs severe(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population</td>
<td>150</td>
<td>RR: 144, RQ: 6, QQ: 0</td>
<td>RR: 0.960, RQ: 0.040, QQ: 0</td>
<td>RR: 294, RQ: 6, QQ: 0</td>
<td>RR: 0.980, RQ: 0.020</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBMN</td>
<td>44</td>
<td>RR: 44, RQ: 0, QQ: 0</td>
<td>RR: 1, RQ: 0, QQ: 0</td>
<td>RR: 88, RQ: 0, QQ: 0</td>
<td>RR: 1, RQ: 0, QQ: 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFHR5</td>
<td>18</td>
<td>RR: 18, RQ: 0, QQ: 0</td>
<td>RR: 1, RQ: 0, QQ: 0</td>
<td>RR: 36, RQ: 0, QQ: 0</td>
<td>RR: 1, RQ: 0, QQ: 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>62</td>
<td>RR: 62, RQ: 0, QQ: 0</td>
<td>RR: 1, RQ: 0, QQ: 0</td>
<td>RR: 124, RQ: 1, QQ: 0</td>
<td>RR: 1, RQ: 0, QQ: 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBMN</td>
<td>58</td>
<td>RR: 55, RQ: 3, QQ: 0</td>
<td>RR: 0.948, RQ: 0.052, QQ: 0</td>
<td>RR: 113, RQ: 3, QQ: 0</td>
<td>RR: 0.974, RQ: 0.026</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFHR5</td>
<td>27</td>
<td>RR: 21, RQ: 6, QQ: 0</td>
<td>RR: 0.778, RQ: 0.222, QQ: 0</td>
<td>RR: 48, RQ: 6, QQ: 0</td>
<td>RR: 0.889, RQ: 0.111</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>85</td>
<td>RR: 76, RQ: 9, QQ: 0</td>
<td>RR: 0.894, RQ: 0.196, QQ: 0</td>
<td>RR: 161, RQ: 9, QQ: 0</td>
<td>RR: 0.947, RQ: 0.053, QQ: 0.056</td>
<td>\textbf{0.010}(^a)</td>
<td>\textbf{0.043}(^b)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

*Statistical significance \((p<0.05)\)

### Filtrin | Nephrin - p.V353M | Pooled Hematuric Cohort (HEMATURIA)

<table>
<thead>
<tr>
<th>Cohort</th>
<th>VV</th>
<th>VM+MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Mild”: 223</td>
<td>220 (99%)</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>“Severe”: 301</td>
<td>276 (92%)</td>
<td>25 (8%)</td>
</tr>
</tbody>
</table>

Samples include:
- TBMN from Cyprus
- Hematuric patients from Australia (Prof. Savidge)
- IgA nephropathy from UK and Crete
- Other hematuric patients from Cyprus & Greece

P value (genotypic association): 0.003 [age & gender correction]
P value (allelic association): 0.004 [kinships correction]
Odds ratio: 6.6 (~6 fold risk for renal failure!)

Voskarides & Deltas, unpublished results
(select as oral presentation at ERA-EDTA conference in Istanbul, 2013)
Altered interaction of mutant podocin with wild type nephrin, in cell culture experiments

Stefanou C et al, NEPHRON 2015
Gross data from 260 whole Exomes of TBMN patients

Table 2. Number of total variants identified in this study, and overlap with public databases

<table>
<thead>
<tr>
<th>Feature</th>
<th>Total</th>
<th>1000G (201305)</th>
<th>ESP6500AA</th>
<th>ESP650CEA</th>
<th>dbSnp141</th>
<th>dbSnp138</th>
<th>ExAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>837,313</td>
<td>502,254</td>
<td>185,963</td>
<td>185,798</td>
<td>438,620</td>
<td>520,894</td>
<td>242,779</td>
</tr>
</tbody>
</table>

Table 3. Genomic location of variants (from KGGSeq)

<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 12-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>Region</td>
<td>Count (Percentage)</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Synonymous</td>
<td>56,551 (6.754%)</td>
<td>Nucleotide substitution does not change amino acid.</td>
</tr>
<tr>
<td>Exonic</td>
<td>25 (0.003%)</td>
<td>Due to loss of sequences in reference database, this variant can only be mapped into exonic region without more precise annotation.</td>
</tr>
<tr>
<td>5'UTR</td>
<td>44,081 (5.265%)</td>
<td>Within a 5' untranslated region</td>
</tr>
<tr>
<td>3'UTR</td>
<td>166,223 (19.85%)</td>
<td>Within a 3' untranslated region</td>
</tr>
<tr>
<td>Intronic</td>
<td>385,777 (46.07%)</td>
<td>Within an intron</td>
</tr>
<tr>
<td>Upstream</td>
<td>22,443 (2.68%)</td>
<td>Within 1-kb region upstream of transcription start site</td>
</tr>
<tr>
<td>Downstream</td>
<td>19,630 (2.34%)</td>
<td>Within 1-kb region downstream of transcription end site</td>
</tr>
<tr>
<td>ncRNA</td>
<td>12,663 (1.51%)</td>
<td>Within a transcript without protein-coding annotation in the gene definition</td>
</tr>
<tr>
<td>Intergenic</td>
<td>139 (0.017%)</td>
<td>Variant is in intergenic region</td>
</tr>
</tbody>
</table>
Carriers of autosomal recessive Alport Syndrome, Thin Basement Membrane Nephropathy, frequently presenting with FSGS

The inheritance paradox | The phenotypic Chameleon

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

Simple statistics
As of 15/11/16

Mutations
COL4A3: 13 mutations (and 3 unclear findings)
COL4A4: 21 mutations (and 4 unclear findings)
COL4A5: 29 mutations

Polymorphisms
COL4A3: 46 polymorphisms,
COL4A4: 58 polymorphisms,
COL4A5: 10 polymorphisms.

Mutation COL4A3-p.G1334E: 20 families, with more than 200 patients
The genetic map of Cyprus

Thin Basement Membrane Nephropathy presenting as FSGS

Villages with patients from 30 families
All patients carry mutations in \textit{COL4A3/COL4A4}
A peasant stranded in Mesaoria
A peasant roaming in Mesaoria
Alport mouse model
Prospects for a pre-clinical trial with a repurposed drug

- The first of its kind for Alport Syndrome (severe hereditary nephropathy)
- Knockin model – introduction of a substitution mutation in the *Col4a3* gene (p.G1332E)
- It provides an opportunity for preclinical studies using repurposed drugs (synthetic chaperones)
Ultrastructural pathology of wild type and mutant knock-in mice. Ultrasturcural pathology of the mutant knockin mice is consistent with Alport syndrome nephritis. Wild type mice (WT/WT) display normal glomerular basement membranes thickness, 280 to 300nm range, while G1332E/G1332E (M/M) homozygous mice demonstrate thin glomerular basement membranes, 140-160nm range (black arrow) with areas of mild (3 mo mice) or severe (7 mo mice) irregular thickening (white arrows), consistent with Alport nephritis (Pieri M, Stephanou C et al, JASN 2014)
Conclusions

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 chronic 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

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 symptoms behaves as a **multifactorial condition**, with a primary genetic defect and additional genetic modifiers.
Funding / Collaborators

European Regional Development Fund & the Republic of Cyprus through the Cyprus Research Promotion Foundation,

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University of Cyprus

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- 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 Christoferos Stavrou, Evangelismos, Pafos