A New Gene for Hereditary Tubulo Interstitial Nephropathies

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Hereditary TIN in Chronic Kidney Diseases

- Chronic TIN # 5 % of ESRD patients
- « Hypertension » and « Vascular » : 25%
- Unknown: 15%

- **TIN (Hereditary) underrecognized?**
  - TIN can be misdiagnosed as « nephroangiosclerosis »
  - Associated clinical features are unspecific
  - Familial disease sometimes unknown or ... forgotten
  - 15-20% of ESRD Pts have a 1st degree relative with ESRD
HTIN:
How many diseases?
How many genes?

« Adult » Nephronoptisis
Familial Juvenile Hyperuricemic Nephropathy FJHN
Medullary Cystic Kidney Disease MCKD

NPH
Towards a molecular classification of HTIN…

FJHN
MCKD
Adult NPH
...

FJHN1/MCKD2
FJHN2
FJHN3
MCKD1
NPHP1/3
HNF1b
Others?
Linkage analysis and positional cloning identified FJHN1/MCKD2 as the UROMODULIN gene.

UMOD mutation

Hyperuricemia and gout

Chronic renal failure

Renal cysts

Autosomal dominant

Hart TC, J Med Genet 2002
Dahan K, JASN 2004
Identification of FJHN2 as the RENIN gene

- autosomal dominant TIN
- Anemia +++
- Hyperkalemia (tendency)
- Gout/Hyperuricemia
- small kidneys (no cysts)
- Low BP
- late onset ESRD

- #12 Families worldwide

Hodanova, Kidney Int, 2005;
Zivna, Am J Hum Genet 2009
Towards a molecular classification of HTIN…

FJHN1/MCKD2

FJHN2

FJHN3

MCKD1

NPHP1/3

HNF1b

Others?

MCKD1 Locus identified more than 10 years ago! (C Deltas)
**Medullary cystic kidney disease type 1: mutational analysis in 37 genes based on haplotype sharing**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Role as a functional gene/ Candidate for MCKD</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MUC1</strong> (Mucin 1) <em>(OMIM 158340)</em></td>
<td>Large cell surface mucin glycoprotein expressed by most glandular and ductal epithelial cells and some hematopoietic cell lineages. It is a type 1 transmembrane protein with a large extracellular tandem repeat domain. The tandem repeat domain is highly O-glycosylated. May play a role in adhesive functions and in cell-cell interactions, metastasis and signaling. May provide a protective layer on epithelial surfaces. Direct or indirect interaction with actin cytoskeleton.</td>
<td>6,953 bps , 8 ex, 1255 aa; 122072 Da, RE (UG,GN,SG) Contains 1 SEA domain.</td>
</tr>
</tbody>
</table>
Mutations causing medullary cystic kidney disease type 1 lie in a large VNTR in \textit{MUC1} missed by massively parallel sequencing

\textit{nature genetics}

6 « MCKD » Families

AD TIN

No gout/hyperuricemia

No or few renal cysts

Linked to MCKD1 (1q21)

> 10 years ago....

No gene identified by Sanger sequencing of more than 30 genes in the region...
Refinement of the MCKD1 locus on chrom 1 in 6 Families

No mutation identified by Whole Exome Sequencing Or Whole Genome sequencing......
Cloning, assembly and sequencing of genomic fragments

Identified a Cytosine insertion in the VNTR of MUC1

(a) F4:IV-2 (identical to F4:V-3 assembly)


(b) F6:IV-3


(c) F2:IV-3

MUCIN GENES AND GLYCOPROTEINS

A Membrane-tethered Mucins

- MUC1
  - Signal Sequence
  - Transmembrane Domain
  - VWF-C-like Domain
  - PTS-region
  - SEA Domain

  - Histadin-like Domain

  - Leucine Zipper

- MUC4

B Secreted Mucins - non-Cysteine Rich

- MUC7
  - VWF-D-like Domain
  - EGF-like Domain
  - Tandem Repeats
  - Nidogen Domain
  - AMOP Domain
  - Cysteine-rich Domain

C Secreted Mucins - Cysteine Rich

- MUC2

  - n = 21

  - n = 51-115

- MUC5B

  - n = 11

  - n = 11

  - n = 17

  - n = 11

  - n = 22

- MUC5AC

  - n ≈ 124

  - n = 17

  - n = 34

  - n ≈ 66
a MUC1

Variable number of tandem repeats (O-glycosylated) (25–125 repeats of 20 aa)

Heavily O-glycosylated region

GSVVV proteolytic cleavage site

N-glycosylation sites

MUCIN GENES AND GLYCOPROTEINS

Threonine

O-Glycan

Serine

MUC Protein Backbone

NH₂ COOH
Fragment N-Terminal

Tandem Repeat

Membrane plasmique

Cytoplasme

Fragment C-Terminal

GalNAc (Tn)

Gal

GlcNAc

Sialic Ac

STn

ST

Core Protéique de MUC1

MUC1 à l'état normal Glycosylation riche

MUC1 à l'état tumoral Surexpression, Glycosylation réduite et aberrante
Wt MUC1 is cleaved to MUC1-N and MUC1-C that dimerize

Figure 1.
Schematic representation of the MUC1 heterodimer positioned at the cell membrane. MUC1-N is the mucin component of the heterodimer that contains the glycosylated variable numbers of tandem repeats. MUC1-N is tethered to the cell surface through binding to the transmembrane MUC1-C subunit (left). Shedding of MUC1-N leaves MUC1-C at the cell membrane as a putative receptor for signaling the presence of extracellular stress to the interior of the cell (right).
MUC1-C signals to the cytoplasm
Role of Mucin 1 in GI Epithelium

@ Protects against Infections:

GastroIntestinal Tract (C Jejuni), Pulmonary Airways,..... Urinary Tract?

MUC1-/- mice

Mc Auley, J Clin Invest 2007
MUC1 Mucin Is a Negative Regulator of Toll-Like Receptor Signaling

Keiko Ueno\textsuperscript{1,2}, Takeshi Koga\textsuperscript{1,3}, Kosuke Kato\textsuperscript{1,2}, Douglas T. Golenbock\textsuperscript{4}, Sandra J. Gendler\textsuperscript{5}, Hirofumi Kai\textsuperscript{2}, and K. Chul Kim\textsuperscript{1}

Am J Respir Cell Mol Biol, 2008
Role of Mucin 1 in the Kidney?

Mucin1/EMA is expressed during kidney development

Muc1 is induced by Ischemia in the rat kidney

MUC1-fs is expressed in the patients’ kidneys.
Mechanisms of MUC1 mutation associated Renal Phenotype

Autosomal Dominant Disease

1) Haploinsufficiency/Loss of Function
   Only 50% of the wild type protein is present

2) Dominant Negative Effect
   MUC1fs heterodimerize with wt MUC1 and blocks/decreases its normal activity

3) Gain of Function of MUC1fs
   extracellular only? Intracytoplasmic?
   Toxic effect of ER retention (ER stress....)
MUC1 mutations seem frequent in non hyperuricemic Families?

Bleyer et al

« same » MUC1 mutation in 13/21 Families (62%)
# MUC1 Mutations Occur Worldwide

Table 1. Characteristics of families with MUC1 mutation

<table>
<thead>
<tr>
<th>Family Number&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ethnicity</th>
<th>MUC1 Mutation (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>Middle Eastern</td>
<td>11</td>
</tr>
<tr>
<td>L2</td>
<td>African American</td>
<td>11</td>
</tr>
<tr>
<td>L3</td>
<td>Native American</td>
<td>22</td>
</tr>
<tr>
<td>L4</td>
<td>European American</td>
<td>7</td>
</tr>
<tr>
<td>L5</td>
<td>European American</td>
<td>9</td>
</tr>
<tr>
<td>L6</td>
<td>European American</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>European American</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>European American</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>European American</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>European American</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>African American</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Finnish</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>European American</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>European American</td>
<td>1</td>
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<tr>
<td>9</td>
<td>European American</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>European American</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>Australian</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>Eastern European</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>Eastern European</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>European American</td>
<td>6</td>
</tr>
<tr>
<td>15</td>
<td>Australian</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>Russian</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>Australian</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>Israeli</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Family Number
MUC1 Detection in the French HTIN cohort

125 NTIH Families tested

14 MUC1 status uncertain

33/101 (32%) MUC1 Positive

68/101 (68%) MUC1 Negative
Modified method to detect MUC1 mutation from genomic DNA

ADN génomique

MUC1-VNTR Ex2 (30 à 80 Répétitions de 60pb)

Digestion MWO1 (Coupe 7C)

Produit de digestion

Recherche de l’ évènement 8C parmi 60 à 160 répétitions par méthode SNaPSHOT

Seq WT 7C : ccGCCCCC/CAGCcc - Coupé
Seq Mut 8C : ccGCCCCCCCCAGCcc – Non coupé

V Morinière, Dép Génétique Necker
Family I

TIN
Cysts
Hyperuricemia/gout in 2 pts
Membranous Nephropathy in III.1!

IRT 51
Go -

IRT 56
Go-; Ky+
PBR (NTIC)

IRT (62 ans)
Ky +

Go + (27 ans)
IRT 46

HyperUA?
Go -; Ky +

IRC
HyperU /Go-; Ky+
PBR: GEM+NTIC

Hyperuricemia/gout in 2 pts
Membranous Nephropathy in III.1!
**5bp Deletion in MUC1 gene in Family IM**

MUC1

MUC1-InsC

MUC1-del 5bp

Nouvelle Protéine

Même effet sur MUC1 que Ins C

Seq WT 7C : ccGCCCCC/CAGCcc - Coupé
Seq Mut 8C : ccGCCCCCCCCAGCcc – Non coupé

**ADN génomique**

Digestion MWO1 (Coupe 7C)

Produit de digestion

Recherche de l’ événement 8C parmi 60 à 160 répétitions par méthode SNaPSHOT

V Morinière, Dép Génétique Necker
## MUC1 Patients: Demographic and Blood Pressure

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>123</td>
</tr>
<tr>
<td>Number of families</td>
<td>33</td>
</tr>
<tr>
<td>Male gender (n, %)</td>
<td>64 (52.0)</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>High blood pressure (n,%) (n=63)</td>
<td>38 (60.3)</td>
</tr>
<tr>
<td>Age at diagnosis of HBP (years)</td>
<td>41 ± 5</td>
</tr>
</tbody>
</table>
## Gout/Hyperuricemia is unfrequent in MUC1 Patients

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early gout (n,%)(n=87)</td>
<td>4 (4.6)</td>
</tr>
<tr>
<td>Age at first gout episode (years)</td>
<td>28.3 ± 4.9</td>
</tr>
<tr>
<td>Under allopurinol therapy (n,%)(n=55)</td>
<td>16 (29.1)</td>
</tr>
<tr>
<td>Early gout or preemptive allopurinol therapy (n,%)(n=56)</td>
<td>17 (30.4)</td>
</tr>
<tr>
<td>Uricemia in men (μmol/L)(n=16)</td>
<td>455 ± 36</td>
</tr>
<tr>
<td>Uricemia in women (μmol/L)(n=26)</td>
<td>365 ± 63</td>
</tr>
<tr>
<td>Uricemia &gt; 90th centile (n, %)(n=40)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Uricemia &gt; 75th centile (n, %)(n=40)</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>UAEF (% mean)(n=10)</td>
<td>8.3 ± 1.8</td>
</tr>
</tbody>
</table>
Renal Cyst are not the rule in MUC1 Patients

<table>
<thead>
<tr>
<th>Renal cysts (n,%)(n=65)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence</td>
<td>21 (32.3)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>16 (76.2)</td>
</tr>
<tr>
<td>Unilateral</td>
<td>5 (23.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Renal cysts localization (n,%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Medullary only</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>Cortical only</td>
<td>10 (47.6)</td>
</tr>
<tr>
<td>Corticomedullary</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>5 (23.8)</td>
</tr>
</tbody>
</table>

« MCKD » is a misleading appellation!
Renal Pathology in MUC1 associated HTIN

- Tubular atrophy
  Tubular cystic dilatations

- Interstitial inflammation
  (often mild but sometimes striking)

- Abnormal thick and duplicated TBM
  (nephronophptisis suggested)

- No immune deposits

- Non Specific Vascular lesions
  (nephroangiosclerosis suggested in several cases....)
EM specific features?

Thick and multilamellated TBM

Ekici, KI 2014
eGFR slopes in MUC1 individuals

<table>
<thead>
<tr>
<th></th>
<th>baseline</th>
<th>Last follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>36 +/- 4</td>
<td>45 +/- 5</td>
</tr>
<tr>
<td>eGFR (median)</td>
<td>53 (29-82)</td>
<td>28 (10-69)</td>
</tr>
</tbody>
</table>

-3.4 ml/min/yr (-13.6-1.6)
Rapid eGFR decline in MUC1 patients after eGFR < 50ml:mn

Bleyer, CJASN, 2014
MUC1 : large variability in CRF progression

eGFR at last follow up (n = 53)
Age at ESRD according to decades

N = 94 patients
Renal Survival in MUC1 Patients

94 patients reached ESRD
Median age 45 yrs (16-82)
MUC1 : Age at ESRD

Intrafamilial Variability

Family 16 :
39 yrs to 82 yrs!
Screening of at risks individuals in MUC1 Families

Often pauci symptomatic

CRF, +/- HBP,

No PU, No HU
<table>
<thead>
<tr>
<th></th>
<th>MUC1 +</th>
<th>MUC1 -</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>123</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Number of families</td>
<td>33</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Male gender (n, %)</td>
<td>64 (52.0)</td>
<td>73 (51.1)</td>
<td>0.9</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>37 ± 3</td>
<td>44 ± 3</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>34 (15 - 79)</td>
<td>43 (16 - 86)</td>
<td></td>
</tr>
<tr>
<td>High blood pressure (n,%) (n=63)</td>
<td>38 (60.3)</td>
<td>51 (71.8)</td>
<td>0.16</td>
</tr>
<tr>
<td>Age at diagnosis of HBP (years)</td>
<td>41 ± 5</td>
<td>41 ± 4</td>
<td>0.9</td>
</tr>
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<td>Early gout (n,%) (n=87)</td>
<td>4 (4.6)</td>
<td>27 (24.8)</td>
<td>&lt; 0.001</td>
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<td>28.3 ± 4.9</td>
<td>27 ± 3</td>
<td>0.8</td>
</tr>
<tr>
<td>Under allopurinol therapy (n,%)(n=55)</td>
<td>16 (29.1)</td>
<td>27 (46.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>Early gout or preemptive allopurinol therapy (n,%) (n=87)</td>
<td>17 (19.5)</td>
<td>40 (36.7)</td>
<td>0.009</td>
</tr>
<tr>
<td>Uricemia in men (µmol/L) (n= 16)</td>
<td>455 ± 36</td>
<td>517 ± 55</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>448 (298 - 702)</td>
<td>515 (303 - 779)</td>
<td></td>
</tr>
<tr>
<td>Uricemia in women (µmol/L) (n= 26)</td>
<td>365 ± 63</td>
<td>446 ± 60</td>
<td>0.09</td>
</tr>
<tr>
<td>Uricemia &gt; 90th centile (n, %)(n=40)</td>
<td>3 (7.5)</td>
<td>8 (20.0)</td>
<td>0.10</td>
</tr>
<tr>
<td>Uricemia &gt; 75th centile (n, %)(n=40)</td>
<td>4 (10.0)</td>
<td>17 (42.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>UAEF (%, mean) (n=10)</td>
<td>8.3 ± 1.8</td>
<td>7.8 ± 2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Renal cysts (n,%) (n=65)</td>
<td>21 (32.3)</td>
<td>33 (45.2)</td>
<td>0.1</td>
</tr>
<tr>
<td>Presence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR at diagnosis (ml/min/1.73m²) (n=52)</td>
<td>47 ± 5</td>
<td>41 ± 3</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>49 (15 - 82)</td>
<td>40 (14 - 75)</td>
<td></td>
</tr>
<tr>
<td>Number of patients At ESRD (n, %)</td>
<td>94 (76.4)</td>
<td>99 (69.2)</td>
<td>0.2</td>
</tr>
<tr>
<td>Age at ESRD (years) (n=94)</td>
<td>45 ± 3</td>
<td>50 ± 3</td>
<td>0.02</td>
</tr>
<tr>
<td>Locus</td>
<td>Chrom</td>
<td>Gene</td>
<td>Frequency</td>
</tr>
<tr>
<td>------------</td>
<td>---------</td>
<td>-----------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>FJHN1/MCKD2</td>
<td>16p12</td>
<td>UMOD</td>
<td>20-40%</td>
</tr>
<tr>
<td>FJHN2</td>
<td>1q31.1</td>
<td>REN</td>
<td>&lt; 2.5%</td>
</tr>
<tr>
<td>FJHN3</td>
<td>2p22-p21</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>MCKD1</td>
<td>1q21</td>
<td>MUC1</td>
<td>30%-60% (selected families)</td>
</tr>
<tr>
<td>HNF1b</td>
<td>17q12</td>
<td>HNF1b/TCF2</td>
<td>5%</td>
</tr>
<tr>
<td>MCKD3</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
HTIN French Cohort

380 Families screened for UMOD

57/380 UMOD (15%)
33/101 MUC1 (32%)
6 REN
6 HNF1b
1 Jagged 1

6 NPHs (NPHP1)
### HTIN/ADTIKD

Common and Specific Features of the main 3 genes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Medullary Cystic Kidney Disease Type 1 (Caused by Mutations in the MUC1 Gene)</th>
<th>Medullary Cystic Kidney Disease Type 2 (Caused by Mutations in the UMOD Gene)</th>
<th>Mutations in the REN Gene Encoding Renin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance</td>
<td>Autosomal dominant</td>
<td>Autosomal dominant</td>
<td>Autosomal dominant</td>
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<tr>
<td>Urinary sediment</td>
<td>Bland</td>
<td>Bland</td>
<td>Bland</td>
</tr>
<tr>
<td>Urinary protein</td>
<td>Absent/minimal</td>
<td>Absent/minimal</td>
<td>Absent/minimal</td>
</tr>
<tr>
<td>Medullary cysts</td>
<td>Rare</td>
<td>Rare</td>
<td>Absent</td>
</tr>
<tr>
<td>Age of ESRD (yr)</td>
<td>Variable: late 20s to &gt;70</td>
<td>Variable: 30–60</td>
<td>Variable: 40–70</td>
</tr>
<tr>
<td>Gout in many affected family members (often occurring early in life)</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Hyperkalemia and low BP (symptoms of low renin state)</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Anemia in childhood</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>
Patient presents with renal insufficiency, bland urinary sediment and family history of kidney disease

- Strong family history with many affected family members
- ESRD onset in 30's – 60's

- Strong family history of gout occurring in many family members prior to severe renal insufficiency

YES

- Consider medullary cystic kidney type 2
- Pursue mutational analysis of gene encoding uromodulin (available commercially)

NO

- Disease present in siblings but not parents
- ESRD in teens to 20's

- Consider autosomal recessive nephronophthisis

- Consider medullary cystic kidney type 1
- Refer family for linkage analysis
Acknowledgements

Service de Néphrologie, Hôpital Necker
Said Lebbah, Guillaume Bollée, Aurélie Hummel

Laboratoire de génétique et INSERM U983, Fondation Imagine, Hôpital Necker
Vincent Morinière, Corinne Antignac

Centre MARHEA: Laurence Heidet

Anatomopathologie, Necker
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