Mutations HUS, DGKE

Véronique Frémeaux-Bacchi

Centre de Recherche des Cordeliers- UMRS 1138, Team : « Complement et maladies »
et
Laboratoire d’Immunologie, HEGP Paris, France

veronique.fremeaux-bacchi@egp.aphp.fr
Disclosure

Alexion : Member of SAB and aHUS registry
Atypical HUS

Ultra rare Kidney diseases
Microangiopathy disease with mechanical anemia, thrombocytopenia and acute renal failure

Fremeaux-Bacchi et al, Blood, 2008

Fremeaux-Bacchi et al., Blood, 2008

Goicoechea de Jorge et al., PNAS 2007
Roumenina et al, Blood 2009

Up to 1000 patients from European and US cohorts (between 100 to 300 patients/cohort)

Complement mediated disease
Glomerular Endothelial cells damaged by complement attack

Complement overactivation

Excessive cleavage of C5 leads TMA:
Eculizumab is recommended as first line therapy in aHUS with pediatric onset

### Frequency of mutations in complement genes

<table>
<thead>
<tr>
<th></th>
<th>France (1)</th>
<th>Italy (2)</th>
<th>US (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>214</td>
<td>273</td>
<td>144</td>
</tr>
<tr>
<td>Age at onset</td>
<td>0 to 83y</td>
<td>0 to 83y</td>
<td>6m to 34y</td>
</tr>
<tr>
<td>Overall identified mutations or Ab (%)</td>
<td>66</td>
<td>49</td>
<td>46</td>
</tr>
<tr>
<td>No identified mutation</td>
<td>34</td>
<td>51</td>
<td>54</td>
</tr>
</tbody>
</table>

- 90% of mutations are heterozygous
- Most frequently the disease is sporadic with incomplete penetrance.
- 15% of familial forms including family without mutations in complement genes

(1) Fremeaux-Bacchi et al CJASN, 2013, (2) Noris et al CJASN, 2010; (3) Maga et al; Human mutation, 2010
Exome sequencing of 2 kindreds with familial aHUS from the French Cohort

- No identified mutations in complement gene
- Pattern suggesting recessive transmission
- Similar phenotype: aHUS presenting < age 1, relapses following by significant PT

- aHUS at age 9 mo then proteinuria. No aHUS recurrence.
- aHUS at age 4 mo, remission, plasma infusion, 2 relapses then proteinuria.
- aHUS at age 7 mo, 1 relapse then nephrotic proteinuria, hypertension. ESRD at age 18 yrs.
- aHUS at age 4 mo, then proteinuria, 4 relapses. CKD5 at 23 yrs.

We hypothesised autosomal recessive transmission in these families. We searched for genes with homozygous/compound heterozygous mutations that were shared by both affected subjects.

We performed exome sequencing of these 4 affected subjects.
Mutations in Diacylglycerol Kinase ε (DGKE) segregate with aHUS in both kindreds

**Kindred 1**
- Trp322Stop
- Trp322Stop

**Kindred 2**
- Arg63Pro
- Ins A - exon 2

**Mutation types**
- Missense
- Nonsense
- Frameshift
6 additional cases in French pediatric cohort (9/50 total) with DGKE mutations

- All mutations novel or rare
- 5 out the 8 patients carried at least one Trp322Stop in DGKE (50% of mutations): we showed that this change originates from a common ancestor from approximately 60 generations ago
- No DGKE mutations in 36 adults
Emirati Family with Early-Onset aHUS

Ha: * 2002
aHUS at age 3 mo. C3, C4 normal. Plasma infusions, remission.
2 relapses in first two years of life, then proteinuria/hematuria
from age 5 yrs nephrotic proteinuria. ESRD at age 13 yrs.

Sa, Brother: * 2005
aHUS at age 6 mo. 2 relapses.
Currently (7 yrs): Proteinuria, CKD

No, Sister: * 2008
aHUS at age 4 mo. 2 relapses.
Currently (4 yrs): Proteinuria, hematuria
Screening candidate locus with Genome-Wide Linkage Analysis and Homozygosity mapping in Emirati Kindred

Locus on Chromosome 17
13 cM interval, 105 genes

→ Targeted NG exome sequencing

→ 3 genes with homozygous mutation in diseased patients, parents and healthy sibling heterozygous

• DGKE (Arg273Pro) Diacyl-glycerol kinase epsilon
• RNF43 (Arg117His) E3 ubiquitin-protein ligase
• MTMR4 (Val297Gly) Dual specificity protein phosphatase
Timing and Types of Clinical Events

- No HUS episodes > 5 y in all
- Persistent proteinuria, microhematuria, hypertension
- Nephrotic syndrome (3/13)
- CKD with age (6/13 so far)
- Renal transplants (3/13) without HUS recurrence (12 person•years)
- No apparent extra-renal phenotype
- Anti-complement therapies: no benefit

Lemaire and Fremeaux-Bacchi et al. Nature Gen 2013
Genetic aHUS: Manifestation Age and Outcome

[Lemaire and Fremeaux-Bacchi et al. Nature Gen 2013]

[Graph showing age at onset for different genes: DGKE, CFH, MCP, CFI, C3/CFB.]

[Survival probability graph with ages and survival curve data for DGKE, CFH, MCP, C3/CFB genes.]

[Table showing number of subjects at risk remaining:]

<table>
<thead>
<tr>
<th>Genes</th>
<th>Number of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGKE</td>
<td>13 8 5 4 2 0 0 0</td>
</tr>
<tr>
<td>CFH</td>
<td>21 5 0 0 0 0 0 0</td>
</tr>
<tr>
<td>MCP</td>
<td>12 10 7 7 6 4 3 3</td>
</tr>
<tr>
<td>C3/CFB</td>
<td>10 6 6 4 3 1 1 1</td>
</tr>
</tbody>
</table>
Phenotypic Expansion of DGKE-Associated Diseases
Rik Westland et al, JASN, 2014

Table 1. Clinical characteristics of individuals 58 and 59 at presentation and last follow-up

<table>
<thead>
<tr>
<th></th>
<th>Individual 58 (Girl)</th>
<th>Individual 59 (Boy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presentation</td>
<td>Last Follow-Up</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>0.8</td>
<td>5.3</td>
</tr>
<tr>
<td>SCr (mg/dl)</td>
<td>1.5</td>
<td>0.29</td>
</tr>
<tr>
<td>Platelet count ($\times 10^9$/L)</td>
<td>64</td>
<td>279</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>1923</td>
<td>414</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>&lt;2</td>
<td>46</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>66</td>
<td>79</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hematuria</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Reference values for C3: 84–192 mg/dl. Proteinuria is defined as a protein-to-creatinine ratio greater than 0.2 mg/mg on a morning urine sample. Hematuria is defined as greater than 3 red blood cells/high power field on urinalysis. SCr, serum creatinine; LDH, lactic dehydrogenase; C3, serum complement C3 level.

From Linosa, Island in the Mediterranean

Aggressive plasma infusion treatment controlled systemic symptoms and prevented renal failure

p.K101X

Unique single nucleotide variants (SNV)
n=88,119

High-quality SNVs and absent in public databases
n=764

Protein altering SNVs
n=194

Homzygous SNVs
n=3

Absent in in-house exomes
n=1

Cys (C)  Leu (L)  Arg (R)  Lys (K)  Ala (A)  Asp (D)
DGKE Variants Cause a Glomerular Microangiopathy That Mimics Membranoproliferative GN

Fatih Ozaltin.*† Binhua Li.‡ Alvsha Rauhauser.‡ Sung-Wan An.‡ Oğuz Soylemezoglu.§

- 3 kindreds with similarly deleterious homozygous DGKE mutations
- Nephrotic phenotype, median age at onset 2 yrs
  No reported evidence of atypical HUS in any of the 9 patients!
- Kidney biopsy results consistent with chronic TMA

… “membranoproliferative-like glomerular microangiopathy”,
DGKe mutations

- **aHUS phenotype**
  - p.K101X
  - p.Ser11*
  - p.Arg63Pro

- **MPGN phenotype**
  - c.610delA
  - c.486insA
  - c.472insT
  - p.Trp322*
  - p.Arg273Pro
  - c.889-2A>G
  - c.889-1G>A
  - p.Gln334*

*(Lehaye and Fremeaux-Bacchi et al; and Wesland et al;)*
What is DGKE?

- DGKe are intra cellular lipid kinases.
- DGKe is the only constitutively active membrane-bound isoform.
- DGKe phosphorylate diacylglycerol (DAG) to phosphatidic acid (PA).

Therefore DGKe terminates DAG associated signaling and may regulate the PKC pathway.
DGKE is Expressed in Endothelium, Platelets and Podocytes

Lemaire and Fremeaux-Bacchi et al. Nature Gen 2013
What are the potential mechanisms linking DGKe deficiency to TMA lesions?

It is plausible that lack of DGKe drives prothrombotic state.

- It is well reported that PKC increases the production of various prothrombotic factors as VWF and Tissu Factor and decreased VEGFR2 signaling in endothelium.

- But chronic activation in PKC drives also increased activity of TRPC6 a podocyte cation channel implicated in familial glomerular disease. Therefore lack of DGKe may drive a complexe glomerulopathy

- But low C3 levels in two children with DGKe deficiency: Is there a link with complement?
Conclusions

✓ DGKe deficiency is a novel genetic causes of atypical HUS: Approximately 25% of patients with HUS below the age 1 and 10% of patients with pediatric onset carry homozygous mutation in DGKe.

✓ The penetrance with the disease is complete.

✓ Patients presented with one to four episodes of aHUS, have persistent hypertension, hematuria and proteinuria and develop chronic kidney disease with age.

✓ Independant study reported three families with recessive mutations in DGKe without episodes of HUS but histologic features of TMA leading to proteinuria and nephrotic syndrome.

✓ DGKe is a lipid kinase implicated in the inactivation of the DAG signaling and expresses in endothelium, platelets and podocytes.

✓ All these results highlight the essential role in regulation in signaling lipids pathway in kidney cells.

✓ Which therapeutic for these patients?
Recessive mutations in DGKE cause aHUS

**French study group of aHUS:**
C. Loirat, M. Le Quintrec, F. Fakhouri

**Yale**
Richard Lifton

**Mathieu Lemaire**
M.Choi, W.H.Tang, J.Hwa, W.Ji, J.D.Overton, G Moeckel

**F. Schaefer**
G.Nürnberg, J. Altmüller S.M.Mane,, H.Thiele, P.Nürnberg

S.Taque, F. Nobili, F. Martinez, D. Morin, G.Deschenes, V.Baudouin, B.Llanas, M.C. Gubler

L.Collard, N. Rioux-Leclerc, L.Collard, N. Rioux-Leclerc,

M.A.Majid, E.Simkova
Acknowledgements

All the Clinicians

The Complement’ team

Lubka Roumenina
Moglie Le quintrec

Prof. Richard Lifton and Mathieu Lemaire (Yale, USA)

Marie-Agnes Dragon-Durey