Membranous nephropathy - an auto-immune disease: clinical and therapeutic implications

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Disclosures:

Consulting:
• Questcor
• Taligen

Grant support:
• Questcor

Patent pending:
• Diagnostics for Membranous Nephropathy
Membranous Nephropathy

- A leading cause of nephrotic syndrome in adults
- Autoimmune disease of the kidney
- Variable clinical course
  - Spontaneous remission (30-40%)
  - Persistent proteinuria (30-40%)
  - Progression to ESRD (15-30%)
- Treatment involves potent immunosuppressive agents
- Current diagnosis relies exclusively on renal biopsy
- No assay available to detect active disease
Membranous Nephropathy

IgG & C3

IgG4 >> IgG1
Idiopathic vs. Secondary MN

Idiopathic (cause undefined)

Secondary
- Systemic lupus erythematosus
- Hepatitis B (rarely C)
- Schistosomiasis, malaria, syphilis
- Anti-rheumatic agents (gold, NSAIDS, penicillamine)
- Solid tumors
Experimental Membranous Nephropathy - Heymann Nephritis

- Rats immunized with tubular brush border (Fx1A) develop proteinuria after 6-8 weeks and an immune complex GN indistinguishable from human MN

- Rats injected with anti-Fx1A develop passive HN and proteinuria within 5 days

- Subepithelial immune deposits form in situ when circulating antibody binds to an intrinsic glomerular antigen (megalin)

- Proteinuria is due to podocyte injury from assembly of the complement membrane attack complex
Does the paradigm established in experimental MN also apply in human membranous nephropathy?

- Megalin is not present on human podocytes
- IgG4, the predominant IgG subclass in the glomerular deposits in human MN, does not bind complement C1q
- No one has consistently identified a glomerular autoantigen in human MN
Antenatal Membranous Glomerulonephritis Due to Anti–Neutral Endopeptidase Antibodies

H Debiec., V Guigonis, B Mougenot, F Decobert, J-P Haymann, A Bensman, G Deschênes, and P M. Ronco

- This proves that the pathogenesis of MN involves reactivity of circulating antibodies with antigen/s expressed on podocytes
- However it is questionable whether anti-NEP antibodies account for idiopathic MN
Is there an intrinsic glomerular antigen in idiopathic MN?
Preparation of Human Glomerular Extract for Western Blot
Discovery of a major target antigen in idiopathic MN

M-type Phospholipase A2 Receptor

Llorca *Cell Mol Life Sci* 2008
Circulating and tissue-deposited anti-PLA2R autoantibodies are predominantly but not exclusively IgG4

Reactivity to PLA2R is Specific for Idiopathic MN

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<tbody>
<tr>
<td>Anti-PLA2R NEG</td>
<td>12</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>11</td>
<td>72</td>
<td>25</td>
<td>32</td>
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<tr>
<td>Anti-PLA2R POS</td>
<td>31</td>
<td>26</td>
<td>15</td>
<td>9</td>
<td>49</td>
<td>0</td>
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</table>
The absence of circulating PLA2R autoantibody at the time of kidney biopsy does not rule out a diagnosis of PLA2R-related membranous nephropathy.
The PLA2R epitope identified by MN autoantibodies is sensitive to reduction.

Mannose receptor family members may exist in extended or bent configurations.
Human anti-PLA$_2$R antibodies recognize an epitope in the N-terminal part of the protein.
Risk HLA-DQA1 and PLA$_2$R1 Alleles in Idiopathic Membranous Nephropathy


N Engl J Med
Volume 364(7):616-626
February 17, 2011
Manhattan Plots for the Joint Genomewide Association Study.


<table>
<thead>
<tr>
<th>Variable</th>
<th>Single-Nucleotide Polymorphism Characteristics</th>
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<tbody>
<tr>
<td></td>
<td>Chromosome 6, rs2187668 (HLA-DQA1)</td>
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<tr>
<td>French study</td>
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<tr>
<td>Odds ratio (95% CI)</td>
<td>4.48 (2.68–7.50)</td>
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<tr>
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<td>Patients</td>
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<tr>
<td>Controls</td>
<td>1.8×10⁻⁸</td>
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<tr>
<td>Dutch study</td>
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<td>Odds ratio (95% CI)</td>
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<td>Patients</td>
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<tr>
<td>Controls</td>
<td>5.6×10⁻⁷</td>
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<td>British study</td>
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<td>Odds ratio (95% CI)</td>
<td>5.33 (4.04–7.02)</td>
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<td>Minor allele frequency (%)</td>
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<td>Patients</td>
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<tr>
<td>Controls</td>
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<td>Joint study</td>
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<td>Odds ratio (95% CI)</td>
<td>4.32 (3.73–5.01)</td>
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<td>Minor allele frequency (%)</td>
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<tr>
<td>Patients</td>
<td>13.0</td>
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<tr>
<td>Controls</td>
<td>8.0×10⁻³</td>
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</table>

rs4664308 is highly correlated with the SNP that encodes M292V in CTLD1

PLA2R1 polymorphisms in idiopathic MN

Single Nucleotide Polymorphisms in the Phospholipase A2 Receptor Gene Are Associated with Genetic Susceptibility to Idiopathic Membranous Nephropathy

Sejoong Kim a, Ho Jun Chin b,c, Ki Young Na b,c, Suhnggwon Kim c,d, Jieun Oh e, Wookyung Chung a, Jung Woo Noh e, Young Ki Lee e, Jong Tae Cho f, Eun Kyoung Lee f, Dong-Wan Chae b,c, Progressive Renal Disease and Medical Informatics and Genomics Research (PREMIER) members 1

Association of phospholipase A2 receptor 1 polymorphisms with idiopathic membranous nephropathy in Chinese patients in Taiwan

Yu-Huei Liu 1,2, Cheng-Hsu Chen 3, Shih-Yin Chen 1,2, Ying-Ju Lin 1,2, Wen-Ling Liao 1,2, Chang-Hai Tsai 4,5, Lei Wan 1,2,6, Fuu-Jen Tsai 1,2,4,7,8,9,10
### PLA₂R Extracellular Region

![Diagram of PLA₂R extracellular region with H300D highlighted]

### Table 3. OR for the presence of idiopathic MN in SNPs of the PLA₂R gene

<table>
<thead>
<tr>
<th></th>
<th>Idiopathic MN vs. controls</th>
<th>Secondary MN vs. controls</th>
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<tbody>
<tr>
<td></td>
<td>OR (95% CI) p</td>
<td>OR (95% CI) p</td>
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<tr>
<td><strong>rs35771982</strong></td>
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<tr>
<td>CC vs. CG or GG(^a)</td>
<td>2.626 (1.816–3.796)</td>
<td>&lt;0.001</td>
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<tr>
<td>CC vs. CG or GG(^b)</td>
<td>2.935 (1.499–5.748)</td>
<td>0.002</td>
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<tr>
<td>CC vs. CG or GG(^c)</td>
<td>2.587 (1.549–4.321)</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>rs3828323</strong></td>
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<td></td>
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<tr>
<td>CC vs. CT or TT(^a)</td>
<td>1.355 (0.953–1.928)</td>
<td>0.091</td>
</tr>
<tr>
<td>CC vs. CT or TT(^b)</td>
<td>1.505 (0.796–2.846)</td>
<td>0.209</td>
</tr>
<tr>
<td>CC vs. CT or TT(^c)</td>
<td>1.550 (0.947–2.537)</td>
<td>0.081</td>
</tr>
</tbody>
</table>

\(^a\) Unadjusted OR in all subjects.
\(^b\) Adjusted OR for covariables such as age, gender, height, weight, systolic blood pressure, eGFR, hemoglobin, and total cholesterol levels in all subjects.
\(^c\) OR in age- and gender-matched subjects.

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PLA2R contains several SNPs in the region of the anti-PLA2R epitope
Does serum reactivity to the MN-Ag correlate with disease activity?
Association of anti-PLA$_2$R with disease status

Hofstra, Beck et al (in press cJASN 2011)
Proteinuria correlates with anti-PLA$_2$R

Hofstra, Beck et al. (in press cJASN 2011)
Time following treatment with rituximab

Disappearance

Persistence

Relapse

Beck, Fervenza et al (in press JASN 2011)
Immunological remission in primary MN precedes clinical remission

Beck, Fervenza et al (in press JASN 2011)
Clinical disease

Immunologic disease

Treatment

Anti-PLA₂R

Proteinuria

Partial remission

Complete remission

Time

100%

0%
Is Recurrent MN in Transplanted Human Kidneys Associated with Circulating Anti-PLA2R?

- Recurrent MN has been reported in up to 50% of cases with a high rate of allograft failure.
- There is presently no way to predict which patients are likely to recur.
- Serial kidney biopsy is the only way to diagnose early recurrence.
PLA2R Autoantibodies and Recurrent Membranous Nephropathy after Transplantation

**Figure 1.** Anti–PLA2R Autoantibody Detected before and after Renal Transplantation.
Anti-PLA2R reactivity at the time of transplantation is associated with a high risk of recurrent MN

Transplant cases (27)

Clinical recurrence (19)

Anti-PLA2R at time of TP

Positive (14)

Negative (5)

No clinical recurrence (8)

Anti-PLA2R at time of TP

Positive (4)

Negative (4)
Clinical and therapeutic implications

- IgG4 anti-PLA2R autoantibodies are highly specific markers of active primary MN
- A negative test for circulating antibodies does not exclude anti-PLA2R-related disease
- Most patients with anti-PLA2R-related MN will have a therapeutic or spontaneous remission
- Loss of serum reactivity for anti-PLA2R precedes clinical remission of proteinuria
- Anti-PLA2R positivity at the time of transplantation is associated with a high risk of recurrent MN
Membranous Nephropathy

Primary (~85%)
- Anti-PLA2R-associated (80%)
- Idiopathic (20%)

Secondary (15%)
- SLE, Hepatitis B, Drugs, Toxins, Cancer, Food/Environmental allergens, Other
- Fetomaternal immunization
- De novo post TP MN
- GVHD post HSCT

Alloimmune (? Frequency)

Recurrent post-transplant
Recurrent post-transplant?
Remaining questions

• Is there another Ag in the 20% of idiopathic MN cases or are they simply inactive?
• What are the antigens in secondary MN?
• How do IgG4 antibodies cause podocyte injury if they can’t activate the classical complement pathway?
• Can anti-PLA2R be used to predict recurrence of MN after transplantation?
• What triggers the development of anti-PLA2R?
• Do variations in *PLA2R1* account for the conformation-dependence of the epitope?
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Patients and volunteers that provided serum

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Richard Quigg

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