Biomarkers in Pediatric Glomerulonephritis and Nephrotic Syndrome

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Overview

• Biomarker Definitions
• Biomarker Development
• Historical Biomarkers
  • Glomerulonephritis
  • Nephrotic Syndrome
• Emerging and Future Biomarker Development
  • Pediatric Nephrotic Syndrome
  • Pediatric and Adult Glomerular Diseases
Biomarker Definitions

• **Prognostic Biomarker**
  - A biological or clinical trait that provides information on the outcome of a disease
  - Used in untreated individuals
  - Most useful at the time of diagnosis

• **Predictive Biomarker**
  - A biological or clinical trait that provides information on the severity of disease and likelihood of benefit of a given treatment
  - Can help identify responders in a population
  - Can support targeted therapy

(Hwang VJ, *Biomarkers in Medicine*, 2014)
Biomarker Definitions

• **Validated Biomarker**
  - Small minority of biomarkers
  - Identified through discovery process
    - Biased (i.e. targeted; suspected to be involved)
    - Unbiased (no preconceived ideas/mechanism)
  - Subsequently validated in larger cohort
    - Preferably a completely separate cohort of patients!

• **Candidate Biomarker**
  - Vast majority of current biomarkers
  - Identified through discovery process
  - NOT yet validated in a separate larger cohort!
Biomarker Development

• Goals
  • **Single** sample
    • Predict outcome of disease
    • Predict severity of disease
    • Predict response to therapy
  • **Sequential** samples
    • *Understand mechanism of response to therapy*
    • *Understand mechanism of resistance to therapy*

• Samples
  • Clinical or pathological finding (i.e. crescents on BX)
  • **Blood or urine**
Biomarkers of IgAN

• Most common primary GN in world!
  • 20-40% develop ESKD within 20 years of diagnosis
  • Pathophysiology based on galactose-deficient IgA1

• Blood biomarkers (NO validated biomarker...)
  • Galactose-deficient IgA1 (Gd-IgA1) levels
    • Serum ELISA assay for Gd-IgA1: 90% specific / 76% sensitive
    • High initial levels $\rightarrow$ faster decline in GFR
  • Anti-glycan Abs (directed at hinge region of Gd-IgA1)
    • High levels $\rightarrow$ proteinuria; faster decline in GFR

• Pathology biomarkers (NONE validated...)
  • Mesangial C4d deposition (+ staining $\rightarrow$ faster GFR decline

• Urine biomarkers (NONE validated...)

Biomarkers of MPGN

• Less common cause of GN in adults and children
  • Often due to other diseases (infection / autoimmune)
  • Pathophysiology based on Ig- vs. non-Ig-mediated causes

• **Blood** biomarkers (NO validated biomarker !)
  • Serum C3 levels
  • Functional analyses of alternative complement pathway
    • C3a / C5a / Soluble membrane attack complex (sMAC)
  • Acquired autoantibodies
    • Stabilize C3 convertase (C3Nef / Anti-CFB)
    • Block action of pathway inhibitors (Anti-Factor H; Anti-Factor I)

• **Urine** biomarkers (NONE validated)

Biomarkers of IMN

• Common cause of NS in adults; Rare in children
  • 1/3 remission; 1/3 sustained symptoms; 1/3 → ESKD
  • Pathophysiology based on Abs to podocyte PLA2R

• Blood biomarkers (One validated biomarker !)
  • Anti-PLA2R antibodies (IgG4 subclass)
    • Serum levels elevated in 60-70% of patients
    • High titers clearly correlated to disease activity !
    • Tissue staining may be positive despite negative serum test…
    • Secondary forms of MN can → positive test…

• Urine biomarkers (NONE validated)

Biomarkers of FSGS

• Common cause of NS in children and adults
  • 20% steroid resistant (in children); ~50% (in adults)
  • Pathophysiology of most cases not yet clear…

• Blood biomarkers (NO validated biomarker !)
  • Many candidates proposed
    • **Hemopexin** (infusion in rats → proteinuria / FP effacement)
    • **Soluble IL-2 receptor** (↑ levels in relapse / NL in remission)
    • **Haptoglobin** (↑ levels prior to treatment predicted SRNS)
    • **Vascular endothelial growth factor (VEGF)**
    • **Glucocorticoid receptor / IL-13** (overexpression → NS)
    • **Angiopoietin-like-4 / Cardiotrophin-like cytokine-1**
Biomarkers of FSGS

• **Blood** biomarkers (continued)
  • **suPAR** (soluble urokinase plasminogen activating receptor)
    • Serum levels increased in 2/3 patients with 1° FSGS
  • *Animal Studies*:
    • Membrane-bound uPAR activates podocyte β3 integrin signaling → foot process effacement
    • False positives: Inflammation / Infection / Malignancy
    • 2 large pediatric clinical trials → conflicting correlation with disease or steroid responsiveness
    • Differentially glycosylated isoforms may have variable effects…

• **Urine** biomarkers (NONE validated)
  • CD80 levels (MCD vs. FSGS / Not treatment response)
“Omic” Approaches to Improve Patient Care

Transcriptomics
Proteomics
Metabolomics
Cytokine profiling

Patient Blood Samples
Clinical Phenotyping (SRNS / SSNS)
Diagnosics and Therapy

Alignments, Clustergrams, Heat Maps, Computation Algorithms
Validation

Biological testing and relevance
Integrated discovery-based analyses of serial clinically-phenotyped blood samples from children with NS can identify novel predictive biomarkers as well as new molecular targets for the treatment of NS.
Multiple “Omic” analyses of paired (Pre and Post 6-12 weeks of GC therapy) blood samples from children with SSNS and SRNS

SSNS vs. SRNS
Pre-treatment
Predictive Biomarkers

Pre vs. Post
SSNS / SRNS
Molecular Steroid Resistance
### Study Design Approaches

<table>
<thead>
<tr>
<th>#</th>
<th>Comparison</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SSNS (Pre) vs. SRNS (Pre)</td>
<td>Predictive biomarkers</td>
</tr>
<tr>
<td>2</td>
<td>SSNS [Post - Pre] vs. SRNS [Post - Pre]</td>
<td>Molecular mechanisms of steroid resistance</td>
</tr>
<tr>
<td>3</td>
<td>SSNS [Post/Pre] vs. SRNS [Post/Pre]</td>
<td>Molecular mechanisms of steroid resistance</td>
</tr>
<tr>
<td>4</td>
<td>SSNS Post vs. SRNS Post</td>
<td>Differences at disease remission/nephrotic</td>
</tr>
<tr>
<td>5</td>
<td>SSNS Pre vs. SSNS Post</td>
<td>Efficacious and steroid responsive pathways</td>
</tr>
<tr>
<td>6</td>
<td>SRNS Pre vs. SRNS Post</td>
<td>Steroid responsive pathways, not necessarily associated with efficacy</td>
</tr>
</tbody>
</table>

SSNS = steroid sensitive nephrotic syndrome  
SRNS = steroid resistant nephrotic syndrome  
Pre = 1\textsuperscript{st} sample, **steroid naïve** (at the time of disease presentation)  
Post = 2\textsuperscript{nd} paired sample, after steroid therapy (~ 8 weeks of steroids)
Predictive Biomarkers with Transcriptomics (SSNS Pre vs. SRNS Pre)

- 25 children presenting with new-onset NS
- Transcriptomes created from leukocytes at presentation
- 13 SSNS and 12 SRNS patients
- Total 15,418 genes after filtering for normalized counts with reads at least 2 counts per million (cpm) in >50% of samples
- 7,907 genes after filtering for values >1 cpm
- Volcano plots and clustergram
Predictive Biomarkers with Transcriptomics
(SSNS Pre vs. SRNS Pre)

<table>
<thead>
<tr>
<th>RANK</th>
<th>GENE</th>
<th>p-value</th>
<th>Expression</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>ZNF528</td>
<td>0.00194</td>
<td>up</td>
</tr>
<tr>
<td>2</td>
<td>ZNF346</td>
<td>0.00577</td>
<td>down</td>
</tr>
<tr>
<td>3</td>
<td>POMT2</td>
<td>0.01892</td>
<td>up</td>
</tr>
<tr>
<td>4</td>
<td>LOC100294145</td>
<td>0.01973</td>
<td>up</td>
</tr>
<tr>
<td>5</td>
<td>ZNF302</td>
<td>0.01979</td>
<td>up</td>
</tr>
<tr>
<td>6</td>
<td>RNF185</td>
<td>0.02032</td>
<td>down</td>
</tr>
<tr>
<td>7</td>
<td>ROPN1L</td>
<td>0.02208</td>
<td>up</td>
</tr>
<tr>
<td>8</td>
<td>CD163</td>
<td>0.02348</td>
<td>down</td>
</tr>
<tr>
<td>9</td>
<td>PLRG1</td>
<td>0.02449</td>
<td>up</td>
</tr>
<tr>
<td>10</td>
<td>DDX26B</td>
<td>0.02713</td>
<td>up</td>
</tr>
<tr>
<td>11</td>
<td>DIS3L2</td>
<td>0.02766</td>
<td>up</td>
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<tr>
<td>12</td>
<td>TCF4</td>
<td>0.03028</td>
<td>up</td>
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<tr>
<td>13</td>
<td>SNORD89</td>
<td>0.03417</td>
<td>down</td>
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<tr>
<td>14</td>
<td>DNAJC27</td>
<td>0.03669</td>
<td>up</td>
</tr>
<tr>
<td>15</td>
<td>PLEKHF2</td>
<td>0.03771</td>
<td>up</td>
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<tr>
<td>16</td>
<td>PMAIP1</td>
<td>0.03911</td>
<td>down</td>
</tr>
<tr>
<td>17</td>
<td>DDX55</td>
<td>0.03925</td>
<td>up</td>
</tr>
<tr>
<td>18</td>
<td>ASB7</td>
<td>0.03970</td>
<td>up</td>
</tr>
<tr>
<td>19</td>
<td>PXMP4</td>
<td>0.04073</td>
<td>up</td>
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<tr>
<td>20</td>
<td>TESK2</td>
<td>0.04115</td>
<td>down</td>
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<td>21</td>
<td>PRPH2</td>
<td>0.04341</td>
<td>down</td>
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<tr>
<td>22</td>
<td>RASGEF1B</td>
<td>0.04349</td>
<td>up</td>
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<td>23</td>
<td>HDDC2</td>
<td>0.04354</td>
<td>up</td>
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<td>24</td>
<td>PPP1R3D</td>
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<td>up</td>
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<td>25</td>
<td>ZNF71</td>
<td>0.04829</td>
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<td>26</td>
<td>MDFIC</td>
<td>0.04856</td>
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<td>27</td>
<td>MCM8</td>
<td>0.04905</td>
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<tr>
<td>28</td>
<td>ZNF611</td>
<td>0.04950</td>
<td>up</td>
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</tbody>
</table>
Molecular Mechanisms of Steroid Resistance (SSNS [Pre/Post] vs. SRNS [Pre/Post])

- 7 SSNS and 4 SRNS patients
- Transcriptomes created from leukocytes at presentation and follow-up visits
- Total >20,000 genes reduced to 7,334 after filtering for little or no expression
- Volcano plots and clustergram
- Candidate gene selection using BCGA-ELM (Binary Coded Genetic Algorithm – Extreme learning Machine)
Molecular Mechanisms of Steroid Resistance

(SSNS [Pre/Post] vs. SRNS [Pre/Post])

Genes up-regulated in SRNS compared to SSNS

Genes up-regulated in SSNS compared to SRNS
## Predictive Biomarkers with Proteomics

### (SSNS Pre vs. SRNS Pre)

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Gene Name</th>
<th>Detection Rate (SSNS)</th>
<th>Detection Rate (SRNS)</th>
<th>P-Value (Ranksum)</th>
<th>Area Ratio (SSNS:SRNS)</th>
<th>Log2 (SSNS:SRNS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen alpha-3(VI) chain</td>
<td>COL6A3</td>
<td>86</td>
<td>50</td>
<td>0.02</td>
<td>9.1</td>
<td>3.2</td>
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<tr>
<td>Insulin-like growth factor-binding protein 2</td>
<td>IGFBP2</td>
<td>100</td>
<td>63</td>
<td>0.04</td>
<td>9</td>
<td>3.2</td>
</tr>
<tr>
<td>72 kDa type IV collagenase</td>
<td>MMP2</td>
<td>86</td>
<td>63</td>
<td>0.03</td>
<td>2.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Apolipoprotein E*</td>
<td>APOE</td>
<td>100</td>
<td>100</td>
<td>0.04</td>
<td>1.9</td>
<td>0.9</td>
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<tr>
<td>Adiponectin</td>
<td>ADIPOQ</td>
<td>100</td>
<td>100</td>
<td>0.01</td>
<td>1.8</td>
<td>0.8</td>
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<tr>
<td>Sex hormone-binding globulin</td>
<td>SHBG</td>
<td>100</td>
<td>100</td>
<td>0.03</td>
<td>1.8</td>
<td>0.8</td>
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<tr>
<td>EGF-containing fibulin-like extracellular matrix protein 1</td>
<td>EFEMP1</td>
<td>100</td>
<td>100</td>
<td>0.01</td>
<td>1.6</td>
<td>0.7</td>
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<tr>
<td>Inter-alpha-trypsin inhibitor heavy chain H4</td>
<td>ITIH4</td>
<td>100</td>
<td>100</td>
<td>0.01</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Hemopexin</td>
<td>HPX</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0.7</td>
<td>-0.5</td>
</tr>
<tr>
<td>Vitamin D-binding protein</td>
<td>GC</td>
<td>100</td>
<td>100</td>
<td>0.03</td>
<td>0.7</td>
<td>-0.5</td>
</tr>
<tr>
<td>Antithrombin-III</td>
<td>SERPINC1</td>
<td>100</td>
<td>100</td>
<td>0.01</td>
<td>0.7</td>
<td>-0.5</td>
</tr>
<tr>
<td>Zinc-alpha-2-glycoprotein</td>
<td>AZGP1</td>
<td>100</td>
<td>100</td>
<td>0.03</td>
<td>0.6</td>
<td>-0.7</td>
</tr>
<tr>
<td>Fetuin-B</td>
<td>FETUB</td>
<td>100</td>
<td>100</td>
<td>0.02</td>
<td>0.5</td>
<td>-1</td>
</tr>
</tbody>
</table>
Cure Glomerulopathy (CureGN) Study

• NIH funded study (UM1 Grant) to recruit 2,400 adults and children with four glomerular diseases
  • MCNS / FSGS / IgA Nephropathy / IMN
  • Detailed longitudinal clinical data
  • Serial blood collections for DNA, RNA, Plasma
  • Serial urine collections
  • Digital Pathology Repository being created…
• ~2,320 patients now recruited (38% children)
• Ancillary studies invited!
Cure Glomerulopathy (CureGN) Study

• Initial ancillary study (R01) now funded

• **Hypothesis:** Integrated proteomic and metabolomic analyses of serial urine samples from children with CureGN diseases can identify:
  • Biomarkers predictive of clinical outcomes
  • Biomarkers of molecular pathways / targets
    • Common among all 4 CureGN diseases
    • Differentiate the 4 CureGN diseases
Summary

- Growing interest in identifying biomarkers able to predict:
  - Outcome of disease / Severity of disease / Response to therapy
- **Sequential** samples offer possibility to also understand:
  - Mechanisms of response to therapy
  - Mechanisms of resistance to therapy
- Many biomarkers for GN and NS have been suggested, but very few have been validated
  - **IgAN** - Galactose-deficient IgA1 levels / Anti-glycan Abs / C4d
  - **MPGN** - C3 levels / Alt complement assays / Acquired auto-Abs
  - **IMN** - Anti-PLA2R antibodies (**validated !**)
  - **FSGS** – suPAR / many other candidates proposed…
- Increasing interest in **unbiased** (i.e. discovery-based) approaches to biomarker development
Future Directions

• Example of creation of initial landscape of pediatric NS using systems biology approaches
  • Transcriptomics
  • Proteomics
  • Metabolomics

• **Integration** of above approaches expected to enhance identification of:
  • Novel molecular **pathways** of disease
  • Novel molecular **targets** for future therapies

• Use of computational algorithms expected to identify **biomarker panels** able to predict and/or define steroid resistance
Thank You!
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Nationwide Children’s
When your child needs a hospital, everything matters.

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