HKUPP Active sites

- Niigata, Japan
- Little Rock, Arkansas, USA
- Louisville, Kentucky, USA
- Davis, California, USA
- Charleston, South Carolina, USA
UAMS Core Proteomics Lab
Urine Samples Collected by Southern Acute Kidney Injury Network (SAKInet)

• Four Centers (MUSC, Duke, GWU, Chattanooga)
• Samples collected from patients with AKI after cardiac surgery
  • AKI Stage 1 at collection
• Goal: Identify and validate prognostic AKI biomarkers of severe AKI.
Discovery Protocol (2017)

AKI Stage 1
Match for Confounders

No Progression
20X
Dialysis

Trypsin Digestion
Reverse Phase Chromatography

Tandem Mass Spectrometry Orbi Fusion
Protein Identification
Comparison Of Abundances

2428 Proteins
IGFBP1 Is Increased in Severe AKI

Best predictor among 2428 proteins
14.8 fold increase in IGFBP1 in RRT patients
p<0.01
Validation of IGFBP1 by ELISA

N=206
22 Events (Stage 3/Death)

AUC
IGFBP 1 0.87
IGFBP 1/uCr 0.86
<table>
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<th></th>
<th>LC-2013</th>
<th>FASP LC</th>
<th>GeLC</th>
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<tbody>
<tr>
<td>Samples</td>
<td>12</td>
<td>30</td>
<td>30</td>
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<tr>
<td>Fractions</td>
<td>7</td>
<td>1</td>
<td>24</td>
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<tr>
<td>Proteins 1% FDR</td>
<td>359</td>
<td>2428</td>
<td>3629</td>
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<tr>
<td>Proteins (2+ Peptides)</td>
<td>339</td>
<td>1808</td>
<td>3421</td>
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<td>Peptides</td>
<td>4866</td>
<td>13,691</td>
<td>44,722</td>
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<td>Sequence Coverage (Median)</td>
<td>15.4%</td>
<td>14.3%</td>
<td>31.0%</td>
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<tr>
<td>Peptides/Protein (Median)</td>
<td>3</td>
<td>3</td>
<td>7</td>
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<tr>
<td>Time</td>
<td>63 hours</td>
<td>55 hours</td>
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The Kidney
Silver stain of glomeruli

Normal

Membranous Nephropathy
Electron Microscopy

Normal

Membranous Nephropathy
IgG Immunofluorescence

Membranous Nephropathy
Laser Capture Microscopy
Proof of concept

• Two known antigens in Membranous nephropathy are PLA2R and THSD7A
• Unknown if they are more abundant or have an uncovered epitope.
• Compared 3 PLA2R+, 2, THSD7A+ and 18 other membranous nephropathy like cases
• Laser capture microdissection of 10-80 glomerular sections per case
Identification of novel antigens

• MGMID is a kidney disease almost exclusively found in young women.
• The light microscopy looks like membranous nephropathy but there are no immunoglobulins on immunofluorescence until unmasking procedures are done. Immunoglobulins are always IgG kappa subtype 1.
• Protein abundance in glomeruli from 14 cases with MGMID and 8 other membranous like cases were compared