Protein Biomarker Discovery
in
Organ Transplantation

A Proteomics Approach

Tara Sigdel, PhD

9/26/2011

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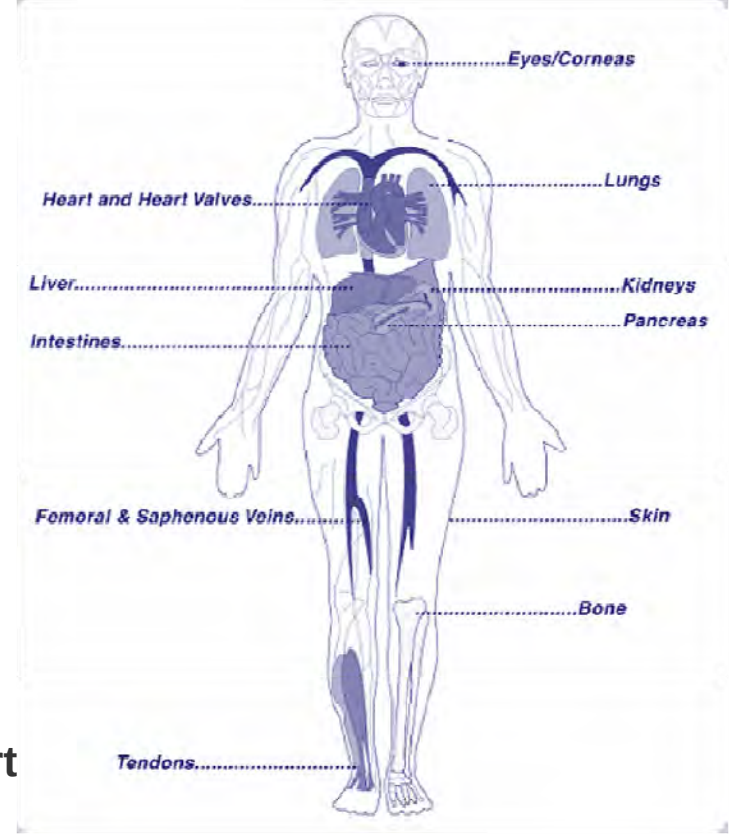
Organ Transplantation

- **Optimal Choice for end stage organ failure**
- **Significant number of transplant occur every year**

  Total transplantation in the US January - June 2011 = 13, 969

  (source: ttp://optn.transplant.hrsa.gov)
- **Causes of organ dysfunction after transplantation:**
  - Donor related
  - Ischemia reperfusion injury
  - Recipient's Immune response
  - Drug toxicity
- **Improvements in organ procurement & Immunosuppressive drugs have contributed to short term outcome**
- **Long-term outcome is still not very satisfactory and needs to be improved**



http://www.mode.org.in/organ_transplantation.html

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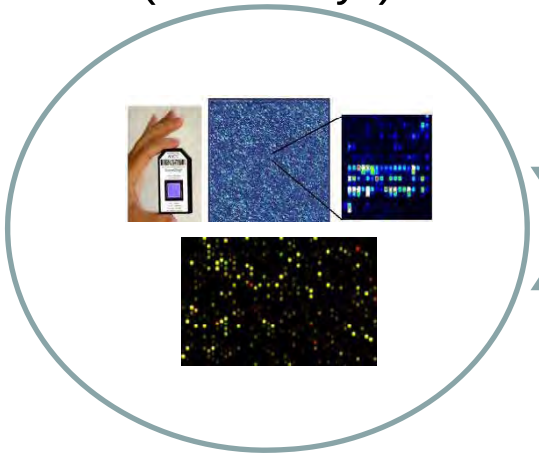
Biomarkers in Transplantation

Biomarker: Substance (molecules such as gene, proteins, metabolites etc) that can be measured to determine the biological, pathological, pharmacological state

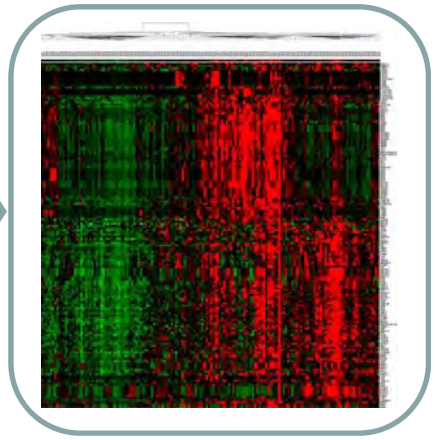
Current monitoring relies on dated technologies

- Serum creatinine level
- Histopathology of biopsy samples

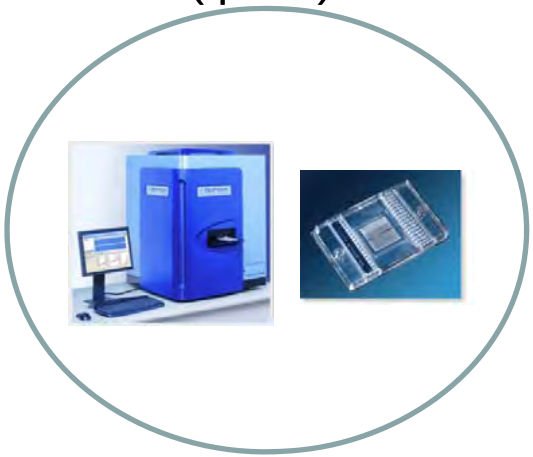
DISCOVERY (microarrays)



Data Analysis



Validation (qPCR)



N Engl J Med. 2003 Jul 10;349(2):125-38.
Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling.
Sarwal M, Chua MS, Kambham N, Hsieh SC, Satterwhite T, Masek M, Salvatierra O Jr.

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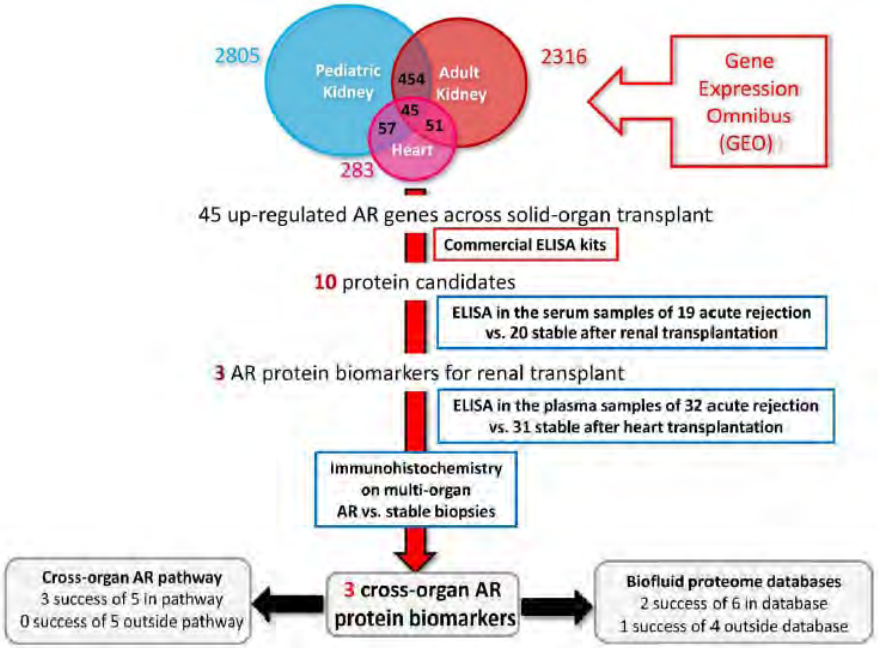
Gene Expression & Protein Biomarkers

OPEN ACCESS Freely available online PLoS COMPUTATIONAL BIOLOGY

Differentially Expressed RNA from Public Microarray Data Identifies Serum Protein Biomarkers for Cross-Organ Transplant Rejection and Other Conditions

Rong Chen[1,2], Tara K. Sigdel[1,2], Li Li[1,2], Neeraja Kambham[3], Joel T. Dudley[1,2], Szu-chuan Hsieh[1,2], R. Bryan Klassen[1,2], Amery Chen[1,2], Tuyen Caohuu[4], Alexander A. Morgan[1,2], Hannah A. Valantine[4], Kiran K. Khush[4], Minnie M. Sarwal[1,2¶]*, Atul J. Butte[1,2¶]*

1 Department of Pediatrics, Stanford University School of Medicine, Stanford, California, United States of America, 2 Lucile Packard Children's Hospital, Palo Alto, California, United States of America, 3 Department of Pathology, Stanford University School of Medicine, Stanford, California, United States of America, 4 Division of Cardiovascular Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, California, United States of America



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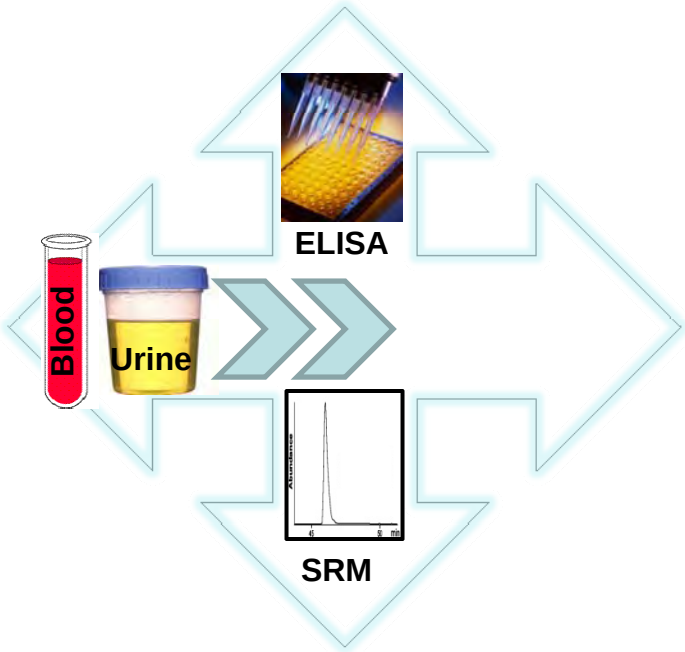
Hypothesis

There is a signature of proteins present in the blood and urine which could serve as non-invasive biomarkers for organ transplant injury

The Goal

Acute Rejection

Chronic Injury



Stable Graft

BK Virus Infection

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Urinary Protein Biomarkers
Urine Proteomics

NIH Public Access
Author Manuscript
Proteomics Clin Appl. Author manuscript; available in PMC 2010 June 10.
Published in final edited form as:
Proteomics Clin Appl. 2010 January 1; 4(1): 32–47. doi:10.1002/prca.200900124.

NATIONAL INSTITUTES OF HEALTH

NIH-PA Author Manuscript

Shotgun Proteomics Identifies Proteins Specific for Acute Renal Transplant Rejection

Tara K. Sigdel[1], Amit Kaushal[2], Marina Gritsenko[3], Angela D. Norbeck[3], Wei-Jun Qian[3], Wenzhong Xiao[2], David G. Camp II[3], Richard D. Smith[3], and Minnie M. Sarwal[1]
[1] Department of Pediatrics, 300 Pasteur Dr, S378 Grant Bldg, Stanford University School of Medicine, Stanford, CA 94305
[2] Department of Biochemis
[3] Battelle, Pacific Northwes

BASIC RESEARCH

www.jasn.org

Integrative Urinary Peptidomics in Renal Transplantation Identifies Biomarkers for Acute Rejection

Xuefeng B. Ling,* Tara K. Sigdel,† Kenneth Lau,* Lihua Ying,† Irwin Lau,* James Schilling,* and Minnie M. Sarwal†
Divisions of *Biotechnology Core and †Nephrology and Department of Pediatrics, Stanford University School of Medicine, Stanford University, Stanford, California

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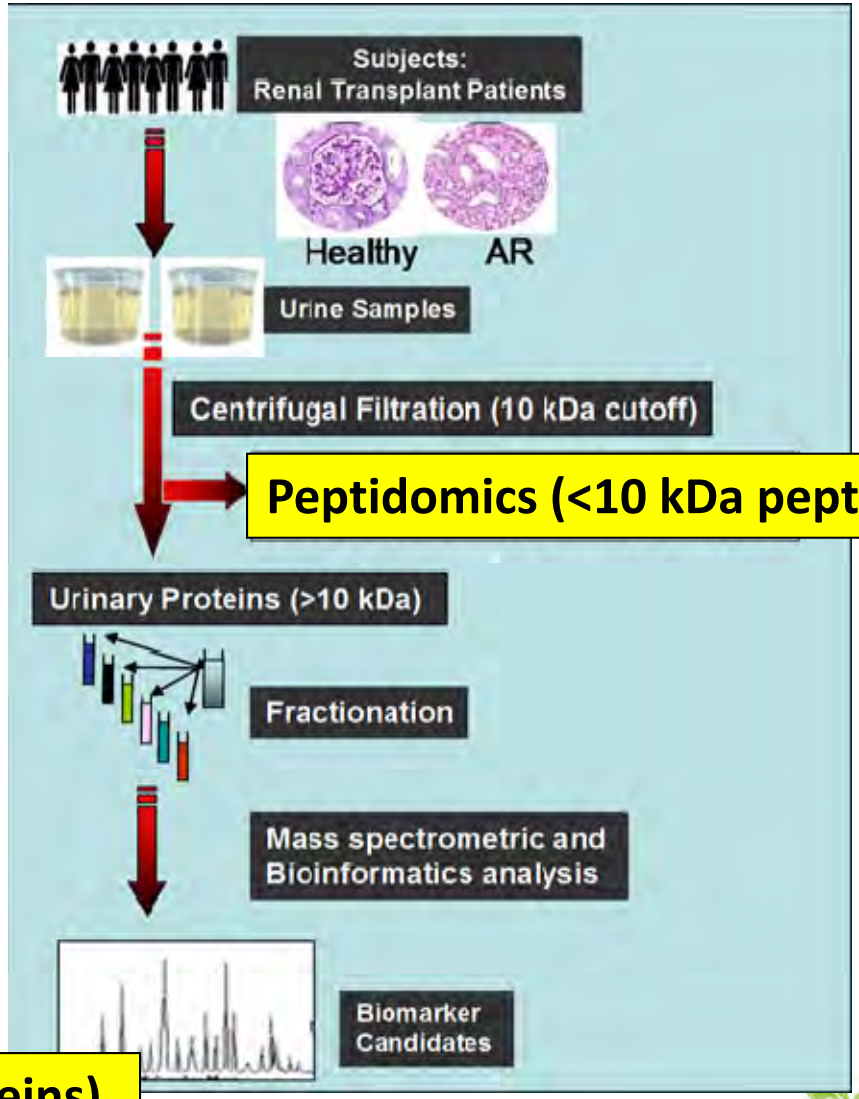
Optimization of Proteomics Protocol

Sigdel et al. Clin Transplant. 2008 (5):617-23



Peptidomics (<10 kDa peptides)

Proteomics (>10 kDa proteins)

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Urine Protein Biomarkers for Kidney Transplantation Rejection (Study Subjects)

Patient demographic information

Patient information on 60 renal transplant patients (30 AR, 30 STA).

	Acute Rejection (AR) (n=30)	Stable Graft Function (STA) (n=30)	*P* value
Mean Age	12±5	14±5	0.21
Age Range	3–19	6–21	
Immunosuppression, %SF[#]	66%	50%	0.19
Race[*]	63%,13%,0%,17%,7%	59%,7%,10%,17%,7%	0.45
Donor, % living donor	40%	53%	0.44
Mean GFR (mL/min/1.73m^2)	87.45±38.46	124±29.86	0.0001

[#]SF: Steroid-free immunosuppression treatment, consisting of daclizumab induction + mycophenolate mofetil + tacrolimus

[*]Race: 1=Caucasian; 2= Hispanic; 3=Asian; 4=African American; 5=Other

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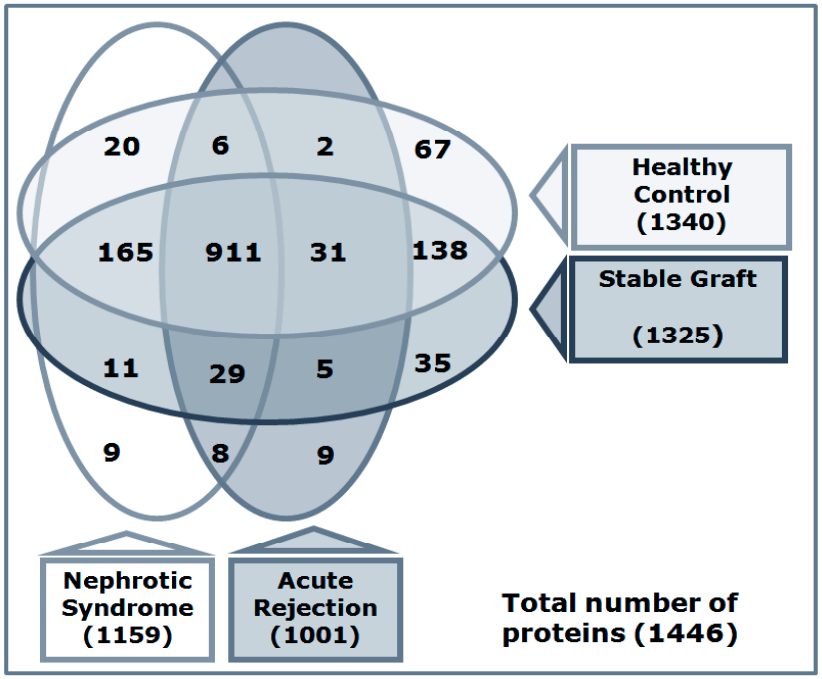
Shotgun Proteomics Analysis of Urine Identifies AR Specific Urine Markers

Sigdel et al Proteomics Clin Appl. 2010 Jan 1;4(1):32-47

High throughput proteomics using shotgun proteomics identified a total of 1446 urinary proteins which included a number of AR specific proteins.



We have identified alterations in a number of specific urinary proteins in AR, primarily relating to MHC antigens, the complement cascade and extra-cellular matrix proteins.

Selected candidates were verified by ELISA in an independent urine sample set

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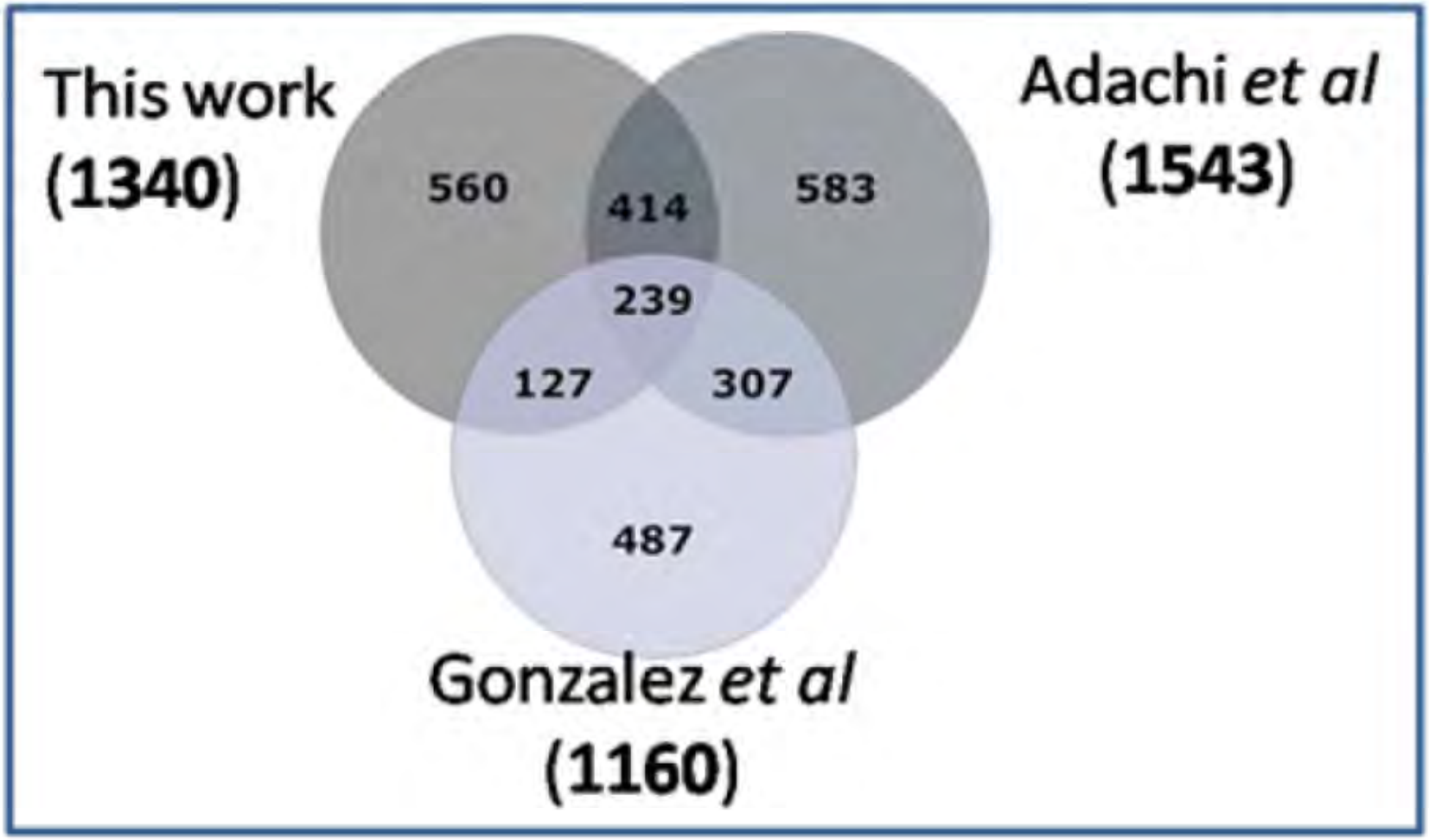
Identification of More Proteins in Urine

Sigdel et al Proteomics Clin Appl. 2010 Jan 1;4(1):32-47



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Acute Rejection Specific Proteins

Sigdel et al Proteomics Clin Appl. 2010 Jan 1;4(1):32-47

Table 4A: List of proteins identified only in AR urine

S. No.	IPI ID	Gene Symbol	Protein Name
1	IPI00103082.7	*HLA-DBP*	HLA class II histocompatibility antigen, DP(W4) beta chain
2	IPI00005180.2	*IgHM*	HLA class II histocompatibility antigen, DRB1-8 beta chain
3	IPI00021727.1	*C4BPA*	C4b-binding protein alpha chain
4	IPI00641889.1	*KIAA1522*	25 kDa protein
5	IPI00746396.1		302 kDa protein
6	IPI00760688.2	*HLA-DR*	MHC class II antigen (Fragment)
7	IPI00027255.1	*MYL6B*	Myosin light chain 1, slow-twitch muscle A isoform
8	IPI00783351.1	*SUMF2*	sulfatase modifying factor 2 isoform d
9	IPI00743218.1	*HLA-DQB1*	HLA class II histocompatibility antigen, DQ(3) beta chain

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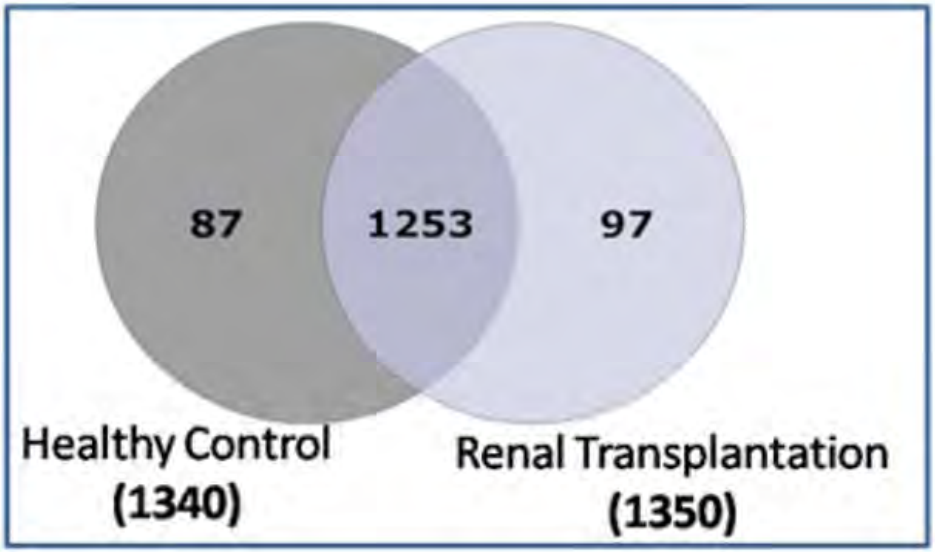
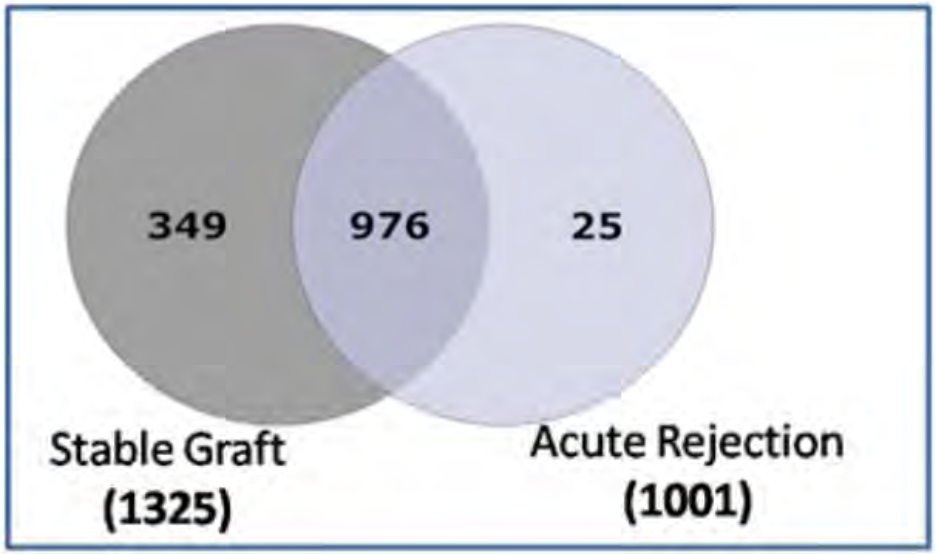
Transplantation and AR Specific Proteins

Sigdel et al Proteomics Clin Appl. 2010 Jan 1;4(1):32-47





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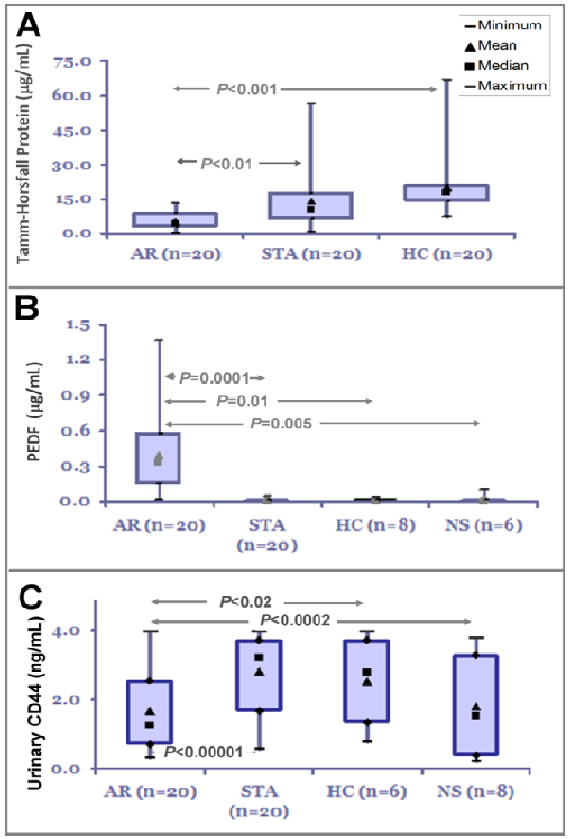
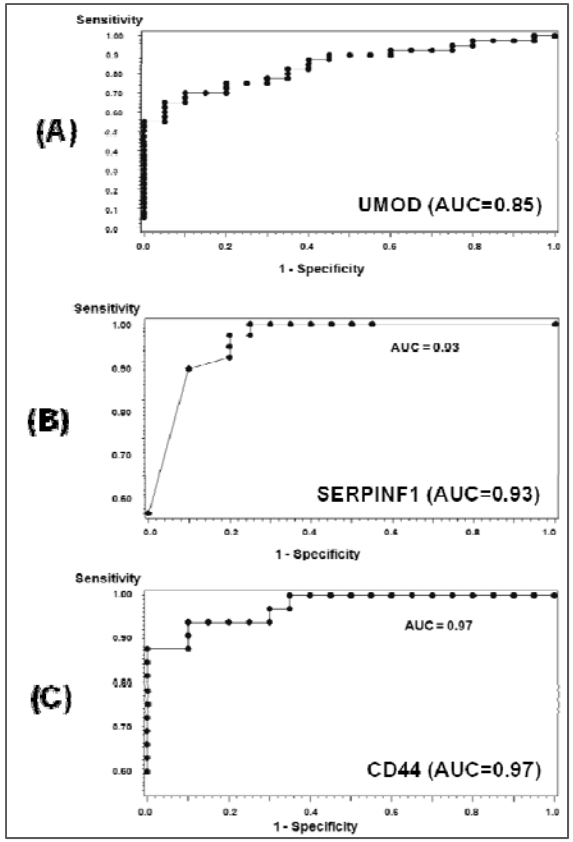
Verification of Urine Protein Markers

UMOD, SERPINF1 and CD44 (ELISA Assay)

Sigdel et al Proteomics Clin Appl. 2010 Jan 1;4(1):32-47

We validated observation made in discovery step by ELISA assay performed on independent set of samples.





Shotgun proteomics approach for analyzing urinary proteome in normal and disease states is a robust and sensitive method for detection of urinary proteins for serial, non-invasive clinical monitoring for graft rejection after kidney transplantation.

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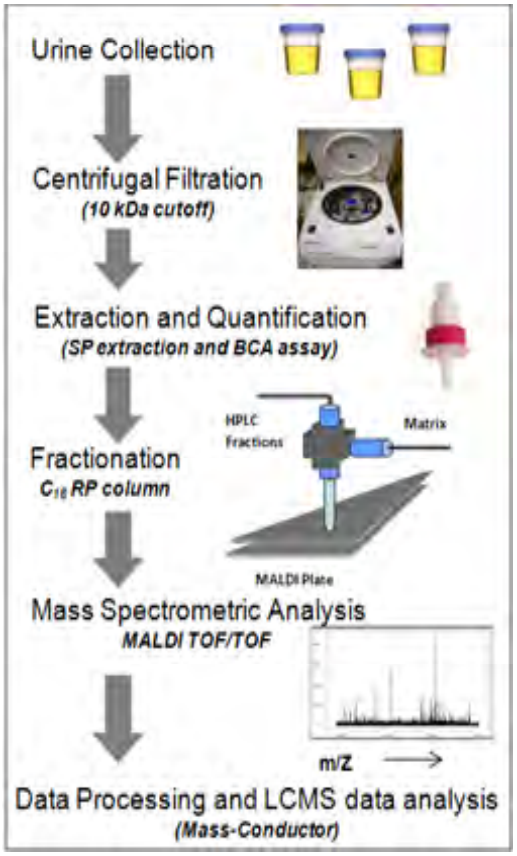
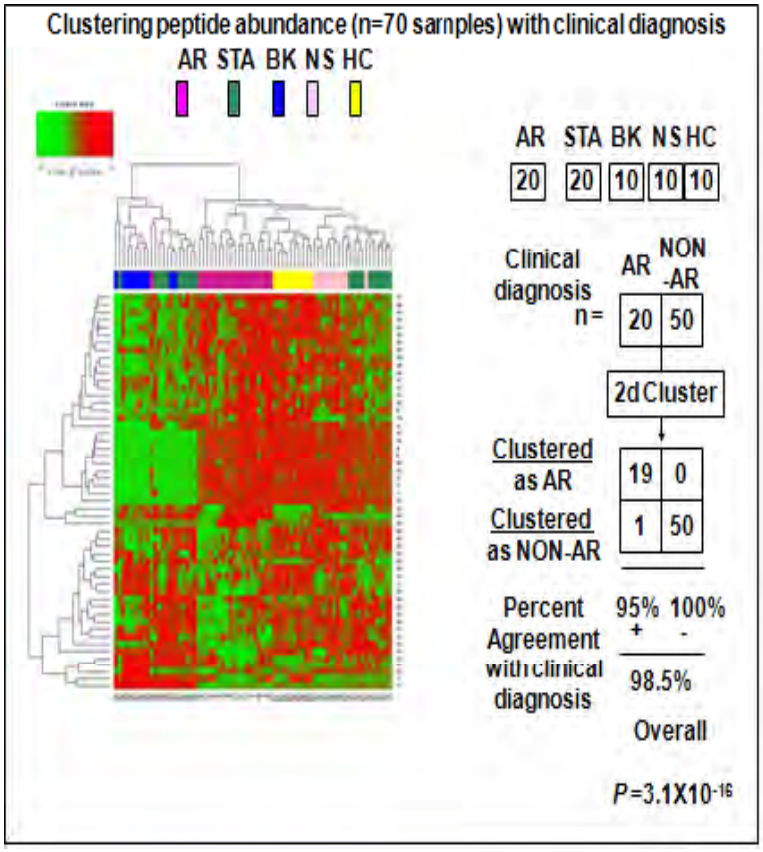
Integrative Urinary Peptidomics in Renal Transplantation Identifies Novel Biomarkers for Acute Rejection

Ling and Sigdel et al JASN 2010 Apr;21(4):646-53

Peptidomics approach for biomarker discovery (70 urine samples)

(50 renal transplant and 20 controls)





We identified a panel of 40 peptides that discriminates AR from non-AR, ROC AUC>0.96

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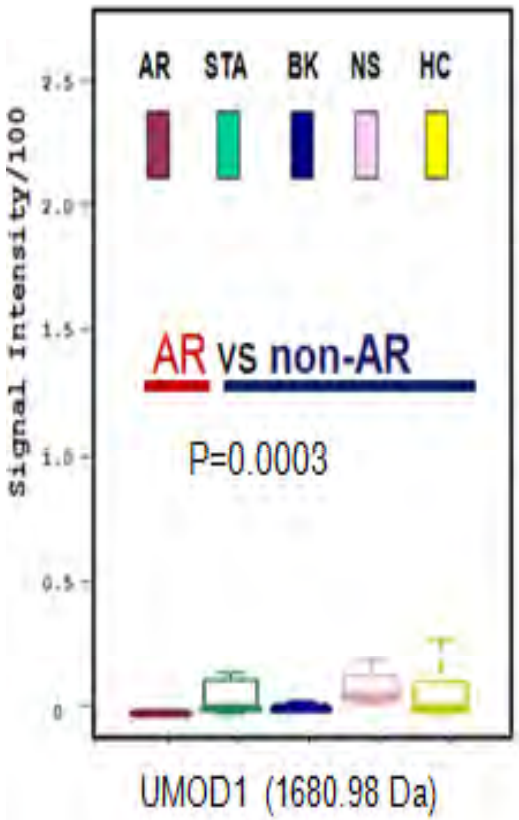
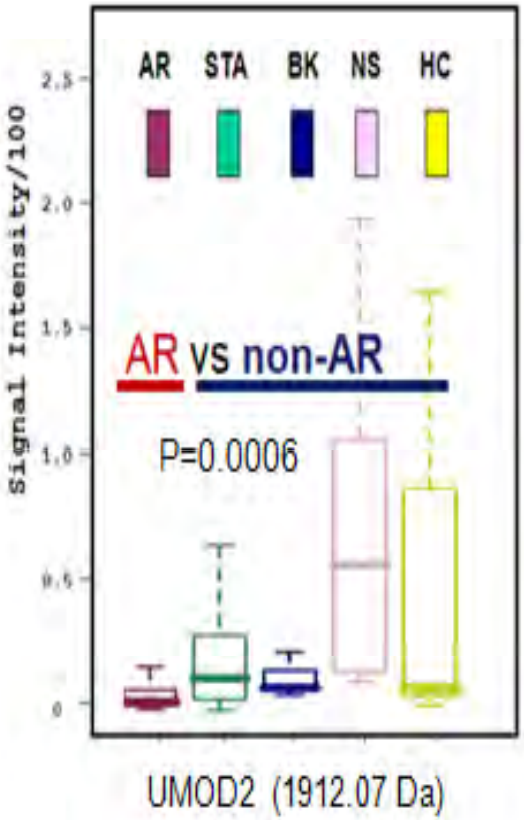
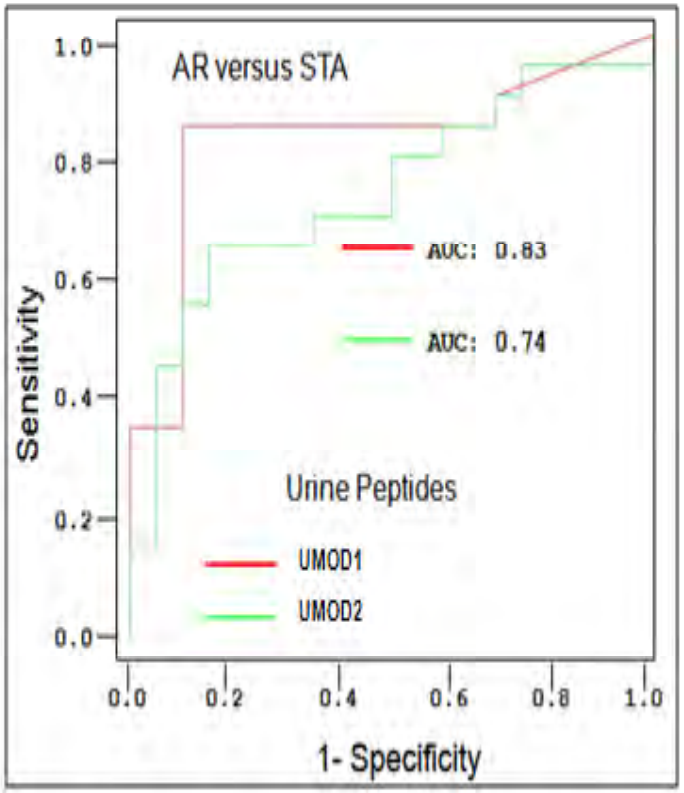
Verification of Urine Peptide Markers

Ling and Sigdel et al JASN 2010 Apr;21(4):646-53

SRM verification of two UMOD peptide biomarkers







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Discovery of Disease Specific Proteolytic Activity

Collegens

1.	COL1A1	1235.56	APGDRGEPGPPGP
2.	COL1A1	1251.55	APGDRGEPGPPGP
3.	COL1A1	1322.57	APGDRGEPGPPGPA
4.	COL1A1	1316.59	DAGPVGPPGPPGPPG
5.	COL1A1	1409.66	GPPGPPGPPGPPGPPS
6.	COL1A1	2048.92	NGDDGEAGKPGRPGERGPPGP
7.	COL1A1	2064.91	NGDDGEAGKPGRPGERGPPGP
8.	COL1A1	2192.97	NGDDGEAGKPGRPGERGPPGPQ
9.	COL1A1	2362.12	GKNGDDGEAGKPGRPGERGPPGPQ
10.	COL1A1	2378.10	GKNGDDGEAGKPGRPGERGPPGPQ
11.	COL1A1	2645.24	GPPGKNGDDGEAGKPGRPGERGPPGPQ
12.	COL1A1	1709.79	PPGEAGKPGEQGVPGDLG
13.	COL1A1	2031.95	PPGEAGKPGEQGVPGDLGAPGP
14.	COL1A1	2221.97	ADGQPGAKGEPGDAGAKGDAGPPGP
15.	COL1A1	2205.99	ADGQPGAKGEPGDAGAKGDAGPPGP
16.	COL1A1	2277.01	ADGQPGAKGEPGDAGAKGDAGPPGPA
17.	COL1A1	2293.01	ADGQPGAKGEPGDAGAKGDAGPPGPA
18.	COL1A1	2617.15	GPPGADGQPGAKGEPGDAGAKGDAGPPGPA
19.	COL1A1	2086.93	EGSPGRDGSPGAKGDRGETGPA
20.	COL1A1	2157.96	AEGSPGRDGSPGAKGDRGETGPA
21.	COL1A1	3014.41	ESGREGAPGAEGSPGRDGSPGAKGDRGETGPA
22.	COL1A1	1266.58	SPGPDGKTGPPGPA
23.	COL1A1	2129.99	DGKTGPPGPAGQDGRPGPPGPPG
24.	COL1A1	2017.93	GRPGEVGPPGPPGPAGEKGSPG
25.	COL1A2	2081.94	DGPPGRDGQPGHKGERGYPG
26.	COL1A2	2195.99	NDGPPGRDGQPGHKGERGYPG
27.	COL2A1	1861.85	SNGNPGPPGPPGPSGKDGPK
28.	COL3A1	1738.76	NDGAPGKNGERGGPGGPGP
29.	COL3A1	2008.93	DGESGRPGRPGERGLPGPPG
30.	COL3A1	2079.92	DAGAPGAPGGKGDAGAPGERGPPG
31.	COL3A1	2565.18	GAPGQNGEPGGKGERGAPGEKGEGGPPG
32.	COL3A1	2743.24	KNGETGPQGPPGPTGPGGDKGDTGPPGPQG
33.	COL4A1	1424.66	PGQQGNPGAQGLPGP
34.	COL4A2	1126.51	GLPGLPGPKGFA
35.	COL4A3	1161.52	GEPGPPGPPGNLG
36.	COL4A4	1218.55	GLPGPPGPKGPRG
37.	COL4A5	1144.52	GPPGPPGPLGPLG
38.	COL4A5	1269.53	PGLDGMKGDPGLP
39.	COL4A5	1733.76	GIKGEKGNPGQPGLPGLP
40.	COL4A6	1158.52	GLPGPPGPPGPPS
41.	COL5A1	1748.82	KGPQGKPGLAGMPGANGPP
42.	COL7A1	1690.80	PGLPGQVGETGKPGAPGR
43.	COL9A1	1732.84	KRPDSGATGLPGRPGPPG
44.	COL11A1	1441.64	GPPGPPGLPGPQGPKG
45.	COL11A1	1828.84	DGPPGPPGERGPQGPQGPV
46.	COL17A1	1368.62	LPGPPGPPGSFLSN
47.	COL18A1	1142.51	GPPGPPGPPGPPS

Uromodulin

1.	UMOD	982.59	VLNLGPITR
2.	UMOD	1047.48	SGSVIDQSRV
3.	UMOD	1211.66	DQSRVLNLGPI
4.	UMOD	1225.69	SRVLNLGPITR
5.	UMOD	1324.76	IDQSRVLNLGPI
6.	UMOD	1423.83	VIDQSRVLNLGPI
7.	UMOD	1468.82	DQSRVLNLGPITR
8.	UMOD	1510.87	SVIDQSRVLNLGPI
9.	UMOD	1567.91	GSVIDQSRVLNLGPI
10.	UMOD	1581.91	IDQSRVLNLGPITR
11.	UMOD	1654.91	SGSVIDQSRVLNLGPI
12.	UMOD	1680.98	VIDQSRVLNLGPITR
13.	UMOD	1755.96	SGSVIDQSRVLNLGPIT
14.	UMOD	1768.01	SVIDQSRVLNLGPITR
15.	UMOD	1912.07	SGSVIDQSRVLNLGPITR
16.	UMOD	2040.16	SGSVIDQSRVLNLGPITRK

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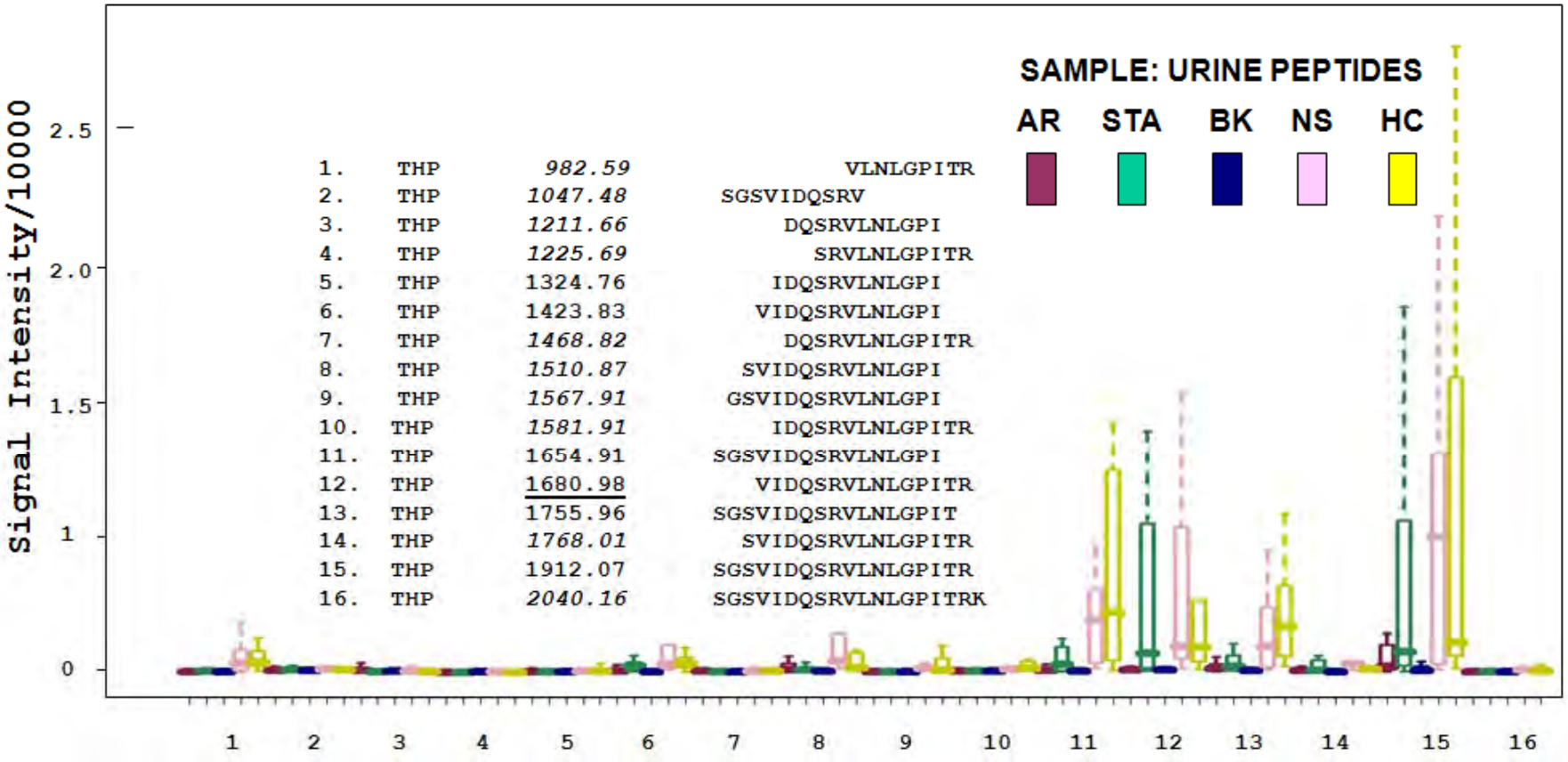
Decreased Fragments of COL and UMOD in AR

Ling and Sigdel et al J Am Soc Nephrol. 2010



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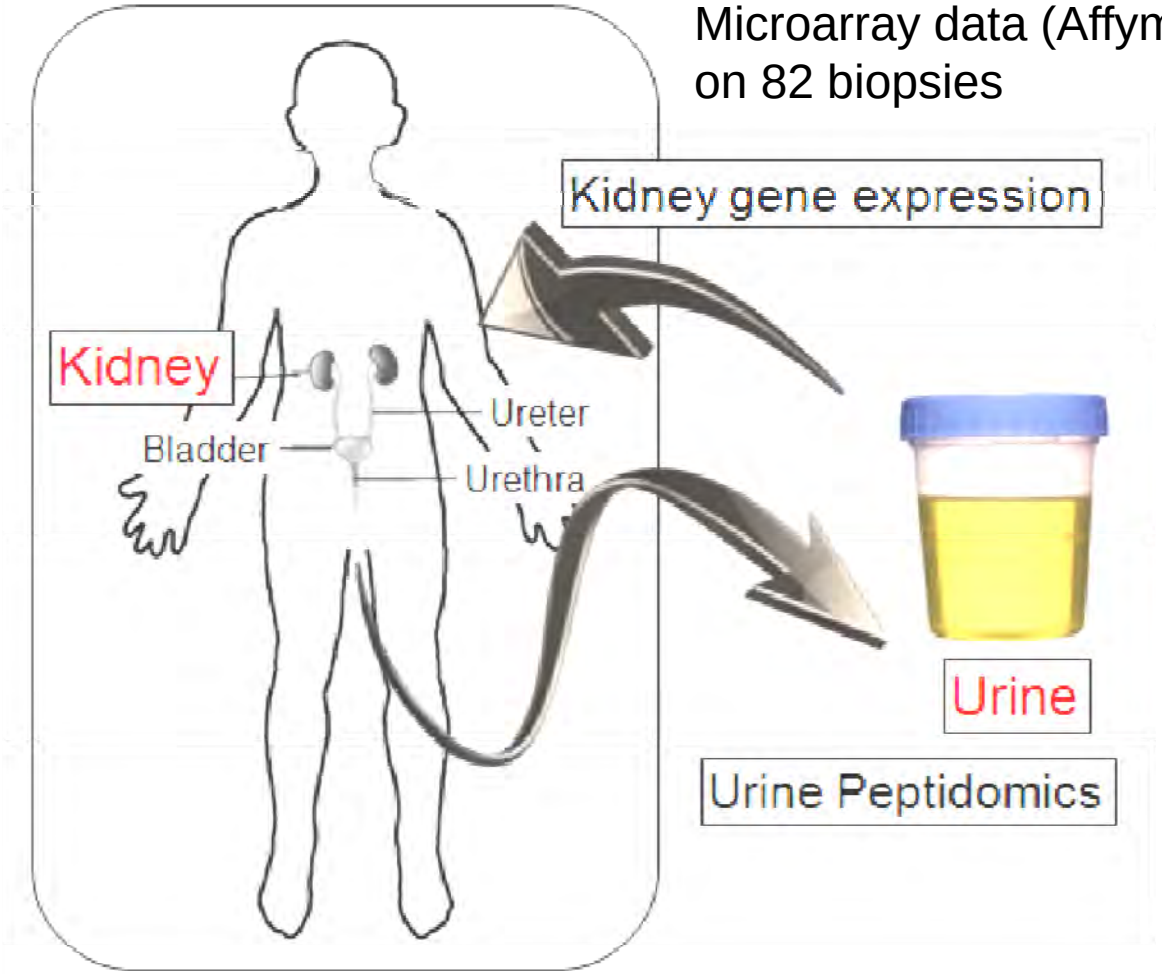
Transcription-Translation-Proteolysis

Is there an altered gene expression for the precursor gene of biomarker peptides in AR?

Ling and Sigdel et al J Am Soc Nephrol. 2010

Microarray data (Affymetrix HU133 plus 2) on 82 biopsies



Biopsy arrays chosen for study on overlapping **urine peptidomic** samples for 20 AR, 20 STA and 10 HC.

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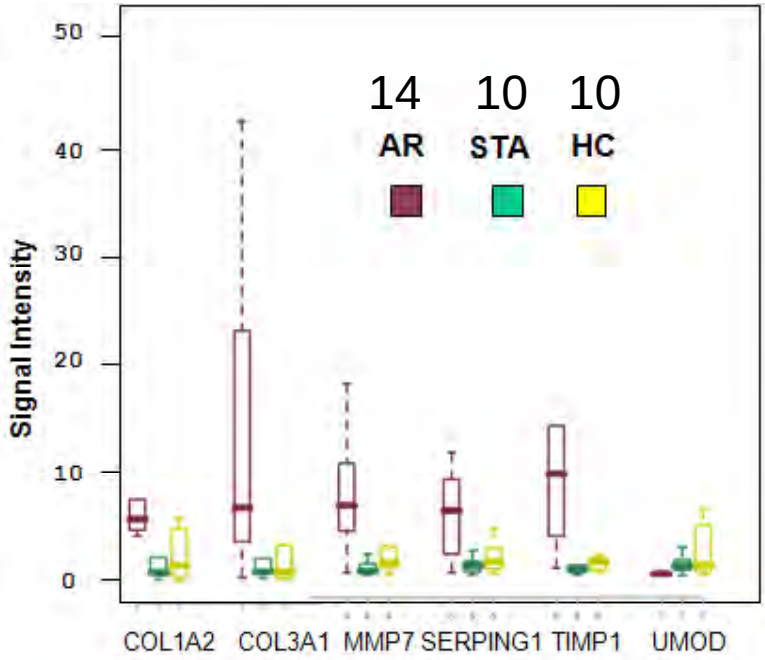
A Panel of genes as biomarkers specific for AR (Q-PCR validation)

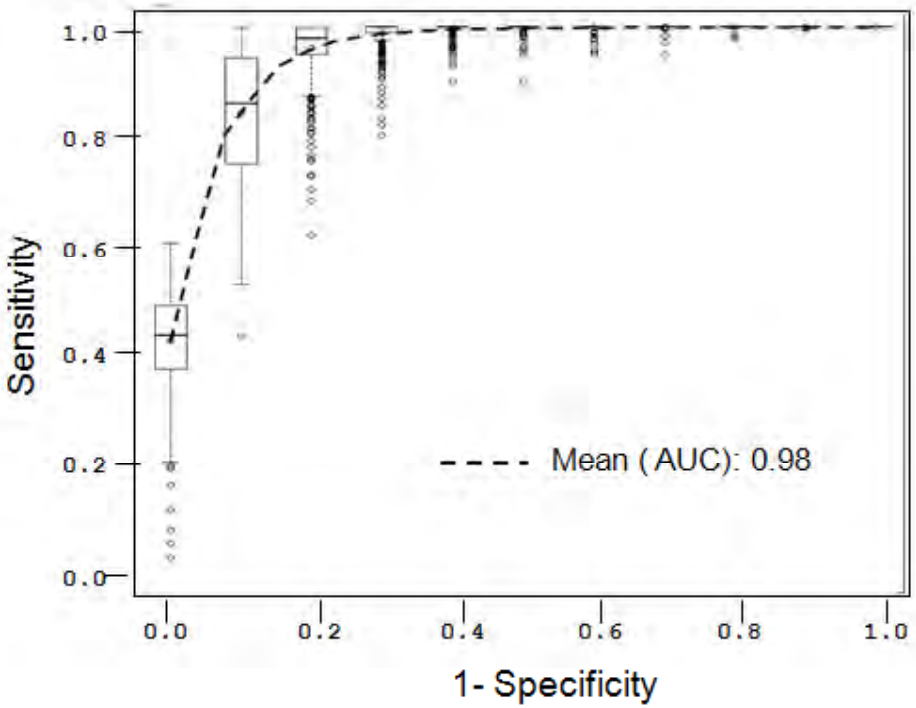
Ling and Sigdel et al J Am Soc Nephrol. 2010

Kidney gene expression analysis:

1. Upregulation of COL1A2, COL3A1, MMP7, SERPING1, and TIMP1

2. Downregulation of UMOD





Q-PCR validation

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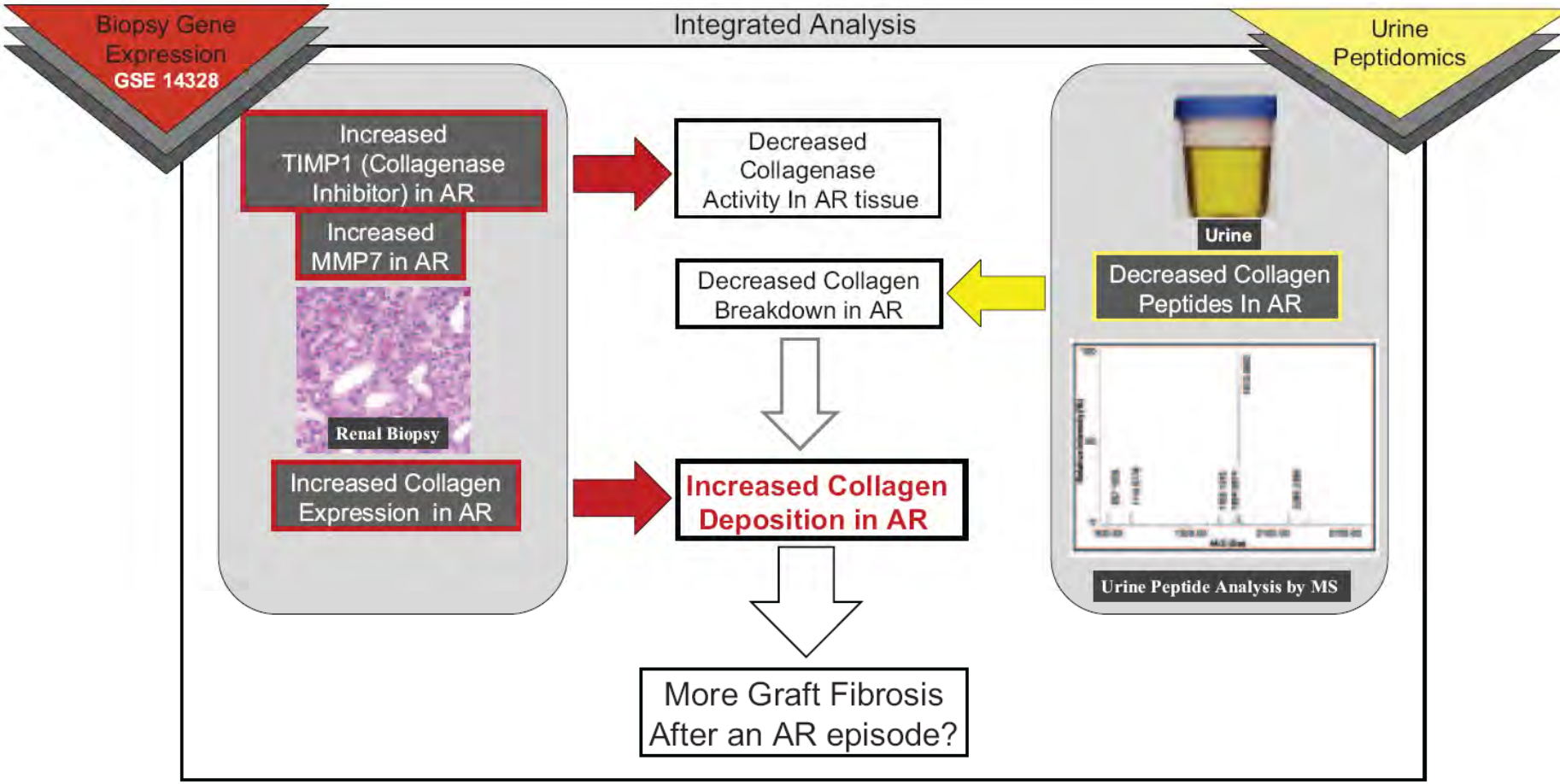
A New Understanding



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Protein Biomarkers in the Blood

Serum Proteomics

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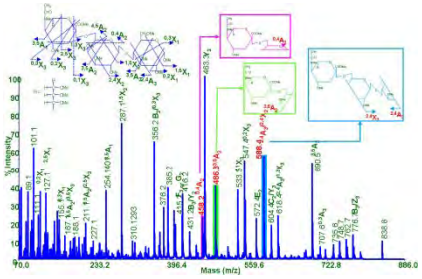
Serum Protein Biomarkers Study Design

Serum Protein Biomarkers for Renal Transplant Injury Samples (n=78)

- Transplant Injury Phenotype
  - AR
  - CAN
  - BKV
- Transplant Non-injury Phenotype
  - STA

Shotgun Proteomics



Data Analysis

Discovery → **Verification** → **Validation**

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Patient Demography

Discovery (n=60) (50 transplantation 10 healthy normal)

Phenotype	AR	STA	CAN	CNIT	BKV
Number of Patients	10	10	10	10	10
Steroid-free/Steroid-based	5/5	4/6	3/7	8/2	6/4
Recipient Gender (M/F)	6/4	7/3	6/4	8/2	7/3
Recipient Age*	12 ± 5 (14; 10-19)	16 ± 3 (16; 10-19)	12 ± 6 (9; 8-18)	11 ± 6 (11; 3-17)	13.5 ± 7 (18; 1-20)
Living/Deceased	3/7	5/5	1/2	3/7	6/4
Donor Gender (M/F)	5/5	6/4	4/6	6/4	7/3
Donor Age*	28 ± 8 (29; 17-37)	28 ± 10 (27; 14-47)	24 ± 8 (25; 16-31)	28 ± 10 (28; 17-37)	29 ± 9 (30; 16-47)

Verification (n=71) (71 transplantation sera)

Number of Patients	16	15	17	10	13
Steroid-free/Steroid-based	9/7	9/6	8/9	5/5	9/4
Recipient Gender	12/4	10/5	6/11	5/5	8/5
Recipient Age*	12 ± 5 (14; 3-19)	13 ± 7 (16; 1-20)	8 ± 6 (7; 1-17)	13 ± 5 (15; 4-20)	11 ± 8 (15; 1-19)
Living/Deceased	13/3	2/13	10/7	6/4	11/2
Donor Gender	8/8	8/7	11/6	7/3	10/3
Donor Age*	24 ± 5 (14; 5-38)	23 ± 6 (23; 14-36)	30 ± 10 (28; 14-44)	35 ± 5 (37; 29-44)	27 ± 16 (29; 1-49)

Heart transplantation sera (n=29)
Lung transplantation sera (n=27)

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Work Flow

Sera Retrieval

⇩

Immunodepletion

To deplete 20 highly abundant proteins from serum

⇩

Protein Assay

⇩

Trypsin Digestion

⇩

MS MS/MS Analysis

Data Analysis

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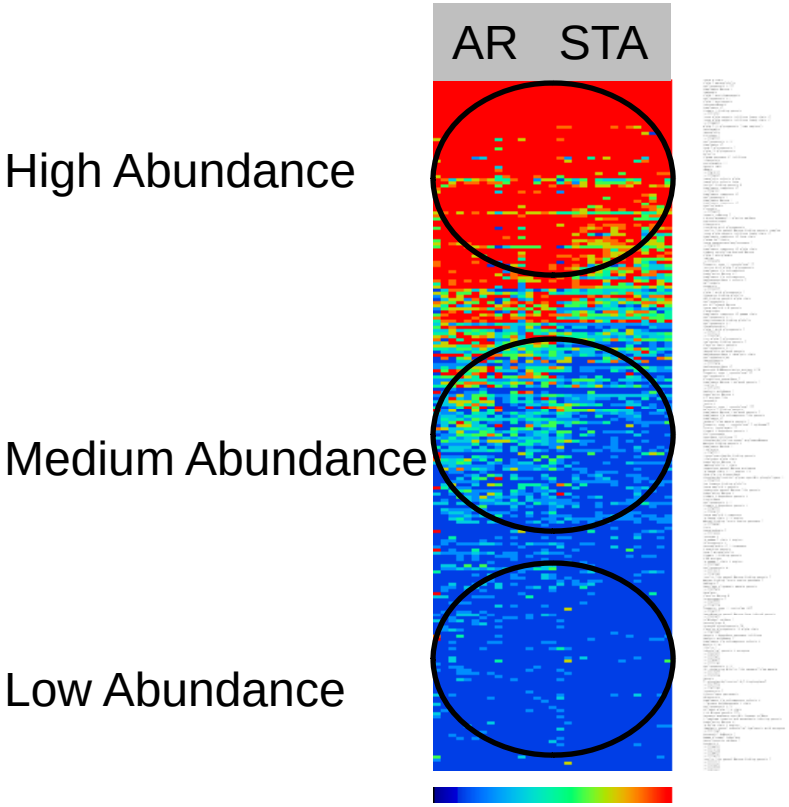
There is slightly increased protein presence in AR Overall



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Antibody Biomarkers in the Blood

High-density Protein Arrays

"Antibiomics"

PNAS

Identifying compartment-specific non-HLA targets after renal transplantation by integrating transcriptome and "antibodyome" measures

Li Li[a], Persis Wadia[b], Rong Chen[c], Neeraja Kambham[d], Maarten Naesens[a], Tara K. Sigdel[a], David B. Miklos[b], Minnie M. Sarwal[a,1], and Atul J. Butte[a,c,1]

[a]Department of Pediatrics, Blood and Marrow Transplantation Division, Departments of [b]Medicine and [d]Pathology, and [c]Center for Biomedical Informatics Research, Stanford University, 300 Pasteur Drive, Stanford, CA 94304

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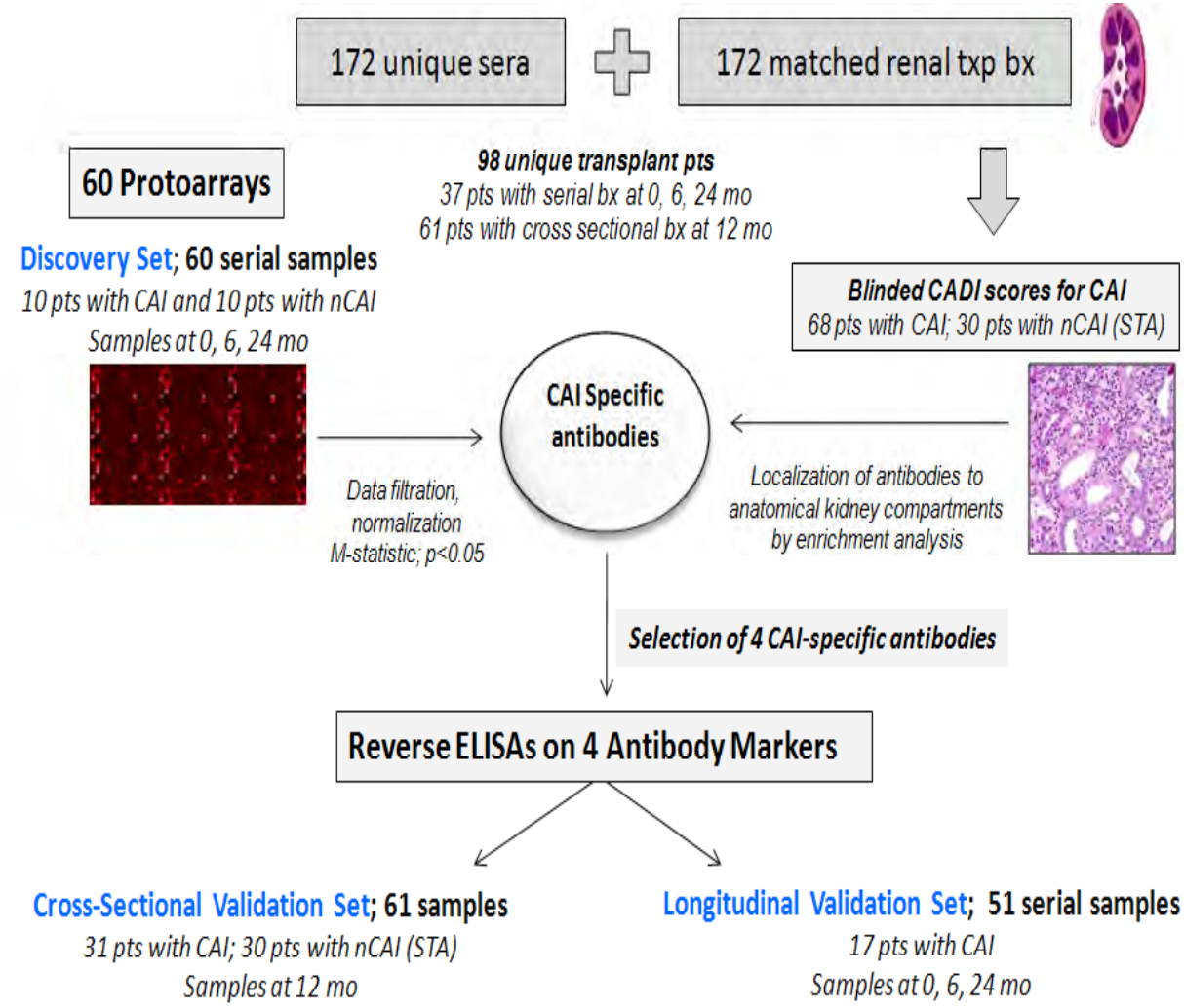
Immune Response Markers for Chronic Allograft Injury



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Conclusion

- There is a dire need of more sensitive and specific biomarkers
- Carefully designed proteomics studies on appropriate samples could provide potential biomarkers for diagnosis and monitoring of transplant dysfunction
- Our effort in this field has yielded a number of potential biomarker proteins and peptides that could provide more specific and sensitive biomarkers that can be used in clinical setting

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