

Biomarkers for Early Chronic Pancreatitis

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Darwin Conwell, Hanno Steen**

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**Boston
Children's
Hospital**

Pathology



THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER



HARVARD MEDICAL SCHOOL

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\$\$\$

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Samples

University of Minnesota

Greg Beilman

Melena Bellin

Brigham and Women's Hospital/

Harvard Medical School

Peter Banks (BWH)

Darwin Conwell (then BWH)

Disclosures

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None is related to any of the presented data.

The Idea

Identifying Body Fluid-based Biomarker for CP

Using for Proteomics for it.

- ☐ Pancreatic Fluid
- ☐ Blood
- ☐ Urine

The Idea

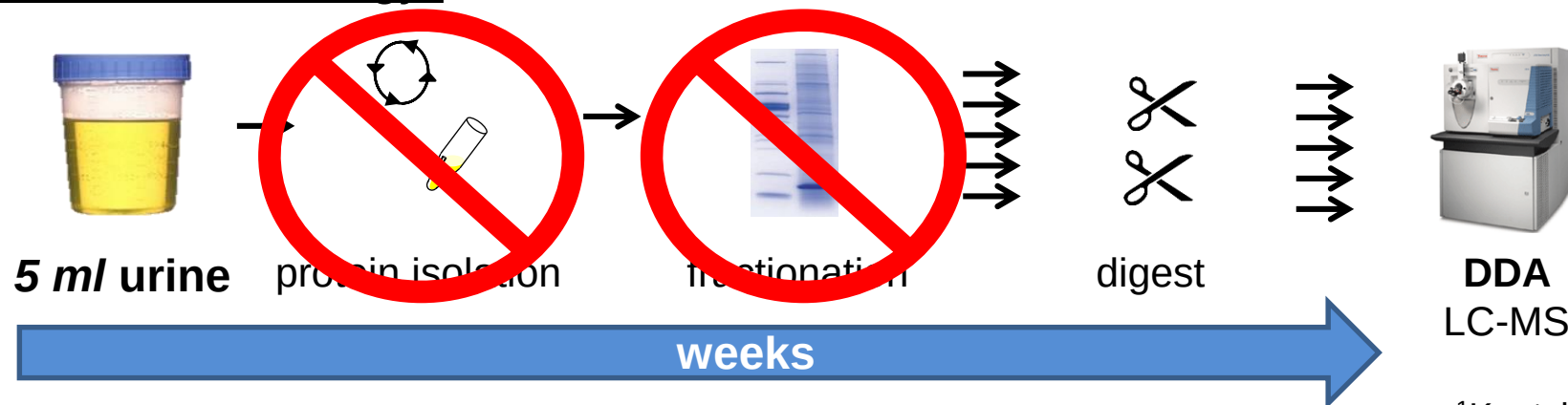
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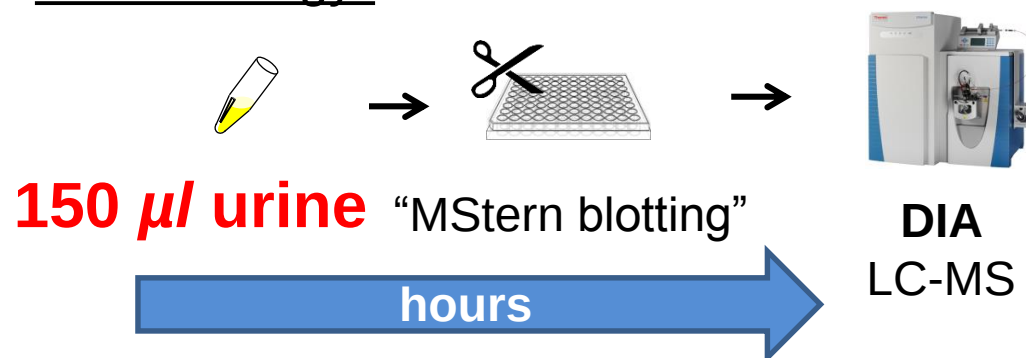
Our Urine Proteomics Platform

Conventional Strategy¹



¹Kentsis et al. *Proteomics Clin Appl.* 2009

New Strategy²



Reduced sample needs:
3% of the original volume!

Reduced processing time:
Complete processing in 8 hours!

²Berger et al. *Mol Cell Proteomics* 2015
Muntel et al. *J. Proteome Res.* 2015

Our (Legacy) Cohorts

Legacy Urine Samples (BWH, Dr. D. Conwell):

Green 1/Healthy Controls:	11 (5 male, 6 female)
Green 2/Symptomatic Controls:	11 (5 male, 6 female)
Yellow/Equivocal-Mild:	19 (14 male, 5 female)
Red/Moderate-Severe:	60 (37 male, 23 female)

Paired Pre-/Post-TPIAT Urine Samples (UMN, Drs. M. Bellin & G. Beilman):
22 (5 male, 17 female)

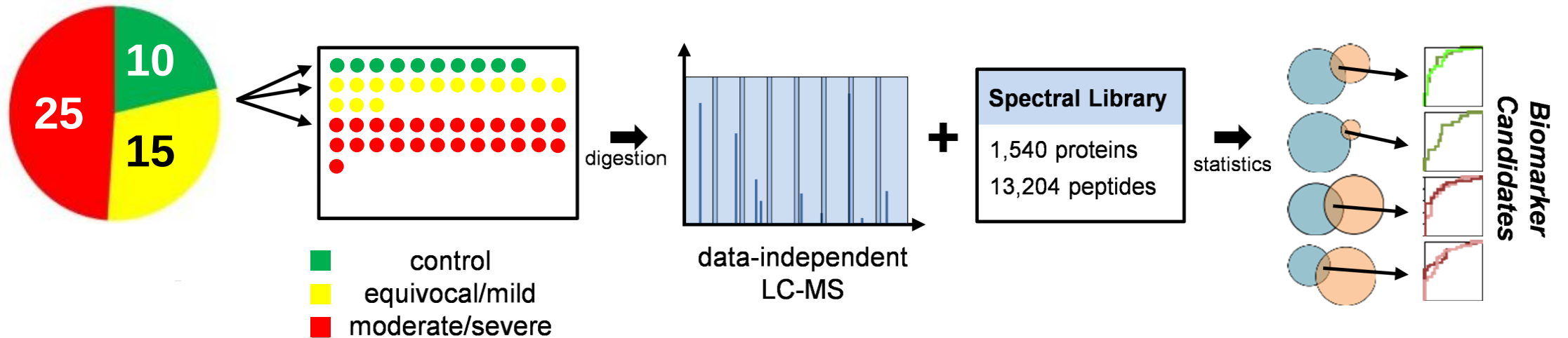
The Aims

- Urinary proteins different between healthy and moderate/severe CP.
- Pancreas dependent changes in the urinary proteome.
- Proteins correlating with peak bicarbonate levels in pancreatic fluid.

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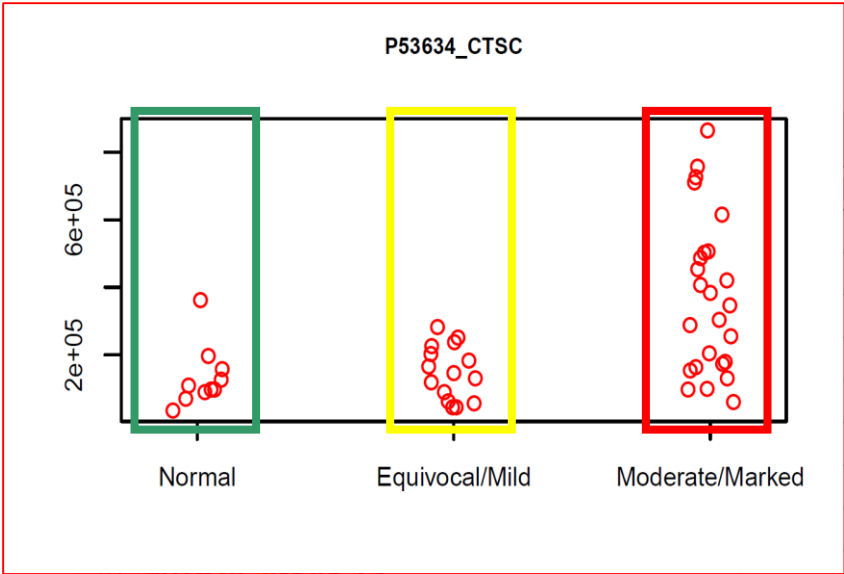
CP-dependent Changes in the Urinary Proteome



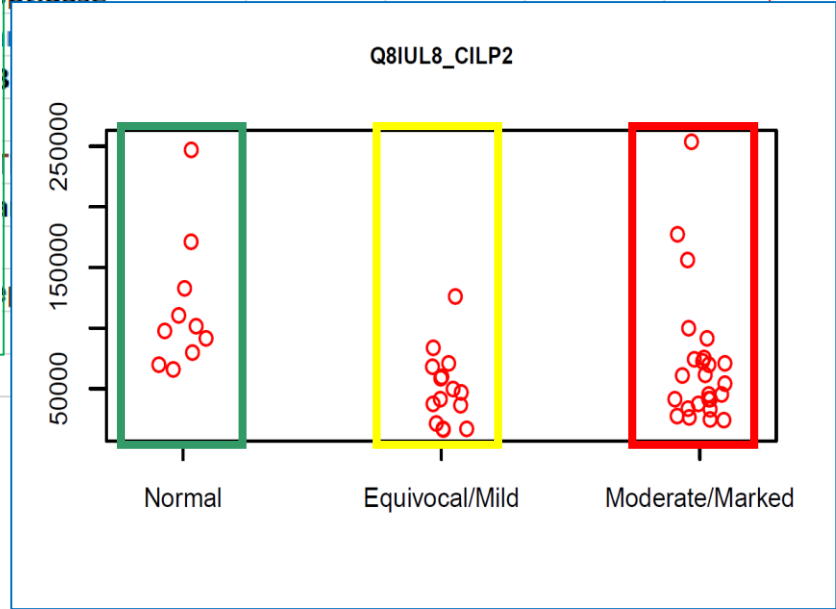
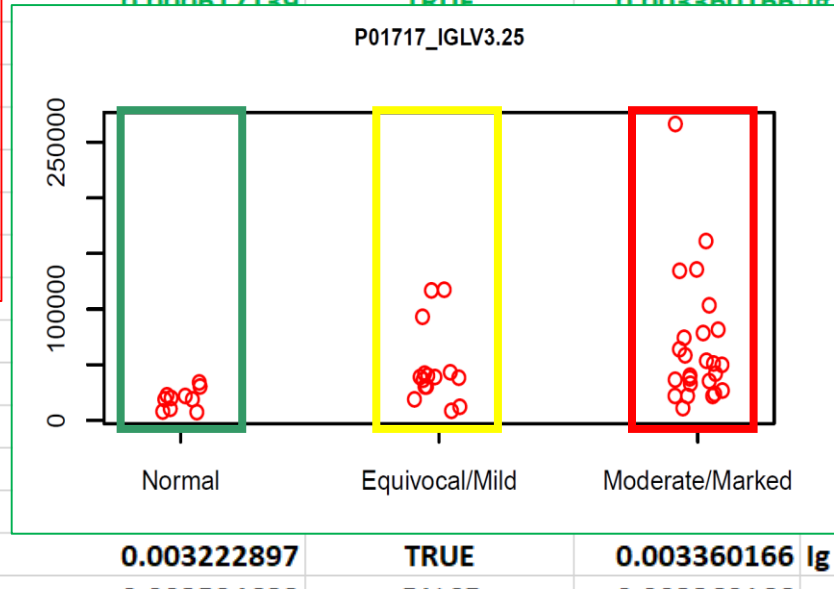
CP-dependent Changes in the Urinary Proteome

	A	B	C	E	G	H	I	J	K	L
1	Protein ID	Kruskal-Wallis Test P-value	Flagged at FDR 20%	20% FDR cutoff						
2	P53634_CTSC	0.000359456	TRUE	0.003360166	peptidase					
3	P01717_IGLV3.25	0.000617139	TRUE	0.003360166	Ig					
4	P0DOY2_IGLC2	0.00077869	TRUE	0.003360166	Ig					
5	A0A087WSY6_IGKV3D.15	0.000838531	TRUE	0.003360166	Ig					
6	Q07075_ENPEP	0.001021857	TRUE	0.003360166	peptidase					
7	Q9UHL4_DPP7	0.001075337	TRUE	0.003360166	peptidase					
8	Q8IUL8_CILP2	0.001445418	TRUE	0.003360166	Cartilage intermediate layer protein 2					
9	Q8TED0_UTP15	0.001524791	TRUE	0.003360166	U3 small nucleolar RNA-associated protein 15 homolog					
10	P01624_IGKV3.15	0.001559063	TRUE	0.003360166	Ig					
11	Q6PL18_ATAD2	0.002111226	TRUE	0.003360166	ATPase family AAA domain-containing protein 2					
12	P17900_GM2A	0.002115702	TRUE	0.003360166	Ganglioside GM2 activator					
13	A0A0C4DH25_IGKV3D.20	0.002475682	TRUE	0.003360166	Ig					
14	P12955_PEPD	0.002826795	TRUE	0.003360166	peptidase					
15	P06312_IGKV4.1	0.002961142	TRUE	0.003360166	Ig					
16	P0DOX7_	0.003222897	TRUE	0.003360166	Ig					

CP-dependent Changes in the Urinary Proteome



B	C	E	G	H	I	J	K	L
Wallis Test P-value	Flagged at FDR 20% 20% FDR cutoff							
0.000359456	TRUE	0.003360166	peptidase					
0.000617139	TRUE	0.003360166	peptidase					



What are the Relevant Comparisons?

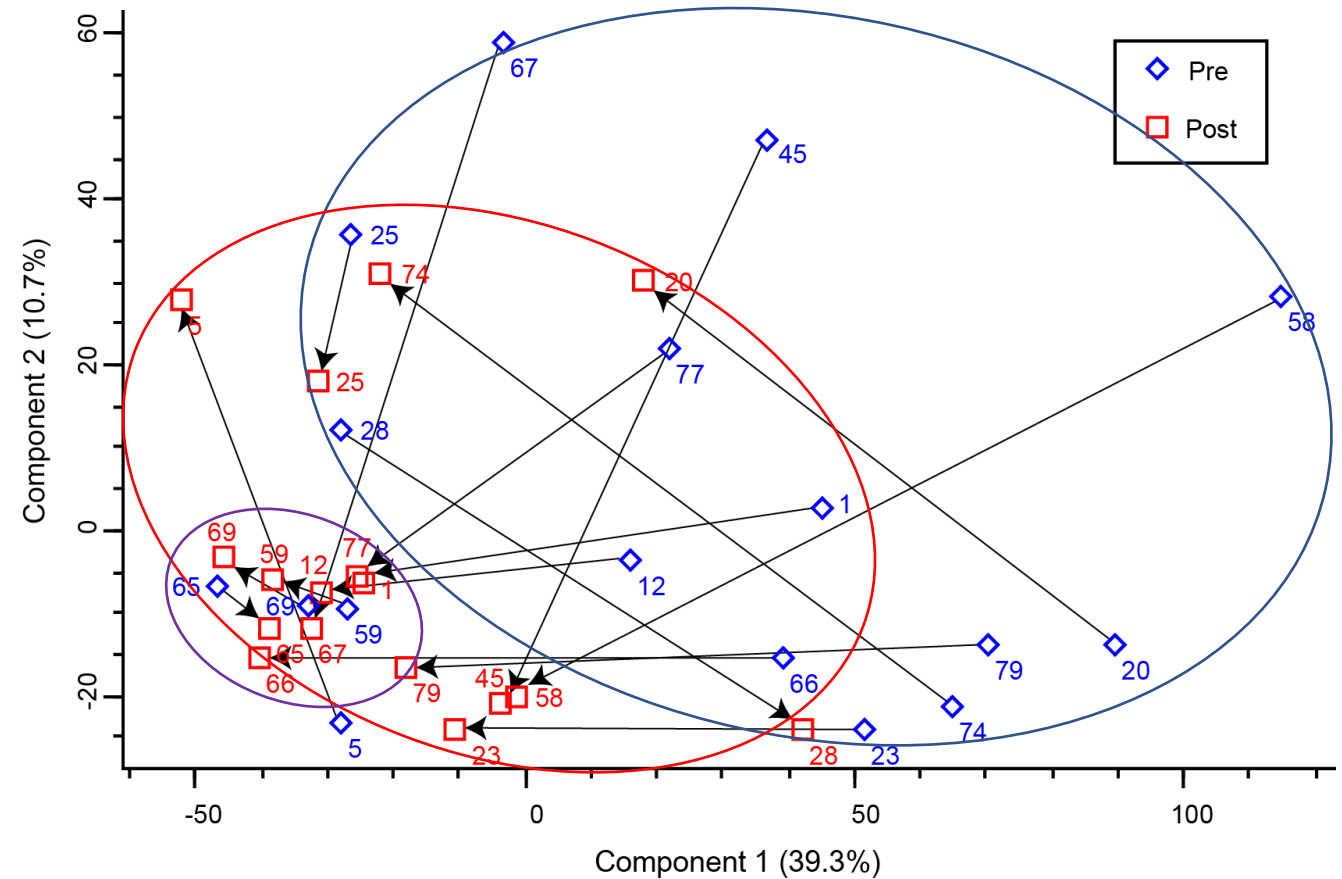
- **N vs. M:** this would define the 'baseline'/starting point of any biomarker analysis.
- **N vs. (E+M):** this would be equivalent to a screening test for the PCP.
- **N vs. E:** this would provide insights into potential biomarker candidates for the detection of early/mild CP.
- **E vs. M:** a test for the pancreatologist/GI specialist to stratify CP patients.

The Aims

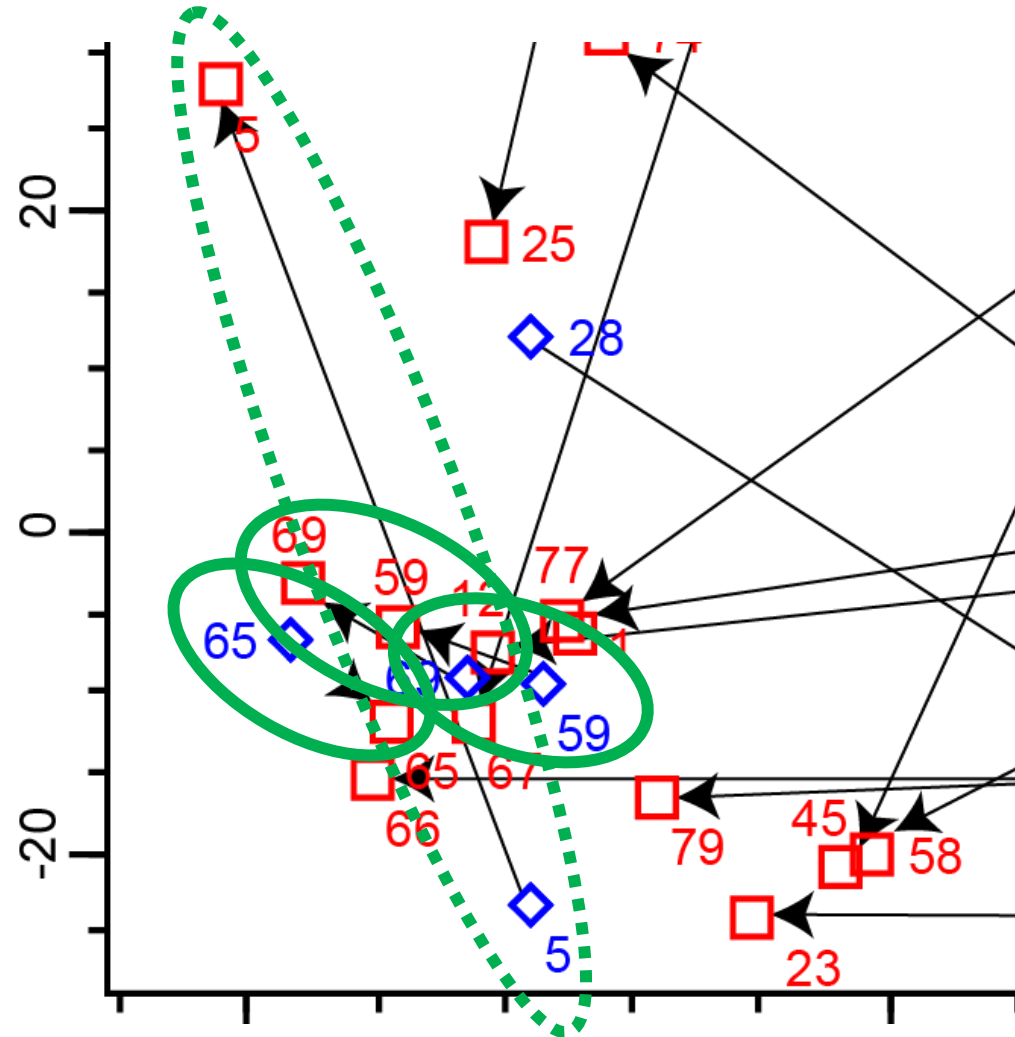
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Pancreas-Dependent Changes in the Urinary Proteome

22 Pre-/Post-TPIAT Sample Pairs
17 Pairs Met our Quality Standards



Pancreas-Dependent Changes in the Urinary Proteome



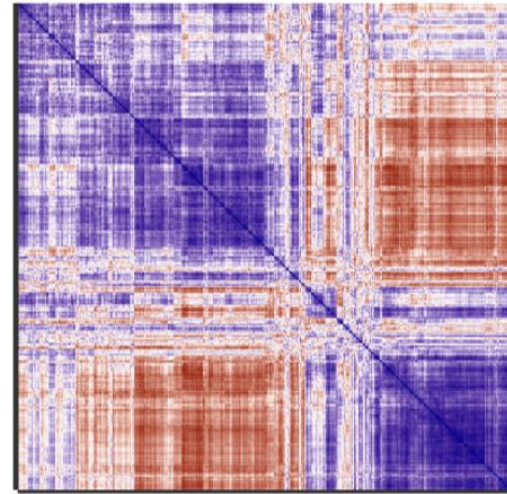
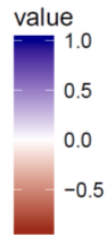
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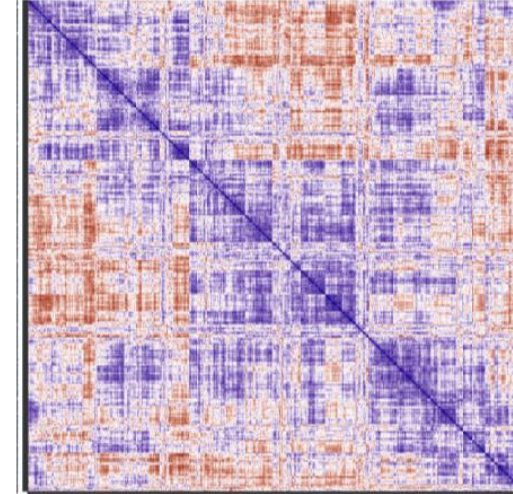


Pancreas-Dependent Changes in the Urinary Proteome

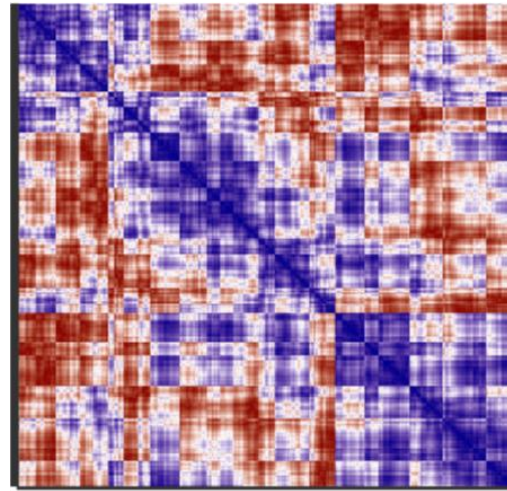
FEMALE:



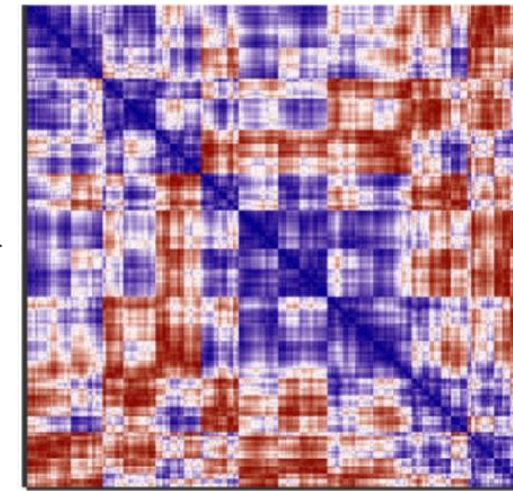
TPIAT



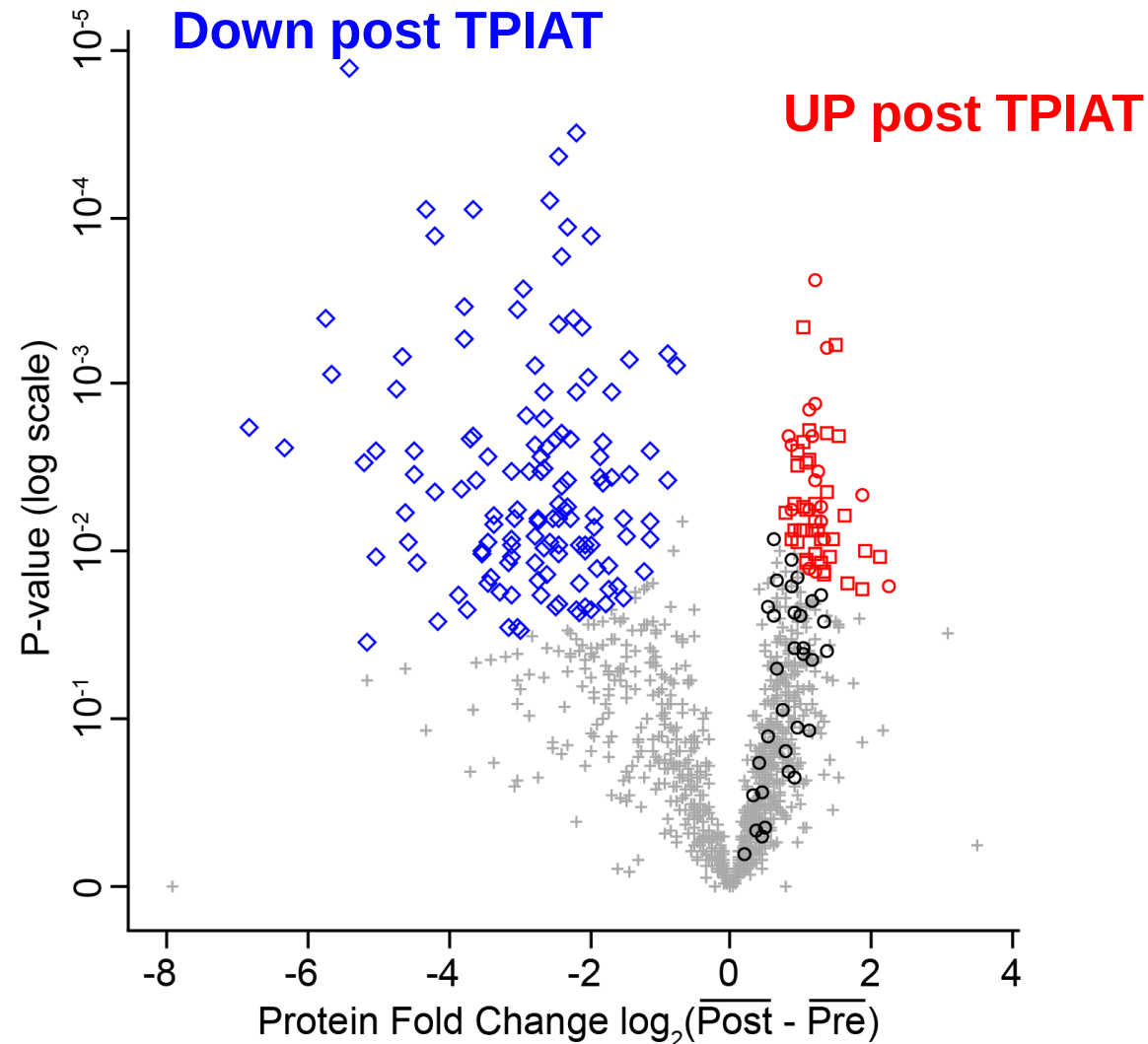
MALE:



TPIAT



Pancreas-Dependent Changes in the Urinary Proteome



***Paired two-samples t-tests,
permutation-based FDR
(q-value < 0.05):***

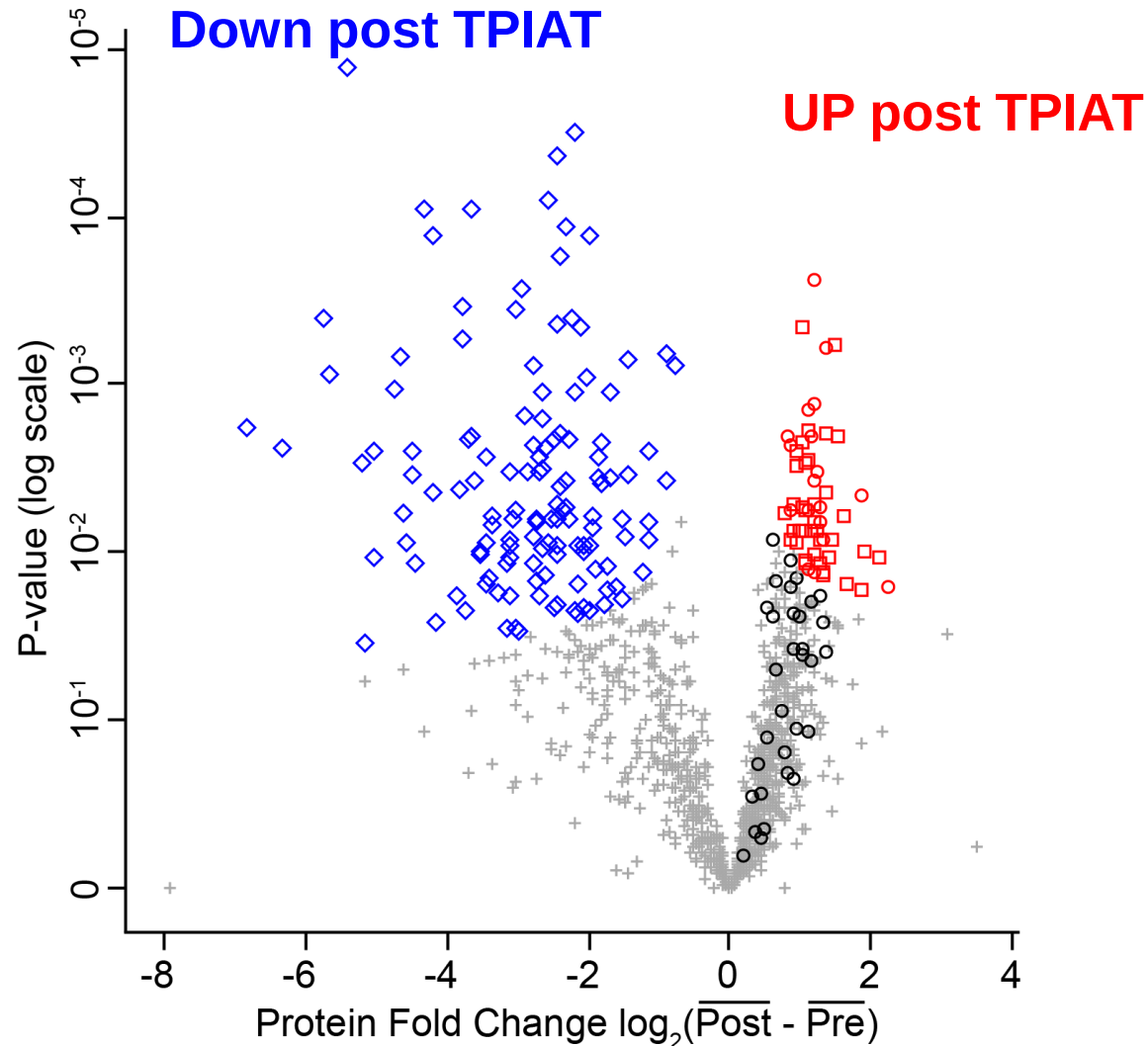
□ *more abundant post*

◇ *less abundant post*

+ *not significantly changed*



Pancreas-Dependent Changes in the Urinary Proteome

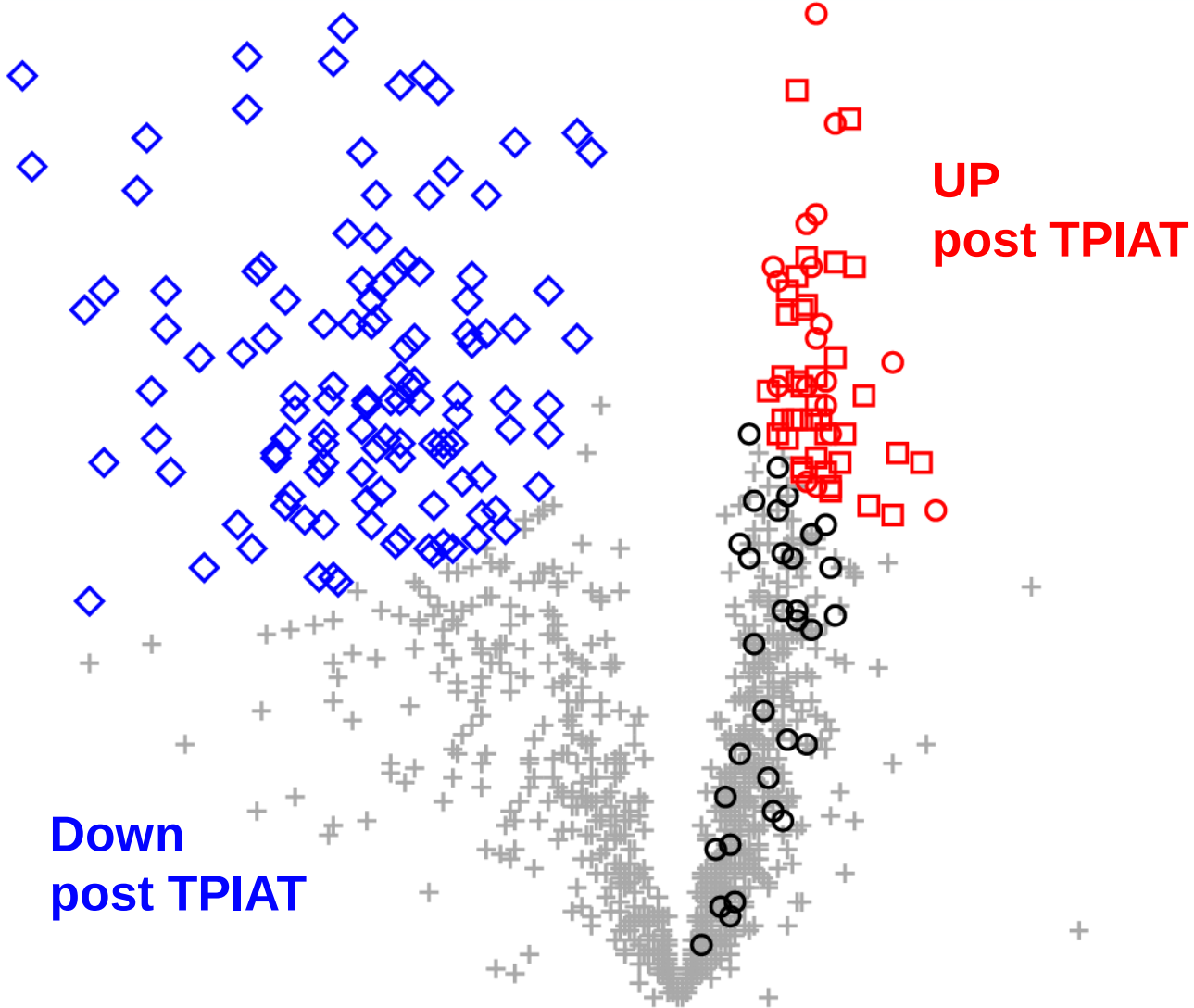


Conclusions:

Unexpected cross-talk between pancreas (function) and kidney function, which seems to be gender-dependent.



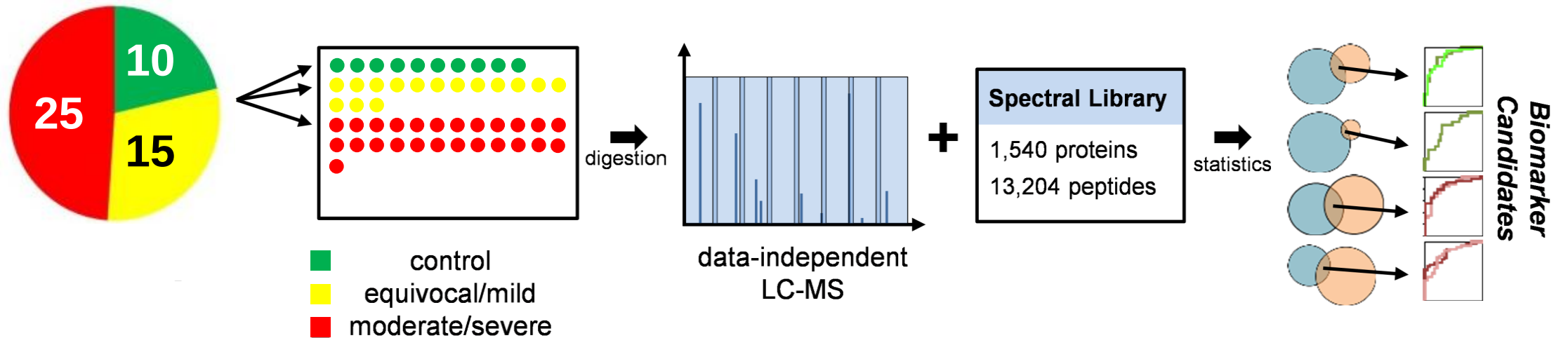
How Do We Know It Is Kidney Function?



***Paired two-samples t-tests,
permutation-based FDR
($q\text{-value} < 0.05$):***

- *more abundant post*
- ◇ *less abundant post*
- + *not significantly changed*
- *immunoglobulins*

CP-dependent Changes in the Urinary Proteome

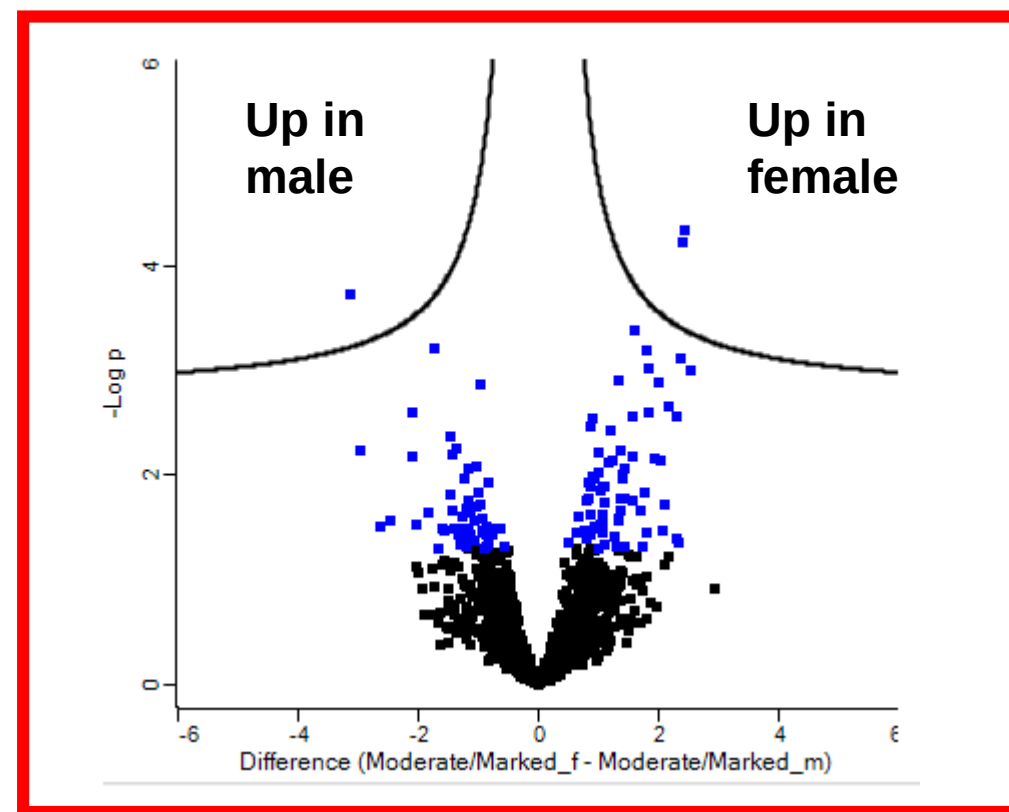
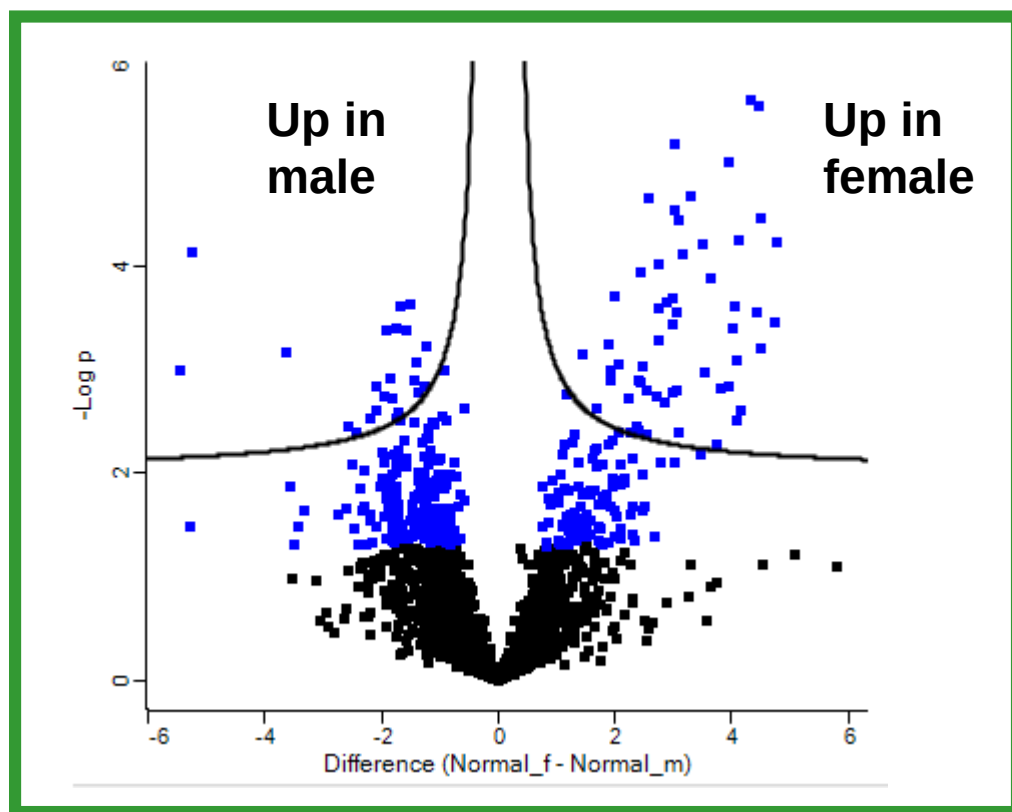


CP-dependent Changes in the Urinary Proteome

Females vs. Males in

Healthy Controls/Green 1

Moderate-Severe/Red

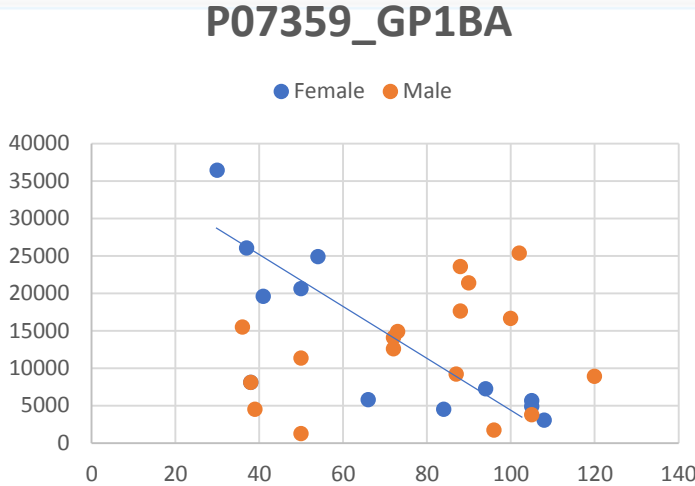
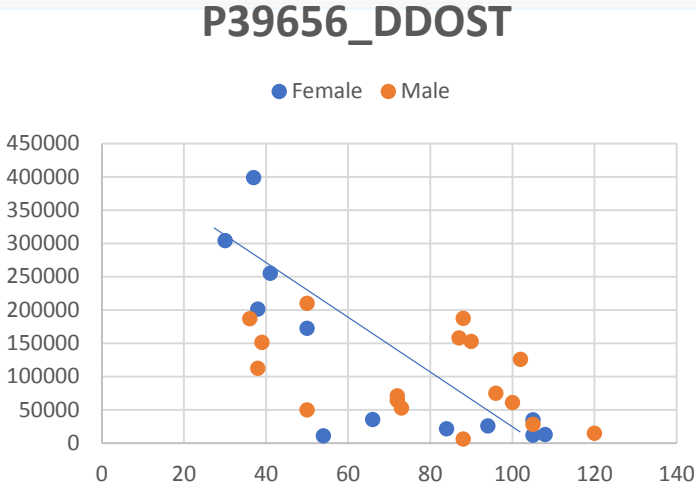


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Proteins Correlating with Peak Bicarbonate Levels in PF

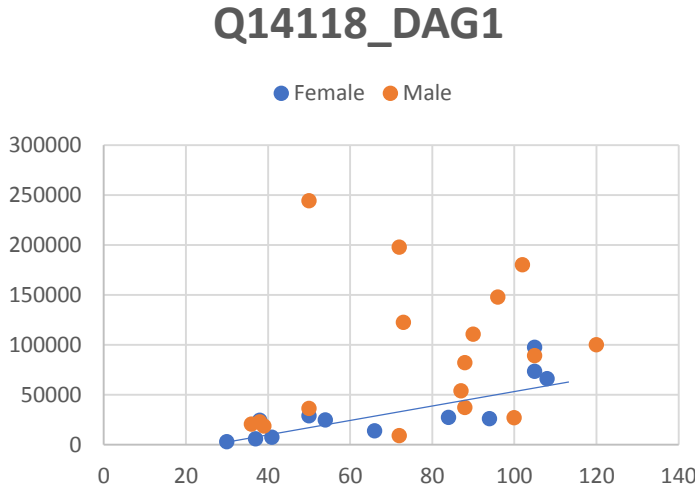
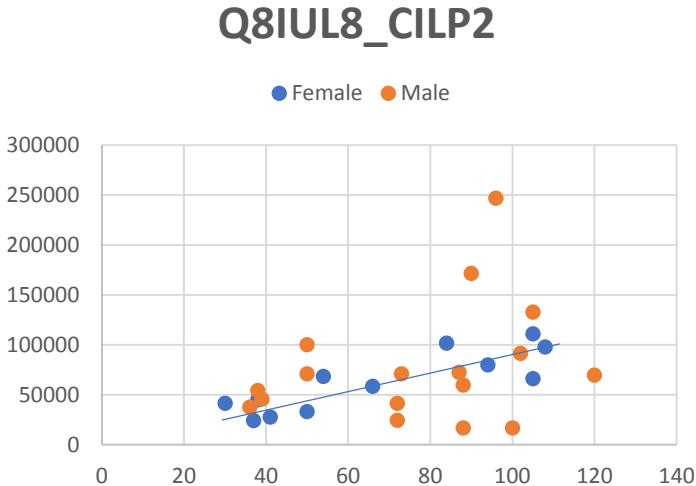
Highest negative correlation for females



Female

Protein	Correlation
P39656_DDOST	-0.81
P07359_GP1BA	-0.79
Q14118_DAG1	0.82
Q8IUL8_CILP2	0.85

Highest positive correlation for females



Male

Protein	Correlation
P04040_CAT	-0.69
Q6PL18_ATAD2	-0.69
P22891_PROZ	0.63
Q13822_ENPP2	0.64

Conclusion and Outlook

- CP-dependent changes in the urinary proteomes are observable.
- Unexpected sex-dependent crosstalk of pancreas (function) and kidney (function) – identified in 2 independent cohorts.
- Indication for pancreatic function dependent changes in urinary proteomes.
- Validation in well annotated independent cohort.
- Accounting for sex as biological variable when moving forward.
- PROCEED will be ideal for all follow-up and validation studies.