



Beth Israel Deaconess
Medical Center



A teaching hospital of
Harvard Medical School



Precision Medicine for Delirium

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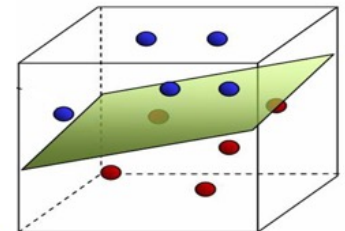
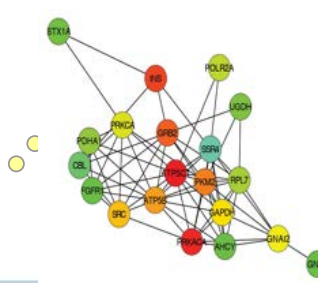
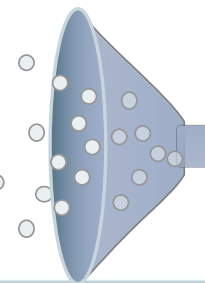
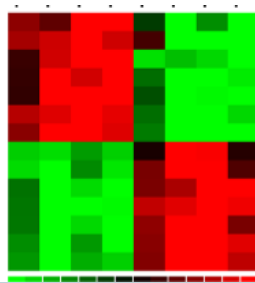
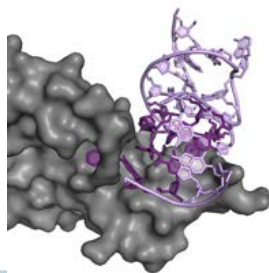
Center and

DF/HCC Proteomics Core

Div. of Interdisciplinary Medicine and Biotechnology

Beth Israel Deaconess Medical Center

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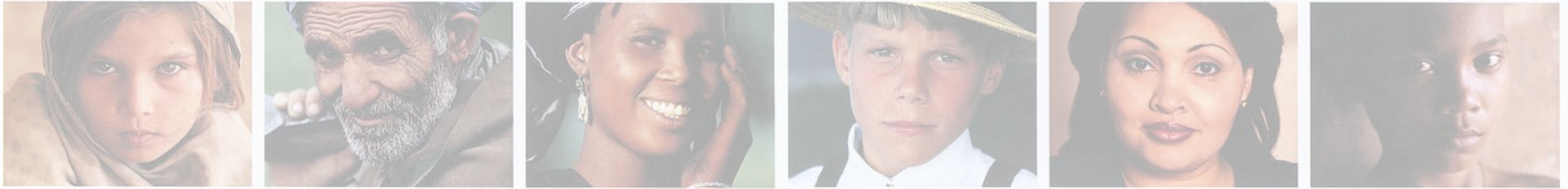
DF/HCC

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A large group of grey 3D human figures, resembling stylized people, are arranged in a dense cluster. In the center of this group, one figure is highlighted in red, standing out from the rest. The figures are rendered with soft shadows, giving them a three-dimensional appearance.

What is Precision Medicine?

Precision Medicine



The Promise: Advanced Molecular Diagnostic Will
Tailor Medical Management & Treatment Based on
the Individual Characteristics of Each Patient



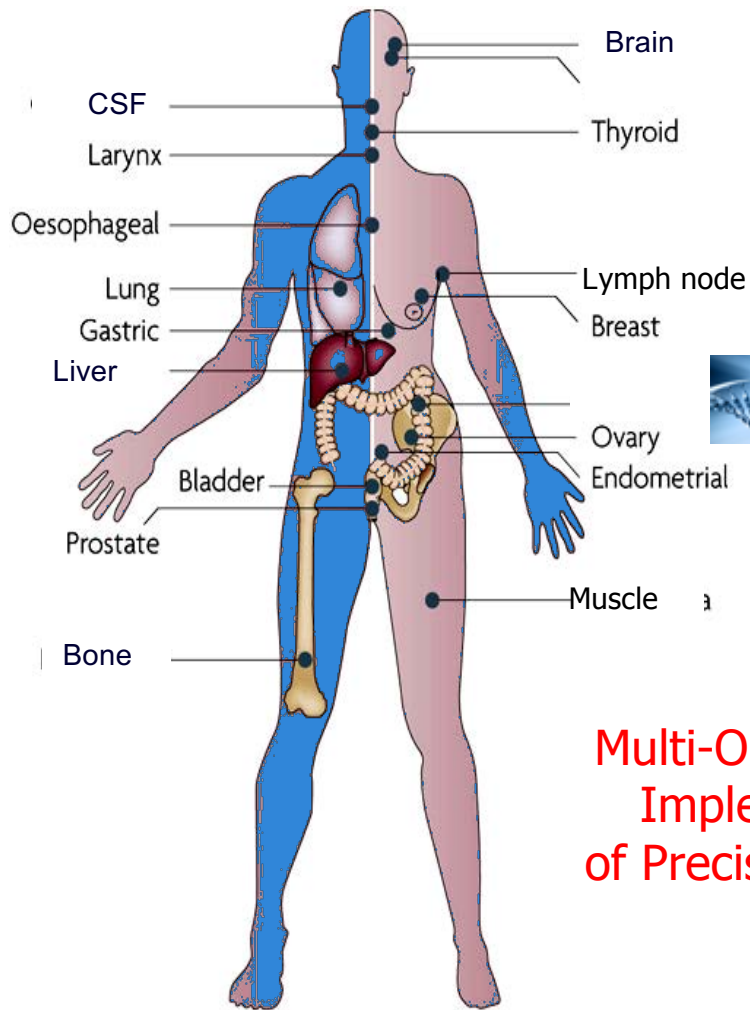
Optimized Therapeutic Benefit
Less Adverse Reactions



The end of “one-size-fits-all”?

New Disease Concepts Transform Medicine

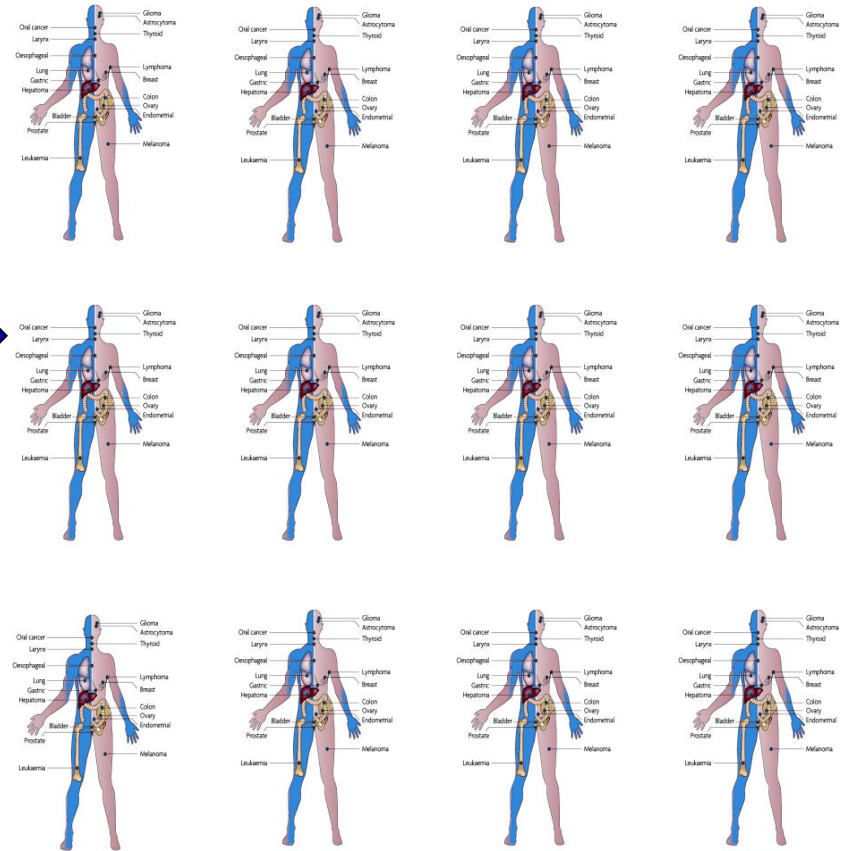
One Size Fits All



Multi-Omics enables
Implementation
of Precision Medicine

Disease-Centric

One Size Fits One



Patient-Centric

Exceptional Success When Treatment Matched to Driver Mutation

Sequencing enables patient-specific recommendation of targeted therapies with improved outcome

Without Precision Medicine
Some benefit, many do not

Lung Cancer Patients



Treatment with
EGFR Inhibitor

Sequencing



With Precision Medicine
Each patient receives right medicine



Normal EGFR
• No response



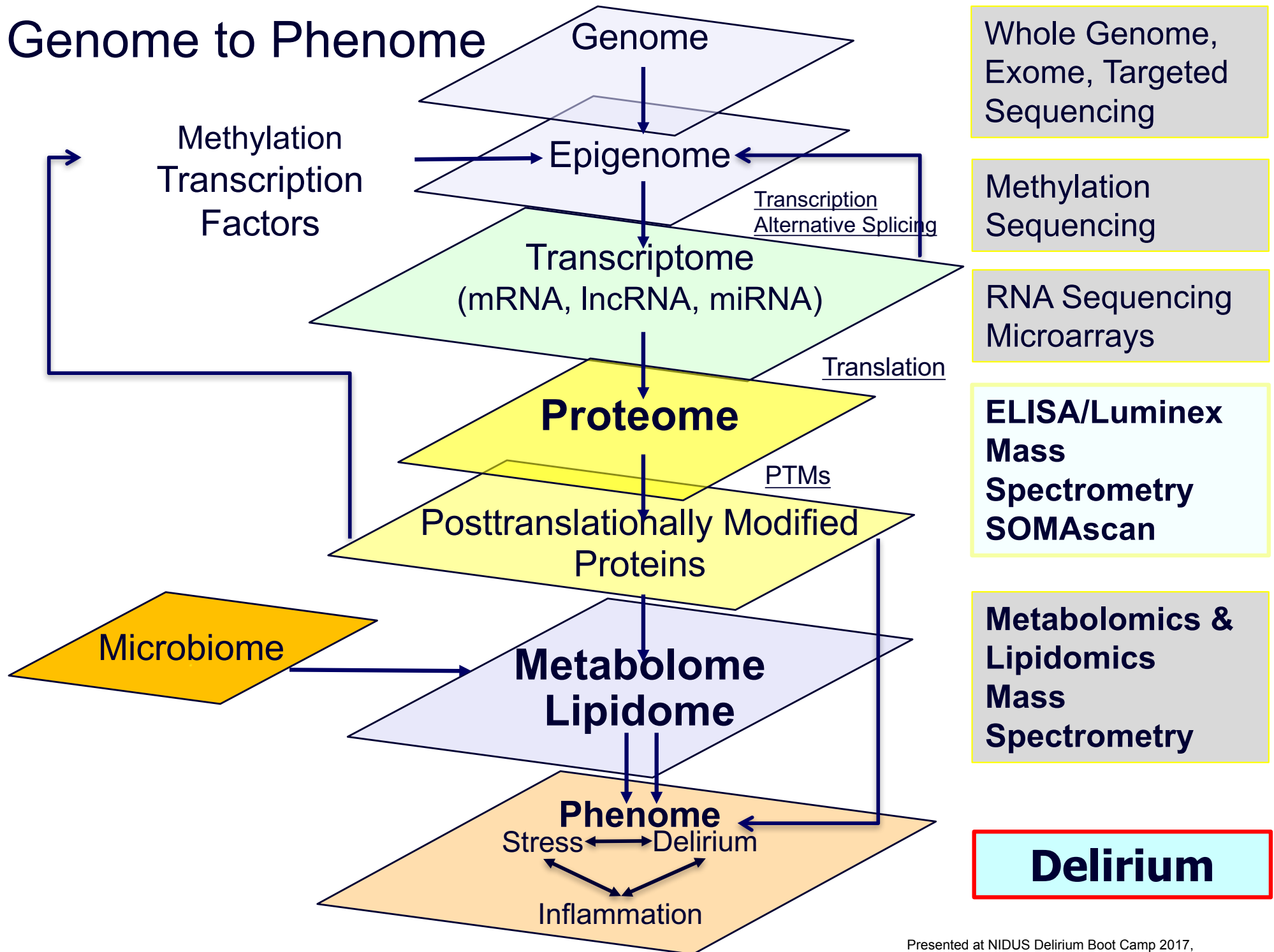
Mutant EGFR, Normal K-Ras/N-Ras
• Response



Mutant EGFR, Mutant K-Ras/N-Ras
• Shorter survival

Approaches for Biomarker Discovery and Precision Medicine for Delirium

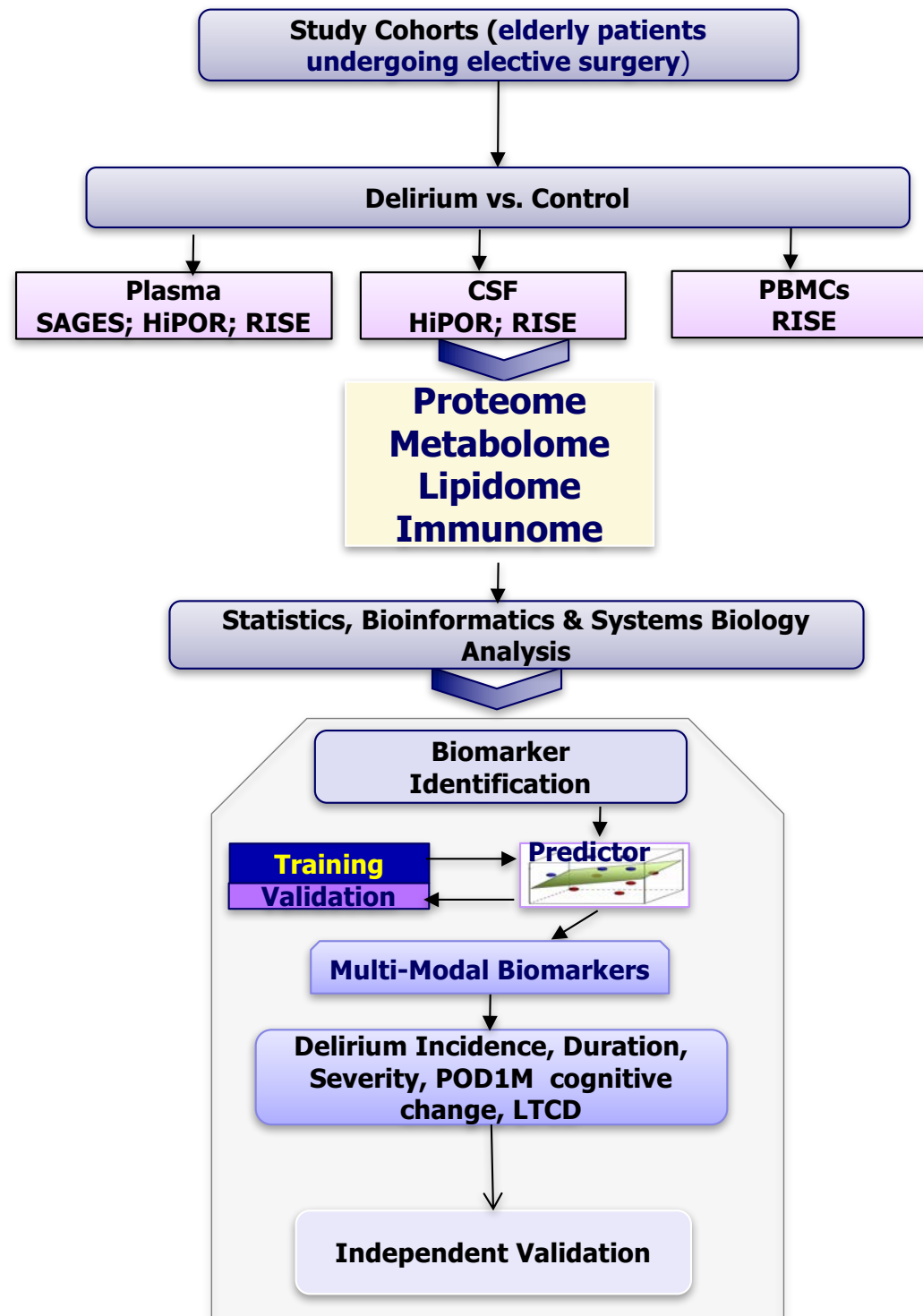
Genome to Phenome



SAGES Study Design

- **Plasma collection at 4 timepoints (before, during and after)**
 - pre-operation (PREOP)
 - post-operation (post-anesthesia care unit) (PACU)
 - post-operation day 2 (POD2)
 - post-operation day 30 (POD30)
- **Matched case:control design**
 - delirium versus no delirium
 - 6 matching factors
- **Carefully selected patient population (N =560; 24% delirium rate)**
 - dementia-free
 - ≥ 70 years
 - elective, non-cardiac surgery
- **Biomarkers to assess**
 - Risk
 - Guide diagnosis
 - Management
 - Pathogenesis
- **Objective:** Identify reliable blood-based postoperative delirium biomarkers, delirium pathophysiology & new therapeutic targets
- **Targeted and untargeted biomarker discovery & validation**

Overall Multi-Omics Approach for Biomarker Discovery for Delirium



What defines a good biomarker?

- Specificity to the disease
- Reliability
 - low false positive rate
 - low false negative rate
- Does it inform about the underlying biological processes involved?
 - Can we predict new therapeutic targets based on revealed pathophysiology?

Potential Uses for Delirium Biomarkers

- Risk predictor:
 - Measurable before delirium onset
 - Identifies individuals at risk
- Disease marker:
 - Changes (up or down) with delirium onset
 - Returns to pre-surgery levels with delirium resolution
- Prognostic marker:
 - Measurable before or after delirium onset
 - Alterations in measured level is proportional to long term “consequences”

Types of Molecules Used as Biomarkers

- Proteins/peptides
 - Post-translational modifications (PTMs)
- Metabolites
- Lipids
- Cells
- DNA sequence
 - Entire genome
 - Specific genes, SNPs

Protein Biomarkers for Delirium

Why use plasma?

- Minimally invasive
- Easily obtained
- Widely used clinically
- A source for good representation of proteins released from many tissues in the body
- Plasma, serum, and urine are being used in the diagnosis of many diseases
- Opportunities for home diagnostics



But: CSF may be more informative

Targeted Proteomics



Research Article

SAGES study paper

Cytokines and Postoperative Delirium in Older Patients Undergoing Major Elective Surgery

Sarinnapha M. Vasunilashorn,^{1,2,3*} Long Ngo,^{1,3*} Sharon K. Inouye,^{1,2,3}
Towia A. Libermann,^{1,3} Richard N. Jones,^{2,5} David C. Alsop,^{2,4}
Jamey Guess,³ Sandra Jastrzebski,⁷ Janet E. McElhaney,⁸ George
A. Kuchel,^{7**} and Edward R. Marcantonio^{1,2,3**}

¹Harvard Medical School, Boston, Massachusetts. ²Aging Brain Center, Institute for Aging Research, Hebrew SeniorLife, Boston, Massachusetts. ³Department of Medicine, and ⁴Department of Radiology, Beth Israel Deaconess Medical Center, Boston, Massachusetts. ⁵Department of Psychiatry and Human Behavior, Warren Alpert Medical School, Brown University, Providence, Rhode Island. ⁷UConn Center on Aging, University of Connecticut Health Center, Farmington. ⁸Advanced Medical Research Institute of Canada, Sudbury, Ontario, Canada.

Patient Characteristics In 2 Matched Cohorts (Discovery And Replication) And Pooled Cohort used for Biomarker Verification

Pooled Cohort (75 Pairs)

Variable	Discovery (39 pairs)		Replication (36 pairs)	
	Delirium (n=39)	No Delirium (n=39)	Delirium (n=36)	No Delirium (n=36)
Age (M, SD)	77.3 (5.0)	76.8 (4.7)	78.0 (4.4)	77.6 (4.2)
Female (%)	54	54	58	58
GCP (M, SD)	55.2 (5.6)	56.4 (5.6)	53.7 (5.0)	54.6 (5.1)
Type of surgery (%)				
Orthopedic	92	92	83	83
Vascular	5	5	6	6
Gastrointestinal	3	3	11	11
Vascular comorbidity (%)	38	38	50	50
ApoE ε4 carrier (%)	13	13	28	28

GCP=general cognitive performance, a composite measure of neuropsychological measures reflecting cognitive domains vulnerable to delirium.

ApoE= presence of an ApoE ε4 allele (i.e., ApoE ε carrier) has been associated with increased risk of Alzheimer's Disease.

Vascular comorbidity: present if patient had a myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, hemiplegia, diabetes, and diabetes with end organ damage.

Luminex Analysis of 12 Cytokines in Plasma

Median paired difference between delirium and matched control

Cytokine (pg/mL)	Pooled Cohort			
	PREOP	PACU	POD2	POD1M
IL-1 β	0.26	0.28	0.31	0.27
IL-2	0.99*	0.77*	1.07**	0.73*
IL-4	7.13	0.54	-1.56	-2.32
IL-5	0.19	0.19	-0.52	0.57
IL-6	1.01	7.17*	39.35**	0.49
IL-8	0.86	0.68	0.89	-0.18
IL-10	0.00	0.10	0.27	-0.11
IL-12	-2.64	-1.73	-2.88	-4.24*
IFN- γ	0.00	0.00	0.00	0.03
GMCSF	-0.58	-0.49	-0.45	-0.22
TNF- α	2.12	2.52	3.22	3.10*
VEGF	3.50	-0.34	4.10*	0.83

* $p < .05$; ** $p < .01$.

Untargeted Proteomics



SAGES study paper

Higher C-Reactive Protein Levels Predict Postoperative Delirium in Older Patients Undergoing Major Elective Surgery: A Longitudinal Nested Case-Control Study

Simon T. Dillon, Sarinnapha M. Vasunilashorn, Long Ngo, Hasan H. Otu, Sharon K. Inouye, Richard N. Jones, David C. Alsop, George A. Kuchel, Eran D. Metzger, Steven E. Arnold, Edward R. Marcantonio, and Towia A. Libermann

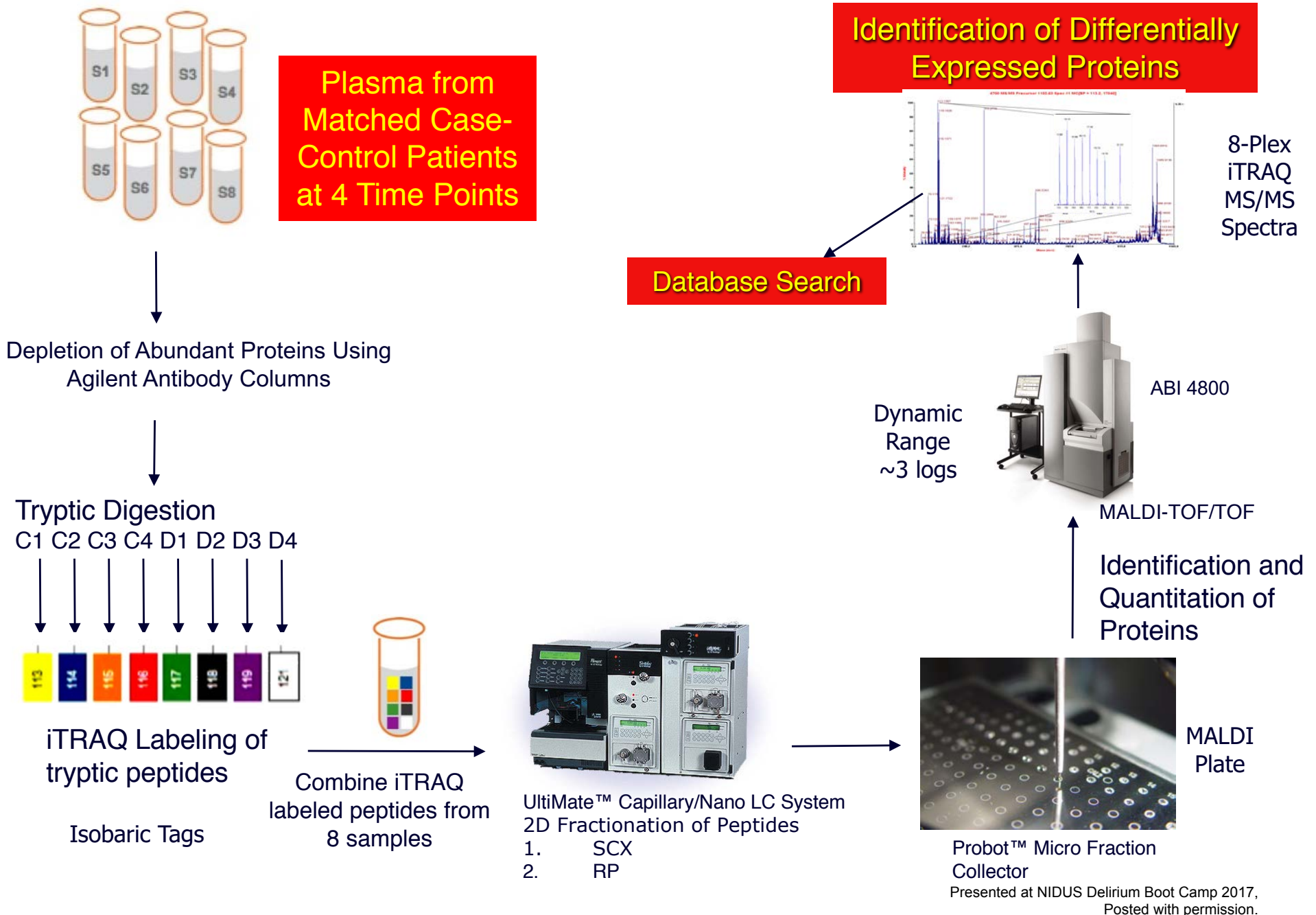
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Biological Psychiatry ■■■, 2016; ■■■–■■■ www.sobp.org/journal

<http://dx.doi.org/10.1016/j.biopsych.2016.03.2098>

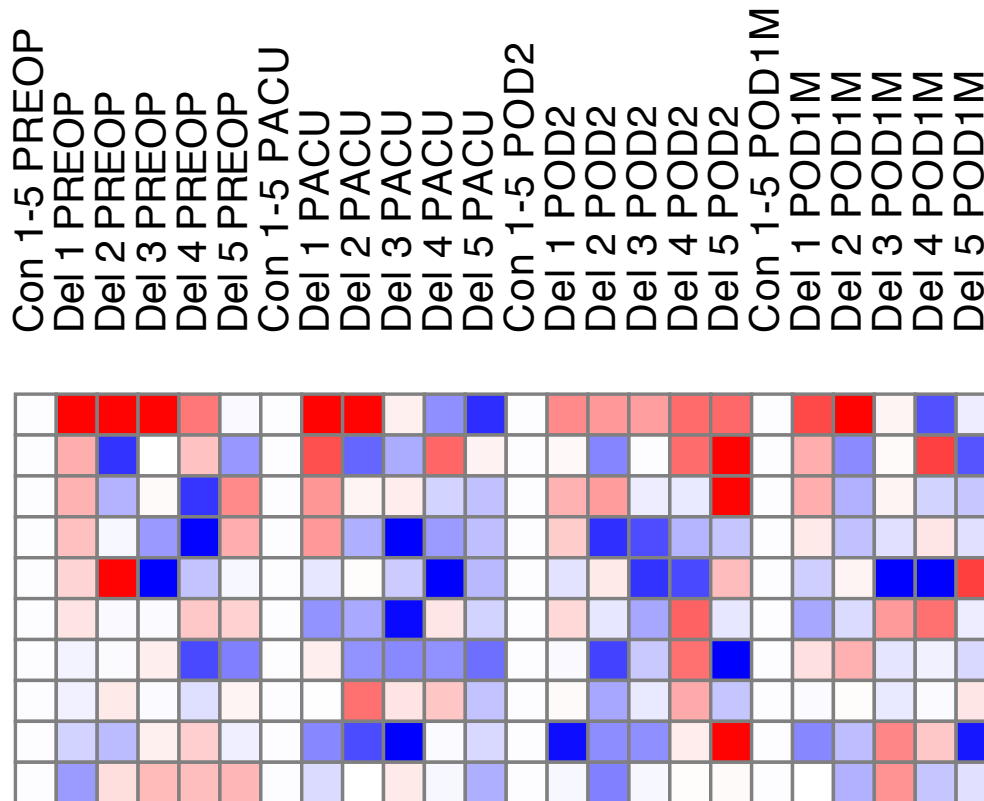
Global Proteomics using Mass Spectrometry

Quantitative Shotgun Proteomics for Unbiased Biomarker Discovery




Biomarker Discovery Phase

iTRAQ Quantitative Mass Spectrometry Identifies Consistently Higher Levels of CRP in Patients who Develop Delirium



Annotation

C-reactive protein (CRP) 
Heparin cofactor 2 (SERPIND1)
Pigment epithelium-derived factor (SERPINF1)
Coagulation factor XII (F12)
Serum amyloid P-component (APCS)
Tetranectin (CLEC3B)
Extracellular matrix protein 1 (ECM1)
CD44 antigen (CD44)
Gelsolin (GSN)
Glutathione peroxidase 3 (GPX3)

Heat map of iTRAQ relative quantitation for 10 proteins in 5 matched case-control samples across four timepoints (PREOP, PACU, POD2 and POD1M)

Biomarker Verification Phase

ELISA of CRP in Whole Matched Case-Control Cohort Confirms Statistically Significant Higher CRP Levels in Patients with Delirium

Time-specific median of paired differences (MPD) of ELISA CRP concentrations between delirium cases and no-delirium controls at 4 timepoints in the discovery, replication, and pooled cohorts

Time of Blood Draw	Discovery (39 pairs)			Replication (36 pairs)			Pooled (75 pairs)		
	MPD (mg/L)	IQ range	P-value	MPD (mg/L)	IQ range	P-value	MPD (mg/L)	IQ range	P-value
PREOP	1.97	(-1.02, 7.75)	0.02	0.29	(-1.68, 9.59)	0.13	0.56	(-1.61, 7.89)	<0.01
PACU	2.83	(-2.29, 10.68)	0.06	2.22	(-0.91, 7.68)	0.01	2.53	(-1.57, 10.33)	<0.01
POD2	71.97	(5.05, 139.82)	<0.01	35.18	(-30.42, 88.90)	0.04	63.76	(-22.29, 126.17)	<0.01
POD1M	2.72	(-1.85, 7.16)	0.06	-0.66	(-3.83, 2.49)	0.63	1.1	(-3.17, 5.45)	0.18



MPD=Median of paired differences (delirium case minus no-delirium control)

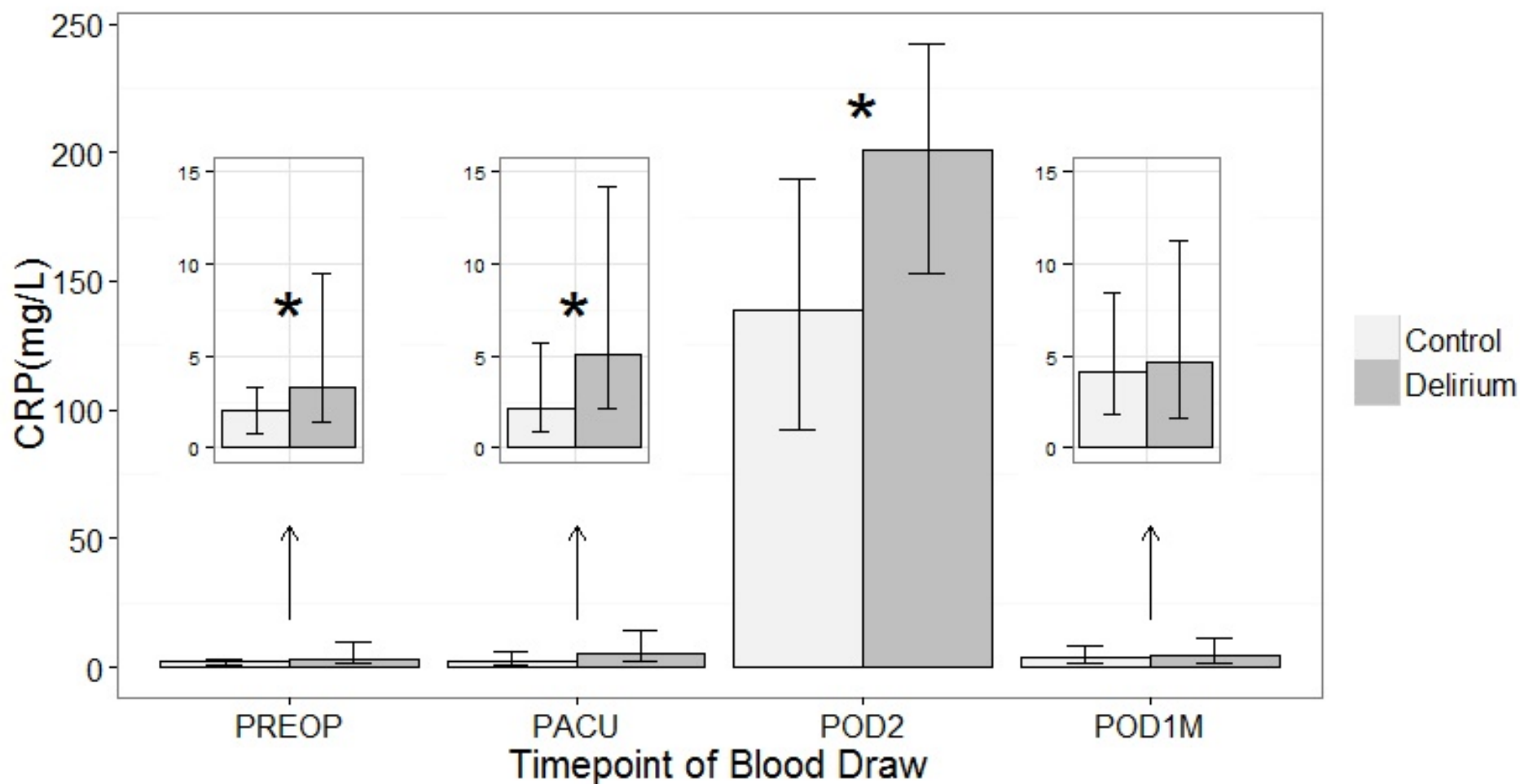
ELISA=enzyme-linked immunosorbent assay

IQ=interquartile

PREOP= preoperative, PACU= postanesthesia care unit, POD2=postoperative day 2, POD1M=30 days postoperation

p-values obtained from nonparametric signed-rank test. Bold indicates significant at p<.05 level

Median CRP Concentrations by Delirium Status at 4 Timepoints in Pooled Cohort (75 Matched Pairs)



CLINICAL INVESTIGATION

High C-Reactive Protein Predicts Delirium Incidence, Duration, and Feature Severity After Major Noncardiac Surgery

Sarinnapha M. Vasunilashorn, PhD,^{a,b,c} Simon T. Dillon, PhD,^{b,d} Sharon K. Inouye, MD, MPH,^{b,c,e}
Long H. Ngo, PhD,^{a,b} Tamara G. Fong, MD, PhD,^{c,f} Richard N. Jones, ScD,^{c,g}
Thomas G. Trivison, PhD,^{b,c,e,h} Eva M. Schmitt, PhD,^c David C. Alsop, PhD,^{b,i}
Steven D. Freedman, MD, PhD,^{b,j} Steven E. Arnold, MD,^{b,k} Eran D. Metzger, MD,^{b,c,l}
Towia A. Libermann, PhD,^{b,d,*} and Edward R. Marcantonio, MD, SM^{a,b,c,e,*}

JAGS 65:e109–e116, 2017

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CRP measure (mg/L)	Delirium Incidence RR (95% CI)	Delirium Duration (per day) ^a Days (95%CI)	Sum CAM-S (per point) ^a Score (95% CI)
CRP PREOP			
Quartiles			
Q1 (≤0.95)	Reference	Reference	Reference
Q2 (0.95-2.56)	1.4 (0.8-2.3)	0.3 (0.2-0.5) ^b	1.5 (0.9-2.1) ^c
Q3 (2.56-6.39)	1.7 (1.0b-2.7)	0.3 (0.2-0.5) ^b	2.5 (1.8-3.2) ^c
Q4 (≥6.39)	1.8 (1.2-2.9)	0.4 (0.2-0.5) ^b	3.6 (2.9-4.3) ^b
p-trend ^d	<.01	<.01	<.01
High-risk cutpoint ^e			
≥3 vs. <3	1.5 (1.1-2.1)	0.2 (0.1-0.4) ^b	2.6 (2.1-3.2) ^b
CRP POD2^f			
Quartiles			
Q1 (≤127.53)	Reference	Reference	Reference
Q2 (127.53-177.05)	1.1 (0.6-1.7)	0.1 (-0.1-0.2)	1.2 (0.6-1.8) ^c
Q3 (177.05-235.73)	1.5 (1.0 ^g -2.3)	0.2 (0.0 ^h -0.4) ^c	3.5 (2.9-4.2) ^b
Q4 (≥235.73)	1.5 (1.0 ⁱ -2.4)	0.2 (0.0 ^j -0.4) ^c	4.5 (3.8-5.2) ^b
p-trend ^d	0.02	0.02	<.01

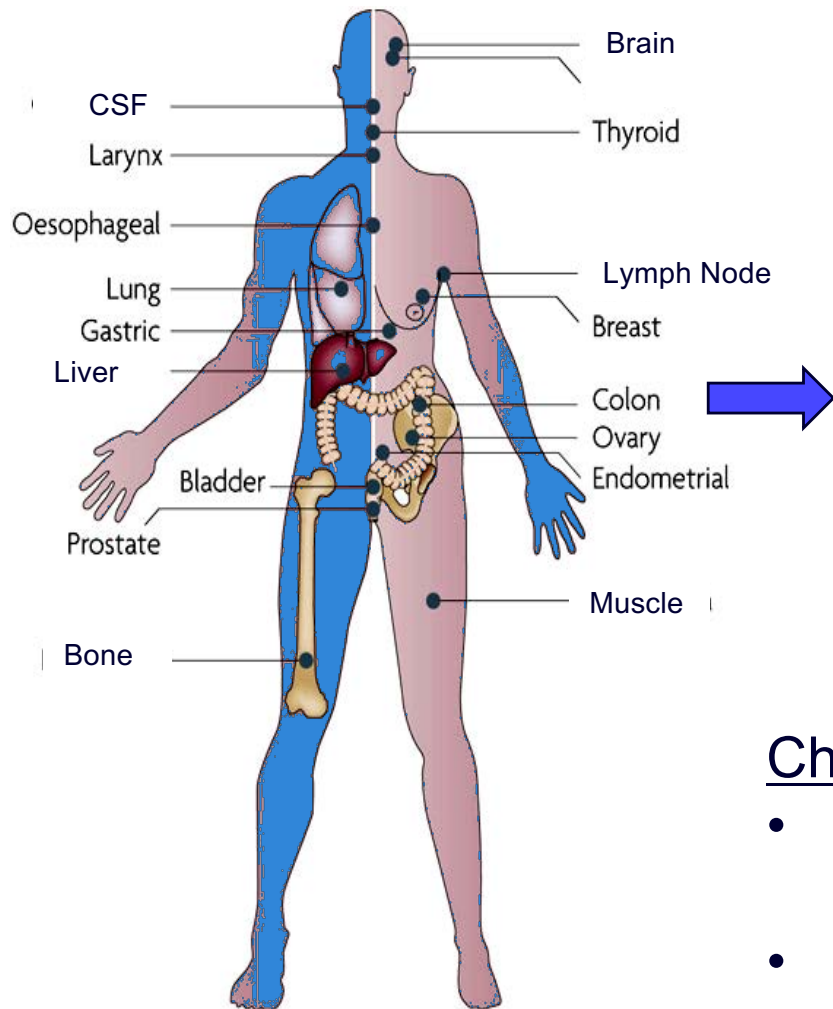
Associations of CRP across the entire SAGES cohort with postoperative delirium, delirium duration, & delirium feature severity (sum of all CAM-S scores)

Summary

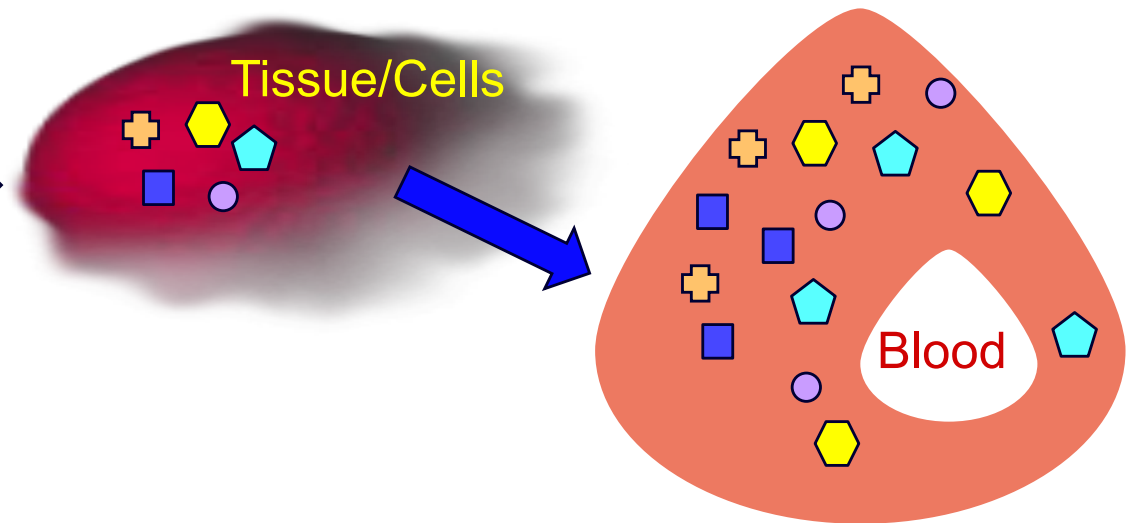
- IL-6 levels increase in patients experiencing delirium
- CRP is elevated before surgery and is a potential predictive biomarker for delirium, delirium duration, & delirium severity
- Pre-Inflammatory status prior to surgery may increase risk of postoperative delirium
- CRP and IL-6 involved in many diseases
- **Can we identify more specific and novel biomarkers for delirium?**

Challenges in Delirium Biomarker Discovery (Serum/Plasma/CSF)

Blood Test for Delirium?



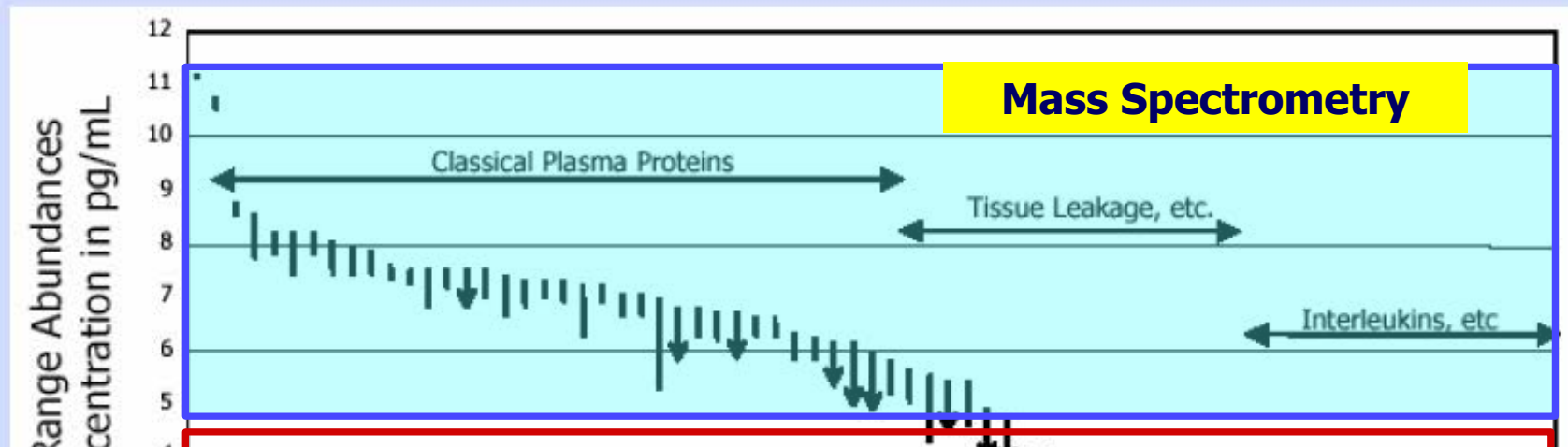
- Proteins from various tissues/cells released into circulation
- Exosomes with proteins from various tissues/cells including brain released into circulation



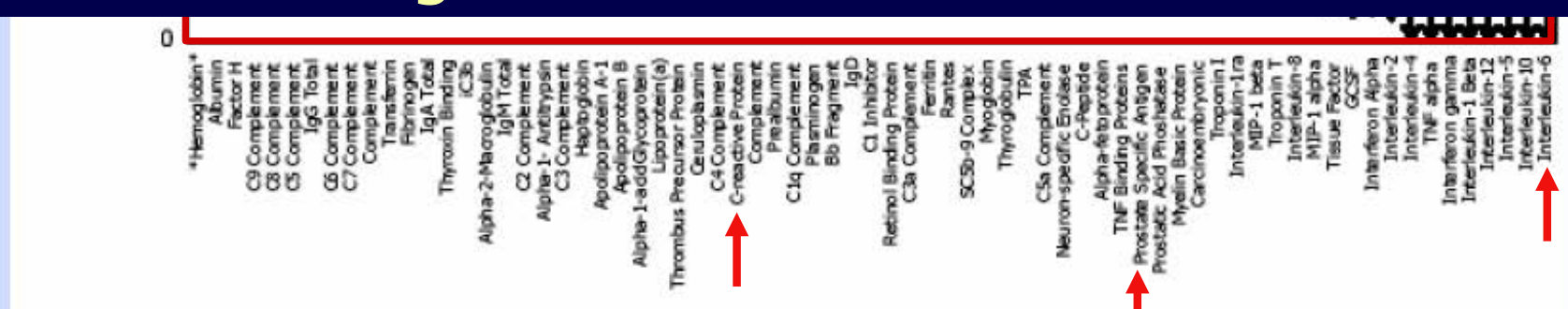
Challenges

- Biomarker proteins present at low concentrations
- Many other more abundant blood proteins
- Exosomes present at low concentrations

Levels of proteins in plasma



How Do We Measure Simultaneously a Broad Range of Low Abundant Proteins?

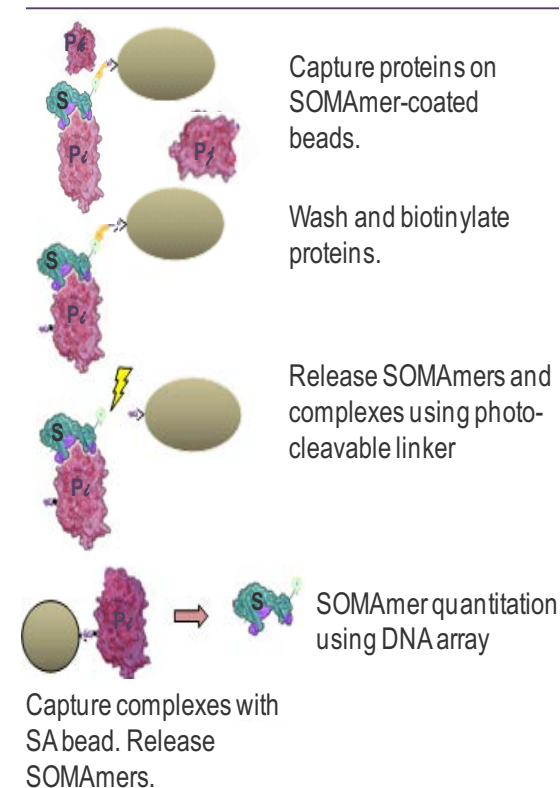
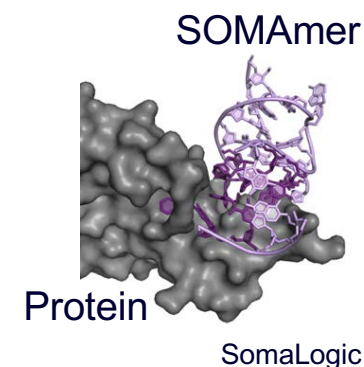


Molecular & Cellular Proteomics 2003 , Anderson and Anderson 2 (1): 50

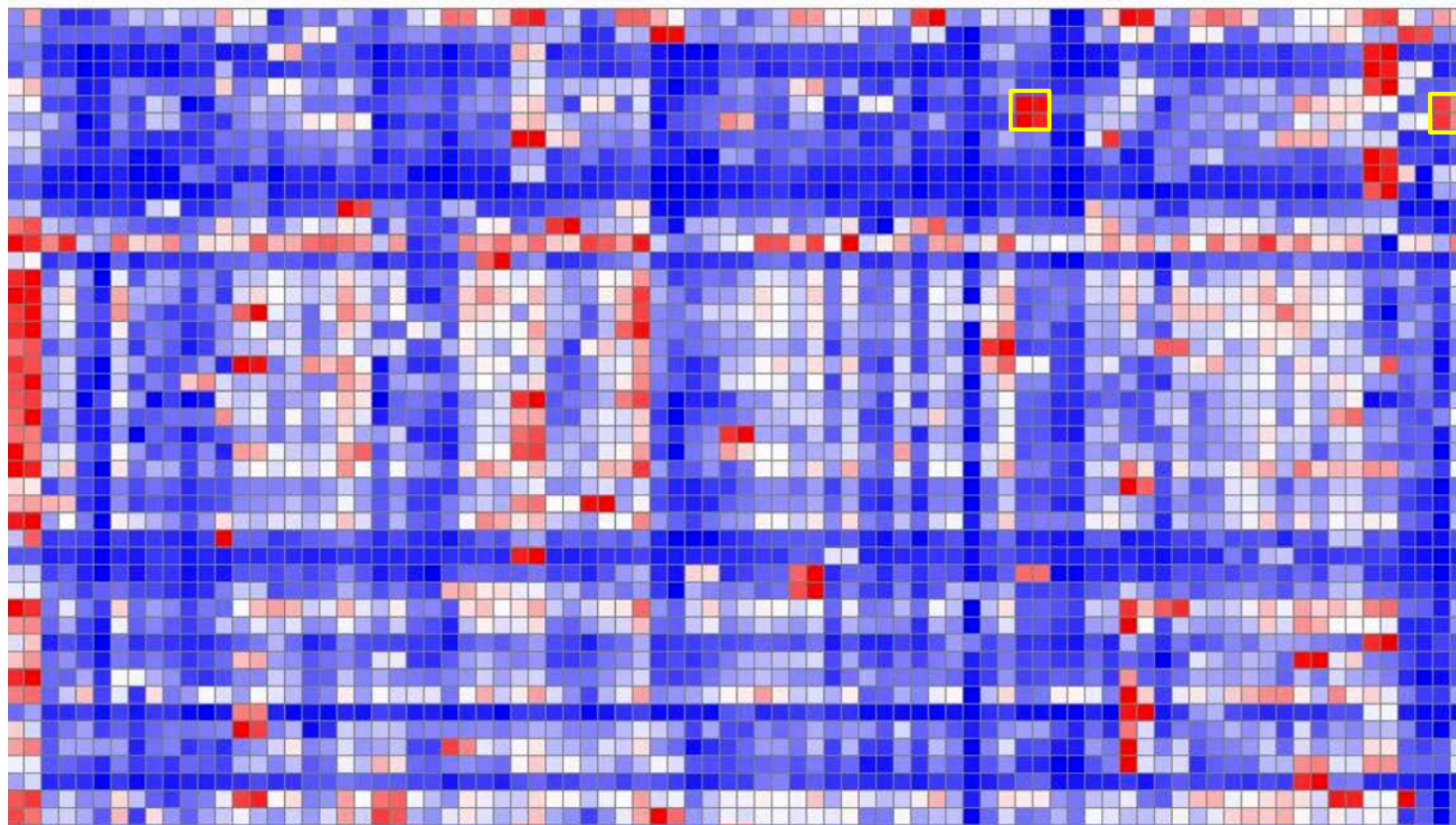
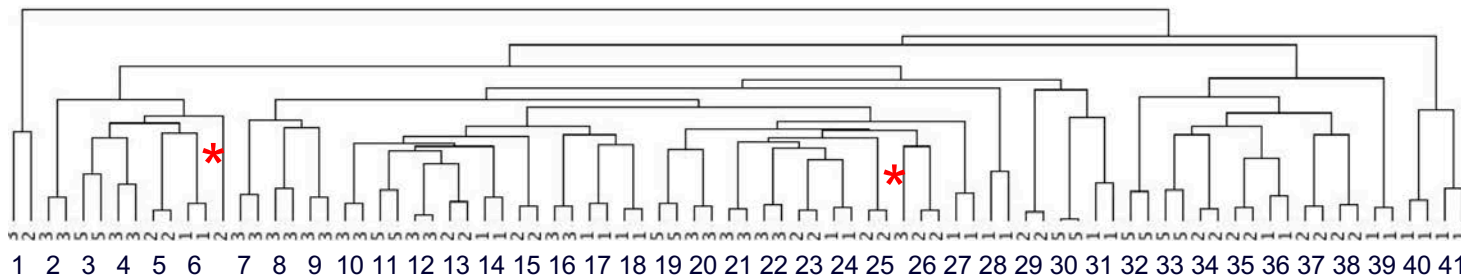
12-13 Logs Differences in Protein Expression

Ideal Proteomics Platform for Protein Biomarker Discovery: SOMAscan

- Highly multiplexed, sensitive, specific, quantitative proteomic tool
- Measures simultaneously 1305 proteins/sample in only 65µl of human serum/plasma/urine; 6µg of protein from tissue/cell lysate/exosomes
- Dynamic range >8 logs (femtomolar to micromolar)
- Reproducibility (~5% median %CV)
- Protein-capture SOMAmer (Slow Off-rate Modified Aptamer) reagents
- SOMAmers: protein affinity-binding reagents and unique nucleotide sequences recognizable by specific DNA hybridization probes



Within-Person Stability of Plasma Protein Expression Patterns Over 1 Year



Annotation

SL005217
SL007049
SL005235
SL000445
SL005437
SL004339
SL014148
SL006892
SL010499
SL004360
SL003104
SL004066
SL000089
SL008158
SL004180
SL000557
SL003332
SL004724
SL006992
SL007806
SL004685
SL001721
SL004346
SL014096
SL010372
SL001999
SL004142
SL007024
SL004349
SL006803
SL008590
SL004851
SL002524
SL008378
SL000345
SL004144
SL003178
SL000007
SL014009
SL004364
SL003753
SL000584
SL010461
SL014130
SL003186
SL000540
SL016563

- Each patient clusters with itself across the 2 time points
- Blood drawn from a patient at different time points is very similar
- Every person has a different fingerprint of proteins

ICC or Spearman $r \geq 0.4$ for 91% of proteins

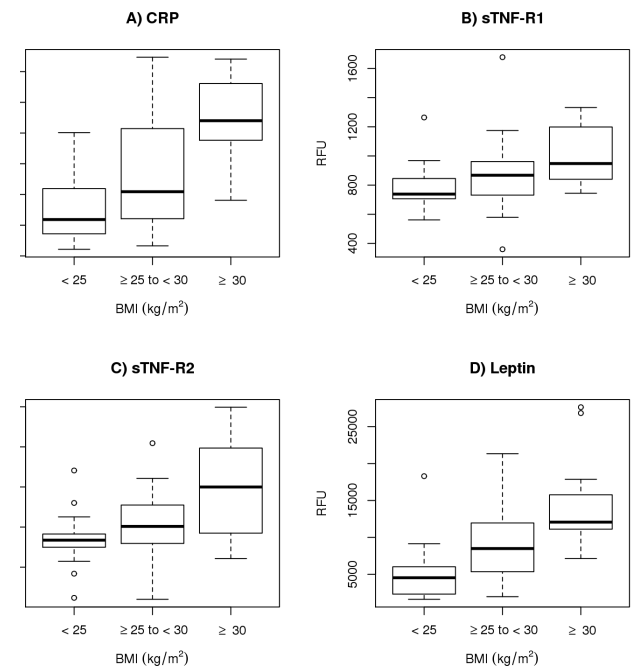
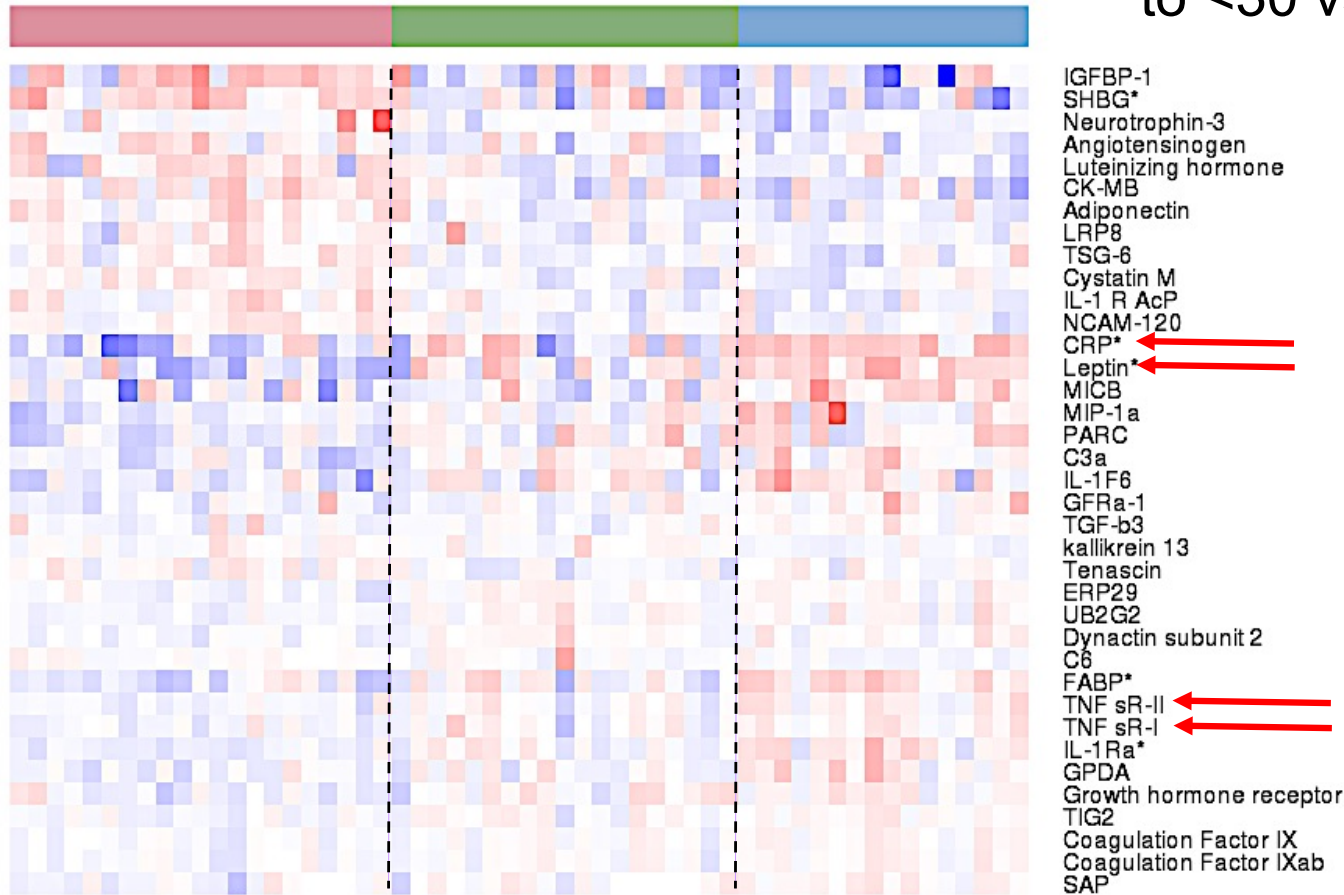
Nurses Health Study Cohort

Differences in BMI Easily Captured by SOMAscan:

Elevated Inflammatory Proteins Correlate with BMI

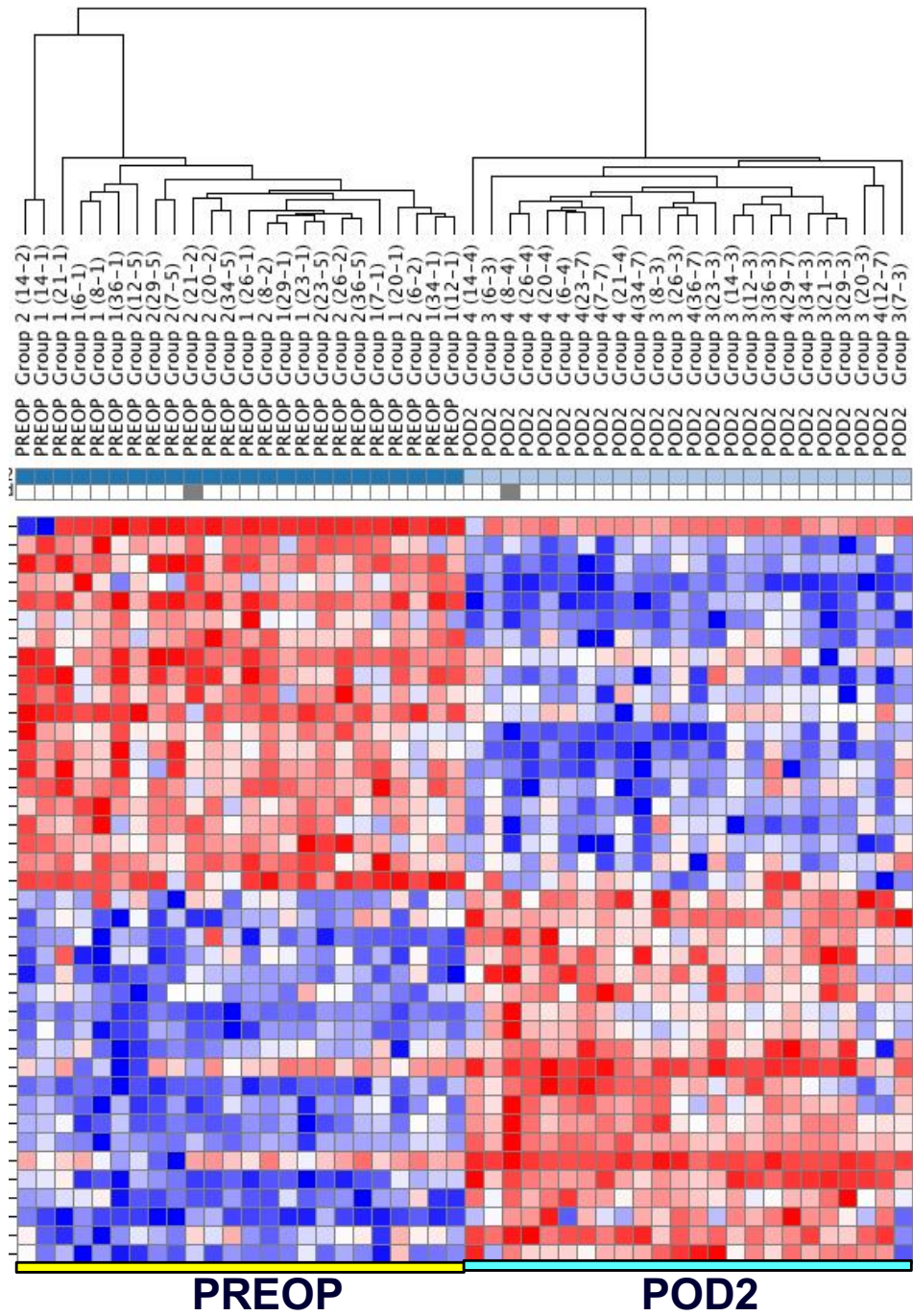
- BMI <25 Kg/m²
- BMI ≥ 25 to <30 Kg/m²
- BMI ≥ 30 Kg/m²

Heatmap of Proteins Comparing Individuals with BMI <25 vs. ≥25 to <30 vs. ≥30 kg/m²



*FDR-adjusted P-value <0.05

SOMAscan perfectly differentiates plasma proteins before (PREOP) and after surgery (POD2)



Stress & Inflammation linked proteins are increased by surgery

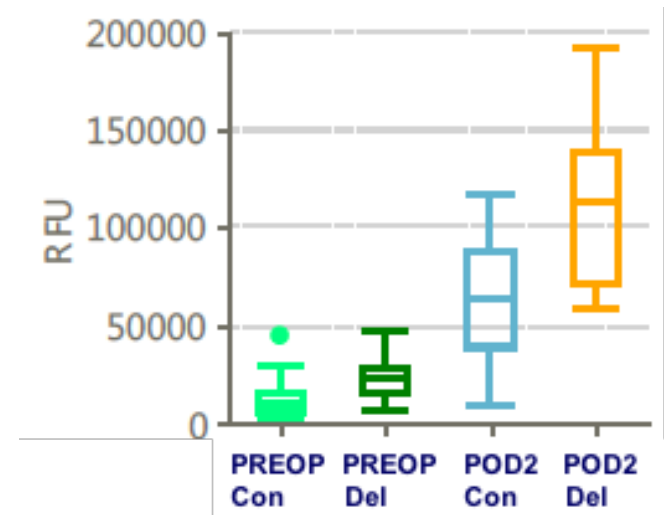
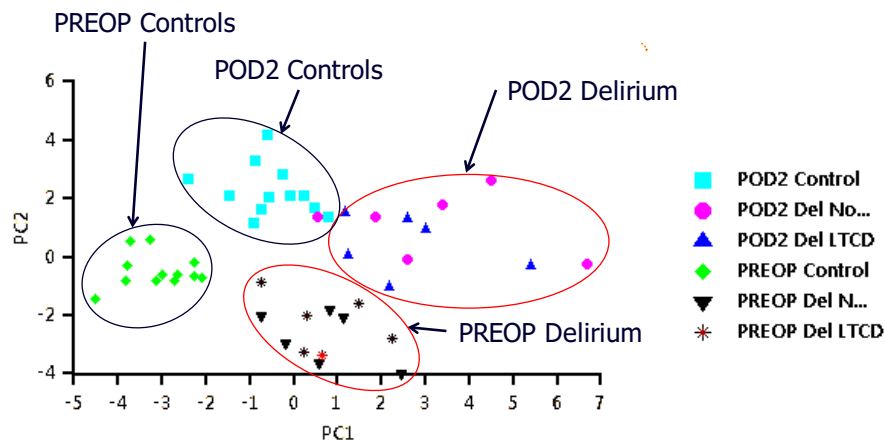
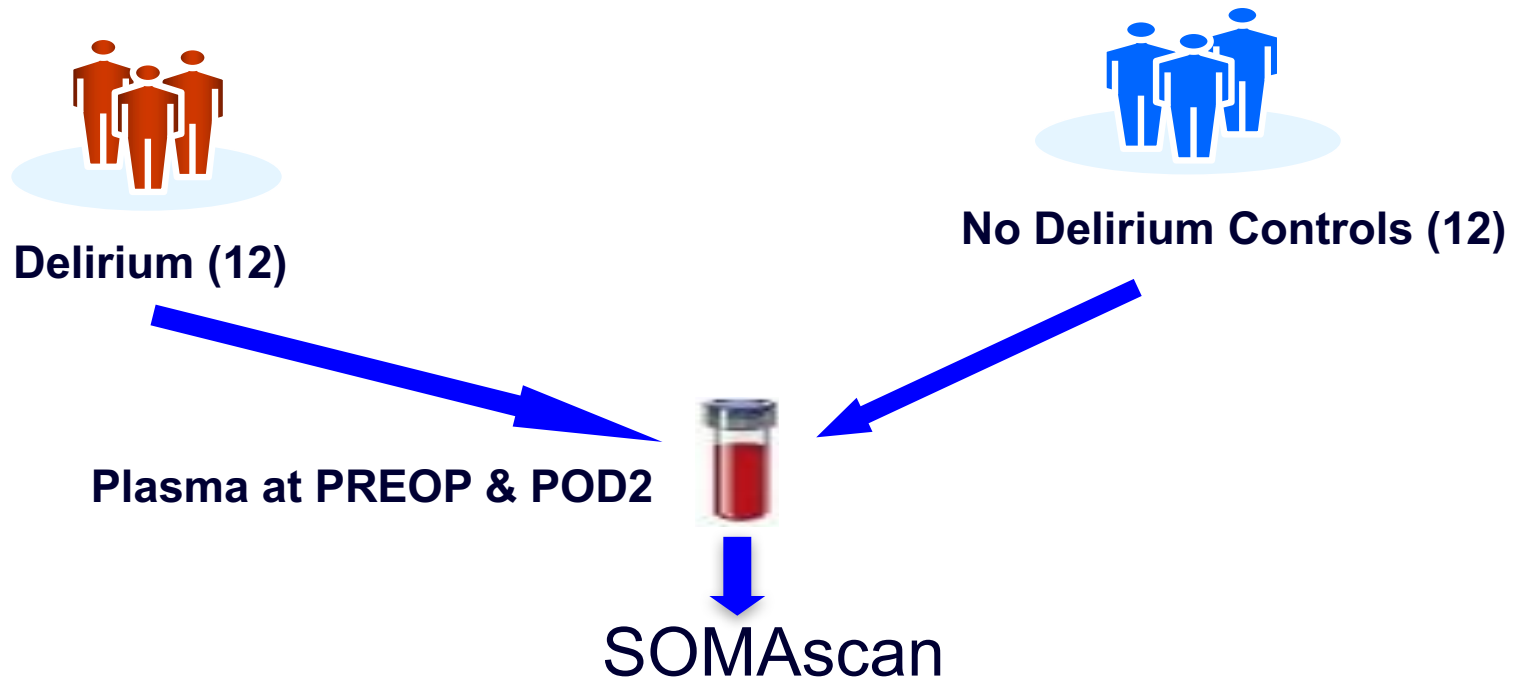
POD2 vs. PREOP (BH corrected paired t-test $p < 0.01$) L1OXV: 100%

- | Annotation | Annotation-1 |
|------------|--|
| SL000550 | Plasma serine protease inhibitor |
| SL000251 | Alpha-2-HS-glycoprotein |
| SL008381 | Cathepsin F |
| SL000508 | Lymphotoxin alpha2:beta1 |
| SL010328 | Mediator of RNA polymerase II transcription subunit 1 |
| SL021043 | Growth/differentiation factor 11/8 |
| SL014092 | Cell adhesion molecule-related/down-regulated by oncogenes |
| SL004876 | Kallistatin |
| SL004183 | Cadherin-3 |
| SL004742 | Afamin |
| SL006777 | Fetuin-B |
| SL000019 | Apolipoprotein A-I |
| SL000541 | Plasminogen |
| SL000268 | Angiostatin |
| SL004060 | Endothelin-converting enzyme 1 |
| SL000566 | Retinol-binding protein 4 |
| SL000358 | Coagulation factor VII |
| SL003300 | C-C motif chemokine 16 |
| SL007237 | Dual specificity mitogen-activated protein kinase kinase 4 |
| SL000426 | Fibronectin |
| SL016555 | Dual 3',5'-cyclic-AMP and -GMP phosphodiesterase 11A |
| SL000325 | Complement component C9 |
| SL011770 | Leucine carboxyl methyltransferase 1 |
| SL004477 | Protein S100-A9 |
| SL000640 | Nidogen-1 |
| SL000598 | Thrombopoietin |
| SL003302 | C-C motif chemokine 23 |
| SL003301 | Ck-beta-8-1 |
| SL003341 | Fibrinogen gamma chain |
| SL004536 | Hepcidin |
| SL002528 | Phospholipase A2, membrane associated |
| SL000424 | Fibrinogen |
| SL000248 | Alpha-1-antichymotrypsin |
| SL003309 | Lipopolysaccharide-binding protein |
| SL000051 | C-reactive protein |
| SL000572 | Serum amyloid A-1 protein |
| SL000249 | Alpha-1-antitrypsin |
| SL000310 | Complement C1r subcomponent |
| SL003340 | Chitinase-3-like protein 1 |
| SL012774 | Cysteine-rich with EGF-like domain protein 1 |

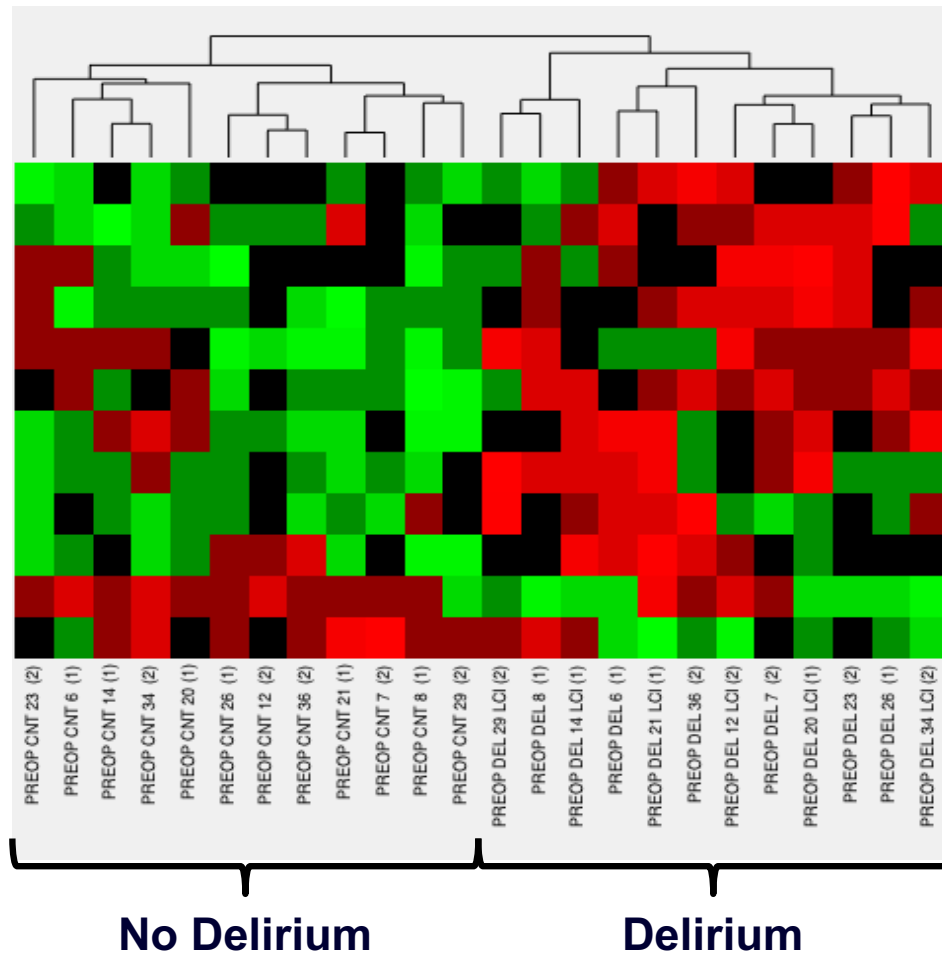
SOMAscan Ideal for Neuroinflammation & Immune System Biomarker Discovery

- Covers large portion of immune system proteins
 - CD antigens
 - Cytokines
 - Chemokines
 - Soluble Receptors
 - Coagulation
 - Complement
 - Checkpoints
- Covers many inflammation and neuroinflammation proteins
 - Cytokines
 - Chemokines
 - Soluble Receptors
 - Acute Phase Proteins

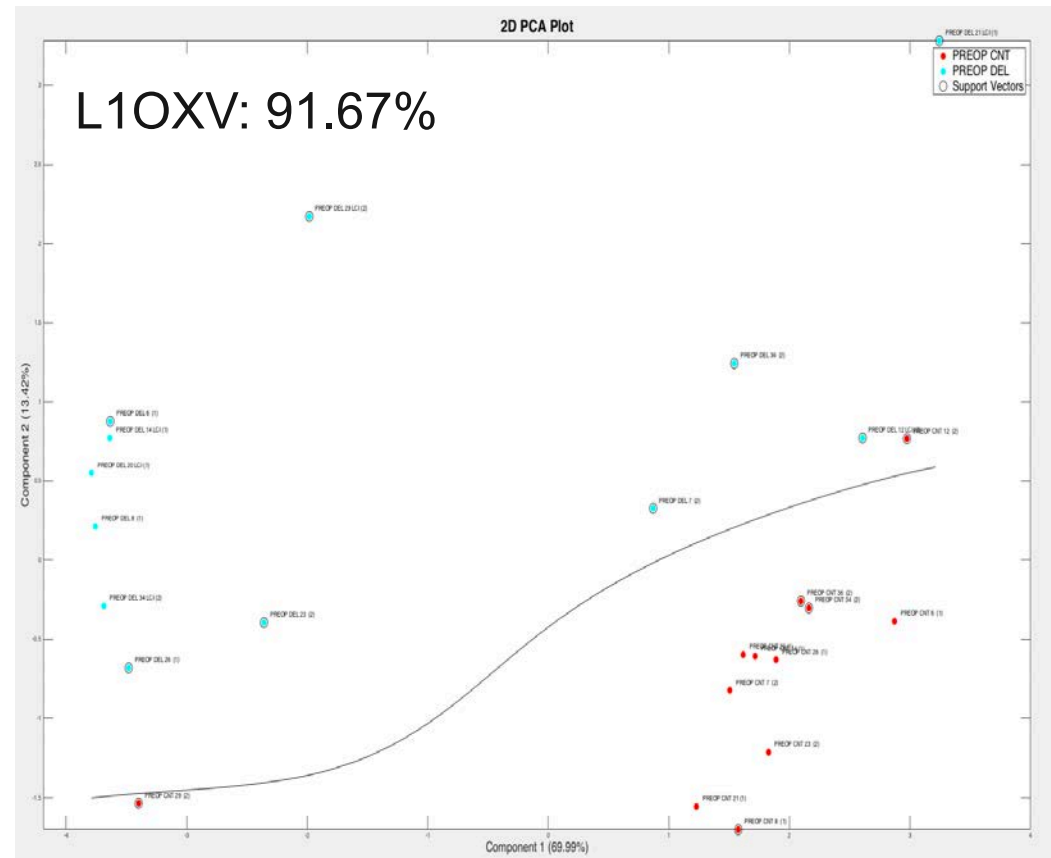
Postoperative Delirium Plasma Biomarker Discovery



SOMAscan Accurately Discriminates Between Delirium & No Delirium at PREOP



Hierarchical Clustering of 12 Proteins



Principal Component Analysis of 12 Proteins

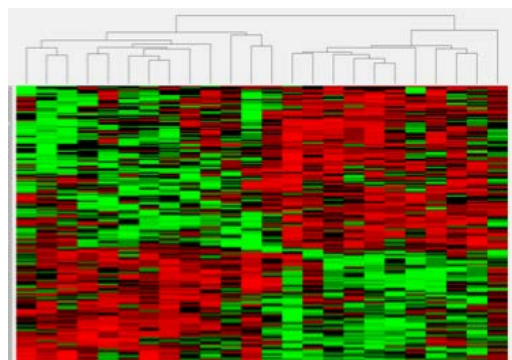
Delirium Metabolome/Lipidome Platforms

Targeted Metabolomics

- AB/SCIEX 5500 QTRAP triple quadrupole



>250 metabolites



MetaboAnalyst
Pathway Enrichment

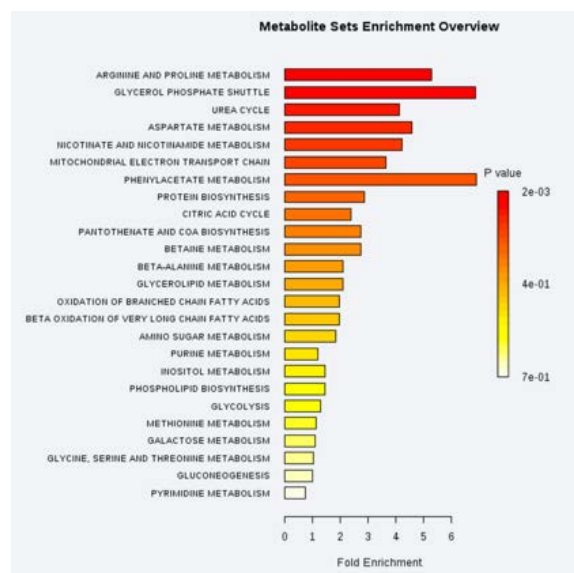
- ✓ Serum
- ✓ Biopsies
- ✓ Stool

Untargeted Metabolomics/Lipidomics

- Thermo Scientific Q Exactive HF/Plus
- Ultra fast & ultra sensitive

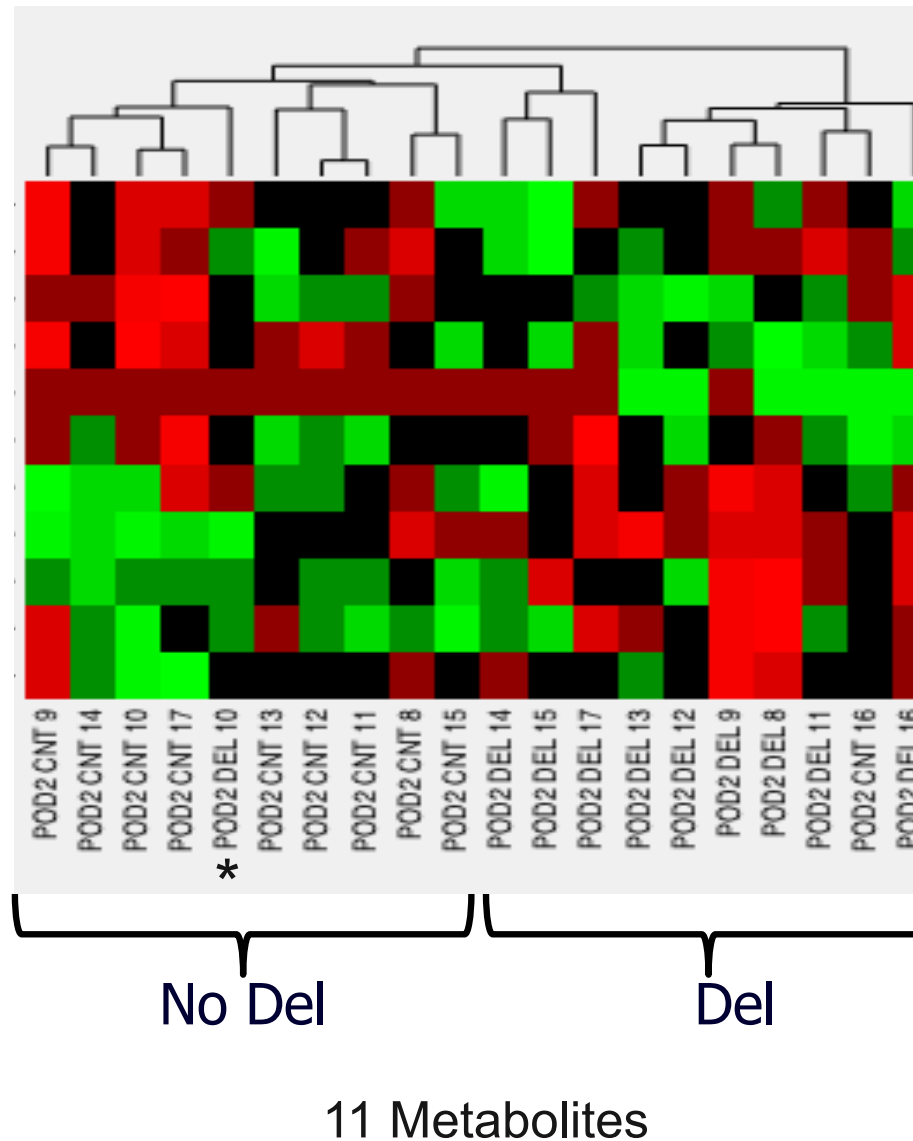


5,000-20,000 metabolites
>2000 lipids



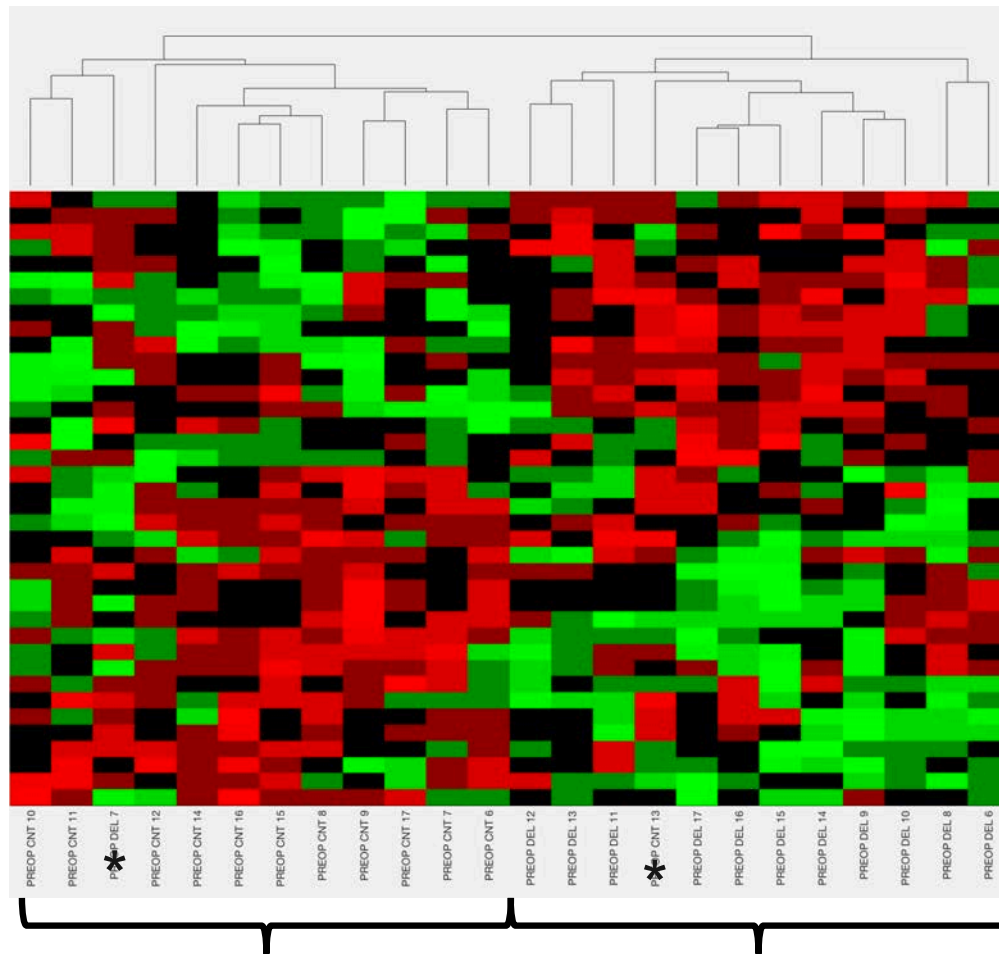
- High resolution
- Extremely fast scan speeds
- Quantitative

Metabolomics Analysis of Plasma Samples at POD2 Reveals Delirium-Specific Alterations



Lipidomics Analysis of 12 Matched Pairs of Plasma Samples at PREOP and POD2 Reveals Delirium-Specific Alterations

PREOP

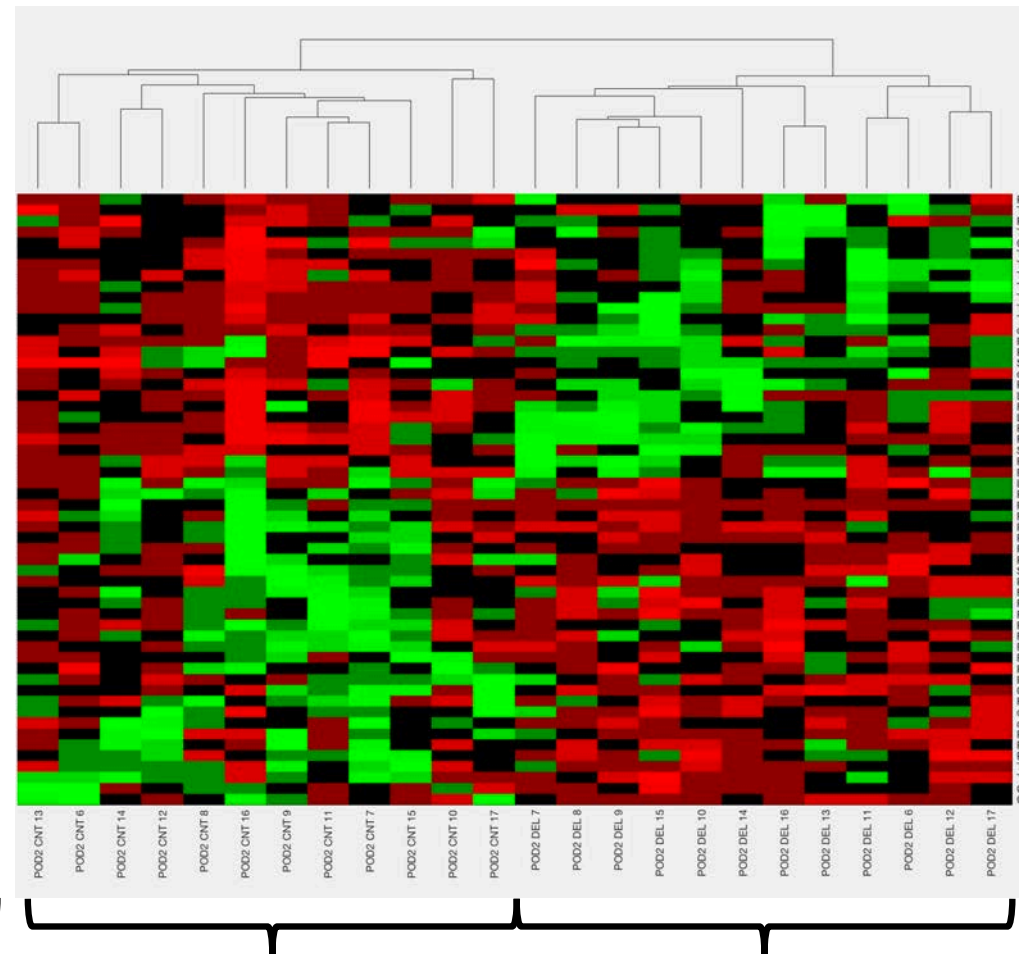


No Del

Del

38 Lipids

POD2



No Del

Del

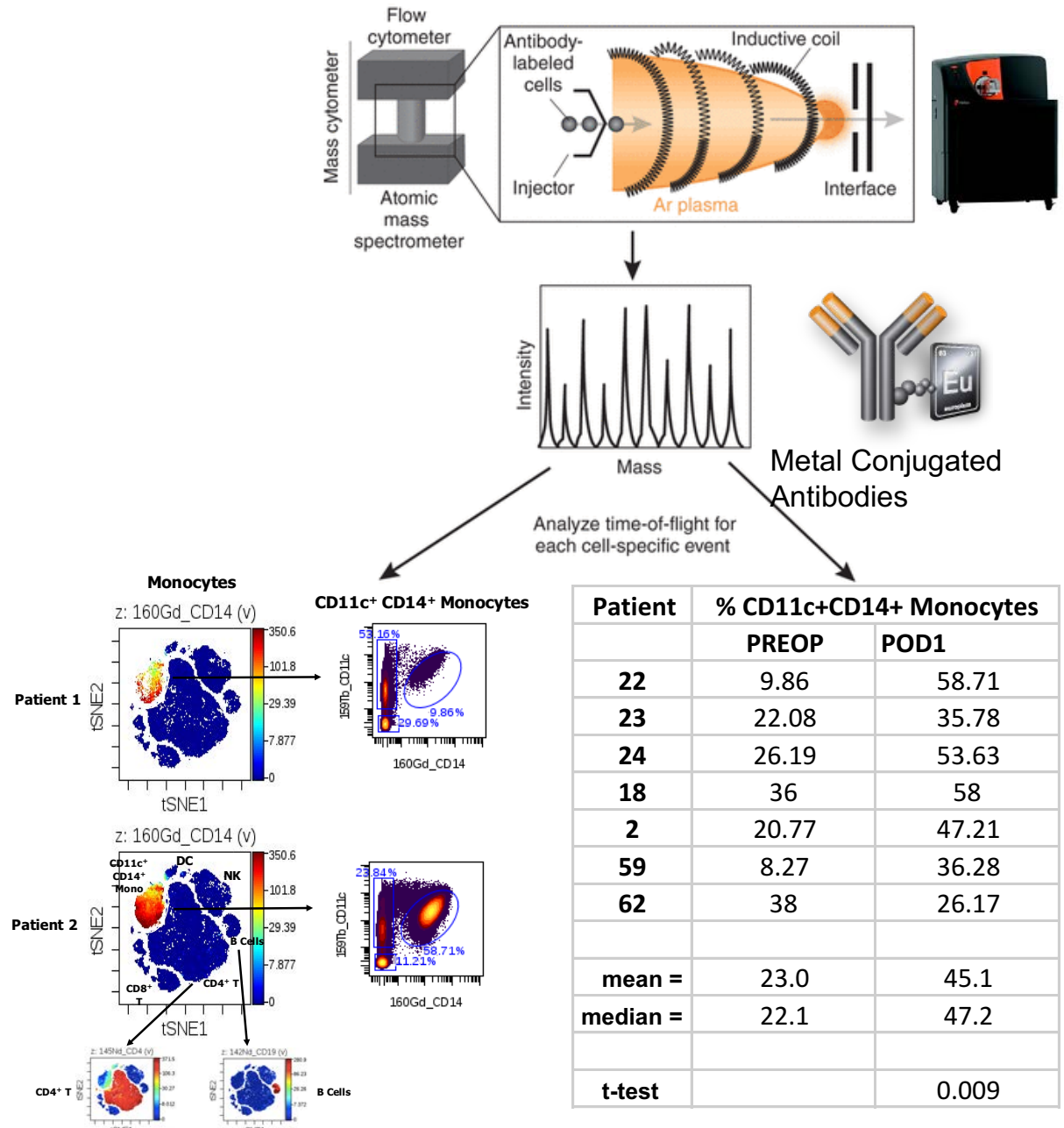
56 Lipids

Delirium Immunome Platform: CyTOF Mass Cytometry

Massively Multi-Parametric Detection System for Single Cell ImmunoPhenotyping

Key Advantages of CyTOF

- Phenotypically & functionally profile all immune cell subsets at single-cell resolution for up to 100 different cell surface and intracellular signaling proteins by using antibodies coupled to metal isotopes
- Discrete isotope peaks without significant overlap, enabling higher multiplexing than FACS
- Innovative software tools (viSNE,, SPADE, Citrus) incorporate pattern recognition approaches to enable detection of finely tuned cell subsets (clusters of single cells with similar expression patterns)



The Next Revolution: Single Use Health & Wellness Chip on Laptop or iPhone

Disease
Predisposition



Early Disease Detection
e.g. Cancer, Cardiovascular

Benefit: Earlier Detection, Precise Diagnosis, & Targeted Treatment

Improved Outcomes

e.g. Cyt P450



