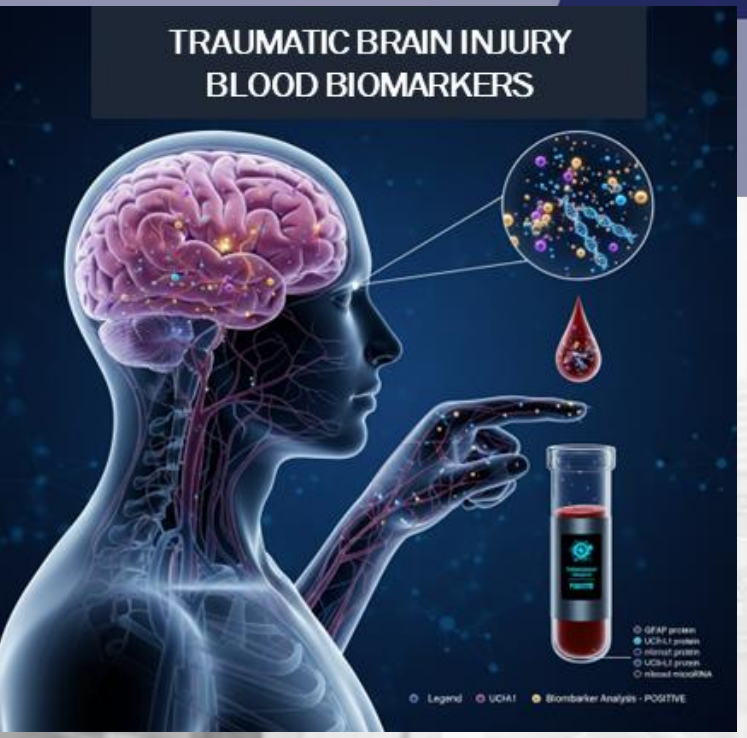

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Walter Reed Army Institute of Research
Defense Health • Global Health

Blood Biomarkers to Distinguish Mild TBI at the Asymptomatic State and Predict the Recovery Time:

AI/ML Empowered Analysis of Multi-Omics Study

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DHA
Defense Health Agency
RESEARCH & DEVELOPMENT

August 2026

Disclaimers

The views expressed in this presentation reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Defense Health Agency, Department of War, nor the U.S. Government.

The study protocol was approved by the Keller Army Hospital, United States Military Academy Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects.

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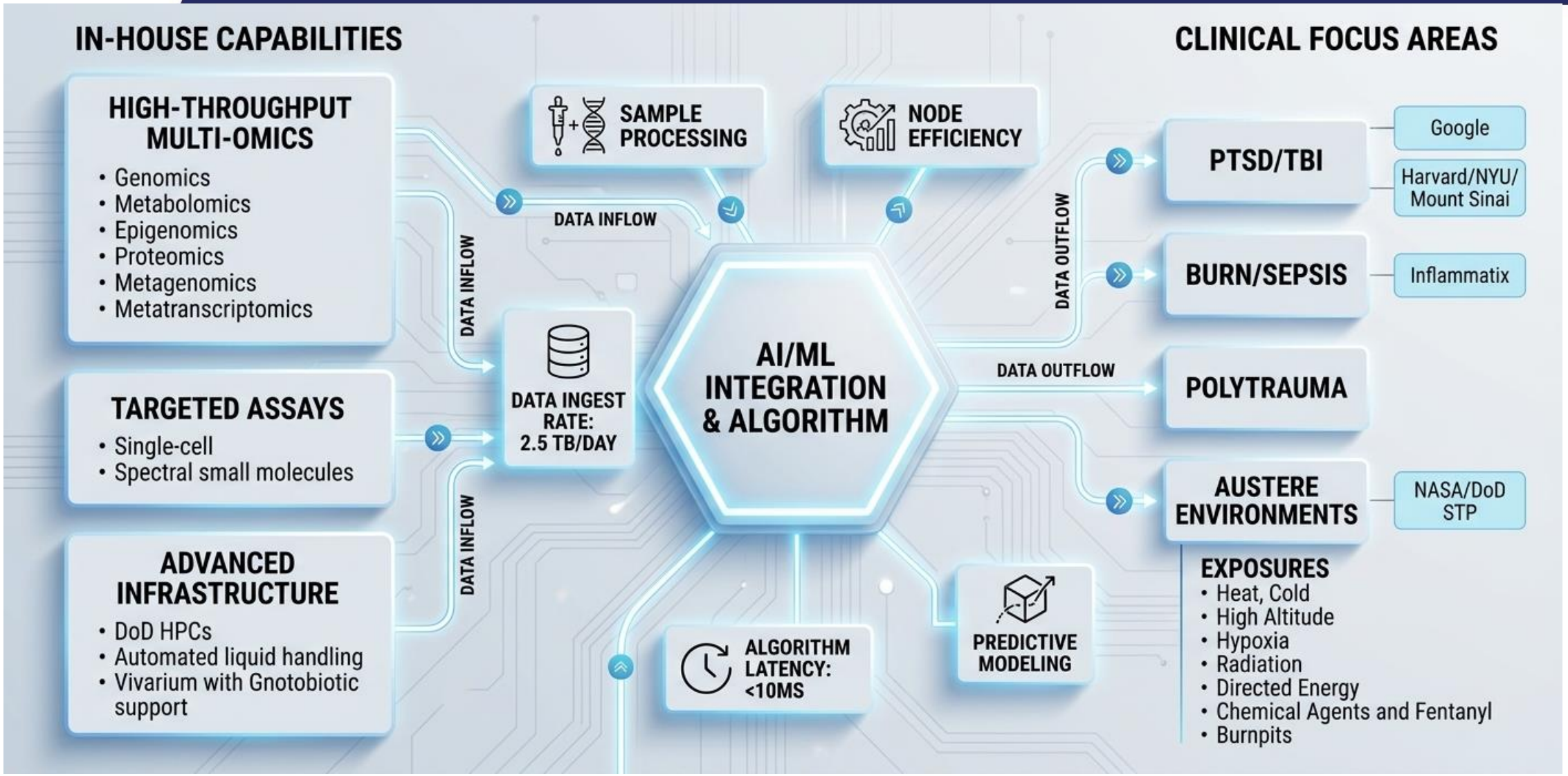

IN-HOUSE CAPABILITIES
HIGH-THROUGHPUT MULTI-OMICS
• Genomics
• Metabolomics
• Epigenomics
• Proteomics
• Metagenomics
• Metatranscriptomics
TARGETED ASSAYS
• Single-cell
• Spectral small molecules
ADVANCED INFRASTRUCTURE
• DoD HPCs
• Automated liquid handling
• Vivarium with Gnotobiotic support
SAMPLE PROCESSING
NODE EFFICIENCY
DATA INFLOW
DATA INFLOW
DATA INFLOW
DATA INGEST RATE: 2.5 TB/DAY
AI/ML INTEGRATION & ALGORITHM
ALGORITHM LATENCY: <10MS
PREDICTIVE MODELING
DATA OUTFLOW
DATA OUTFLOW
CLINICAL FOCUS AREAS
PTSD/TBI
Google
Harvard/NYU/ Mount Sinai
BURN/SEPSIS
Inflammatix
POLYTRAUMA
AUSTERE ENVIRONMENTS
NASA/DoD STP
EXPOSURES
• Heat, Cold
• High Altitude
• Hypoxia
• Radiation
• Directed Energy
• Chemical Agents and Fentanyl
• Burnpits

Study objective

Gaps:

- ❑ Blood biomarker (sex-specific?) to diagnose TBI/ impacts of concussion
- ❑ Objective and scalable matrix to aid in Return-to-duty (RTD) decision
- ❑ Therapeutic targets

Approach:

- ❑ Multi-omics assay of archived blood samples from young athletes, who underwent concussion during contact sports, and followed up towards return to participation (RTP)
- ❑ AI/ML-augmented analysis towards identifying biomarkers of diagnostic, triage and therapeutic values to meets the gaps

NCAA-DoD-CARE consortium, the parent cohort

- 1,751 concussed male (63.2%) and female (36.8%) collegiate athletes were studied. Concussion incidents were noted and scored. A subset of this group (44 test and 44 controls) were used for current project.
- 80% of participants became asymptomatic by day 14 and/or return to participation (RTP) by day 21.
- Slow recovery was thus defined as exceeding 1 or both of those intervals (more likely to be female ($p = 0.05$), and initial postinjury SCAT symptom severity scores ($p < 0.001$).

Mean % (min-max)

	Male	Female
Time to RTP protocol initiation		
By day 7	57% (54-60)	52% (48-56)
By day 14	82% (80-84)	80% (77-87)
By day 21	90% (88-92)	89% (87-91)
By day 28	92% (90-94)	92% (90-94)
Time to RTP protocol initiation		
By day 7	17% (15-19)	14% (11-17)
By day 14	58% (55-61)	56% (52-60)
By day 21	78% (76-80)	74% (71-77)
By day 28	87% (85-89)	82% (79-85)

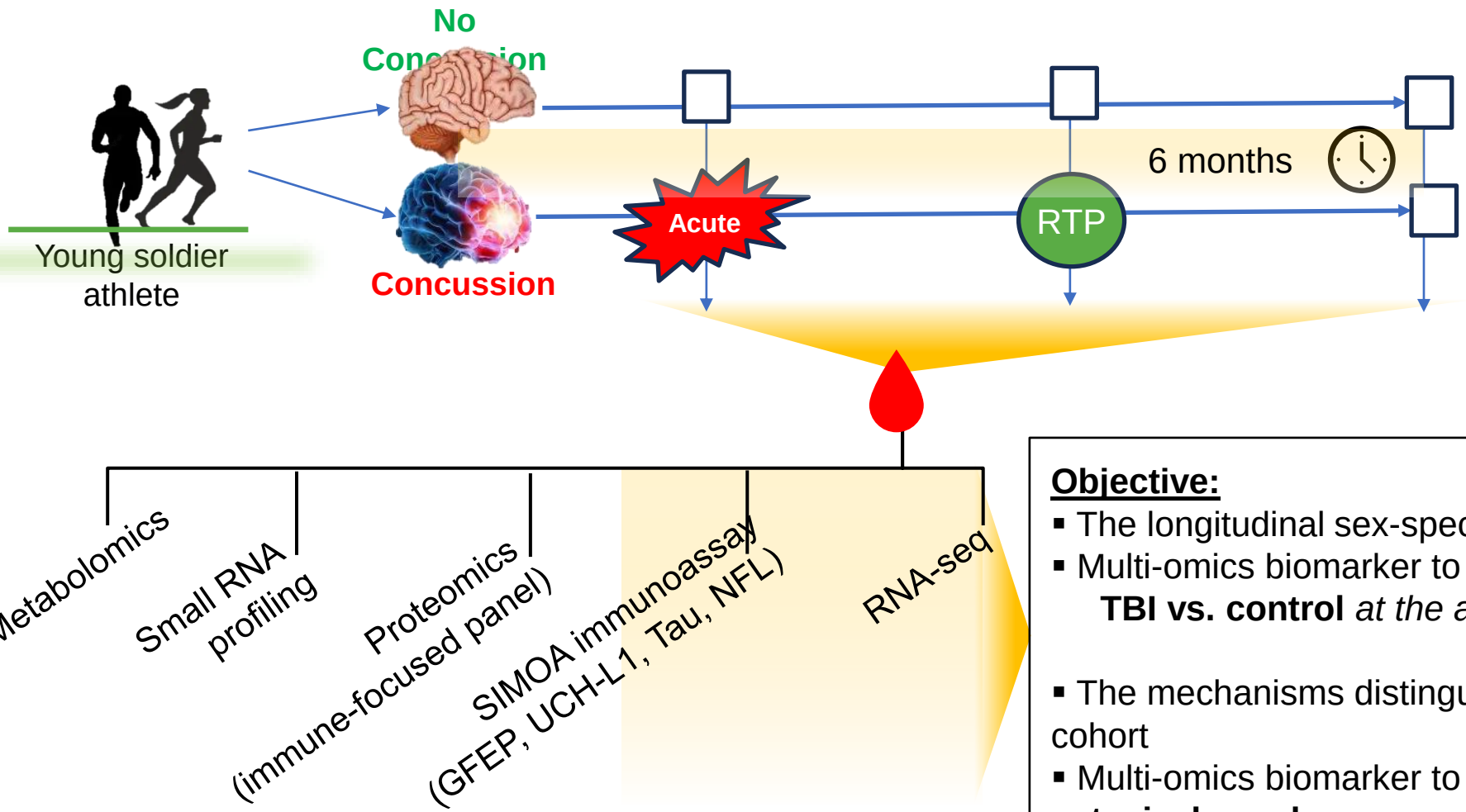
Current study used 14d, 21d and 28d as the cut-off to define **typical** vs. **slow** recovery group

References:

1. Characteristics and Outcomes of Athletes With Slow Recovery From Sports-Related Concussion A CARE Consortium Study, Neurology, 2023
2. The natural history of sport-related concussion in collegiate athletes: findings from the NCAA-DoD CARE Consortium. Sports medicine. 2022 Feb;52(2):403-15.

SCAT: Sport concussion assessment tool

Study design



Objective:

- The longitudinal sex-specific mechanism of TBI progression
- Multi-omics biomarker to differentiate
  TBI vs. control *at the acute timepoint*

- The mechanisms distinguishing typical vs. slow recovery cohort
- Multi-omics biomarker to differentiate
  typical vs. slow recovery cohort *at the acute timepoint*

*Blood collected within ~24h of concussion incidents

Subject metadata for current study: 44 cases and 44 controls

Cohort	Age (y)	Height	Weight (lb)	RTP (d)	Symptom Severity (Acute)	Symptom Severity (at RTP)	Symptom Severity (Delayed)
All	ns	ns	ns		4.8 x 10^-13	ns	0.0025
44 Controls	19.6 ±1.48	68.8±3.95	164.7± 28.3	--	2.4 ±3.37	1.6±2.58	2.8±4.25
44 TBI	19.9 ±1.46	68.7±4.25	164.8± 30.26	25.25± 17.34	28.7±20.32	0.86±3.00	0.8±1.54
Male	0.01	ns	ns		3.2x10^-8	ns	ns
25 Controls	19.8 ±1.24	71.4±2.64	180.6± 22.64	--	2.1±2.82	1.4±2.38	2.7±4.47
25 TBI	20.4 ±1.44	71.4±2.76	180.5± 25.14	23.68± 16.71	28.6 ±16.7	0.6±1.29	0.76±1.54
Female	ns	ns	ns		2.9 x 10^-5	ns	ns
19 Controls	19.3 ±1.73	65.4±2.54	143.7± 20.47	--	2.9±4.01	1.9±2.85	2.9±4.06
19 TBI	19.4 ±1.30	65.2±3.11	144.2± 23.51	27.31± 18.40	35.5±23.15	1.2±4.36	0.8±1.57

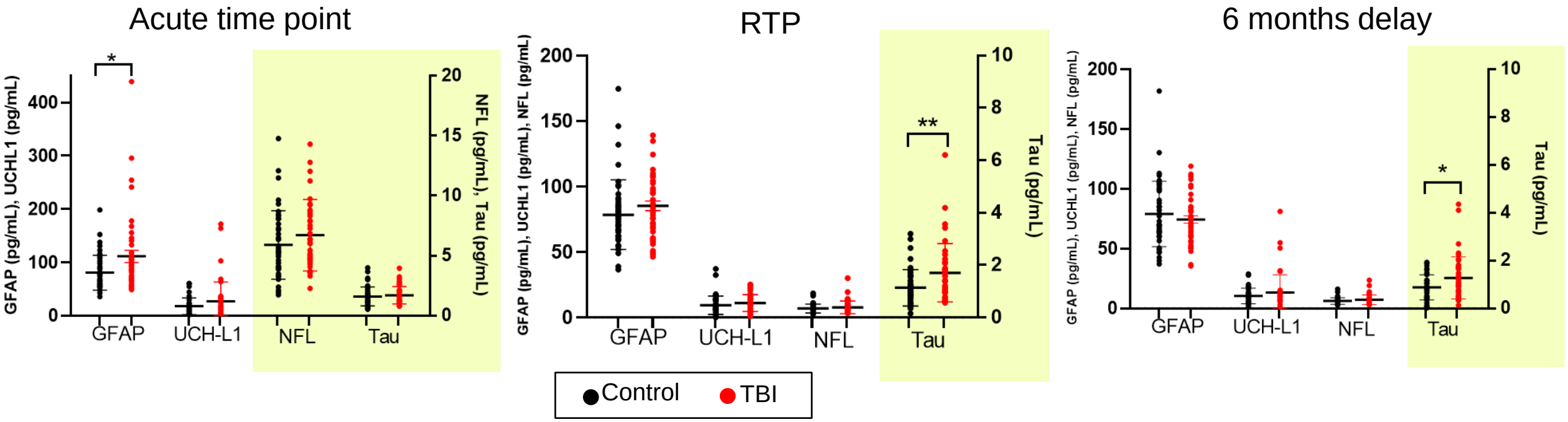
*Symptom severity was estimated by Sport Concussion Assessment Tool (SCAT)

Distribution of 44 cases at three different RTP: 14d, 21d and 28d

Cohort	Age (y)	Height	Weight (lb)	RTP (d)	Symptom Severity (Acute)	Symptom Severity (at RTP)	Symptom Severity (Delayed)
Recovery (14d)	ns	ns	ns	0.0003	ns	ns	ns
12 Typical (M8/F4)	20.0 ±1.27	69.8±3.15	168.4± 18.81	11.5±2.06	20.4±17.24	0.87 ±1.74	0.8±1.77
32 Slow (M15/F17)	19.6 ±1.43	68.3±4.57	163.5± 33.74	30.4± 17.75	31.4±20.78	0.83±3.38	0.7±1.47
Recovery (21d)	ns	ns	ns	6.2 x 10^-8	0.04	ns	ns
24 Typical (M15/F9)	19.7 ±1.44	67.4±4.38	167.2± 21.696	14.3±3.39	23.4±19.40	0.5 ±1.31	0.6±1.46
20 Slow (M10/F0)	20.1 ±1.48	69.4±1.40	161.9± 35.92	38.4± 18.25	35.0±20.13	1.25±4.25	0.9±1.65
Recovery (28d)	ns	ns	ns	1.9 x 10^-11	ns	ns	ns
32 Typical (M18/F14)	20.1 ±1.48	68.6±4.51	162.9± 29.61	16.8±5.32	27.8±21.55	1.1 ±3.48	0.5±1.31
12 Slow (M7/F5)	19.6 ±1.43	69.1±3.63	169.9± 32.73	47.8± 18.21	31.1±16.87	0.33±0.88	1.5±1.96

*Symptom severity was estimated by Sport Concussion Assessment Tool (SCAT)

SIMOA: *TBI vs. Control*

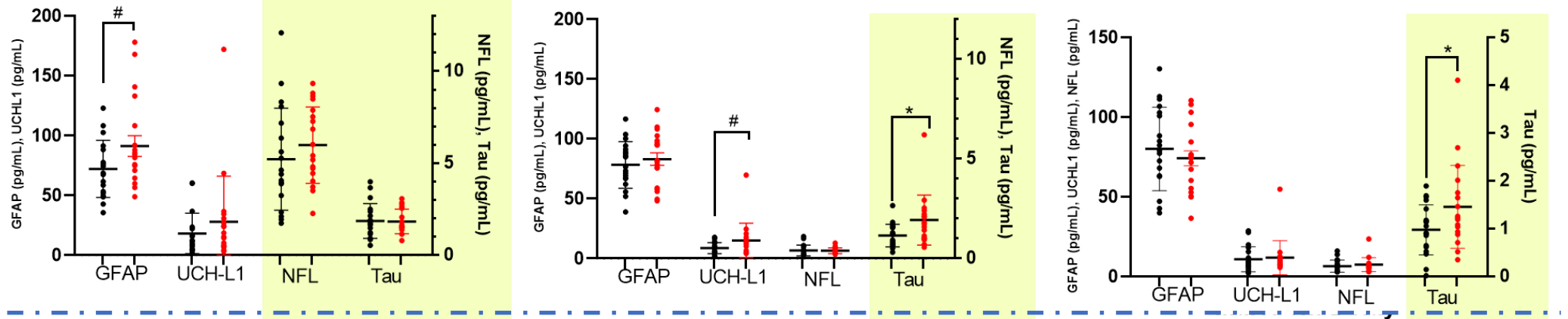


- GFAP emerge as the early marker, *$p<0.05$
- Tau emerged significant at RTP time and beyond

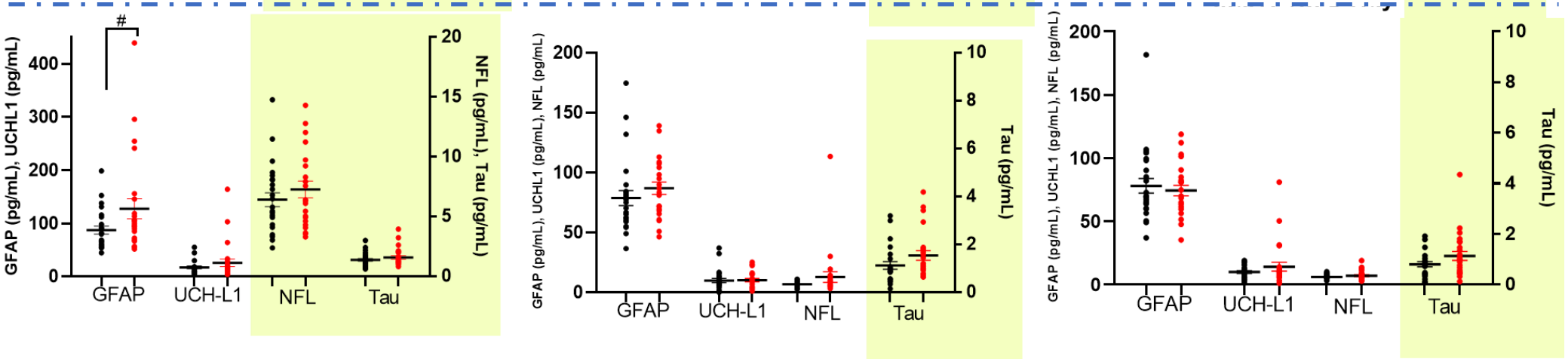
SIMOA: *TBI vs. Control (sex specific)*

Acute time point | RTP | 6 months delay

Female



Male

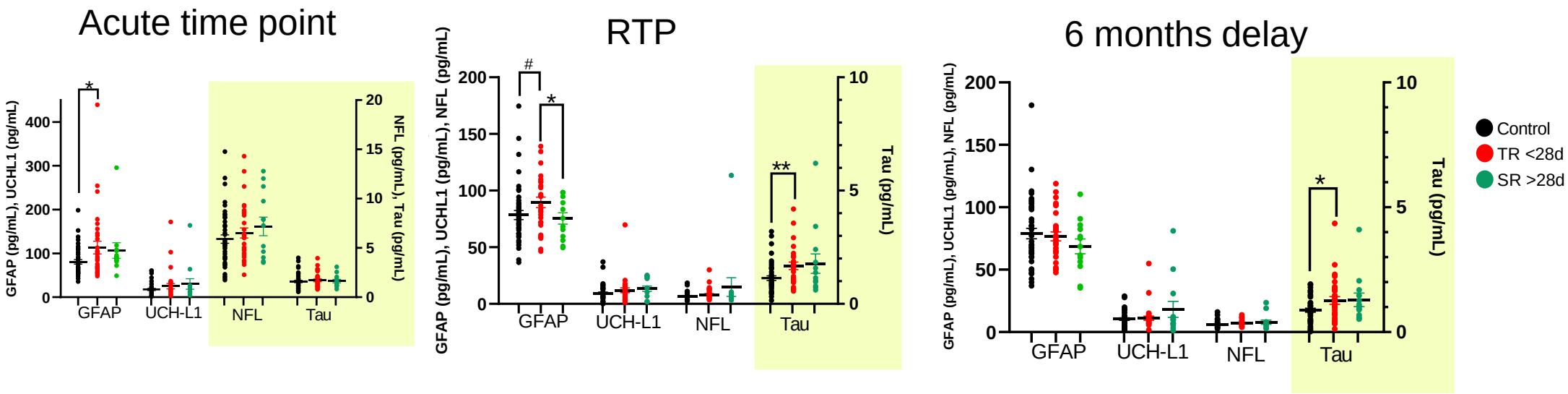


- GFAP's regulation was mostly sex neutral. #0.05<*p*<0.1
- Tau regulation emerged sex-biased <u>in this cohort</u>

Control TBI

SIMOA:

Typical vs. Slow recovery: 28d RTP



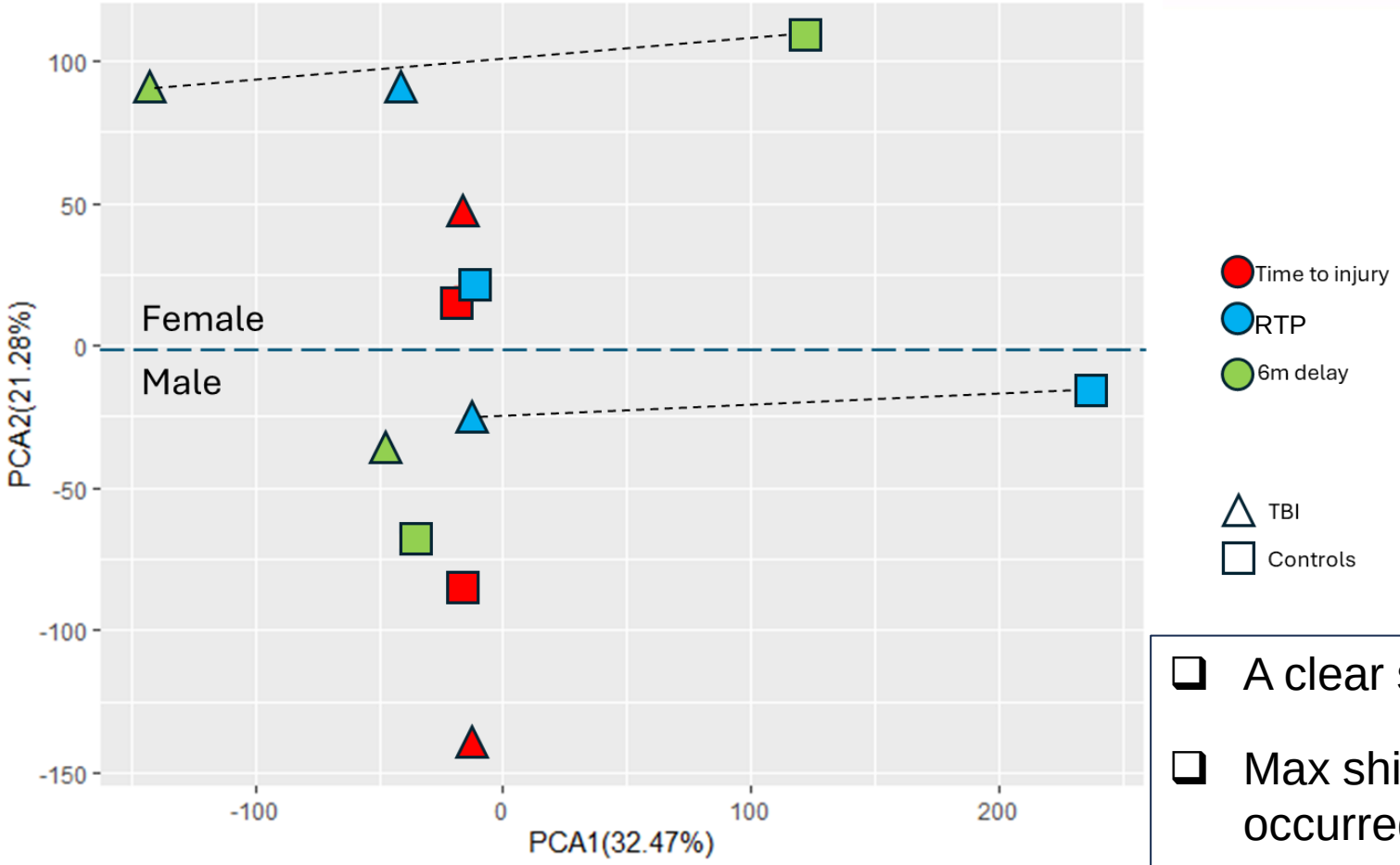
- GFAP separated the control from the typical recovery (TR) group at acute time point and at RTP
- None of the proteins could separate typical recovery from slow recovery (SR) group; although GFAP and Tau separated typical recovery from slow recovery at RTP

- This analysis could not be repeated to check the sex bias, due to low sample size

Conclusion- so far….

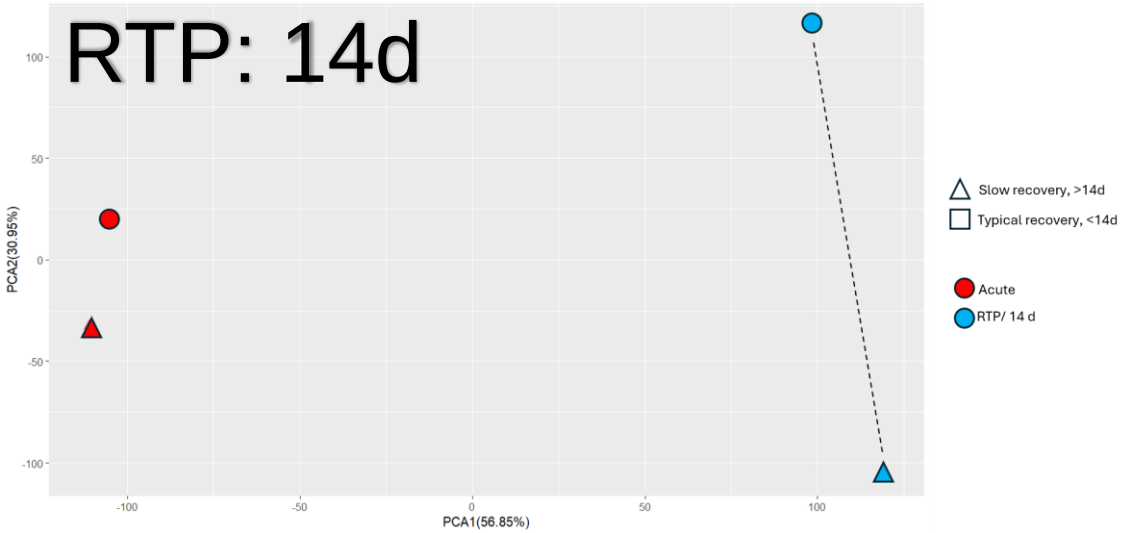
- At the asymptomatic state: only GFAP moderately diagnosed mTBI across male and female
- At the asymptomatic state, no proteins could triage slow vs. typical RTP cohort (RTP= 28d)

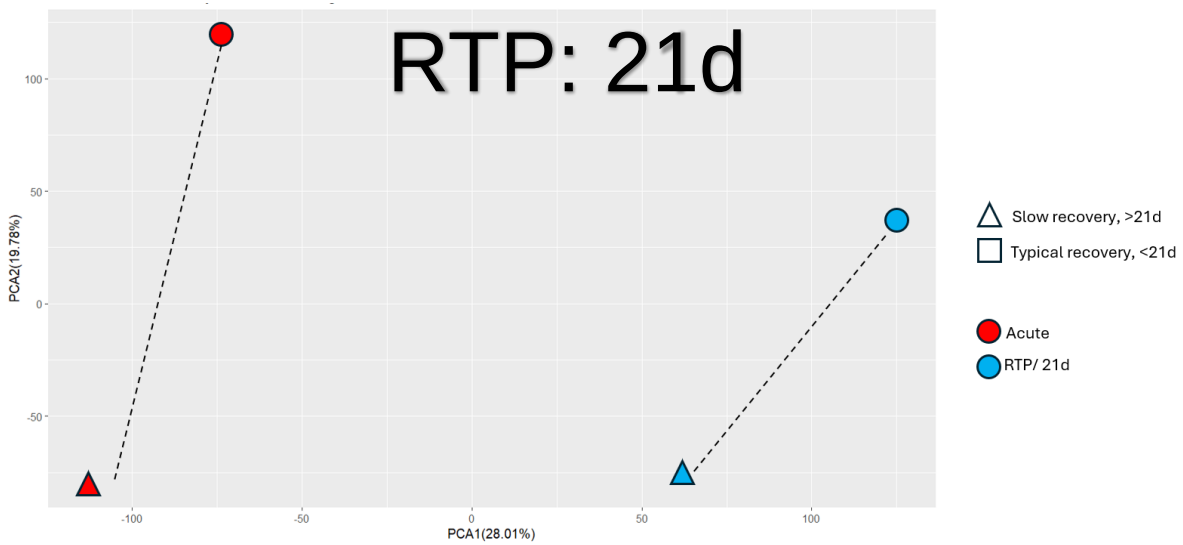
Principal Component Analysis (PCA) of mRNA-seq: *TBI vs. Control*

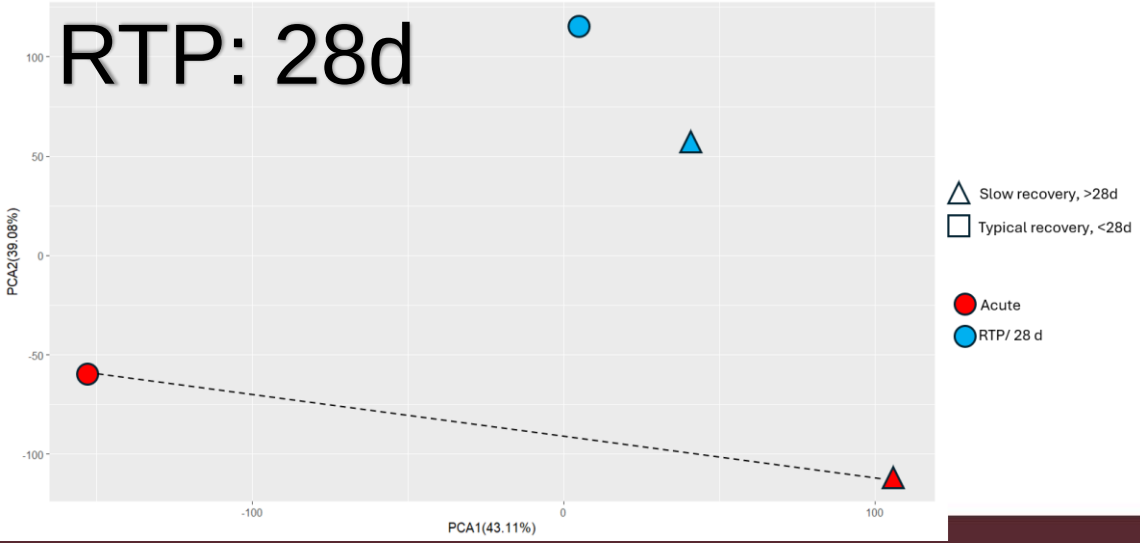


- ❑ A clear sex-bias in overall genomics perturbation
- ❑ Max shift in genomic regulation in female cohort occurred at delayed time points
- ❑ Max shift in genomic regulation in male cohort occurred at RTP

PCA of RNA-seq: *Typical vs. Slow recovery: RTP: 14d, 21d and 28d*







- Separation between typical vs. slow recovery group in Acute state was minimum at 14d RTP and largest in 28d RTP
- Note: SCAT symptom severity score could not separate these two groups
- This analysis could not be repeated to check the sex bias, due to low sample size

Biomarker panel to differentiate mTBI vs. Control at acute time

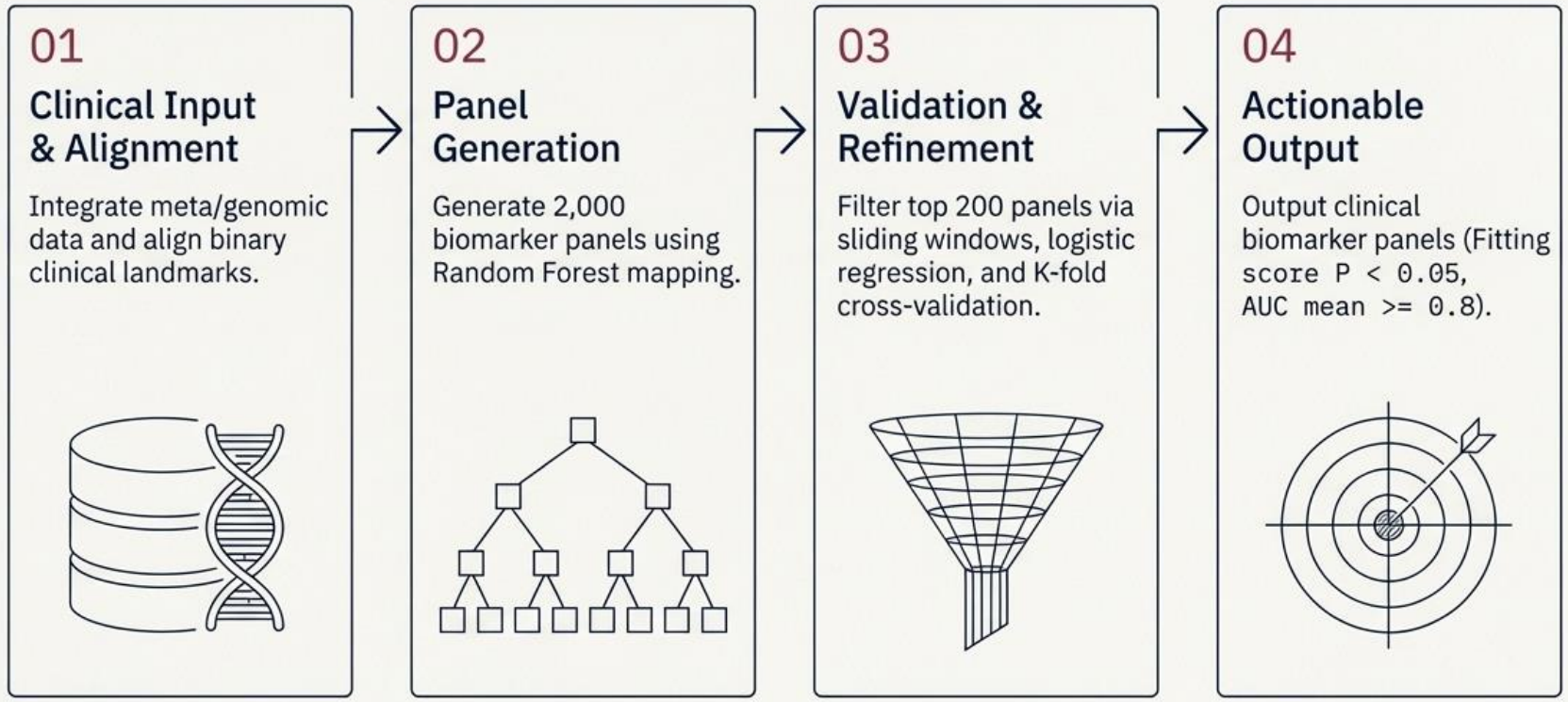
Current trends of biomarker discovery:
A panel of biomarkers instead of a single molecular signature

Justification:
Every stage of disease pathophysiology is associated with multiple molecules with various degrees of involvements; hence a panel of biomarkers would be better candidate

Tools at disposal:
- Rapid handling of a large set of prospective and longitudinal samples
- Next Gen molecular detection tools with high resolution power
- Artificial intelligence/ Machine learning (AI/ML) tools: 2BDP
- Gene transcripts and SIMOA data were processed by 2BDP pipelines for panel development.

Biomarker discovery process at binomial decision point (2BDP): Analytical pipeline to construct biomarker panel. Computational and Structural Biotechnology Journal. 2023 Jan 1;21:4729-42.

The AI/ML Engine: Binomial Decision Point (2BDP) Algorithm



$$logit(P) = a + b X_1 + c X_2 + \cdots + \alpha X_n \ldots\ldots\ldots\ldots$$

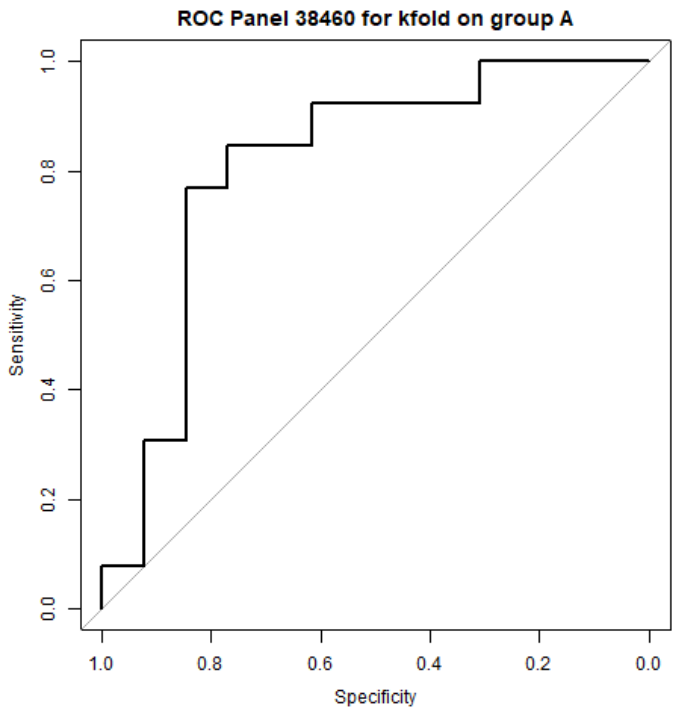
Biomarker discovery process at binomial decision point (2BDP): Analytical pipeline to construct biomarker panel. Computational and Structural Biotechnology Journal. 2023 Jan 1;21:4729-42.

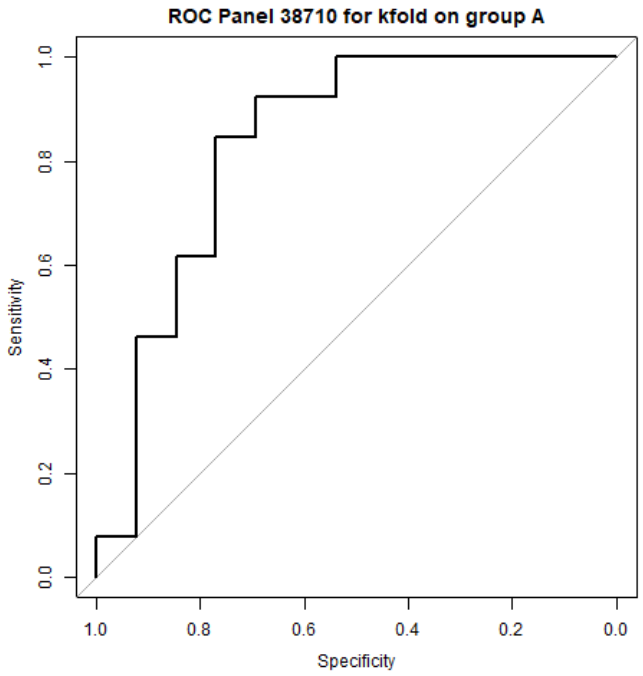
Top performing panels to separate TBI vs. control at acute timepoint

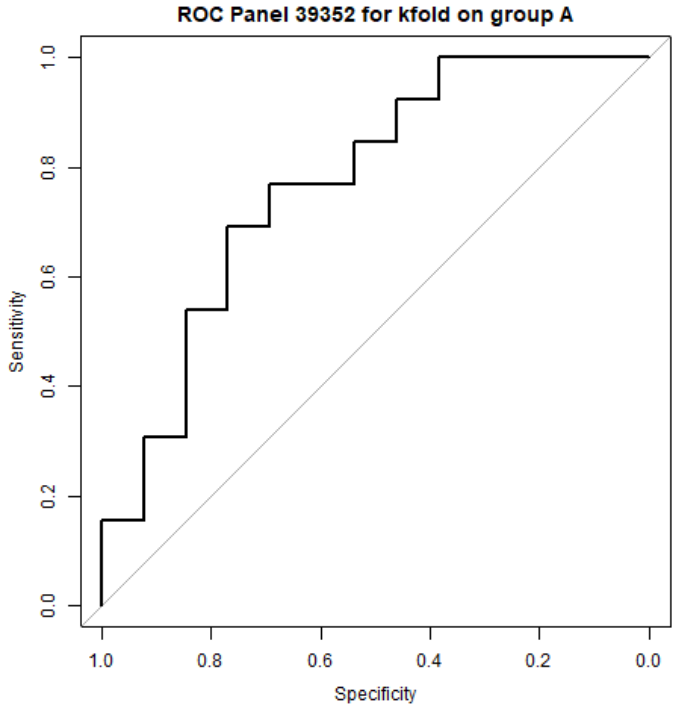
Panel	AUC	Sensitivity	Specificity	Accuracy	Kappa	R2.adj	Features
61636	0.958	0.923	1	0.961	0.923	0.568	GFAP+ TAU+7 probes
11291	0.964	1	0.923	0.961	0.923	0.463	GFAP + (GFAP/Tau) + TAU+ 6 probes
38710	0.964	1	0.923	0.961	0.923	0.426	GFAP+ TAU+5 probes
3434	1	1	0.923	0.961	0.923	0.410	GFAP + (GFAP/Tau) + TAU+ 5 probes
39352	0.994	1	0.923	0.961	0.923	0.405	(GFAP/Tau) + TAU+ 6 probes
38460	0.964	0.846	1	0.923	0.846	0.627	GFAP+ TAU+7 probes
39388	0.988	1	0.846	0.923	0.846	0.594	GFAP+ TAU+7 probes
40820	0.952	1	0.846	0.923	0.846	0.572	GFAP+ TAU+7 probes
59235	0.970	1	0.846	0.923	0.846	0.571	GFAP + (GFAP/Tau) + 7 probes
9141	0.976	0.846	1	0.923	0.846	0.530	GFAP + (GFAP/Tau) + 8 probes

Potential panels to separate mild TBI at the time of asymptomatic state

Representative ROC plots: sex-independent TBI diagnostic panels







Top performing panels to separate TBI vs. control at acute timepoint

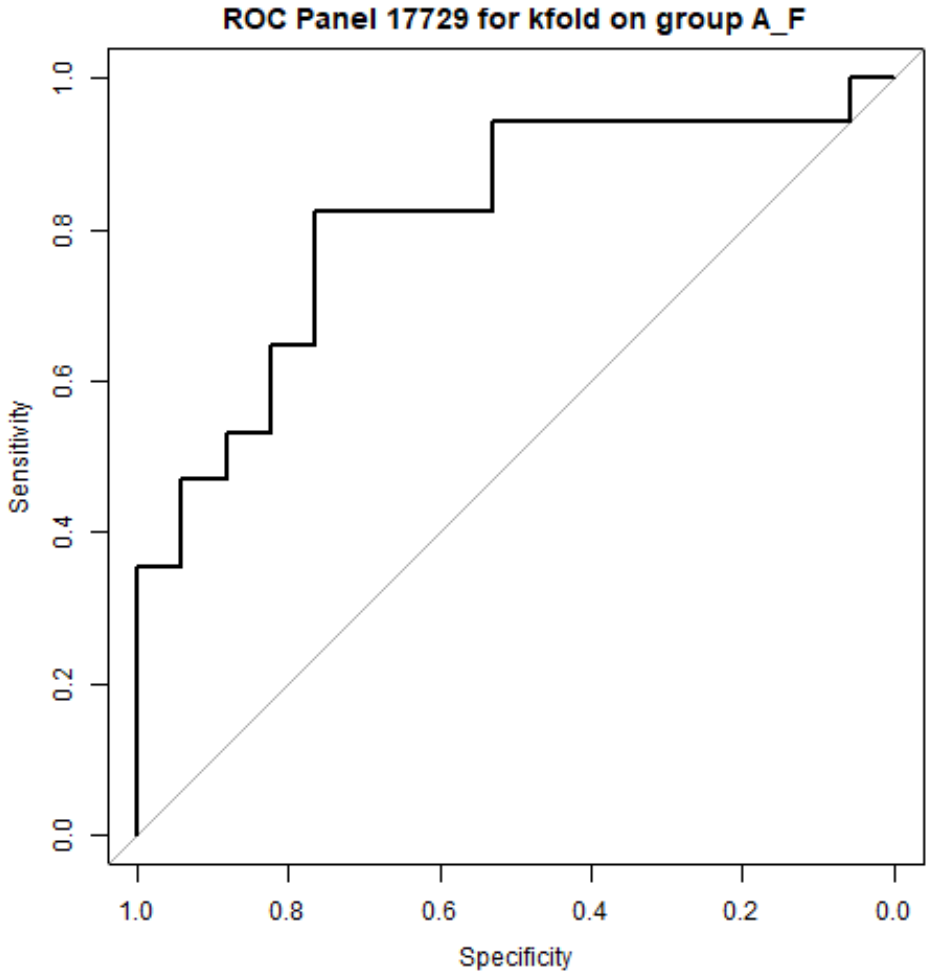
Female:

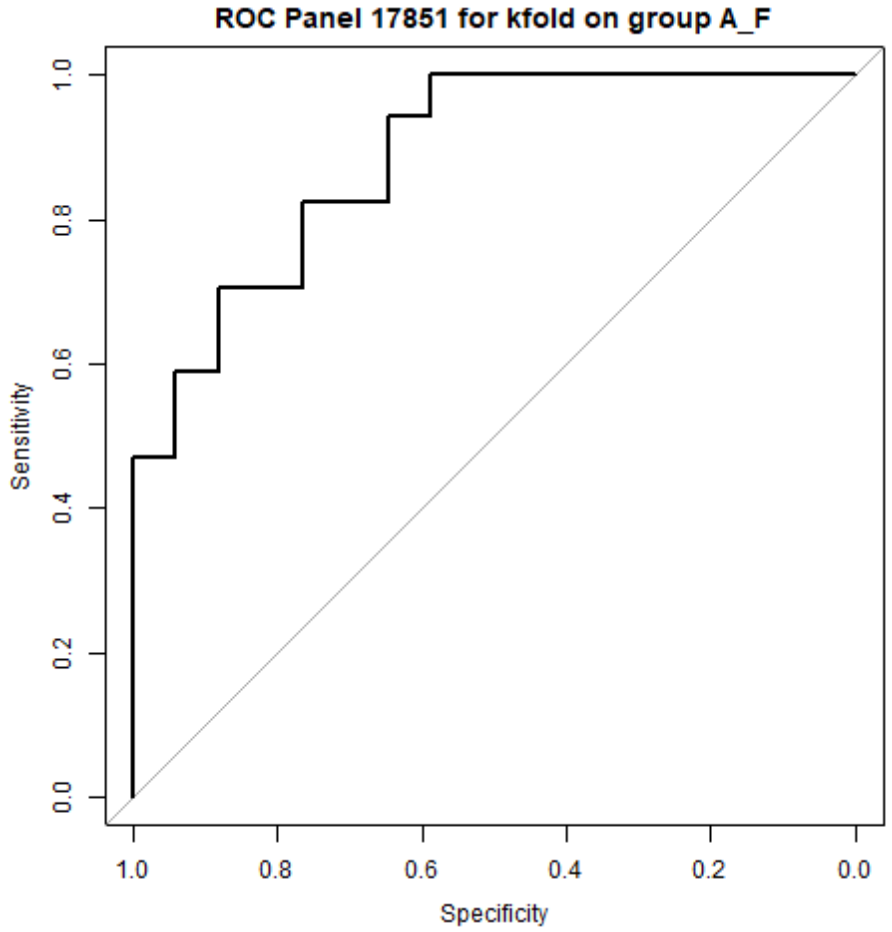
Panel	AUC	Sensitivity	Specificity	Accuracy	Kappa	Accuracy P-Value	McFadden R^2	Features
17816	0.96	0.94	0.94	0.94	0.88	3.47E-08	0.77	GFAP + (GFAP/Tau) + 6 probes
16724	0.95	1	0.82	0.91	0.82	3.83E-07	0.86	(GFAP/Tau) + Tau + 5 probes
17851	0.96	0.88	0.94	0.91	0.82	3.83E-07	0.82	GFAP + (GFAP/Tau) + Tau+ 6 probes
13473	0.95	0.94	0.88	0.91	0.82	3.83E-07	0.78	6 probes
17637	0.96	0.94	0.88	0.91	0.82	3.83E-07	0.77	6 probes
17734	0.95	0.82	1	0.91	0.82	3.83E-07	0.75	(GFAP/Tau) + 6 probes
17739	0.96	0.82	0.94	0.88	0.76	3.08E-06	0.77	Tau + 6 probes
17729	0.95	0.82	0.94	0.88	0.76	3.08E-06	0.77	GFAP + 6 probes
17817	0.96	0.82	0.94	0.88	0.76	3.08E-06	0.75	(GFAP/Tau) + Tau+ 6 probes
16479	0.97	0.94	0.82	0.88	0.76	3.08E-06	0.75	GFAP + (GFAP/Tau) + Tau + 7 probes

Male:

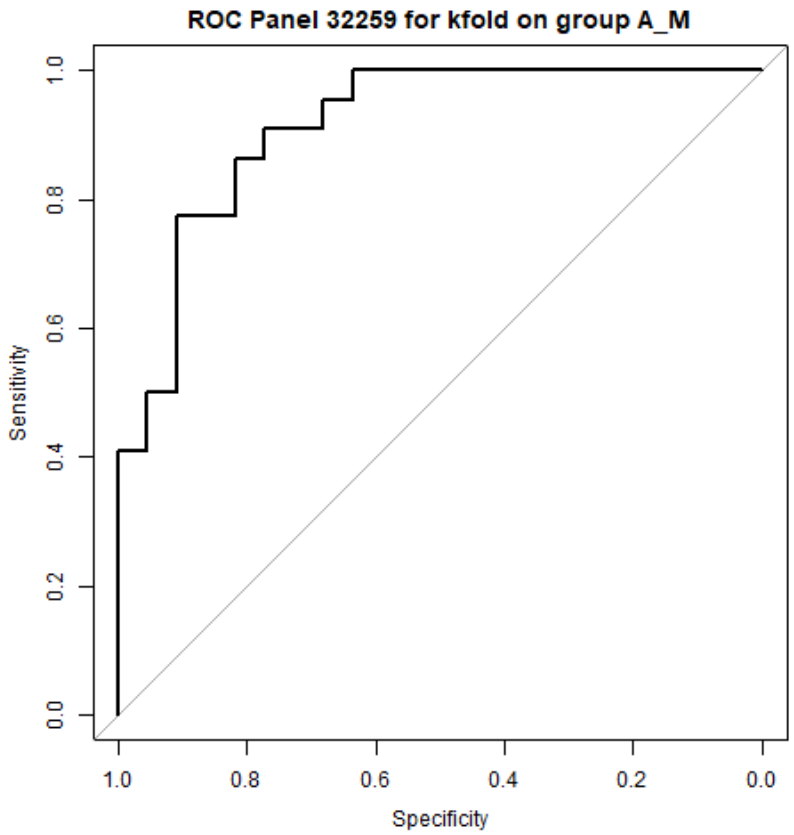
Panel	AUC	Sensitivity	Specificity	Accuracy	Kappa	Accuracy P-Value	McFadden's R^2	Features
20401	0.958	0.909	0.954	0.931	0.863	8.09E-10	0.347	GFAP+ TAU+6 probes
32259	0.960	0.909	0.954	0.931	0.863	8.09E-10	0.318	GFAP+6 probes
37131	0.991	0.909	0.909	0.909	0.818	8.53E-09	0.320	GFAP + (GFAP/Tau) + 4 probes
47197	0.969	0.954	0.863	0.909	0.818	8.53E-09	0.313	TAU + (GFAP/Tau) + 6 probes
47703	0.958	0.909	0.909	0.909	0.818	8.53E-09	0.306	GFAP+ TAU+6 probes

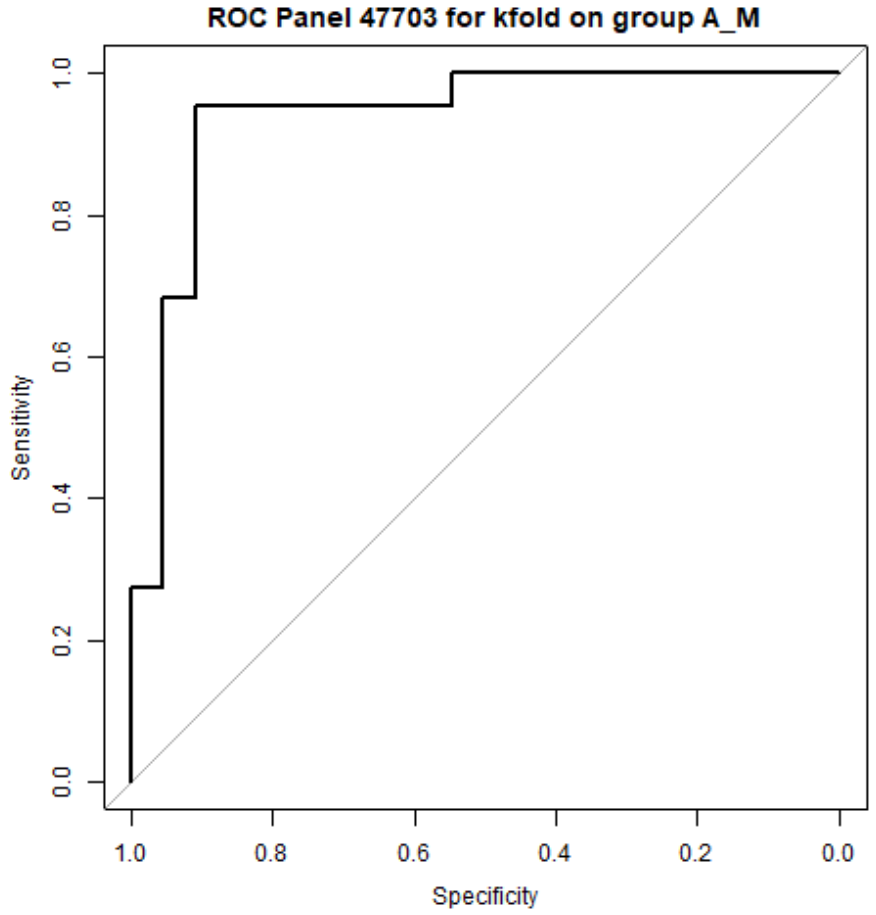
Representative ROC plots: TBI-female





Representative ROC plots: TBI-male





Conversion of 2BDP output to 0-10 scale

Equation to calculate the signal of the panel by 2BDP

$$logit(P) = a + b X_1 + c X_2 + \cdots + \alpha X_n \ldots\ldots\ldots$$

a, b, c… n = constant values

X1, X2, Xn : RT-PCR data

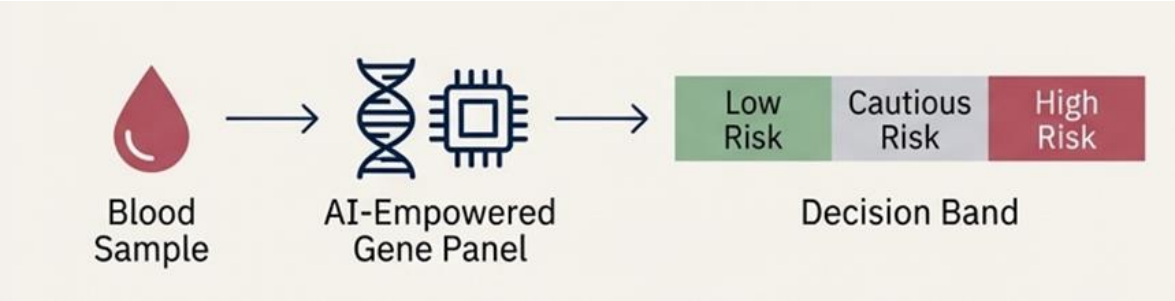
Equation to score from 0 to 10

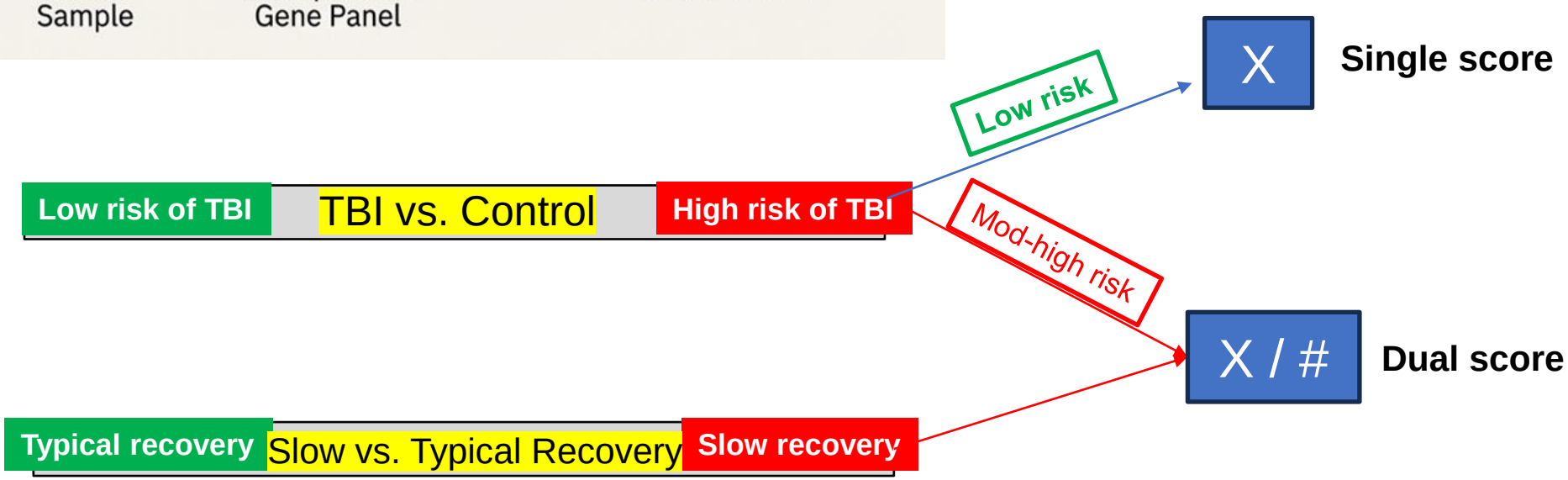
$$x' = to_{min} + \frac{(x - from_{min}) \cdot (to_{max} - to_{min})}{from_{max} - from_{min}}$$

X': Final value; X: Logit(p); $t0_{min} = 0$, $t0_{max} = 10$,

$from_{max}$= highest value in the data, $from_{min}$: lowest value in the data

Data interpretation: Dual scoring system





Protocol outline

Sample collected in PAXgene tube

RNA extraction from Sample collected in Paxgene tube

Total RNA → cDNA

qPCR on QuantStudio Flex 7

Integrate data to determine the score

Plasma extraction

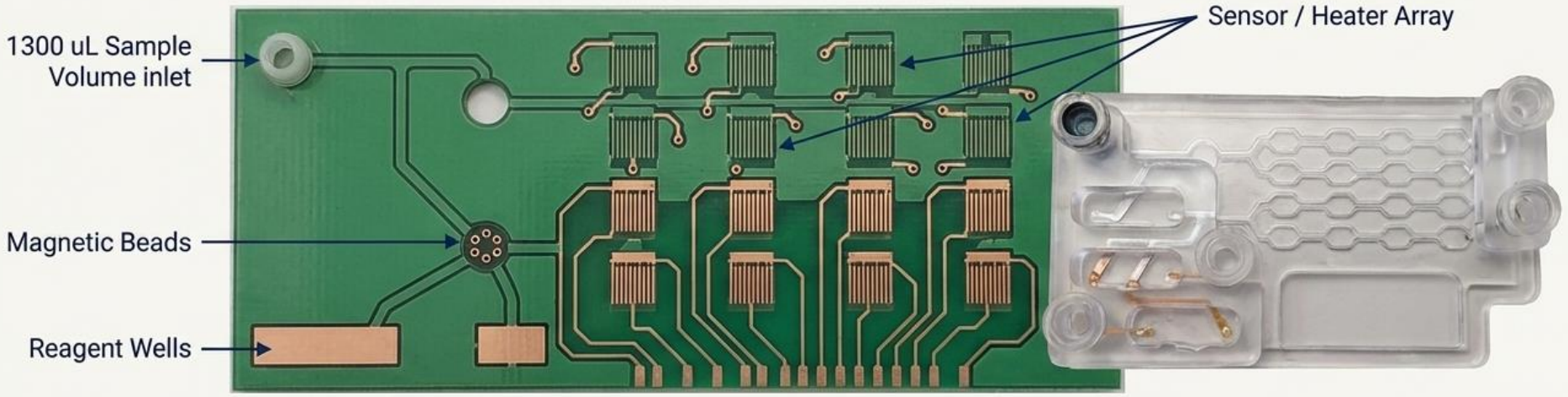
Sample collected in EDTA tube

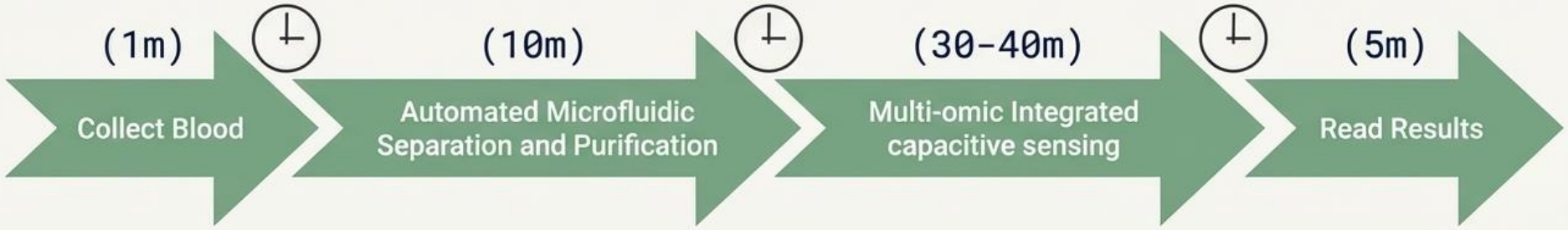
SIMOA/ i-STAT

Handheld Device Developed by STTR Program

RapiOmics: Multiplexed Threat Detection

Hand-held microfluidic device for rapid diagnosis and mass casualty triage. Integrates AI/ML diagnostic predictions directly at Role 1-2. Proposed alignments: CRC2, CRC17, AWG18.





A market comparison

	Input matrix (assay platform)	Hands on time	RTD?/ gender specific?
i-STAT/SIMOA	Plasma (ELISA)	High	No/No
TBI blood omics Biomarker (TBOB)	Whole blood (qPCR ± ELISA)	High-moderate	Ongoing/ Ongoing
rapid TBOB (rTBOB)	Whole blood (RapiOmics)	Minimum	Ongoing/ Ongoing

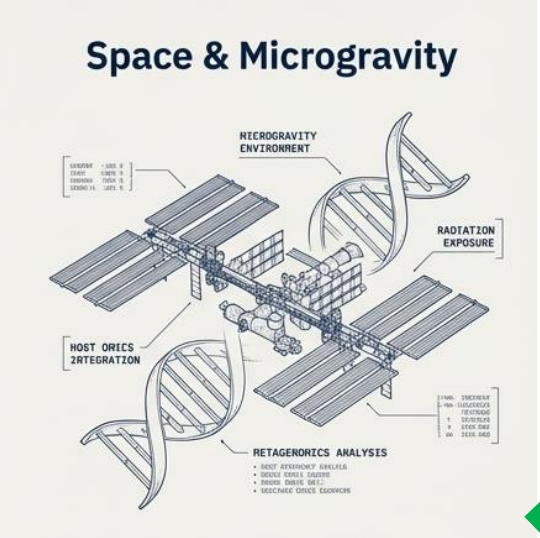
Conclusion

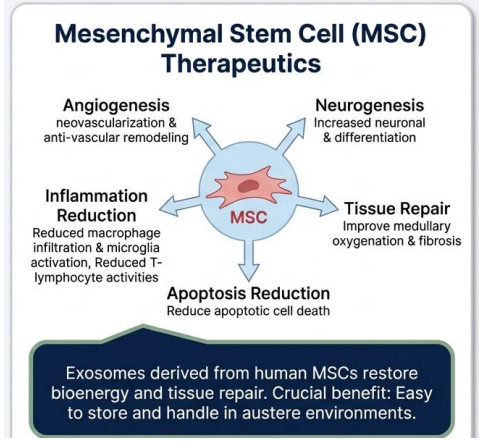
- At the asymptomatic state (acute timepoint), the plasma protein, namely GFAP moderately diagnosed mTBI across male and female. However, *no proteins* could triage slow vs. typical RTP cohort (RTP= 28d).
- Gene PCA plot suggested a strong sex-bias in the developing mTBI and the duration of return to participation (RTP).
- Biomechanism study identified a major perturbations during RTP that justifies further exploration of the time course study to find therapeutic targets.
  - RTP based functional analysis showed changes in biomechanisms related to epigenetic changes between slow vs. typical recovery cohorts.
- Functional analysis suggested some of the key networks linked to mTBI development.

Conclusion (Continued)

- In house AI/ML tool, namely 2BDP identified potential biomarker panels to diagnose mTBI at the acute/ asymptomatic phase from whole blood.
- Sex-specific and sex-independent panels are developed.
- Ongoing study is to develop panels to predict RTP at the acute/ asymptomatic phase from whole blood.
- Pending (funds needed)
  - Validate data using qPCR
  - Validate results with independent cohort
  - IP submission and FDA pre-submission

Thank You





Review of our five Space studies

Session: Current Challenges in DoW Aerospace Medicine
Time: Monday, 1300-1500

hMSC-ex in Cold stress

Session: Human Performance Optimization in Cold-Weather & High-Altitude Environments
Time: Monday, 1530-1730

hMSC-ex in ASD

Session: Cellular Therapeutics for Trauma
Time: Thursday, 1115-1315

TBI Biomarker

Session: Managing the Golden Hour & Beyond: Innovative TBI Care in Contested Combat Environments
Time: Wednesday, 0830-1000

Radiation Therapeutics

Session: Ops in a Nuclear Environment: Ensuring Medical Readiness & Preparing for Complex Injuries
Time: Wednesday, 1530:1730

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