Multi-omics mechanisms tracking the longitudinal path towards return to action from contact sports-induced TBI

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Abstract

Introduction: Understanding the biomechanistic pathways leading to return to action (RTA) is essential for making objective, knowledge-driven decisions. Without such insights, hospital stays are prolonged, healthcare costs increase, and force morale is impacted. This study aims to track incidents up to RTA and beyond, using high-throughput multi-omics analysis to identify functions influencing recovery times toward return to duty (RTD).

Materials and Methods: A cohort of 44 military cadets from the United States Military Academy (USMA), West Point, NY, diagnosed with sport-related concussion, was recruited. The control group consisted of sex-, age-, height-, and weight-matched cadets with recent injuries but no concussion symptoms. Whole blood samples were collected at three timepoints: (i) acute phase, within 48 hours post-injury manifesting mild TBI (mTBI), (ii) at RTA, and (iii) delayed phase, six months post-injury (p.i.). High-throughput RNA-seq assays, miRNA profiling, global metabolomic assays, and SIMOA assays were conducted. Differential expression analysis compared mTBI vs. control and categorized mTBI cadets by RTD timepoints: 14, 21, and 28 days p.i. Nearly 80% of mTBI cadets became asymptomatic by 14 days p.i., while 75% and 85% returned to action by 21 and 28 days p.i., respectively.

Results: Global mRNA profiles revealed sex-based differences in mTBI recovery at all timepoints p.i. Functional analysis showed minimal perturbation at the acute phase but significant immune function and DNA repair network activity at RTA and delayed phases. At the acute phase, metabolomic deficiencies differentiated slow vs. typical recovery cohorts for 14-day and 21-day RTA groups. Epigenetic changes emerged as key factors distinguishing slow vs. typical recovery groups at 28 days RTA.

Conclusion: While functional-level perturbations between mTBI and controls were minimal at the acute phase, deeper analysis revealed significant differences distinguishing slow vs. typical recovery cohorts. These findings enable caregivers to customize treatment during the asymptomatic phase, improving recovery outcomes and reducing time to RTA.

Study Design

Figure 1.

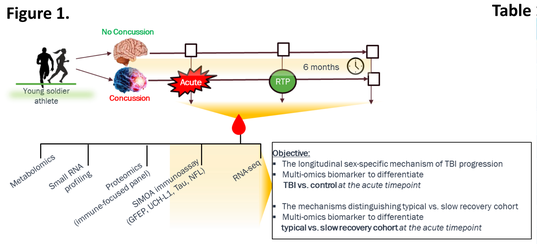

Table 1.

	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)

Figure 1. Overall study design. Blood samples were collected across three time points, namely acute, RTA and at 6 months later. The RTA time points changes for every individual with mTBI. Time dependent controls were recruited across this timeline. A multi-omics assay was conducted. For the present purpose, we used RNA-seq data integrated with SIMOA data.

Table 1. RTA profile (Mean and range), see References 1 and 2. In Care Consortium, 1,751 concussed male and female collegiate athletes were studied. Nearly 80% of participants became asymptomatic by day 14 and/or return to participation (RTA) 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).

Results

Table 2A.

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

Table 2B.

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

Table 2A. Comparative phenotypes of the full cohort. Data from a total of 44 mTBI subjects and matched controls are presented. Significantly altered features are highlighted in yellow. Sport Concussion Assessment Tool (SCAT)–based symptom severity scores differed significantly between TBI participants and controls at the acute phase.

Table 2B. Comparative phenotypes of slow- versus typical-recovery cohorts. Three major RTA timepoints were observed—14, 21, and 28 days. To investigate the biomechanisms underlying each RTA outcome, cohorts were segregated accordingly. Significantly altered features are highlighted in yellow.

Figure 2A.

Figure 2B.

Figure 2C.

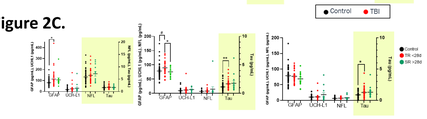

Figure 2. SIMOA data. (A) Plasma proteins in mTBI vs. matched controls. **(B)** Sex-dependent plasma protein profile. **(C)** Plasma protein profile among the control, slow recovery (SR) and typical recovery (TR) cohorts. #0.1> p >0.05; *p <0.05

Figure 3A.

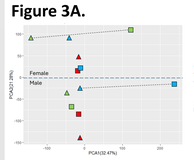

Figure 3B.

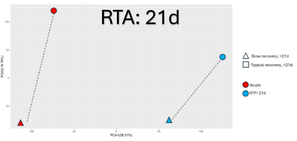

Figure 3C.

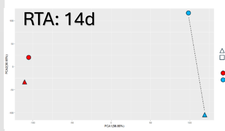

Figure 3D.

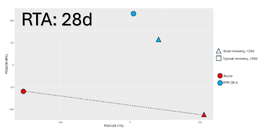

Figure 3E.

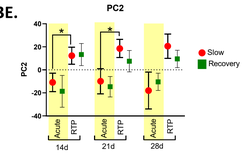

Figure 3. Principal component analysis (PCA) of cohort phenotypes. **(A)** PCA of the entire cohort revealed a strong sex bias. **(B–D)** PCA of the mTBI cohort alone, grouped by slow- and typical-recovery subgroups, with recovery defined by RTA at **(B)** 14, **(C)** 21, and **(D)** 28 days. **(E)** PC2 scores corresponding to panels B–D, showing the features that differed significantly along PC2 between recovery subgroups. No features differed significantly along PC1.

Diagnostic Panel

Figure 4A.

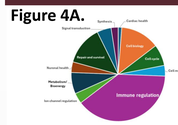

Figure 4B.

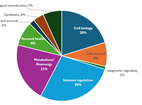

Figure 4C.

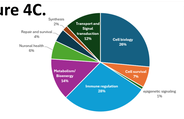

Figure 4D.

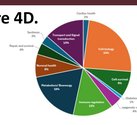

Figure 4. Venn of functional networks. (A) Comparative profile of functional networks differentially enriched between mTBI subjects and controls. **(B–D)** Comparative profiles of functional networks differentially enriched between slow- and typical-recovery cohorts, with recovery defined by RTA at **(B)** 14, **(C)** 21, and **(D)** 24 days. Overlapping regions denote networks shared across comparisons; non-overlapping regions denote networks unique to a given comparison.

Figure 5A.

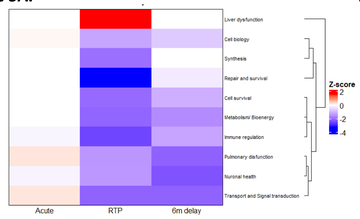

Figure 5B.

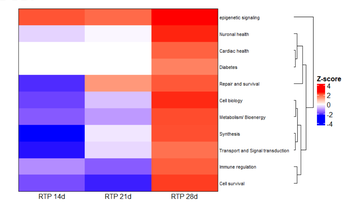

Figure 5. Hierarchical clustering of brain network superfamilies distinguishing mTBI status and recovery trajectory. (A) Hierarchically clustered heatmap of differentially enriched functional networks between mTBI subjects and controls across the three time points. **(B)** Hierarchically clustered heatmap of differentially enriched functional networks at the acute time point that segregate slow- versus typical-recovery cohorts, defined by RTA at 14, 21, and 24 days.

Figure 6A.

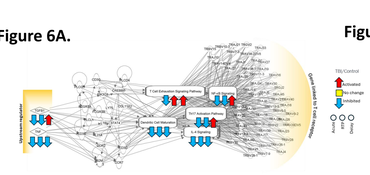

Figure 6B.

Figure 6C.

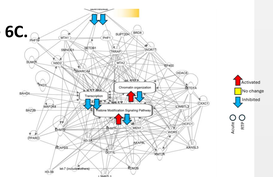

Figure 6. (A) TBI vs. Control: Inhibited dendritic cell maturation. **(B)** TBI vs. Control: Activated BBB breakdown. **(C)** Typical vs. Slow cohort with RTA 28d: Gene signal of epigenetic changes.

Conclusions

- At the asymptomatic (acute) timepoint, the plasma protein glial fibrillary acidic protein (GFAP) showed moderate diagnostic value for mTBI across both males and females. However, no protein could effectively triage the slow- versus typical-recovery cohorts (RTA = 28 days).
- Gene-expression PCA revealed a strong sex bias in both the development of mTBI and the duration of RTA.
- PCA of the TBI cohorts, segregated by RTA, showed a longitudinal trend, particularly along the PC2 axis.
- Only a minimal number of differentially expressed genes were detected at the acute timepoints, underscoring the challenge of identifying early molecular markers of mTBI.
- Mechanistic analysis identified major perturbations during RTA, justifying further time-course studies to uncover potential therapeutic targets.
- RTA-based functional analysis revealed mechanistic changes related to epigenetic regulation distinguishing the slow- versus typical-recovery cohorts.
- Functional analysis identified key networks linked to mTBI development.

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.
3. 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

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