This project was supported in part by the National Collegiate Athletic Association and the Department of Defense. The Defense Health Agency Contracting Activity (DHACA) is the awarding and administering acquisition office. This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs, Defense Health Agency Research and Engineering, and endorsed by the Department of Defense through the Defense Health Agency Research and Engineering Psychological Health and Traumatic Brain Injury Program under Award Nos. W81XWH1420151/W81XWH1820047. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

NCAA•DOD Grand Alliance CARE Consortium

Alternative RNA Splicing 24 Hours Post-Injury: A CARE Consortium Study

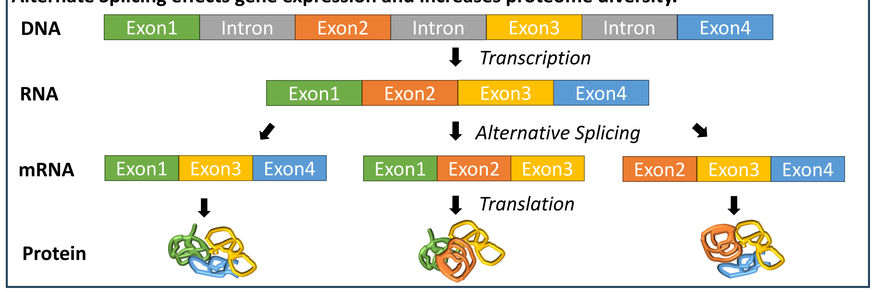
Kelly N. H. Nudelman, PhD[1], Jessie Browne-Michaels, MS[1], Rudong Li, PhD[1,2], Jie Ren, PhD[3], Jill L. Reiter, PhD[1,2], Tatiana M. Foroud, PhD[1], Paul F. Pasquina, MD[4], Steven P. Broglio, PhD, ATC[5], Michael McCrea, PhD, ABPP[6], Thomas W. McAllister, MD[7], Yunlong Liu, PhD[1,2]

[1]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA
[2]Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, Indianapolis, IN, 46202, USA
[3]Department of Biostatistics and Health Data Science, Indiana University, Indianapolis
[4]Uniformed Services University of the Health Sciences, Bethesda, MD
[5]Michigan Concussion Center, School of Kinesiology, University of Michigan, Ann Arbor
[6]Center for Neurotrauma Research, Department of Neurosurgery, Medical College of Wisconsin, Milwaukee
[7]Department of Psychiatry, Indiana University School of Medicine, Indianapolis

Background and Initial Aims

Genomic studies examine inherited DNA variation, whereas RNA sequencing captures the genes actively transcribed under specific biological conditions. Following transcription, RNA may be processed into multiple isoforms through mechanisms such as alternative splicing. These isoforms can differ in stability, regulation, or protein-coding potential, allowing a single gene to contribute to multiple cellular functions [1,2]. Characterizing these changes may therefore provide insight into the acute molecular response to concussion.

Alternate Splicing effects gene expression and increases proteome diversity.



Concussions may induce rapid transcriptomic changes, including alternative splicing, which are not fully characterized in human cohorts. Better understanding of these changes may help elucidate the changes in the brain following injury and identify biological mechanisms as targets for intervention. We investigated genome-wide alternative RNA splicing within 24 hours post-injury in the Concussion Assessment, Research, and Education (CARE) Consortium cohort [3].

Methods

RNA-sequencing data from the CARE phase I cohort were analyzed, restricting to participants with both baseline and 24-hour post-injury samples (N=131 injured; N=98 contact controls). Alternative splicing events were quantified using rMATS to estimate percent splicing inclusion (PSI) across five event types: alternative 3' splice site (A3SS), alternative 5' splice site (A5SS), mutually exclusive exons (MXE), retained introns (RI), and skipped exons (SE) [4]. A zero-one inflated beta regression model assessed associations between PSI at 24 hours and injury status, controlling for baseline PSI and sex. Events were filtered to include those with ≥10 observations per group and 95% credible interval bounds excluding zero.

Five major types of Alternate Splicing events result in mRNA isoforms.

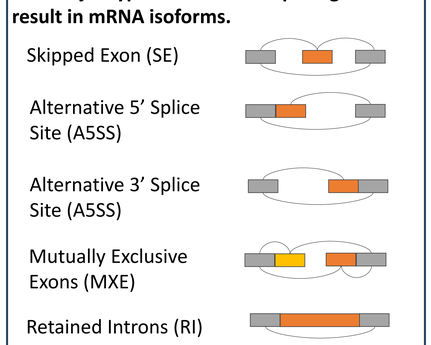


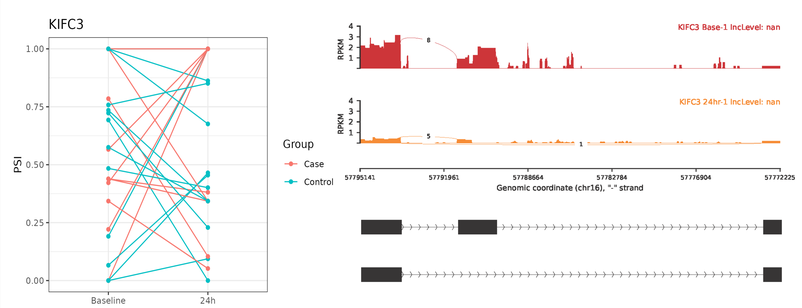
Figure adapted from rmats documentation.

Results

A total of 659,548 splicing events were identified genome-wide, including 33,544 A3SS, 23,483 A5SS, 123,795 MXE, 12,355 RI, and 466,371 SE events. After filtering, 52 events demonstrated significant differences between injured participants and controls (3 A3SS, 1 A5SS, 29 MXE, 1 RI, and 18 SE. These events were further prioritized by identifying those in which mean PSI changed in opposite directions between the injured and control groups. Notable differential splicing events were observed in genes including *KIFC3*, *TOGARAM2*, *ITGB3BP*, *WIZ*, *TAOK3*, and *ZNF652*, suggesting potential involvement of cytoskeletal organization and altered regulation of transcription in early post-injury response.

Event ID	Gene Symbol	Chr	Mean PSI Ctrl, Base	Mean PSI Ctrl, 24hr	Mean PSI, Inj, Base	Mean PSI, Inj, 24hr	N, Ctrl	N, Inj
SE_274235	KIFC3	chr16	0.556	0.517	0.522	0.622	13	10
A5SS_3879	LINC01588	chr14	0.869	0.847	0.880	0.901	22	15
MXE_69966	ENSG00000291103	chr16	0.827	0.787	0.803	0.907	24	17
MXE_31406	NIPA1	chr15	0.123	0.068	0.164	0.169	28	21
MXE_82265	PLB1	chr2	0.293	0.400	0.261	0.189	16	11
MXE_117773	ZNG1C	chr9	0.790	0.724	0.719	0.898	16	11
SE_25455	WIZ	chr19	0.819	0.959	0.848	0.763	18	15
SE_38080	ZNF652	chr17	0.099	0.109	0.090	0.073	54	50
SE_153824	POPDC2	chr3	0.917	0.768	0.944	0.975	13	10
SE_221739	TTN-AS1	chr2	0.909	0.928	0.915	0.874	18	14
SE_233814	ITGB3BP	chr1	0.842	0.899	0.862	0.748	18	11
SE_274230	KIFC3	chr16	0.706	0.711	0.670	0.612	18	12
SE_319732	TOGARAM2	chr2	0.621	0.672	0.789	0.579	25	17
SE_336031	ENSG00000257524	chr9	0.638	0.915	0.746	0.592	14	10
SE_342081	TAOK3	chr12	0.115	0.072	0.084	0.161	17	11
SE_457924	ENSG00000291072	chr1	0.845	0.883	0.802	0.785	34	28

Alternative Slicing Event SE_274235 results in an isoform of KIFC3, a member of the kinesin-14 family of microtubule motors, which is upregulated in the injured group at 24 hr.



Discussion

At 24 hours after concussion, differential alternative splicing was observed in genes involved in cytoskeletal organization, stress-responsive gene regulation, and membrane-associated processes. Splicing changes in genes such as ***KIFC3***, ***TOGARAM2***, and ***ITGB3BP*** may reflect acute remodeling of microtubule-dependent cellular structure and intracellular organization. Changes involving ***WIZ***, ***TAOK3***, and ***ZNF652*** further suggest altered regulation of transcription and cellular stress responses following injury.

Splicing differences were also identified in genes associated with ion transport, lipid metabolism, and muscle-related biology, including ***NIPA1***, ***PLB1***, ***POPDC2***, and ***TTN-AS1*** [5,6]. These findings may indicate that the circulating RNA response to concussion includes both central and systemic components. However, muscle-associated transcripts could also reflect accompanying peripheral injury, recent physical exertion, or differences in circulating cell populations rather than concussion-specific mechanisms [7].

Differential Alternative Splicing 24 hr Post-Injury

- **Cellular Remodeling**
  - KIFC3: Microtubule Motor Protein
  - TOGARAM2: Microtubule Organizer
  - ITGB3BP: Centromere-Associated Protein
- **Stress and Gene Regulation**
  - WIZ: Transcriptional Regulator
  - TAOK3: Stress-Signaling Kinase
  - ZNF652: Transcriptional Regulator
  - ZNG1C: Protein-Maturation GTPase
- **Systemic and Membrane-Associated Response**
  - NIPA1: Magnesium Transporter
  - PLB1: Membrane Lipid Enzyme
  - POPDC2: Membrane Signaling Protein
  - TTN-AS1: Titin-Regulating RNA

The occurrence of several zinc-associated regulatory proteins and a magnesium transporter raises the possibility that metal-ion homeostasis or metal-dependent protein function contributes to the acute injury response. These results provide hypotheses for future studies incorporating serum mineral measurements and clinical outcomes.

Impact and Future Directions

These findings identify widespread alternative splicing changes within 24 hours of concussion and support further investigation of their biological and clinical relevance. Ongoing work will focus on replication in an independent cohort and evaluation of specific isoforms in relation to symptom severity and recovery.

Weighted Gene Co-expression Network Analysis (WGCNA) will be used to identify co-expression modules associated with the observed splicing events, followed by Gene Ontology enrichment analysis to clarify their broader biological context [8].

Because whole blood can be collected repeatedly with relatively low burden, RNA sequencing may provide a practical approach for monitoring warfighters and athletes after injury. With further validation, injury-associated isoforms could contribute to a multivariable biomarker panel that supports recovery monitoring and return-to-activity decisions.

References: **[1]** Marasco, L. E., & Kornblihtt, A. R. (2023). The physiology of alternative splicing. *Nature Reviews Molecular Cell Biology, 24*, 242–254. **[2]** Leclair, N. K., & Anczuków, O. (2022). "Poisoning" of the transcriptome by ultraconserved elements. *Nature Reviews Molecular Cell Biology, 23*, 777. **[3]** Broglio SP, McCrea M, McAllister T, Harezlak J, Katz B, Hack D, Hainline B; CARE Consortium Investigators. A National Study on the Effects of Concussion in Collegiate Athletes and US Military Service Academy Members: The NCAA-DoD Concussion Assessment, Research and Education (CARE) Consortium Structure and Methods. Sports Med. 2017 Jul;47(7):1437-1451. doi: 10.1007/s40279-017-0707-1. PMID: 28281095; PMCID: PMC5488134. **[4]** Wang, Y., Xie, Z., Kutschera, E. *et al.* rMATS-turbo: an efficient and flexible computational tool for alternative splicing analysis of large-scale RNA-seq data. *Nat Protoc* 19, 1083–1104 (2024). doi: 10.1038/s41596-023-00944-2 **[5]** The UniProt Consortium. UniProt: the Universal Protein Knowledgebase in 2025. *Nucleic Acids Research*. 2025;53(D1):D609–D617. doi:10.1093/nar/gkae1010. **[6]** Sayers EW, Beck J, Bolton EE, et al. Database resources of the National Center for Biotechnology Information in 2025. *Nucleic Acids Research*. 2025;53(D1):D20–D29. doi:10.1093/nar/gkae979. **[7]** Sadek A, Petrier M, Fourgeux C, Braud M, Gourain V, Bouras M, Poulain C, Moumni M, Roquilly A, Poschmann J. A conserved monocyte activation program links brain injury to systemic immune adaptation and clinical outcomes. Front Immunol. 2026 Mar 19;17:1731068. doi: 10.3389/fimmu.2026.1731068. PMID: 41939870; PMCID: PMC13043442. **[8]** Langfelder, P., Horvath, S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 9, 559 (2008). Doi: 10.1186/1471-2105-9-559

Contact: *Kelly N. H. Nudelman, khohohan@iu.edu, and Jessie Browne-Michaels, jbmchls@iu.edu , Indiana University School of Medicine, Health Information and Translational Sciences HS 4000, 410 W 10th Street, Indianapolis, IN, 46202*

Acknowledgements: *This project was supported in part by the National Collegiate Athletic Association and the Department of Defense. The Defense Health Agency Contracting Activity (DHACA) is the awarding and administering acquisition office. This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs, Defense Health Agency Research and Engineering, and endorsed by the Department of Defense through the Defense Health Agency Research and Engineering Psychological Health and Traumatic Brain Injury Program under Award Nos. W81XWH1420151/W81XWH1820047. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.*

Funding support is provided by the U.S. Army Medical Research and Development Command (USAMRDC), Combat Casualty Care Research Program (CCCRP) through the Medical Technology Enterprise Consortium (MTEC) as project MTEC-21-01-WBH-001 under Other Transaction Number W81XWH-15-9-0001, with additional funding provided by the NCAA and the Defense Health Agency via a Cooperative Agreement with the NFL. Funding support is also provided by the Cure Alzheimer's Foundation and BrightFocus Foundation.

Visit www.careconsortium.net for more information.

The opinions and assertions expressed herein are those of the authors and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of Defense.