IRONMAN WORLD CHAMPIONSHIP MEDICAL SYMPOSIUM

Biomarkers for Concussion: A New Era?

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Ironman Medical Symposium 2023

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Disclosures

I am an owner of an NFL team

(Dr. Sills can confirm 😉)

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Overview

- The problems of **diagnosis and prognosis** in SRCs
- Potential objective tests
  - What's available/what's in the works
- **miRNA as a possible biomarkers**
  - What are they?
  - How have they been studied?
- The problem of prolonged recovery
  - '**persisting** post-concussive symptoms' (PPCS)'
  - Potential tools to predict this ***(research*** not clinical)

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What this talk is NOT about

Clinical Journal of SPORT MEDICINE
Founded in 1990 by the Canadian Academy of Sport and Exercise Medicine

ORIGINAL RESEARCH

Concussion-Associated Gene Variant *COMT* rs4680 Is Associated With Elite Rugby Athlete Status

Antrobus, Mark R. PhD[*,†]; Brazier, Jon PhD[*,‡]; Callus, Peter PhD[*]; Herbert, Adam J. PhD[§]; Stebbings, Georgina K. PhD[*]; Day, Stephen H. PhD[¶]; Kilduff, Liam P. PhD[‖]; Bennett, Mark A. BSc[‖]; Erskine, Robert M. PhD[**,††]; Raleigh, Stuart M. PhD[‡‡]; Collins, Malcolm PhD[§§]; Pitsiladis, Yannis P. PhD[¶¶]; Heffernan, Shane M. PhD[‖]; Williams, Alun G. PhD[*,††]

Author Information

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The 'problem' with concussion

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- Difficulties with diagnosis
  - Not entirely 'objective' measures
- Difficulties with prognosis
  - Contributes to patient/familial anxiety
  - Who gets PPCS?
- Difficulties with the fundamentals of research
  - 'misclassification'

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BJSM 2017 Systematic Review

- Neuroimaging
  - DTI
  - Task-based, resting fMRI
  - EEG/quantitative EEG
  - MR spectroscopy
- Biomarkers
  - **Glial fibrillary acidic protein (GFAP)**
  - **Ubiquitin c-terminal hydrolase L1 (UCH L1)**
  - Multiple other serum markers
  - Salivary *cortisol*
- *No mention of miRNAs*

Role of advanced neuroimaging, fluid biomarkers and genetic testing in the assessment of sport-related concussion: a systematic review

Michael McCrea,[1] Timothy Meier,[1,2] Daniel Huber,[1] Alain Ptito,[3,4] Erin Bigler,[5] Chantel T Debert,[6] Geoff Manley,[7] David Menon,[8] Jen-Kai Chen,[9] Rachel Wall,[10] Kathryn J Schneider,[11] Thomas McAllister[10]

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Other potential tests

- Optical coherence tomography (OCT)
  - Macula
  - Retinal nerve fiber layer thickness
    - thicknesses

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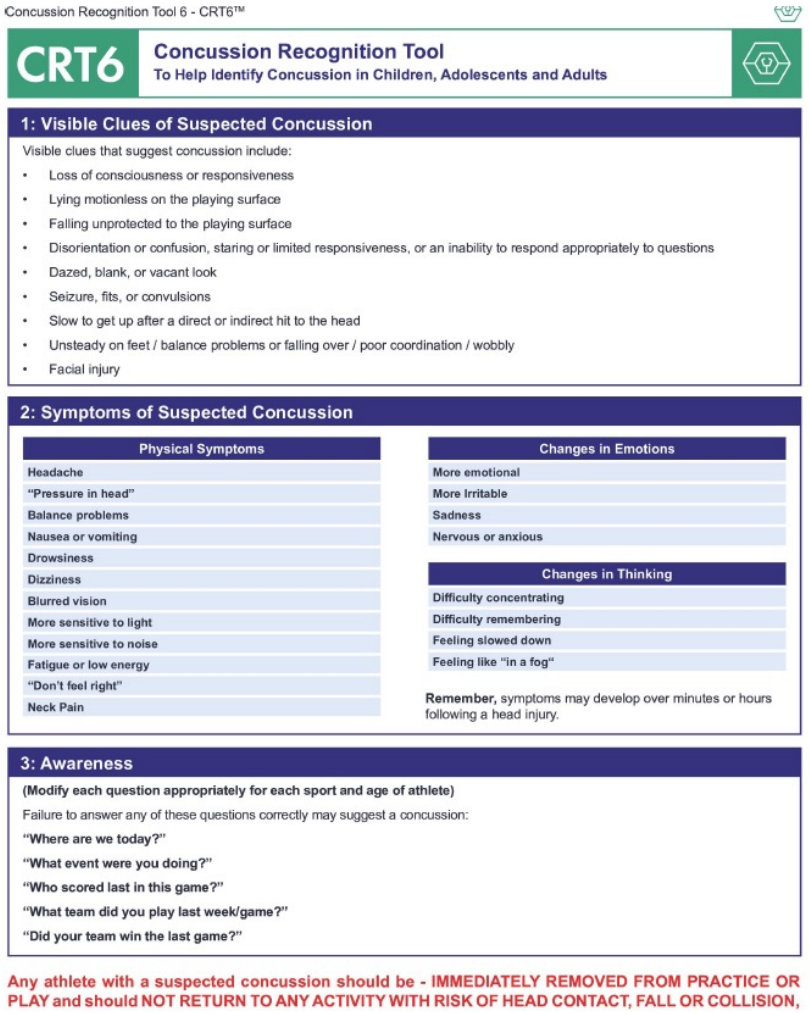
CRT6

- Concussion Recognition Tool 6
- Sign/symptoms
- **'Recognize and remove'**
- How does one identify or 'diagnose' a concussion???



Concussion Recognition Tool 6 - CRT6™

CRT6 — **Concussion Recognition Tool**
To Help Identify Concussion in Children, Adolescents and Adults

1: Visible Clues of Suspected Concussion

Visible clues that suggest concussion include:

- Loss of consciousness or responsiveness
- Lying motionless on the playing surface
- Falling unprotected to the playing surface
- Disorientation or confusion, staring or limited responsiveness, or an inability to respond appropriately to questions
- Dazed, blank, or vacant look
- Seizure, fits, or convulsions
- Slow to get up after a direct or indirect hit to the head
- Unsteady on feet / balance problems or falling over / poor coordination / wobbly
- Facial injury

2: Symptoms of Suspected Concussion

Physical Symptoms
Headache
"Pressure in head"
Balance problems
Nausea or vomiting
Drowsiness
Dizziness
Blurred vision
More sensitive to light
More sensitive to noise
Fatigue or low energy
"Don't feel right"
Neck Pain

Changes in Emotions
More emotional
More Irritable
Sadness
Nervous or anxious

Changes in Thinking
Difficulty concentrating
Difficulty remembering
Feeling slowed down
Feeling like "in a fog"

Remember, symptoms may develop over minutes or hours following a head injury.

3: Awareness

(Modify each question appropriately for each sport and age of athlete)

Failure to answer any of these questions correctly may suggest a concussion:

"Where are we today?"
"What event were you doing?"
"Who scored last in this game?"
"What team did you play last week/game?"
"Did your team win the last game?"

Any athlete with a suspected concussion should be - IMMEDIATELY REMOVED FROM PRACTICE OR PLAY and should NOT RETURN TO ANY ACTIVITY WITH RISK OF HEAD CONTACT, FALL OR COLLISION,

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NEJM May 2023

- CARE Consortium
  - SCAT
  - SAC
  - Brief Sx Inventory 18
  - BESS
  - ImPACT
- Most clinical assessments normalized 2 – 7 d
- Visual memory and reaction time (14d and 18d) took longer

"Concussion diagnoses and clearance for return to play were **determined on the basis of the overall clinical impression,** which was informed in part by the results of the assessments."

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Biomarkers -- the Holy Grail?

- Might this be a POC test on sideline or in clinic like urine HCG? Serum HgbA1c?
- Diagnosis and/or prognosis?

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Potential biomarkers

sources

- Serum
- **Saliva**
- Urine

types

- Proteins
- **miRNAs**

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Biomarkers in evaluation of mTBI

- GFAP UCH-L1 are proteins with FDA-approval
- Measure levels < 12 hours from injury can distinguish individuals with ‘concussion’ who may warrant CT scan (?intracranial lesion)
- Downside for true concussion (mTBI)
  - Proteins large -- may require BBB disruption for detection peripherally
  - May be good acutely (but are degraded by proteases)

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JAMA Neurol 2016 73: 551 – 560

NCAA Dept. of Defense CARE Consortium

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- Concussion (N = 284), contact sport controls (N = 138), non-contact sport controls (N = 102)
- **GFAP, UCHL-1, tau**, neurofilament light chain
- Concussed – significant elevations in GFRP and tau
- AUC for distinguishing concussed vs. contact controls for GFAP and UCHL-1 **0.71 [0.64 – 0.78]**

JAMA Network Open

Original Investigation | Neurology

Association of Blood Biomarkers With Acute Sport-Related Concussion in Collegiate Athletes

Findings From the NCAA and Department of Defense CARE Consortium

Michael McCrea, PhD, ABPP; Steven P. Broglio, PhD; Thomas W. McAllister, MD; Jessica Gill, PhD; Christopher C. Giza, MD; Daniel L. Huber, MPH; Jaroslaw Harezlak, PhD; Kenneth L. Cameron, PhD; Megan N. Houston, PhD; Gerald McGinty, DPT; Jonathan C. Jackson, MD; Kevin Guskiewicz, PhD; Jason Mihalik, PhD; M. Alison Brooks, MD, MPH; Stephan Duma, PhD; Steven Rowson, PhD; Lindsay D. Nelson, PhD; Paul Pasquina, MD; Timothy B. Meier, PhD; and the CARE Consortium Investigators

Abstract

IMPORTANCE There is potential scientific and clinical value in validation of objective biomarkers for sport-related concussion (SRC).

OBJECTIVE To investigate the association of acute-phase blood biomarker levels with SRC in collegiate athletes.

DESIGN, SETTING, AND PARTICIPANTS This multicenter, prospective, case-control study was conducted by the National Collegiate Athletic Association (NCAA) and the US Department of Defense Concussion Assessment, Research, and Education (CARE) Consortium from February 20, 2015, to May 31, 2018, at 6 CARE Advanced Research Core sites. A total of 504 collegiate athletes with concussion, contact sport control athletes, and non-contact sport control athletes completed clinical testing and blood collection at preseason baseline, the acute postinjury period, 24 to 48 hours after injury, the point of reporting being asymptomatic, and 7 days after return to play. Data analysis was conducted from March 1 to November 30, 2019.

MAIN OUTCOMES AND MEASURES Glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCH-L1), neurofilament light chain, and tau were quantified using the Quanterix Simoa multiplex assay. Clinical outcome measures included the Sport Concussion Assessment Tool-Third Edition (SCAT-3) symptom evaluation, Standardized Assessment of Concussion, Balance Error Scoring System, and Brief Symptom Inventory 18.

RESULTS A total of 264 athletes with concussion (mean [SD] age, 19.08 [1.24] years; 211 [79.9%] male), 138 contact sport controls (mean [SD] age, 19.03 [1.27] years; 107 [77.5%] male), and 102 non-contact sport controls (mean [SD] age, 19.39 [1.25] years; 82 [80.4%] male) were included in

Key Points

Question Is sport-related concussion associated with levels of traumatic brain injury biomarkers in collegiate athletes?

Findings In this case-control study of 504 collegiate athletes with concussion, contact sport control athletes, and non-contact sport athletes, the athletes with concussion had significant elevations in multiple traumatic brain injury biomarkers compared with preseason baseline and with 2 groups of control athletes without concussion during the acute postinjury period.

Meaning These results suggest that blood biomarkers can be used as research tools to inform the underlying pathophysiological mechanism of concussion and provide additional support for future studies to optimize and validate biomarkers for potential clinical use in sport-related concussion.

+ Invited Commentary

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JAMA Network Open 2020

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"FDA Approved"

- iStat TBI – plasma test
  - **GFAP**
  - **UCH L-1**
- FDA approved January 2021
- Liken to troponin in chest pain of "Ottawa Ankle Rules" for ankle sprain
- Help distinguish higher level TBI from mTBI
  - (guides in determining need for CT scan)

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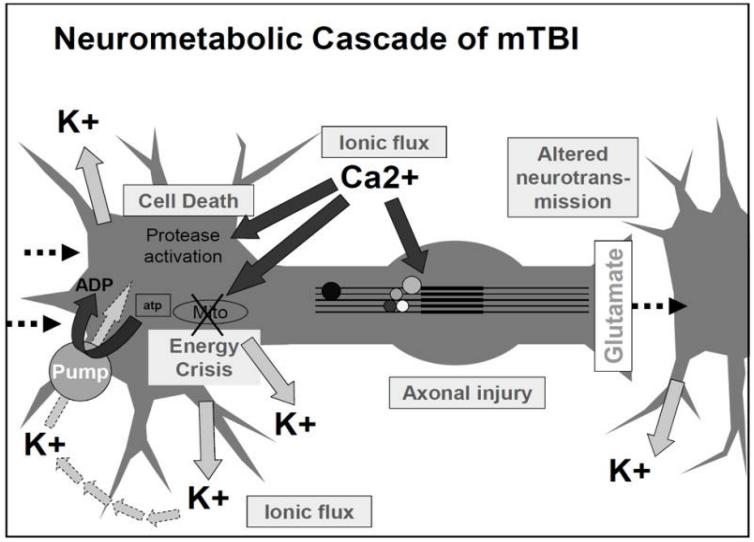
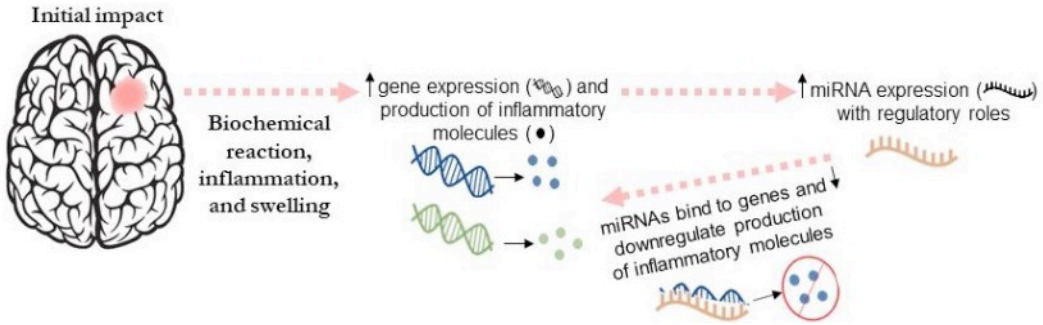
Biology of concussion





Acute cellular biological processes occurring after concussion/mild TBI.

Giza CC, Hovda DA. The new neurometabolic cascade of concussion. *Neurosurgery*. 2014;75 Suppl 4(0 4):S24-S33. doi:10.1227/NEU.0000000000000505

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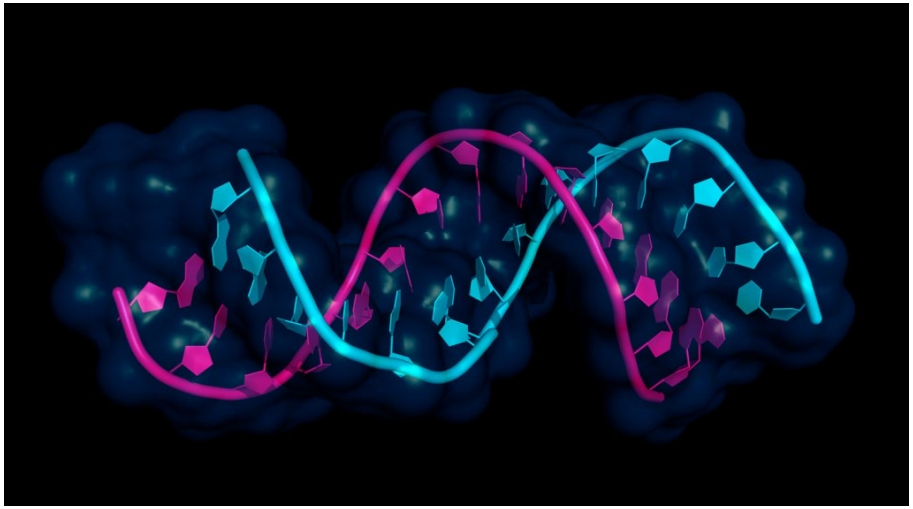
microRNAs – what are they?

- miRNAs – short (19- 28 nucleotides) noncoding molecules found throughout the body (serum, CSF, saliva)
- Function in RNA silencing and post-transcriptional regulation of gene expression



- Transported through extracellular space by exosomes & microvesicles

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More on miRNAs

- A type of SNCRNA
- Have been looked at in
  - Alzheimer's, Parkinson's
  - **Autism**
  - Alcoholism
  - Multiple cancers

- Critical for neurodevelopment and brain function
- Circulating miRNAs elevated after injury
- miRNA expression profiles differ between healthy and disease states.
- 100s of circulating miRNAs

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miRNAs in concussion

- CNS contains highest concentration and diversity of miRNAs in body
- miRNAs are small enough (unlike proteins) that they can cross BBB and be found in peripheral fluids (e.g. saliva, urine) without injury to BBB (as would be expected in mTBIs)
- Implicated in both the 1°and 2° damage responses to TBI
- miRNA profiles may predict the trajectory of recovery from brain injury.

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Atif, Hicks 2019

Table 1.

Putative targets of the 17 miRNAs with the highest TBI biomarker potential.

MicroRNA Targets	miR-181a-5p ↑ (4)	miR-128-3p ↓ (4)	miR-16-5p ↑ (4)	miR-221-3p ↓ (4)	miR-26b-5p ↑ (3)	miR-27b-3p ↑ (6)	miR-29a/c-3p ↓ (4)	miR-30a-5p ↑ (5)	miR-320c ↑ (4)	miR-532-5p ↑ (3)	miR-1307-3p ↑ (3)	miR-151a-3p ↑ (2)	miR-7-5p ↓ (3)	miR-629-5p ↓ (3)	miR-10a/b-5p ↑ (3)	mRNA targets
Total no. of transcripts	49	197	203	20	246	313	168	380	72	7	124	40	105	8	82	2014
ECM-receptor interaction (FDR = 2.3E-39)	1	4	0	0	1	4	13	1	0	0	1	0	0	0	0	ITGA8, COL4A5, COL27A1, ITGA5, COL3A1, SV2A, COL2A1, RELN, COL5A1, COL4A4, COL1A2, LAMC1, COL11A1, COL6A3, LAMA2, COL5A3, COL5A2, SPP1, COL4A1, VEGFA
Pluripotency of stem cells (FDR = 1.5E-06)	1	5	11	0	6	7	2	3	1	0	0	0	1	0	1	BMI1, JARID2, GSK3B, WNT7A, INHBB, APC, WNT10B, REST, MAPK14, RAF1, INHBA, WNT4, ZFHX3, FZD3, ACVR1, ACVR2B, FZD10, LIFR, PIK3R1, ACVR2A, FGF2, ACVR1C, IGF1, AKT3, BMPR1A, WNT3A, ISL1, GRB2, SMAD1, PIK3R2
Amphetamine addiction (FDR = 2.6E-05)	0	4	0	0	1	5	1	5	1	0	2	0	1	0	2	AFT2, CAMK4, CREB5, PPP1CC, PPP3R1, DRD1, GRIA1, CREB1, PPP3CA, SLC6A3, PRKX, PRKCB, CAMK2B, GRIA4, GRIN2D
Cocaine addiction (FDR = 0.020)	0	2	1	0	2	2	0	4	1	0	1	0	0	0	2	ATF2, CREB5, DRD1, GPSM1, GRM3, CDK5R1, BDNF, CREB1, SLC6A3, PRKX, GRIN2D
Neurotrophin signaling (FDR = 0.021)	0	6	4	0	3	6	3	4	1	0	0	0	0	0	1	GSK3B, SH2B3, CAMK4, MAP2K7, RAP1A, MAPK14, RAF1, BCL2, PRKCD, BDNF, KIDINS220, RPS6KA6, PIK3R1, SOS1, IRS1, GAB1, AKT3, CAMK2B, PRDM4, GRB2, RAP1B, NGFR, PIK3R2
Glioma (FDR = 0.0022)	0	1	3	0	2	2	3	1	1	0	3	0	1	0	1	REF1, EGFR, CDK6, E2F3, PIK3R1, SOS1, PRKCB, IGF1, AKT3, CAMK2B, PTEN, GRB2, PIK3R2
ErbB signaling (FDR = 0.0056)	0	4	1	1	2	7	1	3	0	0	1	0	3	0	1	GSK3B, HBEGF, MAP2K7, RAF1, CDKN1B, EGFR, CBLB, NRG3, PIK3R1, SOS1, PRKCB, GAB1, AKT3, CAMK2B, MAP2K4, ABL2, GRB2, PIK3R2
Long-term potentiation (FDR = 0.016)	0	4	3	0	3	7	0	7	1	0	0	0	2	0	1	CAMK4, PPP1CC, RAP1A, GRM5, PPP3R1, RAF1, GRIA1, PPP3CA, PLCB1, RPS6KA6, PRKX, PRKCB, CAMK2B, GRIN2D, RAP1B

ECM, extracellular matrix; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; TBI, traumatic brain injury.

The 17 miRNAs identified in ≥2 human TBI studies, across 3 biofluids (cerebrospinal fluid, saliva, and blood), were interrogated for putative mRNA targets. Together, they targeted 2014 coding transcripts with high confidence (microT-CDS > 0.975) which demonstrated enrichment (FDR < 0.05) for 22 KEGG signaling pathways. Of the 22 pathways, 8 implicated in brain-related processes are shown here (FDR *P*-values in parentheses). The number of mRNAs targeted by each miRNA in the respective pathway is displayed. Arrows denote the general direction of change for each miRNA, along with the number of studies in which it was detected (in parentheses).

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5 P tool from PERC: Predicting Persisting Post-Concussion Sx

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- Follow up 28d post concussion
  - Female sex
  - Age > 13
  - PMH migraine
  - PMH concussion > 1 wk
  - Headache
  - Phonophobia
  - Fatigue
  - Answering questions slowly
  - > 4 errors BESS

- N = 3063 patients
  - Median 12 (IQR 9.2 – 14.6)
- **Clinical Risk Score** (12 pt)
- AUC 0.71 [0.69 – 0.74]
- **"Modest discrimination to stratify PPCS risk at 28d."**

JAMA 2016 "Clinical Risk Score for Persistent Postconcussion Symptoms Among Children with Acute Concussion in the ED

JAMA Johnson et al. (2018)

Association of Saliveary miRNA Changes with Prolonged Concussion Symptoms

- N = 52
- 42% female
- **Age (7 – 21)**
- Heterogenous MOI
- Within 14 d of injury
- Salivary miRNA sample at time of initial clinical presentation (one time point)
- F/U 4 weeks SS > 5 = PPCS

5 biomarkers accurately identifying patients with PPCS

- *miR320c-1*
- *miR-133a-5p*
- *miR-769-5p*
- *let-7a-3p*
- *miR-1307-3p*

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NCH Study (2022)

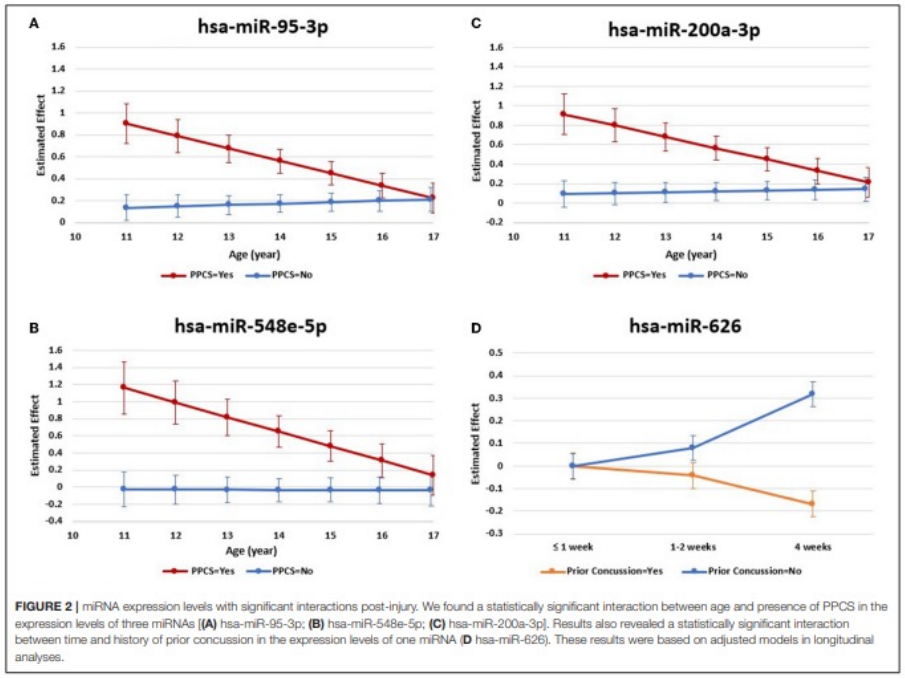

FIGURE 2 | miRNA expression levels with significant interactions post-injury. We found a statistically significant interaction between age and presence of PPCS in the expression levels of three miRNAs [**(A)** hsa-miR-95-3p; **(B)** hsa-miR-548e-5p; **(C)** hsa-miR-200a-3p]. Results also revealed a statistically significant interaction between time and history of prior concussion in the expression levels of one miRNA (**D** hsa-miR-626). These results were based on adjusted models in longitudinal analyses.

frontiers | Frontiers in Public Health

ORIGINAL RESEARCH
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Salivary miRNA Expression in Children With Persistent Post-concussive Symptoms

Katherine E. Miller[1†], James P. MacDonald[2,3†], Lindsay Sullivan[4,5], Lakshmi Prakruthi Rao Venkata[1], Junxin Shi[6], Keith Owen Yeates[7], Su Chen[8], Enas Alshaikh[4], H. Gerry Taylor[3,9], Amanda Hautmann[4], Nicole Asa[4,10], Daniel M. Cohen[3,11], Thomas L. Pommering[2,3], Elaine R. Mardis[1,3,12‡], Jingzhen Yang[3,4‡] and the NCH Concussion Research Group[§]*

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OPEN ACCESS

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Background: Up to one-third of concussed children develop persistent post-concussive symptoms (PPCS). The identification of biomarkers such as salivary miRNAs that detect concussed children at increased risk of PPCS has received growing attention in recent years. However, whether and how salivary miRNA expression levels differ over time between concussed children with and without PPCS is unknown.

Aim: To identify salivary MicroRNAs (miRNAs) whose expression levels differ over time post-concussion in children with vs. without PPCS.

Methods: We conducted a prospective cohort study with saliva collection at up to three timepoints: (1) within one week of injury; (2) one to two weeks post-injury; and (3) 4-weeks post-injury. Participants were children (ages 11 to 17 years) with a physician-diagnosed concussion from a single hospital center. We collected participants' daily post-concussion symptom ratings throughout their enrollment using the Post-concussion Symptom Scale, and defined PPCS as a total symptom score of ≥ 5 at 28 days post-concussion. We extracted salivary RNA from the saliva samples and measured expression levels of 827 salivary miRNAs. We then compared the longitudinal expression levels of salivary miRNAs in children with vs. without PPCS using linear models with repeated measures.

Results: A total of 135 saliva samples were collected from 60 children. Of the 827 miRNAs analyzed, 91 had expression levels above the calculated background threshold and were included in the differential gene expression analyses. Of these 91 miRNAs, 13 had expression levels that differed significantly across the three timepoints

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miRNA Extraction & Analysis

Saliva sample → Oragene RNA RE collection vial → RNA isolation → NanoString miRNA expression assay → Digital readout of each miRNA expression → Statistical analysis

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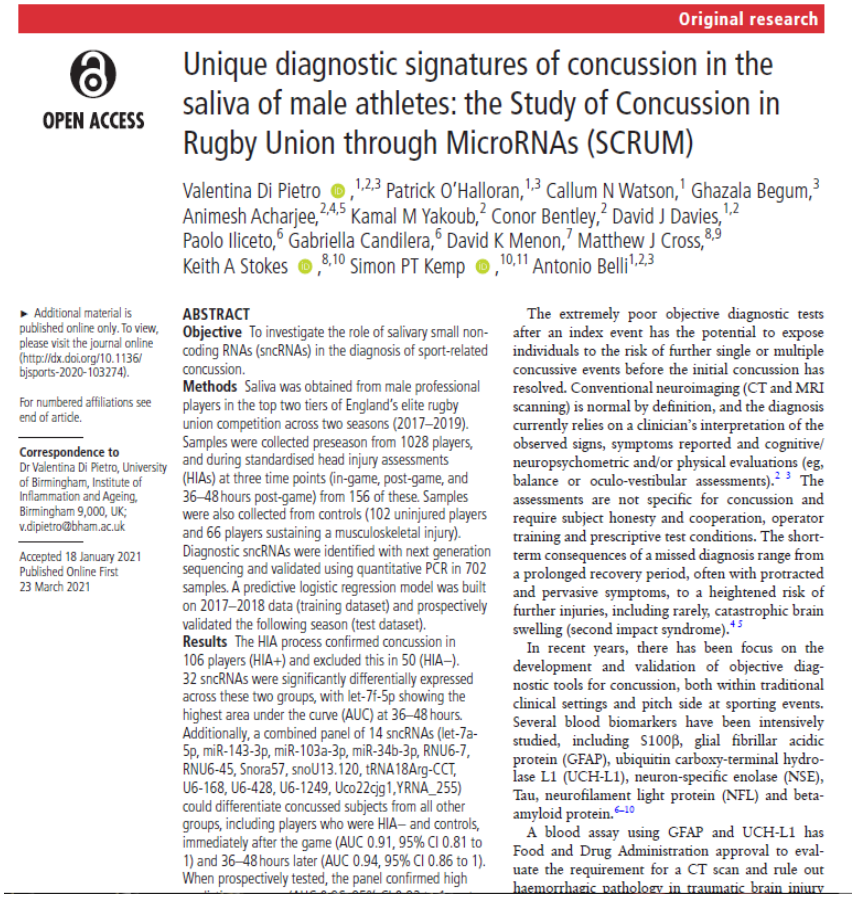
My *favorite* miRNA study

- 2017 – 2019 seasons
- Top 2 tiers English Rugby
- 1028 players pre-season
- 156 HIA players
  - In game, post game, 36 - 48°
- 102 uninjured controls
- 66 MSK injured
- **AUC > 0.9 for 'fingerprint' panel**



Original research

OPEN ACCESS

Unique diagnostic signatures of concussion in the saliva of male athletes: the Study of Concussion in Rugby Union through MicroRNAs (SCRUM)

Valentina Di Pietro ,[1,2,3] Patrick O'Halloran,[1,3] Callum N Watson,[1] Ghazala Begum,[3] Animesh Acharjee,[2,4,5] Kamal M Yakoub,[2] Conor Bentley,[2] David J Davies,[1,2] Paolo Iliceto,[6] Gabriella Candilera,[6] David K Menon,[7] Matthew J Cross,[8,9] Keith A Stokes ,[8,10] Simon PT Kemp ,[10,11] Antonio Belli[1,2,3]

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bjsports-2020-103274).

For numbered affiliations see end of article.

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ABSTRACT

Objective To investigate the role of salivary small non-coding RNAs (sncRNAs) in the diagnosis of sport-related concussion.

Methods Saliva was obtained from male professional players in the top two tiers of England's elite rugby union competition across two seasons (2017–2019). Samples were collected preseason from 1028 players, and during standardised head injury assessments (HIAs) at three time points (in-game, post-game, and 36–48 hours post-game) from 156 of these. Samples were also collected from controls (102 uninjured players and 66 players sustaining a musculoskeletal injury). Diagnostic sncRNAs were identified with next generation sequencing and validated using quantitative PCR in 702 samples. A predictive logistic regression model was built on 2017–2018 data (training dataset) and prospectively validated the following season (test dataset).

Results The HIA process confirmed concussion in 106 players (HIA+) and excluded this in 50 (HIA–). 32 sncRNAs were significantly differentially expressed across these two groups, with let-7f-5p showing the highest area under the curve (AUC) at 36–48 hours. Additionally, a combined panel of 14 sncRNAs (let-7a-5p, miR-143-3p, miR-103a-3p, miR-34b-3p, RNU6-7, RNU6-45, Snora57, snoU13.120, tRNA18Arg-CCT, U6-168, U6-428, U6-1249, Uco22cjg1,YRNA_255) could differentiate concussed subjects from all other groups, including players who were HIA– and controls, immediately after the game (AUC 0.91, 95% CI 0.81 to 1) and 36–48 hours later (AUC 0.94, 95% CI 0.86 to 1). When prospectively tested, the panel confirmed high

The extremely poor objective diagnostic tests after an index event has the potential to expose individuals to the risk of further single or multiple concussive events before the initial concussion has resolved. Conventional neuroimaging (CT and MRI scanning) is normal by definition, and the diagnosis currently relies on a clinician's interpretation of the observed signs, symptoms reported and cognitive/neuropsychometric and/or physical evaluations (eg, balance or oculo-vestibular assessments).[2 3] The assessments are not specific for concussion and require subject honesty and cooperation, operator training and prescriptive test conditions. The short-term consequences of a missed diagnosis range from a prolonged recovery period, often with protracted and pervasive symptoms, to a heightened risk of further injuries, including rarely, catastrophic brain swelling (second impact syndrome).[4 5]

In recent years, there has been focus on the development and validation of objective diagnostic tools for concussion, both within traditional clinical settings and pitch side at sporting events. Several blood biomarkers have been intensively studied, including S100β, glial fibrillar acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), neuron-specific enolase (NSE), Tau, neurofilament light protein (NFL) and beta-amyloid protein.[6–10]

A blood assay using GFAP and UCH-L1 has Food and Drug Administration approval to evaluate the requirement for a CT scan and rule out haemorrhagic pathology in traumatic brain injury

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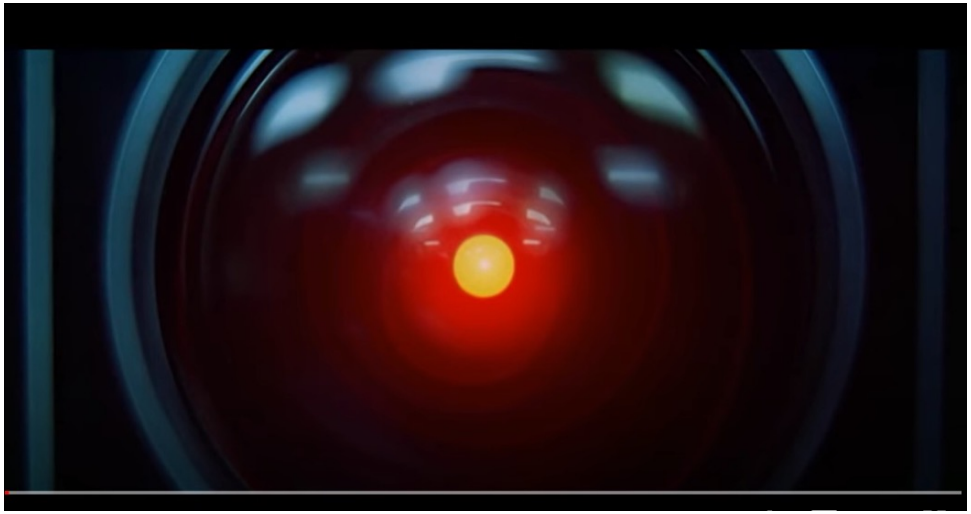
Do you read me HAL?



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Brave New World

- Large data bases shared
  - HIPAA issues
  - Proprietary issues
- Machine learning
- “Literature Based Discovery” (LBD)
- ‘Fingerprints’ of miRNA patterns
  - Diagnosis
  - Prognosis

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Summary

- No current biomarkers are thought by CISG/Amsterdam to be clinically 'prime time' for *SRCs*
- The 1st clinically available biomarker in USA is designed to assess need for CT scan
  - GFAP / UCH-L1
- Salivary miRNAs are a promising biomarker
  - Easy to sample and store
  - Potential for diagnosis and prognosis
- Technology will be a game changer
  - It IS a new era 😉

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Thank you

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