Genome-wide Blood DNA Methylation Profiles for Cognitive Declines among Professional Fighters*

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Closed Head Traumas—*Traumatic Brain Injuries (TBI)*

➤ A leading cause of neurological, cognitive, & psychosocial disability[1,2]

➤ 1/3 of all injury deaths involve TBI[3]

➤ Over 75-80% considered 'mild' (mTBI) or concussion[4]

➤ Annually 1.7 million TBIs occurring in the US[3]

  ❑ >40% with long-term disability[5]

➤ If *repetitive*, persistent functional impairments & higher risk for chronic traumatic encephalopathy (CTE)[6]
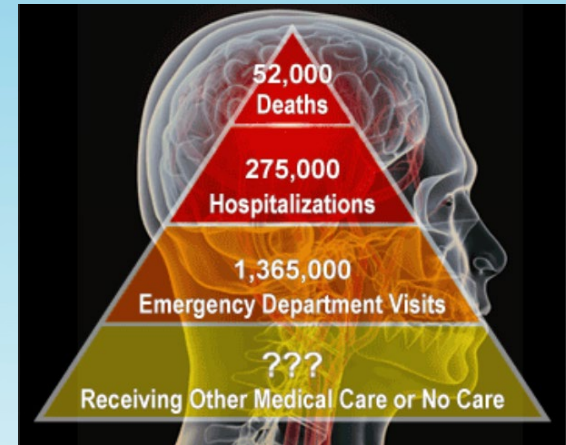
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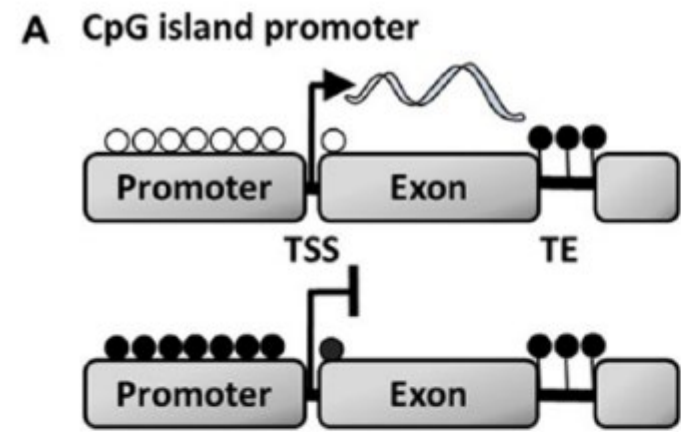6. Bernick C, Banks SJ, Shin W, et al. *Br J Sports Med*. 2015;49(15):1007-1011.

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Persistent Functional Impairments
following Repetitive Head Traumas—*TBIs*

Executive dysfunction

Parietal lobe
Perception, spatial awareness, manipulating objects, spelling

Wernicke's area
Understanding language

Broca's area
Expressing language

Frontal lobe
Planning, organising, emotional and behavioural control, personality, problem solving, attention, social skills, flexible thinking and conscious movement

Occipital lobe
Vision

Temporal lobe
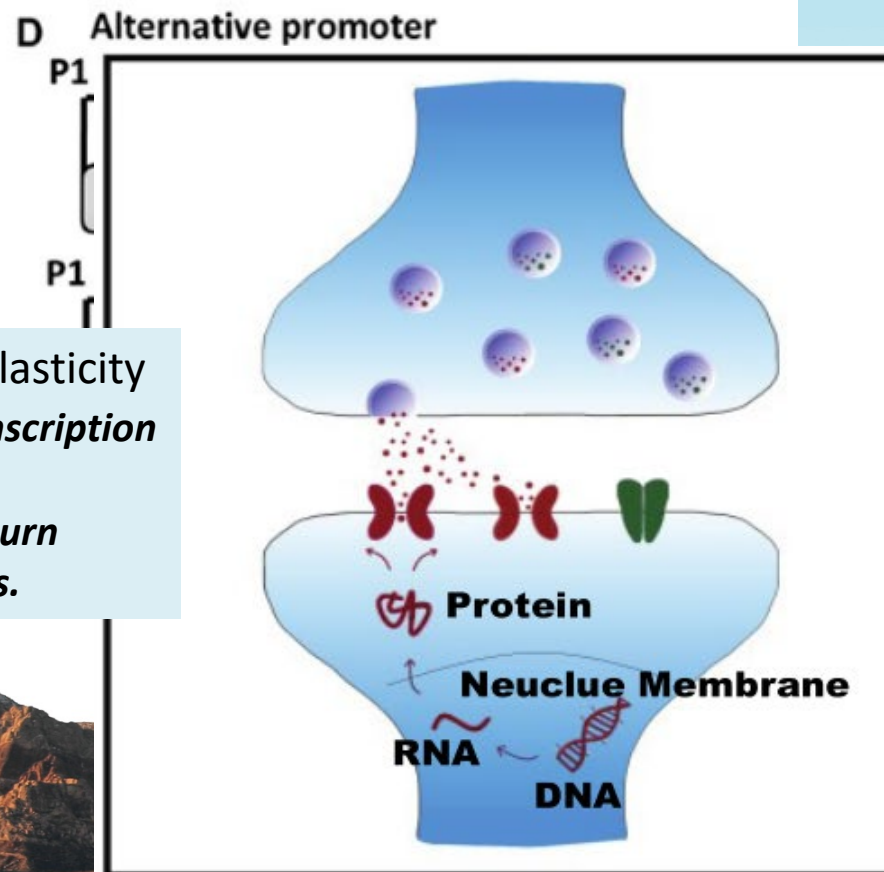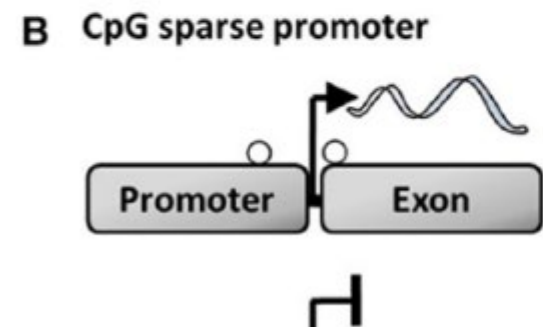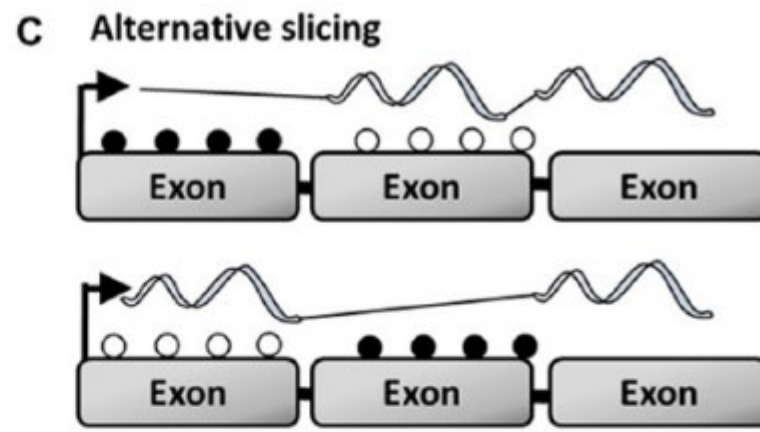Memory, recognising faces, generating emotions, language

➢ Cognitive declines

    ❑ >40% of the affected individuals[1]

    ❑ 25% within mTBIs[2]

➢ Inability to return to work 1-year post-injury of further 25%[3]

➢ ***Cognitive decline variabilities***

    ❑ Physiological responses to the head injury via molecular activities, regulated by epigenetic mechanisms,[4] including DNA methylation[5,6]
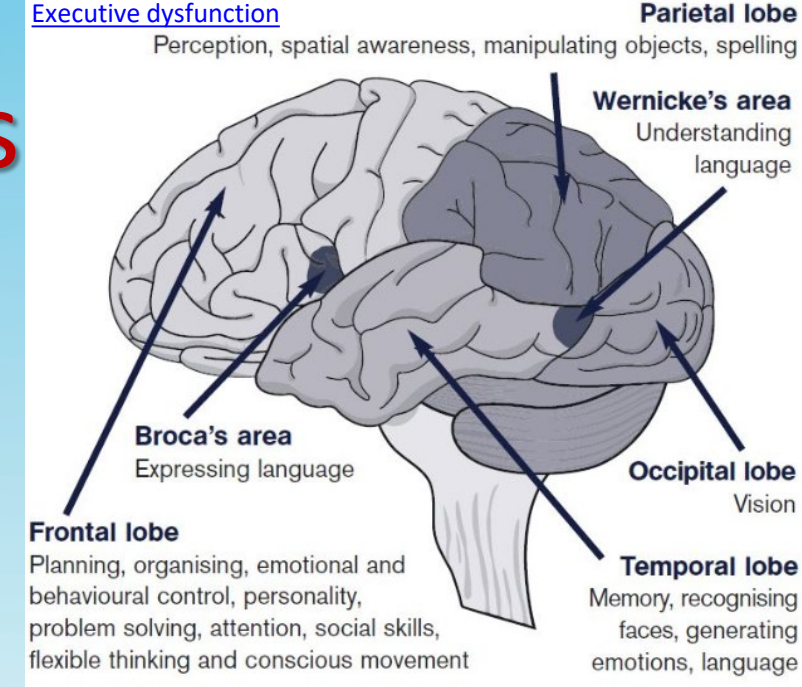
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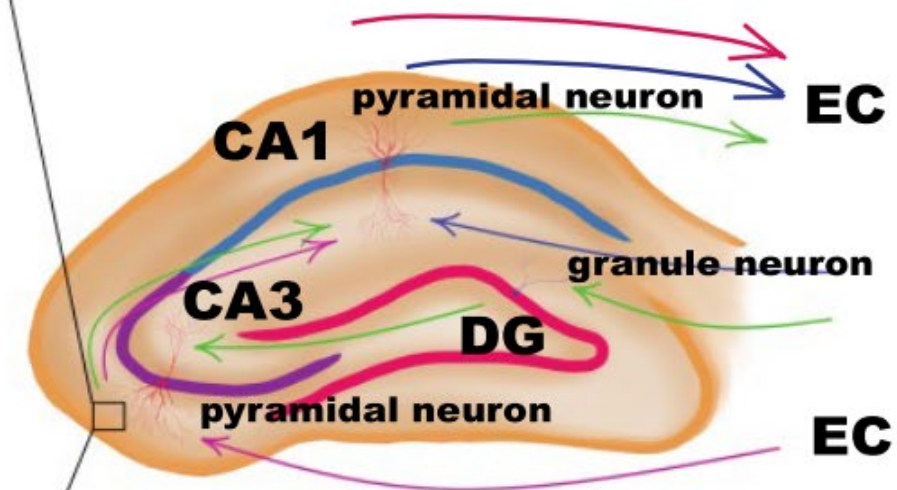6. Chouliaras L, Pishva E, Haapakoski R, et al. *Epigenomics*. 2018;10(5):585-595.

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A CpG island promoter

Promoter | Exon
TSS | TE

Promoter | Exon

B CpG sparse promoter

Promoter | Exon

C Alternative slicing

Exon | Exon | Exon

Exon | Exon | Exon

D Alternative promoter

P1

P1

Barter JD, Foster TC. *Neurosci*. 2018;24(5):516-525.

DNA methylation regulating transcription -
The relationship between DNA methylation and gene expression is dependent on genome location (promoter, enhancer and silencer within introns and exons of the gene). Methylation in DNA promoter regions, particularly for CpG islands, is the canonical mechanism for cell-specific gene silencing during development.

Cui D, Xu X, Cui D, Xu X. *Int J Mol Sci*. 2018;19(5):1315.

Protein
Neuclue Membrane
RNA
DNA

pyramidal neuron — EC
CA1
CA3
granule neuron
DG
pyramidal neuron
EC

Hippocampal Circuits and Synaptic Plasticity
- *DNA methylation regulates RNA transcription of synaptic plasticity genes, further modulating ion channel receptors, in turn mediating synaptic plasticity in circuits.*

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Purpose

➢ To investigate **whole-genome blood DNA methylation** associated to cognitive declines within the following years among male professional fighters

  ❑ Recruited from the Professional Fighters Brain Health (PFBHS) longitudinal cohort study (PI: Bernick), funded by the Lincy Foundation[1,2]

    o Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, Nevada, USA

1. Bernick C, Banks S, Phillips M, et al. *Am J Epidemiol*. 2013;178(2):280-286.
2. Bernick C, Banks SJ, Shin W, et al. *Br J Sports Med*. 2015;49(15):1007-1011.

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Methods

➢ Original PFBHS[1]: 224 male fighters—93 boxers and 131 MMAs, & 22 controls

➢ This study: 15 boxers, 10 MMAs, & 5 controls

➢ Cognitive performance measured by **CNS Vital Signs** (CNSVS)[2]

❑ **Processing speed domain** - its reduction consistent with repeated concussion and considered a component of the clinical manifestation of CTE[3,4]

❑ Changes between baseline (T1) and follow-ups in 1-3 years (T2, T3, or T4)

1. Bernick C, Banks S, Phillips M, et al. *Am J Epidemiol*. 2013;178(2):280-286.
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3. Mendez MF. *Int J Psychiatry Med*. 1995;25(3):249-262.
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Methods: Targeted methylation sequencing (Methyl-Seq)

➢ **Genomic DNA (gDNA) from blood at T1**

  ❑ Methyl-Seq performed at the Nevada Institute of Personalized Medicine (NIPM) Sequencing Lab in the UNLV

   o 2.5 µg of gDNA fragmented down to 250 bp (ultrasonicator)

   o 1µg of frag. DNA containing methylated CpG, enriched using TruSeq-Methyl Capture EPIC kits (Illumina)

   o 10 ng of enriched DNA used for a DNA library prep., using Truseq ChIP sample preparation kit (Illumina)

   o Cluster generated on flow cell

   o All samples duplicated for the quality control

   o NextSeq 500 (Illumina) sequenced 250-300 bp size DNA library
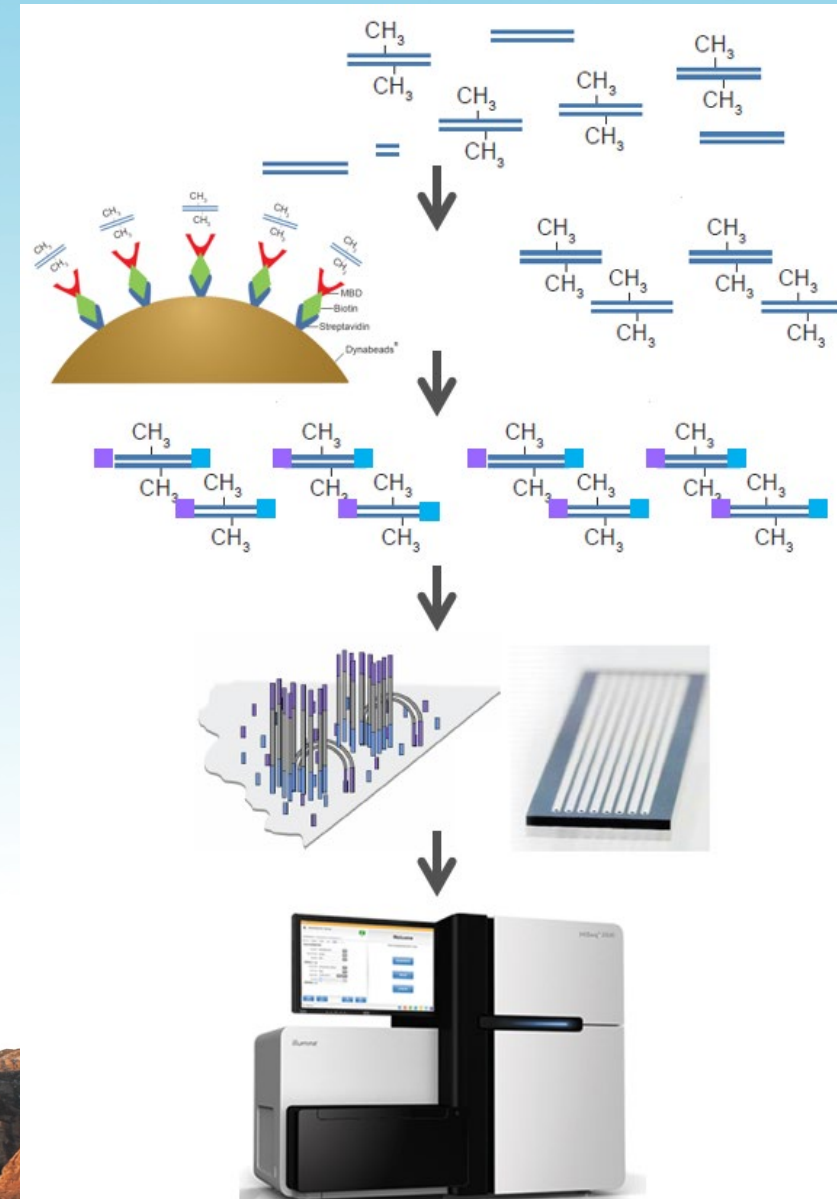
Methods: Methyl-Seq Data Analysis

➢ Quality of the sequencing confirmed by FASTQC version 0.11.5

➢ Fastq reads aligned to the Human Genome version 38 (hg38) by BWA software version 0.7.15-r1140 using MEM algorithm

➢ Peaks called using MACS version 2.1.1.20160309 ➔ *Identifying methylated DNA regions*

➢ Clustering, Principal Component Analysis (PCA), and Differential peak interval analysis by DiffBind software package, version 2.8, with cutoff of 0.05 of False Discovery Rate (FDR) by Benjamini-Hochberg

➢ Network analysis by Ingenuity IPA, version 2018-07-16, Build 478438M
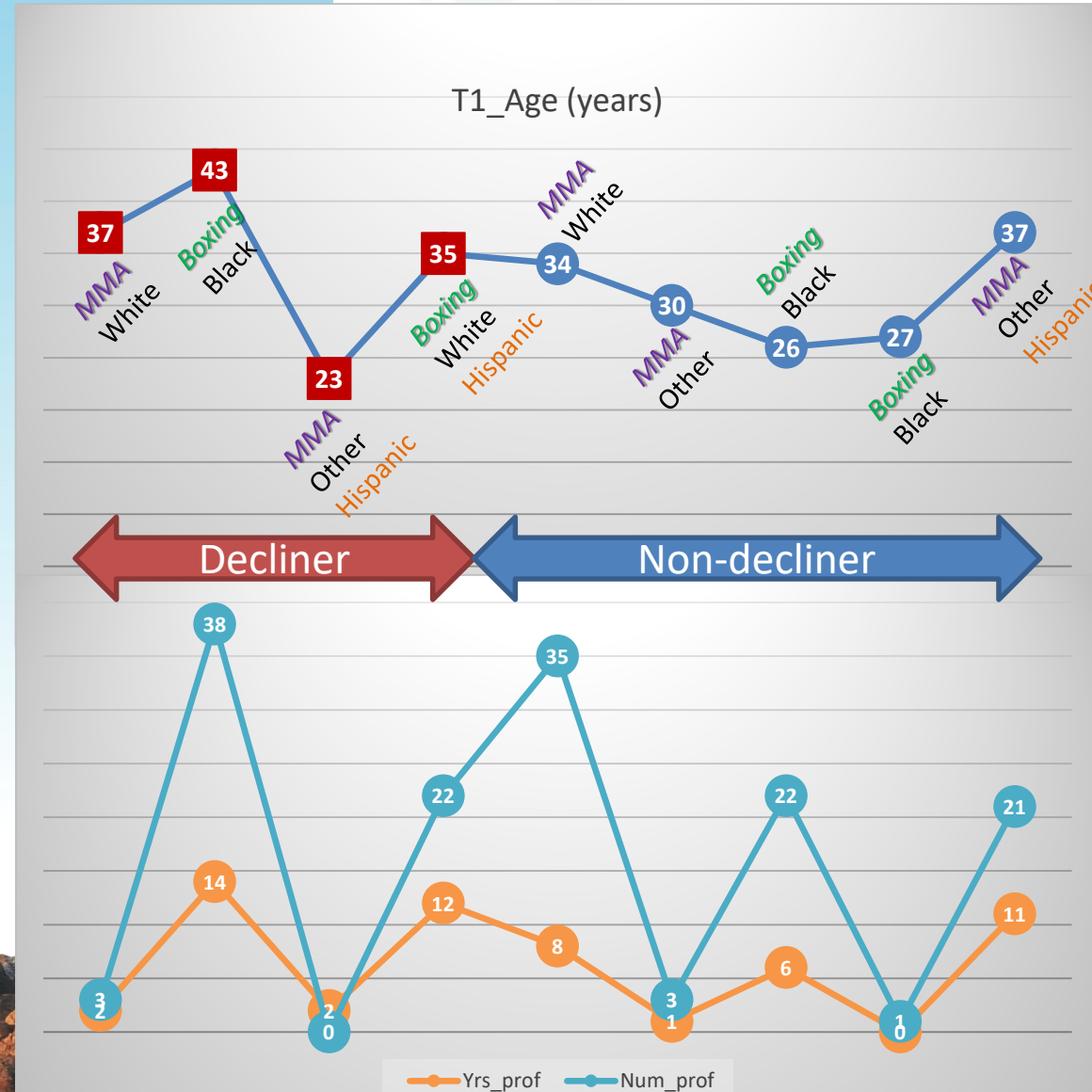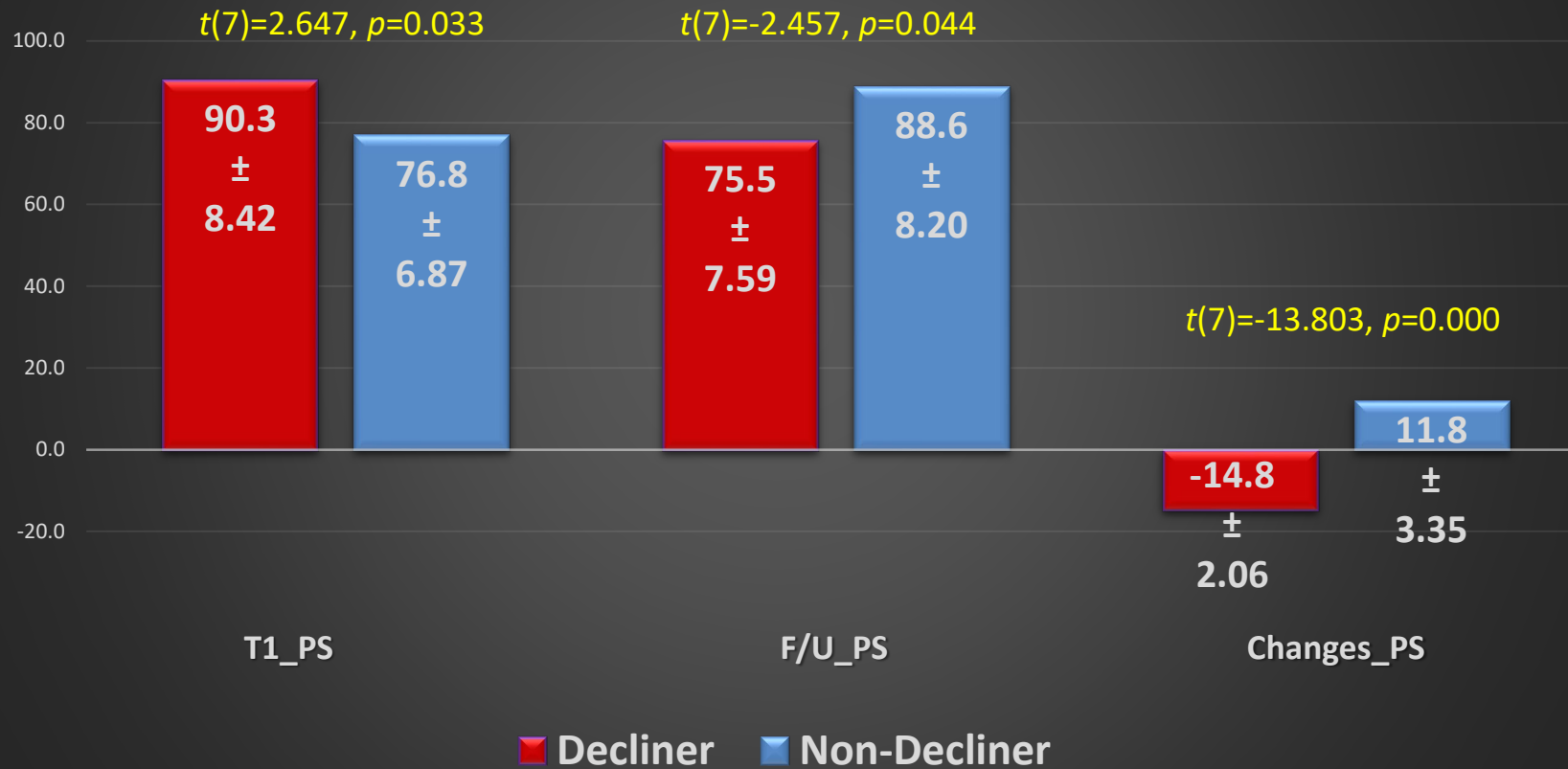
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Results: Demographics

➢ 4 Decliners & 5 Non-decliners

➢ 9 professional male fighters

❑ 4 boxers + 5 mixed martial arts

❑ Age (T1): 32.4 ± 6.41 years old

❑ Years of professional fighting (T1): 6.2 ± 5.26 years

❑ # professional fights (T1): 16.1 ± 14.84 fights

Results: CNSVS-Processing Speed

Processing Speed Standardized Score

$t(7)=2.647, p=0.033$ $t(7)=-2.457, p=0.044$

T1_PS: Decliner 90.3 ± 8.42, Non-Decliner 76.8 ± 6.87

F/U_PS: Decliner 75.5 ± 7.59, Non-Decliner 88.6 ± 8.20

$t(7)=-13.803, p=0.000$

Changes_PS: Decliner -14.8 ± 2.06, Non-Decliner 11.8 ± 3.35

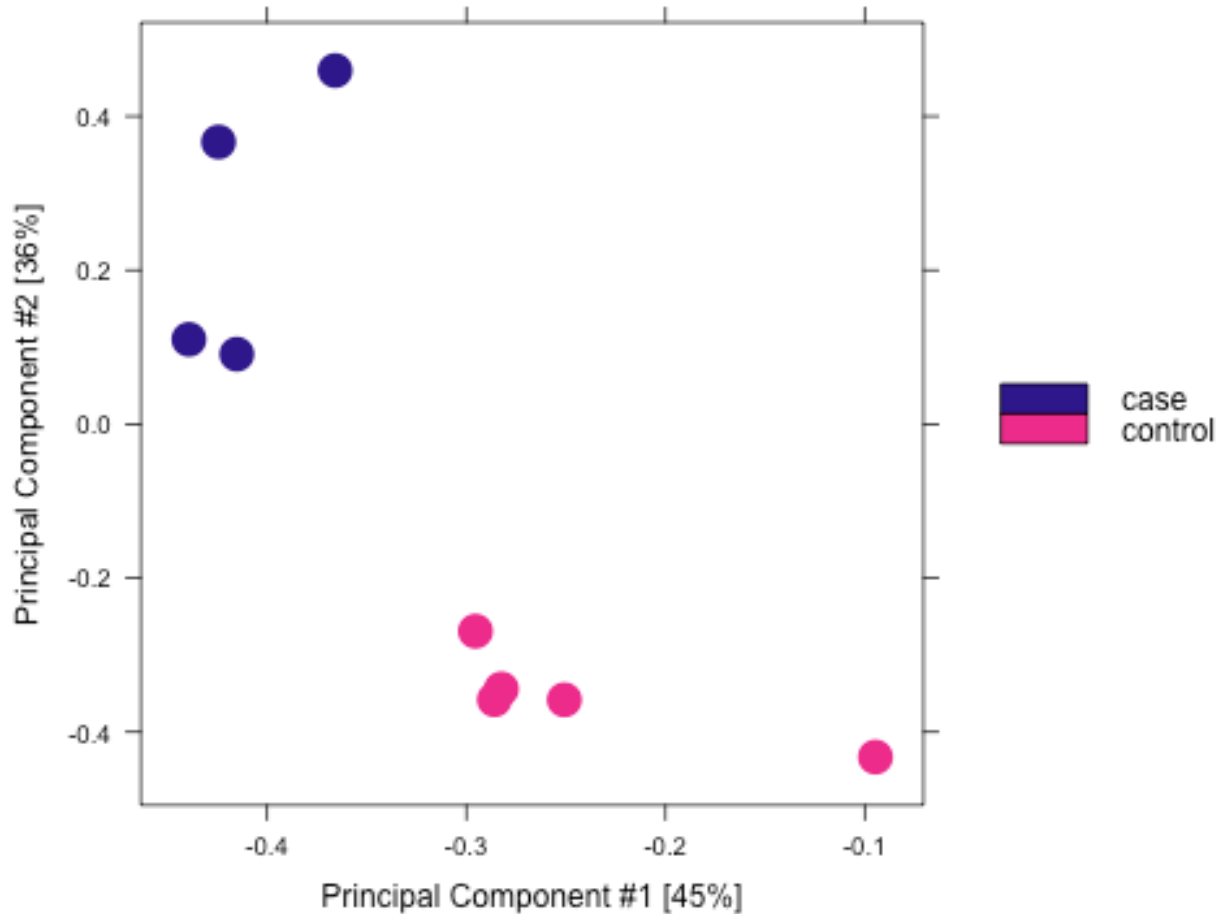■ Decliner ■ Non-Decliner
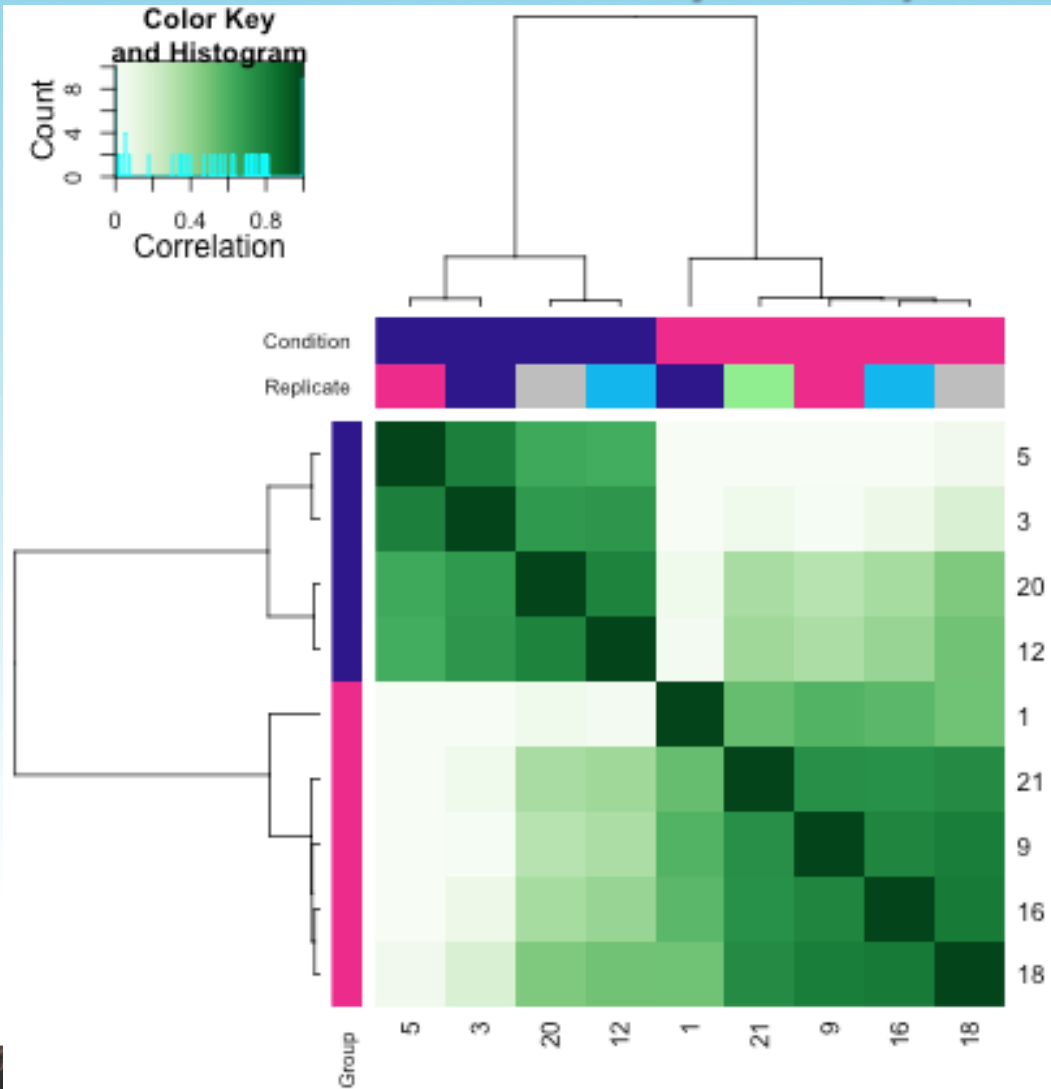
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Results: Methyl-Seq Findings

PCA: ID

Principal Component Analysis by genome-wide methylation profile for 4 cognitive decliners and 5 non-decliners.
Each dot represents a subject. It shows well separated clustering between decliners and non-decliners.

Results: Methyl-Seq Findings

Heatmap plot of count matrix and its hierarchical clustering for most cognitive decliners vs. non-decliners.

Darker green denotes more correlation. Each of the rows and columns represents each of our de-identified subjects.
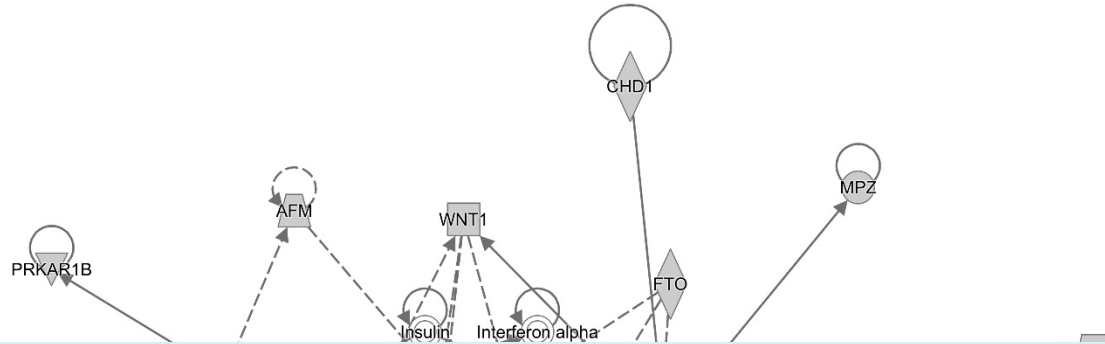
Results: Methyl-Seq Findings

De-regulated network 1 from **hypermethylated genes** related to **organismal injury**.

Gray color denotes hypermethylated genes in decliners. In this network, 25 genes out of 35 were hypermethylated.

Hub genes that could be affected include **ADAM17, GRB10, PRKD1, and FGFR2**.

➔ *Affecting axonal dysfunction*

Up-regulated network 2 from **hypomethylated genes** related to **organismal injury.**

Gray color denotes hypomethylated genes in decliners. In this network, 24 genes out of 35 were hypermethylated.

Hub genes that could be affected include **CDH11, TWIST1, Mmp, GNAQ, and PLC**.

Results: Methyl-Seq Findings

De-regulated network 1 from **hypermethylated genes** related to **neurological disorder.**
Gray color denotes hypermethylated genes in decliners. In this network, 16 genes out of 35 were hypermethylated.
Hub genes that could be affected include **Pka, PI3K, LDL, and Pkc(s)**.
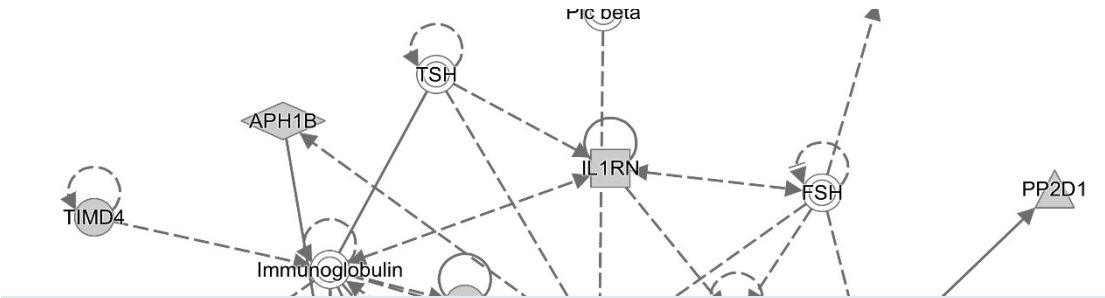
Up-regulated network 2 from **hypomethylated genes** related to **neurological disorder**.
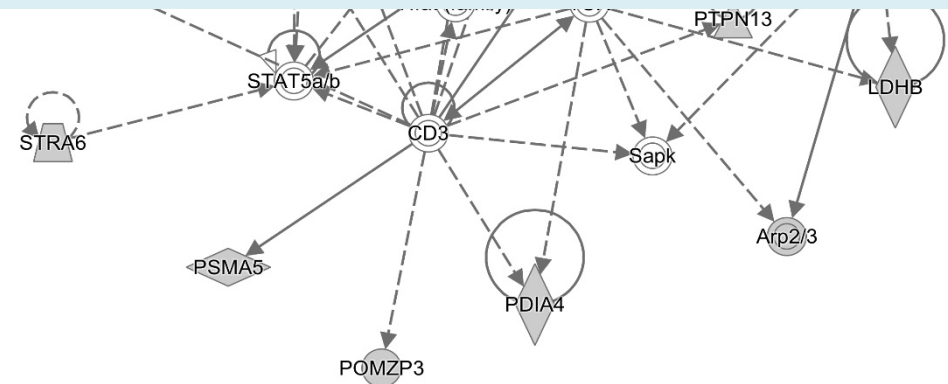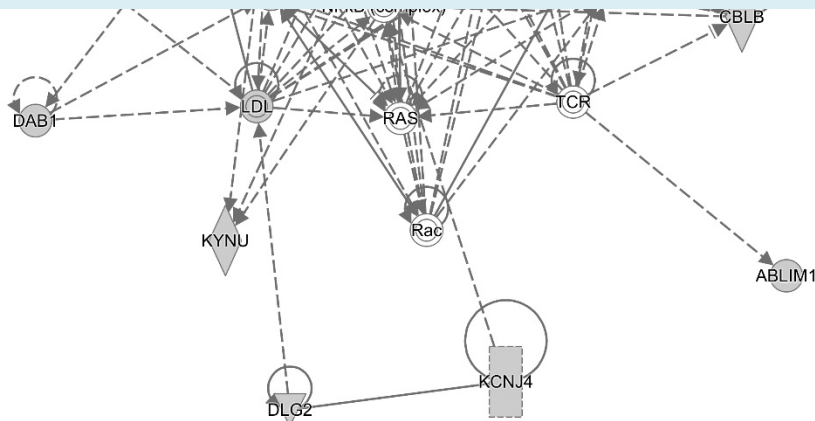Gray color denotes hypomethylated genes in decliners. In this network, 19 genes out of 35 were hypomethylated.
Hub genes that could be affected include **IL1RN, TNC, PTPRC, and GFI1**.

Results: Methyl-Seq Findings

De-regulated (hypermethylated) Post-Injury		Up-regulated (hypomethylated) Post-Injury	
Our Findings	Others	Our Findings	Others
Low density lipoprotein receptor (LDLR)	↑ shortly after injury then ↓ below baseline a week post-injury[1]	Tenascin C (TNC)	Important for recovery shortly after trauma[4]
Protein kinase A (PKA)	↓ cAMP-PKA signaling cascades post-injury[2]	Protein Tyrosine Phosphatase Receptor Type C (PTPRC)	↑ 24hours after TBI[5]
Fibroblast growth factor receptor 2 (FGFR2)	Key role in neuroinflammation → neurogenesis & neurodegeneration[3]	Cadherin 11 (CDH11)	Significant role post-injury[6]
ADAM metallopeptidase domain 17 (ADAM17)	Cell-adhesion proteins, cytokines, and growth factors	Twist Family BHLH Transcription Factor 1 (TWIST1)	Significant for immunology & cellular senescence[7]
Phosphoinositide 3-kinase (PI3K)	Involved in multiple inflammatory processes	Interleukin 1 Receptor Antagonist (IL1RN)	Involved in cerebral hemorrhaging after TBI[8]
Growth factor receptor bound protein 10 (GRB10)	Involved in tyrosine kinase activity and cellular growth	Insulin-like growth factor 1 (IGF-1)	Vital roles in recovery post-injury[9, 10]

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2. Atkins CM, Oliva AA, Alonso OF, et al. *Exp Neurol*. 2007;208(1):145-158.
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6. Lamprecht MR, Elkin BS, Kesavabhotla K, et al. *J Neurotrauma*. 2017;34(1):97-108.
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8. Hadjigeorgiou GM, Paterakis K, Dardiotis E, et al. *Neurology*. 2005;65(7):1077-1082.
9. Rubovitch V, Edut S, Sarfstein R, Werner H, Pick CG. *Neurobiol Dis*. 2010;38(2):299-303.
10. Wong VS, Langley B. *Neurosci Lett*. 2016;625:26-33.

Results: Methyl-Seq Findings

➤ Affecting axonal dysfunction (e.g., ADAM17, GRB10, FGFR2, Pka, PI3K), altered dopamine-DARPP32 feedback in cAMP signaling

➤ Altered methylation of BDNF (brain-derived neurotrophic factor) promoters on cognition

  ❑ Parkinson's disease, CTE[1]

  ❑ Depression-like behaviors, including suicide[2]

1. Sanchez-Mut J V, Heyn H, Vidal E, et al. *Transl Psychiatry*. 2016;6(1):e718-e718.
2. Mirkovic B, Laurent C, Podlipski M-A, Frebourg T, Cohen D, Gerardin P. *Front Psychiatry*. 2016;7:158.

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Discussions

- Limitations
  - Data were not collected directly
  - Only male subjects & not generalizable to females
  - gDNA samples from peripheral cells, which may not be closely associated with the brain pathophysiology post-injury
  - Validation lacking

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Discussions

➢ Significances

❑ Successful profiling of gen[o]
the following cognitive dec[...]

  o Our cognitive declines-related ge[...]
  disorders

❑ Epigenetic mechanisms of [...]
brain injury

❑ Improving diagnostic/scree[...]
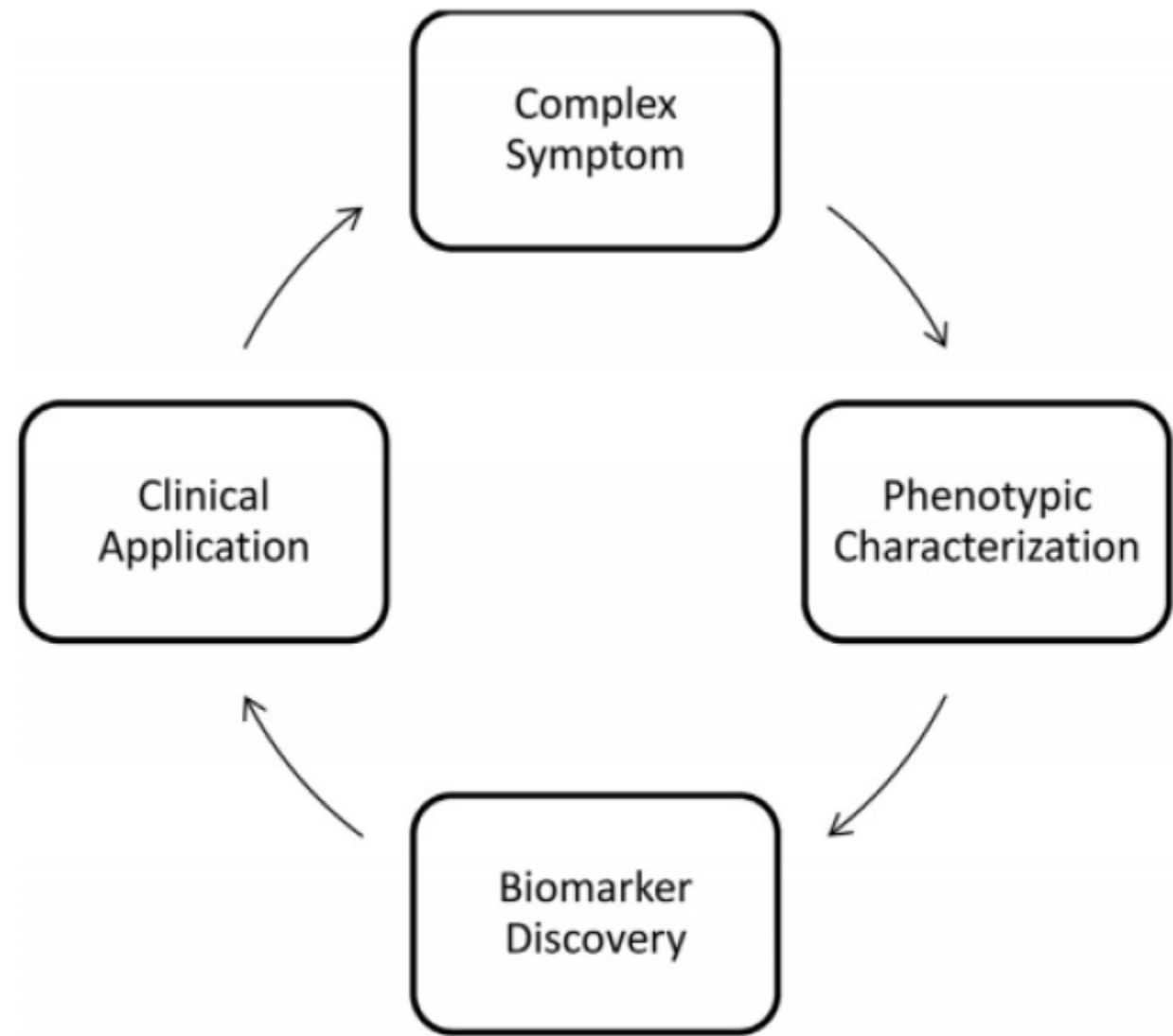
Figure 1. National Institutes of Health Symptom Science Model (NIH-SSM). In our study, we investigated neurodegeneration in TBI through complex phenotypic characterization with the focus on changes in cognitive performance over up to 4 years among professional fighters who had repetitive TBIs. Such clinical characterization synergistically measured with peripheral DNA methylation markers can assist in identifying at-risk groups for developing neurodegeneration after a brain injury.
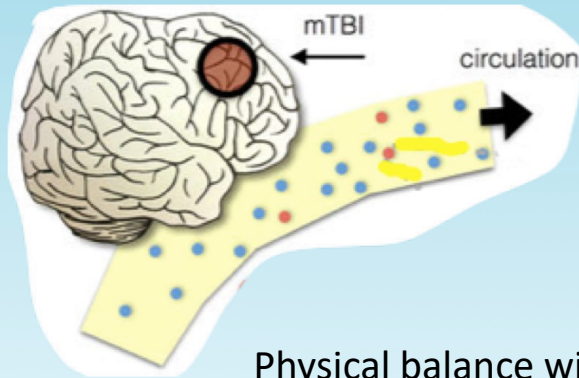
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Implications & Conclusion

➢ Contributing to a change in the clinical paradigm of head injury screening and treatments

➢ Advancing the scientific model to understand the underlying mechanisms and heterogeneous trajectories of head injury progress

➢ Better understanding of the symptoms of chronic illness, minimizing the related morbidity and mortality risks, and improving quality of life in affected individuals
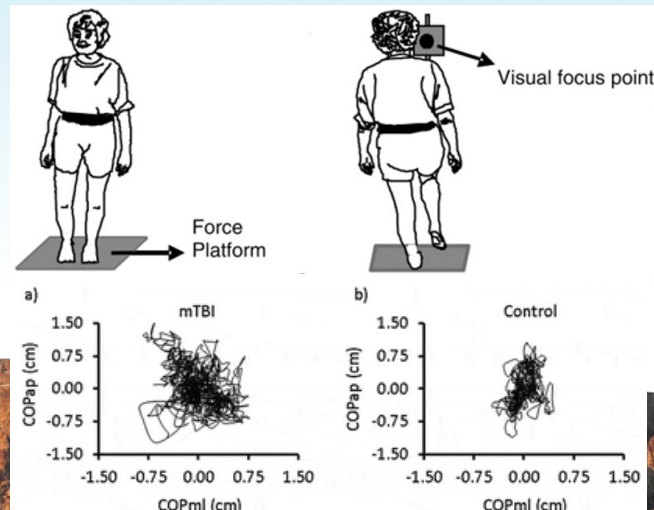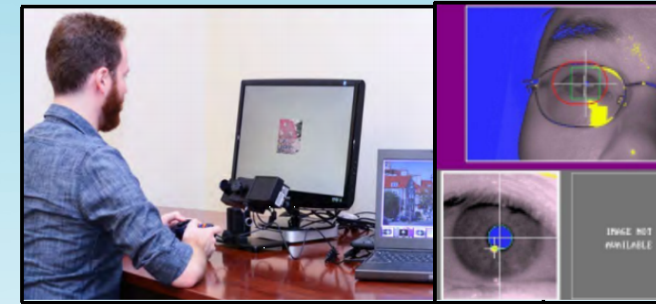
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Peripheral Cellular Senescence Epigenetic Markers

Saccadic Eye Movements

Physical balance with forceplate & stabilometer

Real-time mobile health (mhealth) for dynamic balance

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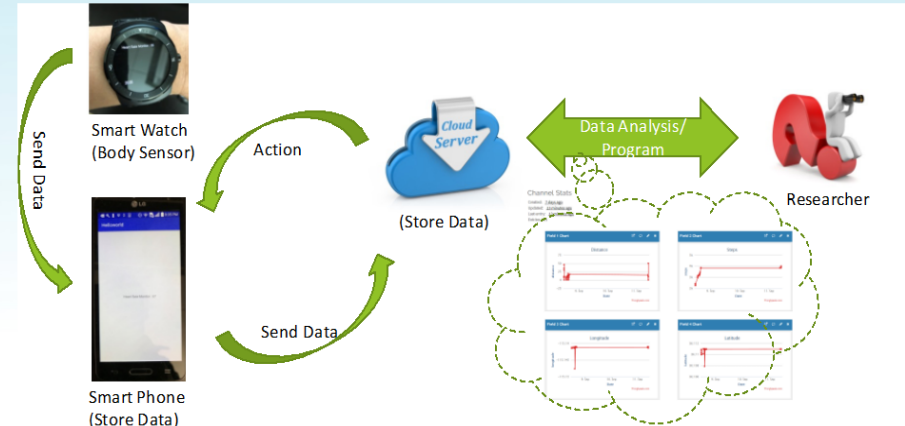
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