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Genome-Wide Blood DNA Methylation Profiles for Cognitive Declines Among Professional Fighters

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Introduction:

Repetitive head traumas produce persistent functional impairments, including cognitive declines, among over 40% of the affected individuals (Seabury et al., 2018), resulting in inability to return to work one-year post-injury of further 25% (Langlois, Rutland-Brown, & Wald, 2006). Cognitive decline variabilities are related to physiological responses to the head injury via molecular activities that are potentially regulated by DNA methylation (Lu, Liu, Deng, & Qing, 2013). The purpose of this study was to investigate whole-genome blood DNA methylation associated to cognitive declines within the following years among male professional fighters who were recruited from the Professional Fighters Brain Health Study (PFBHS) (PI: Bernick) – a longitudinal cohort study (Bernick et al., 2015).

Method:

Nine professional male fighters (5 mixed martial arts fighters and 4 boxers) were selected for our analysis: 4 showed cognitive declines (‘decliners’) and 5 did not (‘non-decliners’) in the next 1 or up to 4 years (T2, T3, T4 or T5), compared to the baseline (T1), based on CNS-Vital Signs – processing speed domain. Genomic DNA from blood was obtained at T1. Targeted methylation sequencing (Methyl-Seq) for whole-genome methylation profiling was conducted at the Nevada Institute of Personalized Medicine Sequencing Lab. DNA fragments containing methylated CpG were enriched using TruSeq-Methyl Capture EPIC kits and sequenced using Targeted Methyl-Seq (Illumina NextSeq500). For data analysis, FASTQC v.0.11.5 to evaluate sequencing quality, BWA v.0.7.15r1140 to align the fastq to hg38 with MEM algorithm, MACS 2.1.1.20160309 to call peaks with a cutoff of 0.05 of False Discovery Rate by Benjamini-Hochberg, DiffBind software package v.2.8 for differential analysis, and Ingenuity IPA v.20180716 Build 478438M for network analysis were used.

Results:

Age ranged from 27 to 43 (mean = 32.4, SD = 6.41) and professional fighting experience ranged from 0 to 14 (6.2 ± 5.26) years. Each third was white, black or other races. No significant differences were found in any of these characteristics between decliners and non-decliners.

Heat map and principal component analysis (PCA) on genome-wide methylation profile correctly discern the two groups. The differential analysis identified 149 hypermethylated and 199 hypomethylated genes in the decliners. Network analysis identified deregulated networks related to organismal injury and neurological disorder, affecting axonal guidance, neuropathic pain, and dopamine-DARPP32 feedback in

cAMP signalings. From upregulated networks, BDNF or brain-derived neurotrophic factor was one of the top common upstream regulators.

Discussions:

We successfully profiled genome-wide blood DNA methylation associated with the following cognitive declines among nine professional fighters. Our key networks confirmed the effect of axonal dysfunction (e.g., ADAM17, GRB10, FGFR2, Pka, PI3K), altered dopamine-DARPP32 feedback in cAMP signaling, and decreased or altered methylation of BDNF promoters on cognition, which is also found in Parkinson's disease, chronic traumatic encephalopathy (Sanchez-Mut et al., 2016), or depression-like behaviors, including suicide (Mirkovic et al., 2016). Validation will follow.

Implications:

Although head trauma or traumatic brain injury is not among the main areas where typical genetic markers have emerged for diagnosing or planning treatments, epigenetic markers will contribute to better understanding the disease progress based on pre-symptomatic and symptomatic conditions and identifying which markers might be associated with or influence changes in cognitive functions over time after the brain injury. Our findings can contribute to (1) a change in the clinical paradigm of head injury screening and treatments, and (2) an advancement of the scientific model to understand the underlying mechanisms and heterogeneous trajectories of head injury progress. In addition, this research may contribute to a better understanding of the symptoms of chronic illness, the minimization of the related morbidity and mortality risks, and the improvement of quality of life in affected individuals.

Title:
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Keywords:
Cognitive decline, Head trauma and Methylation

References:
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Abstract Summary:
Cognitive decline variabilities after head injuries may be regulated by altered DNA methylation. We successfully profiled genome-wide blood DNA methylation associated with cognitive declines among professional fighters. Our key network findings confirmed the effect of axonal dysfunction, altered dopamine feedback in cAMP signaling, altered methylation of BDNF promoters on cognition.

Content Outline:

1. Introduction:
    1. Significance and health impact of repetitive closed head injury
    2. Persistent impairment in cognitive function following the injury
    3. Regulation of cognitive declines by DNA methylation after the injury
2. Body
    1. Methods
        1. Selected subjects from the Professional Fighters Brain Health Study (PFBHS)
        2. CNS-Vital Signs to measure processing speed
        3. Targeted Methylation Sequencing (Targeted Methyl-Seq) and analysis procedures
    2. Results
        1. Demographic and Fighter experience data
        2. Differential analysis & Network analysis findings
            1. Deregulation of axonal function and dopamine feedback
            2. Altered BDNF function
3. Conclusion:
    1. Discussions of the findings: Our cognitive declines-related gene network findings are also found in other neurodegenerative disorders.
    2. Implications: Our epigenetic marker findings may contribute to a better understanding of cognitive declines following head trauma, which may reduce the related morbidity and mortality risks, and improve quality of life in affected individuals.

First Primary Presenting Author
Primary Presenting Author
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Author Summary: My research focus is on epigenetic markers along with other biomarkers (e.g., cognitive and sensorimotor function) associated with neurodegeneration after mild traumatic brain injury (mTBI) or concussion which can be utilized in clinical treatment to facilitate the recovery process from the injury.

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Author Summary: Doctoral graduate assistant who has been working on this project for 1 year along with trainings in human translational laboratories as well as expertise in anthropology.

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Author Summary: Dr. Bernick, the 2016 Inspired Excellence in Healthcare Award Recipient, is a nationally recognized leader and research scientist in the long-term effects of repetitive brain injury and a

neurologist for more than 30 years. Dr. Bernick is the lead researcher of the Professional Fighters Brain Health Study (PFBHS), which measures the effects of long-term head trauma in combat sports.