RESEARCH ARTICLE

Inflammation-related gene expression profiles of salivary extracellular vesicles in patients with head trauma

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Abstract

At present, there is no reliable biomarker for the diagnosis of traumatic brain injury (TBI). Studies have shown that extracellular vesicles released by damaged cells into biological fluids can be used as potential biomarkers for diagnosis of TBI and evaluation of TBI severity. We hypothesize that the genetic profile of salivary extracellular vesicles in patients with head trauma differs from that in uninjured subjects. Findings from this hypothesis would help investigate the severity of TBI. This study included 19 subjects, consisting of seven healthy controls who denied history of head trauma, six patients diagnosed with concussion injury from an outpatient concussion clinic, and six patients with TBI who received treatment in the emergency department within 24 hours after injury. Real-time PCR analysis of salivary extracellular vesicles in participants was performed using TaqMan Human Inflammation array. Gene expression analysis revealed nine upregulated genes in emergency department patients (LOX5, ANXA3, CASP1, IL2RG, ITGAM, ITGB2, LTA4H, MAPK14, and TNFRSF1A) and 13 upregulated genes in concussion clinic patients compared with healthy participants (ADRB1, ADRB2, BDKRB1, HRH1, HRH2, LTB4R2, LTB4R, PTAFR, CYSLTR1, CES1, KLK1, MC2R, and PTGER3). Each patient group had a unique profile. Comparison between groups showed that 15 inflammation-related genes had significant expression change. Our results indicate that inflammation biomarkers can be used for diagnosis of TBI and evaluation of disease severity. This study was approved by the Institutional Review Board on December 18, 2015 (approval No. 0078-12) and on June 9, 2016 (approval No. 4093-16).

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Introduction

Traumatic brain injury (TBI) broadly describes any change or altered brain function or pathology caused by an external impact (Cheng et al., 2019; Menon et al., 2010). Brain functions that are evaluated are loss of consciousness, loss of memory, retrograde amnesia, neurologic deficits (weakness, paralysis, sensory loss, aphasia), and altered mental state (confusion, disorientation) (Menon et al., 2010). In 2013, there were approximately 2.5 million emergency department (ED) visits due to head injuries, approximately TBI-related 282,000 hospitalizations and approximately 56,000 TBI-related deaths (Taylor et al., 2017). Repeated TBI attacks increase the risk of neurodegenerative diseases such as chronic traumatic encephalopathy (CTE) (McKee et al., 2009; Prins et al., 2013; Mez et al., 2017), Alzheimer's disease (Prins et al., 2013; Heneka et al., 2015), and Parkinson's disease (Gyoneva and Ransohoff, 2015). Inflammation is a major feature in neurodegenerative diseases and is considered an underlying cause for chronic neurodegeneration following TBI (Faden et al., 2016; Yang et al., 2018). Inflammatory responses involve multiple components, such as release of intracellular components from damaged cells, microglia and astrocyte activation, cytokine production, and immune cell recruitment to injury (Gyoneva and Ransohoff, 2015). Understanding of inflammatory response can provide insights for drug targeting or other potential therapeutic benefits by preventing further damage.

All cells release extracellular vesicles (EVs) (size ranges from 100 to 1000 nm) (Aliotta et al., 2012) and can be found in all biological fluids, such as blood, urine, and saliva. EVs modulate cell communication and interact with target cells by surface receptors, proteins, mRNA/miRNA, and lipids (Svetlov et al., 2009; Michael et al., 2010; Aliotta et al., 2012; Yokobori et al., 2013; Papa et al., 2016). EVs alter the pheno-

type of target cells by attaching to the target cells, becoming internalized by a cell or fusing with the target cell membrane and releasing its contents into the cells (Tkach and Thery, 2016). EV luminal components are safe from degradation making EVs the ideal repository of biomarkers (Choi et al., 2014). EV secretion is increased during disease state and therefore can be tested for unique protein, mRNA, and miRNA content (Choi et al., 2014).

In the present study, salivary EVs were characterized and analyzed for inflammation-related gene expression in three participant groups: head trauma emergency department patients, patients diagnosed with concussion from an outpatient concussion clinic, and healthy participants. We utilized the Taqman human inflammation array, which contains 92 genes involved in a broad range of inflammatory response. Since healthy central nervous system (CNS) tissue has low (undetectable) levels of inflammatory mediators (Lucas et al., 2006), we hypothesized low expression of inflammation-related genes in the healthy participants. The healthy participants will serve as a normal baseline when compared to the acute and chronic head trauma patients.

The goal of this study is the development of surrogate EV-based biomarkers that will afford clinicians adjuvant metrics, which is correlated to standard neurological testing and imaging. This may be used to confirm the diagnosis of TBI and perhaps grade its severity. In addition, biomarker expression may serve as ancillary data point to grade therapeutic response as and prognostic value.

Participants and Methods

Participant selection

The study is a continuation of previous work done by Cheng et al. (2019). Nineteen participants were included due to availability of mRNA to perform arrays and availability of arrays in this study: six patients with acute head trauma from emergency department (EDPT), six patients with concussion from an outpatient concussion clinic (CCPT) and seven healthy participants. These 19 participants were randomly chosen, and not matched for age or sex. Patients with acute head trauma had trauma-induced impairment of neurological function. Healthy participants denied history of head trauma. The patient demographic data are summarized in **Table 1** and were detailed in Cheng et al. (2019). All participants and/or their family members gave informed consent. Protocol was approved by the Institutional Review Board on December 18, 2015 (approval No. #0078-12) and on June 9, 2016 (#4093-16).

Saliva sample collection

Saliva collection was done as previously described by Navazesh et al. (1993). Briefly, participants rinsed with water and then spit saliva into a 50-mL test tube. At least 5 mL saliva sample was collected from each participant. For patients with acute head trauma, saliva sample was collected within 24 hours after injury. EVs were evaluated and characterized (data not shown) using NanoSight NS500 instrument, transmission electron microscopy (TEM), and western blot analysis (results published in Cheng et al., 2019).

Table 1 Participant demographics

Group	ID	Age at collection	Gender	Duration from onset
Healthy controls	C10	25	F	NA
	C11	33	F	NA
	C12	23	F	NA
	C15	22	M	NA
	C17	U	M	NA
	C18	22	F	NA
	C9	22	F	NA
CCPT	CCPT13	49	M	27 d
	CCPT2	51	F	145 d
	CCPT3	52	F	173 d
	CCPT4	31	F	30 d
	CCPT6	50	F	168 d
	CCPT7	28	M	207 d
EDPT	EDPT2	36	M	< 24 h
	EDPT3	25	F	< 24 h
	EDPT4	31	M	< 24 h
	EDPT6	26	F	< 24 h
	EDPT9	31	F	< 24 h
	EDPT11	27	F	< 24 h

CCPT: Concussion clinic patients (chronic); EDPT: emergency department patients (acute); F: female; M: male; NA: not available; U: unknown.

Salivary EV isolation

Salivary EV was isolated using differential centrifugation (Michael et al., 2010). All salivary samples were kept at –80°C. Saliva samples were diluted with phosphate buffered saline (PBS) (Gibco, Carlsbad, CA, USA). All salivary samples were centrifuged at 1500 × *g* for 10 minutes at 4°C. The supernatant was centrifuged at 17,000 × *g* for 15 minutes. The pellets were discarded, and the supernatant was saved. EVs were isolated via ultracentrifugation at 120,000 × *g* for 1 hour. Pellets were washed with PBS and centrifuged at 120,000 × *g* for 1 hour. All EV samples were resuspended in a final volume of 500-μL PBS.

Real-time PCR and gene expression analysis

RNA was extracted from EVs using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) using the protocol recommended by the manufacturer. RNA quantity and purity were determined utilizing the Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA was reverse transcribed to cDNA using the High Capacity cDNA transcription kit (Applied Biosystems, Carlsbad, CA, USA) in a volume of 20 μL using a 9800 Fast Thermal Cycler (Life Technologies, Carlsbad, CA, USA). Reverse transcription PCR consisted of one cycle for 10 minutes at 25°C, one cycle for 120 minutes at 37°C, and one cycle for 5 minutes at 85°C. cDNA was then pre-amplified using a TaqMan PreAamp Master mix (Life Technologies). Pre-amplification reaction was performed according to manufacturer's guidelines in a final volume of 50 μL prior to real-time PCR. Pre-amplification reaction consisting of an initial 10 minute cycle at 95°C followed by 14 cycles of 15 seconds at 95°C followed by a final cycle at 60°C for 4 minutes. Pre-amplificated cDNA

product was mixed with a TaqMan Universal Master Mix (Life Technologies) then loaded onto the TaqMan® Human Inflammation Array (Life Technologies) (**Additional Table 1**, TaqMan human Inflammation Array gene description). Each array had 92 genes involved in inflammation processes and four housekeeping (endogenous) controls. Arrays were run on the Viia7 Real-Time PCR System (Life Technologies) using comparative cycle threshold (CT) settings and the QuantStudio™ Real-Time PCR system (Life Technologies) was used to analyze the data. CT values were used to calculate fold change of gene expression using GAPDH as the housekeeping gene. Only genes with CT values less than 35 were considered for calculating the fold change in expression. ExpressioSuite Software (Life Technologies) was used to accurately quantify relative gene expression across genes and samples. Only samples with amplification scores higher than 1.2 were considered.

Statistical analysis

Participant ages were analyzed using one-way analysis of variance with a *post-hoc* Tukey's honestly significant difference (HSD) test. Participant inflammation-related gene expression differences were identified by a Wilcoxon sum test (STATA, College Station, TX, USA) on the delta Ct values in EDPT *versus* healthy participants, CCPT *versus* healthy participants, and EDPT *versus* CCPT. A *P*-value of less than 0.05 was determined to be statistically significant.

Results

Age comparison among participants

The average age of the CCPT group was significantly higher than that of healthy participants (43.5 years *vs.* 24.5 years, $P = 0.001$), but it was not significantly different from that of the EDPT group (29.3 years *vs.* 24.5, $P = 0.492$) (**Table 1**).

Comparison of gene expression between EDPT and healthy participants

Gene expression profiles were compared between the EDPT group and healthy participants. Among 92 inflammation-related genes on the array, 46 genes were up-regulated between EDPT and healthy participants. Each EDPT with gene showing two-fold greater change in expression (greater than two considered biologically relevant) compared to healthy participants is represented (**Figure 1**).

The Wilcoxon sum test was performed to compare delta Ct values between EDPT and healthy participants. Results indicate that nine genes (**Figure 1**) were significantly upregulated in EDPT compared with healthy participants ($P < 0.05$): ALOX5 (25.89 ± 7.04), ANXA3 (13.38 ± 13.96), CASP1 (8.03 ± 3.79), ITGB2 (14.53 ± 6.01), IL2RG (57.5 ± 13.75), ITGAM (22.57 ± 13.67), LTA4H (24.37 ± 30.66), MAPK14 (20.23 ± 9.95), and TNFRSF1A (6.59 ± 1.42).

Comparison of gene expression between CCPT and healthy participants

Inflammation-related gene expression profile in the CCPT group showed that 51 genes were upregulated when compared with healthy participants. CCPT with genes showing twofold change in expression are shown in **Figure 2**. The Wilcoxon rank sum test performed on the delta Ct values identified that 13 genes were significantly different ($P < 0.05$) between CCPT and healthy participants (**Figure 2**). The 13 genes that were significantly upregulated are ADRB1 (130.04 ± 101.86), ADBR2 (25.14 ± 10.25), BDKRB1 (125.85 ± 84.9), HRH1 (253.9 ± 140.72), HRH2 (81.09 ± 67.12), LTB4R2 (41.80 ± 20.92), LTB4R (50.23±42.85), PTAFR (22.10 ± 13.72), CYSLTR1 (94.64 ± 55.86), CES1 (56.31 ± 40.73), KLK1 (113.11 ± 0.00), MC2R (37.81 ± 0.89), PTGER3 (62.38 ± 0.00).

Comparison of inflammation-related gene expression profile between EDPT and CCPT

Inflammation-related gene expression profile was compared between EDPT and CCPT. Results showed that 15 genes were significantly altered in expression in EDPT and CCPT (**Figure 3**; $P < 0.05$). ALOX5, ANXA3, CASP1, and ITGB2 were significantly higher in EDPT than in CCPT and ADRB1, ADRB2, BDKRB1, CYSLTR1, HRH1, HRH2, LTB4R2, LTB4R, MC2R, NFKB1, PTAFR were significantly higher in CCPT than in EDPT.

Discussion

TBI begins with an initial injury or impact to the head. Secondary injuries occur when inflammatory cells and cytokines are recruited to the area of injury. Inflammatory responses are advantageous when actived in a regulated manner to fight an infection. In TBI, inflammation alters brain homeostasis by changing cellular functions. Brain inflammation is initiated by microglial activation which leads to the release of cytokines, free radicals, and other macromolecules (Lucas et al., 2006; Paolicelli et al., 2018). The function of microglia is to rescue neuronal cells from damage; however, chronic exposure to inflammation becomes neurotoxic (Paolicelli et al., 2018). Astrocytes help maintain brain homeostasis of the central nervous system. Inflammation activates astrocytes, leading to change in cell phenotype and cell migration to damaged area (Fields and Ghorpade, 2012). TBI induces many cells to react to this damage. EVs released by the activated microglia and astrocytes (as well as other neuronal cells) can be isolated to detect stress level in the brain due to inflammation. EVs are excellent intermediators that can deliver messages to surrounding cells and tissues and eventually to biological fluids that can be isolated for diagnostic purposes.

In this study, saliva was collected from EDPT who received treatment within 24 hours after injury, CCPT that have suffered from long term effects from the injury, and healthy individuals with no history of head trauma. Inflammation-related genes in isolated EVs were detected using the Taqman human inflammation array. The expression levels of ALOX5, ANXA3, CASP1, IL2RG, ITGAM, ITGB2, LTA4H, MAPK14, and TNFRSF1A were increased in EDPT than in healthy participants. Some of these genes have been reported as altered in TBI. ALOX5 metabolizes arachidonic acid to

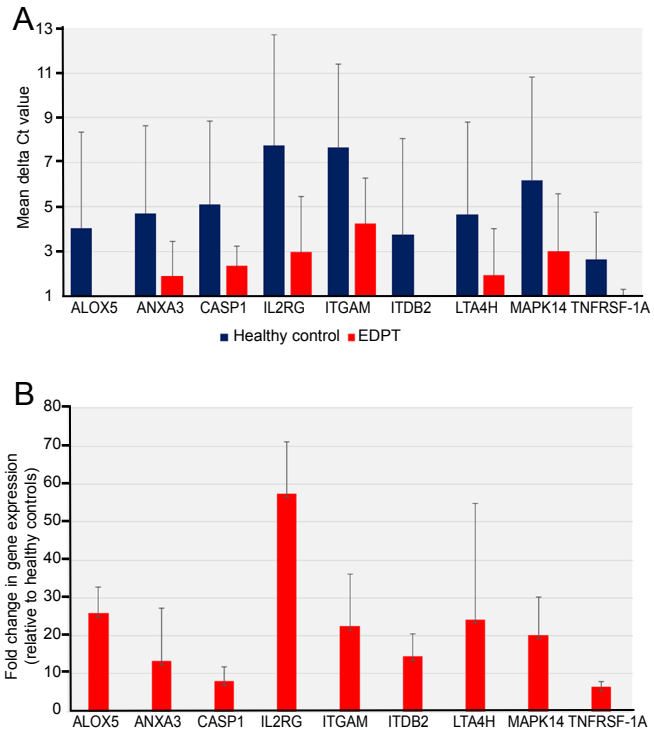

Figure 1 Inflammation-related gene expression information of EDPT.
(A) Mean delta Ct values of inflammation-related genes ($P < 0.05$), comparing EDPT ($n = 6$) with healthy participants ($n = 7$). (B) Genes with two-fold or higher increase in gene expression. Error bars represent standard deviation. CT: Cycle threshold; EDPT: emergency department patients.

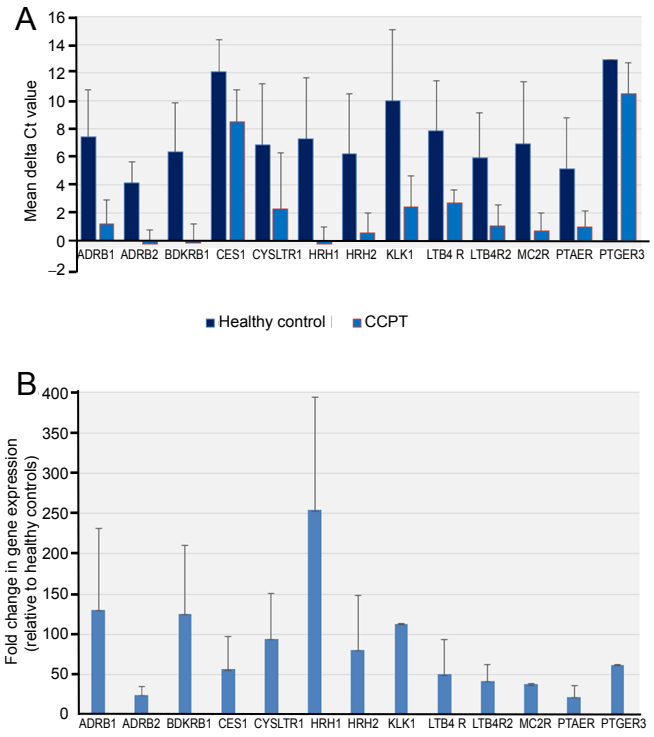

Figure 2 Inflammation-related gene expression information of CCPT
(A) Delta Ct value of inflammation-related genes ($P < 0.05$), comparing CCPT ($n = 6$) with healthy participants ($n = 7$). (B) All genes that had a two-fold or higher increase in expression. Error bars represent standard deviation. CCPT: Concussion clinic patients; CT: cycle threshold.

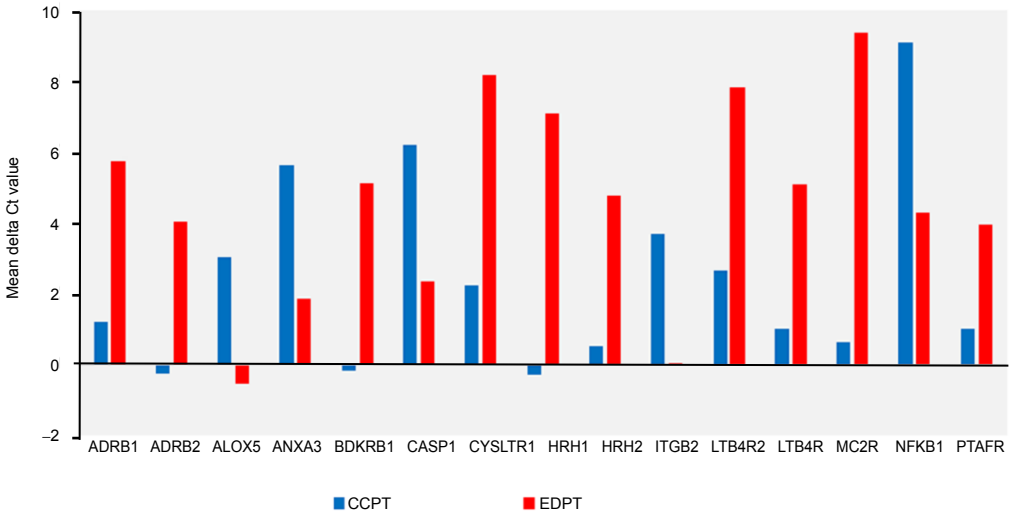

Figure 3 Mean delta Ct value in CCPT *versus* EDPT.
The Wilcoxon test sum test was used to compare delta Ct value between EDPT and CCPT. Fifteen inflammation-related genes were significantly ($P < 0.05$) upregulated. CCPT: Concussion clinic patients; CT: cycle threshold; EDPT: emergency department patients.

leukotrienes. ALOX5 expression increases in the brain after TBI, specifically in glial cells and neutrophils (Zhang et al., 2006; Hijioka et al., 2017). ANXA3 (annexin A3) has been shown to be overexpressed in rodent studies. ANXA3 is upregulated in primary mouse cortical neurons after neuronal injury (Chong et al., 2010) and in rat cerebral ischemia (Junker et al., 2007). CASP1 (caspase 1) is involved in apoptosis. LTA4H (leukotriene A4H) converts LTA4 to LTB4 (Hijioka et al., 2017). LTB4 is a lipid mediator which plays a role in neutrophil infiltration and inflammation in central nervous system disorders (Hijioka et al., 2017). Studies have shown that LTA4H expression was increased in head trauma patients than in controls (Orr et al., 2015; Hijioka et al., 2017). MAPK pathway was reported to be involved in astrocyte activation due to TBI (Li et al., 2017). Thirteen genes were increased in CCPT than in healthy participants: ADRB1, ADRB2, BDKRB1, HRH1, HRH2, LTB4R2, LTB4R, PTAFR, CYSLTR1, CES1, KLK1, MC2R, and PTGER3. BDKRB1 (bradykinin) increases blood-brain barrier permeability and is involved in brain edema relating to ischemic brain injury (Dobrivojevic et al., 2015). Leukotrienes LTB4R2 and LTB4R as discussed previously are believed to be part of inflammatory response. PTAFR (platelet activating factor receptor, aka PAF) is involved in cerebral edema and cerebral ischemia/reperfusion injury through interactions with PAFR (Yin et al., 2017). PTGER3 (prostaglandin E receptor) expression was significantly increased after unilateral TBI (White et al., 2016). All the genes on the array are involved in more than one inflammatory pathway. Results from this study showed both EDPT and CCPT had many genes statistically significantly upregulated compared with healthy participants, and they did not have any of the 92 genes in common. In this study, we found a unique 15-gene profile between two patient groups. This unique transition of gene transcription could give future insights into the progression of inflammation within the central nervous system.

Salivary EVs isolation for diagnosis is a simple and non-invasive process compared with cerebral spinal fluid or blood collection. Membrane-bound EVs are protected from degradation that serum biomarkers face (Cheng et al., 2019). It is difficult to precisely grade the severity of TBI because it is based on subtle examination and neuroimaging findings (Papa et al., 2015). Salivary EV phenotypes and genetic cargo may allow for early diagnosis. An additional clinical relevance of salivary EVs is to monitor inflammatory responses to therapeutic interventions (Cheng et al., 2019).

In this study, CCPT are followed up over a large period of time, during which multiple genes may change in expression. One benefit of EV-based biomarkers is that EVs are free to cross the blood-brain barrier at any time, whereas conventional biomarkers can usually only cross a narrow window following TBI and are also limited by variable kinetics and elimination times. Following the initial insult, TBI begins and progresses as an evolving pathology – at each step EVs packed with unique cargo are shed and likely represent this dynamic process (Lucas et al., 2006; Yang et al., 2018). Following the initial event, the secondary phase of TBI usually involves a wide spread, systemic immune and neuroinflammatory response mediated by numerous cytokines – a phenomenon that we have focus on and captured in our current work.

Our work identifies multiple genes within EVs of neural origin that have varying expression between the three experimental groups. There was considerable difference between multiple gene candidates when comparing the EDPT with healthy controls. This gene palette may represent the acute to sub-acute pathophysiological changes that are triggered following TBI, as many of these genes (**Additional Tables 2** and **3**) are involved in established pathways involved in the development of inflammation, the development of neural plaques, and amyloid precursor proteins, neural apoptosis, and axon regeneration. Genetic variations that were common between the two TBI groups (EDPT and CCPT) but not seen in the EDPT group identify candidate biomarkers that may be related to long-term recovery of patients who have suffered TBI. As prospective studies detected the expression of these biomarkers over time in patients that recover from their injury versus those who have post-concussion syndrome, clinicians can identify those patients who need earlier more aggressive intervention in the early period.

Targeting specific gene candidates is also a future potential of such a study. The current therapeutic landscape for patients with TBI of post-concussion issues is supportive symptom management, including vestibula suppressants for vertigo, or various psychostimulants to address memory and attentions deficits. No therapy aimed at preventing the long-term sequelae of TBI such as the development of post-concussion syndrome, and ultimately abating the possibility of dementia. However, the current therapeutic utility of the EV-based genetic biomarkers in this study comes in the form of grading and stratifying TBI severity. Rather than simply relying on variable and often imperfect examination and neuroimaging findings, purported biomarkers may allow clinicians to diagnose patients earlier, and, depending on the flux of genetic expression, TBI stratification may also be accomplished. Both early detection and accurate stratification can clearly identify patients who need closer monitoring, earlier intervention, and stricter follow-up.

A limitation of this study is the small patient sampling size and that only one sample was taken per participant. Two patient groups: ED head injury (EDPT) and sub-acute/chronic symptomatic concussion (CCPT) groups provided inferential data on the longitudinal course of TBI (Cheng et al., 2019). Future studies of patients suffering from TBI will include sample collection over time. This will provide data on intra-subject patterns of post-TBI gene expression (Cheng et al., 2019). Future studies will also include not only more patients but a focus on closer age matched controls. Biomarker expression and TBI pathophysiology may vary slightly between older and younger individuals, such as those seen in our groups.

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Declaration of participant consent: *The authors certify that they have obtained all appropriate participant consent forms. In the forms the participants have given their consent for their images and other clinical information to be reported in the journal. The participants understand that their names and initials will not be published and due efforts will be made to conceal their identity.*

Reporting statement: *This study followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.*

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Additional files:

Additional file 1: *Open peer review report 1.*

Additional Table 1: *TaqMan human inflammation array gene description.*

Additional Table 2: *Genes and pathways in concussion clinic patients.*

Additional Table 3: *Genes and pathways in emergency department patients.*

References

Aliotta JM, Lee D, Puente N, Faradyan S, Sears EH, Amaral A, Goldberg L, Dooner MS, Pereira M, Quesenberry PJ (2012) Progenitor/stem cell fate determination: interactive dynamics of cell cycle and microvesicles. Stem Cells Dev 21:1627-1638.

Cheng Y, Pereira M, Raukar N, Reagan JL, Queseneberry M, Goldberg L, Borgovan T, LaFrance WC Jr, Dooner M, Deregibus M, Camussi G, Ramratnam B, Quesenberry P (2019) Potential biomarkers to detect traumatic brain injury by the profiling of salivary extracellular vesicles. J Cell Physiol 234:14377-14388.

Choi DS, Kim DK, Kim YK, Gho YS (2015) Proteomics of extracellular vesicles: Exosomes and ectosomes. Mass Spectrom Rev 34:474-490. doi:10.1002/mas.21420

Chong KW, Chen MJ, Koay ES, Wong BS, Lee AY, Russo-Marie F, Cheung NS (2010) Annexin A3 is associated with cell death in lactacystin-mediated neuronal injury. Neurosci Lett 485:129-133.

Dobrivojević M, Špiranec K, Sindić A (2015) Involvement of bradykinin in brain edema development after ischemic stroke. Pflugers Arch 467:201-212.

Faden AI, Wu J, Stoica BA, Loane DJ (2016) Progressive inflammation-mediated neurodegeneration after traumatic brain or spinal cord injury. Br J Pharmacol 173:681-691.

Fields J, Ghorpade A (2012) C/EBPβ regulates multiple IL-1β-induced human astrocyte inflammatory genes. J Neuroinflammation 9:177.

Gyoneva S, Ransohoff RM (2015) Inflammatory reaction after traumatic brain injury: therapeutic potential of targeting cell-cell communication by chemokines. Trends Pharmacol Sci 36:471-480.

Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, et al. (2015) Neuroinflammation in Alzheimer's disease. Lancet Neurol 14:388-405.

Hijioka M, Anan J, Ishibashi H, Kurauchi Y, Hisatsune A, Seki T, Koga T, Yokomizo T, Shimizu T, Katsuki H (2017) Inhibition of leukotriene B4 action mitigates intracerebral hemorrhage-associated pathological events in mice. J Pharmacol Exp Ther 360:399-408.

Junker H, Suofu Y, Venz S, Sascau M, Herndon JG, Kessler C, Walther R, Popa-Wagner A (2007) Proteomic identification of an upregulated isoform of annexin A3 in the rat brain following reversible cerebral ischemia. Glia 55:1630-1637.

Li D, Liu N, Zhao HH, Zhang X, Kawano H, Liu L, Zhao L, Li HP (2017) Interactions between Sirt1 and MAPKs regulate astrocyte activation induced by brain injury in vitro and in vivo. J Neuroinflammation 14:67.

Lucas SM, Rothwell NJ, Gibson RM (2006) The role of inflammation in CNS injury and disease. Br J Pharmacol 147 Suppl 1:S232-40.

McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, Santini VE, Lee HS, Kubilus CA, Stern RA (2009) Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. J Neuropathol Exp Neurol 68:709-735.

Menon DK, Schwab K, Wright DW, Maas AI; Demographics and Clinical Assessment Working Group of the International and Interagency Initiative toward Common Data Elements for Research on Traumatic Brain Injury and Psychological Health. (2010). Position statement: definition of traumatic brain injury. Arch Phys Med Rehabil 91:1637-1640.

Mez J, Daneshvar DH, Kiernan PT, Abdolmohammadi B, Alvarez VE, Huber BR, Alosco ML, Solomon TM, Nowinski CJ, McHale L, Cormier KA, Kubilus CA, Martin BM, Murphy L, Baugh CM, Montenigro PH, Chaisson CE, Tripodis Y, Kowall NW, Weuve J, et al. (2017) Clinicopathological evaluation of chronic traumatic encephalopathy in players of American football. JAMA 318:360-370.

Michael A, Bajracharya SD, Yuen PS, Zhou H, Star RA, Illei GG, Alevizos I (2010) Exosomes from human saliva as a source of microRNA biomarkers. Oral Dis 16:34-38.

Navazesh M (1993) Methods for collecting saliva. Ann N Y Acad Sci 694:72-77.

Orr SK, Butler KL, Hayden D, Tompkins RG, Serhan CN, Irimia D (2015) Gene expression of proresolving lipid mediator pathways is associated with clinical outcomes in trauma patients. Crit Care Med 43:2642-2650.

Paolicelli RC, Bergamini G, Rajendran L (2019) Cell-to-cell communication by extracellular vesicles: focus on microglia. Neuroscience 405:148-157.

Prins M, Greco T, Alexander D, Giza CC (2013) The pathophysiology of traumatic brain injury at a glance. Dis Model Mech 6:1307-1315.

Roozenbeek B, Maas AI, Menon DK (2013) Changing patterns in the epidemiology of traumatic brain injury. Nat Rev Neurol 9:231-236.

Saatman KE, Duhaime AC, Bullock R, Maas AI, Valadka A, Manley GT; Workshop Scientific Team and Advisory Panel Members (2008) Classification of traumatic brain injury for targeted therapies. J Neurotrauma 25:719-738.

Taylor CA, Bell JM, Breiding MJ, Xu L (2017) Traumatic Brain Injury-Related Emergency Department Visits, Hospitalizations, and Deaths - United States, 2007 and 2013. MMWR Surveill Summ 66:1-16.

Tkach M, Théry C (2016). Communication by extracellular vesicles: where we are and where we need to go. Cell 164:1226-1232.

Werner C, Engelhard K (2007) Pathophysiology of traumatic brain injury. Br J Anaesth 99:4-9.

White TE, Surles-Zeigler MC, Ford GD, Gates AS, Davids B, Distel T, LaPlaca MC, Ford BD (2016) Bilateral gene interaction hierarchy analysis of the cell death gene response emphasizes the significance of cell cycle genes following unilateral traumatic brain injury. BMC Genomics 17:130.

Yang Y, Boza-Serrano A, Dunning CJR, Clausen BH, Lambertsen KL, Deierborg T (2018) Inflammation leads to distinct populations of extracellular vesicles from microglia. J Neuroinflammation 15:168.

Yin XJ, Chen ZY, Zhu XN, Hu JJ (2017) Loss of PAFR prevents neuroinflammation and brain dysfunction after traumatic brain injury. Sci Rep 7:40614.

Zhang L, Zhang WP, Hu H, Wang ML, Sheng WW, Yao HT, Ding W, Chen Z, Wei EQ (2006) Expression patterns of 5-lipoxygenase in human brain with traumatic injury and astrocytoma. Neuropathology 26:99-106.

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