Cerebrospinal Fluid Tau, Amyloid, and sTREM2 in Former National Football League Players: Modeling the Relationship between Repetitive Head Impacts, Microglial Activation, and Neurodegeneration

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Abbreviations: CSF = cerebrospinal fluid; CTE = chronic traumatic encephalopathy; AD = Alzheimer's disease; RHI = repetitive head impacts; t-tau = total tau; p-tau = phosphorylated tau; Aβ = beta-amyloid; sTREM2 = soluble triggering receptor expressed on myeloid cells 2

RESEARCH IN CONTEXT

Systematic Review: We reviewed the literature using PubMed and references of research articles. Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with repetitive head impacts (RHI). Cerebrospinal fluid (CSF) markers of total tau (t-tau) for neurodegeneration, phosphorylated tau (p-tau) for tau pathology, beta-amyloid ($A\beta_{1-42}$) for amyloid deposition, and sTREM2 for microglial activation may facilitate detection and study of mechanisms of long-term neurological consequences associated with RHI, including CTE. The utility of CSF protein analysis in this setting remains unclear. This is despite the role that CSF markers of t-tau, p-tau, amyloid, and microglial activation (e.g., sTREM2) play in the diagnosis and study of mechanisms of Alzheimer's disease; these studies are appropriately cited.

Interpretation: In former NFL players, RHI was associated with long-term neuronal injury (i.e., t-tau), and microglial activation (as measured by sTREM2) may be both a cause and a consequence of neuronal injury from RHI.

Future directions: Once CTE can be diagnosed during life, this study should be repeated to clarify the diagnostic role of CSF markers of t-tau, $p\text{-}tau_{181}$, amyloid, and microglial activation in CTE.

HIGHLIGHTS

- Repetitive head impacts (RHI) were positively associated with the neurodegeneration biomarker CSF total tau in former NFL players
- In former NFL players, sTREM2 mediated the relationship between RHI and total tau; however, total tau also mediated the association between RHI and sTREM2
- Microglial activation may be a cause and consequence of long-term neuronal injury associated with RHI from tackle football

ABSTRACT

INTRODUCTION: Cerebrospinal fluid (CSF) protein analysis may facilitate detection and study of mechanisms of neurological consequences from repetitive head impacts (RHI), like chronic traumatic encephalopathy (CTE). We examined CSF biomarkers for neurodegeneration, total tau (t-tau); tau pathology, phosphorylated tau (p-tau); and brain amyloidosis, beta-amyloid ($A\beta_{1-42}$), and their association with RHI in former National Football League (NFL) players. The role of microglial activation (using sTREM2) was examined as a potential pathogenetic mechanism of CTE.

METHODS: Sixty-eight former NFL players and 21 controls underwent lumbar puncture to quantify t-tau, p-tau_{181}, $A\beta_{1-42}$, and sTREM2 in CSF using immunoassays. The cumulative head impact index (CHII) estimated RHI.

RESULTS: No between group differences for CSF analytes emerged. The CHII was associated with t-tau($p=0.009$). sTREM2 mediated the effect of RHI on t-tau($p=0.004$); t-tau also mediated the association between RHI and sTREM2($p=0.0196$).

DISCUSSION: In former NFL players, microglial activation may be both a cause and a consequence of long-term neuronal injury from RHI.

Keywords: Cerebrospinal fluid; tau; amyloid, chronic traumatic encephalopathy; microglial activation; Alzheimer's disease; repetitive head impacts; concussion; subconcussive

INTRODUCTION

CTE is a neurodegenerative disease associated with prior exposure to repetitive head impacts (RHI), such as those incurred through contact sports (e.g., American tackle football, boxing, soccer) and combat military service involving blast exposures.[1-4] In a recent convenience sample of 202 deceased tackle football players, neuropathological evidence of chronic traumatic encephalopathy (CTE) was found in 177 participants; all but one of the 111 former National Football League (NFL) players had CTE.[4] Our understanding of CTE has evolved, but key knowledge gaps remain. Major caveats include that CTE cannot yet be diagnosed during life and the mechanisms by which exposure to RHI transitions to neurodegeneration are unknown. To address these knowledge gaps, current research is focused on the development of *in vivo* biomarkers for CTE.[5,6] *In vivo* biomarkers play a critical role in both the clinical diagnosis and the study of mechanistic pathways of various neurodegenerative diseases, such as Alzheimer's disease (AD).[7] Clinically useful fluid biomarkers for concussion and acute traumatic brain injury (TBI) are emerging.[8]

The pathognomonic diagnostic lesion of CTE is the perivascular deposition of phosphorylated tau (p-tau) at the base of the cortical sulci.[9] If present, beta-amyloid (Aβ) plaques are sparse and diffuse.[4,10] Positron emission tomography (PET) tau radioligands will likely be the optimal diagnostic biomarkers to identify and grade tau pathology in patients with suspected CTE, and may also have value for CTE diagnosis, particularly when used in conjunction with amyloid PET. PET imaging, however, is time-demanding, expensive, and invasive. Cerebrospinal fluid (CSF) can be extracted by lumbar puncture and analyzed for concentrations of tau (total tau [t-tau], p-tau) and Aβ, representing a more pragmatic biomarker that still provides a direct window to the central nervous system (CNS). CSF concentrations of Aβ, t-tau,

and p-tau are indeed core biomarkers for AD.[7,11-13] As in AD,[7] CSF p-tau$_{181}$ may be a specific marker for underlying tangle pathology in CTE. Total tau, in contrast, is a non-specific marker of neurodegeneration and, in addition to CTE, other long-term neurological consequences associated with RHI (e.g., axonal degeneration)[3-5] might be captured by t-tau. Although acute changes in CSF t-tau have been observed following a mild TBI,[14,15] the relationship between RHI and later life CSF tau and amyloid is unknown.

The association between RHI and CTE has been theorized to involve microglial activation.[16,17] In 66 deceased American football players with autopsy-confirmed CTE, more years of football play predicted CD68 (a marker of microglial activation), which partially mediated the effect of RHI on p-tau.[17] Notably, there was a bidirectional relationship between CD68 and p-tau. Tackle football players with autopsy-confirmed CTE have also been shown to exhibit elevated CSF CCL11 (a marker of neuroinflammation), which was related to RHI.[18] Molecular neuroimaging research has further demonstrated chronic microglial activation (e.g., PET measures of TSPO) in active, recently retired, and older former NFL players.[19,20]

Soluble CSF concentrations of the triggering receptor expressed on myeloid cells 2 (sTREM2) may serve as an additional *in vivo* biomarker of microglial activation in CTE. TREM2 is expressed on microglia in the CNS and modulates microglial activation.[21,22] TREM2 variants predict increased risk for other neurodegenerative diseases (e.g., frontotemporal dementia, AD),[23] with odds ratios for AD risk similar to *apolipoprotein E*, although the allele frequency in the population is low.[24,25] sTREM2 can be detected in the CSF and higher concentrations are believed to reflect increased microglial activation.[26-30] sTREM2 has been the focus of several recent clinical research studies of AD, a neurodegenerative disease with clinical and neuropathological similarities as CTE. sTREM2 correlates with CSF markers of AD

neurodegeneration (t-tau, p-tau),[26-28,31] as well as with CSF glial protein YKL-40 concentrations.[26,27] sTREM2 concentrations, however, vary with disease progression. AD cohorts have exhibited elevated,[26,31] reduced,[32] or no changes[33] in sTREM2. When disease stage is considered, sTREM2 levels are higher in the early stages of the AD continuum[27,28,32] and can be increased five years before symptom onset.[34]

Analysis of CSF t-tau, p-tau, and Aβ may be a pragmatic method to detect long-term neurological consequences associated with RHI, including early CTE pathology. Evaluation of sTREM2, in conjunction with CSF markers of neurodegeneration, may provide insight into the potential role of microglial activation in the underlying pathogenetic mechanism of CTE. This study compared CSF t-tau, p-tau$_{181}$, and Aβ_{1-42} between symptomatic former NFL players at high risk for CTE and same-age asymptomatic controls without a history of contact sports or head trauma. The relationship between estimated exposure to RHI (using a cumulative head impact index [CHII][35]) and CSF t-tau, p-tau$_{181}$, Aβ_{1-42}, and sTREM2 was investigated in the former NFL players. This study used simultaneous equations regression models to examine microglial activation (measured by sTREM2) as a potential mechanistic pathway between RHI and neurodegeneration.

METHODS

Participants and Study Design

The current sample included 68 former NFL players who participated in the National Institutes of Health (NIH)-funded study, entitled, "Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests" (DETECT). The purpose of the DETECT study is to identify possible *in vivo* biomarkers for CTE. Inclusion criteria for the former NFL players included: male, ages 40-69, a minimum of two seasons in the NFL and a minimum of twelve years of organized football, and self-reported complaints of cognitive, behavior, and/or mood symptoms at the time of telephone screening. The sample also included 21 controls who were of the same-age as the former NFL players, had no history of TBI or participation in contact sports, and who reported being asymptomatic at the time of telephone screening. The present sample had complete data for all CSF analytes examined. Exclusion criteria for all participants included MRI and/or lumbar puncture contraindications, presence of another CNS disease, and/or a primary language other than English. Participants completed a single two- to three-day study visit, which involved administration of a battery of neuropsychological tests, neurological and psychiatric evaluations, lumbar puncture, and history interview, as well as other examinations not pertinent to the current study. Additional descriptions of the DETECT Study protocol have been reported previously.[5,36] All study protocols were approved by the Boston University Medical Center Institutional Review Board. Participants provided written informed consent prior to participation.

Measures

CSF Analytes

CSF (15-20mL) was obtained by lumbar puncture (LP) in the morning after overnight fasting. LPs were performed by the study neurologist (S.F.) using an atraumatic 25-guage Sprotte needle at either L3/L4 or L4/L5. After aspiration, approximately 10mL of CSF was deposited into a polypropylene transfer tube and frozen at -80°C. Aliquots were shipped to the University of Pennsylvania for batch analysis of t-tau, p-tau$_{181}$, and Aβ_{1-42}. Additional samples of CSF were shipped to University College London (UK) for batch analysis of sTREM2. Methods of CSF analysis of t-tau, p-tau$_{181}$, and Aβ_{1-42},[37-39] and sTREM2[26,28] are described elsewhere. Briefly, p-tau, t-tau$_{181}$, and Aβ_{1-42} levels were measured by flow cytometry using the INNO-BIA Alz Bio3 immunoassay kit (Innogenetics, Ghent, Belgium) with the Luminex xMAP platform (Luminex, Austin, TX). sTREM2 was measured using an in-house electrochemoluminescent assay on an MSD SECTOR imager 6000 (Meso-Scale Discovery, Maryland, US), using a method adapted from Kleinberger et al. (you have this ref no 32.)

Cumulative Head Impact Index (CHII)

The CHII was used to quantify estimated exposure to RHI.[4] The CHII is based on reported number of football seasons played, position[s] played, and levels played (e.g., youth, high school, college), as well as estimated head impact frequencies derived from published helmet accelerometer studies. The CHII was initially developed in only former high school and college football players because helmet accelerometer studies at the professional football level have not been published or made available. For the current sample of former NFL players,

college level estimates of head impact frequencies were applied for estimation of professional level head impact frequencies. A higher CHII reflects greater exposure to RHI.

Cognitive and Neuropsychiatric Function

A neuropsychological test battery, and semi-structured interviews and self-report measures of neuropsychiatric function were administered to all participants. All tests were completed on a separate day from the lumbar puncture. For a full list of the tests administered as part of DETECT, see Alosco et al.[35] To limit the number of analyses, a subset of measures was selected *a priori* to include tests routinely used in the clinical evaluation of neurodegenerative disease, as well as those that assess clinical functions presumed to be impaired in CTE and included in provisional clinical research diagnostic criteria for CTE.[40] This included measures of depression (Center for Epidemiologic Studies Depression Scale [CES-D]), behavioral regulation (Behavior Rating Inventory of Executive Function-Adult version [BRIEF-A] Behavioral Regulation Index [BRI]), episodic memory (Neuropsychological Assessment Battery [NAB] List Learning [LL] Delayed Free Recall), and executive function (Trail Making Test Part B [TMT B]). Raw scores from the neuropsychological tests were transformed to standard scores using normative data that accounted for age, gender, and/or education. For analyses that included clinical measures, the sample size was reduced to 66 former NFL players due to exclusion of two participants for evidence of intentional symptom exaggeration; sample size for TMT B was further reduced to 65 former NFL players due to missing data.

Statistical Analyses

Multivariate linear mixed-effects models examined differences between former NFL players and controls in CSF t-tau, p-tau$_{181}$, p-tau$_{181}$/t-tau, and Aβ_{1-42}. Linear mixed-effect models were performed to reduce type I error, as they account for correlations between groups and outcomes from the same participant. The models were adjusted for age and body mass index (BMI). Bootstrap analysis was performed on 500 replicates to control for type I error, account

for potential non-linear relationships, and increase statistical power. The between group results presented are those from the bootstrap analysis.

Independent regression analyses were used to examine the relationship between exposure to RHI (using the CHII) and the CSF analytes of t-tau, p-tau$_{181}$, p-tau$_{181}$/t-tau, and Aβ_{1-42}. These analyses were adjusted for age and BMI. For relationships that emerged significant, the role of sTREM2 was examined using simultaneous equations regression models. Previous research in participants with autopsy-confirmed CTE found a bidirectional relationship between CTE pathology and microglial activation.[17] Simultaneous equations regression modeling is a form of structural equation modeling that limits endogeneity and evaluates for the presence of potential feedback loops between multiple outcomes.[41] The predictor variables included age, BMI, and the CHII. Outcome variables included sTREM2 and CSF t-tau, p-tau$_{181}$, p-tau$_{181}$/t-tau, and Aβ_{1-42}; again, only the analyte(s) that demonstrated a significant relationship with the CHII in the independent regression model was (were) examined as an outcome, along with sTREM2, in the model. The model tested for direct and total effects (i.e., direct + indirect) of the predictor variables on the outcomes (i.e., the direct and total effects of age, BMI, and CHII on the CSF analytes), as well as for direct effects between the outcomes. In initial models, age, BMI, and the CHII were estimated to predict all outcome variables. However, to maintain model parsimony, only pathways with a $p < 0.10$ were retained in the final model. Regarding clinical function, independent regressions controlling for age and BMI were used to examine the effects of t-tau, p-tau$_{181}$, p-tau$_{181}$/t-tau, Aβ_{1-42}, and sTREM2 on each of the clinical measures (i.e., NAB LL Delayed Recall, TMT-B, BRIEF-A BRI, and CES-D). Results from these analyses were used to guide inclusion of clinical measures as additional outcomes into the simultaneous equations regression models.

RESULTS

Table 1 summarizes sample characteristics. The linear mix-effect models showed no differences between the former NFL players and controls in t-tau ($p = 0.18$), p-tau$_{181}$ ($p = 0.79$), p-tau$_{181}$/t-tau ($p = 0.06$), or A$\beta_{1\text{-}42}$ ($p = 0.08$). In the former NFL players, independent regressions showed a statistically significant association between the CHII and t-tau ($p = 0.009$). Higher CHII scores were associated with higher levels of t-tau (**Table 2).** There were no direct effects between the CHII and p-tau$_{181}$ ($p = 0.29$), p-tau$_{181}$/t-tau ($p = 0.58$), or A$\beta_{1\text{-}42}$ ($p = 0.30$). Thus, only a simultaneous equations regression model was performed for t-tau.

To examine microglial activation (sTREM2) as a potential mechanism for the relationship between RHI and CSF t-tau, a simultaneous equations regression model with t-tau and sTREM2 as the outcome variables was conducted. Age, BMI, and the CHII were included as predictors. In the initial model, the p-values for paths between age and t-tau, as well as between BMI and sTREM2 were greater than 0.10 and were therefore removed from the final model. Based on the independent regression models (see above), the CHII was modeled to have a direct effect on t-tau. The model fit was excellent (Goodness of Fit = 0.9996). There was a statistically significant total effect for the CHII on t-tau ($p = 0.004$) and sTREM2 ($p = 0.0196$), meaning that: 1) sTREM2 mediated the relationship between the CHII and t-tau; and 2) t-tau mediated the association between the CHII and sTREM2 (see **Figure 1**). There was a statistically significant feedback loop between t-tau and sTREM2, such that higher t-tau concentrations predicted greater levels of sTREM2 ($p = 0.006$) and vice versa ($p = 0.016$). See **Table 3**. Independent regressions controlling for age and BMI showed no associations between any of the CSF analytes and the clinical measures (ps > 0.10 for all). Therefore, simultaneous equations regression models with clinical measures included as additional outcomes were not performed.

DISCUSSION

In this sample of 68 symptomatic former NFL players, greater exposure to RHI was associated with higher CSF concentrations of t-tau, but not p-tau$_{181}$, p-tau$_{181}$/t-tau, or Aβ_{1-42}. sTREM2 mediated the relationship between exposure to RHI and t-tau. However, there was a bidirectional mediation relationship, as t-tau also mediated the association between RHI and sTREM2. Total tau and sTREM2 exerted bidirectional effects on each other. The CSF analytes were not associated with cognitive or neuropsychiatric function, and there were no statistically significant differences in concentrations for any of the CSF analytes between former NFL players and same-age asymptomatic controls without a history of contact sports or head trauma. Overall, this study provides initial evidence for microglial activation as both a cause and a consequence of long-term neuronal injury associated with exposure to RHI from tackle football. If confirmed by longitudinal studies and animal models, exposure to RHI may initiate a chronic state of microglial activation to contribute to neurodegeneration in former NFL players. Furthermore, once neurodegeneration from RHI or CTE begins, an additional microglia response may be activated that might initially be reparative in nature but could eventually promote further neuronal injury.

Microglial Activation as a *Cause* of Neuronal Injury from RHI

Chronic microglial activation may be involved in the development of long-term neurological consequences associated with exposure to RHI from tackle football. Tau is predominantly expressed in neuronal axons. An acute mild TBI (concussion) leads to a 'cascade' of neurometabolic events due, in part, to diffuse shearing and tearing of axons.[42] In response to acute CNS injury, microglia are activated for reparative and restorative functions. However, microglia may become chronically over-active with repeated aggravation of CNS injury due to

RHI, which involves repeated concussive and subconcussive brain trauma with minimal recovery between head impacts.[35] Chronic microglial activation can be detrimental due to prolonged exposure to inflammatory cytokines that can result in neuronal toxicity and eventual neurodegeneration.[43] This is evidenced in proposed neuroinflammation models of AD,[44] where neuroinflammation contributes to the formation of neurofibrillary tangles.[44-47] Overall, chronic microglial activation from exposure to RHI may represent the mechanistic trigger for a cascade of pathophysiological events that ultimately leads to neurodegeneration and neurodegenerative diseases, like CTE.

There is existing research that supports the role of microglial activation in the pathogenesis of CTE. In a sample of 66 deceased tackle football players with neuropathologically-confirmed CTE, Cherry et al. found a direct effect between increased microglial activation (measured by CD68) and p-tau, and the association between years of football play and p-tau accumulation was mediated by microglial activation.[17] More years of football play was also found to be associated with elevated CSF concentrations of CCL11 (a marker of neuroinflammation) in tackle football players with autopsy-confirmed CTE.[18] Mice models of RHI and CTE have further linked microglial activation with p-tau pathology.[45-49] Importantly, the current study found specific effects for t-tau and not p-tau$_{181}$. Unlike the autopsy studies from Cherry et al.,[17,18] the presence of CTE neuropathology in this clinical cohort is unknown. Although p-tau$_{181}$ is an established biomarker for tangle pathology in AD,[7] normal levels of CSF p-tau have been reported in other neurodegenerative tauopathies.[50] Each neurodegenerative tauopathy, including CTE, may be characterized by distinct p-tau isoforms or species not uniformly detected by CSF assays. Further, the severity of tau pathology may be too limited to result in changed CSF p-tau levels in this group of former NFL players. Because t-tau

is a non-specific marker of neurodegeneration, the current findings may be capturing p-tau, as well as non-p-tau pathologies associated with concussion and RHI that also accompany CTE, such as axonal injury.[3,4,42] This is consistent with research from our team that found a significant positive relationship between exposure to RHI and *plasma* t-tau.[5]

Microglial Activation as a *Consequence* of RHI-Related Neuronal Injury

Based on our findings, once neurodegeneration from RHI or CTE begins, an additional microglia response may be initiated that could further promote neuronal injury. Cherry et al. also found that p-tau pathology predicted microglial activation (and vice versa) in their sample of 66 deceased tackle football players with CTE.[17] The homeostatic functions of reactive microglial activation can be comprised in the presence of neurodegeneration.[44] In AD, it has been postulated that stimuli-induce microglial activation causes neurotoxicity to promote neurodegeneration and, in turn, the injured neurons propagate further initiation of glial activity.[44] There could thus be multiple sources of neuroinflammation (e.g., tau, RHI) potentially occurring simultaneously or at different times (e.g., initially during active exposure to RHI, and then again later in life in the setting of neurodegeneration) that converge to cause progressive chronic neuronal damage in former NFL players. Indeed, increased microglial activation has now been a rather consistent finding in living and deceased, active and retired, tackle football players[17-20] and seems to contribute to the eventual development of dementia.[17] Modulation of microglia may thus be a key therapeutic target for the management of long-term neurological conditions associated with RHI.

Group Differences and Clinical Function

There were no differences between former NFL players and same-age asymptomatic controls for any of the CSF analytes. Furthermore, within the former NFL group, none of the

CSF analytes were associated with cognitive or neuropsychiatric function. Estimated RHI exposure was not associated with $A\beta_{1-42}$. The lack of findings for $A\beta_{1-42}$ is not surprising, given Aβ is commonly absent in CTE and, if present, plaques are sparse and diffuse.[3,4,10] The relationship between TBI and Aβ accumulation has been examined by several studies using transgenic mice susceptible to AD pathology (due to mutations of the Aβ precursor protein) subjected to controlled cortical impact.[51-53] Some of these studies provide evidence for reduced or no accumulation of Aβ months after the TBI.[51,52] Interestingly, however, other evidence in transgenic mice with mutations of the Aβ precursor protein suggests that repetitive mild TBI may accelerate Aβ deposition,[54] and this is similar to what has been observed in CTE.[10] Overall, $A\beta_{1-42}$ deposition in the setting of CTE continues to be the focus of research by our team.

The current study also did not find group differences or clinical effects for p-tau_{181} or t-tau. There is likely substantial variability across participants in the presence and severity of CTE and/or RHI-related pathology. p-tau_{181} burden and/or neuronal injury may be below clinical threshold or only a small subset have meaningful pathology.[5] The disease stage of the sample (which is unknown) may have particularly important implications for sTREM2. When disease stage is considered, sTREM2 levels are higher in the early stages of the AD continuum, perhaps even before symptom onset.[27,28,32,34] In CTE, sTREM2 may be highest during active exposure to RHI, diminish after exposure to RHI has ended, and increase when neurodegeneration begins. Interestingly, microglial activation seems to have opposing effects on Aβ and p-tau in AD, as it may facilitate clearance of Aβ[55-58] and promote p-tau pathogenesis.[44-47] It could therefore be speculated that early and persistent microglial activation from RHI could partially explain the reduced and often absent fibrillar Aβ pathology in CTE.

Limitations

Although sTREM2 is believed to reflect microglial activation, the exact biological role(s) of sTREM2 remains unclear.[23] There are multiple TREM2 variants that influence ligand binding and each variant may play a unique role in response to pathology and to the different types of pathologies. We found evidence for bidirectional relationships between t-tau and sTREM2. Longitudinal studies that follow former tackle football players throughout their life are needed to elucidate directionality in the relationships among RHI, sTREM2, and t-tau. For example, it is possible that neurodegeneration may begin years after exposure to RHI has ended, but it is unclear whether a persistent state of microglial activation remains even after exposure to RHI has ended. In other words, it remains unclear how microglial activation from the time of exposure to RHI transitions to neurodegeneration. Another possible limitation of this study is the use of CSF analytes. Although lumbar punctures are pragmatic and safe, they are often viewed by patients and some clinicians as invasive. The correspondence of CNS pathology and plasma tau concentrations is unclear, however, blood protein analysis of tau is highly practical and there is preliminary support for plasma t-tau and exosomal tau as biomarkers for CTE.[5,6] Future work should examine plasma sTREM2 in the setting of CTE, particularly given increased expression of TREM2 has been found in plasma of AD dementia patients compared to controls, and has been found to predict cognitive status.[59]

CONCLUSIONS

In this sample of former NFL players, estimated cumulative exposure to RHI was associated with long-term neuronal injury (based on CSF t-tau), which was mediated by microglial activation (based on CSF sTREM2). Our findings support microglial activation as both a potential cause and a consequence of neuronal injury subsequent to exposure to RHI from tackle football. Microglial activation may indeed be involved in the pathogenesis of long-term neurological consequences associated with exposure to RHI, like CTE. This study provides initial evidence for CSF t-tau and sTREM2 as possible *in vivo* biomarkers to assist in the detection and identification of mechanistic pathways of long-term neurological consequences associated with exposure to RHI. Once CTE can be diagnosed accurately during life, this study should be repeated to elucidate the clinical and diagnostic implications of CSF t-tau, p-tau$_{181}$, and sTREM2 in the setting of CTE.

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Table 1. Sample Characteristics

	NFL (n = 68)	**Control (n = 21)**	**p-value**
Age, mean (SD) years	54.50 (7.95)	57.57 (7.10)	0.12
Education, mean (SD) years	16.44 (0.97)	17.38 (2.22)	0.07
[a]African American, n (%)	29 (42.6)	1 (4.8)	**0.001**
Duration of football play, mean (SD) years	18.67 (3.56)	--	--
Years in the NFL, mean (SD)	8.30 (2.89)	--	--
Cumulative Head Impact Index, mean (SD)	20,304.87 (6195.14)	--	--
Primary Position Group, n (%)			--
Offensive line	20 (29.41)	--	--
Running back	5 (7.35)	--	--
Tight end	5 (7.35)	--	--
Offensive skill	0	--	--
Defensive line	12 (17.65)	--	--
Linebacker	14 (20.59)	--	--
Defensive Back	12 (17.65)	--	--
Body mass index, mean (SD) kg/m^2	32.67 (4.43)	28.36 (3.84)	**<0.001**

The control group was required to be asymptomatic and have no history of head trauma.
[a]Independent sample t-tests were performed for continuous outcomes and Fisher's Exact Test was used to compare group differences in race.

Table 2. Summary of Linear Regression Examining the Direct Effects of Exposure to Repetitive Head Impacts on Cerebrospinal Fluid Markers of Tau and Amyloid

	t-tau			**p-tau$_{181}$**			**p-tau$_{181}$/ t-tau**			**Aβ$_{1-42}$**		
	beta	SE	P-value	beta	SE	P-value	beta	SE	P-value	beta	SE	P-value
Age	0.39	0.19	**0.039**	0.06	0.15	0.70	-0.01	0.004	0.25	-0.20	1.18	0.86
BMI	-0.89	0.34	**0.010**	-0.04	0.28	0.90	0.01	0.01	0.10	-1.64	2.15	0.45
CHII	0.64	0.24	**0.009**	0.21	0.20	0.29	-0.003	0.01	0.58	1.59	1.53	0.30

Abbreviations: t-tau = total tau, p-tau$_{181}$ = hyperphosphorylated tau, Aβ = beta-amyloid, BMI = body mass index, CHII = cumulative head impact index, SE = standard error. Beta is unstandardized and bolded values highlight statistical significance.

Table 3. Summary of Direct and Total Effects from Simultaneous Equations Regression Model Examining sTREM2 and Total Tau as Outcomes

	Standardized Direct Effects								**Standardized Total Effects**							
	sTREM2				**t-tau**				**sTREM2**				**t-tau**			
	Beta	**SE**	**t**	**P**	**Beta**	**SE**	**t**	**P**	**Beta**	**SE**	**t**	**P**	**Beta**	**SE**	**t**	**P**
Age	0.20	0.14	1.46	0.15	--	--	--	--	0.39	0.10	3.84	**0.0001**	0.24	0.11	2.24	**0.025**
BMI	--	--	--	--	-0.17	0.13	-1.33	0.18	-0.24	0.10	-2.40	**0.016**	-0.32	0.10	-2.98	**0.003**
CHII	--	--	--	--	0.16	0.12	1.34	0.18	0.22	0.10	2.33	**0.0196**	0.30	0.11	2.85	**0.004**
sTREM2	--	--	--	--	0.63	0.26	2.41	**0.016**	0.89	1.07	0.84	0.40	1.19	1.12	1.06	0.29
t-tau	0.75	0.27	2.76	**0.006**	--	--	--	--	1.42	1.25	1.14	0.26	0.89	1.07	0.84	0.40

In the initial model, the p-values for paths between age and t-tau, as well as between BMI and sTREM2 were greater than 0.10 and were therefore removed from the final model. Based on independent regression, the CHII was only estimated to have a direct effect on t-tau. BMI = body mass index, CHII = cumulative head impact index, t-tau = total tau, SE = standard error. Beta is unstandardized and bolded values highlight statistical significance.

Figure 1 Legend. Relationships Among Exposure to Repetitive Head Impacts, Total Tau, and sTREM2. The Figure is a visual representation of the simultaneous equations regression model examining CSF total tau (t-tau) and sTREM2 as outcomes. Dashed lines are indirect effects and solid lines are direct effects. Values are unstandardized betas and asterisk denotes statistical significance of $p<0.05$. Age, body mass index (BMI), and the cumulative head impact index (CHII) were included as predictors. In initial models, the p-values for paths between age and t-tau, as well as between BMI and sTREM2 were greater than 0.10 and were therefore removed from the final model below. Based on independent regression models, the CHII was only estimated to have a direct effect on t-tau. Model fit was excellent (Goodness of Fit = 0.9996). The Figure shows a bidirectional mediation relationship among the variables. sTREM2 mediated the association between CHII and t-tau ($p = 0.004$); however, t-tau also mediated the association between the CHII and sTREM2 ($p = 0.0196$). There was a statistically significant feedback loop between t-tau and sTREM2. Total tau mediated the association between BMI and sTREM2 ($0 = 0.016$), and the relationship between BMI and t-tau was also mediated by sTREM2 ($p = 0.003$). Age had indirect effects on both t-tau ($p = 0.025$) and sTREM2 ($p = 0.0001$).