



Association of Chronic Pain with Biomarkers of Neurodegeneration, Microglial Activation, and Inflammation in Cerebrospinal Fluid and Impaired Cognitive Function

Angélique Sadlon, MD, PhD ^{1,2,3} Petros Takousis, PhD,¹ Barbara Ankli, MD,^{3,4}
Panagiotis Alexopoulos, MD,^{5,6,7,8} and Robert Perneczky, MD, ^{1,9,10,11,12}
for the Alzheimer's Disease Neuroimaging Initiative[†]

Objective: Debate surrounds the role of chronic pain as a risk factor for cognitive decline and dementia. This study aimed at examining the association of chronic pain with biomarkers of neurodegeneration using data from the Alzheimer's Disease Neuroimaging Initiative.

Methods: Participants were classified using the ATN (amyloid, tau, neurodegeneration) classification. Chronic pain was defined as persistent or recurrent pain reported at baseline. For each ATN group, analysis of covariance models identified differences in cerebrospinal fluid (CSF) levels of amyloid β_{1-42} , phosphorylated tau 181 (ptau₁₈₁), total tau (t-tau), soluble triggering receptor expressed on myeloid cells 2 (sTREM2), and cognitive function between chronic pain states. Differences in CSF levels of inflammatory markers between chronic pain states were further analyzed. Linear mixed effect models examined longitudinal changes.

Results: The study included 995 individuals, with 605 (60.81%) reporting chronic pain at baseline. At baseline, individuals with suspected non-Alzheimer pathophysiology and chronic pain showed increased CSF levels of t-tau and sTREM2. Chronic pain was associated with increased tumor necrosis factor α levels, irrespective of the ATN group. Longitudinally, an increase in ptau₁₈₁ CSF levels was observed in chronic pain patients with negative amyloid and neurodegeneration markers. Amyloid-positive and neurodegeneration-negative chronic pain patients showed higher memory function cross-sectionally. No significant longitudinal decline in cognitive function was observed for any ATN group.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.26804

Received Dec 17, 2022, and in revised form Sep 20, 2023. Accepted for publication Sep 22, 2023.

Address correspondence to Dr Perneczky, Division of Mental Health of Older Adults, Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-Universität München, Nußbaumstr 7, 80336 Munich, Germany. E-mail: robert.perneczky@med.uni-muenchen.de

[†]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

From the ¹Ageing Epidemiology Research Unit, School of Public Health, Imperial College London, London, UK; ²Department of Clinical Chemistry, Inselspital, Bern University Hospital and University of Bern, Bern, Switzerland; ³Pain Clinic Basel, Basel, Switzerland; ⁴Faculty of Medicine, University of Basel, Basel, Switzerland; ⁵Global Brain Health Institute, School of Medicine, Trinity College Dublin, University of Dublin, Dublin, Ireland; ⁶Department of Psychiatry, Patras University General Hospital, Faculty of Medicine, School of Health Sciences, University of Patras, Patras, Greece; ⁷Patras Dementia Day Care Center, Patras, Greece; ⁸Department of Psychiatry and Psychotherapy, Rechts der Isar Hospital, Technical University of Munich, Munich, Germany; ⁹Department of Psychiatry and Psychotherapy, University Hospital, Ludwig Maximilian University of Munich, Munich, Germany; ¹⁰German Center for Neurodegenerative Diseases Munich, Munich, Germany; ¹¹Munich Cluster for Systems Neurology, Munich, Germany; and ¹²Sheffield Institute for Translational Neurosciences, University of Sheffield, Sheffield, UK

Additional supporting information can be found in the online version of this article.

Interpretation: Our study suggests that chronic pain induces neuronal damage and microglial activation in particular subgroups of patients along the AD spectrum. Further studies are needed to confirm these findings.

ANN NEUROL 2023;00:1–12

Pain is a distressing experience involving biological, cognitive and environmental dimensions and is defined as chronic if lasting >3 months.¹ Chronic pain can be a highly disabling condition, affecting >20% of adults in the USA and Europe.² Large longitudinal studies suggest that chronic pain is associated with cognitive decline and incident dementia.^{3,4} However, two recent systematic reviews and meta-analyses failed to show an unequivocal link between the two conditions, highlighting the heterogeneity among the included studies in terms of pain definition and assessment, follow-up time, neuropsychological testing, and definitions of cognitive decline and dementia.^{5,6} Furthermore, analyses were not always adjusted for risk factors known to alter cognitive function such as analgesic intake and depression, highly prevalent in chronic pain.⁷ Finally, so far no study has accounted for the presence of genetic risk factors for Alzheimer disease (AD), the most frequent form of dementia.⁸ Yet, it is now well established that AD has a strong genetic heritability, accounting for 58 to 79% of late onset cases.⁹ It remains therefore unknown whether chronic pain individuals suffering from cognitive decline already had a genetic predisposition for AD.

Nonetheless, the link between chronic pain and cognitive decline deserves further investigation, particularly as accumulating evidence from experimental studies points toward shared alterations between chronic pain and AD such as upregulated microglial activation, and locus coeruleus dysfunction.¹⁰ Also, a recent genome-wide association study in the UK Biobank found that genetic variants significantly associated with multisite chronic pain are located in genes involved in neurogenesis and synaptic signaling, pathways also altered in AD.¹¹ Finally, a recent study in a mouse model of chronic neuropathic pain found that chronic pain is associated with tau-mediated hippocampal atrophy and cognitive deficits.¹²

To our knowledge, no study has examined the association between chronic pain status and biomarkers of neurodegeneration in the cerebrospinal fluid (CSF), and advanced neuropsychological testing. Yet, such results would not only unveil the influence of chronic pain on neuronal function but would also help identify individuals at risk for developing AD, most likely to benefit from targeted preventive measures.

Using data from the well-phenotyped and genotyped Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, we examined the influence on CSF biomarkers of

neurodegeneration, inflammation, and microglial activation cross-sectionally and longitudinally. In a further step, we investigated the effect of chronic pain on cognitive function, both cross-sectionally and longitudinally.

Patients and Methods

Study Design

Data used in the preparation of this article were obtained from the ADNI database (accessed in May 2022). The ADNI was launched in 2003 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD ([ClinicalTrials.gov](https://clinicaltrials.gov) IDs: NCT02854033, NCT01231971). The primary goal of ADNI is to test whether serial magnetic resonance imaging, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. The ADNI cohort includes subjects with AD dementia, MCI, and healthy controls. Using the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria, individuals were classified as having AD if they presented a Mini-Mental State Examination (MMSE) score of 20 to 26 and a Clinical Dementia Rating (CDR) score of 0.5 or 1. Individuals without functional complaints but a MMSE score of 24 to 30, a CDR score of 0.5 (with a memory box score of 0.5 or greater), and memory complaints were classified as having MCI. Finally, healthy controls were defined as being independent in their activities of daily living, reporting no memory complaints and impairment on cognitive testing. Further details on the study characteristics are described elsewhere.¹³ Study protocols can be found on <https://adni.loni.usc.edu/methods/documents/>.

Standard Protocol Approvals, Registration, and Contents

ADNI was reviewed and approved by all host study site institutional review boards, and participants completed written informed consent after receiving a comprehensive description of the ADNI.

ATN Classification

To study the role of chronic pain on biomarkers of neurodegeneration, participants were classified based on their CSF AD biomarker results using the ATN classification from the National Institute on Aging–Alzheimer's

Association research framework. This classification is based on the presence of amyloid deposition (A), tau pathology (T), and neurodegeneration (N) at baseline and is intended for research purposes with the aim of understanding the pathophysiology behind neurodegenerative diseases.¹⁴ As proposed before, A+ was defined as CSF amyloid β ($A\beta$)₁₋₄₂ ≤ 192 pg/ml ($A\beta$ ₁₋₄₂ range = 77.90–327.00pg/ml) and TN+ was defined as phosphorylated tau 181 ($p\tau_{181}$) ≥ 23 pg/ml ($p\tau_{181}$ range = 6.90–213.00pg/ml) and/or total tau (t-tau) ≥ 93 pg/ml (t-tau range = 14.30–479.00pg/ml).¹⁵ Using these cutoffs, we defined 4 distinct groups: 2 groups along the AD continuum (A+TN+ and A+TN-), 1 group with suspected non-AD pathophysiology SNAP (suspected non-Alzheimer disease pathophysiology (SNAP); A-TN+), and 1 group with normal biomarkers (A-TN-). Individuals with other biomarker profiles suggesting a non-AD pathology were excluded.

Chronic Pain Definition

In ADNI, participants were asked at each visit about the presence of pain symptoms as well as symptom duration (episodic, persistent, or recurrent) and severity (mild, moderate, or severe). Definition of severity grades were as follows: mild as discomfort without disruption of normal daily activity, moderate as discomfort with reduction of normal daily activity, and severe as incapacity to perform any normal daily activity. The symptoms were recorded in the *BSXSYMP* or *MHDESC* columns in the *recbllog* and *recmbist* datasets, respectively. We used the International Association for the Study of Pain classification to manually identify patients suffering from recurrent or persistent pain at the screening or baseline visits for a duration of at least 3 months.¹ The duration was calculated as the difference between the examination date and the reported symptom onset date (*BSXONSET* or *MHDTONSET* columns in the *recbllog* and *recmbist* datasets, respectively). Both chronic primary and secondary pain were considered. Chronic primary pain syndromes included chronic widespread pain (eg, fibromyalgia), complex regional pain syndromes, chronic primary headaches (eg, migraine), chronic primary visceral pain (eg, arising from irritable bowel syndrome), and chronic primary musculoskeletal pain (eg, low back pain). Chronic secondary pain syndromes were defined as pain syndromes arising from an underlying condition (eg, rheumatoid arthritis).¹

Outcomes

Neuropsychological Assessments. For neuropsychological assessments we used the composite scores of memory and executive function, described and validated previously, which are provided by ADNI.^{16,17} Briefly, the composite

score of memory (ADNI MEM, range = -2.27 to 3.14) included results from the Auditory Verbal Learning Test, the word list learning and recognition components of the AD Assessment Scale–Cognitive Subscale, word recall items from the MMSE, and Logical Memory I from the Wechsler Memory Test–Revised.¹⁷ The composite score for executive function (ADNI EF, range = -3.02 to 3.00) was derived from the digit symbol substitution and digit span backward tests, Trail Making Test Parts A and B, animal and vegetable Category Fluency, Digit Cancellation, and the Clock Drawing test.¹⁶

CSF and Blood Biomarkers. Biomarkers of neurodegeneration included CSF concentrations of $A\beta$ ₁₋₄₂, $p\tau_{181}$, and t-tau, analyzed by the ADNI Biomarker core laboratory on a multiplex xMAP Luminex platform using specific monoclonal antibodies.¹⁸ To investigate neuroinflammation, we used CSF levels of soluble triggering receptor expressed on myeloid cells 2 (sTREM2), a marker of microglial activation, altered at different stages of the AD spectrum.^{19,20} sTREM2 was measured in the CSF via enzyme-linked immunosorbent assay as described previously.²¹ Inflammatory protein CSF levels (tumor necrosis factor [TNF]-R1, TNF-R2, transforming growth factor [TGF] β 1, TGF β 2, TGF β 3, interleukin [IL]-21, IL-6, IL-7, IL-9, IL-10, TNF α , interferon γ -induced protein 10, IL.12.P40, intercellular adhesion molecule-1, vascular cell adhesion molecule-1) were analyzed using a multiplex immunoassay.²²

Covariates

In addition to age, sex, education, and apolipoprotein E (*APOE*) ϵ 4 status (at risk individuals were defined as carrying at least one risk allele), we considered the Geriatric Depression Scale (GDS) short form score (range = 0–15) for each visit as well as the CDR–Sum of Boxes (CDR-SB; range = 0–18). Moreover, for each visit, analgesic intake by study participant provided in the *recmed* table by ADNI was extracted. We extracted following drug categories recommended in the management of chronic pain: "opioids" (Anatomical Therapeutic Chemical Classification code [ATC]: *N02A*), "anti-inflammatory and anti-rheumatic products, non steroids" (ATC: *M01A*), "pyrazolones" (ATC: *N02BB*) such as metamizole, "anilides" (ATC: *N02BE*) such as paracetamol, "carboxamide derivatives" (ATC: *N03AF*) such as carbamazepine, and finally, pregabalin and gabapentin from the "other antiepileptics" (ATC: *N03AX*) group.²³ The number of chronic conditions as defined in the Charlson Comorbidity Index was extracted for each visit and converted to a binary variable if at least one chronic condition was present.²⁴

Statistical Methods

Descriptive data are presented as frequency (n [%]) for categorical variables and mean (standard deviation [SD]) for continuous variables. Outliers were removed if they fell $>3 \times$ the interquartile range above the third quartile or under the first quartile. Normality was assessed using the Shapiro–Wilk test. Here, all CSF biomarkers showed a significant Shapiro–Wilk test ($p < 0.05$) and were log10 transformed to approach the assumptions of Gaussian normal distribution. Differences between chronic pain status were first investigated using a Pearson chi-squared test or a Fisher's exact test for categorical variables and t test or Wilcoxon test for normally or non-normally distributed continuous variables, respectively.

At baseline, analyses of covariance compared CSF biomarkers as well as neuropsychological outcomes

between chronic pain and non-chronic pain patients for each ATN group, adjusting for age, sex, education, *APOE* $\epsilon 4$ carrier status, and CDR-SB score. Additional covariates (GDS score, intake of analgesic, presence of chronic diseases) were added to the model if they met the significance level of $\alpha = 0.2$ in the univariate model. We further conducted an interaction analysis between pain status and painkiller intake (except for the A–TN+ group, as only one patient in the non-chronic pain group reported painkiller intake).

For the longitudinal analysis, for each ATN group, we used a linear mixed effect regression model using the function *lmer* from the *lme4* package in R and adding the 2-way interaction time of visit \times chronic pain status. The covariates age, sex, education, *APOE* $\epsilon 4$ carrier status,

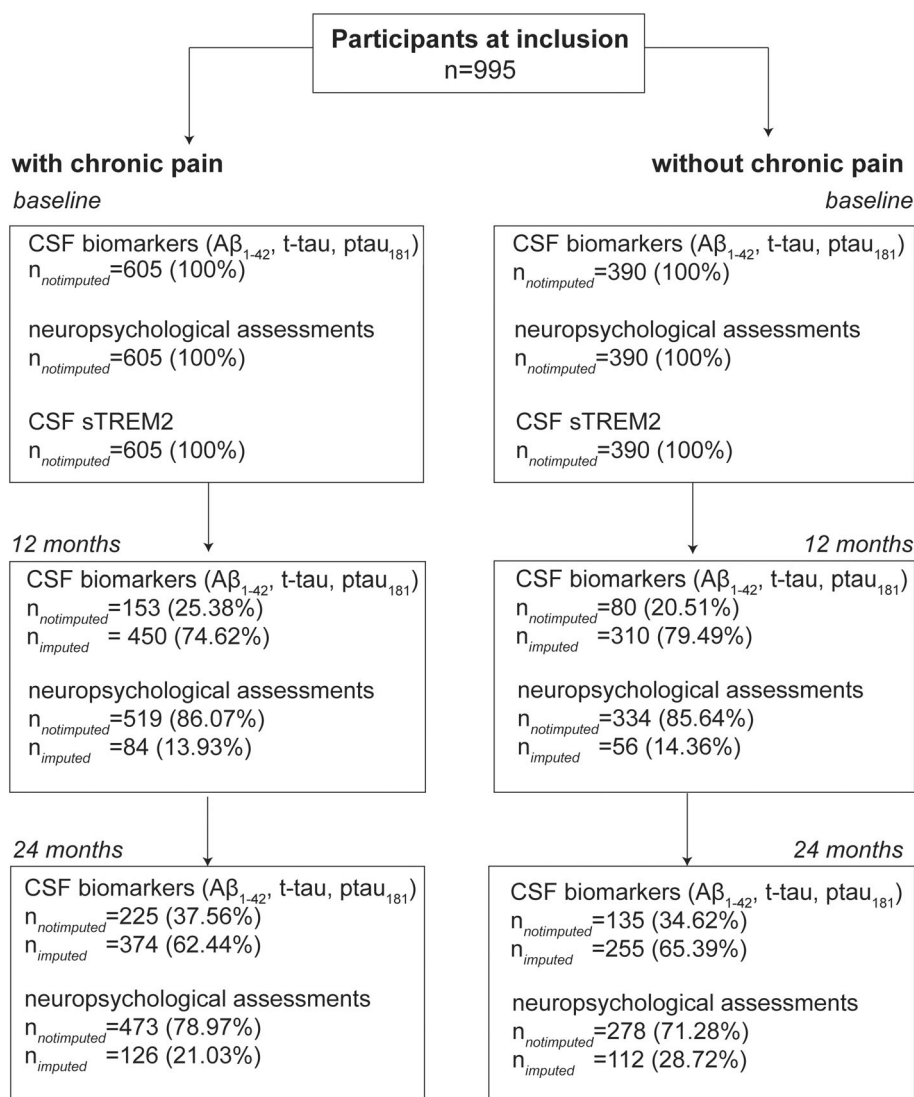


FIGURE 1: Study flowchart. Missing variables were imputed using a random forest algorithm implemented in the *MissForest* package in R. Neuropsychological assessments included Alzheimer's Disease Neuroimaging Initiative (ADNI) composite scores of memory (ADNI MEM) and executive function (ADNI EF). $A\beta$ = amyloid β ; CSF = cerebrospinal fluid; ptau₁₈₁ = phosphorylated tau 181; sTREM2 = soluble triggering receptor expressed on myeloid cells 2; t-tau = total tau.

TABLE. Demographics

Characteristic	Overall, n = 995 ^a	No chronic pain, n = 397 ^a	Chronic pain, n = 605 ^a	p ^b
Age, yr	73.07 (7.33)	72.86 (7.55)	73.20 (7.19)	0.5
Gender				<0.001
Female	435 (43.72%)	144 (36.92%)	291 (48.10%)	
Male	560 (56.28%)	246 (63.08%)	314 (51.90%)	
Education, yr	16.03 (2.79)	16.40 (2.64)	15.78 (2.85)	0.002
<i>APOE</i> ε4				0.092
Noncarrier	528 (53.07%)	194 (49.74%)	334 (55.21%)	
Risk allele carrier	467 (46.93%)	196 (50.26%)	271 (44.79%)	
GDS	1.44 (1.38)	1.32 (1.28)	1.53 (1.44)	0.053
CDR	1.66 (1.80)	1.86 (1.96)	1.53 (1.67)	0.049
Presence of ≥1 chronic conditions	316 (31.76%)	114 (29.23%)	202 (33.39%)	0.2
Painkiller intake	205 (20.60%)	35 (8.97%)	170 (28.10%)	<0.001
Pain type				
Visceral	31 (3.12%)	0 (0.00%)	31 (5.12%)	
Musculoskeletal	284 (28.54%)	0 (0.00%)	349 (57.69%)	
Widespread pain [eg, fibromyalgia]	1 (0.10%)	0 (0.00%)	1 (0.17%)	
Headache or orofacial pain	31 (3.12%)	0 (0.00%)	34 (5.62%)	
Mixed pain types	190 (19.10%)	0 (0.00%)	190 (31.40%)	
ATN				0.026
A–TN–	189 (18.99%)	64 (16.41%)	125 (20.66%)	
SNAP	182 (18.29%)	61 (15.64%)	121 (20.00%)	
A+TN–	76 (7.64%)	27 (6.92%)	49 (8.10%)	
A+TN+	548 (55.08%)	238 (61.03%)	310 (51.24%)	

Abbreviations: *APOE* = apolipoprotein E; ATN = amyloid, tau, neurodegeneration; CDR = Clinical Dementia Rating; GDS = Geriatric Depression Scale; SNAP = suspected non-Alzheimer disease pathophysiology.

^aMean (standard deviation) or n (%).

^bWilcoxon rank-sum test, Pearson chi-squared test, or Fisher exact test.

CDR-SB score, analgesic intake (at visit point), and GDS total (at visit point) were added as fixed effects to the model. We ran a model without random effects and a model including a random intercept and/or a random slope for each participant and then compared the models using the Akaike information criterion. Here, the model including a random intercept showed better performance for each outcome.

To increase the power for our longitudinal analysis, we imputed missing values at 12- and 24-month visits using a random forest algorithm implemented in the

MissForest package in R.²⁵ This method creates a nonparametric imputation for each variable; it was recently described and validated to impute missing values for longitudinal data analysis in the ADNI datasets.^{26,27}

All statistical analyses were undertaken in R version 4.2.1. Due to the hypothesis-generating nature of our paper, statistical significance was set at $p_{raw} < 0.05$. We also carried a Bonferroni correction (p_{adj}) to account for multiple comparison for the CSF biomarker outcomes ($N_{comp} = 4$) and for the cognitive function outcomes ($N_{comp} = 2$).

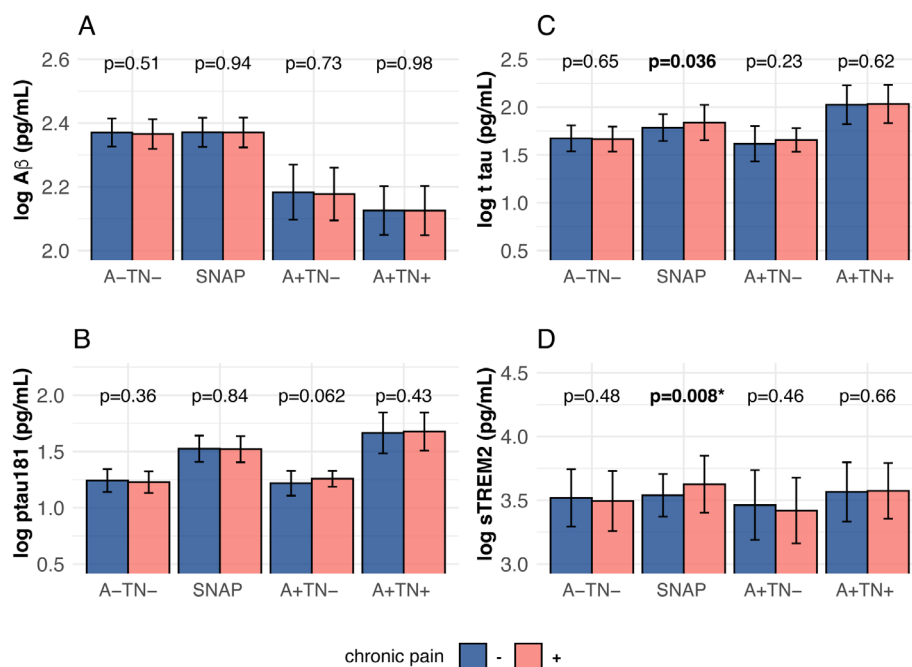


FIGURE 2: Differences in cerebrospinal fluid levels of amyloid β ($A\beta$; A), total tau (t tau; B), phosphorylated tau 181 (ptau181; C), and soluble triggering receptor expressed on myeloid cells 2 (sTREM2; D) between chronic pain patients from different ATN (amyloid, tau, neurodegeneration) groups. Uncorrected p values are shown, with boldface highlighting significant uncorrected p values, and * denoting significant results after Bonferroni correction for multiple comparison. Analysis of covariance model includes age, gender, education, APOE $\epsilon 4$ carrier status, Clinical Dementia Rating–Sum of Boxes, analgesic intake, and Geriatric Depression Scale total score as covariates. Blue = no chronic pain, red = chronic pain. SNAP = suspected non-Alzheimer disease pathophysiology.

Results

Baseline Characteristics

The total sample size at baseline consisted of 995 individuals (Fig 1). The cohort's mean age (SD) was 73.07 (7.33) years, and 560 (56.28%) participants were male. A total of 605 (60.81%) participants reported chronic pain at baseline, with musculoskeletal pain being the most frequent chronic pain type (46.94%); compared to patients without chronic pain, these participants were older (mean age = 73.20 vs 72.86 years, $p < 0.001$), were more frequently male (51.90% male vs 48.10% female, $p < 0.001$), reported lower education years (mean education years = 15.78 vs 16.40 years, $p = 0.002$), and took analgesics more regularly (regular analgesic intake: 28.10% vs 8.97%, $p < 0.001$). The two chronic pain status groups showed differences in ATN group distribution, as well as $A\beta_{1-42}$ CSF levels at baseline (Table).

Cross-Sectional Analysis of Chronic Pain and CSF Neurodegeneration Markers

At baseline, we found that individuals from the SNAP group had increased t-tau CSF levels (log mean [SD] = 1.84 [0.19] pg/ml for chronic pain vs 1.79 [0.14] pg/ml for non-chronic pain, $F_{1,175} = 4.46$, $p_{raw} = 0.036$, $p_{adj} = 0.14$) and increased sTREM2 CSF levels (log mean [SD] = 3.54 [0.17] pg/ml for chronic pain vs 3.52 [0.22]

pg/ml for nonchronic pain, $F_{1,174} = 7.36$, $p_{raw} = 0.008$, $p_{adj} = 0.028$; Fig 2). We found no significant interaction between chronic pain status and painkiller intake.

Cross-Sectional Analysis of Chronic Pain and Cognitive Function

Our analysis revealed that chronic pain patients in the A+TN– group showed higher memory function at baseline (mean [SD] = 0.46 [0.68] for chronic pain vs 0.10 [0.93] for non-chronic pain, $F_{1,69} = 7.65$, $p_{raw} = 0.007$, $p_{adj} = 0.098$; Fig 3). No significant interaction between chronic pain status and painkiller intake was found.

Cross-Sectional Analysis of Chronic Pain and CSF Inflammatory Levels

We investigated the influence of chronic pain on inflammatory proteins in the CSF, irrespective of the ATN group. Our analysis showed that chronic pain is associated with increased CSF TNF α levels ($\beta = 0.16$, 95% CI [0.035–0.29], $p_{raw} = 0.013$, $p_{adj} = 0.19$; Fig 4).

Longitudinal Analysis of Chronic Pain Association with CSF Neurodegeneration Biomarkers and Memory Function

For the A–TN– group, we found an interaction effect between time and chronic pain status for ptau $_{181}$

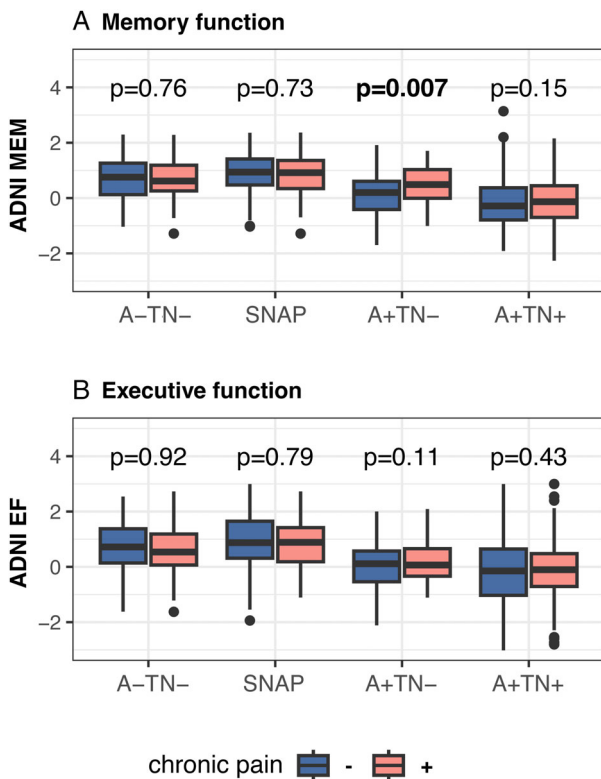


FIGURE 3: Differences in neuropsychological testing between chronic pain patients from different ATN (amyloid, tau, neurodegeneration) groups. Uncorrected *p* values are presented, with boldface denoting significant results after Bonferroni correction for multiple comparison. Analysis of covariance model includes age, gender, education, APOE ϵ 4 carrier status, Clinical Dementia Rating–Sum of Boxes, Geriatric Depression Scale total score, and analgesic intake. Blue = no chronic pain, red = chronic pain. ADNI = Alzheimer’s Disease Neuroimaging Initiative; EF = executive function; MEM = memory; SNAP = suspected non-Alzheimer disease pathophysiology.

($p = 0.038$) and A β CSF levels ($p = 0.016$); chronic pain patients showed an increase in ptau₁₈₁ CSF levels over time ($\beta = 0.014$, 95% CI [0.01–0.028]). In contrast, non-chronic pain patients showed a decrease in A β CSF levels over time ($\beta = -0.01$, 95% CI [-0.02 to -0.01]; Fig 5). For cognitive function, no interaction effect for time and chronic pain status was observed for ADNI EF for any ATN group, whereas a significant effect was observed for ADNI MEM ($p = 0.041$) for A+TN– individuals. In this group, non-chronic pain patients had a significant increase in ADNI MEM over time ($\beta = 0.21$, 95% CI [0.06–0.36]; see Fig 5). The 3-way interactions time \times chronic pain status \times painkiller intake and time \times chronic pain \times baseline sTREM2 were not significant for any outcome.

Discussion

In this study, we assessed the relationship between chronic pain and CSF biomarkers of neurodegeneration in the

context of AD, at baseline and after 24 months. We found that chronic pain induced increased t-tau CSF levels only in patients with SNAP. In the same group, we also observed that chronic pain was associated with increased CSF levels of sTREM2, a biomarker of microglial activation.²¹ Considering that increased t-tau CSF levels reflect neuronal damage and neurodegeneration intensity, our findings suggest an interplay between chronic pain, neuronal damage, neurodegeneration, and microglial activation.²⁸ These results are consistent with previous studies reporting increased microglial activation in chronic pain states such as neuropathic pain, cancer pain, and migraine.²⁹ However, our study is the first to suggest a link between neuronal damage and neurodegeneration with microglial activation in chronic pain states. Additionally, we observed that the difference in sTREM2 CSF levels between chronic pain states in the SNAP group were close to the differences reported between healthy controls and MCI or AD patients.^{21,30} Because the CSF levels of sTREM2 and biomarkers of neurodegeneration were analyzed from the same aliquot in ADNI, we were not able to determinate whether microglial activation preceded neuronal damage. Further studies are needed to establish the temporal relationship between microglial activation and neuronal damage, as such findings have potential therapeutic implications. That our analysis did not yield any significant CSF alterations in other ATN groups indicates that the pathological processes underlying SNAP may favor chronic pain-induced neuronal damage. SNAP is a heterogenous clinical entity, and various pathological drivers have been reported in the literature, including the presence of cerebrovascular diseases (CeVD).³¹ The latter are characterized by alterations in cerebral blood flow (CBF) that may precipitate neuronal damage and subsequent increased CSF t-tau levels.³² Interestingly, past studies found that changes in CBF participate in pain perception alteration and chronic pain states.³³ Hence, based on our findings, we may postulate that chronic pain induces changes in CBF, which induce significant neuronal damage in vulnerable brains, such as in SNAP patients with CeVD.

At the longitudinal level, we observed that amyloid- and neurodegeneration-negative individuals with chronic pain experienced an increase in ptau₁₈₁ CSF levels over time. Considering that past studies have reported increased microglial activation in chronic pain patients,³⁴ our results suggest that chronic pain induces a microglial phenotype more prone to tau seeding and propagation.³⁵ It is noteworthy that a recent study in a mouse model showed that transcription factor NF- κ B, a key player in chronic pain, participates in microglial-driven tau spreading and toxicity.^{36,37} Another hypothesis may be that

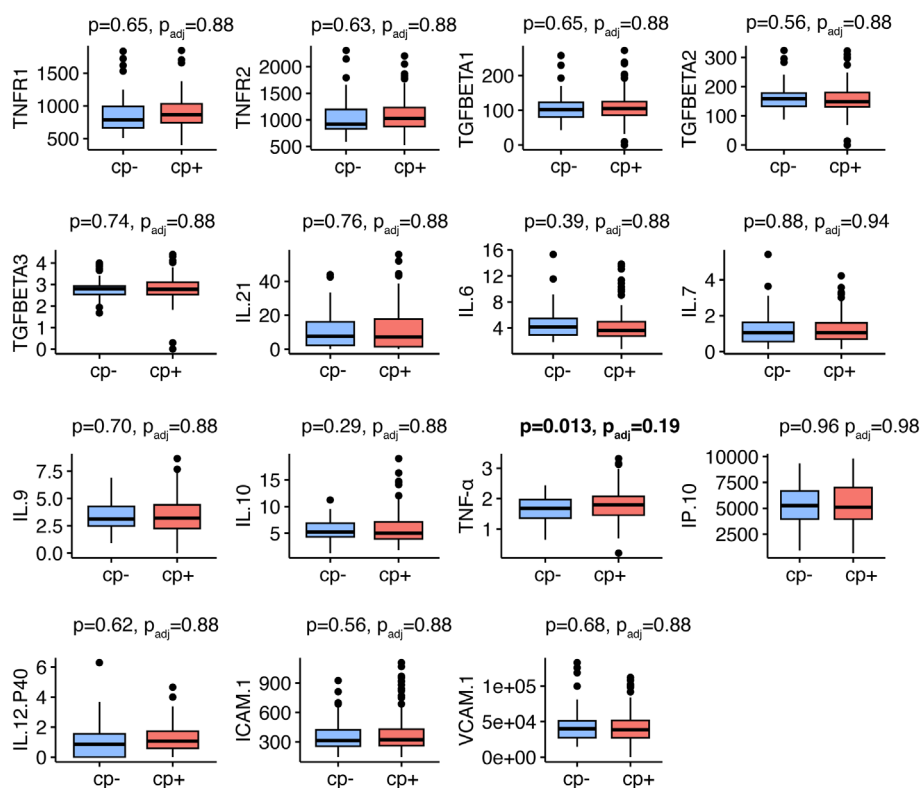


FIGURE 4: Differences in cerebrospinal fluid inflammatory markers between chronic pain patients from different ATN (amyloid, tau, neurodegeneration) groups. Analysis of covariance model includes age, gender, education, *APOE* ϵ 4 carrier status, Clinical Dementia Rating–Sum of Boxes Geriatric Depression Scale total score, and analgesic intake. Blue = no cp, red = cp. cp = chronic pain; ICAM = intercellular adhesion molecule; IL = interleukin; IP = interferon γ -induced protein; TGF = transforming growth factor; TNF = tumor necrosis factor; VCAM = vascular cell adhesion molecule.

pathological processes involved in chronic pain interact with the aberrant tau phosphorylation process typical for AD. Tau is a microtubule-associated protein that participates in the cytoarchitecture by stabilizing the microtubule; hyperphosphorylation of tau leads to decreased binding affinity, eventually resulting in microtubule disruption and later to intracellular neurofibrillary tangles.³⁸ The literature describes a wide range of protein kinase families involved in tau hyperphosphorylation, such as the mitogen-activated protein kinase and Src families.^{39,40} Interestingly, both families are also key players in chronic pain, as they modulate the transmission of pain receptors and interact with neurotrophic factors.^{41,42} These kinase signaling pathways consist of a cascade of reactions involving kinases and phosphatases, interacting through feedback regulatory loops.⁴³ Therefore, in individuals from the A–TN– group, dysregulated protein kinase signaling described in chronic pain may to some extent increase tau phosphorylation either by a feedback loop or by creating an imbalance between the protein phosphatases and kinases. Although the longitudinal increase in ptau₁₈₁ CSF levels was not associated with a deterioration in cognitive function over time, it is possible that

cognitive changes occur at a later stage.²⁸ Surprisingly, in the same group non-chronic pain patients experienced a decrease in $A\beta$ over time. We may hypothesize that the chronic pain-induced microglial phenotype may play a protective role against amyloid deposition, similar to findings in early stages of AD.⁴⁴

An exploratory analysis of CSF levels of inflammatory proteins revealed increased TNF α levels in the CSF of chronic pain patients. This proinflammatory cytokine has been involved in pathological processes underlying chronic pain conditions such as new daily persistent headache⁴⁵ and migraines.⁴⁶ Noteworthy, we previously showed that TNF α is positively correlated with sTREM2 in CSF.¹⁵ It remains to be further determined whether increased TNF α in chronic pain induces microglial activation or whether TNF α is produced by chronic pain-activated microglia.

Our study found no effect of chronic pain on cognitive function over time for any ATN group. These results are in line with two recent meta-analyses, which failed to show a clear association between chronic pain and cognitive decline and advocated for further investigations.^{5,6} Our findings are strengthened in that we adjusted for

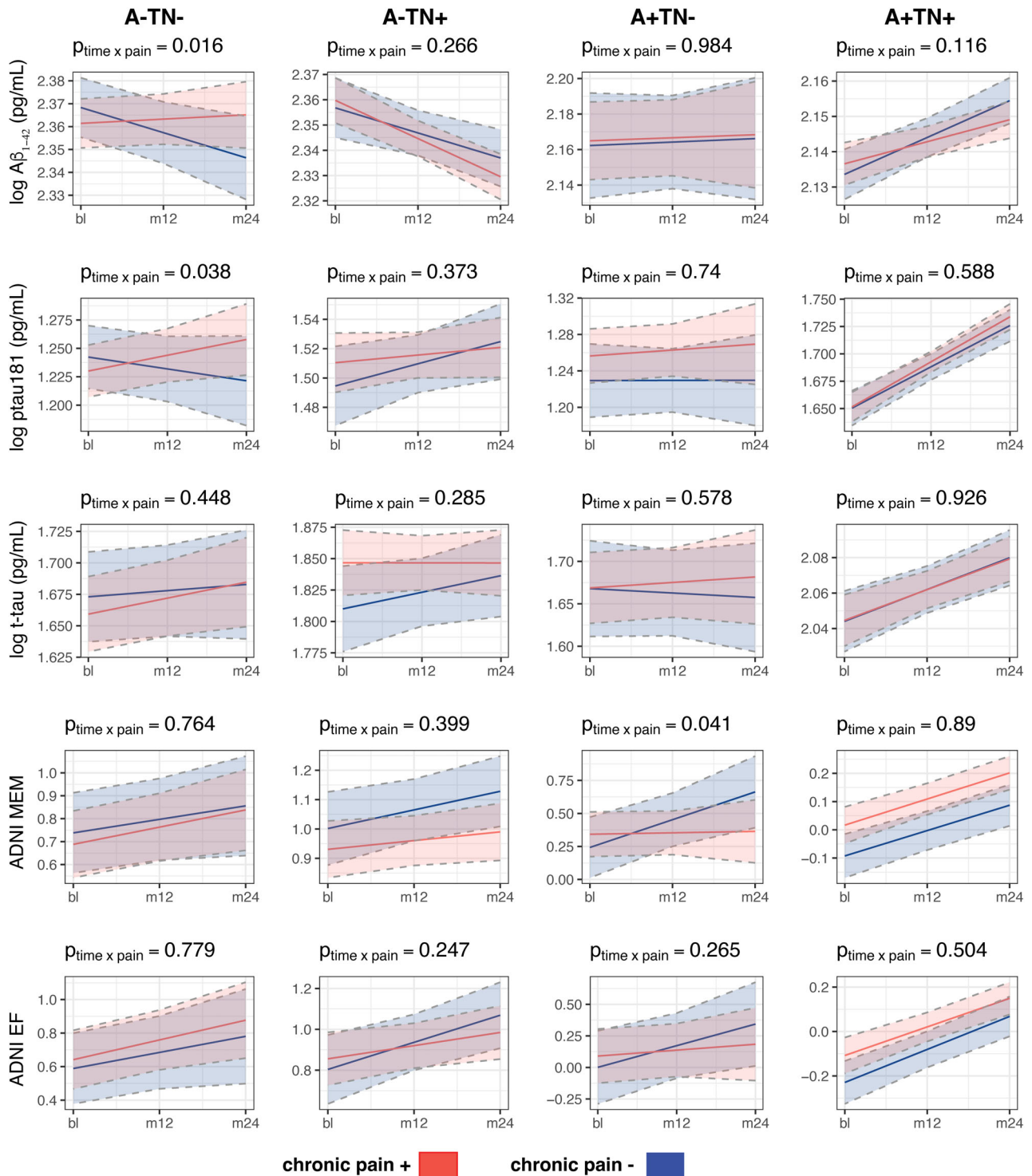


FIGURE 5: Longitudinal changes in cerebrospinal fluid biomarkers of neurodegeneration and cognitive function between chronic pain patients from different ATN (amyloid, tau, neurodegeneration) groups. Linear mixed effect regression model includes age, gender, education, *APOE* $\epsilon 4$ carrier status, Clinical Dementia Rating–Sum of Boxes, Geriatric Depression Scale total and analgesic intake. Blue = no chronic pain, red = chronic pain. $A\beta$ = amyloid β ; ADNI = Alzheimer’s Disease Neuroimaging Initiative; bl = baseline; EF = executive function; m12 = month 12; m24 = month 24; MEM = memory; ptau181 = phosphorylated tau 181; t-tau = total tau.

important confounders known to influence cognitive function, such as *APOE* $\epsilon 4$ carrier status, depressive symptoms, and intake of analgesics.⁴⁷ Moreover, our outcomes consisted

of composite scores of memory and executive function, validated in ADNI, a well-established cohort specifically designed to investigate questions pertaining to AD.^{16,17}

The prevalence of chronic pain in our study was 60.81%, which is in line with previous USA-based epidemiological studies conducted at the time when ADNI data were collected. More than 50% of adults 65 years and older reported suffering from pain in the past months in the National Health and Aging Trends Study 2011.⁴⁸ Surprisingly, in our study, chronic pain was slightly more prevalent in male participants (51.9% in male vs 48.1% in female), which contrasts with past studies.⁴⁸ We have identified two hypotheses to explain our findings. First, prevalence studies on chronic pain are marked by a high heterogeneity in study settings and population, pain definition, and pain assessments and we lack the evidence from a memory clinic-based population. Second, we identified chronic pain patients based on symptoms reported at baseline as well as in the follow-up visits if the given pain duration indicated presence of chronic pain at baseline. It is well established that men are less likely to report chronic pain symptoms than women, due to social, psychological, and cultural constructions.⁴⁹ Hence, our multi-step approach of identifying chronic pain patients at different time points may have increased the possibility of identifying male chronic pain patients who omitted to report chronic pain symptoms at the baseline and screening visits.

Overall, our study suggests an association between chronic pain and various biomarkers of neurodegeneration in a large cohort. Although our work offers a novel perspective on the shared biology between chronic pain and AD, some limitations need to be mentioned. First, due to the relatively small sample size of chronic pain patients, we could not assess the effect of chronic pain severity (ie, mild, moderate, or severe) or chronic pain type on our outcomes of interest. Second, as ADNI was not designed for chronic pain patients, assessment and screening of chronic pain did not follow a specific pain screening instrument. Third, the follow-up time was short compared to epidemiological cohorts (24 months) and it is possible that our described CSF patterns and cognitive function may undergo further changes along the disease spectrum. Fourth, we lacked the power to explore the influence of anti-inflammatory medication intake on neurodegenerative markers. Considering that anti-inflammatory markers have attracted increased attention in the field of neurodegeneration in recent decades, further studies are now needed to investigate their role in chronic pain patients, where anti-inflammatory medication intake is high.⁵⁰ Fifth, the trend of microglial activation over time could not be ascertained due to lack of data. Finally, to improve the power of our analysis, we imputed missing longitudinal data. Further studies, with a specific focus on chronic pain patients, are needed to confirm our results.

Conclusions

Our study suggests that chronic pain is associated with neuronal damage, neurodegeneration, and microglial activation in patients with SNAP and may participate in a long-term aberrant phosphorylation in individuals without biomarker alterations. Future studies targeted at investigating the link between chronic pain and biomarkers of neurodegeneration are needed.

Acknowledgments

We thank the ADNI participants and staff for their support, dedication, and commitment. Data collection and sharing for this project were funded by ADNI (NIH grant U01 AG024904) and Department of Defense (DOD) ADNI (DOD award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging and the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from AbbVie, Alzheimer's Association, Alzheimer's Drug Discovery Foundation, Araclon Biotech, BioClinica, Biogen, Bristol Myers Squibb, CereSpir, Cogstate, Eisai, Elan Pharmaceuticals, Eli Lilly and Company, EuroImmun, F. Hoffmann-La Roche and its affiliated company Genentech, Fujirebio, GE Healthcare, IXICO, Janssen Alzheimer Immunotherapy Research & Development, Johnson & Johnson Pharmaceutical Research & Development, Lumosity, Lundbeck, Merck & Co, Meso Scale Diagnostics, NeuroRx Research, Neurotrack Technologies, Novartis Pharmaceuticals Corporation, Pfizer, Piramal Imaging, Servier, Takeda Pharmaceutical Company, and Transition Therapeutics. The Canadian Institutes of Health Research provide funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. Open Access funding enabled and organized by Projekt DEAL.

Author Contributions

A.S. contributed to the conception and design of the study. All authors contributed to the acquisition and analysis of data. All authors contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data Availability Statement

Data used in preparation of this article were obtained from the ADNI database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the conception, design, and implementation of ADNI and/or provided data but did not participate in the analysis or writing of this report. A complete listing of ADNI investigators can be found at https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf. All data used in this study are available from ADNI (adni.loni.usc.edu).

References

- Treede RD, Rief W, Barke A, et al. Chronic pain as a symptom or a disease: the IASP classification of chronic pain for the international classification of diseases (ICD-11). *Pain* 2019;160:19–27. <https://doi.org/10.1097/j.pain.0000000000001384>.
- Croft P, Blyth FM, van der Windt D, et al., eds. The global occurrence of chronic pain: an introduction. *Chronic pain epidemiology: from Aetiology to public health*. Oxford: Oxford University Press, 2010. <https://doi.org/10.1093/acprof:oso/9780199235766.003.0002>.
- Whitlock EL, Diaz-Ramirez LG, Glymour MM, et al. Association between persistent pain and memory decline and dementia in a longitudinal cohort of elders. *JAMA Intern Med* 2017;177:1146–1153. <https://doi.org/10.1001/jamainternmed.2017.1622>.
- Rong W, Zhang C, Zheng F, et al. Persistent moderate to severe pain and long-term cognitive decline. *Eur J Pain* 2021;25:2065–2074. <https://doi.org/10.1002/ejp.1826>.
- de Aguiar GPCG, Saraiva MD, Khazaal EJB, et al. Persistent pain and cognitive decline in older adults: a systematic review and meta-analysis from longitudinal studies. *Pain* 2020;161:2236–2247. <https://doi.org/10.1097/j.pain.0000000000001932>.
- Zhang X, Gao R, Zhang C, et al. Evidence for cognitive decline in chronic pain: a systematic review and meta-analysis. *Front Neurosci* 2021;15:737874. <https://doi.org/10.3389/fnins.2021.737874>.
- Barnes DE, Yaffe K, Byers AL, et al. Midlife vs late-life depressive symptoms and risk of dementia: differential effects for Alzheimer disease and vascular dementia. *Arch Gen Psychiatry* 2012;69:493–498. <https://doi.org/10.1001/archgenpsychiatry.2011.1481>.
- Knopman DS, Amieva H, Petersen RC, et al. Alzheimer disease. *Nat Rev Dis Primer* 2021;7:33. <https://doi.org/10.1038/s41572-021-00269-y>.
- Sims R, Hill M, Williams J. The multiplex model of the genetics of Alzheimer's disease. *Nat Neurosci* 2020;23:311–322. <https://doi.org/10.1038/s41593-020-0599-5>.
- Cao S, Fisher DW, Yu T, Dong H. The link between chronic pain and Alzheimer's disease. *J Neuroinflammation* 2019;16:204. <https://doi.org/10.1186/s12974-019-1608-z>.
- Johnston KJA, Adams MJ, Nicholl BI, et al. Genome-wide association study of multisite chronic pain in UK biobank. *PLoS Genet* 2019;15:e1008164. <https://doi.org/10.1371/journal.pgen.1008164>.
- Guerreiro SR, Guimarães MR, Silva JM, et al. Chronic pain causes tau-mediated hippocampal pathology and memory deficits. *Mol Psychiatry* Published online September 2 2022;27:1–9. <https://doi.org/10.1038/s41380-022-01707-3>.
- Petersen RC, Aisen PS, Beckett LA, et al. Alzheimer's disease neuroimaging initiative (ADNI): clinical characterization. *Neurology* 2010;74:201–209. <https://doi.org/10.1212/WNL.0b013e3181cb3e25>.
- Jack CR, Bennett DA, Blennow K, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* 2016;87:539–547. <https://doi.org/10.1212/WNL.0000000000002923>.
- Rauchmann BS, Sadlon A, Pernecky R. Initiative for the ADNI. Soluble TREM2 and inflammatory proteins in Alzheimer's disease cerebrospinal fluid. *J Alzheimers Dis* 2020;73:1615–1626. <https://doi.org/10.3233/JAD-191120>.
- Gibbons LE, Carle AC, Mackin RS, et al. A composite score for executive functioning, validated in Alzheimer's disease neuroimaging initiative (ADNI) participants with baseline mild cognitive impairment. *Brain Imaging Behav* 2012;6:517–527. <https://doi.org/10.1007/s11682-012-9176-1>.
- Crane PK, Carle A, Gibbons LE, et al. Development and assessment of a composite score for memory in the Alzheimer's disease neuroimaging initiative (ADNI). *Brain Imaging Behav* 2012;6:502–516. <https://doi.org/10.1007/s11682-012-9186-z>.
- Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol* 2009;65:403–413. <https://doi.org/10.1002/ana.21610>.
- Knapik AB, Henjum K, Idland AV, et al. Cerebrospinal fluid sTREM2 in Alzheimer's disease: comparisons between clinical presentation and AT classification. *Sci Rep* 2020;10:15886. <https://doi.org/10.1038/s41598-020-72878-8>.
- for the Alzheimer's Disease Neuroimaging Initiative, Suárez-Calvet M, Morenas-Rodríguez E, et al. Early increase of CSF sTREM2 in Alzheimer's disease is associated with tau related neurodegeneration but not with amyloid- β pathology. *Mol Neurodegener* 2019;14:1. <https://doi.org/10.1186/s13024-018-0301-5>.
- Suárez-Calvet M, Kleinberger G, Araque Caballero MÁ, et al. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO Mol Med* 2016;8:466–476. <https://doi.org/10.15252/emmm.201506123>.
- Hu W, Howel JC. Hu Lab – ADNI Methods, Published January 31 2011. https://adni.bitbucket.io/reference/docs/HULAB/ADNI_Methods_Hu_Lab.pdf.
- Queremel Milani DA, Davis DD. Pain management medications. *StatPearls*. Florida, USA: StatPearls Publishing, 2022. Accessed October 31, 2022 <http://www.ncbi.nlm.nih.gov/books/NBK560692/>.
- Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. *J Clin Epidemiol* 1994;47:1245–1251. [https://doi.org/10.1016/0895-4356\(94\)90129-5](https://doi.org/10.1016/0895-4356(94)90129-5).
- Stekhoven DJ. Using the missForest Package: 11.
- Shishegar R, Cox T, Rolls D, et al. Using imputation to provide harmonized longitudinal measures of cognition across AIBL and ADNI. *Sci Rep* 2021;11:23788. <https://doi.org/10.1038/s41598-021-02827-6>.
- McCombe N, Liu S, Ding X, et al. Practical strategies for extreme missing data imputation in dementia diagnosis. *IEEE J Biomed Health Inform* 2022;26:818–827. <https://doi.org/10.1109/JBHI.2021.3098511>.
- Self WK, Holtzman DM. Emerging diagnostics and therapeutics for Alzheimer disease. *Nat Med* 2023;29:2187–2199. <https://doi.org/10.1038/s41591-023-02505-2>.
- Chen G, Zhang YQ, Qadri YJ, et al. Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. *Neuron* 2018;100:1292–1311. <https://doi.org/10.1016/j.neuron.2018.11.009>.
- Zhao A, Jiao Y, Ye G, et al. Soluble TREM2 levels associate with conversion from mild cognitive impairment to Alzheimer's disease. *J Clin Invest* 2022;132:e158708. <https://doi.org/10.1172/JCI158708>.

31. Saridin FN, Chew KA, Reilhac A, et al. Cerebrovascular disease in suspected non-Alzheimer's pathophysiology and cognitive decline over time. *Eur J Neurol* 2022;29:1922–1929. <https://doi.org/10.1111/ene.15337>.
32. Hesse C, Rosengren L, Andreasen N, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neurosci Lett* 2001;297:187–190. [https://doi.org/10.1016/S0304-3940\(00\)01697-9](https://doi.org/10.1016/S0304-3940(00)01697-9).
33. Iwabuchi SJ, Xing Y, Cottam WJ, et al. Brain perfusion patterns are altered in chronic knee pain: a spatial covariance analysis of arterial spin labelling MRI. *Pain* 2020;161:1255–1263. <https://doi.org/10.1097/j.pain.0000000000001829>.
34. Kohno K, Tsuda M. Role of microglia and P2X4 receptors in chronic pain. *Pain Rep* 2021;6:e864. <https://doi.org/10.1097/PR9.0000000000000864>.
35. Perea JR, Llorens-Martín M, Ávila J, Bolós M. The role of microglia in the spread of tau: relevance for Tauopathies. *Front Cell Neurosci* 2018;12:12. Accessed October 13, 2022. <https://doi.org/10.3389/fncel.2018.00172>.
36. Wang C, Fan L, Khawaja RR, et al. Microglial NF- κ B drives tau spreading and toxicity in a mouse model of tauopathy. *Nat Commun* 2022;13:1969. <https://doi.org/10.1038/s41467-022-29552-6>.
37. Souza GR, Cunha TM, Silva RL, et al. Involvement of nuclear factor kappa B in the maintenance of persistent inflammatory hypernociception. *Pharmacol Biochem Behav* 2015;134:49–56. <https://doi.org/10.1016/j.pbb.2015.04.005>.
38. Xia Y, Prokop S, Giasson BI. “Don't Phos over tau”: recent developments in clinical biomarkers and therapies targeting tau phosphorylation in Alzheimer's disease and other tauopathies. *Mol Neurodegener* 2021;16:37. <https://doi.org/10.1186/s13024-021-00460-5>.
39. Bhaskar K, Konerth M, Kokiko-Cochran ON, et al. Regulation of tau pathology by the microglial fractalkine receptor. *Neuron* 2010;68:19–31. <https://doi.org/10.1016/j.neuron.2010.08.023>.
40. Maphis N, Xu G, Kokiko-Cochran ON, et al. Reactive microglia drive tau pathology and contribute to the spreading of pathological tau in the brain. *Brain J Neurol* 2015;138:1738–1755. <https://doi.org/10.1093/brain/awv081>.
41. Ge MM, Zhou YQ, Tian XB, et al. Src-family protein tyrosine kinases: a promising target for treating chronic pain. *Biomed Pharmacother* 2020;125:110017. <https://doi.org/10.1016/j.biopha.2020.110017>.
42. Mai L, Zhu X, Huang F, et al. p38 mitogen-activated protein kinase and pain. *Life Sci* 2020;256:117885. <https://doi.org/10.1016/j.lfs.2020.117885>.
43. Sarma U, Ghosh I. Different designs of kinase-phosphatase interactions and phosphatase sequestration shapes the robustness and signal flow in the MAPK cascade. *BMC Syst Biol* 2012;6:82. <https://doi.org/10.1186/1752-0509-6-82>.
44. Butovsky O, Weiner HL. Microglial signatures and their role in health and disease. *Nat Rev Neurosci* 2018;19:622–635. <https://doi.org/10.1038/s41583-018-0057-5>.
45. Rozen T, Swidan SZ. Elevation of CSF tumor necrosis factor α levels in new daily persistent headache and treatment refractory chronic migraine. *Headache J Head Face Pain* 2007;47:1050–1055. <https://doi.org/10.1111/j.1526-4610.2006.00722.x>.
46. Musubire AK, Cheema S, Ray JC, et al. Cytokines in primary headache disorders: a systematic review and meta-analysis. *J Headache Pain* 2023;24:36. <https://doi.org/10.1186/s10194-023-01572-7>.
47. Livingston G, Huntley J, Sommerlad A, et al. Dementia prevention, intervention, and care: 2020 report of the lancet commission. *Lancet* 2020;396:413–446. [https://doi.org/10.1016/S0140-6736\(20\)30367-6](https://doi.org/10.1016/S0140-6736(20)30367-6).
48. Patel KV, Guralnik JM, Dansie EJ, Turk DC. Prevalence and impact of pain among older adults in the United States: findings from the 2011 National Health and aging trends study. *Pain* 2013;154:2649–2657. <https://doi.org/10.1016/j.pain.2013.07.029>.
49. Blackbeard D, Aldous C. Chronic pain and masculine identity: life-world interviews with men at a south African pain clinic. *Int J Qual Stud Health Well-Being* 2021;16:1970303. <https://doi.org/10.1080/17482631.2021.1970303>.
50. Zhang C, Wang Y, Wang D, et al. NSAID exposure and risk of Alzheimer's disease: an updated meta-analysis from cohort studies. *Front Aging Neurosci* 2018;10:83. <https://doi.org/10.3389/fnagi.2018.00083>.