Cerebrospinal Fluid Markers of Alzheimer's Disease Pathology and Microglial Activation are Associated with Altered White Matter Microstructure in Asymptomatic Adults at Risk for Alzheimer's Disease

Kelsey E Melah[a], Sharon Yuan-Fu Lu[b], Siobhan M Hoscheidt[a], Andrew L Alexander[b], Nagesh Adluru[b], Daniel J Destiche[b], Cynthia M Carlsson[a], Henrik Zetterberg[cd], Kaj Blennow[ce], Ozioma C Okonkwo[a,f], Carey E Gleason[a,g], N Maritza Dowling[a,h], Lisa C Bratzke[i], Howard A Rowley[j], Bruce P Hermann[f], Mark A Sager[f], Sanjay Asthana[a,g], Sterling C Johnson[a,f,g], Barbara B Bendlin[a,f]

[a]Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, 600 Highland Avenue J5/1 Mezzanine, Madison, WI 53792

[b]Waisman Laboratory for Brain Imaging and Behavior, Madison, WI, USA.

[c]Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, SE-431 80 Mölndal, Sweden

[d]UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK

[e]The Torsten Söderberg Professorship at the Royal Swedish Academy of Sciences

[f]Wisconsin Alzheimer's Institute, School of Medicine and Public Health, 7818 Big Sky Dr., Madison, WI 53719

[g]Geriatric Research Education and Clinical Center, Wm. S. Middleton Memorial Veterans Hospital, 2500 Overlook Terrace, Madison, WI 53705

[h]Department of Biostatistics and Medical Informatics, University of Wisconsin School of Medicine and Public Health, 600 Highland Avenue, Madison, WI 53705

[i]School of Nursing, Wisconsin School of Medicine and Public Health, Madison, WI

[j]Department of Neuroradiology, School of Medicine and Public Health, 600 Highland Avenue, Madison, WI 53705

Send Correspondence to:
Barbara B. Bendlin, PhD
J5/1M, Clinical Science Center
600 Highland Ave
Madison, WI 53792
Phone: (608) 265-2483
E-mail: bbb@medicine.wisc.edu

ABSTRACT

BACKGROUND: The immune response in Alzheimer's disease (AD) involves activation of microglia which may remove amyloid-β (Aβ). However, overproduction of inflammatory compounds may exacerbate neural damage in AD. AD pathology accumulates years before diagnosis, yet the extent to which neuroinflammation is involved in the earliest disease stages is unknown.

OBJECTIVE: To determine whether neuroinflammation exacerbates neural damage in preclinical AD.

METHODS: We utilized cerebrospinal fluid (CSF) and magnetic resonance imaging collected in 192 asymptomatic late-middle-aged adults (mean age=60.98 years). Neuroinflammatory markers chitinase-3-like protein 1 (YKL-40) and monocyte chemoattractant protein-1 (MCP-1) in CSF were utilized as markers of neuroinflammation. Neural cell damage was assessed using CSF neurofilament light chain protein (NFL), CSF total tau (T-Tau), and neural microstructure assessed with diffusion tensor imaging (DTI). With regard to AD pathology, CSF Aβ42 and tau phosphorylated at threonine 181 (P-Tau181) were used as markers of amyloid and tau pathology, respectively. We hypothesized that higher YKL-40 and MCP-1 in the presence of AD pathology would be associated with higher NFL, T-Tau, and altered microstructure on DTI.

RESULTS: Neuroinflammation was associated with markers of neural damage. Higher CSF YKL-40 was associated with both higher CSF NFL and T-Tau. Inflammation interacted with AD pathology, such that greater MCP-1 and lower Aβ42 was associated with altered microstructure in bilateral frontal and right temporal lobe and that greater MCP-1 and greater P-Tau181 was associated with altered microstructure in precuneus.

CONCLUSION: Inflammation may play a role in neural damage in preclinical AD.

1. INTRODUCTION

Inflammation is a well-established feature of Alzheimer's disease (AD) [1,2]. Several pro-inflammatory cytokines, chemokines, and complement proteins are elevated in the AD brain and both human and animal studies suggest inflammation may occur early in the AD process [3–7]. The underlying cause of inflammation in AD is not entirely known, but is likely secondary to β-amyloid deposition which elicits a pro-inflammatory microglial response via a complex cytokine-signaling cascade (perhaps add a reference here; Jennifer Pocock has written some good ones on the topic). The extent to which neuroinflammation has a negative effect on neural health in the early stages of AD is presently unclear. While microglial activation and inflammation may have a beneficial effect in clearing β-amyloid, overproduction of pro-inflammatory compounds is suspected as a major contributing factor to neuronal damage by causing injury and toxicity to neurons [8].

Microglial activation, along with cell death and degradation of synaptic connections, are found to a greater extent in AD patients post mortem, compared to individuals who were cognitively intact at time of death despite comparable levels of amyloid and tangle pathology [6]. Neurofibrillary tangles and β-amyloid plaques are hallmarks of AD, however post-mortem histological and *in vivo* amyloid imaging studies suggest that a substantial number of healthy individuals harbor significant AD pathology despite normal cognition [6,9,10]. Given that inflammatory mediators released by glial cells can be toxic to neurons, one possible mechanism for this dissociation is the presence of neuroinflammation. In turn, neuronal loss and synaptic pathology tend to be more closely related to cognitive function compared to β-amyloid pathology [11,12], suggesting that inflammation-mediated cell loss may contribute to cognitive dysfunction in AD. While there is growing evidence that microglial activation may play a role in neural injury and cell loss, very little is known about the timing and localization of inflammation-mediated neural damage.

The primary aim of the current study was to determine the effect of neuroinflammation on neural microstructure in asymptomatic adults enriched for AD risk factors including parental family history of AD and apolipoprotein E ε4 (*APOE* ε4) genotype. In this study, cerebrospinal fluid (CSF) markers served as proxies of neuroinflammation, and included YKL-40 and monocyte chemoattractant protein 1 (MCP-1). These two proteins are elevated in pre-clinical AD, mild cognitive impairment (MCI), and early AD [4,13–16]. Neurofilament light chain protein (NFL) in CSF, and diffusion tensor imaging (DTI) measures were used to assess neural injury. NFL is a structural protein of neurons predominantly localized in axons, and is elevated in CSF in several neurodegenerative diseases, including AD [17–20]. In turn, DTI is sensitive to microstructural diffusion of water molecules and has been used extensively to assess tissue damage in AD, MCI, and preclinical AD [21–32]. Two summary measures derived from DTI were used to assess tissue microstructure: fractional anisotropy (FA), a measure of directional water diffusion that is highly sensitive to microstructural features including axonal density, diameter, and myelination, and mean diffusivity (MD), a measure of isotropic diffusion that is sensitive to cellular structure, necrosis, and edema [33,34]. We hypothesized that greater neuroinflammation would be associated with higher levels of CSF NFL and microstructural damage as revealed on FA and MD maps.

Because we were specifically interested in the extent to which neuroinflammation may affect neural health in the presence of existing AD pathology, analyses incorporated measures of CSF $A\beta_{42}$, and T-tau. High T-tau and low $A\beta_{42}$ in CSF have been associated with atrophy, cortical thinning, and altered white matter microstructure in preclinical AD [35–38] but the extent to which neuroinflammation may moderate these effects are unknown. We hypothesized that the negative effects of β-amyloid and tau pathology on neural microstructure would be greater among individuals with higher inflammation. A subsidiary aim was to examine whether age, family history, or *APOE* ε4 genotype moderated the effect of AD pathology and inflammation on microstructure. While being a clear independent risk factor for AD, *APOE* ε4 has also been associated with up-regulation of innate immune factors, including plasma TNF-α and IL-6 [39]. Likewise parental family history of AD has been shown to confer a pro-inflammatory cytokine profile independent of *APOE* ε4 [7]. We hypothesized that individuals at greater risk for AD, indicated by positive family history, *APOE* ε4 genotype, or older age, would have greater changes in tissue microstructure in the presence of higher AD pathology or greater neuroinflammation. Understanding the impact of preclinical inflammation is expected to provide valuable information on the pathobiology of AD and inform prevention and treatment strategies in the disease.

2. METHODS

Study procedures were approved by the University of Wisconsin Health Sciences Institutional Review Board and were in accordance with U.S. federal regulations. All participants provided written informed consent.

2.1 Participants

Participants were asymptomatic late-middle-aged adults (mean age = 61.2 years, SD = 7.58) from the Wisconsin Registry for Alzheimer's Prevention (WRAP) study and the Wisconsin Alzheimer's Disease Research Center (ADRC) clinical core who underwent brain imaging and lumbar puncture as part of studies on memory, aging, and preclinical AD. Both the WRAP and Wisconsin ADRC comprise well-characterized and longitudinally followed participants who are either positive or negative for parental history of AD. Positive parental family history of AD classification was defined as having one or both parents with autopsy-confirmed or probable AD as outlined by research criteria [40,41], and reviewed by a multidisciplinary diagnostic consensus panel. Detailed medical history and phone interviews were conducted to confirm AD negative participants. Absence of family history of AD required that the participant's father survive to at least age 70 years and the mother to age 75 years without diagnosis of dementia or cognitive deterioration. Family history was classified as a binary variable. *APOE* ε4 genetic testing was performed at the University of Wisconsin-Madison, Waisman Center. *APOE* ε4 extraction and isoform classification have been described previously [42]. Participants were categorized using a binary variable as an *APOE* ε4 carrier or non- carrier.

General inclusion criteria consisted of: 1) normal cognitive function determined by neuropsychological evaluation, 2) negative history of psychiatric or neurological disease or untreated depression, and 3) no history of head trauma. Participants were also required to have previously undergone magnetic resonance imaging (MRI) and lumbar puncture for CSF assays.

Participants were excluded from the analysis based on one or more of the following: a) abnormal radiological read on study MRI, including evidence of infection, infarct, or tumor (n=31), b) CSF assay results that were below detection threshold on one or more assays (n=4), c) unsatisfactory DTI data quality after processing, such as major susceptibility artifact (n=12), or d) an incomplete or failed DTI acquisition or transfer (n=11).

Of 248 participants identified for possible inclusion in the study, 190 participants met criteria for inclusion. Overall the sample was largely female, Caucasian, well educated, had a parental family history of AD or were *APOE* ε4 carriers (see Table 1).

2.2 CSF collection and analysis:

CSF was collected with a Sprotte 25-or 24-gauge spinal needle at the L3/4 or L4/5 using gentle extraction into polypropylene syringes. Samples were collected in the morning after a 12h fast. Approximately 22mL of CSF were combined, gently mixed and centrifuged at 2000g for 10 minutes. Supernatants were frozen in 0.5mL aliquots in polypropylene tubes and stored at -80ºC. Samples were analyzed for T-tau and $A\beta_{42}$ using commercially available enzyme-linked immunosorbent assay (ELISA) methods (INNOTEST assays, Fujiurebio, Ghent Belgium) as described previously in detail [43]. MCP-1 levels in CSF were measured using the Meso Scale Discovery technique (MSD Human MCP-1; Meso Scale Discovery, Gaithersburg, MD, USA), and YKL-40 was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, Minn., USA). CSF NFL was measured with a sandwich ELISA method (NF-light ELISA kit, UmanDiagnostics AB, Umeå, Sweden). Board-certified laboratory technicians who were blinded to clinical diagnosis performed all analyses on one occasion. All samples were analyzed according to protocols approved by the Swedish Board of Accreditation and Conformity Assessment (SWEDAC) using one batch of reagents (intra-assay coefficients of variation <10%).

2.3 Magnetic Resonance Imaging:

The average delay between MRI and lumbar puncture was 6.57 days (SD 19.68). Participants underwent scanning on one of two identical General Electric 3.0 Tesla Discovery MR750 MRI systems with 8 channel head coils and parallel imaging (ASSET). DTI was acquired using a diffusion-weighted, spin-echo, single-shot, echo planar imaging (EPI) pulse sequence in 40 encoding directions with b = 1300 s/mm^2, and eight non-diffusion weighted (b = 0) reference images. The cerebrum was covered using contiguous 2.5 mm thick axial slices, FOV = 24 cm, TR = 8000 ms, TE = 67.8 ms, matrix = 96 × 96, resulting in isotropic 2.5 mm3 voxels. High order shimming was performed prior to the DTI acquisition to optimize the homogeneity of the magnetic field across the brain and to minimize EPI distortions. A T1-weighted volume was acquired in the axial plane with a 3D fast spoiled gradient-echo sequence using the following parameters: inversion time (TI) = 450 ms; repetition time (TR) = 8.1 ms; echo time (TE) = 3.2 ms; flip angle = 12°; acquisition matrix = 256 × 256 mm, field of view (FOV) = 256 mm; slice thickness = 1.0 mm. 3D T2-weighted fluid attenuated inversion recovery (FLAIR) scans were acquired in the sagittal plane using the following parameters: TI = 1868 ms; TR = 6000 ms; TE = 123 ms; flip

angle = 90°; acquisition matrix = 256 x 256mm, FOV = 256 mm; slice thickness = 2.0 mm, no gap, yielding a voxel resolution of 1 mm x 1 mm x 2 mm.

2.4 MRI Processing

DTI was processed using a customized pipeline, as described in detail in Adluru et al. [44]. Briefly, images underwent eddy current correction, field inhomogeneity correction, and skull stripping using tools from the FMRIB Software Library (FSL) (http://www.fmrib.ox.ac.uk/fsl/). Tensor fitting was performed using the University College London, Camino Diffusion MRI Toolkit (http://cmic.cs.ucl.ac.uk/camino/). Tensor-based registration was implemented utilizing Diffusion Toolkit for DTI analysis (DTI-TK, http://dti-tk.sourceforge.net/pmwiki/pmwiki.php), whereby images were registered to a population specific template created with DTI-TK. FA and MD maps were calculated using DTI-TK.

In order to control for ischemic lesion burden, total white matter hyperintensity (WMH) volume for each participant was determined using Lesion Segmentation Tool (version 1.2.2) implemented in SPM8 [45]. The T1-weighted and T2FLAIR images were processed according to the method detailed in Birdsill et al. [46]. WMH was adjusted for variability in head size by dividing total WMH by intracranial volume to yield a WMH ratio (WMHr). Intracranial volume was calculated using the "reverse brain masking" method detailed in Keihaninejad et al. [47].

2.5 Statistical Analysis

2.5.1 CSF analyses

To test the effect of neuroinflammation and AD pathology on axonal damage, a linear regression model was implemented in SPSS (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) assessing the effect of YKL-40, MCP-1, $A\beta_{42}$ and T-tau on CSF NFL. Age, sex, family history of AD, *APOE ε4,* and WMHr were entered as covariates.

2.5.2 Voxel-wise DTI analyses

To test the effect of neuroinflammation and AD pathology on tissue microstructure, voxel-wise analyses of FA and MD maps were implemented using statistical parametric mapping software (SPM8; http://www.fil.ion.ucl.ac.uk/spm/). Multiple regression models tested main effects of YKL-40 and MCP-1 on FA and MD, in addition to interactions between YKL-40 and MCP-1 and CSF $A\beta_{42}$, T-tau, family history of AD, *APOE* ε4 status, and age. Models used age, sex, family history, *APOE ε4* status, and WMHr as covariates. Given that anisotropic water diffusion is less interpretable in gray matter compared to white matter, FA analyses were limited to white matter. A white matter mask was constructed by thresholding the FA template to values > 0.15. MD analyses were conducted in both gray and white matter, using a brain mask constructed by thresholding the population based MD template image to 0.10. Both masks were visually inspected to ensure inclusion of tissue of interest. The MD template was solely used for constructing the brain mask and as an underlay for results (i.e. not used for normalization). Results were determined using a cluster extent based thresholding approach, with a primary voxel level threshold of $p < 0.005$ and a cluster level threshold of $p < 0.05$. This method offers increased

sensitivity to spatially extended signals with moderate effect size [48]. To account for spatial non-stationarity of the DTI maps, we used the random field theory based approach documented in Worsley et al. [49]. While the approach may be overly conservative, the large sample size and smoothness of our data met the assumptions of random field theory (see Hayasaka et al. [48]) minimizing this concern.

3. RESULTS

Inflammation, AD pathology, and neural injury: effects on NFL. A linear regression analysis in SPSS revealed a significant effect of YKL-40 on CSF NFL concentration. As predicted, higher YKL-40 was associated with higher CSF NFL (β=.191, p=.005), suggesting axonal degeneration. Linear regression analyses in SPSS revealed that *higher* $A\beta_{42}$ and higher T-Tau were associated with greater concentration of CSF NFL (β=.133, p=.039 and β=.270, p<.001 respectively). MCP-1 was not associated with CSF NFL levels.

Inflammation, AD pathology, and neural injury: effects on DTI. A voxel-wise analysis revealed significant interactions between YKL-40 and MCP-1 and markers of AD pathology although there were no main effects of either YKL-40 or MCP-1 on FA or MD (**Table 2**). Significant interactions were observed between MCP-1 and $A\beta_{42}$ on MD in the bilateral inferior frontal lobe and right inferior temporal lobe (**Table 2B**). The relationship was such that among participants with lower CSF $A\beta_{42}$ (suggesting cerebral amyloid deposition), higher MCP-1 was associated with higher MD, suggestive of tissue damage. The interaction between MCP-1 and $A\beta_{42}$ was non-significant in other brain regions but was trending in right fusiform (p = 0.054) and left inferior temporal gyrus (p = 0.067). The interaction between YKL-40 and $A\beta_{42}$ did not reach significance, but trended toward significance in right posterior orbital gyrus (p = 0.065). Again, among participants with lower CSF $A\beta_{42}$ levels, higher inflammation (CSF YKL-40) was associated with higher MD.

A voxel-wise analysis of FA revealed an interaction between YKL-40 and CSF T-tau (**Table 2B**). Specifically, among participants with higher CSF T-tau, higher YKL-40 was associated with higher FA in both the right and left internal capsule and cerebral peduncles. In the corresponding MD analysis, this interaction was noted in diffuse regions throughout the brain, with peak associations in frontal white matter (**Figure 1**). In both of these interactions, greater neuroinflammation (high YKL-40) in the presence of greater tau pathology (high CSF T-tau) was associated with both higher FA and MD. No significant interactions were observed between MCP-1 and T-tau, although bilateral post-central gyri were trending (**Table 2B**). Brain regions where main effects of T-tau were observed differed from the regions where interactions were noted. For example, as is detailed under main effects in **Table 2A**, higher CSF T-tau was associated with significantly higher FA in fornix and bilateral thalamus, and higher MD in a large portion of lateral parietal lobe and bilateral fusiform gyri.

Inflammation and neural injury: regional localization. Given that our sample was characterized on NFL, a protein that is primarily localized to axons, we conducted voxel-wise analyses to determine whether CSF NFL levels alone, or in combination with neuroinflammation were associated with regional gray and white matter microstructure. The voxel-wise analyses revealed widespread associations between CSF NFL and MD across lateral and medial temporal, parietal, and frontal lobes (**Table 2A, Figure 2**). As detailed in **Table 2C**, YKL-40

showed a significant interaction with CSF NFL on FA in bilateral internal and external capsule, in addition to effects on MD, with a peak association in precuneus. Finally, CSF NFL also interacted with T-tau, where elevated T-tau combined with elevated CSF NFL was associated with higher MD (**Table 2C**).

Inflammation, AD pathology, and neural injury: interactions with AD risk. In order to determine if risk factors for AD moderated the effect of neuroinflammation and AD pathology on neural health, we tested for interactions between CSF markers of inflammation and CSF biomarkers of AD pathology, parental family history status, *APOE ε4*, and age. Neuroinflammation interacted with both *APOE* ε4 and parental family history of AD. Specifically, we found an interaction between MCP-1 and *APOE* ε4, whereby higher MCP-1 in *APOE ε4* carriers was associated with higher MD (**Table 2D**). Neuroinflammation also interacted with parental family history of AD, whereby higher MCP-1 among individuals with a positive family history of AD showed higher FA and MD, a relationship not observed among individuals without parental family history of AD. Neither a linear regression analysis nor voxel-wise analyses yielded significant effects of $A\beta_{42}$ x *APOE* ε4, $A\beta_{42}$ x family history, T-tau x *APOE* ε4 or T-tau x family history interactions on CSF NFL, FA, or MD.

Age showed significant interactions with several variables. While we expected that older age would be associated with greater effects of AD pathology on neural health, across all significant interactions it was among *younger* middle-aged individuals that we found the greatest effects. Brain regions where significant interactions with age were observed are detailed in **Table 2D**. With regard to amyloid pathology, there was a significant $A\beta_{42}$ x age interaction on MD, where younger individuals with higher CSF levels of $A\beta_{42}$ had higher MD, a relationship not observed among older adults. With regard to tau pathology, there was a significant interaction between T-tau and age, whereby younger individuals with high CSF T-tau showed higher FA and higher MD, a relationship not observed in older adults. Finally, YKL-40 also showed an interaction with age, where younger individuals with higher YKL40 had higher FA and higher MD, a relationship not observed in older adults (**Figure 3**). While age was analyzed as a continuous variable, we plotted the interaction using a mean split on age (younger or older than 61.2 years) to determine the direction of the interaction.

4. DISCUSSION

Pathological changes associated with AD appear years before the onset of clinically relevant symptoms [50]. Prospective treatments or therapies may be most beneficial in the preclinical stage, before extensive and permanent neural damage. In the present study, we assessed whether neuroinflammatory processes play a role in preclinical neural injury, particularly in the presence of amyloid and tau pathology. As predicted, a neuroinflammatory response was associated with neural damage, in addition to interacting with both AD pathology and risk factors for AD.

Microglial activation and inflammation play a role in β-amyloid clearance, however, the associated inflammatory cascade is suspected of contributing to neuronal damage. In this study, we show evidence that this may in fact be the case. Higher MCP-1 combined with lower $A\beta_{42}$ was associated with altered neural microstructure in brain regions known to be affected by amyloid pathology, including bilateral frontal cortex, and lateral temporal lobe.

MCP-1 is a chemokine expressed in response to inflammatory signals, and primarily serves to attract immune cells (monocytes) to sites of inflammation. In humans, MCP-1 is increased in MCI and AD and can predict cognitive decline [4,51]. Indeed cellular studies show that human monocytes and microglia produce MCP-1 in response to Aβ fragments and larger amyloid plaques [52,53]. We also found that neuroinflammation, specifically CSF YKL-40 was associated with higher CSF levels of NFL, a structural protein primarily localized to axons. Axonal degeneration has been hypothesized to be an early feature of the AD process [54], although the specific effect of neuroinflammation on axonal degeneration is incompletely characterized [55]. Histological, cellular, and molecular studies show that microglia localize with and are reactive toward amyloid plaques [56,57]. While neuroinflammation is an adaptive response to pathology such as amyloid accumulation or tissue damage, the immune response can in turn lead to a cascade that causes neural injury [1]. Significant interactions between neuroinflammation, amyloid and tau pathology observed in this study suggest that this may be the case in the early stages of developing pathology.

In addition to relationships with amyloid pathology, we also observed interactions between inflammation and tau pathology—specifically a significant interaction between YKL-40 and T-tau. The combination of high CSF T-tau and high CSF YKL-40 was associated with both lower FA and MD. Lower FA has been associated with axonal injury in animal models [58] and across several disorders including traumatic brain injury, multiple sclerosis, and AD [59–61], while lower MD suggests restricted diffusion possibly secondary to microglial proliferation. YKL-40, a 40k da glycoprotein, is expressed by activated microglia and is elevated at all stages of AD. However, as of yet, no studies have specifically linked YKL-40 expression to tau reactivity on a cellular level. Histological studies have found that activated microglia are regionally correlated with extracellular neurofibrillary tangles (NFTs) and dystrophic tangle-bearing neurons even in the early stages of tangle formation [62,63]. Interestingly, the interaction between YKL-40 and T-tau spatially paralleled the interaction of YKL-40 and NFL, perhaps suggesting that CSF tau and NFL protein arising from inflammation-mediated neural damage, originate in similar brain regions undergoing degeneration.

Both CSF markers related to neuroinflammation (YKL-40 and MCP-1) also interacted with factors that increase risk for AD. Parental family history of AD significantly interacted with YKL-40 and trended toward a significant interaction with MCP-1. In both cases, presence of parental family history combined with higher neuroinflammation was more deleterious. Likewise, *APOE* ε4 genotype interacted with MCP-1, where *APOE* ε4 carriers with higher MCP-1 showed higher MD. Given that both parental family history and *APOE* ε4 genotype have been linked with a pro-inflammatory phenotype, it's possible that these risk factors could confer vulnerability to neural damage via inflammatory mechanisms. Expression of specific inflammation-related genes is associated with risk for AD [64], but further work is needed to understand genetic profiles that may link family history of AD to inflammation.

Investigation of CSF biomarkers of AD with DTI measures in a preclinical population provides unique insights regarding brain regions that may show early pathological changes. It is known that tau pathology is associated with disruption of cytoskeletal equilibrium, synaptic dysfunction, and axonal degeneration [65,66] and tau protein

measured in CSF indicates cellular damage, as damaged cells “leak” tau protein [67,68]. In this study, higher CSF T-tau was associated with higher FA and MD in brain regions that are known to undergo pathological changes in early stages of AD such as in the fornix, thalamus, precuneus, cuneus and cingulum. Ryan et al. (2003) reported higher FA among asymptomatic carriers of the PSEN1 mutation that causes autosomal dominant AD, likely due to early and selective loss of axonal fibers [69]. Similarly, associations between higher T-tau and higher FA in this study suggest evidence of fiber loss. Further, we found a robust relationship between CSF levels of NFL, and both FA and MD, involving several brain regions affected in AD. Overall, these findings suggest axonal loss is an early and measurable feature of preclinical AD.

While older age is the strongest predictor of AD, results from the current study indicated that neuroinflammation and AD pathology had a greater deleterious effect among the younger participants in our middle-aged cohort. In the MD analysis, younger individuals with high $A\beta_{42}$ had higher MD in several brain regions including bilateral superior frontal gyri and left transverse temporal gyrus and higher T-tau was associated with higher MD in frontal gray matter. Higher inflammation was also more deleterious in younger individuals, higher YKL-40 was associated with higher MD in diffuse regions including frontal cortex. In some cases, younger participants also showed higher FA, such as higher FA in the internal capsule with higher T-tau, and higher FA in thalamus in association with higher MCP-1. In the context of higher MD and other indicators of preclinical pathology, we suspect that higher FA here suggests loss of fibers. The results are interesting because it well established that AD related pathological changes, inflammation, and structural brain changes increase with age. However, with regard to the timing of AD pathology and inflammation, midlife may be an especially vulnerable period. Several risk factors for AD when appearing in middle-age, but not older age, confer increased risk for AD [70], and the results of this study suggest that inflammation may be a modifiable risk factor that could be targeted in midlife to prevent or delay AD. Indeed, a growing literature suggests that while anti-inflammatory treatment in AD and even MCI is largely ineffective, preclinical use of anti-inflammatory medications may confer a reduced risk for developing AD in addition to preserving gray and white matter volume [30,71,72].

Amyloid in combination with neuroinflammation was associated with neural injury; however, CSF $A\beta_{42}$ alone did not show a significant association with DTI measures. While amyloid deposition is known to be an early feature of AD, it is thought that the toxic effects on neurons only appear after significant levels of amyloid burden are reached [73]. Another possibility for the lack of direct effect of $A\beta_{42}$ may be due to a dynamic fluctuation in CSF $A\beta_{42}$ that has been observed in preclinical AD. While CSF $A\beta_{42}$ generally declines during the progression of AD, studies in individuals with Presenilin 1 mutation show elevated CSF $A\beta_{42}$ levels compared to non-carriers in the asymptomatic stage of the disease [74]. Indeed the CSF analyses in this study showed that higher CSF $A\beta_{42}$ was associated with higher CSF NFL, suggesting greater axonal injury in individuals with higher CSF $A\beta_{42}$. Main effects of $A\beta_{42}$ on DTI were likely not observed in this study because measures used were not sensitive enough to detect this relationship. This notion is supported by recent findings from our group showing that preclinical amyloid deposition as measured with [C11]PiB-PET is associated with altered microstructure as shown on DTI [75].

There are a few limitations that should be noted. Generalizability of the results may be limited. While our study is one of the largest to examine the relationship between CSF markers of pathology and neural injury, the sample was largely Caucasian, female, and highly educated with access to quality healthcare. It is also important to note that the study is cross-sectional. Longitudinal studies will be needed to understand preclinical trajectories of AD pathology, particularly as it relates to neuroinflammation. Finally, the significance of *in vivo* markers of brain pathology in a clinically healthy population is still not well understood. We found that higher CSF markers of AD pathology and neuroinflammatory processes were associated with evidence for greater neural injury among our youngest participants. These findings are novel but will require additional follow-up to be fully understood. Further, while the results of the current study suggest that neuroinflammatory processes are associated with neural damage, it is important to keep in mind that microglia, the resident immune cells of the central nervous system, are needed to control neuropathology. It is possible that the observed association between neuroinflammation and neural damage could be the result of activated microglia responding to damaged tissue [76], Aβ and cellular debris [77]. Interestingly, El Khoury et al. [78] found that in APP transgenic mice deletion of *Ccr2*, a gene for the MCP-1 receptor, resulted in reduction of microglial accumulation around plaques, an increase in Aβ deposition, and decreased survival. Microglial activation may provide a beneficial mechanism for the removal of pathology that is not directly related to neural damage. More work is needed to further understand the timing of neuroinflammation in the AD process, and to determine whether the role of microgila is beneficial or deleterious.

Summary

In conclusion, this study found that CSF biomarkers of AD pathology and neuroinflammatory processes are related to microstructural brain alterations in asymptomatic individuals. Of particular interest, among certain individuals—including those who are younger, are *APOE* ε4 carriers, or harbor parental family history of AD—there appear to be greater neural consequences of AD pathology and inflammation. T-tau and $A\beta_{42}$ have been evaluated extensively and may have diagnostic and prognostic utility in patients with AD and MCI [79–82] and have been associated with cognitive decline and conversion from healthy to impaired cognition [83–85]. The results of this study provide evidence that examining markers of AD pathology together with markers of neuroinflammation may provide greater insight into the pathological processes occurring in preclinical AD.

Acknowledgements

This project was supported by the National Institute on Aging (R01 AG037639, R01 AG027161, ADRC P50 AG033514, R01 AG021155 , the University of Wisconsin Institute for Clinical and Translational Research, funded

through a National Center for Research Resources/National Institutes of Health Clinical and Translational Science Award, 1UL1RR025011, a program of the National Center for Research Resources, United States National Institutes of Health; and Waisman Center Core Grant P30 HD003352-45 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The project was also facilitated by the facilities and resources at the Geriatric Research, Education, and Clinical Center (GRECC) of the William S. Middleton Memorial Veterans Hospital, Madison, WI. The authors gratefully acknowledge Nancy Davenport, LeAnn DeRungs, Amy Hawley, Sandra Harding, Caitlin Cleary, Jay Fruehling, Paul Cary, Jennifer Oh, and Chuck Illingworth. Above all, we wish to thank our dedicated volunteers for their participation in this research.

FIGURE LEGENDS:

Figure 1: MCP-1 x $A\beta_{42}$ interaction on MD.

Higher CSF MCP-1 and lower $A\beta_{42}$ were associated with increased mean diffusivity in several brain regions including the inferior frontal lobe, right fusiform gyrus, and inferior temporal gyri. Results were corrected for non-stationarity using a random field theory based approach with a voxel-level threshold of P<.005 and a cluster level threshold of P<.05. Variations in color map correspond to the size of the t-statistic. Right=Left.

Figure 2: CSF NFL on MD.

Higher CSF NFL was associated with greater MD in a variety of brain regions related to AD, including bilateral precuneus, right superior temporal gyrus, right hippocampus, and right superior parietal lobule. Results were corrected for non-stationarity using a random field theory based approach with a voxel level threshold of P<.005 and a cluster level threshold of P<.05. Variations in color map correspond to the size of the t-statistic. Right=Left.

Figure 3: YKL-40 x Age interaction on MD (warm) and FA (cool)

3A: Higher YKL-40 and younger age were associated with increased FA in the bilateral internal capsule and diffusely increased MD. Results were corrected for non-stationarity using a random field theory based approach with a voxel level threshold of P<.005 and a cluster level threshold of P<.05. Variations in color map correspond to the size of the t-statistic. Right=Left.

3B: Covariate adjusted MD (left) and FA (right) plotted against YKL-40. MD was extracted from superior frontal gyrus, FA was extracted from right internal capsule. While age was a continuous variable in the analyses, we used a mean split (</>61.2) to divide participants in to younger and older groups to visualize the interaction. The blue line indicates the relationship between YKL-40 and FA or MD for younger participants, while the red line indicates the relationship between YKL-40 and FA or MD for older participants. Younger individuals with higher YKL-40 had higher FA and MD.

REFERENCES

[1] Akiyama H. Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21:383–421. doi:10.1016/S0197-4580(00)00124-X.

[2] McGeer EG, McGeer PL. Inflammatory processes in Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:741–9. doi:10.1016/S0278-5846(03)00124-6.

[3] DeKosky ST, Ikonomovic MD, Wang X, Farlow M, Wisniewski S, Lopez OL, et al. Plasma and cerebrospinal fluid ?1-antichymotrypsin levels in Alzheimer's disease: Correlation with cognitive impairment. Ann Neurol 2003;53:81–90. doi:10.1002/ana.10414.
[4] Galimberti D, Fenoglio C, Lovati C, Venturelli E, Guidi I, Corrà B, et al. Serum MCP-1 levels are increased in mild cognitive impairment and mild Alzheimer's disease. Neurobiol Aging 2006;27:1763–8. doi:10.1016/j.neurobiolaging.2005.10.007.
[5] Tarkowski E. Intrathecal inflammation precedes development of Alzheimer's disease. J Neurol Neurosurg Psychiatry 2003;74:1200–5. doi:10.1136/jnnp.74.9.1200.
[6] Perez-Nievas BG, Stein TD, Tai H-C, Dols-Icardo O, Scotton TC, Barroeta-Espar I, et al. Dissecting phenotypic traits linked to human resilience to Alzheimer's pathology. Brain 2013;136:2510–26. doi:10.1093/brain/awt171.
[7] Van Exel E, Eikelenboom P, Comijs H, Frölich M, Smit JH, Stek ML, et al. Vascular factors and markers of inflammation in offspring with a parental history of late-onset Alzheimer disease. Arch Gen Psychiatry 2009;66:1263–70. doi:10.1001/archgenpsychiatry.2009.146.
[8] Wyss-Coray T. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med 2006;12:1005–15. doi:10.1038/nm1484.
[9] Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 1999;45:358–68.
[10] Chételat G, La Joie R, Villain N, Perrotin A, de La Sayette V, Eustache F, et al. Amyloid imaging in cognitively normal individuals, at-risk populations and preclinical Alzheimer's disease. NeuroImage Clin 2013;2:356–65. doi:10.1016/j.nicl.2013.02.006.
[11] Lassmann H, Fischer P, Jellinger K. Synaptic Pathology of Alzheimer's Diseasea. Ann N Y Acad Sci 1993;695:59–64. doi:10.1111/j.1749-6632.1993.tb23028.x.
[12] DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 1990;27:457–64. doi:10.1002/ana.410270502.
[13] Anna A, Alicia M, Lorena R, Albert L, Alex I, Jaume O, et al. Cerebrospinal Fluid Level of YKL-40 Protein in Preclinical and Prodromal Alzheimer's Disease. J Alzheimeraposs Dis 2014:901–8. doi:10.3233/JAD-140624.
[14] Bob O, Joakim H, Ronald L, Henrik Z, Katarina N, Kina H, et al. Microglial Markers are Elevated in the Prodromal Phase of Alzheimer's Disease and Vascular Dementia. J Alzheimeraposs Dis 2013:45–53. doi:10.3233/JAD-2012-120787.
[15] Craig-Schapiro R, Perrin RJ, Roe CM, Xiong C, Carter D, Cairns NJ, et al. YKL-40: A Novel Prognostic Fluid Biomarker for Preclinical Alzheimer's Disease. Biol Psychiatry 2010;68:903–12. doi:10.1016/j.biopsych.2010.08.025.
[16] Rosén C, Andersson C-H, Andreasson U, Molinuevo JL, Bjerke M, Rami L, et al. Increased Levels of Chitotriosidase and YKL-40 in Cerebrospinal Fluid from Patients with Alzheimer's Disease. Dement Geriatr Cogn Disord Extra 2014;4:297–304. doi:10.1159/000362164.
[17] Gaiottino J, Norgren N, Dobson R, Topping J, Nissim A, Malaspina A, et al. Increased Neurofilament Light Chain Blood Levels in Neurodegenerative Neurological Diseases. PLoS ONE 2013;8:e75091. doi:10.1371/journal.pone.0075091.
[18] Scherling CS, Hall T, Berisha F, Klepac K, Karydas A, Coppola G, et al. Cerebrospinal fluid neurofilament concentration reflects disease severity in frontotemporal degeneration: Neurofilament in FTD. Ann Neurol 2014;75:116–26. doi:10.1002/ana.24052.
[19] Sj&#65533;gren M, Blomberg M, Jonsson M, Wahlund L-O, Edman &#65533;, Lind K, et al. Neurofilament protein in cerebrospinal fluid: A marker of white matter changes. J Neurosci Res 2001;66:510–6. doi:10.1002/jnr.1242.
[20] Skillback T, Farahmand B, Bartlett JW, Rosen C, Mattsson N, Nagga K, et al. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology 2014. doi:10.1212/WNL.0000000000001015.
[21] Bozzali M, Falini A, Franceschi M, Cercignani M, Zuffi M, Scotti G, et al. White matter damage in Alzheimer's disease assessed in vivo using diffusion tensor magnetic resonance imaging. J Neurol Neurosurg Psychiatry 2002;72:742–6.
[22] Takahashi S, Yonezawa H, Takahashi J, Kudo M, Inoue T, Tohgi H. Selective reduction of diffusion anisotropy in white matter of Alzheimer disease brains measured by 3.0 Tesla magnetic resonance imaging. Neurosci Lett 2002;332:45–8.
[23] Nierenberg J, Pomara N, Hoptman MJ, Sidtis JJ, Ardekani BA, Lim KO. Abnormal white matter integrity in healthy apolipoprotein E epsilon4 carriers. Neuroreport 2005;16:1369–72.

[24] Medina D, DeToledo-Morrell L, Urresta F, Gabrieli JDE, Moseley M, Fleischman D, et al. White matter changes in mild cognitive impairment and AD: A diffusion tensor imaging study. Neurobiol Aging 2006;27:663–72. doi:10.1016/j.neurobiolaging.2005.03.026.
[25] Stahl R, Dietrich O, Teipel SJ, Hampel H, Reiser MF, Schoenberg SO. White matter damage in Alzheimer disease and mild cognitive impairment: assessment with diffusion-tensor MR imaging and parallel imaging techniques. Radiology 2007;243:483–92. doi:10.1148/radiol.2432051714.
[26] Fellgiebel A, Schermuly I, Gerhard A, Keller I, Albrecht J, Weibrich C, et al. Functional relevant loss of long association fibre tracts integrity in early Alzheimer's disease. Neuropsychologia 2008;46:1698–706. doi:10.1016/j.neuropsychologia.2007.12.010.
[27] Rose SE, Janke AL, Chalk JB. Gray and white matter changes in Alzheimer's disease: a diffusion tensor imaging study. J Magn Reson Imaging JMRI 2008;27:20–6. doi:10.1002/jmri.21231.
[28] Canu E, McLaren DG, Fitzgerald ME, Bendlin BB, Zoccatelli G, Alessandrini F, et al. Microstructural diffusion changes are independent of macrostructural volume loss in moderate to severe Alzheimer's disease. J Alzheimers Dis JAD 2010;19:963–76. doi:10.3233/JAD-2010-1295.
[29] Honea RA, Vidoni E, Harsha A, Burns JM. Impact of APOE on the healthy aging brain: a voxel-based MRI and DTI study. J Alzheimers Dis JAD 2009;18:553–64. doi:10.3233/JAD-2009-1163.
[30] Bendlin BB, Ries ML, Canu E, Sodhi A, Lazar M, Alexander AL, et al. White matter is altered with parental family history of Alzheimer's disease. Alzheimers Dement 2010;6:394–403. doi:10.1016/j.jalz.2009.11.003.
[31] Xiong C. Role of Family History for Alzheimer Biomarker Abnormalities in the Adult Children Study. Arch Neurol 2011;68:1313. doi:10.1001/archneurol.2011.208.
[32] Westlye LT, Reinvang I, Rootwelt H, Espeseth T. Effects of APOE on brain white matter microstructure in healthy adults. Neurology 2012;79:1961–9. doi:10.1212/WNL.0b013e3182735c9c.
[33] Alexander AL, Lee JE, Lazar M, Field AS. Diffusion tensor imaging of the brain. Neurother J Am Soc Exp Neurother 2007;4:316–29. doi:10.1016/j.nurt.2007.05.011.
[34] Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 2002;15:435–55. doi:10.1002/nbm.782.
[35] Bendlin BB, Carlsson CM, Johnson SC, Zetterberg H, Blennow K, Willette AA, et al. CSF T-Tau/Aβ42 Predicts White Matter Microstructure in Healthy Adults at Risk for Alzheimer's Disease. PLoS ONE 2012;7:e37720. doi:10.1371/journal.pone.0037720.
[36] Fjell AM, Walhovd KB, Fennema-Notestine C, McEvoy LK, Hagler DJ, Holland D, et al. Brain Atrophy in Healthy Aging Is Related to CSF Levels of A 1-42. Cereb Cortex 2010;20:2069–79. doi:10.1093/cercor/bhp279.
[37] Fortea J, Vilaplana E, Alcolea D, Carmona-Iragui M, Sánchez-Saudinos M-B, Sala I, et al. Cerebrospinal fluid β-amyloid and phospho-tau biomarker interactions affecting brain structure in preclinical Alzheimer disease: CSF β-Amyloid and p-Tau in AD. Ann Neurol 2014:n/a – n/a. doi:10.1002/ana.24186.
[38] Mattsson N, Insel P, Nosheny R, Trojanowski JQ, Shaw LM, Jack CR, et al. Effects of cerebrospinal fluid proteins on brain atrophy rates in cognitively healthy older adults. Neurobiol Aging 2014;35:614–22. doi:10.1016/j.neurobiolaging.2013.08.027.
[39] Gale SC, Gao L, Mikacenic C, Coyle SM, Rafaels N, Murray Dudenkov T, et al. APOε4 is associated with enhanced in vivo innate immune responses in human subjects. J Allergy Clin Immunol 2014;134:127–34.e9. doi:10.1016/j.jaci.2014.01.032.
[40] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–44.
[41] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–9. doi:10.1016/j.jalz.2011.03.005.
[42] Johnson SC, La Rue A, Hermann BP, Xu G, Koscik RL, Jonaitis EM, et al. The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE ε3/ε3 genotype. Alzheimers Dement J Alzheimers Assoc 2011;7:456–65. doi:10.1016/j.jalz.2010.11.012.
[43] Palmqvist S, Zetterberg H, Blennow K, Vestberg S, Andreasson U, Brooks DJ, et al. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid β-amyloid 42: a cross-validation study against amyloid positron emission tomography. JAMA Neurol 2014;71:1282–9. doi:10.1001/jamaneurol.2014.1358.

[44] Adluru N, Destiche DJ, Lu SY-F, Doran ST, Birdsill AC, Melah KE, et al. White matter microstructure in late middle-age: Effects of apolipoprotein E4 and parental family history of Alzheimer's disease. NeuroImage Clin 2014;4:730–42. doi:10.1016/j.nicl.2014.04.008.
[45] Schmidt P, Gaser C, Arsic M, Buck D, Förschler A, Berthele A, et al. An automated tool for detection of FLAIR-hyperintense white-matter lesions in Multiple Sclerosis. NeuroImage 2012;59:3774–83. doi:10.1016/j.neuroimage.2011.11.032.
[46] Birdsill AC, Koscik RL, Jonaitis EM, Johnson SC, Okonkwo OC, Hermann BP, et al. Regional white matter hyperintensities: aging, Alzheimer's disease risk, and cognitive function. Neurobiol Aging 2014;35:769–76. doi:10.1016/j.neurobiolaging.2013.10.072.
[47] Keihaninejad S, Heckemann RA, Fagiolo G, Symms MR, Hajnal JV, Hammers A, et al. A robust method to estimate the intracranial volume across MRI field strengths (1.5T and 3T). NeuroImage 2010;50:1427–37. doi:10.1016/j.neuroimage.2010.01.064.
[48] Hayasaka S, Phan KL, Liberzon I, Worsley KJ, Nichols TE. Nonstationary cluster-size inference with random field and permutation methods. NeuroImage 2004;22:676–87. doi:10.1016/j.neuroimage.2004.01.041.
[49] Worsley KJ, Andermann M, Koulis T, MacDonald D, Evans AC. Detecting changes in nonisotropic images. Hum Brain Mapp 1999;8:98–101.
[50] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement J Alzheimers Assoc 2011;7:280–92. doi:10.1016/j.jalz.2011.03.003.
[51] Westin K, Buchhave P, Nielsen H, Minthon L, Janciauskiene S, Hansson O. CCL2 is associated with a faster rate of cognitive decline during early stages of Alzheimer's disease. PloS One 2012;7:e30525. doi:10.1371/journal.pone.0030525.
[52] Ishizuka K, Kimura T, Igata-Yi R, Katsuragi S, Takamatsu J, Miyakawa T. Identification of monocyte chemoattractant protein-1 in senile plaques and reactive microglia of Alzheimer's disease. Psychiatry Clin Neurosci 1997;51:135–8. doi:10.1111/j.1440-1819.1997.tb02375.x.
[53] Meda L, Bernasconi S, Bonaiuto C, Sozzani S, Zhou D, Otvos L, et al. Beta-amyloid (25-35) peptide and IFN-gamma synergistically induce the production of the chemotactic cytokine MCP-1/JE in monocytes and microglial cells. J Immunol Baltim Md 1950 1996;157:1213–8.
[54] Terry RD. The cytoskeleton in Alzheimer disease. J Neural Transm Suppl 1998;53:141–5.
[55] Aktas O, Ullrich O, Infante-Duarte C, Nitsch R, Zipp F. Neuronal damage in brain inflammation. Arch Neurol 2007;64:185–9. doi:10.1001/archneur.64.2.185.
[56] Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms Underlying Inflammation in Neurodegeneration. Cell 2010;140:918–34. doi:10.1016/j.cell.2010.02.016.
[57] Kitazawa M. Microglia as a Potential Bridge between the Amyloid -Peptide and Tau. Ann N Y Acad Sci 2004;1035:85–103. doi:10.1196/annals.1332.006.
[58] Mac Donald CL, Dikranian K, Bayly P, Holtzman D, Brody D. Diffusion tensor imaging reliably detects experimental traumatic axonal injury and indicates approximate time of injury. J Neurosci Off J Soc Neurosci 2007;27:11869–76. doi:10.1523/JNEUROSCI.3647-07.2007.
[59] Roosendaal S, Geurts J, Vrenken H, Hulst H, Cover K, Castelijns J, et al. Regional DTI differences in multiple sclerosis patients. NeuroImage 2009;44:1397–403. doi:10.1016/j.neuroimage.2008.10.026.
[60] Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, Little DM. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain 2007;130:2508–19. doi:10.1093/brain/awm216.
[61] Stebbins GT, Murphy CM. Diffusion tensor imaging in Alzheimer's disease and mild cognitive impairment. Behav Neurol 2009;21:39–49. doi:10.3233/BEN-2009-0234.
[62] Filipcik P, Zilka N, Bugos O, Kucerak J, Koson P, Novak P, et al. First transgenic rat model developing progressive cortical neurofibrillary tangles. Neurobiol Aging 2012;33:1448–56. doi:10.1016/j.neurobiolaging.2010.10.015.
[63] Sheffield LG, Marquis JG, Berman NE. Regional distribution of cortical microglia parallels that of neurofibrillary tangles in Alzheimer's disease. Neurosci Lett 2000;285:165–8. doi:10.1016/S0304-3940(00)01037-5.
[64] McGeer PL. Polymorphisms in Inflammatory Genes and the Risk of Alzheimer Disease. Arch Neurol 2001;58:1790. doi:10.1001/archneur.58.11.1790.

[65] Cárdenas AM, Ardiles AO, Barraza N, Baéz-Matus X, Caviedes P. Role of Tau Protein in Neuronal Damage in Alzheimer's Disease and Down Syndrome. Arch Med Res 2012;43:645–54. doi:10.1016/j.arcmed.2012.10.012.
[66] Mandelkow E. Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. Neurobiol Aging 2003;24:1079–85. doi:10.1016/j.neurobiolaging.2003.04.007.
[67] Blennow K, Vanmechelen E, Hampel H. CSF total tau, Abeta42 and phosphorylated tau protein as biomarkers for Alzheimer's disease. Mol Neurobiol 2001;24:87–97. doi:10.1385/MN:24:1-3:087.
[68] Hesse C, Rosengren L, Andreasen N, Davidsson P, Vanderstichele H, Vanmechelen E, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci Lett 2001;297:187–90.
[69] Ryan NS, Keihaninejad S, Shakespeare TJ, Lehmann M, Crutch SJ, Malone IB, et al. Magnetic resonance imaging evidence for presymptomatic change in thalamus and caudate in familial Alzheimer's disease. Brain J Neurol 2013;136:1399–414. doi:10.1093/brain/awt065.
[70] Bendlin BB, Carlsson CM, Gleason CE, Johnson SC, Sodhi A, Gallagher CL, et al. Midlife predictors of Alzheimer's disease. Maturitas 2010;65:131–7. doi:10.1016/j.maturitas.2009.12.014.
[71] In 't Veld BA, Launer LJ, Hoes AW, Ott A, Hofman A, Breteler MM, et al. NSAIDs and incident Alzheimer's disease. The Rotterdam Study. Neurobiol Aging 1998;19:607–11.
[72] Walther K, Bendlin BB, Glisky EL, Trouard TP, Lisse JR, Posever JO, et al. Anti-inflammatory drugs reduce age-related decreases in brain volume in cognitively normal older adults. Neurobiol Aging 2011;32:497–505. doi:10.1016/j.neurobiolaging.2009.03.006.
[73] Jack CR, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol 2013;12:207–16. doi:10.1016/S1474-4422(12)70291-0.
[74] Reiman EM, Quiroz YT, Fleisher AS, Chen K, Velez-Pardo C, Jimenez-Del-Rio M, et al. Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study. Lancet Neurol 2012;11:1048–56. doi:10.1016/S1474-4422(12)70228-4.
[75] Racine AM, Adluru N, Alexander AL, Christian BT, Okonkwo OC, Oh J, et al. Associations between white matter microstructure and amyloid burden in preclinical Alzheimer's disease: A multimodal imaging investigation. NeuroImage Clin 2014;4:604–14. doi:10.1016/j.nicl.2014.02.001.
[76] Lee CYD, Landreth GE. The role of microglia in amyloid clearance from the AD brain. J Neural Transm 2010;117:949–60. doi:10.1007/s00702-010-0433-4.
[77] Bolmont T, Haiss F, Eicke D, Radde R, Mathis CA, Klunk WE, et al. Dynamics of the microglial/amyloid interaction indicate a role in plaque maintenance. J Neurosci Off J Soc Neurosci 2008;28:4283–92. doi:10.1523/JNEUROSCI.4814-07.2008.
[78] El Khoury J, Toft M, Hickman SE, Means TK, Terada K, Geula C, et al. Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease. Nat Med 2007;13:432–8. doi:10.1038/nm1555.
[79] Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. NeuroRX 2004;1:213–25. doi:10.1602/neurorx.1.2.213.
[80] Vemuri P, Wiste HJ, Weigand SD, Shaw LM, Trojanowski JQ, Weiner MW, et al. MRI and CSF biomarkers in normal, MCI, and AD subjects: Diagnostic discrimination and cognitive correlations. Neurology 2009;73:287–93. doi:10.1212/WNL.0b013e3181af79e5.
[81] Visser PJ, Verhey F, Knol DL, Scheltens P, Wahlund L-O, Freund-Levi Y, et al. Prevalence and prognostic value of CSF markers of Alzheimer's disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: a prospective cohort study. Lancet Neurol 2009;8:619–27. doi:10.1016/S1474-4422(09)70139-5.
[82] Andreasen N, Minthon L, Davidsson P, Vanmechelen E, Vanderstichele H, Winblad B, et al. Evaluation of CSF-tau and CSF-Aβ42 as Diagnostic Markers for Alzheimer Disease in Clinical Practice. Arch Neurol 2001;58. doi:10.1001/archneur.58.3.373.
[83] Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal Fluid tau/β-Amyloid42 Ratio as a Prediction of Cognitive Decline in Nondemented Older Adults. Arch Neurol 2007;64:343. doi:10.1001/archneur.64.3.noc60123.
[84] Gustafson DR, Skoog I, Rosengren L, Zetterberg H, Blennow K. Cerebrospinal fluid -amyloid 1-42 concentration may predict cognitive decline in older women. J Neurol Neurosurg Psychiatry 2006;78:461–4. doi:10.1136/jnnp.2006.100529.

[85] Stomrud E, Hansson O, Zetterberg H, Blennow K, Minthon L, Londos E. Correlation of Longitudinal Cerebrospinal Fluid Biomarkers With Cognitive Decline in Healthy Older Adults. Arch Neurol 2010;67. doi:10.1001/archneurol.2009.316.