Higher baseline levels of CSF inflammation increase risk of incident mild cognitive impairment and Alzheimer's disease dementia

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Abstract

Background

Few studies have investigated how inflammation early in the disease course may affect AD progression over time despite converging evidence that elevated levels of inflammation are associated with AD in cross-sectional studies.

Methods

Two-hundred ninety-two research participants with CSF biomarkers from the Alzheimer's Disease Neuroimaging Initiative (ADNI) were included in this study. Cox proportional hazards models were used to investigate whether baseline levels of inflammatory CSF markers were associated with incident mild cognitive impairment or AD and time to conversion. Potential moderating effects of sex and *APOE4* were also examined.

Results

Elevated levels of pro-inflammatory markers TNF- α , IL-9, and IL-12p40 at baseline were associated with higher rates of conversion to MCI/AD. Interactions with sex and *APOE4* were observed, such that women with elevated TNF- α and all *APOE4* carriers with elevated IL-9 levels had shorter times to conversion. Additionally, TNF- α mediated the relationship between elevated IL-12p40 and IL-9.

Conclusion

Elevated inflammation markers are associated with incident MCI or AD, and the factors of sex and *APOE4* status modify the time to conversion.

Background

Alzheimer's disease (AD) is increasingly recognized as a complex disease with heterogenous pathologies that affects populations and individuals differently. Numerous studies have demonstrated the importance of CSF biomarkers A β 42, phosphorylated-tau (p-tau), and total-tau (t-tau) as predictors of conversion to mild cognitive impairment (MCI) or AD. However, relatively little is known about the role of inflammation in conversion, and even less about the interactions between inflammation and sex or *APOE4* status.

A growing body of evidence indicates that neuroinflammatory responses exacerbate AD progression (1–4) and that inflammatory markers colocalize with AD pathology postmortem in AD clinical cohorts (5–9). Post-mortem studies of the AD brain indicate overexpression and/or activation of NF- κ B, particularly in

regions with high levels of A β plaques and neurofibrillary tangles (NFT) (10). NF- κ B is a key transcriptional regulator of inflammation and, more recently, has also been found to have roles in learning and memory via neuronal signaling (11–12). NF- κ B has binding sites in the promoter region of the genes involved in amyloidogenesis and inflammation (10) and as such, inflammatory markers associated with the NF- κ B pathway are of particular interest and relevance to AD.

The cytokine tumor necrosis factor α (TNF- α), implicated in many AD studies, is among the most well-studied and well-known activators of NF- κ B in both neuronal and non-neuronal cells (13–14) and plays an important role in regulating long-term potentiation (LTP) (11). In addition to TNF- α , IL-9 is also a part of the NF- κ B inflammatory pathway and has been associated with AD risk. Interleukin 9 (IL-9) is an understudied pro-inflammatory cytokine known for promoting cell proliferation and prevention of apoptosis and whose expression is regulated by NF- κ B (15). Collectively, this pathway may play a central role in inflammation associated with AD risk.

In this longitudinal study, we examined whether the baseline level of each CSF inflammatory marker was associated with incident MCI or AD, and whether these markers interact to participate in the same inflammatory pathway. We hypothesized that neuroinflammatory markers (TNF- α , IL-9), along with the classic CSF biomarkers of AD (A β 42, t-tau, and p-tau), would predict clinical conversion to MCI/AD. Lastly, accompanying the current literature on sex and *APOE4* differences in AD (16), we investigated the role of these two risk factors on inflammation and conversion in an exploratory analysis.

Methods

Subjects

Data used in the preparation of this manuscript were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). ADNI is a convenience sample predominantly comprised of highly educated, non-Hispanic whites.

Participants were selected by availability of CSF data at baseline, and the availability of at least one additional time point for diagnosis as of January 2021. Additional participant demographics included *APOE* status, age, sex, and diagnosis at time of CSF collection ($n = 295$; 177 females). The combined sample excluded participants with AD diagnosis at the baseline visit. Participants who converted to MCI or AD at the second time point were categorized as converters. Participants without diagnostic change throughout the observation period were categorized as non-converters. The frequency of follow-up visits consisted of a baseline visit, 3-month, 6-month, and annual visits. Local institutional review boards

approved the ADNI study across the United States and Canada, and each subject provided written informed consent.

APOE4 carrier status

The details of *APOE* genotyping have been previously published (17). Participants with at least one copy of the E4 allele were considered *APOE4* carriers which consisted of primarily heterozygous *APOE3/4* genotypes (78% *APOE3/4*; 19% *APOE4/4*; 0.03% *APOE2/4*).

CSF markers

According to the ADNI protocol, the levels of CSF inflammatory markers of interest (TNF- α , IL-9, IL-12p40) were measured examined by William Hu and J. Christina Howell, Department of Neurology, Emory University. All markers were measured in duplicate using commercially available multiplex immunoassays (Millipore Sigma, Burlington, MA, United States) (18). Values are given in pg/ml. Primary analyses examined TNF- α and IL-9 to test the hypothesis that higher levels of inflammatory markers associated with transcription factor NF- κ B would be associated with conversion. Exploratory analyses examined the CSF biomarker IL-12p40.

CSF AD biomarkers A β 42, t-tau and p-tau proteins were analyzed with the multiplex xMAP Luminex platform and Innogenetics/Fujirebio AlzBio3 immunoassay reagents, details of which have been described previously (19).

Statistical Analyses

Analyses were conducted using SPSS Statistics 27 (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.) and R (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>), and PASS 14 (PASS 14 Power Analysis and Sample Size Software (2015). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass).

Baseline differences in CSF inflammatory marker levels between converters and non-converters were assessed by Wilcoxon rank sum. An association was considered significant if the two-sided p value corresponding to the test statistic was less than 0.05. Cox proportional hazards models were used to investigate whether the baseline levels for each CSF inflammatory marker and pathology markers (A β 42, p-tau, and t-tau) were associated with hazard rate of conversion. Lower CSF A β -42 values is an indicator of greater presence of amyloid-beta in the brain, which is contrary to the other CSF markers in which higher levels indicate greater AD risk. Therefore, for A β 42 values only, the inverse HR is reported (1/HR) to align lower A β 42 values with increased AD, which places all CSF markers on the same scale of HR > 1.0 equates to elevated risk of conversion. CSF inflammatory markers were scaled to have a median of 0 and interquartile range (IQR) of 1 for interpretation purposes; effect estimates, expressed as hazard ratios (HRs) are therefore interpreted per IQR. Baseline age, sex, and *APOE4* status were included in the analyses

as covariates. Schoenfeld residuals were visually inspected to test for violation of the proportional hazards' assumption. Plots of Martingale residuals against continuous covariates were used to detect nonlinearity. Potential moderating effects of sex and *APOE4* were tested by assessing CSF marker*sex and CSF marker*APOE4 interactions using females and *APOE4* non-carriers as the corresponding reference groups. Further stratification and repeated analyses were conducted if the p-value corresponding to an interaction term was below 0.15 (20).

Power analyses for Cox regression were carried out using PASS 14 software. The assumption for validity of proportional hazards model was tested before carrying out the analysis. Standard deviations of the variables of interest were calculated for power analyses. The sample size was adjusted based on the variability in the continuous variables of interest explained by the additional covariates in the Cox regression. The sample size was adjusted for the anticipated event rate. We assumed 80% power and a 2-sided alpha of 0.05 and calculated the minimum detectable regression coefficient (log HR) for the analyses at 0.2410. This corresponds to HR of 1.27.

A mediation analysis was carried out using the PROCESS macro (21) version 3.5.2 for SPSS to test the significance of associations between CSF inflammatory markers in NF- κ B pathway. TNF- α was treated as a potential mediator in IL-12p40–IL-9 relationship ($x = \text{IL-12p40}$, $y = \text{IL-9}$) based on *a priori* knowledge (22,23) using age and sex as covariates.

Results

Demographic characteristics of the cohort

292 participants (152 Converters, 140 Non-converters) were included in this study excluding 3 participants due to outlying CSF values. Of the total sample, 118 participants (40%) were female, 123 (42%) were *APOE4* carriers, and 108 (37%) were cognitively unimpaired. CSF markers A β 42, t-tau, and p-tau differed between groups with increased levels of pathology in converters. Between converters and non-converters, there were no significant differences in age or CSF levels of TNF- α , IL-9 or IL-12-p40 at baseline. All variables of interest are summarized in Table 1.

Table 1

Baseline demographic and biomarker values of research participants. Effect size and p-value correspond to group differences between participants who go on to convert (converters) and those that remain at the same impairment stage (non-converters). Medians and interquartile ranges were reported for continuous variables and counts, and corresponding percentages were reported for categorical variables. Continuous variable medians were compared using Wilcoxon rank sum test and effect size was calculated according to Cohen's D. Categorical variable counts were compared using χ^2 test and the respective size was calculated according to Cramer's V.

Baseline variable	All (n = 292)	Converter (n = 152)	Non-converter (n = 140)	Effect Size	p value
Sex (M/F)	174/118	97/55	77/63	0.08	0.16
APOE4 (Carrier/Non-Carrier)	123/169	76/76	47/93	0.16	< 0.01
Age	74.85 (6.78)	74.50 (7.07)	75.23 (6.46)	0.10	0.39
TNF- α	1.74 (0.52)	1.80 (0.53)	1.68 (0.51)	0.23	0.05
IL-9 (n = 212)	3.44 (1.55)	3.58 (1.69)	3.30 (1.38)	0.18	0.19
IL-12 (P40) (N = 212)	1.31 (1.76)	1.52 (2.21)	1.08 (1.06)	0.25	0.07
p-Tau (N = 288)	27.70 (13.10)	31.42 (13.72)	23.76 (11.11)	0.61	< 0.01
Tau (N = 288)	287.48 (114.95)	318.49 (119.16)	252.82 (99.58)	0.60	< 0.01
A β -42	1018.82 (581.56)	870.28 (578.58)	1180.10 (545.27)	0.55	< 0.01

The median follow-up period was 2.13 years (IQR = 3.21) after the baseline visit for converters and 3.22 years (4.44) for non-converters. For converters, median time to conversion to AD was 1.99 years (IQR = 2.97 years), whereas conversion to MCI was 5.32 years (IQR = 3.67 years). 14 (4.79%) CN participants converted to AD, 22 (7.53%) CN participants converted to MCI, and 114 (39.04%) MCI participants converted to AD.

CSF Biomarkers at baseline predict conversion to MCI/AD:

Amyloid Beta-42

Baseline A β 42 was significantly associated with increased rate of conversion to MCI/AD after adjusting for baseline age, sex, and APOE4 carrier status (HR = 2.17 per 1 IQR, 95% CI: (1.59,3.03), $p < 0.001$, Fig. 1). As expected, there was an A β 42*APOE4 interaction on conversion (HR = 1.96 per 1 IQR, CI: (0.92,4.17), $p = 0.08$). Lower A β 42 was significantly associated with increased rate of conversion, in both carriers (HR = 3.33 per 1 IQR, CI: (1.69, 6.67), $p < 0.001$) and non-carriers (HR = 1.72 per 1 IQR, 95% CI: (1.19,2.5), $p = 0.004$). In a subgroup analysis of those who converted to AD, lower CSF A β 42 was significantly associated with increased rate of conversion to AD (HR = 3.23 per 1 IQR, 95% CI: (2.17,4.76), $p < 0.001$).

There was also a significant interaction with sex (HR = 0.55 per 1 IQR, 95% CI: (0.25,0.93), $p = 0.03$), such that the rate of conversion among women was significantly different from men. A β 42 had higher rates of conversion in women (HR = 4.76 per 1 IQR, 95% CI: (2.17, 11.11), $p < 0.001$), which was also true among men (HR = 2.63 per 1 IQR, 95% CI: (1.64,4.17), $p < 0.001$). There was no significant A β 42*APOE4 interaction on conversion (HR = 0.66 per 1 IQR, 95% CI: (0.28,1.59), $p = 0.36$). CSF A β 42 levels at baseline were not significantly associated with conversion to MCI (HR = 0.77 per 1 IQR, 95% CI: (0.45,1.33), $p = 0.35$).

Phosphorylated-tau

Higher baseline p-tau was significantly associated with increased rate of conversion to MCI/AD (HR = 1.64 per 1 IQR, 95% CI: (1.35,1.98), $p < 0.001$, Fig. 1). No sex interactions were observed (HR = 0.9237 per 1 IQR, 95% CI: (0.65,1.32), $p = 0.66$). There was a significant p-tau*APOE4 interaction on conversion (HR = 0.65 per IQR, CI: (0.44,0.97), $p = 0.04$), such that higher p-tau levels were significantly associated with increased conversion among non-carriers (HR = 2.10 per 1 IQR, 95% CI: (1.54,2.87), $p < 0.001$), but not among *APOE4* carriers (HR = 1.28, CI: (0.99,1.66), $p = 0.06$) in *APOE4*-stratified models. There was a significant main effect of p-tau on the rate of conversion to AD (HR = 1.69 per 1 IQR, 95% CI: (1.38, 2.07), $p < 0.001$). There was a significant p-tau*APOE4 interaction on conversion (HR = 0.55, 95% CI: (0.36,0.85), $p = 0.01$), such that *APOE4* carriers had higher risk of conversion (HR = 2.43, 95% CI: (1.72, 3.43), $p < 0.001$) compared to *APOE4* non-carriers (HR = 1.31 (1.00,1.71), $p = 0.05$). P-tau was not significantly associated with the rate of conversion to MCI (HR = 1.58, 95% CI: (0.97,2.57), $p = 0.07$).

Total-tau

Higher baseline t-tau was significantly associated with increased rate of conversion to MCI/AD (HR = 1.64 per 1 IQR, 95% CI: (1.35,1.99), $p < 0.001$, Fig. 1). No sex ($p = 0.5$) or *APOE4* ($p = 0.15$) interactions with t-tau on conversion were observed. T-tau was significantly associated with a higher rate of conversion to AD (HR = 1.73 per 1 IQR, 95% CI: (1.4,2.14), $p < 0.001$). No t-tau*sex interaction on conversion to AD was observed. There was a significant t-tau*APOE4 interaction on conversion to AD (HR = 0.62 per 1 IQR, 95% CI (0.40, 0.96), $p = 0.03$), such that *APOE4* carrier participants had higher hazard ratio for conversion (HR = 2.28 per 1 IQR, 95% CI: (1.63,3.18), $p < 0.001$), compared to non-carriers (HR = 1.34 per 1 IQR, 95% CI: (1.01, 1.78), $p = 0.04$). CSF t-tau levels at baseline were not significantly associated with conversion to MCI (HR = 1.72 per 1 IQR, 95% CI: (0.98,3.03), $p = 0.06$).

Elevated CSF Inflammatory Markers at baseline predict conversion to MCI/AD:

Tumor Necrosis Factor- α

Baseline TNF- α level was significantly associated with rate of conversion to MCI/AD (HR = 1.24 per 1 IQR, 95% CI:(1.01,1.52), $p = 0.04$, Fig. 1, Fig. 2A). A significant TNF- α *sex interaction was observed (HR = 0.81 per 1 IQR change, 95% CI: (0.62,1.06), $p = 0.13$), suggesting that the association between TNF- α and

conversion was higher among women (HR = 1.38, 95% CI: (1.05,1.83), $p = 0.02$), but not among men (HR = 1.06 per 1 IQR, 95% CI: (0.99,1.127), $p = 0.06$) in sex-stratified models. No TNF- α *APOE4 interactions on conversion were observed. There was a significant main effect of TNF- α on the rate of conversion to AD (HR = 1.33 per IQR, 95% CI: (1.04,1.71), $p = 0.02$, Fig. 2C). A TNF- α *sex interaction on conversion (HR = 0.69 per 1 IQR, 95% CI: (0.42,0.86), $p = 0.15$) indicated that the association between TNF- α and conversion was higher among women (HR = 1.66 per 1 IQR, 95% CI: (1.1,2.48), $p = 0.01$) than men (HR = 1.08 per 1 IQR, 95% CI: (0.78,1.5), $p = 0.63$). TNF- α was not significantly associated with rate of conversion to MCI (HR = 1.36 per IQR change, 95% CI: (0.87,2.11), $p = 0.18$, Fig. 2B). There were no significant interactions with *APOE4* carrier status in the models.

Interleukin-9

Baseline IL-9 level was not significantly associated with the rate of conversion to MCI/AD (HR = 1.07 per 1 IQR, 95% CI: (0.84,1.37), $p = 0.56$, Fig. 3A) or with rate of conversion to AD (HR = 1.07 per 1 IQR, 95% CI: (0.81,1.39), $p = 0.65$) (Fig. 3C). No significant interaction with sex or *APOE4* and IL-9 on conversion was observed. Baseline IL-9 levels were significantly associated with a higher rate of conversion to MCI (HR = 1.53 per IQR change, 95% CI: (3.97,5935.36), $p = 0.01$) (Fig. 1, 3B). A significant IL-9*APOE4 interaction (HR = 0.001, 95% CI: (< 0.001 ,0.17), $p = 0.01$) on CN—MCI conversion, indicating that IL-9 conversion was significant in *APOE4* carriers (HR = 1.39 per 1 IQR, 95% CI: (0.37, 52713), $p = 0.10$), but not in non-carriers (HR = 1.04 per 1 IQR, 95% CI: (0.5,2.23), $p = 0.91$) in *APOE4*-stratified models. There was no significant interaction with IL-9*sex on conversion to MCI.

Interleukin-12p40

Baseline levels of IL-12p40 were significantly associated with a higher rate of conversion to MCI/AD (HR = 1.38 per 1 IQR, 95% CI: (1.20,1.59), $p < 0.01$, Figs. 1, 4A). No sex or *APOE4* interactions with IL-12p40 on conversion were observed. IL-12p40 was significantly associated with conversion to AD (HR = 1.36 per 1 IQR, 95% CI: (1.18,1.57), $p < 0.01$, Fig. 4C). IL-12p40 was not significantly associated with conversion to MCI (HR = 1.42 per 1 IQR, 95% CI: (0.72,2.815), $p = 0.31$, Fig. 4B).

TNF- α mediates the association between IL-12p40 and IL-9

Based on *a priori* knowledge of the NF- κ B relationship between the three inflammatory markers (IL-12p40, IL-9, and TNF- α), a bootstrapped mediation analysis was conducted to investigate whether TNF- α (M) was a significant mediator of IL-12p40 (X) and IL-9 (Y). Adjusting for age and sex, IL-12p40 was significantly associated with TNF- α ($\beta = 0.07$ per 1 pg/mL, CI: (0.04, 0.11), $p < 0.001$) and with IL-9 ($\beta = 0.12$ per 1 pg/mL, CI: (0.00, 0.24), $p = 0.04$). TNF- α was a significant mediator in IL-12p40—IL-9 relationship. The indirect effect (average mediated effect) was 0.11 (95% CI: (0.05,0.18), $p < 0.001$). Average direct effect was 0.01 (95% CI: (-0.10,0.12), $p = 0.81$). Total effect was 0.13 (95% CI: (0.01,0.24), $p = 0.04$). TNF- α mediated 88.64% of the relationship (95% CI: (0.33,5.22), $p = 0.04$, Fig. 5).

Discussion

Chronic low-grade inflammation is a hallmark feature of AD, as evidenced by increased expression of proinflammatory cytokines in postmortem AD brains (24–26), yet outside of examining known CSF biomarkers, few studies have examined the contribution of elevated inflammatory levels on future incidence of clinical conversion. In the present study, we evaluated inflammatory markers at baseline to determine differences between those who progressed to MCI or AD and those that remained stable over time. Evaluating individuals before conversion to AD retroactively evaluates early features of AD risk and can provide an outlook on time to conversion. Therefore, the goal of this study was to address whether: i) inflammatory cytokines are involved in incident MCI or AD as compared to canonical CSF AD biomarkers, ii) sex or *APOE4* impact these relationships, and iii) there is a relationship between the inflammatory markers via mediation analysis.

As expected, CSF biomarkers A β 42, p-tau, and t-tau predicted conversion to MCI or AD. This was especially true among *APOE4* carriers for A β 42 and p-tau, which has been previously confirmed (27,28). In line with the primary goal of the study, inflammatory CSF markers TNF- α , IL-9, and IL-12p40 were associated with conversion to MCI/AD with sex and *APOE4* interactions.

While we know that neural degeneration and the chronic presence of pathology can stimulate inflammation, evidence suggests that inflammation itself can both generate and increase pathology. For instance, modulatory proteins associated with innate immunity are directly connected to amyloid-beta production (29) indicating the importance of neuroimmune actions on AD. Therefore, it is possible that inflammatory markers may be important indicators of MCI and AD risk, possibly accelerating decline. Additionally, several interactions involving sex and *APOE4* status among these inflammatory markers were observed, suggesting these genetic factors may influence the relationship between early inflammation and future AD risk, providing key insight for precision medicine approaches. Lastly, the association between these markers suggests a common tie to the NF- κ B inflammatory pathway, which may be activated in AD.

CSF TNF- α , one of the inflammatory markers most consistently implicated in AD, was associated with conversion in individuals who were cognitively normal at baseline. Previous work has shown increased levels of TNF- α in both the brains and plasma of patients with clinical AD (30). This may be partially related to A β , which can directly stimulate microglial production of TNF- α through activation of the transcription factor NF- κ B (31). In addition, early increases in TNF- α can increase A β burden through the upregulation of β -secretase production and increase of γ -secretase activity (32,33), demonstrating how TNF- α and A β can perpetuate a vicious cycle of increasing pathology and chronic inflammation. In corroboration of a role for TNF- α in AD, a strong association between elevated TNF- α levels and decreased functional connectivity in an AD susceptible cohort has been observed at a whole-brain level (34). Aside from TNF- α as a consistently elevated proinflammatory marker in AD, its regulation of the NF- κ B inflammatory cascade is well characterized (35,36).

When decomposing the association between TNF- α levels in cognitively normal individuals and clinical converters, the effect was driven by conversion to AD, not conversion to MCI, indicating that elevated levels of TNF- α resulted in accelerated onset of Alzheimer's dementia. Given that TNF- α both promotes and is regulated by NF- κ B, it could be that TNF- α accumulates at a faster rate than other inflammatory factors. In AD patients who experienced systemic inflammatory events (SIE), rapid cognitive decline was observed over 6 months creating a 2-fold decrease in cognitive function. Additionally, SIE AD patients also exhibited an association between elevated plasma TNF- α levels and a 10-fold greater rate of cognitive decline over a 6-month observation period (37).

Importantly, a sex*TNF- α interaction was observed in which women with higher TNF- α levels had shorter time to conversion to MCI or AD. There is precedence for this result with recent evidence suggesting important sex differences in immune parameters. This includes brain responses to inflammatory mediators and markers of microglial disruption which may contribute to higher risk of AD among women (38). For instance, given an acute endotoxin challenge, females responded with higher levels of pro-inflammatory plasma cytokines like TNF- α , compared to males (39). This may be due to a putative link between levels of TNF- α and sex hormones. A significant positive correlation between luteinizing hormone and TNF- α levels has been observed (albeit only in males) providing some evidence that sex hormones exert their influence on AD by modulating systemic TNF- α levels (40). There are also sex differences among function, morphology, and volume of microglia, with evidence suggesting that women later in life have higher numbers of microglia than men, creating an exaggerated immune response (41). Interestingly, our finding that females with higher TNF- α levels have a shorter time to conversion to MCI/AD suggests that elevated inflammation is associated with AD risk. It has been suggested that elevated inflammatory factors, including TNF- α , are associated with lower brain levels of amyloid-beta and tau in cognitively normal individuals (42), suggesting inflammation may be protective for a time before eventually exhausting this compensatory effect and converting to AD at an accelerated rate. Studies have started showing prevention of cognitive loss with anti-TNF- α therapy in a mouse model of compromised cognition (43) lending additional evidence to an important role of TNF- α in the initiation and amplification of the inflammatory cascade.

Baseline elevated IL-9 levels were associated with conversion to MCI or AD, but in contrast to TNF- α , the results were driven by conversion to MCI and not AD. Additionally, no interaction with sex was observed. IL-9 is an understudied pro-inflammatory cytokine that promotes cell proliferation and whose expression is driven by NF- κ B (44). Its pleiotropic effects have been implicated in both neurological (i.e., multiple sclerosis [45]) and systemic disorders (i.e., psoriasis [46]). Additionally, neurons are thought to possess the ability to induce T cells to release IL-9 [47]. Thus, IL-9 may represent a critical link in the crosstalk between neurons and perivascular T cells in the brain. Changes related to IL-9 have not been consistently reported in AD (48,49), but there is mouse *APOE4* knock-in models showing greater IL-9 production than the wild-type ϵ 3 allele (50). We add to this evidence by reporting an interaction between *APOE4* and IL-9 levels, such that *APOE4* carriers with higher IL-9 levels at baseline had higher rates of conversion.

Lastly, higher baseline levels of the proinflammatory cytokine IL-12p40 were also significantly associated with conversion to AD. IL-12, which has different subunits, including p40 as investigated here, is involved in the differentiation of naive T cells into Th1 cells [51] and plays an important role in the activities of natural killer (NK) cells and T lymphocytes, enhancing their activity. IL-12 also stimulates production of TNF- α from T and NK cells (52). Its role in AD remains unclear, but recent evidence indicates a role for IL-12 in amyloid induced neurodegeneration (53). The p40 subunit of IL-12 is produced by microglia and was increased in transgenic mouse models demonstrating a significant correlation between cognitive performance and CSF IL-12p40 levels (52,53). More support of its role in AD was shown by elevated CSF IL-12p40 levels in AD patients (54). Interestingly, the potential association of plasma IL-12p40 levels with the diagnosis of MCI or AD was also highlighted by a recent plasma multianalyte profiling study (55). Together, these findings imply that IL-12p40 is involved in AD progression.

To investigate interrelationships between cytokines showing significant associations with conversion to MCI/AD, mediation analyses using *a priori* knowledge of the markers and their role in the NF- κ B pathway were conducted. As mentioned previously, IL-12p40 has been shown to stimulate the production of TNF- α (23), a canonical promotor of the NF- κ B inflammatory pathway (33,34), and evidence shows that IL-9 production is mediated by NF- κ B (22). TNF- α was tested as a significant mediator between IL12-p40 and IL-9, and mediation analysis results suggest that TNF- α mediated 89% of the association between IL12-p40 and IL-9. Based on the available literature, this study is the first showing that these cytokines may be involved in the same NF- κ B inflammatory pathway, thereby further implicating the inflammatory cascade's importance in AD detection.

Limitations

Current limitations of this study include an inability to test specific demographic variables, including race, which we know play a significant role in inflammation and AD and yet have not been prioritized in ADNI recruitment. The investigation of longitudinal cognitive data from this sample and other datasets could help determine if differences exist between converters and non-converters, and shed light on specific cognitive domains that may be more closely tied to inflammation. We also note that because this was interval censored data, we are not able to know the actual time of conversion and are restricted to examining data at discrete time points. Competing risks were not discussed (i.e., death occurring prior to follow-up). Additionally, because we only had baseline CSF inflammatory markers, we were restricted to a cross-sectional mediation analysis that limits our confidence in temporality of events, but as more data become available, longitudinal analyses of change in inflammatory markers can be evaluated. Lastly, the analyses include adjusted p-values to explore interactions, so the results should be carefully considered. Future studies aimed at replication of these findings in other independent cohorts, including those which are more nationally representative of the AD population, would help validate these results.

Conclusions

This work demonstrates the need to investigate inflammatory markers and their role in conversion to AD. Investigations focused on inflammation differences between key grouping, based on clinical diagnosis, sex, and *APOE* status may provide insight on key underlying processes relevant to future clinical conversion. As a testament to the importance of longitudinal analysis, no baseline differences in CSF inflammatory marker levels were observed between CN and MCI groups, or between *APOE4* carriers and non-carriers in the current study. The strength of this study comes from the rich longitudinal dataset because changes occurring within individual participants may be more useful for characterizing disease progression than cross-sectional comparisons which are confounded/rendered less sensitive by large amounts of interindividual variation.

These inflammatory biomarkers could prove to be easily accessible targets to gauge the efficacy of therapeutic interventions and be crucial to point to new biological pathways that may play a role in dementia.

Declarations

Ethics approval and consent to participate

The study was approved by the institutional review boards of the participating institutions. All participants gave informed written consent. More details can be found online (<https://adni.loni.usc.edu>, Accessed: 17 September 2021).

Consent for publication

This manuscript meets the guidelines put forth by the Alzheimer's Disease Neuroimaging Initiative Data Sharing and Publication Policy.

Availability of data and materials

The data used in this publication are available through the ADNI website upon user approval: <https://adni.loni.usc.edu>

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: DSA; Implementation and computational methods: JAC & VA; Paper writing: JCA; Paper review and supervision: JP. All authors read and approved the final manuscript.

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Figures

Figure 1

Forest plot highlighting predictive power of CSF inflammatory markers as predictors of conversion. Each model tested is represented by a line in the plot where color and shape differentiate our variables of interest. Line of no effect (HR=1.0) is represented by dotted line. Variable hazard ratios (per IQR) visualize how the ratio magnitude of inflammatory markers TNF- α , IL-9, and IL-12p40 compare to the magnitudes of the ratios of AD biomarkers.

TNF- α *Sex

Figure 2

Survival curves for CSF inflammatory marker, TNF- α . Panel A shows survival probabilities split between sex. Red represents females and blue, males, to better show the interaction effect between sexes of overall conversion to either MCI or AD. Females with high levels of TNF- α tended to have later conversion times. Results were split by conversion to either MCI (panel B) or AD (panel A) with conversion to MCI not significant.

IL-9* *APOE4* Status

Figure 3

Survival curves for CSF inflammatory marker, IL-9. Panel A displays overall conversion to either MCI or AD, split between *APOE4* carriers and noncarriers. Orange represents *APOE4* carriers, and grey, non-carriers, illustrate that *APOE4* carriers convert sooner in both the high and lower groups. When split between conversion status, these results were driven by conversion to MCI (Panel B). Conversion to AD was not significant (Panel C).

IL-12p40* *APOE4* Status

Figure 4

Survival curves for CSF inflammatory marker, IL-12p40. Panel A displays conversion to either MCI or AD, split between *APOE4* carriers and noncarriers. Orange represents *APOE4* carriers, and grey represents non-carriers. When split between conversion status, these results were driven by conversion to AD (Panel C). Conversion to MCI was not significant (Panel B).

Figure 5

TNF- α mediates relationship between IL12-p40 and IL-9. The figure depicts the relationship between the independent, mediator, and dependent variables. The mediation analysis partitions the total variance (total effect) explained by the predictor into a part that is independent of the mediating variable (direct effect) and a part that is accounted for via the mediating variable (indirect effect). a represents the “ a ” path and b represents the “ b ” path. A bootstrapped mediation analysis revealed that TNF- α significantly mediated the relationship between inflammatory cytokines IL12-p40 (independent) and IL-9 (dependent) variables.