Neurodegeneration, synaptic dysfunction, and gliosis are phenotypic of Alzheimer dementia

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Abstract

Objective

To test the hypothesis that cognitively unimpaired individuals with Alzheimer disease (AD) neuropathology differ from individuals with AD dementia on biomarkers of neurodegeneration, synaptic dysfunction, and glial activation.

Methods

In a cross-sectional study, adult participants >70 years old ($n = 79$, age 77.1 ± 5.3 years) underwent comprehensive cognitive evaluation and CSF collection, which was assayed for markers of amyloid, phosphorylated tau (p-tau), neurodegeneration (neurofilament light protein [NFL] and total tau), synaptic dysfunction (neurogranin), and glial activation (chitinase-3-like protein 1 [YKL-40]). Participants were divided into 3 groups based on diagnosis and p-tau/ β -amyloid₄₂ ($A\beta_{42}$): those with low p-tau/ $A\beta_{42}$ and unimpaired cognition were classified as controls ($n = 25$); those with high p-tau/ $A\beta_{42}$ diagnosed with AD-dementia or AD-mild cognitive impairment were classified as AD-Dementia ($n = 40$); and those with high p-tau/ $A\beta_{42}$ but unimpaired cognition were classified as mismatches ($n = 14$). A similar, secondary analysis was performed with no age exclusion criteria ($n = 411$).

Results

In both the primary and secondary analyses, biomarker levels between groups were compared with the use of analysis of covariance while controlling for age and demographic variables. Despite p-tau/ $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ levels comparable to those of the AD-Dementia group, mismatches had significantly lower levels of NFL and total tau. While not significantly lower than the AD-Dementia group on YKL-40 and neurogranin, mismatches were also not significantly different from controls.

Conclusions

These results provide evidence that, in the absence of significant neurodegenerative processes, individuals who harbor AD neuropathology may remain cognitively unimpaired. This finding provides insight into the biological processes phenotypic of dementia and supports monitoring multiple biomarkers in individuals positive for AD neuropathology.

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Glossary

A β = β -amyloid; AD = Alzheimer disease; ADRC = Alzheimer's Disease Research Center; MCI = mild cognitive impairment; NFL = neurofilament light protein; p-tau = tau phosphorylated at threonine 181; t-tau = total tau; WRAP = Wisconsin Registry for Alzheimer's Prevention; YKL-40 = chitinase-3-like protein 1.

The neuropathology of Alzheimer disease (AD), amyloid plaques (β -amyloid [A β]) and neurofibrillary tangles, accumulates in a silent phase years before cognitive decline. Remarkably, some individuals live to older age without developing dementia despite substantial AD neuropathology burden on autopsy, leading some to describe these individuals as mismatches.¹

Postmortem, observed brain differences between individuals with AD dementia and mismatches include neuronal dysfunction and glial activation. Neuronal and synaptic degeneration is strongly correlated with cognitive dysfunction,² and microglial and astrocytic activation is a key feature of the pathogenic process of AD.³

In the present study, we tested whether CSF biomarkers of neurodegeneration, synaptic dysfunction, and glial activation differ between unimpaired older adults with no biomarker evidence of AD neuropathology (controls), individuals with AD dementia (AD-Dementia), and cognitively unimpaired individuals with evidence of significant AD neuropathology (mismatches).

We hypothesized that, compared to mismatches, AD-Dementia participants would exhibit higher neurofilament light (NFL), neurogranin, total tau (t-tau), and chitinase-3-like protein 1 (YKL-40), indicative of neurodegeneration, synaptic dysfunction, and gliosis.

Methods

Participants

All participants were recruited from the Wisconsin Registry for Alzheimer's Prevention (WRAP) and the Wisconsin Alzheimer's Disease Research Center (ADRC). These cohorts are composed of unimpaired middle-age to older adults with and without parents with late-onset AD (WRAP), as well as those with AD-mild cognitive impairment (MCI) and AD-dementia (ADRC). All participants are community dwelling and underwent examination (including lumbar puncture) at the University of Wisconsin Medical Center between 2010 and 2017. The current sample was enriched for AD risk via a parental history of AD (69%) and included participants positive for the known AD genetic risk factor APOE ϵ 4 (42%). Exclusion criteria included any significant neurologic disease other than AD or major psychiatric disorders.

Standard protocol approvals, registrations, and patient consents

The University of Wisconsin's institutional review board approved all portions of this study, and each participant provided written informed consent before all procedures.

Diagnosis

Participants underwent comprehensive cognitive testing, described previously for WRAP⁴ and ADRC.⁵ Diagnosis of dementia due to AD or MCI due to AD was determined with National Institute on Aging-Alzheimer's Association criteria⁶ and reviewed by a multidisciplinary panel of experts at a diagnosis consensus meeting at which cognitive testing findings and supporting information gathered at each study visit were reviewed (e.g., self-reported medical history, depressive symptoms, self-reported memory functioning, social history, and informant reports of cognitive and functional status). Participants included in the final analysis fell into 1 of 3 diagnostic categories: cognitively unimpaired, MCI due to AD, or dementia due to AD.

CSF analyses

CSF collection and assays have been described previously.⁷ CSF biomarkers were used to assess AD-related neuropathology, neuronal dysfunction, and glial activation. We measured A β ₄₂, A β ₄₀, t-tau, and tau phosphorylated at threonine 181 (p-tau), biomarkers that are known to distinguish patients with dementia due to AD from controls⁸ and are indicative of conversion from MCI to dementia.⁹ In addition to these AD biomarkers, we examined markers of axonal and synaptic neurodegeneration: NFL protein and neurogranin, respectively. These biomarkers are associated with cognitive decline and are elevated in patients with AD compared to controls.¹⁰ Finally, we measured a marker of activated microglia and astrocytes, YKL-40. This protein is elevated in AD, likely as a response to amyloid accumulation and cell injury.^{11,12} We computed and conducted analyses using A β ₄₂/A β ₄₀, given that it is more closely associated with amyloid plaque burden,¹³ and p-tau/A β ₄₂, which is a sensitive marker of AD progression.¹⁴

Statistical analysis and group delineation

Group delineations were based on cognitive status and CSF biomarker levels, and 2 analyses were performed. In both analyses, participants were included if they had complete CSF data analyzed and fell into 1 of 3 categories: MCI or dementia due to AD (AD-Dementia), cognitively unimpaired but with AD neuropathology (mismatches), or unimpaired controls without AD neuropathology (controls). The difference between the 2 analyses is that, in the age-matched analysis, groups were limited to ≥ 70 years and therefore did not statistically differ on age (tests described below). The second analysis included individuals of any age, and age was controlled for statistically. This is described in the following sections.

Age-matched analysis (>70 years)

To delineate groups of interest, we started with an initial sample comprising 461 participants with a successful lumbar puncture. We then divided the entire sample into quartiles of p-tau/A β ₄₂,

categorizing the top 25% as phenotypic of AD ($n = 115$) and the remaining 75% of participants as not phenotypic of AD ($n = 346$). Unsurprisingly, the group not phenotypic of AD was substantially younger. To account for age as robustly as possible, we limited our primary analysis to individuals ≥ 70 years of age ($n = 99$ of the original 461) while also including age as a covariate (i.e., the age-matched analysis).

Individuals in the top 25% of p-tau/ $A\beta_{42}$ were then divided again on the basis of diagnosis. Participants with a diagnosis of AD-MCI and AD-dementia were categorized as AD-Dementia. Participants in the top 25% of p-tau/ $A\beta_{42}$ but who were cognitively unimpaired were categorized as mismatches. To examine a relatively pure AD phenotype, we excluded 20 participants who had an MCI or dementia diagnosis that was deemed unlikely to stem from AD (e.g., frontotemporal dementia) or who had a p-tau/ $A\beta_{42}$ ratio in the bottom 75% of the entire sample (e.g., not phenotypic of AD despite a clinical AD diagnosis). This produced a final sample of $n = 79$.

This classification scheme resulted in 3 groups: low p-tau/ $A\beta_{42}$ and unimpaired cognition classified as controls ($n = 25$), high p-tau/ $A\beta_{42}$ diagnosed with AD-dementia or AD-MCI classified as AD-Dementia ($n = 40$), and high p-tau/ $A\beta_{42}$ but unimpaired cognition classified as mismatches ($n = 14$).

Age-controlled analysis (entire cohort)

A secondary analysis comprised the entire initial sample ($n = 461$, including participants from the age-matched analysis). The control, AD-Dementia, and mismatch groups were delineated in an identical procedure as outlined above except that age was not limited to >70 years. To examine a relatively pure AD phenotype, we excluded 50 participants from this 461 who had an MCI or dementia diagnosis that was deemed unlikely to stem from AD (e.g., frontotemporal dementia) or because their p-tau/ $A\beta_{42}$ ratio was in the bottom 75% of the entire sample (e.g., not phenotypic of AD despite a clinical AD diagnosis). This produced a final sample of $n = 411$.

This classification scheme resulted in 3 groups: low p-tau/ $A\beta_{42}$ and unimpaired cognition classified as controls ($n = 291$), high p-tau/ $A\beta_{42}$ diagnosed with AD-dementia or AD-MCI classified as AD-Dementia ($n = 61$), and high p-tau/ $A\beta_{42}$ but unimpaired cognition classified as mismatches ($n = 59$).

Statistical analyses

Analysis of covariance was used to compare the 3 groups on CSF biomarkers while controlling for demographic variables (years of education, *APOE* $\epsilon 4$ status, family history of AD, sex, and age). Significance was inferred at $\alpha < 0.05$. To achieve normality, NFL, neurogranin, and p-tau/ $A\beta_{42}$ data were log-transformed before analysis.

Data availability

For purposes of replicating procedures and results, the data used in this study can be made available on request.

Results

Demographic characteristics

Age-matched analysis (>70 years)

Demographic characteristics are displayed in table 1. An analysis of variance comparing demographic variables revealed that groups did not significantly differ on age (as expected). The AD-Dementia group had a significantly lower percentage of female participants compared to controls ($p = 0.046$), and no other groups differed. The AD-Dementia and mismatch groups had a higher percentage of *APOE* $\epsilon 4$ carriers compared to controls (all $p < 0.001$). Groups did not differ on family history of AD or education, although we observed a trend for education such that the AD-Dementia group had 1.3 fewer years of education than controls ($p = 0.51$).

Age-controlled analysis (entire cohort)

Demographic characteristics are displayed in table 2. The analysis of variance comparing demographic variables revealed that groups differed on age at the time of CSF collection (as expected). The AD-Dementia group was 13.3 years older than controls and 7.8 years older than the mismatch group. The mismatch group was 5.5 years older than controls (all $p < 0.001$). The AD-Dementia group had a significantly lower percentage of female participants compared to both the control and mismatch groups (all $p < 0.001$). The AD-Dementia ($p < 0.001$) and mismatch ($p = 0.003$) groups were more likely to be *APOE* $\epsilon 4$ carriers compared to controls. The AD-Dementia group was less likely to have a family history of AD at the trend level ($p = 0.087$), likely because many of the younger participants who enroll in the WRAP and ADRC studies do so because of a parental family history of AD. Finally, the AD-Dementia group had 0.91 fewer years of education compared to controls ($p = 0.034$).

Biomarker comparisons

As expected from the group classification, analyses of covariance for both the age-matched and age-controlled analyses revealed that the AD-Dementia and mismatch groups had higher levels of p-tau/ $A\beta_{42}$ compared to controls but did not differ from each other. Similarly, the AD-Dementia and mismatch groups had lower levels of $A\beta_{42}/A\beta_{40}$ but did not differ from each other (all $p < 0.001$, plots not shown).

NFL results

The group comparison results for NFL varied between the age-matched and age-controlled analyses. Specifically, levels of NFL did not differ significantly between controls and mismatches in the age-matched analysis (figure 1A), with both groups showing reduced levels compared to AD-Dementia (all $p < 0.001$). In the age-controlled analysis (figure 2A), mismatches showed intermediate levels of NFL that were significantly higher compared to controls but significantly lower compared to AD-Dementia ($p < 0.001$).

Neurogranin results

Neurogranin was consistently elevated in AD-Dementia compared to controls across both analyses (all $p < 0.001$). In the age-

Table 1 Characteristics of age-matched participants

Sample characteristics	Controls	AD-Dementia	Mismatches	Total
No.	25	40	14	79
Age, y	76.0 (6.5)	78.3 (6.0)	75.8 (4.1)	77.1 (5.3)
Female, %	64	35	71	51
APOE ε4, % positive	12	68	71	51
AD parental history, % positive	40	45	71	48
Education, y	17.0 (2.6)	15.3 (2.9)	16.3 (2.7)	16.0 (2.8)
p-Tau/Aβ ₄₂	0.05 (0.01)	0.15 (0.07)	0.13 (0.06)	0.12 (0.08)
Aβ ₄₂ /Aβ ₄₀	0.11 (0.01)	0.06 (0.01)	0.06 (0.01)	0.08 (0.03)
NFL, pg/mL	879 (279)	1,844 (816)	951 (284)	1,380 (771)
Neurogranin, pg/mL	312 (140)	567 (352)	384 (134)	454 (291)
YKL-40, ng/mL	192 (67)	264 (73)	207 (63)	233 (75)
t-Tau, pg/mL	337 (106)	819 (324)	543 (191)	617 (330)

Abbreviations: Aβ = β-amyloid; AD = Alzheimer; p-tau = phosphorylated tau; t-tau = total tau; YKL-40 = chitinase-3-like protein 1. Values are mean (SD) except when otherwise indicated.

matched analysis (figure 1B), mismatches showed qualitatively intermediate values compared to controls and AD-Dementia that did not reach significance. However, in the age-controlled analysis (figure 2B), mismatches showed significantly elevated neurogranin compared to controls ($p < 0.001$), and mismatches were statistically indistinguishable from AD-Dementia.

YKL-40 results

In the age-matched analysis (figure 1C), the AD-Dementia group exhibited higher levels of YKL-40 compared to controls

($p < 0.05$), but there was no statistical difference between mismatches and either the control or AD-Dementia group. In the age-controlled analysis (figure 2C), the AD-Dementia group exhibited higher levels of YKL-40 compared to both the control and mismatch groups ($p < 0.001$), who did not differ from each other.

t-Tau results

In the age-matched analysis (figure 1D), the AD-Dementia group exhibited higher levels of t-tau compared to controls

Table 2 Characteristics of age-controlled participants

Sample characteristics	Controls	AD-Dementia	Mismatches	Total
No.	291	61	59	411
Age, y	59.9 (7.6)	73.2 (8.9)	65.4 (7.5)	62.7 (9.1)
Female, %	69	38	73	65
APOE ε4, % positive	34	72	56	43
AD parental history, % positive	73	59	76	71
Education, y	16.2 (2.5)	15.3 (2.8)	16.0 (2.6)	16.1 (2.6)
p-Tau/Aβ ₄₂	0.05 (0.01)	0.18 (0.08)	0.16 (0.06)	0.09 (0.07)
Aβ ₄₂ /Aβ ₄₀	0.10 (0.02)	0.06 (0.01)	0.06 (0.01)	0.09 (0.02)
NFL, pg/mL	579 (296)	1,412 (799)	814 (307)	790 (540)
Neurogranin, pg/mL	309 (159)	576 (323)	441 (144)	367 (214)
YKL-40, ng/mL	151 (51)	196 (77)	159 (53)	159 (65)
t-Tau, pg/mL	289 (108)	725 (315)	448 (160)	376 (241)

Abbreviations: Aβ = β-amyloid; AD = Alzheimer; p-tau = phosphorylated tau; t-tau = total tau; YKL-40 = chitinase-3-like protein 1.

Figure 1 Biomarker plots for age-matched analysis

Each black dot represents 1 CSF sample from 1 individual. Bottom of the boxplot represents the 25th percentile; top represents the 75th percentile (thereby comprising the interquartile range [IQR]); and middle line represents the median. Whiskers extend 1.5 × IQR above the third quartile and 1.5 × IQR below the first quartile. White diamond within the box represents the mean. (A) The group with Alzheimer disease dementia (AD-Dementia) showed significantly higher neurofilament light protein (NFL) compared to controls and mismatches, who did not differ from each other. (B) AD-Dementia group exhibited higher levels of neurogranin compared to the control group, and the mismatch group did not differ from either the control or AD-Dementia group. (C) AD-Dementia group exhibited higher levels of chitinase-3-like protein 1 (YKL-40) compared to controls, and mismatches did not differ from either controls or AD-Dementia. (D) AD-Dementia group exhibited higher levels of total tau compared to both the control and mismatch groups, who did not differ from each other. n.s. = no significant difference. ** $p < 0.001$; * $p < 0.05$.

($p < 0.001$) and mismatches ($p < 0.05$), who did not differ from each other. In the age-controlled analysis (figure 2D), mismatches showed intermediate levels of t-tau that were significantly higher than those of controls but significantly lower than those of AD-Dementia ($p < 0.001$).

Discussion

Some individuals harbor substantial AD neuropathology in the absence of dementia. This raises the question of whether additional biological factors are informative in staging the disease course. Examining cognitively unimpaired individuals with AD-like levels of amyloid and p-tau (mismatches), our primary analysis revealed that these individuals had significantly lower NFL and t-tau

compared to patients in the AD-Dementia group. While not significantly lower than AD patients on YKL-40 and neurogranin, mismatches did not differ significantly from controls either, suggesting qualitatively intermediate levels of these biomarkers.

NFL, which is expressed principally in large-caliber myelinated axons,¹⁵ is an important component of the neuronal cytoskeleton.^{16,17} Studies indicate that increased NFL predicts reduced cognitive performance on the Mini-Mental State Examination in patients with AD,¹⁸ in line with the facts that these large-caliber myelinated axons are found primarily in temporal and frontal lobes¹⁹ and that NFL is a marker of disease progression.¹⁸ NFL may be a particularly promising biomarker in conjunction with amyloid and tau, given that it

Figure 2 Biomarker plots for age-controlled analysis

Each black dot represents 1 CSF sample from 1 individual. Bottom of the boxplot represents the 25th percentile; top represents the 75th percentile (thereby comprising the interquartile range [IQR]); and middle line represents the median. Whiskers extend $1.5 \times$ IQR above the third quartile and $1.5 \times$ IQR below the first quartile. The white diamond within the box represents the mean. (A) All groups differed on levels of neurofilament light protein (NFL), with the mismatch group exhibiting intermediate levels compared to the Alzheimer disease dementia group (AD-Dementia) and control group. (B) AD-Dementia and mismatch groups did not differ from each other on levels of neurogranin, but both groups had higher levels compared to controls. (C) AD-Dementia group exhibited higher levels of chitinase-3-like protein 1 (YKL-40) compared to both controls and mismatches, who did not differ from each other. (D) All groups differed on levels of total tau, with the mismatch group exhibiting intermediate levels compared to the AD-Dementia and control groups. n.s. = no significant difference. ** $p < 0.001$; * $p < 0.05$.

can be measured in plasma.²⁰ The results presented in this study underscore the fact that loss of axonal integrity, particularly large-caliber, myelinated axons, plays a role in the development of dementia due to AD.

Neurogranin was elevated in AD-Dementia compared to controls, and mismatches exhibited qualitatively intermediate values between the AD-Dementia and control groups in the age-matched analysis. In the larger, age-controlled analysis, the difference between mismatches and controls reached statistical significance, with mismatches showing levels similar to AD-Dementia. Neurogranin is a protein expressed in dendritic spines²¹ and regulates levels of calmodulin after action potentials and calcium influx.²² Expressed highest in

associative cortical regions,²³ it is implicated in plasticity, synaptic regeneration, long-term potential, and learning and memory.²⁴ Synaptic dysfunction is thought to underlie the progression to dementia in AD and may in fact be a harbinger of neuronal loss.²⁵ Indeed, concentration of synapses in the cortex is reduced by up to 30% even in early stages of AD.²⁶ Neurogranin is reduced in postmortem brain tissue of patients with AD compared to age-matched controls,²⁷ supporting the idea that it plays a role in cognitive decline. Indeed, the fact that the mismatch and AD-Dementia groups had similar levels of neurogranin in both analyses presented here suggests that mismatches exhibit significant synaptic degeneration despite remaining cognitively unimpaired and therefore that cognitive impairment may be imminent.

Postmortem studies also implicate glial activation as a defining feature of dementia due to AD.^{1,28} YKL-40 measured in CSF differs between cases with AD and controls²⁹ and may be a marker of disease progression.^{30,31} The fact that higher levels of YKL-40 were observed in the AD-Dementia group while the mismatch group had qualitatively intermediate (in the age-matched analysis) or normal (in the age-controlled analysis) levels suggests that gliosis in concert with AD neuropathology may be necessary for the development of dementia due to AD.

Several hypotheses have been put forward to explain why some individuals with significant AD neuropathology remain cognitively unimpaired. Lifestyle factors and cognitive strategies may contribute to cognitive reserve, allowing individuals to function despite AD neuropathology.³² A related concept is that of brain reserve, in which structural or functional features of the brain confer resilience to AD dementia.³³ It is also possible that mismatches have some genetically conferred immune advantage or environmental influence on immune function that contributes to resilience.^{34,35} Ultimately, it is still unclear why some individuals are more resilient to the damaging effects of AD neuropathology, but elucidating these differences will provide insight into the biological processes underlying resilience and dementia.

Holistically speaking, the AD-Dementia, control, and mismatch groups differ on 1 important characteristic: education. The AD-Dementia group was less educated compared to controls at the trend level in the age-matched analysis and significantly so in the age-controlled analysis. Previous research suggests that education is associated with reduced prevalence of AD-dementia.^{36,37} However, crucially, mismatches and controls did not differ on years of education in either analysis presented here. Therefore, mismatches may be resilient in the face of AD neuropathology compared to the AD-Dementia group as a result of cognitive or brain reserve conferred by education or other associated protective factors. One caveat is that the sample overall was highly educated, as well as racially and ethnically homogeneous. Therefore, these results may not be applicable to the general population. More research is needed in diverse populations, and improved metrics of education quality—beyond simply years of schooling completed—are needed.³⁸

We use the term resilient with caution when describing the mismatch group. Cross-sectional data prevent us from concluding that the mismatch group will remain resilient to dementia as AD neuropathology accumulates. Indeed, the fact that mismatches exhibited intermediate levels of several biomarkers could suggest a progressively worsening disease state. At the same time, mismatches may have had an abnormal AD biomarker profile (p-tau/A β ₄₂) for a shorter length of time than the AD-Dementia group. Still, the results presented here indicate that, at the time of assessment and at ages comparable to the dementia group, mismatches appear resilient to cognitive decline despite comparably high levels of AD neuropathology.

Longitudinal evaluation is needed for mapping biomarker and cognitive trajectories, as well as for determining the duration of time that mismatches remain resilient.

Although results are generally consistent between the age-matched analysis and the age-controlled analysis, it is important to note small differences in the patterns of results generated from the 2 analyses. For example, mismatches had levels of neurogranin that were qualitatively intermediate between the control and AD-Dementia groups in the age-matched analysis but were similar to AD-Dementia and higher than controls in the age-controlled analysis. This discrepancy is likely attributable to differences in sample size and thus statistical power in the 2 analyses. While the age-matched analysis further removes any unaccounted-for bias between groups of different ages, the age-controlled analysis increases statistical power to detect differences between groups. Of course, an age-matched analysis with a larger set of participants would be ideal, and longitudinal studies should be the goal of future work.

While longitudinal data will prove invaluable for confirming these findings, this research suggests that larger panels of biomarkers will be useful for differentiating between groups with and without dementia that harbor similar levels of AD neuropathology. Mechanistically, this research adds support to the hypothesis that amyloid and tau are necessary but not sufficient for the onset of frank dementia.

Author contributions

Andrew P. Merluzzi: study concept/design, analysis of data, statistical analysis, manuscript author. Cynthia M. Carlsson: study concept/design, data acquisition, revision of manuscript. Sterling C. Johnson: study concept/design, study supervision, revision of manuscript. Suzanne E. Schindler: study concept/design, interpretation of data, revision of manuscript. Sanjay Asthana: study concept/design, study supervision, revision of manuscript. Kaj Blennow and Henrik Zetterberg: study concept/design, data acquisition, revision of manuscript. Barbara B. Bendlin: study concept/design, study supervision, interpretation of data, revision of manuscript.

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