Early Selective Vulnerability of the CA2 Hippocampal Subfield in Primary Age-Related Tauopathy

Jamie M. Walker, MD, PhD, Timothy E. Richardson, DO, PhD, Kurt Farrell, PhD, Megan A. Iida, BS, Chan Foong, MS, Ping Shang, HT(ASCP), Johannes Attems, MD, Gai Ayalon, PhD, Thomas G. Beach, MD, PhD, Eileen H. Bigio, MD, Andrew Budson, MD, Nigel J. Cairns, PhD, Maria Corrada, ScD, Etty Cortes, MD, Dennis W. Dickson, MD, Peter Fischer, MD, Margaret E. Flanagan, MD, Erin Franklin, MS, Marla Gearing, PhD, Jonathan Glass, MD, Lawrence A. Hansen, MD, Valhram Haroutunian, PhD, Patrick R. Hof, MD, Lawrence Honig, MD, PhD, Claudia Kasaw, MD, C. Dirk Keene, MD, PhD, Julia Kofler, MD, Gabor G. Kovacs, MD, PhD, Edward B. Lee, MD, PhD, Mirjam I. Lutz, MSc, Qinwen Mao, MD, PhD, Eliezer Masliah, MD, Ann C. McKee, MD, Corey T. McMillan, PhD, M. Marsel Mesulam, MD, Melissa Murray, PhD, Peter T. Nelson, MD, PhD, Richard Perrin, MD, PhD, Thao Pham, BS, Wayne Poon, PhD, Dushyant P. Purohit, MD, Robert A. Rissman, PhD, Kenji Sakai, MD, Mary Sano, PhD, Julie A. Schneider, MD, Thor D. Stein, MD, PhD, Andrew F. Teich, MD, PhD, John Q. Trojanowski, MD, PhD, Juan C. Troncoso, MD, Jean-Paul Vonsattel, MD, Sandra Weinfraub, PhD, David A. Wolk, MD, Randall L. Woltjer, MD, PhD, Masahito Yamada, MD, PhD, Lei Yu, PhD, Charles L. White III, MD, and John F. Crary, MD, PhD
Abstract

Primary age-related tauopathy (PART) is a neurodegenerative entity defined as Alzheimer-type neurofibrillary degeneration primarily affecting the medial temporal lobe with minimal to absent amyloid-β (Aβ) plaque deposition. The extent to which PART can be differentiated pathoanatomically from Alzheimer disease (AD) is unclear. Here, we examined the regional distribution of tau pathology in a large cohort of postmortem brains (n = 914). We found an early vulnerability of the CA2 subregion of the hippocampus to neurofibrillary degeneration in PART, and semiquantitative assessment of neurofibrillary degeneration in CA2 was significantly greater than in CA1 in PART. In contrast, subjects harboring intermediate-to-high AD neuropathologic change (ADNC) displayed relative sparing of CA2 until later stages of their disease course. In addition, the CA2/CA1 ratio of neurofibrillary degeneration in PART was significantly higher than in subjects with intermediate-to-high ADNC burden. Furthermore, the distribution of tau pathology in PART diverges from the Braak NFT staging system and Braak stage does not correlate with cognitive function in PART as it does in individuals with intermediate-to-high ADNC. These findings highlight the need for a better understanding of the contribution of PART to cognitive impairment and how neurofibrillary degeneration interacts with Aβ pathology in AD and PART.

Key Words: Alzheimer disease, CA2, Cognitive status, Cornu ammonis, Hippocampal subfields, Neurodegenerative disease, Primary age-related tauopathy.

INTRODUCTION

Primary age-related tauopathy (PART), previously termed tangle-only dementia, tangle-predominant senile dementia, or regarded as age-related neurofibrillary degeneration (1–3), is defined as the presence of Alzheimer-type neurofibrillary pathology in the medial temporal lobe and other structures in the absence of significant amyloid-β (Aβ) plaque deposition. In contrast and by definition, a diagnosis of Alzheimer disease (AD) neuropathologic change (ADNC) requires the presence of neurofibrillary degeneration alongside neuritic and diffuse amyloid-β (Aβ) plaques (4, 5). The Braak staging system for neurofibrillary degeneration begins with pretangles and neurofibrillary tangles (NFTs) in the locus coeruleus (termed a–c), followed by the transentorhinal and entorhinal cortex at Braak stages I–II and the hippocampal CA1 subregion at Braak stage III–IV, with neocortical neurofibrillary degeneration in Braak stages V–VI (6–9). As compared with AD (10), neurofibrillary degeneration in PART generally has a more limited distribution, with Braak stages typically ranging from I to IV. The diffuse and neuritic plaque burden ranges from Thal phase 0 to 2 and CERAD neuritic plaque score of none to sparse, but exact cutoffs are challenging to delineate (11). Many individuals above the age of 20 display some degree of tau pathology in the brainstem, principally the locus coeruleus, and almost all individuals above the age of 50 display, at minimum, neurofibrillary degeneration in the entorhinal and transentorhinal regions. However, it has also been demonstrated that over 80% of individuals in the 50–60-year age range, and at least 20% of people over the age of 80, have no amyloid plaque deposition (12). Thus, the term PART was created to describe these subjects who display NFTs without Aβ plaques. Whether older individuals with PART will develop amyloid pathology is difficult to ascertain, but Aβ plaque levels plateau in very old age (13). That is to say, despite current debate (14–16), a compelling argument can be made supporting the hypothesis that PART is a distinct neuropathologic entity, and not merely a stage along a pathologic continuum eventually leading to ADNC (4). Distinguishing neuropathological features would be helpful for further advancing our understanding of this issue.

Numerous studies have addressed the regional vulnerability of the hippocampal cornu ammonis (CA) subregions in various neurodegenerative diseases, ischemia, and epilepsy. Neurofibrillary degeneration in AD occurs earliest and is most severe in the entorhinal cortex and CA1/subiculum (9, 17–19); the CA2 subregion of the hippocampus is thought to be spared until Braak stage V in AD (6, 12). Pick disease affects the entorhinal cortex, dentate gyrus, and CA1 most severely (20), while hippocampal neurofibrillary degeneration in chronic traumatic encephalopathy (CTE) is often most severe in the CA2 and CA4 subregions (21). In addition, 4R-tauopathies, such as progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and argyrophilic grain disease (AGD), have a predilection for CA2 (19, 22). Other neurologic disorders affecting the hippocampus similarly have stereotypically affected regions: α-synuclein pathology in Lewy body disease (LBD) tends to occur earliest in the CA2 and CA3 subregions (23, 24), while neuron loss and gliosis associated with ischemia, epilepsy, and TDP-43 pathologies tend to affect select regions of the CA1 subfield (25–29).

As the next step toward understanding the neuropathologic signature of PART, we studied the hippocampal formation from 914 individuals derived from 21 different neurodegenerative disease brain banks and assessed the distribution of p-tau and Aβ pathology. Here, we characterize the neuropathologic and clinicopathologic differences that we observed in PART as compared with ADNC focusing on the hippocampus proper. Delineating these differences will further our understanding of the mechanisms by which individuals with PART differ from those with amyloid plaque deposition,
from all analyses. Clinical cognitive data, such as Clinical De-

Material and methods

Overview of Demographic, Clinical, and Pathologic Features

<table>
<thead>
<tr>
<th>Braak Stage</th>
<th>CERAD NP Score</th>
<th>Thal Phase</th>
<th>Diagnosis</th>
<th>Age Range, Years (Average)</th>
<th>Cognitive Status*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Sparse</td>
<td>0</td>
<td>1-2</td>
<td>3+</td>
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<tr>
<td>0-I</td>
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<td>151</td>
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<td>II</td>
<td>102</td>
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<td>3</td>
<td>62</td>
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<td>III</td>
<td>295</td>
<td>287</td>
<td>8</td>
<td>167</td>
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<td>17</td>
<td>9</td>
<td>8</td>
<td>3</td>
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</tbody>
</table>

*Clinical data not available for all cases.

and how the presence of plaques may promote a more wide-

MATERIALS AND METHODS

Ethics Approval and Consent to Participate

This study was conducted in accordance with the declara-

Neuropathological Analyses

Comprehensive histopathological workups were per-

Histopathological Assessments and Semiquantitative Analysis

All cases were analyzed using Aperio ImageScope (ver-

104
neocortex only; Thal 2 = any parenchymal β-amyloid plaques in the frontal neocortex as well as the transentorhinal/entorhinal cortex or CA1 subregion of the hippocampus; Thal 3 = any parenchymal β-amyloid plaques in the frontal neocortex, transentorhinal/entorhinal or CA1 subregion, and the molecular layer of the dentate gyrus; and Thal 4 = any parenchymal β-amyloid plaques in the frontal neocortex, transentorhinal/entorhinal or CA1 subregion, and CA4 subregion of the hippocampus, as previously reported (32). CERAD neuritic plaque scores were estimated based on the density of neuritic plaques on the Hirano silver stain in the frontal neocortex, as previously reported (31). Cases with a Thal phase of 0 and CERAD neuritic plaque score of none were defined as definite PART, while cases with a Thal phase of 1–2 (or CERAD neuritic plaque score of sparse) were defined as possible PART as per published consensus criteria (11). Furthermore, cases that qualified as intermediate or high ADNC based on the NIA-AA Reagan criteria (5) were defined as such. From the 914 cases in the cohort, 486 cases were designated as definite PART, 329 cases were designated as possible PART, 28 cases displayed minimal neuropathologic changes, 6 were neither PART nor ADNC (for example, PSP and GGT), and 65 were designated as ADNC or were on the AD continuum (for example, cases with Braak stage I or II, but Thal phase of ≥3 were placed in the ADNC category). Supplementary Data Figure S1 demonstrates an overview of the diagnostic categories and distribution of the 1013 cases that were received for analysis.

Hyperphosphorylated tau (p-tau, AT8) IHC stains of the hippocampal and frontal sections were evaluated for each case to reassess p-tau burden and Braak stage (Supplementary Data Fig. S2) (6). A minority of cases with NFTs in the frontal neocortical sections were given a Braak score of at least V, as the primary visual and auditory cortex were unavailable for review. Neurofibrillary degeneration in all hippocampal subregions (CA1–CA4) (Supplementary Data Fig. S3) (26, 33), the entorhinal cortex, and dentate gyrus were given semiquantitative scores of severe = 3, moderate = 2, mild = 1, rare to sparse = 0.5, or none = 0 (Supplementary Data Fig. S4).

**Statistical Analysis**

A Student t-test (unpaired) was utilized to assess statistical significance in the difference of p-tau burden in the CA1 and CA2 subregions of the hippocampus. Differences in the p-tau burden present in all hippocampal subregions were calculated using analysis of variance (ANOVA). ANOVA and Student t-tests were utilized for statistical analyses of the relationship between Thal phase, neuritic plaque score and CA2/CA1 ratio. Clinical correlations with Braak stage were calculated using linear regression. All statistical analyses were calculated using GraphPad Prism version 8.4 (GraphPad, La Jolla, CA).

**RESULTS**

**Hippocampal Subregion Distribution of Neurofibrillary Degeneration in PART**

Semiquantitative analysis of the AT8-immunostained hippocampi revealed that neurofibrillary degeneration in PART was significantly greater in the CA2 subregion of the hippocampus than in CA1 (p = 0.0001, Fig. 1A), unlike ADNC in which no significant difference between CA1 and CA2 subregions (p = 0.3854, Fig. 1B) was observed. We also found that the CA2/CA1 ratios of neurofibrillary degeneration in the PART cases were significantly higher than in the ADNC cases (p = 0.0118, Figs. 1C, 2, and 3). This subregion distribution trend was previously presented as preliminary data (34) prior to analyzing the full cohort. Subsequently, other studies reported similar neuropathologic changes in smaller independent PART cohorts (35, 36).
Many 4R-tauopathies, such as CBD, PSP, and AGD, similarly display a selective vulnerability of the CA2 subregion of the hippocampus for neurofibrillary degeneration (22). It is known that PART harbors 3R/4R+ Alzheimer-type NFTs (11). However, the predilection for CA2 raised the question of whether the tangles present in CA2 in PART are the usual 3R/4R+ Alzheimer-type NFTs or solely 4R-tau-immunopositive tangles. With 3R-tau and 4R-tau immunostains, we confirmed that PART CA2 NFTs are both 3R+ and 4R+ (Fig. 4). Thus, they represent 3R/4R+ Alzheimer-type NFTs in a non-Alzheimer-type distribution. This also demonstrated that the PART CA2 tangles are distinct from the 4R+ pretangles that are observed in AGD.

Analysis of Entorhinal Cortex and All Subregions of the Hippocampus in PART and ADNC

Semiquantitative scoring of neurofibrillary degeneration was performed on the entorhinal cortex, subiculum/CA1, CA2, CA3, CA4, and dentate gyrus. There were significant differences identified between all analyzed subregions in definite and possible PART groups (Fig. 5A, B), however, the only difference found in the ADNC cases was between the entorhinal/CA1/CA2 and CA3/CA4/dentate regions (Fig. 5C). Significant differences were found in every subregion between our ADNC cases and the possible and definite PART groups, with the exception of CA2, in which no significant difference was observed when comparing any group (Fig. 5D). That is to say, all subregions of ADNC cases have higher scores than PART, except the CA2 subregion. The mean CA2 score in ADNC cases is not significantly different from the mean CA2 score in PART, although the trend appears to be higher in ADNC cases. This is most likely because the overall average Braak stage for the ADNC cases is higher than that of the PART cases (Supplementary Data Fig. S2). CA2 neurofibrillary degeneration is present in PART as an early finding, without corresponding elevation of NFT pathology in the entorhinal or CA1 subregions, as is observed in ADNC. In fact, 73 PART cases displayed absent or sparse neurofibrillary degeneration in CA2 and the entorhinal regions.
degeneration in the entorhinal cortex with a corresponding CA2 score of \( \geq 1 \), whereas only 1 ADNC case displayed this pattern.

**Relationship of Thal Phase and CA2/CA1 Ratio**

For this analysis, cases were separated into subgroups based on Thal phase. Significant differences were identified in the CA2/CA1 ratio in all subgroups with decreasing CA2/CA1 ratio as Thal phase increases (Fig. 6A). In the Thal phase 3 subgroup \((n = 30)\), there were 5 cases without neuritic plaques in the hippocampus or frontal neocortex. Four out of 5 cases were clinically categorized as “normal.” The other case was a 101-year-old individual given a clinical diagnosis of AD. On our examination, this individual had Braak stage IV with severe aging-related tau astrogliopathy (ARTAG). The average CA2/CA1 ratio in the Thal phase 3 cases without neuritic plaques was 2.13, whereas the average CA2/CA1 ratio in the Thal phase 3 cases with neuritic plaques was 0.95 \((p = 0.001\), Fig. 6B). Cognitive status did not correlate in these subgroups. However, one would expect that cases without neuritic plaques and with higher CA2/CA1 ratio would have significantly better cognition. Thus, Thal phase 3 cases lie on a border where they could represent AD-related or PART pathology with significant CA2 neurofibrillary degeneration and a higher CA2/CA1 ratio. In addition, the Thal phase 3 cases with the high CA2/CA1 ratio were less cognitively impaired based on clinical diagnoses.

**Clinicopathologic Correlations of NFD in PART and ADNC**

While Braak stage had a significant positive correlation with cognitive function in cases with ADNC in terms of CDR
Braak stage did not correlate with CDR in PART ($r = 0.10; p = 0.1249$) (Fig. 7A). In addition, there was a significant inverse correlation between Braak stage and MMSE scores in ADNC cases ($r = -0.53; p = 0.0224$), whereas there was no correlation between Braak stage and MMSE in PART ($r = -0.01; p = 0.8441$) (Fig. 7B).

**DISCUSSION**

In this report, we demonstrate significant differences in the hippocampal distribution of neurofibrillary degeneration in PART compared with AD. The distribution of NFTs in PART does not follow the typical Braak staging system. Instead, NFTs demonstrate a predilection to affect the CA2 subregion of the hippocampus before the entorhinal cortex and the CA1 subregion in PART (Figs. 2 and 3) and demonstrates a significantly higher CA2/CA1 ratio of neurofibrillary degeneration compared with ADNC (Fig. 1C). This selective vulnerability of CA2 is similar to 4R-tauopathies, however the NFTs in PART are 3R/4R+ Alzheimer-type NFTs (Fig. 4). It is possible that the lack of abundant plaques may drive this altered distribution of neurofibrillary degeneration (4). Consistent with this hypothesis, there was a trend in this cohort for the CA2/CA1 ratio to increase with lower Thal phase (Fig. 6). It would be interesting to expand this analysis with more ADNC cases, to more fully understand the spectrum of...
amyloid-associated tau changes. We also think that individuals with intermediate amyloid pathology (i.e. Thal phase 3) could represent an intermediate state that may display ADNC or PART-like distribution (with a predilection for CA2), and individuals with PART-like pathology may have a significantly milder disease.

CA2 NFTs are also observed in CTE, another mixed 3R/4R tauopathy (21). In CTE, this change is often found in combination with CA4 neurofibrillary degeneration, and these cases often have a low amyloid burden. These cases with CA2/CA4 neurofibrillary degeneration may represent CTE superimposed on PART; however, CTE could be an entirely separate entity that displays a predilection for CA2 and CA4 on its own given the young age of many reported participants with CTE and hippocampal tau pathology (29). It is also known that LBD involves selective vulnerability of the CA2-3 subregions of the hippocampus for \( \alpha \)-synuclein-immunoreactive Lewy bodies and Lewy neurites (23, 24). However, the amount of CA1 pathology in cases of LBD correlates better with cognitive function than the amount of CA2 pathology (37, 38).

Previous studies comparing PART and AD have demonstrated less overall cognitive impairment and less steep rates of decline in PART patients, as well as a later onset of disease symptoms (39). In the current study, we found that unlike AD, there is no significant correlation between Braak stage and cognitive function in PART (Fig. 7). The reasons for this are unclear; however, this is consistent with PART being a more benign aging phenomenon. It is also possible that there are more resilient individuals within the PART cohort. An
alternative hypothesis is that the cognitive tests currently used do not adequately assess the functionality of the regions most severely involved in PART, such as CA2. Studies have demonstrated that CA2 may be involved in social memory (40, 41). This has mostly been shown in animal models with recognition of conspecifics. Functional MRI (fMRI) activation of CA2, CA3, and dentate gyrus has been described in learning (encoding) names of new faces (42, 43). In addition, one study demonstrated that anterior CA2 is used for learning the names and posterior is used for recall (retrieval) (43). Through disorders, such as prosopagnosia, we know that the fusiform gyrus is involved in face recognition, but there could be a network including CA2 and fusiform gyrus.

Limitations of the current study include the paucity of cognitive data, and the imbalance of sample sizes between the PART and ADNC groups, however the clinical and histologic data derived from the smaller number of ADNC cases is consistent with previously published reports on AD. In addition, since the study was designed to include a large number of total cases, only frontal and hippocampal sections were reevaluated for each case, precluding complete reassessment of Braak staging as this would require tau-stained sections of additional brain regions.

In conclusion, PART displays a predilection for CA2 neurofibrillary degeneration, which may be an aging process that is distinct from AD and independent of amyloid pathology, whereas significant CA1 neurofibrillary degeneration in the presence of amyloid plaques may be the initial dementia causing pathologic change in AD. Of course, more often than not, there are comorbidities, such as cerebrovascular disease, limbic predominant age-related TDP-43 encephalopathy (LATE), and LBD that may affect cognitive function. However, when only taking neurofibrillary degeneration into account, it appears that the path toward CA2 may be more benign. In future studies, it would be of interest to investigate the presence of comorbidities in PART, such as AGD, ARTAG, CTE, LATE, LBD, and cerebrovascular disease, and how the presence of these affect cognition. In addition, it is imperative that we develop a better understanding of the role of CA2 and the effect of pathology in the CA2 subregion as they relate to cognition and prognosis.

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