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In aged human brains without detected neuritic amyloid plaques, the severity of NFT pathology, and APOE genotype, are each associated with non-neuritic A β plaques

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Abstract

Data from a large autopsy series were analyzed to address questions pertinent to primary age-related tauopathy (PART) and Alzheimer's disease (AD): what factors are associated with increased severity of neurofibrillary degeneration in brains that lack neuritic amyloid plaques?; is there an association between *Apolipoprotein E* (APOE) alleles and PART pathologic severity independent of A β deposits?; and, how do the stains used to detect AD-type lesions impact the experimental results? Neuropathologic data were evaluated from elderly research volunteers whose brain autopsies were performed at University of Kentucky Alzheimer's Disease Center (UK-ADC; N=145 subjects). All of the included subjects' brains lacked neuritic amyloid plaques according to the CERAD diagnostic criteria and the overall final MMSE score before death was 26.8 $\pm$ 4.6. The study incorporated evaluation of tissue with both silver histochemical stains and immunohistochemical stains to compare results; the immunohistochemical stains (A β and phospho-Tau) were scanned and quantified using digital pathologic methods. Immunohistochemical stains provided important advantages over histochemical stains due to sensitivity and detectability via digital methods. Even when AD-type pathology was in its presumed earliest phases, lacking detected neuritic amyloid plaques, neocortical parenchymal A β deposits were associated with increased medial temporal lobe neurofibrillary tangles (NFTs). The observation confirms the NIA-AA recommendation that even diffuse A β deposits signal AD pathobiologic mechanisms are occurring. Further, the data are most compatible with the

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hypothesis that the *APOE* $\epsilon 4$ allele exerts its effect via driving A β deposition, i.e. “upstream” AD-type mechanisms, rather than being associated directly with the development of tau NFTs or PART pathology.

Keywords

neuropathology; SNAP; hippocampus; Abeta; ScanScope; MAPT; aging; A-beta

Introduction

There is increasing appreciation of the complex nature of brain pathologies seen in aged humans. When evaluating brains that would previously have been designated “pure” Alzheimer’s disease (AD) based on pathologic features, neuropathologists are currently recognizing the presence of additional, often comorbid, pathologies [1, 2]. Primary age-related tauopathy (PART) is a relatively recently recognized pathologic phenomenon that is common in old age [3]. In brains with Definite PART pathology, amyloid plaques are not detected, but tau neurofibrillary tangles (NFTs) are observed (for a more textured description, see below). The NFTs of PART are not associated with underlying frontotemporal lobar degeneration (FTLD) or chronic traumatic encephalopathy [3]. The paper that presented PART diagnostic features [3] states that using A β immunohistochemistry to detect the amyloid plaques is preferable, but leaves open the possibility that “laboratories using the CERAD neuritic plaque density scores may classify subjects with neuritic plaque frequency of ‘None’ as ‘Definite’ [PART]”.

The presence of age-related tauopathy in the absence of substantial A β amyloid plaques has long been appreciated [4–6], but we are now far more aware of the pathology’s prevalence and public health impact. For example, hippocampal NFTs are practically universal in the 20–30% of centenarians whose brains lack detectable A β amyloid plaques [7, 8]. There is inter-individual variation in the severity of PART-type pathology [3], but the mechanism(s) responsible for this variation are mostly unknown. In large autopsy series, the distribution of PART pathology has been shown to be associated with antemortem cognitive impairment [3, 9], and PART also is implicated in the clinical conditions of subjective memory complaints [10], and Mild Cognitive Impairment [11]. There also is abundant evidence of PART (medial temporal lobe tauopathy lacking A β amyloidogenesis) in living individuals, according to biomarker studies [12, 13]. Overall, since its publication in 2014, more than 300 papers in peer-reviewed journals have cited the paper describing PART [3].

Although it has become a recognized disease entity, PART has generated some controversy among experts in the field, and divergent opinions have been expressed [14–16]. There remains much unknown about the disease itself, including how it can best be operationalized for research [17, 18], and how we should define the ‘border zones’ between AD and PART, in theory and in diagnostic practice. Consideration of these unresolved challenges may yield new insights that are fundamental to both PART and AD. Before addressing them, we acknowledge the lack of universally applied terminology to refer to “amyloid plaques” of AD. Here (as in our prior work; see Refs [1, 19, 20]), we make a distinction between

“neuritic” amyloid plaques, which include a dense amyloidogenic core and/or surrounding degenerating neurites that can be detected with silver stains, and “diffuse” amyloid plaques, that contain A β but lack the core and/or degenerating neurites. Terms such as “amyloid plaque” or “A β plaque” would encompass both of these entities.

A study of cases that lack neuritic amyloid plaques may thus provide a basis to address unresolved, and interrelated, questions pertaining to tau/NFT and A β /plaque pathologies. Two general questions are: what factors are associated with increased severity of neurofibrillary degeneration in brains that lack abundant A β ?; and, when evaluating hypotheses related to PART, how do the neuropathologist’s choices of stains used in detecting the brain lesions affect his/her conclusions? More specific topical questions are: 1. Is it acceptable to define PART according to negativity for “neuritic amyloid plaques” (which tend to be detected using a variety of different means including histochemical techniques), or is it necessary to rely on negativity for A β immunohistochemically, as commonly operationalized via Thal A β stages [21]?; 2. Does the burden of tau NFTs in medial temporal lobe structures, in the absence of any detected neuritic amyloid plaques, have any association with the amount of detected “diffuse” A β deposits?; and, 3. In the absence of detected neuritic amyloid plaques, is there any association between *APOE* genotype and the severity of PART pathology that is independent of detected A β deposits?

The goals of the present study were to address the abovementioned questions using analyses of data gathered from research volunteers followed to autopsy. Clinical and neuropathological data were drawn from the University of Kentucky Alzheimer’s Disease Center (UK-ADC) cohort. The results of these analyses indicated that even when AD-type pathology is in its presumed earliest stages, with topographically limited A β deposits but lacking detected neuritic plaques, the A β deposits were still associated with more severe medial lobe tau NFTs. The data are also compatible with the hypothesis that *APOE- ϵ 4* alleles drive A β deposition rather than directly being associated with the severity of NFT pathology in the medial temporal lobe.

Methods

Details of UK-ADC research volunteers’ recruitment, inclusion/exclusion criteria, and clinical and pathological assessments have been described previously [1, 22, 23]. Briefly, community-based older adult volunteers agreed to be followed annually for cognitive, physical, and neurological examination and to donate their brain at the time of death. Protocols were approved by the UK Institutional Review Board, and all participants provided written informed consent. Included subjects were $\geq$ 70 years of age at death and had no detected neuritic plaques (NPs) – i.e., the CERAD score [24] was “none” – and Braak NFT stages [7] of 0 to IV. Research subjects with relatively rare dementia syndromes (e.g. prions, trinucleotide repeat diseases, or FTL), or any brain tumor were excluded. Additionally, since our research questions focused on the association between *APOE* and tau pathology, cases also had to have *APOE* genotyping available.

Cases meeting inclusion and exclusion criteria formed two groups based on the year of autopsy, which corresponded to distinct neuropathological assessment methods used for

evaluation of A β pathology. The first group comprised cases autopsied between 1997–2011 (n=98), where Bielschowsky silver stains were performed to detect NPs and diffuse plaques (DPs), and Gallyas silver stains [25] were used to detect NFTs in medial temporal lobe structures, in accordance with the 1997 NIA-Reagan Institute consensus-based criteria for AD diagnosis [26]. The second group comprised cases autopsied between 2012–2017 (n=51), during which time immunohistochemical (IHC) stains were performed to detect A β deposits and phospho-Tau antibody used to detect NPs and NFTs (in accordance with the 2012 NIA-AA consensus-based criteria for diagnosis of AD neuropathologic changes [27]), with subsequent digital pathologic methods for lesion detection and counting (see below).

Pathological assessments and staining methods.

The following regions were sampled from the left hemisphere: middle frontal gyrus (Brodmann area [BA] 9), superior and middle temporal gyri (BAs 21 and 22), inferior parietal lobule (BAs 39 and 40), and occipital neocortex including the primary visual area (BAs 17 and 18), hippocampus at the level of the lateral geniculate nucleus, entorhinal cortex, and amygdala. The tissue was fixed in 10% formaldehyde and then processed in paraffin blocks. All specimens were cut at 8-micron thickness. The neocortical regions were used to assess Thal staging because the first location for A β deposition (Thal phases > 0) involve neocortex [21]. We did not limit to Thal A β stages <3 (“Possible PART” according to the consensus criteria [3]) because the basic goal was to explore the biological variation in this community-based sample.

1997–2011 cases: Silver stains, with visual detection and manual counting of DPs, NPs, and NFTs.

Methods of silver stains and manual counting at the UK-ADC have been previously described in detail [1, 28]. Briefly, sections were cut and stained using a modified Bielschowsky silver method and a Gallyas silver impregnation stain were performed without pretreatment [1, 25]. Amyloid plaques were counted using Bielschowsky silver stained sections of the neocortex, and the Gallyas-stained sections were used to count NFTs in the hippocampal formation (CA1 and subiculum) and entorhinal cortex. The most severely affected fields were determined by studying the whole section and marking those areas. Amyloid plaques were separated into DPs (plaques without silver-impregnated dark neurites) and NPs (cored plaques with dark, dystrophic-appearing neurites) using the Bielschowsky stain. An arithmetic mean was calculated from the count of the 5 most involved fields for DPs (number of DPs per 2.35 mm²), NPs (number of NPs per 2.35 mm²), and NFTs (number of NFTs per 0.586 mm²) for each region. Plaque counts were capped at 250 for each area representing an extremely high density of plaques.

2012–2017 cases: Immunohistochemical stains, with digital pathology for lesion detection and quantification.

In these cases, the lesions were detected using an Aperio/Leica ScanScope and quantified using the Genie pattern recognition software as previously described in detail [29]. For each case, A β IHC stains were scored using previously described methods, with pretreatment with 97% formic acid (Thermo) for 3 min followed by pepsin treatment (Thermo Fisher) for 10min [29]. Slides were then loaded into an Aperio ScanScope XTTM, scanned at 40 $\times$

magnification via the semi-automated method, and the images stored on a dedicated server. To enable a focused analysis that could be reproducible between users, a square analysis region was created (4 mm²) and placed within the gray matter at the site of highest concentration of pathology. Subsequent boxes (ten total boxes) were then placed as far from the existing boxes as possible without overlapping other analysis regions as described [29]. Once the analysis regions were selected, different quantitation algorithms were applied.

Beta-Amyloid detection and quantitation:

The monoclonal anti-A β (amino acid residues 1–11) NAB 228 [30] was used for A β IHC. Two different parameters were calculated for amyloid pathologies: an overall amyloid burden and an amyloid plaque density. For the *overall amyloid burden*, the Aperio Image Analysis ToolboxTM Positive Pixel Count (PPC), was used, with the following modifications to optimize for our in house staining protocol: hue value 0.1, hue width 0.5, color saturation threshold 0.24, intensity threshold weak (upper limit) 220, intensity threshold weak (lower limit) 118, intensity threshold moderate (upper limit) 118, intensity threshold moderate (lower limit) 70, intensity threshold strong (upper limit) 70, intensity threshold strong (lower limit) 0, and intensity threshold negative pixels –1. The modified PPC was then run on the ten 4mm² boxes selected on each slide as specified above. The amyloid burden was calculated by adding up all the ‘weak’ (1+), ‘moderate’ (2+), and ‘strong’ (3+) pixels from the data and dividing by the overall analysis area (40 mm²).

For the *amyloid plaque density*, the Aperio Image Analysis ToolboxTM Nuclear algorithm was used with the following modifications: curvature threshold 23.5, segmentation type 2, threshold type 1, lower intensity threshold 0, upper intensity threshold 230, minimum nuclear size (um²) 100, minimum nuclear size (pixels) 1638, minimum roundness 0.1, minimum compactness 0, minimum elongation 0.1, remove light objects 0, weak (1+) threshold 207, moderate (2+) threshold 188, strong (3+) threshold 162, and black threshold 0. This modified algorithm was then run on the same 10 boxes chosen for the amyloid burden analysis. The amyloid plaque density was calculated by adding up all the ‘weak’ (1+), ‘moderate’ (2+), and ‘strong’ (3+) positive nuclei and dividing by the overall analysis area (40 mm²).

Phospho-Tau detection and quantitation:

The PHF-1 antibody recognizes an epitope on phosphorylated tau at or near amino acids ser396 and ser404 [31, 32]. Two parameters were quantified for tau pathologies in selected regions of interest: NFT density and overall tau burden. For the NFT quantitation, a single Genie algorithm was first developed to separate NFTs from other tau immunoreactive structures. Methods for developing the NFT/NP Genie algorithm was described previously [29]. The *NFT density* was calculated via a modified nuclear algorithm with the NFT/NP Genie classifier limited to NFTs and the following additional modifications: curvature threshold 24, segmentation type 0, threshold type 1, lower intensity threshold 75, upper intensity threshold 230, minimum nuclear size (um²) 40, minimum nuclear size (pixels) 655, minimum roundness 0.1, minimum compactness 0, minimum elongation 0.1, remove light objects 0, weak (1+) threshold 222, moderate (2+) threshold 166, strong (3+) threshold 147, and black threshold 0. This modified algorithm was run on the ten 4 mm² boxes within the

gray matter on the PHF-1 stain, selected by the method stated above. The NFT density was calculated by dividing the total number of all the 'weak' (1+), 'moderate' (2+), and 'strong' (3+) nuclei counted by the overall analysis area (40 mm²).

The *overall tau burden* was determined using a PPC algorithm, similar to the amyloid burden quantitation, but with the following modifications: hue value 0.1, hue width 0.5, color saturation threshold 4.e-002, intensity threshold weak (upper limit) 132, intensity threshold weak (lower limit) 123, intensity threshold moderate (upper limit) 123, intensity threshold moderate (lower limit) 130, intensity threshold strong (upper limit) 130, intensity threshold strong (lower limit) 0, and intensity threshold negative pixels -1. The modified PPC was then run on the same 10 4mm² boxes selected for NFT density analyses. The tau burden was calculated by dividing the 'strong'(3+) pixels by the overall analysis area (40 mm²), and this parameter includes detection and quantification of neuropil threads and other tauopathic lesions including NFTs.

Analyses and statistical methods

Descriptive and exploratory data analyses were conducted using χ^2 tests and non-parametric tests. Non-parametric tests were used given the skewed distributions of the untransformed pathology variables. Mediation analysis was performed to test the hypothesis that *APOE* genotype is associated with tau pathology through A β deposition rather than a direct effect of *APOE* on tau. Three sets of analyses were completed using negative binomial regression. The negative binomial model, which uses a log link function for the outcome variable, was chosen to reflect that the measures of A β and tau represent count data, and the Poisson distribution requires a strict assumption that the mean and variance of the outcome are equal, which we did not find evidence to support. No offsets were used in the models given the ascertainment methods ensured all cases had the same amount of tissue sampled, and goodness of fit was assessed by the Pearson χ^2 /DF ratio. All analyses were adjusted for age at death and sex.

For the cases autopsied between 1997 and 2011, the dependent variable was mean NFTs in the medial temporal lobe, which included NFT counts from the hippocampal formation and entorhinal cortex. Study variables were *APOE-ε4* carrier status (1=yes, 0=no) and mean DPs in the neocortex, which included the middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, and occipital neocortex. To assess whether the association of *APOE* carrier status with tau pathology was mediated through amyloid deposition, we first fit the model with *APOE* but not DPs, and then a model with both *APOE* and DPs.

For the cases autopsied between 2012 and 2017, two dependent tau/tangle variables were analyzed: mean tau burden and mean NFT density in the medial temporal lobe measured with digital pathology, which included the same areas as above. The same modelling strategy was used to assess the association between *APOE* and medial temporal lobe tau pathology as above. For tau burden, neocortical amyloid burden was used as the independent amyloid variable. For NFT density, neocortical amyloid density was used as the independent amyloid variable. To convey how the pathology and numerical outputs correlate, we show data in Supplemental Figs. 1 and 2.

Sensitivity analyses were conducted to assess the robustness of the model results and to check for an independent association between cerebral amyloid angiopathy (CAA) and tau/tangle pathology since CAA is known to be associated with *APOE* genotype in this autopsy series [33]. In the first set of sensitivity analyses, we included measures of medial temporal amyloid pathology (the hippocampal formation and entorhinal cortex) in the models; these data were collected and coded in the same manner as for the neocortical regions. In the second set of sensitivity analyses we included a measure of neocortical CAA, which was assessed with A β IHC stains in each neocortical region, and graded on a scale from 0 (no CAA detected) to 3 (severe CAA). For these statistical models, CAA was operationalized as the mean of the ratings in parenchymal blood vessels for each of the 4 neocortical regions.

Results

Descriptive and exploratory results.

A perspective on the overall cohort and included cases, and the reasons for exclusions, are depicted in Fig. 1. Overall work flow is depicted in Fig. 2. Distributions of participant age at death, sex, education, final MMSE score, and Braak NFT stage were similar for the cases autopsied between 1997–2011 (n=95) and 2012–2017 (n=51), but the participants who were autopsied in the later period had more severe cognitive impairment based on final clinical diagnosis ($\chi^2=14.68$, 3 df, $p=0.0016$) (Table 1). Age range was 70–105. Note that Thal A β staging was performed on 43/51 (84%) of the cases and 25 (58%) were Thal A β stage <3, whereas 13 cases (30%) were Thal A β stage 3 and 5 (12%) were Thal A β stage 4. The observed proportion of cases who were *APOE- $\epsilon 4$* carriers was also higher in the latter group, but the difference was marginally significant ($\chi^2=3.74$, 1 df, $p=0.053$). Overall, only 18/145 cases were positive for *APOE- $\epsilon 4$* (12.4%).

The absence of DPs was strongly associated with the absence of the *APOE- $\epsilon 4$* allele, such that just two of 68 cases with no detected DPs carried the $\epsilon 4$ allele ($\chi^2=11.83$, 1 df, $p=0.0006$) (Table 2). These two cases were both from the 1997–2011 autopsy series. The IHC stains were provided increased sensitivity and specificity in comparison to the silver stains, as can be appreciated in Fig. 3 where silver stains and IHC were applied to the same case. Examples of findings in quantitative pathology are depicted in Supplemental Figs. 1–3, including an evaluation of a sample of cases (n=103) with a broader spectrum of pathologies, evaluated with both manually counted DPs and A β burden in the same temporal cortex blocks (Supplemental Fig. 1). To illustrate the differences between DPs and NPs using silver stains, Fig. 4 shows representative results in cases with and without NPs. Representative results of digitally quantified immunohistochemically detectable pathologies are shown in Fig. 5 (A β) and Fig. 6 (P-Tau). Showing the distribution of pathologies in the subsample of cases any detectable plaques, Fig. 7 depicts the severity of DP/A β pathologies in the frontal, temporal, parietal, and occipital neocortical regions. Among cases with detected amyloid plaques (n=77, Table 2), there was no evidence that *APOE* was associated with more severe Braak NFT stages ($\chi^2=4.84$, 4 df, $p=0.30$). We note that 38/51 cases (74.5%) from the 2012–2017 series had detected A β plaques (i.e., Thal A β phase > 0), while just 35/94 cases (37.2%) from the 1997–2011 series had detected DPs ($\chi^2=28.3$, 1 df,

$p < 0.0001$) (data not shown), again attesting to the increased sensitivity of IHC staining results.

The association between neocortical amyloid pathology and medial temporal tau pathology was markedly different based on the autopsy series (1997–2011 vs. 2012–2017) (Table 3). For cases evaluated using silver stains, the presence of DPs was not associated with higher burden of NFTs: the observed NFT burden trended lower in cases with any DPs vs. no DPs, but this difference was not significant (Wilcoxon rank-sum test, $p = 0.67$). On the other hand, there was an association between increasing $A\beta$ pathology and increasing tau/tangle pathology in the 2012–2017 cases. Although the pattern of results was similar for both tau burden and NFT density (Table 3), the association was not significant ($F = 2.03$, $p = 0.12$). For NFT density, ANOVA on ranks showed significantly increased NFTs with quantile of amyloid density ($F = 3.35$, $p = 0.027$).

Negative binomial results.—In the 1997–2011 cases, which were evaluated using silver stains, *APOE* carrier status was not significantly associated with mean medial temporal NFTs ($\beta = 0.85$, $p = 0.08$), but this trend was strengthened when mean neocortical DPs were added to the model ($\beta = 1.08$, $p = 0.053$). For the 2012–2017 cases, which were evaluated using IHC (Figs 5, 6), *APOE* carrier status was significantly associated with mean NFT density ($\beta = 0.65$, $p = 0.032$), but this association was attenuated when neocortical amyloid density was added to the model ($\beta = 0.30$, $p = 0.35$). Amyloid density was significantly associated with NFT density ($\beta = 0.011$, $p = 0.013$). Similarly, when tau burden was the outcome, without amyloid burden in the model, *APOE* status was significantly associated with tau burden ($\beta = 0.79$, $p = 0.017$). However, when amyloid burden was included in the model, *APOE* was no longer significantly associated with NFT burden ($\beta = 0.59$, $p = 0.08$) while amyloid burden was marginally significantly associated with tau burden ($\beta = 0.009$, $p = 0.057$).

Table 4 shows least squares mean estimates of tau pathology by *APOE* status based on the negative binomial models. As above, while *APOE-ε4* was associated with higher predicted mean tau pathology in all of the analyses, the differences were only statistically significant when amyloid pathology was not in the model. For the cases from 2012–2017, the inclusion of amyloid pathology clearly attenuated the association between *APOE* and tau. For NFT density, the inclusion of amyloid density resulted in a 29% reduction in the estimated mean density for *APOE-ε4* carriers (12.0 vs 16.8). For tau burden, the inclusion of amyloid burden resulted in a 17% reduction in the estimated mean burden for *APOE-ε4* carriers (44.8 vs. 54.3). These results are consistent with the hypothesis that $A\beta$ pathology at least partly mediates the impact of *APOE ε4* on early NFT pathology. Model results remained stable for all sensitivity analyses (data not shown), which did not support the hypothesis that effect(s) of *APOE ε4* on NFT pathology are mediated through CAA pathology. Point estimates changed little and the significance of model predictors was unchanged.

Discussion

Collectively, these findings (Fig. 8) indicate that even when AD-type pathology is in its presumed earliest phase(s), the “diffuse” $A\beta$ deposits are associated with increased medial

lobe tau NFTs. Moreover, the observed association between *APOE* $\epsilon 4$ alleles and tau pathology appears to be at least partly mediated via A β deposition. However, we did find that cases with *APOE* $\epsilon 4$ alleles tended to have higher levels of tau pathology, even after controlling for amyloid pathology, although the differences were not statistically significant. It is also noteworthy that almost 90% of the cases were not $\epsilon 4$ carriers, as in the general population. Our analytic results were modified by the staining method, such that cases with IHC staining revealed higher levels of amyloid pathology in the absence of neuritic plaques and analyses suggested that the effect of *APOE* $\epsilon 4$ on tau pathology was diminished after controlling for amyloid. Thus, our findings underscore the importance of using IHC-based staining of brain tissue to detect the pathologic biomarkers for research purposes, in order to provide the most sensitive and specific indication of their presence.

The current study has some limitations. While the UK-ADC autopsy cohort sample was drawn from a community-based group of research volunteers who mostly enrolled while cognitively intact [22], the neuropathologic findings are not representative of a population-based cohort in terms of ethnicity, comorbidities, or socioeconomic variables. We also limited our analyses to specific areas of the brain, and the observed relationships may or may not have implications about other brain regions. Further, the sample sizes may have been too small to provide adequate statistical power to test the hypothesis that *APOE* alleles and tauopathy independently are associated with each other. We also acknowledge technical challenges that pertain to digital pathologic methods, which we have discussed before [29, 34–36], but which still apply to efforts in this field: aspects of standardization, calibration, thresholding, segmentation, as well as how one masks or ignores sectioning “chatter” (see Fig. 3). There also may be artifacts inserted by the technical characteristics of the antibodies – for example, NAB228 may not recognize all subtypes of A β .

In a broader sense, autopsy data are intrinsically cross-sectional, and, some of the diagnostic and clinical practices have changed over time. For example, it wasn’t until mid-2013 that systematic Thal A β staging was performed on each case at the University of Kentucky AD Center. On the other hand, an enormous amount of neuropathologic data have been gathered across a wide spectrum of diseases, and the impact of the evolution of the staining techniques used to operationalize pathologic biomarkers was one of the focal points of the current study.

Despite the pertinent caveats, the results of the current study have implications about neurodegenerative disease biology and neuropathologic practices. We highlight four areas where our findings are directly relevant: the clinical-biological impact of “diffuse” A β deposits; the pathogenetic mechanism(s) of *APOE*; the “amyloid cascade hypothesis”; and, the neuropathologic diagnosis of PART.

It has long been appreciated that parenchymal plaques in AD can be differentiated into at least two subtypes: diffuse A β deposits (DPs) and neuritic (“senile”) amyloid plaques (NPs) [37–40]. The latter is distinguished by the presence of misshapen axons and dendrites which often contain tau paired helical filaments and/or phosphorylated neurofilament proteins [41]. Animal models of AD have addressed some of these issues [38, 42–44]. In terms of clinical-pathologic correlation, the presence and density of NPs are more strongly correlated with

cognitive impairment than is the case for DPs: the brains of many cognitively “normal” individuals harbor DPs [45–50]. However, when large autopsy series are evaluated that include sensitive antemortem cognitive tests and cases with both DP+ and DP- pathology, the presence of DPs is associated with (relatively subtle) cognitive manifestations [20, 45, 51]. The mechanism(s) of those cognitive changes, associated with DPs, is not well understood. While A β may exert a direct impact on cognition, the current study indicates that the A β deposited in neocortical DPs is attended by (perhaps causes) increased severity of medial temporal lobe tauopathy. Prior studies have been quite compatible with this hypothesis [45, 51].

Research on the neuropathologic sequelae associated with *APOE* polymorphisms is a related area of study. In terms of impact on public health, *APOE* is the strongest known genetic risk factor for late-onset AD, and a large number of mechanisms have been proposed to help explain how the *APOE* ϵ 4 allele contributes to the clinical and pathological phenotype [52–57]. Without extending our focus deeply into the molecular mechanisms, we note that there is extensive evidence that *APOE* alleles affect the rate of A β deposition and/or clearance [58]. In autopsy series, there has been a consistently observed strong correlation between *APOE* allele status and the amount of detected A β plaques and CAA pathology [33, 59–61]. However, there also has been a smaller set of published studies, including animal model experiments, interpreted to indicate that the *APOE* ϵ 4 allele may contribute directly to tauopathy [62–64]. There is not necessarily an “either/or” answer, and *APOE* may work through both as well as through completely different mechanisms [65]. However, the current study helps to illustrate an important consideration: even if *APOE* status is associated with tau pathology, it could be exerting its impact on A β , upstream of the tauopathy. This potent confounder is absolutely necessary to take into account, preferably using quantitative metrics of the pathology. Indeed, when quantitative A β pathology was factored into our analyses, there was no statistical evidence that *APOE* genotype had any correlative impact on the amount of detected tau pathology.

The analyses of quantitative neuropathology also led to results compatible with the “amyloid cascade hypothesis” [66] which posits a directional progression in which A β promotes accelerated tauopathy and not the reverse. As a rule, NFTs are seen before A β deposition in human brains [8], yet NFTs are not pathognomonic for AD: rather, NFTs are seen in many different diseases that lack A β , including tauopathy-inducing mutations in the *MAPT* gene which do not induce A β pathology (by contrast, *APP* mutations lead to tau pathology) [67–71]. In the present study, we once again do not find evidence that tauopathy “causes” A β deposition or foments the progression to full-blown AD. There is abundant evidence that the brains of 15–25% of centenarians -- at the apparent limit of our species’ chronological longevity -- harbor PART pathology but lack A β deposits [7, 8, 72, 73]. By contrast, the data in the current study are compatible with the hypothesis that, as soon as A β fibrils start to appear in the parenchyma, those A β deposits are associated with accelerated tauopathy.

Another implication of the current study relates to the stains that are used for making the neuropathologic diagnoses. In the paper that established the diagnostic criteria for PART, the following was written: “In keeping with the current guidelines for AD, mild A β plaques defined using the Thal grading system, consistent with low AD neuropathologic changes,

preclude the diagnosis of “Definite” PART...” [3]. However, it also was noted that “laboratories using the CERAD neuritic plaque density scores may classify subjects with neuritic plaque frequency of “None” as “Definite” [PART] and “Sparse” as “Possible” [PART]” [3]. We note that there is no consensus-based definition of a neuritic amyloid plaque, although it is arguably the lesion that comes closest to representing a pathognomonic feature of AD [74]. This is a problem that should be addressed, but the lack of consensus should not lead to stasis in the research field. We have observed that individual pathology labs prefer to visualize NPs using quite different methods: silver stains, thioflavin, or immunohistochemical stains. Presumably, even when there are substantial densities of A β and NPs, and/or additional comorbid pathologies, the biologic factors that affect PART severity may be still occurring (Fig. 8c), so, the concept of “Possible PART” has some merit. Yet the data and analyses presented in the current paper add support for the practice of relying on the presence or absence of A β deposits, rather than NPs, as a more specific criterion to distinguish between AD and PART.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Flowchart of cases included, and bases for exclusions.

The University of Kentucky AD Center (UK-ADC) autopsy cohort is community-based and most patients were recruited while cognitively intact. Only 11% of the study participants were demented at final examination (See Table 1).

Fig. 2. Workflow.

Schematic to depict the study design of the present study that included different UK-ADC cohorts and methods applied. Notably, different stains were used and the neuropathologic lesions were detected and counted separately. For the 1997–2012 cohort, histochemical stains were used with silver impregnation. Bielschowsky stains were used for diffuse amyloid plaques (DPs) and neuritic amyloid plaques (NPs), whereas the Gallyas silver impregnation technique was used for NFTs; for these cases, the output comprised manually tabulated density counts for DPs, NPs, and NFTs. For the 2012–2017 cohort,

immunohistochemical (IHC) stains were used -- A β and P-Tau IHC – and slides scanned using a digital slide scanner, followed by in silico object recognition, with output including A β burden, A β plaque density, P-Tau burden, and P-Tau NFT density.

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90-year old woman died with
“Intermediate severity”
Alzheimer’s disease
neuropathologic changes (A2 B2 C2).
Shown are H&E (Fig. 3a),
Bielchowsky (Fig. 3b),
A β IHC (Figs. 3c, 4), Gallyas (Fig 3d),
P-Tau IHC (Figs. 3e, 5) stains all from
the same case, depicting
hippocampus and nearby cortex.

Fig. 3. To illustrate characteristics of the different stains used, photomicrographs of stained sections from the hippocampus and nearby cortical structures are displayed.

These photomicrographs all depict brain sections from a woman who died at age 90 years with “Intermediate severity” Alzheimer’s disease neuropathologic changes (Thal A β stage 3, Braak NFT stage III, CERAD “moderate” density NPs). This case is only for illustrative purposes and was not included in the other analyses due to the presence of NPs. Adjacent sections from the same case were used for Figs. 3 and 4 also. On the hematoxylin and eosin (H&E)-stained section are indicated the structures in the tissue block corresponding to the hippocampal formation and adjacent structures: dentate granule cells (dg), CA1, subiculum

(Su), and peri- allocortical cortex (Co). From the Co region is shown immunohistochemical stains used to characterize amyloid plaques: Bielschowsky silver stain (b) and A β IHC (c). Note in the Bielschowsky stain that one can discriminate NPs (pale blue arrowhead) from DPs (purple arrowhead), whereas this discrimination is not possible in the A β IHC (c). Nonetheless, note that there is increased sensitivity and enhanced signal:noise ratio in the A β IHC stain compared to silver stains. For staining NFTs in CA1, the Gallyas silver stain (d) and P-Tau IHC (e) were used. Note that the signal: noise ratio is not high for the Gallyas stain (d); some of the structures may or may not represent NFTs (yellow arrowheads). This signal:noise ratio confounds digital assessment. By contrast, in the P-Tau IHC (e), even the pre-tangles (red arrowhead) are far easier to discriminate than the non-affected neurons (green arrowhead). Scale bars = 3mm (a); = 100 microns (b,c); and, = 50 microns (d,e).

Negative for neuritic amyloid plaques, positive for diffuse plaques and A β

Positive for neuritic amyloid plaques, diffuse plaques and A β

Fig. 4. Depiction of stains in a case that lack neuritic amyloid plaques (Panels A-C) in comparison to a case with neuritic amyloid plaques (D-F).

The Bielschowsky silver stain labels amorphous, fleecy material in the brain of an 80 year old woman; immunohistochemistry confirms the presence of A β (B; A β IHC), whereas P-Tau IHC is negative for dystrophic neurites. In AD, by contrast, the Bielschowsky stain highlights plaques invested with degenerating neurites (pale blue arrowhead) and/or ringing a discrete core (dark blue arrowhead). Note that A β IHC is immunopositive (E), and the P-Tau IHC in this case highlights the profiles of neuritic amyloid plaques (green arrowheads in panel F).

A β IHC with digital plaque detection and quantification

Fig. 5. Photomicrographs depict an area used to evaluate A β IHC using digital pathology. Low-power photomicrograph of A β IHC stained section (a) shows where the boxed area of interest was chosen. At higher magnification (b), the A β immunostained structures can be easily observed. The digitally false-colored box (c) shows how the A β plaques are digitally recognized; all of the orange, red, and yellow areas are quantified automatically. Scale bars =3mm (a); = 150 microns (b,c).

P-Tau IHC with NFT detection and quantification

Fig. 6. Photomicrographs depict an area used to evaluate P-Tau IHC using digital pathology. Low-power photomicrograph of P-Tau IHC stained section (a) depicts the size of the boxes (two such boxes are shown here, up to ten are used per slide) that are used to indicate regions of interest for the digital quantification. At higher power (b-c), digital renderings of the lower-right box in panel (a) are presented to show the areas recognized for P-Tau burden (red in panel b); and P-Tau NFTs (orange in panel c). The yellow arrowheads in all 3 panels show the same NPs, and blue arrowheads show two specific NFTs. Note that the P-Tau

burden image recognition algorithm (b) incorporated the NP structures, whereas the P-Tau NFTs highlights only the NFTs, not the NPs. Scale bars = 200 microns (a-c).

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Fig. 7. Quantitative pathology in four different neocortical brain regions for cases that were CERAD-negative for neuritic amyloid plaques. Shown are color-coded (red=high; white=middle; blue=low) representations of the cases counted manually as diffuse amyloid plaques (1997–2012; n=36) and digitally quantified Aβ amyloid burden (2012–2017; n=44), depicting the severity and distribution of those pathologies in the frontal (Fro; Brodmann areas 39/40); superior and middle temporal (Temp; Brodmann areas 21/22); parietal (Par; Brodmann area xx); and, occipital (Occip; Brodmann areas 17/18) neocortical regions. Each row represents one brain. The cases are

ranked from top to bottom for both cohorts in the order from lowest to highest amount of detected pathology in the frontal cortical sections.

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Fig. 8. Overview of the results.

The data and analyses provide support for the hypothesis that A β deposits, even in the earliest "diffuse" phase, are associated with increased severity of medial temporal lobe NFT pathology as assessed by digital pathology of immunohistochemical stains (A). By contrast, when A β pathology was taken into account, there was not evidence for direct and robust association between APOE genotype and the severity of tau/NFT pathology (B). An overarching point, as depicted in schematic form (C), is that in cases with early AD, both AD-related and PART-related pathogenetic mechanisms are probably occurring at the same time. Whereas it may be most specific to use A β immunohistochemistry for Definite PART, our analyses indicate it still is accurate to assume that some PART pathology is probably present despite the presence of A β plaques.

Table 1.

Characteristics of autopsied participants from the University of Kentucky Alzheimer's Disease Center by year of death. All participants have CERAD neuritic plaque rating of "None".

	All (N=145)	Year of death, 1997–2011 (n=94)	Year of death, 2012–2017 (n=51)
Age at death, y	85.6±7.5	85.1±7.8	86.6±6.7
Female	79 (54.5)	51 (54.3)	28 (54.9)
Education, y	16.0±2.8	15.9±2.6	16.1±3.0
Last MMSE	26.8±4.6	26.9±4.5	26.7±4.9
Last Clinical Diagnosis			
Normal	102 (70.3)	73 (77.7)	29 (56.9)
Impaired, not MCI	9 (6.2)	8 (8.5)	1 (2.0)
MCI	18 (12.4)	6 (6.4)	12 (23.5)
Dementia	16 (11.0)	7 (7.5)	9 (17.7)
Braak NFT stage			
0	17 (11.7)	15 (16.0)	2 (9.5)
I	50 (34.5)	32 (34.0)	18 (35.3)
II	51 (35.2)	27 (28.7)	24 (47.1)
III	15 (10.3)	11 (11.7)	4 (7.8)
IV	12 (8.3)	9 (9.6)	3 (5.9)
Thal A β stage			
Absent (A0)		-	12
Grade 1–2 (A1)		-	14
Grade 3 (A2)		-	13
Grade 4 (A3)		-	5
Missing		145	8
Medial temporal LBs			
Present	21 (14.5)	11 (11.70)	10 (19.6)
Hippocampal Sclerosis			
Present	14 (9.7)	8 (8.5)	6 (11.8)
<i>APOE</i> ϵ 4 positive	18 (12.4)	8 (8.5)	10 (19.6)

Table 2.

Braak NFT stage by diffuse plaques (present vs. absent) and *APOE ε4* carrier status. All participants have CERAD neuritic plaque rating of “None”. Absence of diffuse plaques was characterized by diffuse plaque rating of “None” or Thal phase “0”.

	Any diffuse plaques		No diffuse plaques	
	APOE ε4 positive	<i>APOE ε4</i> negative	APOE ε4 positive	<i>APOE ε4</i> negative
Braak NFT Stage	N=16	N=61	N=2	N=66
0	1 (6.3)	6 (9.8)	0	10 (15.2)
I	5 (31.3)	20 (32.8)	1 (50.0)	24 (36.4)
II	4 (25.0)	26 (42.6)	0	21 (31.8)
III	4 (25.0)	5 (8.2)	0	6 (9.1)
IV	2 (12.5)	4 (6.6)	1 (50.0)	5 (7.6)

Table 3.

Quantitative medial temporal tau pathology by quantile of amyloid pathology (range). All participants have CERAD neuritic plaque rating of “None”.

	N	Tau pathology (median [IQR])
Year of death, 1997–2011		
Diffuse plaque counts		NFT counts
None	60	2.9 (0.8–10.2)
> None (0.05–39.1)	34	2.3 (0.6–17.4)
Year of death, 2012–2017		
Neocortical amyloid density		Medial temporal NFT density
Q1 (0.01–0.27)	12	4.1 (2.4–7.3)
Q2 (0.45–9.03)	13	7.2 (3.6–13.2)
Q3 (9.56–42.3)	13	8.2 (2.4–11.2)
Q4 (42.9–114.3)	13	12.1 (8.5–24.8)
Neocortical amyloid burden		
Q1 (0.03–0.73)	12	8.3 (4.8–24.9)
Q2 (0.97–9.03)	13	17.3 (12.1–37.0)
Q3 (10.72–36.4)	13	15.4 (7.2–44.5)
Q4 (36.5–110.35)	13	31.3 (21.3–58.7)

Table 4.

Predicted mean (95% CI) * tau pathology by *APOE* $\epsilon 4$ carrier status

	Year of death, 1997–2011	Year of death, 2012–2017	
	Adjusted Mean NFTs	Adjusted Mean NFT density	Adjusted Mean tau burden
Model 1			
<i>APOE</i> $\epsilon 4$ positive	16.0 (6.5–39.3)	16.8 (9.8–18.6)	54.3 (30.2–97.6)
<i>APOE</i> $\epsilon 4$ negative	6.8 (5.1–9.1)	8.7 (6.8–11.3)	24.6 (18.7–32.3)
Model 2			
<i>APOE</i> $\epsilon 4$ positive	19.4 (7.0–53.9)	12.0 (6.9–21.0)	44.8 (24.7–81.2)
<i>APOE</i> $\epsilon 4$ negative	6.6 (5.0–8.8)	8.9 (7.0–11.4)	24.7 (18.9–32.3)

* Results are based on negative binomial regression. Bolded results are significantly different between APOE positive and negative at 0.05. Model 1 includes *APOE*, Model 2 includes both *APOE* and amyloid pathology. For NFTs, the amyloid measure was diffuse plaque counts; amyloid density was used for NFT density; and amyloid burden was used for tau burden. All models were adjusted for age at death and sex.