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Hippocampal Laminar Distribution of Tau Relates to Alzheimer's Disease and Age of Onset

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Abstract

Background—Cerebral deposition of phospho-tau in Alzheimer's disease (AD) occurs with varying patterns within hippocampus. Lamina-specific tau changes in AD may reflect trans-synaptic propagation of phospho-tau along neuroanatomical pathways.

Objective—To study patterns of tau deposition within inner (IML) and outer (OML) molecular layers of dentate gyrus and their clinical and neuropathological correlates.

Methods—98 consecutive autopsied brains from the Columbia University Brain Bank were stained for phospho-tau using AT-8. Staining density was rated as High versus Low within IML and OML. Four patterns were observed among the 98 brains: High IML&OML, $n = 44$; High OML Only ($n = 35$); High IML Only ($n = 5$); and Low IML&OML ($n = 14$). Demographic, clinical, and neuropathological characteristics of these four groups were compared.

Results—High IML&OML subjects, versus High OML Only, were more likely to fulfill CERAD criteria for Definite AD (93% versus 66%, $p < 0.01$) and to have higher median Braak stage (6 versus 5, $p < 0.01$) and earlier mean age of onset (65.9 versus 73.7 y, $p = 0.02$), with similar symptom duration. Using logistic regression, the association between High IML&OML and AD remained significant after adjustment for demographics but not symptom duration. In the 70 subjects with Definite AD, High IML&OML was associated with younger age of onset (mean difference 3.7 years, 95%CI -6.7 to -0.7 , $p < 0.01$), after adjustment for demographics and symptom duration.

Conclusions—Phospho-tau pathology, when prominent within both IML and OML, is associated with CERAD diagnosis of Definite AD and earlier age of onset in AD. Laminar patterns of tau deposition reflect regional involvements during disease course.

Keywords

Alzheimer's disease; dentate gyrus; hippocampus; perforant pathway; tau proteins

Introduction

Alzheimer's disease (AD) is marked neuropathologically by cerebral deposits of plaques, containing amyloid- β , and tangles and threads containing abnormally phosphorylated tau protein (phospho-tau) [1]. While plaque distributions are more variable, tangles tend to occur specifically in vulnerable projection neurons and frequently occur in a pattern suggesting regional progression [2, 3]. In typical, late-onset AD, hyperphosphorylated tau pathology progresses sequentially from lateral entorhinal cortex to medial entorhinal cortex [4], although in some atypical and early-onset cases of AD, there are differing patterns, including early tangle development in non-hippocampal brain regions [5, 6]. The reasons for regional variability of hyperphosphorylated tau abnormalities are not known.

Tauopathic burden within specific layers of the hippocampus can result from neurodegeneration of neurons that project into that region [7]. In humans, the lateral entorhinal cortex projects to the outer molecular layer of the dentate gyrus (OML) as part of the well-characterized perforant pathway. The medial entorhinal cortex projects to the more inner portions of the molecular layer (IML) [8, 9]. In some patients, autopsy reveals neurofibrillary tau burden in the hippocampus restricted to the OML, with clear sparing of the IML, while in other patients, tau burden affects both IML and OML. By contrast, amyloid tends to deposit in the perforant path target zone, without exhibiting variable distribution across molecular layers among patients [10]. It is not clear to what extent different distributions of tau relate to a neuropathological diagnosis of AD, or reflect variations in disease expression (i.e., age of onset), stage of disease, or spread of tauopathic burden. In a limited number of prior studies, IML tau burden has been associated with higher Braak neurofibrillary tangle stages and higher Clinical Dementia Rating Scale scores [11, 12]. These studies were limited with respect to sensitivity of staining technique, sample size, specificity of neuropathological diagnosis and/or availability of clinical information.

There is increasing interest in mechanisms by which phospho-tau may propagate trans-synaptically along neuroanatomical pathways [13–15]. A better understanding of the significance of differential tau burden within the human dentate gyrus could further our understanding of spread of neuropathology in AD. For example, it is unclear whether tau pathology within IML results from neurodegeneration in the medial entorhinal cortex or from degeneration of other structures, such as locus coeruleus [16].

Here we study the patterns of phospho-tau deposition within the cellular layers of the hippocampus which may have implications regarding the spread of tau. We use a retrospective autopsy case series of 98 Alzheimer Disease Research Center (ADRC) subjects and utilize a reliable, semi-quantitative method for visually rating the severity of tau burden

within IML and OML of the human dentate gyrus. We characterize the clinical, demographic and neuropathological features associated with observed patterns of tau distribution in the hippocampus. We test the hypotheses that IML phospho-tau deposition is directly associated with 1) the presence and severity of neuropathologically-defined AD and 2) younger age of onset of AD. We also explore whether IML phospho-tau deposition is associated with type of symptom onset (memory versus non-memory), apolipoprotein E4 status, or concomitant brain pathology (synucleinopathy and cerebrovascular disease).

Materials and Methods

Case selection

This study is an analysis of 98 sequential autopsy cases from the brain bank of the Alzheimer's Disease Research Center (ADRC) at Columbia University. All cases represent brains of participants at the Columbia University ADRC. Participants enroll in the ADRC under an institutional review board (IRB) approved protocol. ADRC recruitment, participant evaluation, and diagnostic criteria are detailed elsewhere [17]. Participants were followed at approximately yearly intervals with collection of clinical, genetic, radiologic, and neuropsychological tests, including those data required by the National Alzheimer's Coordinating Center Uniform Data Set [18]. Brains included study subjects with diagnoses of mild cognitive impairment, and dementia. For subjects who had clinical dementia, symptom duration was calculated as the difference between reported date of onset of first symptoms and date of death.

Neuropathology

Neuropathological assessment was performed using methods previously described [19]. All cases were rated according to Braak and Braak [20], Consortium to Establish a Registry for Alzheimer's Disease (CERAD) [21], and National Institute on Aging–Reagan Institute (NIA-RI) criteria [22]. The consensus guidelines for the neuropathological diagnosis of Dementia with Lewy Body (DLB) were used to diagnose DLB cases [23]. For this investigation, the brain section depicted in Fig. 1A was used including the cornu Ammonis (hippocampus proper), parahippocampal gyrus, and occipitotemporalis gyrus. This was obtained from a formalin-fixed, paraffin-embedded coronal section at the level of the lateral geniculate body.

Sections were stained with AT8 antibodies [20] directed against phosphorylated tau, using a diaminobenzidine visualization system and counter-stained with hematoxylin. They were photographed digitally using a Zeiss Axioskop 2 plus (objective Plan-Neofluotar 10) to which a Zeiss AxioCam is attached. The region analyzed was at the midpoint along the medio-lateral axis of the dentate gyrus, including in continuity, a portion of the CA4 sector, the three layers of the dentate gyrus (polymorphic, stratum granulosum, and both inner and outer stratum moleculare); and the vestigial hippocampal sulcus, and molecular layer of the Cornu Ammonis.

Semi-quantitative rating system for rating AT-8 Staining

Four neurologists with varying levels of neuropathological experience independently rated the density of AT8 staining in each layer of the dentate gyrus. The four raters had a training session using projected micrographs depicting a subset of slides with various AT8 staining patterns. We used a semi-quantitative visual rating scale similar to one that was used in a prior study [12] to rate the density of tauopathic burden within IML and OML as follows: 0 = no brown-colored staining, 1 = a mild amount of brown-colored staining limited to a few discrete areas, 2 = a moderate amount of brown-colored staining appearing somewhat confluent, and 3 = dense, darker brown staining seen diffusely throughout the layer. After the initial training session, the four raters were simultaneously shown each of the 98 hippocampal sections using computer-displayed micrographs. Raters were done independently, without discussion, with one minute per slide to record ratings. Score data was entered into an IBM SPSS file (version 19 IBM Corp, New York, New York, USA), and the data were merged with ADRC demographic and clinical data.

Categorization of study subjects

We collapsed the four-point rating scale into a two-point rating scale (0 or 1 = Low and 2 or 3 = High) in order to maximize inter-rater reliability. We assigned a binary rating of High versus Low for each layer for each subject. After the reliability analysis (described below), ratings from one reviewer were excluded and ratings from three reviewers were included in the final analysis (described below). If the three reviewers were not in agreement (16% of cases), we assigned a rating based upon the rating of the two reviewers who were in agreement.

We divided the 98 brains into four groups (Low IML&OML, High IML Only, High OML Only, High IML&OML), as depicted in Fig. 1B. The High IML Only group had a High rating in IML and a Low rating in OML. The High OML Only group had a High rating in OML and a Low rating in IML. The High IML&OML and Low IML&OML groups had High and Low ratings in both layers, respectively.

For statistical comparisons, we compared the two largest groups, High OML Only and High IML&OML. We chose these groups for comparison because there were few cases in the other categories (Low IML&OML $n = 14$; high IML $n = 5$), limiting potential analyses. Additionally, these were the groups we wished to compare in our hypothesis, i.e., whether the presence of tau in both layers, as compared to one layer, is indicative of the presence and severity of AD.

Some of the cases had neurofibrillary tangles visible in the granule cell and neuritic plaques visible at the junction of IML&OML (see Fig. 1B). Raters were instructed to note their presence (yes/no), but these data were not included in the final analysis because inter-rater reliability was inadequate to include this data.

Reliability analysis

Inter-rater reliability was assessed region by region. For IML, there was no statistically significant difference in the average rating (over 98 brains) given by any of the reviewers (p

= 0.597, averages 1.5, 1.6, 1.7, and 1.5 for reviewers 1–4). For OML, reviewer #1 gave lower ratings, on average, than the rest of the group ($p = 0.023$, averages 1.7, 2.0, 2.0, 1.9 for reviewers 1–4). Because this reviewer was an outlier, all further analyses included only the other three reviewers. To assess the inter-rater reliability among the three reviewers, an unweighted kappa statistic was calculated for OML and IML. The initial unweighted kappa statistics for all four reviewers, rating tau staining on a four-point scale (none, mild, moderate, severe) were 0.4 for OML and 0.6 for IML. The unweighted kappa statistics for the three remaining reviewers using a two-point scale (Low versus High) were 0.6 for OML and 0.8 for IML.

Statistical analyses

For comparisons between the High OML Only and High IML&OML groups, Chi-square tests were performed for categorical variables and Mann-Whitney or Kruskal Wallis tests for continuous variables.

To determine whether concomitant IML&OML tau pathology is associated with a neuropathological diagnosis of AD, we used binary logistic regression with Definite AD by CERAD neuritic plaque criteria as the primary outcome with High IML&OML (versus High OML Only) as the primary predictor, adjusting for age, gender, ethnicity, education, and symptom duration. We chose CERAD neuritic plaque criteria because, unlike Braak staging or NIA-Reagan criteria, CERAD criteria do not include measures of tau pathology.

To examine the association with severity of AD, we performed binary logistic regression restricted to the group of subjects with Definite AD, using Braak stage as the primary outcome (VI versus < VI), and High IML&OML (versus High OML Only) as the primary predictor, adjusting for age, gender, ethnicity, education and symptom duration. We chose Braak Stage VI and < VI as comparison groups due to the distribution of Braak stages (50 Definite AD subjects were rated as stage VI and 14 were below stage VI).

To determine the association with age of onset, we performed linear regressions restricted to the group of Definite AD subjects using age of onset as the outcome and High IML&OML (versus High OML Only) as the predictor, adjusting for gender, ethnicity, education, and symptom duration.

We also repeated all analyses stratifying by APOE4 status ($\epsilon 4$ allele present versus absent). And we also performed the above logistic and linear regressions comparing subjects with high IML tau ratings to those with low IML tau ratings (regardless of OML status). Statistical analysis was performed using IBM SPSS file (version 19 IBM Corp, New York, New York, USA).

Results

Characteristics of the study sample

The average age of the sample at autopsy was 81.7 y (SD 11.1, range 50 to 100). 51% were female; 84% were non-Hispanic white, 8% were Hispanic, and 5% were non-Hispanic African American. Mean education level was 15.2 years (SD 4.3, range 1 to 20). Dementia

was present clinically in 70% of the cases (See Table 1). Independent of presence or absence of clinical dementia, neuropathologically, 71% of the sample met CERAD criteria for Definite AD. Primary neuropathologic diagnoses were 54% Definite AD ($n = 53$), 5% Probable AD ($n = 5$), 20% AD with Lewy Bodies ($n = 20$), and 19% other (stroke, normal pressure hydrocephalus, possible AD, progressive supranuclear palsy, hippocampal sclerosis, and frontotemporal lobar degeneration). Independent of presence or absence of Definite AD, concomitant neuropathological findings, included pathological evidence of moderate or severe atherosclerosis (54% of the sample), moderate or severe arteriosclerosis (79% of the sample), and presence of some degree of Lewy body pathology (41% of the sample).

AT8 staining patterns

Figure 2 shows the four general patterns of molecular layer tau burden seen in our case series. Most subjects were categorized as either High IML&OML (45%, $n = 44$) or High OML Only (36%, $n = 35$). The remainder of the subjects were categorized as either Low IML&OML ($n = 14$) or High IML Only ($n = 5$).

In univariate analyses, compared to High OML Only, High IML&OML was associated with younger at age of onset (76.1 versus 81.2 years, $p < 0.01$), higher likelihood of clinical dementia (84% versus 59%, $p < 0.05$) and higher likelihood of fulfilling CERAD criteria for Definite AD (93% versus 66%, $p < 0.01$). This group, as compared to the High OML Only group, also had higher median Braak stage (6 versus 5, $p < 0.01$). There was no significant difference in symptom duration, concomitant brain pathology (i.e., cerebrovascular disease and synucleinopathy) or type of symptom onset (i.e., memory versus non-memory). The frequency of having at least one $\epsilon 4$ allele was higher in the High IML&OML group, as compared to High OML Only, but this difference was not statistically significant (63% versus 37%, $p = 0.081$) (see Table 1).

Relationship of tau distribution patterns to presence of AD

In analyses of the 79 subjects with High IML&OML or High OML Only, High IML&OML was associated with higher likelihood of fulfilling CERAD criteria for Definite AD, after adjustment for age, gender, ethnicity, and education (OR 6.1, 95% CI 1.2–31.7). These associations were significantly attenuated when additionally including symptom duration in the model (see Table 2).

Relationship of tau distribution patterns to severity of AD (symptom duration and Braak stage)

In analyses of the 64 subjects who fulfilled CERAD criteria for Definite AD and belonged to the two major hippocampal tau distribution patterns, clinical duration of symptoms was not significantly different between High IML&OML and High OML Only groups (see Table 1). High IML&OML, as compared to High OML Only, was associated with increased likelihood of having Braak stage higher than V (compared to V or below), after adjustment for age, gender, ethnicity, and education. These associations were significantly attenuated when additionally including symptom duration in the model (see Table 3).

Relationship of tau distribution patterns to age of onset of AD

In the 64 subjects who fulfilled CERAD criteria for Definite AD and belonged to the two major hippocampal tau distribution patterns, those with High IML&OML, as compared to High OML Only, had younger age of onset by 3.7 years (95%CI 0.7 to 6.7 years) (as shown in Table 4), in the full model including gender, ethnicity, education, and symptom duration.

Interactions with apolipoprotein $\epsilon 4$ Allele

APOE4 status was available for 57 subjects. There was no difference between subjects with or without available APOE4 data in age, gender, ethnicity (Caucasian versus non-Caucasian), education level, or frequency of Definite AD (data not shown). Among the subjects with available data, 28 subjects had no $\epsilon 4$ alleles, 24 subjects had one, and five subjects had two. Of note, all 5 of the subjects who were homozygous for $\epsilon 4$ were categorized within the High IML&OML group. As seen in Table 4, the association between tau distribution pattern and earlier onset of AD was statistically significant only in the subjects with at least one APOE4 allele (95% CI -11.0 to -1.8 y). Tau distribution pattern did not relate to age of onset in subjects without an $\epsilon 4$ allele (95% CI -9.3 to 5.1 y).

Sensitivity analyses

We performed additional analyses to ensure the robustness of our findings. We repeated all the previous analyses using high IML tau ratings versus low IML tau ratings as the binary outcome, regardless of OML rating. Results were largely unchanged (see Tables 2–4, comparisons for IML Tau Rating).

Discussion

Using a semi-quantitative method for visually rating the density of AT-8 staining within the human dentate gyrus, we describe four distribution patterns of molecular layer tau burden in a sample of older subjects with differing degrees of cognitive impairment. In the majority of cases, tau pathology was either rated as High in both IML and OML (44%) or High in the OML only (36%). A pattern of higher tau burden involving both IML and OML was associated with significantly greater likelihood of neuropathological diagnosis of AD (by CERAD criteria), as well as younger age of onset by approximately 4 years. All subjects who were homozygous for apolipoprotein $\epsilon 4$ allele fell within the High IML&OML group and indeed the association with age of onset was only significant in subjects with at least one $\epsilon 4$ allele.

Compared to prior studies on this topic [11, 12, 24, 25], this study included a larger group of autopsied subjects with and without AD who were extensively characterized with clinical, neuropathological, and genetic data. We confirmed previous findings of an association of IML tau pathology with disease severity (higher Braak stage) [11] but we detected no association with symptom duration. We added to previous findings by including APOE4 in the analysis. Finally, hippocampal molecular layer tau pathology patterns appeared independent of concomitant brain pathologies such as cerebrovascular disease and synucleinopathy and appeared independent of type of symptom onset (memory versus non-memory).

In AD, neurodegeneration in the lateral entorhinal cortex occurs early and therefore we might expect tau pathology to be seen early in the terminal fibers of this region in the OML. Also, neurodegeneration in the medial entorhinal cortex occurs later and we would therefore expect tau pathology to be seen in the IML later in disease course. Indeed, using Alz-50 (a less sensitive stain for tau), Hyman et al. demonstrated heavy involvement of OML, with no involvement of IML, in 15 patients with AD of unknown severity [25]. In another study of 18 subjects with varying degrees of AD dementia, neurofibrillary tangles within deep layers of the entorhinal cortex invariably accompanied tau changes in the IML [24]. In a larger autopsy series of 106 patients (ages 46–93 y) treated for various illnesses, IML tau burden (by AT-8 staining) was noted only in subjects with more advanced AD clinically (CDR 2 or 3) and pathologically (Braak stage V or VI) [11]. That study lacked data regarding cerebral amyloid and excluded subjects with concomitant brain pathology, so no definitive conclusions regarding AD or mixed dementia could be drawn. A second autopsy series of 93 cases (only eight of whom fulfilled CERAD criteria for Definite AD) showed 43% of subjects to have at least mild tau pathology in the OML and 57% in both OML and IML [12]. In that series, no cases had IML burden without OML burden, implying that OML involvement occurs before IML involvement.

These prior studies support the notion that changes in entorhinal cortex neurons progressively expand into axonal endings within target regions of the perforant path, and that presynaptic axonal terminal changes precede postsynaptic changes via anterograde transneuronal mechanisms. It is also possible that medial septum/basal forebrain neurodegeneration contributes to observed IML tau pathology. Because the medial septum projects to the IML, tau burden in the IML may reflect subcortical, medial forebrain neurodegeneration, which could be more common or more extensive in younger patients or in subjects with atypical presentations. Tau pathology within cholinergic forebrain neurons of the medial septum is known to occur early in cognitive impairment and dementia [26, 27]. Cholinergic, serotonergic, and noradrenergic changes have been suggested to be more severe in early-onset cases, not due to differences in the duration of illness or weight of autopsied brain [28]. In fact, there is currently some controversy regarding whether tau change might begin in the brainstem, actually preceding amyloid and tau changes in cortical regions [29]. Brainstem projection neurons such as those in the locus coeruleus and raphe nucleus have robust connections with medial septum and hippocampus [30]. We explored the possibility that subjects with High IML&OML might have symptom onset referable to non-memory structures such as frontal lobe but our limited sample size revealed no significant difference in predominant symptom at onset. Our finding that the age of onset effect was limited to APOE positive subjects are consistent with current findings that apolipoprotein isoforms influence susceptibility to cognitive decline in younger patients [31] and may suggest that these isoforms play a role in the variability of distribution of tau within hippocampus.

Our study had several limitations. We used a semi-quantitative method to rate the phospho-tau burden, which may have introduced random and systematic bias. Also, since younger AD patients tend to have a more rapid decline in cognition and function, they might be further along in their disease course at the time of death. We attempted to control for this by adjusting for symptom duration; however, we were unable to control for rate of decline which remains a potential residual confounder. We did not account for tau changes in the

polymorphic layer. Our visual rating system was not designed for rating tau within granule cells so we could not determine the extent to which IML tauopathy was mediated by granule cell tauopathy. We did not measure thickness of molecular layers; this is an important limitation because atrophy of molecular layers occurs in parallel with tauopathy. Finally, our study did not allow for the distinction of axonal versus dendritic tau changes and for the identification of neurodegeneration of afferent cortical and subcortical structures to the molecular layers.

Important questions for future study include whether large series support the observation that subjects with IML involvement more likely present at younger ages, or to present with symptoms referable to orbitofrontal cortex or ascending neurotransmitter systems. Correlating IML tau pathology with medial septum neurodegeneration could be a means to improve knowledge of the relative contributions from the medial entorhinal cortex and medial septum to IML tau pathology. In addition, the relationship between genetic variants, including tau haplotypes, with hippocampal layering patterns in AD and other tauopathies might be important directions for future study.

Understanding the patterns and significance of tau pathology within the molecular layers of the human dentate gyrus may be helpful in furthering our understanding of the underlying neuropathology of aging, AD, and other dementias. This will be increasingly important as methods for *in vivo* imaging of tau pathology become clinically available [32].

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Fig. 1.

A. Layers of the human dentate gyrus. On the left, a section of the human dentate gyrus is shown stained for AT-8 antibodies. On the right, a magnified view of the granule cells of the hippocampus is visible. Below the granule cell layer of the dentate gyrus, neuritic plaques are visible at the junction between IML&OML, and phospho-tau deposition is depicted as brown staining. B. Patterns of molecular layer tau deposition (AT-8). Four patterns of molecular layer tau pathology were identified. Low IML&OML had low ratings in both IML and OML. High IML Only had high ratings in IML but low ratings in OML. High OML Only had high ratings in OML and low ratings in IML. High IML&OML had high ratings in both layers. In certain cases, such as that in the High IML Only example shown above, tangles are visible in the stratum granulosum. Magnification 100×.

Fig. 2.

Numbers of subjects by molecular layer pattern. The frequency of the four different patterns in our sample is depicted above, with the High OML Only and High IML&OML being the two largest groups.

Table 1
Subject characteristics by molecular layer tau pathology patterns

Low IML&OML (<i>n</i> = 14) High IML Only (<i>n</i> = 5) High OML Only (<i>n</i> = 35) High IML&OML (<i>n</i> = 44) <i>p</i> -value *				
Demographic Features				
Age at death	80.7	83.5	86.2	78.1
Age of onset	73.1 (<i>n</i> = 7)	69.8 (<i>n</i> = 4)	73.7 (<i>n</i> = 22)	65.9 (<i>n</i> = 32)
Symptom duration	11.7 (<i>n</i> = 7)	13.7 (<i>n</i> = 4)	11.4 (<i>n</i> = 22)	12.2 (<i>n</i> = 32)
Education	14.5 (<i>n</i> = 13)	15.0	15.3 (<i>n</i> = 32)	15.3 (<i>n</i> = 39)
Gender				
Male	<i>n</i> = 8	<i>n</i> = 3	<i>n</i> = 14	<i>n</i> = 23
Female	<i>n</i> = 6	<i>n</i> = 2	<i>n</i> = 21	<i>n</i> = 21
Ethnicity				
Caucasian	<i>n</i> = 13	<i>n</i> = 3	<i>n</i> = 33	<i>n</i> = 35
Non-Caucasian	<i>n</i> = 1	<i>n</i> = 2	<i>n</i> = 2	<i>n</i> = 9
APOE ε4				
E4 homozygous	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 0	<i>n</i> = 5
E4 heterozygous	<i>n</i> = 4	<i>n</i> = 1	<i>n</i> = 7	<i>n</i> = 12
E2 heterozygous	<i>n</i> = 5	<i>n</i> = 1	<i>n</i> = 12	<i>n</i> = 10
Neuropathological features:				
Braak NFT Stage (median)	3	6	5	6
CERAD criteria				
Definite	<i>n</i> = 2	<i>n</i> = 4	<i>n</i> = 23	<i>n</i> = 41
Not definite	<i>n</i> = 12	<i>n</i> = 1	<i>n</i> = 12	<i>n</i> = 3
Presence of Lewy bodies [#]				
Yes	<i>n</i> = 3	<i>n</i> = 1	<i>n</i> = 12	<i>n</i> = 24
No	<i>n</i> = 11	<i>n</i> = 4	<i>n</i> = 23	<i>n</i> = 20
Atherosclerosis				
None/mild	<i>n</i> = 7	<i>n</i> = 2	<i>n</i> = 15	<i>n</i> = 20
Moderate/severe	<i>n</i> = 7	<i>n</i> = 3	<i>n</i> = 19	<i>n</i> = 24
Arteriosclerosis				
Absent	<i>n</i> = 3	<i>n</i> = 1	<i>n</i> = 6	<i>n</i> = 11

Low IML&OML (<i>n</i> = 14) High IML Only (<i>n</i> = 5) High OML Only (<i>n</i> = 35) High IML&OML (<i>n</i> = 44) <i>p</i> -value*				
Present	<i>n</i> = 11	<i>n</i> = 4	<i>n</i> = 29	<i>n</i> = 33
<i>Clinical Features</i>				
First predominant symptom				
Memory	<i>n</i> = 5	<i>n</i> = 3	<i>n</i> = 17	<i>n</i> = 22
Non-memory	<i>n</i> = 2	<i>n</i> = 0	<i>n</i> = 2	<i>n</i> = 3
MMSE (unadjusted)	19.3 (<i>n</i> = 12)	8.8 (<i>n</i> = 4)	15.5 (<i>n</i> = 27)	10.6 (<i>n</i> = 37)
Clinically Demented				
Yes	<i>n</i> = 9	<i>n</i> = 5	<i>n</i> = 20	<i>n</i> = 36
No	<i>n</i> = 4	<i>n</i> = 0	<i>n</i> = 14	<i>n</i> = 7

* comparing High IML&OML to High OML Only.

includes brainstem, intermediate, diffuse, and nonspecific types.

^ comparing subjects with at least one ε4 allele to subjects with zero ε4 alleles.

Bold values indicate 0.05 significance.

Table 2
Logistic regression analyses, laminar tau pathology predicting CERAD diagnosis of definite AD

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
<i>Distribution Pattern</i>			
High OML only (<i>n</i> = 35)	–	–	–
High IML&OML (<i>n</i> = 44)	7.1 (1.8 to 27.9)*	6.2 (1.2 to 31.7)*	0.90 (0.1 to 14.3)
<i>IML Tau Rating</i>			
Low (<i>n</i> = 49)	–	–	–
High (<i>n</i> = 49)	10.8 (3.4 to 34.7)*	8.5 (2.3 to 31.0)*	3.0 (0.7 to 13.0)

Model 1: Univariate analysis, Distribution Pattern or IML Tau Rating predicting CERAD stage. Model 2: Model 1, additionally adjusted for age, gender, ethnicity, and education. Model 3: Model 2, additionally adjusted for symptom duration.

*
p-value < 0.05.

Table 3
Logistic regression analyses, laminar tau pathology predicting Braak Stage VI (versus less than VI) in subjects fulfilling CERAD criteria for definite AD

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
<i>Distribution Pattern</i>			
High OML only (<i>n</i> = 23)	–	–	–
High IML&OML (<i>n</i> = 41)	2.7 (1.4 to 5.2)*	2.0 (0.9 to 4.6)	2.0 (0.7 to 5.4)
<i>IML Tau Rating</i>			
Low (<i>n</i> = 25)	–	–	–
High (<i>n</i> = 45)	9.4 (2.6 to 34.4)*	5.2 (1.1 to 25.5)*	4.6 (0.6 to 35.3)

Model 1: Univariate analysis, Tau Group or Tau Ratings predicting CERAD stage. Model 2: Model 1, additionally adjusted for gender, ethnicity, and education. Model 3: Model 2, additionally adjusted for symptom duration.

*
p-value < 0.05.

Table 4
Linear regression analyses, laminar tau pathology predicting age of onset of symptoms in subjects fulfilling CERAD criteria for definite AD

	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
<i>Distribution Pattern</i>			
High OML only ($n = 19$)	–	–	–
High IML&OML ($n = 29$)	–4.1 (–6.9 to –1.3)*	–4.3 (–7.3 to –1.3)*	–4.1 (–7.2 to –1.1)*
$\epsilon 4$ Present ($n = 16$)			–6.4 (–11.0 to –1.8)*
$\epsilon 4$ Absent ($n = 9$)			–2.1 (–9.3 to 5.1)
<i>IML Tau Rating</i>			
Low ($n = 25$)	–	–	–
High ($n = 45$)	–8.5 (–14.1 to –2.8)*	–8.9 (–14.7 to –3.2)*	–8.6 (–14.4 to –2.8)*
$\epsilon 4$ Present ($n = 24$)			–15.3 (–24.5 to –6.2)*
$\epsilon 4$ Absent ($n = 18$)			–5.6 (–19.7 to 8.5)

Model 1: Univariate analysis, Tau Group or Tau Ratings predicting CERAD stage. Model 2: Model 1, additionally adjusted for gender, ethnicity, and education. Model 3: Model 2, additionally adjusted for symptom duration.

* p -value < 0.05.