α-Synuclein aggregation in pathological aging and Alzheimer's disease: The impact of ß-amyloid plaque level

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Abstract

In central nervous system diseases, abnormal aggregation of one protein is often associated with aggregation of other proteins. To begin to assess whether ß-amyloid (Aß) is associated with α-synuclein (AS) aggregation [secondary Lewy body (LB) formation], we used immunohistochemical techniques to compare the amygdala of 11 subjects with pathological aging and 18 with Alzheimer's disease. Overall, Aß-40 plaque level was greater in cases with secondary AS aggregates. Aß-42 plaque level was not associated with AS aggregation. Aß-40 plaque levels cannot be ruled out as a factor involved in secondary LB formation.

Key words: Alzheimer's disease, ß-amyloid, α-synuclein, Lewy body, pathological aging

Introduction

Overlap syndromes are a major cause of diagnostic uncertainty because dual pathology can obscure clinical phenotypes.[1-3] We previously demonstrated that one-half of patients with familial forms of Alzheimer's disease (AD) have numerous fibrillary α-synuclein (AS) aggregates in the form of Lewy bodies (LBs) and Lewy threads in their amygdala.[4] This finding has been replicated in patients with sporadic AD, in which one-half of these subjects have amygdala LBs.[5] Although the direct cause for this is unknown, we demonstrated that LBs are numerous in nearly one-half of subjects with other diseases in which intraneuronal tau aggregates form, including Pick's disease and argyrophilic grain disease.[6] In contrast, secondary LBs did not occur in dementia lacking distinctive histopathology, a condition in which cells degenerate in the absence of abnormal tau or ß-amyloid (Aß) aggregates.[6] Because AD is the condition most commonly associated with secondary AS aggregation and Aß is the major pathologic hallmark of AD, it is reasonable to determine whether Aß also contributes to secondary LB formation. To explore this idea, we evaluated Aß accumulation in the amygdala in AD subjects as well as a group of subjects with pathological aging (PA), a condition in which high quantities of Aß are present, but tau aggregates are minimal.[7]

Methods

Subjects

We examined 11 mid-amygdala sections from pathologically diagnosed cases of PA (i.e., nondemented individuals whose brains lacked neuritic pathology but contained moderate-to-high numbers of Aß plaques[7]) and 18 pathologically confirmed cases of AD.[8] The AD cases all had advanced Braak Stage V or VI pathology. None of the PA cases had neuritic pathology above Braak Stage II. None of the cases had other significant clinical or pathologic neurological diagnoses, and the AD cases did not contain LBs outside of the amygdala.

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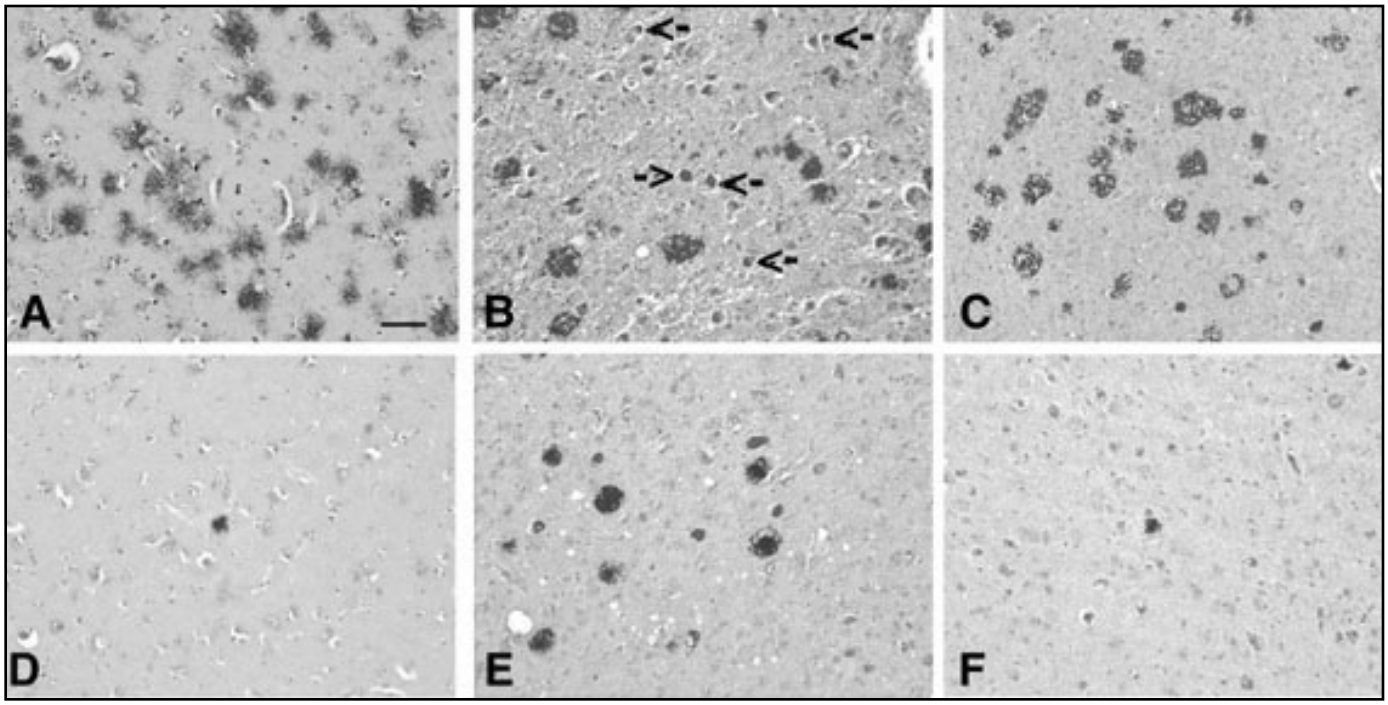

Figure 1. Photomicrographs illustrating the relationship between LBs and Aß plaque level in the amygdala. The images are representative of Aß-42 plaque level (top row) and Aß-40 plaque level (bottom row) in PA (A, D), AD with LBs (B, E), and AD cases lacking LBs (C, F). Double-label immunohistochemistry was used, with DAB as the chromogen for Aß and new fuchsin as the chromogen for α-synuclein-containing LBs. The arrows (B) point out LBs. Aß-42 plaque level was similar between groups (A-C). Aß-40 plaque level was greater in cases with LBs (E) than in cases lacking LBs (D, F). Incidental note is made of the (normal) diffuse neuropil staining for α-synuclein, which is seen in all images. Scale bar = 20 microns.

All AD cases had advanced symptoms at death, requiring assistance with all activities of daily living. PA cases had documented normal cognitive function.

Histology

Formalin-fixed, paraffin-embedded, six-micron-thick sections were cut in the coronal plane through the mid-amygdala. Using well-characterized antibodies,[9] serial sections were immunostained for AS (Zymed, Carlsbad, CA; 1:200, with formic acid pretreatment), the long-tailed form of Aß (Aß-42; 11C; 1:500; monoclonal), and the short-tailed form of Aß (Aß-40; 6A; 1:100; monoclonal). These proteins' immunoreactivity was detected using avidin-biotin complex (ABC) kits (Vector Laboratories, Burlingame, CA) and 3, 3'-diaminobenzidine as a chromogen. Positive and negative control tissues were used, as previously described.[9]

Quantitative assessment

Our semiquantitative scale[6] for rating of LBs was used. Here, 0 refers to no LBs/amygdala, 1 refers to 1 to 5 LBs/amygdala, 2 refers to 6 to 20 LBs/amygdala, 3 refers to more than 20 LBs/amygdala, and 4 means that there were more than 20 LBs per high-power field.

A semiautomated Image-Pro computer system (Media Cybernetics, Silver Spring, MD) was used to determine Aß plaque level (i.e., percent of the amygdala occupied by Aß plaques) as described.[9]

Statistical methods

T-tests were used to determine whether there were differences in Aß level between the groups with and without LBs. To assess whether Aß levels were correlated with LB numbers, Pearson correlation coefficients (PCCs) were used.

Results

As expected, several AD cases (seven of 18) had numerous LBs in the amygdala (Figure 1). The LB burden averaged 1.11 ± 1.64 in these cases. In AD cases with LBs, the average LB grade was 2.86 ± 1.36. No AS aggregates were found in the amygdala of the PA cases.

Table 1. Summary of cases

	Number of cases	Mean age at death (yr)	Gender (percent)	Duration (yr)	Aβ-40 plaque level (percent)	Aβ-42 plaque level (percent)
PA	11	71.1	7 males (64)	N/A	0.46	5.56
AD without LBs	11	74.3	6 males (55)	10.4	3.02	6.44
Overall cases without LBs (calculated from above)	22	72.7	13 males (59)	N/A	1.74*	6.00
Overall cases with LBs	7	72.6	4 males (57)	11.0	3.37*	7.30

AD, Alzheimer's disease; LB, Lewy body; PA, pathologic aging; N/A, not applicable. * Represents a significant difference between the LB group and the group lacking LBs.

When evaluating overall data (PA and AD groups combined), there was a significant difference in Aß-40 plaque level between cases with AS aggregates and cases lacking AS. There was a mean Aß-40 plaque level of 3.37 percent ± 1.44 in cases with LBs and a mean plaque level of 1.73 percent ± 1.72 in cases lacking AS ($t = -2.283$, $p = 0.03$). In contrast, there were no differences between groups in Aß-42 plaque level: mean Aß-42 plaque level was 7.30 percent ± 2.29 and 6.00 percent ± 1.68 in cases with and without AS aggregates, respectively [$t = -1.640$, nonsignificant (NS)].

When solely the AD group was analyzed, the cases with LBs had slightly greater Aß-40 plaque levels than cases lacking LBs; however, this was not significant (Aß-40 plaque level 3.37 percent ± 1.14 and 3.02 percent ± 1.45, respectively; $t = -0.503$; NS). The seven AD cases with LBs did not have a significantly different Aß-42 load from the 11 cases lacking LBs (Aß-42 plaque level 7.30 percent ± 2.30 and 6.64 percent ± 1.68, respectively; $t = -0.711$; NS).

When correlating the intensity of LB pathology with Aß-40 and Aß-42 plaque level, we noted weak correlations. The overall correlation (PCC) between AS aggregates and Aß-40 was 0.466. The overall PCC between AS aggregates and Aß-42 was 0.420. When we restricted the analysis to AD cases only, the correlations remained low, at PCCs of 0.261 and 0.362 for Aß-40 and Aß-42, respectively.

Discussion

We identified an overall positive association between Aß-40 and AS aggregation (LB formation) in the amygdala, with a greater Aß-40 plaque level in individuals with AS aggregates. None of the PA cases had amygdala LBs, and their levels of Aß-40 were considerably lower than those in the AD cases. In AD, however, cases with LBs had minimally greater Aß-40 plaque level than comparable AD cases lacking AS aggregates, suggesting that other factors are also involved in secondary LB formation. From these data we can not exclude the notion that Aß-40 contributes to the process of AS aggregation.

Overall, Aß-42 plaque level was not associated with LB formation. In AD cases, Aß-42 plaque level was not higher when LBs were present. AS aggregates were not found in the PA group. The fact that no AS deposition was found in these cases, which by definition have deposition of the long-tailed form of Aß, would indicate that Aß-42 deposition alone has no relationship to AS aggregation, at least in this population. We cannot exclude the possibility that Aß-42 may play a role in the development of LBs in familial forms of AD, however, because these cases usually have increased Aß-42 levels.

Tau and Aß are the primary pathologic proteins in AD. Therefore, it is logical that these aggregates, or the process that leads to their development, trigger secondary LB formation in AD. Given that AS aggregates occur in the amygdala in other diseases with tau (but not Aß; argyrophilic grain disease and classical Pick's disease),[6] it is likely that amygdala AS aggregation is linked, at least in part, to tau aggregation or the underlying pathological events that result in tau aggregation. Our PA data indirectly support the idea that Aß-40 may also be involved in LB formation because the PA cases lacked AS and had low Aß-40 plaque levels. In this context, it is logical that the short-tailed form of Aß (Aß-40) was associated with AS aggregation because Aß-40 is

the Aß subtype that correlates with the intensity of tau pathology (i.e., neurofibrillary tangle formation) in AD.[10] Further understanding of the association between Aß-40 and tau in central nervous system diseases is needed.

Our study focused on plaque-related Aß. It is possible that soluble aggregates, "oligomers" of Aß that have not aggregated into plaques, also play a role in secondary LB formation. Also, we focused on amygdala LBs because the intensity of pathology is great in this region. These results cannot necessarily be generalized to cases in which secondary LBs occur in widespread brain regions in lower densities (i.e., typical cases of the LB variant of AD).

Previous retrospective screening of our AD cases with amygdala AS aggregates shows that these patients usually lack the classical features of dementia with LBs, including Parkinsonism and visual hallucinations. Because neurologic symptoms are often related to the regional distribution of pathology more than the underlying pathological feature, however, it is likely that amygdala LBs would cause symptoms that reflect amygdala dysfunction. Such symptoms may include affective problems such as impaired emotional-based memories and responses to fear.[11,12] Prospective studies or retrospective studies focused on clinical features representative of amygdala function are necessary to address the question of whether amygdala LBs influence a patient's symptoms, response to treatment, or progression. The relationship between abnormal protein aggregates in degenerative diseases is complex. Overall, the current study found higher Aß-40 levels in cases with secondary LB formation. These findings are important for improving our understanding of the cellular and molecular pathogenesis of AD. However, additional studies are needed to determine whether this association is direct or through an effect of Aß-40 on tau processing. As treatments in development for degenerative diseases are often focused on the abnormal processing of specific proteins such as Aß, AS, and tau, it will be important to continue to define and understand the underlying mechanisms of protein-protein interactions.

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Author note

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