Regional Distribution of α-Synuclein Pathology in Unimpaired Aging and Alzheimer Disease

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Abstract. The amygdaloid complex (AC) was found highly vulnerable to α-synuclein (αS) pathology in both familial and sporadic Alzheimer disease (AD), and recently, incidental Lewy bodies (LBs) were identified primarily in the lower brainstem. This challenges the traditional view that the substantia nigra (SN) is the region that is predominately affected in the spectrum of LB disorders. We examined the immunoreactivity of αS in the SN, the nucleus basalis of Meynert (nbM), and the AC in 904 subjects with or without concomitant AD pathology. αS-positive structures were seen in at least one of the studied brain areas in 121 subjects (13%). The affected regions in the αS-positive subjects included the SN (89%), the nbM (73%), and the AC (67%). This study also included 82 sporadic AD patients diagnosed using CERAD criteria. αS-positive structures were seen in 32% of the AD patients, with the SN and AC being equally affected. In a few subjects the AC was the only affected area. However, this was not inevitably associated with AD pathology, but was related to cognitive decline. Incidental LBs in the SN were described in the occasional subjects, with no αS pathology in the lower brainstem.

Key Words: α-synuclein; Alzheimer disease; Amygdaloid complex; Lower brainstem; Nucleus basalis of Meynert; Substantia nigra.

INTRODUCTION

The 2 main classes of neurodegenerative disorders are known as tauopathies and synucleinopathies (1). They display a significant overlap in both their clinical and pathological features, indicative of a common pathogenesis (2, 3), although this pathogenesis and possible interrelationship remains obscure. It is now well-established that Lewy bodies (LBs) are often found together with classical Alzheimer disease (AD) changes (neuritic plaques and neurofibrillary tangles) in the brains of patients with AD, dementia with Lewy bodies or Parkinson disease (4–7). Furthermore, the main component of LBs, α-synuclein (αS), is frequently found in both familial and sporadic AD as well as in Down syndrome (8–11). In these cases, the amygdaloid complex (AC) has been reported to be most vulnerable, sometimes being the only area affected with αS pathology (10). This finding led to the conclusion that AC is the most consistent area of LB formation in AD. Furthermore, the first changes in incidental LB disease were recently described in the lower brainstem (12). However, in the spectrum of LB disorders, the substantia nigra (SN) has until now been considered to be the region primarily affected. In dementia with Lewy bodies, cortical LBs largely affect the limbic system and also affect the cholinergic forebrain system, including the nucleus basalis of Meynert (nbM) (13–15). Therefore, we wanted to examine the immunoreactivity of αS in the SN, the nbM, and the AC in well-characterized postmortem material and to evaluate the relationship of αS positivity to AD pathology. The lower brainstem was also examined in subjects with αS pathology in either basal forebrain and/or midbrain.

MATERIALS AND METHODS

Case Selection

A total of 904 subjects, aged >40 years at death, were included in this study. The postmortem brains were sampled during the years 1996 to 2000 (Kuopio Brain Bank, Kuopio, Finland) from distinct cohorts previously described in detail (16). These cohorts included a longitudinal follow-up study of Alzheimer-type dementia and a prospective clinical study of aging. The rest of the subjects were derived from a cohort of consecutive clinical autopsy cases collected for 1 year, a cohort of forensic autopsy cases collected for 6 months, and a cohort from Kuopio Brain Bank material that was not part of any of the above studies. In subjects with cognitive decline, the clinical diagnosis of dementia was based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) and those of the Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition, 1987 (17).

Neuropathological Assessment

Following the dissection protocol used at the section of Neuropathology at Department of Neuroscience and Neurology at Kuopio University, the brains were weighed, evaluated for grossly detectable lesions and vessel abnormalities, fixed in 10% buffered formalin for at least 1 week, and cut into coronal slices. Brain specimens taken from 15 cortical and subcortical regions were embedded in paraffin and the sections were routinely stained by applying hematoxylin and eosin (H&E) and modified Bielschowsky silver impregnation. All cases were classified into neuropathological groups as recommended by the Consortium to Establish a Registry for Alzheimer Disease (CERAD) (18).
Fig. 1. Digital photographic images of the basal forebrain (A) and the midbrain (B) sections. Scale bars: 5 mm. Low magnification (×100) photomicrographs of α-synuclein-immunopositive structures in (C) nucleus basalis of Meynert (nbM), (D) amygdaloid complex (AC), and (E) substantia nigra (SN).

Immunohistochemistry

αS expression was visualized using immunohistochemical methodology in brain samples from 904 subjects taken from the basal forebrain, including the nbM and AC (Fig. 1A), and from the midbrain, including the SN (Fig. 1B). For the 121 subjects that showed αS-positive structures in either of these brain regions, additional blocks were also taken from the lower brainstem from the pons at the level of locus ceruleus and from medulla at the level of glossopharyngeal nerve. Seven-μm-thick sections were deparaffinized, rehydrated according to routine procedure, and pretreated with 80% formic acid for 1 hour. For the used antibody, this pretreatment was assessed to be a better antigen retrieval method than autoclaving in citrate buffer or protease pretreatment. Moreover, this method labeled not only inclusions and neurites but also pale bodies. Following pretreatment, the sections were incubated with normal goat serum for 30 min at room temperature to block nonspecific reactions. Monoclonal antibody to rat synuclein-1 (Transduction Laboratories, Lexington, KY) at a dilution of 1:1,000 was applied. The slides were then incubated overnight at 4°C. On the following day, the sections were incubated with biotinylated secondary antibody for 30 min followed by streptavidin-alkaline phosphatase conjugate (LAB-SA; Zymed Laboratories, South San Francisco, CA) for another 30 min at room temperature. The reaction product, streptavidin-biotin complex, was visualized using Vector-Red (Vector Laboratories, Burlingame, CA).

RESULTS

Overall, αS pathology was found in 121 (mean age at death was 76 ± 1 years, 36% demented) of our 904 subjects (mean age at death 70 ± 0.5 years, 17% demented) in either basal forebrain (n = 13), midbrain (n = 23), or in both (n = 85). αS-positive structures were seen in the SN in 108 subjects, in the nbM in 88 subjects, and in the
AC in 81 of 121 subjects (Fig. 1C–E). Further results related to the regional distribution of αS pathology in relation to AD pathology are given in the Table. Positive staining was seen in the 3 regions investigated (i.e. the SN, nbM, and AC) in over half of the cases (n = 66) with αS pathology (Table). Two of 3 investigated regions were affected in 24 subjects. In 19 subjects, αS pathology was seen in the SN and in nbM or AC, whereas in 5 subjects αS pathology was seen only in the basal forebrain (nbM and AC). In those subjects where αS pathology was found in only 1 brain area (n = 31), the SN was predominantly affected (n = 23). There was an equal number of subjects (n = 4) in whom αS-positive structures were found only in the nbM or AC.

This study included 82 patients that fulfilled the CERAD criteria for definite or probable AD, and none of these were familial. Twenty-six of these patients (32%) also had concomitant αS pathology (Table). Most showed widespread distribution of αS-positive structures in all or at least 2 of the 3 regions examined. In 1 subject, αS-positive structures were found only in SN, whereas in 2 subjects the αS immunopositivity was restricted to the AC.

Furthermore, half of the subjects with αS-positive structures (n = 59) showed AD pathology to some extent (Table). In cognitively unimpaired subjects with a moderate or frequent AD pathology (CERAD class of possible AD b), αS-positive structures were seen in 16 (14%) of 116 subjects that fulfilled the criteria in total material. Thus, if one excludes the clinical symptoms, concomitant αS-positive structures were seen in 21% of the 198 subjects with pronounced AD pathology.

Clarification

Most of the subjects in which the SN or nbM were the sole affected regions presented no clinical signs of dementia (87% and 100%, respectively). In contrast, when the AC was the only affected region, 75% of the subjects showed clinical signs of cognitive impairment (Table).

The lower brainstem was affected in 91 of 116 subjects that had αS-positive structures in either basal forebrain or midbrain. Five subjects were excluded from the analysis due to lack of sampled material. The predisposed sites included the dorsal vagus nucleus (n = 71), the reticular formation (n = 71), the locus ceruleus (n = 65), and the raphe nuclei (n = 61). In the subjects in whom only the nbM or AC or basal forebrain (both nbM and AC) were affected (n = 13), 2 subjects had few αS-positive structures in the vagus. In the subjects in whom only the SN was affected (n = 23), 17 had αS-positive structures also in lower brainstem nuclei. Thus, 6 subjects had αS pathology in the SN only.

DISCUSSION

To our knowledge, this is the first study that has analyzed the regional distribution of αS pathology in both unimpaired and demented aged individuals with or without concomitant AD changes. αS pathology in the form of LBs and Lewy neurites (LN s) was found in 13% of our total 904 subjects by screening the basal forebrain and midbrain structures. Overall, the most vulnerable region was the SN (89%), followed by the nbM (73%) and AC (67%). Most laboratories routinely use H&E staining or αS immunohistochemistry to detect LB pathology in the SN, followed, if necessary, by cortical assessment. Our results confirm that αS-positive structures are most readily found in the SN. However, there are also other predilection sites that should be recognized in the postmortem assessment (10, 12).

An intense debate about the most vulnerable region(s) affected with αS pathology was initiated by the studies that reported the AC to be the most consistent area of LB formation in AD (8–11). Another striking discovery was that a vast number of AD patients had αS pathology. The presence of LBs determined by αS immunohistochemistry was detected in the AC of 60% of early-onset familial AD patients and in 50% of Down syndrome patients with AD (8, 9). Furthermore, a study by Hamilton reported αS-positive structures in the AC of 61% of a large cohort of sporadic AD cases diagnosed using CERAD criteria for “definite AD” (10). Also, Araújo et al found that 48% of sporadic AD cases possessed αS-positive structures, most frequently found in the AC (11). In this study, 32% of sporadic AD cases showed αS-positive structures in any of the regions examined, which is rather similar to the results (35%) recently reported by Togo et al (19). The AC was affected in 30% of our AD cases, which is only half of what was previously reported by Hamilton (10). Furthermore, complete staining results for the SN were not reported in Hamilton’s study, although in none of the examined cases were LBs detected only in the brainstem. In contrast, our study included 1 patient fulfilling the CERAD criteria for “probable AD” in whom αS-positive structures were restricted to the SN. In agreement with the study by Hamilton (10), we found 4 subjects in whom the only region affected with αS-positive structures was the AC. Admittedly, most of these “AC only” subjects were cognitively impaired in contrast to those where the αS pathology was restricted to the SN or nbM. However, it is noteworthy that not all “AC only” αS-positive cases had concomitant AD changes. Thus, the selective vulnerability of the AC is not inevitably associated to AD pathology, whereas it seems to be related to cognitive impairment.

The issue of the relationship between αS and AD pathology is complex. Indeed, the present study confirms that αS pathology frequently coexists with AD pathology. However, our results do not support the finding that this occurs as a result of some common interrelated pathogenesis. In contrast to previous reports, αS-positive structures were not seen in the majority (68%) of sporadic AD
Regional Distribution of αS Pathology in Relation to Cognitive Impairment and AD Pathology

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Abbreviations: AC, amygdaloid complex; AD, Alzheimer disease; CERAD, Consortium to Establish a Registry for Alzheimer Disease; norm a, no histological evidence of AD/no clinical history of dementia; norm b, histological evidence of AD is uncertain/no clinical history of dementia; possADb, histological evidence is suggestive or indicative of AD/no clinical history of dementia; norm c, no histological evidence of AD/c clinical history of dementia; possADa, histological evidence of AD is uncertain/clinical history of dementia; prAD, histological evidence is suggestive of AD/clinical history of dementia; defAD, histological evidence is indicative of AD/clinical history of dementia; nbM, nucleus basalis of Meynert; SN, substantia nigra.

cases. Thus, the fact that these 2 entities often coexist does not necessarily mean that one is caused by the other. Interestingly, there was an over 2-fold increase in the prevalence of αS pathology among the subjects with a similar burden of AD pathology, but with or without cognitive impairment (32% vs 14%, respectively). This further emphasizes the role of αS pathology as a substrate for dementia. Recently, coexisting neuritic AD pathology was significantly associated with dementia and poor survival in patients with Parkinson disease (20). Our impression is that in subjects with extensive AD pathology those with concomitant αS pathology rather than those without are cognitively impaired.

A recent study reported that extranigral sites in the lower brainstem are affected with αS pathology prior to SN involvement (12). The first αS-positive LBs and LNs appeared to show a preference for the dorsal motor and pigmented nuclei of the glossopharyngeal and vagal nerves, intermediate reticular zone, gain-setting nuclei, and anterior olfactory nuclei. Therefore, we analyzed the lower brainstem in our αS-positive subjects in order to estimate whether these areas were affected, which was the case for most of them (79%). Even in some of the subjects in whom αS-positive structures were restricted to the basal forebrain, few positive LBs and LNs in the vagus were identified. However, we also discovered that 6 subjects with αS-positive structures only in the SN lacked LBs or LNs in the lower brainstem nuclei. All of these subjects were free of AD pathology and showed no history of psychiatric or neurological disorder. Therefore, in contradiction to the former study (12), we found subjects with incidental LB pathology solely in the SN.

In our study, we could not identify a single predilection or induction site for αS pathology. The basal forebrain, midbrain, and lower brainstem seem to be equal candidates so far. Remaining issues to be resolved are whether there is a specific induction site for the aggregation of αS, and whether a hierarchical progression of αS pathology in the brain can be identified. Furthermore, it is of crucial importance to determine whether a specific induction site defines the developing clinical symptoms, i.e. dementia versus parkinsonism.

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REFERENCES


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