J Neuropathol Exp Neurol
Vol. 70, No. 11
November 2011
pp. 960–969
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ORIGINAL ARTICLE

Stages of the Pathologic Process in Alzheimer Disease: Age Categories From 1 to 100 Years

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Abstract

Two thousand three hundred and thirty two nonselected brains from 1- to 100-year-old individuals were examined using immunocytochemistry (AT8) and Gallyas silver staining for abnormal tau; immunocytochemistry (4G8) and Campbell-Switzer staining were used for the detection of β-amyloid. A total of 342 cases was negative in the Gallyas stain but when restaged for AT8 only 10 were immunonegative. Fifty-eight cases had subcortical tau predominantly in the locus coeruleus, but there was no abnormal cortical tau (subcortical Stages a–c). Cortical involvement (abnormal tau in neurites) was identified first in the transentorhinal region (Stage 1a, 38 cases). Transentorhinal pyramidal cells displayed pretangle material (Stage 1b, 236 cases). Pretangles gradually became argyrophilic neurofibrillary tangles (NFTs) that progressed in parallel with NFT Stages I to VI. Pretangles restricted to subcortical sites were seen chiefly at younger ages. Of the total cases, 1,031 (44.2%) had β-amyloid plaques. The first plaques occurred in the neocortex after the onset of tauopathy in the brainstem. Plaques generally developed in the 40s in 4% of all cases, culminating in their tenth decade (75%). β-amyloid plaques and NFTs were significantly correlated ($p < 0.0001$). These data suggest that tauopathy associated with sporadic Alzheimer disease may begin earlier than previously thought and possibly in the lower brainstem rather than in the transentorhinal region.

Key Words: Alzheimer disease, β-amyloid, Brainstem, Hyperphosphorylated tau protein, Locus coeruleus, Pretangles/neurofibrillary tangles, Neuropil threads.

INTRODUCTION

From beginning to end, the pathologic processes underlying sporadic (versus familial) Alzheimer disease (AD) are confined to the human CNS and chiefly include intraneuronal formation of abnormal tau protein and extracellular deposition of β-amyloid protein (1). Alzheimer disease–related lesions develop at given predilection sites within the brain and progress according to a predictable sequence from there to hitherto uninvolved areas (2–7).

Once initiated, the process progresses for decades without remission until it crosses a threshold to clinically recognizable dysfunction (6). Recently, we found that intraneuronal lesions associated with AD occur before puberty or in early young adulthood and most often affect noradrenergic projection neurons of the locus coeruleus, one of several subcortical nuclei that generate diffuse projections to the cerebral cortex (8). Abnormal tau can be visualized by immunohistochemistry with the antibody AT8, which recognizes a phosphate-dependent epitope at serine 202 and threonine 205 (9). Alzheimer disease may begin with misfolded and abnormally phosphorylated tau protein in the proximal axon of caeruleus projection neurons (8). Thereafter, similar material fills the somatodendritic compartment of involved cells. This soluble and nonargyrophilic ''pretangle'' material gradually aggregates into insoluble fibrillary and argyrophilic neuropil threads (NTs) in dendritic processes and into neurofibrillary tangles (NFTs) in neuronal somata. These inert neurofibrillary changes of the Alzheimer type are resistant to autophagy and other endogenous cellular removal mechanisms (10–15).

To obtain greater insight into the AD-related pathologic processes in a large cohort, tau lesions were reexamined in 2,332 nonselected autopsy cases ranging in age from 1 to 100 years. Particular attention was paid to a subpopulation consisting of 342 cases, which, when they were studied with only the Gallyas silver technique, lacked argyrophilic intraneuronal lesions (NFTs/NTs) at the typical cortical predilection sites. Subsequently, all 342 cases were assessed by immunohistochemistry for abnormally phosphorylated tau protein (AT8). Sections of 100 μm thickness were made through medial portions of the temporal lobe and through the midbrain and pontine tegmentum to assess brainstem nuclei. The new findings presented here supplement data from an earlier report regarding the frequency of AD-associated argyrophilic lesions in the cerebral cortex in individuals ranging from 26 to 95 years (3).

MATERIALS AND METHODS

Study Cohort

This retrospective autopsy study was performed in compliance with university ethics committee guidelines as well as

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This study was supported by the German Research Council (Deutsche Forschungsgemeinschaft, Grant Nos. TR1000/1-1 and TH624/4-1) and the Alzheimer Research Initiative (Alzheimer Forschung Initiative, Grant No. 10810).

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German federal and state law governing human tissue usage. Consent for autopsy was obtained for all cases. Brains had been obtained at autopsy and included nonselected cases from affiliated university hospitals. One individual (17-year-old adolescent male) had Down syndrome. The only exclusionary criterion was the presence of tauopathy other than AD or argyrophilic grain disease (AGD), such as Niemann-Pick disease type C, subacute sclerosing panencephalitis, progressive supranuclear palsy, Pick disease, or corticobasal degeneration (16). Neuropathologic diagnoses for all cases were made for AD and AGD, as described (2, 4, 17–19). The 2,332 cases were grouped into 10 age categories by decade (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273). The ratio of females to males in each age category is shown in Table 1.

Tissue Fixation, Embedding, and Sectioning

Brainstems and at least a single hemisphere from all individuals were fixed by immersion in 4% buffered aqueous formaldehyde. A set of 2 tissue blocks was excised, embedded in polyethylene glycol (PEG 1000; Merck, Darmstadt, Germany), and sectioned at 100 μm, as previously described (4, 8, 20). This section thickness allows for the superimposition of multiple structures, including nerve cells with their entire dendritic tree. The first block was cut at miduncal level through medial portions of the temporal lobe and encompassed anterior (i.e. uncal) portions of the hippocampal formation and the parahippocampal gyrus (entorhinal region), including the adjoining transentorhinal region as well as portions of the occipitotemporal gyrus and additional gyri of the basal temporal neocortex. The second block was cut through the occipital lobe perpendicular to the calcarine fissure and included high-order visual association areas (peristriate region), a first-order visual association area (parastriate area), and the primary visual field (striate area) (4).

From 342 cases that were negative in the Gallyas stain (i.e. they displayed no argyrophilic NFTs/NTs in the cerebral cortex, particularly in the transentorhinal region), a set of additional blocks was cut perpendicular to the brainstem axis of Meynert. One block was cut through posterior portions of the medulla oblongata at the level of the dorsal motor nucleus of the vagal nerve. A second block showed the pontine tegmentum and contained portions of the locus coeruleus and the dorsal raphe nucleus. The third block was excised at the level of the inferior colliculus and contained posterior portions of the substantia nigra and supratrochlear portions of the dorsal raphe nucleus (8, 21).

TABLE 1. Distribution of All Cases by Sex and Age Groups

Age Category, y	Females (n = 1,049) N	Females (n = 1,049) %	Males (n =1,283) N	Males (n =1,283) %	Total (n = 2,332)
1–10	3	37.5	5	62.5	8
11–20	5	20.8	19	79.2	24
21–30	28	45.9	33	54.1	61
31–40	47	47.0	53	53.0	100
41–50	90	47.9	98	52.1	188
51–60	112	33.9	218	66.1	330
61–70	182	37.0	310	63.0	492
71–80	276	48.4	294	51.6	570
81–90	259	53.5	225	46.5	484
91–100	47	62.7	28	37.3	75

Staining and Immunocytochemistry

Collections of free-floating sections from all blocks for each case were processed with the following techniques: Pigment-Nissl staining served to show the presence and extent of lipofuscin deposits (aldehyde fuchsin) and basophilic material (Darrow red) (20) and silver staining with the Campbell-Switzer and Gallyas methods to exploit the physical development of nucleation sites. These advanced silver techniques are reliable and are used to visualize β-amyloid deposition (Campbell-Switzer) and argyrophilic neurofibrillary lesions (Gallyas) (4, 17, 19, 20, 22).

Immunohistochemistry included the use of the following antibodies: monoclonal antibody PHF-Tau (1:2000; Clone AT8; Pierce Biotechnology, Rockford, IL) detected hyperphosphorylated tau protein in pretangle material and Alzheimer-type neurofibrillary changes; monoclonal anti–β-amyloid antibody (1:5000; Clone 4G8; Covance, Dedham, MA) was used for the detection of β-amyloid deposition. β-amyloid plaque phases were assessed as previously published (5). All immunohistochemistry procedures could be performed on the material that had been stored for long periods in formaldehyde (23).

Tissue sections for immunoreactions were treated for 30 minutes in a mixture of 10% methanol plus 10% concentrated (30%) H_2O_2 and 80% Tris. After pretreatment with 100% formic acid for 3 minutes to facilitate the β-amyloid immunoreactions, blocking with bovine serum albumin was performed to prevent nonspecific binding. Subsequently, each of the various sets of free-floating sections was incubated for 18 hours at 20°C using the primary antibodies. After incubation with secondary biotinylated antibody (anti-mouse IgG, 1:200; Vector Laboratories, Burlingame, CA) for 1.5 hours, immunoreactions were visualized with the avidin-biotin complex (ABC, Vectastain; Vector Laboratories) for 2 hours and the chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB D5637; Sigma, Taufkirchen, Germany). Omission of the primary antibody resulted in nonstaining. Positive and negative control sections were routinely included. The tissue sections were cleared and mounted in a synthetic resin (Permount; Fisher, Fair Lawn, NJ). All sections were viewed, and AD staging was performed with a Vanox AHB53 Olympus microscope (Olympus Optical Co., Tokyo, Japan). Digital micrographs were obtained using the Soft Imaging System (Münster, Germany).

Statistics

Statistical analysis was performed using the Student *t*-test for independent samples to compare normally distributed continuous variables between 2 groups. The Mann-Whitney *U* test was used to analyze between-group comparisons of ordered variables, and the Spearman ρ statistics was used for measuring rank correlations. Calculations for males and females were performed separately. Partial correlation analysis was performed to examine the relationship among different pathologic variables after adjusting for the effects of age. Computations were performed with the aid of IBM SPSS, release 19, 2010

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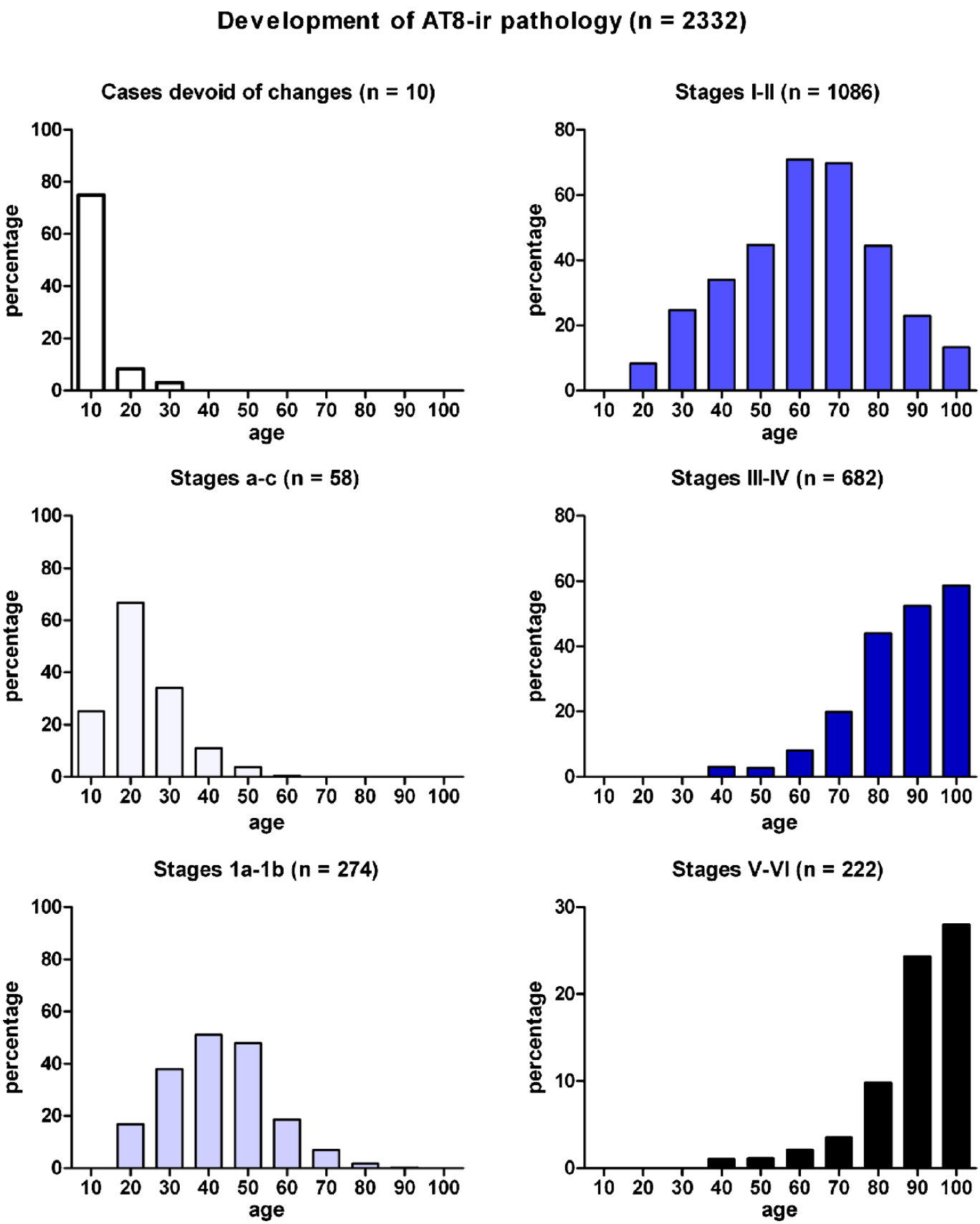

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(SPSS, Inc, Chicago, IL) and GraphPad prism V5.0 (GraphPad Software, San Diego, CA).

RESULTS

Absence of Intraneuronal Lesions

Complete absence of intraneuronal inclusions of AD-related abnormally phosphorylated tau protein within the brain was found in 10 of 2,332 cases; these are designated as ''cases devoid of changes'' in Figure 1 and ''no changes'' in Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273. These ''AD-free'' cases were encountered in young age categories; 6 persons were younger than 10 years; 2 were younger than 20 years; and the eldest were 22 and 23 years (Fig. 1, Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273). These cases did not exhibit β-amyloid plaques.

Intraneuronal AT8-Immunoreactive Abnormal tau Protein (Pretangle Material)

Pretangle Stage a

The first AD-related intraneuronal brain changes were seen in brainstem nuclei with diffuse projections to the cerebral cortex (24–29), most frequently in the locus coeruleus (8, 30–38). Alterations visible by light microscopy developed first in nerve cell processes, possibly the proximal portions of the axon close to the soma but outside the initial segment, where abnormally phosphorylated tau appeared abruptly without precursor or intermediate forms in those areas. The present cohort contained only cases in which immunoreactive neurites were limited to the pontine tegmentum in or close to the locus coeruleus; these are designated ''a'' in Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273. Forty of 2,332 cases were classified as pretangle Stage a cases and occurred mainly in young age groups.

Pretangle Stage b

Twelve cases (designated Stage b) showed involvement confined to the locus coeruleus; however, in these cases, involvement included somatodendritic compartments of locus coeruleus noradrenergic projection cells (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273). Normal projection neurons in the locus coeruleus have smoothly contoured cell bodies. By contrast, they often displayed spiked protrusions along their outer somatic rim in the Stage b cases. In addition, the AT8-immunoreactive (ir) pretangle material extended further into the axon and was also observed in more remote portions of the central tegmental tract (i.e. in the superior cerebellar peduncle and in the midbrain tegmentum lateral to the medial longitudinal fascicle). On the other hand, AT8-ir axons at the level of the dorsal motor nucleus of the vagal nerve within fiber bundles descending to the spinal cord were infrequently seen (8, 39).

All pretangle Stage a and Stage b cases exhibited AT8-ir material in the locus coeruleus in the absence of lesions in the anteromedial temporal cortex. Notably, none of these cases had isolated tau-ir neurons in any other subcortical nuclei with diffuse projections to the cerebral cortex (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273).

Pretangle Stage c

Six of 2,332 cases displayed more widely distributed subcortical lesions in the absence of cortical tau pathology. In this group, AT8-ir nerve cells were found not only in the locus coeruleus but also in other nonthalamic nuclei with cortical projections, such as nuclei of the upper raphe system or magnocellular nuclei of the basal forebrain. These cases are designated ''c'' (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273).

In summary, 58 of 2,332 cases (i.e. pretangle Stages a–c) did not show any cortical AT8-ir projection neurons (or portions thereof) in AD predilection sites of the temporal lobe, including the transentorhinal region (Figs. 1 and 2A, Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273) (8). Cases characterized by these 3 lesional distribution patterns usually occurred in young age groups. As anticipated, tissue sections from these pretangle stage cases stained for lipofuscin pigment and basophilic material did not reveal any obvious pathologic alterations, such as loss of basophilic material or displacement of cell nuclei to the periphery.

Pretangle Stages 1a and 1b

In addition to subcortical pathologic findings, 38 of 2,332 cases displayed mild cortical lesions consisting only of AT8-ir pretangle material in nerve cell processes; such lesions originally had escaped recognition in Gallyas silver–stained sections. These subtle lesions occurred in medial portions of the temporal lobe (particularly in the transentorhinal region) in structures that were most probably axons. Such cases are designated pretangle Stage 1a (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273). Remarkably, no cases in this group displayed even slight cortical pathologic findings in the absence of subcortical AT8-ir lesions.

Finally, in addition to the pretangle lesions described previously, 236 of 2,332 cases showed the presence of AT8-ir nonargyrophilic pyramidal cells in the cerebral cortex. Some cases displayed only a single affected pyramidal cell, whereas others exhibited greater numbers of involved neurons. The pretangle material filled the entire somatodendritic domain of the pyramidal cells; no intermediary abnormal forms of the pathologic changes were detectable. Affected neurons occurred preferentially in the transentorhinal region, in which abnormal tau material was confined to cortical projection cells and was not present in local circuit neurons or nonneuronal cells. Such cases are designated in Table 2 as pretangle Stage 1b. All 1a and 1b cases (n = 274) had subcortical lesions similar to those

FIGURE 1. Development of abnormal intraneuronal tau deposits in 2,332 nonselected autopsy cases. White columns represent the relative frequency of cases devoid of any tau deposits. Pale blue columns show the development of subtle subcortical lesions in cases with Stages a to c pathology. Columns in medium blue show an extension of these nonargyrophilic lesions into portions of the cerebral cortex (Stages 1a and 1b). Development of the pretangle material into argyrophilic neurofibrillary lesions characterizes Stages I to VI as follows: deep blue for Stage I and II cases, dark blue for Stage III and IV cases, and black for Stage V and VI cases.

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seen in Stages a to c. Nonargyrophilic lesions in the cerebral cortex did not occur in the absence of AT8-ir subcortical pathologic finding. Their prevalence increased during the second and third decades and was maximal in the fourth decade (Fig. 1). From the fourth decade onward, all individuals in the cohort had some degree of AD-associated lesions (Fig. 2A).

Intraneuronal Gallyas-Positive Abnormal tau Protein: Neurofibrillary Stages I to VI

Cases displaying AT8-ir and Gallyas-positive material are indicated by roman numerals in Figure 2A (see also Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273). The somatodendritic pretangle material had become insoluble and argyrophilic, forming neurofibrillary lesions, i.e. dendritic NTs and somatic NFTs. The first lesions were usually seen in the transentorhinal region (2). Argyrophilic lesions at subcortical sites were observed only in cases with advanced NFT stages. In NFT Stages I to VI, nerve cells filled with pretangle material were also present. Gallyas-positive neurons alone (i.e. absence of cells with pretangle material) were not observed in this cohort. It is known that nerve cells with NFTs/NTs survive for years (40, 41). However, they die prematurely, and extraneuronal remnants of the argyrophilic material remain as ''ghost'' tangles in the neuropil thereafter (42). Here, ghost tangles were only present in combination with recently formed intraneuronal NFTs and never in isolation. Moreover, ghost tangles were observed only in cases with advanced NFT stages.

The 589 NFT Stage I cases were characterized by low numbers of Gallyas-positive pyramidal cells that were predominantly in the transentorhinal region. The 497 NFT Stage II cases had additional lesions in both the entorhinal region proper and hippocampal formation. Early (i.e. pretangle Stages a to c and Stages 1a and 1b, NFT Stages I and II) AD-related intraneuronal lesions were observed chiefly in young age categories (Fig. 2A; Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273). The intraneuronal pathologic findings during these early stages were not accompanied by insoluble extracellular amyloid deposits with the exception of a 17-year-old adolescent male with Down syndrome (8).

There were 491 NFT Stage III cases that displayed a progression of the intraneuronal lesions into the basal neocortical areas of the temporal lobe; in 191 NFT Stage IV cases, they reached insular and basal frontal areas. The 138 NFT Stage V cases displayed involvement of nearly the entire prefrontal cortex as well as the high-order sensory association neocortex, whereas in the 84 NFT Stage VI cases, the premotor and primary motor areas as well as sensory first-order association areas and primary fields were affected. The prevalence of late NFT Stages (V and VI) increased with age (Fig. 1A; Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273). NFT Stages V and VI (222 cases) were frequently combined with late stages of β-amyloid deposition (Table 2 and Fig. 2B) (3).

Extracellular β-Amyloid Protein Aggregation

All 1,031 cases that displayed the presence of β-amyloid plaques also showed abnormal tau protein in specific nerve cell populations. Notably, in this cohort of 2,332 individuals, no β-amyloid deposits were observed in the 10 cases with no AD-related intraneuronal tau protein changes. With the exception of the 17-year-old adolescent male with Down syndrome with advanced β-amyloid pathologic lesions and stage a pretangle lesions, β-amyloid plaque deposition began to appear between ages 30 and 40 years, a point when such cases already exhibited pretangle Stage 1a or 1b (Fig. 2B and Table 2). The prevalence of β-amyloid plaques assessed according to Thal et al (5) increased in higher age groups (Fig. 2B and Table 2; Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A274) (2, 5, 6).

Statistical Analyses

Males were overrepresented across all age decades ranging from 1 to 80 years (58% males), whereas females were only predominant at ages older than 80 years (54.7% females) (Fig. 2B and Table 1). The cohort contained a greater number of older females than older males (Student *t*-test, $p < 0.0001$), and there were more younger males than younger females. The severity of AD-related pathologic findings (AT8 pathologic findings, NFTs, and β-amyloid) was significantly higher in females than in males (all $p < 0.0001$, Mann-Whitney *U* tests), possibly attributable to the predominance of females at higher ages. There was a significant correlation between β-amyloid deposits and NFT pathologic findings in both females and males (females, $r = 0.69$, $p < 0.0001$; males, $r = 0.5$, $p < 0.0001$), between β-amyloid deposition and AT8 pathology (females, $r = 0.64$, $p < 0.0001$; males, $r = 0.52$, $p < 0.0001$), and between AT8 and AGD pathologic findings (females, $r = 0.51$, $p < 0.0001$; males, $r = 0.56$, $p < 0.0001$), even after controlling

TABLE 2. Distribution of Amyloid-β (Aβ) Pathology by AT8-Immunoreactivity Pathology

	Aβ Pathology					
AT8-Immunoreactivity Pathology	**Phase 0 (n = 1,301, 55.8%)**	**Phase 1 (n = 350, 15.0%)**	**Phase 2 (n = 418, 17.9%)**	**Phase 3 (n = 177, 7.6%)**	**Phase 4 (n = 86, 3.7%)**	**Total (n = 2,332, 100%)**
0	10 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (100)
a–c	57 (98.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.7)	58 (100)
1a–1b	252 (92.0)	14 (5.1)	8 (2.9)	0 (0.0)	0 (0.0)	274 (100)
I	448 (76.1)	89 (15.1)	48 (8.1)	4 (0.7)	0 (0.0)	589 (100)
II	275 (55.3)	112 (22.5)	98 (19.7)	10 (2.0)	2 (0.4)	497 (100)
III	203 (41.3)	96 (19.6)	156 (31.8)	25 (5.1)	11 (2.2)	491 (100)
IV	49 (25.7)	28 (14.7)	69 (36.1)	33 (17.3)	12 (6.3)	191 (100)
V	7 (5.1)	9 (6.5)	30 (21.7)	64 (46.4)	28 (20.3)	138 (100)
VI	0 (0.0)	2 (2.4)	9 (10.7)	41 (48.8)	32 (38.1)	84 (100)

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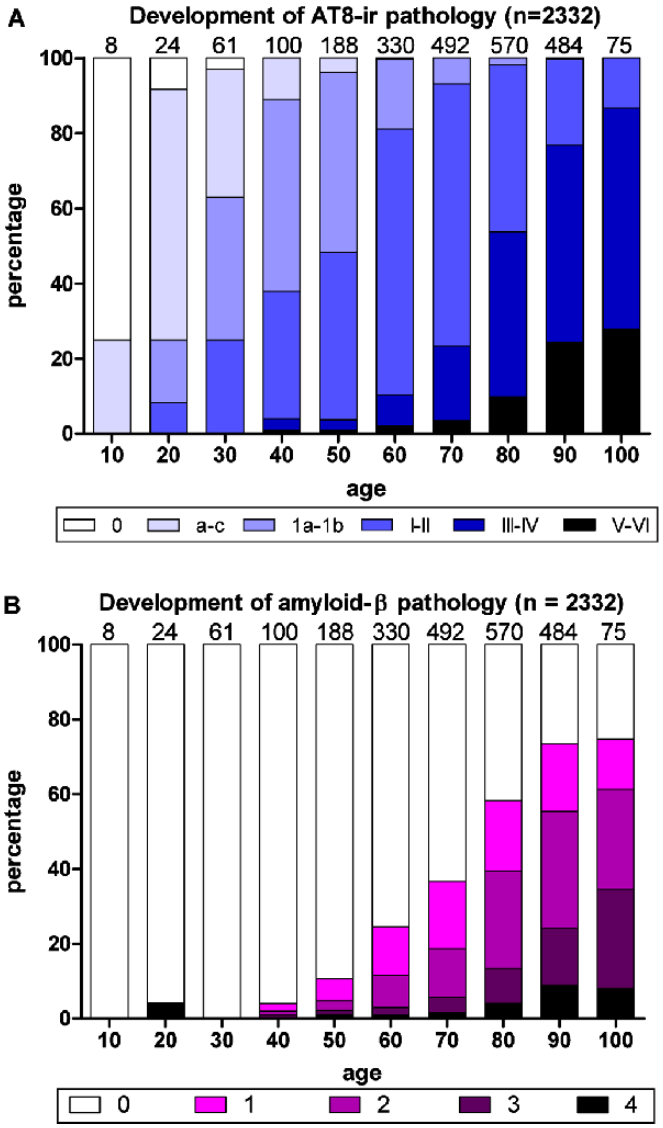

FIGURE 2. Development of AT8-immunoreactivity (ir) versus β-amyloid pathologic findings. **(A)** White columns indicate the relative frequency of 2,332 nonselected autopsy cases devoid of any abnormal intraneuronal tau deposits. Columns in shades of blue indicate the relative frequency of cases with all types of intraneuronal lesions. **(B)** Development of extracellular β-amyloid deposits. Purple areas within the columns indicate subgroups of cases showing plaque-like β-amyloid deposits in temporal neocortex (Phase 1, light purple), allocortex and neocortical association areas (Phases 2 and 3, middle purple and dark purple), or in virtually all cerebral cortical regions (Phase 4, black). Note the relatively late appearance of β-amyloid plaques.

for the effects of age (data not shown). In contrast, the correlation between argyrophilic NFT and AGD pathologic findings was diminished after controlling for age ($r = 0.07$, $p = 0.1$).

There was a significant increase of both AT8 and β-amyloid pathologic findings with age (Fig. 3). The slopes of the regression lines between age and AT8 pathologic findings were similar for both sexes ($p = 0.23$; Fig. 3). However, the slope of the regression line between age and β-amyloid deposition was greater ($p < 0.001$) in females than in males, indicating that the age-associated increase in β-amyloid burden is greater in females (Fig. 3; Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A274).

DISCUSSION

Clinically recognizable AD has long been viewed as a disorder closely associated with old age. In fact, many consider it to be caused by the aging process itself or, at the very least,

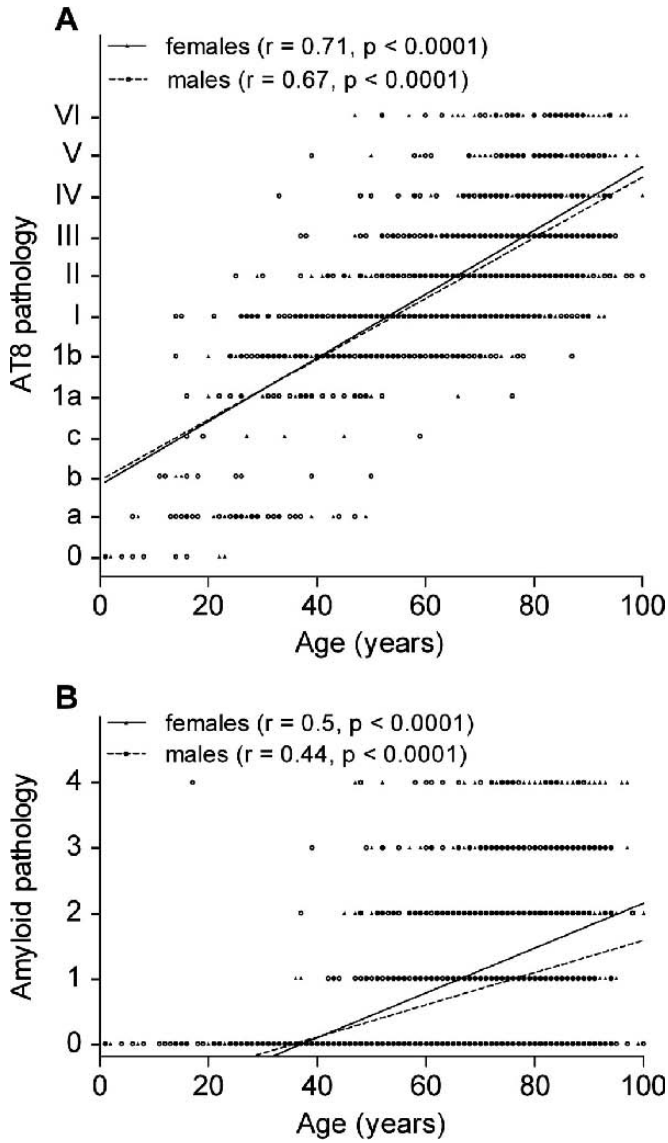

FIGURE 3. Scatter plots of AD-related pathologic findings (AT8 and β-amyloid) by age with separate regression lines for males and females. **(A)** There is a significant correlation between AT8 pathology and age, but there is no significant difference between the slopes of the regression lines of males and females ($p = 0.23$). **(B)** There is a significant correlation between β-amyloid pathology and age in males and females; the slope of regression line between age and β-amyloid deposition was greater ($p < 0.001$) in females than in males.

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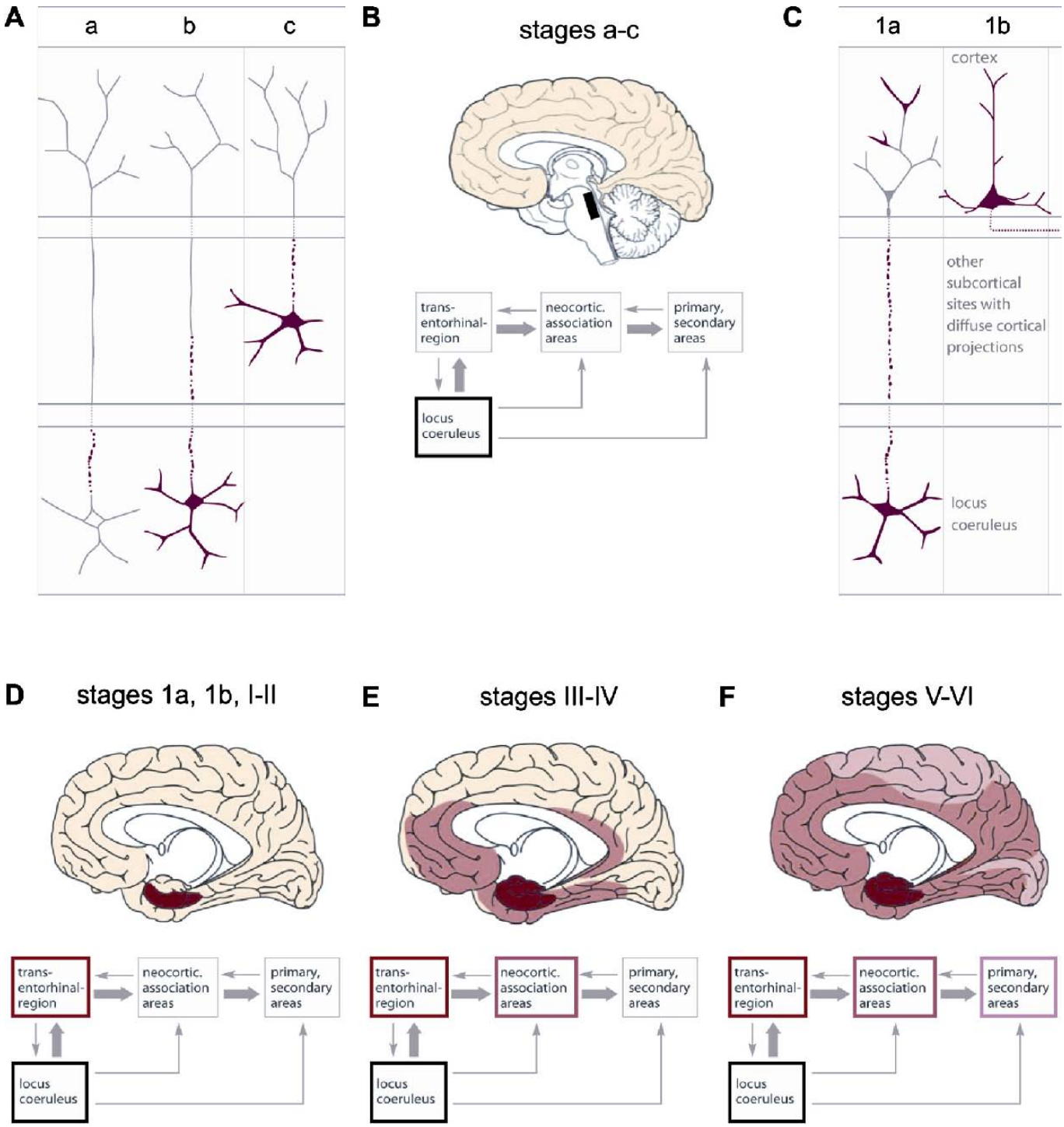
indirectly attributable to aging (43–45). Age-related factors that can damage postmitotic cells or can exert noxious influences on them are thought to play a central role in the pathogenesis of AD (13, 46–48). Yet it should be emphasized in this context that, in AD, not all of the known types of postmitotic cells inside and outside the CNS become involved in the disease process. Even when the discussion is confined to the brain, it quickly becomes clear that AD does not indiscriminately involve all neuronal types; the pathologic process is a remarkably selective one in that it develops in only a minority of neuronal types while sparing others (49, 50).

It also has been pointed out that the disease process in AD requires an inordinately long prodromal period that lasts for 5 or more decades (51–54). Our current findings indicate that the AD-associated process may manifest itself in young individuals of both sexes as well as in the absence of



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any harmful influences clearly attributable to ''old age'' (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273). In addition, pretangle and NFT-bearing nerve cells are capable of surviving into the ninth decade (40, 41). Thus, advanced age per se is not a precondition for the formation of the AD-associated tau lesions; rather, there is now strong evidence that the pathologic process underlying AD is not ''age-dependent'' but an uncommonly slowly progressive one that frequently extends into old age (55).

We observed 52 cases in younger age groups with abnormal tau only in noradrenergic projection neurons of the locus coeruleus and, initially, only in portions of neuronal processes, probably proximal axons (pretangle Stage ''a''; Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273). In these cases, the first pretangle material was identified without immunohistochemically detectable precursor or intermediary forms. During formation, neither the axonal initial segment nor the somatodendritic compartment of affected nerve cells showed any traces of pretangle material. In other words, there is no patent evidence for a transfer of pretangle material from the soma of involved cells into the affected neuronal process. As such, our findings are in accord with the assumption that the pretangle material that purportedly occurs in axons originates from normal tau proteins bound to axonal microtubules. Nevertheless, inasmuch as the shape and diameter of the involved nerve cell processes remained unchanged, the present results do not support the tau microtubule hypothesis, which claims that hyperphosphorylated tau becomes detached from microtubules and induces failed microtubule transport owing to the accumulation of improperly transported material (56–59).

Moreover, the soluble pretangle material did not seem to be incompatible with or toxic to cell functions because nerve cell nuclei or somatodendritic compartments of affected neurons did not display signs of acute reactive responses that would have indicated a life-threatening event (i.e. neuronal cell death) (60). Neurons do not undergo cell death during pretangle Phases a to c and 1a and 1b when they are producing large amounts of AT8-ir pretangle material (42, 61). The pretangles underwent modification during the lifetimes of the individuals examined until nonbiodegradable Gallyas-reactive NFTs/NTs became evident and, in the long run, the argyrophilic NFTs/NTs result in dysfunction and death of at least some neuronal types, for example, neurons of nuclei with extensive projections to the cortex and neurons in layer II of the entorhinal cortex and the first sector of the Ammon's horn (2).

The pretangle stages proposed here rest, in part, on the assumption that the AT8-immunoreactive lesions are the earliest (up to now immunohistochemically detectable) ones along a disease continuum. If one views sporadic AD as a dynamic process, it follows that pretangle pathology in individuals without clinically manifest or pathologically confirmed AD may represent neuropathologic markers of a condition that ultimately leads to the manifestations of clinical disease. A further assumption for staging of the pretangle lesions is that the pathologic process associated with NFT Stages I to VI increases in extent with disease duration. A potential methodological drawback to this approach is that the development of the pathology can only be reconstructed with the help of cross-sectional data obtained at autopsy. As such, the inferences drawn from these data permit only (but, arguably, admissible and reasonable) assumptions. Validated biologic markers that can be measured longitudinally could help to confirm, correct, or refute the existence and significance of the proposed pretangle phase (Fig. 4).

In conclusion, the results presented here corroborate those of a very recent study performed on a much smaller cohort, namely, the pathologic process associated with sporadic AD commences with intraneuritic formation of pretangle material in the lower brainstem rather than in the transentorhinal region (Fig. 4; Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A273) (8). That abnormal tau protein occurred in pretangle stages or early NFT stages without the presence of insoluble β-amyloid plaques (1,291/2,332 cases) means that not only a rethinking of currently existing neuropathologic staging NFT categories for AD is necessary but also a rethinking of the hypothesis that β-amyloid drives AD pathogenesis and secondarily induces the formation of abnormal tau protein (46, 62–68). Sporadic AD may be the result of two separate assaults: first, a tauopathy, possibly beginning in childhood; and second, negative influences of β-amyloid after a given threshold is crossed. β-amyloid might be capable of exacerbating the underlying tauopathy so that it develops into clinical AD (69–71). If the pretangle material is not regressive or transient (72), our findings may indicate that the pathologic process leading to abnormal tau pathology and ultimately capable of inducing NFT formation does not begin

FIGURE 4. Summary of stages in the development of Alzheimer disease (AD)–associated tau pathology. **(A)** Postulated phases in the development of early AD-associated tau pathology. Cellular processes of brainstem nerve cells (e.g. caeruleus neurons) are the earliest structures that display AT8-immunoreactive pretangle material (Stage a). The material fills the soma and dendritic processes of a few neuromelanin-containing caeruleus neurons (Stage b). In Stage c, pretangle material occurs in nerve cells of other nonthalamic brainstem nuclei with diffuse cortical projections (upper raphe nuclei, magnocellular nuclei of the basal forebrain, hypothalamic tuberomammillary nucleus). **(B)** The regions involved in Stages a to c are illustrated schematically and accompanied by a block diagram of key regions and their interconnectivity. Involvement of the locus coeruleus is indicated by black framing. **(C)** In Stage 1a, portions of neuronal processes containing pretangle material appear in the transentorhinal region. These processes may represent pathologically altered terminals of caeruleus axons. In Stage 1b, isolated pyramidal cell somata of the transentorhinal region together with their cellular processes become filled with pretangle material and, thereafter, increase in number. **(D–F)** Schemata as in **B** but without the brainstem illustrating the medial temporal regions. The 4 brain schemata are accompanied by color-coded boxes showing subcortical pretangle Stages a, b, and c (black); cortical pretangle Stages 1a and 1b (dark red); NFT/NT Stages I and II (dark red); NFT/NT Stages III and IV (medium red); and NFT/NT Stages V and VI (light red). The close interconnections by axonal projections between the locus coeruleus and transentorhinal region permits speculation as to whether disease progression of tau lesions in AD could be attributable to anterograde induction of tau pathology from one nerve cell to the next in the neuronal chain.

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in the transentorhinal region but in select subcortical nuclei; it may commence before puberty or in early young adulthood. Currently, too little is known about the pace at which the pathologic process develops. Some individuals were still at NFT Stages I and II at 90 years and older (Fig. 2A). Thus, although all individuals in this study who were 40 years and older exhibited pretangles (owing to a considerable variability in the rate of progression), only a proportion of them would have gone on to develop AD had they lived longer.

If other groups replicate our present findings and confirm that the AD-related tauopathy begins in noradrenergic projection neurons of the locus coeruleus, it might be possible to intervene with therapeutic means earlier than at present, that is, during the first decades of life, by protecting caeruleus projection cells and/or preventing them from developing the pretangle material (73–75).

ACKNOWLEDGMENTS

Autopsy material was supplied by the Braak Collection (Goethe University Frankfurt). The skillful technical assistance of Ms. Simone Feldengut (immunohistochemistry) and Mr. Stephan Mayer (Fig. 4) is gratefully acknowledged.

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