The relationship between subcortical tau pathology and Alzheimer’s disease

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Abstract

The stepwise progression of tau pathology [NFTs (neurofibrillary tangles) and NTs (neuropil threads)] in AD (Alzheimer’s disease) is generally assumed to begin in the transentorhinal region (entorhinal stage) from which it progresses to the hippocampus (limbic stage) and to neocortical regions (neocortical stage). This stepwise progression is reflected in the NFT Braak stages. However, it has been shown recently that tau pathology is frequently seen in subcortical nuclei, in particular the LC (locus coeruleus) in over 90% of individuals under 30 years of age, suggesting that AD-associated tau pathology begins in the LC and not in the transentorhinal region. On the other hand, only minimal amounts of tau pathology are seen in the LC in cases with considerable entorhinal tau pathology, while the severity of tau pathology in the LC significantly increases with increasing NFT Braak stages. These findings suggest that the LC becomes increasingly involved during AD progression rather than representing the site initially affected. Further studies are warranted to answer the question of whether tau pathology in the LC of young individuals is associated with AD or whether it rather reflects non-specific neuronal damage.

Introduction

AD (Alzheimer’s disease) is neuropathologically characterized by the presence of extracellular Aβ (amyloid β-peptide) as Aβ (senile) plaques and aggregates of hyperphosphorylated microtubules-associated protein tau in neuronal somata and neuronal processes in the form of NFTs (neurofibrillary tangles) and NTs (neuropil threads) respectively. The term neuritic plaques refer to Aβ plaques with dystrophic neurites that contain hyperphosphorylated tau. Of note, the presence of neuritic plaques is necessary for the neuropathological diagnosis of AD (for a review on AD neuropathology, see [1]), but neuritic plaques are not a feature of other tauopathies such as Pick’s disease, progressive supranuclear palsy, corticobasal degeneration and tangle-predominant dementia.

Although NFTs and NTs represent aggregated hyperphosphorylated tau that is stained by silver impregnation (e.g. Gallyas), abnormally phosphorylated non-aggregated tau is considered to be a precursor of NFTs/NTs and referred to as pre-tangles/pre-tangle material (pre-tangle). Immunohistochemical methods using appropriate antibodies (e.g. AT8) stain both NFTs and NTs, as well as pre-tangles.

The stepwise progression of NFTs and NTs in AD is assumed to follow a topographical pattern and is reflected in the NFT Braak stages [2,3]. Briefly, NFTs and NTs progress from the transentorhinal region (Braak stage I) to the entorhinal cortex (Braak stage II), the hippocampus and the tempo-occipital gyrus (Braak stage III), the temporal cortex (Braak stage IV), the parietal cortex (Braak stage V) and the occipital cortex (Braak stage VI). However, the concept of this stepwise progression beginning in the transentorhinal region has been challenged recently by the notion that tau pathology may be present in subcortical nuclei including SN (substantia nigra), LC (locus coeruleus) and dmX (dorsal motor vagal nucleus) of individuals under 10 years of age, and tau pathology in the LC is a constant feature in individuals over 30 years of age [4,5].

Here, we review data on the prevalence of tau pathology in various subcortical nuclei and discuss the relationship between subcortical tau pathology and AD.

Prevalence of subcortical tau pathology

On neuropathological post-mortem examination using immunohistochemistry with the AT8 antibody, hyperphosphorylated or abnormally phosphorylated tau is frequently detected in the LC of neurologically unimpaired individuals with the youngest ones being under 10 years of age [4–9]. Investigating a cohort of 42 individuals under 30 years of age [4,5],...
age, Braak and Del Tredici [4] recently demonstrated that AT8-ir (AT8 immunoreactivity) was confined to the LC in 14 individuals (33.3%), while the LC and up to three other selected subcortical nuclei (magnocellular nuclei of the basal forebrain, n=7; SN, n=5; upper raphe, n=2; dmX, n=1) were involved in eight (19.0%) individuals respectively. Sixteen (38.1%) individuals showed AT8-ir in the trans-/entorhinal cortex and subcortical nuclei that invariably included the LC. Taken together, 90.5% of individuals under 30 years of age in this study showed AT8-ir in the LC. Of note, Gallyas-positive NFTs that contain aggregated hyperphosphorylated tau was exclusively seen in the trans-/entorhinal cortex of four cases, but absent from other regions, including the LC [4].

In a subsequently published similar study on 95 cognitively unimpaired individuals (age range 22–50 years) Elobeid et al. [7] found tau pathology limited to the LC in 28 (29.5%) cases and to the transentorhinal cortex in three (3.2%) cases respectively, whereas another three (3.2%) cases showed tau pathology in both the LC and trans-/entorhinal cortex. It has to be emphasized that AT8-ir was assessed on 7-μm-thick sections in this study [7], whereas Braak and Del Tredici [4] used 100-μm-thick sections; sparse AT8-ir in the LC that would have been detected on 100-μm-thick sections might have been missed on sections that are 14 times thinner, thereby probably contributing to the much lower prevalence of 32.6% AT8-ir in the LC in the study by Elobeid et al. [7] compared with 90.5% in Braak and Del Tredici's study. This assumption is further supported by data from our own study [6] in which we used 5-μm-thick sections but stained five additional serial sections if the initial section did not show any AT8-ir, as we found AT8-ir in the LC in 44.4% of cases (total study cohort, n=239; for further details, see the Subcortical tau pathology in different NFT Braak stages section) [6].

In another very large cohort (n=2332; age range, 1–100 years; 55% male) Braak et al. [5] found that ten (0.4%) individuals completely lacked any AT8-ir (aged ≤23 years), whereas 58 (2.5%; aged <51 years) had AT8-ir pre-tangle material limited to subcortical nuclei, most frequently the LC, and 274 (11.7%) showed additional AT8-ir pre-tangle material in the cortex predominantly involving the transentorhinal region (age range 11–80 years). In the remaining 1990 (85.3%) individuals (age range 41–100 years) additional aggregated hyperphosphorylated tau was seen in cortical regions consistent with NFT Braak stage I or higher (I, 29.6%; II, 25.0%; III, 24.7%; IV, 9.6%; V, 6.9%; VI, 4.2%) [5].

In addition to the LC, other subcortical nuclei may show AT8-ir in individuals lacking cortical tau pathology. Grinberg et al. [8] investigated the prevalence of tau pathology in the DR (dorsal raphe) nucleus of 118 individuals and found that 21.1% of cases with NFT Braak stage 0 (n=38) and all cases with NFT Braak stage ≥I showed AT8-ir in the DR. Similarly, other authors suggested that earliest tau pathology is not seen in the cerebral cortex but in LC and DR [9–11]. Data on the frequency of AT8-ir in the SN in cases with Braak stage 0 range from 11.9% [4] to 44.4% [6], whereas respective data on the involvement of the dmX are limited (one out of two in [6] and one out of 42 in [4]).

### Subcortical tau pathology in different NFT Braak stages

Recently, we investigated both prevalence and severity of subcortical (SN and LC) tau pathology in 239 post-mortem brains (mean age, 82.8 years; S.D. ±9.7 years; range, 55–102 years; 102 male; 137 female) and compared these findings with respective NFT Braak stages [6]. In addition, the OB (olfactory bulb) and dmX were assessed in a subset of 156 and 46 cases respectively. The whole study cohort included 107 neuropathologically confirmed AD cases (44.8%), 76 non-demented controls (31.8%), 12 PD (Parkinson’s disease) cases without clinical dementia (5.0%) and six DLBs (dementia with Lewy bodies; 2.5%), whereas 38 clinically demented individuals (15.9%) showed neuropathological lesions consistent with both AD and Lewy body disease (i.e. PD or DLB, but excluding amygdala-only α-synuclein pathology and cases where α-synuclein pathology did not follow either Braak Lewy body staging [12] or McKeith criteria [13]).

AT8-ir structures (pre-tangles, NTs and NFTs) were semi-quantitatively scored. Of note, we used 5-μm-thick sections but from blocks of all of those regions that initially did not show any AT8-ir; an additional five slides of 5-μm thickness were assessed. Th-sc (thread scores) were determined for NTs and thread-like pre-tangle material and ta-sc (tangle score) for NFTs and pre-tangles. In brief (for details, see [6]), the severity of NTs and thread-like pre-tangle material was assessed semi-quantitatively using a five-tiered scale and called the th-sc: 0, absent (no positivity); 0.1, sparse (some dot or thread-like positivity <3 μm in diameter/length). It is to be noted that, 0.1 was usually only scored in serial sections of cases scoring 0 at primary assessment; 1, mild; 2, moderate; 3, severe. Similarly a semi-quantitative four-tiered scale was used to assess the severity of NFTs and pre-tangles and called the ta-sc: 0, absent; 1, mild; 2, moderate; 3, severe. A total tau score was calculated by adding individual th-sc and ta-sc values (Figure 1).

The region affected most frequently by tau pathology in the total cohort was the trans-/entorhinal cortex (92.5%) as reflected by the NFT Braak stage ≥I. With regard to the regions investigated that are not assessed by NFT Braak staging, tau pathology was most frequent in the OB (92.3%) followed by the LC (92.1%), SN (88.7%) and dmX (84.8%). In total, 44.4% of cases with NFT Braak stage 0 (n=18) showed minimal AT8-ir with a total tau score of 0.1 in both the SN and LC, except for one case that scored 5. The OB showed AT8-ir in nine out of 17 cases (52.9%) with Braak stage 0 and the dmX in one out of two. The prevalence of AT8-ir increased in all regions investigated with increasing NFT Braak stages being above 80% in NFT Braak stages III/IV and reaching 100% in Braak stages V/VI. Similarly,
Sparse tau pathology (tau score, 0.1 indicated by arrows) as reflected by AT8-ir is present in cases with NFT Braak stage 0 and I in the OB (A) and LC (B), whereas severe tau pathology (tau score 6) is usually seen in Braak stages V and VI (C, OB; D, LC).

All sections are stained with antibody AT8; original magnification: (A, B and C) \( \times 600; \) (D) \( \times 200. \) Scale bars, 20 \( \mu \text{m} \) (A, B and C); 50 \( \mu \text{m} \) (D).

Mean tau scores in both LC and OB increase with increasing NFT Braak stages

Spearman rank correlation revealed a statistically significant increase in tau scores (LC: \( \rho=0.592, P<0.001; \) OB: \( \rho=0.787, P<0.001 \)). Rings, outliers; asterisks, extreme values.

Total tau scores significantly increased with increasing NFT Braak stages, but they were low, usually not scoring above 2 in Braak stages I/II, and only in Braak stages IV or higher more severe AT8-ir was seen in subcortical nuclei (Figure 2). No influence of concomitant \( \alpha \)-synuclein pathology on AT8-ir in subcortical nuclei was detected [6].

**Implications for the progression of AD-related tau pathology**

The presence of tau pathology as pre-tangles in the LC in young individuals without any detectable form of hyperphosphorylated tau in the trans-/entorhinal cortex prompted the suggestion that AD-associated tau pathology begins in the LC. Consequently, this would imply a revision of the NFT Braak stages. Indeed, such a revision that includes pre-tangle stages has been introduced recently by Braak et al. [5]. According to this new staging system the earliest tau pathology is seen in pre-tangle stage ‘a’ where AT8-ir neurites are limited to the pontine tegmentum in or close to the LC, in pre-tangle stage ‘b’ AT8-ir extends to the somata of LC neurons and to cell processes of other subcortical nuclei, the neuronal somata of which become involved in pre-tangle stage ‘c’. Pre-tangle stages ‘a–c’ are followed by
pre-tangle stage ‘1a’ that is characterized by the presence of AT8-ir in cell processes of the trans-/entorhinal cortex and in pre-tangle stage ‘1b’ trans-/entorhinal neuronal somata become additionally involved. These pre-tangle stages are subsequently followed by the NFT Braak stages in which hyperphosphorylated and aggregated tau manifest as NFTs and NTs primarily in the transentorhinal region (Braak stage I) and finally reaching the occipital cortex (Braak stage VI).

This new staging system implies a pathogenetic link between AT8-ir in subcortical brainstem nuclei with diffuse projections to the cerebral cortex and cortical tau pathology in the form of NFTs and NTs that are associated with AD. A tempting explanation for this pathogenetic link would be the controversial hypothesis of a neuron-to-neuron transfer of hyperphosphorylated tau via seeding, maybe in a prion-like mode [14,15].

It has been suggested that sporadic AD might be the result of two primarily unrelated pathogenetic events [5]. According to this hypothesis the first pathogenetic process would be the tauopathy that begins in early childhood and the second one the accumulation of intracerebral Aβ over a threshold that exacerbates underlying tau pathology, ultimately leading to clinically overt AD. This hypothesis is supported by the finding of an Aβ-dependent increase in tau pathology in transgenic tau mice [16,17] and the significant correlation between Aβ and both NFTs and AT8-ir in the study by Braak et al. [5]. It is to be noted that this correlation remained statistically significant after controlling for age.

However, if AD-associated tau pathology primarily affects the LC, considerable amounts of tau pathology could be expected in the LC in stages where tau pathology has already spread to the cortical regions. This assumption is made in analogy to the findings in the NFT Braak stages: severe tau pathology is invariably present in the transentorhinal region, the region involved in NFT Braak stage I, in cases that show tau pathology in the entorhinal cortex (NFT Braak stage II) and this pattern is observed in all subsequent NFT Braak stages. Moreover, a continuous increase in the amount of hyperphosphorylated tau, as assessed by biochemical methods in individual regions with the progression of AD, has been demonstrated [18,19]; hence if the LC is the first region affected by AD-associated tau pathology, one would expect considerable levels of tau pathology in early subclinical AD as reflected by NFT Braak stages. In contrast, we have observed only minimal AT8-ir in the LCs in cases with NFT Braak stage I, and tau pathology in subcortical nuclei reached severe levels in cases with NFT Braak stages V/VI only. Moreover, in our study cohort tau pathology in the OB showed an almost identical pattern with the one seen in subcortical nuclei. Our findings thereby suggest that both subcortical nuclei and the OB become increasingly affected by tau pathology with increasing NFT Braak stages [6,20,21].

An alternative explanation for the frequent finding of AT8-ir material in the LC of individuals that completely lack tau pathology in cortical regions would be that here AT8-ir might reflect transient disturbances of the physiological equilibrium of dephosphorylated and phosphorylated tau, or indeed non-specific neuronal damage that is not automatically associated with AD pathogenesis; e.g. acute immobilization stress induces transient tau hyperphosphorylation in various brain regions including the LC, hippocampus and neocortex in C57B1/6 (wild-type) mice, but not in mice deficient in corticotropin-releasing hormone [22]. In line with these findings, AT8-ir in the LC could potentially reflect pre-mortem stress.

In conclusion, recent studies suggest that the pathological process of AD-associated tau pathology begins in the LC as early as in the first decade of life and spreads via a hitherto unknown mechanism to the cortical regions. However, further studies are warranted, in particular to answer the question of why LC tau pathology is very limited in stages where it is assumed to already have spread from the LC to trans-/entorhinal regions but increases in severity in stages that show neocortical involvement. We suggest that it is too early to draw any respective conclusions for the pathogenesis of AD and hypothesize that subtle LC tau pathology might also reflect non-specific neuronal damage that is not related to AD-associated tau pathology.

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