Alzheimer Disease: Curly Fibers and Tangles in Organs Other Than Brain

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Abstract. The filamentous brain lesions that define Alzheimer disease (AD) consist of senile plaques and neurofibrillary tangles. Undulated pathological filaments—curly fibers or neuritipil threads—also occur in the neuropil. Beta-amyloid precursor proteins are synthesized by many cells outside the central nervous system and recently, deposition of beta-amyloid-protein was reported to occur in non-neuronal tissues. In addition, increasing data claim the importance of chronic inflammation in the pathogenesis of AD. These observations suggest that AD may be a widespread systemic disorder.

Here we report that pathological argyrophilic filaments with histochemical properties of amyloid showing striking morphological similarity to curly fibers and/or tangles accumulate not only in ependymal layer and in epithelial cells of choroid plexus, but also in several other organs (e.g., liver, pancreas, ovary, testis, thyroid) in AD. The ependyma, choroid plexus, and various organs of 39 autopsy cases were analyzed. In search of curly fiber and tangle-like changes in organs other than brain, 395 blocks from 21 different tissues of 24 AD cases, 5 cases with discrete or moderate AD-type changes, and 10 control cases were investigated. We found in non-neuronal cells “curly fibers” or “tangles” immunoreactive with antibodies to P component, tau-protein, ubiquitin, fibroactin, and Apolipoprotein-E, but lacking immunoreactivity with antibodies to neurofibrilament proteins. Ultrastructurally they consist of densely packed straight and paired helical filaments and closely resemble neurofibrillary tangles and neuritipil threads.

These observations indicate that the formation of “curly fibers” and “tangles” is not unique to the central nervous system. The results suggest that AD might be a systemic disorder or that similar fibrillar changes to tangles and curly fibers may also be associated with other amyloidosis than beta-amyloidosis. Further investigations are necessary to understand the pathogenetic interest of these fibrillar changes outside the CNS.

Key Words: Alzheimer disease; Amyloid; Choroid plexus; Curly fibers; Ependyma; Neurofibrillary tangles; Systemic disorder.

INTRODUCTION

The most important histological changes of Alzheimer disease (AD) are senile plaques and neurofibrillary tangles (NFT) as well as accumulation of beta-amyloid protein (beta-A4) in the brain. Recently, intracellular tangle-like inclusions were also demonstrated in astrocytes in AD (1). An electron microscopic study revealed the presence of paired helical filaments (PHF) and straight tubules in these glial tangles that were indistinguishable from those seen in neurons (1). In addition to tangles and plaques, argyrophilic filamentous structures named “curly fibers” or “neuritipil threads” accumulate in the neuropil in AD. According to the description of Braak et al. (2), the slender silver-stained structures follow a tortuous course, and even in 100-μm thick preparations they could be traced for only short distances. Using morphometric analysis, the mean length of neuritipil threads in AD was found to be 22 μm (3). They are independent structures and do not form continuous networks. The accumulation of similar, helically shaped, slightly undulating filaments, sometimes with a curved end or forming ring-shaped structures in the ependymal layer of the cerebral ventricles was described at the beginning of this century (4, 5). Similar pathological argyrophilic structures (called Bioni-di filaments, rings, or bodies) were also described in the epithelial lamina of the choroid plexus (6). All these pathological argyrophilic filaments in the cerebral cortex, ependymal layer, and choroid plexus show a staining affinity for Thioflavin S and Congo red, indicating that they contain amyloid.

Recently, the presence of beta-A4 immunoreactive deposits in organs other than brain in AD patients suggested that beta-A4 amyloidosis is a systemic disorder (7). This is in line with the increasing number of observations establishing the role of chronic inflammation in the pathogenesis of AD (8). Therefore, we expected to find tangle and thread-like pathological filaments also in extraneuronal tissues in AD. Here, we report that pathological filaments that are morphologically similar to curly fibers and/or tangle-like inclusions also accumulate in several organs other than brain.

MATERIALS AND METHODS

Autopsy Cases Investigated

For this study 39 autopsy cases were analyzed. Using the histological criteria proposed by Khachaturian (9), a neuropathological diagnosis of AD was confirmed in 24 cases. The severity of the cortical AD-type changes corresponded to stages
V and VI of Braak (10), indicating that abundant tangle formation was present not only in the entorhinal cortex and hippocampus but also in the frontal and parietal associative cortex. The semiquantitative analysis and staging procedure that was used for the neuropathological diagnosis of AD was previously described in detail (11). The clinical record mentioned dementia in all 24 AD cases, which included 22 sporadic and 2 familial (FAD) cases. Based on the clinical history, the 2 youngest AD patients, ages 29 years and 55 years respectively, were FAD cases and the others, all older than 68 years, were sporadic AD cases. Genomic DNA obtained from brain tissue of the 2 FAD cases was used for the detection of Presenilin-1 (PSE-1) mutations. The coding region of the Presenilene-1 gene (PS-1) was analyzed by using intronic primers to amplify each coding exon of PS-1 through polymerase chain reaction (PCR) and direct sequencing as previously described (12). PS1 mutations by complete analysis of the coding region of the PS-1 revealed a novel mutation (Y256S) in the 29-year-old FAD case. Genetic analysis to detect mutations in the Presenilin-2 and APP genes is in progress.

In 5 nondemented patients (aged 68–73 years), the neuro-pathological examination revealed discrete or moderate AD-type cortical changes, which were insufficient for the neuropathological diagnosis of AD. The severity of the cortical changes following Braak corresponded to stages I–IV, indicating that neurofibrillary tangles were present in entorhinal cortex and hippocampus but not in associative cortical areas.

As in a previous study (11), cases with early β-amyloid deposits or rare tangles in the entorhinal cortex were not considered as controls. Ten nondemented cases, where the histological analysis did not show any AD-type histological changes in the brain, were used as controls. There were 2 young control cases aged 34 years and 41 years, respectively, and all others were older than 64 years. In all 39 cases we looked for histological changes similar to the Alzheimer neurofibrillary tangles and neuropil threads, not only in the ependymal layer and choroid plexus epithelial cells, but also in several other organs (Table 1).

Histochemistry, Immunohistochemistry, and Electron Microscopy

For the neuropathological diagnosis of AD, blocks were taken from at least 3 different cortical areas (frontal, parietal, and temporal) of all 39 formalin fixed brains. The blocks taken from the temporal lobe included the hippocampus, part of the entorhinal cortex, as well as the ependymal region and the choroid plexus of the temporal horn of the lateral ventricle. In 17 cases (12 AD cases, 3 cases with discrete to moderate AD-type cortical changes, and 2 control cases), similar samples were taken at autopsy from unfixed brains, including the ependymal region and the choroid plexus, and were frozen in liquid N₂ and stored at −80°C until processing. Paraffin and frozen sections cut from these blocks were stained with the Gallyas silver technique, Thioflavin S, Congo red, Jones' silver techniques(13), and Grocott silver stain, and were immunostained with monoclonal antibodies to β-amyloid protein (DAKO, M 872, dil. 1:100), neurofilament proteins (Bio-Science, 010020, dil. 1:50), and apolipoprotein-E (Chemicon MAB1062, dil. 1:200).

<table>
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<tr>
<th>Organs with NT/Total number of organs studied</th>
<th>AD + FAD</th>
<th>Discrete AD-type cortical changes</th>
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In 22 sporadic (AD) and 2 familial (FAD) cases we found curvilinear or tangle-like inclusions (NT) in organs other than brain in 20 cases. Of the sporadic AD cases, 19 of 22 were positive. We also found them in organs of 1 of the 2 FAD cases and in 4 of 5 cases with discrete or moderate AD-type cortical changes. No curvilinear or tangle-like inclusions were found in the organs of the 10 control cases.

In these 39 autopsy cases, (24 AD cases, 5 cases with discrete or moderate AD-type cortical changes, and 10 control cases). tissue samples taken from several organs were processed. Paraffin sections were cut from 35 blocks representing 21 different extraneural tissues listed in Table 1. One sample from each organ with a size of approximately 2 × 1 × 0.5 cm was available for analysis. Frozen material from 12 sporadic AD cases, 3 cases with discrete to moderate AD-type cortical changes, and 2 control cases was also available. The samples taken from the unfixed organs were frozen in liquid N₂ and stored at −80°C until processing. About 7-μm-thick frozen sections were used for the analysis. Both the paraffin and frozen sections taken from various organs were stained with the Gallyas technique, the thionine silver method of Jones’ (13), Thioflavin S, and Congo red for amyloid.
Frozen sections of the cortex, choroid plexus, and of other organs were immunostained with polyclonal antibodies to P component (DAKO, A-302, dil. 1:500), fibronectin (DAKO, A 245, dil. 1:3,000), ubiquitin (DAKO, Z 0458, dil. 1:500), and with monoclonal antibodies to neurofilament proteins (Bio-Science, 010020, dil. 1:30) and apolipoprotein-E (Chemicon MAB1062, dil. 1:200). Frozen sections were also immunostained for Tau protein. Three monoclonal antibodies (Sigma T-5530, Clone Tau-2, dil. 1:1,000, Chemicon MAB375, Clone: Tau-2, dil. 1:500; Endotellin BR-03, clone AT-8, dil. 1:50) and a polyclonal antibody (Sigma, T-6402, dil. 1:500) were used. The monoclonal anti-Tau-antibody (Sigma T-5530) reacts with chemically heterogenous Tau (55 kD-62 kD) and binds Tau proteins in either their phosphorylated or nonphosphorylated forms. These antibodies do not show cross-reaction with other microtubule-associated proteins. The monoclonal mouse anti-human PHF-Tau, clone AT-8 (Endotellin BR-03) is directed against a phosphatase sensitive epitope and does not cross-react with normal tau.

For immunostaining the avidin-biotin-peroxidase technique was used. The immuno reaction was revealed by diaminobenzidine (DAB) alone or with nickel-ammonium sulfate. Control sections without primary antibody or with irrelevant mono- or polyclonal antibodies (e.g. anti-Cytomegalovirus monoclonal antibody, DAKO M757, dil. 1:100) were also used. Sections of cerebral cortex of known AD cases were employed as positive controls.

Laser scanning confocal microscopy (Leica TCS NT) was used to obtain a more detailed morphology of extraneuronal "curly fibers" and "tangles" on Thiolfavin S stained sections. Excitation was obtained with an Argon-Krypton laser with lines set at 488 nm for FITC excitation. Images of 512 x 512 pixels were taken using a x40 or x63 objectives. For each field, a digitized series of 500-nm-thick optical sections taken at different planes of focus were collected on the host computer; the full dynamic range of the photomultipliers was used with the help of a special look up table from Leica. The sections were processed using Imaris software (Bitplane AG, Zurich) and the 3D visualization was made on a Silicon Graphics computer.

For ultrastructural analysis small samples taken from the temporal cortex, the choroid plexus, the ependymal layer of brain ventricles, as well as from the liver and the renal cortex of 4 AD and 2 control cases were processed using the standard technique. The ultrathin sections were contrasted with uranyl acetate and lead citrate and were examined using a Philips CM-10 electron microscope.

RESULTS

In all 24 AD cases, including the 2 FAD cases as well as the 5 cases with discrete or moderate AD-type cortical changes, we found accumulation of neuropil threads in the ependymal layer (Figs. 1D, 2A) and intracellular congophilic inclusions in the epithelial cells of the choroid plexus (Figs. 1B, 2A). These inclusions were not found in choroid plexus or ependymal layer of the 10 control cases. In addition, in 19 of the 22 sporadic AD cases, in 1 FAD case, and in 4 of the 5 cases with discrete or moderate AD-type cortical changes, we observed helically shaped amyloid inclusions in organs other than brain similar to those of the neuropil threads and tangles (Table 1; Figs. 1C, E, F, 2B, C, 3E, F). The youngest FAD case, which had a PS-1 mutation (Y256S), had no fibrillary changes outside the CNS. The distribution of fibrillary changes in different organs was similar in the non-PS-1 mutant case to that of sporadic AD cases.

The size and shape of these structures varied (Fig. 4). Some resembled "silver grains," others had "curly" fiber, "tangle-like," ring, or globular appearance. In individual tissues, including the cerebral cortex, all forms could be found, but a particular morphological variant is usually dominated in an organ. In the pancreas, the morphology was similar to that of the inclusions in the epithelial cells of the choroid plexus (compare Fig. 1B with 1F). The morphological similarity between "curly fibers" of the liver and the cortical neuropil threads was striking (compare Fig. 1A with 1C). Tangled masses of intracellular fibrils were also observed. As in brains with AD, the accumulation of "tangles" and "curly fibers" in other organs was associated with accumulation of lipofuscin granules.

Curly fiber or tangle-like inclusions were frequently found in the adrenal (Fig. 2B, C) and in the pancreas (Figs. 1E, 3B, D, E), but we found them also in liver (Fig. 1C), spleen, ovary (Fig. 1E), testis, pituitary, striated muscle (Fig. 3F), myocardium, thyroid, bowel, and in the wall of the aorta and large arteries (Table 1). In the pancreas, fibrillary changes were found particularly in the intra- and interlobular duct cells (Fig. 3E), but also in pancreatic acinar cells. Fine silver granules were found in some Langerhans islet cells. In the liver, fibrillary changes were found in hepatic cells and in Kupffer cells. In the adrenal, intracellular tangle-like inclusions were observed particularly in the subcapsular cortical cells (Fig. 2B, C), but clustered group of cells containing fibrils or silver granules were found in the zona glomerulosa fasciculata and also reticularis. We had no samples from adrenal medulla. Few curly fiber-like fibrils were observed in the fibrous tissue of the adrenal capsule. Fibrillar changes were observed in follicular epithelial cells and in perifollicular collagensous tissue of the thyroid. In the parathyroid they were localized in small polyhedral chief cells but also in large polyhedral oxyphil cells. In striated muscle they were localized in the sarcoplasm of myofibrils, frequently in the subsarcolemmal region, but also in the central part of the sarcoplasm lying parallel to the myofibril (Fig. 3F). In the myocardium, these "threads" were shorter than in striated muscle, but their disposition was the same. We did not examine samples of the endocardium and pericardium. In the spindle-shaped fibroblast-like stromal cells of the ovary, the amyloid fibrils were also disposed parallel to the cells. It was difficult to determine their intracellular
Fig. 1. Photomicrographs illustrating the presence of curly fiber or tangle-like structures in the cerebral cortex, the ependymal layer, epithelial cells of the choroid plexus, as well as in organs other than brain in AD. A: Curly fibers in the neuropil of the temporal cortex of a familial AD case. The frozen section was stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI), a sensitive DNA stain (32, 33). Curly fibers exhibit the same yellow fluorescence as cell nuclei. B: Biondi filaments and rings in the epithelial cells of the choroid plexus of 1 of the AD cases investigated in this study. The small arrow points to helically shaped curved Biondi filaments. Thioflavin S technique. C: "Curly fibers" accumulating in the liver. Notice the striking morphological similarity between the "curly fibers" of the liver and those of the cortical neuropil seen in A. They exhibit a strong yellow fluorescence when stained with Thioflavin S. Arrow points to a small group of Thioflavin S positive fibrils. D: "Curly fibers" accumulating in the ependymal layer of the lateral ventricle. Thioflavin S stain. E: Neuropil thread-like, Thioflavin S positive filaments in the ovary of 1 of the AD cases investigated. F: Thioflavin S positive, pathological filaments similar to those
or extracellular position. We did not have samples to analyze the cortical cells of the ovary. Fibrillary changes were seen in cells of the stratified epithelium of seminiferous tubules including in Sertoli cells in the testis. They were frequently observed lying in connective tissue in the capsule of several organs.

The organs affected were not always the same in the AD cases, though some organs were more frequently affected than others (Table 1). The severity of fibrillary changes of a given organ varied from one case to another. There was a regional variability of the distribution of fibrillary changes in different parts of the same section. In cases illustrated on Table 1, organs with low (even few) or high-density fibrillary changes were both considered as positive, and those without any fibrillary changes as negative.

In extraneural tissues these thread- and tangle-like filaments exhibited a strong yellow fluorescence when stained with Thioflavin S (Figs. 1B–F 2B). Part of them bound Congo red and then exhibited a bright green-red birefringence (Fig. 2C, inset). They stained with the Gal-lyas silver technique, particularly on frozen sections, but more consistently with Jones methenamine silver method (13). The Jones' technique also demonstrated AD-type changes in the brain. The amyloid inclusions of the plexus were easily visualized by the Grocott silver staining. These non-neuronal thread-like structures were found to be immunoreactive with antibodies to P component, ubiquitin (Fig. 2E), fibronectin (Fig. 3H), apolipoprotein E, and also with the monoclonal and polyclonal anti-Tau antibodies (Figs. 2D, 3G). They lacked immunoreactivity to neurofilament protein (Table 2). We did not find immunoreactivity when the immunoreaction was performed with the specific primary antibody omitted or when an irrelevant antibody was used. We did not find fibrillary changes in ependyma, plexus, or in organs other than brain using the histochemical and immunohistochemical techniques mentioned before.

In our experience it was the Thioflavin S stain that was the most useful on paraffin (and particularly on frozen sections) to find fibrillary changes in different organs. Even a few tangle or curly fiber-like inclusions were clearly visible. A careful examination of all parts of the sections was necessary because sometimes the number of fibrillary changes was very low. When a large number of fibrils accumulate in tissues such as ependyma, plexus, and adrenal, the Jones' stain was very useful. Sometimes, because of the strong silver impregnation of fibrous tissue, the detection of few fibrillary changes may be difficult with silver techniques. The Jones' stain was also useful in detecting single intracellular amyloid inclusions. For this purpose, the use of high magnification (×63) was necessary (Table 2).

Electron microscopic analysis of the "curly fiber" or "tangle-like" inclusions found in the liver (Fig. 5A, B), in the adrenal cortical cells, and in the epithelial cells of the choroid plexus (Fig. 5C, D) showed paired helical filaments 20–25 nm in diameter, similar to those of neurofibrillary tangles (compare Fig. 5E with 5F). Twisted and straight forms were both observed. The twisted filaments were predominant in the plexus and the straight form in the liver. They were distributed in the cytoplasm generally in small bundles (∼0.3–0.5 μm-wide) of dense, broad groups of oriented fibrils. They were frequently in close relation with small lipid granules. Sometimes less close-packed, more dispersed filaments were also seen. We did not find similar ultrastructural changes in the choroid plexus, liver, and adrenal of the control cases.

Using laser scanning confocal microscopy (Fig. 3A–D), the digitized series of optical sections taken at different planes of focus of the fluorescent (Thioflavin S stained) amyloid inclusions of plexus and pancreas showed that the globular amyloid inclusions in plexus and pancreas correspond to enrolled helical filaments (not illustrated here).

DISCUSSION

In all the 24 AD cases, including the FAD cases and in the 5 cases with discrete or moderate AD-type cortical changes, we found accumulation of curly fiber or tangle-like inclusions in ependyma and choroid plexus. In addition, in the majority of the sporadic AD cases (19 of 22), in the non-P5-1 mutant FAD case, and in 4 of 5 cases with discrete or moderate AD-type cortical changes, accumulation of curly fibers were also found in several other organs besides the brain.

In addition to neurons, intracellular tangle-like inclusions have been demonstrated in glial cells (1, 14) and in epithelial cells of the choroid plexus in AD, and were reported immunopositive for anti-tau and anti-ubiquitin. A positive reaction of the Biondi filaments for β-amyloid protein has been recently reported (15), and the β-A4 protein was biochemically isolated from the choroid plexus (16). These findings together with a recent statistical

of the epithelial lamina of the choroid plexus accumulating in the pancreas. Note the similar morphology of the Biondi body of the choroid plexus (large arrow in B) with the Thioflavin S positive inclusions lying in the pancreas (arrow on F). Scale bars: A, C–F = 20 μm. B = 10 μm.
Fig. 2. "Tangles" and "curly fibers" in choroid plexus, ependyma (A), and adrenal (B–E). A–C are paraffin sections, and D and E are frozen sections. A: Tangle-like fibrillary inclusions in epithelial cells of the choroid plexus and "curly fibers" in the ependyma of the temporal horn of the lateral ventricle visualized with the Jones' technique. B and C show similar tangle-like fibrillary changes in adrenal cortical cells on sections stained with Thioflavin S and Jones' silver techniques, respectively.
analysis showing a strong association between the frequency of the AD-type cortical lesions and Biondi inclusions (11) suggest that the pathogenesis of the degenerative changes in neurons and plexus epithelial cells may be similar.

Intracellular amyloid inclusions in the adrenal cortical cells, in Sertoli cells, and in the pituitary gland has been previously observed (17). Here we describe curly fiber and tangle-like inclusions in several other organs not previously observed. These amyloid inclusions were found to be immunoreactive with antibodies to ubiquitin, P component, fibronectin, apolipoprotein-E, and hyperphosphorylated Tau, thus exhibiting similar immunohistochemical features such as neurofibrillary tangles (14, 18). Our results on the occurrence of curly fiber and tangle-like inclusions not only in the ependymal layer, but also in choroid plexus epithelial cells and in organs other than brain in AD suggest a pathogenetic relationship between the formation of these fibrillary changes in brain and outside the CNS.

Both paired helical and straight filaments are components of neurofibrillary tangles and neuropil threads in the AD brain (19) and were reported to be present in astrocytes (1), epithelial cells of the choroid plexus, and in the adrenal cortical cells (17). In addition, twisted tubulofilaments of inclusion body myositis muscle resemble helical filaments of Alzheimer brain and contain hyperphosphorylated tau (20). These observations are in agreement with our electron microscopic analysis showing that the curly fiber and tangle-like inclusions found in the liver, in the adrenal cortical cells, and in the epithelial cells of the choroid plexus showed paired helical filaments (both twisted and straight tubulofilaments) similar to those of neurofibrillary tangels.

The cytoskeletal changes in several organs described here, including in endocrine organs (adrenal, pancreas, pituitary, ovary, testis, etc.), like neurofibrillary degeneration, may lead to cellular dysfunction. This would be in agreement with recently reported observations concerning impaired endocrine and neuroendocrine functions in AD (21–25). Indeed, abnormal levels of insulin and glycogeneration at the site of androgen after injection of ACTH (23), and significantly lower plasma ACTH levels after CRH stimulation (24) were all reported to occur in AD.

Curly fibers or neuropil threads accumulating in the AD brain were suggested to correspond to dystrophic neurites or dendrites (26). The accumulation of thread-like filaments morphologically similar to the neuropil threads in the epithelial layer of the choroid plexus in AD renders this interpretation unlikely. In particular, finding that similar argyrophilic, helically shaped structures occur in several organs other than brain was striking. The fact that they express histochemical, immunohistochemical, and ultrastructural properties similar to neurofibrillary tangles and curly fibers in the AD brain indicates that the formation of these structures is not unique to the CNS, and raises questions concerning the use of the terms "curly fibers" or "neuropil threads" as synonymous to dystrophic dendrites or neurites. Since one may find them in other organs than the brain, it seems difficult to consider their appearance as regenerative reaction of neurites (27). We therefore prefer the statement used by Braak et al (28), that curly fibers occur in dendrites and neurites. Our findings are in agreement with those of Wang et al (29) who reported that generated monoclonal antibodies against isolated neurofibrillary tangles do not react with microtubules or neurofilaments. The fact that the thread-like and tangle-like inclusions of plexus and other extraneural tissues do not show immunoreactivity to antibodies to neurofilament proteins seems to reinforce the notion that hyperphosphorylated neurofilament proteins are associated with (but are not the building blocks of) tangles. Their positivity in tangle bearing neurons is proposed to be secondary to the degenerative process, as phosphorylated neurofilament proteins in perikaryon occur also in other neurodegenerative diseases and in retrograde degeneration of nerve cells.

Our results show that the highly phosphorylated microtubule-associated protein Tau, the major antigenic component of paired helical filaments of neurofibrillary tangles (16), was also expressed in the affected extraneural cells. Similar to neurofibrillary degeneration, the Tau protein could possibly participate in the formation of amyloid inclusions in plexus and in other organs than the brain. This would be in agreement with recent reports that non-neuronal cells may well show immunoreactivity to hyperphosphorylated Tau. Namely, in inclusion body myositis vacuolated muscle fibers containing paired helical filaments were reported to be immunoreactive (19,
Fig. 3. Amyloid inclusions in the plexus, pancreas, and striated muscle. A–D: Confocal microscopy images after 3-D reconstruction showing a more detailed morphology of extraneural "curly fibers" and "tangles" in the plexus (A and C) and in the pancreas (B and D). Thread-like and globular (arrow) amyloid inclusions in A and B are seen at a higher magnification on C.
20). Further molecular analyses are necessary for a more precise characterization of these thread and tangle-like inclusions outside the CNS.

In the present study only 1 sample from each organ was analyzed for the detection of fibrillary changes. In order to answer the question of whether the distribution of fibrillary changes are homogenous in an affected organ, a prospective study with well defined protocol with respect to the sites of tissue samples of each organ will be necessary. A study of the distribution as well as the degree of fibrillary changes in each type of organ may help to better correlate fibrillary changes of different organs and those of the brain. Analysis of a large number of cases, as in the choroid plexus study (11), will be necessary to answer these questions.

In the majority of AD cases, the clinical data available were insufficient to determine the duration of the degenerative process correctly. Consequently, we were unable to analyze the correlation between the duration of disease and the accumulation of “curly fibers” or “tangles” outside the CNS. To consider this point in future studies would be of interest.

![Image](http://jnen.oxfordjournals.org/)

**Table 2**

Some Histochemical and Immunohistochemical Properties of Neurofibrillary Tangles, Curly Fibers, and Amyloid Inclusions of Ependyma, Plexus, and Organs Other than Brain in AD Cases

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<td>Jones'</td>
<td>+++</td>
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<td>+++</td>
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<tr>
<td>Grocott</td>
<td>+</td>
<td>+++*</td>
<td>+</td>
</tr>
<tr>
<td>Tau</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>Ubiquitin</td>
<td>+++</td>
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<td>Apo-E</td>
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<td>P component</td>
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<td>Fibronectin</td>
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<tr>
<td>Neurofilament proteins</td>
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The histochemical and immunohistochemical properties of amyloid inclusions of ependyma, plexus, and organs other than brain were similar to neurofibrillary tangles and curly fibers. However, fibrillary changes of plexus, epithelial cells, and organs other than brain are negative with antibody to neurofilament protein.

* For the immuno-detection of “curly fibers” in ependyma, the use of high magnification is necessary. A fine granular staining of the thread-like structure is visible, +++ = strong; ++ = mild; + = weak staining; * = Variability in consistency.

and D, respectively. Comparison of A and C with B and D shows a morphological similarity of the amyloid intracellular inclusions found in the plexus epithelial cells (A and C) to those found in intralobular duct cells of the pancreas. The 3-D reconstruction of the digitized series of optical sections suggests that the globular structures correspond to enclosed filamentous elements (not shown here). E: Intracellular amyloid inclusions (arrows) in intralobular duct cells of the pancreas stained with Jones’ silver technique. F: Arrows point to “curly fibers” in the sarcoplasm of myofibril. Jones’ silver stain. G and H: Immunostained frozen sections showing Tau and fibronectin positivity of affected intralobular and acinar cells, respectively. G: Cytoplasmic Tau-positivity was revealed in black with ammonium-nickel-sulfate. The section was not counterstained with hematoxylin. Arrowheads point to unstained cell nuclei, and arrow to Biondi body-like inclusion. H: Some affected acinar cells in the pancreas showing fibronectin positivity. Frozen section postfixed with acetone. Scale bars: A = 10 μm; B = 20 μm; C = 1 μm; D = 2 μm; E = 20 μm; F = 30 μm; G and H = 50 μm.

*J Neuropathol Exp Neurol, Vol 58, August, 1999*
The process of normal aging is relatively poorly understood (9). The brains of some elderly patients have no plaques or tangles and the brains of some young patients, like our 2 FAD cases, are severely affected by the degenerative process. Dementia in AD is a slow progressive process and one may assume that the evolution of the severity of the degenerative cortical changes will strongly correlate with the evolution of dementia. For this reason we consider that cases with discrete or moderate AD-type changes correspond with earlier stages of the degenerative process. Therefore, we classified cases as controls only if we did not find any AD-type cortical changes in the brain. In 4 of 5 cases with discrete or moderate AD-type changes we have also found fibrillar changes in organs other than the brain, suggesting that fibrillar changes outside the CNS may appear in early stages of AD, as they appear in the choroid plexus.

It is difficult to predict the utility of the present findings for the clinical diagnosis of AD. If a pathogenetic relationship between the cortical and the extraneural “tangles” and “curly fibers” would be established, some tissues might be reasonable sites for biopsy. Detecting the presence of fibrillar changes outside CNS would useful in confirming the clinical diagnosis of AD.

Conclusions

The findings reported here, that in AD patients pathological argyrophilic filaments, with morphological and ultrastructural similarity to curly fibers and tangles and with histochemical properties of amyloid, occur in non-neuronal tissues support the view of Schwartz (30) and Joachim et al (7), that AD might be a widespread systemic disorder affecting several organs. The present observations together with those of other authors indicate that the formation of curly fiber and tangle-like inclusions is not unique to the nervous tissue. This fits recent observations that cells of other organs than brain, such as thyroid epithelial cells, produce large amounts of β-APP and generate potentially amyloidogenic APP fragments (31). An answer to the question whether AD is a purely neurodegenerative disease or a widespread systemic disorder affecting several organs is important and may contribute to the understanding of the pathogenesis of AD. One must also to consider that fibrillar changes similar to those of neurofibrillary tangles and curly fibers may also be associated with amyloidosis other than β-amyloidosis, for example, with senile amyloidosis of organs other than brain. Further analysis of the biochemical and molecular properties of these non-neuronal “curly fibers” or “tangles” are necessary to understand the pathogenetic interest of these extraneural amyloid inclusions.

ACKNOWLEDGMENTS

We thank S. Gros, A. Moentoro, and S. Trepay for technical assistance, and S. Burki and J. Milardet for photography. We are particularly grateful to P. Darekar, S. Testuz, and I. Favre for their helpful and useful contribution. We thank S. Kasas for helpful discussions and R. Berger for editorial advice.

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**Fig. 5.** Ultrastructure of paired helical filaments (PHF) of “curly fibers” in the liver (A and B) and of Biendi inclusions in the epithelial cells of the choroid plexus (C and D) in a sporadic AD case. They were distributed in the cytoplasm as densely packed oriented fibrils forming long bundles ~0.3–0.5 μm in diameter. At a higher magnification compare the ultrastructure of PHF of choroid plexus (E) with those of neurofibrillary tangles (F). Scale bars: A and C = 0.4 μm; B and D = 0.2 μm; E and F = 100 nm.
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Received October 16, 1998
Revision received April 2, 1999
Accepted April 5, 1999

J Neuropathol Exp Neurol, Vol 58, August, 1999