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Frequency of Stages of Alzheimer-Related Lesions in Different Age Categories

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BRAAK, H. AND E. BRAAK. Frequency of stages of Alzheimer-related lesions in different age categories. NEUROBION AGING 18(4) 351–357, 1997.—Alzheimer’s disease is a relentlessly progressing dementing disorder. Major pathological hallmarks include extracellular deposits of amyloid protein and intraneuronal neurofibrillary changes. No remissions occur in the course of the disease. Initial amyloid deposits develop in poorly myelinated areas of the basal neocortex. From there, they spread into adjoining areas and the hippocampus. Deposits eventually infiltrate all cortical areas, including densely myelinated primary fields of the neocortex (stages A–C). Intraneuronal lesions develop initially in the transentorhinal region, then spread in a predictable manner across other areas (stages I–VI). At stages I–II, neurofibrillary changes develop preferentially in the absence of amyloid deposits. A proportion of cases shows early development of amyloid deposits and/or intraneuronal changes. Advanced age is thus not a prerequisite for the evolution of the lesions. Alzheimer’s disease is an age-related, not an age-dependent disease. The degree of brain destruction at stages III–IV frequently leads to the appearance of initial clinical symptoms. The stages V–VI representing fully developed Alzheimer’s disease are increasingly prevalent with increasing age. The arithmetic means of the stages of both the amyloid-depositing and the neurofibrillary pathology increase with age. Age is a risk factor for Alzheimer’s disease. © 1997 Elsevier Science Inc.

Alzheimer’s disease  Amyloid-protein  Neurofibrillary change  Aging

The insidious onset of Alzheimer’s disease (AD) is characterized by a subtle decline in memory functions. As time passes, changes in personality, deterioration of language functions, and eventually motor dysfunction are added to the initial symptoms. The gradual progress in the clinical picture mirrors the development of AD-related brain destruction. Major pathological hallmarks include extracellular deposits of Aβ-amyloid protein and intraneuronal neurofibrillary changes. The predictable alteration in the pattern and severity of the pathology permits the distinction of stages in amyloid-deposition (A–C) and neurofibrillary changes (I–VI) (8,10). The possible relationships among the three factors (age, amyloid-depositing, and neurofibrillary changes) have not been sufficiently explored. Goal of this study is to partially fill in these gaps in knowledge through assessment of nonselected autopsy cases using a staging procedure for AD-related lesions.

MATERIALS AND METHODS

A total of 2,661 brains obtained at autopsy was examined in this study. The brains were collected between 1986 and 1996 and were sent by three Departments of Pathology and one Department of Forensic Pathology belonging to three Universities in Germany. The material included cases from all the affiliated University clinics. It did not originate in special gerontopsychiatric institutions and does not correspond to such preselected material as might have been provided by a Department of Neuropathology. All cases received by our laboratory during this time period were examined for AD-related changes. The relatively few cases below the age of 25 and above the age of 95 were excluded from the study. The material was checked for, but did not include, cases with diseases known to be associated with the development of neurofibrillary changes but different from AD (Down’s syndrome, type C of Niemann-Pick disease, subacute sclerosing panencephalitis, progressive supranuclear palsy, corticobasal degeneration). Staged cases at our disposal from prospective studies on the development of Alzheimer’s disease were not included. Age distribution and the number of cases permit division into age categories encompassing a range of 5 years. The number of cases and the male or female predominance in each age category is displayed in Table 1 and Fig. 1.

Brains were fixed by immersion in a 4% aqueous formaldehyde solution. Blocks of tissue including anteromedial portions of the temporal lobe were removed at the mid-uncal level, and portions of

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neurofibrillary changes were performed using published criteria. Diagnoses of stages in the development of amyloid deposits and argyrophilic grains, and silver techniques for neurofibrillary changes and Lewy bodies are nearly identical to those seen in immunostained sections for silver-pyridine method for amyloid deposits and Lewy bodies, and a silver-iodide technique for neurofibrillary changes and argyrophilic grains, and a silver-pyridine method for amyloid deposits and Lewy bodies (7,12). Modern silver techniques taking advantage of physical development of the hilar or dentate area were applied: a silver-iodide technique for neurofibrillary changes and argyrophilic grains, and a silver-pyridine method for amyloid deposits and Lewy bodies (9,12,15,23). These techniques demonstrate the pathological material reliably, even if the tissue has been stored for decades in formaldehyde. The results obtained by applying the advanced silver techniques for neurofibrillary changes and amyloid deposits are nearly identical to those seen in immunostained sections for Aβ-protein and abnormally phosphorylated tau protein (9,13,23). Diagnoses of stages in the development of amyloid deposits and neurofibrillary changes were performed using published criteria and were achieved without knowledge of clinical and pathological data or age and gender of the individuals (8,10).

### RESULTS

The proportion of cases exhibiting no amyloid deposits or neurofibrillary changes decreases with advancing age (Figs. 2 and 3, upper row). Neither amyloid deposits nor neurofibrillary changes necessarily accompany old age. The evolution of amyloid deposits is shown in Fig. 2 and Table 2. The subpopulations devoid of neurofibrillary changes are represented by the blank areas in the columns in Fig. 2, and the dark areas indicate the subgroups expressing any degree of neurofibrillary changes. Figure 3 and Table 3 are similarly designed and display the evolution of neurofibrillary changes. Here, the blank areas stand for the subgroups devoid of amyloid deposits and the dark areas represent the subgroups exhibiting them.

### Amyloid Deposits

Cortical deposits of amyloid protein appear gradually, distributed more or less symmetrically, at specific predilection sites. Initially, extensive cloud-like formations with ill-defined boundaries appear. With advance of the disease, they are replaced by sharply outlined globular plaques of variable sizes. Intensely stained plaques occur preferentially in neocortical layers II and III and Va. Layers containing a myelinated plexus (IV and Vb) generally show less dense precipitations. Subpial portions of layer I contain conluent plaques, and layers II and VI are frequently free of deposits. Often, band-like precipitations are seen in the outer and inner pyramidal cell layers of the hippocampal CA1 sector and in layers pre-β and pre-γ of the entorhinal region. The number of deposits increases until a certain level of density is reached. Even at maximum density, a notable amount of the gray matter remains free of deposits. An inverse relationship is observed between the degree of myelination and the density of amyloid deposition, with sparsely myelinated cortical areas and/or layers displaying a denser depositing than those rich in myelin (13).

### Stages in the Evolution of the Amyloid Deposits

Three stages can be distinguished in the gradual development of cortical amyloid deposits (8,10). Typically, the initial patches are seen in the basolateral sector, most frequently in poorly myelinated temporal areas such as the perirhinal and/or ectorhinal fields (stage A, Fig. 2, second row). Some individuals develop initial deposits in young adulthood. The depositions increase in number and spread into the adjoining neocortical areas and the hippocampal formation (stage B, Fig. 2, third row). At this stage, the perforant path is decorated with deposits as it pierces the subiculum and travels through the molecular layers of CA1 and the fascia dentata. Eventually, deposits are found in all areas of the cortex, including the densely myelinated primary areas of the neocortex (stage C, Fig. 2, fourth row). The diagrams of stages A to C show a shift towards higher age-categories (Fig. 2). The prevalence of stage C cases increases with age. More and more individuals arrive at a higher stage of amyloid-depositing with advancing age. The arithmetic means of the relative frequency in the occurrence of amyloid-stages increase with age (A = 1, B = 2, C = 3; Fig. 4, upper portion of the right half).

### Neurofibrillary Changes of the Alzheimer Type

Neurofibrillary tangles (NFTs), neuropil threads (NTs), and argyrophilic dystrophic neurites of neuritic plaques (NPs) are different types of the intraneuronal changes. In general, the first
FIG. 2. Development of amyloid deposits in 2,661 nonselected autopsy cases. The first line displays the frequency of cases devoid of changes in relation to the total number of cases in the various age categories. The second, third, and fourth lines are similarly designed, and show the evolution of the AD-related changes. The dark areas of the columns refer to subgroups showing the presence of neurofibrillary changes.
FIG. 3. Development of neurofibrillary changes in 2,661 nonselected autopsy cases. The first line displays the frequency of cases devoid of changes in relation to the total number of cases in the various age categories. The second, third, and fourth lines are similarly designed, and show the evolution of the AD-related changes. The dark areas of the columns represent the subgroups displaying amyloid deposits.
The intraneuronal changes develop at specific predilection sites. From there, the changes spread in a predictable manner across the cerebral cortex. Six stages can be distinguished in the gradual development of NFTs/NTs (8,10). Specific projection cells in the transentorial region (temporal lobe) are the first cortical neurons to show the changes (stage I). The lesions then extend into the entorhinal proper (stage II). These changes develop preferably in the absence of amyloid deposits (Fig. 3). A significant number of cases show early development of stage I/II pathology (Fig. 3, second row). Proceeding toward higher age categories, the relative frequency of stage I/II cases increases, reaches a culmination point, then decreases. Note in separate illustrations that the culmination point of stage I cases is reached at a younger age than that of stage II cases (Fig. 4, left half). The pathological process then proceeds into both the hippocampus and the temporal pronoecortex (stage III), then reaches association areas of the adjoining neocortex (stage IV). Eventually, the lesions spread superolaterally (stage V) and extend into the primary areas of the neocortex (stage VI). This pattern bears a resemblance to the inverse sequence of cortical myelination (11). The limbic stages III/IV (Fig. 3, third row) and neocortical stages V/VI (Fig. 3, fourth row) display only an ascending portion of the age distribution. The prevalence of stage V/VI cases increases with age. The arithmetic means of the relative frequency in the occurrence of NFT/NT-stages increases with age (Fig. 4, lower portion of the right half).

Combinations between Amyloid- and NFT/NT-Stages in Three Age Ranges

Sixteen combinations are possible among cases without amyloid deposits or neurofibrillary changes, the amyloid stages A, B, C, and the NFT/NT-stages I–II, III–IV, and V–VI (Table 4). Some of these occur more frequently than others. The pattern of the prevailing combinations changes with age. A rough division into three age categories permits recognition of the major trend. The frequently encountered combination of absence of both amyloid deposits and neurofibrillary changes in the younger group decreases with age, and the combination of amyloid-stage C with NFT/NT-stages V–VI increases. In general, initial neurofibrillary changes (stages I–II) develop in the absence of amyloid deposits, while stages V–VI are associated with amyloid-stage C (Table 4).

DISCUSSION

The clinical diagnosis of AD is fraught with difficulty and definite diagnoses cannot presently be made. Both false positive and false negative diagnoses occur in a significant proportion of cases, underlining the necessity for postmortem evaluation. Epidemiological studies often focus on avoiding selection bias, yet still face the problem of inaccurate diagnoses (1,7,10,20,22,25,27). Postmortem assessments offer the advantage of unambiguous diagnoses, however, they unavoidably fail to be representative of a living population (21).

The slow progression in the clinical symptoms of AD reflects the gradual development of the brain changes (4,14,18,19,24). Most conventional criteria for a postmortem diagnosis permit recognition of fully developed AD, but fail to correctly distinguish the broad spectrum of cases with less severe changes. Application of the staging system takes into consideration the gradual progression of AD, provides accurate diagnoses in the initial phases, and even takes note of the brain changes preceding the appearance of clinical symptoms. The basic assumptions underlying the proce-
dure are corroborated by the finding that early stages occur preferably in relatively young individuals, and the more advanced stages appear with increasing age (28). It is important to note that not a single stage I–VI case showed “ghost” tangles alone; all demonstrated fresh NFTs/NTs, indicating that the pathological process was continuing at the time of death. Spontaneous remission does not occur. Once the destructive process has begun it immutably progresses.

A small proportion of cases develop amyloid deposits and/or NFTs/NTs at a surprisingly young age (Figs. 2 and 3). Advanced age is no prerequisite for the evolution of the changes. Development of the changes are frequently attributed to noxious influences expected to increase in severity in old age (peroxidative stress, mitochondrial dysfunction, imbalance of glucose metabolism) (2,5,16,29,30). The fact that initial lesions may develop in a young and otherwise healthy brain should stimulate studies to determine whether or not such influences can be demonstrated even in these very early stages.

As in many other neurodegenerative diseases, the pathological process underlying AD can be recognized over extended periods of time before the onset of clinical symptoms. Data on the appearance of stage I cases are of particular interest, because they provide information about the actual spread of the disease. To find the first neurofibrillary changes in a voluminous organ such as the human brain might seem to be an almost hopeless undertaking. However, with knowledge of the predilection sites of the initial NFTs/NTs, the task can be accomplished. Many of the stage I cases die of causes other than AD, and only a proportion of them live long enough to arrive at end stages V–VI. In higher age categories, the relative frequency of stage I/II cases increases, reaches a culmination point, then decreases. At stages III–IV, severe destruction of the cortex is already observed; however, it is focused on the entorhinal region and adjoining areas. Because of the probable

![Graphs showing the relative frequency of cases showing stage I and stage II in the development of NFTs/NTs (same design as in Fig. 3). Initial neurofibrillary changes usually develop in the absence of amyloid deposits. Some individuals develop stage I pathology early in life. Old age is not a prerequisite for the evolution of the neurofibrillary changes. Note the shift towards higher age-categories with progress from stage I to stage II. Right: The arithmetic means of stages of amyloid deposits (A = 1, B = 2, C = 3) and neurofibrillary pathology increase with age. Age is a risk factor for AD.]

### Table 4

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presence of initial symptoms and the characteristic brain destruction, stage III–IV cases are considered to correspond to clinically incidental AD (4, 14, 18, 24). Stages V/VI correspond to the clinical picture of fully developed AD. They are increasingly prevalent with increasing age (Fig. 3, fourth row).

REFERENCES


