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Argyrophilic grain disease: frequency of occurrence in different age categories and neuropathological diagnostic criteria

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Summary. Argyrophilic grain disease is a progressive degenerative disorder of the human brain which becomes increasingly prevalent with advancing age. The disease entails multiple neuronal systems and results from cytoskeletal degeneration in only a few neuronal types and in oligodendrocytes. Immunoreactions for abnormally phosphorylated tau protein permit identification of the changes. Only a fraction of the emerging abnormal fibrillary material shows a pronounced argyrophilia. Essential for neuropathological diagnosis is assessment of the presence of small spindle-shaped argyrophilic grains in neuronal processes. The anteromedial portion of the temporal lobe bears the brunt of the lesions. Grains generally can be found in abundance in the entorhinal region, the first Ammon's horn sector, the subcortical nuclear complex of the amygdala, and the hypothalamic lateral tuberal nucleus. Frequently, the lesions co-exist with those typically found in Alzheimer's disease or other tauopathies. Owing to the characteristic grains, the disorder easily can be differentiated from other tauopathies. 2661 non-selected brains obtained at autopsy included 125 cases of argyrophilic grain disease (5%) from individuals between 51 and 96 years of age (mean 79 years) . The fact that the same material contained 146 cases of fully developed Alzheimer's disease (6%) supports the view that argyrophilic grain disease is not a rare disorder. Its prevalence with and without concomitant neurofibrillary changes of the Alzheimer type grows with increasing age. Argyrophilic grain disease merits attention because of its frequent occurrence and its potential to cause severe brain dysfunction.

Keywords: Argyrophilic grain disease, tau protein, tauopathies, argyrophilic grains, argyrophilic glial inclusions, cytoskeletal alterations.

Introduction

The pathological process underlying argyrophilic grain disease (AGD) is associated with the formation of abnormal tau protein in a few select neuronal

types and in a subpopulation of oligodendroglial cells. Small spindle-shaped argyrophilic grains (AGs) in the cellular processes of nerve cells characterize this disease (Braak and Braak, 1987, 1989; Itagaki et al., 1989; Ulrich, 1989; Cras and Perry, 1991; Masliah et al., 1991; Yamada et al., 1992; Ikeda et al., 1995; de Vos et al., 1996; Davis et al., 1997; Martinez-Lage and Munoz, 1997; Tolnay et al., 1997a,b). At the present time it can only be identified by means of a neuropathological analysis.

An initial turning point in the pathological process underlying AGD is hyperphosphorylation of the microtubule-associated tau protein. Microtubules play an important role in the transport of substances among individual compartments of cells and the tau protein normally stabilizes the microtubules. With the hyperphosphorylation of this protein the microtubules become destabilized and a hydrophilic material emerges which, at the outset, is non-argyrophilic in nature (Goedert et al., 1991, 1997). Immunoreactions for demonstration of this material display the altered neurons and glial cells with all of their cellular processes. In a second facultative step the abnormal fibrillary material aggregates – most probably by cross-linking – and then rapidly adopts a pronounced argyrophilia. A number of other degenerative illnesses share this sequence in the modification of the tau protein. In the course of Alzheimer's disease (AD) most of the altered tau protein converts into argyrophilic fibrils (Bancher et al., 1989; Cras et al., 1995; Goedert et al., 1997). By contrast, only a small fraction of the abnormal material in AGD eventually consists of argyrophilic fibers which, nontheless, constitute the central feature, the argyrophilic grains (AGs).

The aim of the present investigation is to draw clinicians' attention to the existence of AGD and to acquaint neuropathologists with the morphological hallmarks of this disorder. For this purpose, data of 148 AGD cases are presented and the differences between AGD-related lesions and those associated with other degenerative diseases are described. It is to be expected that correct neuropathological diagnosis of AGD will reduce considerably the number of enigmatic and unexplained cases of adult onset dementia.

Material and methods

Brains from 2684 individuals (2661 brains from a department of general patholoy and 23 from a gerontopsychiatric clinic) of both sexes ranging in age from 25 to 96 years were studied. They were obtained at autopsy and conventionally fixed by immersion in a 4% aqueous formaldehyde solution. For immunocytochemical investigations part of the tissue was fixed in an admixture of 4% para-formaldehyde and picric acid (pH 7, see Braak E et al., 1994). Blocks of tissue from anteromedial portions of the temporal lobe were removed at the mid-uncal level, including the entorhinal and transentorhinal regions, amygdala and/or hippocampal formation. Additional portions of the occipital lobe were cut perpendicular to the calcarine fissure. The blocks were embedded in polyethylene glycol and cut at 50–100 μm. Successive frontal sections were processed using three staining techniques.

The aldehyde fuchsin/Darrow red method was used for topographic orientation (Braak, 1980; Braak et al., 1988). Modern silver techniques exploiting physical development of the nucleation sites were applied: a silver-iodide technique for AGs, argyrophilic glial inclusions, neurofibrillary changes and other tau-associated cytoskeletal alterations, and a silver-pyridine method for β-amyloid deposits as well as for Lewy bodies and Lewy

neurites (Gallyas, 1971; Gallyas and Wolff, 1986; Campbell et al., 1987; Braak and Braak, 1991b; Iqbal et al., 1991, 1993; Braak et al., 1996). Immunoreactions were carried out in only a select number of the AGD cases (marked by an "i" in Table 1). Hyperphosphorylation of the tau protein was ascertained chiefly by reaction with the monoclonal antibody AT8 (INNOGENETICS, Ghent, Belgium), which selectively showed the abnormally phosphorylated protein without crossreactions with normal tau epitopes (Biernat et al., 1992; Mercken et al., 1992; Goedert et al., 1995). Incubation of free-floating sections was carried out applying the monoclonal antibody AT8 for about 40–44 hours at 4°C (1:2,000, Mercken et al., 1992). Incubation with the second biotinylated antibody was performed for 2 hours (anti-mouse IgG). Immunoreactions were visualized with the ABC-complex (Vectastain) and 3,3-diaminobenzidine-tetra-HCl/H_2O_2 (DAB, D5637 Sigma).

In silver-stained sections all cases were classified according to a procedure permitting differentiation between six stages in the development of AD-related neurofibrillary tangles (NFTs) and neuropil threads (NTs) as well as three stages of β-amyloid deposits (Tables 1–3; NFT/NT stages I–II: transentorhinal stages, III–IV: limbic stages, V–VI: neocortical stages; and β-amyloid stages A: a few amyloid deposits in basal neocortex, B: many such deposits in basal neocortex and additional ones in allocortex, C: large numbers of amyloid deposits in all parts of the cerebral cortex; see: Braak and Braak, 1991a, 1994; Samuel et al., 1996; Hansen and Samuel, 1997; Hyman and Trojanowski, 1997; Nagy et al., 1997). Lesions related to Parkinson's disease (PD) were evaluated in sections stained with the Campbell-Switzer silver-pyridin technique (Braak et al., 1996) and/or with immunoreaction against ubiquitin (anti-ubiquitin 1:500; DAKO). Diagnoses were performed using published criteria and were achieved without knowledge of clinical data, age, or gender of the individuals.

The AGD cases included 23 cases sent by gerontopsychiatric hospitals (marked by G in Table 1) and 125 cases which were part of a large number of non-selected cases received from Departments of General Pathology (n = 2661). These AGD cases were controlled against the background of the non-selected cases n = 2661 – 125 = 2536). A few cases below the age of 25 and above the age of 95 were excluded from the study. The material did not include cases with diseases known to be associated with the development of non-AD-related neurofibrillary changes (Braak and Braak, 1997).

Results

Frequency of occurrence, age range, and gender distribution of AGD cases

Pathological lesions associated with AGD were not found to occur regularly in non-demented individuals of advanced age. Table 1 documents all of the AGD cases (n = 148). The diagnoses were achieved solely at post mortem evaluation (not a single case was diagnosed clinically). Often encountered were cases of AGD in combination with NFT/NT stages I–III (130 from a total of 148 cases, Fig. 1). Only 4 cases were devoid of AD-related neurofibrillary changes and 4 showed concomitant fully-developed AD at stage V. The most frequently co-occurring pathology was NFT/NT stage II (n = 58, Fig. 1). Two cases showed the presence of cortical and subcortical Lewy bodies and Lewy neurites (marked by PD in Table 1). Many of the clinical protocols provided no useful documentation regarding possible impairment of intellectual capabilities. However, some protocols definitely noted severe cognitive decline (32 from a total of 148 cases = 22%, marked by "d" or "de" in Table 1).

Only one of the 32 AGD cases with clinically ascertained histories of dementia turned out to be devoid of AD-related lesions, and 17 of this

Table 1. List of AGD cases giving age range, gender distribution, and stage of concomitant AD-related pathology according to Braak and Braak (1991a). Roman numbers refer to NFT/NT-related stages I–VI and capitals to β-amyloid stages A–C. "d" or "de" refers to cases, the clinical protocols of which note a history of severe cognitive decline, "de" marks AGD cases with no or only subtle concomitant AD-related pathology (NFT/NT stages I–II); "i" show cases in which immunoreactions for hyperphosphorylated tau (AT8) have been carried out; PD indicates co-occurrence of PD-related pathology. "G" marks cases from geronto-psychiatric hospitals

n.	age (years)	gender	NFT	βA4	Comm.
1	51	f	I	A	
2	52	f	O	O	de
3	57	m	I	O	i
4	58	f	V	C	G, d
5	59	m	I	O	i
6	60	f	II	O	i
7	61	f	I	O	G
8	61	m	II	O	i
9	62	m	III	O	i
10	63	f	O	O	G
11	63	m	O	O	i
12	63	m	I	O	
13	64	m	II	A	
14	65	f	I	A	G, de
15	65	m	I	A	i
16	66	f	II	B	i
17	66	f	II	O	G, de
18	67	f	I	A	
19	67	f	II	B	d
20	67	f	II	O	G
21	68	f	I	A	i
22	68	f	II	A	i, de
23	68	m	II	O	i
24	68	m	III	B	
25	69	m	I	O	i, de
26	69	m	II	O	i
27	69	m	III	C	i
28	69	m	III	A	
29	70	f	I	O	G, de
30	70	f	I	B	G
31	70	f	II	A	
32	70	f	II	A	i
33	70	m	II	B	i
34	71	f	I	B	i, de
35	71	m	II	A	i
36	71	m	III	A	d
37	72	f	I	O	i
38	72	f	I	O	i, de
39	72	m	II	O	i
40	72	m	II	O	G, de
41	73	f	I	A	i
42	73	f	II	O	i
43	73	f	II	O	i
44	73	f	II	O	G
45	73	f	III	A	

(continued)

Table 1. *Continued*

n.	age (years)	gender	NFT	βA4	Comm.
46	73	m	I	A	i, de
47	73	m	I	A	i, de
48	73	m	III	A	
49	73	m	III	B	d
50	74	f	II	O	i
51	74	m	O	O	
52	75	f	I	B	i
53	75	f	II	A	i
54	75	f	III	B	i
55	75	f	III	B	i
56	75	f	V	C	i,d
57	75	m	I	O	i
58	75	m	II	O	i
59	75	m	II	A	i
60	75	m	II	C	G, de
61	75	m	III	A	i
62	76	f	II	B	i
63	77	f	II	A	i
64	77	m	I	B	i
65	77	m	III	A	
66	78	f	I	A	i, de
67	78	f	II	O	
68	78	f	II	B	G
69	78	f	III	A	
70	78	f	III	B	i, d
71	78	f	III	B	G, d
72	78	f	III	O	i
73	78	m	I	B	i
74	78	m	I	A	i, de
75	78	m	II	A	i
76	79	f	II	A	G, de
77	79	f	IV	B	G, d
78	79	m	II	A	G, de
79	79	m	II	B	i
80	80	f	I	A	i
81	80	f	I	O	
82	80	f	III	O	i
83	80	f	IV	O	i, d
84	80	m	I	O	G, de
85	80	m	II	O	i
86	80	m	II	B	i
87	80	m	III	O	
88	81	f	II	A	i
89	81	f	III	B	i
90	81	f	III	O	
91	81	f	IV	A	i
92	81	m	I	A	i
93	81	m	I	A	G
94	81	m	II	O	PD
95	81	m	III	O	i
96	82	f	I	O	i
97	82	f	I	A	i

(continued)

Table 1. *Continued*

n.	age (years)	gender	NFT	βA4	Comm.
98	82	f	II	B	i
99	82	f	II	B	i
100	82	f	III	B	i
101	82	f	IV	B	i, d
102	82	m	II	B	i
103	82	m	II	B	i
104	83	f	II	O	i
105	83	f	III	B	i
106	83	f	IV	B	G, d
107	83	m	I	O	i
108	83	m	II	A	i
109	83	m	II	O	i
110	83	m	II	O	G
111	83	m	V	C	G, d
112	84	f	I	A	
113	84	f	IV	B	i
114	85	f	I	O	i
115	85	f	III	A	i
116	85	f	IV	B	d
117	85	m	I	A	i
118	85	m	II	B	i
119	85	m	II	A	i
120	85	m	II	O	G
121	85	m	III	A	i
122	86	f	II	B	i
123	86	f	II	B	i
124	86	f	II	A	i
125	86	f	II	O	G
126	86	f	III	A	PD
127	86	f	IV	B	i
128	86	m	I	A	i
129	86	m	II	B	i
130	87	f	V	C	d
131	88	f	I	B	i
132	88	f	IV	B	i
133	88	m	II	A	i
134	88	m	III	C	i
135	88	m	III	B	i
136	88	m	III	B	i
137	89	f	III	B	i
138	89	f	III	B	i
139	89	m	III	B	i
140	90	f	II	O	i
141	91	f	II	A	i
142	91	m	II	A	i
143	91	m	II	O	
144	91	m	III	B	d
145	92	f	III	O	i
146	93	f	I	B	i
147	93	f	II	A	i
148	96	m	IV	B	

Table 2. Gender distribution and number for a total of 125 AGD cases either devoid of neurofibrillary changes of the Alzheimer type or with concomitant NFT/NT-stages I–VI in various age categories. Cases with no signs of AGD in brackets

Age	NFT 0	NFT I/II	NFT III/IV	NFT V/VI	Sum	female	male
26–30	0 (50)	0 (11)	0 (0)	0 (0)	0 (61)	0 (19)	0 (42)
31–35	0 (46)	0 (12)	0 (0)	0 (0)	0 (58)	9 (25)	0 (33)
36–40	0 (61)	0 (22)	0 (0)	0 (0)	0 (83)	0 (36)	0 (47)
41–45	0 (47)	0 (31)	0 (0)	0 (0)	0 (78)	0 (28)	0 (50)
46–50	0 (72)	0 (55)	0 (2)	0 (0)	0 (129)	0 (42)	0 (87)
51–55	1 (75)	1 (86)	0 (3)	0 (1)	2 (165)	2 (61)	0 (104)
56–60	0 (74)	3 (141)	0 (4)	0 (5)	3 (224)	1 (66)	2 (158)
61–65	1 (65)	4 (176)	1 (5)	0 (2)	6 (248)	1 (91)	6 (157)
66–70	0 (46)	10 (216)	3 (32)	0 (6)	13 (300)	8 (147)	6 (153)
71–75	1 (27)	16 (200)	7 (53)	1 (10)	25 (290)	14 (146)	11 (144)
76–80	0 (12)	13 (185)	7 (97)	0 (21)	20 (315)	12 (160)	9 (155)
81–85	0 (3)	19 (210)	11 (147)	0 (45)	40 (405)	17 (234)	13 (171)
86–90	0 (4)	8 (108)	9 (84)	1 (39)	18 (235)	11 (153)	7 (82)
91–96	0 (0)	5 (27)	3 (26)	0 (17)	8 (70)	4 (50)	4 (20)
	3 (582)	79 (1480)	41 (453)	2 (146)	125 (2661)	70 (1258)	58 (1403)

Table 3. Age range, gender distribution, mean and median of age for a total of 125 AGD cases as compared to non-selected non-AGD cases either devoid of neurofibrillary changes of the Alzheimer type or with concomitant NFT/NT-stages I–VI

Line	Morphol. changes	n	Age range	Mean/median	female/male
1	AGD	125	51–96	79/78	66/59
2	AGD-female	66	51–93	80/79	
3	AGD-male	59	57–96	77/77	
4	AGD + AD O	3	52–74	63/63	2/1
5	AGD + AD I	32	52–93	75/75	17/15
6	AGD + AD II	47	60–93	78/78	23/24
7	AGD + AD III	33	62–92	80/80	16/17
8	AD O	582	26–90	51/51	220/362
9	AD I	882	26–95	65/65	386/496
10	AD II	598	29–94	76/75	294/304
11	AD III	359	50–94	80/80	216/143
12	AGD + AD I/II	79	51–93	77/77	40/39
13	AGD + AD III/IV	41	62–96	80/81	23/18
14	AGD + AD V/VI	2	75–86	82/81	2/0
15	AD I/II	1480	26–95	70/71	680/800
16	AD III/IV	453	46–95	81/81	270/183
17	AD V/VI	146	51–95	84/83	88/58

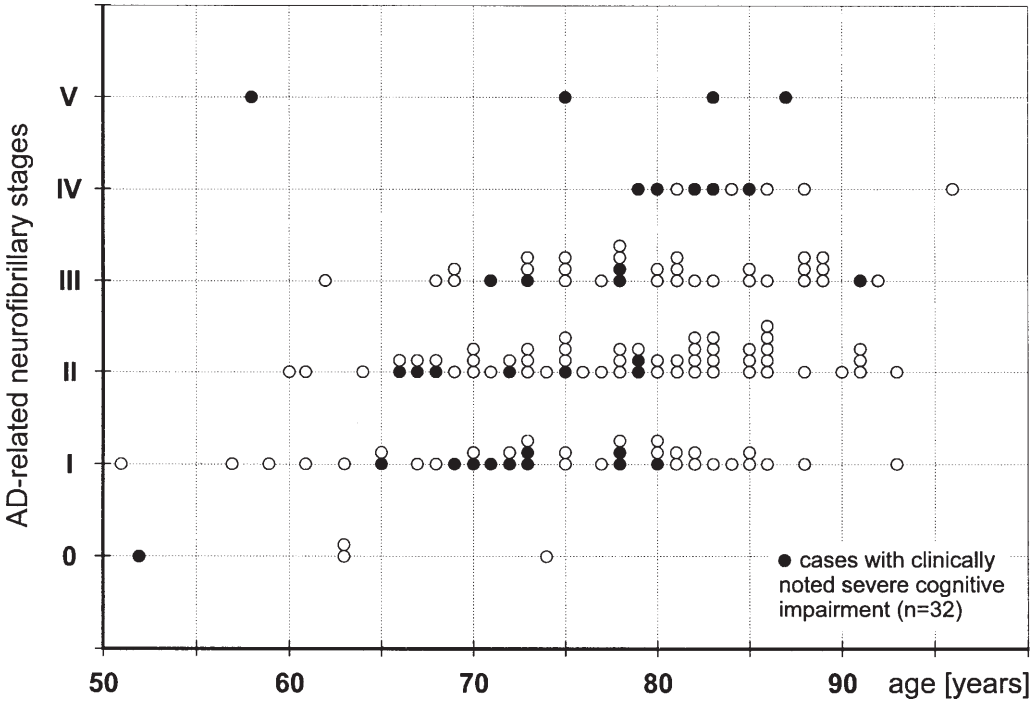

Fig. 1. Age distribution of AGD cases (n = 148) with and without cocomitant AD-related neurofibrillary changes. Most AGD cases exhibit co-occurrence of mild AD-associated lesions corresponding to stages I–III. Note that AGD cases with more advanced AD-stages become more prevalent with increasing age

subgroup showed mild alterations corresponding to NFT/NT stages I or II. All of these cases were free of cortical Lewy bodies and/or other major destructions which could have contributed to the clinical picture of intellectual decline. These cases are marked by "de" in Table 1. The clinical records of such relatively homogeneous AGD cases generally noted an early onset of inappropriate social conduct and personality changes preceding memory failure and cognitive decline. The emotional imbalance overshadowed the decline in intellectual capacities. Patients occasionally exhibited marked restlessness and psychotic episodes. As time passed, dementia predominated and the patients soon became doubly incontinent. Gait disturbances, rigidity, resting tremor, and hypokinesia were not observed. In general, the illness followed a rapid downhill course and death usually was caused by bronchopneumonia.

The material consisted of 23 AGD cases sent by gerontopsychiatric hospitals and 125 cases which were part of a large number of non-selected cases received from Departments of General Pathology (n = 2661). The prevalence of AGD cases (without and with concomitant AD-pathology) increased considerably with growing age (Fig. 2, Tables 2, 3). The fact that the non-selected material (n = 2661) included 146 cases of fully-developed AD at stages V or VI (6%), as well as 125 AGD cases (5%) supports the assertion that AGD is a disorder with a relatively high frequence of occurrence (Fig. 2).

Table 2 displays the number of AGD cases and their gender distribution in various age categories. These cases (n = 125) occurred in the non-selected material and either were found to be devoid of neurofibrillary changes of the Alzheimer type or had concomitant NFT/NT-lesions corresponding to stages I–VI. The total number of non-selected cases including the AGD cases is shown in brackets.

Table 3 compares the age range, age mean and median, and gender distribution in a total of 125 AGD cases found in the non-selected material (n = 2661) with the respective values of the remaining cases (AD 0 to AD V/VI). Note that the mean/median age at death of AGD cases devoid of AD related neurofibrillary changes (line 4) or with mild AD-related neurofibrillary changes of stage I and II (lines 5–6) are remarkably higher than that of the cases devoid of AGD-lesions (lines 8 and 9–10).

The frequency of occurrence of AGD cases in various age categories as compared to similarly achieved data of the remaining cases in the non-select material either devoid of NFT/NT-related pathology or showing lesions corresponding to stages I–VI is shown in Fig. 2.

Histopathological features of AGD

Upon gross examination, the brains of AGD cases appeared virtually unchanged or only mildly atrophic and apparently did not differ from that of age-matched, mentally unimpaired controls.

The key feature of the brain lesions associated with AGD consisted of spindle-shaped small grains, part of which could be detected in sections stained with a suitable silver method. The bulk of the pathology, nevertheless, first was rendered visible by immunoreaction for abnormally phosphorylated

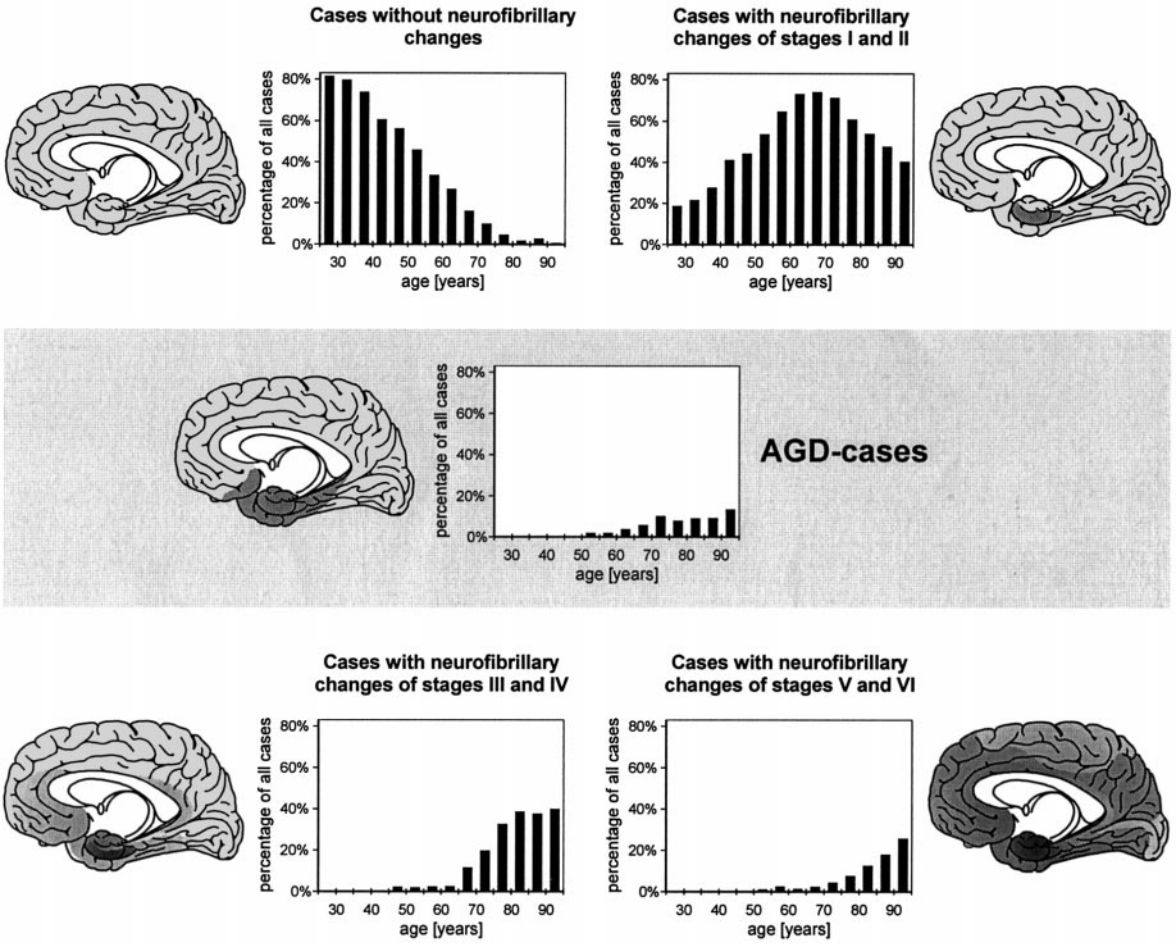

Fig. 2. Frequency in the occurrence of AGD cases in different age categories (center) as compared to the frequency of cases devoid of AD-related neurofibrillary changes (upper left corner), or showing NFT/NT stages I and II (upper right corner), stages III and IV (lower left corner), and stages V and VI – fully developed AD (lower right corner). Data are derived from a total of 2661 non-selected autopsy cases. Note the increase in prevalence in AGD cases with advancing age

tau protein. Most of this material remained in a hyperphosphorylated, non-argyrophilic status and occurred in a few types of nerve cells and in oligodendrocytes located in the vicinity of the afflicted nerve cells.

Abnormal tau in cell bodies and cellular processes of specific neuronal types

Immunostaining for abnormal tau protein revealed a strikingly uniform lesional distribution pattern in all of the AGD cases. The pathological changes were concentrated most highly in anteromedial portions of the temporal lobe and in the hypothalamic lateral tuberal nucleus. All of the afflicted cortical

nerve cells were projection neurons. The overall density of lesions varied from mild to severe among individual cases. However, this did not affect or alter the typical lesional pattern. The transentorhinal and entorhinal regions consistently bore the brunt of the pathology (Fig. 3b). The hippocampal formation, the amygdala, and the hypothalamus generally were a step less severely involved. From the allocortical core, the lesions usually encroached upon the adjoining neocortical areas, but advanced no further thereafter.

The projection cells of the transentorhinal and entorhinal layers pre-α and pri-α, the principal cells of the lateral tuberal nucleus, the projection cells in both the central nucleus and the basolateral nuclei of the amygdala, the pyramidal cells in the first sector of the Ammon's horn (CA1), as well as the hilar mossy cells, and the granule cells of the fascia dentata particularly were prone to develop the abnormal tau protein (Figs. 3, 4). Ballooned achromatic pyramidal cells richly endowed with the pathological material were encountered in layers V and VI of basal temporal neocortical areas.

Sections stained with reduced silver techniques for demonstration of neurofibrillary changes of the Alzheimer type (Bielschowsky, Gallyas) displayed an only small fraction of the pathology seen in immunoreactions. Small segments of nerve cell processes appeared in both immuno- and silver-stained sections as oat-shaped AGs. Individual AGs typically displayed a spindle-shaped, often slightly bent body with a long axis of up to 9 μm and a diameter of up to 4 μm (Fig. 4c–g). Variations in shape included rod- or drumstick-like structures, occasionally with small excrescences along the surface. Occasionally, filiform appendages were seen arising from both poles. AGs in the entorhinal cortex generally were larger than those in the CA1 sector.

The transentorhinal and entorhinal regions were most susceptible to the development of AGs. Layer pre-β bore the brunt of the change, but a scattering of AGs also occurred within the deep entorhinal layer pri-α and occasionally in the cellular islands of layer pre-α (Fig. 4b). The highest AG density was found close to the transentorhinal/entorhinal border (Braak and Braak, 1989, 1992b). AGs typically continued into the upper portions of layer III of the temporal neocortex and eventually extended into anterobasal portions of the insula, as well as into the temporopolar and frontoorbital neocortex.

Characteristically, the fascia dentata showed the presence of granule cells immunoreactive for abnormal tau protein only, but an absence of argyrophilic or immunoreactive grains, nor did AGs regularly appear in sectors CA4, CA3, and CA2 of the Ammon's horn. AGs appeared in both the external and internal pyramidal layers of sector CA1 with a marked decrease in density towards the stratum radiatum-lacunosum-moleculare and stratum oriens. The wedge-shaped prosubicular portion of CA1 usually exhibited the highest AG density in the hippocampal formation, while the subiculum contained only a sparse number. The presubiculum remained without AGs but some occurred in the parasubiculum.

At the subcortical level, a dense scattering of AGs distinguished the basolateral nuclei of the amygdaloid complex. On rare occasions, AGs were found in basal portions of the claustrum, whereas the striatum and the magnocellular nuclei of the basal forebrain remained devoid of AGs. The

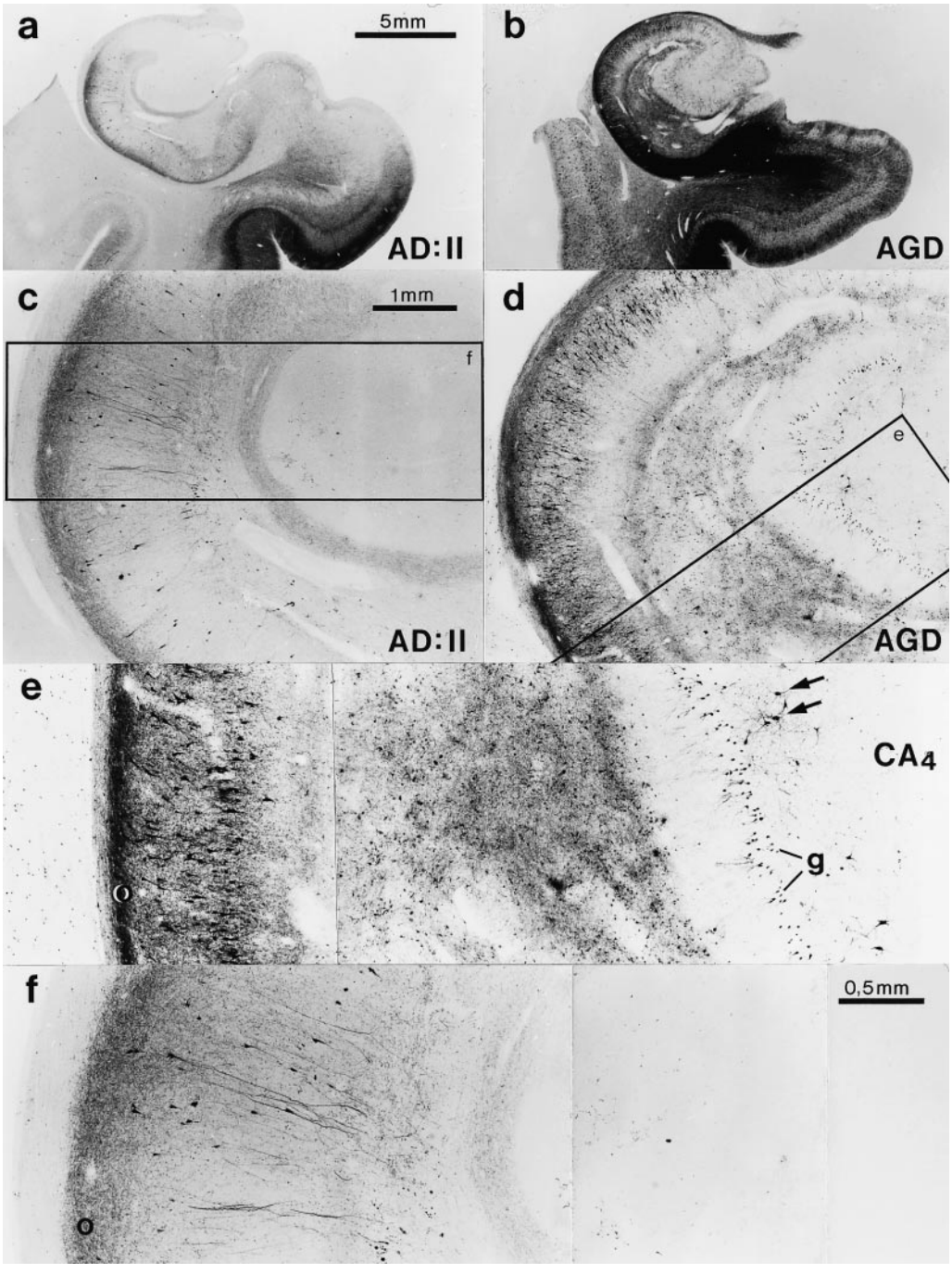

Fig. 3. Comparison between a typical case of AGD with concomitant AD-related stage II pathology (**b,d,e**) and a case exhibiting solely stage II pathology (**a,c,f**), both seen in immunoreactions for abnormally phosphorylated tau protein (AT8) and in sections through the hippocampal formation at the level of the lateral geniculate nucleus (100 μm). Note the much more severe affliction of nerve cells, in particular the remarkable involvement of the granule cells (g) of the dentate fascia in AGD (**e**). Arrows point to afflicted hilar mossy cells (CA4). Note the dense immunoreaction in the stratum oriens (o) of CA1. Abundant immunoreactive oligodendrocytes occur in the white matter and in the course of the perforant path in AGD (**e**). Framed areas in Figs. 3c and 3d are displayed at higher magnification in Figs. 3e and 3f

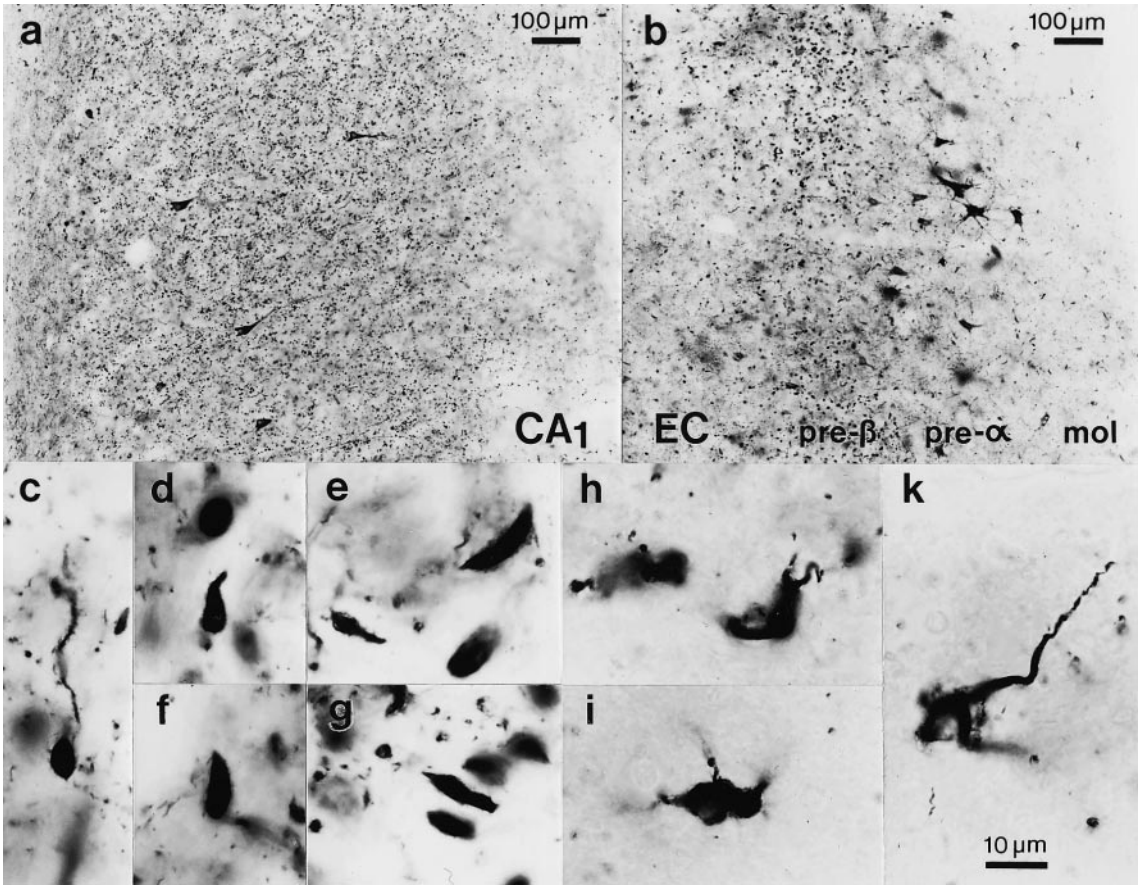

Fig. 4. **a–b** Typical distribution pattern of abundant AGs displayed by immunoreaction for abnormally phosphorylated tau protein (AT8) in CA1 (**a**) and the entorhinal region (**b**). Note the presence of a few immunoreactive pyramidal cells in CA1 and projection cells in the entorhinal layer pre-α (100 µm). **c–g** Characteristic argyrophilic grains. **h–k** Oligodendrocytes immunoreactive for hyperphosphorylated tau protein (antibody AT8)

hypothalamic lateral tuberal nucleus consistently showed high concentrations of AGs, whereas the adjoining hypothalamic nuclei and the entire thalamus appeared nearly free of lesions (Braak and Braak, 1989, 1992a). The lower brain stem displayed a few grains in the periaqueductal grey matter, anterior raphe nuclei, locus coeruleus, and dorsal vagus area. Diencephalic and mesencephalic components of the basal ganglia (external, internal, and ventral pallidum, subthalamic nucleus, substantia nigra, tegmental pedunculopontine nucleus), as well as cerebellum, pons, inferior olive, and spinal cord usually remained devoid of AGs.

Abnormal tau in oligodendrocytes and astrocytes, argyrophilia in oligodendrocytes

Immunoreactive and partially argyrophilic oligodendrocytes occurred predominantly in the white matter close to involved cortical areas and subcortical

nuclei. On occasion, however, they could be encountered within the cortex exclusively in association with bundles of myelinated fibers. The abnormal tau aggregates often showed a loop- or hook-like formation in close vicinity to the cell nucleus (Fig. 4h–k). Altered oligodendrocytes typically accompanied the perforant path throughout its entire course. Furthermore, they were seen in large numbers in the alveus. Only a small portion of the total number of oligodendroglial cells within a given volume of white matter showed the pathological change. From time to time, hyperphosphorylated tau protein was encountered in astrocytes as well. The degree and topographic location of astrocytic involvement was subject to considerable individual variation.

Discussion

Pathological lesions associated with AGD were not found to occur regularly in non-demented individuals of advanced age and therefore cannot be regarded as typical age-changes of the human brain.

Nontheless, the prevalence of AGD cases grew with increasing age. The non-selected material investigated in this study (n = 2661) included 125 AGD cases (5%). This correlates with values reported by Martinez-Lage and Munoz (1997) who found 11 AGD cases in a total of 300 non-selected autopsies (4%). These values show that AGD is a disorder with a relatively high frequence of occurrence. Martinez-Lage and Munoz (1997) noted intellectual deterioration in 2 of their 11 cases (18%). In the present material a progressive mental deterioration was documented clinically in 32 subjects out of a total of 148 AGD cases (22%). Tolnay et al. (1997) recently provided data from 35 clinically well-documented cases of AGD, 19 of which were demented (54%). All of the demented cases showed only mild concomitant AD pathology corresponding to NFT/NT stages I–III. Apparently, the accompaning AD-related lesions were not chiefly responsible for the marked mental deterioration noted in these cases. Rather, the data support the view that the pathological process underlying AGD has the potential to result in severe brain dysfunction as reflected in a corresponding decline of intellectual capabilities.

The brain pathology associated with AGD closely resembles that seen in a number of well-known degenerative diseases of the human nervous system which are marked by the formation of an abundance of abnormal tau phosphoprotein (Goedert et al., 1997). AD, Pick's disease (PID), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD) belong to this group of tauopathies. A critical event at the outset of each of these illnesses is hyperphosphorylation of the tau protein which initially results in the formation of a soluble non-argyrophilic material. In a second step, the material converts into nearly insoluble fibrils which then adopt a notable argyrophilia. In AD, PSP, and CBD, a considerable amount of the material eventually develops a strong argyrophilia and thus is easily detectable in sections routinely stained with suitable silver methods. In PID and AGD, on the other hand, the material largely remains in a non-argyrophilic status and, accordingly, immunoreactions are required for delineation of the full

picture of PID- and AGD-related lesions (Probst et al., 1996; Tolnay et al., 1997b).

Characteristically, many granule cells of the fascia dentata are markedly immunoreactive for abnormal tau protein in AGD. Interestingly, neither dendritic segments nor portions of the granule cell axon develop AGs since both the molecular layer of the fascia dentata and the CA4 and CA3 sectors (target zones of the mossy fibers) remain devoid of AGs. One might speculate that AGs mainly develop in terminal ramifications of extrinsic axons. It is unclear, however, whether this holds true for all locations showing the presence of AGs.

The occurrence of hyperphosphorylated tau protein in oligodendroglial cells is not a prominent feature of AD. Astrocytes containing the abnormal material in AD are seen much more frequently to develop at the induction sites of neuritic plaques and foreshadow the gradual formation of these lesions. Other tauopathies (AGD, PID, PSP, CBD and various forms of multisystem atrophy) usually exhibit a marked development of abnormal tau protein in both oligodendroglial cells and astrocytes (Papp et al., 1989; Horoupian, 1990; Nakazato et al., 1990; Yamada and McGeer, 1990; Yamada et al., 1992; Probst et al., 1993; Papp and Lantos, 1994; Iwatsubo et al., 1994).

Key features of different tauopathies frequently co-occur in one and the same individual. AGD cases preferably develop concomitant AD-pathology. The prevalence of stage I–III pathology increases with age and, accordingly, homogeneous cases of AGD are encountered rarely (Figs. 1, 2, Table 1). Attempts to correlate the clinical picture of AGD with the neuropathological findings become increasingly difficult with growing severity of concomitant lesions. In addition, the clinical records of patients with predominant AGD pathology record symptoms closely resembling those of AD with the subtle difference that personality changes tend to precede memory failure in AGD. Extended prospective clinico-pathological studies are needed to document in detail specific features which would permit a clinical diagnosis of AGD.

At neuropathological examination, AGD not only shares a number of characteristics with AD, PID, PSP, and CBD but also exhibits features allowing easy differentiation from these illnesses. The distinctive AGs are much smaller than AD-related NFTs and never occur in cell bodies. Extraneuronal "ghost" tangles are larger and less argyrophilic than AGs. Neuropil threads (Braak et al., 1986) are slender filiform structures without spindle-shaped thickenings and are much more variable in size and shape than the sturdy grains, which exhibit little variation. The grains normally maintain their distance from each other and only rarely form small aggregations which may – at first glance – resemble neuritic plaques. Again, the dystrophic neurites of neuritic plaques vary greatly in size and shape in comparison to the uniform AGs, and the diameter of neuritic plaques generally exceeds that of AG aggregations. In addition, β-amyloid deposits are not encountered in AG aggregations. All of these differences argue against the inclusion of AGs in the spectrum of AD-related lesions or the classification of AGD as an extreme variant of AD (Cras and Perry, 1991).

An important feature which AGD and PID have in common is the early appearance of abnormal tau protein in the granule cells of the dentate fascia. In PID, Pick bodies typically form in these cells but seldom occur in cases of AGD. Interestingly, ballooned achromatic pyramidal cells in deep layers of some neocortical areas, a prominent feature in PID, are seen in cases of AGD as well albeit in small numbers (Dickson et al., 1986; Mackenzie and Hudson, 1995). However, the general lack of Pick bodies as well as the lack of a focal frontal and temporopolar cortical atrophy contradicts the classification of AGD as a variant of PID (Munoz-Garcia and Ludwin, 1984, 1986).

The very severe PSP-associated involvement of both the external and internal pallidum, the subthalamic nucleus, and the substantia nigra permits a clear distinction from the lesional pattern developing in AGD. There remains the final possibility that PSP may co-occur with AGD. However, none of the 148 cases showed a lesional pattern comparable to that described by Masliah et al. (1991). Specific involvement of subcortical nuclei and the cerebellum, which would indicate a disease process similar to PSP, striatonigral degeneration, Shy Drager syndrom, or olivopontocerebellar degeneration were not encountered in our material, nor were such changes found in the cases presented by Yamada et al. (1992) and Tolnay et al. (1997a,b).

Selective vulnerability of neuronal types is a feature common to all of the tauopathies. Nearly all of the types of nerve cells afflicted in AGD also are prone to develop lesions in the course of AD. Closer inspection, however, reveals subtle differences. In AD, some neuronal types become involved early, e.g., the projection cells in the entorhinal layer pre-α, while others develop late changes, e.g., the granule cells of the fascia dentata. Such a predetermined order in the sequence of involvement is not a characteristic of AGD. From the very beginning there is a more or less simultaneous development of alterations in all of the afflicted cell types. The early and marked involvement of the granule cells of the fascia dentata is a feature that AGD has in common with PID. Indeed, it can be used to distinguish AGD from early stages of AD.

In conclusion, AGD is a disease which easily can be differentiated from other tauopathies. It merits attention because of its frequent occurrence and its potential to cause eventual brain dysfunction, which clinically may appear as personality changes and/or deterioration of intellectual capabilities.

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