J Neuropathol Exp Neurol
Vol. 75, No. 4, April 2016, pp. 316–325
doi: 10.1093/jnen/nlw002

ORIGINAL ARTICLE

Altered Proteins in the Aging Brain

Adila Elobeid, MD, Sylwia Libard, MD, Marina Leino, PhD, Svetlana N. Popova, MD, PhD, and Irina Alafuzoff, MD, PhD

Abstract

We assessed the prevalence of common altered brain proteins in 296 cognitively unimpaired subjects ranging from age 50 to 102 years. The incidence and the stage of hyperphosphorylated-τ (HPτ), β-amyloid, α-synuclein (αS), and transactive response DNA (TDP) binding protein 43 (TDP43)-immunoreactivity (-IR) increased with age. HPτ-IR was observed in 98% of the subjects; the locus coeruleus was solely affected in 46%, and 79% of the subjects were in Braak stages a to II. β-Amyloid was seen in 47% of subjects and the Thal phase correlated with the HPτ Braak stage and age. Intermediate Alzheimer disease-related pathology (ADRP) was seen in 12%; 52% of the subjects with HPτ-IR fulfilled criteria for definite primary age-related tauopathy (PART). The incidence of concomitant pathology (αS, TDP43) did not differ between those with PART and those with ADRP but the former were younger. TDP43-IR was observed in 36%; the most frequently affected region was the medulla; αS-IR was observed in 19% of subjects. In 41% of the subjects from 80 to 89 years at death, 3 altered proteins were seen in the brain. Thus, altered proteins are common in the brains of cognitively unimpaired aged subjects; this should be considered while developing diagnostic biomarkers, particularly for identifying subjects at early stages of neurodegenerative diseases.

Key Words: α-Synuclein, β-Amyloid, Aging, Cognition, Hyperphosphorylated-τ, Immunohistochemistry, Transactive response DNA binding protein 43.

INTRODUCTION

The aggregation of misfolded proteins is a characteristic feature of aging as well as aging-related neurodegenerative disorders (1, 2). The most common altered proteins observed in aging brains are hyperphosphorylated-τ (HPτ) and β-amyloid (Aβ) (ie, hallmark lesions of Alzheimer disease [AD]), α-synuclein (αS) (ie, hallmark lesions of Parkinson disease [PD]/dementia with Lewy bodies [DLB]), and transactive response DNA binding protein 43 (TDP43) (ie, hallmark lesions of frontotemporal lobar degeneration with TDP [FTLD-TDP], and amyotrophic lateral sclerosis) (3–9). Each of these protein alterations seem to progress neuroanatomically in an orderly manner from a presumed initiation/predilection site. When the most severe stages of the progression are reached, these altered proteins are considered to be causative of neurodegenerative diseases such as AD, PD, DLB, FTLD-TDP, and amyotrophic lateral sclerosis (10–16). Previous postmortem studies have indicated that these altered proteins are also frequently seen in the brains of nondemented aged subjects (17–33) (Table 1). HPτ has been reported in 30% to 100% of cognitively unimpaired aged subjects (26, 30). Recently, a new entity, primary age-related tauopathy (PART), which describes neurologically unimpaired aged subjects with HPτ pathology in the hippocampus, has been defined (34). It has even been debated whether subjects with PART represent an early stage of AD or merely reflect a neurodegenerative process at an early stage (35). Also, "occult or incidental" αS pathology has been reported to be seen in 5% to 31% of the cognitively unimpaired aged subjects (21, 22), and Aβ has been reported in the cortex of 39% to 82% of cognitively unimpaired aged subjects (30, 31). TDP43 pathology has been reported in 3% to 40% of cognitively unimpaired aged subjects (23, 33); the latter is frequently associated with hippocampal sclerosis (36). Previous postmortem studies have also demonstrated that these altered proteins can be seen concomitantly in cognitively unimpaired aged subjects (19, 26, 29–31).

There are considerable variations on the reported incidences of altered proteins in cognitively unimpaired subjects and there is increasing use of these altered proteins as diagnostic biomarkers. The variable assessment strategies in previous studies, that is, silver stain versus Immunohistochemistry (IHC) and staging of neuroanatomical distribution versus quantitative assessment of the load of pathology, make comparisons of results difficult. Thus, the objectives of this study were to assess the prevalence of the most common altered proteins (ie, HPτ, Aβ, αS, and TDP43) in a well-characterized, large cohort of cognitively unimpaired aged subjects, implementing sensitive assessment methodologies. We also assessed the distribution pattern of these proteins (stage/phase) and looked at whether they coexisted.

From the Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden (AE, SL, IA); and Department of Pathology, Uppsala University Hospital, Uppsala, Sweden (AE, SL, ML, SNP, IA).
Send correspondence to: Irina Alafuzoff, MD, PhD, Rudbeck Laboratory, Department of Immunology, Genetics and Pathology, Uppsala University/Uppsala University Hospital, Dag Hammarskjölds väg 20, 751 85 Uppsala, Sweden; E-mail: irina.alafuzoff@ipg.uu.se
This study was funded by local grants from Uppsala University Hospital, Hans Gabriel and Alice Trolle-Wachtmeister Foundation, and by L'ORÉAL-UNESCO for Women in Science. The authors have no duality or conflicts of interest to declare.
Supplementary Data can be found at http://www.jnen.oxfordjournals.org.

TABLE 1. Review of the Literature Regarding Age-Related Pathology Seen in the Brain of Cognitively Unimpaired Subjects Assessed Primarily Applying Immunohistochemistry

Author	Subjects	Pathology Assessed	Method Applied	Staging Applied
Davis et al, 1999 (17)	59	ADRP	Silver stain	Braak ADRP and CERAD
		CAA	Aβ/IHC	
		LBRP	Ubiquitin/IHC	McKeith LBRP
Parkkinen et al, 2001 (18)	565	ADRP	Silver stain	Braak ADRP and CERAD
			Aβ/IHC	Quantitative assessments
		LBRP	αS/IHC	Quantitative assessments
Knopman et al, 2003 (19)	39	ADRP	Silver stain	Braak ADRP and CERAD
		CAA	Aβ/IHC	
		LBRP	αS/IHC	McKeith LBRP
Parkkinen et al, 2003 (20)	78	ADRP	Silver stain	CERAD
		LBRP	αS/IHC	Quantitative assessments
Jellinger, 2004 (21)	26	ADRP	Silver stain	Braak ADRP and CERAD
		LBRP	αS/IHC	Braak LBRP
Mikolaenko et al, 2005 (22)	56	ADRP	Silver stain	Braak ADRP and CERAD
		LBRP	αS/IHC	Semiquantitative assessments
Nakashima-Yasuda et al, (23)	63	TDP43	TDP43/IHC	
White et al, 2009 (24)	190	ADRP	Silver stain	Braak ADRP
		LBRP	αS/IHC	McKeith LBRP
Price et al, 2009 (25)	97	ADRP	Silver stain	Braak ADRP and CERAD
			HPτ/and Aβ/IHC	Quantitative assessments
Aho et al, 2009 (26)	54	ADRP	HPτ/IHC	Braak ADRP
		LBRP	Aβ/IHC	Quantitative assessments
			αS/IHC	Braak LBRP
Markesbery et al, 2009 (27)	139	ADRP	Silver stain	Braak ADRP and CERAD
		LBRP	αS/IHC	McKeith LBRP
Bennett et al, 2012 (28)	296	ADRP	Silver stain	Quantitative assessments
		LBRP	Aβ and HPtau-IHC	Quantitative assessments
			αS/IHC	McKeith LBRP
Arnold et al, 2013 (29)	110	ADRP	Silver stain	Braak ADRP and CERAD
		TDP43	TDP43/IHC	Quantitative assessments
Boyle et al, 2013 (30)	382	ADRP	HPτ- and Aβ/IHC	Quantitative assessments
		LBRP	αS/IHC	McKeith LBRP
Kovacs et al, 2013 (31)	51	ADRP and CAA	HPτ- and Aβ/IHC	Braak ADRP
		LBRP	αS/IHC	Braak LBRP
		TDP43	TDP43/IHC	
Dugger et al, 2014 (32)	87	ADRP	Silver stain	Braak ADRP and CERAD
		LBRP	αS/IHC	Beach LBRP
Uchino et al, 2015 (33)	136	ADRP	Silver stain	Braak ADRP
		LBRP	Hematoxylin and eosin	McKeith LBRP
		TDP43	TDP43/IHC	

ADRP, Alzheimer disease-related pathology; CAA, cerebral amyloid angiopathy; IHC, immunohistochemistry; LBRP, Lewy body-related pathology; TDP43, transactive response DNA binding protein 43; HPτ, hyperphosphorylated-τ; Aβ, β-amyloid; αS, α-synuclein; CERAD, The Consortium to Establish a Registry for Alzheimer's Disease (56); Braak ADRP (6, 57), McKeith LBRP (5), Braak LBRP (4).

MATERIALS AND METHODS

Study Subjects

A total of 608 neuropathological assessments were carried out at Uppsala University Hospital during a 6-year period (2009–2014). A clinical autopsy was carried out and recent medical records were available for all included cases. Two hundred ninety-six subjects fulfilled the following inclusion criteria: cognitively unimpaired, age at death ≥50 years, and postmortem delay being less than or equal to 120 hours. The clinical data regarding the cognitive status were obtained from the referral records. It should be noted that some of the patients might have displayed mild cognitive impairment that had not been recognized during their lifetime, although it is unlikely that the presence of moderate or severe dementia would have been overlooked. In most of the subjects, no neurological or psychiatric symptoms were registered. In 32% (n = 95) of the subjects, cerebrovascular lesion, primary or secondary brain tumors, brain infection, or brain trauma was

confirmed by a neuropathological investigation. In 6% (n = 19), a psychiatric disorder or epilepsy was registered in the medical records (Table 2).

Immunohistochemistry

Details regarding the immunohistochemical staining methods applied and antibodies used are provided in Supplementary Data 1. For IHC, 7-µm-thick sections were used. For detection, horseradish peroxidase-IHC detection kit with Romulin-3-amino-9-ethylcarbazol chromogen (Biocare Medical, Concord, CA) or DakoEnVision FLEX (DAKO Denmark AS, Glostrup, Denmark) detection system were used.

Neuropathological Assessment

At autopsy, the brains were weighed and immersed in 10% buffered formalin for at least 1 week and cut into 1-cm-thick coronal slices. Macroscopic lesions and vascular abnormalities were assessed, and brain samples were taken in a standardized manner from 16 brain regions (Table 3). Each section was stained with hematoxylin and eosin stain for the assessment of vascular lesions (ie, seen/not seen) and hippocampal sclerosis following recent recommendations (36). Further IHC stains were applied as summarized in Table 3. The IHC outcome, that is, immunoreactivity (IR), was assessed following the recommendations for HPτ Braak stage (6), Aβ Thal phase (3), αS BrainNet Europe (BNE) stage (4, 5, 12), and TDP43 regions recommended to be assessed by Josephs et al (amygdala, hippocampal formation, and frontal cortex), Brettschneider et al (medulla), and Uchino et al (amygdala, hippocampal formation, and medulla) (10, 16, 33). Cerebral amyloid angiopathy (CAA) was looked for in the Aβ/IHC-stained parietal cortex section and typed as either type 1 or 2 (37).

Assessment of AD-Related Pathology

Each case was assessed following the National Institute of Aging and AD Association (NIA-AA) recommendations. Based on the HPτ Braak stage and Aβ Thal phase, a level of AD-related neuropathological stage was given. When needed for the differentiation between the low/intermediate or intermediate/high level of AD-related pathology (ADRP), a modified Bielschowsky silver impregnation method was used to assess the score of neuritic plaques (38, 39).

Statistical Methods

IBM SPSS statistics software (Armonk, NY) was used, and standard error of means was used when applicable. The correlation between the studied variables was assessed using the Spearman correlation. For assessment of the statistical difference between the studied groups, Mann-Whitney U (2 groups) test and Kruskal-Wallis H test (several groups) were implemented.

TABLE 3. Assessment of the Altered Protein Expression

Neuroanatomic Region, Section	Staining				
	H&E	HPτ	Aβ	αS	TDP43
Frontal cortex, gyrus medius	X				3
Temporal cortex, gyrus medius	X	2		4	2
Gyrus cinguli, anterior	X			3	
Parietal cortex, inferior	X		1*	4	
Pre-/post central cortex	X				
Occipital cortex	X	3			
Hippocampus anterior	X	1			1
Hippocampus posterior	X	1	2	2	1
Basal forebrain, incl. amygdala	X		2	1	1
Striatum	X				
Thalamus	X				
Mesencephalon, incl. substantia nigra	X		3	1	
Pons, incl. locus coeruleus	X	1			
Medulla, incl. motor nucleus of vagus	X			1	1
Vermis	X				
Cerebellum	X		4		

H&E, hematoxylin and eosin; HPτ, hyperphosphorylated-τ; A*β*, *β*-amyloid; αS, α-synuclein; TDP43, transactive response DNA binding protein 43.

Screening of the protein expression was initiated with the assessment of a region numbered as 1 in the table. If immunoreactivity was observed, staining was then carried out on the other regions in ascending order as numbered.

*Assessment of cerebral amyloid angiopathy.

TABLE 2. Demographics of the Included Cognitively Unimpaired Subjects

Age Groups (years)		Gender		Neurologically Unimpaired	Cerebrovascular Lesion	Other Brain Disorders*
		F	M			
	n	n	n	n (%)	n	n
50–59	28	8	20	13 (46)	10	5
60–69	66	25	41	45 (68)	10	11
70–79	89	32	57	57 (64)	22	10
80–89	93	36	57	55 (59)	30	8
≥90	20	10	10	12 (60)	6	2
All subjects (%)	296	111 (38)	185 (62)	182 (61)	78 (26)	36 (12)

n, number; F, female; M, male.

*Psychiatric disorders (n = 16), primary or secondary brain tumor (n = 13), brain infection (n = 2), epilepsy (n = 3), and status postbrain trauma (n = 2).

RESULTS

The mean age at death of the study subjects was 75 ± 1 years. Sixty-two percent were male with a mean age at death 74 ± 1 years; 38% were female (mean age at death 76 ± 1). The mean postmortem delay was 72 ± 2 hours. The subject demographics are summarized in Table 2. The brain weights decreased significantly with age ($p = 0.0001$; Table 4).

Hyperphosphorylated-τ

HPτ-IR was seen in the brain in 98% of the whole cohort (Table 4). HPτ-IR was seen in 98% of the 182 study subjects lacking concomitant brain disease (Table 2). This percentage was not significantly influenced by other brain diseases registered, such as cerebrovascular alterations (96%) or psychiatric disorders, primary or secondary brain tumors, infections, epilepsy, or brain trauma (100%).

HPτ-IR was seen in the locus coeruleus (LC) in 95% of the subjects, increasing from 89% to 95% with age (Table 4). HPτ-IR was seen in the hippocampal section in 91% of the subjects, increasing from 71% to 100% with age. In 9 subjects, a Braak stage could not be given while assessing the section (Table 5). In these cases, HPτ-IR was sparse in the hippocampal formation and was not seen in the LC. The mean age at death of these subjects was 77 ± 4 years; 5 were female, 1 of the subjects had suffered from a psychiatric disorder, and 2 had cerebrovascular alterations.

Concomitant LC and hippocampal HPτ-IR was noted in 260 subjects; thus, 21 subjects displayed only HPτ-IR in the LC. In 95% of the cohort, a Braak stage was assigned (Table 5). A significant correlation was noted between the age at death and the Braak stage ($r = 0.25$, $p = 0.001$); most (46%) were in the Braak stage a/b. Forty subjects fulfilled the criteria for Braak stage a, having a mean age at death of 66 ± 2 years; 95 were in Braak stage b, having a mean age at death of 71 ± 1 years. Thirty-four percent of the subjects were in Braak stage I–II, 10% were in Braak stages III, and 4% in Braak stage IV; most of these subjects were over 80 years at death. Two subjects were in Braak stage V. One of these was a male patient who died at the age of 80 years; his medical history revealed a head trauma at the age of 20 years; he was operated on for cancer in the kidney and had a history of hypertension, hyperlipidemia, and diabetes. The second subject was an 82-year-old man with a history of prostate and lung cancer, hypertension, and cardiac insufficiency. Both of these subjects had been hospitalized prior to death for more than 1 week, and no cognitive impairment was registered.

Primary Age-Related Tauopathy

There were 146 subjects who fulfilled criteria for definite PART, ie, HPτ pathology with a Braak distribution but lacking concomitant Aβ-IR (Table 6). There was a significant correlation between the age at death and the Braak stage ($r = 0.34$, $p = 0.001$). The HPτ-IR ranged from a to IV; the majority were in a Braak stage below III. There were 2 subjects in Braak stage IV. One subject was a 79-year-old man with a clinical history of diabetes, hyperlipidemia, and lung cancer with brain metastasis. He was hospitalized prior to death and there were no records of cognitive impairment. The second subject was an 89-year-old man with myeloma and prostate cancer and atrial flutter. He was living at home by himself, and no record of cognitive impairment was registered. Five out of the 296 cognitively unimpaired subjects displayed HPτ-IR lesions in glial cells in the ventral midline of the medulla oblongata.

β-Amyloid

Aβ aggregates were seen in the cortex in 47% of the subjects (Table 4). Aβ-IR was seen in 50% of the 182 study subjects lacking concomitant brain disease (Table 2). This percentage was not significantly influenced by other brain diseases registered, such as cerebrovascular alterations (46%) or psychiatric disorders, primary or secondary brain tumors, infections, epilepsy, or brain trauma (33%). Thirty-four percent of the subjects were in Thal phase I (Table 7). A significant correlation was noted between the Thal phase and the age at death ($r = 0.32$, $p = 0.001$). One subject, an 82-year-old man,

TABLE 4. Altered Protein Expression in the Neuroanatomical Predilection Areas

Age at Death (Years)		BW (g)	Protein Expression Seen With Immunohistochemistry in n (%)							
			HPτ		Aβ	αS		TDP43		
	n	Mean ± SE	LC	HC	Cortex	Mdl	AC	Mdl	AC	HC
50–59	28	1494 ± 45*	25 (89)	20 (71)	5 (18)			6 (21)		
60–69	66	1450 ± 20*	64 (97)	57 (86)	23 (35)	4 (6)	4 (6)	14 (21)		2 (3)
70–79	89	1376 ± 16*	83 (93)	81 (99)	37 (42)	17 (19)	4 (4)	21 (24)	9 (10)	8 (9)
80–89	93	1357 ± 13*	90 (84)	91 (98)	58 (62)	20 (22)	5 (15)	17 (18)	25 (27)	18 (19)
≥90	20	1324 ± 26*	19 (95)	20 (100)	16 (80)	3 (15)		6 (30)	5 (25)	4 (20)
All subjects (%)	296	1394 ± 10	281 (95)	269 (91)	139 (47)	44 (15)	13 (4)	64 (22)	39 (13)	32 (11)
Subjects with IR (%)	290		290 (98)		139 (47)	57 (19)		105 (36)		

n, number; BW, brain weight; HPτ, hyperphosphorylated-τ; Aβ, β-amyloid; αS, α-synuclein; TDP43, transactive response DNA binding protein 43; LC, locus coeruleus; HC, hippocampal formation; Mdl, medulla; AC, amygdala; IR, immunoreactivity.
*$p = 0.0001$.

TABLE 5. Distribution of Hyperphosphorylated-τ Pathology

Age at Death (Years)		Braak Stage (6) n (Percent of Subjects in the Same Age Group)							
	n	0	a, b	I–II	III	IV	V	a–V	Not staged
50–59	28	2 (7)	22 (79)	3 (11)				25 (89)	1 (4)
60–69	66	2 (3)	45 (68)	14 (21)	4 (6)	1 (2)		64 (97)	
70–79	89	1 (1)	41(46)	32 (36)	5 (6)	5 (6)		83 (93)	5 (6)
80–89	93	1 (1)	22 (24)	44 (47)	15 (16)	7 (6)	2 (1)	90 (96)	2 (2)
≥90	20		5 (25)	7 (35)	7 (35)			19 (95)	1 (5)
All subjects	296	6 (2)	135 (46)	100 (34)	31(10)	13 (4)	2 (1)	281 (95)	9 (3)

n, number; Spearman correlation Braak HPτ stage/age: r = 0.25, p = 0.0001.

TABLE 6. Characteristics of Subjects With Primary Age-Related Pathology and Subjects With Alzheimer Disease-Related Pathology

	n	Gender M/F	Mean age at death (range)	HPτ Braak stage (6) in %						+ αS in %	+ TDP43 in %
				a,b	I	II	III	IV	V		
Definite PART (34)	146	57/89	72.1 ± 0.9 (50–94)*	62	17	19	1	1		14	32
ADRP	135	47/88	78.3 ± 0.9 (50–102)*	33	19	17	21	8	1	24	42

n, number; M, male; F, female; PART, primary age-related tauopathy = subjects with hyperphosphorylated-τ (HPτ); AD, Alzheimer disease; AD-related pathology (ADRP) = subjects with HPτ and β-amyloid; αS, α-synuclein; TDP43, transactive response DNA binding protein 43; Mann-Whitney U test.

*p < 0.0001. Spearman correlation test Braak HPτ stage/age for 146 subjects with PART: r = 0.34, p = 0.0001; Spearman correlation test Braak HPτ stage/age for 135 subjects with AD-related changes: r = 0.10, p = 0.05.

TABLE 7. Distribution of β-Amyloid Pathology

Age at Death (Years)		Thal β-Amyloid Phase (3) n (Percent of Subjects of the Same Age Group)					
	n	0	1	2	3	4–5	1–5
50–59	28	23 (82)	5 (18)				5 (18)
60–69	66	43 (65)	17 (26)	2 (3)		4 (6)	23 (35)
70–79	89	52 (58)	30 (34)	2 (2)	2 (2)	3 (3)	37 (42)
80–89	93	35 (38)	41 (44)		6 (6)	11 (12)	51 (55)
≥90	20	4 (20)	8 (40)	1 (5)	4 (10)	3 (15)	16 (80)
All subjects	296	157 (53)	101 (34)	5 (2)	12 (4)	21 (7)	131 (44)

n, number; Spearman correlation Thal β-amyloid phase/age: r = 0.32, p = 0.0001.

displayed only Aβ pathology; he had suffered from hypertension, atrial flutter, and prostate cancer prior to death.

CAA was observed in the parietal cortex in 44 subjects; 19 subjects (6%) displayed type I; 25 subjects (9%) displayed type II CAA. There were 3 subjects that displayed type I; and 5 cases displayed type II CAA without concomitant Aβ aggregates in the neuropil.

Subjects With AD-Related Pathology

Concomitant HPτ and Aβ-IR were seen in 46% (135/296). A significant correlation was observed between the Braak HPτ stage and the Thal Aβ phase (r = 0.44, p = 0.001). Subjects with concomitant HPτ and Aβ-IR were significantly older compared to subjects with PART (Table 6).

When applying the NIA-AA criteria, 35% were classified as having a low level of AD pathology, 12% an intermediate level, and only 1 case was classified as having a high level of AD pathology (Table 8). In 15 out of the 296 subjects, a silver stain had to be carried out in order to give a NIA-AA level of ADRP. There were significant differences in the mean age at death of the patients with different levels of ADRP.

α-Synuclein

αS-IR was observed in 19% of the subjects (Table 4). ADRP αS-IR was seen in 20% of the 182 study subjects lacking concomitant brain disease (Table 2). This percentage was not significantly influenced by the other brain diseases registered, such as cerebrovascular alterations (18%) or psychiatric

TABLE 8. Level of Alzheimer Disease Pathology, Applying 2012 National Institute of Aging and Alzheimer's Association (NIA-AA) Recommendations (38, 39)

Thal Stage of Aβ Pathology (3) A	CERAD NP Score (56) C	Braak Stage of HPτ Pathology (6) B					Level of AD Pathology (11)	Gender M/F	Mean Age at Death Mean ± SE	Percent of the 287 Cases
			1		2	3				
		0	a, b	I–II	III–IV	V				
0 = none	0	5	80	62	4		None	92/59	72 ± 1[1, 3]	53
1 = 1–2	0–1	1	42	45	7		Low	61/39	77 ± 1[1, 2]	34
	2–3				8		Intermediate	26/8	82 ± 2[2, 3]	12
2 = 3	0–3		2	2	8					
3 = 4–5	0–1		1	1	17	1				
	2–3					1	High	1/0	80	

Aβ, β-amyloid; AD, Alzheimer disease; CERAD, Consortium to Establish Registry of AD (56); NP, neuritic plaques; HPτ, hyperphosphorylated-τ; M, male; F female; C/0, none, C/1, sparse, C/2-moderate, C/3-frequent; Out of the 296 subjects, 9 cases were not classified due to unusual distribution of HPτ pathology. Significant differences Kruskal-Wallis H test: [1, 3] $p < 0.001$, [2] $p < 0.02$.

TABLE 9. Distribution of α-Synuclein Pathology

Age at Death (Years)	n (Percent of Subject in the Same Age Group)							
McKeith stage (5)		0		1		2		1, 2
BNE stage (12)	n	0	1	2	3	4	5	1–5
50–59	28	28 (100)						
60–69	66	62 (94)	3 (4)			1 (6)		4 (6)
70–79	89	72 (81)	8[1] (9)		1(1)	7[2] (8)	1 (1)	17 (19)
80–89	93	73 (78)	3[3] (3)	1(1)		12[1] (12)	4 (4)	20 (22)
≥90	20	17 (85)				3[1] (15)		3 (15)
All subjects	296	252 (85)	14[4] (8)	1(1)	1(1)	23[4] (8)	5(2)	44[8] (15)

n, number; BNE, BrainNet Europe; Spearman correlation test BNE α-synuclein stage/age: $r = 0.2$, $p = 0.0001$. The number of amygdala-predominant cases in each BNE stage is given as suffix.

disorders, primary or secondary brain tumors, infections, epilepsy, or brain trauma (19%). In 13 subjects, αS-IR was seen only in the amygdala. BNE stage was applicable in 44 cases: 10% were in the brainstem (BNE stages 1–3) and 10% were in the limbic stages (BNE stages 4–5) (Table 9). Amygdala-predominant αS pathology was observed in 8 subjects. A significant correlation was noted between the age at death and the αS/BNE stage ($r = 0.2$, $p = 0.001$). There was 1 male patient (age at death 61 years) who displayed only αS pathology in his brain (BNE stage 3).

Transactive Response DNA Binding Protein 43

TDP43-IR was seen in 36% of the subjects (Table 4). TDP43-IR was seen in 34% of the 182 study subjects lacking concomitant brain disease (Table 2). This percentage was not significantly influenced by other brain diseases such as cerebrovascular alterations (37%) or psychiatric disorders, primary or secondary brain tumors, infections, epilepsy, or brain trauma (42%). In 22% of the subjects, TDP43-IR was seen in the medulla, in 13% in the amygdala, and in 11% in the hippocampal formation. Josephs staging was carried out on subjects with AD-related and TDP43 pathology (Table 10). There was a significant correlation between the age at death and the TDP43 Joseph stage ($r = 0.3$, $p = 0.001$).

In 56 subjects, TDP43-IR was seen only in the medulla (36 PART and 20 subjects with ADRP), in 8 subjects only in the hippocampal formation (2 PART and 6 subjects with ADRP), and in 1 PART subject TDP43-IR was seen in both these regions.

Concomitant Pathologies

A summary regarding the concomitant expression of the altered proteins is provided in Table 11. The most common combinations were Aβ/HPτ-IR (46%), followed by TDP43/HPτ-IR (35%). There were 44 subjects (15%) displaying concomitant HPτ/Aβ/TDP43-IR, mean age at death 80 ± 1 years; 33 subjects (11%) displaying concomitant HPτ/αS/Aβ-IR, mean age at death 83 ± 3 years; and 22 subjects (7%) displaying concomitant HPτ/αS/TDP43-IR, mean age at death 81 ± 1 years. There were 16 subjects (5%) that displayed all 4 altered proteins in their brain concurrently; their mean age at death was 81 ± 2 years and 12 of these subjects were male. The Braak HPτ stage ranged from a to V, Thal Aβ phase from 1 to 4, and BNE αS stage from 0 to 5.

TABLE 10. Distribution of Transactive Response DNA Binding Protein 43 in Subjects With Alzheimer Disease-Related Pathology

	Age at Death (Years)		Josephs Stages (10) n (Percent of Subjects Within Each Age Group)			
		n	I	II, III	IV, V	I–V
ADRP	50–59	5				
	60–69	23				
	70–79	36	2 (6)	3 (8)	2 (6)	7 (19)
	80–89	56	8 (14)	3 (5)	9 (16)	19 (34)
	≥ 90	15	2 (13)		2 (13)	4 (27)
	All subjects	135	12 (9)	6 (4)	13 (10)	31 (23)

n, number; ADRP, Alzheimer disease; related pathology, ie, both hyperphosphorylated-τ and β-amyloid detected; Spearman correlation test: Josephs stage/age for the 135 subjects with AD-related pathology, r = 0.3, p = 0.001.

TABLE 11. Subjects With Concomitant Expression of the Altered Proteins

	β-Amyloid n (%)	α-Synuclein n (%)	TDP43 n (%)
Hyperphosphorylated-τ	138 (46)	56 (19)	105 (35)
β-Amyloid		33 (11)	57 (19)
α-Synuclein			22 (7)

n, number; TDP43, Transactive response DNA binding protein 43.

DISCUSSION

Here, we report that the altered proteins such as HPτ, Aβ, αS, and TDP43 assessed by IHC are frequently seen in the brains of cognitively unimpaired subjects. We implemented the methods that have been shown by BNE to yield high agreement rates in an interlaboratory setting (11–14). Neurodegenerative changes in aging brains have certainly been previously reported (Table 1), but the methods applied have varied. The recommended staging strategies while assessing the severity has not been always applied or have differed, and a systematic assessment of each alteration in the same brains has not been carried out. Moreover, the selection criteria, the cohort size, and the mean age of the cohorts have been variable. Thus, conducting a systematic comparison of all previously reported data with our results has been challenging.

HPτ in Cognitively Unimpaired Subjects

HPτ-IR was seen in 98% of the 296 subjects with a mean age at death of 74 ± 1 year, when both the LC and the hippocampal section were assessed. Thus far, Braak et al carried out assessment of LC pathology in cognitively unimpaired subjects, either in a cohort including young (4- to 29-year-old subjects) or a cohort that was clinically unselected (demented and cognitively unimpaired) (8, 9). Both the work by Braak et al and our present results indicate that LC is involved prior to the hippocampal formation. Thus, we recommend that a section obtained from the pons at the level of the LC should be included for assessment of HPτ pathology.

Ninety-one percent of our subjects displayed HPτ-IR in the hippocampus, a region that has been assessed in most prior studies. An incidence of 100% was reported by Boyle et al for 467 cognitively unimpaired subjects, with a mean age at death of 87 ± 7 years (30). The difference in the obtained result of 91 versus 100 percent is probably due to factors such as the number of the brain regions assessed (2 by us and 8 by Boyle and colleagues), different methods used (7-μm-thick vs 20-μm-thick sections), and the differing mean age of the studied cohorts (75 ± 1 vs 87 ± 7 years). However, based on previous results as well as ours, it is clear that HPτ is the most common alteration seen in the aging brain.

In this study, most of the cognitively unimpaired subjects were in Braak stage a, b (46%), and only 2 subjects were in Braak stage V. The same Braak staging strategy based on the HPτ-stained sections was previously implemented by Kovacs et al. They assessed 51 unimpaired subjects, age ranging from 77 to 87 years. They reported that most subjects in their cohort were in Braak stage I–IV (91%) and 7% were in Braak stage V–VI (31). Our cohort included 100 subjects within the same age range of 77 to 87 years at death, 72% were in Braak stage I–IV, and only 2% in Braak stage V. These 2 studies are comparable regarding the age of subjects and the assessment strategy applied; thus, one can conclude that in a cognitively unimpaired subject, HPτ pathology is not expected to be seen in excess in the occipital cortex, which is required for designating stages V and VI.

There were only 2 subjects in our study in Braak stage V; both were hospitalized for some time prior to death and no history of cognitive impairment was reported. It should be noted that both subjects suffered from a malignancy and a possible depression might have masked mild cognitive symptoms. It should be noted, however, that medical records in Sweden are in an electronic format, which facilitates that all observations obtained by various medical personnel regarding a patient are registered. Thus, the medical records can be considered to be quite reliable sources of information.

PART

In 2014, the new entity of PART was defined and 52% of our study subjects fulfilled definite criteria for this entity (34). It is noteworthy that the PART subjects were significantly younger, compared with the subjects with AD-related

pathology. Thus, the "young" subjects with PART might have evolved into subjects with ADRP if they had lived longer. However, it is possible that PART is a different entity from ADRP (Table 6), and a possible difference between these 2 "groups" should be investigated. Issues that are certainly of interest are all those known to be a risk factor regarding the Aβ accumulation but various factors regarding genetics and environmental exposures might also be of interest.

While assessing 23 subjects fulfilling criteria for PART, Nelson et al reported that 35% displayed HPτ-IR lesions in the medulla (40). We were unable to confirm this observation because only 2% of PART cases and 4% of the subjects with ADRP within the same age range displayed HPτ-IR lesions in the medulla. Thus, the etiopathogenesis, incidence, and clinical significance of these pathological findings should be further studied.

Aβ in Cognitively Unimpaired Subjects

Aβ-IR was seen in the cortex in 47% of subjects with age ranging from 50 to 102, increasing from 18% in the youngest to 80% in the oldest age group. Thus, our results are in line with those reported by Boyle et al (82%) (30). Most of our subjects were in the Thal phase 1 (34%), and the number of subjects in the Thal phase 4–5 increased from 6% to 15% with age. Although not previously reported for a cognitively unimpaired cohort, we observed a significant correlation between the Thal phases and age and between the Thal phase and the HPτ stage. This supports the notion that if a subject would live long enough, he/she would display ADRP. Thus, PART would only be a phase of the aging phenomenon rather than a distinct entity.

In this study, 15% of subjects displayed CAA. Within the age range of 77 to 87 years, CAA was seen in 28%, a finding in line with the report of Kovacs et al (25%) (31). Contrary to the above, Davis et al reported an incidence of 62% with CAA when studying 59 cognitively unimpaired subjects with a mean age at death of 84 ± 8 years. This significant difference is probably due to the number of sections assessed: 1 by us and 4 by Davis et al (17).

NIA-AA Criteria

When NIA-AA criteria were applied, 34% displayed a low level of ADRP and 12% an intermediate level. Only 1 subject displayed a high level of AD pathology. Our results are in line with previous results reporting that only 15% of cognitively unimpaired subjects were in the intermediate level of ADRP (41). The only case having a high level of ADRP was in Braak stage V, Thal phase 4, and CERAD scores 2. Without knowledge of the clinical status, this case would certainly have received a neuropathological diagnosis of AD. Lack of cognitive impairment in a subject with this level of pathology might be explained by the cognitive reserve theory (42–44). The observation that only 12% fulfilled the criteria for the intermediate level and 1 subject fulfilled the criteria for the high level are in line with previous studies reporting that advanced Braak stages are rare in individuals without documented dementia (45–48).

αS in Cognitively Unimpaired Subjects

Nineteen percent of the subjects in this neurologically unimpaired cohort displayed αS-IR. Previously, αS-IR has been reported to be seen in 8% to 31% of the neurologically unimpaired subjects (18, 20–22, 24, 27, 31). The factors that influenced the results were case selection, number of cases included, regions screened, and methodology applied as previously reported (18, 49, 50). In this study, there was a significant correlation between the αS-IR lesions and the age of the subjects, a result in line with a previous report (18). This is also in line with epidemiological studies reporting an increase in the incidence of PD with age (51). In most of the subjects with αS-IR, the distribution was within the range of BNE stages 1 to 5 (McKeith midbrain/limbic stage), a result in line with previous reports (27). Eighteen percent of our cognitively unimpaired subjects with αS pathology ranging from BNE stage 1 to 5 displayed an amygdala-predominant pattern. Interestingly, a percentage in line with our observation was reported by Leverenz et al while assessing subjects with dementia (52). In 4% out of the 296 subjects and in 15% of the 93 subjects within the age range of 80 to 89 years, αS-IR was seen only in the amygdala, a result in line with what was reported by Markesbery et al (10% in subjects with mean age at death 85 ± 8) (27). Thus, the amygdala should certainly always be assessed regarding αS pathology. It has been suggested that incidental αS pathology represents preclinical PD or DLB (53, 54), and based on our results, close to every fifth subject from this cognitively unimpaired cohort might represent preclinical stages of α-synucleinopathy.

TDP43 in Cognitively Unimpaired Subjects

In this study, TDP43-IR was seen in 36% of the total cohort, a result in line with what was reported by Arnold et al (29) and Uchino et al (33). Here, we screened 3 brain regions: the medulla, amygdala, and the hippocampal formation. To our surprise, and not previously reported, the most frequently affected region was the medulla and TDP43-IR was already seen in the youngest group. Recently, Uchino et al also assessed the medulla, amygdala, and the hippocampus and reported that the TDP43-IR lesions were most frequently seen in the hippocampus (33). Thus, the neuroanatomical region where the TDP43-IR pathology is most frequently seen varies, that is, the hippocampus (Uchino assessing Asian population), the medulla (our results assessing the Caucasian population), and the amygdala (Josephs et al assessing AD patients). Differing neuroanatomical distributions of pathology related to the racial differences or concomitant AD have previously been reported for αS alterations (55). Thus, based on our results, all 3 regions—the medulla, amygdala, and the hippocampus—should be assessed for TDP43 pathology.

Concomitant Pathologies

In this study, the concomitant HPτ and Aβ-IR were seen in 46% of the subjects. Nineteen percent of our study subjects displayed concomitant HPτ and αS-IR, a result in line with what was reported by Kovacs et al (31). HPτ and TDP43-IR were seen in as many as 35% when 3 brain regions

were screened. In 2014, Josephs et al reported that 57% of their AD patients displayed TDP43-IR (10). Here, in 23% of the subjects with ADRP, we were able to give a Josephs stage of TDP43-IR. With age, we observed an increase in the TDP43-IR from 19% to 27% in the subjects with ADRP. It is of note that most of our cases were in Braak stages a to II, compared with the AD cases included in the study by Josephs et al (10). This probably explains the significant difference in the percentage of the TDP43-IR observed. In our cognitively unimpaired cohort, we observed 10% increase in the number of subjects with 3 altered proteins in the brain with advancing age (60–69 years: 24%; 70–79 years: 32%; and 80–89 years: 41%). It is notable that 5% of the subjects in this cohort displayed all 4 altered proteins. Thus, multiple pathologies should certainly be looked for in the predilection regions in the brain of aged subject, that is, age at death >60 years.

In conclusion, we demonstrate that a considerable percentage of cognitively unimpaired subjects, with an age range from 50 to 102 years, displayed abnormal protein aggregates. The most frequent altered protein we observed was HPτ-IR; furthermore, in 46% of the whole cohort, HPτ-IR was seen solely in the LC, supporting the notion that this region is affected prior to the hippocampus and thus should be routinely assessed. Most of our cases were within the Braak stage range of a to II. Aβ-IR was less common, the deposition followed a pattern described by Thal et al and correlated with both the age of the subject and the stage of HPτ-IR. NIA-AA criteria were applicable on all cases and only 12% of the subjects were classified as intermediate regarding the level of AD. A substantial number of subjects fulfilled the definite criteria for PART, but subjects with PART and subjects with AD-related pathology did not differ regarding the incidence of concomitant pathologies such as TDP43-IR or αS-IR. The only difference between subjects with PART and those with AD-related pathology was the mean age at death; thus, we cannot confirm that PART subjects and those with AD-related pathology represent distinct entities. TDP43-IR was most frequently observed in the medulla when compared with a study from Asia reporting the hippocampus as the most frequently affected. This observation suggests that the neuroanatomical vulnerability regarding the TDP43 alteration might be genetically (ie, racially) determined. In as many as every fifth patient, an incidental αS-IR was observed and the amygdala was affected in a considerable proportion suggesting that this region should be routinely assessed. In as many as 41% of the subjects within the age range 80 to 89 years, a combination of 3 altered proteins was observed, indicating that concomitant altered proteins are indeed common in the aged and should certainly be investigated. Out of our cohort, only 4 subjects lacked any of the assessed misfolded proteins in their brain; 1 subject displayed only Aβ and 1 displayed only αS. As many as 16 subjects displayed all of the 4 alterations assessed here.

ACKNOWLEDGMENTS

We thank the medical laboratory technologists Maud Salomonsson, Karin Staxäng, and Pere Giné i Gras for their skillful technical assistance and Meena Strömqvist for her critical reading of the manuscript. The subjects included and/or their relatives had given their consent for the use of the tissue, and the study was authorized by the regional Ethics Committee of Uppsala, Sweden, # 2011/286.

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