

Table 5 Case profiles of CDR 0.5 with "hippocampal sclerosis"

Age/ gender	Brain weight (g)	Stages of senile changes				CVD lesion	Atherosclerosis
		NFT	SP	LB	AG		
94/F	850	II	0	0	1	–	Mild
89/M	1296	0	B	0	0	–	Mild

SP, senile plaque; LB, Lewy body; AG, argyrophilic grain; CVD, cardiovascular disease; NFT, SP: Braak stage.¹⁰ LB, AG: our stage.^{6,7}



Fig. 3 Neuropathology of hippocampal sclerosis. (A) Macroscopic pathology of hippocampal sclerosis with serial unfixed coronal sections. Prominent atrophy of posterior hippocampus (bar = 1 cm). (B) Myelin-stained sections, demonstrating marked atrophy involving the CA1 and the subiculum (arrows). (Klüver-Barrera stain, bar = 0.5 cm). (C) Higher magnification of the hippocampus, indicated by a rectangle in section B, showing severe neuronal loss of CA1, relatively sparing CA2 (Klüver-Barrera stain, bar = 100 µm).

Neurofibrillary tangle predominant change in CDR 0.5

The case profiles are summarized in Table 3. The mean age of this group was highest (91.6 years).

Dementia with Lewy bodies – type change in CDR 0.5

The case profiles are summarized in Table 4. All cases showed amnesia as the chief complaint. All cases exhibited the presence of cortical Lewy bodies, fulfilling DLB limbic type.

Hippocampal sclerosis in CDR 0.5

The case profiles are summarized in Table 5. Both cases showed degeneration restricted to the anterior to posterior hippocampus (Fig. 3) without degenerative or vascular changes.

Neuropathologically unremarkable cases in CDR 0.5

The case profiles are summarized in Table 6. There was one case accompanied with severe compression myelopathy causing a bed-ridden state.

DISCUSSION

This study examined the pathological background of CDR 0.5 cases from serial autopsy cases from a general geriatric

Table 6 Case profiles of CDR 0.5 without specific neuropathological changes

Age/ gender	Brain weight (g)	Stages of senile changes				ApoE genotyping
		NFT	SP	LB	AG	
76/F	1175	II	A	0	1	33
76/F	1280	I	A	0	0	33
79/M	1180	II	0	0	0	33
95/M	1032	II	B	0	0	34
97/F†	969	I	B	0	0	33

SP, senile plaque; LB, Lewy body; AG, argyrophilic grain; NFT, SP: Braak stage.¹⁰ LB, AG: our stage.^{6,7}

†Bedridden state due to cervical compression myelopathy.

hospital, which roughly represent a general geriatric cohort. The results showed considerable differences from those obtained in memory clinics, where cases with AD represent the majority.

The pathological background of CDR 0.5 in our series consisted of AC, AGC, NFTC and DLBC, as well as vascular change and trauma. Special mention should be made of hippocampal sclerosis, which in Japan, has been neglected as secondary hypoxic/ischemic changes. Since these patients apparently survived the hypoxic/ischemic insults causing hippocampal sclerosis in recent advanced medical care, this pathology should be considered as a cause of cognitive decline, as stressed in the US.

A considerable number of cases showed non-specific pathology, making it difficult to explain the cognitive

decline. These cases may represent depression or reversible MCI. Many CDR 0.5 cases presented with various pathologies and it was difficult to determine which pathology most strongly contributed to cognitive decline with this type of retrospective study. Nevertheless, this study of cases with single neuropathological alterations definitely demonstrates that there were multiple pathological bases for CDR 0.5.

When the pathological background of CDR 0.5 was compared with that of dementia, they both shared each pathological component, but the incidences were different. The reason why the frequency of senile tauopathy was higher in CDR 0.5 than in CDR ≥ 1 was probably related to the speed in the progression of cognitive decline. In other words, AC/DLBC groups progress rapidly, while AGD/NFTC groups present with a more benign course, and thus the latter stay in CDR 0.5 longer. We are now examining cases of CDR 0.5 with MRI VSRAD, CSF biomarkers and PET scan, including fluorodeoxyglucose (FDG) and Pittsburgh Compound B (PIB). The results show that the cases presenting with normal A β 42 in the CSF and lacking cerebral cortical deposition of PIB show a slowly progressing course (data not shown).

From clinical longitudinal studies, MCI cases were classified into progressive, non-progressive and recovering subgroups. Our pathological study indicates that the first group may represent an AC/DLBC group, the second, an AGC/NFTC group and the last, unremarkable pathology.

Recently, neuropathological studies of mild cognitive impairment and cases of dementia followed from MCI stages were reported from the longitudinal prospective studies.^{23,24} The results were similar to our findings, with highest frequency of AD, but definitely included cases of AGC/NFTC changes.

Our study confirmed that MCI or CDR 0.5 represents a stage of cognitive decline and can be explained by different pathological backgrounds. So MCI or CDR 0.5 should receive a diagnosis of early AD, DLB, DG, NFTD, VD or unremarkable pathology and the most appropriate method for intervention should be employed. For this purpose, we are now conducting multi-institutional prospective longitudinal studies.

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Molecular Chaperone-Mediated Tau Protein Metabolism Counteracts the Formation of Granular Tau Oligomers in Human Brain

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Intracellular accumulation of filamentous tau proteins is a defining feature of neurodegenerative diseases termed *tauopathies*. The pathogenesis of tauopathies remains largely unknown. Molecular chaperones such as heat shock proteins (HSPs), however, have been implicated in tauopathies as well as in other neurodegenerative diseases characterized by the accumulation of insoluble protein aggregates. To search for in vivo evidence of chaperone-related tau protein metabolism, we analyzed human brains with varying degrees of neurofibrillary tangle (NFT) pathology, as defined by Braak NFT staging. Quantitative analysis of soluble protein levels revealed significant positive correlations between tau and Hsp90, Hsp40, Hsp27, α -crystallin, and CHIP. An inverse correlation was observed between the levels of HSPs in each specimen and the levels of granular tau oligomers, the latter of which were isolated from brain as intermediates of tau filaments. We speculate that HSPs function as regulators of soluble tau protein levels, and, once the capacity of this chaperone system is saturated, granular tau oligomers form virtually unabated. This is expressed pathologically as an early sign of NFT formation. The molecular basis of chaperone-mediated protection against neurodegeneration might lead to the development of therapeutics for tauopathies. © 2007 Wiley-Liss, Inc.

Key words: tau; oligomer; heat shock protein; molecular chaperone; Braak stage

Intracellular accumulation of filamentous tau proteins is a defining feature of neurodegenerative diseases, including Alzheimer's disease (AD), progressive supranuclear palsy, corticobasal degeneration, Pick's disease, and frontotemporal dementia with Parkinsonism linked to chromosome 17, all known collectively as *tauopathies* (Lee et al., 2001). Tau is a highly soluble and natively unfolded protein dominated by a random coil structure in solution (Schweers et al., 1995). It is believed that aberrant modifications of tau, including phosphorylation,

truncation, and conformational changes, induce filamentous aggregation (Alonso et al., 1996; Gamblin et al., 2003; Garcia-Sierra et al., 2003). In AD and related tauopathies correlated with dementia, tau generates insoluble aggregates (Arriagada et al., 1992). Although the mechanism underlying the conversion of tau protein from a soluble state to one of insoluble aggregates remains unclear, molecular chaperones such as heat shock proteins (HSPs) have been implicated in these tauopathies (Dabir et al., 2004; Nemes et al., 2004).

Molecular chaperones have essential roles in many cellular processes, including protein folding, targeting, transport, degradation, and signal transduction (Hartl and Hayer-Hartl, 2002). In addition to molecular chaperones, cells have evolved two mechanisms for degrading misfolded proteins, the ubiquitin-proteasome pathway and lysosome-mediated autophagy. The collective activities of molecular chaperones, the ubiquitin-proteasome system, and lysosome-mediated autophagy are normally sufficient to prevent the accumulation of misfolded proteins. In AD, Parkinson's disease, familial amyotrophic lateral sclerosis, and polyglutamine diseases, plaques and/or inclusion bodies that characterize these diseases colocalize with various molecular chaperones (Muchowski and Wacker, 2005) and components of the ubiquitin-proteasome degradation system (Sherman and Goldberg, 2001).

Recently, the carboxyl terminus of Hsc70-interacting protein (CHIP) was demonstrated to regulate tau

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ubiquitination and degradation (Hatakeyama et al., 2004; Petrucelli et al., 2004; Shimura et al., 2004b). CHIP is a key molecule in protein quality control processes that link the ubiquitin-proteasome and chaperone systems. We previously found increased levels of CHIP in AD brains that were inversely proportional to the amount of accumulated tau (Sahara et al., 2005). Deletion of CHIP in mice, however, failed to promote tau aggregation (Sahara et al., 2005; Dickey et al., 2006b). Thus, these observations could not clarify the precise roles of the chaperone and ubiquitin-proteasome systems in the pathogenesis of tauopathies. Immunohistochemical studies also showed that AD brains contain very few accumulations of CHIP within neurofibrillary tangle (NFT)-bearing neuron (Petrucelli et al., 2004). Indeed, the colocalization of tau pathology and chaperones varies, as demonstrated by immunostaining for tau, Hsp27, α B-crystallin (Dabir et al., 2004), Hsp70, and Hsp90 (Dou et al., 2003). These observations suggest that tau in a misfolded conformation or state, not tau in an irreversible filamentous state, may exist and be directly regulated as a chaperone client. For this reason, we continue to search for intermediate forms of tau fibrils by examining assembled recombinant tau and tau in human brain extracts. Atomic force microscopy (AFM) has revealed a granular-shaped prefibrillar form of tau in both in vitro and in vivo preparations (Maeda et al., 2006, 2007).

In the present study, human brains with varying degrees of Braak-staged NFT pathology were analyzed to assess tau protein quality control in vivo. Biochemically, HSPs and related proteins were quantitatively analyzed to examine the relationship between tau and HSPs. A positive correlation was revealed between tau and certain HSPs in terms of soluble protein levels. Moreover, we discovered an inverse correlation between the levels of granular tau oligomer and those of HSPs. These findings suggest that molecular chaperones interact with soluble tau protein to down-regulate the formation of granular tau oligomer.

MATERIALS AND METHODS

Post-Mortem Brains

Brain tissue was obtained from the Brain Bank for Aging Research at Tokyo Metropolitan Institute of Gerontology (TMIG) and Tokyo Metropolitan Geriatric Hospital (TMGH). Frontal and temporal cortices were examined from each brain specimen. Senile plaques and NFTs were classified based on quantitative pathological features according to Braak criteria (Braak and Braak, 1991). Demographic information for all brain specimens analyzed is presented in Table I and in a previous report (Maeda et al., 2006). This study was approved by the Institutional Review Board (IRB) of TMIG and TMGH as well as the RIKEN IRB.

Tissue Extraction

Frontal and temporal cortices from each subject were homogenized separately in three volumes of Tris-buffered

TABLE I. Demographic and Neuropathological Characteristics of Subjects*

Case no.	Age (years)	Sex	PMI (hr:min)	Braak stage ^a		Insoluble tau ^b	
				NFT	SP	Frontal	Temporal
1	52	M	15:51	0	0	0.86	0.70
2	69	F	11:48	0	A	0.45	1.06
3	82	F	39:04	0	0	2.34	1.23
4	87	M	70:10	0	0	0	1.86
5	78	M	2:02	0	0	0	9.73
6	66	F	9:51	0	B	0	2.44
7	81	M	3:00	I	B	1.00	10.82
8	97	F	2:40	I	B	7.00	3.34
9	84	M	47:25	I	B	0	4.48
10	87	M	4:25	I	C	1.94	19.1
11	93	M	20:49	I	C	3.38	69.5
12	86	F	6:50	III	C	1.93	100.4
13	94	M	13:00	III	C	2.56	73.3
14	87	F	4:21	III	C	6.81	209.5
15	82	F	10:32	III	C	31.1	184.8
16	89	F	16:11	III	C	7.03	88.6
17	90	F	64:07	V	C	182.5	2189.6
18	86	F	19:51	V	C	86.2	1026.5
19	93	F	13:28	V	C	169.8	1091.2
20	70	M	35:42	V	C	582.8	1580.1
21	80	F	6:41	V	C	9.60	223.4

*NFT, neurofibrillary tangle; SP, senile plaque; PMI, post-mortem interval.

^aFor the staging of NFTs and SPs, Braak and Braak criteria were applied (Braak and Braak, 1991).

^bThe relative score of sarkosyl-insoluble tau was measured by quantifying PHF1 immunoreactivity.

saline (TBS) containing protease and phosphatase inhibitors [25 mM Tris/HCl, pH 7.4; 150 mM NaCl; 1 mM EDTA; 1 mM EGTA; 5 mM sodium pyrophosphate; 30 mM β -glycerophosphate; 30 mM sodium fluoride; and 1 mM phenylmethylsulfonyl fluoride (PMSF)]. The homogenates were centrifuged at 27,000g for 15 min at 4°C to obtain supernatant (TBS sup) and pellet fractions. Pellets were rehomogenized in three volumes of high-salt/sucrose buffer (0.8 M NaCl; 10% sucrose; 10 mM Tris/HCl, pH 7.4; 1 mM EGTA; 1 mM PMSF) and centrifuged as described above. The supernatants were collected and incubated with sarkosyl (Sigma, St. Louis, MO; 1% final concentration) for 1 hr at 37°C, followed by centrifugation at 150,000g for 1 hr at 4°C to obtain salt and sarkosyl-soluble and sarkosyl-insoluble pellets (srk-ppt fractions).

Western Blotting

Fractionated tissue extracts were dissolved in sample buffer containing β -mercaptoethanol (0.01%). The boiled extracts were separated by gel electrophoresis on 10% or 4–20% gradient SDS-PAGE gels and transferred onto nitrocellulose membranes (Schleicher & Schuell Bioscience, Dassel, Germany). After blocking with a solution of 5% nonfat milk and 0.1% Tween-20 in PBS, the membranes were incubated with various antibodies, washed to remove excess antibodies, and then incubated with peroxidase-conjugated goat anti-

rabbit IgG (1:5,000; Jackson Immunoresearch, West Grove, PA) or anti-mouse IgG (1:5,000; Jackson Immunoresearch). Bound antibodies were detected by using an enhanced chemiluminescence system, SuperSignal West Pico (Pierce Biotechnology, Rockford, IL). Quantitation and visual analysis of immunoreactivity were performed with a computer-linked LAS-3000 Bio-Imaging Analyzer System (Fujifilm, Tokyo, Japan) in the software Image Gauge 3.0 (Fujifilm).

Antibodies

Polyclonal antibody E1, which is specific for human tau (aa 19–33; Sahara et al., 2005), and polyclonal antibody tauC, which is specific for the C-terminus of tau (aa 422–438; Sato et al., 2002), were produced in our laboratory. Monoclonal antibody Tau5, which is against an epitope located within the middle region of tau, was purchased from Biosource International (Camarillo, CA). PHF1, which is specific for tau phosphorylated at Ser396/Ser404, was provided by Dr. Peter Davies (Jicha et al., 1999). Polyclonal CHIP antibodies (R1) were produced in rabbits (Imai et al., 2002). Monoclonal antibodies to Hsp90 (Santa Cruz Biotechnology, Santa Cruz, CA), Hsp70 (Chemicon, Temecula, CA), Hsp60 (Stressgen, San Diego, CA), Hsp27 (Stressgen), α B-crystallin (Stressgen), β -tubulin (Sigma), β -actin (Sigma), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Chemicon), and neuron-specific enolase (NSE; Upstate, Charlottesville, VA) were purchased. Polyclonal antibodies against Hsc70 and Hsp40 were purchased from MBL and Stressgen. For Western blotting, antibodies were used at the following dilutions in blocking solution: E1, 1:5,000; tau5, 1:2,000; tauC, 1:5,000; PHF1, 1:2,000; CHIP, 1:5,000; Hsp90, 1:2,000; Hsp70, 1:1,000; Hsc70, 1:1,000; Hsp60, 1:5,000; Hsp40, 1:10,000; Hsp27, 1:1,000; α B-crystallin, 1:1,000; Akt, 1:2,000; β -tubulin, 1:5,000; β -actin, 1:10,000; GAPDH, 1:5,000; NSE, 1:1,000.

Coimmunoprecipitation Analysis

TBS-soluble fractions were incubated with anti-tau antibody JM (an antibody against the longest isoform of recombinant human tau; Takashima et al., 1998) for 2 hr at 4°C. Protein G-sepharose (GE Healthcare Bioscience, Piscataway, NJ) equilibrated in TBS was added to the mixture, which was then rotated overnight at 4°C. The resin was separated by centrifugation, washed four times with TBS, and then boiled in SDS sample buffer. Samples were subjected to SDS-PAGE, with subsequent immunoblotting with antibodies against Hsp90, Hsp70, β -tubulin, tau (anti-tau antibody HT7; Innogenetics, Zwijndrecht, Belgium), and NSE.

Purification of Granular Tau Oligomers

Enriched granular tau oligomer preparations were prepared as previously described (Maeda et al., 2006, 2007). Briefly, soluble fractions of human brain extracts (starting wet weight was ~12 g) were loaded onto an immunoaffinity column containing anti-tau antibody-conjugated Sepharose. Eluted samples were further purified by sucrose-step gradient centrifugation. Granular tau oligomer-enriched fractions were morphologically characterized by AFM.

Atomic Force Microscopy

Samples were dropped onto freshly cleaved mica and left in place for 30 min prior to AFM assessment. After washing the mica with water, we examined the tau-containing samples in solution using a Nanoscope IIIa (Digital Instruments, Santa Barbara, CA) set to tapping mode. OMCL-TR400PSA (Olympus, Tokyo, Japan) was used as a cantilever. The resonant frequency was about 9 kHz. We examined four different areas (4 μm^2) of the mica surface covered with granular aggregates. These areas were analyzed with NIH Image 1.63 (developed at The National Institute of Mental Health and available on the Internet at <http://rsb.info.nih.gov/niimage/>), and summations of four different areas were demonstrated.

Incubation of Recombinant Tau and Fluorescence Spectroscopy

Recombinant human tau (2N4R) was expressed in *Escherichia coli* BL21(DE3) and partially purified as described previously (Hasegawa et al., 1998). The degree of tau aggregation was determined using thioflavin T (ThT). The tau stock solution was diluted to 10 μM in a solution containing PBS (pH 7.4) and 10 μM ThT (Aldrich Chemical, Milwaukee, WI), then loaded into 96-well Black Cliniplates (Thermo Labsystems, Yokohama, Japan). Tau assembly was initiated by adding 10 μM heparin (Acros Organics, Geel, Belgium) and incubating mixtures (final volume of 50 μl per well) at 37°C. To analyze the effects of HSPs, 0.1 μM or 1 μM Hsp70 (Sigma) and 0.1 μM or 1 μM Hsp40 proteins (Stressgen) were added alone or in combination to the aggregation reaction in the presence of 1 mM ATP at time zero. Fluorescence was measured with an ARVO MX Multilabel counter (PerkinElmer, Waltham, MA) at an excitation wavelength of 440 nm and an emission wavelength of 486 nm. Measurements were carried out at the time points indicated.

Fig. 1. Immunoblotting of sarkosyl-insoluble and TBS-soluble tau in frontal and temporal cortices from aged human brains. **A:** Western blots of sarkosyl-insoluble fractions in human frontal and temporal cortices. Samples were categorized as Braak NFT stage 0 (lanes 1–6), stage I (lanes 7–11), stage III (lanes 12–16), and stage V (lanes 17–21). Samples derived from brains (2–20 mg wet weight) were separated by SDS-PAGE, blotted, then probed with PHF1 antibody. Blot of frontal cortical samples was obtained from longer exposure time than that of temporal cortical samples. **B:** Western blots of TBS-solu-

ble tau in human frontal and temporal cortices. Equal volumes of TBS-soluble fraction derived from 0.33 mg wet weight of brain were separated by SDS-PAGE, blotted, then probed with E1 (specific for N-terminus region of tau), tau5 (specific for the middle region), and tauC (specific for C-terminus region) antibodies. **C:** Western blots of TBS-soluble tau in human frontal and temporal cortices. Equal volumes of TBS-soluble fraction were separated by SDS-PAGE, blotted, then probed with the indicated panel of antibodies.

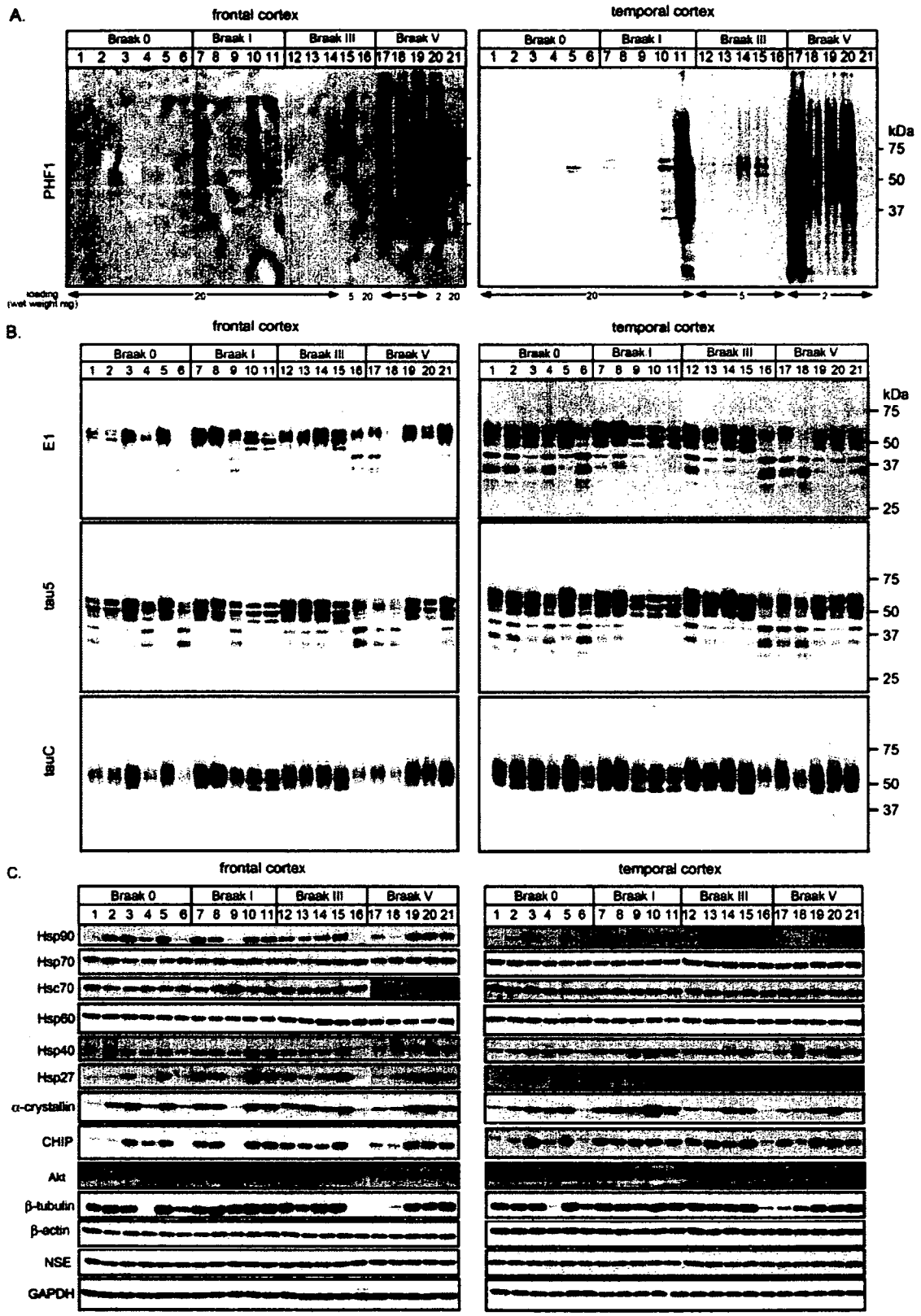


Figure 1.

Statistical Analysis

The statistical significance between groups was assessed by the Mann-Whitney test. The correlation between the level of tau and other proteins was tested using Spearman correlation. Data were analyzed with Prism for Macintosh version 4.0b (Graphpad, San Diego, CA), and significance levels were set at $P < 0.05$.

RESULTS

Sarkosyl-Insoluble Tau in Human Brain

The neuropathological diagnoses of 21 cases were determined by using criteria for degenerative dementia as previously reported (Saito et al., 2002). To verify NFT pathology, we performed Western blotting on sarkosyl-insoluble fractions obtained from these 21 cases. The amounts of sarkosyl-insoluble tau in both frontal and temporal cortices correlated well with various Braak NFT stages (Fig. 1A, Table I). Immunoblots of fractions from Braak V-staged brains displayed the typical triplet bands and smear patterns of PHF1-positive tau. Although low sarkosyl-insoluble tau levels correlated well with Braak 0 and I stages—stages seen during normal brain aging characterized by the absence of NFT pathology in frontal and temporal cortices—weak PHF1-positive tau immunostaining was observed in one Braak 0 brain (case 5) and three Braak I brains (cases 7, 10, and 11; Fig. 1A, Table I). Indeed, some discrepancies have been reported regarding insoluble tau levels and NFT scores of brains staged at Braak stages I–III (Katsuno et al., 2005). Together, these results indicated that the biochemical analysis was more sensitive in detecting precursors of NFTs (i.e., PHF1-positive tau) than was the Braak neuropathological classification method. Nonetheless, for one Braak V brain (case 21), we detected very low levels of sarkosyl-insoluble tau. In case 21, these levels were equivalent to those detected in Braak III brains (Table I), suggesting that, despite reliable neuropathological diagnosis, an unknown tau modification might have occurred in this case. In any case, it is important to identify an early biochemical indicator of AD rather than to measure levels of sarkosyl-insoluble tau.

TBS-Soluble Tau Protein Profiles in Human Brains

As shown in Figure 1B, a wide range of TBS-soluble tau levels and variable Western blot profiles were

observed among the human brains that we analyzed. Although we suspected that protein degraded during post-mortem intervals, the amounts of TBS-soluble tau in frontal and temporal cortices did not correlate with different postmortem intervals (Fig. 2A,B). For most of the samples, E1 and tau5 antibodies, which recognize the amino terminus and the middle regions of tau, respectively, immunostained low-molecular-weight bands ranging from 30 to 40 kDa (Fig. 1B). These bands, however, were not detectable with tauC antibody, which recognizes the carboxyl terminus of tau. This suggests that carboxyl-terminal-truncated tau fragments 30–40 kDa in size were present in these samples. For each case, the profile of tau protein in frontal cortex and that in temporal cortex appeared quite similar (Fig. 1B). Moreover, the relative levels of tau protein in frontal and temporal cortices were closely correlated ($P < 0.001$, Spearman correlation analysis). This result indicated that tau protein modification was conserved in different brain regions. Comparison of the amounts of TBS-soluble tau in brains of different Braak stages revealed significant differences in the frontal and temporal cortices of Braak I and V brains (Fig. 2G,H). These differences might be due to a conversion in tau protein solubility that results from excessive posttranslational modifications in Braak V brains, leading to the formation of filamentous insoluble tau. However, we did not observe a significant inverse correlation between TBS-soluble tau and sarkosyl-insoluble tau levels. Further study will be needed to clarify why Braak I and V brains contain different levels of soluble tau protein.

Correlation Between Tau Protein and HSPs

We used a panel of antibodies to identify HSPs (Hsp90, Hsp70, Hsc70, Hsp60, Hsp40, Hsp27, α -crystallin) and cochaperone CHIP in human frontal and temporal cortices (Fig. 1C). As with TBS-soluble tau levels, Hsp90 and NSE protein levels in frontal and temporal cortices did not correlate with different post-mortem intervals (Fig. 2C–F). Quantitative analysis of soluble protein levels in frontal cortices revealed significant positive correlations between tau and some HSPs (Hsp90, Hsp40, Hsp27, α -crystallin), CHIP, Akt, and β -tubulin (Table II). We obtained comparable results for soluble protein levels in temporal cortices (Fig. 1, Table II). Hsp90, Hsp40, Hsp27, α -crystallin, CHIP, Akt, and β -tubulin levels varied across different Braak stages, with

Fig. 2. Effect of post-mortem time and Braak NFT stage on relative protein levels. **A–F**: Relationship between postmortem interval (hours) and soluble protein levels. The relative amount of TBS-soluble tau in frontal (A) and temporal (B) cortices was calculated by dividing the intensity of tauC-immunoreactive bands by the intensity of GAPDH-positive bands. The relative amounts of Hsp90 in frontal (C) and temporal (D) cortices and NSE in frontal (E) and temporal (F) cortices were calculated by dividing the appropriate band intensity

by the band intensity of GAPDH. The relative amount of protein in case 7 was always normalized to 1. **G,H**: The relative levels of TBS-soluble tau in frontal (G) and temporal (H) cortices were compared for each Braak NFT stage. The relative amounts of TBS-soluble tau, as shown in A and B, were categorized accordingly into Braak NFT stage 0, I, III, or V. The results are shown as scatterplots of mean values. * $P < 0.05$, ** $P < 0.01$.

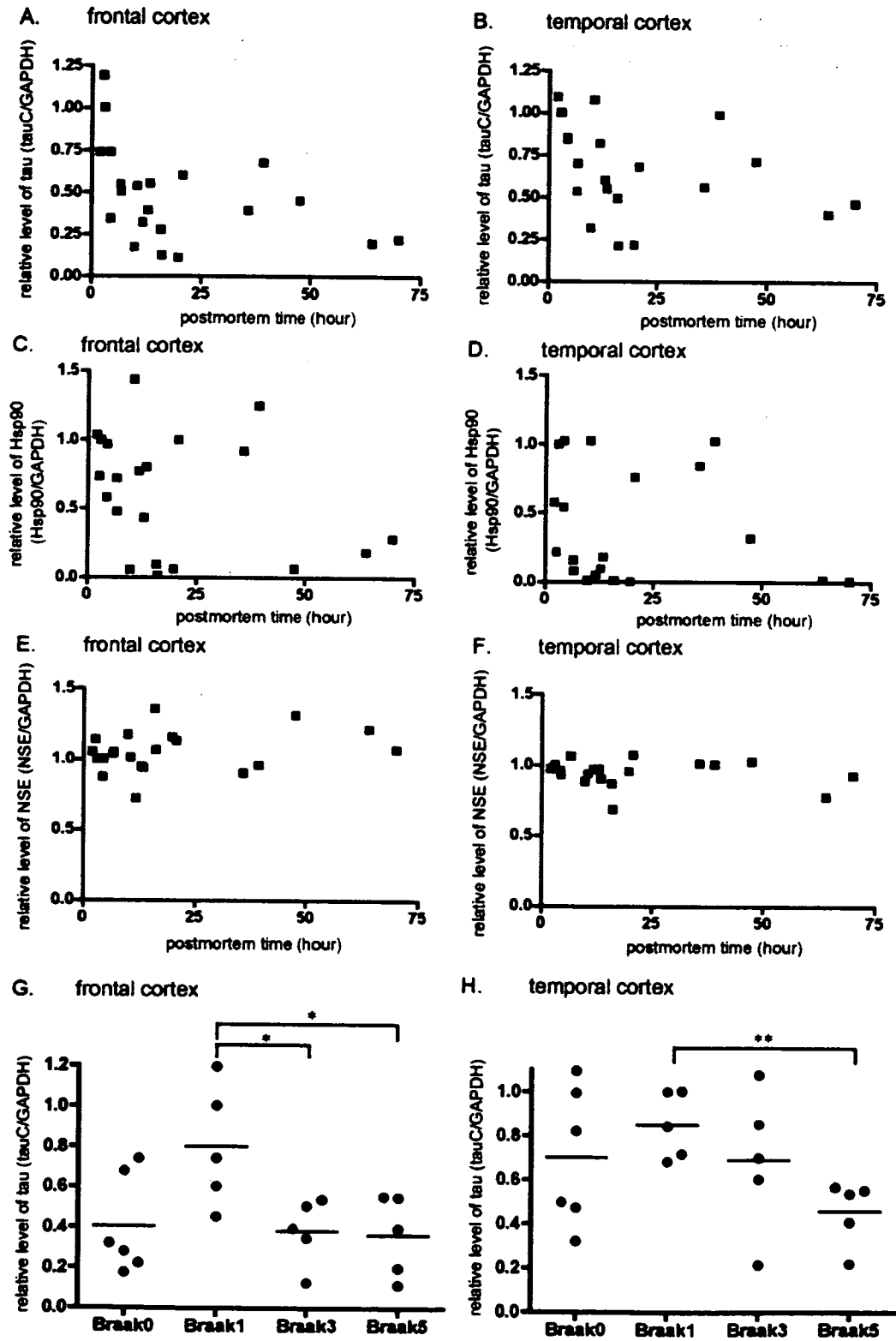


Figure 2.

TABLE II. Regional Correlation Analysis Between Tau and Other Proteins*

	Frontal cortex		Temporal cortex	
	Spearman regression	<i>P</i> value	Spearman regression	<i>P</i> value
Hsp90	0.789	<0.001	0.794	<0.001
Hsp70	-0.242	0.291	-0.186	0.420
Hsc70	-0.190	0.410	-0.143	0.537
Hsp60	0.085	0.714	-0.251	0.273
Hsp40	0.828	<0.001	0.723	<0.001
Hsp27	0.757	<0.001	0.562	0.008
α-Crystallin	0.801	<0.001	0.707	<0.001
CHIP	0.739	<0.001	0.751	<0.001
Akt	0.869	<0.001	0.768	<0.001
β-Tubulin	0.833	<0.001	0.779	<0.001
β-Actin	0.400	0.072	0.197	0.391
NSE	-0.283	0.214	0.449	0.041

*Soluble tau levels were obtained by dividing the intensity of tauC-immunopositive bands by the intensity of GAPDH-positive bands.

samples from Braak I-staged brains tending to contain more of these proteins. In contrast, Hsp70, Hsc70, Hsp60, β-actin, NSE, and GAPDH levels remained steady across different Braak stages and within each cortical region. Quantitative analysis clearly showed that the levels of all soluble proteins did not correlate with the levels of sarkosyl-insoluble tau, more of which was recovered in temporal cortices than in frontal cortices. Moreover, we did not observe any significant correlation between HSP levels and senile-plaque stage (Table I). These data suggest that a molecular chaperone complex (Hsp90, Hsp40, Hsp27, α-crystallin, and CHIP) interacts with Akt, β-tubulin, and tau as client proteins in aged human brains and that this complex is elevated during the Braak I stage.

To determine whether tau and the chaperone proteins physiologically interact in human brain, we analyzed brain extracts using a coimmunoprecipitation assay. Regardless of Braak stage, tau coimmunoprecipitated with Hsp90, Hsp70, and β-tubulin but with not NSE (Fig. 3). Surprisingly, Hsp70 was more efficiently recovered by anti-tau immunoprecipitation than Hsp90. Although the direct interaction between tau and these HSPs remains to be demonstrated, these data support the hypothesis that tau and Hsp90/Hsp70 physiologically interact.

Correlation Between HSP Levels in Soluble Fraction and Granular Tau Oligomer

Previously, the identification of granular tau oligomers as intermediates of tau filaments from human frontal cortices was reported (Maeda et al., 2006). Granular tau oligomer levels in frontal cortex were increased even in brains displaying Braak I characteristics (Fig. 4A,B), suggesting that granular tau oligomers may form before NFTs form. To evaluate these oligomers further, we compared oligomer levels from each specimen to

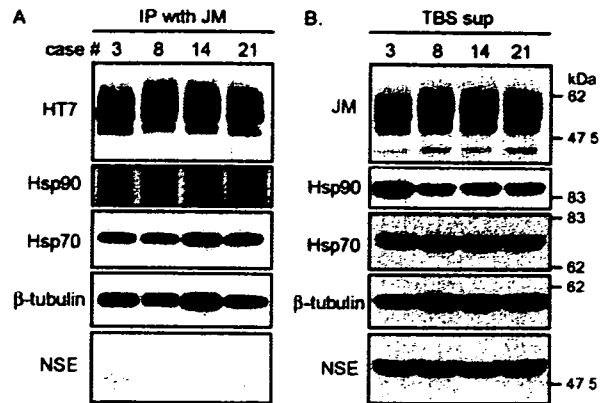


Fig. 3. Coimmunoprecipitation assay of human brain extracts. **A:** TBS-soluble fractions derived from temporal cortices (cases 3, 8, 14, and 21) were immunoprecipitated with anti-tau antibody JM. The resulting samples were immunoblotted with antibodies against HT7, Hsp90, Hsp70, β-tubulin, and NSE. **B:** TBS-soluble fractions were also subjected to SDS-PAGE and immunoblotted with the indicated antibodies.

HSP levels and observed a significant inverse correlation between oligomer and Hsp90 levels (Fig. 4D; oligomers vs. Hsp90, $P = 0.045$) and between oligomer and α-crystallin levels (oligomers vs. α-crystallin, $P = 0.029$). When data from Braak 0 samples were excluded from the correlation analyses, we found that Hsp40, Hsp27, α-crystallin, CHIP, and Akt levels also showed a significant inverse correlation with oligomer levels (Hsp40 $P < 0.001$, Hsp27 $P < 0.001$, α-crystallin $P < 0.001$, CHIP $P = 0.0015$, Akt $P = 0.0033$). Because the levels of these HSPs positively correlate with the levels of TBS-soluble tau, we were able to determine the relationship between tau oligomers and TBS-soluble tau in Braak-staged aged brains. Indeed, oligomer levels inversely correlated with TBS-soluble tau levels (Fig. 4C; oligomers vs. tauC-positive tau, $P = 0.015$). These findings suggest that TBS-soluble tau is a target of the HSP-mediated refolding complex, whereas granular tau oligomers are counterparts of this complex.

HSPs Prevent In Vitro Tau Self-Aggregation

To study the biochemical interaction between tau protein and HSPs, we analyzed in vitro tau polymerization in the presence and absence of Hsp40 and/or Hsp70 by using a ThT-binding assay. The thioflavin assay is the most commonly used assay to assess real-time tau assembly, because ThT binds β-sheet structures. With increased binding, more intense fluorescent signals are emitted (Kuret et al., 2005). As we previously described (Maeda et al., 2007), tau self-assembly was induced by adding heparin, after which ThT fluorescence levels plateaued after 100 hr (Fig. 5A). Tau prepared in the absence of heparin displayed no measurable

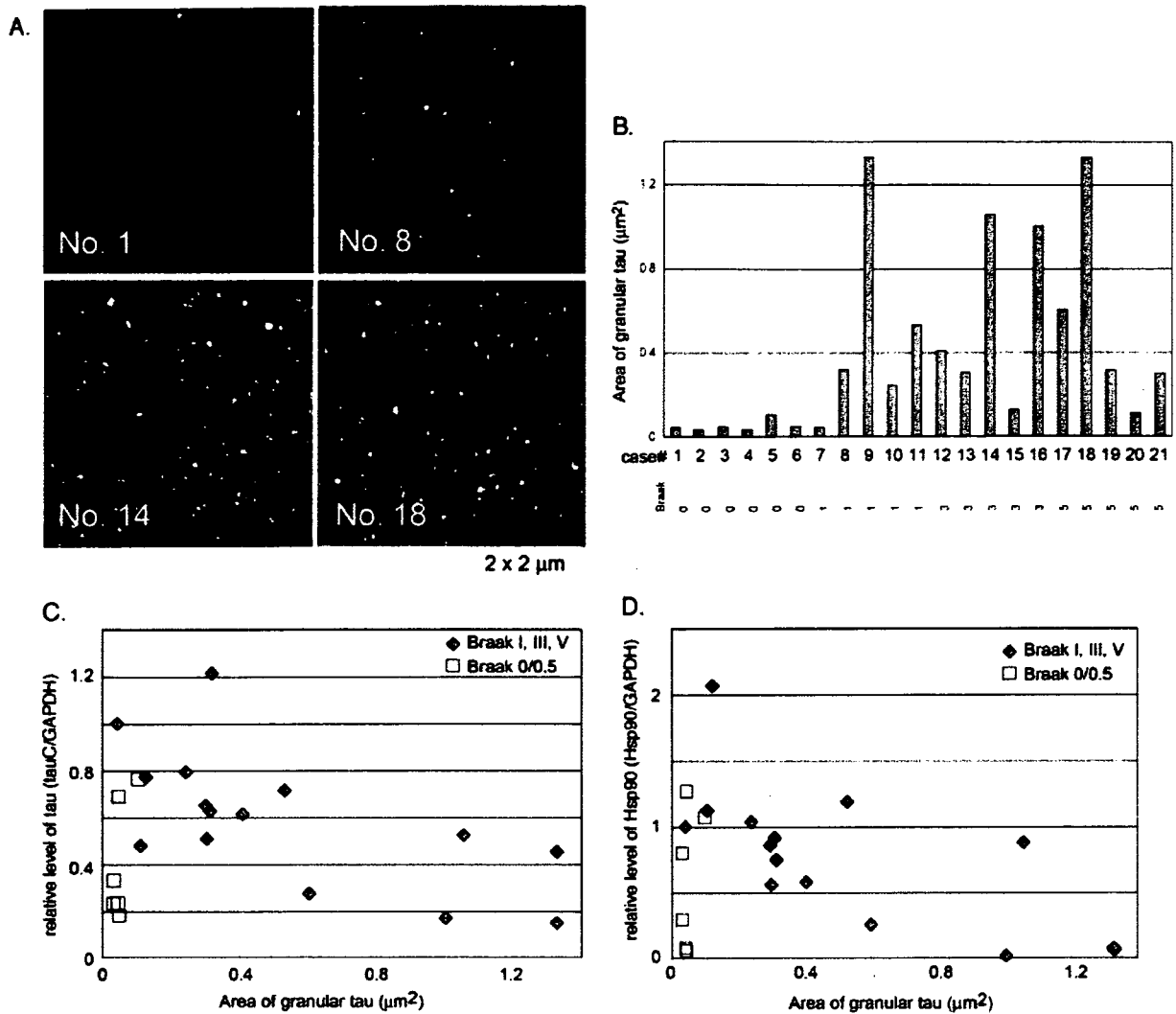


Fig. 4. Quantitative analysis of granular tau oligomer in human frontal cortices. **A:** AFM images of granular tau oligomers from representative Braak NFT-staged samples. Cases 1, 8, 14, and 18 were staged at Braak 0, I, III, and V, respectively. The size of each image size is $2 \mu\text{m}^2$, and the height range is 30 nm. **B:** Quantitative data of granular

lar tau oligomer is represented as the area (μm^2) occupied by tau granules. Each case and Braak NFT stage is indicated. **C,D:** Correlations between granular tau oligomer and tau levels (**C**) and oligomer and Hsp90 levels (**D**) are shown. Samples were grouped into Braak NFT stage 0 (squares) and Braak stages I, III, and V (diamonds).

fluorescent signals (data not shown). Because Hsp40 and Hsp70 form the initial recognition complex during the processing of client proteins of the chaperone network (Dickey et al., 2007), we added these HSPs to heparin-induced tau assembly preparations. Hsp40 and Hsp70 significantly reduced the ThT signals, but adding both Hsp40 and Hsp70 to the preparation reduced fluorescence even more, affecting tau assembly during the entire incubation period (Fig. 5). Interestingly, Hsp40 had a dose-dependent effect, whereas Hsp70 did not (Fig. 5B). These results indicate that Hsp40 and/or Hsp70 directly prevented the aggregation of tau protein.

Further studies will be needed to clarify the refolding and degradation of tau protein related to molecular chaperones.

DISCUSSION

More than 20 neurodegenerative disorders are characterized by the presence of aggregates of the microtubule-binding protein tau (Lee et al., 2001). Tau protein forms insoluble NFTs that accompany and may promote neurodegeneration in these brain diseases. Details of the steps involved in the conversion of solu-

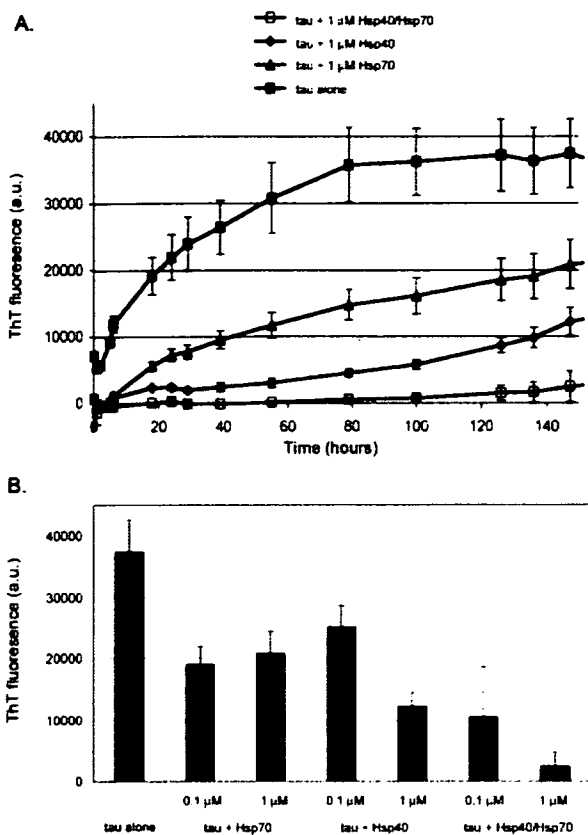


Fig. 5. Effects of Hsp40 and/or Hsp70 on in vitro tau self-assembly (solid squares). **A:** Heparin-induced tau aggregation was assessed by measuring ThT fluorescence at the indicated time points (mean \pm SD; $n = 5$). Hsp40 (diamonds), Hsp70 (triangles), and Hsp40 + Hsp70 (open squares) were added to the aggregation reaction mixture at time zero. **B:** Tau aggregation levels are shown as a function of ThT fluorescence intensity (mean \pm SD; $n = 5$). Tau was incubated with heparin in the presence of Hsp40, Hsp70, or Hsp40 + Hsp70 at the indicated concentrations for 147 hr.

ble tau to insoluble tau having a β -sheet conformation remain unclear. Recent studies have suggested the involvement of multiple degradation pathways in this process that require the action of molecular chaperones (Dou et al., 2003; Petrucelli et al., 2004). Here, we found that molecular chaperone-mediated tau protein metabolism plays a possible role in converting soluble tau to granular tau oligomer (intermediates of tau filaments) in human brain. Indeed, the levels of TBS-soluble tau correlated well with those of Hsp90, Hsp40, Hsp27, α -crystallin, and CHIP. In contrast to the levels of TBS-soluble tau, the levels of granular tau oligomer were inversely proportional to those of HSPs. In addition, our in vitro study showed that Hsp40 and Hsp70 proteins prevented tau aggregation. Taken together, these findings indicate that a molecular chaperone-tau

protein processing pathway likely controls the initial phase of tau aggregation.

Together with comparative biochemical analyses of tau aggregates, electrophoretic profiles of pathological tau protein have allowed the molecular classification of tauopathies. Indeed, tauopathies differ in both tau phosphorylation and tau isoform content (Lee et al., 2001; Sergeant et al., 2005). Although electrophoretic tau protein profiles in various tauopathies have been well characterized, variations in tau protein levels have not been examined extensively, because studies of autopsy samples can be complicated by variability in disease severity, agonal state, age, environmental factors, and post-mortem delays. In the present study, we observed that TBS-soluble tau levels varied across different Braak stages, whereas β -actin, GAPDH, and NSE levels remained steady across different Braak stages. Moreover, the levels of TBS-soluble tau correlated well with those of Hsp90, Hsp40, Hsp27, α -crystallin, and CHIP. These variations were not related to post-mortem interval times, so it was unlikely that these protein levels were affected by post-mortem interval. In addition to chaperone protein levels, Akt and β -tubulin levels also positively correlated with TBS-soluble tau levels. Akt is known to be an Hsp90 client protein (Basso et al., 2002), and β -tubulin is known to be a component of microtubules that are stabilized by tau protein. Therefore, Akt, β -tubulin, and tau protein levels may also be regulated by the chaperone complex through a similar processing pathway.

Recently, our group described the quantitative analysis of a granular-shaped prefilamentous form of tau, the granular tau oligomer, in human brain (Maeda et al., 2006, 2007). We observed increased levels of granular tau oligomer in the frontal cortices staged at Braak I, suggesting that tau dysfunction had already occurred in frontal cortex by the time NFTs formed in entorhinal cortex. In the present study, we observed an inverse correlation between granular tau oligomer and HSP levels, the latter of which positively correlated with soluble tau protein levels. This inverse correlation was more significant when Braak 0 samples were excluded from our analysis. Braak NFT staging traces the gradual development of neurofibrillary changes in brain (Braak and Braak, 1991; Braak et al., 1994). Stage I is characterized by neurodegeneration of specific projection cells in the transentorhinal region, whereas Braak 0 is characterized by the absence of NFTs. Although we did not detect NFTs in the frontal cortices of Braak 0 and Braak I brains, the levels of granular tau oligomer and Hsp27 in Braak I samples were elevated. Because Hsp27 preferentially interacts with a hyperphosphorylated tau variant in human samples (Shimura et al., 2004a), it is possible that hyperphosphorylated prefilamentous tau was already induced in the frontal cortices of Braak I brains. With regard to the inverse correlation between granular tau oligomer and HSP levels, reduction of stress response can explain the increase in granular tau oligomers after the start of neurofibrillary changes. Because aging is a risk factor for various neurodegenerative disorders,

including AD, and the aging process is associated with gradual accumulation of oxidative stress factors (Coyle and Puttfarcken, 1993), it seems reasonable that the overriding of oxidative insults disables molecular chaperones.

Dickey et al. (2006a) reported that HSP induction mediates proteasome-dependent tau degradation. They found that Hsp90 inhibitors reduced the levels of tau phosphorylated at proline-directed Ser/Thr sites (pS202/T205, pS396/S404) and conformationally altered (MC-1) tau species that are induced by Hsp70, Hsp40, and Hsp27. Although Dickey et al. used an *in vitro* experimental system comprising P301L mutated tau-expressing H4 and CHO cells (Dickey et al., 2006a), a system that is completely different from our *in vivo* experimental system involving the protein profiling of human brains, Dickey et al.'s data were consistent with our results showing an inverse correlation between HSPs and MC-1-immunoreactive granular tau oligomers (Maeda et al., 2007). Still, we do not know whether the activation of multiple chaperone systems or inactivation of Hsp90 is induced by stress responses that occur during brain aging. However, because Hsp90 client proteins, such as Akt, are also related to HSP levels in human brain, it is likely that the Hsp90 chaperoning cycle mediates tau protein metabolism. Results from coimmunoprecipitation and *in vitro* tau assembly assays demonstrated that physiological interactions occur between tau and Hsp70. Hsp70 is well known to be a prominent HSP in eukaryotic cytosol (Hartl and Hayer-Hartl, 2002). Hsp70 function not only is modulated by Hsp40 to initiate protein refolding but is also associated with CHIP to process the ubiquitin-proteasome pathway (Dickey et al., 2007). Therefore, it seems likely that Hsp70 is also involved in chaperone-mediated tau protein metabolism.

Tau protein has been reported to be a substrate for a number of proteases, such as trypsin, chymotrypsin, cathepsin D, calpains, caspases, proteasomal proteases, and thrombin. Decreased proteasome activity in AD brains has been previously reported (Keller et al., 2000; Lopez Salon et al., 2000; Keck et al., 2003). The inhibition of Hsp90-mediated tau clearance by a proteasomal inhibitor has been also reported (Dickey et al., 2006a). The lysosomal pathway may represent a secondary pathway for HSP-mediated tau degradation. An alternative tau degradation pathway is chaperone-mediated autophagy (CMA). Recently, α -synuclein has been shown to be a substrate for CMA (Cuervo, 2004) and to be modulated by cochaperone CHIP for degradation via CMA (Shin et al., 2005). Our findings strongly suggest that molecular chaperone-mediated tau protein metabolism is a major regulator of the formation of abnormal oligomeric complexes of tau. Therefore, molecular chaperones might be promising targets in the development of therapeutics for diseases characterized by neurofibrillary degeneration as well as for other diseases characterized by protein conformational disorders. Further studies are necessary to determine the precise status of tau protein as a molecular chaperone client.

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アルツハイマー病

—基礎研究から予防・治療の新しいパラダイム—

III. 臨床編

MCI

病態と病理

村山繁雄 齊藤祐子

III. 臨床編

MCI

病態と病理

Neuropathology of mild cognitive impairment

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Key words : ブレインバンク, アルツハイマー病, パーキンソン病, レビー小体型認知症, 嗜銀顆粒性疾患, 神経原線維変化優位型疾患

はじめに

軽度認知障害(MCI)は, 認知症予備群として, 早期薬物介入による認知症進展予防の可能性を示した Petersen らの発表で脚光を浴びた。しかし, 生理学的退行現象, うつと, 進行性病理学的背景をもつ病態を分離することの困難さが問題となっている。

臨床症状の解析は, 本人の状態の評価には必須であるが, 診断に関する特異度・感度は高くなく, 特に治験を行うときには, 背景病理の不一致が, 結果にブレを生じることが問題となっている。

本誌でも取り上げられている, Alzheimer disease neuroimage initiative (ADNI) は, この問題を解決するために, 生物学的マーカーを駆使し, 診断のための共通基盤を構築する試みであり, 特に MRI 画像に重点が置かれている。

著者らは, 剖検時文書同意の下, 東京都老人医療センター連続開頭剖検例を, 東京都老人総合研究所神経病理担当部門が解析・蓄積してきた, 高齢者ブレインバンクを用い, 後方視的に MCI を呈した症例の病理学的解析をまず試みた。次いで, 東京都老人医療センターもの忘れ外来で, 記憶障害型 MCI (aMCI) にあたる症例

を操作的に分離し, 健康保険適用の検査群に髄液バイオマーカーを組み合わせることで, どの程度背景病理を推定可能であるかを試みたので報告する。

1. 方法

a. MCI の後方視的神経病理研究

臨床症状として, 生前 MMSE と IADL 値を含む病歴・看護記録を参考に, 蓄積剖検例に対し, 神経内科専門医 2 人が個々に clinical dementia rating (CDR) を算出, 一致する場合は採用, 不一致の場合は主治医, 介護者に確認し CDR を決定した。

神経病理学的には, 高感度鍍銀染色 (Gallyas-Braak, 改良メセナミン銀), 各種免疫染色 (抗アミロイドβ蛋白, リン酸化タウ, リン酸化αシヌクレイン, ユビキチン抗体) を適用, 老人斑, 神経原線維変化については Braak 分類, レビー小体, 嗜銀顆粒, アミロイドアンギオパチー, グリア原線維変化については著者らのステージ分類 (www.mci.gr.jp/BrainBank) を用い半定量化した。臨床症状, 経時的画像変化と病理所見を対応させ, 一次情報をすべてデジタルデータベース化し, 高齢者ブレインバンク構築を継続した。これらの基礎データを背景に, CDR

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**表 1 MCI の推定背景病理に基づく認知症
進展予防最適介入法の開発**

-
- ・一次スクリーニング
CT, MMSE/HDSR
 - ・二次スクリーニング
RBMT/FAB/SDS,
髄液タウ・リン酸化タウ・アミロイドβ蛋白
apo E
MRI (VSRAD)
SPECT 3D SSP/eZis 3.0 (2006.10～)
 - ・三次スクリーニング
PET (FDG/PIB)
 - ・治療的介入と, 1年ごとのフォロー
 - ・不慮の転帰をとったとき, 高齢者ブレインバンク
登録をできるかぎり試みる
-

0.5 例の神経病理所見を抽出した。

b. MCI の縦断臨床研究

もの忘れ外来対象者に, 一次スクリーニングで, 介護者から物忘れが確認できる症例に加え, 本人が物忘れによる障害のエビデンスを示せるものを含めた。頭部 CT スキャンで粗大病変なく, MMSE (24/30), HDSR (21/30) とともにカットオフ値以上の症例を対象とした。特に三語再生が MMSE 1/3 以下, HDSR 2/6 以下の症例はこの時点で操作的に aMCI に分類した。

二次スクリーニングは, 記憶検査として Rivermead 行動記憶検査 (RBMT), そのほかに frontal assessment battery (FAB), self assessment depression scale (SDS), MRI (VSRAD), 脳血流 SPECT (ECD eZis 3.0), 髄液バイオマーカー (タウ, リン酸化タウ, アミロイドβ蛋白, 5HIAA, HVA) を行った。RBMT のカットオフ値以下の症例を aMCI に分類し, VSRAD, eZis との関係を見た。

更に, 三次スクリーニングとして, FDG (fluor deoxy glucose)-PET, MIBG 心筋シンチをできるかぎり行うよう努力し, ドーパミン合成能 (CFT)・結合能 (raclopride) PET, アミロイドプローブ (Pittsburgh compound B: PIB)-PET を選択例に施行した (表 1)。

以上の結果を基に, アルツハイマー病極早期 (eAD), レビー小体型認知症極早期 (eDLB), 嗜銀顆粒性疾患 (AGD), 神経原線維変化優位型疾

患 (NFTD), 血管障害性認知症極早期 (eVD) 例を分離することを試みた。

治療としては, 変性型例ならびに Papetz 回路の障害を伴う eVD 例にはコリンエステラーゼ阻害薬を投与し, 反応性をみた。高齢者タウオパチー (AGD と NFTD) には薬物療法に加え, 芸術を基本とするリハビリテーションを指導した。eDLB には転倒防止指導を行い, eVD には危険因子の厳密なコントロールを行った。

非侵襲検査は半年おき, 侵襲検査は 1 年ごとを目安とし, 認知症進展予防効果の検討を開始した。また, 追跡患者が不慮の転帰をとったときは, できるかぎり剖検をとり, 診断の確認を行うこととした。

2. 結 果

都市型在宅高齢者を代表する, 高齢者ブレインバンク蓄積例の, 臨床・画像・病理データベースからは (図 1), CDR 0.5 の, MCI 該当症例の背景病理は, 高齢者タウオパチー (嗜銀顆粒性疾患 (AGD), 神経原線維変化優位型疾患 (NFTD), 進行性核上性麻痺 (PSP)) 14 例, 血管障害性認知症 (VD) 極早期 (eVD) 9 例, レビー小体型認知症 (DLB) 極早期 (eDLB) 9 例, アルツハイマー病 (AD) 極早期 (eAD) 8 例 (含重複例) であり, 高齢者タウオパチーが多い結果となった (図 2-a, b)。

一方, CDR 1 以上の認知症剖検例では AD が 1/4 を占め, 次いで VD, AGD, DLB, NFTD の順で, もの忘れ外来受診者に限ると, AD 次いで DLB で, 併せると 2/3 を占めた (図 3-a, b)。

2006 年 10 月より上記プロトコールを開始し, 2007 年 2 月までに二次スクリーニングが完了した 36 例を解析した。操作的分類により, 11 例が aMCI に分類された。

これらの症例の一覧を示す (表 2)。髄液バイオマーカーは, タウ, リン酸化タウ, アミロイドβ蛋白のどれか 2 つが異常であれば, 特異度・感度 80% で AD であると, スウェーデンより報告されているのを受け, それらの症例を AD とし, eZis 3.0, VSRAD との対応を試みた。既往報告どおり, eZis 3.0 は若年者を抽出,

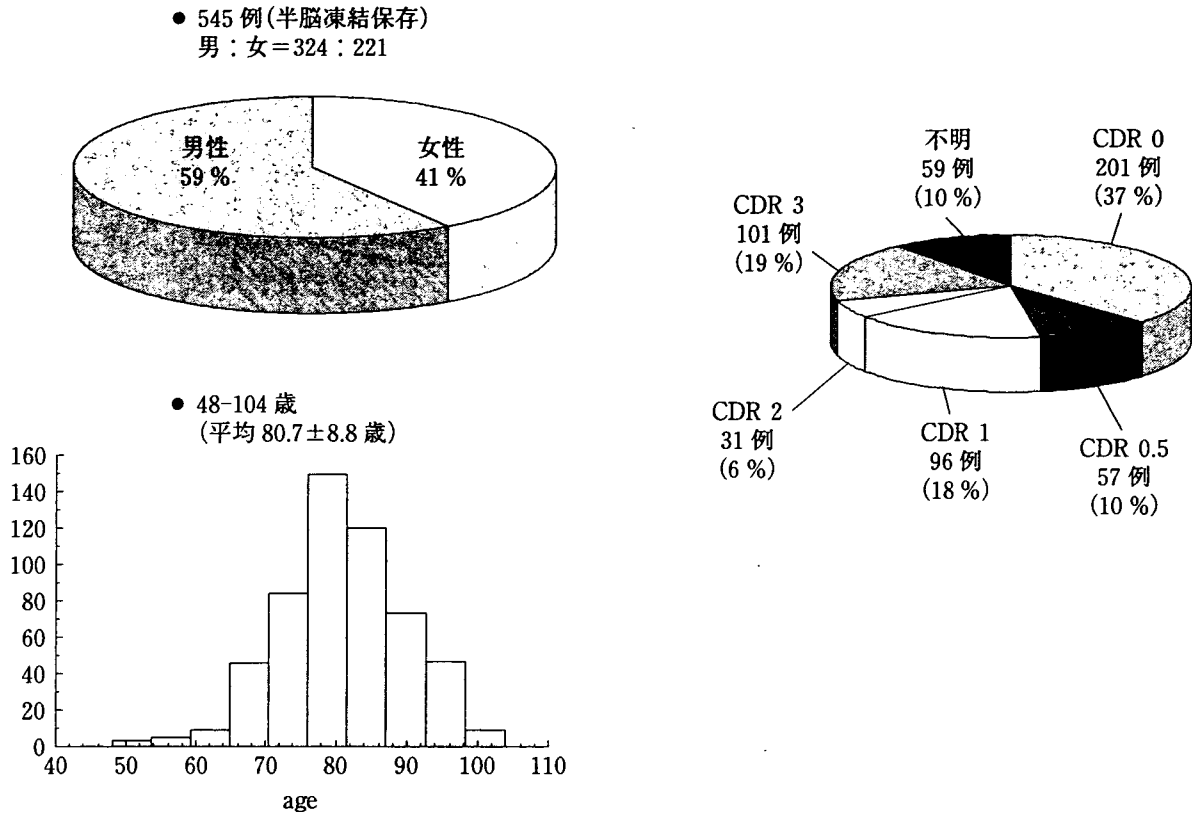


図1 高齢者ブレインバンク(2001.7-2006.3)のCDRの割合(文献¹⁾より引用)

VSRADは高齢者を抽出し、併せると、11例中6例が、ADに該当する結果を得た。残り5例については、現在更に検討中である。

3. 考 察

CDR 0.5例で高齢者タウオパチーが多く、CDR 1以上の症例ではAD/DLBが多くなる理由は、高齢者タウオパチーは進行が遅くMCIにとどまる症例が多いのに対し、AD/DLBは進行が速いので認知症に早期に移行するためと考えた。また、もの忘れ外来では物忘れが主体で進行が速く、介護に問題を生じた群が優位となるのに対し、高齢者コホート全体では、進行の遅い高齢者タウオパチーや、全身内科合併症を通常有するため生命予後が悪いeVDの頻度が相対的に高くなることが予想された。

付け加えるべき点として、高齢になればなるほど、変性型病理と血管障害の組み合わせだけでなく、他の変性型病理との組み合わせが増加し、一つの病理だけで認知障害の原因を説明で

きない症例が増加していく点である。この点は、治療においても、それぞれの要素を考慮した、多角的アプローチが重要であることを示している¹⁾。

一方、前方視的縦断研究ではADの相対的頻度が高いが、VSRADは高齢者AD、eZis 3.0は若年者ADの検出に有効であるという知見を支持する結果が得られたものの、検出率は2つ併せても、55%であった。現在三次スクリーニングを試行中である。これらの症例は、臨床症状・CTスキャンを前提とする現行の治験では、すべてADに分類され、エントリー可能である。しかし、その背景病理は多彩である可能性があり、治験結果に影響を与えることが予想される。J-ADNIは、限られた症例に対する適応になるが、これらの問題点に、一定の解決を提供するものと注目される。それに加え、Rotterdam Studyや久山町スタディーのような長期縦断研究に、剖検所見が加わったものの重要性が、今後ますます強調されることが予想される。

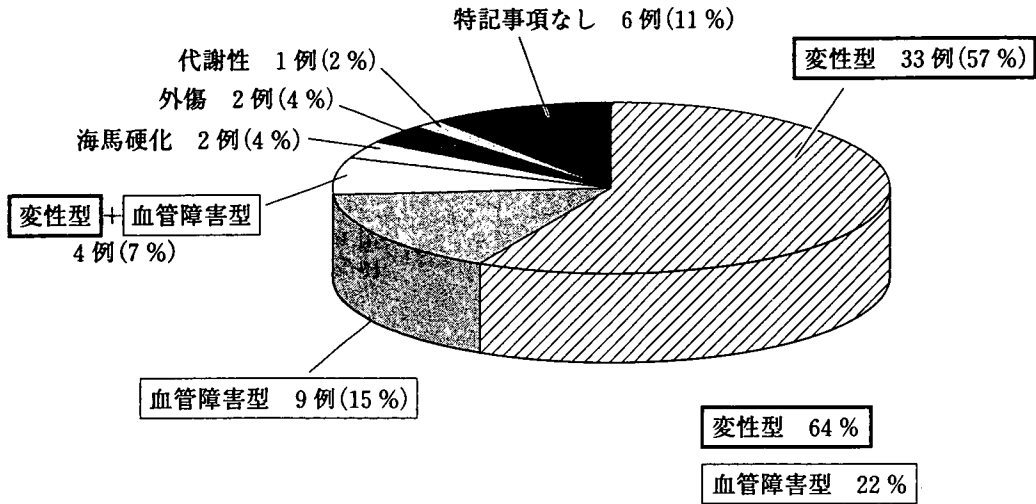


図 2-a CDR 0.5 の背景病理(文献¹⁾より引用)

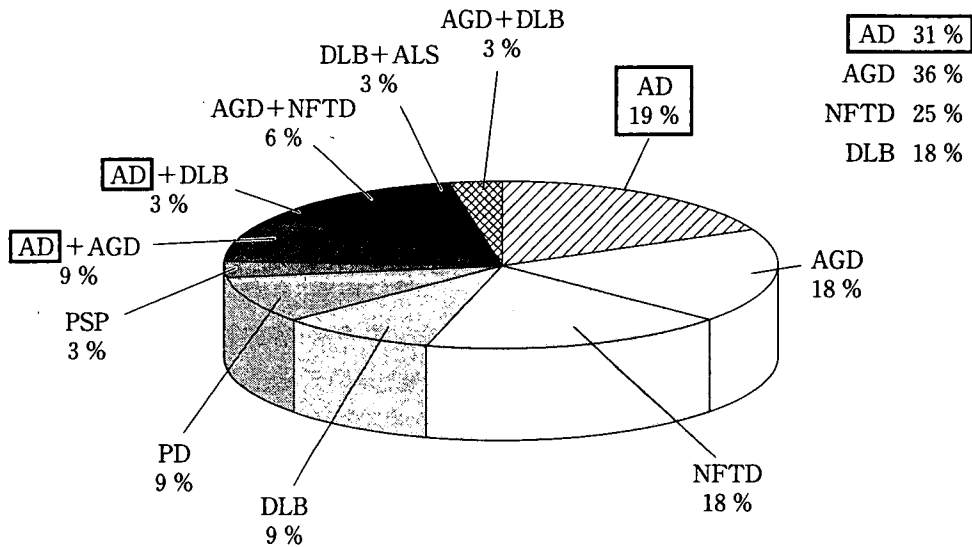


図 2-b CDR 0.5 群 '変性型' の内訳(文献¹⁾より引用)

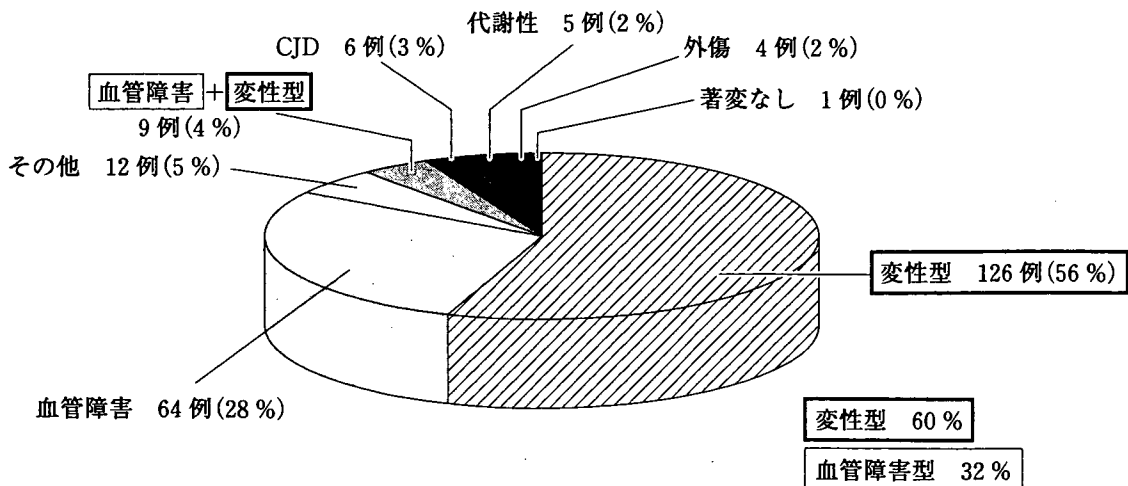


図 3-a CDR 1 以上 'dementia 群' の背景病理(文献¹⁾より引用)

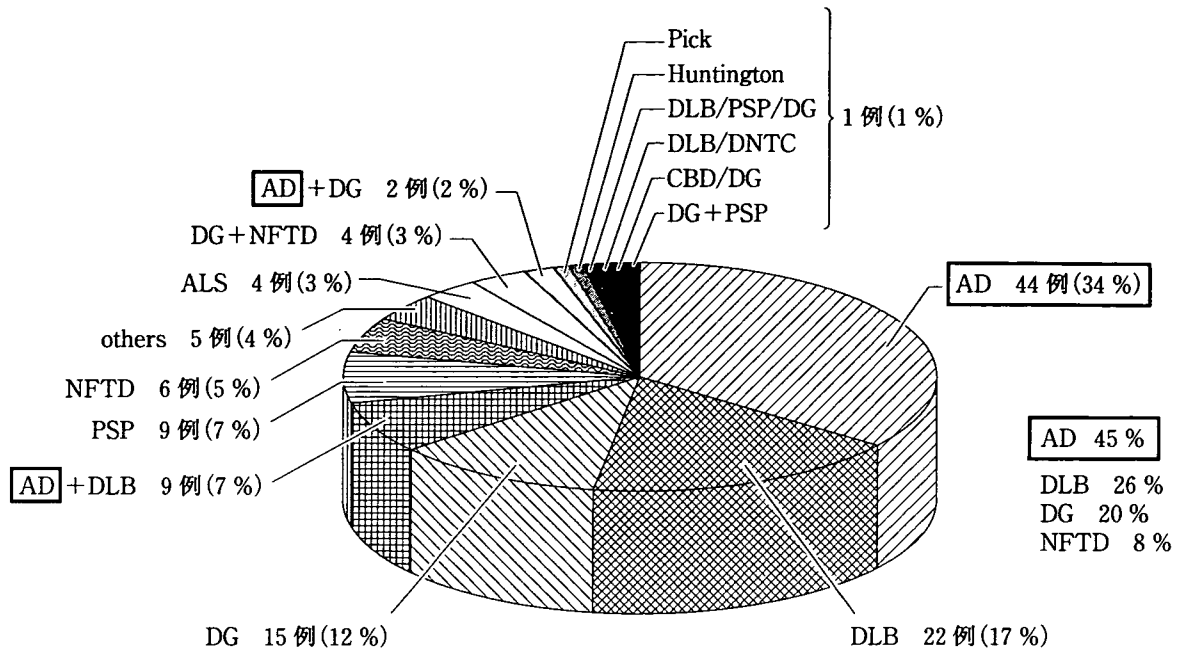


図3-b CDR 1以上 '変性型' の内訳(文献¹⁾より引用)

表2 MCI例

年齢	性別	最終学歴	神経心理学的検査				髄液			eZis 3.0			MRI
			MMSE	三語	RBMT	展望	tau	ptau	Abeta	sever	extent	ratio	VSRAD
72	M	大学	28	1	14	3	43.9	33.3	814	1.01	6.82	1.5	<1.3
69	M	高校	27	2	14	5	55.8		744	1.01	3.32	0.6	<1.3
73	M	高等小学校	27	1	18	3	530.1	109	209	0.97	5.68	1.3	2.08
79	F	女子商業	26	1	2	0	468.2	125	326	0.91	3.32	0.3	3.2
77	F	師範学校	26	2	12	3	418.5	74.3	46.5	1.12	9.34	1.2	1.98
57	M	高校	25	0	11	4	160.9	115	279	1.14	14.1	2.6	1.35
80	F	職業学校	25	0	2	0	219.5	56.4	209	0.72	1.64	0.3	error
77	F	高等女学校	25	0	12	1	27.9		395	1.7	9.8	1.6	1.06
81	F	高等女学校	25	2	6	1	13.9		907	0.62	0.8	0.1	1.8
72	M	高校	24	0	1	0	166.1	93.7	226	1.03	4.54	1.2	2.86
84	M	工業学校	24	1	8	0	181.2	47	1,029	0.69	0	0	0.36

■ 文 献

- 1) Saito Y, Murayama S: Neuropathology of mild cognitive impairment. Neuropathology (in press)