

Accumulation of Intracellular Amyloid- β Peptide (A β 1-40) in Mucopolysaccharidosis Brains

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Abstract. To evaluate whether *in vivo* accumulations of heparan sulfate caused by inborn errors in the metabolism of glycosaminoglycans lead to the formation of neurofibrillary tangles and/or senile plaques, as seen in Alzheimer disease (AD), we studied postmortem brains from 9 patients, ages 1 to 42 years, with mucopolysaccharidosis (MPS). The brains of patients with Hurler's syndrome (MPS I; $n = 5$) and Sanfilippo's syndrome (MPS III; $n = 4$) as well as from caprine MPS IIID and murine MPS VII models were evaluated by thioflavine-S staining and by immunohistochemistry using antibodies directed against heparan sulfate proteoglycans, hyperphosphorylated tau, amyloid- β peptide precursor proteins (APP), and amyloid- β peptides (A β [1-40], and A β [1-42]). A two-site sandwich enzyme-linked immunosorbent assay (ELISA) was also utilized to compare levels of total soluble and insoluble A β (1-40) and A β (1-42) obtained from temporal cortex of MPS patients. Although no neurofibrillary tangles, senile plaques, or tau-positive lesions were detected in any of the MPS brains studied here, antibodies directed against A β (1-40) intensely and diffusely stained the cytoplasm of cells throughout the brains of the MPS patients and the caprine MPS model. The ELISA assay also demonstrated a significant 3-fold increase in the level of soluble A β (1-40) in the MPS brains compared with normal control brains. Thus, at least some of the metabolic defects that lead to accumulations of glycosaminoglycans in MPS also are associated with an increase in immunoreactive A β (1-40) within the cytoplasmic compartment where they could contribute to the dysfunction and death of affected cells in these disorders, but not induce the formation of plaques and tangles. Models of MPS may enable mechanistic studies of the role A β and glycosaminoglycans play in the amyloidosis that is a neuropathological feature of AD.

Key Words: Amyloid- β peptides; Glycosaminoglycans; Heparan sulfate proteoglycan; Neurofibrillary tangles; Senile plaques; Tau protein.

INTRODUCTION

Neurofibrillary tangles (NFTs) consisting primarily of paired helical filaments (PHFs), and senile plaques (SPs) comprised predominantly of amyloid fibrils, are the pathological hallmarks of Alzheimer disease (AD) and related neurodegenerative disorders (1-3). *In vitro* studies have demonstrated that hyperphosphorylated tau (PHFtau) can self-aggregate into fibrillar polymers that resemble PHFs and straight filaments (4-10). The region of tau that is necessary for polymerization consists of the microtubule-binding domain (11, 12), and PHF formation is believed to hamper the binding of tau to microtubules, leading to microtubule disassembly, loss of axonal transport, and

subsequent neuronal degeneration (13). Amyloid- β peptides (A β), the major constituent of amyloid fibrils in SPs (14, 15), also self-aggregate into filaments under appropriate *in vitro* conditions (16-20). A β terminating at amino acid residue 42 (A β [1-42]) is deposited initially in SPs, and aggregates at a faster rate than the more soluble A β terminating at amino acid residue 40 (A β [1-40]) (21-24). However, the *in vivo* mechanism(s) by which tau and A β aggregate, form insoluble deposits, and lead to AD remain unknown.

Although self-aggregation of recombinant tau and A β has been demonstrated, nonphysiological quantities of these peptides are necessary for polymerization (9, 12, 25). Therefore, other molecules have been hypothesized to function as pathological chaperones to facilitate fibril formation. Recent studies suggest that pathological chaperones involved in PHF and amyloid fibril formation may include sulfated glycosaminoglycans (GAGs), notably heparan sulfate (HSGAG) and its most sulfated form, heparin (25-32). GAGs, or mucopolysaccharides, are sugar moieties that are bound to a protein core to form proteoglycans (i.e. heparan sulfate proteoglycan [HSPG]), molecules found in the extracellular matrix and basement membranes (33, 34). HSPGs and HSGAGs have been observed in association with NFTs in the AD brain (27, 29, 31). Moreover, HSPGs stimulate tau phosphorylation by several protein kinases (35, 36), and promote PHF formation from recombinant tau proteins *in vitro* under near physiologic concentrations (25-27, 32, 37). Similarly, HSPGs and HSGAGs have been observed

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in association with SPs in AD brains (31, 38, 39), bind to the amyloid- β peptide precursor protein (APP) and A β in vitro, and accelerate A β fibril formation and stability (28, 30, 40–42).

To test the hypothesis that the accumulation of HSGAGs due to metabolic defects in vivo may lead to the formation of NFTs and/or SPs, we examined sections from the brains of subjects with autosomal recessive mucopolysaccharidosis (MPS), including MPS I and MPS III syndromes in which specific lysosomal hydrolase deficiencies lead to the accumulation of HSGAGs (43, 44). HSGAGs are the sole uncatabolized substrates in MPS III, whereas HSGAGs accumulate along with dermatan sulfate in MPS I. Tissue sections were evaluated by thioflavine-S (TS) staining and by immunohistochemistry using antibodies specific for HSPGs, PHFtau, APP, and A β . In addition, caprine (MPS IIID; a genetic disease in which goats are deficient in activity of N-acetylglucosamine 6-sulfatase) (45) and murine (MPS VII; an hereditary disease in which homozygous mice are β -glucuronidase deficient) (46) models of MPS also were evaluated for evidence of tangle and plaque pathology using the same methodology. A two-site sandwich enzyme-linked immunosorbent assay (ELISA) was utilized to compare levels of total soluble (extracted in Tris-buffered saline [TBS]) and insoluble (extracted in formic acid) A β (1-40) and A β (1-42) obtained from tissue homogenates of temporal neocortex of MPS, normal control, and AD brains. This previously characterized ELISA for soluble and insoluble A β (1-40) and A β (1-42) (47–51) allows for quantitative analysis of even small amounts (e.g. <100 pmol/g wet tissue) of A β (1-40) and A β (1-42) due to the specificity of the monoclonal antibodies (MAbs) used for the ELISA.

MATERIALS AND METHODS

Postmortem brain samples from neocortex, hippocampal formation, cerebellum, and striatum were obtained (n = 9 MPS; n = 6 normal controls [CTR]; disease controls: n = 3 neuronal ceroid lipofuscinosis [NCL]; n = 1 Gaucher's disease; n = 6 AD; n = 4 Down syndrome [DS]; [Table 1]). The samples were fresh-frozen or immersion fixed in either 10% neutral buffered formalin (NBF) or 70% ethyl alcohol plus 150 mM sodium chloride (EtOH), embedded in paraffin, and 6- μ m-thick serial sections were cut.

Brains from nubian goats with a MPS IIID disorder (n = 4; 2 stillborn; one 19 month and one 25 month) and 2 normal goat brains were processed as described previously (45). Mice with MPS VII (n = 4) and unaffected mice (n = 7) were perfused transcardially with 4% paraformaldehyde plus chloral hydrate (52, 53) at 3 or 5 months of age and their brains were embedded in paraffin, and 6- μ m-thick serial sections were cut. Two series of tissue sections were stained with hematoxylin and eosin and periodic acid-Schiff (PAS), respectively, to examine morphology. Deparaffinized tissue sections were blocked in 2% horse serum in Tris/HCl buffer (pH 7.6) for 1 h and incubated with

TABLE 1
Summary of Cases

Case No.	Diagnosis	Gender	Age	PMI	Brain Wt.	Fixation
1	MPS I	M	2.5	48	1,260	NBF
2	MPS I	M	6	48	1,250	NBF
3	MPS I	M	6	1.5	1,050	NBF
4	MPS I	F	6.5	2	*	NBF/F
5	MPS I	M	42	3	*	NBF/F
6	MPS III	F	1	12	*	NBF/F
7	MPS III	F	16	6	600	NBF
8	MPS III	M	14	4	*	NBF
9	MPS III	M	34	8	1,030	EtOH/F
10	GAU	F	6	12	790	NBF
11	NCL	F	5	4	660	NBF/F
12	NCL	*	*	6	1,110	F
13	NCL	M	6	20	*	NBF
14	CTR	M	19	16.5	1,500	NBF
15	CTR	M	62	5	1,360	EtOH/F
16	CTR	M	24	15	1,410	F
17	CTR	F	36	6	1,280	EtOH/F
18	CTR	M	71	13	1,300	EtOH/F
19	CTR	F	92	9	1,090	EtOH
20	AD	F	72	4.5	1,220	EtOH/F
21	AD	M	88	9	1,380	EtOH
22	AD	M	87	7	1,220	EtOH
23	AD	M	79	4	980	EtOH/F
24	AD	M	81	16.5	1,340	EtOH/F
25	AD	M	89	14	1,100	EtOH/F
26	DS	M	58	13	910	EtOH
27	DS	F	53	9	1,100	EtOH/F
28	DS	F	64	5	630	NBF/F
29	DS	F	67	16.5	1,080	EtOH/F

Abbreviations: AD = Alzheimer disease; CTR = control subjects; DS = Down syndrome; EtOH = 70% ethanol plus 150 mM NaCl; F = fresh-frozen tissue; Gau = Gaucher's syndrome; MPS I = Hurler's syndrome; MPS III = Sanfilippo's syndrome; NBF = 10% neutral buffered formalin; NCL = neuronal ceroid lipofuscinosis; PMI = postmortem interval; wt = weight in grams; * = data not available.

primary antibodies (Table 2) in Tris buffer overnight at 4°C in a humidified chamber. Sections were processed with the ABC kit (Vector Labs, Burlingame, CA) and developed with 0.05% diaminobenzidine, 0.03% hydrogen peroxide, and 0.01 M imidazole in Tris buffer for 10 min (54, 55). For TS staining, deparaffinized tissue sections were immersed in 0.0125% TS (Sigma, St. Louis, MO) in a 40% ethanol/60% phosphate-buffered saline solution in the dark for 2 min. Tissue sections were visualized with a Nikon FXA photomicroscope equipped with epifluorescence and brightfield optics. For TS observation, a Nikon B-2E FITC filter was used. Microscope images were digitized to a computer workstation with image analysis software (Northern Exposure, Glen Mills, PA) and printed on a digital printer (Fuji, Japan) as described previously (54, 55).

For the ELISA assay, 2 extraction procedures were utilized to quantitate soluble and insoluble brain levels of A β (1-40) and A β (1-42). This ELISA assay does not cross react with APPs, and is specific for A β (1-40) and A β (1-42) (50). Soluble A β was measured from TBS extracted temporal neocortex, and

TABLE 2
Antibody Characterization

Antibody	Antigen	Species	Dilution	Reference	Source
2332	A β (1-10?)	rabbit	1:4,000	(82)	CNDR
6E10	A β (1-14)	mouse	1:700	(83)	Senetek
4G8	A β (16-20?)	mouse	1:2,000	(82)	Senetek
10D5	A β (1-28)	mouse	1:50	(84)	Athena
BA27	A β (1-40)	mouse	1:1,000	(49)	Takeda
BAN50	A β amino	mouse	1:1,000	(49)	Takeda
BC05	A β (1-42)	mouse	1:1,000	(49)	Takeda
654-39	rat A β (1-28)	rabbit	1:1,000	—	Athena
LN39	APP (1-100)	mouse	1:10	(85)	CNDR
10E4	HSGAG	mouse	1:100	(86)	U.S. Biological
3G10	HSPG	mouse	1:100	(86)	U.S. Biological
PHF6	tau pThr231	mouse	1:5,000	(87)	CNDR
T3P	tau Ser396	rabbit	1:50	(88)	CNDR

This table summarizes the antibodies (first column), antigen and corresponding epitope (question marks denote the approximate epitope) used for antibody generation (second column), species the antibody is raised in (third column), dilution used for immunohistochemistry (fourth column), citation of antibody characterization (fifth column), and source of the antibody (sixth column). The source denoted "CNDR" indicates the antibody was generated in the Center for Neurodegenerative Disease Research, University of Pennsylvania School of Medicine.

insoluble A β was assessed in formic acid extracted tissue homogenates. Briefly, 0.15 g cortex (with meninges stripped away) was Dounce-homogenized (20 strokes) in 4 ml of TBS (pH 7.4) or in 1 ml 70% formic acid with a protease inhibitor cocktail (1 μ g/ml each in 5 mM EDTA: leupeptin, pepstatin A, soybean trypsin inhibitor, N α -p-tosyl-L-lysine chloromethyl ketone [TLCK], and N-tosyl-L-phenylalanine chloromethyl ketone [TPCK]) at 4°C. All tissue homogenates were centrifuged at 125,000 g for 1 h at 4°C. Formic acid extracted samples were diluted 1:20 with 1 M Tris base. All samples were then diluted 1:2, 1:5, and 1:25 with EC buffer (20 mM sodium phosphate buffer pH 7.0, 2 mM EDTA, 400 mM NaCl, 0.2% bovine serum albumin, 0.05% CHAPS, 0.4% Block Ace [Snow Brand Milk Products, Sapporo, Japan], 0.05% NaN₃, pH 7.0) prior to loading onto ELISA plates precoated with the capturing antibody BAN50 (A β amino-terminal specific) and reporter antibodies BA27 (A β [1-40] specific; 30 μ g/ml) or BC05 (A β [1-42] specific; 15 μ g/ml) for measurement as described previously (47–49). Statistical analyses consisted of analysis of variance (ANOVA) with post-hoc Neuman-Keuls test for significance.

RESULTS

MPS Cases

PAS staining revealed swollen neurons throughout the neuraxis in both the Hurler's (MPS I; n = 5) and Sanfilippo's (MPS III; n = 4) cases, consistent with previous observations (56–59). Moreover, the majority of neurons observed within these MPS brains contained abundant cytoplasmic HSPG immunoreactivity (Fig. 1A, D), demarcating perikaryal profiles distended with uncatabolized substrates as expected for MPS I and MPS III. In contrast, HSPG immunoreactivity was not abundant in the cytoplasmic domain of neurons in the brains of

normal and disease control (AD, DS, NCL, and Gaucher) cases where HSPG immunoreactivity was localized principally to blood vessels and parenchymal surfaces (Fig. 1G). HSPG-immunoreactive NFTs and SPs were also observed in AD and DS brains (Fig. 1G), but not in MPS, NCL, or Gaucher brains.

TS staining revealed no NFTs or SPs within the MPS brains, but intensely autofluorescent, punctate lipid accumulations were observed in the cytoplasm of neurons throughout cortical and subcortical regions (data not shown), consistent with previous observations (56, 60, 61). Little or no PHFtau immunoreactivity was detected in the brains of the 9 MPS cases (Fig. 1B, E). Robust PHFtau-immunoreactive NFTs, neuropil threads, and dystrophic neurites were observed, however, in AD and DS cases processed in parallel with the MPS cases (Fig. 1H). Immunohistochemistry using antibodies directed against A β peptides did not reveal extracellular SPs within the brains of the MPS cases. However, abundant intracellular A β immunoreactivity was detected in the MPS brains, notably within the somatodendritic domain of neurons within the neocortex and hippocampal formation (Figs. 1C, F, 2A–C).

Neither intracellular A β immunoreactivity or SPs were observed in the brains of NCL or Gaucher's disease patients. By using a panel of MAbs and polyclonal antibodies specific for several different epitopes within A β (Table 2), we were able to determine that A β (1-40) immunoreactivity was the most abundant species in intracellular stores in MPS brains. In contrast, antibodies specific for A β (1-42) did not reveal intracellular labeling in the MPS brains. Qualitatively, cytoplasmic A β (1-40)

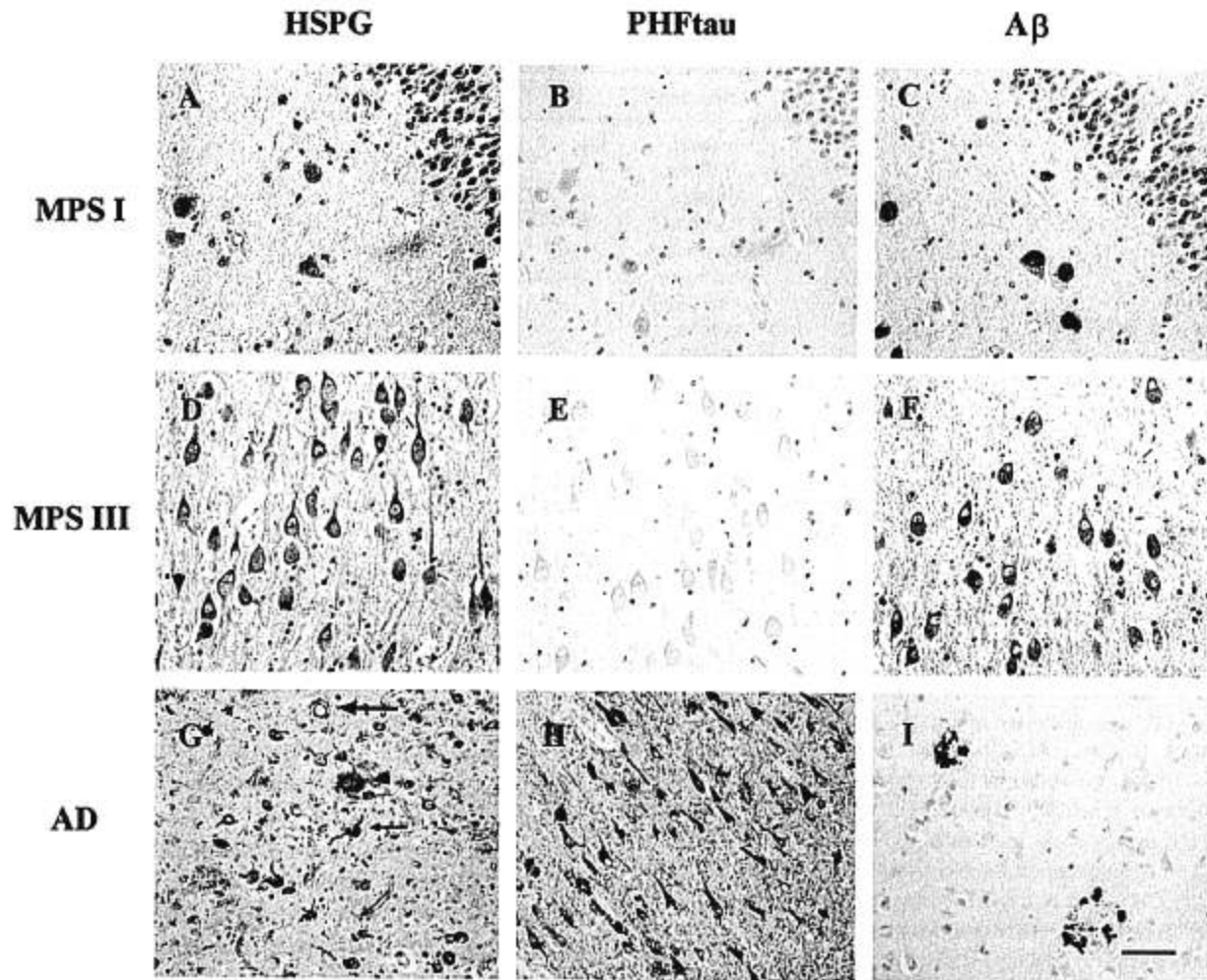


Fig. 1. Differential distribution of HSPG, PHFtau, and A β immunoreactivity within MPS and AD brains. Sections (A–I) are counterstained with hematoxylin. All panels are at the same magnification and the scale bar in I = 30 μ m. A: HSPG immunoreactivity within the granule cell layer of the dentate gyrus and hippocampal hilus of an MPS I (Hurler's syndrome) brain. Cytoplasmic HSPG immunoreactivity delineates swollen storage neurons. B: Adjacent section to (A), stained with an antibody directed against PHFtau. Note the paucity of cellular staining within the hippocampal formation. C: Adjacent section to (B), stained with an antibody directed against A β peptides. Abundant cytoplasmic labeling is detected within the granule cell layer and hilar neurons, similar to observations with HSPG immunoreactivity. D: HSPG immunoreactivity within CA1 subfield of the hippocampus of an MPS III (Sanfilippo's syndrome) brain. Similar to (A), abundant cytoplasmic labeling of neurons is observed. E: A paucity of PHFtau immunoreactivity is observed in a tissue section adjacent to (D). F: A β -immunoreactive neurons throughout the CA1 subfield in a tissue section adjacent to (E). Similar to (C), abundant cytoplasmic labeling of intracellular A β peptides is detected in the MPS III brain. G: HSPG immunoreactivity within the CA1-subicular region of an AD brain. HSPG-immunoreactive blood vessels (thick arrow), SPs (arrowhead), and NFTs (thin arrow) are observed within this field. H: PHFtau-immunoreactive NFTs and neuropil threads are observed within the CA1 region. I: Two A β -immunoreactive SPs are observed within the CA1-subiculum, with little or no intracellular A β immunoreactivity within neuronal profiles.

immunoreactivity appeared similar in staining intensity and distribution to HSPG immunoreactivity within neurons of the MPS brains. In contrast, A β -immunoreactive SPs were observed within the brains of AD and DS cases, with relatively little intracellular A β immunoreactivity detected in neurons (Fig. 1I).

ELISA assays using an amino-terminal capturing antibody and end-specific A β reporter MAbs combined with TBS extracted soluble and formic acid extracted insoluble temporal cortex tissue homogenates detected

both A β (1-40) and A β (1-42) in CTR, MPS, and AD brains. Although the total amount of A β peptides quantitated in MPS and CTR brains was low, we detected a statistically significant ($p < 0.001$), 3-fold increase in the amount of soluble A β (1-40) in the MPS temporal cortex homogenates compared with CTR homogenates (0.92 ± 0.05 (sem) pmol/g wet tissue vs 0.30 ± 0.03 pmol/g wet tissue; Fig. 3A). No significant differences were found between MPS and CTR homogenates for insoluble A β (1-40) (0.28 ± 0.03 pmol/g wet tissue vs



Fig. 2. Detection of intracellular A β immunoreactivity in MPS brains. Sections are counterstained with hematoxylin. All panels are at the same magnification and the scale bar in C = 10 μ m. A: A β -immunoreactive granule cells from a MPS I brain. B: Perinuclear, somatodendritic A β immunoreactivity is observed in CA1 neurons from the same MPS I brain as (A). C: Punctate, A β -immunoreactive labeling of pyramidal cells from temporal neocortex of an MPS III brain further illustrate cytoplasmic storage accumulations in these neurons.

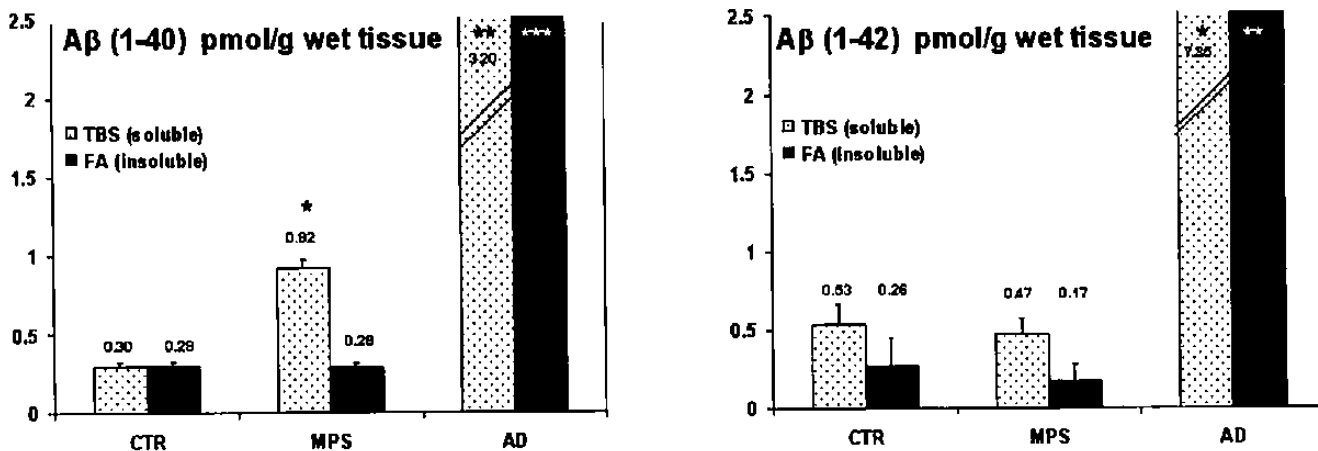


Fig. 3. Quantitation of soluble and insoluble A β (1-40) and A β (1-42) obtained from temporal neocortex of CTR, MPS, and AD brains using a sensitive two-site sandwich ELISA with end-specific monoclonal A β antibodies. Results are reported in units of pmol/g wet tissue for soluble (Tris-buffered saline [TBS] extracted; gray shading) and insoluble (formic acid [FA] extracted; black shading) A β (1-40) and A β (1-42). The scale for the AD homogenates has been split (hatched lines) to reflect that the actual values are larger than the y-axis scale. A: Although the total abundance of A β peptides is low in MPS brains, a 3-fold increase in soluble A β (1-40) was observed in the MPS homogenates as compared with CTR homogenates (asterisk; $p < 0.001$). No differences were observed between MPS and CTR brains for insoluble A β (1-40). As a positive control, soluble A β (1-40) (double asterisk; $p < 0.0001$) and insoluble A β (1-40) (white triple asterisk; $p < 0.00001$) were significantly elevated in AD homogenates. B: No significant differences were observed between MPS and CTR homogenates for soluble or insoluble A β (1-42). In contrast, significant elevations in both soluble A β (1-42) (single asterisk; $p < 0.0001$) and insoluble (1-42) (double white asterisk; $p < 0.00001$) were found for AD homogenates.

0.29 ± 0.03 pmol/g wet tissue). In addition, no differences were detected between MPS and CTR homogenates by ELISA for either soluble or insoluble A β (1-42) (Fig. 3B). To further validate the efficacy of the A β ELISA assay, homogenates obtained from the temporal neocortex of AD patients were used as a positive control. Similar to previous reports (47, 50), both soluble and insoluble A β (1-40) and A β (1-42) were elevated in AD temporal neocortex homogenates compared with CTR homogenates.

In addition, abundant cytoplasmic APP immunoreactivity was detected within neurons in the MPS brains (Fig. 4A–D), within neurons, NFTs, neuropil threads, SP cores in AD and DS brains (Fig. 4E, F), and within neurons in control brains (not shown). In summary, HSPG, PHFtau, A β , and APP immunoreactivity were similar in expression, distribution, and staining intensity in both MPS I and MPS III brains, and the expression of HSPG and A β differed from those observed in CTR, AD, and DS brains.

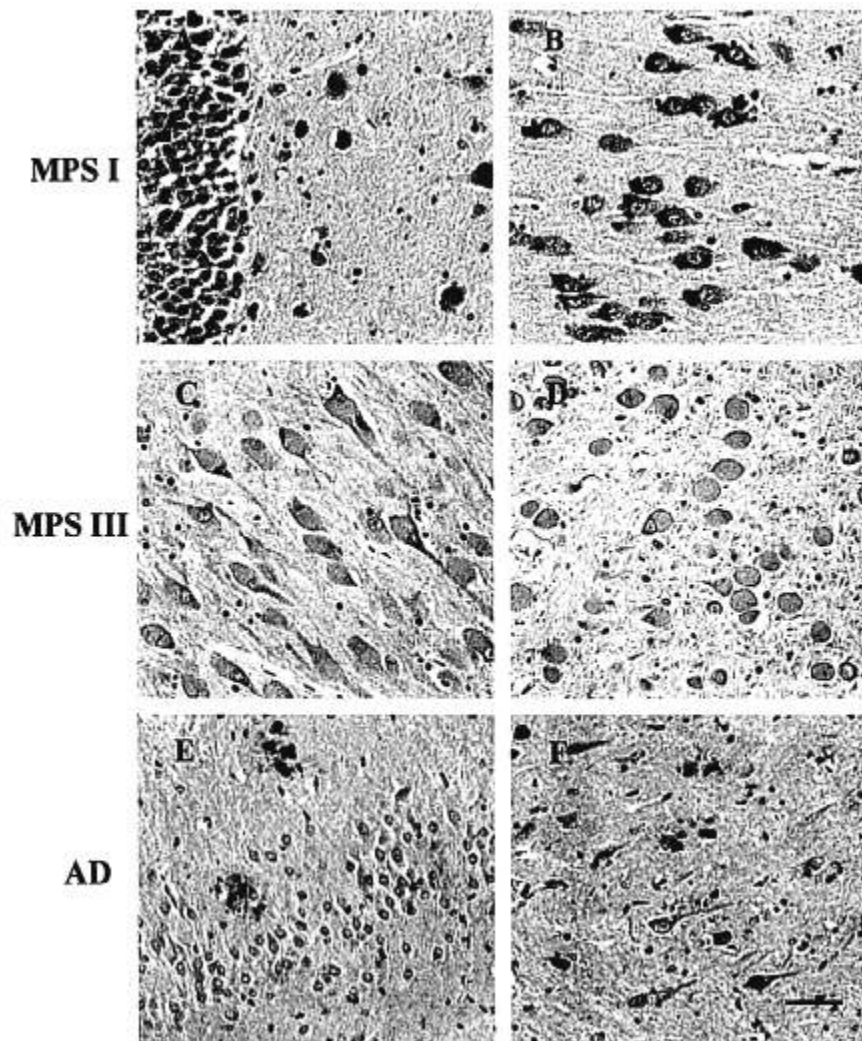


Fig. 4. Differential distribution of APP immunoreactivity within MPS and AD brains. Sections are counterstained with hematoxylin. All panels are at the same magnification and the scale bar in F = 30 μ m. A, B: Abundant perinuclear, cytoplasmic APP immunoreactivity is detected in granule cells and hilar neurons (A) and temporal neocortex (B) of an MPS I brain. C, D: Neuronal APP immunoreactivity observed within the CA1 region (C) and temporal neocortex (D) of an MPS III brain. E, F: Moderate cytoplasmic APP labeling of neurons is observed in an AD brain. APP-immunoreactive SP cores within the hilar region (E) and APP-immunoreactive NFTs and dystrophic processes within temporal neocortex (F) display intense labeling.

Murine and Caprine Models of MPS

PAS staining revealed swollen neurons within the brains of both MPS IIIID goats and MPS VII mice similar to previous observations (45, 52, 53). Cytoplasmic HSPG immunoreactivity was abundant in the goat MPS IIIID brains (Fig. 5A), similar to the human MPS cases. In contrast, moderate to weak HSPG immunoreactivity was observed in the murine MPS VII cases and unaffected goat controls (Fig. 5D). TS staining of brain sections from MPS IIIID goats and MPS VII mice did not reveal NFTs or SPs, and there was no intracytoplasmic PHFtau immunoreactivity in either of the caprine or murine MPS models (Fig. 5B, E). Intracellular accumulation of A β immunoreactivity within neurons was observed in the MPS IIIID goat brains, similar to the human MPS cases

(Fig. 5C) but not in the unaffected caprine controls. In contrast to the MPS IIIID goats, MPS VII mouse brains did not display abundant A β immunoreactivity (Fig. 5F), even when antibodies directed against rat A β (1–28) were used.

DISCUSSION

The neuropathology of MPS I (Hurler's syndrome) and MPS III (A–D) (Sanfilippo's syndrome) has been well described in extensive detail (56–59). However, evidence of PHFtau and A β pathology within the MPS brain has not been reported previously. Because GAGs, notably HSGAGs, have been shown by several criteria to be potential pathological chaperones in the formation of tau-positive PHFs and A β -containing SPs (27–29, 31, 37,

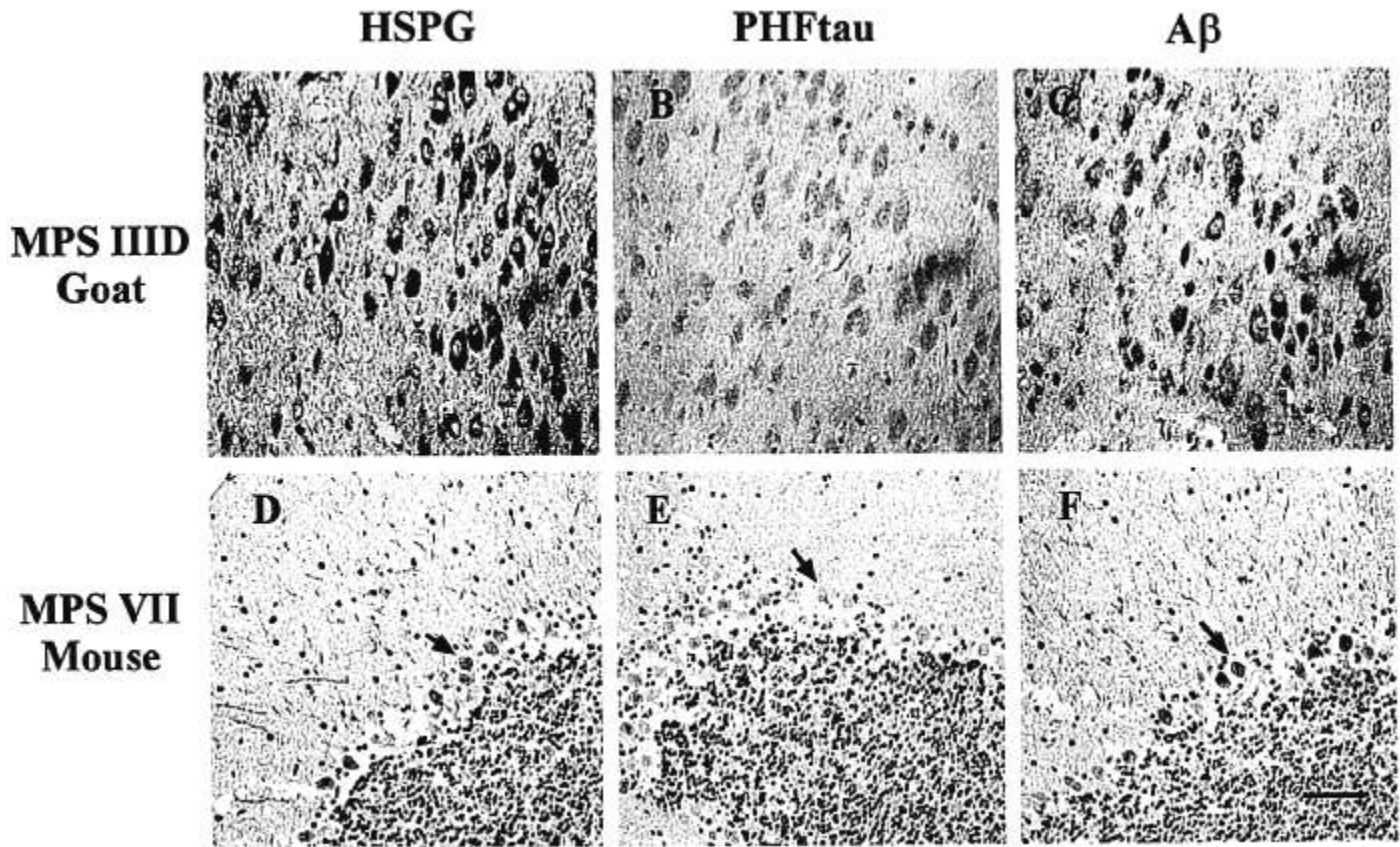


Fig. 5. Distribution of HSPG, PHFtau, and A β immunoreactivity within the brains of caprine (A–C) and murine (D–F) MPS models. Sections are counterstained with hematoxylin. All panels are at the same magnification and the scale bar in F = 30 μ m. A: Tissue section from the CA1 region of hippocampus of an MPS III D goat stained with an antibody directed against HSPG. Note the intense perinuclear, somatodendritic labeling similar to human MPS cases. B: Adjacent section to (A), illustrating the paucity of PHFtau immunostaining. C: Adjacent section to (B), demonstrating strong to moderate A β immunoreactivity. D: Tissue section from the cerebellum of an MPS VII mouse stained with an antibody directed against HSPG. Moderate to weak HSPG immunoreactivity is detected within Purkinje cell bodies (arrow) and dendrites, and contrasts the intense HSPG immunoreactivity observed in the MPS III D goat brains. E: Adjacent section to (D), demonstrating a lack of PHFtau immunoreactivity (arrow). F: Adjacent section to (E), illustrating moderate to weak A β immunoreactivity within Purkinje cells (arrow).

39), we hypothesized that accumulations of uncatabolized HSGAGs in MPS brains due to lysosomal enzyme deficiencies could induce the formation of NFTs and SPs. Notwithstanding, SPs and NFTs are typically associated with progressive, late-onset disorders such as AD, and the majority of MPS patients die before AD typically begins (43, 44), SPs have been observed in the brains of some adolescent DS patients (62, 63), and in most DS patients surviving past the third decade of life (64). In addition, NFTs are observed in the brains of adolescents with subacute sclerosing panencephalitis (65–67), as well as in the brains of adolescent patients with Niemann-Pick type C disease, a cholesterol storage disorder (68–70). Thus, even though NFT and SP pathology was not observed by TS and immunohistochemistry for PHFtau and A β peptides in the present set of MPS cases (ages 1–42 years), tangle and plaque pathology has been observed (albeit rarely) in patients with other disorders who were as young as the MPS patients in the present report.

The detection of intense and diffuse APP and increased A β (1-40) immunoreactivity within the cytoplasm of neurons throughout MPS brains is intriguing because insights into this phenomenon may clarify mechanisms of amyloidosis in AD. For example, it will be important to determine if uncatabolized accumulation of HSGAGs in MPS syndromes lead to an increase in the synthesis of soluble A β , or a decrement in the secretion or degradation of A β . Three pathways have been identified in the proteolytic processing of APPs into A β peptides, including an endoplasmic reticulum/intermediate compartment pathway that generates principally A β (1-42) species (71–73), a trans-Golgi network pathway that generates A β (1-40) (major species) and A β (1-42) species (74, 75), and an endosomal/lysosomal pathway that also generates secreted A β (1-40) (major species) and A β (1-42) (76–79). The latter pathway may be most relevant in MPS, because APP and A β , like HSGAGs, are degraded (or recycled) in part by an endosomal-lysosomal pathway

(76, 77, 79). Moreover, GAGs, including HSGAG, have been reported to inhibit (>20) lysosomal glycolipid and acid hydrolases (80, 81). Thus, overall lysosomal efficiency may be compromised in MPS, and the accumulation of uncatabolized HSGAGs may impair the degradation of many macromolecules that are normally proteolyzed within these organelles, including APP and/or A β peptides.

This is the first report of robust intraneuronal A β (1-40) immunoreactivity in the human MPS brain or the brain of an experimental animal model of MPS. Thus, our findings here provide *in vivo* support for *in vitro* studies demonstrating that A β peptides accumulate within the cytoplasm of cultured cells, especially cells overexpressing neuron-predominant APP695 constructs (48, 72, 73). Moreover, studies of intracellular trafficking and processing of A β peptides leading to increased intracellular levels of A β (1-40) versus A β (1-42) linked to the lysosomal storage defects in MPS may provide insights into mechanisms of amyloidosis in AD. We hypothesize that the lack of SPs observed in MPS brains may be associated with the lack of increased levels of A β (1-42), which has a higher propensity to form insoluble aggregates compared with A β (1-40). Because heparin binding domains have been detected in APPs and HSPGs interact directly with A β peptides (28, 30, 40-42), the intracellular A β immunoreactivity in MPS brains leads to the speculation that a pathological increase in intracellular HSPGs due to MPS may promote interactions between HSPGs and A β peptides that lead to accumulations of these peptides within the somatodendritic domain.

In addition to human MPS brains, we also studied the brains of 2 animal models of HSGAG accumulation. MPS IIID goats accumulate HSPGs and the neuropathology of this model is well characterized (45), but it does not include NFTs and SPs. However, the caprine MPS IIID brain does show intracellular A β accumulations and little or no PHFtau immunoreactivity, similar to our observations in the human MPS I and MPS III brains. Thus, this is an excellent animal model to probe the basis of HSGAG overexpression-A β peptide interactions. The murine MPS VII model also has well-characterized neuropathological changes (46), but no NFTs and SPs. The mouse MPS VII model has many experimental advantages, including potential amelioration of storage deficiencies by transplantation or somatic gene transfer (52, 53), however, the moderate to weak expression of HSPG (as other uncatabolized GAGs, such as chondroitin sulfate and dermatan sulfate accumulate in MPS VII) and A β immunoreactivity does not recapitulate our human MPS I and MPS III observations. Moreover, it would be relevant now to determine if other mouse or large animal models (transgenic and spontaneous mutations) of MPS might be suitable for studies of GAG/A β interactions.

In conclusion, the lack of plaque and tangle pathology in the presence of *in vivo* HSGAG accumulation does not exclude the possibility that GAGs (notably the highly sulfated heparin and HSPG) may serve as pathological chaperones for PHF formation and amyloidosis as demonstrated by several groups *in vitro* (25, 27, 32, 37). Rather, our findings suggest that complex interactions of genetic, developmental, and environmental factors contribute to the accumulation of insoluble filaments in the form of NFTs, dystrophic processes, and SPs. Moreover, the observation that intracellular soluble A β (1-40) is increased in the diseases studied here implies that deficits in lysosomal degradation and subsequent accumulations of HSGAGs may cause an increase in APP and/or A β in the cytoplasm of storage neurons. Thus, MPS patient samples and MPS animal models may enable mechanistic studies of the role of A β peptides and HSPGs/HSGAGs in the amyloidosis that characterizes AD.

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