Articles

Increased Aβ Peptides and Reduced Cholesterol and Myelin Proteins Characterize White Matter Degeneration in Alzheimer's Disease†

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ABSTRACT: Relative to the gray matter, there is a paucity of information regarding white matter biochemical alterations and their contribution to Alzheimer's disease (AD). Biochemical analyses of AD white matter combining size-exclusion, normal phase, and gas chromatography, immunoassays, and Western blotting revealed increased quantities of Aβ40 and Aβ42 in AD white matter accompanied by significant decreases in the amounts of myelin basic protein, myelin proteolipid protein, and 2′,3′-cyclic nucleotide 3′-phosphodiesterase. In addition, the AD white matter cholesterol levels were significantly decreased while total fatty acid content was increased. In some instances, these white matter biochemical alterations were correlated with patient apolipoprotein E genotype, Braak stage, and gender. Our observations suggest that extensive white matter axonal demyelination underlies Alzheimer's pathology, resulting in loss of capacitance and serious disturbances in nerve conduction, severely damaging brain function. These white matter alterations undoubtedly contribute to AD pathogenesis and may represent the combined effects of neuronal degeneration, microgliosis, oligodendrocyte injury, microcirculatory disease, and interstitial fluid stasis. To accurately assess the success of future therapeutic interventions, it is necessary to have a complete appreciation of the full scope and extent of AD pathology.

The neurons of the cerebral cortex and subcortical nuclei are involved in the initiation and processing of information vital to cognitive, sensory, and motor activity. No less important, however, are the WM[1] axonal projections extending from cortical and subcortical neurons, which form the commisural and association fibers that conduct action potentials throughout the cortex. The WM axons play a major role in the modulation of mental activities such as cognition and emotion, and all cerebral functions are fully expressed only when myelinization is complete (*1*). White matter dyscraises are associated with decreased performance in the processing of immediate and delayed memory, executive functions, and global cognitive functions (*2*).

Alzheimer's disease has been conventionally considered to be a disease of the brain GM. Despite the fact that during AD progression the WM tissue is substantially reduced and is severely abnormal (*3*−*7*), there is a comparative paucity of information regarding the WM biochemical alterations and their contribution to this dementia (*8*−*14*). Cerebral WM changes consisting of decreased signal intensity on CT scans, hyperintensities on MRI, and rarefactions and gliosis at the histological level are present in approximately two-thirds of AD cases (*15*−*17*). It is generally believed that the atrophic changes observed in the WM are the consequence of axon retraction caused by Wallerian degeneration due to neuronal cell body loss. However, this contention has been challenged by the lack of correlation between GM and WM damage

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[1] Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; ApoE, apolipoprotein E; BSA, bovine serum albumin; CNP, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; FA, fatty acid; FAME, fatty acid methyl ester; GDFA, glass-distilled formic acid; GM, gray matter; H and E, hematoxylin and eosin; MBP, myelin basic protein; MPP, myelin proteolipid protein; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PLP, proteolipid protein; TBS, Tris-buffered saline; TTBS, Tween-enhanced TBS; WM, white matter.

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(*15*, *18*). In addition, patients with preclinical AD exhibit WM atrophy before GM degeneration, suggesting that axonal or myelin chemical abnormalities provoke neuronal body degeneration (*18*).

Studies using diffusion tensor MRI, a technique that provides an in vivo quantitative assessment of cerebral WM integrity, have shown that AD patients have significantly reduced association and commisural fiber integrity (*19*) as well as decreased white matter in the frontal lobes, the superior longitudinal fasciculus, and the temporal stem (*20*). Regardless of whether the WM changes in AD are primary or secondary, it is improbable that they are benign clinical correlates since similar, albeit more severe, changes are known to be devastating in other diseases, such as Biswanger's disease, the leukodystrophies, and amylotrophic lateral sclerosis.

Recently described cytological alterations in oligodendroglia in AD, including *tau* immunoreactive glial fibrillary tangles (*21*) and complement-activated oligodendroglia and microglia (*22*, *23*), reveal that these critical cells are actively involved in this disease. Alzheimer's disease neurofibrillary tangle pathology follows an apparently inverse sequence of developmental cortical myelination (*24*−*26*). This critical and provocative retrogenesis observation suggests that oligodendroglial pathology must be a primary event in AD pathogenesis (reviewed in ref *27*). Moreover, the β-amyloid precursor protein is expressed in oligodendrocytes (*28*), and the Aβ peptides have been found to be toxic to these cells in culture (*29*). Oligodendrocytes receive information from neurons and concurrently produce factors that promote neuronal structural integrity as well as molecules capable of inhibiting neuritic outgrowth (*30*, *31*). Neuron stability is enhanced with axon myelination, and this key structural element may be damaged or reduced with aging. If oligodendrocyte structural and functional integrity were maintained in old age, thereby averting axonal cytoskeleton degeneration, AD onset may be delayed or prevented entirely (*24*).

In this paper we present the results of quantitative biochemical analyses of the WM of individuals who died with autopsy-diagnosed AD in comparison to nondemented controls. Our studies revealed that the WM chemical characteristics, in particular, the total amount of protein and lipids, were significantly altered in AD patients. In addition, increased amounts of Aβ40 and Aβ42 exist in AD WM and are accompanied by significant proportional decreases in the amount of cholesterol, MBP, MPP, and CNP. Our observations suggest that extensive WM axonal demyelination underlies AD pathology. In some instances, these biochemical deficiencies correlated with patient ApoE genotype, Braak stage, and gender. The relevance of these findings and their potential contribution to AD are discussed in relation to the pathogenesis and pathophysiology of this dementia.

MATERIALS AND METHODS

Human Subjects. Brain tissue was obtained from the Brain Donation Program at Sun Health Research Institute. Tissue donors or their legal representatives signed an informed consent document allowing for post-lividum brain removal. The cases in this study were chosen so as to provide a mixture of ApoE genotypes in both nondemented and Alzheimer's disease groups. Characteristics of the study subjects are given in Table 1. The subject's age, ApoE

Table 1: Study Subjects and Control Cases

(A) Study Subjects

	age/ sex	brain wt (g)	ApoE	white matter score[a]	plaque density[b]	Braak stage	NIA range score[c]
1	89/F	1040	3/3	0.25	mod	IV	N/D
2	76/F	1070	3/3	0.50	mod	IV	N/D
3	91/M	1160	3/3	2.00	mod	II	N/D
4	63/M	1370	3/3	N/A	none	I	N/D
5	92/F	925	3/3	1.25	none	II	N/D
6	91/M	1200	3/3	0.25	sparse	II	N/D
7	79/M	1260	3/3	0.00	none	II	N/D
8	81/F	1274	4/4	0.00	sparse	II	N/D
9	91/M	1050	3/4	0.00	mod	III	N/D
10	78/M	1225	3/4	1.75	mod	III	N/D
11	77/F	1235	3/4	0.00	mod	III	N/D
12	73/F	1180	3/4	0.00	none	I	N/D
13	96/F	1120	3/4	0.25	sparse	II	N/D
51	80/M	1060	3/3	1.00	freq	VI	high
52	93/F	790	3/3	1.25	freq	VI	high
53	79/F	1000	3/3	1.00	freq	VI	high
54	82/F	860	3/3	1.00	freq	VI	high
55	84/M	1020	3/3	1.50	mod	III	high
56	80/F	765	4/4	0.50	freq	VI	high
57	91/F	860	4/4	3.00	freq	VI	high
58	84/M	1050	4/4	1.25	freq	VI	high
59	83/M	940	4/4	1.00	freq	VI	high
60	84/F	940	4/4	0.50	freq	VI	high

(B) Control Cases: Clinical and Neuropathologic Diagnoses

	clinical diagnoses[d]	neuropathologic diagnoses[d]
1	cardiac and/or respiratory failure, COPD	control; old lacunar infarct of left thalamus
2	COPD	control; old microinflorets, left middle frontal gyres
3	DM, asthma, CAD, PVD, CHF, CRF, probable CVA, UTI	control; CAA
4	lung CA and respiratory insufficiency	control; CAA
5	NPH, possible early dementia, pneumonia with dysphagic	control
6	CVA's, CHF, HTN, CRF, cardiac and/or respiratory failure, renal failure	control; incidental Lewy bodies; CAA, old cerebral infarcts
7	HTN, CVA, MI, CAD, intracerebral hemorrhage	control; acute infarction, brainstem
8	aortic stenosis, CHF, unstable margins, cardiac and/or respiratory failure	control
9	CVA, CAD, CRF, CHF, prostate CA, heart failure, valvular heart disease	control
10	colon resection for colon CA	control; old lacunar infarctions, basal ganglia
11	arthritis, strial fibrillation, cardiac and/or respiratory failure	control
12	ovarian CA	control; Alzheimer type II astrocytosis
13	failing memory, confusion, cardiac and/or respiratory failure	control; incidental Lewy bodies

[a] For white matter score, see text. [b] mod = moderate; freq = frequent. [c] N/D = nondemented. [d] Abbreviations: CA = cancer, CAA = cerebral amyloid angiopathy, CAD = coronary artery disease, CHF = congestive heart failure, COPD = chronic obstructive pulmonary disease, CRF = chronic renal failure, CVA = cerebrovascular accident, DM = diabetes mellitus, HTN = hypertension, MI = myocardial infarction, NPH = normal pressure hydrocephalus, PD = Parkinson's disease, PVD = peripheral vascular disease, RA = rheumatoid arthritis, UTI = urinary tract infection.

genotype, neocortical neuritic plaque density, Braak stage, and diagnostic status with respect to AD, as determined using the National Institutes of Aging−Reagan Institute consensus criteria, are listed. The mean age of nondemented cases was 82.8 years, while that of AD cases was 84.0 years. The nondemented cases were made up of seven females and six males while the AD cases were six females and four males. The post-mortem interval for nondemented subjects was 3.75 h while for AD subjects it was 2.92 h (difference not significant with a two-tailed unpaired *t*-test).

Tissue Processing. Cerebral hemispheres were sliced into 1 cm thick slabs in the coronal plane; the right hemisphere slices were frozen between sheets of dry ice and kept thereafter in a freezer at −80 °C. Left hemisphere slices were fixed in 4% paraformaldehyde for 48 h and then dissected into small tissue blocks for paraffin embedding and large tissue blocks for frozen sectioning. Paraffin blocks were sectioned at 5 μm intervals and stained with H and E. Frozen sections (40 μm) were taken after cryoprotection in 2% DMSO/20% glycerol using a sledge-type microtome. Frozen sections were stained with H and E for general white matter morphology and for Alzheimer's changes using Campbell-Switzer, Gallyas (*32*), and thioflavin S methods.

Neuropathologic Grading. Fresh-frozen white matter was dissected at −20 °C from right hemisphere centrum semiovale of the parietal and occipital lobes. The entire centrum semiovale was sampled with the exception of the U-fiber region where senile plaques are known to be typically abundant. As can be appreciated from the data presented in Table 1A, WM rarefaction in each cerebral lobe was graded as mild, moderate, or severe on the basis of the fraction of centrum semiovale affected on thick (40 μm) quarter-hemisphere H and E stained sections (mild, <25%; moderate, 25−50%; severe, >50%). Individual scores for each lobe were converted to a numeric code (mild = 1; moderate = 2; severe = 3) from which a mean score for each brain was obtained. Neuritic plaque density in the cerebral cortex was graded according to CERAD criteria (*33*). Neurofibrillary tangles were staged according to the method of Braak (*32*). The diagnosis of Alzheimer's disease was assigned if a subject was demented and had at least a "moderate" plaque density in combination with a Braak stage of at least III. The protocol developed by the National Institutes of Aging and the Reagan Institute (*34*) was used to assign to each AD case a probability estimate that dementia was due primarily to AD. Probabilities were referred to as being either "intermediate" or "high".

Quantification of WM and GM Total Protein. Proteins were quantified with the Micro BCA protein assay reagent kit (Pierce) using Nunc (Nalge, Rochester, NY) 96-well plates. BSA standards were prepared and the assays performed in triplicate following the manufacturer's instructions. The resulting colorimetric reactions were read at 570 nm on a Wallac Victor 1420 multilabel counter (Perkin-Elmer Life Sciences, Gaithersburg, MD). Protein concentrations were calculated using Graphpad Prism and Excel programs with reference to BSA standard concentration curves.

FPLC Separation and Quantification of Aβ Peptides. One hundred sixty milligram samples of gray and white matter from the brains of 10 AD and 13 nondemented control individuals (Table 1) were dissected and homogenized in 1.5 mL of 90% GDFA using a glass Dounce homogenizer. The homogenized samples were centrifuged at 485000*g* for 30 min using a Beckman SW60Ti rotor at 5 °C. The acid-soluble supernatant was collected, avoiding the lipid layer condensed at the top of the tube and the small acid-insoluble pellet at the bottom of the tube, and divided into 500 μL samples. These specimens were fractionated on a Superose 12 size-exclusion column (30 cm × 1 cm; Amersham Pharmacia Biotech, Piscataway, NJ) equilibrated with 80% GDFA. The chromatography was developed with 80% GDFA at a flow rate of 15 mL/h and monitored at 280 nm. The 3−8 kDa fractions were collected into tubes containing 5 μL of 2% betaine, dried by vacuum centrifugation, and stored at −80 °C until analysis. Amyloid peptides were quantified by suspension in 50 μL of 80% GDFA, diluted with 1 mL of distilled water and 1 mL of 5× TBS (Tris-buffered saline, pH 7.5). The pH was adjusted to 7.4 with 2 M NaOH using a pH meter with microelectrode, and the volume was brought to 5 mL with distilled water. The control samples were diluted 10-fold with distilled water, and the AD samples were diluted 20-fold with distilled water. Samples of 100 μL were plated and submitted to europium immunoassay for quantitation as described previously (*35*). Capture antibodies R208 and R321 used for the Aβ immunoassay were kindly donated by Dr. P. Mehta of The Institute for Basic Research and Mental Disabilities, Staten Island, NY. These antibodies recognize the C-terminal domains of Aβ40 (Aβ residues 34−40) and Aβ42 (Aβ 36−42), respectively. The Aβ peptide antigens used for rabbit antibody induction were synthesized commercially (Ana Spec, San Jose, CA) and conjugated to keyhole limpet hemocyanin in PBS with 0.5% glutaraldehyde. The specificity of rabbit antisera R208 and R321 was examined by sandwich ELISA. For the R208 antibody, there was a strong response with 1 ng/mL Aβ40 but no detectable response with 10 ng/mL Aβ42. Likewise, the R321 antibody was found to be specific for Aβ42 with no reactivity to Aβ40 detectable in our assays. In addition, Western blot experiments confirmed the specificity of these antibodies. The biotinylated monoclonal antibody 4G8 raised against Aβ residues 17−24 (Senetek, Maryland Heights, MO) was used as a reporter antibody for immunoassays. Quantitation experiments were conducted in triplicate assays, and Aβ concentrations were calculated with reference to a standard curve that was generated for each experiment.

Separation and Quantification of Cholesterol in WM and GM. The brain GM and WM cholesterol content was quantified in 66 AD patients (36 males, average age 80.8 years, range 65−94 years, and 30 females, average age 82.4 years, range 63−94 years) and 36 nondemented control individuals (23 males, average age 79.7 years, range 63−94 years, and 13 females, average age 80.1 years, range 67−92 years). In both populations, the post-mortem interval prior to sampling averaged 2.1 h. Brain lipids were extracted by the method of Folch and Lees (*36*). For cholesterol quantification, 200 mg of cerebral cortex was homogenized in 5 mL of chloroform/methanol (2:1 v/v) containing 100 μg of 4-cholesten-3-one as an internal concentration standard. The specimens were filtered through Whatman No. 1 paper, another 2 mL of chloroform/methanol (2:1 v/v) was used to extract the residues, and the specimens were filtered again. The two filtrates were combined, 1.5 mL of distilled water was added, and the organic layer was collected after

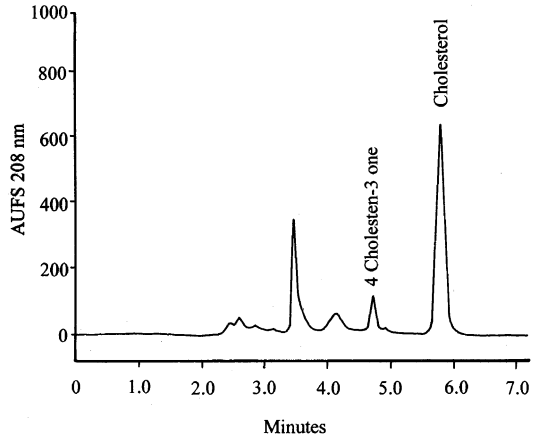

FIGURE 1: High-performance liquid chromatographic elution profile of cholesterol purification. A silica normal phase column (Zorbax SIL, 4.6 mm × 250 mm; MAC-MOD Analytical, Inc., Chadds Ford, PA) was used for the purification of cholesterol. The chromatography was isocratically developed with a mixture of 50% 2-propanol and 50% acetonitrile at a flow rate of 1.0 mL/min and monitored at 208 nm. The representative profile shown in the figure originated from an AD WM case. The column was precalibrated with pure cholesterol which demonstrated a 5.75 min retention time. The 4-cholesten-3-one internal standard was used to calculate brain cholesterol levels. The areas under the peaks were automatically estimated using the PC1000 software provided by the HPLC manufacturer (Thermo Separation Products, San Jose, CA).

centrifugation at 12000*g* for 10 min to separate the two phases. The lipid extracts were completely dried under a fine stream of N_2. The dried specimens were dissolved in 1.2 mL of hexane/2-propanol (95:5 v/v), transferred to an Eppendorf microfuge tube, and centrifuged at 12000*g* for 10 min. For the chromatographic purification of cholesterol a 10 μL supernatant sample was loaded onto a silica normal phase column (Figure 1).

Quantification of WM Fatty Acids. The white matter specimens were lyophilized overnight in tared borosilicate tubes and the sample dry weights calculated by difference. The samples were transferred into a Dounce homogenizer and the tubes rinsed with 1.2 mL of water followed by the addition of 2.8 mL of methanol−water (4:3 v/v). After homogenization, the samples were transferred to borosilicate glass tubes followed by the addition of 1.2 mL of the methanol−water mixture and 4.6 mL of chloroform. The samples were vortexed for 1 min and centrifuged at 1500*g* for 10 min, and the lower organic layer was removed and transferred to tared glass tubes. The solvent was removed by evaporation under a stream of N_2 and the weight of the organic extract recorded. The specimens were dissolved in 100 μL of chloroform−methanol−water (8:4:3 v/v/v), and 1 mg of the soluble lipid mixture was pipetted into a 1 mL glass vial along with 10 μg of nonadecanoic acid (C19:0). After being dried under a stream of N_2, 400 μL of 1 N methanol−HCl was added, the vials were capped, and the samples were heated at 80 °C for 2 h. After being cooled the sample was dried under N_2, 250 μL of dry methanol was added, and the samples were dried again. This entire procedure was repeated twice. The final residues were dissolved in 200 μL of dichloromethane for GC−MS analysis. Analysis of FAMEs was carried out on Hewlett-Packard 5890 GS−MS coupled to a Quadrex OV-225 (Phenomenex, Torrance, CA) column (30 m × 0.25 mm, 0.2 mm film) with an injection volume of 2 μL (split mode, 10:1) as described previously (*37*). Briefly, the chromatography was carried out using ultra-high-purity helium at a flow rate of 2 mL/min with an injection port temperature of 250 °C. The oven temperature was programmed so that it was maintained at 100 °C for 3 min and then raised at a rate of 5 °C/min to a final temperature of 210 °C (45 min total time). Detection was carried out with a mass detector using electron impact ionization. Peaks displaying mass and time-of-flight patterns consistent with those of FAME standards were quantified by integration, taking into account response factors previously determined with respect to the C19:0 internal standard. Results were normalized to micrograms of fatty acid per milligram of dried sample. Following the elimination of outliers (*38*), means for the individual fatty acid components for the AD population were compared to corresponding components of the control group using one-way ANOVA. Bias between groups, simultaneously taking all of the separate fatty acid components into account, was assessed using two-way ANOVA.

Apolipoprotein E Genotyping. Genomic DNA was extracted from approximately 50 mg of cerebellar tissue (*39*). The DNA was PCR amplified using allele-specific oligonucleotide probes spanning positions 112 and 158 of the ApoE gene sequence. The PCR products were cleaved with the restriction enzyme *Hha*I and the fragments separated by polyacrylamide electrophoresis (*40*).

Western Blotting. Two hundred milligrams of tissue was homogenized in 800 μL of PBS, containing 8 μL of a stock solution (160 mg/mL) of protease inhibitor cocktail (PIC; Roche, Mannheim, Germany), using a Potter-Elvehjem tissue grinder with a Teflon pestle. SDS−PAGE samples were prepared with 4× NuPAGE-LDS sample buffer (Invitrogen, Carsbald, CA) and 0.05 M dithiothreitol. Samples were heated to 70 °C for 10 min, run on a 4−12% Bis-Tris NuPAGE gradient gel (Invitrogen) for 2 h at 100 V, and transferred onto a 0.2 μm nitrocellulose membrane using a 25 V electric field for 2 h. The membranes were blocked by incubation in 5% nonfat dried milk and TBS (0.5 M NaCl, 20 mM Tris, pH 7.4) for 1 h at room temperature. The blocking solution was removed, and the membranes were incubated with diluted primary monoclonal antibodies recognizing myelin basic protein (1:1000 dilution; Serotec, Kidlington, U.K.), myelin proteolipid protein (1:750; Serotec), or cyclic nucleotide 3′-phosphodiesterase (CNPase, 1:5000; Sigma, St. Louis, MO) in 1% nonfat milk in TBS overnight. Membranes were subjected to four washes of 10 min duration with TTBS (0.5 M NaCl, 20 mM Tris, pH 7.4, 0.5% Tween), followed by two 5 min rinses with TBS. The washed membranes were incubated with goat anti-mouse IgG secondary antibody (Pierce, Rockford, IL) for 1 h at room temperature, followed by a second set of four TTBS washes and two TBS rinses. Protein bands recognized by antibody were detected using ECL Western blotting detection reagent (Amersham Pharmacia Biotech). The relative band intensity was quantified using a scanning ChemiImager (Alpha Innotech Corp., San Leandro, CA).

RESULTS

AD WM Contains Less Protein than Control Subjects. An evaluation of the total protein concentration by the Micro

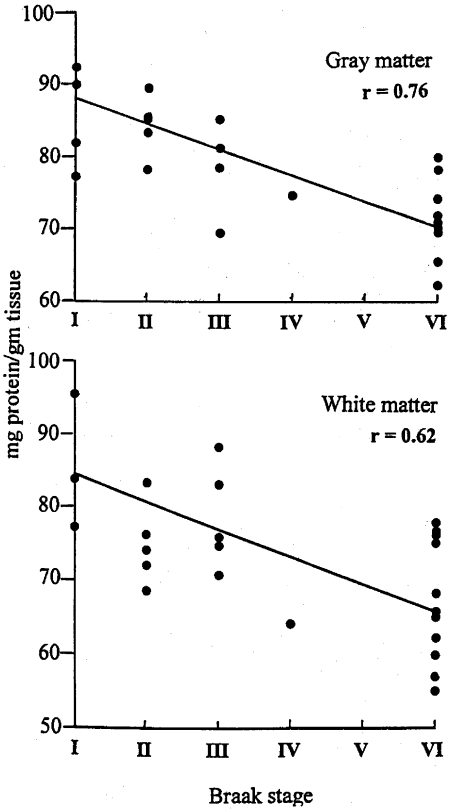

FIGURE 2: Protein level as a function of Braak stage. Correlation between Braak stage and protein levels in GM and WM of control nondemented and AD brains using a linear least-squares analysis.

BCA technique was consistent with the visual observation of tissue samples. The AD subjects had a mean value of 69.4 mg/g WM tissue while the control subjects had a mean value of 78.0 mg/g tissue. Thus, the WM total protein of the AD group was about 11% less compared to the control cohort ($p = 0.025$). The mean WM protein contents of the Braak I and II and Braak VI groups were 75.1 and 66.3 mg/g, respectively, representing a 12% decrease in WM protein in the latter group ($p = 0.05$). No ApoE subgroup of the AD or control subjects was found to have a statistically significant difference in total WM mean protein levels. However, when the GM total protein content was considered, the AD subjects had a mean value of 72.5 mg/g tissue while the control subjects had a mean value of 82.6 mg/g tissue. Again, as with the WM, the GM total protein content of the AD cases was about 11% less than that of the control subjects ($p = 0.003$). Furthermore, the amount of GM protein was found to correlate with the Braak score. The mean GM protein contents for the Braak I and II and Braak VI groups were 83.4 and 70.8 mg/g, respectively. The Braak VI group had ~15% lower GM protein level than did the Braak I and II group ($p = 0.01$). When AD and control ApoE subgroups were examined, the GM protein of those individuals in the control group with ApoE $\epsilon3/\epsilon3$ had 15% more protein than those individuals with AD carrying the ApoE $\epsilon3/\epsilon3$ genotype. Notable positive correlations were demonstrated when the GM and WM protein levels were compared with the Braak stage (Figure 2). As the Braak stage value increased, both the GM and WM protein levels declined ($r = 0.76$ and $r = 0.62$, respectively).

White Matter Aβ Peptides Are More Abundant in AD. To avoid an underestimation of the Aβ peptides in the WM resulting from steric hindrance of antigenic determinants by sequestering proteins (41) prior to immunoassay quantification, we separated the Aβ peptides contained in the 3−8 kDa region by size-exclusion chromatography under denaturing conditions (Figure 3). Quantifying WM Aβ peptides by europium immunoassay revealed significant differences between AD and control groups (Table 2). These assays were performed using samples obtained from the deep WM, away from the boundaries of the cortical GM and the subjacent

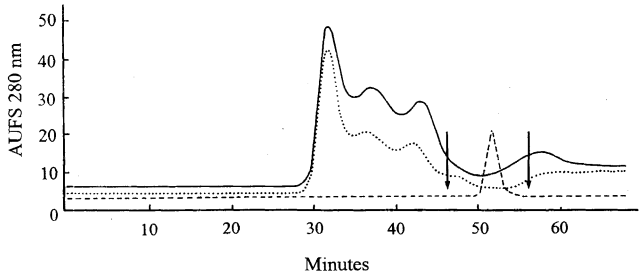

FIGURE 3: Fast-performance liquid chromatographs of WM GDFA homogenates. The size-exclusion chromatographic elution profiles developed in 80% GDFA correspond to control WM (solid line), AD WM (dotted line), and the Aβ marker reversed sequence 40−1 (hyphenated line) which was used as a molecular marker to avoid contamination of the columns by either Aβ 1−40 or Aβ 1−42. The amount of eluted protein under the chromatographic profiles at 280 nm, which originated from 50 mg of starting tissue in all cases, was always less in the AD WM when compared to the control WM. The two arrows represent the eluant that was collected for immunoassay quantification of Aβ peptides and includes molecules ranging from 8 to 3 kDa.

Table 2: White Matter Aβ Levels in Alzheimer's Disease and Nondemented Control

case no.	ApoE	40 ng/g	42 ng/g	total
		Nondemented Control		
1	3/3	53	687	739
2	3/3	4	122	126
3	3/3	79	420	499
4	3/3	125	552	678
5	3/3	37	479	516
6	3/3	165	130	295
7	3/3	*a*	204	204
mean		77	371	437
8	4/4	147	441	588
9	3/4	272	630	902
10	3/4	190	880	1070
11	3/4	70	203	273
12	3/4	48	236	284
13	3/4	141	447	588
mean		145	473	618
total mean		111	418	520
		Alzheimer's Disease		
51	3/3	360	679	1039
52	3/3	5	323	328
53	3/3	437	424	862
54	3/3	108	747	856
55	3/3	402	584	986
mean		263	552	814
56	4/4	606	1711	2317
57	4/4	4149	1624	5773
58	4/4	4030	3313	7343
59	4/4	354	912	1267
60	4/4	236	1034	1270
mean		1875	1719	3594
total mean		1069[b]	1135[c]	2204[c]

[a] The reading was less than the 10 pg/mL standard. [b] $p = 0.05$. [c] $p = 0.02$.

WM, since there were numerous amyloid plaques in the superficial layer of the latter. On the average, the A$\beta n-40$ and A$\beta n-42$ peptides were 9.6 and 2.7 times, respectively, more abundant in AD than in the control cases. The total Aβ ($n-40$ plus $n-42$) was on the average 4.2 times increased in AD compared to the nondemented control group.

In the WM, significant differences were evident between the control and AD cohorts with respect to both A$\beta n-40$ levels (means: 111 and 1069 ng/g of tissue, respectively; $p = 0.05$) and A$\beta n-42$ levels (means: 418 and 1135 ng/g of tissue, respectively; $p = 0.01$). For individuals with the ApoE $\epsilon 3/\epsilon 3$ genotype, a significant difference was evident between the control and AD groups with respect to Aβ $n-40$ levels (means: 77 and 263 ng/g of tissue, respectively; $p = 0.05$). The difference was less significant between those control individuals carrying at least one ApoE $\epsilon 4$ (mean: 145 ng/g of tissue) and those AD subjects with ApoE $\epsilon 4/\epsilon 4$ (mean: 1875 ng/g of tissue; $p = 0.06$). Interestingly, the results in terms of significance were reversed for A$\beta n-42$. The A$\beta n-42$ amounts in the control and AD populations were not significantly different for the ApoE $\epsilon 3/\epsilon 3$ genotype (means: 371 and 552 ng/g of tissue, respectively; $p = 0.16$). However, there was a significant difference in A$\beta n-42$ between the control groups with at least one ApoE $\epsilon 4$ gene (mean: 473 ng/g of tissue) and the AD group with the ApoE $\epsilon 4/\epsilon 4$ genotype (mean: 1719 ng/g of tissue; $p = 0.013$).

The amounts of A$\beta n-40$ and A$\beta n-42$ in the white matter were also examined with respect to Braak stage score. For A$\beta n-40$, the mean values for the Braak I and II and Braak VI groups were 87 and 1175 ng/g, respectively. The Braak VI group had about 13.4 times more A$\beta n-40$ in the WM than did the Braak I and II group ($p = 0.05$). For A$\beta n-42$, however, the mean values for the Braak I and II and Braak VI groups were 415 and 1178 ng/g, respectively. Thus, the Braak VI group had about 2.8 times more white matter A$\beta n-42$ than did the Braak I and II group ($p = 0.03$).

White Matter Cholesterol Is Decreased in AD. The AD subjects had a mean cholesterol value of 6.00 mg/g of GM tissue while the control individuals had a mean value of 5.84 mg/g of GM tissue, and the difference in means was not statistically significant. In contrast, the WM cholesterol levels, due to the high myelin content of this tissue, were some 5.5 times greater than the GM cholesterol level in the control subjects. Alzheimer's disease subjects had a WM cholesterol mean value of 28.6 mg/g of tissue while the control subjects had a mean value of 32.4 mg/g of tissue. Thus, the WM cholesterol of the AD subjects was decreased about 12% compared to that of the controls (Figure 4A), the difference being statistically significant ($p = 0.0003$). Furthermore, within the AD group, the magnitude of the decrease was gender specific with female AD subjects having a 12% lower WM cholesterol level than did their male counterparts ($p = 0.006$). When the ApoE genotype was considered for AD and control groups, the WM cholesterol was about 20% greater in the control ApoE $\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$ subjects compared to AD subjects with the same genotypes ($p = 0.006$).

White matter cholesterol was examined as a function of age. As can be seen in Figure 4B, if the ApoE genotype is disregarded, a continuous decline in WM cholesterol occurred with increasing age in the control cohort ($r = 0.54$). For the AD subjects, the WM cholesterol correlated with

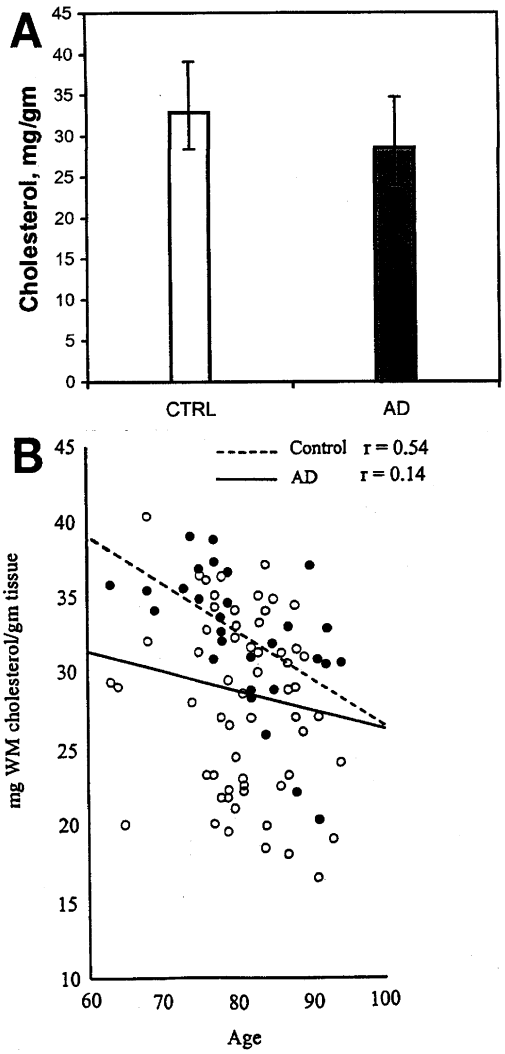

FIGURE 4: Quantification of cholesterol and fatty acid methyl esters in WM. (A) The difference between the average cholesterol content observed in the control ($n = 67$) and AD ($n = 36$) groups in the WM was 3.8 mg, which was statistically significant ($p = 0.0003$). (B) A noticeable continuous decline in cholesterol with age was occurring in the control group. The decline was less pronounced in the AD group, but the averages tend to merge at an advanced age.

age although this correlation was not nearly as robust as for the control group ($r = 0.14$). The correlation lines in Figure 4B revealed that, at any age, the expected WM cholesterol levels would always be lower for the AD subjects. Nonetheless, the WM cholesterol averages for the AD and control groups appeared to be merging with advancing age.

White Matter Fatty Acid Ratios Are Altered in AD. Table 3 shows the FA composition of a crude lipid fraction from the WM of AD and control subjects. The total amount of FAME for the AD group (64 mg/g of WM) was found to be significantly greater than that of the control group (50 mg/g of WM; $p = 0.001$). These absolute values are in reasonable agreement with previous observations (*42*) for total WM phospholipid content of brain from aged subjects (60 mg/g of WM). However, there is considerable disagreement in the literature over the phospholipid content of WM in AD and control subjects. In one study, the control subjects had 10–20% greater amounts of WM lipids of all classes compared to AD subjects (*8*), while in a second study (*43*) no

Table 3: Fatty Acid Composition of the White Matter

FAME	control (mg/g WM)	AD (mg/g WM)
C16:0	6.64 ± 1.83	8.33 ± 2.57
C16:1n7	0.22 ± 0.08	0.26 ± 0.11
C16:1n9	0.32 ± 0.12[a]	0.43 ± 0.14
C17:0	1.33 ± 0.66	1.43 ± 0.64
C18:0	9.45 ± 2.48	12.23 ± 4.58
C18:1n9c	12.57 ± 3.93	16.50 ± 7.30
C18:1n9t	2.15 ± 0.71	2.78 ± 1.13
C18:2n6c	0.21 ± 0.12	0.28 ± 0.36
C20:0	0.05 ± 0.13	0.06 ± 0.11
C20:1	1.68 ± 0.46	1.95 ± 1.40
C18:3n3	0.64 ± 0.20	0.95 ± 0.53
C20:2n9	0.43 ± 0.23[a]	0.67 ± 0.33
C20:2n6	0.60 ± 0.35[a]	1.08 ± 0.64
C20:3n6	0.74 ± 0.51	0.85 ± 0.58
C20:4n6	2.20 ± 0.65[a]	3.10 ± 1.01
C22:0	0.29 ± 0.18	0.41 ± 0.15
C22:4n6	6.28 ± 1.99	7.72 ± 2.53
C23:0	0.48 ± 0.24	0.67 ± 0.35
C22:6n3	1.59 ± 0.60	2.20 ± 0.88
C24:0	1.86 ± 0.61	2.23 ± 0.65
sum	49.71 ± 5.64	64.12 ± 9.72

[a] AD group significantly different from control group ($p < 0.05$).

differences were found between the two groups when absolute amounts of lipids were measured relative to DNA.

The compositions of the lipid fractions in terms of their individual components show that the most abundant component is oleic acid (C18:1n9c, 25%; C18:1n9t, 4%), followed by stearic acid (C18:0, 19%), palmitic acid (C16:0, 13%), adrenic acid (C22:4n6, 12%), and arachidonic acid (C20:4n6, 5%). Similar percentages of palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), arachidonic (C20:4n6), and adrenic (C22:4n6) acids had previously been observed in crude WM extracts (*44*). When AD subjects were compared to controls, there were in the former significantly higher percentages of palmitoleic acid and significantly lower percentages of adrenic acid (*44*). Other studies have found significantly higher fractions of palmitic, stearic, or oleic acids and lower percentages of arachidonic, adrenic, or docosahexaenoic acids (C22:6n3) in purified WM lipid preparations (*42*, *43*). It is possible that the discrepancies which exist between our results and those of previously published work arise from the different methodologies used in preparation of samples or from real individual variations. We found that all of the individual FA components on a milligram per gram of WM tissue basis were greater for the AD compared to control subjects (Table 3). These differences were significant for palmitoleic acid (C16:1), eicosadienoic acid (C20:2n6 and C20:2n9), and archidonic acid (C20:4n6) ($p < 0.05$).

White Matter Myelin and Oligodendrocyte Protein Levels Are Diminished in AD. (A) Myelin Basic Protein. The major protein isoforms of MBP were separated by Western blots (Figure 5A). When the WM average levels of MBP were compared between AD and control groups, there was a decrease of about 16% in the demented group ($p = 0.035$). Separation of the WM MBP values by the ApoE genotype revealed 14% and 17% decreases in the AD groups carrying no $\epsilon4$ or one or two $\epsilon4$ alleles ($p = 0.17$ and $p = 0.12$), respectively. Interestingly, at the same level of total protein load MBP was not detectable in the gray matter.

(B) Myelin Proteolipid Protein. A Western blot of PLP is shown in Figure 5B. Overall, in the WM there was a 10%

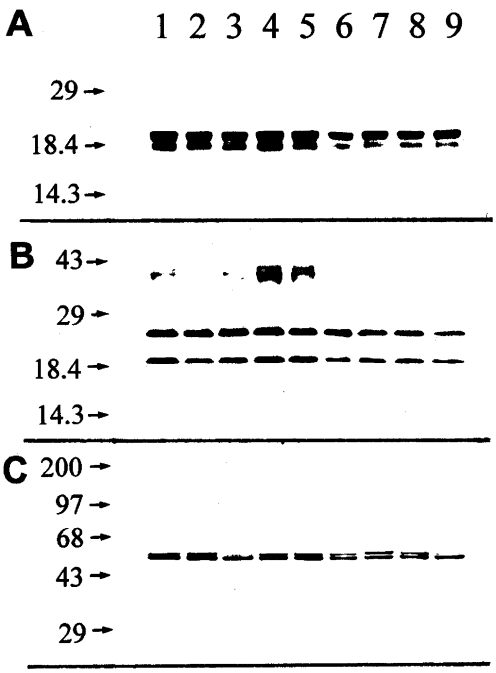

FIGURE 5: Representative Western blots of myelin proteins. Lanes 1−5 and 6−9 contain control nondemented and AD WM homogenates, respectively. (A) Myelin basic protein was separated into two bands that corresponded to the two major isoforms observed in human adults: 18.8 and 17.2 kDa (*81*). Although both isoforms are decreased in the AD cohort, the lower molecular mass band is apparently more deficient than the high molecular mass form. (B) Proteolipid proteins (PLP and DM-20). On SDS−PAGE the 25 kDa PLP is the predominant band relative to the 20 kDa DM-20 isoform which when compared to the PLP has a deletion of 35 amino acids (*82*). Both bands are decreased in AD and apparently lack the dimeric forms observed in control individuals. (C) 2′,3′-Cyclic nucleotide 3′-phosphodiesterase. On SDS−PAGE this enzyme is represented by two very close isoforms, CNP1 ∼46 kDa and CNP2 ∼48 kDa (*83*), that are deficient in the AD WM relative to the controls.

decrease in MPP in the AD cases when compared to the nondemented group ($p = 0.018$). The AD individuals homozygous for ApoE $\epsilon3$ had 15% less MPP than the control group with the same genotype ($p = 0.04$). The AD $\epsilon4$-carrying individuals had a modest 4% decrease when compared with the control group with the same genotype ($p = 0.32$). The mean MPP values for the Braak I and II and Braak VI groups were 0.99 and 0.88, respectively, resulting in a decrease of about 10.5% in the WM MPP level ($p = 0.03$). In contrast, in the GM the MPP was increased by 9% in AD versus the control values, although the bands were not as intense as those observed for the WM. The GM MPP differences between AD and control cohorts were not statistically significant ($p = 0.42$).

(C) 2′,3′-Cyclic Nucleotide 3′-Phosphodiesterase. Western blots of CNP (Figure 5C) revealed that, on the average, this specific myelin protein was 17% less abundant in the WM of the AD individuals when compared to the WM of the nondemented cohort ($p = 0.017$). When the ApoE genotype is considered, those individuals with $\epsilon3/\epsilon3$ and AD had 20% less CNP than the control counterpart ($p = 0.10$). Moreover, AD subjects carrying ApoE $\epsilon3/\epsilon4$ and $\epsilon4/\epsilon4$ genotypes also demonstrated a 16% CNP decrease ($p = 0.07$). No significant differences in CNP were found when the GM of AD and control individuals were compared ($p = 0.30$). Interestingly, the mean CNP values for the Braak I and II and Braak VI

groups were 0.96 and 0.80, respectively. The Braak VI group had approximately 17% lower WM CNP level than did the Braak I and II group, with the difference being statistically significant ($p = 0.05$).

DISCUSSION

Oligodendrocyte myelin sheath integrity is indispensable for axon viability and for the maintenance of axonal flow (*45*). Intact myelin sheaths are essential for axonal insulation and critical for the rapid transmission of the saltatory action potentials between the nodes of Ranvier. A defective or breached myelin sheath will result in loss of capacitance and impede the spread of the action potential and progression of nerve conduction, thereby gravely disrupting interneuronal communication and severely damaging brain function. Brain imaging and morphometric technologies such as MRI and computed stereology have demonstrated that in normal aging there is a proportionately greater loss of WM relative to GM (*46*−*49*), and these differences are even more pronounced in AD patients. In this dementia there is a remarkable WM atrophy accompanied by an enlargement of the lateral ventricles (*50*). These morphological alterations may be due to decreases in the diameter and number of axons and to the combined effects of reduced myelin synthesis and degenerative damage. The loss of GM protein, as expected, was substantial and was consistent with Braak staging, a measure of neuronal breakdown, i.e., neurofibrillary pathology. However, the Braak stage also correlated with WM protein level declines, suggesting that axonal, oligodendrocyte, and myelin injury occur in parallel with GM damage.

In general, the WM tissue of AD cases was soft, friable, and easily deformable under pressure. In contrast, for the WM from the nondemented control cases, the tissue was firmer and denser and, when pressed, had a relatively resilient consistency. To our knowledge, there are no published electron microscopic studies describing the morphological conditions of myelin in the AD brain WM. However, ultrastructural observations in aged nonhuman primates with memory deficits revealed myelin breakdown without axonal degeneration. These observations suggest that cognitive decline in monkeys may be due to pathologic changes in myelinated axon biochemistry rather than an age-related loss of cortical neurons (*51*, *52*). Preliminary ultrastructural examination has revealed degenerative changes in myelin packing such as separation of lamellae, reduced number of axons, and astrogliosis in AD WM tissue. Some AD-related ultrastructural pathological changes have been described in WM astrocytes which showed extensive swelling and vacuolation with beading and disintegration of their cytoplasmic processes (*53*). These alterations could be the result of incorporated edema fluid and cellular debris. In AD there are also signs of WM inflammation as revealed by the presence of immunoglobulins, fibrinogen, and complement proteins (*53*). The blockage of the periarterial spaces in GM parenchymal and leptomeningeal vessels by vascular amyloid, commonly observed in AD (*54*) but also noted in some aged nondemented individuals, may be responsible for the interstitial fluid stagnation observed in the dilated WM perivascular spaces. While some deep WM arteries are direct tributaries of the perforating arteries derived directly from the circle of Willis (*55*), most of the arteries that perfuse the WM originate from a subset of the leptomeningeal penetrator arteries, which continue into the WM as long medullary arteries. White matter rarefaction is observed in two-thirds of the AD brains and is a typical feature of the ischemic vascular dementias (*56*). The pallor of this WM ischemic pathology, evident under the majority of histological stains and observed by radiological techniques, suggests a loss of the formed and densely packed elements common to WM, i.e., oligodendrocytes and myelin which have been replaced with interstitial water and astrocytes. The levels of Aβ peptides in the AD patient WM were significantly elevated relative to those detected in nondemented individuals. This is the first time that Aβ peptides have been quantified in AD patient WM. On the average, A$\beta n-40$ and A$\beta n-42$ peptides were 9.6 and 2.7 times, respectively, more abundant in AD than in the control cases. The total Aβ ($n-40$ plus $n-42$) was on the average 4.2 times increased in AD compared to the nondemented group. As was the case for the GM, the quantities of A$\beta n-42$ peptides were higher in those individuals with a ApoE $\epsilon 4/\epsilon 4$ genotype. The relatively higher levels of A$\beta n-40$ in the WM may be due to this peptide's solubility and ability to diffuse as compared to A$\beta n-42$. Preliminary experiments have revealed that an Aβ peptide concentration gradient exists from the very elevated values characteristic of the GM, encompassing both the soluble Aβ and the insoluble amyloid deposits, to the deepest parts of the centrum semiovale and periventricular areas which contain the lowest Aβ peptide levels. These observations suggest that the Aβ source is in the GM, probably originating from the neuronal bodies, and that a form of soluble Aβ undergoes anterograde diffusion along the axons (*57*). Moreover, the expression of the amyloid-β-precursor protein in oligodendrocytes suggests that a portion of the Aβ peptides could also originate in the WM itself. Interestingly, amyloid plaques are localized in the WM in areas immediately subjacent to the GM which in serial sections appear to be distributed along the path of single blood vessels (*58*). Despite the presence of substantial Aβ levels, in the low microgram per gram of tissue range, there are no microscopically visible Aβ deposits in the deep WM parenchyma or vascular walls. It is possible that a form of soluble oligomeric Aβ is free in the interstitial fluid or alternatively bound to cellular membranes, i.e., myelin, or both. It is well established that Aβ peptides interact avidly with and destabilize membranes (*59*−*61*) and that they readily associate with the GM_1 ganglioside (*62*, *63*), a relatively abundant myelin glycolipid. A loss of WM gangliosides has been reported in early onset AD, suggesting a decreased axodendritic arborization (*8*). The extent and potential pathological interactions of Aβ with the myelin molecules and with oligodendrocytes are being actively investigated.

The chemical composition of WM myelin lipids and proteins appears to be profoundly altered in AD patients compared to age-matched nondemented individuals. In AD, there is a significant decrease in the amount of WM cholesterol (12%). In a previous smaller scale study, WM cholesterol was found to be reduced by 13.5% in AD brains (*8*). In addition, our study revealed that, in the AD subjects, the females had a significantly lower WM cholesterol level than did the males ($p = 0.006$). However, examination of the AD group GM revealed that there was no gender-linked difference in cholesterol level ($p = 0.12$). Previous studies

(*64*) have shown that post-menopausal females have inherently higher serum cholesterol levels than do similarly aged males and that these higher levels have recently been linked to associated memory deficiencies (*65*). Therefore, the total body of evidence indicates that AD females have elevated serum cholesterol levels accompanied by a relative deficiency in WM cholesterol.

The low WM cholesterol levels may be the result of (1) a deficiency in WM cholesterol synthesis or (2) cholesterol depletion as it is shifted to GM neuronal membrane synthesis. No associations were evident between WM cholesterol content and ApoE genotype, but it has been found that the severity of the WM lesions in AD are correlated with female gender (*66*). Cholesterol is the principal lipid component of myelin membranes, where it represents up to 40% of the myelin lipids, and regulates the thickness, fluidity, and permeability of membranes (*67*−*69*). Furthermore, it has been recently found that neuronal membranes enriched in cholesterol promote, through hydrophobic interactions, Aβ accumulation (*70*) and that this association results in a decrease in the overall fluidity of membranes (*71*). A reduction in membrane cholesterol levels significantly alters the content of intrinsic and extrinsic membrane proteins (*72*). It is possible that Aβ peptide deposits are not visible at the microscopic level in the WM due to dispersion within the cholesterol-rich membranes (*73*), which prevent Aβ fibrillogenesis. In addition, the chemical interactions between Aβ and cholesterol or other WM molecules may hinder antigenic determinants.

The total amount and composition of fatty acids also deviated in AD subjects from that observed in the WM of nondemented individuals. In the WM of AD individuals there was an elevation in the average values of most of the fatty acids that may represent a compensatory effect due to a reduced amount of cholesterol in AD subjects. Therefore, per unit volume, the myelin of AD patients contains a larger ratio of phospholipids and glycolipids relative to cholesterol, suggesting an imbalance in the synthesis or incorporation of myelin lipids. Alternatively, the relative increase in fatty acid content in the WM of AD individuals may result from ceramide accumulation due to the activation of the sphingomyelinase in oligodendrocytes induced by enhanced Aβ peptide levels (*74*). Interestingly, in multiple sclerosis, a demyelinating disease, the myelin fatty acid content is altered in the WM with "normal appearance" having more C16 and less C18, C22, and C24 species than in normal non-MS individuals (*75*). The overall effect of the depletion of cholesterol and elevation of fatty acids in myelin may be to cause defective nerve insulation, electrical capacitance, and nerve transmission, which could be important events in the pathogenesis of AD.

In addition to the lipid alterations, there was a significant loss of proteins MBP and PLP and its isoform D20. These structural molecules, representing up to 80% of the myelin proteins, play a pivotal role in maintaining the tight architecture of the compact myelin sheaths. The oligodendrocytic marker CNP was also significantly depleted in the WM of AD subjects. This molecule, representing 4% of the total myelin proteins, is localized in the noncompacted oligodendroglial axon sheaths and in the myelin paranodal loops (*76*, *77*). Although the function of this molecule is unknown, it may play a key catalytic and structural role since it binds up to three molecules of GTP and, when overexpressed, produces aberrant myelination (*78*). Overall, there was a statistically significant decrease in myelin structural proteins in AD WM, which represented an amount 15% less than that measured in the nondemented group.

Several reasons can be advanced to explain the physical atrophy and biochemical alterations observed in the WM of AD patients. (1) White matter changes may be secondary to primary GM changes. "Secondary WM degeneration" is a common concept in neuropathology (e.g., some metabolic congenital encephalopathies) and is based on the fact that WM axons originate from neuronal cell bodies in the GM. In the event the neuronal cell bodies die, the axons also degenerate, and the myelin sheaths will degenerate in response to loss of their axons. Neuronal cell body pathology short of cell death can also cause WM degeneration, as a dysfunctional neuronal cell body may not be able to adequately maintain a long axon. This results in "dying back" of the axon, another common concept in neuropathology (e.g., some vitamin deficiency disorders). This "secondary degeneration" hypothesis of WM degeneration in AD has been specifically tested in only two studies. In one (*15*), histologically apparent cerebral WM rarefaction did not correlate with the density of plaques and tangles in the cortical GM. In the other, WM atrophy, as measured on postmortem brain slices, did not correlate with cortical GM atrophy (*18*). Therefore, neither study supports the secondary degeneration hypothesis. However, our own data from two separate studies, using much larger numbers of cases, show a significant correlation between Braak stage and WM total protein concentration ($N = 25$, $p = 0.01$, $r = 0.62$) and a nearly significant correlation between histologically apparent WM rarefaction and a semiquantitative tangle score ($N = 71$, $p = 0.09$, $r = 0.20$) revealing a significant, but relatively weak, correlation between measures of GM degeneration and measures of WM degeneration in AD. This suggests that secondary degeneration probably contributes to, but does not fully account for, the full magnitude of WM loss seen in AD. (2) The atrophy of the WM may be directly due to a primary disease of the oligodendrocytes resulting in deficient myelin synthesis altogether or in specific defective synthesis of myelin carbohydrates, fatty acids, or proteins. In both circumstances demyelination or dysmyelination would result in axonal injury. Limited information is available regarding the toxic effects of Aβ on oligodendrocytes in culture (*29*) that could be specifically mediated by the noxious interaction of Aβ with the synthesis of myelin (*74*). (3) A defective perfusion of WM has been postulated as the possible cause of WM atrophy in AD. In a large number of AD cases the WM vessels suffer arteriosclerosis and atheromatosis and, not infrequently, multiple micro-embolic episodes leading to lacunar infarcts (*79*) causing ischemia/hypoxia of glial cells and axons, with defective maintenance of the myelin sheaths resulting in altered axonal metabolism and conduction. (4) A deficiency in the return of the interstitial fluid of the WM in AD patients could be caused by the partial or total blockage of perivascular spaces by amyloid deposits localized at the level of the leptomeningeal and cortical perforating arteries (*80*). In humans the interstitial fluid is efficiently removed by the periarterial spaces that drain into the deep lymphatics of the neck and venous circulation (*54*). Periarterial amyloid obstruction, which reaches its maximum

expression in ApoE $\epsilon4/\epsilon4$ AD patients, would cause interstitial fluid stasis−edema and dilation of the perivascular spaces around the deep penetrating arteries of the WM. While this lesion, known as *état criblé* in neuropathology, is commonly observed in normal aging, it occurs to a far greater degree in AD.

Our experiments have revealed that WM tissue is severely affected in AD. In addition to detection of elevated amounts of Aβ40 and Aβ42 peptides in WM, it is clear that fundamental structural aspects of glial cells and myelin are significantly altered in AD patients. These alterations undoubtedly contribute to AD pathology and may represent the combined effects of neuronal degeneration, microgliosis, oligodendrocyte injury, microcirculatory disease, interstitial fluid stasis, and edema. While the most evident clinical manifestations of AD obviously involve activities linked to the cerebral cortex, the idea that all biochemical alterations associated with this dementia are primary and only confined to the GM may be simplistic. To accurately assess the success of future mitigation agents and protocols, it is necessary to have the fullest possible appreciation of the true scope of Alzheimer's disease pathology.

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