K. Hagiwara, MD
H. Ochi, MD
S. Suzuki, MD
Y. Shimizu, MD
T. Tokuda, MD
H. Murai, MD
H. Shigeto, MD
Y. Ohyagi, MD
M. Iwata, MD
T. Iwaki, MD
J.-i. Kira, MD

HIGHLY SELECTIVE LEPTOMENINGEAL AMYLOIDOSIS WITH TRANSTHYRETIN VARIANT ALA25THR

Leptomeningeal amyloidosis is a rare phenotype of hereditary transthyretin (TTR) amyloidosis, characterized by preferential amyloid deposition in the pia-arachnoid and leptomeningeal vessels. Although several mutations in TTR genes have been reported to be associated with this phenotype,¹ they also exhibit considerable variations in systemic amyloid deposition. Here, we describe a patient with the TTR variant Ala25Thr, the purest form of leptomeningeal amyloidosis.

Case report. The patient was a 52-year-old Japanese man without familial history of amyloidosis who had been diagnosed with leptomeningeal amyloidosis with TTR variant Ala25Thr.^{2,3} His initial presentation at age 48 years was chronic progressive polyradiculoneuropathy resulting in severe sensory ataxia. Other neurologic signs included bilateral sensorineural hearing loss and cerebellar ataxia. Autonomic dysfunction was not identified and there was no visceral organ involvement. At age 52 years, he was admitted because of a new-onset seizure. Gadolinium-enhanced T1-weighted brain MRI showed striking leptomeningeal enhancement (figure, A). T2*-weighted images revealed hypointensity along the brain surface, especially in the frontal lobe bases and the upper folia of the cerebellar hemispheres (figure, B). The CSF protein level was 1,099 mg/dL with xanthochromia. He subsequently died of multiple intracranial hemorrhages with local extension into the subarachnoid space (figure, C).

At autopsy, macroscopic examination showed diffusely thickened rusty-brown leptomeninges, especially around the brainstem, cerebellum, and spinal cord (figure e-1, A and B, on the *Neurology*[®] Web site at www.neurology.org). There were multiple lobar intracerebral hemorrhages in the bilateral frontal lobes (figure e-1C), right parietal lobe, and left occipital lobe. These hemorrhages were located in the superficial lobar areas. Microscopic examination of the brain revealed dense hyaline materials in the pia-arachnoid and leptomeningeal vessels. They were strongly stained with Dylon (figure, D) and were positive for the anti-TTR antibody (figure, E). The adventitia and media of the leptomeningeal vessels

were preferentially involved by the amyloid deposits (figure, F). The external elastic lamina was severely disrupted in some vessels bearing a significant amount of amyloid deposits (figure, G). Significant hemosiderin deposition was observed in the pia-arachnoid and subpial layers (figure, H), consistent with superficial siderosis. There were fewer amyloid deposits in the subpia, and some subependymal deposits were observed in the lateral ventricles. However, there was virtually no amyloid deposition in the deep parenchyma (figure, I). Bilateral acoustic nerves were entrapped by the amyloid-laden leptomeninges at their root exit zone (figure, J).

The entire spinal cord was compressed by the thickened leptomeninges, in which massive amyloid deposits and reactive connective tissue formation were observed (figure, K). The proximal portions of the spinal nerve roots were severely entrapped by the thickened leptomeninges (figure, L). There were varying degrees of demyelination and axonal degeneration depending on the nerve fascicles. No amyloid deposits were identified within the spinal cord, nerve roots, dorsal root ganglia, or peripheral nerves. There were also no amyloid deposits in the visceral organs except for minimal deposition in some small vessels.

Discussion. Several mutations in TTR genes are known to cause leptomeningeal amyloidosis with predominant CNS manifestations; however, pathologic studies have shown varying magnitude of amyloid deposition in the peripheral nerves and visceral organs. In contrast, the present case with TTR Ala25Thr was pathologically determined as the purest form of leptomeningeal amyloidosis reported to date, because virtually no amyloid deposition was identified outside the CNS.

TTR is a homotetrameric protein and is a thyroxine (T₄) transporter. TTR amyloid fibril formation requires dissociation of the unstable tetramer followed by tertiary structural changes that enable self-assembly of the monomer. TTR variants of the leptomeningeal phenotype are reported to have faster dissociation rates of the tetramer compared with other TTR variants.^{3,4} These variants are not efficiently secreted into the blood by hepatic cells, but

Supplemental data at
www.neurology.org

Figure Neuroradiologic and histopathologic findings

(A) In an axial T1-weighted image after contrast administration, leptomeningeal enhancement is seen at the surface of the Sylvian fissures, longitudinal fissure, midbrain, and cerebellum. (B) The axial T2*-weighted image shows low signal areas in the bilateral frontal lobe bases, Sylvian fissures, brainstem, and the upper folia of the cerebellar hemispheres. (C) The CT scan shows a left frontal lobe hemorrhage with a superficial location and extension into the subarachnoid space. (D) Amyloid deposition in the pia-arachnoid and leptomeningeal vessels. Some blood vessels in the subpial region are also amyloid-positive. Dylon stain (bar = 100 μ m). (E) The amyloid fibrils are positively immunolabeled with the anti-TTR antibody (bar = 100 μ m). (F) In the leptomeningeal vessels, the adventitia and outer media are preferentially infiltrated by the amyloid deposition. Dylon stain (bar = 50 μ m). (G) The leptomeningeal vessels with high amyloid deposition have severely disrupted elastic laminae. Elastica-van Gieson stain (bar = 20 μ m). (H) Dense hemosiderin deposits are present in the pia-arachnoid. Berlin-blue stain (bar = 50 μ m). (I) No amyloid deposition is observed in the blood vessels penetrating the brain parenchyma (arrow). Dylon stain (bar = 50 μ m). (J) Intense amyloid deposition is seen infiltrating the root exit zone of the acoustic nerve. Dylon stain (bar = 100 μ m). (K) Semi-macroscopic image of the thoracic cord shows intense thickening of the pia-arachnoid with the amyloid deposition and reactive connective tissue formation severely compressing the spinal cord. Dylon stain (bar = 1 mm). (L) The spinal nerve roots (arrows) are diffusely entrapped by the extensive amyloid deposition and reactive connective tissue formation. Dylon stain (bar = 500 μ m).

choroid plexus cells could increase their secretion into the CSF by a T₄-chaperoning mechanism.^{3,4}

Because the Ala25Thr variant was reported to have the second fastest rate of tetramer dissociation of the known disease-associated TTR vari-

ants,^{3,4} it is possible that this marked kinetic alteration leads to the characteristic leptomeningeal phenotype. Consistent with this hypothesis, the Asp18Gly variant, which has the fastest dissociation rate and cannot tetramerize, presents with

a similar leptomeningeal phenotype.⁴⁻⁶ However, in contrast with the present case, asymptomatic amyloid deposits were found outside the CNS in a family of Hungarian descent with the Asp18Gly variant in their fifth decade.⁶ Thus other pathomechanisms may be operative in the predisposition toward organ-selective amyloid deposition.

The Ala25Thr variant presents as highly selective leptomeningeal amyloidosis. The case presented here may help elucidate the pathogenesis of the wide clinical spectrum of TTR amyloidosis.

From the Departments of Neurology (K.H., H.O., H.M., H.S., Y.O., J.-i.K.) and Neuropathology (S.S., T.I.), Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka; Department of Neurology (Y.S., M.I.), Neurological Institute, Tokyo Women's Medical University School of Medicine; and Department of Neurology (T.T.), Kyoto Prefectural University of Medicine, Japan.

Disclosure: The authors report no disclosures.

Received September 24, 2008. Accepted in final form December 16, 2008.

Address correspondence and reprint requests to Dr. H. Ochi, Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; ochi@neuro.med.kyushu-u.ac.jp

Copyright © 2009 by AAN Enterprises, Inc.

1. Connors LH, Lim A, Prokaveva T, Roskens VA, Costello CE. Tabulation of human transthyretin (TTR) variants, 2003. *Amyloid* 2003;10:160–184.
2. Shimizu Y, Takeuchi M, Matsumura M, Tokuda T, Iwata M. A case of biopsy-proven leptomeningeal amyloidosis and intravenous Ig-responsive polyneuropathy associated with the Ala25Thr transthyretin gene mutation. *Amyloid* 2006;13:37–41.
3. Sekijima Y, Hammarström P, Matsumura M, et al. Energetic characteristics of the new transthyretin variant A25T may explain its atypical central nervous system pathology. *Lab Invest* 2003;83:409–417.
4. Sekijima Y, Wiseman RL, Matteson J, et al. The biological and chemical basis for tissue-selective amyloid disease. *Cell* 2005;121:73–85.
5. Hammarström P, Sekijima Y, White JT, et al. D18G transthyretin is monomeric, aggregation prone, and not detectable in plasma and cerebrospinal fluid: a prescription for central nervous system amyloidosis?. *Biochemistry* 2003;42:6656–6663.
6. Garzuly F, Vidal R, Wisniewski T, Brittig F, Budka H. Familial meningocerebrovascular amyloidosis, Hungarian type, with mutant transthyretin (TTR Asp18Gly). *Neurology* 1996;47:1562–1567.

L.R. Snyder, MA
R. Cruz-Aguado, PhD
M. Sadilek, PhD
D. Galasko, MD
C.A. Shaw, PhD
T.J. Montine, MD, PhD

LACK OF CEREBRAL BMAA IN HUMAN CEREBRAL CORTEX

Bioaccumulation of β -methylamino-L-alanine (BMAA) through Cyanobacteria-contaminated food or water supplies has been suggested as a possible contributor to Parkinson-dementia complex (PDC) among the indigenous Chamorro of Guam as well as Alzheimer disease (AD) worldwide,¹ a speculation that has been widely commented on in news and editorial sections of premier scientific and medical journals,² and that has led to product recalls and government-sponsored assessments of public health in Europe. However, quantification of BMAA to date has been based on indirect high performance liquid chromatography methods. Given the potential global health significance, we developed a stable-isotope dilution assay for BMAA to more rigorously test its proposed bioaccumulation in cerebral cortex.

Methods. All studies were conducted following appropriate informed consent and approval by institutional review boards. We synthesized D₃-BMAA by a published method and confirmed product as $\geq 98\%$ pure by ¹H and ¹³C NMR.³ Samples for gas chromatography–mass spectrometry were prepared with ethyl chloroformate; fragmentation patterns for both D₃-BMAA and H₃-BMAA were analyzed.⁴ Selected ion monitoring (SIM) focused on m/z 116 and 245 for H₃-BMAA, and m/z 119 and 248 for D₃-BMAA: m/z 116 and 119 for quantification, m/z 245 and 248

for qualifying ions. Frozen human cerebral cortex was analyzed in triplicate following established homogenization protocols.⁵ H₃-BMAA spiked into cerebral cortex homogenates had limit of detection for free H₃-BMAA = 0.10 $\mu\text{g/g}$ tissue, limit of quantification = 0.25 $\mu\text{g/g}$ tissue, and $r^2 = 1.00$ for 0.25 to 10.0 $\mu\text{g/g}$ tissue using six concentrations. D₃-BMAA and H₃-BMAA were stable to acid hydrolysis conditions; under these conditions to measure protein-associated H₃-BMAA our limit of detection = 5 $\mu\text{g/g}$ tissue, limit of quantification = 10 $\mu\text{g/g}$ tissue, and $r^2 = 1$ for 10 to 1,000 $\mu\text{g/g}$ tissue using seven concentrations.

Frozen tissue from autopsies was obtained following appropriate informed consent as part of NIH-sponsored research programs. Samples were from Chamorro PDC patients on Guam ($n = 8$, 5 women, 64 to 89 years) who underwent a detailed clinical evaluation and were confirmed by neuropathologic examination; Chamorros on Guam who were free of neurologic symptoms during life and lacked neurofibrillary changes in brain ($n = 2$ women, 61 and 93 years); patients from the Seattle region who were diagnosed with definite AD ($n = 5$, 3 women, 74 to 83 years); and individuals from the Seattle region who were seen by a physician and had neuropsychological testing within the last 2 years of life with all results within normal ranges and who showed only age-related changes by neuropathologic examination ($n = 5$, 3 women, 82 to 91 years). All

Supplemental data at
www.neurology.org

Selective ion monitoring chromatograms from stable isotope dilution assay for BMAA. Cerebral cortex from an individual with PDC shows that H_3 -BMAA (m/z 116 and 245) was not detected, whereas its deuterated standard (m/z 119 and 248) was detected.

Seattle area residents in this study were Caucasian. Specific clinical and neuropathologic criteria for these cases have been published previously.⁶ On Guam, tissue from one half of the brain was fixed in formalin for histologic examination, while tissue from the other half was frozen and stored at -80°C ; the postmortem interval ranged from 10 to 40 hours. In Seattle, all tissue was obtained by the Neuropathology Core of the AD Research Center at the University of Washington. Tissue from one half of the brain was dissected at the time of autopsy, flash frozen in liquid nitrogen, and then stored at -80°C . Tissue from the other half was fixed in formalin and used for histopathologic evaluation. The postmortem interval ranged from 3.5 to 9.0 hours.

Results and discussion. No free or protein-associated H_3 -BMAA was detected in any autopsy sample of frontal or temporal cortex, despite our ability to detect other hydrolyzed amino acids (figure). Flash-frozen liver from mice fed BMAA was used as a positive control (figure e-1 on the *Neurology*[®] Web

site at www.neurology.org). While these data cannot address the possibility of earlier exposure in life and clearance of BMAA from tissue, they do seriously challenge the proposal that BMAA, a nonproteinogenic amino acid, accumulates as a free form or is stored in a protein-associated form in human cerebral cortex. Although products of Cyanobacteria may have other health effects, our new data support the considered review of others which concluded that cycad products are unlikely contributors to PDC.⁷ Moreover, our findings do not support a global health concern for BMAA contributing to AD.

From the Departments of Chemistry (L.R.S., M.S.) and Pathology (T.J.M.), University of Washington, Seattle; Department of Ophthalmology and Visual Sciences (R.C.-A., C.S.), University of British Columbia, Vancouver, Canada; and Department of Neurosciences (D.G.), University of California at San Diego.

Supported by the NIH (AG14382 and AG05136) and the Nancy and Buster Alvord Endowment.

Disclosure: The authors report no disclosures. Christopher A. Shaw is the founder and chief operating officer of Neurodyn, a Canadian-based company examining early phase neurological disease.

Received October 21, 2008. Accepted in final form December 16, 2008.

Address correspondence and reprint requests to Laura R. Snyder, Department of Chemistry, Box 351700, University of Washington, Seattle, WA 98195-1700; lrsnyder@u.washington.edu

Copyright © 2009 by AAN Enterprises, Inc.

ACKNOWLEDGMENT

The authors thank Professor Gelb, Mr. Kruse, and the Mass Spectrometry Facility in the Department of Chemistry at the University of Washington.

1. Cox PA, Banack SA, Murch SJ. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. *Proc Natl Acad Sci USA* 2003;100:13380–13383.
2. Miller G. Neurodegenerative disease: Guam's deadly stalker: on the loose worldwide? *Science* 2006;313:428–431.
3. Hu YL, Ziffer H. Synthesis and optical resolution of the neurotoxin 2-amino-3-([N-15]-methylamino)propanoic acid (BMAA). *J Labelled Compounds Radiopharmaceuticals* 1990;28:581–586.
4. Guo T, Geis S, Hedman C, Arndt M, Krick W, Sonzogni W. Characterization of ethyl chloroformate derivative of beta-methylamino-L-alanine. *J Am Soc Mass Spectrom* 2007;18:817–825.
5. Banack SA, Murch SJ, Cox PA. Neurotoxic flying foxes as dietary items for the Chamorro people, Marianas Islands. *J Ethnopharmacol* 2006;106:97–104.
6. Montine TJ, Li K, Perl DP, Galasko D. Lack of beta-methylamino-L-alanine in brain from controls, AD, or Chamorros with PDC. *Neurology* 2005;65:768–769.
7. Steele JC, McGeer PL. The ALS/PDC syndrome of Guam and the cycad hypothesis. *Neurology* 2008;70:1984–1990.

S. Debiais, MD
I. Bonnaud, MD
N. Ferreira-Maldent, MD
E. Mercier, MD
B. de Toffol, MD, PhD

NEUROPSYCHIATRIC DISORDERS REVEALING PRIMARY ANTIPHOSPHOLIPID SYNDROME IN AN ELDERLY PATIENT

Antiphospholipid syndrome (APS) is defined by the presence of clinical signs of recurrent venous or arterial thrombosis, associated or not with spontaneous miscarriage, and biologic abnormalities, particularly the persistence of antiphospholipid antibodies (aPL) such as lupus anticoagulant (LA) or anticardiolipin antibodies (aCL).^{1,2} It usually occurs in young patients before the fifth decade.³ APS can be isolated (primary APS [PAPS]) or associated with an underlying connective tissue disease, most often systemic lupus erythematosus (SLE).^{1,2} APS may present with neurologic syndromes.⁴ Although only stroke is accepted as a diagnostic criterion for APS,² other neurologic symptoms, such as chorea or dementia, have been anecdotally observed.^{4,5} We report an unusual, well-documented case of PAPS in a 72-year-old woman that presented with neuropsychiatric symptoms.

Case report. A 72-year-old woman was admitted for subacute encephalopathy. Her medical history included portal venous thrombosis associated with the presence of LA 6 years previously. She was receiving oral anticoagulants (international normalized ratio [INR] 2.5 to 3). Upon admission, the clinical examination revealed a bilateral pyramidal syndrome and confusion. She was afebrile. The Glasgow Coma Scale score was E3 V4 M4. Examination of CSF and routine blood tests were normal; 14.3.3 protein was not detected. Bacterial and viral serologic tests were negative. Cerebral CT scan and MRI with fluid-attenuated inversion recovery and diffusion-weighted sequences were normal. The EEG only showed diffuse slowing. Five days after admission, the patient presented choreiform movements in both upper extremities and disturbed behavior with logorrhea and euphoria. The neuropsychological evaluation showed a dysexecutive syndrome. There were no clinical signs suggesting SLE or any other systemic disease. Laboratory results revealed normal thyroid function, erythrocyte sedimentation rate, and complement titer, and negative rheumatoid factor and anticardiolipin and antinuclear antibodies. Venereal Disease Research Laboratory was strongly positive (false positive) as were the classic assays for LA. Immunoassay for immunoglobulin G antibodies against beta-2 glycoprotein 1 (B2GP-I) was also positive (ratio patient/threshold: 4.9; normal ratio: <1). The Coombs test was negative. There was no proteinuria. Six weeks and 3 months later, laboratory tests revealed that LA was present and plasma anti B2GP-I

was elevated. CT scan of the thorax and abdomen was normal. IV methylprednisolone was begun (500 mg per day), associated with oral anticoagulation (INR: 2 to 3). After 48 hours, the clinical signs improved dramatically and oral steroid therapy was reintroduced. A few days later, the patient was asymptomatic. One year later, when the dosage was reduced to 5 mg of prednisone/day, she presented an acute psychosis with auditory hallucinations and persecution delusion. Anti-B2GP-I antibodies were elevated and LA was detected, but no antinuclear antibody was found. IV steroid therapy (1 g per day) was administered for 3 days followed by 1 mg/kg/day of prednisone per os. The symptoms rapidly improved and the patient was asymptomatic at discharge. Four years after the last episode, the patient remained asymptomatic with 15 mg of prednisone/day and well-controlled anticoagulant therapy. Repeated diagnostic procedures failed to demonstrate any clinical or biologic abnormality suggesting SLE, cancer, or hemopathy.

Discussion. We report a case of primary APS in an elderly patient who initially presented with progressive encephalopathy, chorea, and disturbed cognition whose acute psychiatric episode later relapsed. Our patient fulfilled both the clinical and biologic criteria for APS¹ and 10 years after the first clinical manifestation (portal vein thrombosis), there was no clinical or biologic finding suggesting concomitant SLE or any other systemic disease.

APS usually affects young patients between the ages of 20 and 30 years and is rarely observed after the sixth decade.^{3,4} Furthermore, the majority of the rare cases of APS diagnosed in elderly patients were related to solid or hematologic cancers,³ ruled out in our patient. The most common neurologic manifestations of APS are stroke and cerebral venous thrombosis.¹ Psychiatric or neurocognitive symptoms can be observed in systemic autoimmune diseases, in particular in SLE, Sjögren syndrome, and primary vasculitis, but have only been reported anecdotally in isolated PAPS.¹⁻³ Choreiform movements have been associated with PAPS, but only in children or young adults.⁵ In our case, the normal MRI, including a normal diffusion-weighted MRI, strongly suggest a nonvascular mechanism underlying the symptoms. The pathophysiology of these non-thrombotic neurologic manifestations remains unclear but a direct toxic interaction between aPL and the CNS is probably involved.

The treatment of APS remains empiric although long-term oral anticoagulant therapy is usually prescribed after venous or arterial thrombosis.¹ In the absence of controlled studies, steroid therapy is not

recommended but has been used successfully in several cases involving choreiform movements.⁵

The diagnosis of PAPS should be considered in elderly patients. The neuropsychiatric symptoms we encountered may represent a corticosteroid-responsive clinical expression of PAPS.

From the Department of Neurology (S.D., I.B., B.d.T.), Department of Internal Medicine (N.F.-M.), and Intensive Care Unit (E.M.), CHRU Tours, France.

Disclosure: The authors report no disclosures.

Received August 5, 2008. Accepted in final form December 17, 2008.

Address correspondence and reprint requests to Dr. Severine Debiais, Department of Neurology, CHRU Tours, 2 Boulevard Tonnelle, 37044 Tours cedex, France; debiais@med.univ-tours.fr

Copyright © 2009 by AAN Enterprises, Inc.

ACKNOWLEDGMENT

The authors thank Dr. Donald Schwartz for help in revising the manuscript.

1. Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. *N Engl J Med* 2002;346:752–763.
2. Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum* 1999;42: 1309–1311.
3. Piette JC, Cacoub P. Antiphospholipid syndrome in the elderly: caution. *Circulation* 1998;97:2195–2196.
4. Cervera R, Piette JC, Font J, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1000 patients. *Arthritis Rheum* 2002;46:1019–1027.
5. Masala C, Morino S, Zangari P, Antonini G. Chorea in primary antiphospholipid syndrome. *Clin Neurol Neurosurg* 1996;98:247–248.

2009 AAN Annual Meeting

- Cutting-Edge Science
- Comprehensive Education
- Practice Improvement Solutions
- Excellent Value

Plus, save up to \$545 when you register

Before you register on-site, bring your CV/Resume to the Membership Booth on Level 4 of the convention center and apply for an AAN membership **first**. You'll instantly save up to \$545 off your Annual Meeting registration fees.

Register on-site at the Washington State Convention and Trade Center.

m.AAN.com: Put the AAN in the Palm of Your Hand

AAN.com, the Academy's award-winning site for neurology resources, has gone mobile. Read more about the special mobile website exclusively available for Academy members at www.aan.com/mobile.

Log on to m.aan.com and access your Academy's web resources on the go.

Visit m.aan.com—it's as close as your phone.