Ectopic White Matter Neurons, a Developmental Abnormality That May Be Caused by the PSEN1 S169L Mutation in a Case of Familial AD with Myoclonus and Seizures

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Abstract. We report clinical, neuropathologic and molecular genetic data from an individual affected by a familial Alzheimer disease (AD) variant. The proband had an onset of dementia at age 29 followed by generalized seizures a year later. He died at age 40. Neuropathologically, he had severe brain atrophy and characteristic histopathologic lesions of AD. Three additional neurologic features need to be emphasized: 1) severe deposition of Aβ in the form of diffuse deposits in the cerebral and cerebellar cortices, 2) numerous Aβ deposits in the subcortical white matter and in the centrum semiovale, and 3) numerous ectopic neurons, often containing tau-immunopositive neurofilament bundles, in the white matter of the frontal and temporal lobes. A molecular genetic analysis of DNA extracted from brain tissue of the proband revealed a S169L mutation in the PSEN1 (PSEN1) gene. The importance of this case lies in the presence of ectopic neurons in the white matter, early-onset seizures, and a PSEN1 mutation. We hypothesize that the PSEN1 mutation may have a causal relationship with an abnormality in neuronal development.

Key Words: Alzheimer disease; Amyloid beta protein; Epilepsy; Myoclonus; Neuronal migration; Presenilin mutation; Tau protein.

INTRODUCTION

Mutations in the Presenilin 1 (PSEN1) gene are the most common genetic abnormality associated with familial presenile Alzheimer disease (AD), and to date more than 100 mutations have been found (1, 2). As the number of mutations known has rapidly increased, it has become apparent that the clinical phenotypes associated with some PSEN1 mutations are characterized by a very early onset of signs and symptoms, some of which differ from those associated with typical AD (3–16). These include seizures in the early stage of the clinical course, spastic paraparesis, parkinsonism, or ataxia, all of which may occur alone or in combination with each other. The occurrence of seizures is frequent in AD during the late stage of the disorder; however, the presence of myoclonus and/or generalized seizures concomitant with a memory disorder at the onset of the clinical manifestations is rare. Among the mutations in PSEN1 reported thus far, at least 8 (P117L, N135D, I143T, L166P, S169L, S169P, L235P, A434C) are associated with this clinical phenotype (4–10, 12–15).

We present the clinical and neuropathologic phenotypes of an individual with early-onset familial AD associated with mutation S169L of the PSEN1 gene. This disorder was characterized clinically by an early onset of myoclonus, seizures, and dementia. Neuropathologically, the gray and white matter had a severe Aβ burden and the cerebral white matter contained numerous ectopic neurons, some of which underwent neurofibrillary degeneration. This report is the first to describe in detail the neuropathology associated with the PSEN1 S169L mutation and to highlight specific neuropathologic alterations that may represent the substrate for the seizure disorder associated with some PSEN1 mutations.

Like the S169L mutation, another recently discovered PSEN1 mutation, L166P, is associated with a very early onset of symptoms and is located in the transmembrane III interface region of the PSEN1 protein (1, 14). A study just completed has shown the L166P mutation to have a profound effect on Aβ42 production and impair Notch1 signaling in a cell culture system (manuscript in preparation). An impairment of Notch signaling has also been seen in Drosophila with mutations in Drosophila presenilin. These mutant Drosophila also had an early pupal-lethal phenotype characterized by defects in eye and wing development and incomplete neuronal differentiation within the larval CNS (17, 18). Therefore, the clinical and neuropathologic phenotypes associated with PSEN1 S169L may be important evidence of a causal relationship between PSEN1 mutations and neuronal maturation abnormalities.

MATERIALS AND METHODS

Pedigree

The family of the proband is identified here as family “G.” We were able to reconstruct a pedigree consisting of 73 members over 5 generations. Figure 1 shows an abbreviated pedigree.

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of this family. The maternal grandparents (I-1 and I-2) of the proband (III-8) are originally from Eastern Europe. The proband first presented neurological signs affecting activities of daily living at 29 yr of age. Throughout the 11-yr course of the disease, he underwent several neurological, psychiatric, neuropsychological, electrophysiological, and neuroradiological examinations.

Neuropathology

A brain autopsy of the proband was carried out 5 h after death. The fresh brain was perfused with 0.1 M phosphate buffer and hemisected along the sagittal plane. Coronal slices of the left frontal lobe and the right frontal, parietal, and temporal lobes as well as slices of the right cerebellar hemisphere were frozen and stored at −70°C for genetic and biochemical analyses.

Neurohistology: Most of the left cerebral hemisphere, multiple coronal slices of the right hemisphere, left cerebellar hemisphere, the brainstem, and the upper cervical spinal cord were fixed in 4% paraformaldehyde. Fixed tissue samples from the cerebral cortex, hippocampus, caudate nucleus, putamen, claustrum, globus pallidus, thalamus, hypothalamus, cerebellum, midbrain, pons, and medulla were dehydrated in graded alcohols, cleared in xylene, embedded in paraffin, and cut into 8-μm-thick sections using a Jung Biocut 2035 rotary microtome (Leica, Wetzlar, Germany). In addition, several coronal and axial slices of the entire cerebral hemisphere were taken at various levels, processed for histology using a similar method, and cut for whole mount on a Jung Polycut E microtome (Leica). These slices allow for a full exploration of the deep cerebral white matter. All of the sections were then stained with hematoxylin and eosin (H&E), Gallocyanin method for Nissl substance, Heidenhain-Woelcke method for myelin, and Bodian method for fibrils. Thioflavin S fluorescence was used to show amyloid.

Quantification of Neurons in the Cerebral White Matter: Ten-micron-thick sections were cut from paraffin-embedded coronal hemispheric slices (section 10a of the Atlas of the Human Brain, Mai et al [19]) of the brains of the proband as well as 2 other individuals that were neuropathologically confirmed to have Alzheimer disease. The 2 comparison cases were selected based on one having an autosomal dominant inheritance pattern (APP V717F) (20) and the other not (late-onset, no known family history of AD). Using the CERAD (21) and the NIA-Reagan criteria (22), both cases were classified as having the same severity of AD. In addition, both individuals had an APOE ε3/3 genotype. These sections were stained with H&E and Nissl. Using an Aristoplan microscope (Leitz, Wetzlar, Germany) with a ×40 objective, 100 fields measuring 0.17 mm² each were selected from the centrum semiovale. All neurons within each field were counted.

Immunohistochemistry: Antibodies raised against Aβ, tau, neurofilament proteins, GFAP, and α-synuclein were used for this study as shown in Table 1 (23–34). Polyclonal antibodies were detected using avidin-biotin with goat anti-rabbit immunoglobulin as the secondary antibody and horseradish peroxidase-conjugated streptavidin visualized with chromogen diaminobenzidine or tetramethylbenzidine. Monoclonal antibodies were detected using avidin-biotin with goat anti-mouse immunoglobulins as secondary antibody and streptavidin conjugated with alkaline phosphatase.

Measurement of Aβ Immunoreactivity: The Aβ immunoreactivity was measured in the neocortex of all 3 cases. Ten-micron-thick sections were cut from paraffin-embedded coronal hemispheric slices of each case (section 10a of the Atlas of the Human Brain Mai et al, [19]). These sections were simultaneously immunolabeled using antibodies 21F12. Using a ×20 objective, 10 to 15 fields (~0.371 mm² each) of the middle frontal, cingulate, and superior temporal gyri from each case were selected and digitally captured using a Spot RT digital camera (Diagnostic Instruments, Inc. Sterling Heights, MI) attached to a Leica DMLB microscope (Leica). The software PAX-it (MIS, Inc, Franklin Park, IL) was used to carry out morphometric analyses on the digital image. After establishing a color and density threshold, the software calculated the percentage of the field affected by Aβ by dividing the area of Aβ immunoreactivity by the total area.

Using the same slices immunolabeled with 21F12 described above, we measured Aβ immunoreactivity in the cerebral white matter. This was carried out through a visual counting of discrete deposits in the centrum semiovale and the subcortical white matter adjacent to the cortices of the middle frontal gyrus, cingulate gyrus, and superior temporal gyrus. A subjective scale was used to represent the number of deposits counted (~none, + mild [0–5/0.317mm²], ++ moderate [6–20/0.317mm²], +++ severe [>21/0.317mm²]).

Molecular Genetics

DNA was obtained from the brain of the proband using a previously described method (35). The DNA was analyzed by direct sequencing of exons 3 through 13 of the PSEN1 gene and exons 16 and 17 of the APP gene. Standard amplification reactions were done with 20 ng/μl of genomic DNA. The amplified products were then gel-purified. To generate a single-stranded template for sequencing, asymmetric amplification was carried out. The amplified products were subjected to Qiaquick...
PCR purification spin columns (QIAGEN, Valencia, CA), which removed remaining primers and deoxynucleotides. Standard dideoxynucleotide sequencing was performed using the U.S. Biochemicals Sequenase kit, 35S-dATP (Amersham, Piscataway, NJ) and modified T7 DNA polymerase (Sequenase Version 2.0, U.S. Biochemicals Sequenase kit, 35S-dATP (Amersham, Pis-
catway, NJ) and modified T7 DNA polymerase (Sequenase

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<tr>
<td>EM3(ABX43)</td>
<td>r</td>
<td>1:1000</td>
<td>Dr Frangione (28)</td>
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Anti-tau antibodies

AT8(p-tauSer202/Thr205) | m | 1:400 | Polymedco (29) |
12E8(p-tauSer202/Thr205) | m | 1:5000 | Elan Pharmaceuticals (30) |

Other antibodies

SMI-32(np-NF160kDa) | m | 1:2000 | Sternberger Monoclonals (31) |
SMI-34(p-NF160kDa) | m | 1:2000 | Sternberger Monoclonals (31, 32) |
AB1981(NF150kDa) | r | 1:200 | Chemicon (33) |
AB1983(NF130kDa) | r | 1:200 | Chemicon (33) |
GFAP | r | 1:100 | Dako Corporation |
α-synuclein | r | 1:300 | IADC (34) |

Abbreviations: AB = amyloid β protein, p-tau = phosphorylated tau, np-NF = non-phosphorylated neurofilament, p-NF = phosphorylated neurofilament, NF = neurofilament, GFAP = glial acidic fibrillary protein, m = mouse monoclonal, r = rabbit polyclonal, IADC = Indiana Alzheimer Disease Center.

RESULTS

Clinical Findings and Family Anamnesis

Clinical History: The proband, a Caucasian male, was reported to have had a head injury at age 8. At age 24, he had difficulty maintaining a job and his marriage failed. At age 29, he began to experience memory loss, periods of confusion and disorientation. Family members noticed bizarre behaviors and personality changes at age 30. He got lost while driving in familiar locations. At age 31, he began to experience memory loss, periods of confusion and disorientation. He was admitted to the hospital at age 38 because of aspiration pneumonia. A neurological examination revealed an increase in patellar and Achilles’ tendon reflexes as well as ankle clonus. He did not have a Babinski sign, but did have a snout reflex.

At age 33, he was placed in nursing home because his symptoms were worsening. He was admitted to the hospital at age 38 because of aspiration pneumonia. A neurological examination revealed that he was able to respond to painful stimuli, but not to verbal stimuli. His tendon reflexes were exaggerated in all extremities and a Babinski sign and Chaddock reflex were noted on the right side. He died at age 40.

Neuropsychology: At age 31, a psychologist reviewed several standardized cognitive test scores from the proband and concluded not only that he was currently at the level of a 10-yr-old, but also that a deterioration in school...
performance began around the age of 15. This is of particular interest, since the family reports that the proband was encouraged to enroll in accelerated programs at the age of 10.

The proband was examined at age 32 with the Halstead-Reitan Neuropsychological test battery (38). He was unemployed at the time of the testing having been referred for the examination by his vocational rehabilitation counselor for suggestions on job training and placement. He had last worked as a clothing salesman for 3 days where he forgot customers as he went to help others. Prior to that, he had worked for several different accounting firms for 1 to 2 yr each in various capacities as an accountant, assistant tax manager, and auditing clerk. He was a high school graduate and intermittently attended college with average grades.

On examination, he was noted to be neatly dressed and clean but with slumped posture, often holding head downward, awkward gait, stiff limbs, and constant right hand tremor. Occasionally, he would forget instructions midway through a test. He had difficulty maintaining attention. On sensory discrimination tasks, he appeared to drift off each time he closed his eyes and he responded inconsistently to stimuli.

According to the Wechsler Adult Intelligence Scale (WAIS Full Scale IQ = 80) (39) his intelligence measured in the low average range with a significant discrepancy between verbal abilities that were in the average range (Verbal IQ = 92) and nonverbal skills that were moderately defective (Performance IQ = 65). The proband’s ability to recite facts, define words, and find similarities among objects and concepts was in the average range (WAIS Similarities = 50th percentile, Vocabulary = 63rd percentile, and Similarities = 50th percentile), which suggests that premorbidly he had at least average intellectual ability. In contrast, novel problem solving (Category Test <1st percentile), visuomotor skill (WAIS Block Design = 1st percentile), tactile perceptual learning (Tactual Performance Test = <1st percentile), and psychomotor speed (WAIS Digit Symbol and Trail Making Test Part A both <1st percentile) were moderately to severely defective. Attention was low average as measured by digit repetition and mental arithmetic (WAIS Digit Span = 16th percentile; WAIS Arithmetic = 25th percentile). For motor skills, hand strength was average bilaterally, finger tapping mildly slowed bilaterally, and manual dexterity was grossly impaired bilaterally.

The test results indicated generalized cerebral dysfunction consistent with mild to moderate dementia. There was an indication of difficulty with level of consciousness and sustained attention at some points during the examination.

Electrophysiology: An electroencephalogram (EEG), which was performed at age 27 to investigate the cause of his headaches, was normal. A second EEG was carried out at age 31 and showed frequent short runs of generalized bilaterally symmetric isoelectric synchronous 2 or 3 cycles per second spikes and waves. A third EEG was performed at age 32 and showed background activities to have diffuse slow waves in the θ range and epileptic activities arising bilaterally from the temporal regions.

Neuroradiology: A brain computed tomographic scan (CT) was carried out when the proband was age 30 and again at age 32. No abnormalities were reported in either CT.

Brain Biopsy: At age 32, the proband underwent a brain biopsy that revealed numerous neuritic plaques and neurofibrillary tangles as well as a congophilic angiopathy. By electron microscopy, it was shown that the neurofibrillary tangles were composed of paired helical filaments. These analyses resulted in the pathologic diagnosis of AD.

Family Anamnesis: The pedigree is shown in Figure 1 where the proband is listed as subject III-8. Only limited medical information is available on most family members, except for the proband’s mother (II-3). She was apparently in good health until age 29 when her husband began to notice changes in her personality. Her ability to maintain tidiness decreased, as seen by the accumulation of bottles, cans, and newspapers throughout the house. In addition, she developed carelessness toward her personal habits and appearance. For example, she would go out in public with disheveled hair and dirty feet. During this same time period, she began to experience forgetfulness several times throughout the day. Seizures began at age 30 and in less than a year, the frequency increased from once every 2 months to 7–8 times a month. Seizures

Fig. 2. Photomicrographs of the proband’s temporal cortex. A: Numerous fluorescent plaques and neurofibrillary tangles are seen. Note the varying degree of intensity of fluorescence of the plaques. One vessel wall is intensely fluorescent indicating the presence of an amyloid angiopathy. Thioflavin-S method. B: Numerous Aβ-immunoreactive plaques and a segment of a vessel (arrow) are seen. Note that the Aβ immunoreactivity is prominent in the molecular and lower cortical layers. Immunohistochemistry using monoclonal antibody 1D05. C: Neurofibrillary tangles are seen in the parahippocampal gyrus. Bodian stain. D: Tau-immunoreactive intracytoplasmic deposits and neuropil threads. Immunohistochemistry using monoclonal antibody AT8. Magnifications: A, B, ×112; C, ×1,000, D, ×700.
would typically last 1 to 2 min though she might remain limp and unconscious for longer periods of time. Upon regaining consciousness, she typically would have a headache. These episodes ceased after starting phenytoin treatment. EEGs obtained at ages 31 and 32 showed dysrhythmia and the latter also showed a slow wave focus in the tracing recorded from the right temporal region.

On neurological examination at age 32, her behavior was described as “silly” and her demeanor was reminiscent of someone with hebephrenic schizophrenia. She also had a left Babinski sign. A pneumoencephalogram revealed negligible atrophy of the cerebral cortex. Cerebrospinal fluid tests were normal, including a Wassermann test. Blood tests were normal, including the level of plasma ceruloplasmin. At age 33, a neurological examination found myoclonus, dysarthria, hyperreflexia, intention tremor, gait ataxia, and a Romberg’s sign. An ophthalmological examination was unremarkable. A psychological examination was conducted during a two-and-a-half week hospital admission for evaluation of personality change and neurological symptoms. A Wechsler-Bellevue test showed her intelligence to be in the defective range. Fund of information and verbal abstraction were relatively preserved while marked deficits were seen in recent memory, rote learning, visuospatial skill, and psychomotor speed consistent with moderately advanced dementia. She was dysarthric and unable to write her name. An EEG showed epileptic discharges. A liver punch biopsy was negative for Lafora bodies and cellular inclusions. She was diagnosed as having possible Unverricht-Lundborg disease and eventually died at age 38.

The proband and his mother are the only 2 individuals in this family known to have suffered from an illness characterized by an onset of myoclonus, seizures, and dementia late in the third decade of life. In fact, both of the proband’s maternal grandparents (I-1, I-2) lived beyond the age of 80 and were not reported to have suffered from any neurological disorder. Of the remaining relatives, only the proband’s great-aunt (I-3) and her son (II-4) suffered from dementia. Subject I-3 developed dementia in her late 60s, which progressively worsened over the next decade. Her functional decline required that she be cared for in a nursing home facility until she died in her 70s. Subject II-4 suffered a stroke in his late 40s and exhibited a progressive decline in cognitive functions thereafter. By age 56, his cognitive function, particularly memory, was severely impaired and he required nursing home care from that age forward. A sibling (III-7) of the proband had cleft palate and a congenital urological defect. Subject IV-3 was born with clubfoot. The proband’s paternal relatives were not known to have suffered from any neurological or dementing illnesses.

Neuropathology

Gross Neuropathology: The proband’s brain weighed 1,060 g; it was diffusely atrophic with the cortical ribbon and hippocampus being the most severely affected. The lateral ventricles were enlarged. No atherosclerosis was seen in the major cerebral arteries.

Microscopic Neuropathology: Both the gray matter and white matter bore the brunt of the disease process (Figs. 2–8). Neuritic plaques, diffuse plaques, neurofibrillary tangles, and neuronal rarefaction were seen throughout the gray matter; amyloid deposits and ectopic neurons with and without neurofibrillary tangles were seen in the cerebral white matter (Fig. 2–6). In the medulla, mild pallor of myelin stain was seen at the level of the pyramids.

Gray Matter

Neurohistology and Immunohistochemistry: The following lesions were seen in large numbers throughout the cerebral cortex: 1) neuritic plaques, 2) amyloid cores not accompanied by degenerating neurites, 3) diffuse plaques, and 4) neurofibrillary tangles (Figs. 2, 3). Using thioflavin S, the cortical and leptomeningeal vessel walls showed a severe amyloid angiopathy (Figs. 2A, 3B). In addition, neuritic plaques were present in multiple subcortical nuclei of the cerebrum, midbrain and medulla (Table 2). Furthermore, amyloid cores not accompanied by degenerating neurites and diffuse plaques were present in numerous other regions of the cerebrum, cerebellum, brainstem, and spinal cord as shown in Table 2. Neurofibrillar tangles were numerous throughout all layers of the cortex of the frontal, temporal, entorhinal, cingulate, insular, parietal, and occipital regions as well as the hippocampus (Table 2; Fig. 2C, D). Granulovacuolar degeneration was seen in the pyramidal neurons of the hippocampus.

The number of neuritic plaques and the distribution of neurofibrillary tangles are consistent with a neuropathologic diagnosis of definite AD using the CERAD criteria.

Fig. 4. Photomicrographs of the white matter of the frontal lobe. A, B: Neurons of various sizes (arrows) among glial cells. Note the pyramidal shape of these neurons and their prominent nucleus and nucleolus. Gallocyanin method. C: Tau immunoreactive neurofibrillary tangles. Immunohistochemistry using monoclonal antibody AT8. D: Neurofibrillary tangle in a neuron and apical dendrite. Bodian stain. Magnifications: A, ×241; B, ×1,000; C, ×1,000; D, ×1,000.
Fig. 5. Photomicrographs of the cerebellar cortex. A: Aβ immunoreactive plaques and vessel walls in the molecular layer (A), granule cell layer (B), and infratentorial white matter (C). Immunohistochemistry using monoclonal antibody 21F12. Magnifications: A, ×110; B, ×550; C, ×690.

Neuronal rarefaction and gliosis were severe throughout the cerebral cortex and hippocampus, but they varied in severity in the basal ganglia, thalamus, cerebellar cortex, dentate nucleus, substantia nigra, and locus coeruleus.

The severity of the pathology throughout the neocortex, including the occipital cortex, precluded the precise distinction of individual cortical layers and the assessment whether the layers had developed normally. In the left middle temporal gyrus, a cluster of large ballooned GFAP-immunopositive astrocytes was seen (Fig. 6B). Gliosis and loss of myelin fibers were evident throughout the white matter of the frontal, temporal, and parietal lobes.

Antibodies raised against tau labeled tangle-bearing neurons, neurons apparently free of neurofibrillary changes and neuropil threads throughout the neocortex, hippocampus, as well as several subcortical nuclei in the cerebrum and brainstem (Table 2; Fig. 2D). Antibodies raised against the 200 kDa and 150 kDa neurofilament proteins sparsely labeled cortical neurons, and antibodies to the 68 kDa neurofilament protein labeled many axons and dendrites. Using antibodies raised against α-synuclein, immunopositivity was found in neurites of the CA2 region of the hippocampus. A-synuclein-immunopositive
Lewy bodies or neurites were not found in any other brain region.

**Aβ Pathology:** Antibodies against Aβ labeled 3 distinct types of lesions: 1) diffuse deposits, 2) amyloid cores, and 3) deposits in the walls of blood vessel of the leptomeninges and parenchyma (Figs. 2B, 3C, D, 5A–C). Except for antibody EM2, all the other anti-Aβ antibodies labeled diffuse Aβ deposits and amyloid cores; however, the strongest labelling was obtained using monoclonal antibodies 10D5 and 21F12 (Figs. 2B, 3C, 5A–C). Antibodies EM2 (Aβ1–40) and 10D5 (Aβ1–28) labeled by far the largest number of deposits in the walls of blood vessels of the leptomeninges and parenchyma as compared to all the other anti-Aβ antibodies, including 21F12, an antibody that has only a negligible cross-reactivity with Aβ1–40 (Figs. 2B, 5A) (23).

Diffuse deposits and amyloid cores were present in all areas of the cerebral cortex and in most gray matter regions of the cerebrum, cerebellum, brainstem, and spinal cord (Figs. 2A, B, 3B–D, 5A–C). A striking feature was the extent of diffuse Aβ deposits observed in the cerebellar molecular layer (Fig. 5A). Overall, the Aβ deposition was more abundant than in most cases of AD, including the 2 comparison cases. In fact, a morphometric analysis revealed that the Aβ immunoreactivity (21F12 antibody) in the proband’s middle frontal, cingulate, and superior temporal gyri was nearly double that seen in the other 2 AD cases (autosomal dominant AD [APP V717F])
Fig. 7. Bar graph showing \( \alpha\beta_{x42} \) burden quantitative data gathered by image analysis of \( \alpha\beta_{x42} \) immunoreactivity in 3 cortical regions from 3 AD subjects. In the proband, the \( \alpha\beta_{x42} \) burden is higher (1.4–2.7 times) than that of the other 2 AD cases. Data presented are the mean of 10 to 15 fields per anatomical region per subject.

Fig. 8. Bar graph showing the number of neurons in the cerebral white matter. Data presented are the total number of neurons in 100 fields (0.17 mm\(^2\) each) per subject.

and non-autosomal dominant AD) (Fig. 7). It should be noted that these results are the total \( \alpha\beta_{x42} \) immunoreactivity and do not discriminate between diffuse and fibrillary \( \alpha\beta \).

### TABLE 2

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<td>Upper cervical cord</td>
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Abbreviations: NP = neuritic plaques, AC = amyloid cores without degenerating neurites, DP = diffuse plaques, NFT = neurofibrillary tangles; – = none, 1+ = sparse, 2+ = moderate, 3+ = frequent.

### White Matter

**Neurohistology, Immunohistochemistry and Neuronal Quantification:** The cerebral white matter was abnormal due to the presence of numerous nerve cells and \( \alpha\beta_{x42} \) deposits that were located in both subcortical and deep regions (Figs. 4A–D, 6A). These neurons often contained argyrophilic and tau-immunopositive neurofibrillary tangles (Fig. 4C, D). The number of neurons in the deep white matter of the proband (26/100 fields) was higher than that of an autosomal dominant AD case (6/100 fields) and a non-autosomal dominant AD case (7/100 fields) (Fig. 8). The subcortical white matter and that of the centrum semiolare in the proband had abundant \( \alpha\beta_{x42} \) immunopositivity in the form of diffuse deposits and...
amyloid cores (Table 3; Fig. 6A). The Aβ deposits without cores were particularly numerous. In contrast, only a small amount of Aβ diffuse deposits were seen in the 2 cases used for comparison and no amyloid cores were seen in the brain of the individual with non-autosomal dominant AD.

Following the observation of ectopic neurons in the white matter, the slides prepared at the time of the biopsy were re-examined. Two neurons were seen in the subcortical white matter; however, they did not contain neurofibrillary tangles. Furthermore, no amyloid deposits were seen. It should be kept in mind that these slides were prepared prior to the availability of antibodies to Aβ and tau, and at the time of this writing the blocks were not available for re-examination.

### DISCUSSION

This report presents the clinical and neuropathologic phenotypes of an individual with the S169L mutation in the PSEN1 gene. The study of families with mutations in PSEN1 has shown that syndromes caused by PSEN1 mutations are inherited in an autosomal dominant manner. The syndrome seen in the proband was characterized clinically by early-onset dementia concomitant with myoclonus and seizures. The proband was the only subject of family “G” to be analyzed genetically; however, his mother had suffered from a similar syndrome that had been diagnosed as progressive myoclonus epilepsy, Unverricht-Lundborg type (41). Based on the similarities of the clinical presentations and the age at onset of symptoms, it appears likely that the proband and his mother had suffered from the same genetic disease. The fact that neither of the proband’s grandparents had early-onset AD leads us to consider the possibility of incomplete penetrance of the genetic defect in the proband’s grandparents, non-paternity, or a new mutation in the proband’s mother. Since no genomic DNA was available from either the proband’s mother or her parents, it was not possible to distinguish between these possibilities.

During the course of the proband’s illness, a brain biopsy of the right frontal cortex revealed the presence of numerous neurofibrillary tangles and senile plaques and led to the diagnosis of Alzheimer disease. This diagnosis was confirmed at autopsy and the availability of frozen postmortem tissue made possible the identification of the PSEN1 S169L mutation. Prior to the discovery of the PSEN1 S169L mutation in family “G,” the mutation had been found in 3 members of a family residing in Australia (10). In that family, the proband had the onset of clinical signs at 31 yr of age. In addition to cognitive dysfunction, he presented myoclonus and seizures at age 33. Neuropathologically, marked diffuse and neuritic plaques as well as neurofibrillary tangles were reported.

The presence of seizures early in the course of familial AD has been seen in association with PSEN1 mutations P117L, N135D, I143T, L166P, S169L, S169P, L235P, A434C; however, at this time, the pathological substrate of the myoclonus and seizures is not known (4–15). The present report describes in detail the neuropathology associated with the PSEN1 S169L mutation and highlights specific neuropathologic alterations that may be one of the substrates involved in the seizure disorder seen in some individuals with PSEN1 mutations.

The presence in the cerebral cortex of Aβ deposits in the form of diffuse and neuritic plaques as well as tau-immunopositive neurofibrillary tangles makes the
neuropathologic phenotype of the PSEN1 S169L mutation similar to previously reported cases of autosomal dominant AD (4–16, 42). However, fibrillary and non-fibrillary Aβ deposits as well as the neurofibrillary tangles were much more numerous and more widely distributed in this case than that of AD with onset later in life, regardless of genetic etiology.

Recent biochemical data have shown that an overrepresentation of N-terminally truncated Aβ forms may be characteristic of AD associated with PSEN1 mutations (43, 44). Neuropathologic studies of PSEN1-associated phenotypes have shown that Aβ deposition may occur as diffuse deposits, amyloid cores with or without degenerating neurites, or “cotton wool” plaques (5, 7, 10, 14, 16, 45–48). The S169L mutation is associated with extensive deposition of diffuse plaques and amyloid cores with and without degenerating neurites, but not with the “cotton wool” plaques that are seen in cases with PSEN1 mutations ΔI83/ΔM84, L166P, G217D, V261F, ∆9, and P436Q (14, 16, 45–48). This is of interest since both the L166P and S169L mutation are located in the transmembrane III interface region of PSEN1 and yet they are associated with Aβ deposits having various morphological characteristics. Our findings show that AβX₄₂ burden in the cerebral cortex of the proband was greater than that seen in the comparison AD cases. In addition, AβX₄₂ deposits in the form of diffuse plaques and amyloid cores were present in many gray matter areas and in the cerebral white matter. Thus, it is possible that the S169L mutation also has an effect on Aβ₄₂ production as has been shown in vitro in cells transfected with the L166P mutation (manuscript in preparation).

A finding that had no precedent in the human neuropathologic literature related to PSEN1 mutations or AD was the presence of numerous ectopic neurons scattered throughout the white matter. These neurons were not only abnormal for their location, but also they showed intracellular tau deposits and neurofibrillary tangles. The presence of ectopic neurons in the white matter has been shown in cases of generalized epilepsy and early myoclonic encephalopathy and has been interpreted as the result of the failure of neuronal migration (49–52). In humans, neuronal migration occurs between the 8th and 20th wk of gestation. The human cerebral cortex has 6 layers by the middle of gestation; however, many neurons are still present in the intermediate zone, the future white matter. The number of neurons in the intermediate zone normally decreases during the first postnatal year (53–54).

Whether the presence of white matter pathology is a coincidental finding in the proband or a part of the neuropathologic phenotype associated with the S169L mutation is yet to be determined. Only 1 member of the S169L family from Australia reported by Taddei et al was studied neuropathologically; however, no information concerning the pathology of the white matter was
provided (10). We believe that this finding is more than coincidental, especially in light of our recent neuropathologic studies of another individual who had a syndrome characterized by an early onset of seizures, spastic paraparesis, ataxia, and dementia. This individual was found to have the L166P mutation in the PSEN1 gene and numerous ectopic neurons in the cerebral white matter (manuscript in preparation). At this time, it is not clear whether ectopic neurons in the white matter are a neuropathologic feature of all PSEN1 mutations or only some of them. In order to better understand this issue, we are currently analyzing brain tissue available in our brain bank that was harvested from individuals with PSEN1 mutations.

An important factor in determining cell survival and cell fate during development is the Notch1 protein, which regulates neurite growth during and after development (55–58). In Drosophila, it has been shown that presenilin is required for neuronal differentiation and that the signaling through the Notch receptor is reduced in Drosophila presenilin mutants (17, 18). In presenilin 1-deficient mice, a partial Notch-deficient phenotype has been observed. In these mice, the CNS is characterized by a loss of the ventricular zone, loss of Cajal-Rezius cells, and an overmigration of cortical neurons (59–61). Recent studies have shown that the ΔE83/ΔM84, L166P, and G384A PSEN1 mutations affect Notch1 signaling; however, it remains to be determined if the S169L mutation has a similar affect (45, 62, manuscript in preparation). Mutant presenilins have also been linked to mechanisms of apoptosis (63). It has been speculated that the apoptotic effects of Drosophila presenilin and perhaps those caused by mammalian presenilin proteins may reflect the normal elimination of aberrant cells.

Thus, the presence of numerous ectopic neurons in the white matter of individuals with PSEN1 mutations S169L and L166P may be the result of a failure in cell migration, and/or a failure in the mechanisms of normal elimination of aberrant cells via apoptosis.

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