

MISCELLANEOUS

Encephalopathy with Neuroserpin Inclusion Bodies Presenting as Progressive Myoclonus Epilepsy and Associated with a Novel Mutation in the *Proteinase Inhibitor 12* Gene

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Abstract

Neuroserpin encephalopathy is an autosomal-dominant degenerative disease associated with mutations in the *Proteinase Inhibitor 12* (*PII2*) gene. A 26-year-old male presented with progressive myoclonus epilepsy and declining mental status. He had failed in university studies because of impaired attention, memory and concentration. Generalized seizures started to occur approximately once a month, and he developed myoclonus and progressive gait disturbances. Neuroimaging revealed mild atrophy and multiple periventricular white matter lesions, consistent with demyelination. He progressively declined and died at age 34. Neuropathologic examination revealed widespread involvement of the cerebral cortex by numerous round eosinophilic inclusions in neuronal perikarya and neuropil, predominantly within the deep cortical layers. Numerous inclusions were also found in the basal ganglia, thalamus, hippocampus, brain stem, spinal gray matter, and dorsal root ganglia. They were essentially absent from the cerebellum. The inclusions were immunopositive for antibodies raised against neuroserpin. The white matter lesions showed histologic features compatible with multiple sclerosis. Genetic analysis revealed a nucleotide substitution in codon 47 in one allele of the *PII2* gene, resulting in a proline for leucine amino acid substitution (L47P). In summary, we report a case of neuroserpin encephalopathy associated with a novel *PII2* mutation and complicated by coexistent multiple sclerosis.

INTRODUCTION

Mutations in the *Proteinase Inhibitor 12* gene (*PII2*; *SERPINI1*), located on chromosome 3q26, have been found to produce an autosomal-dominant neurodegenerative disease that has been termed familial encephalopathy with neuroserpin inclusion bodies (FENIB) (4). Neuroserpin, the protein encoded by the *PII2* gene, is a serine protease inhibitor that is predominantly expressed within the brain. It is widely expressed during the late stages of development but is present in regions of high synaptic plasticity in adulthood. One of its known functions is as an inhibitor of tissue-type plasminogen activator (tPA) (15).

Five different mutations in the *PII2* gene have previously been described (S49P, S52R, H338R, G392E, G392R) (3–6, 24). Mutations in the neuroserpin gene are associated with the accumulation of the mutant neuroserpin protein within neurons, forming intracytoplasmic inclusions (26). The clinical features, age of onset of symptoms and severity of the neurological deficits associated with neuroserpin mutations vary considerably, depending upon the site

of the mutation. Clinical manifestations include progressive myoclonus epilepsy (PME), focal or generalized seizures, dysarthria, tremors and dementia. Here we report a patient with progressive myoclonus epilepsy and dementia associated with neuroserpin inclusion bodies produced by a previously unreported mutation in the *PII2* gene (L47P).

MATERIALS AND METHODS

Clinical evaluation of the patient

Clinical history, physical examination findings, electroencephalogram (EEG) and neuroradiologic reports were obtained from neurological and neurogenetic outpatient records, as well as the inpatient chart from the Montreal Neurological Hospital.

Autopsy and anatomic pathology

An autopsy, carried out on the patient 4 h after death, included the examination of visceral organ systems, central nervous system

(CNS), dorsal root ganglia and bone marrow. Tissue samples from the lung, heart, liver, pancreas, spleen, adrenal glands, kidneys, bladder, testes, prostate, thyroid, parathyroid, skeletal muscle and bone marrow from a lumbar vertebral body were fixed with 10% formalin. Following formalin fixation, the tissue samples were dehydrated in graded alcohols, cleared in xylene and embedded in paraffin. Five-micron-thick sections were cut for histology and stained with hematoxylin and eosin (H&E) and periodic acid schiff (PAS).

Neuropathology

The brain and spinal cord were fixed with 10% formalin. Following fixation, the cerebrum, cerebellum and spinal cord were sliced. Tissue samples were taken from the right frontal cortex, right nucleus basalis, left globus pallidus and putamen, left posterior hippocampus, right thalamus, right corpus callosum, left occipital cortex, right parietal cortex, pons, midbrain, medulla, right cerebellum and spinal cord (cervical, thoracic, lumbar, sacral and cauda equina).

Neurohistology

The sampled tissues were dehydrated in graded alcohols, cleared in xylene and embedded in paraffin. Eight-micron-thick sections were cut with a Leica rotary microtome (Leica microsystems, Wetzlar, Germany). The sections were then stained with H&E, PAS, the Heidenhain–Woelcke method for myelin, the Bodian method for fibrils and thioflavin S for amyloid.

Immunohistochemistry

Polyclonal antibodies raised against human neuroserpin (1:2000) (11, 22), glial fibrillary acidic protein (GFAP) (Dako, Carpinteria, CA, USA; 1:100), β -amyloid (21F12; Elan Pharmaceuticals, San Francisco, CA, USA; 1:1000) and a synthetic peptide corresponding to residues 119–137 of human alpha-synuclein (1:200) were used. A phosphorylation-dependent anti-tau monoclonal antibody recognizing phosphorylated Ser202/Thr205 (AT8; Pierce Endogen, Rockford, IL, USA; 1:300) was also used.

DNA Extraction from Brain Tissue and Genetic Analysis

DNA was extracted from fresh blood and fixed brain tissue from the patient using methods previously described (18, 19). The DNA was analyzed by direct sequencing of exons 2–9 of the *PII2* gene. Amplification and sequencing were done using primers previously reported (5).

RESULTS

Clinical history

The patient, a right-handed Ashkenazi Jewish male from Belarus, presented at the age of 26 with seizures and myoclonus. Prior to the onset of seizures, he had a two-year history of mental deterioration. He was born at term following an uncomplicated pregnancy. His motor and speech development were normal. At the age of 12, he

was hospitalized for “arachnoiditis” following a mild head trauma with no significant sequelae. His family history was positive only for Parkinson’s disease, and his mother was noted to have severe migraine headaches.

He completed three semesters at Leningrad Medical School prior to entering military service. At the age of 21, he immigrated to Canada and enrolled in university but failed in his studies because of impaired concentration, memory and attention. He became increasingly socially withdrawn, unable to maintain a job and had an overall decrease in activities. He developed paroxysmal episodes of myoclonic jerks while standing, which were generalized to all four extremities and associated with falls. He also had generalized tonic–clonic seizures that would occur about once per month. An EEG showed generalized polyspike wave activity that was photosensitive and was also enhanced by hyperventilation. Magnetic resonance imaging (MRI) revealed multiple white matter lesions compatible with demyelination.

From ages 27 to 28, he had progressive deterioration of gait with difficulty going up- and downstairs. By age 28, he also developed a new type of paroxysmal episode, which was characterized by falls associated with generalized stiffening and brief losses of consciousness lasting several seconds. They occurred every few days but up to three times in a day.

Neurological examination at age 28 revealed a withdrawn, apathetic male with some imitative and automatic behavior. A few spontaneous movements and occasional myoclonic jerks of the extremities were noted. Mental status examination revealed dementia with poor long-term recall, short-term recall of only one of three objects with hints and serial 7’s correct to only 93. He had gaze-evoked horizontal nystagmus bilaterally but full and conjugate gaze. He had a marked tremor of the tongue but no palatal myoclonus. The remainder of the cranial nerve examination was normal. Motor exam revealed *Gegenhalten* and a slight spastic catch in his extremities. He was diffusely hyperreflexic with clonus in his ankles. He had some decreased position sense in his toes and decreased vibration sense in his feet. Occasional postural myoclonus, postural tremor that increased with action, end extension tremor on finger–nose exam, and marked tremor with heel–shin testing were also noted. His gait was notable for a paucity of arm swing and wide turn. He was unable to do tandem walking. A Romberg test was marked by myoclonic jerks and a tendency to fall to the right.

His seizures were initially treated with carbamazepine (Tegretol), but he was later switched to valproic acid (Epival), which resulted in better control. Once his seizures were more effectively treated, his ability to concentrate improved significantly, and his falls became less frequent. He reported periodic deficits with attention; however, he felt that his memory and general cognition had improved but not necessarily to the level prior to the onset of his symptoms. His neurological examination showed improvement, though he still had a tremor of the tongue, gaze-evoked horizontal nystagmus, suggestions of a spastic catch in all four extremities and diffuse hyperreflexia with four to six beats of clonus at the ankles. He continued to have a postural tremor that increased with action, but this showed significant improvement.

Initial clinical considerations included progressive myoclonus epilepsy, Lafora disease, mitochondrial disease, celiac disease or a combination of multiple sclerosis and generalized idiopathic epilepsy (14). Multiple MRIs demonstrated multi-focal white matter hyperintensities (Figure 1), compatible with demyelinating

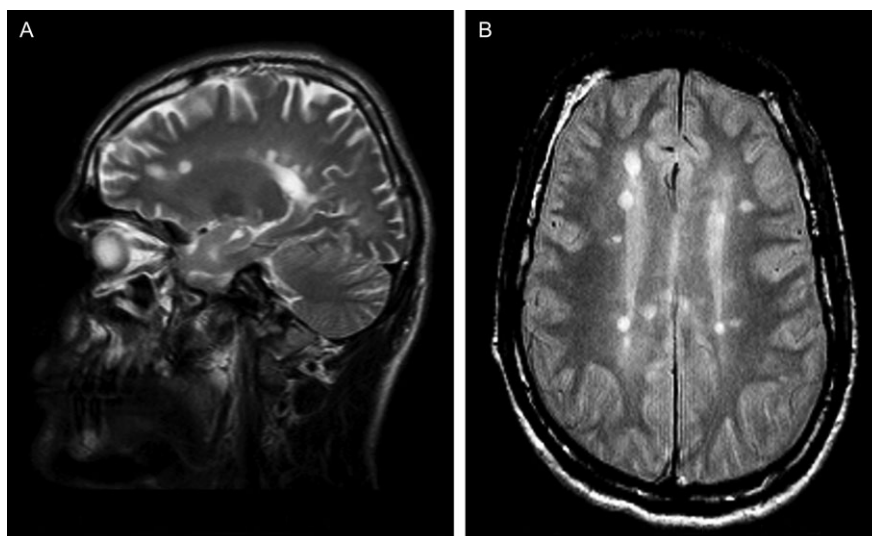


Figure 1. Magnetic resonance imaging (MRI) showing multiple hyperintense lesions within the periventricular white matter. **A.** Sagittal T2-weighted image at age 28. **B.** Axial proton density sequence at age 30.

disease, and mild cerebral atrophy that appeared to progress over time. Examination of his cerebrospinal fluid (CSF) revealed oligoclonal bands. Phosphorus magnetic resonance spectroscopy (^{31}P -MRS) of his gastrocnemius muscle at rest and following ischemic exercise showed results suggestive of mitochondrial disease; however, serum and CSF lactate levels were normal. A muscle biopsy showed no evidence of ragged red fibers. No mitochondrial genetic mutations were identified. Additional serological tests for metabolic and autoimmune diseases were negative. A skin biopsy showed no evidence of Lafora bodies.

Despite his initial improvement, his neurological status continued to deteriorate. By age 30, his coordination, strength, and balance declined to the point where he required the use of a wheelchair for mobility. He was paraplegic by age 32 and dependent upon his parents for all activities of daily living. High doses of piracetam (Nootropyl) were added, which controlled his resting, but not action, myoclonus. By age 33, he was largely confined to bed. He was able to speak but was confused and disoriented. He died at the age of 34 of sepsis and respiratory distress secondary to aspiration pneumonia.

AUTOPSY

A full autopsy was performed with microscopic examination of tissue samples from the lung, heart, liver, pancreas, spleen, adrenal glands, kidneys, thyroid, parathyroid, testes, prostate, lumbar vertebral body, skeletal muscle and bladder. The autopsy confirmed the extensive acute and organizing bronchopneumonia, consistent with aspiration. Acute cystitis was also noted. No abnormal intracellular inclusions were identified within any of the sections from the body other than the nervous system.

NEUROPATHOLOGICAL EXAMINATION

Macroscopic

The weight of the brain was 1147 g. The cerebral hemispheres were symmetrical. No gross abnormalities were noted on the external

examination of the brain, brainstem, spinal cord or cerebral vasculature. Coronal sections of the cerebrum revealed a mild enlargement of the lateral ventricles. Multiple plaques, consistent with demyelination, were noted in the periventricular white matter, focally involving the corpus callosum. Additional plaques were noted in the right parietal white matter, left occipital white matter and pons. No other gross abnormalities were seen.

Microscopic

Within sections from the cerebral cortex, numerous round, eosinophilic, intracytoplasmic inclusions were identified by H&E (Table 1). They were predominantly located in layers III through VI, although sparse to moderate involvement of layer II was seen (Figure 2). The inclusions ranged in size from a few microns to more than 40 μm in diameter. The inclusions were present within the neuronal perikarya as well as present within the neuropil. Often multiple inclusions were identified within affected neurons. No inclusions in cell nuclei were seen. Outside of the neocortex, numerous inclusions were also found in the substantia innominata, subiculum, substantia nigra, oculomotor nuclei and locus coeruleus (Figure 3). Moderate to frequent inclusions were found in the dorsal root ganglia and spinal gray matter, particularly the anterior horns (Figure 3). They were less numerous within the basal ganglia, thalamus, hippocampus and other brainstem nuclei. Other than a few inclusions within the dentate nucleus, they were essentially absent from the cerebellum. These inclusions were PAS-positive and diastase-resistant. They also stained darkly with the Heidenhain–Woelcke method. They were positively labeled by polyclonal antibodies raised against human neuroserpin (22). Immunohistochemical stains for α -synuclein, β -amyloid and hyperphosphorylated tau did not stain the inclusions or reveal any other abnormal deposits.

Also visible within the microscopic sections were areas of pallor and decreased oligodendrocytes within the white matter. These areas demonstrated a loss of myelin by Heidenhain–Woelcke stain (Figure 4). Within the demyelinated areas, some preservation of axons was noted in Bodian stained sections. Surrounding the zone

Table 1. Anatomical distribution of neuroserpin bodies.

| Anatomical region | Semiquantitative assessment of NBs |
|----------------------------------|------------------------------------|
| Frontal cortex | 3+ |
| Cingulate cortex | 3+ |
| Temporal cortex | 3+ |
| Parietal cortex | 3+ |
| Insular cortex | 3+ |
| Occipital cortex | 3+ |
| Caudate nucleus | 3+ |
| Putamen | 3+ |
| Globus pallidus | 1+ |
| Clastrum | 3+ |
| Thalamus | 1+ |
| Hypothalamus | 3+ |
| Hippocampus—CA1 | 3+ |
| Hippocampus—CA2/3 | 1+ |
| Hippocampus—CA4 | 1+ |
| Hippocampus—Dentate gyrus | — |
| Subiculum | 3+ |
| Entorhinal cortex | 3+ |
| Substantia innominata | 2+ |
| Cerebellar cortex | — |
| Dentate nucleus | rare |
| Midbrain | |
| Colliculi | 2+ |
| Periaqueductal gray | 2+ |
| Substantia nigra | 3+ |
| Red nucleus | 2+ |
| Oculomotor nucleus | 3+ |
| Pons | |
| Locus coeruleus | 3+ |
| Dorsal nucleus of Raphe | 2+ |
| Trigeminal nucleus | 3+ |
| Pontine nuclei | 1+ |
| Medulla | |
| Hypoglossal nerve nucleus | 2+ |
| Dorsal motor nucleus of vagus | 3+ |
| Lateral reticular nucleus | 1+ |
| Inferior olivary nucleus | — |
| Vestibular nuclei | 1+ |
| Spinal cord | |
| Anterior horn cell | 3+ |
| Intermediolateral column nucleus | 1+ |
| Nucleus of Clark | 2+ |
| Posterior horn | 1+ |
| Dorsal root ganglia neurons | 2+ |

Abbreviation: NBs = neuroserpin bodies.

of demyelination was reactive gliosis. Perivascular lymphoplasmacytic infiltrates were also seen within or nearby the areas of demyelination. Microscopic examination revealed multiple foci of demyelination within the cerebrum, predominantly in periventricular white matter, in the frontal, parietal, temporal and occipital lobes. Focal involvement of the corpus callosum was also observed. Additional areas of demyelination included the anterior

commissure, right cerebral peduncle, pons, medulla, posterior columns of the cervical spinal cord and corticospinal tract in the thoracic spinal cord.

Genetic analysis

DNA extracted from fresh blood and fixed brain tissue was used to amplify and sequence exons 2–9 of the *PII2* gene. A single nucleotide (T to C) substitution at nucleotide 140, the second position of codon 47 located in exon 2, was found in one allele of the patient's *PII2* gene (Figure 5). This nucleotide change would result in a proline for leucine amino acid substitution (L47P).

DISCUSSION

We describe a patient with a previously unreported mutation in the *PII2* gene that is associated with accumulation of neuroserpin in neurons and a clinical phenotype of progressive myoclonus epilepsy and dementia. Mutations in the neuroserpin gene have previously been associated with progressive myoclonus epilepsy and also with familial presenile dementia. This case appears to be a *de novo* mutation, as neither of the patient's parents or other family members were noted to have a similar type of neurological disease; although genetic confirmation of paternity was not available. A previous *de novo* mutation has been reported with genetic confirmation (3).

Five other mutations in the neuroserpin gene have been previously reported (Table 2) (3–6, 8, 24). Neuroserpin, like many of the other serine protease inhibitors with which it shares structural homology, normally displays conformational mobility in order to stably bind the target molecule that it is inhibiting. This structural mobility makes the molecule susceptible to point mutations, predominantly within two domains, encoded by exons 2 and 8. Using protein models, the regions most affected by mutations appear to be located near strands 3 and 5 of the molecule's main β -sheet. These strands display a shutter-like opening and closing during conformational transitions (6, 21), which is necessary for the proper function of the protein. The S49P mutation is thought to produce instability of the molecule because of the presence of two neighboring proline residues, whereas the substitution of a larger arginine at residue 52 appears to physically disrupt the movement of the β -sheet strands (21). Ultimately, mutations in these key regions cause the molecules to lose their normal functionality and to form abnormal intracellular aggregates (6). Biochemical analysis of the aggregates has revealed that they are composed entirely of the mutant neuroserpin protein (26). *In vitro* studies have shown that the mutant neuroserpin proteins readily form loop-sheet polymers under physiological conditions (1). Cell models have demonstrated that the mutant proteins are retained within the endoplasmic reticulum shortly after synthesis (16).

The exact effects of the L47P mutation on the neuroserpin molecule is not known; however, this amino acid is in close proximity to the mobile 3 and 5 strands and may also affect the movement or stability of this region. The L47P mutation shows greater similarity, both clinical and neuropathologic, to the S52R mutation. Both mutations are associated with an age of onset in the third decade, clinical manifestations of progressive myoclonus epilepsy, progressive cognitive decline and death in the fourth to fifth decade. The neuroserpin inclusion bodies are profusely distributed

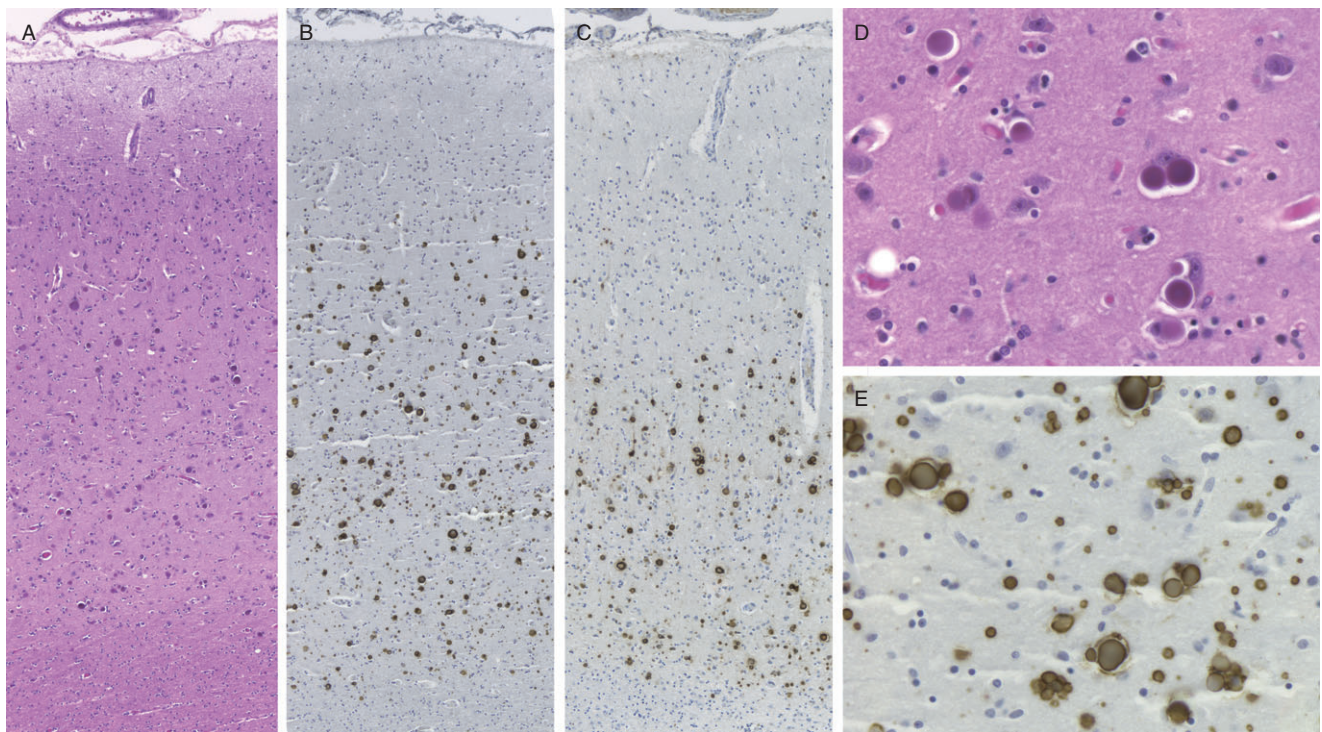


Figure 2. Cerebral cortex with neuroserpin inclusion bodies. The round, eosinophilic inclusions are more numerous with the deeper cortical layers. **A.** Frontal lobe, hematoxylin–eosin (H&E). **B.** Frontal lobe, neuroserpin immunohistochemistry (IHC). **C.** Occipital lobe, neuroserpin IHC.

The inclusions are present within the neuronal perikaryon and neuropil. One or multiple inclusions may be present within affected neurons. **D.** H&E, magnification $\times 400$. **E.** Neuroserpin IHC, magnification $\times 400$.

through much of the gray matter of the cerebrum, brainstem and spinal cord with relative sparing of the cerebellum. This is in contrast to the reported findings of the S49P mutation, where the patients develop a progressive dementia in the fifth to sixth decade with tremors and only rare seizures (2, 4–6, 8). The three mutations that have been reported involving exon 8 display a more severe phenotype, typically with onset in the second decade of life (3, 6, 8).

The clinical severity of the mutations in the neuroserpin gene has been correlated with the predicted degree of molecular instability in protein models of the mutations (6, 21). The severity of the clinical phenotypes of four of the mutations (S49P, S52R, H338R, G392E) was recently found to also correlate with the degree of polymerization, slowed intracellular trafficking and retention of the

mutant neuroserpin proteins in the endoplasmic reticulum within transfected cell lines (17). Additionally, transgenic drosophila models showed differences in onset of locomotor deficits that paralleled the clinical severity of these mutations (17). A transgenic mouse model expressing human neuroserpin with the G392E mutation showed aggregation of neuroserpin in neurons with a similar anatomical distribution as found in humans harboring the mutation. The aggregates were found in the endoplasmic reticulum and lysosomes. Additionally, the transgenic mice showed a lowered seizure threshold to intraperitoneal kainate administration (25). The accumulation of neuroserpin inclusions within the endoplasmic reticulum of neurons was also observed by ultrastructural examination of post-mortem tissue from an individual with the S52R mutation (24).

Table 2. Neuroserpin mutations.

| Mutation: Protein | Mutation: Nucleotide | Age at onset (years) | Age at death (years) | Clinical features | Ref. |
|-------------------|----------------------|----------------------|----------------------|--|------|
| Leu47Pro (L47P) | c.140 C > T | 24 | 34 | Progressive myoclonus epilepsy with dementia | |
| Ser49Pro (S49P) | c.145 T > C | 48 | 57 | Dementia, tremor, rare seizures | (5) |
| Ser52Arg (S52R) | c.154 A > C | 24 | 43 | Progressive myoclonus epilepsy with dementia, status epilepticus | (24) |
| His338Arg (H338R) | c.1013 A > G | 15 | 23 (biopsy) | Progressive myoclonus epilepsy with dementia, tremor, dysarthria | (6) |
| Gly392Glu (G392E) | c.1175 G > A | 13 | 19 | Progressive myoclonus epilepsy with dementia, status epilepticus, dysarthria | (6) |
| Gly392Arg (G392R) | c.1174 G > A | 8 | N/A | Severe dementia, status epilepticus | (3) |

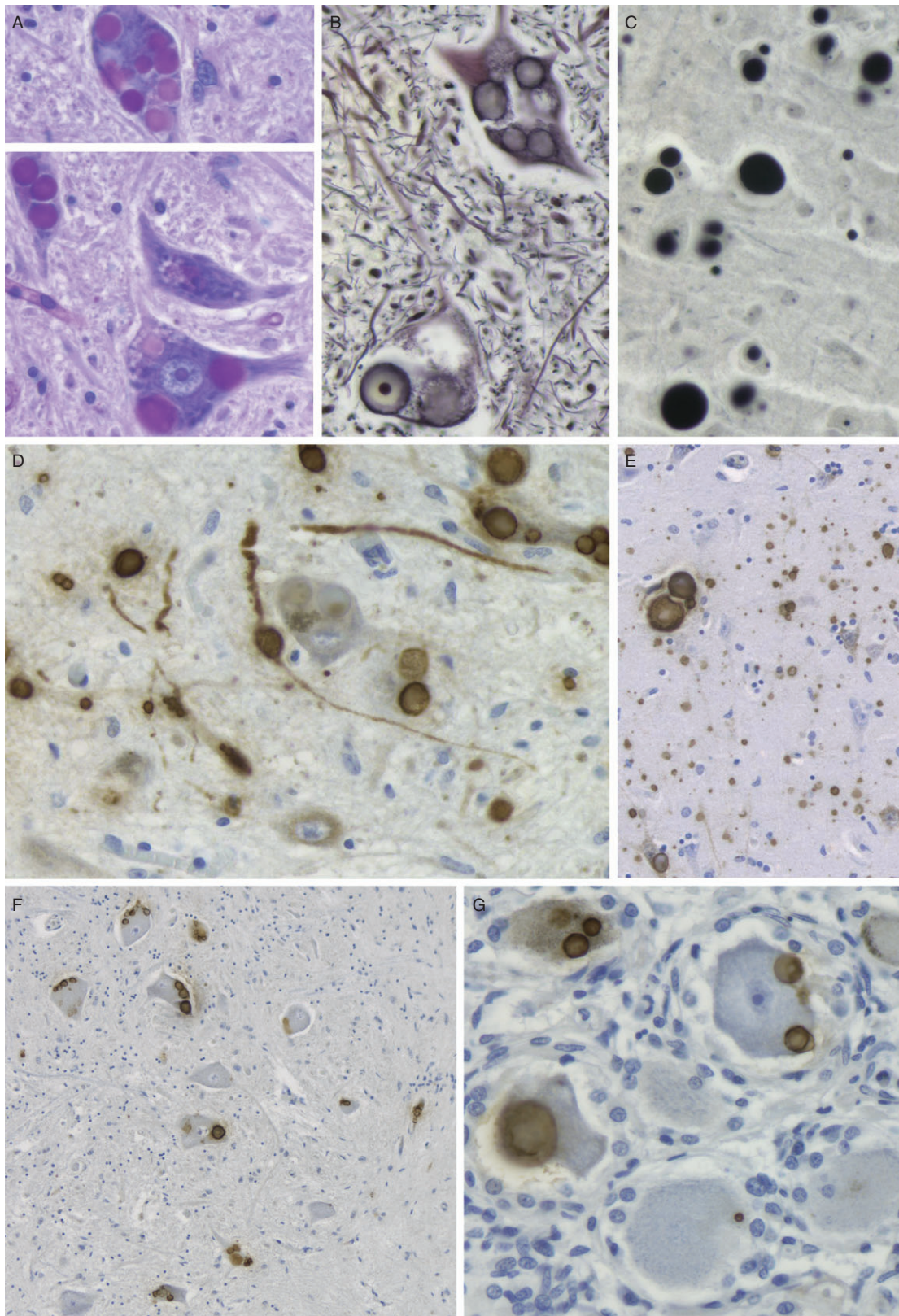


Figure 3. *Neuroserpin inclusion bodies.* (A) PAS and (B) Bodian stains of locus coeruleus. (C) Heidehain–Woelcke stain of occipital cortex. Immunohistochemical stain for neuroserpin in (D) substantia nigra, (E) CA1 region of hippocampal pyramidal cell layer, (F) lumbar spinal cord, anterior horn and (G) dorsal root ganglion. Magnification: A–D and G, $\times 400$; E, $\times 200$; F, $\times 100$.

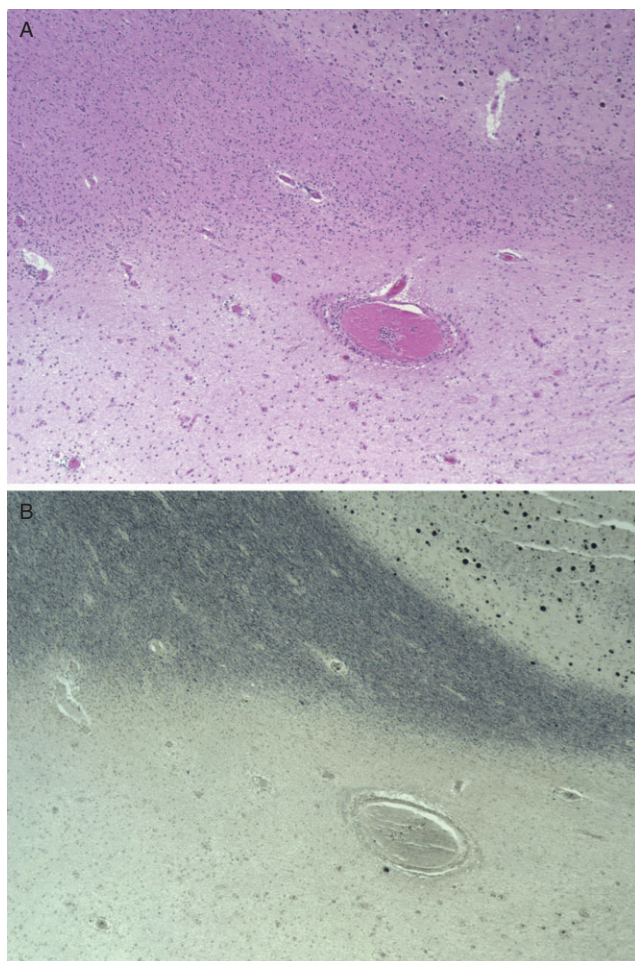


Figure 4. Demyelination within occipital white matter. There is pallor of the white matter by H&E (A), which corresponds to loss of myelination seen with Heidenhain–Woelcke stain (B). Note the mild perivascular inflammation around the large vessels towards the center of the micrograph. The neuroserpin inclusions are evident in the adjacent cortex (upper right side of images). Magnification: $\times 50$.

Although the intracellular accumulation of the mutant neuroserpin appears to cause a toxic gain of function that may lead to cell death, the loss of the normal function of neuroserpin may contribute to the neurodegenerative process. Neuroserpin is an inhibitor of tPA, which has been shown to be a serine protease that is widely expressed within the CNS during events that involve neuronal plasticity, such as long-term potentiation, kindling and seizures (20). Neuroserpin has been found to have an inhibitory effect on seizure progression in a mouse model, indicating its effects are through the regulation of tPA (27). *In vitro* and *in vivo* studies have also demonstrated neuroprotective effects of neuroserpin against N-Methyl-D-aspartate (NMDA) receptor-mediated excitotoxicity following experimental ischemia (13). Overexpression of neuroserpin was also found to decrease neuronal cell loss and reduce the phenotype of a transgenic mouse model of motor neuron disease (23). Neuroserpin has also been shown to bind β -amyloid and appears to reduce the cytotoxicity of $A\beta_{42}$ (12).

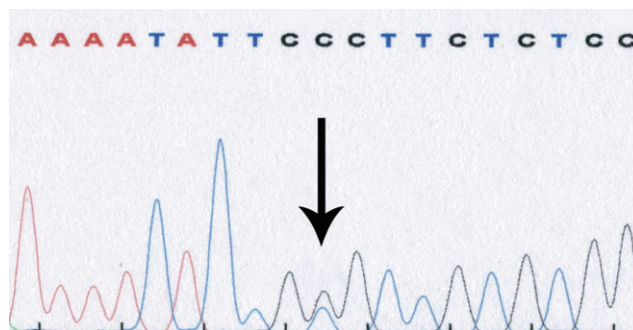


Figure 5. Single nucleotide substitution within *PII2* gene. DNA sequence showing codons 44 through 50. A single nucleotide (T to C) substitution at the second position of codon 47 (arrow) located in exon 2 was found in one allele, which would produce a proline for leucine amino acid change at that site (L47P).

Although the patient's demyelinating disease appears to be an independent finding unrelated to his neuroserpinopathy, there is a possibility that the *PII2* mutation may have contributed to the development of demyelination. tPA has been investigated in experimental models of multiple sclerosis, such as allergic encephalomyelitis (7), with suggestions that deregulation of tPA may play a role in demyelination. A generalized decrease in tPA has been found in brain tissue from patients diagnosed with multiple sclerosis, with focally increased deposition around active plaques and demyelinated axons (9). As neuroserpin is a key regulator of tPA within the CNS, disruption of its normal function may have indirectly contributed to the patient's demyelinating process. This association, however, is not supported by the finding that tPA tends to form complexes with plasminogen activator inhibitor-1, but not neuroserpin, in brain tissue from patients with multiple sclerosis (10). The presence of multiple sclerosis in a patient with a neuroserpin mutation has not been previously reported.

Given the widespread involvement of the gray matter by the neuroserpin inclusions and the multiplicity of the plaques of demyelination, segregation of the patient's signs and symptoms resulting from each process is problematic. Both may have contributed to his general cognitive impairment. His progressive myoclonus epilepsy parallels what has been found in other cases with a mutation in the *PII2* gene. Although a patient with the S52R mutation exhibited hypoalgesia in a stocking-glove distribution (24), loss of vibratory and position sensation has not been reported in other neuroserpinopathy patients. The sensory loss, in this case, was likely secondary to the demyelination identified in the dorsal columns of the spinal cord.

In conclusion, we describe for the first time a patient with encephalopathy with neuroserpin inclusion bodies who had a novel L47P mutation in the *PII2* gene. This appears to be a *de novo* mutation, as no other family members are known to have a similar neurological disorder. The clinical and neuropathological findings in this case were discussed in relationship to the other known mutations. The L47P mutation appears to show the greatest clinical and pathological similarity to the S52R mutation. A unique feature seen in this patient was the coexistence of a demyelinating process consistent with multiple sclerosis.

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