

Clinical and Serial MRI Findings of a Sialidosis Type I Patient with a Novel Missense Mutation in the *NEUI* Gene

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Abstract

The case of a Japanese sialidosis type I patient with a novel *NEUI* gene mutation is described. The patient developed an unsteady gait at age 14 and was referred to our hospital at age 16. On admission, subnormal intelligence, dysarthria, myoclonus, intentional tremors, limb and gait ataxia, hyperreflexia and macular cherry-red spots were observed. An enzymological analysis revealed a primary deficiency of neuraminidase. An *NEUI* gene analysis identified two heterozygous missense mutations: p.P80L and p.D135N. The p.D135N mutation is a novel mutation that is considered to be associated with the mild clinical phenotype of sialidosis. Serial brain MRI showed diffuse brain atrophy progressing rapidly over the 41-month observation period.

Key words: sialidosis, neuraminidase, ataxia, cherry-red spot, myoclonus, magnetic resonance imaging

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Introduction

Sialidosis (MIM# 256550) is an autosomal recessive lysosomal storage disease (1) caused by mutations in the *NEUI* gene encoding the enzyme lysosomal sialidase (neuraminidase, EC 3.2.1.18) (2-4), which leads to decreased enzymatic activity and accumulation of sialyloligosaccharides in tissues. Sialidosis is divided into two main clinical variants with different ages of onset and severity. Sialidosis type I, also known as “cherry-red spot myoclonus syndrome,” is a relatively mild form of the disease with a late onset. Patients usually develop gait abnormalities, progressive impaired vision, bilateral macular cherry-red spots, myoclonus, ataxia and seizures in the second or third decade of life (1, 5-7). Sialidosis type II is the early-onset form and is associated with macular cherry-red spots, the Hurler-like phenotype, dysostosis multiplex, short stature, developmental delays, mental retardation and hepatosple-

nomegaly (1, 8, 9). The age of onset and severity of the clinical manifestations are correlated with *NEUI* mutations (10, 11) and the level of residual neuraminidase activity (10, 12, 13), indicating the existence of considerable genotype-phenotype correlation in this disease. To date, more than 40 mutations within the *NEUI* gene have been identified in patients with sialidosis type I or type II (2, 3, 10-24).

We herein report the clinicopathological, neuroradiological, enzymological and molecular biological findings of a Japanese sialidosis type I patient with a novel missense mutation in the *NEUI* gene.

Case Report

The patient, a 17-year-old Japanese young man, was the second child of non-consanguineous healthy parents and had a healthy sibling. He was born at term after a normal pregnancy with a birth weight of 3,550 g (Apgar scores: 9 at 1

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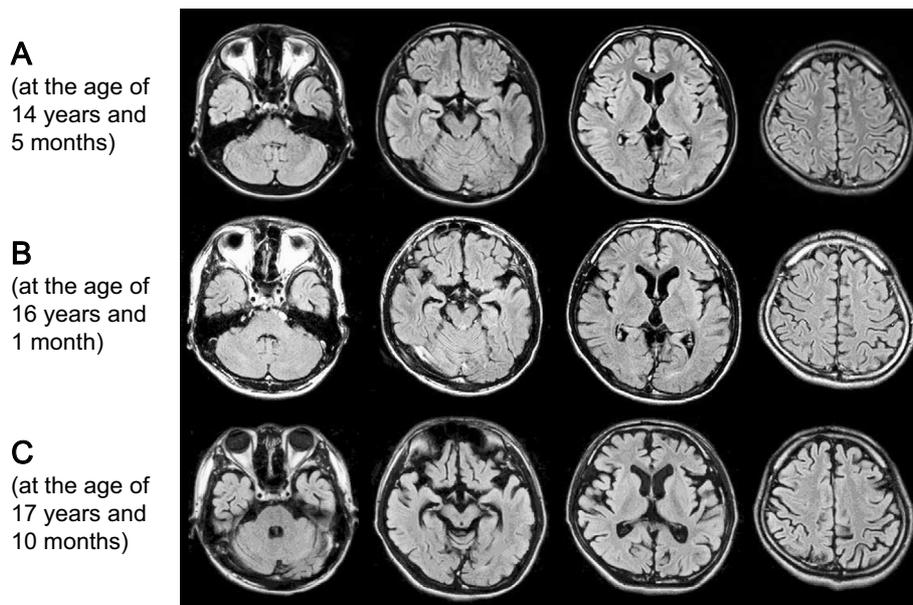


Figure 1. Serial brain MRI of the patient (FLAIR image, axial view). A (top row), B (middle row) and C (bottom row) show MRI findings obtained at 14 years and five months, 16 years and one month and 17 years and 10 months of age. Diffuse brain atrophy became more evident with age. Brain atrophy was more obvious in the inferior parts of the temporal lobe, cerebellum and brainstem region.

minute and 10 at 5 minutes). He showed normal development up to the age of 14 when he noticed an unsteady gait and frequent falls precipitated by sudden movements. He was admitted to a local hospital where he was suspected of having familial paroxysmal kinesigenic dyskinesia. At that time, mild enlargement of the lateral ventricles was detected on brain magnetic resonance imaging (MRI) (Fig. 1A). The patient was treated with carbamazepine, which did not improve his symptoms. At age 15, he noticed frequent jerky involuntary movements when walking, writing and eating and was admitted to another local hospital. On admission, dysarthria, action myoclonus, intentional tremors, cerebellar ataxia and hyperreflexia were observed. His intelligence was assessed using the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III), which revealed the following results: Full Scale IQ: 86, Verbal IQ: 97, Performance IQ: 76, Verbal Comprehension Index: 97, Perceptual Organization Index: 79, Working Memory Index: 91 and Processing Speed Index: 66. Brain MRI showed mild diffuse brain atrophy, including the cerebral hemispheres, cerebellum and brainstem (Fig. 1B). Clonazepam reduced the myoclonus; however, the patient's unsteady gait gradually deteriorated. He was suspected of having hereditary spinocerebellar ataxia and referred to our hospital at the age of 16 years.

On admission, the findings of a general examination of the patient were unremarkable. He was fully conscious, although his intelligence was subnormal (Full Scale IQ: 80, Verbal IQ: 97, Performance IQ: 64, Verbal Comprehension Index: 109, Perceptual Organization Index: 70, Working Memory Index: 69 and Processing Speed Index: 63 on WAIS-III). He had slurred and scanning speech; however,

there were no abnormal findings in the cranial nerves. The muscles in the extremities were generally hypotonic, although their strength was normal and without atrophy. Intention tremors, action myoclonus and limb and gait ataxia were present. The tendon reflexes were increased without laterality. Babinski and Chaddock signs were negative on both sides. An ophthalmological examination demonstrated bilateral macular cherry-red spots (Fig. 2), although the patient's visual acuity and visual field were both normal.

The findings of routine blood examinations, cerebrospinal fluid, chest roentgenography, electrocardiography, echocardiography, electroencephalography and nerve conduction studies were normal. The somatosensory evoked potential (SEP) evoked by right median nerve stimulation showed a giant cortical response. The visual evoked potential (VEP) showed no response following pattern-reversal visual stimulation in both eyes. Brain MRI revealed moderate diffuse brain atrophy (Fig. 1C), which was more evident in comparison with that observed on the patient's previous MRIs (Fig. 1A, B). MRI of the spinal cord was normal. N-Isopropyl-(iodine-123) *p*-iodoamphetamine (^{123}I -IMP) single-photon emission computed tomography (SPECT) showed slightly decreased uptake in the right occipital and bilateral frontotemporal lobes. An electron microscopic study of a rectal mucosa biopsy showed numerous lamellar storage bodies in the ganglion cells of Meissner's plexus (Fig. 3).

The skin fibroblast neuraminidase activity was significantly decreased (0 nmol/h/mg protein) and other lysosomal enzyme activities, including those of α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -hexosaminidase, β -hexosaminidase A, α -mannosidase, α -

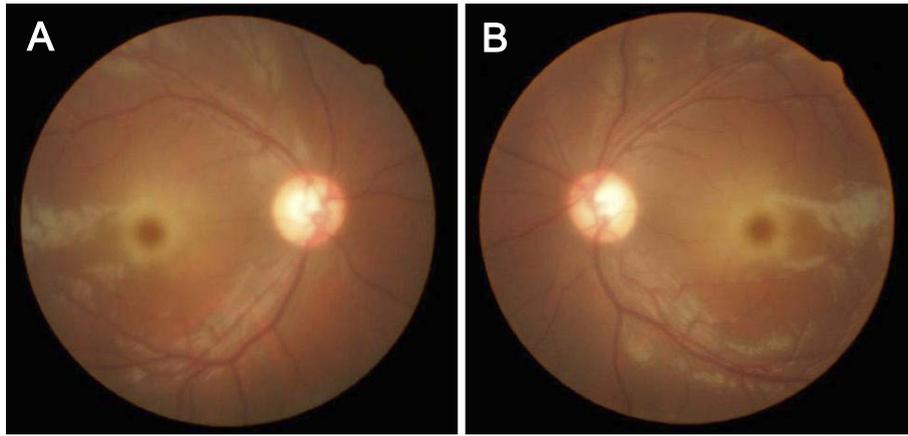


Figure 2. Color fundus photographs of the right (A) and left (B) eyes demonstrating the classic appearance of a cherry-red spot in the maculae.

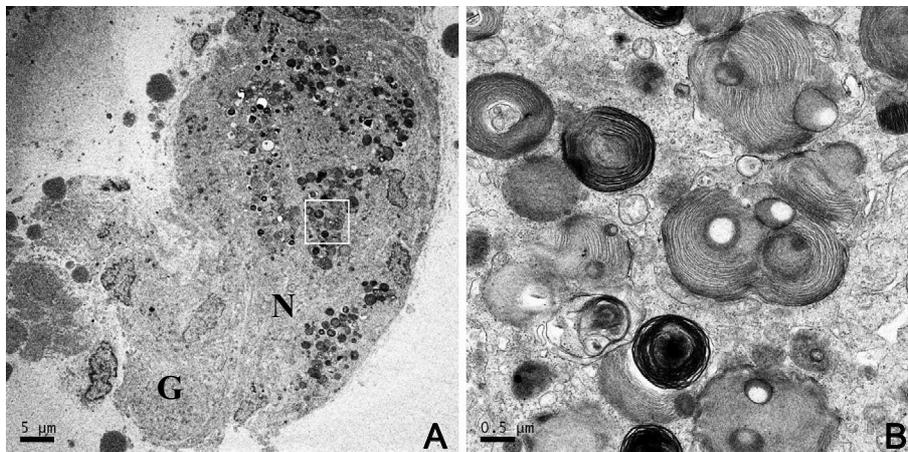


Figure 3. An electron microscopic study of a rectal mucosa biopsy. (A) Rectal ganglion cells (N) of Meissner's plexus contained many small electron-dense inclusions. The glia cells (G) had a normal appearance. Bar = 5 μ m. (B) A high-power view of the rectangle in A. Electron-dense inclusions were composed of osmiophilic lamellar structures lined by a unit membrane. Bar = 0.5 μ m.

fucosidase, β -glucuronidase and arylsulfatase A, were normal, which was compatible with a diagnosis of sialidosis (Table 1).

As the clinical findings and results of an enzymological analysis of the patient were suggestive of sialidosis type I, a genetic analysis of this disorder was performed with informed consent. DNA was extracted from peripheral leukocytes obtained from the patient according to the standard protocol. All 6 exons and the flanking intronic sequences of the *NEU1* gene were amplified using polymerase chain reaction (PCR). The primer sequences and PCR conditions are summarized in Table 2. A direct sequence analysis of the PCR-amplified DNA identified two heterozygous missense mutations, c.239C>T and c.403G>A (Fig. 4), which resulted in amino acid alterations of p.P80L and p.D135N, respectively. Although the *NEU1* genes of the parents were not analyzed, the results suggested that the patient was compound heterozygous for p.P80L and p.D135N. The c.239C>T (p.P80L) mutation has been reported previously in a Japanese sialidosis type II patient (12). On the other hand, the

c.403G>A (p.D135N) mutation has not been previously described (HGMD Professional 2011.3, Human Gene Mutation Database, Biobase, Beverly, MA, USA and SNPs reported in the database (dbSNP) of the National Center for Biotechnology information (NCBI) (<http://www.ncbi.nlm.nih.gov/SNP/>)). The possible impact of a novel p.D135N mutation on the structure and function of neuraminidase was assessed using a bioinformatics tool, Polymorphism Phenotyping-2 (PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>) (25), and was predicted to most likely be damaging (score: 0.991; sensitivity: 0.45, specificity: 0.97).

Discussion

Lysosomal neuraminidase requires carboxypeptidase protective protein/cathepsin A (PPCA, EC 3.4.16.1) for intracellular transport and lysosomal activation (26). Human lysosomal neuraminidase is deficient in two genetic disorders: sialidosis (MIM# 256550), caused by a mutation in the *NEU1* gene (1-4), and galactosidosis (MIM# 256540), in

Table 1. Results of Skin Fibroblast Lysosomal Enzyme Activities

Lysosomal enzyme	Present patient* (nmol/h/mg protein)	Normal control* (n=1)	Normal controls** (n=25, mean ± SD)
Neuraminidase	0.0	176.1	25.0 ± 17.0
α-galactosidase	66.4	143.9	29.6 ± 17.6
β-galactosidase	780.8	1,333.6	401 ± 184.8
α-glucosidase	118.6	264.1	50.6 ± 32.0
β-glucosidase	249.6	265.4	84.7 ± 30.5
β-hexosaminidase	9,017.8	17,060.9	4,678 ± 2,246
β-hexosaminidase A	1,458.7	2,690.1	629 ± 450
α-mannosidase	325.4	485.3	48.7 ± 27.8
α-fucosidase	166.8	332.5	64.6 ± 40.7
β-glucuronidase	51.5	142.9	19.3 ± 12.1
Arylsulfatase A	387.9	526.0	265.7 ± 129.5

* Lysosomal enzyme activities of the patient and a control were analyzed at the same time to rule out technical error. ** Lysosomal enzyme activities were analyzed previously. *** Lysosomal enzyme activities depend on condition of fibroblast, resulting in variation in enzyme activity levels among controls.

Table 2. Sequences of PCR Primers, PCR Product Size, and PCR Annealing Temperatures (T_A) Used for Analysis of the *NEUI* Gene

	Primer sequence 5'→3'	PCR product size (bp)	T _A (°C)
Exon 1F	gcttaagggtgacatctgcgcttt	373	60
Exon 1R	tgggagaagaaaaggctctgtc		
Exon 2F	aactcccctctgttctcttttc	443	60
Exon 2R	caaccaaccctctaagtccctctatc		
Exon 3F	ctagcagaaggtggaaattaacgg	427	60
Exon 3R	gaaaggagtcattgggggtatc		
Exon 4F	attgggaagtggtgggttctctg	500	60
Exon 4R	agtgttagttgttctgtttcggg		
Exon 5F	agatgttccctaccatttgacc	525	60
Exon 5R	catgaggtaccattgctgaagctc		
Exon 6F	cattgtcttcttccaaccage	514	60
Exon 6R	gatttccttggtaaagggaaggtg		

PCR primers complementary to intronic sequences flanking each of the 6 exons were designed based on the published genomic sequence of the *NEUI* gene (NCBI Reference Sequence: NG_008201.1).

which the loss of neuraminidase activity is secondary to a deficiency of PPCA (2). An enzymological analysis of our patient revealed a primary neuraminidase deficit with intact β-galactosidase activity. In addition, a molecular genetic analysis of the *NEUI* gene revealed that this patient was compound heterozygous for the p.P80L and p.D135N mutations, thus confirming the diagnosis of sialidosis.

The c.239C>T (p.P80L) mutation has been previously reported in a Japanese patient with severe congenital sialidosis type II (12). The P80 residue is situated in a conserved FRIP motif that is located at the N-terminus of the first strand of the first β-sheet unit. The side chain of the P80 residue is adjacent to the active site residue R78, as well as to Y370 and E394, which are located in the first strand of the sixth β-sheet unit in the wild-type neuraminidase protein. The amino acid substitution of P80 to L causes conformational changes in the main chain that affect the position of the active site residues R78, Y370 and E394 (12). In ad-

dition, a transient expression study of lysosomal neuraminidase cDNA revealed that p.P80L proteins do not show enzymatic activity and do not exhibit the typical lysosomal distribution in cultured cells, compatible with the severe congenital phenotype (12). On the other hand, c.403G>A (p.D135N) is a novel missense mutation. The D135 residue is one of the active sites of human lysosomal neuraminidase (4, 12), and amino acid substitution of D135 to N was predicted to alter the structure and function of the enzyme using the bioinformatics tool, PolyPhen-2. Based on the clinical findings of our patient and the biological properties of the second mutation (p.P80L), the novel p.D135N mutation was considered to be associated with a relatively mild clinical phenotype of sialidosis (sialidosis type I).

Diffuse brain atrophy is commonly observed in the advanced stage of sialidosis type I; however, the results of neuroradiological imaging can be normal on the first examination in affected patients (19, 20, 27-29). There have been very few studies regarding long-term changes in the findings of neuroimaging (20, 29), and it is largely unknown how rapidly brain atrophy progresses in patients with sialidosis type I. Chen et al. (19) reported the results of a long-term follow-up study of sialidosis type I siblings homozygous for the p.S182G missense mutation. The ages at onset in these patients were 14 and 17 years. The brain MRI findings of the younger brother remained normal even 18 years after onset, while those of the older sister showed mild cerebellar atrophy 11 years after onset. Palmeri et al. (29) reported the case of a 40-year-old woman who developed sialidosis type I at 17 years of age. A brain computed tomography (CT) scan of the patient performed at 21 years of age showed slight enlargement of the fourth ventricle and cisterna ambiens, whereas severe atrophy of the cerebellum, pontine region, cerebral hemispheres and corpus callosum were observed on MRI at the age of 40. Pathologically, cytoplasmic accumulation of sialyloligosaccharides has been observed in many neurons in the central nervous systems of sialidosis

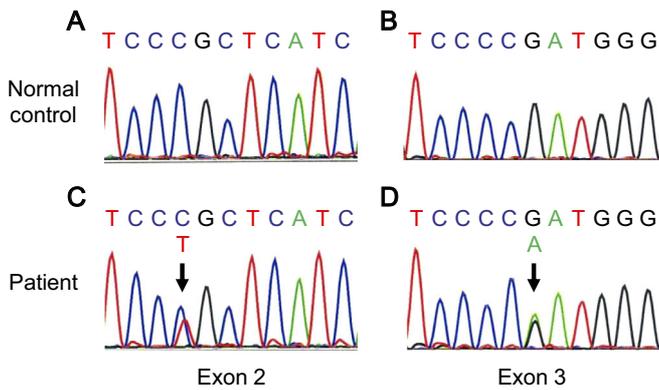


Figure 4. Direct nucleotide sequencing of the PCR-amplified *NEU1* gene DNA of a control (A, B) and the patient (C, D). The vertical arrow indicates nucleotide 239 (exon 2), where a C→T transversion (c.239C>T) resulted in an amino acid substitution, p.P80L (A, C). The arrow head indicates nucleotide 403 (exon 3), where a G→A transversion (c.403G>A) resulted in an amino acid substitution, p.D135N (B, D). The patient was compound heterozygous for the p.P80L and p.D135N mutations.

type I patients (30, 31). However, neuronal loss in these nuclei is not frequent despite massive accumulation of sialyloligosaccharides even 15 years after the onset of the disease (31), which may explain why the results of neuroradiological imaging can remain normal for a considerable period of time in sialidosis type I patients. In contrast to the findings of previous reports (19, 20), mild dilatation of the lateral ventricles was observed at the onset of disease in our patient (Fig. 1A), and his brain atrophy progressed rapidly over the 41-month observation period (Fig. 1B, C). Although the pathological mechanisms underlying this phenomenon remain to be elucidated, our observations demonstrate that brain atrophy can progress rapidly, even in the early stage of sialidosis type I.

In our patient, VEP showed no response in either eye, although the patient's visual acuity and visual field were normal. Lai et al. (20) reported that, although only four of 17 (23.5%) sialidosis type I patients initially complained of visual disturbance, 16 (94.1%) showed abnormal findings on VEP, suggesting that VEP can be a valuable neurophysiological test in the diagnosis of patients with sialidosis type I who do not present with the typical manifestations of the disease.

The authors state that they have no Conflict of Interest (COI).

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