Clinical Case Reports

CASE REPORT

The first genetically confirmed Japanese patient with mucolipidosis type IV

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Key Clinical Message

Mucolipidosis type IV (MLIV) is a rare neurodegenerative disorder characterized by severe psychomotor delay and visual impairment. We report the brain pathology in the first Japanese patient of MLIV with a novel homozygous missense mutation in *MCOLN1*. We detected the localized increase in p62-reactive astrocytes in the basal ganglia.

Keywords

Autophagy, *MCOLN1*, mucolipidosis type IV, neuropathology, p62.

Mucolipidosis type IV (MLIV) is a neurodegenerative disorder characterized by severe delay in psychomotor development, progressive visual impairment, and achlorhydria. MLIV is an autosomal recessive condition caused by mutations in *MCOLN1*, which encodes for mucolipin-1, a member of the transient receptor potential (TRP) cation channel family [1]. To the best of our knowledge, MLIV has rarely been reported in Japan, indicating a potential for misdiagnosis arising from the little understanding of this disorder. We detected a novel mutation in *MCOLN1* in a 68-year-old man with a protracted course of MLIV, whose third elder brother was diagnosed with protracted juvenile neuronal ceroid lipofuscinosis (JNCL) erroneously [2]. In the autopsy brain of the third elder brother, the neurons in the cerebral cortex and basal ganglia demonstrated accumulation of lipofuscin-like materials, being immunoreactive for mitochondrial ATP synthase subunit C, leading to the erroneous diagnosis of JNCL.

He was born from consanguineous parent. His elder sister died of undetermined cause in the neonatal period, and his eldest brother had chronic kidney disease from the age of 70 years. His second and third elder brothers developed progressive visual impairment and psychomotor disability in their second decade who died in their fifties. His motor development was normal, and he was able to run and managed to use chopsticks although he had mild mental retardation. At the age of 6, he showed visual impairment, which gradually progressed, and he became completely blind at the age of 12. He developed spastic gait in his thirties and could not walk at the age of 40. Seven years later, he was diagnosed with JNCL based on the presence of fingerprint structures in the nerve biopsy. He was admitted to our center at the age of 48. He crawled and uttered two- and three-word sentences with dysarthria. His cognitive development corresponded to that of a 3-year-old boy. In addition to blindness, he had severe cataract. His deep tendon reflexes were exaggerated in the lower extremities. Ankle clonus and Babinski's reflex were positive. He showed absent gag reflex and his jaw jerk was exaggerated. Electroretinogram showed no

responses. He had hypertension and his electrocardiogram showed hypertrophy of the left ventricle. He developed chronic kidney disease and liver dysfunction at the age of 50. Crawling and uttering of words vanished at the age of 55. He developed jaw opening dystonia and worsening of rigidity in the upper extremities when he was 58 years old. Trihexyphenidyl hydrochloride was effective for oromandibular dystonia. Tube feeding was required at the age of 66, and he died of chronic renal failure at the age of 68. He had neither epileptic seizures nor iron-deficiency anemia. Vacuolated lymphocytes were detected, comprising about 1% of lymphocytes. In auditory brainstem responses, the latency time between the first and fifth waves became elongated gradually and the amplitude

of wave components reduced with age. Brain magnetic resonance imaging (MRI) performed when he was 54 years old, demonstrated cerebral and cerebellar atrophy, hypoplasia of the corpus callosum, and high signals on T2-weighted images in the periventricular and subcortical white matters (Fig. 1A).

Genomic DNA was obtained from the blood leukocytes. Whole exome sequencing (WES) was performed as previously described [3]. The average mean read depth of the protein-coding regions of RefSeq genes was 104×, such that 96.4% of target coding sequences were covered by 10 reads or more. We found a homozygous missense mutation [c.1292G>A (p.Cys431Tyr)] in *MCOLN1*, which encodes mucolipin-1. This mutation was not found in
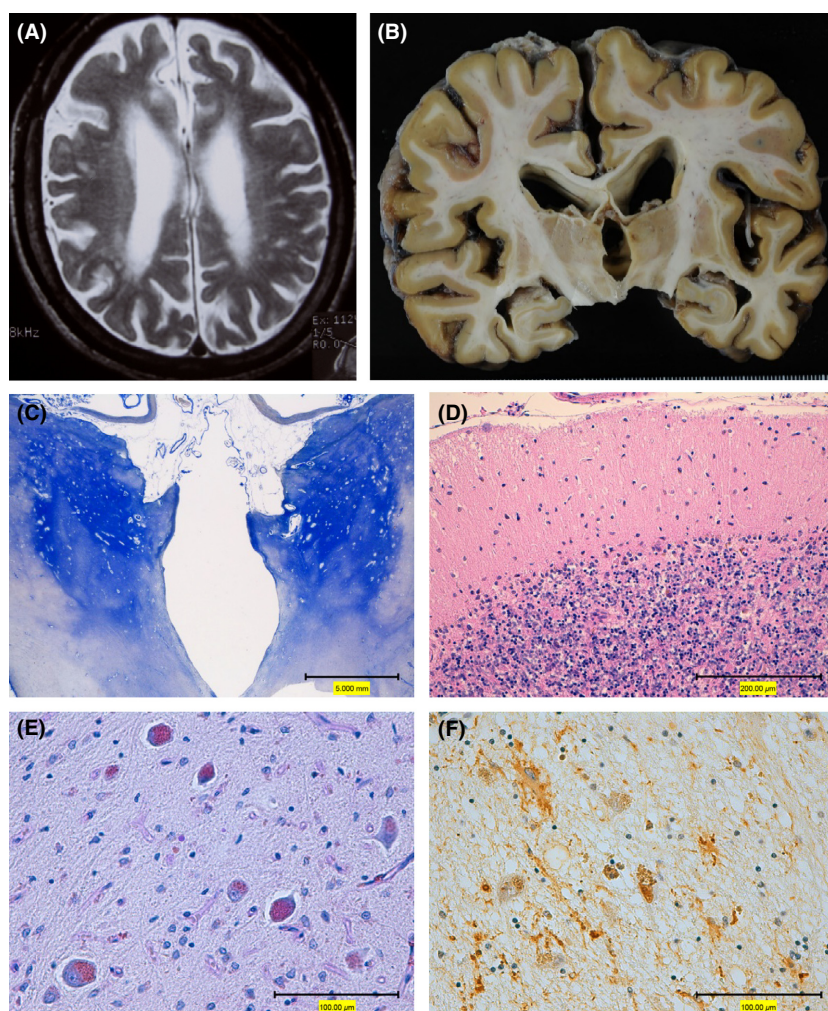

Figure 1. A. T2-weighted axial image revealed signal changes in the periventricular and subcortical white matters. B. The cross section of the brain showed thin corpus callosum and mild enlargement of lateral ventricles and the third ventricle. C. The bilateral thalamus showed moderate fibrillary gliosis (Holzer staining, bar = 5.000 mm, denoting 5 mm). D. The Purkinje and granule cells were reduced in the cerebellar cortex (hematoxylin-eosin staining, bar = 200 μm). E. The dentate neurons had accumulation of lipofuscin-like materials (c) (periodic acid Schiff staining, bar = 100 μm). F. The number of astrocytes immunoreactive for p62 was increased in the substantia nigra (bar = 100 μm).

dbSNP137 data or among our 408 in-house Japanese control exomes. The p.Cys431Tyr mutation has not been previously reported in other patients with mucolipidosis type IV, and was predicted as damaging/disease-causing by SIFT, PolyPhen2, and Mutation Taster.

The brain weighed 910 g at autopsy, and the cerebrum, cerebellum, brainstem, and spinal cord were proportionally small. There was no focal dysplasia or sclerosis in the cerebral surface. On the cross section, the corpus callosum appeared thin from the genu to the splenium. The volume of white matter was reduced in the middle and inferior temporal lobes and the insular lobe (Fig. 1B). Histologically, neurons and the six-layer structure in the cerebral cortex were comparatively spared; however, the pyramidal neurons in the cerebral cortex and hippocampus demonstrated accumulation of lipofuscin-like materials (LFM) positive for periodic acid Schiff and oil red O staining. Fibrillary gliosis was found in the white matter of the middle and inferior temporal lobes and the insular lobes. Mild fibrillary gliosis was seen in the globus pallidus, more predominantly in the lateral segment. Although neurons were comparatively well preserved in the striatum, globus pallidus, and subthalamic nucleus, moderate fibrillary gliosis (Fig. 1C) and patchy neuronal loss was observed in the anterior, medial, and lateral nuclei in the thalamus. In the cerebellar cortex, except in the tonsil, the Purkinje cells and granule cells were moderately reduced (Fig. 1D), Bergmann glia was increased. The number of dentate neurons was preserved, but they had accumulation of LFM (Fig. 1E). The astrocytes immunoreactive for p62, interacting with LC3 on the autophagosome membrane, were increased in the globus pallidus, subthalamic nucleus, medial nucleus in the thalamus, and substantia nigra (Fig. 1F).

The clinical manifestations of typical MLIV consist of severe psychomotor delay, muscle tonus abnormalities, corneal clouding, and retinal dystrophy, resulting in visual impairment, and cranial nerve involvement. Most patients are unable to speak and walk without support [1] and demonstrate slowly progressive neurological disorders. Our case developed ocular manifestations in the first decade. Nevertheless, he showed motor functions such as running or walking independently, and he conversed during his childhood and adolescence. His psychomotor delay was not severe at the early stage of disease, although motor abnormalities were aggravated around the third decade gradually, leading to spastic paraplegia. He developed jaw opening dystonia and worsening of rigidity in the upper extremities in his fifties. The neurological manifestations seem to be variable and complex, hindering clinicians from establishing the diagnosis of MLIV [4].

The common mutations in *MCOLN1* in Ashkenazi Jewish population are rarely found in non-Jewish patients

[5]. Hence, more comprehensive WES was performed in our case and succeeded to disclose the unreported mutation, c.1292G>A (p.Cys431Tyr), encoding the amino acid, which is included in the trans-membrane domain 5 [6], and adjacent to the TRP channel pore. The phenotype in our case was milder than that in Ashkenazi Jewish patients with the mutations of residues, which encode the amino acids composing the intraluminal loop. Recent investigations have suggested that the autophagy process is defective in MLIV cells, such as mucolipin-1-deficient mouse neurons [7] and patient fibroblasts [8]. Alterations in mucolipin result in the accumulation of heterogeneous lipids and proteins in the cytoplasmic vacuoles derived from lysosomes [9]. Regulation of the rate of lysosomal metabolism is clearly associated with mucolipin function [4]. Defective lysosomal function caused by mutations in *MCOLN1* may disturb the fusion of autophagosomes with lysosomes, and the subsequent degradation, in macroautophagy. The delayed fusion of autophagosomes with lysosomes seemed to lead to the accumulation of autophagosomes in the fibroblasts of MLIV patients [8]. Accumulation of mis-folded proteins may not be deleterious in rapidly dividing cells; however, the accumulation of protein inclusions is harmful for neurons, most of which do not divide in the adult brains [8].

P62/SQSTM1 is an LC3- and ubiquitin-binding protein thought to play a role in targeting of ubiquitinated proteins for lysosomal degradation in the macroautophagy pathway [10]. In MLIV, p62 was found to accumulate in the patient fibroblasts as protein inclusions [8]. In the brains of *Mcoln1*$^{-/-}$ mice, p62-immunoreactive inclusions were found throughout the central nervous system, suggesting the occurrence of disturbed macroautophagy and impaired protein degradation in the brains [11]. We immunohistochemically investigated the accumulation of p62 in our case, in addition to the expression of LC3. Although there were no abnormalities in LC3 immunohistochemistry, the number of p62-immunoreactive astrocytes was increased in the basal ganglia and substantia nigra, regardless of neuronal loss, gliosis, or LFM accumulation. Possibly, the localized increase of p62-immunoreactive astrocytes in the basal ganglia may be involved in the occurrence of remarked dystonia in our case. Accumulation of p62 is related to not only dysfunction of autophagy but also lysosomal membrane permeability (LMP). Although the relationship between p62 and LMP and neuronal viability requires additional studies, recent studies show the sequestration of released lysosomal contents by p62 may represent a physiological response to the potentially deleterious event [12]. We believe that the involvement of p62 in autophagy disturbance and/or LMP should be investigated in other child-onset neurodegenerative and congenital metabolic errors.

Conflict of Interest

None declared.

References

1. Bach, G. 2001. Mucolipidosis type IV. Mol. Genet. Metab. 73:197–203.

2. Anzai, Y., M. Hayashi, N. Fueki, K. Kurata, and T. Ohya. 2006. Protracted juvenile neuronal ceroid lipofuscinosis-an autopsy report and immunohistochemical analysis. Brain Dev. 28:462–465.

3. Saitsu, H., T. Nishimura, K. Muramatsu, H. Kodera, S. Kumada, K. Sugai, et al. 2013. De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. Nat. Genet. 45:445–449.

4. Wakabayashi, K., A. M. Gustafson, E. Sidransky, and E. Goldin. 2011. Mucolipidosis type IV: an update. Mol. Genet. Metab. 104:206–213.

5. Geer, J. S., S. A. Skinner, E. Goldin, and K. R. Holden. 2010. Mucolipidosis Type IV: a subtle pediatric neurodegenerative disorder. Pediatr. Neurol. 42:223–226.

6. Dong, X. P., X. Wang, D. Shen, S. Chen, M. Liu, Y. Wang, et al. 2009. Activating mutations of the TRPML1 channel revealed by proline-scanning mutagenesis. J. Biol. Chem. 284:32040–32052.

7. Curio-Morelli, C., A. C. Florie, M. C. Micsenyi, Y. Cao, B. Venugopal, M. F. Browning, et al. 2010. Macroautophagy is defective in mucolipin-1-deficient mouse neurons. Neurobiol. Dis. 40:370–377.

8. Vergarajauregui, S., and R. Puertollano. 2008. Mucolipidosis type IV: the importance of functional lysosomes for efficient autophagy. Autophagy 4:832–834.

9. Venugopal, B., M. F. Browning, C. Curio-Morelli, A. Varro, N. Michaud, N. Nanthakumar, et al. 2007. Neurologic, gastric, and ophthalmologic pathologies in a murine model of mucolipidosis type IV. Am. J. Hum. Genet. 81:1070–1083.

10. Pankiv, S., T. H. Clausen, T. Lamrk, A. Brech, J. A. Bruun, H. Outzen, et al. 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J. Biol. Chem. 282:24131–24145.

11. Micsenyi, M. C., K. Dobrenis, G. Stephney, J. Pickel, M. T. Vanier, S. A. Slaugenhaupt, et al. 2009. Neuropathology of the $Mcoln1^{-/-}$ knockout mouse model of mucolipidosis type IV. J. Neuropathol. Exp. Neurol. 68:125–135.

12. Micsenyi, M. C., J. Sikora, G. Stephney, K. Dobrenis, and S. U. Walkley. 2013. Lysosomal membrane permeability stimulates protein aggregate formation in neurons of a lysosomal disease. J. Neurosci. 33:10815–10827.