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ORIGINAL COMMUNICATION

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L-2-Hydroxyglutaric aciduria: two Japanese adult cases in one family

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Abstract We report two adult Japanese sisters with L-2-hydroxyglutaric aciduria (acidemia), both of whom were much older (aged 57, 47 years old) than previously reported patients (from neonate to 44 years old), and who presented with differing severity. Magnetic resonance imaging revealed typical subcortical white matter lesions in both cases and showed brainstem atrophy and thickness of the calvarium in the elder sister. L-2-Hydroxyglutaric acid levels were increased in urine, plasma, and cerebrospinal fluid. These cases suggest that organic acid analysis is necessary even in elderly patients who seem to have neurodegenerative disorders.

Key words L-2-Hydroxyglutaric aciduria · Adult case · Magnetic resonance imaging · Japanese · Elderly

Introduction

L-2-Hydroxyglutaric (L-2-OHG) aciduria is a newly defined and rare inherited metabolic disorder. Duran et al. [7] in 1980 reported the first case in a Moroccan boy with mental and motor retardation; the clinical entity was established when Barth et al. [2] reported eight cases in 1992. Several other cases have since been reported [1, 3, 5, 6, 9, 10, 13, 14, 16, 23, 24]. In Japan only one case has been described, that by Inoue et al. [11, 12] in 1990. These cases have exhibited developmental delay, seizures, cerebellar signs, dystonia, pyramidal signs, and macrocephaly in general; their age range was from neonate to 44 years. Autosomal recessive inheritance was strongly suggested by the family histories. Increased L-2-OHG acid was found in the urine, plasma, and cerebrospinal fluid (CSF) of all investigated patients.

We report two Japanese sister cases with L-2-OHG aciduria who, at 57 and 47 years of age, are much older than previously reported patients, and present new magnetic resonance imaging (MRI) findings in the elder sister.

Case reports

The two sisters were born of nonconsanguineous Japanese parents who had no neurological symptoms; the patients' six siblings also lacked neurological symptoms.

The elder sister (case 1, 57 years old) had generalized tonic-clonic seizure and was found to have psychomotor retardation in childhood. Her mental and motor impairments were slowly progressive, especially after 25 years of age; she became unable to walk at the age of 46. She was admitted to a local hospital at the age of 54;

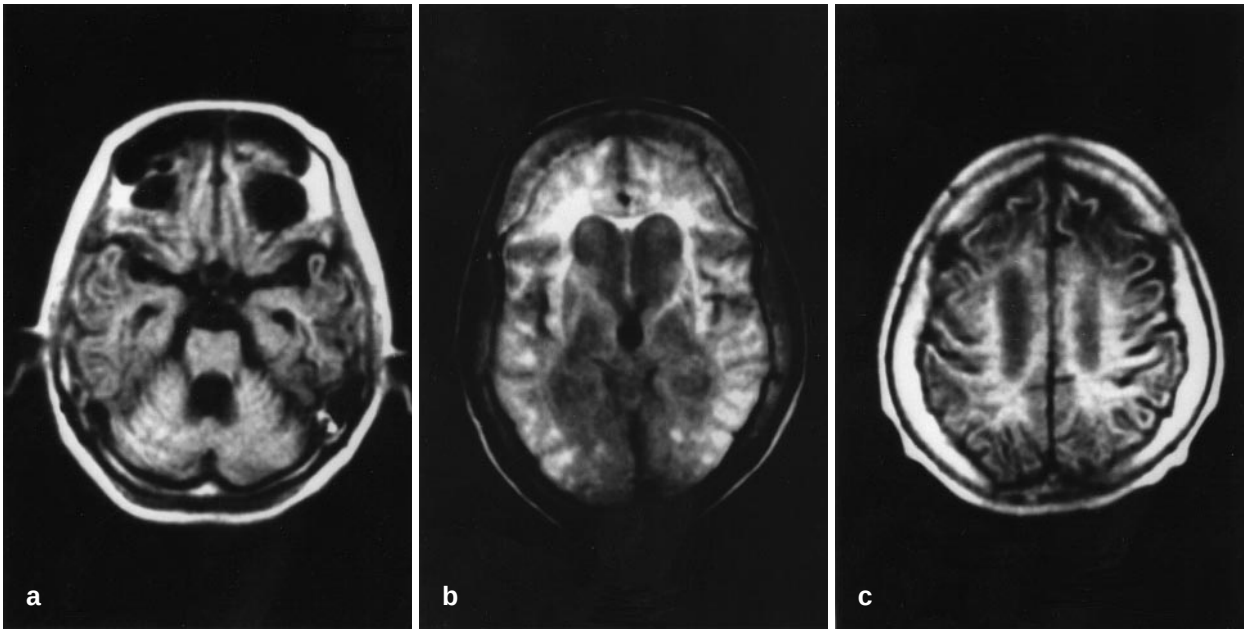

Fig. 1 a–c MRI of case 1 (examined at 55 years of age). **a** Transaxial T1-weighted image (TR 500, TE 30) showing marked enlargement of fourth ventricle and moderate atrophy of pons. Cerebellar vermis is severely atrophic; cerebellar hemispheric folia are mildly atrophic. **b** Transaxial T2-weighted image (TR 2000, TE 80) showing marked ventricular enlargement, and high signal intensities in subcortical white matter, spreading to periventricular white matter in frontal lobe. Genu of corpus callosum is involved. Caudate nuclei, putamina, and globi pallidi are atrophic. Internal capsules are also atrophic; external capsules show high signal intensities. **c** Transaxial T1-weighted image showing low signal intensity in subcortical white matter. Gyri are shrunken to some extent. Calvarium, especially diploë, is markedly thickened

she was neurologically examined by us at the age of 57. She had normal stature and did not have macrocephaly or dysphagia. However, she could not communicate or obey simple verbal commands. She presented mild intention tremor of the fingers and dystonic posture of the bilateral hands and toes. She showed rigidity and spasticity in the neck and the four extremities, and tetraplegia in flexion in bed, but was able to extend her arms somewhat. She did not show pseudobulbar palsy or tongue and skeletal muscle fasciculations.

The younger sister (case 2, 47 years old) seemed normal until the age of 3 years, when she became unable to walk for 3 months after her first seizure. She was diagnosed with very mild psychomotor impairment at school age but was able to work as an assistant in her father's factory after graduation from elementary school. At the age of 41, upon admission to another hospital for surgery of an atrial septal defect, she was found to have nocturnal myoclonus. She was subsequently referred to our hospital for neurological examination, which disclosed mild mental retardation and cerebellar speech, horizontal nystagmus and saccadic eye movement, dystonia of the right arm and bilateral toes, mild limb and truncal ataxia, and ataxic gait. Her deep tendon reflexes were hyperactive. She did not have macrocephaly, and was of normal stature. She did not show pseudobulbar palsy or tongue and skeletal muscle fasciculations. Her symptoms have slowly progressed over the past 6 years, but she remains able to walk to hospital by herself. Results of her routine blood analysis (complete blood count, liver enzyme activities, electrolytes, glucose, urea nitrogen, lactate and pyruvate levels, etc.) were normal. Lysosomal enzyme activities, including arylsulfatase A, α-galactosidase, β-galactosidase, β-hexosaminidase A and B were normal. CSF showed increased protein level (80 mg/dl). Chromosomal analysis, EEG, and somatosensory evoked potentials were normal. Brainstem auditory evoked potentials were also normal. Visual evoked potentials showed low amplitude and delayed latency of P100.

MRI findings

MRI was performed with 0.2-T Toshiba MRT22A in case 1, and 1.0-T Siemens Magnetom Impact in case 2. The images revealed cerebellar, especially vermian, atrophy, and showed high signal intensity on the T2-weighted image of the dentate nuclei in both cases (Figs. 1, 2). The brainstem was moderately atrophic in case 1 and mildly atrophic in case 2, along with fourth ventricular dilatation. MRI also disclosed ventricular enlargement, which was more severe in case 1 than in case 2. Also shown were characteristic subcortical white matter lesions including U fiber primarily, which appeared as low signal intensities on T1-weighted images and high signal intensities on T2-weighted images in both cases. In case 1 these white matter lesions extended to the periventricular white matter in the frontal lobe, and the genu of the corpus callosum was involved, whereas the deep white matter was almost intact in case 2. Caudate nuclei, putamina, and globi pallidi were also atrophic, and more severe in case 1 than case 2. Internal capsules were atrophic and external capsules showed high signal intensity on T2-weighted images in both cases. Thalamic nuclei appeared to be normal. In case 1 the calvarium, especially diploë, was markedly thickened, and the gyri were shrunken to some extent.

Organic acid and amino acid investigations

Urinary organic acids were analyzed as trimethylsilyl derivatives by gas chromatography–mass spectrometry (GC-MS). Total 2-OHG acids of urine, plasma, and CSF were quantified by selected-ion monitoring GC-MS analysis (m/z 129 for 2-OHG acid and m/z 131 for $^{2}H_{2}$-2-OHG acid, the internal standard), as described previously [12]. Absolute configuration of D- and L-2-OHG acids was analyzed by the use of (+)-2-butanol/acetyl derivatives, as de-

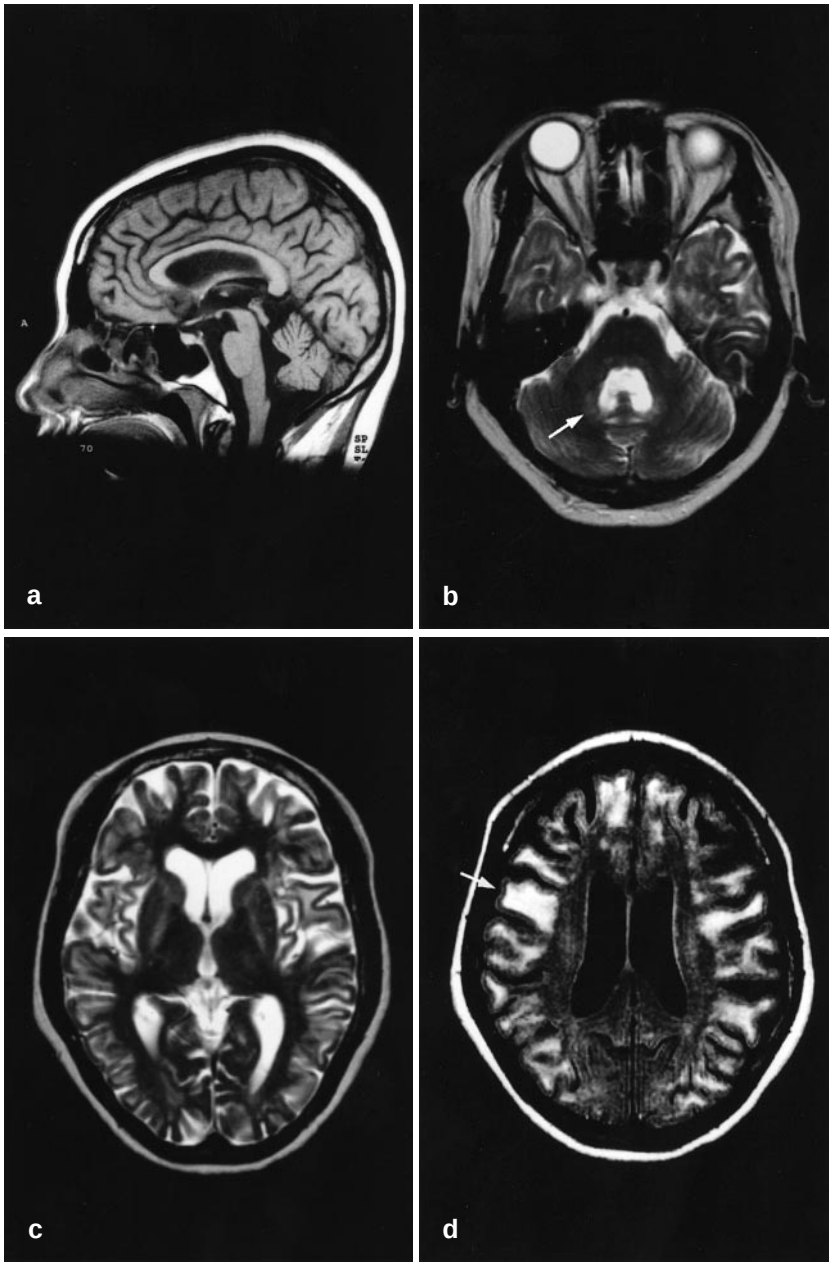

Fig. 2 a–d MRI of case 2 (examined at 46 years of age). **a** Sagittal T1-weighted image (TR 578, TE 15) showing enlargement of fourth ventricle, and cerebellar vermian atrophy. **b** Transaxial T2-weighted image (TR 4535, TE 128) showing same findings and high signal intensities in bilateral dentate nuclei (*arrow*). **c** Transaxial T2-weighted image showing high signal intensities in the subcortical white matter. Periventricular white matter is almost intact. Lateral ventricles are enlarged. Caudate nuclei, putamina, globi pallidi, and internal capsules are atrophic. External capsules show high signal intensities. **d** Transaxial fluid-attenuated inversion recovery image (TR 9999, TI 2300, TE 150) showing characteristic subcortical white matter lesions including U fiber primarily. Some broadened gyri with white matter abnormality are seen (*arrow*)

scribed by Chalmers et al. [4]. Amino acids were quantified with automatic amino acid analyzer.

GC-MS analysis of urinary organic acids revealed a large peak of 2-OHG acid in both cases. Other organic acids were normal. 2-OHG acid concentrations in urine, plasma, and CSF were elevated (Table 1). The CSF/plasma ratio was 0.73 in case 2. Analysis of the enantiomer of 2-OHG acid of urine revealed L-configuration in both cases. Plasma lysine levels in both cases, and CSF in case 2, were elevated (Table 1). The levels of other amino acids were normal. *N*-Acetylaspartic acid was not increased.

Table 1 2-OHG acid and lysine concentrations in body fluids. (Concentrations in urine as μmol/mg creatinine; in plasma and CSF as μmol/l; *ND* below detection level of method (1 μmol/l)

	Case 1	Case 2	Control
2-OHG acid			
Urine	7.00	5.44, 7.15	0.01–0.12
Plasma	22.5	25.7	ND
CSF	–	18.8	ND
Lysine			
Plasma	408.0	364.5	142.5–208.3
CSF	–	99.9	10–30

Discussion

The clinical picture was consistent with previous reports of L-2-OHG aciduria. However, these two patients were

much older than previously reported cases, and the severity of the disorder differed between them. The younger sister showed mild neurological impairments, even after a long clinical history, her impairments also being milder than those of her elder sister at the same age.

In most patients with this disease the initial symptoms are delay of unsupported walk, speech delay, febrile seizure, and macrocephaly, which appear insidiously during the first or second years of life. Over the years mental retardation and cerebellar ataxia develop with or without dystonia, pyramidal signs, and seizures. Adolescent cases with these symptoms have been reported relatively frequently; however, progression shows no overall pattern. Barth et al. [2] reported a 39-year case with spastic ataxic gait, truncal and limb ataxia, and dystonic posture. Larnaout et al. [14] reported a severe case of a Tunisian who was bedridden at 28 years and died at 30 years. Barbot et al. [1] reported a Portuguese patient who lost independent walking at 5 years, and Chen et al. [5] reported a severe neonatal case leading to death in the perinatal period. Our patients, aged 57 and 47 years, are cases of unusually long survival. Their onset was during childhood, which was slightly later than usual. Motor dysfunction became prominent after 20 years in case 1 and 40 years in case 2, which was extraordinarily late in comparison with previously reported cases, and progression was very slow. Our cases can thus be considered adult cases.

Our MRI studies showed characteristic subcortical white matter lesions which were consistent with the previous reports [1–3, 6, 9–11, 16, 21, 24]. MRI findings, especially in case 2, which were much milder than those of case 1, were typical and consistent with previous reported adolescent cases. This may reflect the benign clinical course in this case. On the other hand, the MRI findings in case 1 were severe, that is, ventricular enlargement was prominent, the periventricular white matter in the frontal lobe and genu of corpus callosum were also involved, the brainstem was moderately atrophic, and the calvarium was thick. Brainstem atrophy and calvarial thickening have previously not been reported in this disease.

The typical MRI findings in case 2, that is, symmetrical supratentorial subcortical white matter lesions including U fibers, infratentorial atrophy of cerebellum (vermis more than hemispheres), and lesions in both dentate nuclei, have a diagnostic value in this disease [21]. However, Canavan disease has similar MRI findings. It also has subcortical white matter lesions including U fibers. However, Canavan disease has much more widespread white matter lesions than L-2-hydroxyglutaric aciduria, and the globus pallidus and thalamus are frequently involved [16, 20]. In widespread white matter lesions such as those in case 1, further differential diagnosis is required. Alexander disease, maple syrup urine disease, leukoencephalopathy with swelling and a discrepantly mild clinical course etc. must be considered in addition to Canavan disease. Characteristic MRI findings of Alexander disease are the frontal dominant white matter lesions, the tendency to form cysts, and the peculiar pattern of contrast enhancement [19]. In the milder variant of maple syrup urine disease MRI studies show diffuse abnormality of the white matter of both the cerebellar and the cerebral hemispheres. The internal and external capsules, brainstem, thalamus, and globus pallidus are involved [22]. MRI findings of leukoencephalopathy with swelling and a discrepantly mild clinical course are diffuse abnormality in signal intensity and swelling of the cerebral hemispheric white matter with cystlike spaces in the frontoparietal and anterior-temporal subcortical area [18]. MRI findings of our cases and these diseases differ in several points; however, organic acid analysis and another biochemical analysis are necessary for the final diagnosis.

In many neurodegenerative diseases brainstem atrophy reflects a secondary degeneration related to involvement of superior cerebellar peduncles, the descending motor pathway, and, perhaps in long-surviving patients, to impairment of the ascending sensory tract. Brainstem atrophy found in case 1 (moderate) and case 2 (mild) may reflect a secondary degeneration related to long clinical history. Such findings would be reported more frequently if more patients survived as long as ours.

Macrocephaly was the common clinical manifestation in many reported cases in this disease. However, in case 1 the calvarium, especially diploë, was markedly thickened and the brain was very atrophic. Calvarial thickening is a common feature of the cranial vault in the infants with a severe microcephaly secondary to anoxic/ischemic encephalopathy or to severe developmental brain injury. Brain growth is the principal determinant of calvarial growth. Moreover, it is generally thought that diffuse overgrowth of the diploic space is a common feature of the cranial vault in cerebral atrophy in early life [8]. Calvarial thickening is also encountered occasionally in some neurometabolic diseases such as mannosidosis [15] and Sanfilippo syndrome [17]. These conditions are not associated with cerebral atrophy but may be an expression of dysostosis multiplex. Therefore the calvarial thickening found in patient 1 may be an incidental radiological finding, but the possibility that the brain atrophy began in early life cannot be excluded. Attention must thus be paid to whether calvarial thickening and brain atrophy is seen in this disease.

The elevated levels of L-2-OHG acid in the urine and plasma in our cases were similar to the levels in previous reports, but the CSF/plasma ratio (0.73) of 2-OHG acid in case 2 was lower than that in previously reported cases (>1) [1–3, 12, 16, 24]. The one case reported by Divry et al. [6] had a ratio (0.74) similar to that of our case 2. Although this ratio in case 2 is only a one-point examination, it may be related to the relatively mild CNS involvement seen in the case. The lysine levels of plasma and CSF were also elevated in both cases, as in previous reports.

It is remarkable that these 57- and 47-year-old patients are alive, although progression in this disease is generally

slower than that in other organic acid disorders. These cases suggest that organic acid analysis is necessary even in elderly patients who seem to have neurodegenerative disorders. The MRI findings in our cases provide a clue to the correct diagnosis; similar cases may increase in number as the use of MRI becomes more widespread.

The pathophysiological mechanisms and the enzyme responsible for this disease have not been elucidated, nor has DNA analysis yet been established. The clinical evaluation of many cases with this disease as well as DNA analysis and elucidation of organic acid metabolic pathway, should provide further advances in our understanding and may lead to the optimum treatment of this rare disease.

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