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The effect of dietary protein restriction in a case of molybdenum cofactor deficiency with *MOCS1* mutation

Yu Abe [a,*], Yu Aihara [a], Wakaba Endo [a], Hiroshi Hasegawa [b], Kimiyoshi Ichida [b,c], Mitsugu Uematsu [a], Shigeo Kure [a]

[a] Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan
[b] Department of Pathophysiology, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan
[c] Division of Kidney and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Minato-ku, Tokyo, Japan

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ABSTRACT

Molybdenum cofactor deficiency (MoCD) is an autosomal recessive inborn error of metabolism that results from mutations in genes involved in molybdenum cofactor (Moco) biosynthesis. MoCD is characterized clinically by intractable seizures and severe, rapidly progressing neurodegeneration leading to death in early childhood in the majority of known cases. We report on a patient with an unusual late disease onset and mild phenotype, characterized by delayed development and a decline triggered by a febrile illness and a subsequent dystonic movement disorder. Magnetic resonance imaging showed abnormal signal intensities of the bilateral basal ganglia. Blood and urine chemistry tests demonstrated remarkably low serum and urinary uric acid levels. A urine sulfite test was positive. Specific diagnostic workup showed elevated levels of xanthine and hypoxanthine in serum with increased urinary sulfocysteine (SSC) levels. Genetic analysis revealed a homozygous missense mutation at c.1510C > T (p.504R > W) in exon 10 of the *MOCS1* in isoform 7 (rs1387934803). At age 1 year 4 months, the patient was placed on a low protein diet to reduce cysteine load and accumulation of sulfite and SCC in tissues. At 3 months after introduction of protein restriction, the urine sulfite test became negative and the urine SCC level was decreased. After starting the protein restriction diet, dystonic movement improved, and the patient's course progressed without regression and seizures. Electroencephalogram findings were remarkably improved. This finding demonstrates that the dietary protein restriction suppresses disease progression in mild cases of MoCD and suggests the effectiveness of dietary therapy in MoCD.

1. Introduction

Molybdenum cofactor deficiency (MoCD) is a rare autosomal recessive disorder that results in the combined deficiency of molybdenum-dependent enzymes, including xanthine oxidoreductase, sulfite oxidase (SO), and aldehyde oxidase (AO) [1]. Four different genes are involved in molybdenum cofactor (Moco) biosynthesis: *MOCS1* (OMIM*603707), *MOCS2* (OMIM*603708), *MOCS3* (OMIM*609277), and *GPHN* (OMIM*603930) [2]. *MOCS1* mutations are responsible for more than half of the patients, followed by *MOCS2* [3]. *GPHN* mutations have been described in two families of Danish and Algerian origin, respectively [4,5]. To date, to our knowledge, only one patient has been reported with *MOCS3* gene mutation [6].

The incidence is estimated at 1 in 100,000 to 200,000 live births and more than 100 patients from multiple ethnic groups have been identified to date [7–9]. Untreated MoCD results in loss of activity of all four Moco–dependent enzymes and is characterized by feeding difficulties, dysmorphic signs (mainly facial dysmorphisms), lens dislocation, rapidly progressive neurodegeneration, and intractable seizures, which all result mainly from dysfunctional SO [10]. Loss of SO activity results in accumulation of sulfite, a strong reductant that cleaves disulfide bridges in proteins and cystine. Sulfite-mediated cysteine reduction results in cysteine/cysteine deficiency, which is accompanied by formation of S-sulfocysteine (SSC), which has been demonstrated besides sulfite [11] to mediate neurotoxicity [12] (Fig. 1). SSC can be quantified in urine and serum, representing a very reliable biomarker in diagnosing MoCD and isolated SO deficiency [13].

The classical form manifests in the neonatal period with severe encephalopathy, including intractable seizures, magnetic resonance imaging (MRI) changes that resemble hypoxic-ischemic injury,

* Corresponding author at: Department of Pediatrics, Tohoku University School of Medicine, 1-1 Seiryomachi, Aobaku, Sendai 980-8574, Japan.
E-mail address: aveyou@nifty.com (Y. Abe).

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microcephaly, and early death [13]. In late-onset MoCD patients, the onset is within the first 2 years of life. Usually, the clinical manifestations include developmental delay, lens dislocation, extrapyramidal, and pyramidal symptoms often arising abruptly after an intercurrent illness. Seizures are less common compared to the classical form [14].

In the attempt to reduce sulfite accumulation, several treatments have been suggested with limited benefit for MoCD. To our knowledge, cyclic pyranopterin monophosphate (cPMP) is the only effective treatment for MOCS1 patients, even though it cannot reverse brain injuries already established before treatment [15]. To date few reports exist of dietary efficacy including dietary protein restriction.

We describe a relatively mild phenotype in a patient with novel *MOCS1* homozygous mutation treated with dietary protein restriction. Protein restriction treatment restrains the progression of symptoms. Effectiveness in patients with a mild clinical course was suggested.

2. Material and methods

2.1. Determination of biomarkers

2.1.1. SSC in urine

SSC concentrations in urine were determined by high-performance liquid chromatography (HPLC) according to the method of Johnson and Rajagopalan [16] with some modifications. In brief, a urine sample (20 μL) was diluted 5 times with 100 mM sodium carbonate buffer (pH 8.9). Then, 100 μL of 3 mg/mL Dabsyl chloride in acetonitrile was added and the resulting solution was heated at 70 °C at 15 min. After cooling, the sample was diluted with 200 μL of HPLC mobile phase. The resulting solution was filtered through a 0.45-μm membrane filter and the filtrate (20 μL) was injected into HPLC. HPLC was performed on a Mightysil RP-18 aqua column (5 μm, 150 × 4.6 mm ID; Kanto Chemicals, Tokyo, Japan). The mobile phase was 25 mM potassium phosphate buffer (pH 6.8)-acetonitrile-2-propanol (76:18:6, vol/vol/vol) and the flow rate was 1.2 mL/min. After 20 min, the column was washed at 2 mL/min with a solution of 25 mM potassium phosphate buffer (pH 6.8)-acetonitrile-2-propanol (2:3:1, vol/vol/vol) for 15 min. The column was re-equilibrated with the original solvent mixture for the next run. The wavelength of the detector (UV-2075 UV/Vis detector; Jasco, Tokyo, Japan) was set at 436 nm.

2.1.2. Hypoxanthine, xanthine, and uric acid in plasma

Plasma concentrations of hypoxanthine, xanthine, and uric acid were determined by the method of Kojima et al. [17] with some modifications. In brief, plasma samples (50 μL) were deproteinized with 150 μL of 3% perchloric acid on a vortex mixing. After centrifugation at 5000*g* for 10 min, the supernatant was filtered through a 0.45-μm membrane filter and the filtrate (50 μL) then was injected into HPLC. HPLC was performed on a Mightysil RP-18 aqua column. The mobile phase was 0.1% trifluoroacetic acid and the flow rate was 1 mL/min. The wavelengths of the detector (Jasco UV-970 M 4λ-intelligent detector) were set at 284 nm for uric acid and 254 nm for hypoxanthine and xanthine, respectively.

2.1.3. Methionine, cysteine, and homocysteine in plasma

Plasma concentrations of methionine, total homocysteine, and total cysteine were determined by the isotope dilution method as described previously [18,19] with some modifications. In brief, to 50 μL plasma samples were added 2.5 nmol [3,3,4,4-$^{2}H_4$]methionine ([$^{2}H_4$]methionine), 5.0 nmol [2,2′,3,3,3′,3′-$^{2}H_6$]cystine ([$^{2}H_6$]cystine), and 1.0 nmol [3,3,3′,3′,4,4,4′,4′-$^{2}H_8$]homocystine ([$^{2}H_8$]homocystine) as the internal standards. The plasma samples then were subjected to gas chromatography–mass spectrometry with selected ion monitoring (GC–MS-SIM) measurements after reduction of the disulfide bond with dithiothreitol, precipitation of proteins with trichloroacetic acid, purification by cation exchange chromatography using the BondElut SCX cartridge, and derivatization with isobutyl chloroformate in water-ethanol-pyridine.

GC–MS-SIM was made with a Shimadzu QP2010 quadrupole GC–MS. The GC column was a fused-silica capillary column SPB-1 (Supelco,

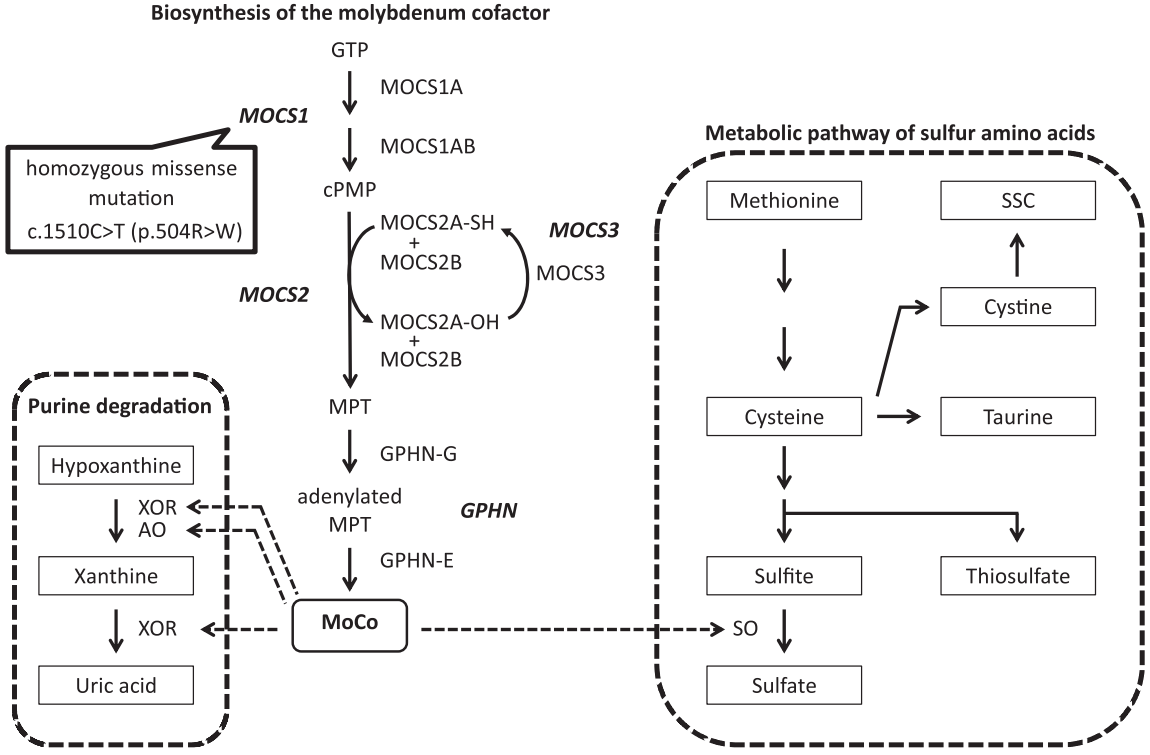

Fig. 1. Biosynthesis of the molybdenum cofactor (Moco) and metabolic pathway of sulfur amino acids and purine degradation. The position of the *MOCS1* mutation in our patient is indicated by the bold square. GTP, guanosine triphosphate; cPMP, cyclic pyranopterin monophosphate; MPT, molybdopterin (metal-binding pterin); AO, aldehyde oxidase; SO, sulfite oxidase; XOR, xanthine oxidoreductase; SCC, S-sulfocysteine.

Bellefonte, PA, USA) and the mass spectrometer was operated in chemical ionization mode with isobutane as the reagent gas. SIM was performed for the protonated molecular ions at m/z 278 and 282 for N (O,S)-isobutyloxycarbony ethyl ester (IBC-OEt) derivatives of methionine and [$^2H_4$]methionine, at m/z 350 and 353 for the IBC-OEt derivatives of cysteine and [$^2H_3$]cysteine, and at m/z 364 and 368 for the IBC-OEt derivatives of homocysteine and [$^2H_4$]homocysteine.

2.2. MRI and spectroscopy

MRI and point-resolved spectroscopy [20] were performed on a 3 T system using a head coil (Siemens, Munich, Germany) with the following parameters: echo time 30 ms, repetition time 3 s, 64 acquisition, voxel size 2·2·2 cm^3. Spectroscopy data were quantified with LC model [21].

3. Results

3.1. Case report

The patient is the first child of nonconsanguineous Japanese parents. She was born at week 36 of gestation by cesarean section due to poor blood pressure control associated with pregnancy-induced hypertension in her mother. The Apgar score was 8 points for 1 min and 9 points for 5 min. Birth body weight was 2420 g (−0.21 standard deviations [SD]), body height 47.5 cm (+0.26 SD), and head circumference 33.5 cm (+0.72 SD). Neonatal asphyxia was not observed. Postnatal blood glucose level was 30 mg/dL and glucose infusion was required for 3 days. No neurological abnormalities were noted during the neonatal period.

At age 5 months, she was referred for close examination for floppy infant syndrome. At age 8 months, she could not hold up her own head. She always showed opisthotonus, limb tremor, and intermittent conjugate deviation of the eyes. At age 9 months, she had a high fever and showed convulsive status epilepticus followed by general hypertonia, dystonic movement, and continuous conjugate deviation of the eyes.

Brain MRI at age 9 months revealed bilateral increased signal intensities (T2 and diffusion weighted images) of the ventral globus pallidus. At age 12 months, T2-weighted images showed clearly increased signal intensities of bilateral putamen and caudate nucleus. The FLAIR image showed a faint high signal at their edges and the inside appeared to be necrotic. Bilateral globus pallidus showed slightly increased signal intensities (T2-weighted images). At age 21 months, increased signal intensities in the basal ganglia disappeared and the regions atrophied (Fig. 2).

Blood and urine chemistry tests showed remarkably low serum and urinary uric acid levels. A urine sulfite test was positive. Selective screening for inborn errors of metabolism was performed, which comprised the determination of urinary purine and pyrimidine levels. This showed elevated levels of xanthine and hypoxanthine. Together

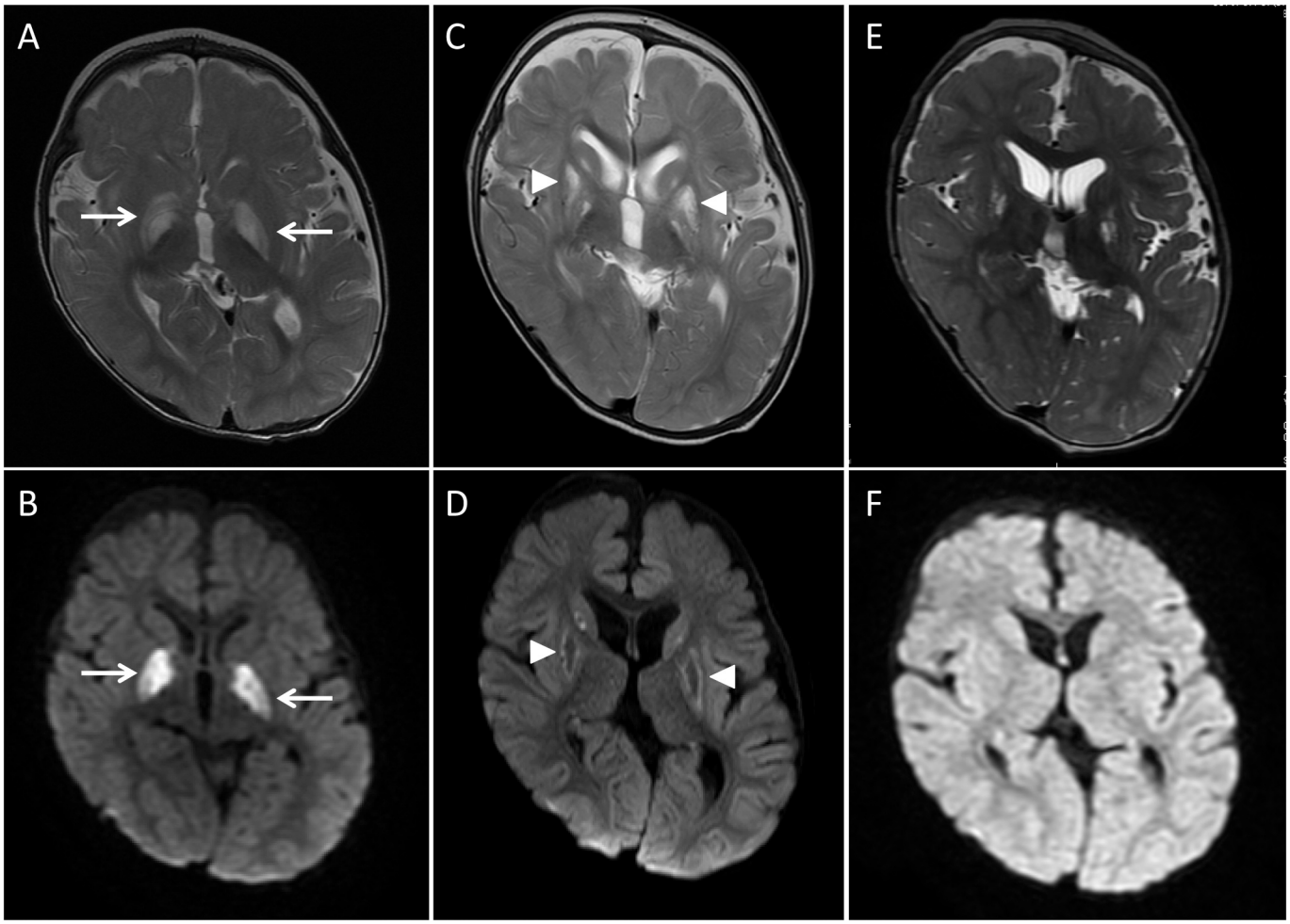

Fig. 2. MRI at diagnosis and follow-up. (A, B) Axial T2-weighted (T2WI) and diffusion weighted (DWI) images at age 9 months showed bilateral increased signal intensities (arrows) of the globus pallidus. (C, D) Axial T2WI and DWI at age 12 months showed increased signal intensities of bilateral putamen and caudate nucleus (arrowheads). DWI showed high signal intensities at the edge of the putamen and the inside appeared to be necrotic. (E, F) Axial T2WI and DWI at age 21 months showed that hyperintense lesions disappeared. Although the bilateral basal ganglia slightly atrophied, the cerebral cortex and white matter showed no atrophy.

with low concentrations of homocysteine in serum, an extremely high level of urinary SSC was observed (Table 1). This prompted a specific diagnostic testing for MoCD that included mutation analysis of Moco genes.

The patient had a homozygous missense mutation at c.1510C > T (p.504R > W) in exon 10 of the *MOCS1* gene in isoform 7 (rs1387934803) (Fig. 1). Heterozygosity for the same variant was confirmed in both parents. Although the c.1510C > T is an unreported mutation to date, it is located in the N-terminal of the catalytically essential MOCS1B domain and is expected to affect the catalytic activity of MOCS1AB protein (Supplementary Fig. S1). A definitive diagnosis of MoCD was made at age 1 year and 2 months.

3.2. Dietary protein restriction suppresses disease progression in a mild case of MoCD

At age 16 months the girl was placed on a low protein diet to reduce cysteine load and accumulation of sulfite and SCC in tissues. Whole natural protein was dietary restricted, with essential amino acids monitoring so as not to be insufficient in essential amino acids. We used protein-free milk (S-23; Yukijirushi, Tokyo, Japan) combined with parenteral nutrition (Ensure Liquid; Abbott Japan, Tokyo, Japan). At initiation of treatment, the content of protein was 1.75 g/kg/day for 1 week. In the following 1 month, the content of protein was decreased to 1.4 g/kg/day. After that, it was maintained to 1.25 g/kg/day. At age 42 months, the content was decreased further to 1.0 g/kg/day.

At 4 months after initiation of protein restriction (at age 20 months), the urine SCC level was decreased and serum uric acid level was increased (Table 1). Urine sulfite test became negative. Methionine and total cysteine in serum were decreased. Isoleucine was used as an index of essential amino acid to check for excessive protein restriction and it was maintained within the normal range. She grew up along the normal growth curve after starting protein restriction. At 14 months after starting protein restriction (at age 30 months) with the content of protein maintained to 1.25 g/kg/day, the urine SCC level was slightly increased, although it was maintained lower than before the protein restriction diet. At age 42 months, protein content was lowered to 1.0 g/kg/day to make parameters normalize further. At age 44 months, the urine SCC level was maintained in half compared to before protein restriction diet.

After initiation of the dietary protein restriction, improvements in hypertonia, opisthotonus, and dystonic movement were observed. Although she showed severe intellectual disabilities at age 44 months, she had progressed without regression or seizures. Electroencephalogram (EEG) at age 12 months (2 months before initiation of protein restriction) showed frequent interictal epileptic discharges. However, no obvious epileptic discharges were noted at age 43 months (27 months after initiation of protein restriction; Fig. 3).

Table 1
Relevant laboratory findings at age of diagnosis and follow-up.

Age (before and after starting protein restriction diet)	13 months (3 months before)	20 months (4 months after)	30 months (14 months after)	44 months (28 months after)	Reference range
Restricted protein (g/kg/day)		1.25	1.25	1	
Urine parameters					
Sulfite test	Positive	Negative	Negative	Negative	
Uric acid (mmol/mol creatinine)	178.4				220–790[a]
SSC (μmol/mmol creatinine)	188.7	96.3	127.8	102.2	0–9[a]
Serum parameters					
Uric acid (μmol/l)	32.2	41.6	45.6	61.3	156–432[a]
Xanthine (μmol/l)	6.60	7.49	6.78	11	0.7–1.2[b]
Hypoxanthine (μmol/l)	6.83	2.69	3.90	6.75	1.1–3.0[b]
Methionine (μmol/l)	16.3	7.2	42.1	11.3	12.1–20.1[c]
Total cysteins (μmol/l)	51.3	43.5	67.8	55.7	
Total homocysteins (μmol/l)	0.51	0.64	1.53	1.03	0.4–7.5[d]

[a] Mayr et al. J Inherit Metab Dis 41:187–196 (2018).
[b] Inoguchi et al. Gout and Nucleic Acid Metabolism Vol. 33 No.2 (2009).
[c] Tagami et al. Annual Report of Sapporo City Institute of Public Health 30: 35–40 (2003).
[d] Accinni et al. J Chromatogr B 785, 219–226 (2003).

4. Discussion

We report a mild case of MoCD with *MOCS1* mutation treated efficaciously with dietary protein restriction. Our patient was placed on a low protein diet to reduce cysteine load and accumulation of sulfite and SCC in tissues at age 16 months. The whole natural protein was diet-restricted so as to be careful not to be insufficient in essential amino acids. A sulfite test became negative 4 months after starting dietary protein restriction. Surprisingly, the biochemical profile improved not only with a significant reduction in serum SSC levels, but also an increase in serum uric acid and reduction in serum hypoxanthine despite no change in total purine body intake. Reduction in serum hypoxanthine might be explained in dietary protein restriction, but the mechanism of the increase in serum uric acid is unknown. In addition to maintaining sufficient essential amino acids, we adjusted the diet to obtain enough calories. Total calories were maintained at 800 to 900 kcal/day after the start of protein restriction, and she grew along a normal growth curve for her age. Neurotoxic sulfite accumulates and triggers a progressive neurologic disease, which is lethal if untreated. According to Mechler et al., [3] the median survival is 36 months. Age at onset of the disease is the first day of life and initial cardinal disease features are intractable seizures and feeding difficulties. Although our patient showed severe intellectual disabilities at age 44 months, she had progressed without regression or seizures. EEG at age 12 months (2 months before initiation of protein restriction) showed frequent interictal epileptic discharges. However, no obvious epileptic discharges were noted at age 43 months (27 months after initiation of protein restriction). Few studies have focused on the changes on EEG with MoCD, especially before and after dietary protein restriction. The EEG improvement in our case showed the possibility of improved neurologic outcome with protein restriction.

In the attempt to reduce sulfite accumulation, several treatments have been suggested with limited benefit for MoCD. To our knowledge, cPMP is the only effective treatment for MOCS1 patients, even though it cannot reverse brain injuries already established before treatment [15]. In Japan, treatment with cPMP for MoCD has not been officially approved, and there is no preparation for cPMP, so it was not possible to introduce treatment with cPMP for our patient. Treatment with cPMP supplementation is indicated only in cases with MOCS1 mutations, but protein restriction may be useful not only in *MOCS1* but also in *MOCS2* and *MOCS3*. To date few reports exist of dietary efficacy. Touati et al. [22] treated two children with SO deficiency using a low-protein diet with reduced intake of methionine, cystine, and taurine. The biochemical profile improved, with significant reductions in urinary thiosulfate and SSC levels, and both children grew well physically. Development was considered normal for one child and relatively mildly delayed for the other. In the other two reports of dietary intervention in isolated SO deficiency with low-protein diets plus limited methionine and cysteine, the patients became less irritable, but there was no perceptible

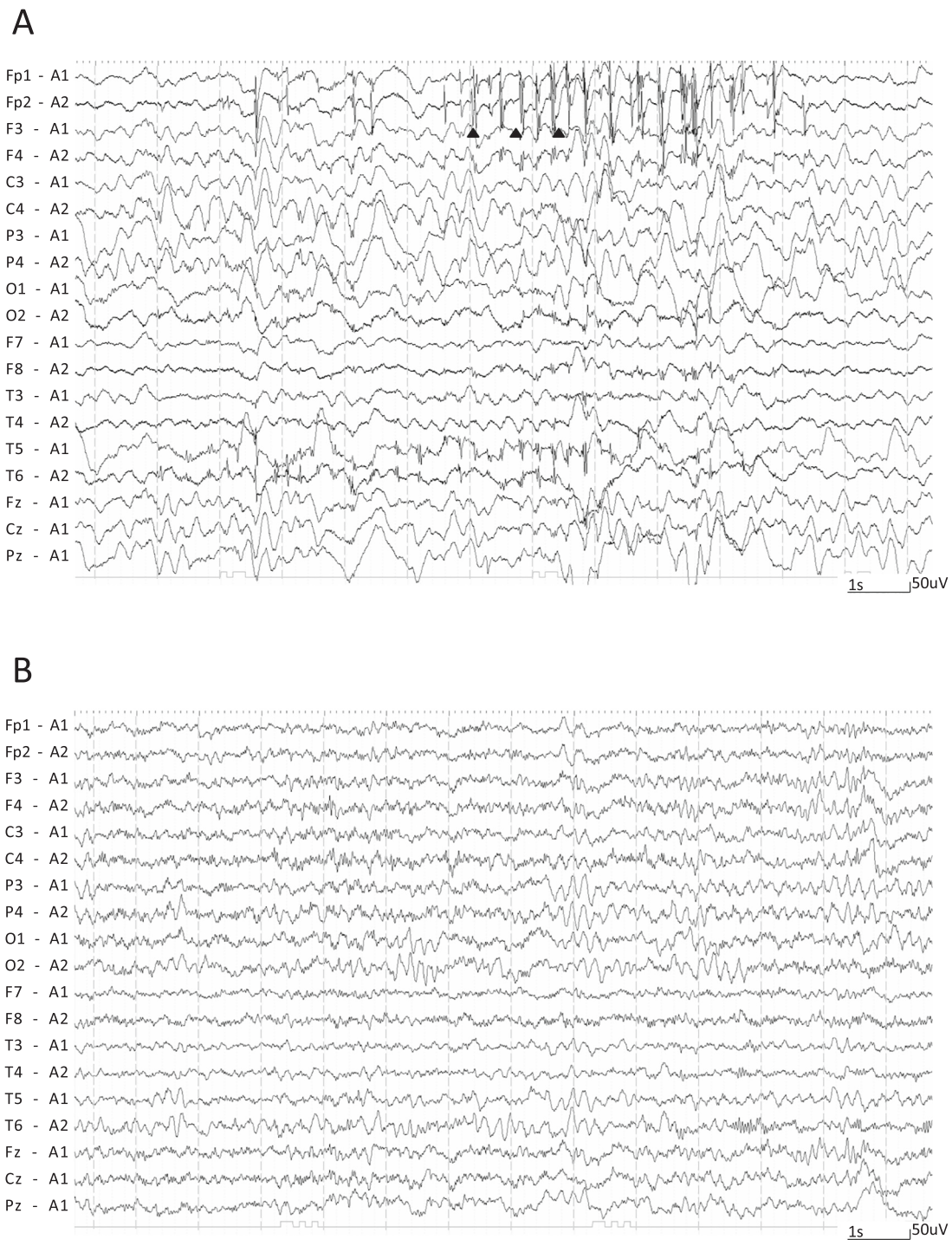

Fig. 3. EEG before and after dietary protein restriction. (A) EEG at age 12 months (2 months before initiation of protein restriction) showed frequent interictal epileptic discharges (arrowheads). (B) EEG at age 43 months (27 months after initiation of protein restriction) showed no obvious epileptic discharges. EEG montage: unipolar induction. Filter settings: 1–60 Hz. Vertical scale bar: 50 μV.

improvement in their neurologic function or development [23,24]. In contrast, Boles et al., [25] reported that dietary restriction of methionine and supplementation of cysteine in a 5-month-old girl with MoCD led to rapid clearance of urinary sulfites, improvement in neurodevelopment, and resumption of head growth 1 month after initiation of the diet. When dietary restrictions were abandoned 2 months later, urinary sulfites reappeared, the patient regressed developmentally, and her head stopped growing. They suggested that cysteine supplementation might be important because cysteine can form disulfide bonds with toxic sulfur-containing metabolites and promote their excretion through an alternative "salvage pathway."

The disorder presents classically with neonatal onset refractory seizures, marked global developmental delay, microcephaly, abnormal muscle tone, and markedly reduced lifespan. Clinical signs of progressive pyramidal and extrapyramidal dysfunction ensue. Dysmorphic features include long face, puffy cheeks, widely spaced eyes, elongated palpebral fissure, thick lips, long philtrum, and small nose. Feeding difficulty, failure to thrive, and screaming episodes are common.

Table 2
Review of patients described in the literature with mild phenotype of MoCD.

	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8
	Shih et al. (1977) - Johnson et al. (1988)	Mize et al. (1995)	Graf et al. (1998)	Hughes et al. (1998) and Alkufri et al. (2013)		Johnson et al. (2001)	Arenas et al. (2009)	Vijayakumar et al. (2011)
				Case 1	Case 2 (Sister)			Case 1
Age at onset (months)	17	24	6	12	NA	15	24	8
Molecular analysis	NP	NP	NP	*MOCS2* heterozygous variants: NM_004531.4: c.377 + 1G > C; and NM_004531.4:c.539_ 540del, p. Lys180fs	*MOCS2* heterozygous variants: NM_004531.4: c.377 + 1G > C; and NM_004531.4: c.539_540del; p. Lys180fs	*MOCS2* heterozygous variants: NM_176806.3: c.16C > T; p.Gln6ter and NM_176806.3: c.19G > T; p. Val7Phe	*MOCS1* homozygous variant: NM_001075098.3: c.[a]7 + 6 T > C	*MOCS1* C > T (Arg91Trp)
Uric acid (plasma)	Normal	↓	↓	↓	↓	Normal	Normal	↓
S-sulfocysteine	↑	↑	↑	NA	NA	↑	↑	NA
Sulfi test	+	+	+	+	+	+	+	NP
MRI	NP	LN	BG-SWM	BG-DN	BG	BG-cortical dysplasia	DN-parietal cleft	LN-A
Treatment	Antiepileptic drugs	None	Antiepileptic drugs	Antiepileptic drugs	Levodopa Prednisolone	None	None	Antiepileptic drugs
Seizure	Yes	No	No	Yes	No	No	No	Yes
Psychomotor development	Delay	Delay	Delay	Delay	Normal, Deterioration at 23 years of age	Delay	Delay	Delay
Pyramidal signs	Yes (hemiplesia)	Yes (hemiplesia)	Yes	No	No	No	No	Yes
Extrapyramidal signs	Yes	Yes (cordination deficit)	Yes	Yes	Yes (at 23 years of age)	Yes	No	Yes
Independent walking	Yes	Yes	No	No	Unstable gait after 23 years of age	Yes	Yes	No
Feedng difficulities	No	No	NA	Yes	Yes after 23 years of age	No	No	Yes
Language development	Episode of aphasia	Delay	NA	Regression after 17 months-absent at last examination	Normal - Regression after 23 years of age (severe dysphonia -anarthria)	Delay (expressive language)	Delay (expressive and receptive language)	Delay
Behavioral disorders	Yes (intermittent)	No	No	Yes (irritability)	Yes (in infancy mild attention deficit, in adulthood apathy)	No	Yes (hyperkinesia)	No
Ophthalmological findings (age-years)	Lens dislocation (4)	Lens dislocation (8)	Lens dislocation (2)	No	Lens dislocation (6)	No	Lens dislocation-myopia (4)	No
Outcome at last examination (age-years)	Mild-moderate (4)	Moderate (22)	Severe (2)	Severe (3)	Severe (23)	Mild (4.5)	Mild (7)	Severe (4)

NA: not available; NP: not performed; BG: basal ganglia lesions; DN: dentate nuclei leisions; LN: lentiform nuclei lesions; SWM: abnormality of subcortical white matter; A: atrophy

No.9	No.10	No.11	No.12	No.13	No.14	No.15
Vijayakumar et al. (2011)	Zaki et al. (2016)	Megahed et al. (2016)	Hujimans et al. (2018)	Mayr et al. (2018)	Scelsa et al. (2019)	Current study (2020)
Case2						
24	6	1	14	7	16	5
MOCS1 homozygous variant: NM_001075098.3: c.[a]7 + 6 T > C	*MOCS2* homozygous variant: NM_176806.3: c.3G > A p.Met1?	*MOCS2* homozygous variant: NM_176806.3: c.3G > A p.Met1?	*MOCS3* homozygous variant: NM_014484.4: c.769G > A, p.Ala257Thr	*MOCS1* Homozygous c.1338delG	*MOCS2* homozygous variant: NM_176806.3: c.19G > T; p.Val7Phe	*MOCS1* homozygous variant: c.1510C > T p. Arg504Trp
Normal	Normal	NA	Normal -↓	↓	↓	↓
NA	↑	↑	↑	↑	↑	↑
+	NP	NA	–	+	+	+
DN	BG-SWM-A	A	SWM	BG-SWM	BG-DN-LN	BG
None	Antiepileptic drugs	Antiepileptic drugs Omega3 B-vitamins	Low-methionine diet	Low-methionine diet	Acetylsalicylic acid	Antiepileptic drugs Dietry protein restriction
No	Yes	Yes	No	No	Yes	Yes
Delay	Delay	Delay	Delay	Delay	Delay	Delay
No	Yes	Yes (hemiplesia)	Yes	No	Yes	Yes
No	Yes	No	No	Yes	Yes	Yes (dystonic movement)
Yes	No	Yes (unstable)	Yes (unstable)	Yes	Yes	No
No	Yes	No	No	No	No	Yes
Delay (verbal dyspraxia)	Delay	Delay	Delay (expressive language)	Delay (expressive language)	Delay (expressive language)	Delay (No meaningful word)
No	No	Yes	Yes (autistic features)	Yes (autistic features)	No	Unknown
Lens dislocation (5)	No	No	Strabismus	Hyperopia	No	No
Mild-moderate (7)	Severe (death at 5.5 years)	Moderate (6)	Moderate (17)	Mild (14)	Mild (6)	Severe (3)

Cortical damage, myopia, and lens dislocation contribute to impaired vision, which is universal [7,9]. Although our patient showed severe intellectual disabilities, she had survived the neonatal period and progressed without regression or seizures. She also has no dysmorphic features and Ophthalmic abnormalities. From these points of view, her phenotype was considered to be milder than the classical cases.

Our patients showed relatively slow progress compared to the previous reports of MoCD. Usually, seizures start shortly after birth and feeding difficulties are reminiscent of amino acid intolerance. Within the first week, feeding difficulties are observed accompanied by tonic-clonic seizures refractory to anticonvulsants with a prominent opisthotonus and an exaggerated startle reaction. Patients who survive the neonatal period in most cases show severe mental retardation as well as dislocated lenses and usually do not learn to sit or speak. Our patient had noticeable developmental delay at age 5 months due to hypotonia. She showed no seizure or feeding difficulty until age 9 months when she showed encephalopathy-like symptoms with fever. In the literature, an atypical phenotype with late onset has been recognized and to date only 14 patients have been reported (Table 2) [1,6,7,14,26–35].The clinical course is variable including patients with predominant extrapyramidal signs, occurring even in adulthood, and those with catastrophic neurologic deterioration. Basal ganglia and dentate nuclei changes often are recognized as an isolated finding in MRI of patients with a late onset and mild clinical course. Those findings are similar to the early-onset form of MoCD (diffuse brain atrophy, gliosis, arrested development of myelination, and cystic necrosis of cerebral white matter). Recently, Mayr et al. (2018) reported on a patient with an unusually late disease onset and mild phenotype, characterized by a lack of seizures, normal early development, decline triggered by a febrile illness, and a subsequent dystonic movement disorder. Genetic analysis revealed a homozygous c.1338delG *MOCS1* mutation causing a frameshift (p.S442fs) with a premature termination of the MOCS1AB translation product at position 477 lacking the entire MOCS1B domain. Urinalysis detected trace amounts (1% of control) of the Moco degradation product urothione, suggesting a residual Moco synthesis in the patient, which was consistent with the mild clinical presentation. They found an unusual mechanism of translation reinitiation in the MOCS1 transcript, which results in trace amounts of functional MOCS1B protein being sufficient to partially protect the patient from the most severe symptoms of MoCD. Our patient had a homozygous missense mutation at c.1510C > T (p.504R > W) in exon 10 of the *MOCS1* gene. Although this mutation is unreported to date, it located in the N-terminal of the catalytically essential MOCS1B domain and is expected to affect the catalytic activity of MOCS1AB protein. Although the mutations in our patient and the patient of Mayr et al. (2018) were both located in the N-terminal of the MOCS1B domain, the phenotype of our patient was more severe than that reported by Mayr et al. (2018). Of the 14 cases reported so far as mild MoCD, serum uric acid levels were normal in 6 cases and decreased in 8 cases (not described in 1 case). Among the mild cases with decreased uric acid levels, our case had the lowest uric acid level (Table 2). These findings could be related to the amounts of functional MOCS1B protein. If we would have access to patient fibroblast, they might be able to determine sulfite oxidase activity in those cells, but we couldn't get the consent of her family.

The observation of this patient with a *MOCS1* mutation should encourage further investigation of the effect of dietary protein restriction in a case of MoCD. The description of additional patients also is very important to define the effect of dietary protein restriction and sophisticate the protocol of the dietary protein restriction.

Details of the contributions of individual authors

Y.A. devised the project, the main conceptual ideas and proof outline. M.U. supervised the project. Y.A. and W.E. assisted with medical care of the patient. H.H. and K·I carried out the determination of biomarkers and genetic analysis. S.K. encouraged Y.A. to investigate MoCD

and supervised the findings of this work.

Details of funding

This study is not particularly funded.

Details of ethics approval

Such approval was not required for this study.

A patient consent statement

Informed consent was obtained from the patient included in this study.

Availability of data and material

Raw data will be available when requested.

Author statement

19th January 2021
Dr. Edward RB McCabe
Dr. Gerard T. Berry
Co-Editors-in-Chief
Molecular Genetics and Metabolism Reports
Dear Dr. McCabe and Dr. Berry,

Please find enclosed our manuscript entitled "The effect of dietary protein restriction in a case of molybdenum cofactor deficiency with *MOCS1* mutation", which we once submitted as an *Original Article in Molecular Genetics and Metabolism Reports* on November 24, 2020, and was decided to revise on January 8, 2021. We would like to resubmit for publication as an *Original Article in Molecular Genetics and Metabolism Reports* because we revised according to the reviewer's comment and also follows all the instructions for authors. We attached the document of responses to reviewer. Please see the attached document for further details.

Molybdenum cofactor deficiency (MoCD) is an autosomal recessive inborn error of metabolism that results from mutations in genes involved in molybdenum cofactor biosynthesis. MoCD is characterized clinically by intractable seizures and severe, rapidly progressing neurodegeneration leading to death in early childhood in the majority of known cases. In this study, we report on a patient with an unusual late disease onset and mild phenotype, characterized by delayed development and a decline triggered by a febrile illness and a subsequent dystonic movement disorder. Magnetic resonance imaging showed abnormal signal intensities of the bilateral basal ganglia. Blood and urine chemistry tests demonstrated remarkably low serum and urinary uric acid levels. A urine sulfite test was positive. Specific diagnostic workup showed elevated levels of xanthine and hypoxanthine in serum with increased urinary sulfocysteine (SSC) levels. Genetic analysis revealed a homozygous missense mutation at c.1510C > T (p.504R > W) in exon 10 of the *MOCS1*. At age 1 year 4 months, the patient was placed on a low protein diet to reduce cysteine load and accumulation of sulfite and SCC in tissues. At 3 months after introduction of protein restriction, the urine sulfite test became negative and the urine SCC level was decreased. After starting the protein restriction diet, dystonic movement improved, and the patient's course progressed without regression and seizures. Electroencephalogram findings were remarkably improved. Our finding demonstrates that the dietary protein restriction suppresses disease progression in mild cases of MoCD and suggests the effectiveness of dietary therapy in MoCD.

This manuscript has not been published elsewhere and is not under consideration by another journal. We have approved the manuscript and agree with submission to *Molecular Genetics and Metabolism Reports*. There are no conflicts of interest to declare.

We believe that the findings of this study are relevant to the scope of your journal and will be of interest to its readership. The manuscript has been carefully reviewed by an experienced editor whose first language is English and who specializes in editing papers written by scientists whose native language is not English.

We look forward to hearing from you at your earliest convenience.
Sincerely,
Yu Abe.
Department of Pediatrics, Tohoku University School of Medicine,
1-1 Seiryomachi, Aobaku, Sendai 980-8574, Japan.
Telephone: +81-22-717-7287
FAX: +81-22-717-7290

CRediT authorship contribution statement

Y.A. played a role in Conceptualization, Data curation, Investigation and Writing - original draft. Y.A. and W.E. played a role in Formal analysis and Investigation.H.H. and K.I. played a role in Formal analysis and Methodology. M.U. played a role in Supervision and Writing - review & editing.S.K. played a role in Supervision.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2021.100716.

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