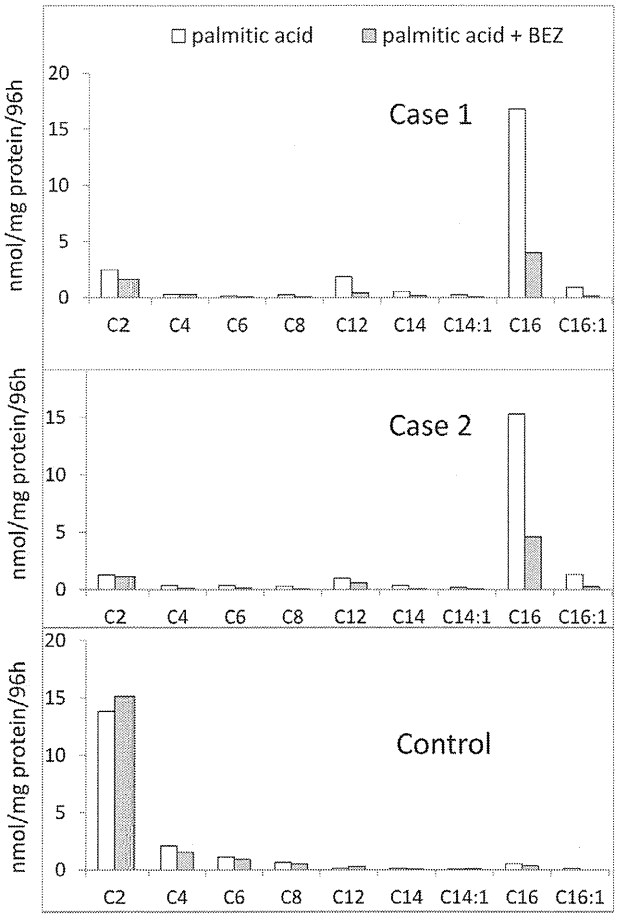

Fig. 2. Acylcarnitine profiles of IVP assay in the presence and absence of bezafibrate (BEZ) of cases 1, 2, and normal control respectively. Unit of vertical lines, nmol/mg protein of acylcarnitines (ACs); the horizontal lines represent acylcarnitines from C2, C4, C6, C8, C12, C14, C14:1, C16, and C16:1. The experiments for each were performed in triplicate, and the mean values of ACs are illustrated with bars.

showed some improvement in acylcarnitine profiles with bezafibrate. We used a dosage of 17–25 mg/kg/day as previously described [11]. Monitoring of liver functions, lactate dehydrogenase (LDH), creatine kinase (CK), and lipid profiles showed no adverse effects of bezafibrate. A short-term evaluation, after 6 months of the trial, did not show clinical improvement except for slightly increased back muscle strength noted by the mother. An echocardiography showed stable but no improvement in a left ventricular mass index. Acylcarnitine profiles in dried blood spots and other biochemical parameters did not show improvement (data not shown). Case 2 died before a clinical trial was considered.

4. Discussion

We report 2 unrelated cases of CACT deficiency with molecular confirmation first identified in Thailand. The c.199-10T>G (IVS2-10T>G) nucleotide change was the most prevalent mutation and identified in 14 out of 76 mutant alleles [15]. This mutation was homozygously identified in three Vietnamese and three Chinese patients. In the present study, in spite that two families had no consanguineous history, both patients were also a homozygotes of the c.199-10T>G mutation. In Japan, three CACT deficient patients have been described. Among them the same mutation was identified heterozygously in only one patient [14]. We propose that this mutation is a founder mutation in Asian populations. Clinical history of the three Chinese patients with homozygous c.199-10T>G mutation were reported [16]. All of them developed cardiac arrest within two days of age, as well as our two patients. Hence the phenotype of homozygotes of c.199-10T>G mutation is severe. This mutation was suggested to reside at a consensus lariat branch point sequence resulting in skipping of exons 3 and 4 or exon 3 alone, which leads to truncation of the protein [17].

Although our cases 1 and 2 were homozygotes of the same mutation, Case 1 survived until 2 years and 8 months and Case 2 died at 4 months of age. Several factors might attribute to their different clinical outcomes: (1) Thalassemia disease in case 2 which required repeated blood transfusions might affect cardiac functions by chronic hypoxia, iron overload, or decreased carnitine [18]; (2) differences in possible modifier genes such as *SLC25A29* gene (CACT-like, CACL) which has palmitoyl-carnitine transporting activity [19]; and (3) different formulas using in our cases, one is a synthetic modular formula and the other is a commercial formula. However, the rationale of both special formulas for diet therapy is a reduction in long-chain fatty acids together with supplementation of medium-chain triglyceride oil to be a caloric source shunting an obstruction of long-chain fatty acid β-oxidation.

Although increased FAO flux induced by bezafibrate was clearly shown in fibroblasts only from patients with mild phenotypes of FAO disorders, increased mRNA expression after bezafibrate exposure also occurred in cell lines from patients with severe phenotypes [20]. This could explain *in vitro* response to bezafibrate observed in fibroblasts of patient 1 and 2. Despite the severe genotype leading to barely detectable enzyme activity [21], we believe that there should be some FAO flux which could be enhanced by bezafibrate in these patients. Our hypothesis is if there is entirely absent FAO flux in these patients, they should have anomalies like those found in a lethal neonatal form of CPT2 deficiency or GA2 [22], even though there has been no report of such findings in CACT deficiency. To our knowledge, patient 1 is the first case of neonatal-onset CACT deficiency who underwent a clinical trial of bezafibrate after showing an *in vitro* response by IVP assay. However, no beneficial short-term effect was shown. This might indicate the irreversible damage of the affected organs esp. the cardiac and skeletal muscles, and liver. Moreover, the difference between the *in vitro* and *in vivo* responses is

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probably due to the difference of bezafibrate concentration used in the IVP assay (400 μmol/L) and typical concentrations obtained in patients on bezafibrate therapy (50–200 μmol/L) [23]. Another possible reason is inadequate acetyl-CoA production despite bezafibrate treatment. This hypothesis is supported by persistently low C2 acylcarnitines in IVP assays of our cases and a previous case with CACT deficiency [11]. Moreover, C16 acylcarnitine did not decrease to the control level after bezafibrate treatment. Overall, although some improvement of acylcarnitine profile was shown in the patient 1 and 2's fibroblasts in IVP assay with bezafibrate, the effect of bezafibrate was less than those in fibroblasts from patients with mild forms of FAO disorders [11,24]. Hence clinical improvement in this patient was thought to be limited. Since CACT-deficient patients who developed metabolic decompensation in early neonatal period had poor prognosis with routine management [7], we decided to use bezafibrate treatment in patient 1. He survived until two years of age with bezafibrate treatment. However, it is uncertain whether this longer survival owed to the effect of bezafibrate treatment or not, since no apparent improvement of clinical laboratory data was obtained.

In conclusion, CACT deficiency may be a common FAO disorder in East Asian populations probably from a founder effect. IVP assay of fibroblasts could determine a response to bezafibrate treatment. A long-term clinical trial and more enrolled patients are required for evaluation of this therapy.

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References

[1] Stanley CA, Hale DE, Berry GT, Deleeuw S, Boxer J, Bonnefont JP. A deficiency of carnitine–acylcarnitine translocase in the inner mitochondrial membrane. N Engl J Med 1992;327:19–23.

[2] Huizing M, Iacobazzi V, Ijlst L, Savelkoul P, Ruitenbeek W, van den Heuvel L, et al. Cloning of the human carnitine–acylcarnitine carrier cDNA and identification of the molecular defect in a patient. Am J Hum Genet 1997;61:1239–45.

[3] Iacobazzi V, Naglieri MA, Stanley CA, Wanders RJA, Palmieri F. The structure and organization of the human carnitine/acylcarnitine translocase (CACT) gene. Biochem Biophys Res Commun 1998;252:770–4.

[4] Saudubray JM, Martin D, de Lonlay P, Touati G, Poggi-Travert F, Bonnet D, et al. Recognition and management of fatty acid oxidation defects: a series of 107 patients. J Inherit Metab Dis 1999;22:488–502.

[5] Hui J, Tang NL, Li CK, Law LK, To KF, Yau P, et al. Inherited metabolic diseases in the Southern Chinese population: spectrum of diseases and estimated incidence from recurrent mutations. Pathology 2014;46:375–82.

[6] Rubio-Gozalbo ME, Bakker JA, Waterham HR, Wanders RJ. Carnitine–acylcarnitine translocase deficiency, clinical, biochemical and genetic aspects. Mol Aspects Med 2004;25:521–32.

[7] Rubio-Gozalbo ME, Vos P, Forget PP, Van Der Meer SB, Wanders RJ, Waterham HR, et al. Carnitine–acylcarnitine translocase deficiency: case report and review of the literature. Acta Paediatr 2003;92:501–4.

[8] Iacobazzi V, Invernizzi F, Baratta S, Pons R, Chung W, Garavaglia B, et al. Molecular and functional analysis of *SLC25A20* mutations causing carnitine–acylcarnitine translocase deficiency. Hum Mutat 2004;24:312–20.

[9] Pierre G, Macdonald A, Gray G, Hendriksz C, Preece MA, Chakrapani A. Prospective treatment in carnitine–acylcarnitine translocase deficiency. J Inherit Metab Dis 2007;30:815.

[10] Bonnefont JP, Bastin J, Behin A, Djouadi F. Bezafibrate for an inborn mitochondrial beta-oxidation defect. N Engl J Med 2009;360:838–40.

[11] Yamaguchi S, Li H, Purevsuren J, Yamada K, Furui M, Takahashi T, et al. Bezafibrate can be a new treatment option for mitochondrial fatty acid oxidation disorders: evaluation by *in vitro* probe acylcarnitine assay. Mol Genet Metab 2012;107:87–91.

[12] Okun JG, Kölker S, Schulze A, Kohlmüller D, Olgemöller K, Lindner M, et al. A method for quantitative acylcarnitine profiling in human skin fibroblasts using unlabelled palmitic acid: diagnosis of fatty acid oxidation disorders and differentiation between biochemical phenotypes of MCAD deficiency. Biochim Biophys Acta 2002;1584:91–8.

[13] Djouadi F, Aubey F, Schlemmer D, Ruiter JP, Wanders RJ, Strauss AW, et al. Bezafibrate increases very-long-chain acyl-CoA dehydrogenase protein and mRNA expression in deficient fibroblasts and is a potential therapy for fatty acid oxidation disorders. Hum Mol Genet 2005;14:2695–703.

[14] Fukushima T, Kaneoka H, Yasuno T, Sasaguri Y, Tokuyasu T, Tokoro K, et al. Three novel mutations in the carnitine–acylcarnitine translocase (*CACT*) gene in patients with CACT deficiency and in healthy individuals. J Hum Genet 2013;58:788–93.

[15] Wang GL, Wang J, Douglas G, Browning M, Hahn S, Ganesh J, et al. Expanded molecular features of carnitine acyl-carnitine translocase (CACT) deficiency by comprehensive molecular analysis. Mol Genet Metab 2011;103:349–57.

[16] Lee RS, Lam CW, Lai CK, Yuen YP, Chan KY, Shek CC, et al. Carnitine–acylcarnitine translocase deficiency in three neonates presenting with rapid deterioration and cardiac arrest. Hong Kong Med J 2007;13:66–8.

[17] Ogawa A, Yamamoto S, Kanazawa M, Takayanagi M, Hasegawa S, Kohno Y. Identification of two novel mutations of the carnitine/acylcarnitine translocase (CACT) gene in a patient with CACT deficiency. J Hum Genet 2000;45:52–5.

[18] El-Beshlawy A, El Accaoui R, Abd El-Sattar M, Gamal El-Deen MH, Youssry I, Shaheen N, et al. Effect of L-carnitine on the physical fitness of thalassemic patients. Ann Hematol 2007;86:31–4.

[19] Sekoguchi E, Sato N, Yasui A, Fukada S, Nimura Y, Aburatani H, et al. A novel mitochondrial carnitine–acylcarnitine translocase induced by partial hepatectomy and fasting. J Biol Chem 2003;278:38796–802.

[20] Gobin-Limballe S, Djouadi F, Aubey F, Olpin S, Andresen BS, Yamaguchi S, et al. Genetic basis for correction of very-long-chain acyl-coenzyme A dehydrogenase deficiency by bezafibrate in patient fibroblasts: toward a genotype-based therapy. Am J Hum Genet 2007;81:1133–43.

[21] Costa C, Costa JM, Slama A, Boutron A, Vequaud C, Legrand A, et al. Mutational spectrum and DNA-based prenatal diagnosis in carnitine–acylcarnitine translocase deficiency. Mol Genet Metab 2003;78:68–73.

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[22] Distelmaier F, Vogel M, Spiekerkötter U, Gempel K, Klee D, Braunstein S, et al. Cystic renal dysplasia as a leading sign of inherited metabolic disease. Pediatr Nephrol 2007;22:2119–24.

[23] Monk JP, Todd PA. Bezafibrate. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in hyperlipidaemia. Drugs 1987;33:539–76.

[24] Li H, Fukuda S, Hasegawa Y, Kobayashi H, Purevsuren J, Mushimoto Y, et al. Effect of heat stress and bezafibrate on mitochondrial beta-oxidation: comparison between cultured cells from normal and mitochondrial fatty acid oxidation disorder children using *in vitro* probe acylcarnitine profiling assay. Brain Dev 2010;32:362–70.

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Case Report

Myocerebrohepatopathy spectrum disorder due to *POLG* mutations: A clinicopathological report

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Abstract

We report on the clinical, neuropathological, and genetic findings of a Japanese case with myocerebrohepatopathy spectrum (MCHS) disorder due to polymerase gamma (*POLG*) mutations. A girl manifested poor sucking and failure to thrive since 4 months of age and had frequent vomiting and developmental regression at 5 months of age. She showed significant hypotonia and hepatomegaly. Laboratory tests showed hepatocellular dysfunction and elevated protein and lactate levels in the cerebrospinal fluid. Her liver function and neurologic condition exacerbated, and she died at 8 months of age. At autopsy, fatty degeneration and fibrosis were observed in the liver. Neuropathological examination revealed white matter-predominant spongy changes with Alzheimer type II glia and loss of myelin. Enzyme activities of the respiratory chain complex I, III, and IV relative to citrate synthase in the muscle were normal in the biopsied muscle tissue, but they were reduced in the liver to 0%, 10%, and 14% of normal values, respectively. In the liver, the copy number of mitochondrial DNA compared to nuclear DNA was reduced to 3.3% of normal values as evaluated by quantitative polymerase chain reaction. Genetic analysis revealed compound heterozygous mutations for *POLG* (I1185T/A957V). This case represents the differential involvement of multiple organs and phenotype-specific distribution of brain lesions in mitochondrial DNA depletion disorders.

Keywords: Alpers syndrome; Mitochondrial DNA depletion; Myocerebrohepatopathy spectrum disorder; *POLG*

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1. Introduction

Mitochondrial DNA (mtDNA) depletion syndrome (MDDS), first described in 1991, is defined as a reduction in the mtDNA copy number in different tissues, leading to insufficient synthesis of respiratory chain complexes (RCC) [1]. Clinical manifestations of MDDS involve many organ systems including the central and peripheral nervous system, liver, muscle, and gastrointestinal tract [2]. Human polymerase gamma (*POLG*) is the common causative gene involved in MDDS, whose mutations result in a diverse group of phenotypes, such as Alpers syndrome and myocerebrohepatopathy spectrum (MCHS) disorders, which typically show disease onset during early childhood. Further, several *POLG*-related phenotypes manifesting during adolescence and adulthood are recognized, including progressive external ophthalmoplegia, ataxia-neuropathy spectrum disorders, myoclonus epilepsy myopathy sensory ataxia, and sensory ataxic neuropathy with dysarthria/dysphagia and ophthalmoplegia. Some overlaps in the symptoms between these adult phenotypes exist, and can be additionally accompanied by tremor, parkinsonism, hearing loss, stroke-like episodes, and gastrointestinal symptoms, which are reminiscent of symptoms of mitochondrial diseases with pathomechanisms other than MDDS [3,4].

MCHS, the most severe phenotype of POLG disorders, was recently identified and is defined by the clinical triad of (1) myopathy or hypotonia, (2) developmental delay or dementia, and (3) liver dysfunction [3,5]. Severe, intractable epilepsy is included in the diagnostic hallmarks of Alpers syndrome, but is not characteristic of MCHS. As the number of patients with MCHS disorders is small and detailed clinicopathological findings are unavailable, we herein report the case of a girl with MCHS disorders due to *POLG* mutations. As far as we know, this is the first Japanese case of MCHS disorders with *POLG* mutation.

2. Case report

A girl was born at 40 weeks of gestation to healthy non-consanguineous parents without any abnormalities. The birth weight, height, and head circumference were normative. Early development and growth were unremarkable. At 4 months of age, she developed poor weight gain, emesis, hypotonia, developmental delay, and lethargy. She was admitted to our hospital because of recurrent vomiting at 6 months of age.

On admission, body length was 60.9 cm [−2.2 standard deviation (SD)], body weight was 5600 g (−2.3 SD), and head circumference was 42 cm (+0.2 SD). Hepatomegaly of a hard consistency was observed approximately 3 cm under the costal margin with no associated splenomegaly. She was alert and could establish good eye contact and smile. She showed severe hypotonia and proximal dominant muscular weakness. She could hold neither her head nor limbs up. All deep tendon reflexes were weak.

Although complete blood count and urinalysis were unremarkable, hepatocellular dysfunction was obvious at the time of hospitalization, with the following laboratory test values: aspartate aminotransferase, 390 U/L; alanine aminotransferase, 218 U/L; total bilirubin, 1.6 mg/dL; total bile acids, 172 μmol/L; γ-glutamyl transpeptidase, 179 IU/L; leucine aminopeptidase, 268 IU/L; and cholinesterase, 73 IU/L. Levels of serum creatine kinase and blood glucose were normal. Cerebrospinal fluid (CSF) examination showed elevated protein levels of 304 mg/dL and normal cell count and glucose levels. Lactic acid was elevated in both plasma and CSF, at 15.9 mg/dL and 30.3 mg/dL, respectively. Pyruvic acid was normal in both plasma and CSF. Metabolic screening tests, including urine organic acids, plasma, and urine amino acids, were unremarkable. Initial brain computed tomography (CT) and magnetic resonance imaging performed at 6 months of age were unremarkable. The electroencephalogram showed generalized slow wave activity. Only wave I was identifiable on auditory evoked potentials. Motor nerve and sensory conduction were mildly delayed.

Muscle biopsy findings at 6 months of age showed a variation in fiber type; ragged-red fiber was not observed. Lipid and glycogen storage were not observed. Cytochrome c oxidase staining showed normal findings. Analysis of the RCC enzyme activity revealed no abnormality. No mtDNA mutations were identified.

Soon after admission, difficulty in feeding and vomiting aggravated, and tube feeding along with parenteral nutrition was required. She experienced bouts of diarrhea. Consciousness level decreased progressively, and myoclonic jerks of the right and left arms were infrequently observed. Follow-up CT revealed mild cerebral atrophy at 7 months of age. Hepatocellular dysfunction exacerbated progressively, and she died of multiple organ failure caused by hepatic failure at 8 months of age, despite supplementation of multiple vitamins and coenzyme Q 10, and was autopsied. Two years later, another girl was born to the parents. She had the same clinical course and laboratory findings observed in the present patient and died at 7 months of age. Valproic acid was not used in either patient.

2.1. Postmortem examinations

Body weight was 6.0 kg (mean ± SD, 8.0 ± 0.88 kg). The weight of the atrophic liver was 200 g, and the surface was yellowish, irregular, and hard. The lungs were congested and adrenal glands were atrophic. The other visceral organs were unremarkable on macroscopic

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examination. The brain weighed 760 g and showed massive edema and caudal necrosis. Microscopically, hepatocytes and adrenal cortical cells were swollen, and renal tubular cells contained phospholipids and diffuse foam cells. Similar foam cells were also seen in the lungs and cardiac muscle fibers. In the liver, hepatic fibrosis, microvesicular steatosis, and fatty degeneration were observed (Fig. 1). In the central nervous system, a spongy change was noted predominantly in the cerebral white matter, and neuronal loss in the cerebral and cerebellar cortex was mild. Alzheimer type II glia was observed in massive numbers in the cerebral and cerebellar white matter, with a smaller amount in the cerebral cortex and deep gray matter. Neuronal loss, capillary proliferation, and sponginess were prominent in the substantia nigra (Fig. 2). Recent linear necrosis was present in the bilateral caudate nucleus.

2.2. Assay of respiratory chain complex enzyme activity in the liver

The liver samples were immediately frozen at autopsy and stored at −70 °C. Activities of RCC I, II, III and IV were assayed as described previously [6,7]. The percentages of RCC I, II, III and IV activities relative to that of citrate synthase (CS) as a mitochondrial enzyme marker were calculated. Relative enzyme activities of RCC I, III, and IV to CS in the liver were reduced to 0%, 10%, and 14% of normal values, respectively, while that of RCC II was reduced to 29%.

2.3. Analysis of quantitative polymerase chain reaction of mtDNA and DNA sequence of POLG gene

Written informed consent was obtained from the patient's parents in order to perform gene analysis. The quantitative estimation of mtDNA was performed by real-time amplification of fragments of *ND1* in the mtDNA genome, as previously described [7,8]. To determine the overall abundance of mtDNA, we compared the real-time amplification of *ND1* with a single-copy nuclear reference gene (exon 24 of the *CFTR* gene) [7,9]. The ratio of *ND1* to *CFTR* in the liver was reduced to 3.3% (SD, 1.2%) as compared to the control.

Mutation analysis was performed on the genomic DNA using primers designed to amplify the coding exons and the exon-intron boundaries of *POLG* (NM_002693.2). Fragments were analyzed by direct sequencing using ABI 3130XL (Applied Biosystems, Tokyo, Japan). The genetic analysis revealed compound heterozygous mutations in *POLG* (c.2870C>T, p.A957V and c.3554T>C, p.I1185T). The two DNA mutations

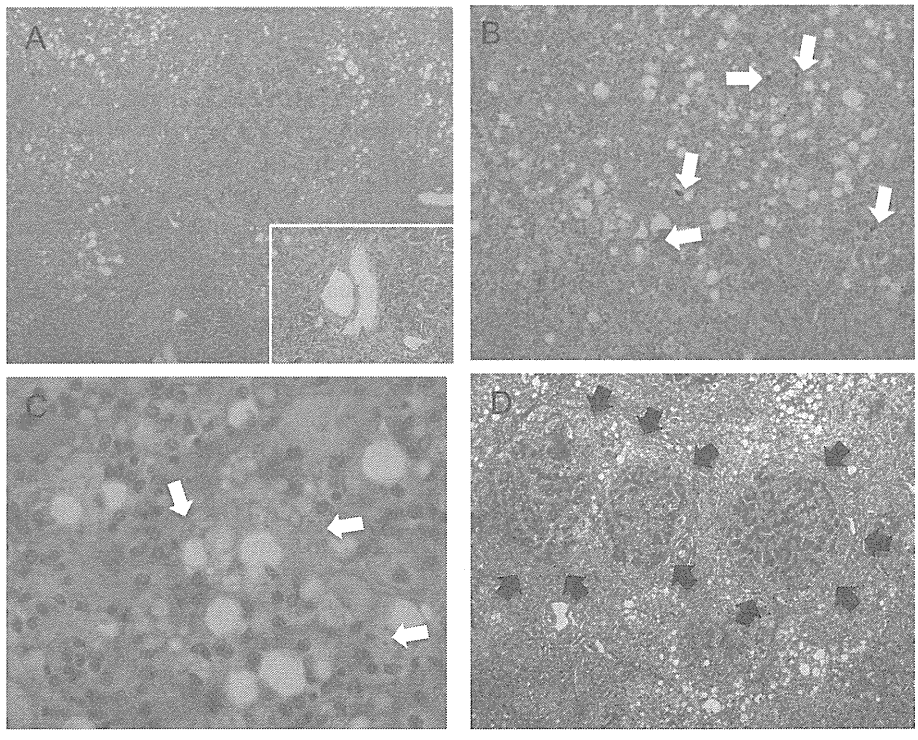

Fig. 1. Pathological findings of the postmortem liver (A–C: hematoxylin & eosin staining, D: Masson trichrome staining). (A) Moderate inflammatory cell infiltration (inset) with destroyed limiting plates and a rather progressive fibrosis with bridging formation in the portal tracts were observed (original magnification, ×40). (B) Swollen hepatocytes containing lipid droplets of various sizes were found. Bile plugs (white arrows in B and C) were noted in the cytoplasm of hepatocytes and dilated canaliculi (×100). (C) Swollen hepatocytes containing lipid droplets of various sizes were found. Bile plugs were noted in the cytoplasm of hepatocytes (×400). (D) A rather progressive fibrosis with bridging formation (arrows) in the portal tracts was found (×40).

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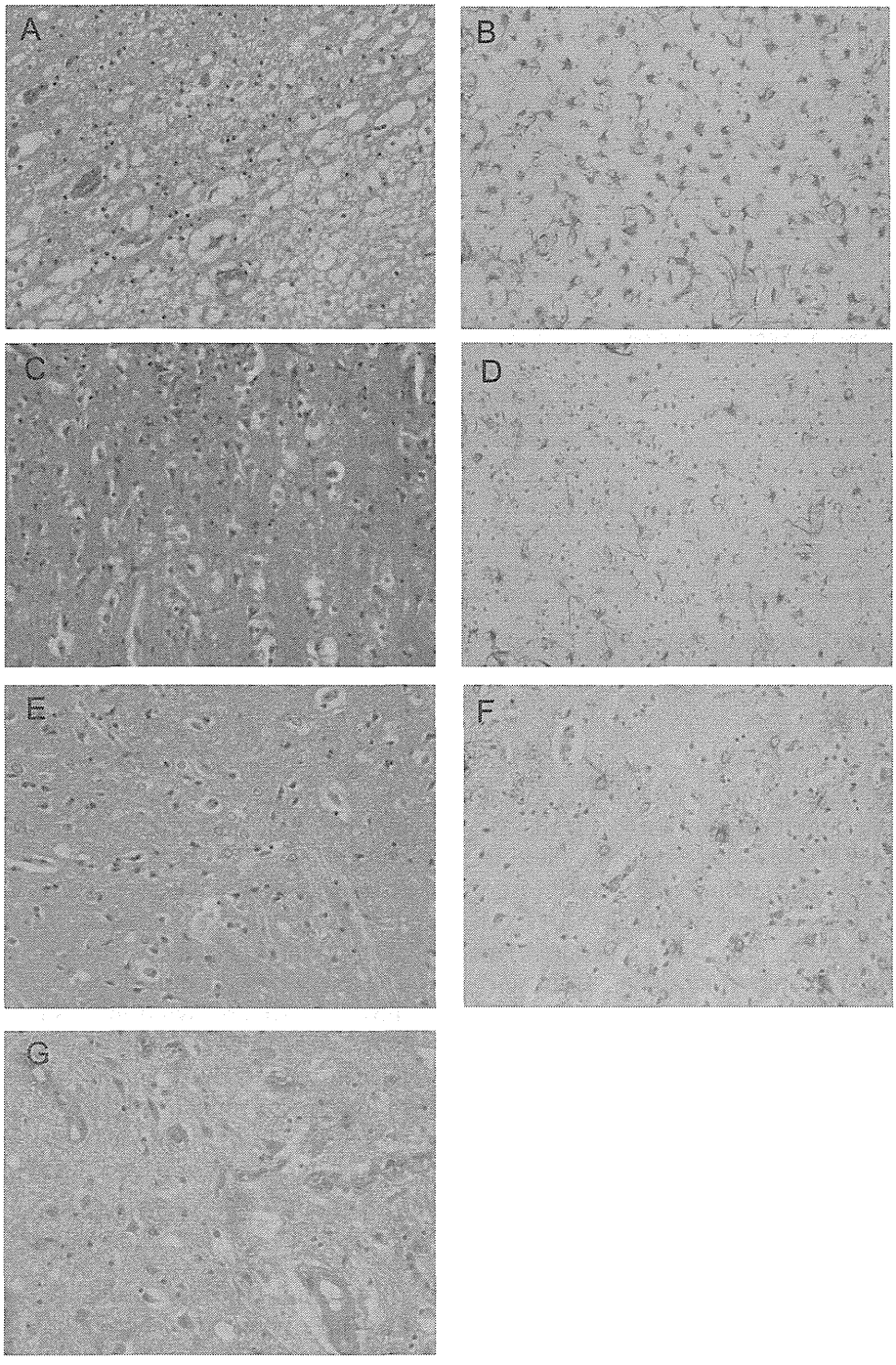

Fig. 2. Pathological findings of the postmortem brain (A, C, E, and G: hematoxylin & eosin staining; B, D, and F: immunohistochemical staining against glial fibrillary acidic protein; original magnification, ×400). Marked spongy changes (A) with Alzheimer type II astrocytosis (B) was observed in the cerebral white matter, and less prominently in the cerebral cortex (C and D) and striatum (E and F). Neuronal loss, sponginess, and capillary proliferation, which were reminiscent of the findings of Leigh syndrome, were noted in the substantia nigra (G).

were not registered in neither of the 1000 Genomes Project Database (http://www.1000genomes.org/), ESP6500 database (http://evs.gs.washington.edu/EVS/) or HGVD (http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html). The amino acid sequences of these two sites (p.A957V and p.I1185T) are well conserved across species, suggesting their importance (Fig. 3). *In silico* analyses were performed using the prediction algorithms SIFT (http://sift.jcvi.org) and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/). These mutations are predicted to be deleterious by SIFT (0 and 0, respectively) and PolyPhen2 (0.985 and 0.991, respectively) programs. The results of mutation analysis have been reported previously (patient 6 in Ref. [9]).

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Fig. 3. Conservation analysis of mutation sites in *POLG*. The sites of compound heterozygous amino acid mutations (p.957A and p.1185I) are well conserved across species.

3. Discussion

The hetero compound mutations in *POLG* were not found in either of the 1000 Genomes Project Database, ESP6500 database nor HGVD, suggesting that these are pathogenic mutations. The amino acid sequences of these two sites (p.A957V and p.I1185T) are well conserved across species including *Saccharomyces cerevisiae*, indicating their importance (Fig. 3). *In silico* analyses also predicted that these two amino acid mutations are deleterious. Furthermore A957V has been reported by Tang et al. [10]. They reported A957V allele was shared in three unrelated patients and concluded this mutation is pathogenic. The pathogenic mutations in the flanking region of p.1185I; p.1184D [11,12] and p.1186D [13] have been reported, suggesting this region is also important. Thus, we conclude the compound heterozygous mutations of this patient cause the disease.

Alpers syndrome is defined as the clinical triad of (1) refractory, mixed-type seizures that often include a focal component, (2) psychomotor regression, often triggered by intercurrent infection, and (3) hepatopathy with or without acute liver failure. There is an overlap between the phenotypes of MCHS and Alpers syndrome; however, the former usually shows an earlier onset age and more rapid disease progression, while the latter is characterized by intractable epilepsy. Using the "myo-" prefix in MCHS may be confusing since the pathological findings of muscles in this disorder often shows no evidence of mitochondrial myopathy; instead, the hypotonia observed in the triad can be regarded as a symptom of brain dysfunction. Thus, the clinical features of the patient discussed herein were typical of MCHS.

Although Wong et al. [3] "*...excluded classical Alpers hepatopathy by liver biopsy*" in MHCS, exact pathological findings were not provided by the authors. Differences in the hepatopathy observed in these two phenotypes have not been established; pathological characteristics of the liver in Alpers syndrome include fibrosis, regenerative nodules, hepatocyte dropout, bile duct proliferation, fatty changes, and bile stasis [14]. The findings of the present patient were compatible with those of Alpers syndrome, similar to the case of POLG-related MDDS previously observed [15]. As for the neuropathological findings, Alpers syndrome usually shows a preferential involvement of gray matter, characterized by gliosis, nerve cell loss, spongy degeneration, and accumulation of neural lipids in the cerebral cortex [16]. Alzheimer type II glia, representing hepatic encephalopathy, was also distributed predominantly in the gray matter [17].

A patient exhibiting a clinical evolution from MCHS to Alpers phenotype showed gray matter involvement and microscopic findings similar to those in Leigh syndrome [5], and brain biopsy in another Alpers patient with prominent white matter signal change revealed pathological characteristics typical of Alpers disease with intractable seizures [18]. On the other hand, marked gliosis and sponginess of the white matter without pathological changes in the cerebral cortex was observed in a patient with probable MCHS [17]. Apart from these, we could not find any MCHS cases with a neuropathological description in the literature. The white matter-predominant spongy degeneration with Alzheimer type II astrocytosis in the present patient may therefore be characteristic of MCHS.

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POLG disorders often show elevated levels of lactate both in the serum and CSF as well as elevated levels of hepatic enzymes. However, these findings are not specific for POLG disorders; rather, they are hallmarks of mitochondrial disorders. Analysis of the RCC enzyme activity is the most valuable test for diagnosis of MDDS. However, RCC enzyme activity varies among muscle, liver, kidney, and brain tissues in the same patient [1,19], presumably due to the differential degree of DNA depletion among individual organs. The constituents of complex II are coded by genes in the nuclear, not mitochondrial DNA. In the present patient, the decreased complex II enzyme activity in the biopsied liver may either result from augmented activity of control CS enzyme due to an increase of mitochondria in number, or may be secondary to the damage of hepatocytes with necrotic and fibrotic changes [19]. It is very important to keep in mind that morphological findings and RCC enzyme activities in the muscle are sometimes unremarkable in MCHS patients, even though they show hypotonia or muscular weakness, as in the present case [5,15,20]. Therefore, analysis of RCC enzyme activities in the liver should be considered when Alpers syndrome or MCHS disorders are suspected, even when the morphological findings of muscle or enzyme assay results are unremarkable.

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References

[1] Moraes C, Shanske S, Tritschler HJ, Aprille JR, Andreetta F, Bonilla E, et al. mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. Am J Hum Genet 1991;48:492–501.

[2] Sarzi E, Bourdon A, Chrétien D, Zarhrate M, Corcos J, Slama A, et al. Mitochondrial DNA depletion is a prevalent cause of multiple respiratory chain deficiency in childhood. J Pediatr 2007;150(531–4):e6.

[3] Wong L-J, Naviaux RK, Brunetti-Pierri N, Zhang Q, Schmitt ES, Truong C, et al. Molecular and clinical genetics of mitochondrial diseases due to *POLG* mutations. Hum Mutat 2008;29:150–72.

[4] Cohen BH, Naviaux RK. The clinical diagnosis of POLG disease and other mitochondrial DNA depletion disorders. Methods 2010;51:364–73.

[5] Scalais E, Francois B, Schlesser P, Stevens R, Nuttin C, Martin J-J, et al. Polymerase gamma deficiency (POLG): clinical course in a child with a two stage evolution from infantile myocerebrohepatopathy spectrum to an Alpers syndrome and neuropathological findings of Leigh's encephalopathy. Eur J Paediatr Neurol 2012;16:542–8.

[6] Kirby DM, Crawford M, Cleary MA, Dahl HH, Dennett X, Tourburn DR. Respiratory chain complex I deficiency. An underdiagnosed energy generation disorder. Neurology 1999;52:1255–64.

[7] Kaji S, Murayama K, Nagata I, Nagasaka H, Takayanagi M, Ohtake A, et al. Fluctuating liver functions in siblings with MPV17 mutations and possible improvement associated with dietary and pharmaceutical treatments targeting respiratory chain complex II. Mol Genet Metab 2009;97:292–6.

[8] Pagnamenta AT, Taanman JW, Wilson CJ, Anderson NE, Marotta R, Duncan AJ, et al. Dominant inheritance of premature ovarian failure associated with mutant mitochondrial DNA polymerase gamma. Hum Reprod 2006;21:2467–73.

[9] Yamazaki T, Murayama K, Compton AG, Sugiana C, Harashima H, Amemiya S, et al. Molecular diagnosis of mitochondrial respiratory chain disorders in Japan: focusing on mitochondrial DNA depletion syndrome. Pediatr Int 2014;56:180–7.

[10] Tang S, Wang J, Lee NC, Milone M, Halberg MC, Schmitt ES, et al. Mitochondrial DNA polymerase gamma mutations: an ever expanding molecular and clinical spectrum. J Med Genet 2011;48:669–81.

[11] Martikainen MH, Hinttala R, Majamaa K. Novel POLG1 mutations in a patient with adult-onset progressive external ophthalmoplegia and encephalopathy. BMJ Case Reports 2010; doi:10.1336/bcr.01.2010.2604.

[12] González-Vioque E, Blázquez A, Fernández-Moreira D, Bornstein B, Bautista J, Arpa J, et al. Association of novel POLG mutations and multiple mitochondrial DNA deletions with variable clinical phenotypes in a Spanish population. Arch Neurol 2006;63:107–11.

[13] Cheldi A, Ronchi D, Bordoni A, Bordo B, Lanfranconi S, Bellotti MG, et al. *POLG1* mutations and stroke like episodes: a distinct clinical entity rather than an atypical MELAS syndrome. BMC Neurol 2013;13:8.

[14] Nguyen KV, Sharief FS, Chan SS, Copeland WC, Naviaux RK. Molecular diagnosis of Alpers syndrome. J Hepatol 2006;45:108–16.

[15] Kurt B, Jaeken J, Van Hove J, Lagae L, Löfgren A, Everman DB, et al. A novel POLG gene mutation in 4 children with Alpers-like hepatocerebral syndromes. Arch Neurol 2010;67:239–44.

[16] Boustany R, Zucker A. Degenerative disease primarily gray matter. In: Swaiman KF, Ashwal S, Ferriero D, editors. Pediatric neurology: principles & practice. Philadelphia: Mosby; 2006. p. 1326–8.

[17] Kollberg G, Moslemi AR, Darin N, Nennesmo I, Bjarnadottir I, Uvebrant P, et al. POLG1 mutations associated with progressive encephalopathy in childhood. J Neuropathol Exp Neurol 2006;65:758–68.

[18] Bao X, Wu Y, Wong LJ, Zhang Y, Xiong H, Chou PC, et al. Alpers syndrome with prominent white matter changes. Brain Dev 2008;30:295–300.

[19] de Vries M, Rodenburg RJ, Morava E, van Kaauwen E, ter Laak H, Mullaart R, et al. Multiple oxidative phosphorylation deficiencies in severe childhood multi-system disorders due to polymerase gamma (*POLG1*) mutations. Eur J Pediatr 2007;166:229–34.

[20] Ferrari G, Lamantea E, Donati A, Filosto M, Briem E, Carrara F, et al. Infantile hepatocerebral syndromes associated with mutations in the mitochondrial DNA polymerase-gamma A. Brain 2005;128:723–31.

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Case report

Leigh syndrome with Fukuyama congenital muscular dystrophy: A case report

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Abstract

We report the first case of Leigh syndrome (LS) with Fukuyama congenital muscular dystrophy (FCMD). A neonate suffered from lactic acidosis and subsequently presented with poor feeding, muscle weakness, hypotonia, cardiopulmonary dysfunction, and hydrocephalus. He died at 17 months. The findings of brain magnetic resonance imaging indicated some specific features of both LS and FCMD, and FCMD gene mutation was detected. Decreased mitochondrial respiratory complex I and II activity was noted. Mitochondrial DNA sequencing showed no pathogenic mutation. A case with complex I + II deficiency has rarely been reported, suggesting a nuclear gene mutation.

Keywords: Leigh syndrome; FCMD; Mitochondria; Complex I + II deficiency

1. Introduction

Fukuyama congenital muscular dystrophy (FCMD), one of the most common autosomal recessive disorders in the Japanese population, is characterized by congenital muscular dystrophy with cortical dysgenesis. The gene responsible for FCMD is located on 9q31. Most FCMD-bearing chromosomes (87%) have a 3-kb retrotransposal insertion in the 3′-untranslated region of the gene [1].

Leigh syndrome (LS) is a progressive neurodegenerative disorder with psychomotor retardation, signs and symptoms of brain stem and/or basal ganglia involvement, and raised lactate levels in blood and/or cerebrospinal fluid (CSF). In majority of the cases, dysfunction of the mitochondrial respiratory chain is responsible for the disease. LS is caused by either mitochondrial or nuclear gene mutations with large genetic heterogeneity [2]. Here, we report the first case of LS with FCMD.

2. Case report

2.1. Index case

A Japanese boy was born at term as the third child to non-consanguineous healthy parents. His serum creatine

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