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BCS1L mutations produce Fanconi syndrome with developmental disability

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Abstract

Fanconi syndrome is a functional disorder of the proximal tubule, characterized by pan-aminoaciduria, glucosuria, hypophosphatemia, and metabolic acidosis. With the advancements in gene analysis technologies, several causative genes are identified for Fanconi syndrome. Several mitochondrial diseases cause Fanconi syndrome and various systemic symptoms; however, it is rare that the main clinical symptoms in such disorders are Fanconi syndrome without systematic active diseases like encephalomyopathy or cardiomyopathy. In this study, we analyzed two families exhibiting Fanconi syndrome, developmental disability and mildly elevated liver enzyme levels. Whole-exome sequencing (WES) detected compound heterozygous known and novel *BCS1L* mutations, which affect the assembly of mitochondrial respiratory chain complex III, in both cases. The pathogenicity of these mutations has been established in several mitochondria-related functional analyses in this study. Mitochondrial diseases with isolated renal symptoms are uncommon; however, this study indicates that mitochondrial respiratory chain complex III deficiency due to *BCS1L* mutations cause Fanconi syndrome with developmental disability as the primary indications.

Keywords

BCS1L, Fanconi syndrome, mitochondrial respiratory chain complex III deficiency, GDF-15

Introduction

Fanconi syndrome is a functional disorder of the proximal tubule, characterized by pan-aminoaciduria, glucosuria, hypophosphatemia, proximal tubular acidosis, and low molecular weight proteinuria[1]. Fanconi syndrome can be congenital or acquired. With the advancements in gene analysis technologies, such as WES and next generation sequencing, genetic diagnosis is possible for renal tubulopathies; several causative genes are identified for Fanconi syndrome. Congenital Fanconi syndrome occurs as an associated symptom of cystinosis, caused by *CTNS*

abnormality (OMIM#219800), and Lowe syndrome, caused by *OCRL* abnormality (OMIM#309000). In addition, it is also caused by pathogenic variants of *SLC34A1* (OMIM#613388)[2], *EHHADH* (OMIM#615605)[3], *SLC2A2* (OMIM#227810)[4], *HNF4A (OMIM#616026)*[5] and *GATM* (OMIM#134600)[6].

Mitochondrial diseases also cause Fanconi syndrome, which subsequently develop into encephalomyopathy or cardiomyopathy[7,8]. Mitochondrial diseases usually present as disorders of the nerves and muscles; however, they may also present as isolated organ disorders, such as mitochondrial cardiomyopathy and hepatopathy[9]. The kidney, like the heart and liver, is an organ with large energy demands; however, mitochondrial disease with isolated nephropathy is relatively uncommon. Fanconi syndrome of Acadian, resulting from the abnormality of *NDUFAF6* (OMIM#618913), which modulates the assembly of the mitochondrial respiratory chain complex I, is known but rare; however, there are several cases of mitochondrial diseases mainly associated with renal Fanconi syndrome[10,11].

Here, we indicate that mitochondrial respiratory chain complex III deficiency due to *BCS1L* mutations causes Fanconi syndrome with developmental disability.

Materials and Methods

Patients

We analyzed the patients clinically diagnosed with Fanconi syndrome of unknown cause. Details regarding the clinical findings and laboratory data were obtained from the referring clinician or the patient's hospital records.

Patient 1

Patient 1 was a 3-year-old girl. She was born to non-consanguineous parents at 37 weeks and 5 days of gestation. Her birth weight was 2125 g (-1.9 SD), and her birth height was 43.5 cm (-2.0 SD). She was able to hold her head up at 3 months, roll over at six months, and pull herself up at nine months

of age; but at the age of one and half years, she was pointed out for developmental disability, because she could not walk by herself and could not speak meaningful words. She was diagnosed with Fanconi syndrome based on renal tubular dysfunction and rickets, and elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Details of the laboratory data are shown in Supplementary Table 1. At the age of 3 years, she had severe developmental disability (developmental quotient: 47) and growth failure (height 81.7 cm; -2.6 SD, weight 10.5 kg; -1.7 SD). She was administered with phosphorus and alfacalcidol to control her Fanconi syndrome.

She has twin brothers. Both were diagnosed with Fanconi syndrome, but one of the twin brothers died of pulmonary hypertension at the age of 1 year (II-1). The other is now 8 years old (II-2) and has developmental disability. She also has a younger brother, who is 1-year-old (II-5) and does not present with any clinical abnormalities. Her pedigree is shown in Figure 1a.

Patient 2

Patient 2 was a 10-months-old boy. He was born to non-consanguineous parents at 38 weeks and 3 days of gestation. His mother achieved pregnancy through microfertilization and delivered via cesarean section. His birth weight was 2458 g (-1.3 SD), and birth height was 46.7 cm (-0.9 SD). At approximately 7 months of age, his height and weight gain were retarded. At the 10-month medical check-up, he was diagnosed with developmental disability.

A detailed examination was performed, and he was diagnosed with Fanconi syndrome based on the findings of renal tubular dysfunction and rickets, as well as elevated AST and ALT levels. Details of the laboratory data are shown in Supplementary Table 1. He exhibited severe developmental disability (developmental quotient: 52) and growth failure (height, 64.5 cm; -3.3 SD, weight, 7.4 kg; -1.8 SD). Moderate hearing loss was also recorded. Liver biopsy revealed lymphocytic infiltration and fibrosis in the portal area, accumulation of fat, and an increase in the number of mitochondria (Figure 2). His pedigree is shown in Figure 1b.

Genetic testing

Genetic testing was performed after obtaining written informed consent. Genomic DNA was isolated from the peripheral blood leukocytes of patients and their family members using the QuickGene-Mini80 system (Wako Pure Chemical Industries, Ltd., Tokyo, Japan), in accordance with the manufacturer's instructions. WES was performed as follows. Target regions were concentrated using the SureSelectXT Human All Exon Kit V6 (Agilent Technologies, Santa Clara, CA, USA). The captured DNA samples were amplified using PCR and sequenced using the HiSeq 2500 platform (Illumina, San Diego, CA, USA). WES data were analyzed using SureSelect software (Agilent Technologies, Santa Clara, CA, USA). Mapping of the sequence reads to the human reference genome (GRCh37) was performed. We extracted variants of minor allele frequencies that were less than 0.03 in Japanese and were consistent with the Segregation analysis. Further, those that did not match the phenotype were excluded. Variants were confirmed using Sanger sequencing. Pathogenicity predictions were performed in accordance with the American College of Medical Genetics guidelines[12]. Prediction of the pathogenicity of mutations was performed using Alamut Visual software (Interactive Biosoftware, Rouen, France), including Align GVGD (http://agvgd.iarc.fr/), SIFT (https://sift.bii.a-star.edu.sg/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.pathogenic varianttaster.org/). The cDNA reference number for *BCS1L* is NM_001257343.1.

Measurement of serum GDF-15 and FGF-21

Enzyme-linked immunosorbent assay was used for measuring the levels of growth differentiation factor 15 (GDF- 15) and fibroblast growth factor 21 (FGF-21) in the patient serum samples. Human GDF-15 Quantikine ELISA Kit (R&D systems, Minneapolis, MN, USA) and the Fibroblast Growth Factor 21 Human ELISA kit (BioVendor, Brno, Czech Republic) were used[13]. The mean ± SD values of control, measured for 146 individuals, were 462.5 ± 141.0 pg/ml (range, 152.5–1,010) for

GDF-15 and 156.0 ± 203.5 pg/ml (range, 0–1,645) for FGF-21. The calculated threshold values for GDF-15 and FGF-21 were 707.0 and 343.8 pg/ml, respectively. The sensitivity and specificity of measurement of GDF-15 level for the diagnosis of mitochondrial disorders were 97.9% and 95.2%, respectively, and were substantially higher than the values of 77.1% and 87.7%, respectively, for FGF-21.

Activities of mitochondrial respiratory chain complexes I, II, III, and IV

To analyze the functional consequences of the mutation, the activity of mitochondrial respiratory chain complexes I, II, III, and IV was assayed in mitochondria isolated from skin fibroblasts of the patient's skin samples, as described previously[14,15]. The enzyme activity of each complex was presented as a percentage of the mean value obtained for 35 healthy controls and was calculated as a percentage relative to citrate synthetase (CS), a mitochondrial enzyme marker, or Co II activity. When enzyme activity decreased below 40%, it was defined as a decrease, as reported [16].

Cellular oxygen consumption rate (OCR)

To analyze the functional consequences of the mutation, oxygen consumption rate (OCR) of mitochondria was measured by detecting changes in the concentrations of oxygen and hydrogen, using microscale oxygraphy (Seahorse XF96 system; Seahorse Bioscience, Billerica, MA, USA). Skin fibroblasts isolated from patient skin samples were used. Fibroblast cell lines were seeded in Dulbecco's modified Eagle's medium and cultured in a 5% CO_2 incubator for 24 h at 37°C. The basal respiration rate was measured; oligomycin (ATP synthase inhibitor), carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone, and rotenone (a mitochondrial complex I inhibitor) were added sequentially, and the maximum respiration was recorded after each addition. The maximum respiration rate (MRR) was calculated as the OCR after the addition of carbonyl cyanide phenylhydrazone minus the rotenone-insensitive OCR. OCR was expressed as the ratio of the rates

158 in the patient's sample and the average of the control samples. We analyzed five control samples,
159 with at least five measurements taken for each sample. Based on the distribution of MRR in the five
160 control samples, a reduction in the rate to a value <71.6% was considered a significant decline ($p < 0.05$). In each run, we performed measurement for one or two control samples along with patient
161
162 samples. OCR was expressed as a percentage relative to the average of control(s).
163
164 **Blue native polyacrylamide gel electrophoresis (BN-PAGE)**
165 The expression of the mitochondrial respiratory chain complex I, II, III, and IV proteins was
166 examined using western blotting and blue native polyacrylamide gel electrophoresis (BN-PAGE), as
167 described previously [17,18]. The primary antibodies used in this study were: NDUFA9 (Life
168 Technologies, 459100) for detection of complex I deficiency, SDHA (Life Technologies, 459200)
169 for detection of complex II deficiency, UQCRC1 (Life Technologies, 459140) for detection of
170 complex III deficiency, and COXI (Life Technologies, 459600) for detection of complex IV
171 deficiency.
172
173 **Statistical analysis**
174 Statistical analysis was performed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA).
175 The Kruskal–Wallis H test was used to evaluate differences in continuous variables between
176 groups, chi-squared and Fisher's tests were used to evaluate differences between categorical
177 variables, and Wilcoxon test was used to evaluate differences between control and patient samples.
178 All statistical tests were two sided, and p values <0.05 were considered statistically significant.
179
180 **Results**
181 **Genetic testing**

In patient 1, we identified compound heterozygous missense mutation and frameshift mutation of *BCS1L*, c.268C>T, p.(Arg90Cys) [19] and c.821del, p.(Pro274Argfs*26) [20]. These mutations are also present in each parent in a heterozygous state. Her elder brother (II-2) had the same compound heterozygous mutations; whereas her younger brother (II-5) is a heterozygous carrier of the frameshift mutation (Figure 3a). These mutations have been reported previously.

In patient 2, we also identified compound heterozygous novel missense mutations of *BCS1L*, c.167G>A, p.(Arg56Gln) and c.1195T>G, p.(Tyr399Asp), which were present in each parent in a heterozygous state (Figure 3b).

The results of evaluation of the pathogenicity of missense mutations using in silico analysis are shown in Supplementary Table 2. Other variants identified in the patients by WES are shown in Supplementary Table 3.

Mitochondria-related test

The level of GDF-15 in Patient 1 was 1025.5 pg/ml (normal: <707.4 pg/ml). The elder brother with the same *BCS1L* mutations (II-2) showed a high GDF-15 level of 1152.1 pg/ml, whereas the younger brother (II-5), who was a healthy heterozygous carrier, had a normal GDF-15 level of 219.2 pg/ml.

In patient 2, the level of FGF-21 was 1592.8 pg/ml (normal: <281.0 pg/ml) and the level of GDF-15 was 2035.2 pg/ml (normal: <707.4 pg/ml).

Patient 2 had novel mutations; therefore, the function of mitochondria was evaluated. Because both the mutations in Patient 1 are known mutations and are likely pathogenic, functional analysis was not performed. The activities of CO I-IV in skin fibroblasts were normal (Table 1), whereas the MRR was reduced to 69%, and the OCR in skin fibroblasts was significantly lower than that in the control (Figure 4), suggesting mitochondrial respiratory dysfunction. BN-PAGE analysis of mitochondrial respiratory chain complexes I, II, III, and IV proteins from liver lysate indicated a decreased expression of complex III (Figure 5).

Discussion

We describe two cases of mitochondrial respiratory chain complex III deficiency associated with *BCS1L* mutations, in which renal Fanconi syndrome and developmental disability were recognized as the primary symptoms.

BCS1L encodes a member of the AAA family of ATPases, which is a chaperone necessary for the assembly of mitochondrial respiratory chain complex III[21]. Disease phenotypes of *BCS1L* mutations vary, and disease severity cannot be predicted based on their location within the protein[22]. Mutations in *BCS1L* cause congenital syndromes, such as Bjornstad syndrome (OMIM #262000), a relatively mild phenotype characterized by pili torti and sensorineural hearing loss, and GRACILE syndrome (OMIM #603358), a severe phenotype characterized by growth retardation, fulminant lactic acidosis during the initial days of life, Fanconi syndrome, and liver hemosiderosis. Patients with GRACILE syndrome contract severe Fanconi syndrome in the early postnatal period[23]. In addition, although not applicable to the two diseases, mitochondrial complex III deficiency (OMIM #124000), characterized by multiple mitochondrial symptoms may occur during the neonatal period.

The two mutations, c.268 C>T, p.(Arg90Cys) and c.821del, p.(Pro274Argfs*26) observed in Patient 1 were known mutations. The evaluation of the pathogenicity of c. 268 C>T has been reported in detail. Specifically, significant reductions in enzyme activity of complex III were observed in skeletal muscle, liver, and skin fibroblasts. Cross-species conservation by conservation analysis and degradation of mRNA and protein by quantitative PCR and Western blotting also supported the pathogenicity of this variant. The other mutation, c. 821del was obviously pathogenic due to truncating mutation. The reported patient with the c.166 C>T, p.(Arg56*) and c.268 C>T mutation had severe global developmental delay, tachypnea, renal tubular acidosis, failure to thrive, and other problems and was symptomatic before 1 week of age. On the contrary, the other reported patient

with the c.821del and c.917G>A, p.(Arg306His) had a phenotype of Bjornstad syndrome and did not appear to have severe systemic symptoms.

Patients with *BCS1L* mutations do not always present with Fanconi syndrome; however, in a meta-analysis, at least one-third of the 36 patients studied presented with Fanconi syndrome[24]. Mitochondrial diseases are often associated with Fanconi syndrome, secondary to encephalomyopathy or cardiomyopathy, but mitochondrial diseases that are primarily associated with 'nephropathy' are rare[7,8]. There is a possibility that it remains undiagnosed as a mitochondrial disease in the case of mitochondrial nephropathy. In addition, the symptoms of mitochondrial nephropathy have a variety of clinical manifestations. Some cases of steroid-resistant nephrotic syndrome and focal segmental glomerulosclerosis are caused by mitochondrial aberrations. For example, patients with abnormalities in CoQ2, CoQ6, and ADCK4, which code for enzymes of the CoQ10 cascade of the mitochondrial respiratory chain, present with steroid-resistant nephrotic syndrome [25, 26, 27]. However, none of them had renal tubular dysfunction. In the future, as comprehensive analysis methods such as WES and next-generation sequencing become more popular, the number of cases diagnosed with mitochondrial nephropathy will increase.

In this study, the levels of GDF-15, a mitochondrial disease biomarker with high sensitivity and specificity, were evaluated. GDF-15 levels are not only useful for the diagnosis of mitochondrial diseases, but also useful for the evaluation of disease severity, because they are correlated with the severe mitochondrial disease rating scale[28]. In our cases, GDF-15 levels were relatively low, compared to that in other mitochondrial diseases, consistent with the milder clinical phenotype, with no abnormalities noted during the neonatal period.

Objective measures of pathogenicity, such as functional analysis, are required, especially for novel mutations, as per the ACMG guideline[12]. Functional analyses, such as measurements of mitochondrial respiratory chain complex activity and OCR, are useful for diagnosis; however, their use has limitations in mitochondrial nephropathy because of the difficulty in culturing

kidney-associated cells. We attempted culturing cells derived from the urine of Patient 1. We were able to establish cells, but they were difficult to passage; therefore, we discontinued the analysis.

In Patient 2, although OCR indicated mitochondrial dysfunction, there was no decrease in the mitochondrial complex activity in the fibroblasts assay. It has been reported that OCR was decreased in fibroblast, even in normal enzyme activity. This also occurred in cases with *BCS1L* mutations~~this is in line with a previous reports~~, suggesting that OCR assay is more sensitive than the enzyme activity assay[29, 30]. Mitochondrial respiratory chain disorders are tissue-specific, and even when the enzyme activity is normal in the fibroblast assay, decreased enzyme activity is observed in the affected organ [29, 31].

A simple latex reagent (LTIA Device) for GDF-15 measurement would be sufficient for clinical diagnosis[28] and the cost will be covered by universal insurance in Japan. This makes the diagnosis of mitochondrial diseases easier and may increase the number of cases diagnosed with mitochondrial nephropathy.

In this study, we have described cases of mitochondrial respiratory chain complex III deficiency due to *BCS1L* mutations, primarily presenting with Fanconi syndrome. We propose that *BCS1L* mutations should be considered in patients with renal Fanconi syndrome.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

Ethical approval

All procedures involving human participants were in accordance with the ethical standards of the Institutional Review Board of Kobe University Graduate School of Medicine (IRB approval number 301), the Kurume University Institutional Review Board (IRB approval number 273), the Kyushu University Institutional Review Board (IRB approval number 667-00), and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all individuals participating in this study.

References

1. Klootwijk ED, Reichold M, Unwin RJ, Kleta R, Warth R, Bockenhauer D. Renal Fanconi syndrome: taking a proximal look at the nephron. Nephrol Dial Transplant. 2015;30:1456-60.
2. Magen D, Berger L, Coady MJ, Ilivitzki A, Militianu D, Tieder M, et al. A loss-of-function mutation in NaPi-IIa and renal Fanconi's syndrome. N Engl J Med. 2010;362:1102-9.
3. Klootwijk ED, Reichold M, Helip-Wooley A, Tolaymat A, Broeker C, Robinette SL, et al. Mistargeting of peroxisomal EHHADH and inherited renal Fanconi's syndrome. N Engl J Med. 2014;370:129-38.
4. Santer R, Schneppenheim R, Dombrowski A, Götze H, Steinmann B, Schaub J. Mutations in GLUT2, the gene for the liver-type glucose transporter, in patients with Fanconi-Bickel syndrome. Nat Genet. 1997;17:324-6.
5. Hamilton AJ, Bingham C, McDonald TJ, Cook PR, Caswell RC, Weedon MN, et al. The

HNF4A R76W mutation causes atypical dominant Fanconi syndrome in addition to a β cell phenotype. J Med Genet. 2014;51:165-9.
6. Reichold M, Klootwijk ED, Reinders J, Otto EA, Milani M, Broeker C, et al. Glycine Amidinotransferase (GATM), Renal Fanconi Syndrome, and Kidney Failure. J Am Soc Nephrol. 2018;29:1849-58.
7. Niaudet P, Rötig A. Renal involvement in mitochondrial cytopathies. Pediatr Nephrol. 1996;10:368-73.
8. Govers LP, Toka HR, Hariri A, Walsh SB, Bockenhauer D. Mitochondrial DNA mutations in renal disease: an overview. Pediatr Nephrol. 2021;36:9-17.
9. Murayama K, Shimura M, Liu Z, Okazaki Y, Ohtake A. Recent topics: the diagnosis, molecular genesis, and treatment of mitochondrial diseases. J Hum Genet. 2019;64:113-25.
10. Kuwertz-Bröking E, Koch HG, Marquardt T, Rossi R, Helmchen U, Müller-Höcker J, et al. Renal Fanconi syndrome: first sign of partial respiratory chain complex IV deficiency. Pediatr Nephrol. 2000;14:495-8.
11. Morris AA, Taylor RW, Birch-Machin MA, Jackson MJ, Coulthard MG, Bindoff LA, et al. Neonatal Fanconi syndrome due to deficiency of complex III of the respiratory chain. Pediatr Nephrol. 1995;9:407-11.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-24.
13. Yatsuga S, Fujita Y, Ishii A, Fukumoto Y, Arahata H, Kakuma T, et al. Growth differentiation factor 15 as a useful biomarker for mitochondrial disorders. Ann Neurol. 2015;78:814-23.
14. Kirby DM, Crawford M, Cleary MA, Dahl HH, Dennett X, Thorburn DR. Respiratory chain complex I deficiency: an underdiagnosed energy generation disorder. Neurology. 1999;52:1255-64.
15. Rahman S, Blok RB, Dahl HH, Danks DM, Kirby DM, Chow CW, et al. Leigh syndrome: clinical features and biochemical and DNA abnormalities. Ann Neurol. 1996;39:343-51.
16. Bernier FP, Boneh A, Dennett X, Chow CW, Cleary MA, Thorburn DR. Diagnostic criteria for respiratory chain disorders in adults and children. Neurology. 2002;59:1406-11.
17. Van Coster R, Smet J, George E, De Meirleir L, Seneca S, Van Hove J, et al. Blue native polyacrylamide gel electrophoresis: a powerful tool in diagnosis of oxidative phosphorylation defects. Pediatr Res. 2001;50:658-65.
18. Dabbeni-Sala F, Di Santo S, Franceschini D, Skaper SD, Giusti P. Melatonin protects against 6-OHDA-induced neurotoxicity in rats: a role for mitochondrial complex I activity. FASEB J. 2001;15:164-70.
19. Calvo SE, Compton AG, Hershman SG, Lim SC, Lieber DS, Tucker EJ, et al. Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. Sci

Transl Med. 2012;4:118ra10.
20. Shigematsu Y, Hayashi R, Yoshida K, Shimizu A, Kubota M, Komori M, et al. Novel heterozygous deletion mutation c.821delC in the AAA domain of BCS1L underlies Björnstad syndrome. J Dermatol. 2017;44:e111-e12.
21. Cruciat CM, Hell K, Fölsch H, Neupert W, Stuart RA. Bcs1p, an AAA-family member, is a chaperone for the assembly of the cytochrome bc(1) complex. EMBO J. 1999;18:5226-33.
22. Jackson CB, Bauer MF, Schaller A, Kotzaeridou U, Ferrarini A, Hahn D, et al. A novel mutation in BCS1L associated with deafness, tubulopathy, growth retardation and microcephaly. Eur J Pediatr. 2016;175:517-25.
23. Ezgu F, Senaca S, Gunduz M, Tumer L, Hasanoglu A, Tiras U, et al. Severe renal tubulopathy in a newborn due to BCS1L gene mutation: effects of different treatment modalities on the clinical course. Gene. 2013;528:364-6.
24. Baker RA, Priestley JRC, Wilstermann AM, Reese KJ, Mark PR. Clinical spectrum of BCS1L Mitopathies and their underlying structural relationships. Am J Med Genet A. 2019;179:373-80.
25. Diomedi-Camassei F, Di Giandomenico S, Santorelli FM, Caridi G, Piemonte F, Montini G, et al. COQ2 nephropathy: a newly described inherited mitochondriopathy with primary renal involvement. J Am Soc Nephrol. 2007;18:2773-80.
26. Heeringa SF, Chernin G, Chaki M, Zhou W, Sloan AJ, Ji Z, et al. COQ6 mutations in human patients produce nephrotic syndrome with sensorineural deafness. J Clin Invest. 2011;121:2013-24.
27. Ashraf S, Gee HY, Woerner S, Xie LX, Vega-Warner V, Lovric S, et al. ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ10 biosynthesis disruption. J Clin Invest. 2013;123:5179-89.
28. Koga Y, Povalko N, Inoue E, Ishii A, Fujii K, Fujii T, et al. A new diagnostic indication device of a biomarker growth differentiation factor 15 for mitochondrial diseases: From laboratory to automated inspection. J Inherit Metab Dis. 2021;44:358-66.
29. Invernizzi F, D'Amato I, Jensen PB, Ravaglia S, Zeviani M, Tiranti V. Microscale oxygraphy reveals OXPHOS impairment in MRC mutant cells. Mitochondrion. 2012;12:328-35.
30. Ogawa E, Shimura M, Fushimi T, Tajika M, Ichimoto K, Matsunaga A, et al. Clinical validity of biochemical and molecular analysis in diagnosing Leigh syndrome: a study of 106 Japanese patients. J Inherit Metab Dis. 2017;40:685-93.
31. Arakawa C, Endo A, Kohira R, Fujita Y, Fuchigami T, Mugishima H, et al. Liver-specific mitochondrial respiratory chain complex I deficiency in fatal influenza encephalopathy. Brain Dev. 2012;34:115-7.

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Titles and legends to figures

Figure 1.

a. Pedigree of Patient 1. An arrow denotes the proband (Patient 1). Both II-1 and II-2 were diagnosed with Fanconi syndrome, but II-1 died of pulmonary hypertension at 1 year of age. The other is 8 years old and had developmental disability. b. Pedigree of Patient 2.

Figure 2.

Liver pathology of Patient 2. a. Lymphocytic infiltration and fibrosis. b. Deposition of micro fat droplets in hepatic lobules. c. Enlarged and activated Kupffer's cells in the sinusoids. d. Increased number of mitochondria.

Figure 3.

Results of genetic analysis confirmed using Sanger sequencing. a. Patient 1 and her family members. b. Patient 2 and his parents.

Figure 4.

Oxygen consumption rate (OCR) of skin fibroblasts. Vertical axis; oxygen consumption rate. Horizontal axis; elapsed time (min). OCR was significantly lower in the fibroblasts of Patient 2, compared to that of the control. FCCP; carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone

Figure 5.

Blue native polyacrylamide gel electrophoresis (BN-PAGE) analysis of the mitochondrial respiratory chain complex I, II, III, and IV proteins from liver lysate. Protein expression of complex III was decreased in Patient 2.

Figure 1.

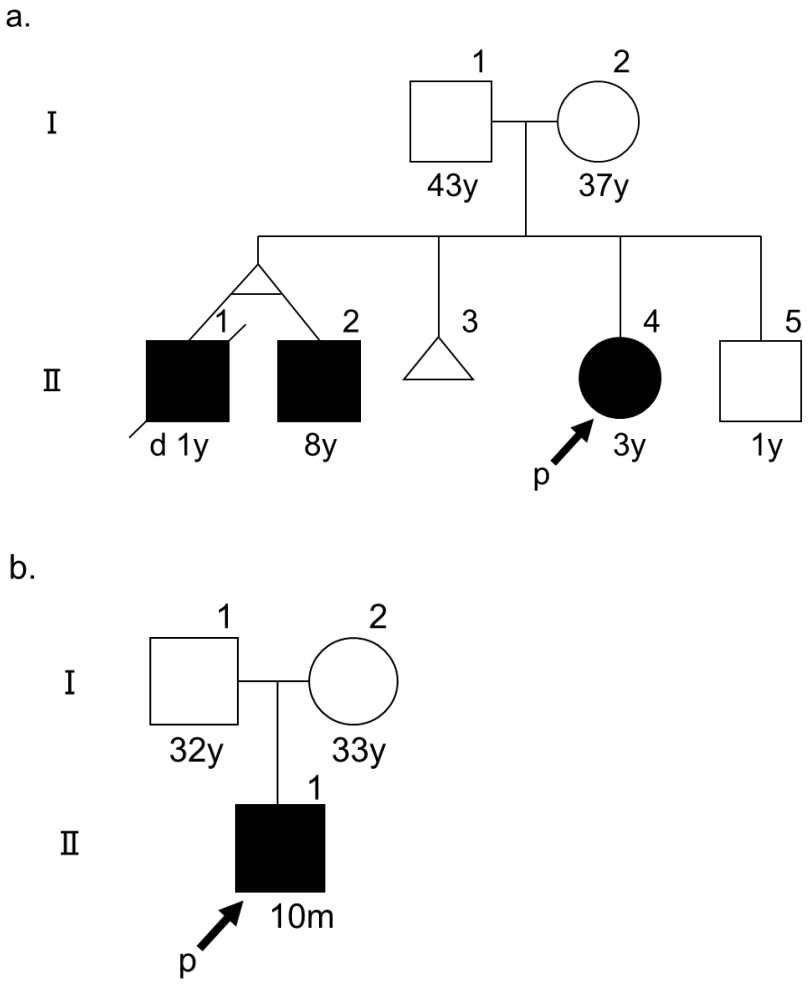

Figure 2.

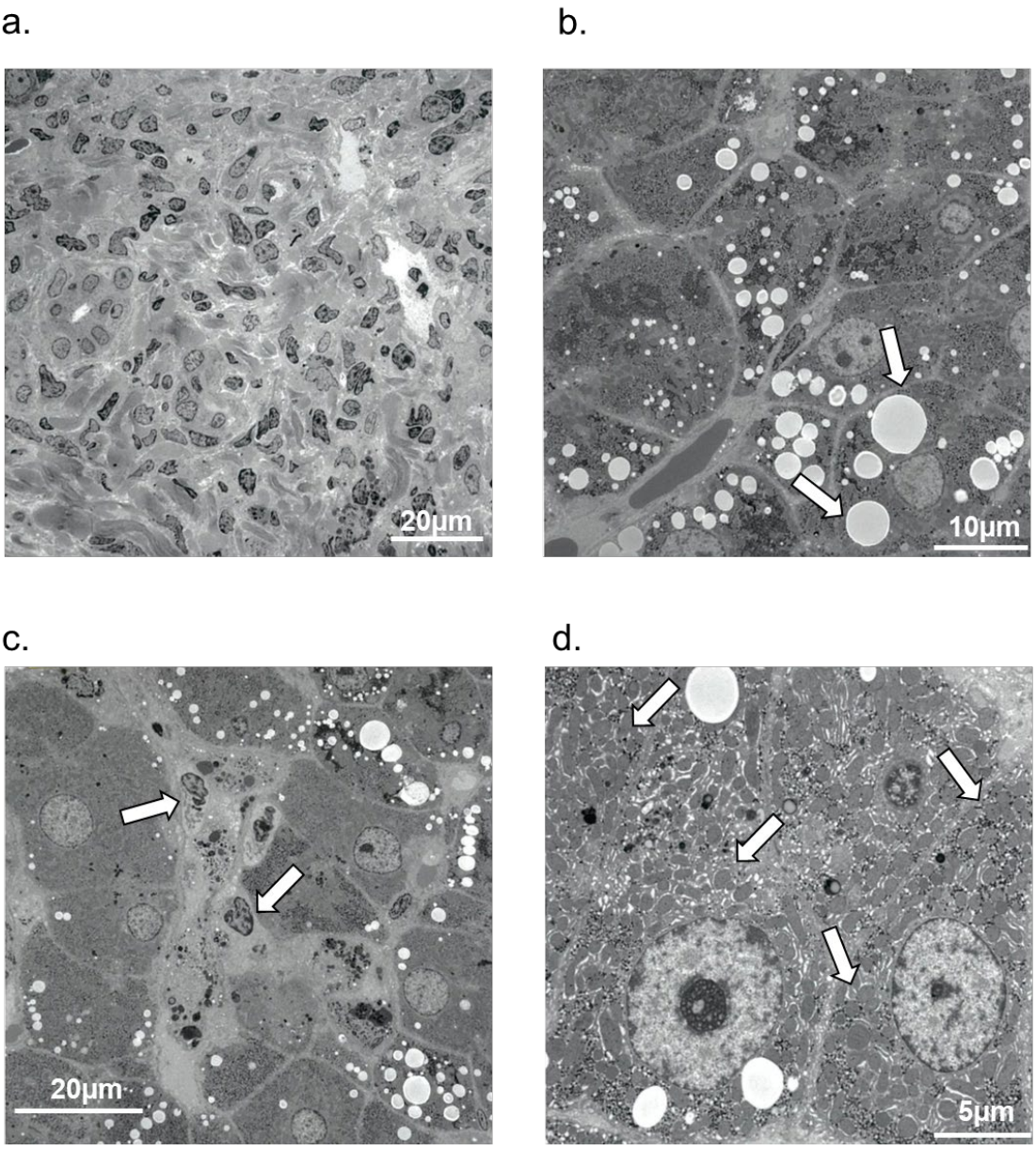

Figure 3

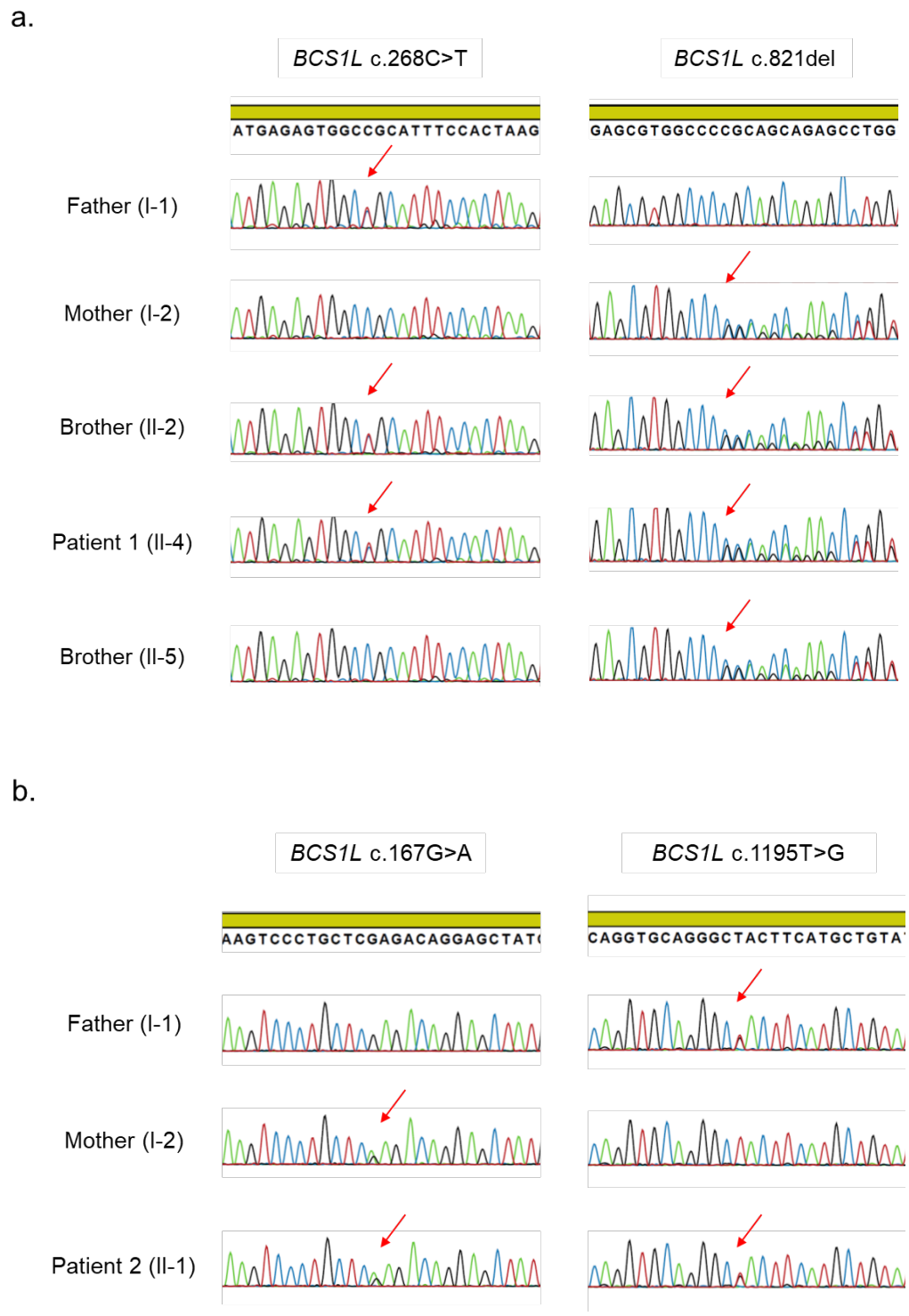
a.
BCS1L c.268C>T
BCS1L c.821del
ATGAGAGTGGCCGCATTTCCACTAAG
GAGCGTGGCCCCGCAGCAGAGCCTGG
Father (I-1)
Mother (I-2)
Brother (II-2)
Patient 1 (II-4)
Brother (II-5)
b.
BCS1L c.167G>A
BCS1L c.1195T>G
AAGTCCCTGCTCGAGACAGGAGCTATC
CAGGTGCAGGGCTACTTCATGCTGTA
Father (I-1)
Mother (I-2)
Patient 2 (II-1)

Figure 4.

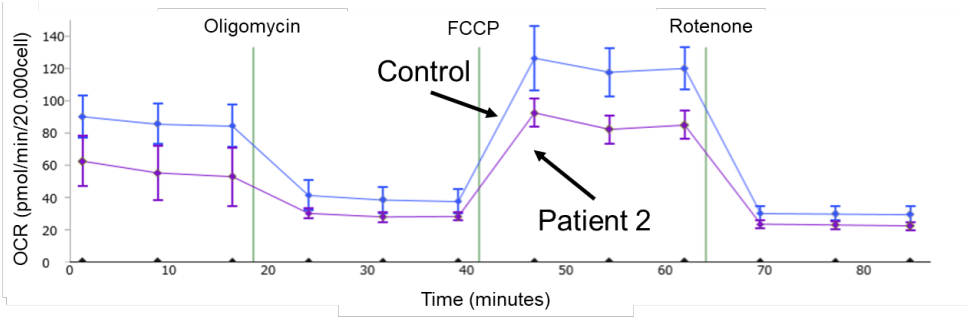
Oligomycin
FCCP
Rotenone
Control
Patient 2
OCR (pmol/min/20.000cell)
Time (minutes)
0
20
40
60
80
100
120
140
0
10
20
30
40
50
60
70
80

Figure 5.

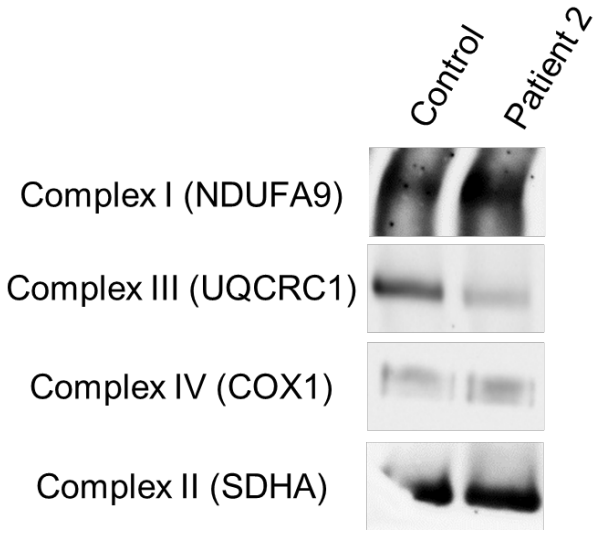
Control
Patient 2
Complex I (NDUFA9)
Complex III (UQCRC1)
Complex IV (COX1)
Complex II (SDHA)

Table 1. Enzyme assay of respiratory chain complexes in skin fibroblasts

	Co I	Co II	Co II+III	Co III	Co IV	CS
Control						
% of normal	31.3	33.9	35	22.9	44.2	34.4
CS ratio (%)	90	97.7	99.2	64.3	126.6	
Co II ratio (%)	91.1		98.7	65	126.3	
Patient						
% of normal	25.6	23.7	16.7	19	25.2	26.6
CS ratio (%)	95.1	88.1	61.1	68.8	93.4	
Co II ratio (%)	106.9		67.5	77.1	103.3	

Co I ; complex I, Co II ; complex II, Co III ; complex III , Co IV ; complex IV, CS ; citrate synthase. Enzyme activities are expressed as a % of the mean relative activity of the normal control and relative to CS and Co II.