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A girl with intragenic variants in *MARS2* and a chondrodysplasia phenotype

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ABSTRACT

Background: The human mitochondrial methionyl-tRNA is crucial for mitochondrial translation, serving as both initiator and elongator in polypeptide chains. The *MARS2* gene is responsible for binding methionine to mitochondrial tRNA. The clinical characteristics of *MARS2* intragenic variants are still largely unknown, since only a pair of siblings has been reported. The present patient presented with psychomotor developmental delay, growth failure, and spondylar dysplasia, which attracted attention in infancy and deteriorated with age.

Case presentation: A 7-month-old Japanese girl presented with failure to thrive, feeding difficulties, and psychomotor developmental delay. Radiological examination showed generalized skeletal alterations including mild spondylar dysplasia and abnormal ilia, which resembled mucopolysaccharidosis; however, the urinary glycosaminoglycan levels and alpha-L-iduronidase activity in the filter paper blood were normal. At age 33 months, she showed hyperlactatemia, and genetic analysis showed compound heterozygous novel variants (NM_138395.4: c.[277G > A]; [409C > T]: p.([Asp93Asn]; [Arg137Cys])) in the *MARS2* gene. After starting vitamin supplementation, her growth and development improved. Radiological examination at ages 2 and 4 years demonstrated a skeletal phenotype: platyspondyly with anterior beaking of the vertebral bodies; large proximal femoral epiphyses; and mild brachymesophalangy. The results of the mitochondrial respiratory chain activity examination using skin fibroblasts were within the normal range.

Conclusion: The skeletal phenotype may be a syndromic component of this disorder associated with *MARS2* intragenic variants.

1. Introduction

Mitochondrial transfer RNA (tRNA) contributes to the synthesis of mitochondrial proteins, including subunits of the oxidative phosphorylation (OXPHOS) system. As mitochondria produce more than 90 % of cellular ATP in nearly all tissues, the clinical manifestations of mitochondrial tRNA dysfunction are extremely diverse [1]. Human mitochondrial methionyl-tRNA is particularly important in mitochondrial translation because it is the only molecule that acts as both a polypeptide chain initiator and chain elongator. The human mitochondrial methionyl-tRNA synthetase 2 gene (*MARS2*, MIM*609728) contributes to the covalent binding of methionine to mitochondrial tRNA. The pathogenic relationship between copy number variants in *MARS2* and autosomal recessive spastic ataxia with leukoencephalopathy (ARSAL) has been reported in a cohort of French-Canadian families [2]; however, a disorder due to biallelic intragenic variants in *MARS2* was reported only once in a pair of siblings with mitochondrial dysfunction. The clinical manifestations of the siblings were distinctly different from those of individuals with ARSAL [3].

The case of a girl with failure to thrive, psychomotor developmental delay, and hyperlactatemia with novel compound heterozygous variants in *MARS2* is reported. She also had generalized skeletal changes.

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2. Case report

The patient was a Japanese girl, the second child of non-consanguineous parents. The family history was not noteworthy. Up to now, her seven-year-old sister has been asymptomatic. The girl was born at 38 weeks' gestation after an uncomplicated pregnancy. Birth height and weight were 45.0 cm (−1.66 SD) and 1956 g (−2.89 SD), respectively. The placenta was approximately half the standard size. She gained head control at 4 months, and her growth rate was normal until 5 months of age, when the growth rate slowed down progressively. At 7 months, she was referred to our hospital because of short stature and poor weight gain, feeding difficulty, and inability to turn over. Physical examination was normal except for central hypotonia. Radiological examination showed mild spondylar dysplasia (ovoid-shaped, mildly flat vertebral bodies with anterior beaking of L2) and abnormal ilia (flared iliac wings, constricted distal ilia, and steep acetabular angles) (Fig. 1). The skeletal alterations raised a suspicion of mucopolysaccharidosis. However, the urinary glycosaminoglycan levels (dermatan sulfate 2 %, heparan sulfate 6 %, keratan sulfate 0 %, chondroitin sulfate 92 %) and alpha-L-iduronidase activity in the filter paper blood were normal. Brain magnetic resonance imaging (MRI), abdominal ultrasonography, and echocardiography showed no abnormal findings. G-banded chromosome examination showed a normal karyotype (46, XX). At this time, whole-exome sequencing and chromosomal microarray analysis did not identify any causative genetic abnormalities. Her subsequent motor development included turning over at 8 months, sitting independently at 15 months, and pulling up to stand at 20 months. She often became lethargic with each viral infection.

At 33 months, increased lactate and pyruvate levels in plasma were noted (7.0 mmol/L and 0.35 mmol/L, respectively) when she was admitted to the hospital due to lethargy caused by parainfluenza virus type 1 infection. The laboratory data showed normal levels of serum transaminases (AST 47 IU/L and ALT 23 IU/L), creatine phosphokinase (56 U/L), ammonia (40 μmol/L), and glucose (86 mg/dL), along with elevated free fatty acids (1895 μEq/L), whereas total ketone bodies (3164 μmol/L) were not abnormal. The levels of insulin-like growth factor-1 (75 ng/mL), thyroid-stimulating hormone (2.64 μIU/mL), free thyroxine (1.08 ng/dL), and free triiodothyronine (4.52 pg/mL) were also within the normal ranges. The acylcarnitine profile showed low free carnitine (20.9 μmol/L; reference interval, 32–52 μmol/L) and slightly high acylcarnitines (C4-OH, C5-DC, C6, C10, C14, C14:1, C16, C16-OH, and C18:1). The amino acid analysis showed a high plasma level of alanine (0.63 mmol/L). A urine organic acid analysis showed elevated metabolites of the citric acid cycle (fumarate, malate, and α-ketoglutaric acid), together with massive excretion of lactate, pyruvate, and ketone bodies, pointing to mitochondrial disease. Radiological examinations at ages 2 and 4 years showed skeletal manifestations, including the development of platyspondyly (flattening of vertebral bodies) with pronounced anterior beaking of the lumbar vertebral bodies, large, round proximal femoral epiphyses, and mild brachymesophalangy (short middle phalanges) in the index and little fingers (Fig. 2). Brain MRI examination, including magnetic resonance spectroscopy, showed no abnormal findings.

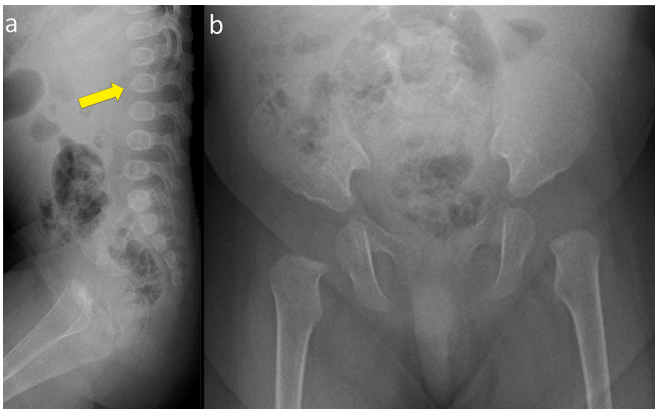

Fig. 1. Plain radiographs of the lumbar spine (a) and pelvis (b) at 8 months of age show mild spondylar dysplasia and iliac deformity. The lumbar vertebral bodies are rounded. Anterior bone projection is seen at L2 (arrow). The iliac wings are flared. The distal ilia are constricted along with steep acetabular angles.

After starting vitamin supplementation (vitamin B_1 (250 mg/day), vitamin B_2 (200 mg/day), vitamin B_{12} (2 mg/day), vitamin C (500 mg/day), biotin (20 mg/day), L-carnitine (46 mg/kg/day), coenzyme Q10 (10 mg/day), she became more active and less prone to lethargy, even when contracting infections. She could stand without support at 43 months and walk independently at 44 months. The lactate levels decreased from 5.3 to 11.0 mmol/L before treatment to 2.3–5.0 mmol/L. Activities of mitochondrial respiratory chain complexes in skin fibroblasts were low normal (complex I, 66.4 %; complex II, 55.0 %; complex III, 76.6 %; complex IV, 66.8 % of the normal control mean relative to citrate synthase). The oxygen consumption rate in the fibroblasts was 145 % in the glucose medium and 124 % in the galactose medium.

The whole mitochondrial DNA sequence and second whole-exome sequencing showed compound heterozygous missense variants (NM_138395.4: c.277G > A [p.(Asp93Asn), located in exon 1] and NM_138395.4: c.409C > T [p.(Arg137Cys), located in exon 1] of the *MARS2* gene) in the patient. The former variant was detected in her mother, whereas the latter was detected in her father, both heterozygous variants. These variants were confirmed by Sanger sequencing. The variant c.277G > A was deposited in dbSNP as an SNP (rs1269449956) with a frequency of 0.00001029 in GnomAD and not recorded in ExAC. The variant c.409C > T was deposited in dbSNP as an SNP (rs138469286) with a frequency of 0.000007686 and 0.000008454 in GnomAD and ExAC, respectively. On PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://asia.ensembl.org/index.html) analyses, p.(Asp93Asn) and p.(Arg137Cys) were predicted as “probably damaging” and “deleterious”, respectively. Written informed consent was obtained from the patient's parents for all procedures, tests, and publication.

3. Discussion

The current patient presented with psychomotor developmental delay, growth failure, and generalized skeletal alterations. Spondylar dysplasia attracted attention in infancy and deteriorated with age, leading to a suspicion of mucopolysaccharidosis. Subsequently, however, examinations for mucopolysaccharidosis were negative, and she was diagnosed with mitochondrial disease following hyperlactatemia. Genetic analyses showed novel biallelic variants, c.277G > A; p.(Asp93Asn) and c.409C > T; p.(Arg137Cys) in the *MARS2* gene. According to the ACMG/AMP classification [4], these variants are likely pathogenic based on the PM1, PM2, PP3, and PP4 scores.

The *MARS2* gene encodes the human mitochondrial methionyl-tRNA synthetase, which belongs to the class I aminoacyl-tRNA synthetases. Class I synthetase contains a Rossmann fold characterized by two highly conserved sequences, His-Ile-Gly-His (HIGH) and Lys-Met-Ser-Lys-Ser (KMSKS) [5]. The first study of *MARS2* variants was based on 54 French-Canadian patients belonging to 38 families. The investigation showed that complex genomic *MARS2* re-arrangements cause a neurodegenerative disease named Autosomal Recessive Spastic Ataxia with Leukoencephalopathy (ARSAL) and reduction in mitochondrially translated proteins and mitochondrial Complex I activity [2]. The mean age of onset of individuals with ARSAL was 24.4 (2–59) years, and their main manifestations of ataxia (100 %), spasticity (100 %), cerebellar atrophy (93 %), and dysarthria (78 %) were not seen in the present patient.

To date, only a pair of siblings with biallelic intragenic variants

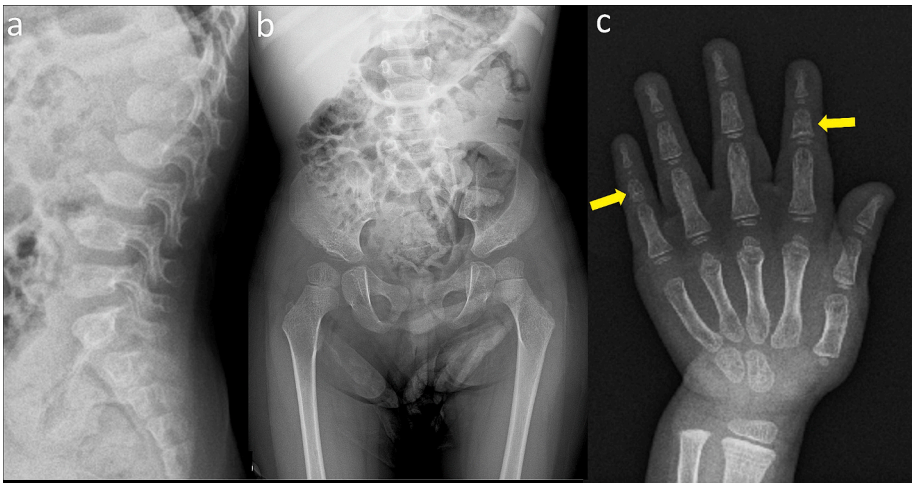

Fig. 2. Plain radiographs of the lumbar spine (a), pelvis (b), and left hand (c) at 4 years of age: Progression of spondylar dysplasia associated with platyspondyly (flattening of the vertebral bodies) is noted. Anterior central bone projections are more prominent at L2, L3, and L4. The proximal femoral epiphyses appear large and round. Flared ilia and acetabular dysplasia are seen again. Mild brachymesophalangy (shortening of the middle phalanges) is seen in the index and little fingers (arrows).

(c.424C > T; p.(Arg142Trp) and c.550C > T; p.(Gln137*)) in *MARS2* has been reported [3]. The older brother had hyperlactatemia and 3-methylglutaconateuria, and the younger brother had decreased mitochondrial respiratory chain complex I and IV activities in fibroblasts. They both showed psychomotor developmental delay from infancy, failure to thrive, and hypotonia, but not the ataxia, spasticity, or dysarthria seen in ARSAL. The siblings shared many clinical manifestations in common with the girl in the present report. However, unlike the siblings, she had no abnormal signals in the cerebral white matter on MRI and sensorineural hearing loss, did not require nasogastric tubes, and had normal mitochondrial respiratory chain activity in skin fibroblasts. Excluding nonsense mutation, the three mutations identified in the present patient (p.(Asp93Asn) and p.(Arg137Cys)) and the previously reported p.(Arg142Trp) are all located away from the HIGH and KMSKS motifs. Though the relative importance of each amino acid remains unclear, the mutations in the present patient might have a lesser impact on the protein structure. The clinical features of patients with alterations in *MARS2* are summarized in supplemental Table 1.

It was intriguing that the present patient had generalized skeletal changes. A previous study showed that the affected siblings with *MARS2* variants had marked pectus carinatum, but not other skeletal alterations, nor were shown any radiographs. Although generalized skeletal abnormalities are not typically features of mitochondrial diseases, several studies have shown that impairment of mitochondria-related genes, *LONP1*, *HSPA9*, *IARS2*, and *AIFM1,* causes skeletal dysplasias, termed CODAS syndrome, EVEN-plus syndrome, CAGSSS syndrome, and spondyloepimetaphyseal dysplasia with hypomyelinating leukodystrophy (SEMDHL), respectively [6–9]. These disorders belong to a broad category of spondylo-epi-metaphyseal dysplasia, but the skeletal phenotypes vary among the disorders. The skeletal hallmarks are summarized in supplemental Table 2. CODAS syndrome and EVEN-plus syndrome are characterized by wide vertebral coronal clefts, severe epiphyseal dysplasia, and “bifid” distal femora (severe metaphyseal cupping). CAGSSS syndrome manifests as mild spondylar dysplasia and asymmetric epiphyseal dysplasia of the knee. SEMDHL presents with severe spondylar and epimetaphyseal dysplasias. The skeletal manifestations of these disorders were different from those of the present girl. Her initial skeletal changes, including mild spondylar dysplasia and abnormal ilia, were mistaken for those of mucopolysaccharidosis. However, she developed a skeletal phenotype with age, including platyspondyly with anterior beaking, large round proximal femoral epiphyses, and brachymesophalangy. It appears that the *MARS2* gene is also involved in skeletal development, and its aberration can cause constitutional skeletal phenotypes.

4. Conclusion

This is the second report of a patient with biallelic variants in *MARS2*, who presented with failure to thrive, psychomotor developmental delay, and hyperlactatemia, as well as generalized skeletal changes. These results expand the phenotype of *MARS2* variants and demonstrate that copy number rearrangements and biallelic variants in *MARS2* have different clinical presentations. We recommend that investigations for mitochondria-related genes, such as the *MARS2* gene, should be considered for children with unclassifiable skeletal dysplasias.

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CRediT authorship contribution statement

Hiroyuki Iijima: Writing – original draft, Visualization, Project administration, Investigation, Data curation, Conceptualization. **Yuko Tsujioka:** Writing – review & editing, Visualization, Data curation, Conceptualization. **Yoshiyuki Tsutsumi:** Writing – review & editing, Visualization, Investigation, Conceptualization. **Gen Nishimura:** Writing – review & editing, Visualization, Data curation. **Yasushi Okazaki:** Resources, Methodology, Investigation, Funding acquisition. **Kei Murayama:** Resources, Methodology, Investigation, Funding acquisition. **Mitsuru Kubota:** Writing – review & editing, Supervision, Conceptualization. **Akira Ohtake:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2025.101198.

Data availability

Data will be made available on request.

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