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Novel Insights from Clinical Practice

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A Case of Combined Oxidative Phosphorylation Deficiency 35 Associated with a Novel Missense Variant of the *TRIT1* Gene

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Established Facts

- Combined oxidative phosphorylation deficiency 35 (COXPD35; OMIM #617873) is a rare autosomal recessive disorder associated with homozygous or compound heterozygous mutations in the tRNA isopentenyltransferase (*TRIT1*) gene.
- Patients with COXPD35 demonstrate a variable heterogeneous clinical phenotype such as seizures, microcephaly, developmental delay, intellectual disability, failure to thrive, vision problems, EEG abnormalities, cardiac involvement, polyuria-polydipsia, endocrinopathies, and structural brain anomalies.
- To date, only 10 types of allelic variants in the *TRIT1* gene have been previously reported in 9 patients with COXPD35.

Novel Insights

- Our case is the first report describing strabismus, ketotic hypoglycemia, nephrolithiasis, and bicuspid aortic valve in *TRIT1*-related COXPD35.
- This study expands the genotype-phenotype spectrum of *TRIT1*-related COXPD35.

Keywords
Combined oxidative phosphorylation deficiency · *TRIT1* · Whole-exome sequencing · Mitochondrial disorders · Genotype-phenotype correlation

Abstract
Combined oxidative phosphorylation deficiency 35 (COXPD-35) is a rare autosomal recessive disorder associated with homozygous or compound heterozygous mutations in the tRNA isopentenyltransferase (*TRIT1*) gene in chromosome 1p34.2. To date, only 10 types of allelic variants in the *TRIT1* gene have been previously reported in 9 patients with COXPD35. Herein, we describe a case with a novel homozygous missense variant in *TRIT1*. A 6-year, 6-month-old boy presented with global developmental delay, microcephaly, intractable seizures, and failure to thrive. The other main clinical manifestations were intellectual disability, spastic tetraparesis, truncal hypotonia,

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malnutrition, polyuria and polydipsia, ketotic hypoglycemia, dysmorphic facial features, strabismus, bicuspid aortic valve, and nephrolithiasis. The detailed biochemical, radiological, and metabolic evaluations were unremarkable. Chromosomal analysis confirmed a normal male 46,XY karyotype and the array comparative genomic hybridization analysis revealed no abnormalities. We identified a novel homozygous missense variant of c.246G>C (p.Met82Ile) in the *TRIT1* gene, and the variant was confirmed by Sanger sequencing. The present case is the first report describing strabismus, ketotic hypoglycemia, nephrolithiasis, and bicuspid aortic valve in *TRIT1*-related COXPD35. This study expands the genotype-phenotype spectrum of *TRIT1*-related COXPD35.

Introduction

Mitochondrial dysfunctions typically lead to a wide spectrum of clinically heterogeneous often devastating characteristics. Mitochondrial energy production is a complicated process involving the organized enzymatic activity of the mitochondrial respiratory chain, which includes 5 complexes and over 85 proteins. Mitochondrial disorders have broad and often overlapping clinical features due to the genetic and biochemical complexity of mitochondrial functions. Therefore, clinical diagnosis of mitochondrial diseases is extremely challenging [Vafai and Mootha, 2012; Lightowlers et al., 2015].

Disorders of oxidative phosphorylation include heterogeneous infantile, childhood, and adult-onset diseases characterized by variable involvement of high-energy requiring organs such as central and peripheral nervous systems, muscles, eyes, ears, lungs, endocrine organs, kidneys, and liver [Vafai and Mootha, 2012; Lightowlers et al., 2015]. Combined oxidative phosphorylation deficiencies (COXPD) are multisystem disorders with variable manifestations resulting from biochemical defects in the mitochondrial oxidative phosphorylation enzyme activity [Yarham et al., 2014; Kernohan et al., 2017]. There are many subtypes and genotypic/phenotypic heterogeneity. Combined oxidative phosphorylation deficiency 35 (COXPD35; OMIM #617873) is a rare autosomal recessive disorder associated with homozygous or compound heterozygous mutations in the tRNA isopentenyltransferase (*TRIT1*) gene in chromosome 1p34.2 [Yarham et al., 2014; Kernohan et al., 2017; Takenouchi et al., 2019; Yoo et al., 2021].

Herein, we describe a case with a novel homozygous missense variant in the *TRIT1* gene.

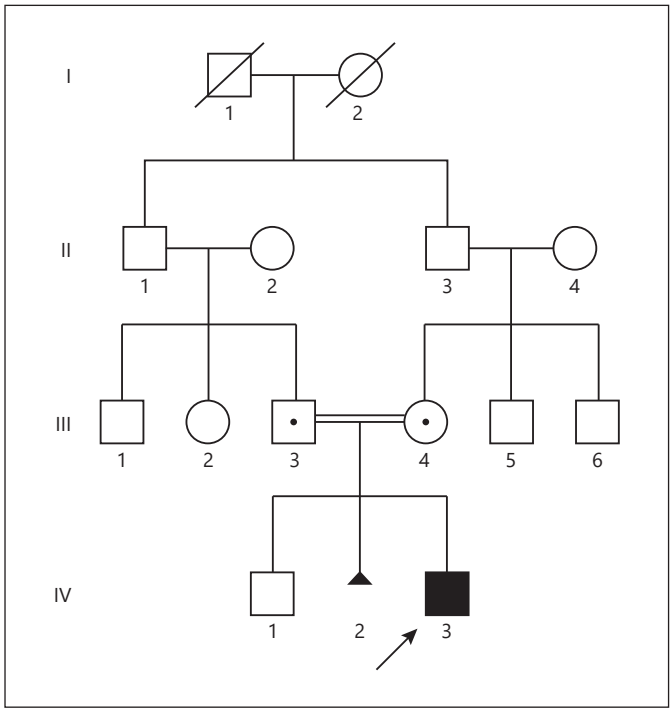

Fig. 1. Pedigree of the family carrying the *TRIT1* variant-related combined oxidative phosphorylation deficiency 35.

Case Presentation

A 6-year, 6-month-old boy presented with global developmental delay, microcephaly, intractable seizures, and failure to thrive. He was born after an uneventful pregnancy and delivery to second-degree consanguineous parents (Fig. 1). He was born at 39 weeks of gestation and had a birth weight of 2,270 g (−3.14 SD) and a head circumference of 33 centimeter (−1.32 SD). There was no family history of neurogenetic or neurometabolic diseases. His brother (Fig. 1; VI-1) has no clinical findings similar to the present case. The developmental milestones were reported as normal up to the age of 6 months. At this age, his parents noticed that he could not sit without support. At the age of 7 months, he developed nephrolithiasis. He experienced his first episode of seizures as a febrile convulsion at the age of 14 months. Subsequently, he developed repetitive episodes of febrile seizures over the course of the next 6 months. He also exhibited myoclonic jerks during infancy and early childhood. Generalized tonic-clonic convulsions and myoclonic jerks persisted despite combinations of anticonvulsant therapy such as levetiracetam, topiramate, valproic acid, and clobazam. The motor milestones were delayed: sitting independently at 11 months and walking independently at 4 years of age. The patient also manifested acquired microcephaly, intellectual disability, spastic tetraparesis, failure to thrive, malnutrition, polyuria and polydipsia, ketotic hypoglycemia, dysmorphic facial features, and strabismus.

On physical and neurological examinations at 6 years, his weight, height, and head circumference were below the 3rd percentile. He had acquired microcephaly [head circumference at 3 years of age: 43,5 cm (−3,59 SD), at 4 years and 6 months of age:

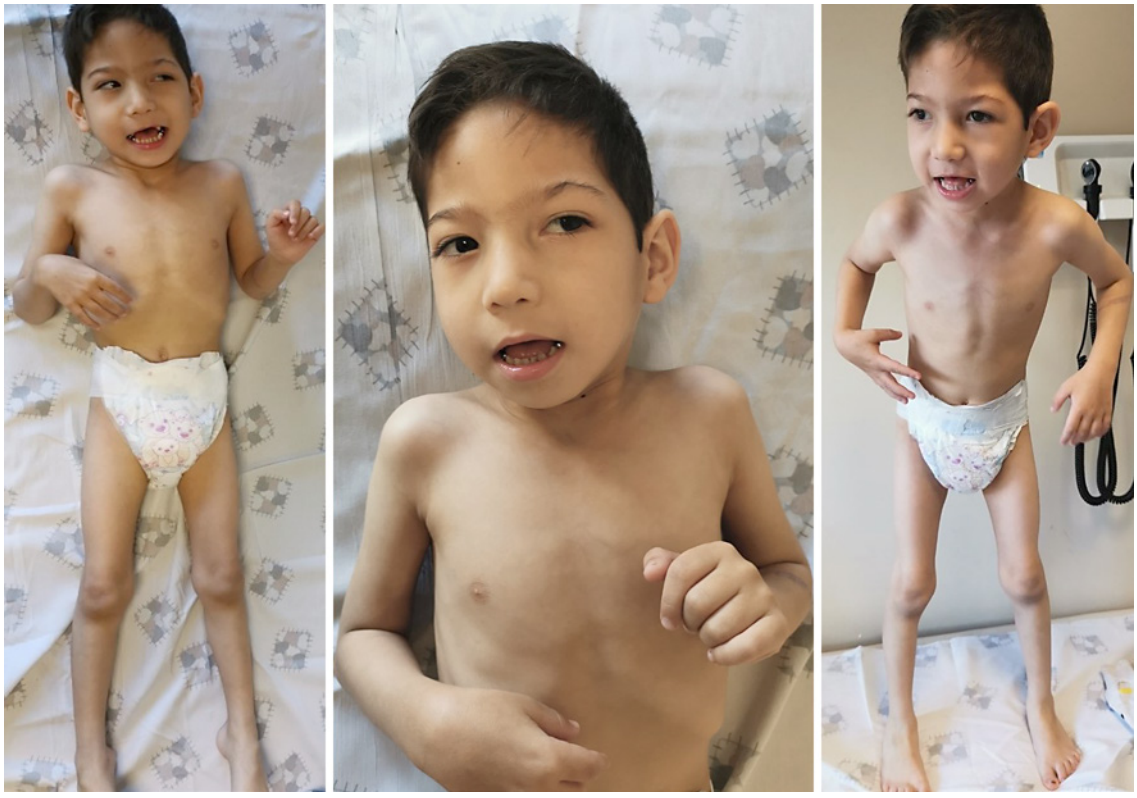

Fig. 2. Photographs and clinical characteristics of the 6-year and 6-month-old boy, showing microcephaly, global developmental delay, strabismus, dysmorphic facial features, malnutrition, limb spasticity, and wide-based gait.

45 cm (−3.88 SD), and at 6 years of age: 46,2 cm (−3.94 SD)], strabismus, dysmorphic features (hypertelorism, epicanthal fold, prominent ear, high-arched palate, mild retrognathia), truncal hypotonia, upper and lower limb spasticity, hyperreflexia, bilateral Babinski sign, and a wide-based ataxic gait (Fig. 2). He was able to comply some simple commands and spoke several meaningful words. Endocrinological, dermatological, and hearing evaluations were normal. Cardiological evaluation revealed bicuspid aortic valve and gastrointestinal evaluation revealed failure to thrive, malnutrition, and constipation. Moreover, nephrological evaluation revealed nephrolithiasis and ophthalmological evaluation revealed strabismus.

First, at 5 years of age, our case presented with fatigue and altered consciousness after a prolonged fasting period, while blood glucose was 45 mg/dL, blood ketone was 3.3 mmol/L (reference range: 0–0.6) and plasma free carnitine was 9.3 nmol/mL (reference range: 24–63). Then, similar episodic ketotic hypoglycemia attacks repeated 2 more times. The biochemical tests such as hepatic, thyroid, and renal function tests, electrolytes, creatine kinase (77 U/L, reference range: 0–171), levels of thyroid stimulating hormone (TSH: 1.67 μIU/mL, reference range: 0.6-4.84), parathormone (30 pg/mL, reference range: 12–88), cortisol (10.2 μg/dL, reference range: 6.7–22.6), and growth hormone were unremarkable. Metabolic tests such as plasma and urine amino acids, tandem mass spectrometry, urine organic acids, serum ammonia level, blood gas analysis, and very long-chain fatty acids tests were unremarkable. Lysosomal enzymes such as alpha-galactosidase, beta-galactosidase, alpha-glucosidase, beta-glucosidase, and sphingomyelinase were normal. Lactic acid and pyruvic acid were 12.5 mg/dL and 0.53 mg/dL, respectively, and the ratio was 23.6. At 3 years and 6 months of age, cranial magnetic resonance imaging (MRI) was normal. At 4 years of age, electroencephalogram (EEG) demonstrated epileptic discharges with a high incidence of generalized spike-polyspike wave activity without a photoparoxysmal response. Chromosomal analysis confirmed a normal male 46,XY karyotype and the array comparative genomic hybridization (aCGH) analysis revealed no abnormalities.

Based on the clinical manifestations, examination, and laboratory findings, we suspected the patient to have a neurometabolic disorder. Whole-exome sequencing revealed a novel homozygous missense variant of the *TRIT1* gene NM_017646.6:c.246G>C (p.Met82Ile) via using xGen Exome Research Panel v2 (Integrated DNA Technologies, Coralville, IA, USA), and the mutation was confirmed by Sanger sequencing (Fig. 3). The present missense mutation, NM_017646:6c.246G>C (p.Met82Ile), was classified as a variant of uncertain significance according to the American College of Medical Genetics and Genomics (ACMG) criteria [Richards et al., 2015]. This missense variant has not been previously reported in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), the ClinVar archive, or in population studies (gnomAD, ESP, or 1000 Genome Project). In the Genome Aggregation Database (gnomAD) v2.1.1, the allele frequen-

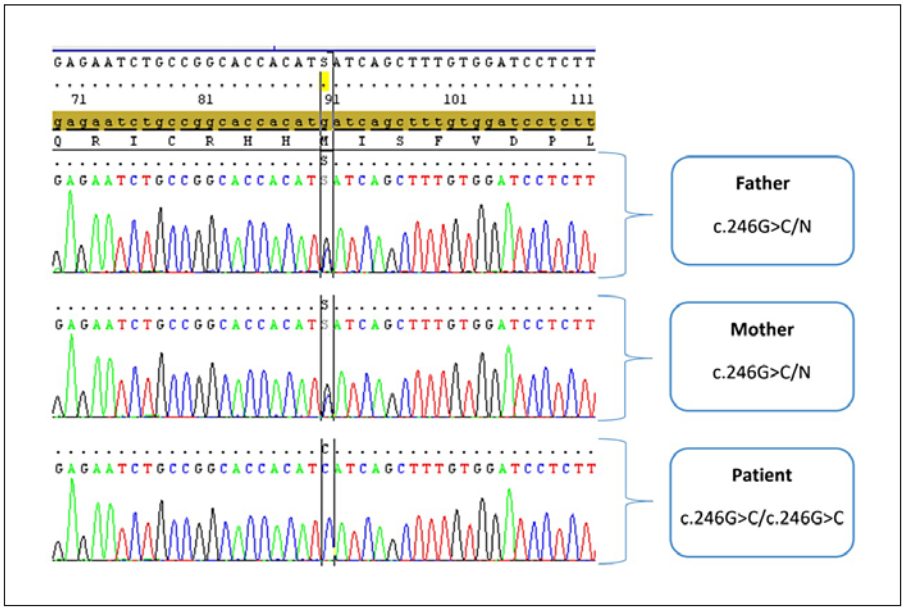

Fig. 3. Sequencing of the *TRIT1* variant c.246G>C with Sanger sequencing.

cy of this variant is 0.000003980, and it is considered rare because its minor allele frequency score is below 1%. However, our case had the same altered amino acid as a case with the compound heterozygous allele previously reported by Takenouchi et al. [2019]. His parents were both heterozygous for the same mutation in the *TRIT1* gene. Moreover, whole-exome sequencing revealed a heterozygous variant of the *TRAF7* gene, NM_032271.3:c.353C>T (p.Pro118Leu). It was classified as a variant of uncertain significance according to the ACMG criteria. The clinical findings of the present case were not compatible with *TRAF7* gene-related cardiac, facial, and digital anomalies with developmental delay. Our case was supplemented with coenzyme Q10, riboflavin, thiamine, and L-carnitine with conservative treatments. There has been no improvement or worsening of symptoms due to this conservative treatment.

Discussion

We present the first Turkish case with COXPD35 due to a novel homozygous missense variant in *TRIT1*, presenting with acquired microcephaly, intractable seizures, global developmental delay, failure to thrive, malnutrition, strabismus, limb spasticity, ataxic gait, bicuspid aortic valve, polyuria and polydipsia, ketotic hypoglycemia, and nephrolithiasis. Based on the clinical manifestations, examination, and genetic test results, we suggest that the present case is a typical COXPD.

To date, only 10 types of allelic variants in *TRIT1* gene have been previously reported in 9 patients with COXPD35 (Table 1) [Yarham et al., 2014; Kernohan et al., 2017; Takenouchi et al., 2019; Yoo et al., 2021]. The onset of symptoms occurs in early infancy (range 3–13.5 months). The main clinical manifestations were febrile and/or myoclonic seizures, microcephaly, developmental delay, intellectual disability, failure to thrive, vision problems, and EEG abnormalities (Table 1). Moreover, cardiac involvement, polyuria-polydipsia, endocrinopathies such as elevated TSH and diabetes, skin abnormalities, structural brain anomalies, and biochemical abnormalities were detected less frequently [Yarham et al., 2014; Kernohan et al., 2017; Takenouchi et al., 2019; Yoo et al., 2021]. Abnormal neuroimaging findings such as cerebral atrophy, mega cisterna magna, Dandy-Walker syndrome, hydrocephalus, septo-optic dysplasia, and partial agenesis of the corpus callosum have been reported in patients with COXPD35 [Kernohan et al., 2017; Yoo et al., 2021]. To date, only 2 cases have shown normal brain MRI findings [Yarham et al., 2014; Takenouchi et al., 2019]. Similarly, the present case also had a normal brain MRI. To the best of our knowledge, our case is the first report describing strabismus, ketotic hypoglycemia, nephrolithiasis, and bicuspid aortic valve in COXPD35. On the other hand, this study has a limitation as we were not able to perform a muscle biopsy to investigate biochemical or histological features and analyze the respiratory chain complexes.

A minority of children with ketotic hypoglycemia are diagnosed with endocrine, genetic, or metabolic disorders such as growth hormone deficiency, ACTH deficiency, glucagon deficiency, Prader-Willi syndrome, Silver-Russell syndrome, glycogen storage disease, and

Table 1. Characteristics of patients with *TRIT1* gene variants

	Present case	Yoo et al. [2021]		Takenouchi et al. [2019]	Kernohan et al. [2017]				Yarham et al. [2014]	
Patient	1	2	3	4	5	6	7	8	9	10
Gender	M	F	M	F	F	F	F	M	M	F
Age at onset	6 months	3 months	3 months	4 months	3 months	6 months	5 months	NA	13.5 months	NA
Age at diagnosis	6 years	16 years	13 years	NA	4 years	9 years	NA	NA	NA	16 years
Consanguinity	Y	N	N	N	N	N	N	N	Y	Y
Country	Turkey	Korea	Korea	NA	USA	Canada	USA	USA	UK-Pakistan	UK-Pakistan
Inheritance	Homozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Homozygous	Homozygous
Microcephaly	Y	Y	NA	Y	Y	Y	Y	Y	Y	Y
Failure to thrive	Y	Y	Y	NA	Y	Y	Y	NA	NA	NA
Hypotonia	Y	NA	NA	Y	Y	Y	Y	NA	NA	NA
Developmental delay	Y	Y	Y	Y	Y	Y	Y	NA	Y	Y
Intellectual disability	Y	Y	Y	Y	Y	Y	NA	NA	Y	Y
Cardiac evaluation	Bicuspid aortic valve	N	N	NA	ASD	ASD and VSD	NA	NA	N	N
Ophthalmological evaluation	Strabismus	Cataract, retinal and optic disc hypoplasia	Optic disc hypoplasia	NA	Optic disc hypoplasia	No tracking	No tracking	NA	N	N
Hearing	Normal	NA	NA	NA	Normal	Normal	Normal	NA	Normal	Normal
Gastrointestinal evaluation	Malnutrition, constipation	Malnutrition	Malnutrition	NA	N	G-tube	Constipation	NA	NA	NA
Seizure	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Febrile convulsions	Y	NA	NA	Y	Y	Y	Y	NA	Y	NA
Myoclonus	Y	N	Y	Y	Y	Y	Y	NA	Y	Y
Polyuria/polydipsia	Y	NA	NA	NA	N	NA	NA	NA	Y	Y
Endocrinopathy	Ketotic hypoglycemia	NA	Transient subclinical hypothyroidism, vitamin D deficiency	NA	Elevated TSH	N	NA	NA	Diabetes	Diabetes
Brain MRI	Normal	Reduced volume of periventricular white matter, megacisterna magna	Dandy-Walker syndrome, hydrocephalus	Normal	Septo-optic dysplasia, partial agenesis of corpus callosum	Generalized cerebral atrophy	Frontal atrophy and increased fluid	NA	Normal	NA
EEG	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	NA	Abnormal	NA
TRIT1 variants	c.246G>C (p.M82I)	c.979G>A (p.Q327K) and c.682+2T>C	c.979G>A (p.Q327K) and c.682+2T>C	c.244A>G p.(M82V) and c.1034A>G p.(Y345C)	c.1256A>C (p.H419P) and c.848T>G (p.I283S)	c.1256A>C (p.H419P) and c.1204C>T (p.R402*)	c.856A>G (p.K286E) and c.22C>T (p.R8*)	c.856A>G (p.K286E) and c.22C>T (p.R8*)	c.968G>A (p.R323Q)	c.968G>A (p.R323Q)

NA, not applicable; N, no; Y, yes; ASD, atrial septal defect; VSD, ventricular septal defect; TSH, thyroid stimulating hormone; MRI, magnetic resonance imaging; EEG, electroencephalogram; *TRIT1*, tRNA isopentenyltransferase 1.

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glucose or ketone body transport and metabolism disorders [Drachmann et al., 2021]. The rest of patients are diagnosed as idiopathic ketotic hypoglycemia. On the other hand, normal children may also evolve ketotic hypoglicemia during acute illness, after vomiting, diarrhea, or prolonged starvation [van Veen et al., 2011]. Moreover, ketotic hypoglycemia can be frequent in conditions with chronic malnutrition [Monde et al., 2010]. We suggest that the present case suffered ketotic hypoglycemia due to prolonged starvation and chronic malnutrition. On the other hand, ketotic hypoglycemia has also been rarely described in mitochondrial diseases. It was reported in a patient with complex deficiency due to an in-frame MT-CYB deletion in a recent case report [Mori et al., 2015]. Moreover, strabismus, nephrolithiasis, and structural cardiac anomalies are relatively more common findings in mitochondrial disorders. Valproic acid we used in the treatment of our case is known to cause potential hepatotoxicity in mitochondrial disorders [Finsterer and Scorza, 2017]. However, we experienced neither serious adverse events nor worsening of seizure control from taking valproic acid for epilepsy in the present case. But then, we discontinued the therapy because we did not detect any improvement in seizure control.

Strabismus, ketotic hypoglycemia, and nephrolithiasis have been associated with other mitochondrial diseases, but not associated with COXPD35 in previous reports [Vafai and Mootha, 2012; Lightowlers et al., 2015; Mori et al., 2015]. Therefore, our case contributes to the spectrum of *TRIT1*-related COXPD35. Further case reports are required to expand the genotype-phenotype correlations. Evolving genotype-phenotype correlations for extremely rare diseases is essential for determining various organ involvements, predicting clinical prognosis, and providing families with genetic counseling.

Acknowledgement

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Statement of Ethics

Informed consent for genetic analysis and publication of clinical reports and photographs were obtained from patient's parents in compliance with the national ethics regulation. There is no name or number indicating the patient's identity. This paper is exempt from ethical committee approval: Institutional review board approval was not required for this type of publication.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.Y., N.Y.S., and Y.S. wrote the manuscript and designed the figures. Ö.B. and S.T. devised the main conceptual ideas and critically reviewed the manuscript. Ü.Ö. evaluated the metabolic tests and drafted the manuscript. E.T. carried out and evaluated the genetic tests such as whole-exome sequencing analysis, designed the pedigree and drafted the manuscript. All authors approved the final version of this manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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