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Novel Insights from Clinical Practice

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A Patient with a Novel *RARS2* Variant Exhibiting Liver Involvement as a New Clinical Feature and Review of the Literature

Selin Sevinç[a] Aslı İnci[b] Fatih S. Ezgü[b] Fatma T. Eminoğlu[c]

[a]Department of Pediatrics, Ankara University Faculty of Medicine, Ankara, Turkey; [b]Division of Pediatric Metabolism and Nutrition, Gazi University Faculty of Medicine, Ankara, Turkey; [c]Department of Pediatric Metabolism, Ankara University Faculty of Medicine, Ankara, Turkey

Established Facts

- Pontocerebellar hypoplasia type 6 (PCH6) is a mitochondrial disease associated with autosomal recessive inheritance that results from mutations in the *RARS2* gene.
- The typical clinical picture of PCH6 includes neonatal lactic acidosis, severe encephalopathy, persistent seizures, hypotonia, spastic quadriplegia, microcephaly, nutritional problems, and severe developmental delay.

Novel Insights

- Our patients are the first cases with liver involvement in PCH6 and a novel homozygous *RARS2* pathogenic variant to be reported in literature.
- *RARS2* pathogenic variants should be considered in the differential diagnosis of diseases that lead to cholestasis in the neonatal period.

Keywords
Liver involvement · Mitochondrial arginyl tRNA · Pontocerebellar hypoplasia type 6 · *RARS2*

Abstract
Pontocerebellar hypoplasia (PCH) is a heterogeneous neurodevelopmental disorder that is characterized by decreased brainstem and cerebellum volume. Pontocerebellar hypoplasia type 6 (PCH6) is a mitochondrial disease associated with autosomal recessive inheritance that results from mutations in the *RARS2* gene. In this case report, we describe a new clinical presentation with a novel *RARS2* pathogenic variant. We report here on 2 siblings who presented with neonatal lactic acidosis, microcephaly, growth retardation, persistent seizures, and cholestasis with a previously undefined *RARS2* pathogenic variant. In our literature review, we evaluated the clinical features and pathogenic variants of 34 patients reported in 16 publications since the initial identification of *RARS2* pathogenic variants in PCH6 in 2007. Both siblings were detected with c.1564G>A (p.Val522Ile), a novel homozygous pathogenic variant of

karger@karger.com
www.karger.com/msy

Correspondence to:
Selin Sevinç, slnsvnc@hotmail.com

Karger

the *RARS2* gene. Imaging revealed advanced cerebral atrophy and cerebellar hypoplasia, while the basal ganglia and pons were preserved. At follow-up, the elevations in liver function test results and cholestasis had regressed while the LDH and GGT elevations persisted. Both siblings showed microcephaly on follow-up and started to suffer seizures. Severe developmental delay and nutritional problems were observed, and both died in infancy. *RARS2* pathogenic variant is a mitochondrial disease that causes severe mental, motor, and developmental retardation, as well as short life expectancy. Our patients are the first cases with liver involvement in PCH6 and a novel homozygous *RARS2* pathogenic variant to be reported in the literature. This additional phenotype can be considered as making a valid contribution to the literature.

Introduction

Cerebellar and brain stem hypoplasia is referred to as pontocerebellar hypoplasia (PCH). Presentations of severe supratentorial white matter involvement and cerebral atrophy in particular result in pontocerebellar hypoplasia type 6 (PCH6) [Rankin et al., 2010]. PCH6 is a mitochondrial disease that is associated with autosomal recessive inheritance and is caused by pathogenic variants in the *RARS2* gene. *RARS2* encodes the mitochondrial arginyl transfer RNA (tRNA) synthetase. As reported by Glamuzina et al. [2012], these encoded proteins play an important role in protein synthesis by binding to the tRNA molecules in amino acid synthesis. The *RARS2* pathogenic variant was first detected in a relative Sephardic Jewish family [Edvardson et al., 2007], and 34 further cases have been recorded by October 2020. The typical clinical picture of PCH6 includes neonatal lactic acidosis, severe encephalopathy, persistent seizures, hypotonia, spastic quadriplegia, microcephaly, nutritional problems, and severe developmental delay [Kastrissianakis et al., 2013; Joseph et al., 2014]. Most patients have cerebellar hypoplasia and progressive cerebral and pontocerebellar atrophy [Joseph et al., 2014].

In the present study, we report on 2 siblings with microcephaly, neonatal lactic acidosis, persistent seizures, and cholestasis with a previously undefined *RARS2* pathogenic variant. Imaging revealed advanced cerebral atrophy and cerebellar hypoplasia, while the basal ganglia and pons were preserved.

Case Report

We report here on 2 siblings, 1 male and 1 female, born to a 3rd degree consanguineous marriage.

Patient 1

Patient 1 was born at 38 gestational weeks, G3P2 with a birth weight of 2,550 g (10th–25th percentile) and a 30.8 cm head circumference (<3rd percentile). The patient's sister (Patient 2) had died at the age of 6 months in another hospital with signs of microcephaly, seizure, and transient cholestasis. DNA of Patient 1 was isolated, although no genetic testing had been carried out previously. He was transferred to our hospital with respiratory problems and metabolic acidosis on the postnatal 1st day. A physical examination revealed microcephaly, a syndromic appearance, scooped and low ears, a long philtrum, and a prominent nasal bridge. An antenatal ultrasonography revealed only microcephaly in Patient 1. At the postnatal 12th hour he became hypoglycemic, and a diagnostic work-up revealed metabolic acidosis with elevated lactate. Initial laboratory studies recorded high total bilirubin (13.89 mg/dL, normal: <1.1 mg/dL), direct bilirubin: (1.11 mg/dL, normal: <0.3 mg/dL), AST (146 U/L, normal: <41 U/L), ferritin (621 ng/mL, normal range: 24–336 ng/mL), GGT (762 U/L, normal range: 8–61 U/L), LDH (745 U/L, normal range: 125–220 U/L), lactate (18.17 mg/dL, normal range: 10–14 mg/dL), and ammonia (261 μmol/L, normal range: 11–32 μmol/L) levels. Furthermore, alanine (2,198 μmol/L, normal range: 116–450 μmol/L) and tyrosine (461 μmol/L, normal range: 40–160 μmol/L) levels were high in a blood amino acid analysis. In an analysis of urine organic acid, carnitine-acyl carnitine and VLCFA were normal. A systemic, abdominal ultrasound was normal, while peripheral pulmonary stenosis and PFO were detected on echocardiography. A cranial MRI on postnatal day 8 showed microcephaly, cerebellar and cerebral atrophy, and pathologic signal changes that cause cerebral parenchyma and gyrus expansion in the subcortical white matter that suggested a metabolic disease. In the postnatal 1st month, the elevations in the ALT, AST, total and direct bilirubin levels were seen to improve. The LDH and GGT elevations decreased, although mild elevations persisted. The patient began to suffer seizures lasting 1–2 seconds at 3 months of age, and the frequency of the seizures increased at 4 months of age. The patient was uninterested in the environment. The findings of an electroencephalogram examination were consistent with epileptic encephalopathy with a burst suppression pattern, and phenobarbital and levetiracetam were added to the patient's treatment. A BAER examination indicated bilateral hearing loss, also there was a prolongation of bilateral latencies in the VEP examination. Microcephaly and a syndromic appearance were evident after 6 months, having been also prominent at birth; and the head circumference of the patient was 41 centimeters, under the rd percentile for that month (Fig. 1). At 7 months of age, a cranial MRI revealed a marked loss of white matter, deep cerebellar hypoplasia and cerebral atrophy, and an increase in the T2 intensities of cerebral white matter, identified from an MRI examination carried out on the postnatal 8th day (Fig. 2).

The patient began to be fed with a nasogastric tube at 8 months, and secretions and the need for aspiration began to increase at the age of 12 months, culminating in death at 18 month of age. The final blood lactate levels remained high, but with mild regression.

Fig. 1. Patient 1 with microcephaly, syndromic appearance, scooped and low ears, long philtrum, and prominent nasal bridge.

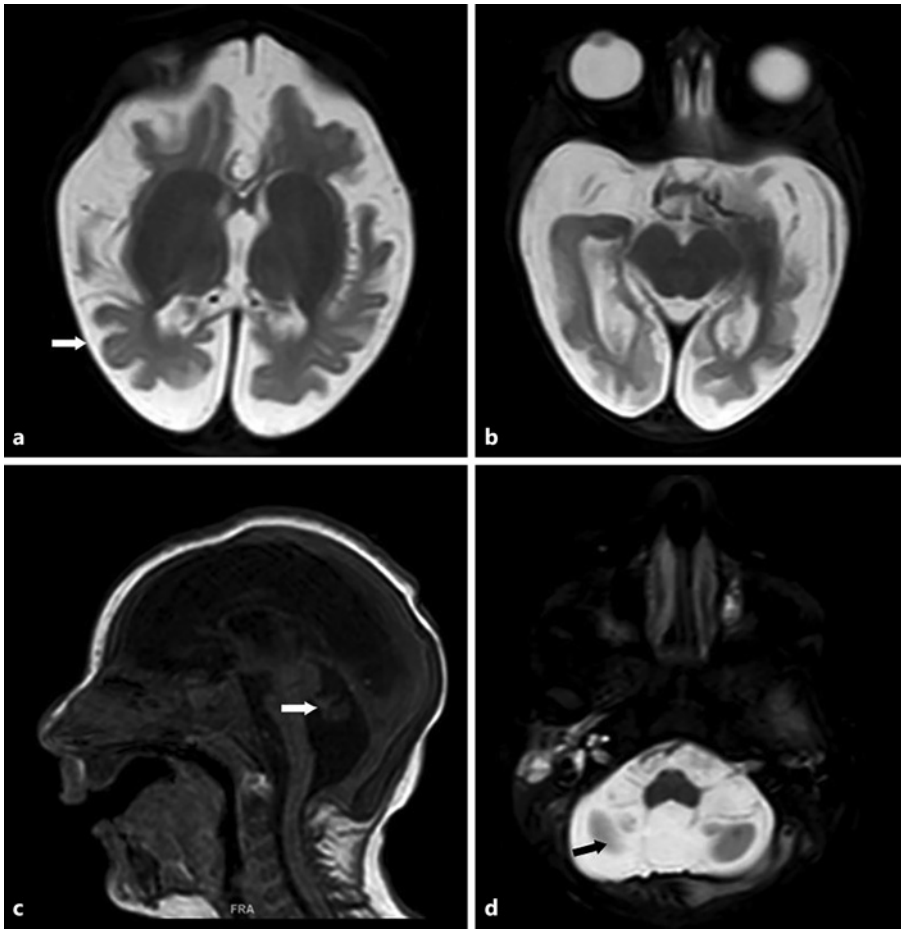

Fig. 2. Cranial MRI of Patient 1. **a** Significant cerebral atrophy, extra-axial areas prominent with effusion. **b** Preserved basal ganglia. **c**, **d** Cerebellum atrophy at an advanced level.

In the final stages of life, the patient had severe developmental retardation and was spastic quadriplegic. In a genetic analysis of the gene panel for inborn metabolic disease, a homozygous c.1564G>A (p.Val522Ile) novel change in the *RARS2* gene was detected.

Patient 2

Patient 2, the first child of the same parents, was a girl born at 38 gestational weeks to an uncomplicated cesarean section delivery with a birth weight of 2,840 g. On the postnatal 2nd day, the patient

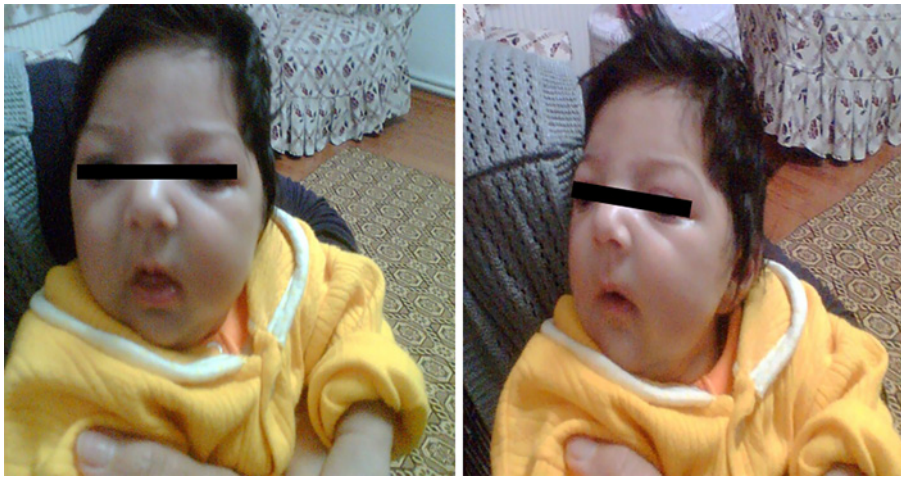

Fig. 3. Patient 2 with microcephaly, micrognatia, syndromic appearance, prominent occipital bone, narrow forehead, low anterior hairline, and thin upper lip.

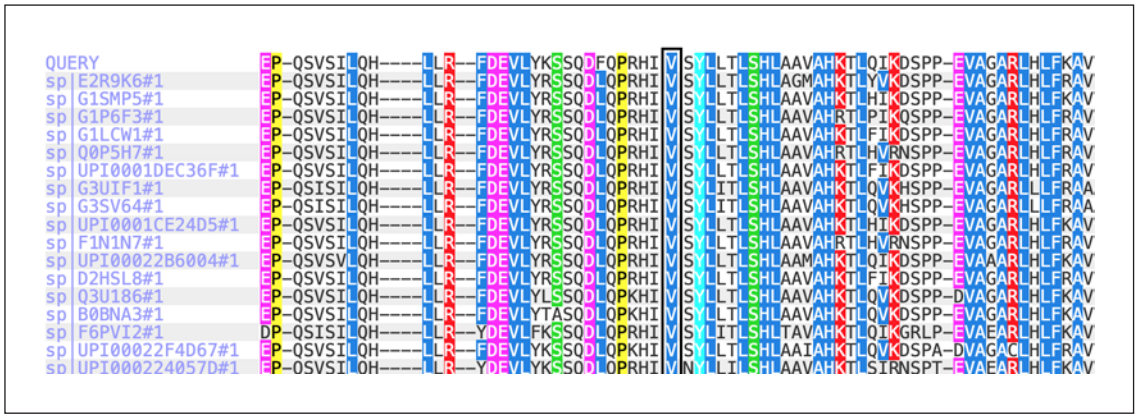

Fig. 4. The valine amino acid at position 532 is a very highly conserved region among species.

developed cholestatic jaundice and was monitored in another hospital for 20 days. Basic metabolic screening, alpha fetoprotein, and sweat test results were found to be normal in a preliminary diagnosis of cholestatic jaundice.

At the postnatal 2nd month examination, her body weight, length and head circumference were under the 3rd percentile (2,965 grams, 50 cm, and 33 cm, respectively). She had microcephaly, micrognatia, jaundice, syndromic appearance, closed fontanels, overriding metopic suture, prominent occipital bone, narrow forehead, low anterior hairline, and thin upper lip (Fig. 3). Laboratory tests revealed ALT (50 U/L, normal: <40 U/L) and AST (57 U/L, normal: <40 U/L) to be mildly high; GGT (561 U/L, normal range: 12–122 U/L) to be significantly high; and total protein (3.9 g/dL, normal range: 5.1–7.3 g/dL) and albumin (2.9 g/dL, normal range: 3.5–5.4 g/dL) to be low. She had direct hyperbilirubinemia (total bilirubin: 4.8 mg/dL, direct bilirubin: 3.5 mg/dL); and lactate was high (4.14 mmol/L, normal range: 0–2 mmol/L). Other metabolic screening and chitotriosidase test results were normal. Leber's congenital amaurosis and chorioretinal atrophy were detected in an eye examination. A transfontanelle and abdominal sonography was normal. Chromosome analysis resulted in 46,XX. BAER was prolonged. Patient 2 had no head control or object-tracking and started to suffer seizures at 3 months of age. A cranial CT for craniosynostosis revealed pontocerebellar and pontocerebral subarachnoid wide margins and cerebral atrophic parenchyma.

At 6 months of age, intractable seizures and microcephaly were evident in the patient, and a neurological examination revealed no head control, no hearing and no object tracking. Death occurred at 6 month of age and the patient's DNA was separated for further analysis. Due to the similarities with the sibling's history and similar laboratory findings, a gene analysis was carried out to identify any *RARS2* gene pathogenic variant, and the same homozygous c.1564G>A (p.Val522Ile) change was detected. The parents were found to be heterozygous for the variant.

Methods

An Ion Torrent S5™ (Life Technologies, Gilford, CT, South San Francisco, CA, USA) platform was used for DNA sequencing analysis, along with a custom-targeted Ion AmpliSeq™ panel that included 7,219 amplicons covering 450 genes associated with Inborn Metabolic Diseases. The sequence variant was described in accordance with the Human Genome Variation Society Nomen-

clature [Den Dunnen and Antonarakis, 2000]. A total of 473 variants were present without any filters. With the filter of 1% allele frequency the number of variants were reduced to 32. The filters of homozygous variants and clinical overlap revealed one variant that is c.1564G>A (p.Val522Ile). The identified pathogenic variant was classified as "Probably Damaging, Disease Causing and Damaging" according to Polyphen [Adzhubei et al., 2010], Mutation Taster [Schwarz et al., 2014], and SIFT [Vaser et al., 2016] in silico prediction tools, respectively, as it existed in a highly conserved region of the protein. According to the ACMG classification, this variant was classified as "Variant of Unknown Significance" (PM2, PP2, PP3). However, the valine amino acid at the position 532 of the protein seems to be completely conserved among different species suggesting that this is a pathogenic variant (Fig. 4).

Discussion

Pathogenic variants in *RARS2* have been reported in patients with pontocerebellar hypoplasia type 6 (PCH6; OMIM 611523). Mitochondrial aminoacyl-tRNA synthetase (ARS) and arginyl transfer RNA synthetase enzymes are encoded by the *RARS2* gene (MIM 611524). ARSs attach amino acids to their tRNA molecules from the same lineage and play an important role in mRNA translation. Missense mutations, deletions, and splice-site variant mutations are identified in *RARS2* [Lühl et al., 2016]. Since the initial identification of *RARS2* mutations in PCH6 by Edvardson et al. [2007], a total of 34 patients have been reported in 16 publications, and a total of 28 mutations have been reported to date. The patients, references, consanguinity statuses, and clinical features identified in these studies are summarized in Table 1. Nucleotide changes of all the patients with *RARS2* mutation in the literature are listed in Table 2.

As is the case with other mitochondrial diseases, the brain is the most involved organ, which can be attributed to the fact that it is more sensitive to oxidative substrates. The earliest clinical manifestations of PCH6 are seizures, which may not occur only in the first days of life, but also in later periods. Zhang et al. [2018] analyzed 23 patients in the literature and identified initial presentations of symptoms such as hypotonia (43.48%; 10/23), epileptic seizures (34.78%; 8/23), encephalopathy (26.08%; 6/23), and feeding difficulties (17.39%; 4/23), while other less common initial presentations included hypoglycemia (8.70%; 2/23), tachypnea (13.04%; 3/23), and cyanosis (8.70%; 2/23). A literature review in the present study found seizures to be reported in the vast majority of cases (88%; 30/34; Table 1), and most of these cases also reported difficulties in controlling the seizures, despite the use of multiple anti-epileptic treatments. Our index patient (Patient 1), started to suffer seizures in the 3rd month of life, and the seizure frequency increased at 4 months of age, while the elder sister also began to suffer seizures in the 3rd month of life. The seizures of both patients were resistant to multiple anti-convulsants.

Hypotonia is another potential initial symptom of this disease. In our review of 34 cases, 56% (19/34; Table 1) of the patients had a history of hypotonic infancy. Feeding difficulty, as another accompanying condition, was reported by Zhang et al. [2018] to be an initial symptom in 17% of cases, while our own review indicated a prevalence of 53% (18/34; Table 1), and most developed the condition not as an initial symptom, but within the course of the disease. Our index case (Patient 1) began to be fed via a nasogastric tube at 8 months, while the elder sister (Patient 2) had died at the age of 6 months, but difficulties in feeding were noted in the patient's history.

Hypoglycemia can also develop in the first hours of life. Symptoms such as changes in neurological behavior, exaggerated Moro reflex, irritability, jitteriness, tremors, high-pitched crying, seizures, lethargy, or poor feeding should arouse suspicions of hypoglycemia. Hypoglycemia was reported in 7 of the 34 patients (20%; Table 1) in the literature and was the first complaint seen at the 12th hour of Patient 1 in the present study. After the hospitalization and regulation of feeding, no further hypoglycemia was observed in Patient 1.

The majority (79%, 27/34; Table 1) of cases in our review presented with severe developmental delay, with the exception of 7 cases (4 died before reaching the age of 2 months and were unavailable for assessment; the other 3 patient reports made no mention of developmental milestones). Microcephaly is another common physical examination finding in cases with *RARS2* pathogenic variants, and the detection of microcephaly at birth or percentile drops in head circumference measurements in long-term follow-up were observed in 17 of the 34 cases (Table 1) analyzed in the present study. Patient 1 had prominent microcephaly since birth, and after 4 months of age he developed seizures that were resistant to multiple anti-epileptic drugs. Patient 2 also had microcephaly.

Besides such neurological symptoms as microcephaly, hypotonia, and severe developmental delay, the cases presented here suffered also losses of vision and hearing. Loss of vision frequently accompanies this disease, being reported in 12 of the 34 (35%; Table 1) patients reviewed in the present study, while hearing loss was reported in only 2 patients in our literature review [Glamuzina et al.,

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Table 1. Clinical features and laboratory findings of all patients with *RARS2* mutation in the literature

Patient	Year	Reference	Ethnicity	Sex	Clinical features									Investigations	
					hypo-glycaemia	hypotonia	spasticity	micro-cephaly	hearing loss	vision loss	feeding difficulty	seizures	develop. delay	lactate↑	LFT↑
1	2007	Edvardson et al. (1)	Sephardic Jewish	F		+	+	+			+		+	–	
2		Edvardson et al. (2)	Sephardic Jewish	M		+					+	+	+	+	
3		Edvardson et al. (3)	Sephardic Jewish	F		+	+	+			+	+		–	
4	2010	Rankin et al	British	F	+	+		+		+	+	+	+	+	
5	2012	Glamuzina et al	British Caucasian	F	+	+	+	+	+	+	+	+	+	+	
6	2013	Casandrini et al. (1)	Italian	M		+	+	+			+	+	+	+	
7		Casandrini et al. (2)	Italian	M		+	+	+			+	+	+	+	
8		Casandrini et al. (3)	Italian	F	+	+	+	+			+	+	+	+	
9		Casandrini et al. (4)	Italian	F		–	+	+			+	+	+	+	
10		Casandrini et al. (5)	Italian	M		+	+	+			+	+	+	+	
11	2013	Kastrissianakis et al. (1)	-	F		Hypertonic	+	+		+	+	+	+		
12		Kastrissianakis et al. (2)	-	M	+		+			+		+	+	+	
13*	2014	Joseph et al. (1)*	Canadian	M		+						+			
14*		Joseph et al. (2)*	Canadian	F								+			
15*		Joseph et al. (3)*	Canadian	F								+			
16	2015	Li et al. (1)	Hispanic	M		+		–		+	+	+	+		
17		Li et al. (2)	Hispanic	F		+							+		
18*	2015	Lax et al. (1)*	British Caucasian	F											+
19		Lax et al. (2)	British Caucasian	F		+		–				+		+	
20	2016	Nishri et al. (1)	Moroccan-Libyan	F			+	+		+		+	+		
21		Nishri et al. (2)	Moroccan-Libyan	M		+	+			+		+	+	–	
22	2016	Alkhateeb et al. (1)	Jordanian Arab	F			+					+	+		
23		Alkhateeb et al. (2)	Jordanian Arab	F			+					+	+		
24		Alkhateeb et al. (3)	Jordanian Arab	F			+					+	+		
25	2016	et al. (1)	British Caucasian	M	+		+	+		+	+	+	+	+	
26		Ngoh et al. (2)	British Caucasian	M		+	+	+		+	+	+	+	–	
27	2016	Van Dijk et al. (1)	-	F		Hypertonic		+		+	+	+	+	+	
28		Van Dijk et al. (2)	-	F		+		–			+	+	+	–	
29	2016	Lühl et al. (1)	Saudi Arabian	M	+	+		+		+		+	+	+	
30		Lühl et al. (2)	Saudi Arabian	F	+									+	
31	2018	Zhang et al	Chinese	M		+	+	+	+		+	+	+	+	
32	2018	Mathew et al. (1)	-	F			+		–	–		+	+	–	
33		Mathew et al. (2)	-	M			+		–	–		+	+	–	
34	2019	Nevaninna et al	Belgian	M		+	+	+		+	+	+	+	+	

Numbers in parentheses next to the references refer to patients in the publications in which more than one case is presented. * For patients lost in the early period with a lack of detailed information about their follow-up, their life time and positive findings were written in brief. –, Denoting evaluated symptoms, especially those identified as absent (unmentioned symptoms in publications are left blank).

Table 2. Consanguinity status and nucleotide change of all patients with *RARS2* mutation in the literature

Patient	Year	Reference	Ethnicity	Sex	Consanguinity	Nucleotide and amino acid change
1	2007	Edvardson et al. (1)	Sephardic Jewish	F	Yes	IVS2+5A>G
2		Edvardson et al. (2)	Sephardic Jewish	M	Yes	IVS2+5A>G
3		Edvardson et al. (3)	Sephardic Jewish	F	Yes	IVS2+5A>G
4	2010	Rankin et al	British	F	No	c.1024A>G, M342V and c.35A>G, Q12R
5	2012	Glamuzina et al	British Caucasian	F	No	c.1211T>A, M404K
6	2013	Casandrini et al. (1)	Italian	M	No	c.25A>G/p.I9V and c.1586+3A>T
7		Casandrini et al. (2)	Italian	M	No	c.25A>G/p.I9V and c.1586+3A>T
8		Casandrini et al. (3)	Italian	F	No	c.734G>A/p.R245Q and c.1406G>A/p.R469H
9		Casandrini et al. (4)	Italian	F	No	c.734G>A/p.R245Q and c.1406G>A/p.R469H
10		Casandrini et al. (5)	Italian	M	No	c.35A>G/p.Q12R and c.721T>A/p.W241R
11	2013	Kastrissianakis et al. (1)	–	F	No	c.773G>A, R258H and c.1651–2A>G
12		Kastrissianakis et al. (2)	–	M	No	c.773G>A, R258H and c.1651–2A>G
13*	2014	Joseph et al. (1)*	Canadian	M	No	–
14*		Joseph et al. (2)*	Canadian	F	No	c.997C>G, p.Arg333Gly and c.1432G>A, p.Gly478Arg
15*		Joseph et al. (3)*	Canadian	F	No	c.997C>G, p.Arg333Gly and c.1432G>A, p.Gly478Arg
16	2015	Li et al. (1)	Hispanic	M	No	c.-2A>G
17		Li et al. (2)	Hispanic	F	No	c.-2A>G
18*	2015	Lax et al. (1)*	British Caucasian	F	No	c.613–3927C>T
19		Lax et al. (2)	British Caucasian	F	No	c.613–3927C>T
20	2016	Nishri et al. (1)	Moroccan-Libyan	F	No	c.878+5G>T and c.110+5A>G
21		Nishri et al. (2)	Moroccan-Libyan	M	No	c.878+5G>T and c.110+5A>G
22	2016	Alkhateeb et al. (1)	Jordanian Arab	F	Yes	c.1588C>T, p.H530Y
23		Alkhateeb et al. (2)	Jordanian Arab	F	Yes	c.1588C>T, p.H530Y
24		Alkhateeb et al. (3)	Jordanian Arab	F	Yes	c.1588C>T, p.H530Y
25	2016	Ngoh et al. (1)	British Caucasian	M	No	c.848T>A, p.L283Q and c.472_474del, p.K158del
26		Ngoh et al. (2)	British Caucasian	M	No	c.848T>A, p.L283Q and c.472_474del, p.K158del
27	2016	Van Dijk et al. (1)	-	F	No	c.297+2T>G and c.1544A>G, p.(Asp515Gly)
28		Van Dijk et al. (2)	-	F	No	c.297+2T>G and c.1544A>G, p.(Asp515Gly)
29	2016	Lühl et al. (1)	Saudi Arabian	M	Yes	c.392T>G; p.Phe131Cys
30		Lühl et al. (2)	Saudi Arabian	F	Yes	c.392T>G; p.Phe131Cys
31	2018	Zhang et al	Chinese	M	No	c.1718C>T (p.Thr573Ile) and c.991A>G (p.Ile331Val)
32	2018	Mathew et al. (1)	-	F	Yes	c.848T>A; p.Leu283Gln
33		Mathew et al. (2)	-	M	Yes	c.848T>A; p.Leu283Gln
34	2019	Nevanlinna et al	Belgian	M	No	c.8C>T, p.Ser3Leu

Numbers in parentheses next to the references refer to patients in the publications in which more than one case is presented. * For patients lost in the early period with a lack of detailed information about their follow-up, their life time and positive findings were written in brief.

2012; Cassandrini et al., 2013]. Hyperlactacidemia is the earliest abnormality to be identified in laboratory findings in patients with *RARS2* and is a consequence of respiratory chain failure. It usually occurs in the neonatal period and is of critical importance. Hyperlactacidemia has been reported in 16 patients of the total 34 *RARS2* patients to date (Table 1). In the cases in the present study, high lactate levels were a significant laboratory finding in both siblings in the neonatal period. At follow-up, the hyperlactacidemia in Patient 1 degraded, as was the case in the other patients reported in the literature, but in spite of this decrease, it never declined to normal

levels. The last period blood lactate levels of Patient 2 could not be accessed as she was followed-up in another hospital.

Both of the siblings presented with cholestasis, which, when accompanied by high lactate levels, first indicated mitochondrial depletion syndrome as a diagnosis. However, the cholestasis had improved spontaneously at 6 months. To date, there has been only one case in the literature with cholestasis, having been born with severe cardiomyopathy and hydrops fetalis [Lax et al., 2015]. The patient was ventilator-dependent from the first minutes of life, and died on the 14th day of life. An autopsy revealed centrilobular congestion with focal cholestasis in the liver, microsteatosis, and the lipid vacuolation of some hepatocytes. This case was reported to have been diagnosed with hydrops fetalis, based on an antenatal USG series, and elevated LFT was reported within the first 14 days, although the test results were not reported in detail [Lax et al., 2015]. When this condition is accepted as secondary to multiple organ dysfunction caused by severe hydrops fetalis, our patients can be considered to be the first cases to present with cholestasis in the literature.

In this case report, we describe a new clinical presentation with a novel *RARS2* pathogenic variant. The cases presented with cholestasis and lactic acidosis in the neonatal period, similar to the presentations expected with a *TRMU* gene defect, another mitochondrial translation defect [Gaignard et al., 2013]. The neonatal cholestasis spontaneously regressed, and liver function and hypoglycemia improved, as observed in *TRMU* mutations; however, severe neurological development retardation, microcephaly, and seizures were identified as the dominant clinical findings at follow-up. Both patients were lost in early infancy due to persistent epilepsy. We speculate that in *RARS2*, a translation mutation like *TRMU*, cholestasis can improve spontaneously with age, which can be seen also in patients with *TRMU* mutations.

A total of 28 different *RARS2* mutations have been reported in a total of 34 patients, of which 20 (59%) were identified with compound heterozygous pathogenic variants and 14 (41%) with homozygous pathogenic variants. In the cases presented here, a novel homozygous pathogenic variant in the *RARS2* gene was detected that was carried both by the mother and the father. The protein position of this variant is a very highly conserved region with a Polyphen Score of 99% (Fig. 4).

Although the signs and symptoms of our cases overlap with that of other cases with *RARS2* pathogenic variants, this disorder did not exist among our preliminary diagnoses. Next-generation sequencing including a panel of genes accounting for inborn errors of metabolism has facilitated this rapid diagnosis. Next-generation sequencing with panels of genes or whole exomes or genomes clearly increased the diagnostic rate for patients presenting with non-specific signs and symptoms. However, the interpretation/validation of the variants of unknown significance and the cost seem to be the current disadvantages.

In conclusion, *RARS2* mutations are responsible for a mitochondrial disease that causes severe mental, motor, and developmental retardation as well as short life expectancy. Our 2 sibling cases both had liver involvement and cholestasis in the neonatal period that spontaneously improved, as with *TRMU* mutations. Our patients are the first cases with liver involvement in PCH6 and a novel homozygous *RARS2* pathogenic variant to be reported in the literature. This additional phenotypic can be considered as making a valid contribution to the literature. *RARS2* pathogenic variants should be considered in the differential diagnosis of diseases that lead to cholestasis in the neonatal period.

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Statement of Ethics

Informed consent in compliance with the Helsinki Declaration for genetic analysis and publication of clinical reports and photographs were obtained from the patient or his parent/guardian in compliance with the national ethics regulation. This study protocol was reviewed and the need for approval was waived by Ankara University Ethic Committee.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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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.

Author Contributions

S.S., F.T.E., and A.I. managed the patient and prepared the figures. S.S., F.T.E., and F.S.E. performed the literature search, collected, analyzed, and interpreted data, and wrote the initial draft of the manuscript. F.S.E. performed the genetic analysis and wrote the genetic analysis section. All authors reviewed the final manuscript and endorsed the findings and the scientific content.

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