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Rescue of respiratory failure in pulmonary alveolar proteinosis due to pathogenic *MARS1* variants

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Abstract

Background: Pulmonary alveolar proteinosis (PAP) is a heterogeneous condition with more than 100 different underlying disorders that need to be differentiated to target therapeutic options, which are generally limited.

Methods: The clinical course of two brothers with pathogenic variants in the methionyl-tRNA synthetase (*MARS)1* gene was compared to previously published patients. Functional studies in patient-derived fibroblasts were performed and therapeutic options evaluated.

Results: The younger brother was diagnosed with PAP at the age of 1 year. Exome sequencing revealed the homozygous *MARS1* variant p.(Arg598Cys), leading to interstitial lung and liver disease (ILLD). At 2 years of age, following surgery

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hypoglycemia was detected, the pulmonary condition deteriorated, and the patient developed multiorgan failure. Six therapeutic whole lung lavages (WLL) were necessary to improve respiratory insufficiency. Methionine supplementation was started and a high protein diet ensured, leading to complete respiratory recovery. The older brother, homozygous for the same *MARS1* variant, had a long-known distinct eating preference of methionine-rich food and showed a less severe clinical phenotype. Decreased aminoacylation activity confirmed the pathogenicity of p.(Arg598Cys) in vitro. In agreement with our review of currently published ILLD patients, the presence of hepatopathy, developmental delay, muscular hypotonia, and anemia support the multisystemic character of the disease.

Conclusions: Catabolic events can provoke a severe deterioration of the pulmonary situation in ILLD with a need for repetitive WLL. Although the precise role of oral methionine supplementation and high protein intake are unknown, we observed an apparent treatment benefit, which needs to be evaluated systematically in controlled trials.

KEYWORDS

interstitial lung and liver disease, *MARS1*, methionine, pulmonary alveolar proteinosis, whole lung lavage

1 | INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is defined by increased accumulation of surfactant in the alveolar space. Deficient removal leads to impaired gas exchange and respiratory failure.[1,2] More than 100 different underlying conditions can cause PAP.[3] Symptomatic treatment with whole lung lavages (WLL) removes the abundant material from the alveolar space and restores gas exchange. Recently, we described together with a group in Paris a new specific type of PAP mostly prevalent around La Réunion and nearby islands caused by pathogenic biallelic variants in the methionyl-tRNA synthetase (*MARS)1 gene*, encoding for cytosolic MARS1.[4] MARS1 belongs to the class 1 family of cytosolic aminoacyl-tRNA synthetases (ARSs), which play a pivotal role in protein translation catalyzing the aminoacylation of tRNA by their cognate amino acid, thereby linking it with the correct nucleotide anticodon.[5-7] While monoallelic pathogenic variants in cytosolic ARSs genes have been identified to cause neurodegenerative diseases,[8,9] biallelic pathogenic variants in cytosolic ARSs genes are typically associated with multisystemic phenotypes. Biallelic pathogenic variants in *MARS1* lead to a disease characterized by an early onset PAP with rapid progression to pulmonary fibrosis and structural hepatopathy,[4,10] known as "interstitial lung and liver disease" (ILLD, MIM #615486). However, a systematic analysis of the clinical spectrum has not been performed till date. Of note, the introduction of pathogenic *MARS1* variants into yeast caused attenuation of enzyme activity and growth retardation, which could be alleviated by methionine supplementation in cell culture.[4,11] In one patient diagnosed with PAP caused by pathogenic *MARS1* variants, the respiratory condition improved after methionine supplementation was initiated.[12] No details, however, were given describing the further clinical course of the infant. We provide detailed data on the clinical course of two brothers affected by ILLD due to a novel homozygous *MARS1* variant in comparison with the published patients suffering from this condition, along with functional data on MARS1 activity in fibroblasts of both brothers. Therapeutic options like WLL, a diet rich in proteins, and oral methionine supplementation are discussed. Our data expand the clinical and genetic phenotype of the disease and will be helpful for future treatment decisions of PAP caused by pathogenic variants in *MARS1*.

2 | MATERIALS AND METHODS

2.1 | Study design, recruitments of patients and data acquisition

Patients were studied in detail by thoroughly evaluating the medical history and re-evaluating liver histology. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013. Informed consent to participate in the study was obtained from the parents. The study was approved by the ethical committee of the University Hospital Heidelberg. Regarding previously published patients, data were retrieved from the published reports identified by a comprehensive bibliographic search via PubMed and Google Scholar. Queries were based on the terms "MARS," "MARS1," "Interstitial lung and liver disease," and "ILLD." Phenotyping was performed according to Human Phenotype Ontology terminology. The cut-off date for data analysis was 6 April 2020.

2.2 | Exome sequencing and variant filtering

MARS1 variants of hitherto unreported individuals were identified using exome sequencing. The carrier status of the parents was confirmed via trio-based exome sequencing (details available upon request).

2.3 | In-silico effect prediction of MARS1 missense variants

To assess the deleterious effect of missense variants, six commonly used prediction scores (CADD v1.4, M-CAP v.3.5a, PolyPhen-2 v.2.2.2, PROVEAN v.3.5a, REVEL v.3.5.a, and SIFT v.5.2.2) were calculated for all possible *MARS1* missense variants and subsequently mapped onto a two-dimensional representation of the MARS1 protein, as methodically shown by Hebebrand et al[13] We generated all possible base exchanges referring to the *MARS1* coding sequence (transcript NM_020117.9) and matched the resulting variant call format file to the GRCh37/hg19 reference genome using the Mutalyzer Position Converter. Prediction scores of resulting genomic sequence variants were subsequently calculated using the Ensembl variant effect prediction tool.[14] We used the ggplot2 package within the RStudio framework (Version 1.2.1335, RStudio Team, 2018) to build a generalized additive model by a quadratically penalized likelihood type approach (formula = y ~ s(x, bs = "cs")) of the *geom_smooth* function to enable improved pattern detection by plotting a smoothened line and the confidence interval around the calculated scores for each alternate amino acid position.

2.4 | In vitro studies in patient fibroblasts and controls

Fibroblasts were collected from affected individuals after informed consent was obtained. A fibroblast control cell line was purchased from Merck (SCC058, Darmstadt, Germany). All cells were tested for mycoplasma contamination. Fibroblasts were cultivated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C and 5% CO_2. All studies were performed as biological triplicates.

2.5 | MARS1 enzyme assay

Aminoacylation was assessed by measuring MARS1 activity in cultured fibroblasts. Fibroblast lysates (cytosolic fraction) were incubated at 37°C for 10 minutes in a reaction buffer containing 50 mmol/L Tris buffer pH 7.5, 12 mmol/L $MgCl_2$, 25 mmol/L KCl, 1 mg/mL bovine serum albumin, 0.5 mmol/L spermine, 1 mmol/L ATP, 0.2 mmol/L yeast total tRNA, 1 mmol/L dithiothreitol, 0.3 mmol/L [^{13}C,D_3]-methionine, [$^{13}C_2$]-leucine and [$^{13}C_6$,^{15}N]-isoleucine. The reaction was terminated using trichloroacetic acid. After sample washing with trichloroacetic acid, ammonia was added to release the labeled amino acids from the tRNAs. [D_3]-leucine and [^{13}C]-valine were added as internal standards, and the labeled amino acids were quantified by LC-MS/MS. Intra-assay and interassay variation were <15%. Isoleucyl-tRNA synthetase (IARS1), and leucyl-tRNA synthetase (LARS1) activities were simultaneously detected as control enzymes. Statistical significance of differences between activities of controls and individual patients were assessed using the Student *t* test, confidence interval (CI) 95% (SPSS 26.0.0.1).

2.6 | Western blot

For immunoblotting, cells were washed in phosphate buffered saline with tween (0.1%), and resolved in radioimmunoprecipitation assay buffer. Twenty µg of protein of every sample were separated on a 10% polyacrylamide gel. Primary antibodies against MARS1 (Sigma, Darmstadt, Germany) and β-actin (Santa Cruz Biotechnology, Heidelberg, Germany) were incubated overnight. Secondary hrp-coupled antibodies (goat-antirabbit or rabbit-antimouse) were obtained from Dianova (Hamburg, Germany). Enhanced chemiluminescence of proteins was detected using Vilberscan Fusion FX7.

3 | RESULTS

3.1 | Case presentations including liver histology

The index patient was born to healthy, consanguineous parents (first-grade cousins) as the third child of four siblings (Figure 1A). Birth of the index patient was at term (38 + 4 weeks of gestational age) via Cesarean section with low body weight (2120 g; z − 2.97) and height (44 cm; z − 3.29),[15] hence presenting small for gestational age (SGA). He was admitted to the neonatology ward due to hypoglycemia (min. 2.17 mmol/L). He was discharged in good clinical conditions after some days of intensive feeding. Aged 4 months, the boy was admitted to hospital with pneumonia (Figure 2A), suffering from diarrhea and failure to thrive. Apart from a respiratory infection, CMV colitis was diagnosed and treated with ganciclovir. The diagnostic work-up revealed no signs for immunodeficiency, cystic fibrosis or celiac disease. Testing regarding metabolic disorders remained unremarkable. Increased alanine aminotransferase(ALAT) and total bilirubin levels were noticed for the first time. At 5 months of age, the boy experienced another respiratory tract infection with the detection of Corona-virus, Respiratory syncytial virus, and Pneumocystis jirovecii (PCR). Due to normocytic anemia (Hb 6.6 g/dL) iron supplementation was started. At 7 months of age, psychomotor developmental delay and severe dystrophy (BMI 11.7 kg/m², z − 5.1) were noticed. The MRI of the brain was normal, but work-up revealed hepatomegaly and elevated hepatic transaminases. Liver biopsy showed lobular disarray, a moderate mixed periportal steatosis, mild ballooning, and scattered inflammatory foci; a pattern consistent with mild nonalcoholic steatohepatitis and overlapping chronic extra-acinar cholestasis. There was a mild ductular reaction, while the interlobular bile ducts revealed only subtle irregularities. Progressive liver disease was indicated by the initial development of liver cirrhosis

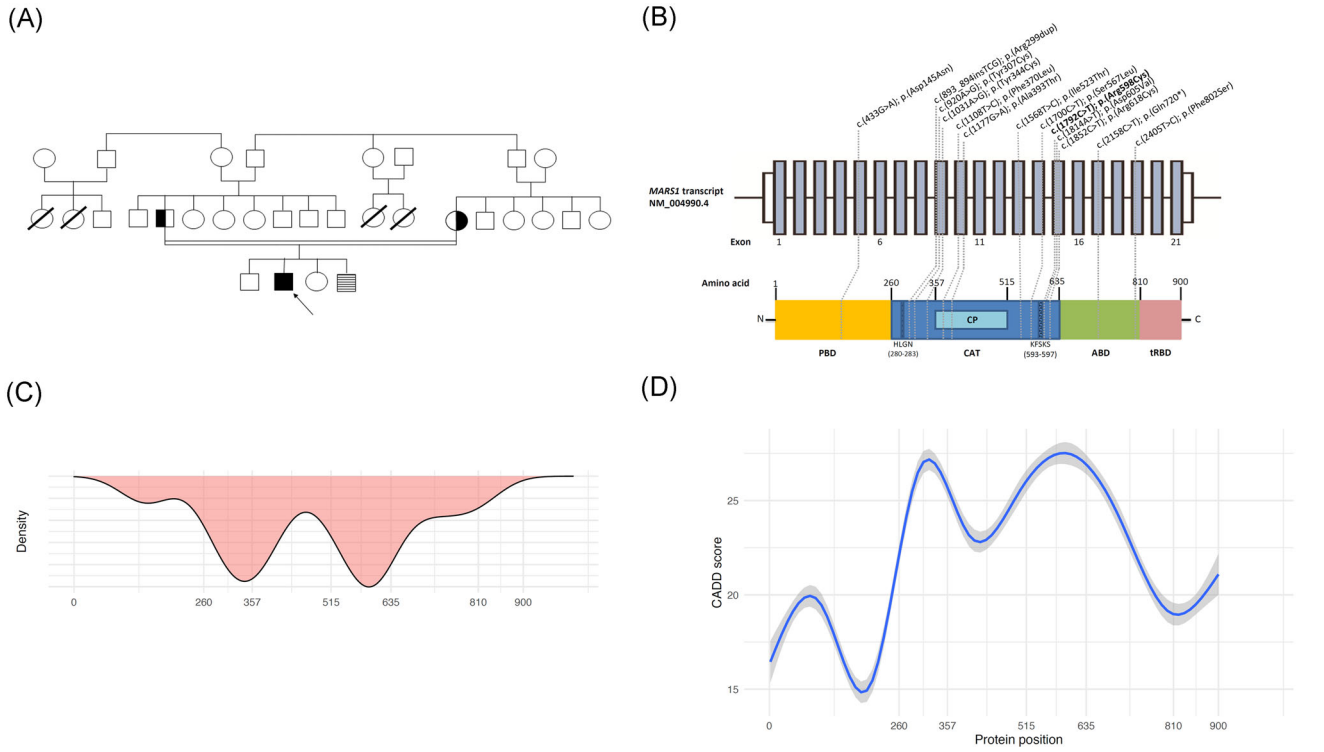
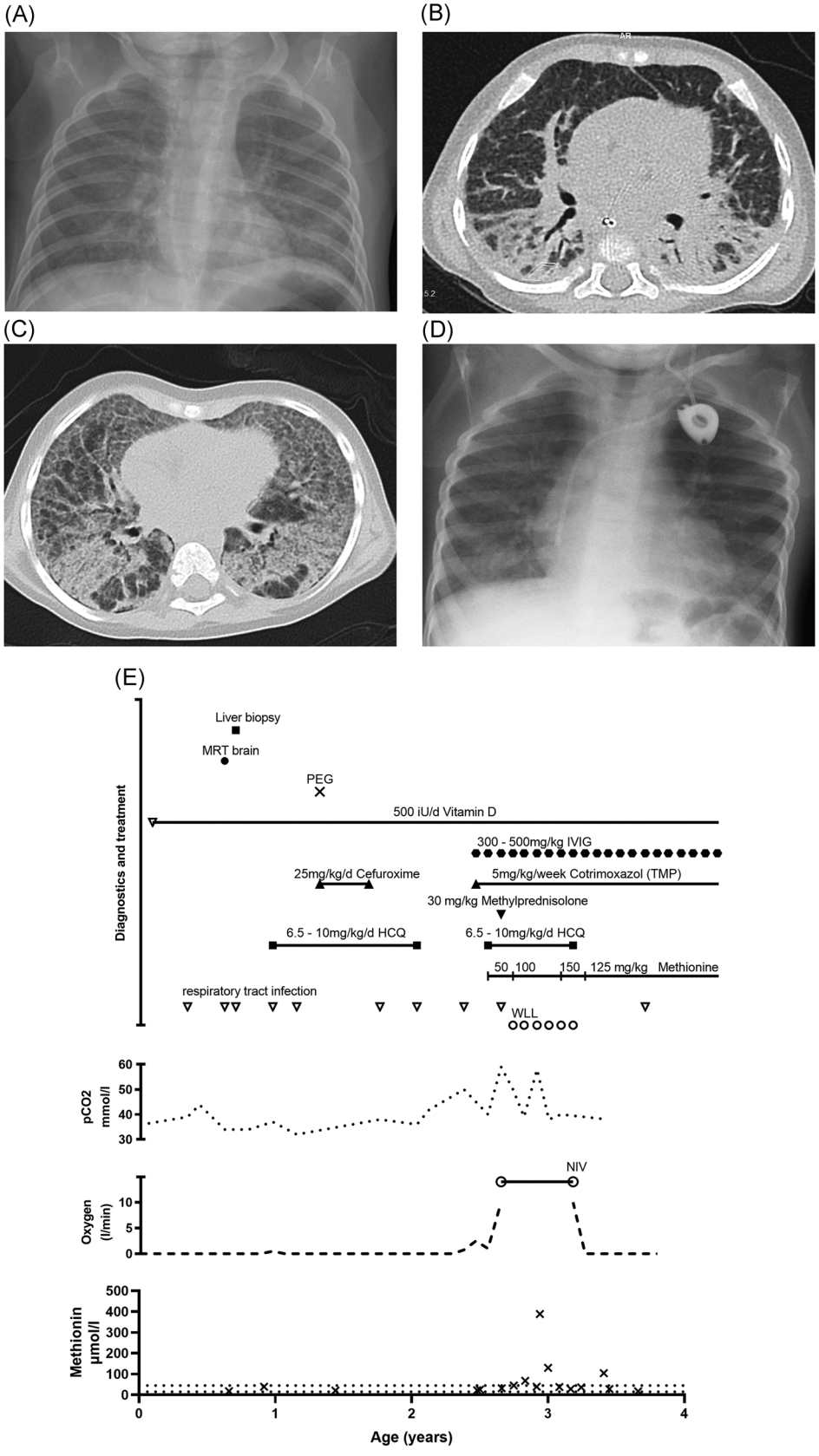


FIGURE 1 Genetic background of interstitial lung and liver disease (ILLD) caused by pathogenic biallelic variants in *MARS1*. A, Pedigree of the patient's family. The index patient (filled black square) is the third child of four living siblings. He is marked with an arrow. In the oldest child the same genetic variant was found (striped black square). The sister in the middle and the youngest brother are healthy. Both parents were heterozygous carriers of the genetic variant (half-filled black square and circle) and first cousins. The father's brother and the mother's father were diagnosed with familial Mediterranean fever. Deceased family members are slashed. B, All known pathogenic *MARS1* variants including the affected region of MARS1 protein and its domains. The novel variant is displayed in bold. C, Density plot of known pathogenic variants with respect to affected protein domains. D, In-silico effect prediction of *MARS1* missense variants using CADD score. ABD, adapter-binding domain; CAT, catalytic domain; CP, protein coding; *MARS1*, methionyl-tRNA synthetase; PBD, p21 binding domain; tRBD, tRNA-binding domain [Color figure can be viewed at wileyonlinelibrary.com]

with demonstrable perisinusoidal collagen deposition (Figure 3 A-F). Persistent tachypnea triggered chest CT showing ground-glass opacities in combination with extensive interlobular septal thickening (crazy-paving pattern) in all lobes (Figure 2B), leading to the diagnosis of childhood interstitial lung disease (chILD) and treatment with hydroxychloroquine since the age of 11 months. A gastric tube was inserted, improving weight and height development in the following months (Figure S1 A-C). At 2 years of age, the boy had experienced eight hospital admissions due to respiratory tract infections. Except for one episode, no prolonged oxygen supplementation was necessary (Figure 2E). After peer review by the child-EU management platform (www.childeu.net),[16] exome sequencing was conducted to search for variants in the *MARS1* gene, revealing the homozygous variant NM_004990.4: c.(1792C > T); NP_004981.2: p.(Arg598Cys) (Figure 1B). Segregation analysis confirmed both parents as heterozygous carriers. The older brother was diagnosed as homozygous (F1:II.1). An older sister (F1:II.2) and a younger brother (F1:II.4), born after the initial presentation of the index patient, did not show biallelic variants in *MARS1*. Aged 2 years and 7 months, the index patient underwent fundoplication and gastric and jejunal catheter insertion. Sixteen hours after surgery, the boy suffered from severe, rapid hypoglycemia (min. 0.56 mmol/L) and developed multiorgan failure with pulmonary edema, renal and liver failure within the next 24 hours. Invasive high-frequency oscillation ventilation was started including NO-therapy. Renal failure necessitated 20 hours of hemofiltration and showed tubulo-interstitial damage. Coagulopathy was treated by substitution of vitamin K and fresh-frozen plasma. Amino acids were supplemented (2 g/kg/d) and high glucose intake (8 g/kg/d) was assured. After 5 days of invasive ventilation, the boy was extubated and therapy was continued with supplemental oxygen by nasal cannula. Chest-CT (Figure 2C) revealed an increasing crazy paving pattern in line with severe PAP with respiratory insufficiency. For immediate relief of respiratory compromise, therapeutic WLLs were performed.[17,18] Furthermore, oral methionine was initiated at the increasing dosage and protein intake of 2 to 3 g/kg/d was assured (Figure 2E). To prevent frequent respiratory infections, treatment with intravenous immunoglobulins (IVIG) was started. With this therapeutic approach, the clinical course bettered, no more ventilation or supplemental oxygen was needed and chest imaging improved (Figure 2D). At last visit, the boy had learned to walk and run without dyspnea. Growth curves had normalized (Figure S1A-C). There was still psychomotor delay with muscular hypotonia, and the patient had persistently elevated liver transaminases.


(A)
(B)
(C)
(D)
(E)
Liver biopsy
MRT brain
PEG
500 iU/d Vitamin D
300 - 500mg/kg IVIG
25mg/kg/d Cefuroxime
5mg/kg/week Cotrimoxazol (TMP)
30 mg/kg Methylprednisolone
6.5 - 10mg/kg/d HCQ
6.5 - 10mg/kg/d HCQ
50 100 150 125 mg/kg Methionine
respiratory tract infection
WLL
Diagnostics and treatment
pCO2 mmol/l
60
50
40
30
NIV
Oxygen (l/min)
10
5
0
Methionin µmol/l
500
400
300
200
100
0
0
1
2
3
4
Age (years)

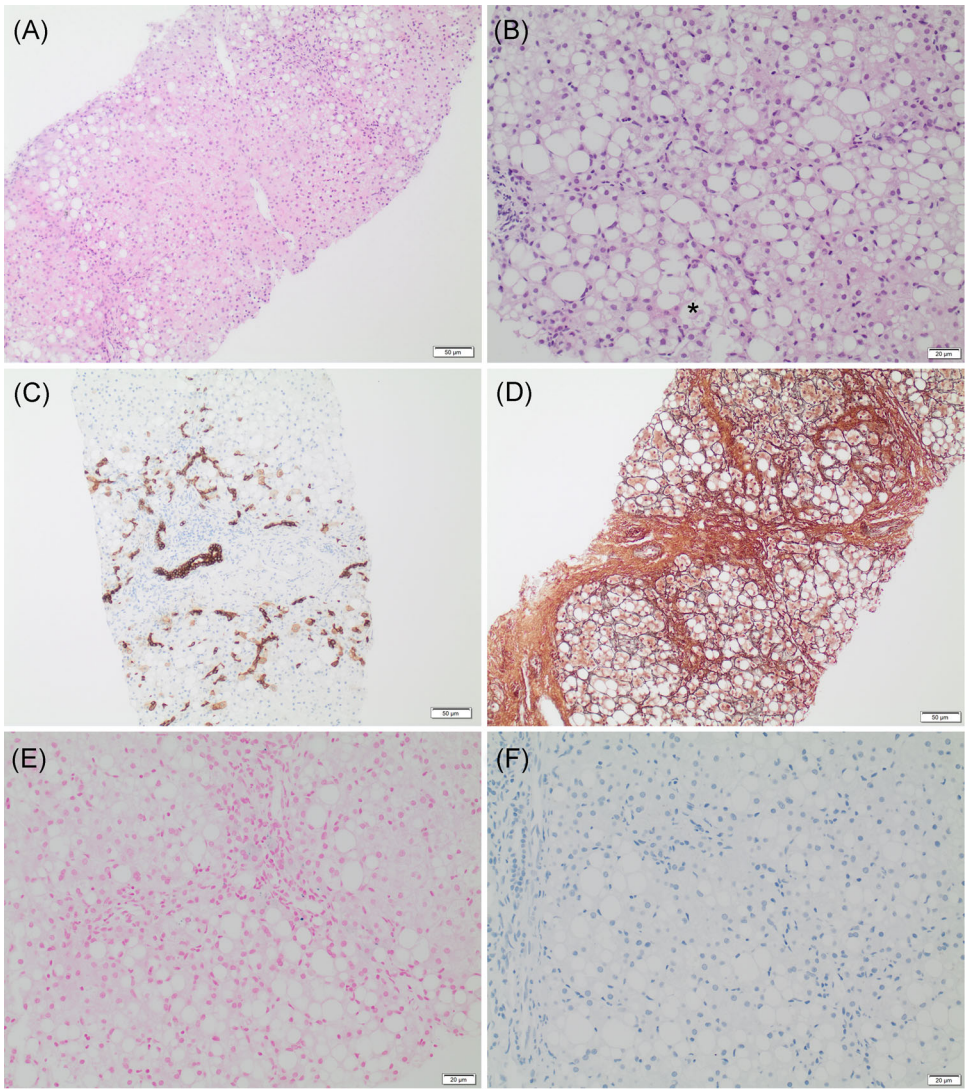


FIGURE 3 Representative images of the index patient's liver biopsy taken at the age of 7 months. A, The liver histology shows mild trabecular disarray of the liver parenchyma and moderate periportal steatosis. B, Higher magnification reveals mixed-type steatotis, few ballooned hepatocytes (asterisk), and scattered single cell necrosis. C, Keratin 7 immunostaining shows ductular metaplasia of periportal hepatocytes, a mild ductular reaction and only mild irregularities of the interlobular bile duct. D, There is prominent septal fibrosis with perisinusoidal collagen deposition and the initial formation of pseudolobules indicating initial cirrhosis development (modified Gomori's). E, No iron deposition (Prussian blue). F, CMV immunostaining is negative [Color figure can be viewed at wileyonlinelibrary.com]

The older brother of the index patient (F1:II.1; Figure 1A), carrying the same homozygous variant, is 8 years old at the time of this report. Until genetic family screening for *MARS1*, he had been considered healthy and only reverse phenotyping revealed the presence of mild symptoms: He was born at 35 + 1 weeks of gestation via Cesarean section due to a HELPP-syndrome of the mother (birthweight 2040 g, z − 1.39).[15] In the neonatal period, the boy developed apnea, tachypnea, and hypoglycemia (min. 1.78 mmol/L). Hyperbilirubinemia (max. 270 µmol/L) was treated by

FIGURE 2 Long-term clinical course, treatments, and radiological pulmonary findings in the index patient. A, First chest X-ray at the time the patient was diagnosed with pneumonia showing bilateral perihilar infiltrates (4 months of age). B, High resolution chest CT (HRCT) at the time of diagnosis of interstitial lung disease showing diffuse ground-glass opacities (GGO) and extensive interlobular septal thickening in all lobes. No enlargement of the heart or pleural effusion (1.2 years of age). C, At time of multiorgan failure including respiration, HRCT showed deterioration with severe PAP, marked crazy paving pattern (diffuse GGO, inter- and intralobular thickening), and pectus excavatum (2.5 years). D, X-Ray showing clinical improvement of the respiratory situation (3.5 years). E, The clinical course of the index patient including respiratory infections and therapeutic interventions. Dotted lines represent the target range of methionine plasma levels. HCQ, hydroxychloroquine; IVIG, intravenous immunoglobulins; NIV, noninvasive ventilation; PEG, percutaneous endoscopic gastrostomy; WLL, whole lung lavage

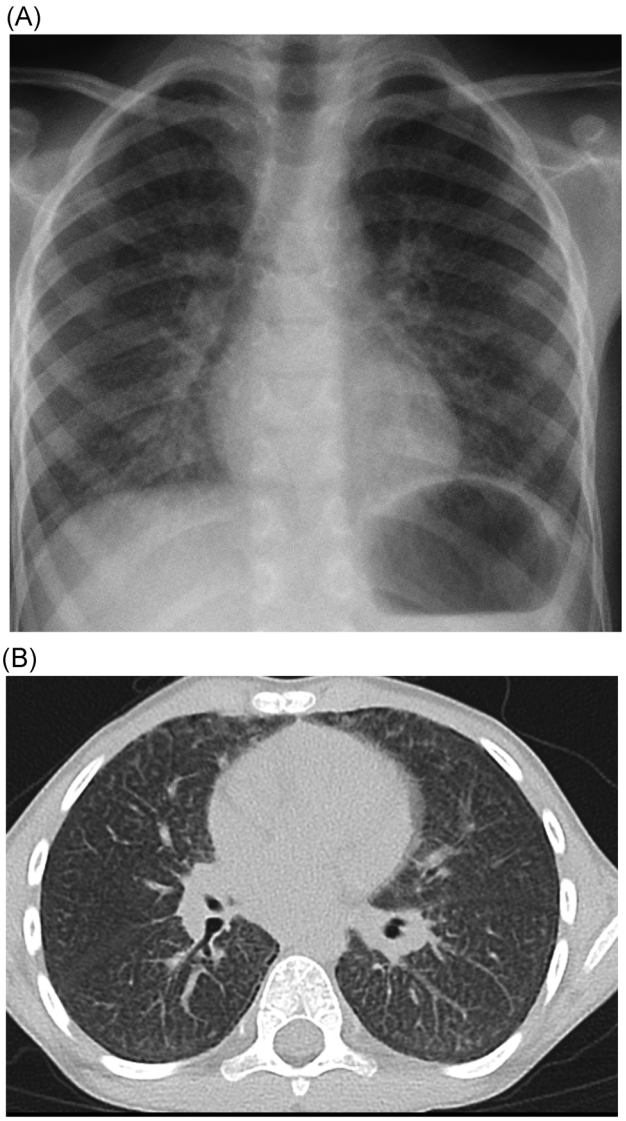


FIGURE 4 Pulmonary findings in the older brother of the index patient. A, Initial chest x-ray showing diffuse bilateral perihilar infiltrates and reticulations (8 years of age). B, High resolution chest CT (HRCT) showing diffuse ground glass opacities (GGO) and peripheral cysts (8 years of age)

phototherapy, anemia (Hb 9.1 g/dL) with iron supplementation. He was admitted to hospital three times within his first 2 years of life (twice due to gastroenteritis and once due to an Influenza infection). Basic diagnostic work-up showed elevated lactate in serum without further hints of an underlying metabolic disorder. Normocytic anemia was reluctant to the iron treatment. In the following years, the boy was in good health, except weight gain in the lower range for age (BMI 13-14 kg/m², z −1.8 to −1.3; Figure S1 D-H). The parents reported that the boy had a clear preference for a high protein diet, including up to five eggs per day and typically preferring chicken meat and other protein-rich nutritional products to other food. Examinations performed after the genetic diagnosis revealed PAP (Figure 4A,B), impaired lung function, and a slight gross and fine motor coordination disorder. However, no signs of liver disease were detected. Based on the positive experience in his younger brother, a protein-rich diet was assured (2-3 g/kg/d) and methionine supplementation initiated (see below).

Find more details on the clinical course of both patients in the supplement.

3.2 | Methionine supplementation and monitoring in both brothers

The plasma levels of methionine of the two brothers were closely monitored. Blood sampling occurred 4 hours postprandially, in case of supplementation samples were taken before intake of methionine (target range 80-150 µmol/L). Before the start of supplementation (initially 50 mg/kg/d), methionine levels of the index patient (F1:II.3) were in the normal range. Due to insufficient increase of methionine in plasma, dosage was adapted several times as follows: 86 mg/kg/d, 100 mg/kg/d, 150 mg/kg/d, 125 mg/kg/d. Methionine levels, however, remained fluctuating, lately ranging between normal concentrations and concentrations within the target rang. Before the start of supplementation (50 mg/kg/d) of the older brother (F1:II.1) methionine levels were in the normal range. After supplementation, plasma methionine rose to 177 µmol/L at the last visit (Figure 5A,B).

3.3 | Phenotypic and genetic specturm of ILLD

The literature search identified five peer-reviewed publications on individuals with biallelic pathogenic *MARS1* variants, published since October 2013; 34 patients from seven families with seven different genotypes and a total of 12 different pathogenic variants were reported. Herein, we present two novel patients from one family harboring a novel homozygous variant. For an overview on the overall clinical phenotype, see Table S1. The lung was the predominantly affected organ present in all 36/36 individuals, whereas liver affection was reported for 31/36, ranging from hepatomegaly and elevated hepatic transaminases to cholestasis and early cirrhosis. Other organ affections were noticed when data was available: at least 17 patients were born small for gestational age, 29 of 35 presented with failure to thrive, anemia was present in 7/7. There is limited information on the neurological phenotype, such as developmental delay or muscular hypotonia (the latter found in 4/5 patients). Three patients were reported to have hypothyroidism. 14/36 patients died at a median age of 16.4 months (range 5 months-25 years) which was associated to respiratory failure in all cases.

Altogether, these 36 patients carried 13 different variants, including 11 missense variants, 1 nonsense variant, and 1 duplication triplet insertion leading to an aminoacid duplication. Variants were distributed throughout the gene with a clearly increased density in the catalytic domain, in line with higher prediction scores compared to variants affecting other domains of the protein (Figures 1C,D). As the variants carried by the patients from the Réunion Island are attributed to one founder,[4] they were counted only once for the density plot (Figure 1C).

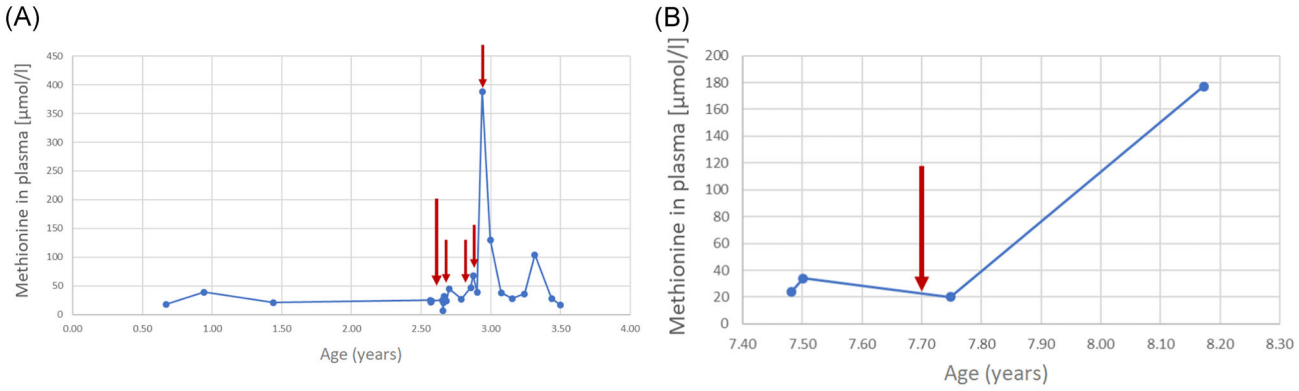


FIGURE 5 A, Individual F1:II.3, the index patient. Before the start of supplementation with methionine (50 mg/kg/d), indicated by the long red arrow, methionine levels were in the normal range (Ref 15-45 µmol/L). The dosage was adapted several times (smaller red arrows). Please note the different ranges of the Y-axis between panels. B, Individual F1:II.1, the older brother of the index patient. Before start of supplementation with methionine (50 mg/kg/d), indicated by the long red arrow, methionine levels were in the normal range (Ref 15-45 µmol/L). After supplementation, plasma methionine rose to 177 µmol/L at last visit [Color figure can be viewed at wileyonlinelibrary.com]

3.4 | In-vitro analyses

Western blot analysis showed normal levels of MARS1 in fibroblasts of patient F1:II.3 (Figure 6A). However, the aminoacylation activity was significantly reduced in F1:II.3 to about 5% of controls ($P < .01$). In the older brother (F1:II.1), MARS1 activity was reduced to 22% of that of controls, still significant ($P < .01$). To demonstrate that the reduced enzyme activity was specific for MARS1, we analyzed the activity of IARS1 and LARS1 in parallel and found no difference to the control cells (Figure 6B).

4 | DISCUSSION

Recently we have described biallelic pathogenic variants in *MARS1* to cause an early-onset and severe disease named interstitial lung and liver disease (MIM #615486).[4] In this study, we present two brothers homozygous for a previously unreported variant in *MARS1* and further detail the phenotype in particular with respect to metabolic vulnerability and additional organ-involvement of this condition.

Following a catabolic situation, the index patient experienced hypoglycemia and in a second episode a respiratory deterioration with liver and kidney failure. This observation is of specific interest, as acute deterioration triggered by catabolism has not been reported in ILLD patients so far, although acute deterioration is known in other cytosolic ARS deficiencies.[19-21] To stabilize the boy temporarily, several WLLs were necessary. Such symptomatic treatment is sometimes necessary for many years.[17] Therefore, alternative and less invasive treatments are urgently needed. Based on mutationally reduced MARS1 enzymatic activity which can be overcome in vitro by methionine substrate supply, and anecdotal evidence from patients on La Réunion and surrounding islands indicating a benefit from the consumption of large amounts of eggs, we supplemented methionine and ensured a high protein intake. We argue that this intervention contributed to a significant improvement in growth, pulmonary, and neurodevelopmental state. Of note, MARS1-04 (Table S1) was also reported to improve his clinical condition after methionine supplementation, but no details on dosage or methionine levels were given, nor was follow-up data available.[12] However, methionine supplementation should be handled carefully with monitoring of methionine plasma concentrations (suggested target range 80-150 µmol/L), as high levels (>600 µmol/L) are neurotoxic leading to severe brain edema.[22] The index patient showed a considerable fluctuation of methionine concentrations despite standardized measurement regimens. Interestingly, high daily protein intake is also

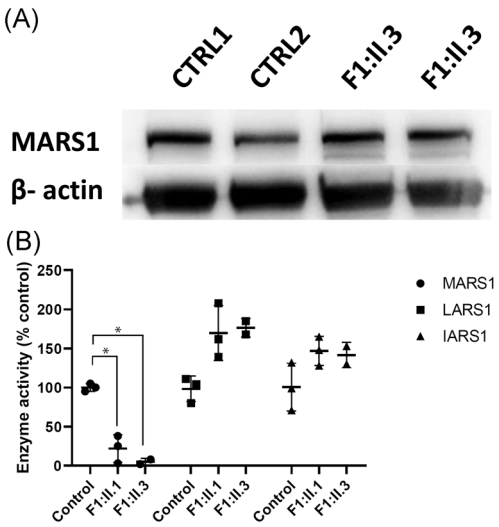


FIGURE 6 Expression and activity of MARS1. A, MARS1 protein expression by Western blot analysis in fibroblasts of individual F1:II.3 (index patient) and controls (CTRL). B, Aminoacylation measured in fibroblasts of F1:II.1 and F1:II.3 and controls for MARS1, LARS1, and IARS1. Aminoacylation activity of MARS1 was significantly reduced in F1:II.3 and F1:II.1 compared to controls ($P < .01$), whereas the activity of LARS1 and IARS1 is normal or even elevated compared to controls. LARS1, leucyl-tRNA synthetase; MARS1, methionyl-tRNA synthetase

recommended in LARS1 deficiency[20,21] and isoleucine supplementation appeared to be beneficial in IARS1 deficiency.[19] However, data are lacking to judge this effect and further studies are needed to evaluate the benefit of nutritional therapeutic approaches in the group of ARS deficiencies.

The homozygous variant of the index patient was also found in his older brother. As his clinical course was milder the question was raised whether disease severity was influenced by environmental factors. In fact, the older brother had special dietary habits, for example, the intensive consumption of eggs in everyday life since early childhood and hence an increased protein and methionine intake. In addition, the aminoacylation activity in fibroblasts was higher in this brother than in the index patient, an observation that cannot be explained by nutritional habits but pointing at a higher residual activity as a potential cause of the milder clinical phenotype. Scrutinizing exome data of the index patient did not reveal further suspicious variants neither in *MARS1* nor in genes encoding for direct interaction partners of MARS1 in the multi synthetase complex. This complex consists of eight cytosolic ARSs together with three auxiliary proteins and regulates transcription, translation, and various signaling pathways.[23,24]

Analysis of the distribution of genetic variants found in ILLD patients demonstrates a clear accumulation of variants in the region of the catalytic domain, a finding which well reflects the in silico analysis of disease severity scores related to variant location. This provides further evidence that the pathomechanism in ILLD is linked to defective aminoacylation.

MARS1 belongs to the group of cytosolic aminoacyl-tRNA synthetases playing a pivotal role in protein translation in all kinds of cells. In line with this, defects of those enzymes affect growth and multiple organ systems, mainly lungs, liver, nervous system, hematopoietic system, and musculature.[25] Besides intense pulmonary affection and progressive cirrhotic liver disease, our index patient F1:II.3 suffered from severe global developmental delay, dystrophy, muscular hypotonia, and anemia. Systematically analyzing all currently published patients with biallelic pathogenic *MARS1* variants, our study emphasizes that human disease related to biallelic *MARS1* variants is not restricted to lung and liver, but also comprises abnormalities of the nervous system, growth, and hematopoietic system. Deep phenotyping is crucial to understand the natural history of the disease and to improve care for affected individuals and families. We recommend that patients with biallelic *MARS1* variants should be thoroughly analyzed regarding multisystemic signs and symptoms, especially monitoring psychomotor development, growth, and blood abnormalities, even if lung disease with recurrent respiratory infections is the clinically predominant and leading sign. As in other forms of PAP, we assume that those respiratory infections, in particular when they affect the alveolar region, result in disturbance of alveolar macrophage surfactant clearance, leading to the accumulation of alveolar surfactant, that is, PAP, if there is a predisposition from genetic or other factors for PAP. In conclusion, our study points to the fact that biallelic pathogenic variants in *MARS1* not only provoke interstitial lung and cholestatic liver disease, but cause a multisystemic phenotype including growth retardation, neurodevelopmental delay, anemia, and muscular hypotonia. Catabolic events like infections or surgery can lead to acute deterioration and multiorgan failure. WLL can stabilize the pulmonary condition, if PAP is predominant. High protein intake together with methionine supplementation may improve PAP, but also other organ manifestations in ILLD and should be considered to be started early in life. Controlled clinical trials are necessary to investigate the role of protein and methionine supplementation in individuals with biallelic pathogenic variants in *MARS1*.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

1. Griese M. Pulmonary surfactant in health and human lung diseases: state of the art. *Eur Respir J*. 1999;13:1455-1476. https://doi.org/10.1183/09031936.99.13614779
2. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. *N Engl J Med*. 2003;349:2527-2539. https://doi.org/10.1056/NEJMra023226
3. Griese M. Pulmonary alveolar proteinosis: a comprehensive clinical perspective. *Pediatrics*. 2017;140:e20170610. https://doi.org/10.1542/peds.2017-0610
4. Hadchouel A, Wieland T, Griese M, et al. Biallelic mutations of methionyl-tRNA synthetase cause a specific type of pulmonary alveolar proteinosis prevalent on Reunion island. *Am J Hum Genet*. 2015;96:826-831. https://doi.org/10.1016/j.ajhg.2015.03.010
5. Casina VC, Lobashevsky AA, McKinney WE, Brown CL, Alexander RW. Role for a conserved structural motif in assembly of a class I aminoacyl-tRNA synthetase active site. *Biochemistry*. 2011;50:763-769. https://doi.org/10.1021/bi101375d
6. Mechulam Y, Schmitt E, Maveyraud L, et al. Crystal structure of *Escherichia coli* methionyl-tRNA synthetase highlights species-specific features. *J Mol Biol*. 1999;294:1287-1297. https://doi.org/10.1006/jmbi.1999.3339
7. Schmitt E, Meinnel T, Blanquet S, Mechulam Y. Methionyl-tRNA synthetase needs an intact and mobile 332KMSKS336 motif in catalysis of methionyl adenylate formation. *J Mol Biol*. 1994;242:566-576. https://doi.org/10.1006/jmbi.1994.1601

8. Gonzalez M, McLaughlin H, Houlden H, et al. Exome sequencing identifies a significant variant in methionyl-tRNA synthetase (MARS) in a family with late-onset CMT2. *J Neurol Neurosurg Psychiatry*. 2013;84:1247-1249. https://doi.org/10.1136/jnnp-2013-305049
9. Novarino G, Fenstermaker AG, Zaki MS, et al. Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders. *Science*. 2014;343:506-511. https://doi.org/10.1126/science.1247363
10. Enaud L, Hadchouel A, Coulomb A, et al. Pulmonary alveolar proteinosis in children on La Réunion Island: a new inherited disorder? *Orphanet J Rare Dis*. 2014;9:85. https://doi.org/10.1186/1750-1172-9-85
11. Comisso M, Hadchouel A, de Blic J, Mirande M. Mutations in MARS identified in a specific type of pulmonary alveolar proteinosis alter methionyl-tRNA synthetase activity. *FEBS J*. 2018;285:2654-2661. https://doi.org/10.1111/febs.14510
12. Rips J, Meyer-Schuman R, Breuer O, et al. MARS variant associated with both recessive interstitial lung and liver disease and dominant Charcot-Marie-Tooth disease. *Eur J Med Genet*. 2018;61:616-620. https://doi.org/10.1016/j.ejmg.2018.04.005
13. Hebebrand M, Hüffmeier U, Trollmann R, et al. The mutational and phenotypic spectrum of TUBA1A-associated tubulinopathy. *Orphanet J Rare Dis*. 2019;14:38. https://doi.org/10.1186/s13023-019-1020-x
14. McLaren W, Gil L, Hunt SE, et al. The ensembl variant effect predictor. *Genome Biol*. 2016;17:122. https://doi.org/10.1186/s13059-016-0974-4
15. Voigt M, Fusch C, Olbertz D, et al. Analysis of the neonatal collective in the Federal Republic of Germany. *Geburtsh Frauenheilk*. 2006;66:956-970. https://doi.org/10.1055/s-2006-924458
16. Griese M, Seidl E, Hengst M, et al. International management platform for children's interstitial lung disease (chILD-EU). *Thorax*. 2018;73:231-239. https://doi.org/10.1136/thoraxjnl-2017-210519
17. Griese M, Ripper J, Sibbersen A, et al. Long-term follow-up and treatment of congenital alveolar proteinosis. *BMC Pediatr*. 2011;11:72. https://doi.org/10.1186/1471-2431-11-72
18. Reiter K, Schoen C, Griese M, Nicolai T. Whole-lung lavage in infants and children with pulmonary alveolar proteinosis. *Paediatr Anaesth*. 2010;20:1118-1123. https://doi.org/10.1111/j.1460-9592.2010.03442.x
19. Kopajtich R, Murayama K, Janecke AR, et al. Biallelic IARS mutations cause growth retardation with prenatal onset, intellectual disability, muscular hypotonia, and infantile hepatopathy. *Am J Hum Genet*. 2016;99:414-422. https://doi.org/10.1016/j.ajhg.2016.05.027
20. Casey JP, Slattery S, Cotter M, et al. Clinical and genetic characterisation of infantile liver failure syndrome type 1, due to recessive mutations in LARS. *J Inherit Metab Dis*. 2015;38:1085-1092. https://doi.org/10.1007/s10545-015-9849-1
21. Lenz D, Smith D, Crushell E, et al. Genotypic diversity and phenotypic spectrum of infantile liver failure syndrome type 1 due to variants in LARS1 [online ahead of print]. *Genet Med*. 2020.
22. Schwahn BC, Scheffner T, Stepman H, et al. Cystathionine beta synthase deficiency and brain edema associated with methionine excess under betaine supplementation: four new cases and a review of the evidence. *JIMD Rep*. 2020;52:3-10. https://doi.org/10.1002/jmd2.12092
23. Park SG, Ewalt KL, Kim S. Functional expansion of aminoacyl-tRNA synthetases and their interacting factors: new perspectives on housekeepers. *Trends Biochem Sci*. 2005;30:569-574. https://doi.org/10.1016/j.tibs.2005.08.004
24. Lee SW, Cho BH, Park SG, Kim S. Aminoacyl-tRNA synthetase complexes: beyond translation. *J Cell Sci*. 2004;117:3725-3734. https://doi.org/10.1242/jcs.01342
25. Fuchs SA, Schene IF, Kok G, et al. Aminoacyl-tRNA synthetase deficiencies in search of common themes. *Genet Med*. 2019;21:319-330. https://doi.org/10.1038/s41436-018-0048-y

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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