Deep phenotyping of *MARS1* (interstitial lung and liver disease) and *LARS1* (infantile liver failure syndrome 1) recessive multisystemic disease using Human Phenotype Ontology annotation: overlap and differences. Case report and review of literature.

Charlotte La Fay [a,*], Celia Hoebeke [b], Marine Juzaud [a], Anne Spraul [c], Pauline Heux [d], Jean-Christophe Dubus [e,f], Alice Hadchouel [g], Alexandre Fabre [h,i]

a *Department of Pediatric Gastroenterology and Hepatology, Multidisciplinary Pediatric, Aix Marseille University, La Timone Children Hospital, AP-HM, 13005 Marseille, France*

b *Department of Neuropediatrics and Metabolism, Reference Center of Inherited Metabolic Disorders, La Timone Children Hospital, Marseille, France*

c *AP-HP, Hospital Bicêtre, DMU15, Service de Biochimie, Le Kremlin Bicêtre, France*

d *Aix Marseille University, INSERM, MMG, Marseille, France*

e *Service de Médecine Infantile et Pneumologie Pediatrique, CHU Timone-Enfants, 264 Rue Saint-Pierre, 13385, Marseille Cedex 5, France* f *Aix-Marseille Université, IRD, AP-HM, MEPHI, IHU Méditerranée-Infection France*

g *Pediatric Pulmonology, AP-HP, University Hospital Necker-Enfants Malades, Paris, France*

h *Pediatric Multidisciplinary Pediatric APHM, Timone Enfant, Marseille, France*

i *Aix-Marseille Université, INSERM, GMGF, Marseille, France*

ABSTRACT

INTRODUCTION Aminoacyl transfer RNA (tRNA) synthetases are associated with diseases when mutations occur in their encoding genes. Pulmonary alveolar proteinosis can be caused by mutation in the methionyl-tRNA synthetase (*MARS*) gene while mutations in the leucine-tRNA synthetase (*LARS*) gene lead to infantile liver failure syndrome type 1.

We report the case of a patient with *LARS1* pathogenics variants and two patients with *MARS1* pathogenics variants.

The aim of this study was to analyze the phenotypes of our three patients in detail and classify cases in the literature using Human Phenotype Ontology (HPO) terms

RESULTS The first patient has two previously undescribed heterozygous variants in *LARS1* (c.1818dup and c.463A>G). The other two patients' *MARS1* variants (c.1177G>A and c.1700C>T) have already been described in the literature.

All three patients had anemia, hepatomegaly, feeding difficulties, failure to thrive and hypoalbuminemia.

Including ours, 65 patients are described in total, for whom 117 phenotypic abnormalities have been described at least once, 41.9% of which both in patients with *LARS1* and *MARS1* mutations.

CONCLUSION Patients with *LARS1* and *MARS1* mutations seem to share a common phenotype but further deep phenotyping studies are required to clarify the details of these complex pathologies.

KEYWORDS: tRNA synthetase deficiency, Infantile liver failure syndrome type 1, Pulmonary alveolar proteinosis, LARS, MARS, MARS1, LARS1

INTRODUCTION

Aminoacyl transfer RNA synthetases (ARSs) are a highly conserved ubiquitously expressed class of enzymes that covalently attach amino acids onto transfer RNA (tRNA) as part of the protein synthesis process. (1,2) *MARS1* and *LARS1* respectively encode cytoplasmic methionyl and cytoplasmic leucine-tRNA synthetase. Homozygous or compound heterozygous mutations in the *MARS1* gene cause interstitial lung and liver disease (ILLD; OMIM #615486) including a specific form of pediatric pulmonary alveolar proteinosis (PAP) (3,16), while recessive mutations in the *LARS1* gene have been identified as the cause of a multisystem disorder referred to as infantile liver failure syndrome 1 (ILFS1; OMIM # 615438) (4).

An outstanding question raised by Fuchs et al. for *LARS1* and *MARS1* is whether tRNA synthetase deficiencies have a common phenotype (1). The aim of this study was to address this question by comparing the phenotypes of three patients diagnosed in our hospital: two children respectively 8 months and 12 years of age with homozygous pathogenic variations in *MARS1* and a 10 month-old infant with compound heterozygous variants in *LARS1*.

METHODS

Three cases of recessive *LARS1* or *MARS1* disease are described and these are analyzed with previous case reports of recessive *LARS1* or *MARS1* recessive disease in the literature using Human Phenotype Ontology (HPO) codes (15). HPO provides a standardized vocabulary of phenotypic abnormalities encountered in human diseases, with each term describing a phenotypic abnormality. HPO coding reveals phenotypic similarities between diseases.—Venn diagrams were drawn using Van de Peer et al's online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/.)

Cases in the literature were only considered if they had been described in peer-reviewed articles (i.e. excluding theses and congress abstracts) with a confirmed genetic diagnosis. Some previously described cases of PAP were therefore excluded (16). One of our patients with *MARS1* mutations (Patient 3) was also included in Hadchouel et al.'s study (ID: P6). Here, we analized his phenotype in more detail and provide updated information.

All three patients provided written consent for publication of their case details. For Patient 1, western blot analysis using LARS1 *(D7Q4Q) Rabbit mAb 35509 Cell Signaling* Technology is shown in Supplementary Figure (S3)

PATIENT DESCRIPTION

Patient 1

The first patient is a boy born at 37^{+6} weeks of gestation with a birth weight of 1995 g (1^{st} percentile) after labor was induced because of intrauterine growth retardation. He is the third child of a non-consanguineous couple with an unremarkable medical history. Routine clinical examination at two months of age revealed icteric skin and sclera and failure to thrive (10 g per day) with normal stool color. Blood tests showed acute hepatic failure with severe coagulopathy: prothrombin rate, 11%; cofactors II and X < 0.05 IU/L (normal range (NR), > 0.7 IU/L); Factor V, 66% and unresponsive to vitamin K; elevated transaminase levels (AST, 122 IU/L (NR, 5–87 IU/L); ALT, 74 IU/L (IU/L 5–60)); hyperbilirubinemia (total bilirubin, 82 µmol/L (NR, 2–17 µmol/L), direct bilirubin, 62 µmol/L (NR, 2–5 µmol/L)); hypoalbuminemia (serum albumin, 34 g/L (NR, 38–54 g/L)) and hypochromic microcytic anemia (hemoglobin, 8.8 g/dl; unresponsive to iron therapy). The patient was hospitalized and had lactic acidosis (pH, 7.25; lactates, 2.8 mmol/L) on admission. The plasma ammonia level was normal. Abdominal ultrasound findings were normal and there was no sign of hepatomegaly.

Neurological clinical examination was normal. Metabolic disorders were ruled out and all tests were normal except urine amino acid chromatography, which showed elevated levels of all amino acids, suggesting a tubulopathy, and urine organic acid analysis, which showed isolated elevation of 3-hydroxyisovaleric acid. Viral hepatitis was excluded by serological testing. Further evaluations excluding other diagnoses are listed in Table S1.

Sequencing of genes associated with cholestasis (MiSeq, Illumina) revealed two heterozygous variants in *LARS1* (NM_020117), respectively in exon 19 (c.1818dup, p.(Ala607Cysfs*8,) and exon 6 (c.463A>G, p.(Lys155Glu). The patient's parents were asymptomatic heterozygous carriers (c.463A>G, p.Lys155Glu in father and c.1818dup, p.Ala607Cysfs*8 in mother). The missense variant is predicted as pathogenic by Mutation Taster (www.mutationtaster.org) and not reported in gnomAD. The other is a nonsense variant not reported in gnomAD Western blot analysis using LARS1 *(*D7Q4Q*) Rabbit mAb 35509 Cell*

Signaling Technology showed decreased LARS1 signal intensity in parents and in proband versus control (29.1% of signal in proband, 48.5% in mother and 97.3% in father) S3.

At 6 months of age, growth was improving (weight, −1 SD). The patient had moderate axial hypotonia between 3 and 5 months of age but his head circumference and neurodevelopment were subsequently normal with no seizure or encephalopathy and normal muscle tone. Concentrations of bilirubin, albumin and coagulation factors normalized, and transaminase levels decreased(< 2×NR). He still had mild tubulopathy and anemia, but transfusion had not been considered necessary. Hepatomegaly had developed. Treatment consisted of vitamin (A, C, D, E, K), ursodesoxycholic acid, sodium bicarbonate, MCT oil and iron supplementation.

Patient 2

The second patient, a girl, was born prematurely at 34^{+5} weeks of gestation to a non-consanguineous couple from Mayotte with an unremarkable medical history. There was no intrauterine growth retardation. In the first months of life, the patient had malnutrition due to feeding difficulties, recurrent vomiting and failure to thrive, leading to the placement of a gastrostomy tube for enteral nutrition.

She showed neurodevelopmental delay at two months of age (poor head control).

MRI showed hypoplasia of the corpus callosum and bilateral subependymal pseudocysts.

At 6 weeks of age, the patient developed respiratory distress with tachypnea (80 /min), cough, chest retraction, crackles, and hypoxia requiring oxygen supplementation for over a month. Chest CT showed interstitial lung disease. Pulmonary alveolar proteinosis was considered the most likely diagnosis given the patient's ethnic origin, CT images (extensive ground-glass opacities, and peribronchovascular thickening with a crazy paving pattern), and respiratory condition.

Blood tests showed hypoalbuminemia, thrombocytosis and microcytic hypochromic nonregenerative anemia, which required three blood transfusions then resolved with iron supplementation.

Liver enzymes suggested transitory cholestasis (maximum GGT, 182 IU/L (NR, 0–40)) and abdominal ultrasonography showed hepatomegaly with steatosis and persistent hydronephrosis. She had sinus tachycardia but echocardiography and Holter results were normal.

At 2 months of age, the patient had CMV infection (PCR positive, up to 72000 copies/ml), treated with valganciclovir, having tested negative at birth.

Sequencing of genes implicated in interstitial lung disease (MiSeq, Illumina) revealed a homozygous missense mutation c.1700C>T, p.(Ser567Leu) and a heterozygous variant c.1177G>A, (p.Ala393Thr) of the *MARS1* gene, both of which are associated with pulmonary alveolar proteinosis and have already been described as pathogenic by Hadchouel et al. (6). Bronchoalveolar lavage confirmed the diagnosis of PAP. The patient is currently 10 months old, her respiratory symptoms have improved and she no longer needs oxygen supplementation. She still has feeding and eating disorders, with enteral nutrition through a gastrostomy tube and moderate undernutrition. Her neurological development is normal.

Patient 3

The third patient is a boy referred to the hospital at 2 months of age for failure to thrive, hepatomegaly with hepatic cytolysis and respiratory distress. He was born at term with a normal birth weight and is the first child of a non-consanguineous couple; his father is of Comorian descent and his mother, Malagasy.

He had tachypnea without need for oxygen supplementation. CT showed alveolar-interstitial syndrome in the lower lobes and bronchoalveolar lavage findings were consistent with pulmonary alveolar proteinosis. Digital clubbing developed over the following years.

He had slight splenomegaly and significant hepatomegaly with cytolysis and intermittent cholestasis without jaundice. Liver biopsy showed severe macrovesicular steatosis.

He had several episodes of cholecystitis without gallstones and major feeding difficulties with recurrent vomiting, abdominal flatulence and no weight gain leading to parenteral nutrition then enteral nutrition through a gastrostomy tube.

Blood tests showed normocytic normochromic nonregenerative anemia, hypoalbuminemia and hypergammaglobulinemia. Bone marrow biopsy showed erythroblastopenia and granulocytic lineage maturation disorder. The patient also had fasting intolerance and recurrent hypoglycemia and required continuous enteral feeding. All endocrine and metabolic tests were normal however.

A primary CMV infection resolved without antiviral treatment and the patient also had several bacterial infections with septicemia. Between 1 and 6 months of age, he developed severe eczema that disappeared with topical steroid treatment. He also had moderate psychomotor delay in infancy (he could not stand alone at 14 months of age) that resolved over time. The patient is currently 12 years old, with steady growth (height -1.5 SD, weight between the 3rd and 10th percentile) and has enteral nutrition through a gastrostomy tube at night. His neurological status is normal and he has no learning delay.

As described by Hadchouel et al. (6) sequencing of the *MARS1* gene in this patient revealed two homozygous missense variants (c.1177G>A, (p.Ala393Thr) and c.1700C>T).

RESULTS

Our review of the literature identified 14 articles, 7 on *LARS1* mutations and 7 on *MARS1* mutations, such that including our 3 patients, 65 patients with *LARS1* or *MARS1* mutations (respectively 27 and 38) have so far been described in the literature. (1-14)

Twenty-one of these patients (32.3%) patients died: 6/27 of the patients with *LARS1* mutations (4 boys and 2 girls) and 15/38 of the patients with *MARS1* mutations (11 boys and 4 girls). One of the patients with a *LARS1* mutation underwent liver transplantation. Mean age of death was 4.5 year (range 51 days- 25.2 years).

Using HPO terms, the mean number of phenotypic abnormalities identified per article was 26.1 for *LARS1* mutations and 20.4 for *MARS1* mutations (range, 11–45). The number of phenotypic abnormalities described only exceeded 30 in articles describing more than one patient. In single case reports, the mean number of phenotypic abnormalities described was 18 for *LARS1* and 20.5 for *MARS1* mutations. We described 23 clinical phenotypic abnormalities for our patient with *LARS1* mutation, and 24 phenotypic abnormalities for each of our patients with *MARS1* mutations.

In total, 117 HPO phenotypic abnormalities have been described at least once in patients with *MARS1* or *LARS1* mutations (Table S2), among which 41 (35%) have only been described in patients with *LARS1* mutations, 27 (23.1%) only in patients with *MARS1* mutations, and the remaining 49 (41.9%) for both *MARS1* and *LARS1* mutations.

Table 1 lists all the phenotypic abnormalities associated with both *LARS1* and *MARS1* mutations and the most frequent phenotypic abnormalities that differ between the two populations. Among the phenotypic abnormalities described for both *LARS1* and *MARS1* mutations, 4/49 were observed in more than 50% of cases, 8/49 in 30–50% of cases, 17/49 were observed in 10–30% of cases, and 20/49 in less than 10% of cases.

Fig. 1 shows a Venn diagram of the different phenotypic abnormalities described in the literature for *MARS1* and *LARS1* mutations. The proportion of phenotypic abnormalities shared between these two groups of patients was 34% and 39% after including our three patients. Only 9 (24.3%) of the phenotypic abnormalities

described in the first two single case reports of patients with *MARS1* and *LARS1* mutations were common to both, whereas now 44 (37.9%) of the phenotypic abnormalities described in the literature have been reported for mutations in both genes. Our patient with LARS mutations had 7 phenotypic abnormalities in common with the first of our patients with *MARS1* mutations, and 5 with the second, among which 4 were observed in all three patients. Interestingly, our two patients with *MARS1* mutations only had 9 phenotypic abnormalities (23%) in common.

Classifying the abnormalities in terms of the affected organ (Fig. 1e) shows that while there is no overlap between phenotypes in the two groups for the kidney and lungs, for other organs (e.g. the liver and gastrointestinal tract) most of the phenotypic abnormalities overlap.

DISCUSSION

Aminoacyl-tRNA synthetase deficiencies are very complex and polymorphic pathologies. This work is an attempt to clarify the phenotypes of *LARS1* and *MARS1* mutations using HPO terms (15), which are a means to describe and compare disease phenotypes more precisely.

Among the three patients treated in our hospital whose clinical phenotypes we describe, one had compound heterozygous *LARS1* variants while the two others had homozygous mutations in *MARS1*. Decompensation (hepatic, neurological or pulmonary) triggered by an infection, mainly in infancy, is described for most of the cases in the literature and was observed here in all three of our patients. Both our patients with *MARS1* mutation had CMV infection in the first months of life. This had not been described until a recent report of a boy with *MARS*1 mutation who presented CMV colitis at 4 months of age. (12) Regarding phenotypes, all three of our patients have 12–15 HPO terms specific to their condition, and 5–9 terms in common with the other two. In these three patients, *LARS1* and *MARS1* phenotypes largely overlap.

This is generally the case for *LARS1* and *MARS1* mutations. Indeed, blood, metabolism, gastrointestinal and growth abnormalities are described in more than half of patients with these mutations and are frequent in both phenotypes. However, certain characteristics seem to be more specific to one or other of the phenotypes. For instance, nervous system and muscle abnormalities (global or motor developmental delay, hypotonia, encephalopathy, MRI findings) are described in less than 10% of patients with *MARS1* mutations but in more than half of those with *LARS1* mutations. Conversely, *LARS1* mutations are rarely associated with respiratory abnormalities but these are a characteristic feature of *MARS1* mutations. For the kidney and lung finally, none of the phenotypic abnormalities overlap.

The number of common phenotypic abnormalities has increased with the number of cases and phenotypes described in the literature. One would indeed expect the number of specific abnormalities to either decrease as more cases are described, or stay constant as they are confirmed as characteristic of a specific mutation.

The relative frequency of shared phenotypic abnormalities may also differ in the two groups of patients. Respiratory abnormalities are indeed far more frequent in patients with *MARS1* mutations and precise

diagnoses as alveolar proteinosis or interstitial pulmonary disease are well described. However, most recent reports of *LARS1* mutations (1,8,14) also describe associated respiratory abnormalities.

It therefore seems as if no condition is specifically associated with mutations in either of the two genes. Many phenotypic abnormalities have been described in patients with either *LARS1* or *MARS1* mutations but based on the deep phenotyping of our patients, it appears that all these abnormalities may in fact be seen in both populations.

These complex and rare diseases having only recently been described, their phenotypic description still needs to be completed. The description of our three cases increases the number of phenotypic abnormalities associated with both *LARS1* and *MARS1* mutations. The number of phenotypic abnormalities described and the proportion of common phenotypic abnormalities have both increased (from 37 to 117 and from a quarter to more than a third, respectively) since the publication of the first case reports of *LARS1* and *MARS1* mutations. This suggests that *LARS1* and *MARS1* mutations have a mainly common phenotypic expression. Continued description of these patients is important for a more precise understanding of these phenotypes, one limitation being that in most cases, only identified abnormalities are reported while negative tests for other abnormalities are rarely mentioned.

Aminoacyl transfer RNA synthetases attach amino acids to tRNA. Leucine and methionine have different sidechains (aliphatic for leucine and sulfur-containing for methionine) but both are hydrophobic and uncharged at neutral pH. This raises the question whether phenotyping studies of other recessive aminoacyl transfer RNA synthetase diseases would return similar results to ours or if some phenotypes stand apart.

CONCLUSION

Precise descriptions of the clinical phenotypes of *LARS1* and *MARS1* mutations patients are in our view essential. A few of the phenotypic abnormalities described here have only been reported in one or two patients and while they may therefore not be actually part of the phenotype of these mutations, they may also be found in future patients.

In complex diseases such as those due to *LARS1* and *MARS1* mutations, the more phenotypic abnormalities are described, the more the proportion found to be common to the two pathologies increases. *LARS1* and *MARS1* phenotypes seem to fit on a continuum, but with certain specificities.

Since the first descriptions of patients with *MARS1* and *LARS1* mutations appear to have been only partial, further studies and case reports are needed to clarify the common and specific features of aminoacyl-tRNA synthetase deficiency phenotypes.

ACKNOWLEDGEMENTS:

This article was edited by Paul Guerry (Green Grow Scientific).

Conflicts of interest: none

BILBIOGRAPHY

1. Fuchs SA, Schene IF, Kok G, Jansen JM, Nikkels PGJ, van Gassen KLI, et al. Aminoacyl-tRNA synthetase deficiencies in search of common themes. Genet Med. 2019;21(2):319-30.

2. van Meel E, Wegner DJ, Cliften P, Willing MC, White FV, Kornfeld S, et al. Rare recessive loss-of-function methionyl-tRNA synthetase mutations presenting as a multi-organ phenotype. BMC Med Genet. 2013;14:106.

3. Sun Y, Hu G, Luo J, Fang D, Yu Y, Wang X, et al. Mutations in methionyl-tRNA synthetase gene in a Chinese family with interstitial lung and liver disease, postnatal growth failure and anemia. J Hum Genet. 2017;62(6):647-51.

4. Casey JP, McGettigan P, Lynam-Lennon N, McDermott M, Regan R, Conroy J, et al. Identification of a mutation in LARS as a novel cause of infantile hepatopathy. Mol Genet Metab. 2012;106(3):351-8.

5. Casey JP, Slattery S, Cotter M, Monavari AA, Knerr I, Hughes J, 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(6):1085-92.

6. Hadchouel A, Wieland T, Griese M, Baruffini E, Lorenz-Depiereux B, Enaud L, et al. Biallelic Mutations of Methionyl-tRNA Synthetase Cause a Specific Type of Pulmonary Alveolar Proteinosis Prevalent on Réunion Island. Am J Hum Genet. 2015;96(5):826-31.

7. Lin W-X, Zheng Q-Q, Guo L, Cheng Y, Song Y-Z. [Clinical feature and molecular diagnostic analysis of the first non-caucasian child with infantile liver failure syndrome type 1]. Zhongguo Dang Dai Er Ke Za Zhi. 2017;19(8):913-20.

8. Lenz D, Smith DEC, Crushell E, Husain RA, Salomons GS, Alhaddad B, et al. Genotypic diversity and phenotypic spectrum of infantile liver failure syndrome type 1 due to variants in LARS1. Genet Med. 2020;22(11):1863-73.

9. Rips J, Meyer-Schuman R, Breuer O, Tsabari R, Shaag A, Revel-Vilk S, 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(10):616-20.

10. Alzaid M, Alshamrani A, Al Harbi AS, Alenzi A, Mohamed S. Methionyl-tRNA synthetase novel mutation causes pulmonary alveolar proteinosis. Saudi Med J. 2019;40(2):195-8.

11. Abuduxikuer K, Feng J-Y, Lu Y, Xie X-B, Chen L, Wang J-S. Novel methionyl-tRNA synthetase gene variants/phenotypes in interstitial lung and liver disease: A case report and review of literature. World J Gastroenterol. 2018;24(36):4208-16.

12. Lenz D, Stahl M, Seidl E, Schöndorf D, Brennenstuhl H, Gesenhues F, et al. Rescue of respiratory failure in pulmonary alveolar proteinosis due to pathogenic MARS1 variants. Pediatr Pulmonol. 2020;55(11):3057-3066.

13. Peroutka C, Salas J, Britton J, Bishop J, Kratz L, Gilmore MM, et al. Severe Neonatal Manifestations of Infantile Liver Failure Syndrome Type 1 Caused by Cytosolic Leucine-tRNA Synthetase Deficiency. JIMD Rep. 2018;45:71-6.

14. Hirata K, Okamoto N, Ichikawa C, et al. Severe course with letal hepatocellular injury and skeletal muscular dysgenesis in a neonate with infantile liver failure syndrome type 1 caused by novel LARS1 mutations. *Am J Med Genet Part A.* 2020;1-5

15. Köhler S, Vasilevsky NA, Engelstad M, Foster E, McMurry J, Aymé S, et al. The Human Phenotype Ontology in 2017. Nucleic Acids Res. 2017;45(Database issue):D865-76.

16. Enaud L, Hadchouel A, Coulomb A, Berteloot L, Lacaille F, Boccon-Gibod L, et al. Pulmonary alveolar proteinosis in children on La Réunion Island: a new inherited disorder? Orphanet J Rare Dis. 14 juin 2014;9:85.

HPO	Patient LARS	Literature LARS (N/26)	Patients MARS (N/2)	Literature mars (N/37)	
HP:0003073	1	24	2	7	Hypoalbuminemia
HP:0002240	1	7	2	31	Hepatomegaly
HP:0002910	1	23	1	22	Elevated hepatic transaminases
HP:0001508	1	22	2	30	Failure to thrive
HP:0004840	1	18	1		Hypochromic microcytic anemia
HP:0001622		16	1	3	Premature birth
HP:0001396	1	1	2	24	Cholestasis
HP:0001392	1	14		6	Abnormality of the liver
HP:0012758		21	2	5	Neurodevelopmental delay
HP:0001270		16	1	4	Motor delay
HP:0001511	1	21		1	Intrauterine growth retardation
HP:0001518		13		17	Small for gestational age
HP:0001935		17		2	Microcytic anemia
HP:0001894	1	2	1	4	Thrombocytosis
HP:0004897	1			2	stress induced lactic acidosis
HP:0030363		2	2	3	Primary cesarian section
HP:0006554	1	16		1	Acute hepatic failure
HP:0004396		10	1		Poor appetite
HP:0001403		7	1	7	Macrovascular hepatic steatosis
HP:0001394		5		11	Cirrhosis
HP:0011968		3	2	4	feeding difficulties
HP:0002572		5	2	2	Episodic vomiting
HP:0002719		7	1		Recurrent infection
HP:0001252		18		1	Muscular hypotonia
HP:0006846		14		1	Encephalopathy
HP:0012443		9	1	1	abnormality of brain morphology
HP:0001217		1	1	10	Clubbing
HP:0001530	1	6		2	Mild postnatal growth retardation
HP:0008897		6	1	3	Postnatal growth retardation
HP:0001891	1	1	1		Iron deficiency anemia
HP:0100807		1		1	Long fingers
HP:0000023	1			1	Inguinal hernia
HP:0001987		1		1	Hyperammonemia
HP:0002909	1			1	Generalized aminoaciduria
HP:0002908	1	2		2	Conjugated hyperbilirubinemia
HP:0001988		2		1	Recurrent hypoglycemia
HP:0001998		2		1	Neonatal hypoglycemia
HP:0100602		1	1		Preeclampsia
HP:0008071		1	1		Maternal hypertension
HP:0001395		2		4	Hepatic fibrosis
HP:0011471		2	2	1	Gastrostomy tube feeding in infancy
HP:0001744		2	1	2	Splenomegaly
HP:0001627		2		1	Abnormal heart morphology
HP:0000832		1		3	Primary hypothyroidism
HP:0003162		1	1		Fasting hypoglycemia
HP:0009062	1	2		2	Infantile axial hypotonia
HP:0006849		3	1		Hypodysplasia of the corpus callosum
HP:0000252		3		2	Microcephaly
HP:0006538		1		2	Recurrent bronchopulmonary infections

Table 1a: Phenotypic abnormalities associated with both *LARS* and *MARS* mutations

Patient LARS	Literature LARS	Patients MARS /2	Literature MARS	
			HP :0002094	Dyspnea
		HP:0006530	HP:0006530	Interstitial pulmonary disease
			HP :0012735	Cough
			HP :0002206	Pulmonary fibrosis
			HP :0012418	Hypoxemia
	HP:0012444			Brain atrophy
	HP:0011342			Mild global development delay
	HP:0001250			Seizures

	<10%
	10-30%
	30-50%
	>50%

Table 1b: most frequent phenotypic abnormalities specifically associated with either *MARS* or *LARS* mutations

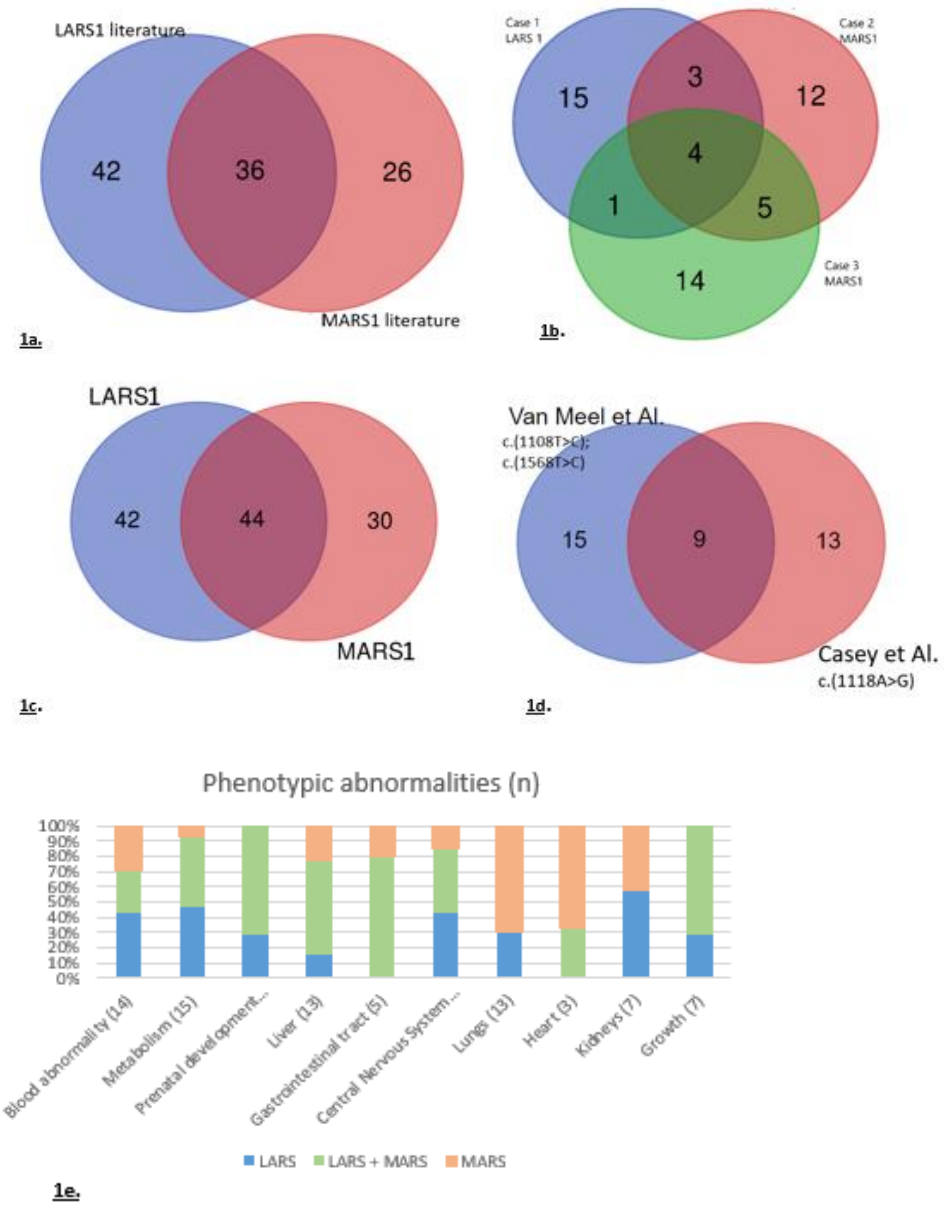

Figure 1: 1a–c. Venn diagrams. 1a. Phenotypic abnormalities described in the literature for patients with *LARS1* or *MARS1* mutations. **1b.** Phenotypic abnormalities of our three patients. **1c**. Phenotypic abnormalities described for *LARS1* and *MARS1* mutations either in the literature or in our 3 patients (+8 abnormalities in common vs considering the literature only). **1d.** Phenotypic abnormalities described in the first case reports of *MARS1* (Van Meel et Al.2013) and *LARS1* mutations (Casey et Al.2012). **1e**. Bar chart showing the proportions of phenotypic abnormalities associated with *LARS1* mutations only, *MARS1* mutations only, or common to both, classified in terms of the affected organ, tissue or system.