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Novel partial loss-of-function variants in the tyrosyl-tRNA synthetase 1 (*YARS1*) gene involved in multisystem disease

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Abstract

Cytoplasmic aminoacyl-tRNA synthetases (ARSs) are emerging as a cause of numerous rare inherited diseases. Recently, biallelic variants in *tyrosyl-tRNA synthetase 1 (YARS1)* have been described in ten patients of three families with multi-systemic disease (failure to thrive, developmental delay, liver dysfunction, and lung cysts). Here, we report an additional subject with overlapping clinical findings, heterozygous for two novel variants in *tyrosyl-tRNA synthetase 1* (NM_003680.3(*YARS1*):c.176T>C;p.(Ile59Thr) and NM_003680.3(*YARS1*):c.237C>G;p.(Tyr79*) identified by whole exome sequencing. The p.Ile59Thr variant is located in the highly conserved aminoacylation domain of the protein. Compared to subjects previously described, this patient presents a much more severe condition.

Our findings support implication of two novel *YARS1* variants in these disorders. Furthermore, we provide evidence for a reduced protein abundance in cells of the patient, in favor of a partial loss-of-function mechanism.

Key words:

YARS1, exome sequencing, aminoacylation, hepatic cirrhosis, intestinal lung disease, developmental delay.

Introduction

Aminoacyl-tRNA synthetase (ARS) family proteins are enzymes that are ubiquitously expressed and highly evolutionarily conserved that catalyze the aminoacylation of tRNAs by their cognate amino acid. They link amino acids with the correct nucleotide triplets and ensure the correct translation of the protein code for protein synthesis in the cytoplasm and mitochondria. Each amino acid has a specific ARS enzyme. The human genome encodes 17 cytoplasmic ARSs, 17 mitochondrial ARSs enzymes and 3 bifunctional enzymes charging tRNAs in both cytoplasm and mitochondria¹. Many human tRNA synthetases display alternative functions outside of protein synthesis. Recent studies have shown that some

ARSs have essential non-translational functions². For example, WARS1 and YARS1 play major roles in regulating angiogenesis³⁻⁶, HARS1 is involved in the inflammatory response⁷ and GARS1 acts in tumorigenesis defense⁸.

As suggested by their essential biochemical functions, ARSs are emerging as a cause of numerous rare inherited diseases: Pathogenic variants in most of the cytoplasmic aminoacyl-tRNA synthetases have previously been reported as causes of dominant peripheral neuropathy (like Charcot-Marie-Tooth disease) or mitochondrial disorders, such as variants in Mitochondrial ARS. A total of 31 out of the 37 human ARSs have been linked to genetic diseases. Since 2012, more and more ARSs have been implicated in multi-organ phenotypes: *LARS1* (MIM 151350; phenotype MIM number 615438)⁹, *MARS1* (MIM 156560; phenotype MIM 615486)^{10,11} and *IARS1* (MIM 600709; phenotype MIM 617093)¹²⁻¹⁴. Notably, heterozygous variants in the *tyrosyl-tRNA synthetase 1* (*YARS1*; MIM 603623) are well known to cause Charcot-Marie-Tooth Disease, Dominant Intermediate C; (CMTDIC) (phenotype MIM 608323). *YARS1* variants were involved in a multi-systemic disease in two siblings of Polish origin, with compound heterozygous *YARS1* variants associated with defects in liver, muscle and brain¹⁵. A study by Tracewska-Siemiatkowska et al. also reported a novel YARS phenotype associated with rapid retinal degeneration, hearing impairment, agenesis of corpus callosum, primary amenorrhea and liver disease in a patient of Swedish origin with a homozygous missense variant in *YARS1*¹⁶. More recently, Williams et al. described homozygosity for a variant in the catalytic domain in seven related children with multisystem disease, including sensorineural hearing loss, nystagmus, developmental delay, growth failure, chronic anemia, hypoglycemia, progressive cholestatic liver disease and renal dysfunction¹⁷.

In this study, we report a patient with a severe multi-organ phenotype, including failure to thrive (FTT), liver disease, chronic diarrhea, developmental delay, and pulmonary fibrosis.

We identify compound heterozygous variants within the *YARS1* gene using whole exome sequencing approach and extend the spectrum of pathogenic variants in the *YARS1* gene.

Materials & Method

Patient Recruitment

We obtained free and informed written consent from parents under a protocol approved by our institutional review board.

Array-CGH

The genomic screening of chromosomal imbalanced rearrangements by array-CGH was performed using the Agilent 4x180K chromosomal microarray platform (Agilent Technologies, Santa Clara, CA). Microarray slides were scanned with a DNA Microarray Scanner (Agilent Technologies, Santa Clara, CA). Data were obtained using the Agilent Feature Extraction software 10.7.3.1 and Agilent DNA Analytics software 4.0.85. Genomic positions were estimated using the human genomic reference sequence GRCh37/hg19.

Whole Exome Sequencing (WES)

Genomic DNA was extracted from peripheral blood samples from the proband, her sister and both parents via standard procedures using the Gentra Puregene blood kit (Gentra

Systems, Inc., Minneapolis, MN) and the Autopure LS instrument. The Biobank CRB Assistance Publique des Hôpitaux de Marseille (CRB TAC (CRB AP-HM TAC), Marseille, France [BIORESOURCES]) proceeded with the DNA extraction and ensured the long-term storage of samples.

The whole-exome capture and sequencing were performed with the AROS Applied Biotechnology platform (AROS Applied Biotechnology A/S, Aarhus N, Denmark) from 1.5 µg of genomic DNA per individual using XT Capture Exome V5 (Agilent). The resulting libraries were sequenced on HiSeq 2000/2500 platforms (Illumina) according to the manufacturer's recommendations for paired-end 100-bp reads. Reads were aligned to the human genome reference sequence (GRCh37/hg19 build of UCSC Genome Browser), and potential duplicate paired-end reads were marked with Picard v.1.109. The Genome Analysis Toolkit (GATK) v.3.3-0 was used for base quality score recalibration, indel realignment, and variant discovery (both single-nucleotide variants and indels). Read mapping and variant calling were used for data analysis and interpretation.

The variants were filtered with the in-house Variant Analysis and Filtration Tool software (VarAFT; varaft.eu). Variant filtering adhered to the following criteria: i) variants affecting the coding sequence, including splicing and ii) rare variants (<1% allele frequency) from public databases (see URLs), iii) homozygous, compound heterozygous or *de novo* variants. The candidate variants in *YARS1* were confirmed by Sanger sequencing.

Sanger direct sequencing confirmation of *YARS1* variants

Genomic DNA was extracted from blood samples via a standard manufacturer's protocol (QIAamp DNA Blood Mini Kit; Qiagen, Chatsworth, CA). Sanger sequencing was performed

to confirm both the presence of the two candidate variants in *YARS1* gene in our patient and the heterozygous status of the unaffected parents. Direct Sanger sequencing was performed on an ABI 3130XL (Applied Biosystems, Foster City, CA) after PCR amplification by primers targeting exons 2 and 3 containing the variants found in our patients by Exome Sequencing (Forward primer: 5'-tgctgtgttttccttgca-3'; reverse primer: 5'-cacgccagacagcttcta-3'). The *YARS1* genomic sequence from GenBank accession number NM_003680.3 was used as the reference sequence.

Western blotting

Fibroblasts of patient (and unrelated healthy control) and the entire family lymphoblastoid cells were lysed by NP40 cell lysis buffer (Thermo Scientific, Rockland, IL, USA) complemented with Halt Proteases and Phosphatases Inhibitor Cocktail 100X (Thermo Scientific, Rockland, IL, USA). After incubation on ice for 30 min, lysed cells were sonicated (4 cycles 15 s on/15 s off) and then centrifuged at 16,000 x g for 10 min at 4°C. The supernatant was collected and analyzed for protein concentration by Pierce BCA Protein Assay (Thermo Scientific, Rockland, IL, USA). Whole lysates of fibroblasts and lymphoid cells (40 µg per lane) were loaded onto SDS-PAGE 10% Bis-Tris gels (Bio-Rad, Hercules, CA, USA) for electrophoresis and transferred to PVDF membranes. The membranes were hybridized with rabbit monoclonal anti-Human YARS1 antibody (cat # ab154819, dilution 1/1000, Abcam) and with mouse anti-human glyceraldehyde-3-phosphate dehydrogenase monoclonal antibody as a loading control (MAB374, dilution 1/10,000, Millipore Merck, Darmstadt, Germany). Quantification of protein levels was made with Fiji software.

Results

Clinical report

The proband was the second daughter of non-consanguineous parents. The pregnancy was complicated by intrauterine growth retardation (IUGR) and oligoamnios. She was born by emergency caesarean section delivery for chorioamnionitis suspicion, anomaly of the fetal heart rate, decrease in fetal movements and premature rupture of membranes at 29 Weeks of Amenorrhea (WA). The proband's birth weight was 785 grams in an IUGR context (z-score:-1.95). The Apgar scores were 9 and 10. At 8 days of life, she presented with infant respiratory distress syndrome (IRDS), which was treated over 10 days by continuous positive airway pressure (CPAP). She was admitted to the Neonatal Intensive Care Unit for low birth

weight at 15 days of life. During her admission, at 23 days of life, she had an ulcerative-necrotic enterocolitis episode requiring conservative treatment. She required nutritional support (enteral and parenteral) over 70 days. Complete blood count (CBC) showed microcytic anemia, and the proband needed three blood transfusions. At 2 months of age, ultrasound imaging showed a mass on her suprarenal gland (7 mm), which disappeared 5 months later. Tumoral markers (carcinoembryonic antigen (CEA), alpha fetoprotein, beta human chorionic gonadotropin (beta-hCG)) were negative. She developed cholestasis, increased alkaline phosphatase and gamma-glutamyl transferase (GGT) (two times higher than normal).

She was discharged from the hospital with oral nutrition (hydrolyzed milk) and without oxygen at 73 days of age and a weight of 2.130 kg (z-score:-7.02) The regular follow-up visits after her release from the hospital showed global hypotonia and persistent FTT (Fig. 2).

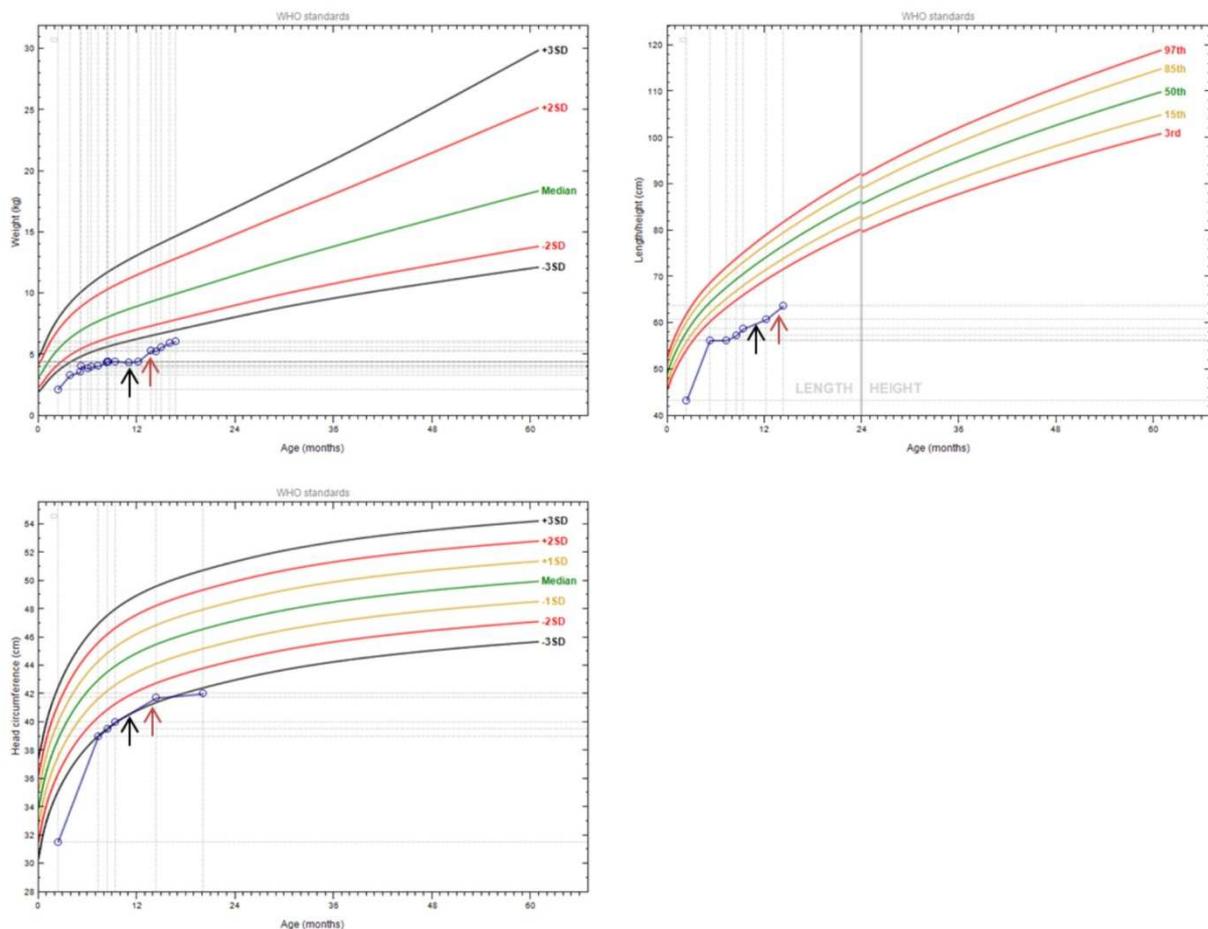


Figure 1: Growth curves of weight, length and head circumference of patient II.2 between 1 month and 22 months

At 11 months of life, she was admitted to the hospital for FTT (Fig. 2) and anemia. She had transient hypoglycemia and required 24-h/24-h enteral feeding. Regenerative anemia with dyserythropoietic signs and bone marrow hyperplasia was diagnosed (Fig. 2 (J)(K)). She received blood transfusions. She presented hepatosplenomegaly with weight stagnation (4.4 kg, z-score: -5.96) and chronic diarrhea. Liver biopsy at 13 months of age showed cholestasis, steatosis with severe steatohepatitis (Grade 6, fibrosis F4), and portal-tract fibrosis (Fig. 2 (D) (E)). Mitochondrial disease was suspected, and respiratory chain complexes were measured in muscle, fibroblasts, liver and muscle. Activities of complexes were normal. Moreover, the whole mtDNA (from liver biopsy) was sequenced. These analyzes did not show any pathogenic point variants or deletions. array-CGH showed only two microdeletions of unknown significance on 3p and 7q. The first variation was a deletion of 844kb of the region 3p26.1. This deleted region included exon 2 to 10 of the *GRM7* gene encoding a metabotropic glutamate receptor involved in neurogenesis¹⁸. Pathogenic missense and nonsense variants of this gene are associated with autosomal recessive Neurodevelopmental disorder with seizures, hypotonia and brain abnormalities (OMIM *604101). Because we didn't found a second variant in this gene, this disorder is unlikely the cause of the patient's phenotype. The second Copy Number Variation (CNV) involves a region of 140kb in 7q31.1 region which contains exons 1 to 3 of *IMMP2L*. Moreover loss of function structural variant of *GRM7* and *IMMP2L* are described in gnomAD in gnomAD SVs2 (3 times and 20 times respectively). .. In the present state of knowledge these two microdeletions are unlikely linked to the phenotypic traits observed in our patient.

Clinical and metabolic investigations were without specific findings in favor of mitochondrial disease. She was discharged from the hospital at 13 months of life.

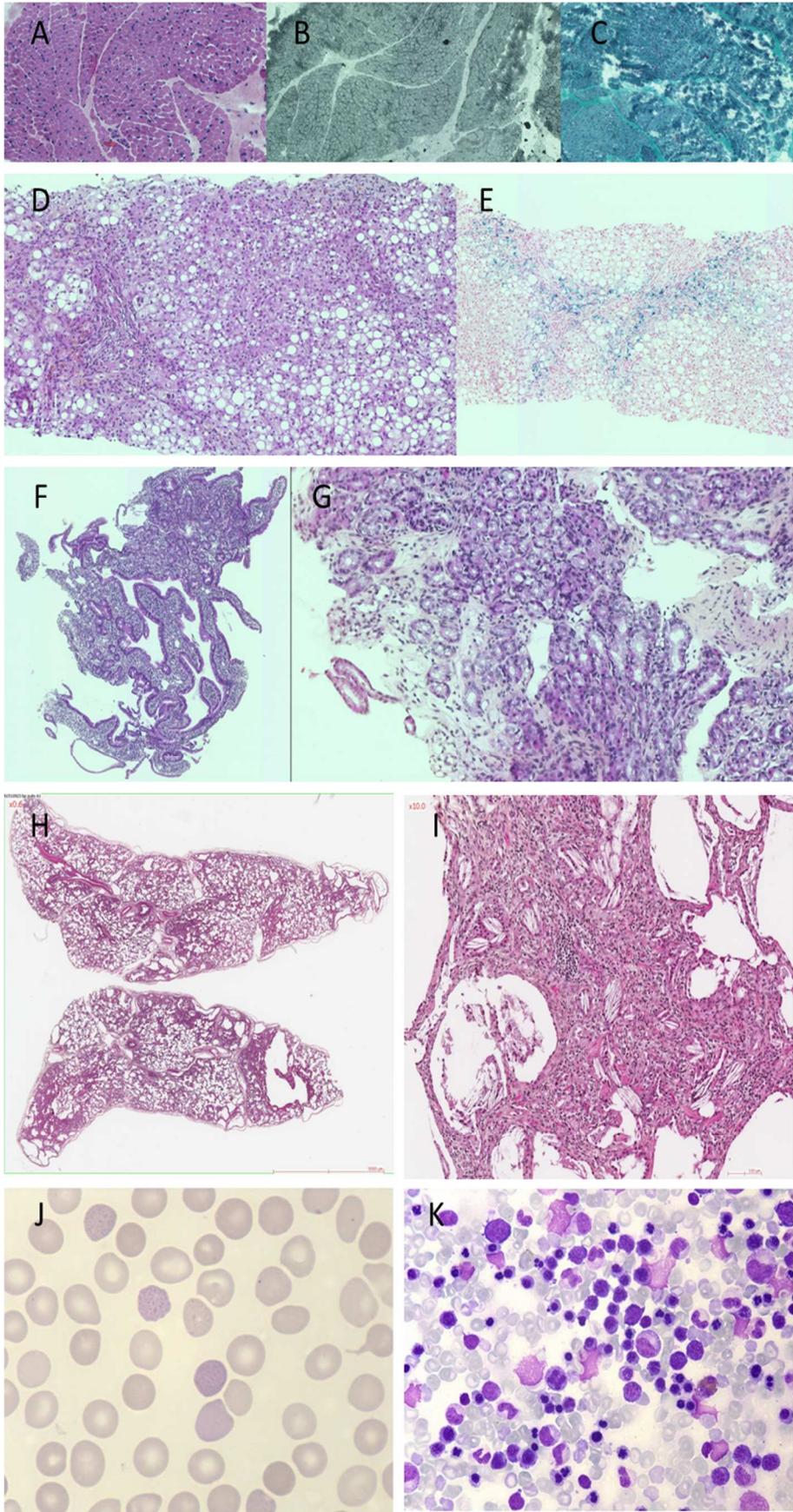


Figure 2: Histologic findings

She was admitted again to the hospital at 14 months of age in the Intensive Care Unit suffering from acute lung distress in the context of a urinary tract infection. Lung imaging (Fig. 3) and histology showed lung fibrosis (interstitial lung disease) (Fig. 2 (H) (I)). At the same time, the patient's liver phenotype became worse, with portal hypertension and liver failure. She had several perfusions of albumin. Total parenteral nutrition and tracheostomy ventilation were started at this time.

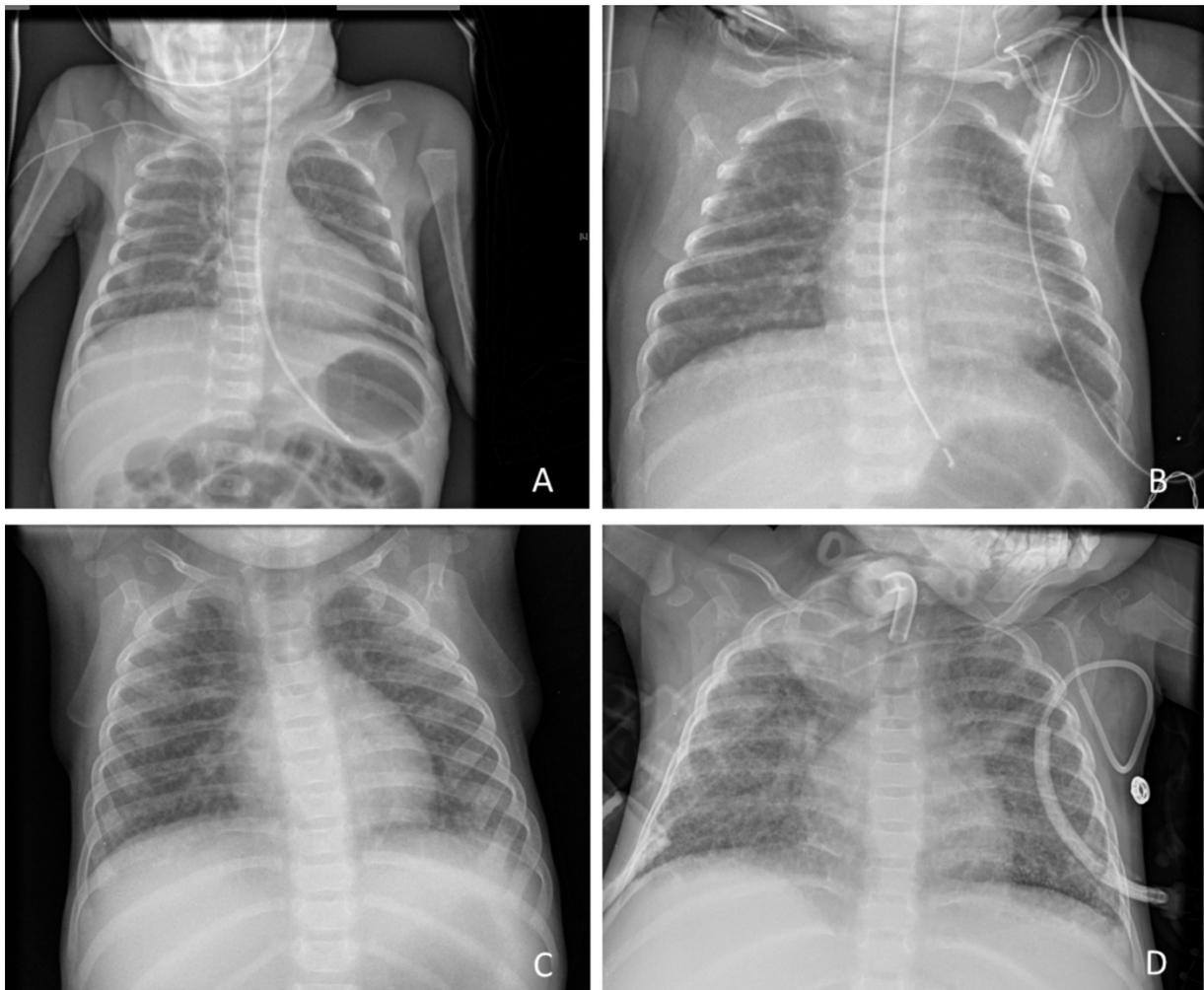


Figure 3: Chest X-rays

The patient presented global hypotonia (axial and peripheral hypotonia): she was not able to hold her head up and could not sit by herself at the age of 23 months. It was difficult to assess her psycho-motor development because of her prematurity and the severity of the disease.

The patient had no specific facial dysmorphic features: she had protruding ears with narrow rims and her mouth was slightly small, as were the extremities. She presented a congenital hemangioma on the left thigh. She presented CNS (Central Nervous System) defect with microcephaly with a head circumference of 24cm (z-score:-1.6207)(however, this measurement was consistent with her height and weight). Cerebral Magnetic Resonance Spectroscopy showed myoinositol increase within the white matter compatible with metabolic disorders, such as galactosemia or lysosomal diseases. On the morphological plane, MRI (Magnetic Resonance Imaging) at 15 months showed enlarged lateral ventricles and sub-arachnoid spaces reflecting brain volume loss. The brainstem showed hypomyelination.

Extensive genetic and metabolic workups were negative, leading to the realization of the need for exome sequencing (no pathogenic variants were found in *GALT*, *PMM2*, *MPV17*, *POLG*, *BCS1L*, *NCP1*, *NCP2*, and *ALDOB*, and extensive metabolic studies were negative).

After the identification of pathogenic variants in the *tyrosyl-tRNA synthetase 1* gene, the medical team decided to initiate tyrosine supplementation with no improvement (100 mg/kg/day and then 200 mg/kg/day). This treatment was started after the agreement of the Hospital Committee of Health Commodities Security (COSEPS). After 7 months in the unit, home hospitalization was set up with 24-h/24-h ventilation. She needed emergency admittance due to aggravation of her respiratory ailments. From an ethical standpoint, regarding the severity and the incurable character of the disease, it was decided to progressively restrict treatments, to administrate palliative care and to limit intensive therapies. The patient's death occurred at the age of 23 months due to multiple organ failure.

Whole exome sequencing identified variants in *YARS1*

The sporadic case was found in a cohort of patients with liver disease without genetic diagnosis. We performed whole exome sequencing on genomic DNA from the patient and her parents. The data were analyzed following a trio strategy under an autosomal recessive hypothesis. A search for homozygous and compound heterozygous rare variants (<0.1% in ExAC Browser and gnomAD) allowed us to identify two variants in the *YARS1* gene. The two identified *YARS1* variants were confirmed by Sanger sequencing, and their segregation was consistent with an autosomal recessive trait. The variants were present in the healthy parents and absent in the healthy sister (Fig. 4). The missense NM_003680.3(*YARS1*):c.176T>C;p.(Ile59Thr) (ClinVar accession number VCV000576327.4) and the nonsense NM_003680.3(*YARS1*):c.237C>G;p.(Tyr79*) (ClinVar accession number VCV000635008.1) variants. Both are predicted to be damaging by UMD Predictor software¹⁹ and “probably

damaging” and “deleterious” in Polyphen and SIFT software programs, respectively. They are rare in the Genome Aggregate Database (gnomAD; <http://gnomad.broadinstitute.org>) (5/277196) or never seen and have only been reported in the heterozygous state. Further conservation analysis showed that the missense variant affects the amino acid residue at position 59, which is conserved among evolutionarily diverse eukaryotic species (Fig. 4). The physico-chemical properties of isoleucine (non-polar) and threonine (polar) differ significantly. Our exome findings and the high predicted expression levels of *YARS1* in the lung, liver, brain, blood and the spleen, which were the affected organs in our patient (Proteomics DB, PaxDb, MaxQB and MOPED), rendered this locus a strong candidate.

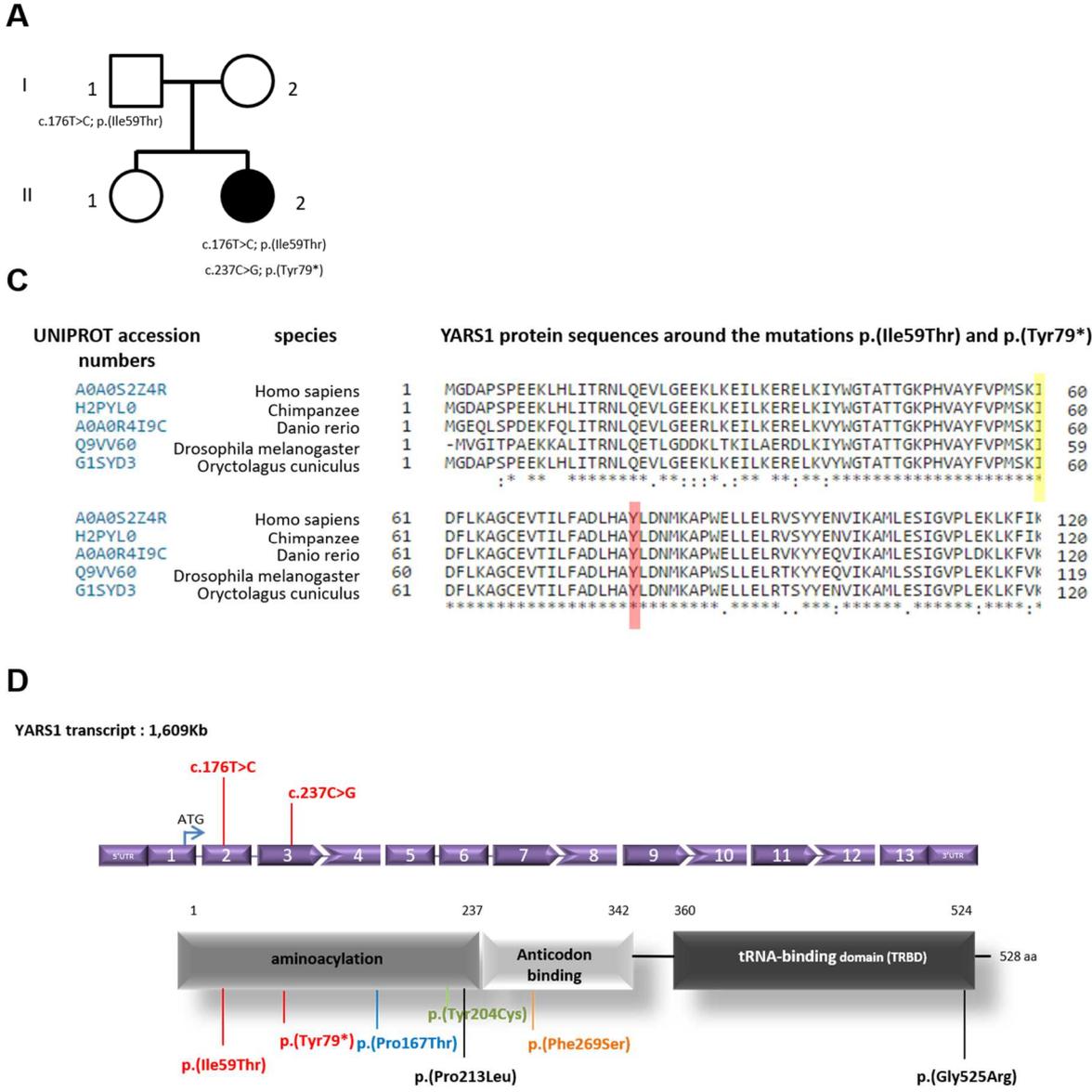


Figure 4: *YARS1* variants and gene structure

Recently, this locus was associated with human genetic disorders that partially overlap^{15,16} the phenotype of our patient. Taken together, all of these data are in favor of the pathogenicity of these variants.

Functional impact of variants found in *YARS1* at the protein level

To investigate whether these alleles have an effect on protein abundance, we performed Western blot using a monoclonal antibody against the *YARS1* protein in lymphoblastoid cell lines of all members. We detected *YARS1* at the predicted size of 59 kDa. Quantification of the observed signal in the patient, the parents and the sister showed a decrease of *YARS1* abundance in the patient's cells. Specifically, we showed a significant 80% decrease in protein abundance in the affected child compared to control cells (Fig. 5 A and B). Furthermore, we were not able to detect any signal at ~8 kDa, which would have corresponded to the truncated protein translated from the *YARS1* allele carrying the nonsense variant p.Tyr79*. Moreover, a polyclonal antibody directed against the N-terminal part of the protein did not detect any truncated protein (data not shown). However, we cannot formally exclude the possibility of a truncated protein, which would probably be degraded by nonsense mediated mRNA decay (NMD). The mother, who is heterozygous for p.Tyr59*, also had reduced *YARS1* levels (~65%), indicating that this allele is associated with reduced protein levels. Together, all of these studies suggest a loss of protein abundance and probably activity and, thus, at least a partial loss-of-function disorder.

We next asked whether the reduction of *YARS1* expression was restricted to lymphoblastoid cells. Using whole-cell lysates from patient II.2 fibroblasts, we observed a 90% decrease of *YARS1* compared to control cells (Fig. 5 C and D).

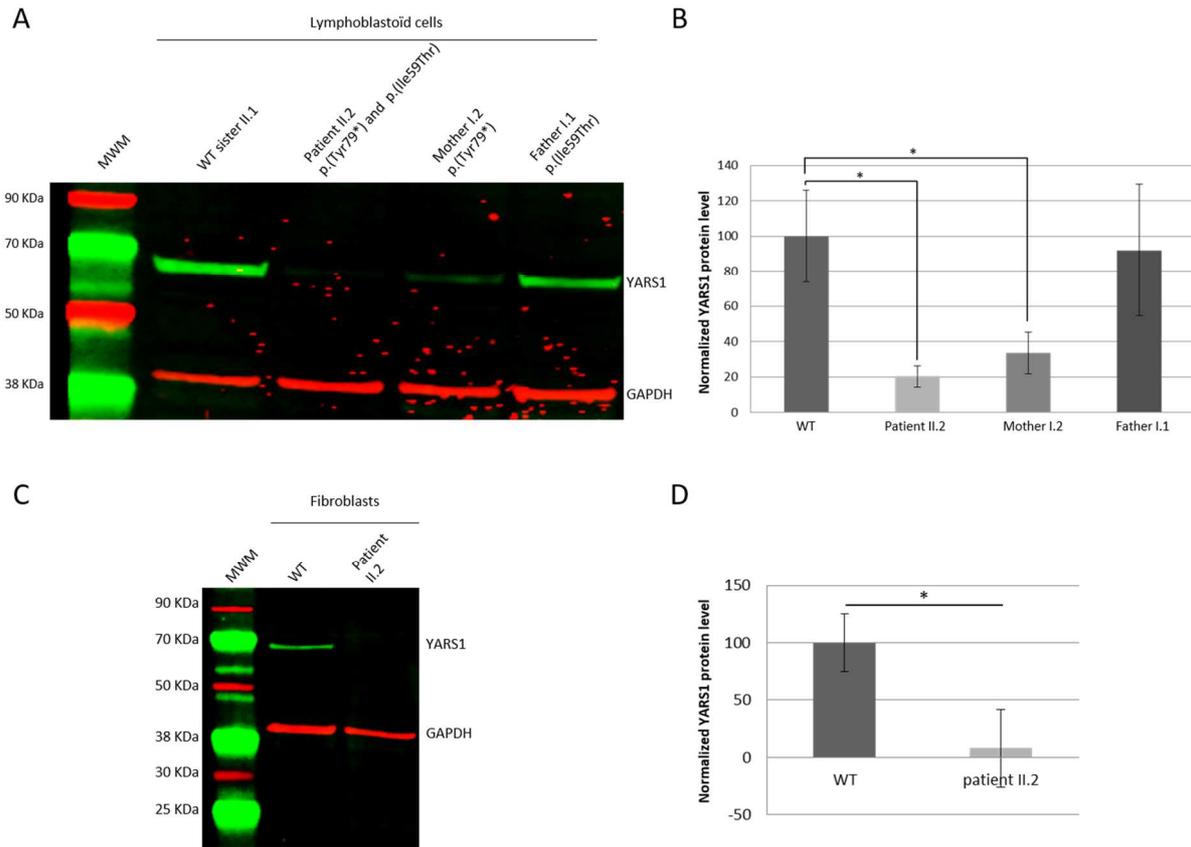


Figure 5: Western blot detection and quantification of the YARS1 protein in controls and the patient

Discussion

We report a genetic syndrome associated with compound heterozygous variants in the *YARS1* gene. The pathology is characterized by IUGR, failure to thrive, hypotonia, liver disease, lung fibrosis, chronic diarrhea, anemia, elevated triglycerides and developmental delay.

We identified two variants in the *YARS1* gene that segregate with the pathology in the family. *YARS1* encodes the tyrosyl-tRNA synthetase 1 known for its essential function in the first step of protein translation in the cytoplasm. Immunoblotting analysis of lymphoblastoid and fibroblast cell extracts from the affected individual showed reduced levels of YARS1 protein, providing strong evidence that a reduced YARS1 function resulted in the multisystem disease we observed in our patient.

Nowaczyk et al. described molecular defects causative for a multisystem disease in *YARS1*¹⁵. Their study reported 2 missense variants transmitted in a recessive pattern in two siblings presenting a similar multisystem syndrome characterized by failure to thrive,

hyperglyceridemia, developmental delay, liver dysfunction, lung cyst and abnormal subcortical white matter. A few months later, Tracewska-Siemiatkowska et al. reported a distinct phenotype characterized by liver disease, rapid retinal degeneration, profound hearing impairment, agenesis of corpus callosum, and primary amenorrhea caused by a homozygous missense variant in *YARS1*¹⁶. More recently, Williams et al. described seven related children with multisystem disease, including sensorineural hearing loss, nystagmus, developmental delay, growth failure, chronic anemia, hypoglycemia, progressive cholestatic liver disease and renal dysfunction¹⁷ (Table 1).

There is a core phenotype shared by the reported patients: FTT, developmental delay, IUGR, hypotonia, steatosis, corpus callosum defect, gastrointestinal disorders and hypertriglyceridemia are the eight main signs seen in at least three patients.

However, our patient had a much more severe phenotype than the patients described in Nowaczyk's and Tracewska-Siemiatkowska's studies^{15,16}: she died at the age of 2 years. The clinical severity is different from patient to another, even in the same family^{15,16}. This suggests that the phenotype of autosomal recessive (AR) *YARS1* variants is highly heterogeneous. However, the clinical severity was difficult to assess in our patient, who was a very preterm infant. We could not exclude the possibility that the premature birth worsened the clinical presentation due to *YARS1* deficiency. Conversely, premature birth could be a consequence of reduced *YARS1* function. Indeed, three of the seven affected Amish children described by Williams et al were born prematurely¹⁷. In this family, 3 patients presented lung disease despite term delivery. Most of the published autosomal recessive *YARS1* variants are located in the catalytic domain (4/6): The missense variants found in our patient, the missense variant described by Williams and colleagues and one of the two missense variants of the patients report by Nowaczyk et al. are also located in this domain (Fig. 4)^{15,17}. These data suggest a link between the severity of the translation defect and the severity of the phenotype. To further test this hypothesis, it would be interesting to assess the impact of all other variants on the *YARS1* protein levels. The patient described by Tracewska-Siemiatkowska et al., who presented with a distinct and mild phenotype, is the only individual presenting a homozygous missense variant in the anticodon-binding site without any variant in the catalytic aminoacylation domain¹⁶. Whether variants located within specific domains of *YARS1* lead to different functional outcomes is still unknown. However, except for the patient described by Tracewska-Siemiatkowska and colleagues¹⁶, who had no variant in the catalytic domain, all of the patients have presented some variable developmental delay^{15-17,20}.

An essential role of *YARS1* is underlined by the fact that none of the human subjects have two nonsense variants (nor other aminoacyl-tRNA synthetase (ARS) deficiencies).

Homozygosity or compound heterozygosity for *YARS1* or other *ARS* null alleles would likely be lethal, as demonstrated in *Gars* homozygous mouse models²¹ and in *Yars* homozygous mouse models from the International Mouse Phenotyping Consortium (IMPC), probably because of the essential roles of the encoded enzymes.

The pathomechanism of *YARS1* deficiency is not yet understood. However, we hypothesized that the clinical symptoms observed in our patient were due to insufficient aminoacylation and inefficient translation because we observed a strong reduction in protein level. Moreover, the patient's growth restriction could be explained by the alteration of translation caused by reduced aminoacylation activity. *YARS1* is only active as a homodimer, and Williams et al showed that the p.Pro167Thr substitution impairs *YARS1* homodimerization¹⁷. In this report, a non-polar residue was also replaced by a polar residue Thr in the catalytic domain. Thus, we hypothesized that the p.Ile59Thr substitution could likely cause a defect *YARS* homodimerization and its aminoacylation activity as the p.Pro167Thr variant¹⁷.

Assuming there was residual enzyme activity in our patient (complete loss of activity seems to be lethal), we tried to improve the patient's symptoms with supplementation of tyrosine (100 mg/kg/day and then 200 mg/kg/day). However, we saw no improvements in growth or other symptoms. However, methionine supplementation improved *MARS1* aminoacylation activity and proliferation in *Mars* mutant yeasts¹¹. Moreover, Fuchs and colleagues emphasized that the supply of proteins or amino acids is crucial in the therapeutic care of cytoplasmic *ARS* deficiencies, especially during periods of increased translational demand, such as in the first year of life or following infection²². Nevertheless, the inefficiency of the tyrosine supplementation observed could be explained by several points: due to the extreme severity of the disease at the time of treatment, we might have missed the time window of efficiency. Another hypothesis could be that the disease mechanism is not due to an impairment in cellular translation. To test whether a variant affects the cellular translation machinery, it would be interesting to evaluate overall protein synthesis. Xu and colleagues performed a puromycin incorporation assay and demonstrated that their proband's cells harboring *FARSB* (MIM609690; phenotype MIM number 613658) biallelic missense variants (another cytoplasmic aminoacyl-tRNA synthetase) had no decline of protein synthesis²². Indeed, *ARSs* are also known to perform non-canonical functions in vertebrates, including translational control, transcription regulation, signal transduction and modulation of cell migration, angiogenesis, inflammation and tumorigenesis²³⁻²⁵.

Even if *YARS1* variants are also known to cause CMTDIC in an autosomal dominant way, neither of the parents exhibits obvious symptom of CMTDIC as muscle weakness, high-stepped gait or loss of muscle mass in the lower leg (neither did the grand-parents

according to the familial interview). It would be interesting to deeply assess the parents neuromuscular phenotype with nerve biopsies or electromyogram. But at the time of the *YARS1* variants identification the condition of the patient worsened and the phenotypic exploration of the parents was not the priority. Nowaczyk and colleagues deeply explored the neuromuscular phenotype of the parents of their patient and did not find any evidence of neuromuscular defect¹⁵. Although some of the mutant alleles listed in OMIM that are associated with Dominant Intermediate C Charcot-Marie-Tooth disease are located in the same aminoacylation domain. CMTDIC causing variants seem to be gain-of-function variants²⁶. In this report, we describe new variants leading to a partial loss-of-function of the protein.

Cytoplasmic aminoacyl-tRNA synthetases (ARSs) are emerging as a cause of numerous rare inherited diseases. Variants in ARSs cause considerably distinct clinical features with tissue-specific phenotypes in humans^{1,23,26}. Interestingly, there is an overlap of clinical features in the investigated individuals harboring *YARS1* biallelic variants with the phenotypic spectrum of other autosomal recessive ARS defects. Indeed, all ARS deficiencies are associated with failure to thrive, feeding and gastrointestinal problems, liver disease and central nervous system symptoms²⁶. Fuchs et al hypothesized that organs with the highest amino acid incorporation rates, such as the liver, the lung and the brain, are the more affected, especially in the first year of life²⁶. This involvement could also explain the fact that a highly proliferative organ, such as the intestine, was affected in our patient (chronic diarrhea). However, some clinical features may be related to non-canonical functions of *YARS1* protein.

In conclusion, our *in vitro* data provide further strong evidence that partial loss of *YARS1* function results in a multisystem disease. The main clinical features of this syndrome include IUGR, FTT, hypotonia, liver disease, lung cystic fibrosis, chronic diarrhea, anemia, steatosis, elevated triglycerides and developmental delay. For the first time, we report a nonsense variant in the *YARS* gene and provide evidence in favor of a partial loss-of-function mechanism. Nevertheless, further analyses using animal models or supplemental functional *in vitro* tests are required to investigate the underlying mechanisms involved and to identify potential therapeutic targets.

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Note

During the revision process of the article a new patient was described. Clinical and genetical data were added in the table 1. The phenotype was consistent with previously described patients²⁰.

Figure Titles and Legends

Figure 1: Growth curves of weight, length and head circumference of patient II.2 between 1 month and 22 months

Arrows represent periods of feeding by nasogastric tube (black) and parenteral nutrition (red).

Figure 2: Histologic findings

Muscle fibers are of normal caliber, with no necrosis or regenerated lesions, and the interstitial area is normal. However, there are microvacuolated aspects of muscle fibers. (HES, magnification x 200) (A). There is an increase in lipids, in agreement with the microvacuolized aspect of the muscle fibers. (Sudan black, magnification x 200) (B). No mitochondrial accumulation or "ragged red" fiber (Gomori trichrome, magnification x 120) (C). The hepatic histology shows severe steatohepatitis activity grade 6 and fibrosis stage F4 (cirrhosis histology) according to Kleiner and mixed systematized steatosis, (HES, magnification x 150) (D) and systemic mixed periportal hepatosclerosis score 3 SEARLE / SCHEUR (Pearls staining, magnification x 100) (E). Digestive biopsies were aspecific for the duodenum exam, (hematein eosin saffron (HES), magnification x 50) (F) but show a discrete fundic gastritis, without activity, atrophy or intestinal metaplasia, (HES, magnification x 200) (G). Lung biopsy shows interstitial lung disease with emphysema and areas of tissue densification preferentially distributed in sub-pleural and peri-lobular spaces. (H). These areas are characterized by macrophage accumulation, some of which are vacuolated, and numerous cholesterol clefts (I). No pulmonary alveolar proteinosis was seen. Peripheral blood tests showed hemolytic anemia (hemoglobin=9,4 g/dL; reticulocytes= 516 x 109/L),

normal platelet count ($379 \times 10^9/L$) and hyperleukocytosis with a white blood cell count of $14 \times 10^9/L$. Polychromatophilic cells and basophilic stippling were detected with light microscopy (magnification $\times 100$) on the peripheral blood smear with May-Grünwald-Giemsa stain (J). Bone marrow aspirate showed erythroid hyperplasia and important dyserythropoiesis with multinucleate erythroblasts (K).

Figure 3: Chest X-rays

Chest X-rays of the proband were taken at the ages of 1 day (A) 1 months (B) 11 months (C) and 20 months (D) and showed intestinal lung disease with reticulomicronodular opacities (B) (C) (D).

Figure 4: *YARS1* variants and gene structure

(A) Pedigree with *YARS* variants. The proband is compound heterozygous and inherited the variant c.237C>G; Tyr79* from her mother and the variant c.176T>C; p.Ile59Thr from her father. (B) *YARS1* sequence alignment between different species. The amino acids mutated in the patient are highlighted in yellow for Ile59 and red for Tyr79. (C) Structural organization of *YARS1* transcript (ENST00000373477) and protein with known conserved protein domains and localization of amino acid residues affected by variants. The two novel variants we found are indicated on the transcript and the protein in red. Previously reported variants associated with multisystem disease are in black¹⁵ (Nowaczyk et al.), orange¹⁶ (Tracewska-Siemiatkowska et al.), blue¹⁷ (Williams et al.) and green (Zeiad et al.)²⁰. Exonic regions (in purple) are not drawn to scale.

Figure 5: Western blot detection and quantification of the *YARS1* protein in controls and the patient

Detection of *YARS1* by Western blot analysis in lymphoblastoid cells (A) and fibroblasts (C) using a monoclonal antibody. GAPDH was used as a loading control. Quantification of the *YARS1* protein levels shows significantly reduced expression levels of *YARS* in the lymphoblastoid cells of the patient ($p= 0.015$) and her mother ($p= 0.019$) (B) and in the fibroblasts of the patient ($p= 0.029$) (D). Values are mean ratios \pm SD, $n=3$; Welch's two sample t-test (patient vs control).

Table 1: Clinical findings in individuals with *YARS* variants

All of the patients presenting multisystemic disease with biallelic variants in *YARS* described in the literature (NA: not available; yrs: years; mths: months).

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