JIMD Reports
DOI 10.1007/8904_2015_515

RESEARCH REPORT

LARS2 Variants Associated with Hydrops, Lactic Acidosis, Sideroblastic Anemia, and Multisystem Failure

Lisa G. Riley · Joëlle Rudinger-Thirion · Klaus Schmitz-Abe · David R. Thorburn · Ryan L. Davis · Juliana Teo · Susan Arbuckle · Sandra T. Cooper · Dean R. Campagna · Magali Frugier · Kyriacos Markianos · Carolyn M. Sue · Mark D. Fleming · John Christodoulou

Received: 29 July 2015 / Revised: 08 October 2015 / Accepted: 08 October 2015 / Published online: 5 November 2015

Abstract Pathogenic variants in mitochondrial aminoacyl-tRNA synthetases result in a broad range of mitochondrial respiratory chain disorders despite their shared role in mitochondrial protein synthesis. *LARS2* encodes the mitochondrial leucyl-tRNA synthetase, which attaches leucine to its cognate tRNA. Sequence variants in *LARS2* have previously been associated with Perrault syndrome, characterized by premature ovarian failure and hearing loss (OMIM #615300). In this study, we report variants in *LARS2* that are associated with a severe multisystem metabolic disorder. The proband was born prematurely with severe lactic acidosis, hydrops, and sideroblastic anemia. She had multisystem complications with hyaline membrane disease, impaired cardiac function, a coagulopathy, pulmonary hypertension, and progressive renal disease and succumbed at 5 days of age. Whole exome sequencing of patient DNA revealed compound heterozygous variants in *LARS2* (c.1289C>T; p.Ala430Val and c.1565C>A; p.Thr522Asn). The c.1565C>A (p.

Communicated by: Carlo Dionisi-Vici, MD

Competing interests: None declared

Electronic supplementary material: The online version of this chapter (doi:10.1007/8904_2015_515) contains supplementary material, which is available to authorized users.

L.G. Riley (✉) · J. Christodoulou
Genetic Metabolic Disorders Research Unit, Kids Research Institute, Children's Hospital at Westmead, Locked Bag 4001, Westmead, NSW 2145, Australia
e-mail: lisa.riley@health.nsw.gov.au

L.G. Riley · S.T. Cooper · J. Christodoulou
Discipline of Paediatrics & Child Health, Sydney Medical School, University of Sydney, Sydney, NSW, Australia

J. Rudinger-Thirion · M. Frugier
Architecture et Réactivité de l'ARN, Université de Strasbourg, CNRS, IBMC, Strasbourg, France

K. Schmitz-Abe · K. Markianos
Division of Genetics and Genomics, Department of Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

K. Schmitz-Abe · K. Markianos
Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, MA, USA

K. Schmitz-Abe
Broad Institute, Cambridge, MA, USA

D.R. Thorburn
Department of Paediatrics, Murdoch Children's Research Institute and Victorian Clinical Genetics Services, Royal Children's Hospital, University of Melbourne, Melbourne, VIC, Australia

R.L. Davis · C.M. Sue
Department of Neurogenetics, Kolling Institute of Medical Research, University of Sydney and Royal North Shore Hospital, Sydney, NSW, Australia

J. Teo
Department of Haematology, Children's Hospital at Westmead, Sydney, NSW, Australia

S. Arbuckle
Department of Pathology, Children's Hospital at Westmead, Sydney, NSW, Australia

S.T. Cooper
Institute for Neuroscience and Muscle Research, Kids Research Institute, Children's Hospital at Westmead, Sydney, NSW, Australia

D.R. Campagna · M.D. Fleming
Department of Pathology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

J. Christodoulou
Discipline of Genetic Medicine, Sydney Medical School, University of Sydney, Sydney, NSW, Australia

Thr522Asn) *LARS2* variant has previously been associated with Perrault syndrome and both identified variants are predicted to be damaging (SIFT, PolyPhen). Muscle and liver samples from the proband did not display marked mitochondrial respiratory chain enzyme deficiency. Immunoblotting of patient muscle and liver showed LARS2 levels were reduced in liver and complex I protein levels were reduced in patient muscle and liver. Aminoacylation assays revealed p.Ala430Val LARS2 had an 18-fold loss of catalytic efficiency and p.Thr522Asn a 9-fold loss compared to wild-type LARS2. We suggest that the identified *LARS2* variants are responsible for the severe multisystem clinical phenotype seen in this baby and that mutations in *LARS2* can result in variable phenotypes.

Introduction

Mitochondrial respiratory chain (RC) disorders are a heterogeneous group of disorders caused by sequence variants in mitochondrial DNA or in nuclear genes encoding proteins required for RC biogenesis or function (Menezes et al. 2014). Many of these disorders present in infancy with highly variable clinical presentations, usually with multisystem involvement. An increasing number of defects have been shown to impair mitochondrial protein translation, including pathogenic variants in many of the genes encoding mitochondrial aminoacyl-tRNA synthetases (aaRS2) (Diodato et al. 2014). aaRS2 family proteins are nuclear encoded, translated in the cytosol, and imported into mitochondria where they conjugate amino acids to cognate tRNAs during protein translation. They contain a catalytic domain (class I or class II) and an anticodon-binding domain involved in tRNA recognition (Schimmel et al. 1993). A wide range of clinical phenotypes are associated with variants in different aaRS2 proteins, despite their similar roles in the synthesis of mitochondrial DNA-encoded subunits of the RC (Diodato et al. 2014). In some cases, overlapping clinical features have been observed from mutations in different genes. For instance, mutations in *HARS2* and *LARS2* are both associated with sensorineural hearing loss and progressive ovarian failure (OMIM 614926) (Pierce et al. 2011; Pierce et al. 2013). However, in other cases, different mutations in the same gene result in distinct phenotypes. For example, *AARS2* mutations result in either infantile cardiomyopathy (OMIM 614096) (Gotz et al. 2011) or leukoencephalopathy with premature ovarian failure (POF; OMIM 615889) (Dallabona et al. 2014). In this study we expand the clinical spectrum associated with *LARS2* variants to include a patient with hydrops, lactic acidosis, sideroblastic anemia, and infantile multisystem failure.

Methods

Clinical Information

The Human Research Ethics Committee of the Children's Hospital at Westmead approved this research and all procedures followed were in accordance with ethical standards. Informed consent was obtained for all individuals sequenced in the study, and we also had ethics approval for the use of control muscle samples in this study.

The female proband was the first born child of unrelated Pakistani parents. The pregnancy was complicated by oligohydramnios and fetal growth restriction, hydrops, and anemia, with antenatal scans showing fetal pericardial effusion, ascites, and scalp edema. Fetal cardiotocography at 29 weeks' gestation was abnormal and led to an emergency caesarean section. Apgar scores were 4 and 8 at 1 and 5 min respectively, and she had a very low birth weight of 1,010 g. She was intubated and ventilated from birth. Her arterial cord pH was 7.27 and base excess was −6.7 mEq/L, indicative of metabolic acidosis. Soon after birth, an arterial blood gas showed acidosis with pH to 6.8 and lactate to 16 mmol/L (reference range 0.7–2.0 mmol/L). The acidosis continued with lactate of 13 mmol/L, despite management with sodium bicarbonate infusions and despite normal mean blood pressures and presumed adequate perfusion. A urine metabolic screen showed gross lactic aciduria. She had a normal acylcarnitine profile and a normal female karyotype.

The proband developed multisystem complications, including hyaline membrane disease, for which she received two doses of surfactant and remained mechanically ventilated. She also had impaired cardiac function confirmed on echocardiogram that was associated with structural abnormalities, including a patent ductus arteriosus, a moderate ventricular septal defect, and an overriding aorta. She had associated tachyarrhythmias and possible second-degree heart block. She also developed pulmonary hypertension that required treatment with nitric oxide and multiple inotropic agents. It is unclear whether the pulmonary hypertension was secondary to unrestrictive shunting through the ventricular septal defect and/or patent ductus arteriosus, neither of which was surgically repaired, or whether the pulmonary hypertension might have been a primary distinctive feature.

The proband was severely anemic at birth with hemoglobin levels of 19 g/L (reference range 121.8–145.2 g/L) and elevated MCV at 133 fL (reference range 110–125 fL). She was treated by extensive fluid resuscitation with blood products. The Kleihauer test was negative. There was no hemolysis evident and TORCH serology was normal. She had thrombocytopenia that was managed with platelet transfusions. A bone marrow aspirate revealed moderate

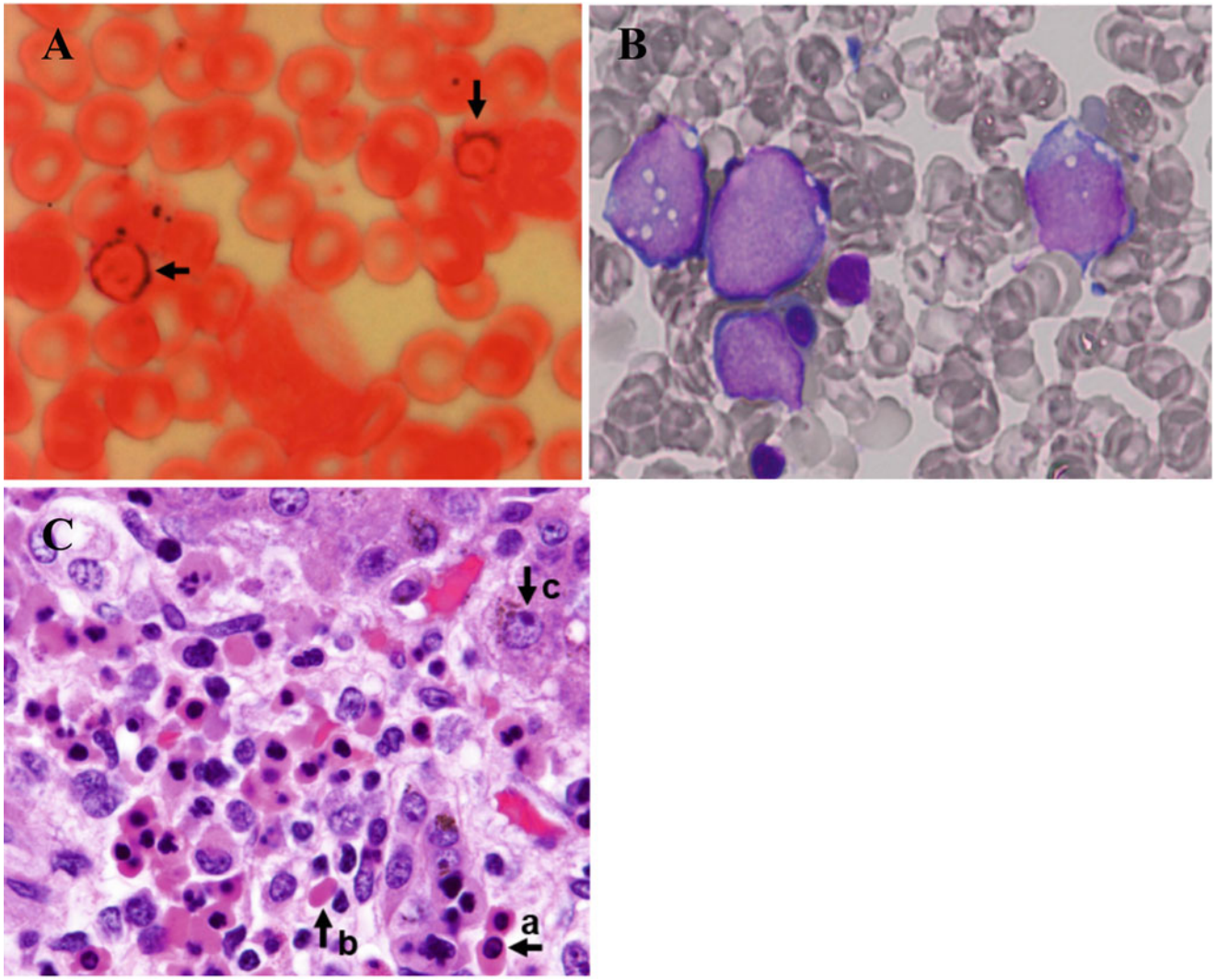

Fig. 1 Histological abnormalities in patient blood and liver. (**a**) Perl's iron stain of bone marrow aspirate showing ringed sideroblasts (*arrows*). (**b**) May–Grunwald–Giemsa stain of bone marrow aspirate showing vacuolation of proerythroblasts. (**c**) Hematoxylin and eosin staining of the portal area of the liver with extramedullary hematopoiesis (mainly erythroid) showing dyserythropoiesis with irregular, crenated nuclei and binucleate cells with variable eosinophilic cytoplasm. *Arrows* indicate: (*a*) normal nucleated red blood cell. Most of the others present are abnormal. (*b*) Anucleate red blood cell. (*c*) Hepatocyte with hemosiderin

erythroid hyperplasia and vacuolization of red cell precursors with the Perl's iron stain showing ringed sideroblasts (Fig. 1a). May–Grunwald–Giemsa staining of bone marrow showed vacuolation of proerythroblasts (Fig. 1b). Hematoxylin and eosin staining of liver samples showed extramedullary hematopoiesis with dyserythropoiesis (Fig. 1c).

There was no liver enlargement but liver dysfunction with disordered coagulation was treated with fresh-frozen plasma and cryoprecipitate, and a low albumin level required albumin infusion. Her renal function showed rising creatinine levels with hematuria, although her kidneys were found to be structurally normal on ultrasound. The proband also had clinical and electrical seizures needing multiple anticonvulsants. An electroencephalogram showed an abnormal background consistent with diffuse cerebral dysfunction, despite normal structures apparent on head ultrasound. No neuroimaging was performed.

Worsening cardiac function and hypotension led to a decision by the family for withdrawal of active intensive care management and she succumbed at 5 days of age. The parents have since had a second child who is currently 14 months old and apparently healthy, but not available for examination.

RC Enzyme Assays

Mitochondrial respiratory chain enzyme assays were performed as previously described (Frazier and Thorburn 2012) on muscle and liver samples collected at autopsy.

DNA Sequencing and Analysis

Initially, *YARS2* coding exons were PCR amplified from patient gDNA and Sanger DNA sequencing performed by Macrogen, Korea. Quantitative PCR was performed to check for deletions in *YARS2* and mtDNA depletion in patient liver (Pagnamenta et al. 2006).

Subsequently, whole exome sequencing, using a 62 Mb SureSelect capture, was performed on patient DNA by Macrogen, Korea. The sequence was analyzed at the Boston Children's Hospital, USA, using a custom-built, rule-based "variant explorer" pipeline capable of integrating SNP chip, linkage, sequencing, and functional database information. In this case only the sequencing and database components were used. Due to the severity of the phenotype and the presence of ringed sideroblasts, we prioritized variants that were: (1) rare, with less than 1% frequency in the 6,500 exome database compiled by the National Heart, Lung, and Blood Institute Exome Sequencing Variant Project (http://evs.gs.washington.edu/EVS/); (2) recessive, either homozygous or compound heterozygous mutations; and (3) within a list of genes related to mitochondrial disease, either genes previously implicated in sideroblastic anemia or among mitochondrial proteins annotated in the MitoCarta database (Pagliarini et al. 2008). *LARS2* variants identified by WES were confirmed by Sanger sequencing of patient and parental DNA samples. *LARS2* was screened by Sanger sequencing DNA from a further 14 patients with syndromic sideroblastic anemia.

Mitochondrial DNA sequencing was performed on patient liver DNA via amplification of nine overlapping fragments. The amplicons were sequenced using BigDye Terminator v.3.1 using a total of 62 forward and reverse primers (sequences available on request). Capillary separation of sequencing products was performed on an ABI 3730*xl* genetic analyzer (Life Technologies) by the Ramaciotti Centre for Genomics (Sydney, Australia). Raw sequences were aligned to the revised Cambridge Reference Sequence for mitochondrial DNA (NC_012920) using SeqScape v.2.5 software (Applied Biosystems).

Immunoblotting

Immunoblotting and densitometry were performed as previously described (Riley et al. 2013), with the following modifications: Membranes were probed with a 1:250 dilution of anti-LARS2 (Abcam ab97439) or a 1:5,000 dilution of anti-VDAC1 (Abcam ab14734), overnight at 4°C.

Cloning, Aminoacylation Assays, and Editing Assays

Recombinant LARS2 proteins were synthesized to assess aminoacylation and editing activity of the identified variants. *LARS2* variants were introduced into the pET22b/LARS2 construct (Yao et al. 2003). WT and variant LARS2 were expressed and purified as previously described (Yao et al. 2003). The sequence of *Escherichia coli* $tRNA_5^{Leu}$(UAA) was cloned, transcribed, and purified according to established procedures (Perret et al. 1990). In vitro leucylation of the $tRNA^{Leu}$ transcript was performed in the presence of 30 μM L-[^{14}C]leucine at 37°C as previously described (Sohm et al. 2003). Kinetic parameters were determined from Lineweaver–Burk plots in the presence of 3–30 nM WT or variant LARS2 and concentrations of *E. coli* $tRNA^{Leu}$ transcript ranging from 0.3 to 5.6 μM. Experimental errors for k_{cat} and K_m varied by ≤20%. Data were expressed as the averages of at least three independent experiments.

In vitro deacylation assays were performed as described (Mursinna et al. 2001) in the presence of 70 nM of WT or variant LARS2. Radioactive Leu–$tRNA^{Leu}$ and Ile–$tRNA^{Leu}$ transcripts were prepared by aminoacylating $tRNA^{Leu}$ either with L-[^{14}C]leucine using WT LARS2 or with L-[^{14}C]isoleucine using an editing defective *E. coli* LARS (Sarkar et al. 2012). Aminoacylation reactions were stopped by phenol extraction under acidic conditions and aminoacylated transcripts were ethanol precipitated in the presence of 0.3 M sodium acetate (pH 4.5).

Mitochondrial Protein Synthesis Assays

Mitochondrial protein synthesis assays were performed as previously described (McKenzie et al. 2009) with the following modifications: Samples were analyzed on precast 10% Tricine protein gels (Life Technologies).

Results

We investigated the cause of a lethal infantile multisystem disease in a premature infant born to non-consanguineous parents. The presence of severe lactic acidosis, sideroblastic anemia, and multiple organ involvement led to the suspicion of a mitochondrial RC disorder. RC enzyme activities for complexes I and IV were low in muscle (Table 1), consistent with levels typically found in premature infants and not sufficiently low to be diagnostic for a mitochondrial RC disorder. Complex IV activity was also reduced in liver (Table 1), but again, not to a level that

Table 1 Respiratory chain enzyme activities in patient skeletal muscle and liver

	Muscle			Liver		
	Activity (ref range)	% activity	% CS[a] ratio	Activity (ref range)	% activity	% CS[a] ratio
Complex I (nmol/min/mg)	23 (19–72)	55	40	9 (8–11)	95	61
Complex II (nmol/min/mg)	50 (26–63)	111	78	**48** (54–73)	79	50
Complex III (/min/mg)	22.8 (12.8–50.9)	78	53	7.3 (5.2–10.3)	96	61
Complex IV (/min/mg)	3.46 (3.3–9.1)	52	38	**0.35** (0.5–0.9)	49	32
Citrate synthase (nmol/min/mg)	**182** (85–179)	141		**43** (26–31)	154	

Values in bold are outside the ref range
[a] *CS* citrate synthase

would support the diagnosis of a mitochondrial RC disorder. Citrate synthase levels were elevated in both muscle and liver relative to controls although normative data from premature infants are not available.

The baby shared some clinical features with a severe congenital presentation of *YARS2* MLASA2 (Riley et al. 2013). However, genetic screening of *YARS2* for sequence variants and deletions was negative. Patient DNA was then analyzed by whole exome sequencing, identifying two heterozygous variants in a related gene, *LARS2*. No other rare homozygous or compound heterozygous variants were identified in any other gene related to mitochondrial function within the MitoCarta database (Supplementary Table 1). The patient harbored a novel *LARS2* c.1289C>T (p.Ala430Val) variant and a *LARS2* c.1565C>A (p.Thr522Asn) variant previously reported in Perrault syndrome. Sanger sequencing confirmed the presence of these variants in the proband (Fig. 2a). The mother was heterozygous for the c.1289C>T variant and the father heterozygous for the c.1565C>A variant, consistent with the expectation of recessive inheritance. DNA was not available from the unaffected sibling. The identified *LARS2* variants were not present in 6,500 exomes of the Exome Variant Server (EVS) nor 345 exomes we have analyzed for a broad spectrum of Mendelian disorders at Boston Children's Hospital (100 unaffected). *LARS2* Thr522Asn has an allele frequency of 0.0002 with p.Ala430Val not detected in 60,000 exomes (http://exac.broadinstitute.org). The variants affect semi-conserved and conserved positions within LARS2 (Fig. 2b). They were identified by gene annotation algorithms as highly deleterious with near maximal scores: SIFT = 0.01 and 0.00, PolyPhen2 = 0.993 and 0.999, for c.1289C>T; p.Ala430Val and c.1565C>A; p.Thr522Asn, respectively. Neither variant was predicted to affect splicing (Alamut v2.4). Mitochondrial DNA sequencing analysis showed there were no rare variants likely to be pathogenic (Supplementary Table 2), and qPCR showed there was no mtDNA depletion in patient liver. We did not identify *LARS2* variants in fourteen other probands with syndromic sideroblastic anemia.

We examined the effect of the *LARS2* variants on endogenous levels of LARS2 protein and representative subunits of each RC complex. Immunoblotting of LARS2 from patient muscle showed levels that were similar to age-matched controls (Fig. 2c). The results clearly show that RC complex levels in control muscle (C1–C4) increase with gestational age (Fig. 2c). Complex I levels in patient muscle (29 week fetal muscle) were ~57% of a fetal control of a similar gestational age (C2, 25 week fetal muscle). Immunoblotting of patient liver showed LARS2 levels were ~50% of those in a 30-week-old control (Fig. 2c). Levels of the RC complexes were also reduced in patient liver with complex I being the most markedly reduced at ~25% of the control level. Immunoblotting of patient fibroblasts showed no change in LARS2 protein level and no effect on mitochondrial RC complex protein levels relative to pediatric control fibroblasts (results not shown). Consistent with this, no defect in mitochondrial protein synthesis was detected in an in vitro assay in patient fibroblasts or induced myotubes (results not shown), indicating the *LARS2* variants most likely only affect tissues with higher energy demands.

The effect of the *LARS2* variants on LARS2 aminoacylation activity was investigated. In vitro aminoacylation activity of purified recombinant LARS2 p.Ala430Val and p.Thr522Asn variants was measured by the incorporation of [^{14}C]-leucine into an *E. coli* tRNA^{Leu} substrate. LARS2 p.Ala430Val demonstrated an 18-fold loss of catalytic efficiency and LARS2 p.Thr522Asn showed a 9-fold reduction compared to WT LARS2, essentially due to decreased catalytic rates (Table 2). In this study, catalytic values for WT LARS2 aminoacylation differ from those previously published (Yao et al. 2003). This is explained by the use of different tRNA substrates. In the present work, we used *E. coli* tRNA$_5^{Leu}$(UAA) transcript, whereas the Wang group used native *E. coli* tRNA$_1^{Leu}$(CAG).

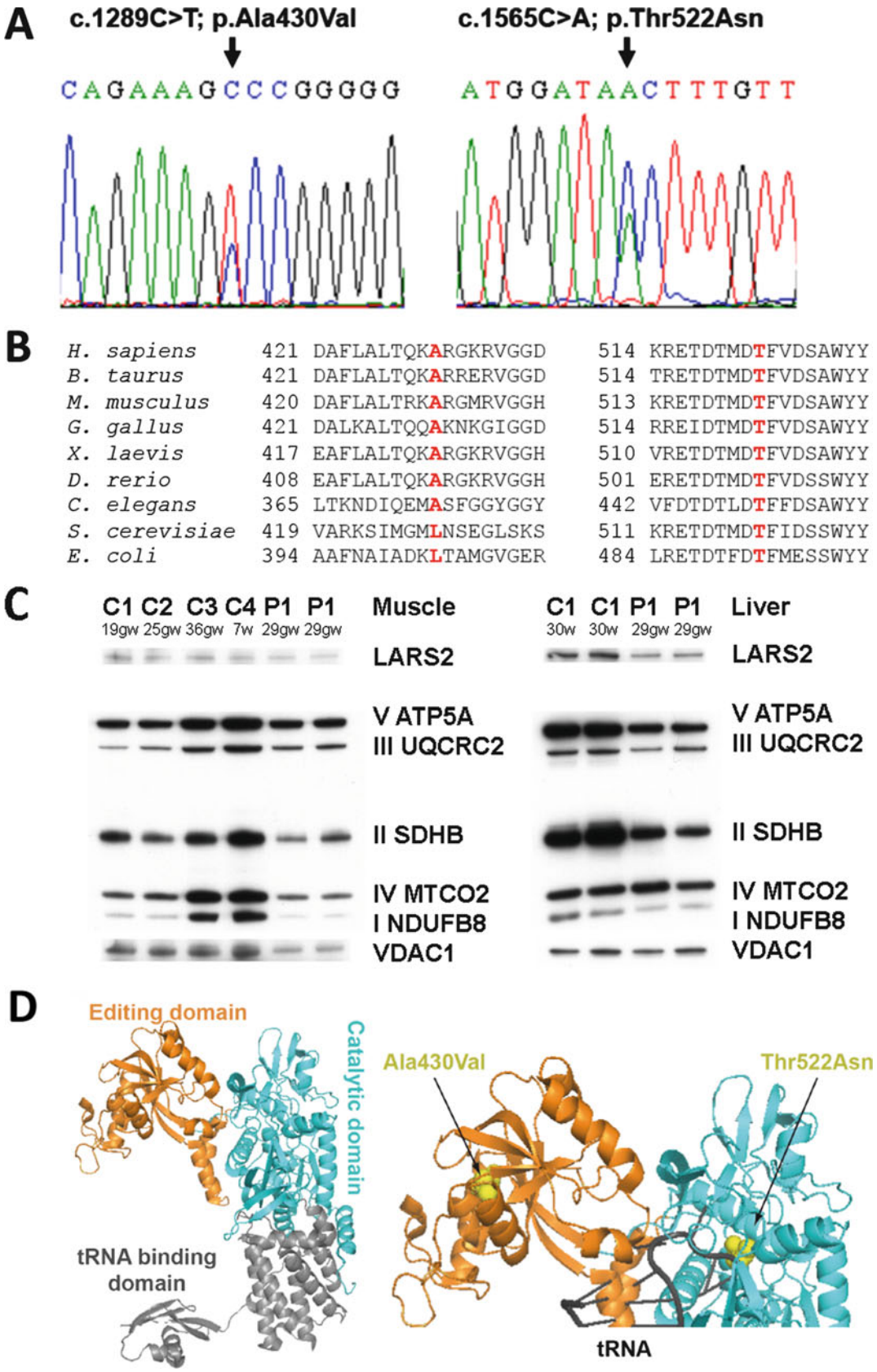

Fig. 2 *LARS2* variants and their effect on LARS2 and RC complex levels. (**a**) Patient DNA sequence identifying the c.1289C>T; p.Ala430Val and c.1565C>A; p.Thr522Asn *LARS2* variants. (**b**) LARS2 sequence alignments showing the location of p.Ala430 and

Table 2 Kinetic parameters for leucylation of *E. coli* $tRNA_5^{Leu}$(UAA) transcript by LARS2 wild-type and variant recombinant proteins

LARS2 variant	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (efficiency)	Loss of efficiency[a] (fold change)
WT	1.35	0.430	0.320	1
p.A430V	1.70	0.031	0.018	18
p.T522N	1.35	0.050	0.037	9

[a] Loss of efficiency is calculated relative to wild-type (WT) LARS2

In control experiments, all enzymes were assayed for $tRNA^{Leu}$ misacylation with isoleucine; however we did not detect any evidence of Ile–$tRNA^{Leu}$ formation (data not shown). Moreover, as expected, all three enzymes were also inactive in Leu–$tRNA^{Leu}$ and Ile–$tRNA^{Leu}$ deacylation (data not shown).

Discussion

Here we report compound heterozygous *LARS2* sequence variants in an infant with hydrops, lactic acidosis, sideroblastic anemia, and severe multisystem failure, which we propose is a novel phenotype for *LARS2* variants. *LARS2* variants have previously been reported in association with sensorineural hearing loss and premature ovarian failure (Pierce et al. 2013) in three siblings of Palestinian origin who were homozygous for the LARS2 p.Thr522Asn variant and a Slovenian child with compound heterozygous LARS2 p. Ile360fs*15 and p.Thr629Met variants (see Supplementary Table 3 for a comparison of phenotypes).

The LARS2 variants identified in our patient result in reduced $tRNA^{Leu}$ aminoacylation efficiency. The recombinant LARS2 p.Thr522Asn had a 9-fold loss of catalytic efficiency, consistent with yeast complementation studies that showed the yeast ortholog of LARS2 p.Thr522Asn retained partial function (Pierce et al. 2013). Modeling of LARS2 on the *E. coli* leucyl-tRNA synthetase crystal structure indicates that Thr522 is located in the active site where the 3′ end of the tRNA binds (Pierce et al. 2013) (Fig. 2d). Thus a substitution at this site is likely to impact on leucylation. The recombinant LARS2 p.Ala430Val had an 18-fold loss of catalytic efficiency. In silico analysis revealed that the Ala430 residue is not strictly conserved and is replaced by a leucine residue in the *E. coli* LARS sequence. Furthermore, Ala430 is located in the LARS2 CP1 (connective peptide 1) domain (Lue and Kelley 2005) (Fig. 2d). The CP1 domain is an editing domain, which clears toxic mischarged tRNAs in some cytosolic ARSs. Indeed, cytosolic LARSs can activate and promiscuously transfer isoleucine onto $tRNA^{Leu}$, but specifically edit this mistake (Starzyk et al. 1987). On the contrary, LARS2 has a more specific active site and does not mischarge $tRNA^{Leu}$ with isoleucine and thus its CP1 domain has lost its editing activity (Lue and Kelley 2005; Sarkar et al. 2012). However, we verified that LARS2 p.Ala430Val (as well as LARS2 p.Thr522Asn) did not restore editing activity as it was unable to (1) form mischarged Ile–$tRNA^{Leu}$ and (2) promote deacylation of Ile–$tRNA^{Leu}$ or even Leu–$tRNA^{Leu}$. Thus, our results suggest that the LARS2 p.Ala430Val mutation perturbs leucylation via some long-range effects that may induce structural changes in the active site. This view is supported by the fact that in *E. coli* LARS, a unique mutation in CP1 affects leucylation activity (Du and Wang 2003). In addition, structural data show that CP1 is directly involved in positioning $tRNA^{Leu}$ on *E. coli* LARS for efficient charging (Palencia et al. 2012). However, because the editing-inactive CP1 domain of yeast LARS2 is required for splicing of group I introns (Sarkar et al. 2012), it is possible that an unknown alternative function could be altered in the CP1 domain of human LARS2 p.Ala430Val.

The combined effect of the two LARS2 variants on aminoacylation activity in the patient most likely results in reduced mitochondrial protein synthesis in affected organs and hence mitochondrial RC dysfunction. While the RC deficiencies seen in the muscle and liver were only mild,

Fig. 2 (continued) p.Thr522 in *red* and their conservation among species. (**c**) Immunoblot analysis of LARS2 and the RC complexes (I–V) in patient (P1) and control (C1–4) muscle and liver. Age of individuals at sample collection is shown in gestational weeks (gw) or postnatal weeks (w). VDAC1 was used as a loading control. (**d**) Localization of LARS2 pathogenic mutations on the *E. coli* LARS crystal structure (PDB: 4AQ7). *Left panel*: LARS2 is a class I ARS homologous to *E. coli* LARS which displays a catalytic domain (*cyan*), an editing domain (*orange*), and a C-terminal tRNA-binding domain (*gray*). The catalytic domain binds ATP, leucine, and the 3′ extremity of $tRNA^{Leu}$ and releases Leu–$tRNA^{Leu}$ after catalysis. In *E. coli*, the editing domain is involved in the hydrolysis of mischarged $tRNA^{Leu}$ (mainly with isoleucine). This domain is present but not functional in LARS2. *Right panel*: mutations equivalent to LARS2 residues 430 and 522 are emphasized in yellow. They are found in the editing-like domain and the catalytic domain, respectively. Ala430 corresponds to Leu403 and Thr522 corresponds to Thr492 in the *E. coli* LARS sequence. The 3′ end of the $tRNA^{Leu}$ acceptor helix is shown in dark gray and is in close proximity to the mutated residue at position 522 (Thr492 in *E. coli* LARS)

the heart and brain may be more severely affected with tissue-specific RC deficiency being reported in other aaRS2 mutations (Konovalova and Tyynismaa 2013). The more severe multisystem disease in our patient may be due to the severity of the LARS2 p.Ala430Val variant compared to the variants in previous *LARS2* cases, consistent with the greater loss of aminoacylation activity detected. There did not appear to be any contribution from mtDNA variants (Supplementary Table 1), although other genetic modifiers may be involved.

Our patient shares some phenotypic features with symptoms caused by mutations in other members of the aaRS2 family. Initially, this patient was suspected of having a *YARS2* defect as we previously reported in an infant with lactic acidosis, sideroblastic anemia, and multisystem disease (cardiac hypertrophy) who succumbed in infancy (Riley et al. 2013). Pulmonary hypertension, elevated serum lactate, anemia, premature birth, and death in infancy are also characteristics of patients with *SARS2* mutations; patients also display progressive renal disease (Belostotsky et al. 2011). For a growing number of aaRS2 family genes, genetic variants have resulted in two distinct phenotypes, usually a more moderate form and a severe, lethal infantile form. Most *YARS2* defects result in a more moderate MLASA2 than seen in the patient mentioned above (Riley et al. 2013; Shahni et al. 2013); however another lethal infantile case has recently been reported (Nakajima et al. 2014). In the case of *AARS2*, two missense variants have been associated with a lethal infantile cardiomyopathy (Gotz et al. 2011), while another 11 variants have resulted in leukoencephalopathy and premature ovarian failure (Dallabona et al. 2014). A range of compound heterozygous variants in *EARS2* result in leukoencephalopathy with thalamus and brainstem involvement and high lactate (Steenweg et al. 2013). In one case with a homozygous *EARS2* variant in the catalytic domain, the patient had a multisystem fatal disease characterized by severe failure to thrive, intractable lactic acidosis, mitochondrial myopathy, and hepatopathy, resulting in death at 3 months of age (Talim et al. 2013). For some genes there appears to be a correlation between the severity of the variant and the outcome of the disease, while in others it appears that other genetic and/or environmental factors contribute to the phenotypic variability.

In addition to *LARS2*, variants in several other genes encoding components of the mitochondrial protein translation apparatus result in sideroblastic anemia, including *YARS2*, *PUS1* (MLASA1; OMIM 600462), *TRNT1* (SIFD; OMIM 616084), and deletions of genes encoding mt-tRNAs responsible for Pearson syndrome (OMIM #557000) (Bottomley and Fleming 2014; Chakraborty et al. 2014). However, the mechanistic basis as to why variants in genes involved in mitochondrial protein translation cause sideroblastic anemia and such a broad phenotypic spectrum remains elusive.

In conclusion, LARS2 p.Ala430Val and p.Thr522Asn variants result in reduced mitochondrial $tRNA^{Leu}$ aminoacylation activity and are associated with hydrops, lactic acidosis, sideroblastic anemia, and multisystem failure, thus expanding the clinical phenotype for *LARS2* variants.

Acknowledgments We are indebted to Enduo Wang (Shanghai) and Susan Martinis (Urbana) for their generous gifts of clones encoding LARS2 and editing deprived *E. coli* LARS, respectively. This research was supported by a National Health and Medical Research Council of Australia (NHMRC) Project Grant APP1026891. RLD is an NHMRC Early Career Fellow, DRT is an NHMRC Principal Research Fellow, STC is an NHMRC Career Development Fellow, and CMS is an NHMRC Practitioner Fellow. MDF and KM are supported by RO1 DK 087992 from the US National Institutes of Health. We are grateful to the Crane and Perkins families for their generous financial support.

Synopsis (1 Sentence)

LARS2 variants that result in reduced LARS2 aminoacylation activity were identified in a patient with a novel phenotype: hydrops, lactic acidosis, sideroblastic anemia, and multisystem failure.

Compliance with Ethics Guidelines

Conflict of Interest

Lisa G. Riley, Joëlle Rudinger-Thirion, Klaus Schmitz-Abe, David R. Thorburn, Ryan L. Davis, Juliana Teo, Susan Arbuckle, Sandra T. Cooper, Magali Frugier, Kyriacos Markianos Carolyn M. Sue, and Mark D. Fleming declare that they have no conflict of interest.

John Christodoulou is a communicating editor of the *Journal of Inherited Metabolic Disease*.

Informed Consent

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 2000 (5). Informed consent was obtained for all individuals sequenced in the study. This article does not contain any studies with animals.

Author Contributions

LGR performed Sanger sequencing, immunoblotting, and cloning and wrote the manuscript; JR-T and MF designed

and performed in vitro aminoacylation assays; KS-A performed WES analysis; DRT provided mitochondrial RC enzyme activities and mtDNA depletion results; RLD and CMS performed mtDNA sequencing and analysis; JT performed bone marrow aspiration and described sideroblastic anemia; SA provided hematology and histopathology images; STC supervised the Western analysis and provided the developmental muscle samples; KM supervised WES analysis and interpretation; MDF and DRC participated in WES analysis and surveyed additional phenotypically similar patients for mutations. JC was involved in the initial diagnosis and management of the patient, obtaining consent and samples for the study, and contributed to the overall conception and progression of the study. All authors contributed to editing the manuscript.

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