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The expanding *LARS2* phenotypic spectrum: HLASA, Perrault syndrome with leukodystrophy, and mitochondrial myopathy

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ABSTRACT

LARS2 variants are associated with Perrault syndrome, characterised by premature ovarian failure and hearing loss, and with an infantile lethal multi-system disorder: hydrops, lactic acidosis, sideroblastic anemia (HLASA) in one individual. Recently we reported *LARS2* deafness with (ovario) leukodystrophy. Here we describe five patients with a range of phenotypes, in whom we identified biallelic *LARS2* variants: three patients with a HLASA-like phenotype, an individual with Perrault syndrome whose affected siblings also had leukodystrophy, and an individual with a reversible mitochondrial myopathy, lactic acidosis and developmental delay.

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Three HLASA cases from two unrelated families were identified. All were males with genital anomalies. Two survived multisystem disease in the neonatal period; both have developmental delay and hearing loss. A 55 year old male with deafness has not displayed neurological symptoms while his female siblings with Perrault syndrome developed leukodystrophy and died in their 30's. Analysis of muscle from a child with a reversible myopathy showed reduced LARS2 and mitochondrial complex I levels, and an unusual form of degeneration. Analysis of recombinant LARS2 variant proteins showed they had reduced amino-acylation efficiency, with HLASA-associated variants having the most severe effect. A broad phenotypic spectrum should be considered in association with *LARS2* variants.

Key words: Perrault syndrome; hydrops; lactic acidosis; sideroblastic anemia; leukodystrophy

INTRODUCTION

LARS2 encodes mitochondrial leucyl-tRNA synthetase which is responsible for the ATP-dependent conjugation of leucine to its cognate tRNA to support mitochondrial protein synthesis. Variants in mitochondrial aminoacyl-tRNA synthetases are associated with a broad range of mitochondrial disorders despite their common role in mitochondrial protein synthesis. The central nervous system (CNS) is affected in most mitochondrial aminoacyl-tRNA synthetase-related disease, with or without involvement of other organs, however a few of these disorders exclusively affect non-CNS tissues (Gonzalez-Serrano, Chihade, & Sissler, 2019). For some of the mitochondrial aminoacyl-tRNA synthetases two different phenotypes have been associated with biallelic variants e.g., *AARS2* infantile cardiomyopathy (MIM# 614096) (Götz et al., 2011), and leukoencephalopathy with premature ovarian failure

(MIM# 615889) (Dallabona et al., 2014), or a wide range of disease severity has been reported e.g., *YARS2* MLASA2 (MIM# 613561) where some cases manifest isolated myopathy or sideroblastic anemia (Riley et al., 2018; Sommerville et al., 2017).

Most reports of disorders associated with *LARS2* variants report sensorineural hearing loss and premature ovarian failure (Perrault syndrome 4; MIM# 615300) (Demain et al., 2017; Lerat et al., 2016; Pierce et al., 2013). However, we have previously reported an individual with a lethal multi-system disorder comprising hydrops, lactic acidosis, and sideroblastic anemia (HLASA; MIM# 617021) with *LARS2* variants (Riley et al., 2016). More recently, individuals with Perrault syndrome and neurological symptoms caused by biallelic *LARS2* variants have been reported (Kosaki, Horikawa, Fujii, & Kosaki, 2018; van der Knaap et al., 2019). Here we report three additional HLASA cases from two unrelated families who begin to bridge the phenotypic spectrum of *LARS2*-associated disease, where one individual from each family has survived the neonatal period but has sensorineural hearing loss and developmental delay. We also report a family where a male has biallelic *LARS2* variants and sensorineural hearing loss and two female siblings who had deafness and ovarioleukodystrophy. In a fourth family, an individual with biallelic *LARS2* variants had a reversible myopathy, lactic acidosis phenotype which has not previously been reported in association with *LARS2*, but has some features in common with *YARS2* MLASA2.

MATERIALS AND METHODS

Editorial policies and ethical considerations

This research and all procedures followed were in accordance with ethical standards and was approved by the Human Research Ethics Committee of the Children's Hospital at Westmead (ID number 10/CHW/114) and of the Royal Children's Hospital (ID numbers HREC/16/MH251 and HREC34228). Informed consent was obtained for all individuals sequenced in the study, and ethics approval for the use of control muscle samples.

Clinical information

Clinical histories for affected individuals are supplied in Supp. Materials and family pedigrees in Supp. Figure S1.

DNA sequencing and analysis

Patient 1- The proband had clinical genetic testing through GeneDx, a commercial genetic testing company. Patient and parental DNA underwent trio exome sequencing and data analysis by GeneDx. Variants were confirmed by Sanger sequencing.

Patient 2b – The proband (2b) was ascertained through the Australian Genomics Acute Care Study. Patient 2b and parental DNA underwent rapid trio exome sequencing using an Ampliseq RDY exome kit, analysed on a Life Technologies Proton instrument using a P1 v3 chip at the New South Wales (NSW) Health Pathology Randwick Genomics Laboratory. Reads were aligned to the Human Genome Reference Sequence Hb19/GRCh37, and single nucleotide and short insertion/deletion variants identified using TorrentSuite v5.0.5 software. Data filtering

was performed using a NATA-approved in-house pipeline (v2.0) based on Gemini v18 with annotation from the Variant Effect Predictor (VEP) and dbNSFP databases, and leverages family structure and known inheritance patterns.

Targeted sequencing of *LARS2* on stored DNA from the proband's deceased sibling (2a) was performed by the NSW Health Pathology Randwick Genomics Laboratory.

Patient 3 - Singleton exome sequencing of patient 3 was performed on a commercial basis at the Victorian Clinical Genetic Service laboratory on a Illumina HiSeq4000 using a Nextera Rapid Capture Exome Kit. Data was processed using Cpipe (Sadedin et al., 2015).

Patient 4 - This patient was included in a previously studied cohort of Complex I deficiency patients (Calvo et al., 2010). His DNA underwent singleton exome sequencing at the Broad Institute of MIT and Harvard using Illumina Capture Exome technology (v.1) supplemented with additional baits to capture mitochondrial DNA (mtDNA), in conjunction with Prof Vamsi Mootha and Dr Sarah Calvo. Data was mapped to NCBI hg19/GRCh37 human genome reference sequencing using BWA (Li & Durbin, 2009), and then targeted gene analysis was performed using GATK Best Practices recommendations (McKenna et al., 2010), HaplotypeCaller (DePristo et al., 2011; Van der Auwera et al., 2013), Variant Effect Predictor (McLaren et al., 2016), and Seqr (<https://seqr.broadinstitute.org/>). The exome data was analysed for high quality, autosomal recessive variants predicted as having high/moderate impact on protein with a $MAF < 0.005$. Analysis of mtDNA was performed as described previously (Lieber et al., 2013).

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Patient genomic data are available from the corresponding author on reasonable request.

In silico predictions of the effect of variants on protein function were made using SIFT (v6.2.0; Kumar et al., 2009) and PolyPhen-2 (HumVar; Adzhubei et al., 2010). For all reported SIFT analyses, the final set of aligned sequences had a median conservation score of 3.0.

Cloning and leucyl-tRNA synthetase assays

Recombinant LARS2 proteins were produced to assess amino-acylation of the identified variants. *LARS2* (NM_015340.3) variants were introduced into the pET22b/LARS2 construct using site-directed mutagenesis (Yao, Wang, Wu, & Wang, 2003). N-terminal 6-His tagged wild-type (WT) and variant LARS2 were expressed and purified as previously described (Yao et al., 2003). Since leucylation in mitochondria follows rules similar to those for *E. coli* leucylation (Sohm et al., 2004), *E. coli* tRNA₅^{Leu}(UAA) was used as the amino-acylation substrate in these experiments. The sequence of *E. coli* tRNA₅^{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 as previously described (Sohm et al., 2003). Kinetic parameters for tRNA leucylation were determined from Lineweaver-Burk plots by varying *E. coli* tRNA^{Leu} transcript (from 0.2 to 2.4 μ M) and WT or variant LARS2 (from 3 to 30 nM). Further, for WT and p.(Arg103His) LARS2, amino-acylation plateaus were obtained in the presence 0.65 μ M of tRNA^{Leu} transcript and 20 nM of each LARS2 variant and two different ATP concentrations (0.5 and 2.5 mM). Data were expressed as the mean of at least two independent experiments $\pm$ SEM.

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Immunoblotting

Immunoblotting and densitometry were performed as previously described, with the following modifications: membranes were probed with a 1:250 dilution of anti-LARS2 (Abcam ab96221), 1:500 anti-OXPHOS (Abcam ab110411) or a 1:1000 dilution of anti-VDAC1 (Abcam ab14734), overnight at 4°C (Riley et al., 2013). Quantitation was performed using Image Studio Lite (Li-cor Biosciences). LARS2 and RC complex subunit intensities were normalized to VDAC1 intensities.

RESULTS

Clinical features of the *LARS2* phenotypic spectrum

Four probands with potentially pathogenic biallelic *LARS2* variants were identified by independent exome sequencing analyses. More detailed clinical histories are provided in the Supp. Materials and family pedigrees in Supp. Figure S1. Patients from two unrelated families (patient 1 and patients 2a & 2b) had similar clinical features and resembled those of a previously reported individual with HLASA (Table 1) (Riley et al., 2016). The severity of hydrops varied among cases. Lactic acidosis and anemia were present in all cases, with sideroblastic anemia confirmed in the two patients who had a bone marrow aspirate. Pulmonary hypertension was a common feature. These new HLASA cases were all male and had hypospadias. There were variable effects on liver, cardiac and renal systems. Patient 1 had neonatal cholestasis (Supp. Figure S2), while patients 2a and 2b had hepatosplenomegaly. Patient 1 and 2b were not as severely affected as the first reported HLASA case and both survived the neonatal period. Lactic acidosis and anemia resolved in both children. They have sensorineural hearing loss and developmental delay.

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Patient 3, a male, had sensorineural hearing loss and two sisters in their 30's with hearing loss, primary amenorrhoea, and onset of leukodystrophy in adulthood. His sisters died without a genetic diagnosis but their clinical features are similar to the recently reported *LARS2* deafness and ovarioleukodystrophy phenotype (van der Knaap et al., 2019).

Patient 4 with reversible myopathy, lactic acidosis, and developmental delay, had a unique *LARS2* phenotype with some features overlapping with mild *YARS2* MLASA2 presentations (Riley et al., 2018; Sommerville et al., 2017). He had an unusual form of muscle degeneration predominantly seen in neck muscle (Figure 1a).

Identification of *LARS2* variants

Exome sequencing identified biallelic variants in *LARS2* (NM_015340.3) in all patients. The *LARS2* variants, their allele frequency, inheritance, and *in silico* predictions of pathogenicity are shown in Table 2, and their conservation among species in Supp. Figure S3. Variants have been submitted to ClinVar [https://www-ncbi-nlm-nih-gov.ezproxy2.library.usyd.edu.au/clinvar/](https://www.ncbi.nlm.nih.gov/ezproxy2.library.usyd.edu.au/clinvar/); (submission ID: SUB6018179; SCV000994653-SCV000994660). The c.683G>A [p.(Arg228His)] variant identified in patient 1 has previously been reported *in trans* with another *LARS2* missense variant in an individual with deafness and ovarioleukodystrophy (van der Knaap et al., 2019). It is noteworthy that the c.1552G>A [p.(Asp518Asn)] variant is not rare and 6 homozygotes have been reported in the gnomAD database, all of whom are adults >45 years of age (Patient 4, Table 2). All results were confirmed by Sanger sequencing. Sanger sequencing of DNA from patient 2a who died at day 1 confirmed that the same *LARS2* variants as 2b, the proband, were present. DNA was

not available from the parents or affected siblings of patient 3, however Sanger sequencing of unaffected siblings confirmed the *LARS2* variants were in *trans*.

LARS2 encodes mitochondrial leucyl-tRNA synthetase, which is responsible for attaching leucine to its cognate tRNA for mitochondrial protein synthesis. Six of the identified missense variants lie within the catalytic domain of *LARS2*, p.(Asp438Gly) is localized in the non-functional editing domain which enhances the catalytic efficiency (Ye et al., 2015), while p.(Thr700Ile) lies in the anticodon binding domain (Figure 2).

Amino-acylation activity of *LARS2* variants

The effect of the *LARS2* variants on *LARS2* amino-acylation activity was investigated by measuring the attachment of radiolabelled leucine to an *E. coli* tRNA^{Leu} substrate by purified recombinant *LARS2* variants. No significant differences in the yield or solubility of the variant proteins were observed during their production, suggesting that none of the recombinant *LARS2* variants were misfolded during their production in *E. coli* (data not shown). The *LARS2* variants identified in these patients result in reduced tRNA^{Leu} amino-acylation efficiency (Table 2). The k_{cat}/K_m ratio is proportional to the catalytic efficiency of the enzyme and thus allows comparison of each variant with the wild-type *LARS2*. For the HLASA patients (patients 1, 2a, 2b), there was one *LARS2* variant with a severe effect on amino-acylation (>10-fold loss in catalytic efficiency) in combination with a variant with less effect on amino-acylation. The *LARS2* variants in patients 3 and 4 had milder effects on amino-acylation activity compared to the HLASA patients, consistent with their less severe phenotypes.

The p.(Arg228His) LARS2 variant identified in patient 1 caused a 48-fold loss in catalytic efficiency (Table 2), mainly due to a decreased k_{cat} value (Supp. Table S1) suggesting the variant hinders efficient transfer of the leucyl moiety from the active site to the 3' end of the tRNA (Figure 2). p.Arg228 is highly conserved, including in bacterial leucyl-tRNA synthetase (LeuS) alignments (Supp. Figure S3). LARS2 p.Arg228 is equivalent to p.Arg178 in *Thermus thermophilus* LeuS that binds the leucyl-adenylate analog and is important for the activity of the enzyme. In contrast, LARS2 p.Asp438 is not highly conserved, and the p.(Asp438Gly) variant found in patient 1 had little effect on amino-acylation efficiency (< 2-fold) compared to wild-type LARS2 (Table 2).

The p.(Ala130Thr) LARS2 variant found in patients 2a and 2b had a 16-fold decrease in amino-acylation efficiency (Table 2), again mainly due to a decreased k_{cat} value (Supp. Table S1). p.Ala130 lies in the catalytic domain and is highly conserved (Supp. Figure S3). In the crystal structure of the homologous *E. coli* LeuS-tRNA^{Leu} complex, the equivalent amino acid of p.Ala130 is located in close vicinity of A76, the last tRNA nucleotide where the leucyl moiety is attached after amino-acylation, and is probably involved in the catalytic process (Figure 2) (Palencia et al., 2012). LARS2 p.Thr700, located in the anticodon binding domain, is conserved in mammals but not in invertebrates and prokaryotes and the p.(Thr700Ile) variant found in patients 2a and 2b led to a 3-fold loss in catalytic efficiency (Table 2).

The p.(Pro536Leu) variant identified in patient 3, led to a 6-fold loss in catalytic efficiency. p.Pro536 is conserved in most species (Supp. Table S1). The p.(Gln147Pro) variant identified in patient 3 caused a 2-fold loss in amino-acylation

efficiency compared to the WT LARS2 (Table 2). p.Gln147 is located in the catalytic domain but is not conserved.

For variants identified in patient 4, the recombinant LARS2 p.(Arg103His) had a <2-fold loss of catalytic efficiency and p.(Asp518Asn) had a 6-fold loss of efficiency (Table 2). p.Asp518 is located in the catalytic domain (Figure 2) and conserved in eukaryotes but may be replaced by Asn in some bacteria (e.g. *B. subtilis*, *S. aureus*, *M. mobile*). Moreover, there are six reported healthy homozygotes with the p.(Asp518Asn) variant in the gnomAD database. Given this, it was unexpected that p.(Arg103His) only showed a 1.6-fold loss in catalytic efficiency. Since p.Arg103 is highly conserved and located next to the HIGH (His-Ile-Gly-His) motif, responsible for ATP binding (Figure 2), additional experiments were performed to investigate the effect of the ATP concentration on the amino-acylation efficiency of the LARS2 p.(Arg103His) variant. The aminoacylation of tRNA^{Leu} by WT LARS2 was not affected in the presence of low ATP concentration *in vitro* (Figure 3). On the contrary, tRNA^{Leu} amino-acylation with the LARS2 p.(Arg103His) variant was reduced when ATP concentration was lowered to 0.5 mM (Figure 3). Reports of mitochondrial ATP concentrations are sparse and varied but have been reported at 2.5 mM in cultured human cells and as low as 0.5 mM in isolated rat hepatocytes (Soboll, Grundel, Schwabe, & Scholz, 1984; Yoshida, Kakizuka, & Imamura, 2016).

Muscle histopathology and immunoblotting

Further studies were undertaken for patient 4 who had a phenotype not previously reported in association with *LARS2* variants: myopathy, lactic acidosis and mitochondrial complex I enzyme deficiency in muscle, with improvement by adulthood. Muscle histopathology on biopsies taken at two years of age showed

evidence of a mitochondrial myopathy (Figure 1a). A neck extensor muscle biopsy showed marked variation in muscle fiber size with many small degenerate fibers and some regenerating fibers. There was a mild increase in the number of central nuclei, and mild peri-and endomysial fibrosis was seen. There was evidence of an unusual form of degeneration, with the Gomori trichome stain showing a flocculent grey granular appearance and accumulation of mitochondria, whilst the acid phosphatase stain also showed changes consistent with degeneration. Fiber typing showed a marked reduction of Type I fibers. Oil Red O staining revealed increased numbers of large droplets, many of which had irregular shapes. Quadriceps muscle showed mild variation of muscle fiber size, probably within normal limits (Figure 1a). There was no increase in central nuclei, but the Gomori trichome staining revealed subsarcolemmal accumulation of mitochondria, with other stains normal and indicative of a normal fiber type distribution.

The effect of the *LARS2* variants identified in patient 4 on endogenous levels of *LARS2* protein and representative subunits of each of the respiratory chain (RC) complexes was also examined. Immunoblotting of *LARS2* from patient 4 muscle showed levels of *LARS2* were reduced to ~20% of age-matched control levels (Figure 1b). Complex I subunit NDUF8 levels in patient 4 muscle were reduced to ~12% of the control level, (Figure 1b), consistent with the reported isolated complex I enzyme deficiency in muscle. All other RC complex subunits were moderately reduced (II-SDHB 40%, III-UQCRC2 75%, IV-COX II 70%, V-ATP5A 65% of control). Immunoblotting of patient fibroblasts showed no change in *LARS2* protein level and no effect on mitochondrial RC complex protein levels relative to control fibroblasts (results not shown).

DISCUSSION

Here we report the phenotypic spectrum of individuals from four families with biallelic *LARS2* sequence variants. The additional HLASA cases reported in this study confirm HLASA as a *LARS2* phenotype and suggest that the three known *LARS2* phenotypes represent a spectrum of disease severity.

Patients 1, 2a and 2b in this study have clinical features consistent with HLASA, although hydrops was not as severe as the previously reported case. Abnormalities were detected *in utero* by ultrasound in all four HLASA cases, ranging from increased nuchal translucency, suspected fetal anemia, shortening of long bones and suspected micropenis to severe hydrops, and 3 of 4 cases were delivered prematurely due to fetal distress. All had respiratory distress and pulmonary hypertension at birth and required respiratory support. All four had severe lactic acidosis in the newborn period and were anemic. Many of the presenting clinical features of HLASA resolved in the two individuals who survived the neonatal period. Interestingly, micropenis/hypospadias was evident in three of these new cases, suspected on antenatal ultrasound. Hypospadias has only been previously reported in one *LARS2* Perrault syndrome case (Demain et al., 2017). Whereas ovarian dysgenesis is a defining feature of Perrault syndrome in females, most males are not reported to have genital anomalies or subfertility. Endocrine investigations in patient 2b at age 4 months were normal for age with no evidence of gonadal failure. These additional HLASA patients confirm hypospadias as a *LARS2* phenotype and the surviving patients who have sensorineural hearing loss, developmental delay, and male external genital anomalies suggest HLASA may represent the severe end of the Perrault syndrome spectrum.

Recently cases of *LARS2*-associated Perrault syndrome with neurological symptoms have been reported (Kosaki et al., 2018; van der Knaap et al., 2019). In five of six of these patients developmental delay was present, as in the surviving HLASA cases, and one patient was reported to be small for gestational age. Leukodystrophy was confirmed in three patients. These patients developed neurological features anywhere from early childhood to 45 years of age. In this study, the female siblings of patient 3 had Perrault syndrome and leukodystrophy, with neurological features presenting at 28 and 34 years of age while patient 3 has no neurological symptoms at age 55 years. While the female siblings did not have genetic testing, it is probable that they have the same genotype as their brother but with a more severe phenotype, suggesting *LARS2*-associated disease may be affected by genetic and/or environmental modifiers, and that long-term monitoring for new neurological features is required in all patients. The phenotypic features of families 1, 2 and 3 are all on the *LARS2* phenotypic spectrum and the severity of disease correlates somewhat with the degree of loss of aminoacylation activity caused by the variants involved.

Phenotypic variability is common in Perrault syndrome, which is caused by variants not only in *LARS2* but also *HARS2* (MIM# 614926), *TWINK* (MIM# 616138), *CLPP* (MIM# 614129), *ERAL1* (MIM# 617565), and *HSD17B4* (MIM# 233400) (Newman, Friedman, & Conway, 1993). Most of these genes are involved in mitochondrial proteostasis, suggesting common pathways may be impacted. A wide phenotypic spectrum has also been observed in *HSD17B4*-associated disease that ranges from Perrault syndrome to D-bifunctional protein deficiency (MIM# 261515) which is a severe, usually infantile lethal disease (Pierce et al., 2010). The severity of disease is related to the level of dysfunction caused by *HSD17B4* variants and the functional domain in which the variants are located. Similarly, in this study *LARS2* variants

associated with HLASA had a more severe effect on amino-acylation than those in the less severe phenotypes. Neurological symptoms have been reported in Perrault syndrome associated with variants in *TWNK*, *CLPP*, and *HSD17B4* as well as *LARS2* (Demain et al., 2017; Kosaki et al., 2018; Lerat et al., 2016; van der Knaap et al., 2019). A few cases of male infertility (azoospermia) have been reported in males with *CLPP* and *HSD17B4* variants, and both male and female *CLPP* knockout mice are sterile (Demain et al., 2017; Lerat et al., 2016). A male with *LARS2* Perrault syndrome had hypospadias while his sister had oligomenorrhoea with a small uterus and ovaries (Demain et al., 2017). A strain of *C. elegans* with a homozygous protein-truncating *LARS2* variant was sterile, and had smaller gonads than wild-type controls (Pierce et al., 2013). This study indicates that male external genital anomalies can occur in *LARS2*-associated disease, and male gonadal dysgenesis could also occur but may be underreported in *LARS2* Perrault syndrome.

There is also phenotypic overlap between *LARS2*-associated disorders and those caused by other mitochondrial aminoacyl-tRNA synthetases. *HARS2* variants are also associated with Perrault syndrome (Pierce et al., 2011). There is some phenotypic overlap between some *LARS2* cases and patients with variants in *MARS2* and *AARS2*, encoding mitochondrial methionyl- and alanyl-tRNA synthetases respectively.

Variants in *MARS2* cause autosomal recessive spastic ataxia with leukoencephalopathy (ARSAL; MIM# 611390) and developmental delay and sensorineural hearing loss (MIM# 616430) (Bayat et al., 2012; Webb et al., 2015).

Variants in *AARS2* have been associated with leukodystrophy and premature ovarian failure (Dallabona et al., 2014). *YARS2* variants cause myopathy, lactic acidosis and sideroblastic anemia (MLASA2) and severe cases bear some similarity to HLASA while mild cases bear some similarity to the phenotype of patient 4 (Riley et al., 2018;

Riley et al., 2013; Sommerville et al., 2017). In some MLASA2 cases sideroblastic anemia resolved, as seen in patient 1 and 2b (Riley et al., 2018).

The phenotype of patient 4 included early developmental delay and lactic acidosis in common with patients 1 and 2b, but he also had reversible infantile-onset myopathy which has not previously been reported in association with *LARS2* variants.

Interestingly, the myopathy was most severe in the neck muscles, which are derived from a different lineage and regulated by distinct genetic programs compared to other muscle types (e.g., quadriceps) (Heude et al., 2018). The unusual features of this patient may be related to the combination of *LARS2* missense variants, one of which has reduced amino-acylation efficiency at low ATP concentrations, or could be a result of genetic and/or environmental factors. However, it is difficult to assess the limit at which a variant causes a phenotype *in vivo* based on the loss of efficiency as it does not always correlate with disease severity (Gonzalez-Serrano et al., 2019). It should therefore be kept in mind that in addition to a slight effect on amino-acylation, these variants could also alter the stability of the protein *in vivo* or its transport into the mitochondria and worsen the defect. Indeed, the immunoblot of patient 4 muscle showed a reduction in *LARS2* protein level while there was no observable difference in protein expression or stability of the recombinant variants. Similarly, we cannot exclude that some *LARS2* variants may differentially affect the amino-acylation of the two mt-tRNA^{Leu} isoacceptors encoded by the mitochondrial genome which may explain some discrepancies in the observed phenotypes. The patient's symptoms had largely resolved by adolescence, raising the possibility that the individuals in the gnomAD database homozygous for the p.(Asp518Asn) variant may have a very mild phenotype which has gone clinically unrecognised. Some other reversible infantile

mitochondrial diseases associated with mitochondrial translation defects have been described (Boczonadi, Bansagi, & Horvath, 2015).

In conclusion, we expand the clinical spectrum of *LARS2*-associated mitochondrial disease to include infantile lethal HLASA, infantile HLASA resolving to developmental delay and hearing loss, hypospadias in males, hearing loss with premature ovarian failure (Perrault syndrome), hearing loss with ovarioleukodystrophy, and reversible infantile-onset myopathy. We recommend these possible phenotypes be considered in association with *LARS2* variants and monitoring of patients for development of these clinical features.

DATA AVAILABILITY STATEMENT

Patient genomic data are available from the corresponding author on reasonable request. Variants reported in this study have been deposited in ClinVar [https://www-ncbi-nlm-nih-gov.ezproxy2.library.usyd.edu.au/clinvar/](https://www.ncbi.nlm.nih.gov/ezproxy2.library.usyd.edu.au/clinvar/); submission ID: SUB6018179.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

AUTHOR CONTRIBUTIONS

LGR performed patient 4 Sanger sequencing, *in silico* predictions, immunoblotting, cloning and wrote the manuscript; JR-T and MF designed and performed *in vitro* aminoacylation assays; KW and SP provided clinical history, diagnosis and management of patient 1; MWilson, ML, TIA, provided clinical history, diagnosis and management of patient 2a/b, CYN, TR, EK, SL, ZS provided genomic analysis of patient 2a/b; MWalsh and EH coordinated research consents and sample collection,

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and provided clinical history, diagnosis and management of patient 3; ST and AGC extracted patient 4 DNA for exome sequencing, curated the exome data and performed some confirmatory experiments; SA muscle histopathology for patient 4; DRT provided mitochondrial RC enzyme activities for patient 4; JC provided clinical history, diagnosis and management of patient 4, obtained consent and samples for the study and contributed to the overall conception and progression of the study. All authors contributed to editing the manuscript.

REFERENCES

- Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A. Bork, P.,... Sunyaev, S.R. (2010). A method and server for predicting damaging missense mutations. *Nature Methods*, 7, 248-249.
- Bayat, V., Thiffault, I., Jaiswal, M., Tetreault, M., Donti, T., Sasraman, F.,... Bellen, H. (2012). Mutations in the mitochondrial methionyl-tRNA synthetase cause a neurodegenerative phenotype in flies and a recessive ataxia (ARSAL) in humans. *PLoS Biology*, 10, 1-19.
- Boczonadi, V., Bansagi, B., & Horvath, R. (2015). Reversible infantile mitochondrial diseases. *Journal of Inherited Metabolic Disease*, 38, 427-435.
- Calvo, S. E., Tucker, E. J., Compton, A. G., Kirby, D. M., Crawford, G., Burt, N. P.,... Mootha, V. K. (2010). High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency. *Nature Genetics*, 42, 851-858.

- Dallabona, C., Diodato, D., Kevelam, S., Haack, T., Wong, L. J., Salomons, G.,... van der Knaap, M. (2014). Novel (ovario) leukodystrophy related to AARS2 mutations. *Neurology*, 82, 2063-2071.
- Demain, L. A., Urquhart, J. E., O'Sullivan, J., Williams, S. G., Bhaskar, S. S., Jenkinson, E. M.,... Newman, W. G. (2017). Expanding the genotypic spectrum of Perrault syndrome. *Clinical Genetics*, 91, 302-312.
- DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C.,... Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, 43, 491-498.
- Gonzalez-Serrano, L. E., Chihade, J. W., & Sissler, M. (2019). When a common biological role does not imply common disease outcomes: Disparate pathology linked to human mitochondrial aminoacyl-tRNA synthetases. *Journal of Biological Chemistry*, 294, 5309-5320.
- Götz, A., Tynismaa, H., Euro, L., Ellonen, P., Hyötyläinen, T., Ojala, T.,... Suomalainen, A. (2011) Exome sequencing identifies mitochondrial alanyl-tRNA synthetase mutations in infantile mitochondrial cardiomyopathy. *American Journal of Human Genetics*, 88, 635-642.
- Heude, E., Tesarova, M., Sefton, E. M., Jullian, E., Adachi, N., Grimaldi, A.,... Tajbakhsh, S. (2018). Unique morphogenetic signatures define mammalian neck muscles and associated connective tissues. *Elife*, 7. doi:10.7554/eLife.40179

- Kosaki, R., Horikawa, R., Fujii, E., & Kosaki, K. (2018). Biallelic mutations in LARS2 can cause Perrault syndrome type 2 with neurologic symptoms. *American Journal of Medical Genetics A*, 176, 404-408.
- Kumar, P., Henikoff, S., & Ng, P.C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature Protocols*, 4, 1073-1082.
- Lerat, J., Jonard, L., Loundon, N., Christin-Maitre, S., Lacombe, D., Goizet, C.,... Marlin, S. (2016). An Application of NGS for Molecular Investigations in Perrault Syndrome: Study of 14 Families and Review of the Literature. *Human Mutation*, 37, 1354-1362.
- Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 25, 1754-1760.
- Lieber, D. S., Calvo, S. E., Shanahan, K., Slate, N. G., Liu, S., Hershman, S. G.,... Mootha, V. K. (2013). Targeted exome sequencing of suspected mitochondrial disorders. *Neurology*, 80, 1762-1770.
- McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A.,... DePristo, M. A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, 20, 1297-1303.
- McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R., Thormann, A.,... Cunningham, F. (2016). The Ensembl Variant Effect Predictor. *Genome Biology*, 17, 122.

Newman, W. G., Friedman, T. B., & Conway, G. S. (2014). Perrault Syndrome. In R. A. Pagon, M. P. Adam, H. H. Ardinger, S. E. Wallace, A. Amemiya, L. J. H. Bean, T. D. Bird, N. Ledbetter, H. C. Mefford, R. J. H. Smith, & K. Stephens (Eds.), *GeneReviews(R)*. Seattle (WA): University of Washington, Seattle; 1993-2020.

Palencia, A., Crépin, T., Vu, M. T., Lincecum, T. L., Jr., Martinis, S. A., & Cusack, S. (2012). Structural dynamics of the aminoacylation and proofreading functional cycle of bacterial leucyl-tRNA synthetase. *Nature Structural and Molecular Biology*, 19, 677-684.

Perret, V., Garcia, A., Grosjean, H., Ebel, J. P., Florentz, C., & Giegé, R. (1990). Relaxation of a transfer RNA specificity by removal of modified nucleotides. *Nature*, 344, 787-789.

Pierce, S. B., Chisholm, K. M., Lynch, E. D., Lee, M. K., Walsh, T., Opitz, J. M.,... King, M. C. (2011). Mutations in mitochondrial histidyl tRNA synthetase HARS2 cause ovarian dysgenesis and sensorineural hearing loss of Perrault syndrome. *Proceedings of the National Academy of Sciences U S A*, 108, 6543-6548.

Pierce, S. B., Gersak, K., Michaelson-Cohen, R., Walsh, T., Lee, M. K., Malach, D.,... Levy-Lahad, E. (2013). Mutations in LARS2, encoding mitochondrial leucyl-tRNA synthetase, lead to premature ovarian failure and hearing loss in Perrault syndrome. *American Journal of Human Genetics*, 92, 614-620.

Pierce, S. B., Walsh, T., Chisholm, K. M., Lee, M. K., Thornton, A. M., Fiumara, A.,... King, M. C. (2010). Mutations in the DBP-deficiency protein HSD17B4

cause ovarian dysgenesis, hearing loss, and ataxia of Perrault Syndrome.

American Journal of Human Genetics, 87, 282-288.

Riley, L. G., Heeney, M. M., Rudinger-Thirion, J., Frugier, M., Campagna, D. R., Zhou, R.,... Fleming, M. D. (2018). The phenotypic spectrum of germline YARS2 variants: from isolated sideroblastic anemia to mitochondrial myopathy, lactic acidosis and sideroblastic anemia 2. *Haematologica*, 103, 2008-2015.

Riley, L. G., Menezes, M. J., Rudinger-Thirion, J., Duff, R., de Lonlay, P., Rotig, A.,... Christodoulou, J. (2013). Phenotypic variability and identification of novel YARS2 mutations in YARS2 mitochondrial myopathy, lactic acidosis and sideroblastic anaemia. *Orphanet Journal of Rare Diseases*, 8, 193.

Riley, L. G., Rudinger-Thirion, J., Schmitz-Abe, K., Thorburn, D. R., Davis, R. L., Teo, J.,... Christodoulou, J. (2016). LARS2 Variants Associated with Hydrops, Lactic Acidosis, Sideroblastic Anemia, and Multisystem Failure. *Journal of Inherited Metabolic Disease Reports*, 28, 49-57.

Sadedin, S. P., Dashnow, H., James, P. A., Bahlo, M., Bauer, D. C., Lonie, A.,... Thorne, N. P. (2015). Cpipe: a shared variant detection pipeline designed for diagnostic settings. *Genome Medicine*, 7, 68. doi:10.1186/s13073-015-0191-x

Soboll, S., Grundel, S., Schwabe, U., & Scholz, R. (1984). Influence of fatty acids on energy metabolism. 2. Kinetics of changes in metabolic rates and changes in subcellular adenine nucleotide contents and pH gradients following addition of octanoate and oleate in perfused rat liver. *European Journal of Biochemistry*, 141, 231-236.

- Sohm, B., Sissler, M., Park, H. King, M.P. & Florentz, C. (2004). Recognition of human mitochondrial tRNA^{Leu(UUR)} by its cognate leucyl-tRNA synthetase. *Journal of Molecular Biology*, 339,17-29.
- Sohm, B., Frugier, M., Brulé, H., Olszak, K., Przykorska, A., & Florentz, C. (2003). Towards understanding human mitochondrial leucine aminoacylation identity. *Journal of Molecular Biology*, 328, 995-1010.
- Sommerville, E. W., Ng, Y. S., Alston, C. L., Dallabona, C., Gilberti, M., He, L.,... Gorman, G. S. (2017). Clinical Features, Molecular Heterogeneity, and Prognostic Implications in YARS2-Related Mitochondrial Myopathy. *JAMA Neurology*, 74, 686-694.
- Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A.,... DePristo, M. A. (2013). From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Current Protocols in Bioinformatics*, 43, 11.10.11-33.
- van der Knaap, M. S., Bugiani, M., Mendes, M. I., Riley, L. G., Smith, D. E. C., Rudinger-Thirion, J.,... Mochel, F. (2019). Biallelic variants in LARS2 and KARS cause deafness and (ovario)leukodystrophy. *Neurology*, 92(11), e1225-e1237. doi:10.1212/wnl.00000000000007098
- Webb, B., Wheeler, P., Hagen, J., Cohen, N., Linderman, M., Diaz, G.,... Schadt, E. (2015). Novel, compound heterozygous, single nucleotide variants in MARS2 associated with developmental delay, poor growth, and sensorineural hearing loss. *Human Mutation*, 36, 587-592.

- Yao, Y., Wang, L., Wu, X., & Wang, E. (2003). Human mitochondrial leucyl-tRNA synthetase with high activity produced from *Escherichia coli*. *Protein Expression and Purification*, 30, 112-116.
- Ye, Q., Wang, M., Fang, Z., Ruan, Z., Ji, Q, Zhou, Z & Wang. E. (2015). Degenerate connective polypeptide 1 (CP1) domain from human mitochondrial leucyl-tRNA synthetase. *Journal of Biological Chemistry*, 290, 24391-24402.
- Yoshida, T., Kakizuka, A., & Imamura, H. (2016). BTeam, a Novel BRET-based Biosensor for the Accurate Quantification of ATP Concentration within Living Cells. *Science Reports*, 6, 39618. doi:10.1038/srep39618

FIGURE LEGENDS

Figure 1. Analyses of Patient 4 muscle biopsies. **(a)** Histological findings.

Haemotoxylin and eosin staining of neck extensor (i) and quadriceps (ii) Gomori trichrome staining of neck extensor (iii) and quadriceps (iv) NADH staining of neck extensor (v) and quadriceps (vi). Acid phosphatase staining of neck extensor (vii).

Images taken at 20× magnification except (v) and (vii) at 40×. **(b)** Immunoblot for LARS2 and subunits of the RC complexes (I –NDUFB8, II-SDHB, III-UQCRC2, IV-COX II, V-ATP5A) in patient 4 (P4) and age-matched control (C1) muscle replicates.

Note that these RC complex subunits are labile if their complex is not assembled.

VDAC1 was used as a loading control.

Figure 2. Localisation of LARS2 variants on the crystal structure of the *E. coli*

LeuS/tRNA^{Leu} complex (PDB: 4AQ7). **(a)** LARS2 is a class I ARS homologous to *E. coli* LeuS which displays a catalytic domain (cyan), an editing domain (orange) and a C-terminal tRNA binding domain (grey). The catalytic domain binds ATP (red) and leucine (green) to form leucyl-adenylate. The leucyl moiety is then transferred to the 3' end of tRNA^{Leu} (magenta). In *E. coli*, the editing domain is involved in the hydrolysis of mischarged tRNA^{Leu} (mainly with isoleucine) to ensure translation fidelity. This domain does not function as an editing domain in LARS2 but is crucial in enhancing the catalytic efficiency. Similarly, the zinc (dark blue) binding domain is not functional in LARS2. *E. coli* LeuS nucleotides equivalent to LARS2 variants are underlined by yellow spheres. They correspond to LARS2 p.(Arg103) (*E. coli* Arg54), LARS2 p.(Ala130) (*E. coli* Ala81), LARS2 p.(Gln147) (*E. coli* Ala98), LARS2 p.(Arg228) (*E. coli* Arg178), LARS2 p.(Asp438) (*E. coli* Gly409), LARS2 p.(Asp518) (*E. coli* Asp488), LARS2 p.(Pro536) (*E. coli* Pro506) and LARS2 p.(Thr700) (*E. coli* Tyr678). **(b)** Close up of the catalytic domain of *E. coli* LeuS highlighting variants that affected amino-acylation with a >3-fold loss in catalytic efficiency.

Figure 3. Effect of ATP concentration on WT and p.(Arg103His) LARS2 enzymatic activities. Leucylation reactions were carried out using 0.5 (●) or 2.5 mM (○) ATP in the presence of 0.65 μ M tRNA^{Leu} transcript and 20 nM of wild-type (blue) and p.(Arg103His) (red) enzymes. Values are mean (SEM), n = 3.

Table 1. Clinical features of *LARS2* HLASA cases

Clinical Feature	Patient 1	Patient 2a	Patient 2b	Previous Report (Riley et al., 2016)
<i>LARS2</i> variants (NM_015340.3)	c.[683G>A];[1313A>G] p.[(Arg228His)];[(Asp438Gly)]	c.[388G>A];[2099C>T] p.[(Ala130Thr)];[(Thr700Ile)]	c.[388G>A];[2099C>T] p.[(Ala130Thr)];[(Thr700Ile)]	c.[1289C>T];[1565C>A] p.[(Ala430Val)];[(Thr522Asn)]
Sex	M	M	M	F

Age	18 months	Deceased at Day 1	8 months	Deceased at Day 5
IUGR	Yes			Yes
Fetal Distress	No	Yes	Yes	Yes
		Delivered at 33 weeks	Delivered at 32 weeks	Delivered at 29 weeks
Evidence of hydrops	Pericardial effusion, ascites	Increased nuchal translucency	Increased nuchal translucency, fetal anemia, mild pleural effusions, polyhydramnios	Pericardial effusions, ascites, scalp edema, oligohydramnios
Lactic Acidosis	Yes	Yes	Yes	Yes
		24 mmol/L	11 mmol/L	16 mmol/L
Sideroblastic Anemia	Yes	Anemia (no bone marrow smear) Hb 53 g/L	Anemia (no bone marrow smear) Hb 71 g/L	Yes Hb 19 g/L
Sensorineural hearing loss	Yes	ND	Yes	ND
Developmental delay	Yes	ND	Yes	ND
Gonadal Dysgenesis	Hypospadias	Micropenis	Penoscrotal hypospadias, undescended testes	ND

Pulmonary Hypertension	Yes	ND	Yes	Yes
Respiratory	Hypoxic respiratory failure	Hypoxic respiratory failure	Hypoxic respiratory failure	Hyaline Membrane Disease
Cardiac	Neonatal low blood pressure	ND	PDA resolved spontaneously	PDA, ventricular septal defect, overriding aorta, tachyarrhythmias
Liver	Neonatal cholestasis	Hepatosplenomegaly	Hepatosplenomegaly, liver cyst	Disordered coagulation, low albumin, extramedullary hematopoiesis, dyserythropoiesis
Renal	No	ND	No	Rising creatinine, hematuria
Other	Exocrine pancreatic insufficiency	Hypoglycaemia, hyperinsulinism, low cortisol, fetal long limb shortening	Cerebral atrophy. Hypoglycaemia, hyperinsulinism, low cortisol, fetal long limb shortening	Seizures

ND not determined

Table 2. *LARS2* variants identified, their predicted pathogenicity, and aminoacylation efficiency measured *in vitro*

Patient	<i>LARS2</i> Variant (NM_015340.3) & location	Inheritance	gnomAD Allele Frequency	SIFT prediction; score; (median)	PolyPhen 2 prediction; score; (sensitivity; specificity)	Loss of catalytic efficiency†
1	c.683G>A p.(Arg228His) Catalytic domain	Maternal	-	Deleterious; 0; (3.0)	Probably Damaging; 1.000; (0.00; 1.00)	48
1	c.1313A>G p.(Asp438Gly) Editing	Paternal	-	Tolerated; 0.69; (3.0)	Benign; 0.082; (0.92; 0.67)	1.8

	domain						
2a, 2b	c.388G>A p.(Ala130Thr) Catalytic domain	Maternal	-	Tolerated; 0.11; (3.0)	Possibly Damaging ; 0.653; (0.79; 0.84)	16	
2a, 2b	c.2099C>T p.(Thr700Ile) Anticodon binding domain	Paternal	-	Tolerated; 0.05; (3.0)	Possibly Damaging ; 0.770; (0.76; 0.86)	2.7	
3	c.440A>C p.(Gln147Pro) Catalytic domain	-	-	Tolerated; 0.26; (3.0)	Benign; 0.06; (0.92; 0.65)	2.2	
3	c.1607C>T p.(Pro536Leu	-	-	Deleteriou s; 0.03; (3.0)	Probably Damaging ; 0.979; (0.92; 0.65)	6.2	

	)			(3.0)	(0.57;	
					0.94)	
	Catalytic					
	domain					
4	c.308G>A	Maternal	0.000016	Deleteriou	Probably	1.6
	p.(Arg103His			s; 0;	Damaging	
	)			(3.0)	; 1.000;	
	Catalytic				(0.00;	
	domain				1.00)	
4	c.1552G>A	Paternal	0.0039	Deleteriou	Probably	5.9
	p.(Asp518As			s; 0;	Damaging	
	n)			(3.0)	; 1.000;	
	Catalytic				(0.00;	
	domain				1.00)	

†Loss of efficiency is calculated as a fold-change relative to wild-type (WT) LARS2.

Corresponding data are found in supplementary Table 1.

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