COLD SPRING HARBOR
Molecular Case Studies

RAPID COMMUNICATION

Biallelic variants in *VARS* in a family with two siblings with intellectual disability and microcephaly: case report and review of the literature

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Abstract Two male siblings ages 15 and 10 yr old had similar features of intellectual disability, developmental delay, severe speech impairment, microcephaly, prematurity, and transient elevation of liver enzymes in infancy. Exome sequencing revealed one novel (c.65C>A; p.Ala22Asp) and one ultra-rare (c.3214T>C; p.Phe1072Leu) predicted damaging missense variant in *trans* in the gene encoding cytoplasmic valyl-tRNA synthetase (*VARS*). Biallelic variants in *VARS* have previously been associated with a neurodevelopmental disorder characterized by microcephaly, seizures, and cortical atrophy (NDMSCA; MIM #617802). Although our patients have no history of seizures or cortical atrophy, we suggest that the biallelic variants in *VARS* p.Ala22Asp and p.Phe1072Leu in this family are likely pathogenic and associated with NDMSCA, expanding the clinical phenotype of the condition.

[Supplemental material is available for this article.]

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CASE PRESENTATIONS

Index case (II-1, Supplemental Fig. 1) is a 15-yr-old male with intellectual disability (ID), severe speech impairment, acquired microcephaly, and hypotonia (Table 1). The pregnancy was unremarkable except for elevated α-fetoprotein (AFP) levels on maternal serum screening, which were later found normal in amniotic fluid analysis. He was born at 31 wk of gestation with normal weight (1.47 kg [25%–50%]), length (40.6 cm [50%]), and head circumference (28 cm [25%–50%]). He was admitted to the neonatal intensive care unit (NICU) because of prematurity and spent 45 d in the hospital with no major complications. He was noted to have neonatal tooth eruption. At 3 mo of age he was severely hypotonic. He had a transient mild elevation of his liver enzymes without an identifiable etiology during infancy, which resolved spontaneously. He sat without support at 4 yr of age, started crawling at ~4.5 yr of age, and now can walk with a gait trainer but usually scoots around on his bottom. He is nonverbal. He has had no regression of his milestones nor has he had problems with weight gain or short stature, but his head circumference fell >2 SDs below the mean at ~18 mo of age, and he remained microcephalic afterward. He has no problems with feeding and sleeping. He has repetitive movements and rocks his legs back and forth when seated and kicks his legs repeatedly when sitting on the floor. There is no history of seizures and an

[4]These authors contributed equally to this work.

Table 1. Clinical findings of patients with biallelic variants in *VARS*

	This study		Karaca et al. 2015			Stephen et al. 2018
Patient ID	II-1	II-2	BAB3643	BAB3186	BAB3187	II-2
Gender	Male	Male	Female	Female	Female	Male
Age	15-yr-old	10-yr-old	?	?	?	32-mo-old
Genotype	p.Ala22Asp p.Phe1072Leu	p.Ala22Asp p.Phe1072Leu	p.Arg1058Gln p.Arg1058Gln	p.Leu885Phe p.Leu885Phe	p.Leu885Phe p.Leu885Phe	c.1577-2A>G p.Met1064Ile
Intellectual disability	Yes	Yes	Yes	Yes	Yes	Yes
Speech	Nonverbal	Nonverbal	NA	NA	NA	Nonverbal
Microcephaly (congenital vs. acquired)	Yes (acquired)	Yes (NA)	Yes (NA)	Yes (NA)	Yes (NA)	Yes (congenital)
Seizures	No	No	Yes	Yes	Yes	Yes
Hypotonia	Yes	Yes	NA	NA	NA	Yes
Cortical atrophy	No	NA	Yes	Yes	Yes	Yes
Other						
Prematurity	Yes	Yes	NA	NA	NA	No
Elevated liver enzymes	Yes	Yes	NA	NA	NA	No
Stereotypic behavior	Yes	No	NA	NA	NA	No
Neonatal tooth	Yes	No	NA	NA	NA	No
Volvulus	No	Yes	NA	NA	NA	No
Dysmorphic features	Yes	No	NA	NA	NA	No

NA, not available.

overnight ambulatory EEG at age 4–5 yr showed no epileptiform discharges. A brain MRI was also found normal. Previous genetic studies including karyotype, chromosomal microarray, fragile X, MECP2 sequencing, and metabolic screening (urine organic acids, very long chain fatty acids, and lysosomal storage disorders) were all normal.

Case 2 (II-2, Supplemental Fig. 1) is the 10-yr-old brother of the proband, who is similarly affected with ID, severe speech impairment, microcephaly, hypotonia, transient elevation of liver enzymes in infancy, and prematurity. He was born at 27 wk of gestation with intestinal volvulus which was repaired surgically in the first week of life. He has always been underweight, and his normalized height has just recently started to decline. He is also nonverbal and nonambulatory.

There is no history of consanguinity between parents and the remaining family history is unremarkable. The parents are both of European ethnicity.

VARIANT INTERPRETATION

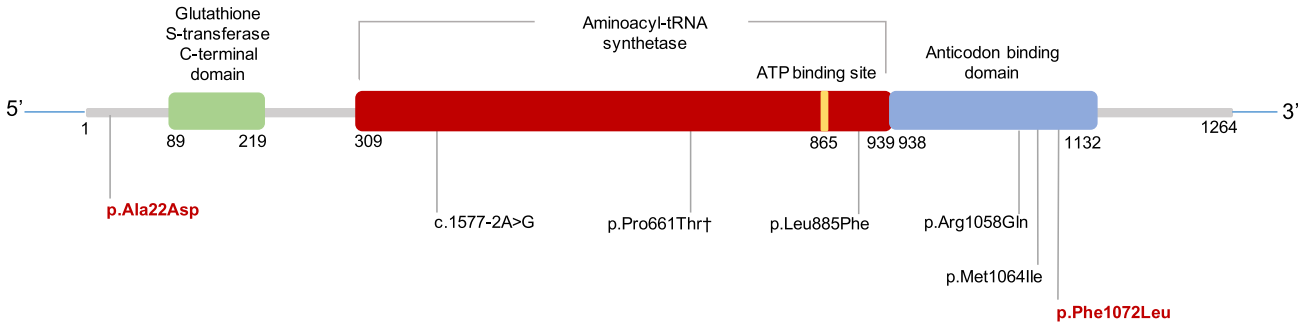
Our patients are compound heterozygous for one novel and one ultra-rare missense variant, NM_006295:c.65C>A:p.Ala22Asp and NM_006295:c.3214T>C:p.Phe1072Leu, in the gene encoding cytoplasmic valyl-tRNA synthetase protein (*VARS*). Although the p.Ala22Asp variant is not present in any population databases, p.Phe1072Leu is observed in one NonFinnish European allele in gnomAD. Computational prediction algorithms favor pathogenicity for both variants. Variants are evaluated as of uncertain significance (VUS) according to American College of Medical Genetics and Genomics (ACMG) variant interpretation guidelines fulfilling PM3_Supporting, PP1, and PP4 criteria (Richards et al. 2015), although because of the overlapping clinical features to the previously reported cases they are likely

Table 2. Genomic findings and variant interpretation

Gene	Genomic location	HGVS cDNA	HGVS protein	Zygosity	Parent of origin	Variant interpretation
VARS	Chr 6:31762930 (hg19) Chr 6:31795153 (hg38)	c.65C>A	p.Ala22Asp	Heterozygous	Maternal	Variant of uncertain significance
VARS	Chr 6:31747459 (hg19) Chr 6:31779682 (hg38)	c.3214T>C	p.Phe1072Leu	Heterozygous	Paternal	Variant of uncertain significance

causative of the patients' phenotypes (Table 2). No other likely pathogenic variants were identified that segregated with the phenotype in the family.

Aminoacyl-tRNA synthetases catalyze the aminoacylation of tRNA by their cognate amino acid. There are 37 aminoacyl-tRNA synthetases (18 cytoplasmic, 17 mitochondrial, 2 bifunctional) in human cells, and biallelic variants in many aminoacyl-tRNA synthetases have been reported in individuals with neurodevelopmental disorders (Meyer-Schuman and Antonellis 2017). Variants in *VARS* were first identified in patients with microcephaly, ID, and cortical atrophy (Karaca et al. 2015), and a recent study reported additional variants in patients with similar clinical findings associated with biallelic variants in *VARS* (Table 1; Stephen et al. 2018). VARS has three important conserved domains: glutathione S-transferase, carboxy-terminal domain (GST_C), tRNA synthetases class I (tRNA_synt_1), and anticodon-binding domain of tRNA (Anticodon_1). Four predicted likely pathogenic variants reported in the literature and in ClinVar are missense variants, and they all are located in either tRNA_synt_1 or Anticodon_1 domain. The only reported variant that is located outside of those domains is a canonical splice site variant (Fig. 1). The p.Phe1072Leu variant is also

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HUMAN 18 ALIAARYGEAG 28 ... 660 VPLCN 665 ... 880 ISLQGLHNQLL 890 ... 1050 YTCLDVGLRLLSPFMPFVTEELFQRLPRRMP 1080
PANTR 18 ALIAARYGEAG 28 ... 660 VPLCN 665 ... 880 ISLQGLHDQLL 890 ... 1050 YTCLDVGLRLLSPFMPFVTEELFQRLPRRMP 1080
MACMU 18 ALIAARYGEAG 28 ... 660 VPLCN 665 ... 880 VSLQGLHDQLL 890 ... 1050 YTCLDVGLRLLSPFMPFVTEELFQRLPRRMP 1080
MOUSE 18 ALIAARYGEAG 28 ... 659 VPLCN 664 ... 879 VSLQGLYDQLL 889 ... 1049 YTCLDVGLRLLSPFMPFVTEELFQRLPRRTP 1079
CANLF 18 ALIAARYGEAG 28 ... 660 VPLCN 665 ... 880 VSLQGLHDQLL 890 ... 1050 YTCLDVGLRLLSPFMPFVTEELFQRLPRRTL 1080
LOXAF 18 ALIAARYGEAG 28 ... 658 VPLCN 663 ... 878 VSLQGLHDQLL 888 ... 1048 YTCLDVGLRLLSPFMPFVTEELFQRLPRRTP 1078
RAT   18 ALIAARYGEAG 28 ... 660 VPLCN 665 ... 880 VSLQGLHDQLL 890 ... 1050 YTCLDVGLRLLSPFMPFVTEELFQRLPRRTP 1080
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Figure 1. Distribution of reported *VARS* variants in the literature and ClinVar over 2D representation of VARS domains given on InterPro for P26640 (SYVC_HUMAN) and the mutated residues' sequence alignment across species. Mutated residues are shown in bold black font for previously reported variants and in bold red font for variants reported in our study. HUMAN (P26640); *Homo sapiens*, PANTR (H2QSM5); *Pan troglodytes* (chimpanzee), MACMU (G7MRK4); *Rhesus macaque*; MOUSE (G3UY93); *Mus musculus*; CANLF (E2RTJ7), *Canis lupus familiaris* (dog); LOXAF (G5E7F5), African elephant; RAT (Q04462), *Rattus norvegicus*. †This variant is deposited into ClinVar but not reported in any publication.

located in the Anticodon_1 domain (SIFT: Damaging; PolyPhen-2: Benign; CADD v1.3: 24.8). The p.Ala22Asp variant is not located in any of the domains, but the prediction scores for this variant are supportive of pathogenicity (SIFT: Damaging; Polyphen-2: Damaging; CADD v1.3: 32). All of the previously reported variants and the variants we report are located in highly conserved residues (Fig. 1).

SUMMARY

Here we report biallelic missense variants in *VARS* in two similarly affected siblings with ID, severe speech impairment, acquired microcephaly, hypotonia, prematurity, and transient elevation of liver enzymes in infancy, consistent with previously reported findings in patients with biallelic *VARS* variants. Our patients did not have seizures or cortical atrophy, and this is different from some other reported patients in the literature, expanding the phenotypic spectrum of NDMSCA. Our patients share overlapping clinical features reported in other autosomal recessively inherited aminoacyl-tRNA synthetase disorders (i.e., IARS; MIM #617093, LARS; MIM #615438) including elevated liver enzymes along with microcephaly and hypotonia. Future clinical and functional studies are needed to better define the genotype–phenotype correlations and assess molecular mechanism of disease.

METHODS

Clinical exome sequencing was performed with peripheral blood DNA samples from the two affected brothers and their mother and was nondiagnostic. Raw data was requested from the diagnostic laboratory and reanalysis was performed. Variants were filtered by their population allele frequencies in Exome Aggregation Consortium (ExAC) (Lek et al. 2016), Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS/), 1000 Genomes Samples (Auton et al. 2015), and Genome Aggregation Database (gnomAD) (Lek et al. 2016) using 1% and 3% thresholds for autosomal dominant and autosomal recessive inheritance models, respectively. Biallelic predicted pathogenic variants that fit to an autosomal recessive inheritance pattern were identified. Variants were confirmed by Sanger sequencing, and they segregated with the affected individuals in the family (Supplemental Fig. 1).

ADDITIONAL INFORMATION

Data Deposition and Access

All sequence data and interpreted variants have been deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under accession numbers SCV000803173 (for c.65C>A) and SCV000803174 (for c.3214T>C).

Ethics Statement

Written informed consent was obtained for all individuals in this study. The study is approved by the Institutional Review Board of Columbia University under protocol #AAAJ8651.

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Competing Interest Statement

The authors have declared no competing interest.

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Author Contributions

V.O. and M.G. analyzed the data and drafted and critically reviewed the manuscript. A.W. provided the clinical data and critically reviewed the manuscript. W.K.C. conceived of the study, provided clinical data, analyzed the data, and drafted and critically reviewed the manuscript.

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