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Supplemental Data

Mutations in *QARS*, Encoding Glutaminyl-tRNA Synthetase, Cause Progressive Microcephaly, Cerebral-Cerebellar Atrophy, and Intractable Seizures

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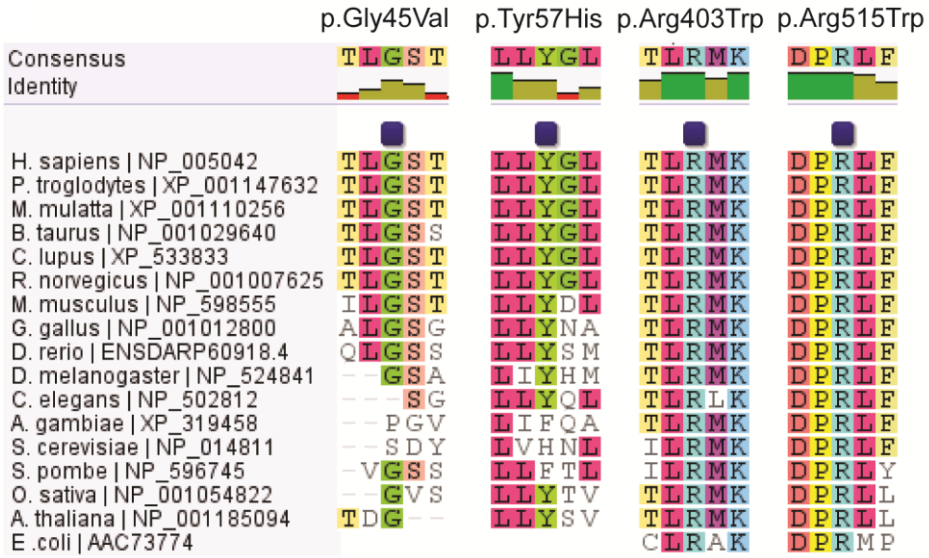

Figure S1. Amino acids affected by *QARS* mutations are highly conserved.

All four amino acids affected by human mutations are conserved in vertebrates and plants and two of them (Arg403 and Arg515) are conserved in all species examined.

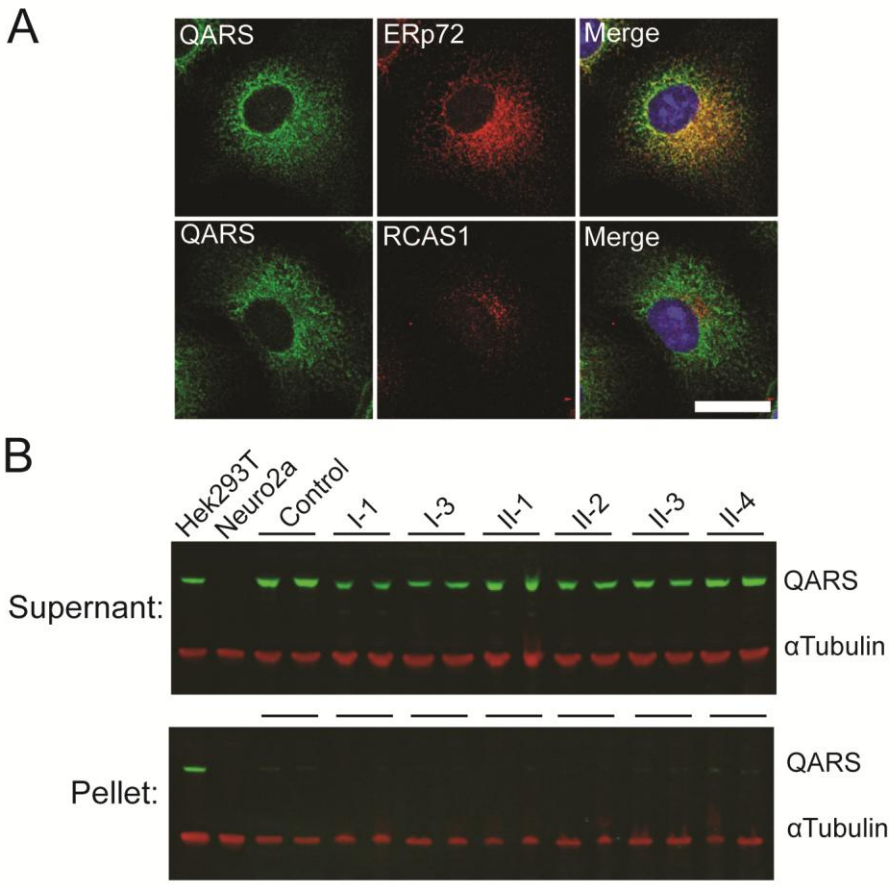

Figure S2. QARS subcellular localization, and protein levels in individual cell lines.

(A) Double labeling of endogenous QARS proteins with ER (ERp72) and Golgi (RCAS1) markers in Cos7 cells (monkey). Scale bar, 20 μm.

(B) (Top) Western-blot showing that anti-QARS antibody recognizes QARS protein (green) in human cell lines but not Qars protein in a mouse cell line (Neuro2a), and that QARS protein level is similar among tested control and individual lymphoblastoid cell lines in the supernant. (Bottom) Western-blot showing QARS protein level is low in the insoluble fraction of each lymphoblastoid cell lysate. Total protein lysates from HEK293T (human) and Neuro2a (mouse) represent positive and negative controls. Loading control, anti-αTubulin (red).

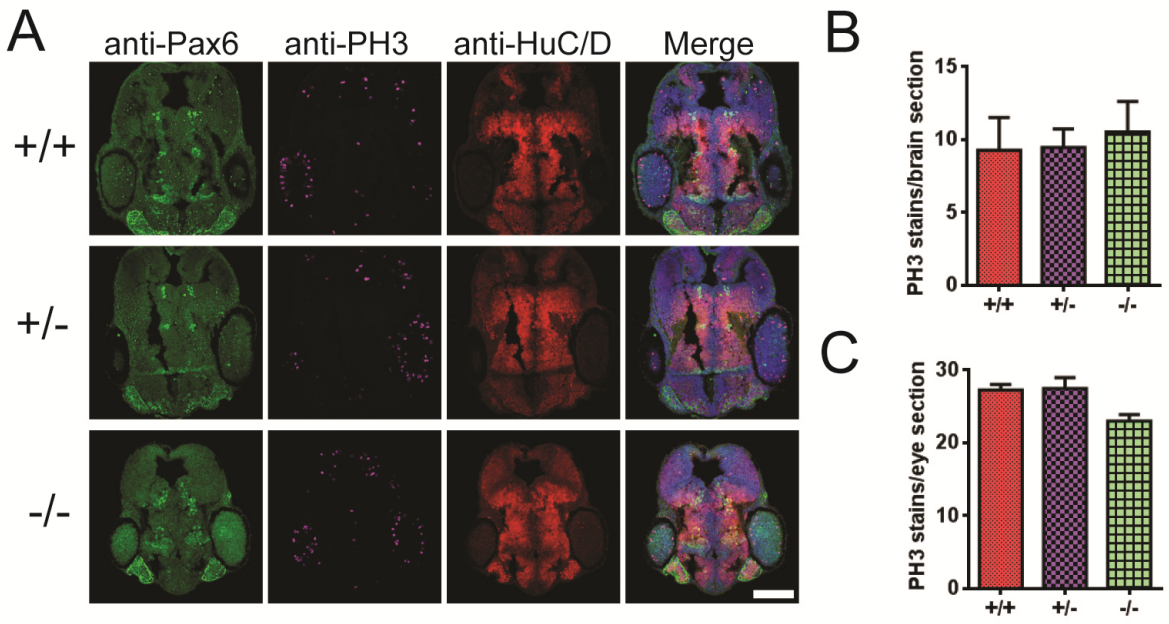

Figure S3. Neurogenesis in the brains and eyes are preserved in 2 dpf *qars* mutants.

(A-C) Immunostaining results of fish brain sections (A) showing neural progenitors (anti-Pax6), mitotic cells (anti-PH3) and postmitotic neurons (anti-HuC/D) display similar patterns in 2 dpf *qars* mutants when compared to their (+/+) and (+/-) siblings. Scale bar, 300 μm. Bar diagrams (B, C) showing that numbers of mitotic cells (anti-PH3 positive) are largely preserved in the brains and eyes of *qars* mutants when compared to their (+/+) and (+/-) siblings, mean ± SEM values are presented.

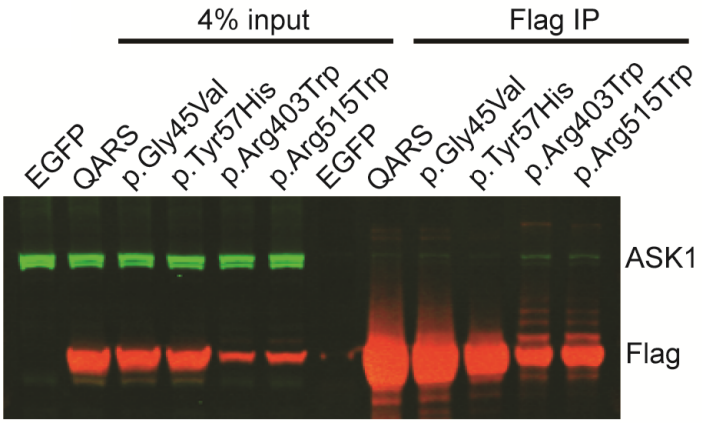

Figure S4. Effects of human variants on QARS-ASK1 interaction.

Individual recombinant QARS variant as well as wild-type protein were expressed and immunoprecipitated (mouse anti-Flag, clone M2) from a human cell line (HEK293T). Enrichment of recombinant proteins was confirmed by blotting with a rabbit anti-Flag antibody (red). Weak interactions with endogenous ASK1 (green) are detected for all recombinant QARS proteins.

Table S1. Oligo names and sequences.

Name	Sequence
CH87	5'-CCATTCTACACCCACCATTTG-3'
CH90	5'-GGGCTCAGTGTGGATCTTCTT-3'
CH91	5'-GACTCCCTGTCGCTCTTCAC-3'
CH92	5'-TGCCTGTGCAGAACTAGGTG-3'
CH93	5'-CACTGCTGCTCTTTGAGGTG-3'
CH94	5'-GTGCTCGATGGAGTCACAGA-3'
CH184	5'-CGGACGTGGTGTCTTCTTTC-3'
CH185	5'-CCAGTGATCTTGTCAATGGCAG -3'
CH186	5'-GCTAGCTTGCCAAACCTACAGGT -3'
CH188	5'-CAGCAGACCCTGGTTTCCACCATTGAC-3'
CH189	5'-GTCAATGGTGGAAACCAGGGTCTGCTG-3'
CH190	5'-CGAGGCCACACTATGGATGAAGCTGG-3'
CH191	5'-CCAGCTTCATCCATAGTGTGGCCTCG-3'
CH379	5'-ACGGTACCATGGAGCAGAAGCTGATCTCAGAAGAGGACCTGGACTACAAAGACGATGACGACAAGATGGCGGCTCTAGACTCCC-3'
CH380	5'-ATCTCGAGAGCTCACACCTTTCCTGGGTC-3'
CH381	5'-CGGGATCCTGTTACATGGCTTGGCCTC-3'
CH382	5'-GAGGCCAAGCCATGTAACAGGATCCCG-3'
CH383	5'-CTGGGATGACCCATGGCTCTTTACACTC-3'
CH384	5'-GAGTGTAAAGAGCCATGGGTCATCCCAG-3'
KCNT1-Ex1/2 F	5'-CATTGGTCAGCGAGTGAA-3'
KCNT1-Ex1/2 R	5'-GAACTGGCAGGACAGGTA-3'
KCNT1-Ex16/17 F	5'-TGGCTCCTGCCTGGTTCC-3'
KCNT1-Ex16/17 R	5'-AAAGTTCAGCATCAGTCA-3'

Table S2. Additional clinical findings and developmental measurements.

Individual	**I-1**	**I-2**	**II-1**	**II-2**
Gender	Male	Male	Male	Female
Year of birth	2008	2009	2008	2009
Age at most recent assessment	4 years	3 years	4.5 yrs	15 months
Delivery	Term (38wk)	Term	Term (41wk)	Term
Birth Weight	6.2lb (12%)	6.8lb (22%)	6lb (9%)	6.2lb (12%)
Birth Height	19.75" (50%)	19.5" (41%)	20.18" (66%)	19.29" (42%)
Birth Head Circumference	11" (-3.9SD)	12.25" (-2.1SD)	13.2" (-1.0SD)	13.2" (-1.0SD)
Seizure Onset	One hour after birth	First day of life	One hour after birth	One month old
Developmental Features	Profound delays, cortical visual impairment, normal hearing, chronic constipation, tracheomalacia, possible tapetoretinal degeneration as seen in Leber's congential amaurosis, no meaningful visual response in either eye, nutrition by Gtube.	Profound delays, no constipation, can bubble, nystagmus, nutrition by Gtube.	Lack of visual contact from birth. Profound psychmotor delay at 4.5 years.	Normal tone and eye contact until onset of seizures, then profound delays by 15 months.
Muscle tone	Mixed hypotonia and hypertonia	High tone with brisk reflexes	Severe hypotonia	Severe hypotonia
Dysmorphisms	Sloping forehead, bitemporal narrowing, hypotelorism, bilateral epicanthal folds, broad flat nasal bridge, high arched palate. At age 5 months skin exam with slightly raised red rash across his chest and abdomen.	Less of sloping forehead than brother, has bitemporal narrowing, epicanthal folds, hyptelorism, low set and posteriorly rotated ears, broad nasal bridge, high palate. Unremarkable skin exam.	Coarse facies, hypoplastic helix of ear and prominent upper lip.	N/A
Microcephaly	-4.8SD at 1.5 months; -10.4SD at 21months	-5.8SD at 3 months; -7.8SD at 7 months	-3SD at 4.5 years	-2.5SD at 15 months

Table S3. *QARS* mutations are rare and predicted to cause deleterious amino acid substitutions.

Person	Position (hg19)	Allele Frequency (6503 samples in EVS)	Amino acid change	SIFT	PolyPhen-2
I-3	chr3: 49141888 C>A	0	p.G45V	0.01	1
II-4	chr3: 49141853 A>G	0	p.Y57H	0.14	1
I-4	chr3: 49137482 G>A	0	p.R403W	0	1
II-3	chr3: 49136848 G>A	0	p.R515W	0	1

QARS mutations identified in both families were not seen in Exome Variant Server (EVS), and the amino acid substitutions are predicted to be damaging to protein functions by SIFT and PolyPhen-2.