Supporting Information

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SI Methods

Subjects. The family was referred by the diagnosing physician (J.M.O.). Control subjects were 982 individuals of self-described Caucasian ancestry without deafness or ovarian dysgenesis. Lymphoblast cell lines were established for all relatives, and DNA was extracted from blood and from cell lines of all participants using standard methods (1). The project was approved by the University of Washington Human Subjects Division (protocol 33468).

Linkage Analysis. Linkage analysis was carried out as previously described (1). Short tandem repeat (STR) markers spaced every 5–10 Mb throughout the genome were genotyped on an ABI 3100 Genome Analyzer and evaluated using Sequencing Analysis (v3.3) and Sequencher (v4.2) software. Lod scores for linkage of the phenotype to each marker and each pair of adjacent markers were calculated under a recessive model of full penetrance. For fine mapping of the linked region, additional STR markers were developed using the Simple Repeats track of the University of California, Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu/).

Sanger Sequencing. For the 58 genes in the region of linkage, PCR primers were designed to flank all exons and neighboring regulatory regions. Primers for *HARS2* are listed in Table S2. Amplified products were sequenced on an ABI 3100 Genome Analyzer.

Genomic Sequencing. The entire 4.142-Mb linkage region was tiled with overlapping cRNA oligonucleotide bait probes, using previously described methods (2). Genomic DNA from individual II-1 was hybridized to the baits and sequenced to a median 150-fold coverage, with 97% of targeted bases having a minimum of 20-fold coverage. Sequence variants were filtered against previously described polymorphisms in dbSNP131, the 1,000 Genome Project, and 60 exome sequences determined in our laboratory.

Control Genotyping. Genotyping of Caucasian control individuals was performed using custom Taqman genotyping assays (Applied Biosystems) according to manufacturer's instructions.

Analysis of Alternate Transcripts. Proportions of transcripts encoding the L200V and Δ200–211 mutant forms of HARS2 were determined by amplifying and cloning the critical regions of *HARS2* from lymphoblast cDNA, using PCR primers 5′-TCCCTTTGCTCGTTATCTGG-3′ and 5′-CCTTTCTTCACCACCATCTC-3′. Individual clones were sequenced.

cDNA Cloning and Mutagenesis. Human *HARS* and *HARS2* full-length cDNA sequences and yeast *HTS1* genomic sequence with −478 bp and +417 bp flanking sequence were obtained by PCR. Mutations were introduced using primers designed with the QuikChange Kit (Stratagene) guidelines and Phusion DNA Polymerase (Finnzymes). For mammalian cell expression, wild-type *HARS* and *HARS2* and mutant *HARS2* cDNAs were cloned into pCMV-3Tag-3 (FLAG) or pCMV-3Tag-9 (Myc) C-terminal epitope tagging vectors (Stratagene). For bacterial expression, wild-type and mutant *HARS2* cDNAs were cloned into pQE60 (Qiagen). For yeast transformation, wild-type and mutant *HTS1* were cloned into pRS316 (URA3) or pRS314 (TRP1) low-copy plasmids (3). The accuracy of all constructs was verified by sequencing.

Transfection, Cell Fractionation, and Western Blotting. 293T cells were transfected with Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Cytoplasmic and mitochondrial fractions were isolated with the Qproteome Mitochondria Isolation Kit (Qiagen) according to manufacturer's instructions. Proteins were immunoprecipitated from 56 μg mitochondrial extract with 0.4 μL anti-Myc antibody (no. 2276; Cell Signaling Technology) and Protein G Dynabeads (Invitrogen). Immunoprecipitates (IP) and total cytoplasmic and mitochondrial extracts (25% of IP input) were separated by SDS/PAGE, transferred to Immobilon-FL membrane (Millipore), and blotted with antibodies against Myc (0.5 μg/mL, A00704; Genscript), FLAG (0.5 μg/mL, A00170; Genscript), β-actin (1:10,000, A2228; Sigma), or VDAC (1:1,000, no. 4866; Cell Signaling Technology). Proteins were detected with IRDye-conjugated secondary antibodies, followed by analysis with an Odyssey infrared imaging system (LI-COR Biosciences). Myc and FLAG blots were stripped and reprobed for β-actin and VDAC, which served as cytoplasmic and mitochondrial controls, respectively, for fractionation and loading.

Purification of Recombinant HARS2 Proteins. Expression of wild-type and mutant *HARS2* in M15[pREP4] cells (Qiagen) was induced with 1 mM IPTG for 4 h at 37 °C. Proteins were purified by Ni^{2+}-affinity chromatography according to manufacturer's instructions (Qiagen). Protein concentrations were determined by Bradford assay (Bio-Rad), using BSA as the standard.

Pyrophosphate Exchange Assay. Pyrophosphate exchange assays were performed by a modification of published procedures (4, 5) in a 150-μL reaction containing 100 mM Tris (pH 8.0), 10 mM β-mercaptoethanol, 10 mM KF, 10 mM $MgCl_2$, 2 mM ATP, 10 μM histidine, 2 mM [^{32}P]Na_4PP_i, and 200 nM enzyme. Reactions were incubated at 37 °C for up to 40 min. Aliquots (20 μL) were quenched in 3.5% perchloric acid, 1% activated charcoal, followed by collection of the charcoal on GF/C filters (Whatman) and washing with 10 mM Na_4PP_i, pH 2.0. The amount of ^{32}P incorporated into ATP adsorbed onto the charcoal was determined by scintillation counting.

Yeast *HTS1* Deletion Mutant and Complementation Assay. The ORF of HTS1 was deleted in the diploid strain KGY315 (6) by PCR-mediated gene replacement with the Kan^R gene (7), which was generated from plasmid pFA6a-3HA-KanMX6 (8). Cells were transformed with plasmid pRS316-HTS1 (*HTS1*, *URA3*, *CEN*), to rescue *hts1Δ*, followed by sporulation and selection of the haploid *hts1Δ* strain on media containing G418 and lacking uracil. This strain failed to grow on media containing 5-fluoroorotic acid (5-FOA), which is toxic to Ura^+ yeast cells. Wild-type or mutant *HTS1*, expressed under the control of its endogenous promoter on a *TRP1* plasmid, was introduced into the rescued *hts1Δ* strain and tested for growth on media without tryptophan containing 5-FOA. Growth of 5-FOA resistant colonies indicated loss of the *HTS1-URA3* rescuing plasmid and complementation of the *HTS1* deletion by the gene carried on the *TRP1* plasmid.

***C. elegans* Strains.** *C. elegans* were maintained using standard methods (9). The *hars-1(tm4074)* allele contains a 662-bp out-of-frame deletion beginning at codon 41 or 42 of the cytoplasmic (*hars-1a*) or mitochondrial (*hars-1b*) isoform, respectively, which is predicted to truncate the protein upstream of the catalytic domain (http://www.shigen.nig.ac.jp/c.elegans). *hars-1(tm4074)* was

outcrossed with N2 four times before analysis and the *hars-1* deletion was verified by PCR and sequencing.

***C. elegans* RNAi by Feeding.** RNAi by feeding was performed essentially as described (10), using clones from the Ahringer RNAi libraries (11, 12). L4440 empty vector was used as a negative control. Plates were seeded with 0.35 mL liquid bacteria and dried overnight at room temperature. Adults were allowed to lay eggs for 2 h on the seeded bacteria expressing dsRNA and plates were incubated at 15 °C for 4 d. L4 progeny were transferred to new RNAi plates, incubated at 15 °C for 1 d, and then photographed or transferred individually to plates seeded with standard OP50 bacteria. F_2 progeny of individual F_1 animals were counted.

Measurement of *hars-1* Message Expression. *C. elegans* were harvested 48 h after egg deposition onto plates seeded with RNAi bacteria. Total RNA was isolated using TRIzol reagent (Invitrogen) and treated with Turbo DNA-free (Applied Biosystems). RNA was reverse transcribed using random primers and SuperScript III Reverse Transcriptase (Invitrogen). Quantitative PCR was performed on an ABI 7900HT Real-Time PCR System, using manufacturer's instructions and default machine settings. A custom assay for *hars-1b* and the following predesigned Taqman Gene Expression Assays (Applied Biosystems) were used: *hars-1a*, Ce02459560; *cdc-42*, Ce02435137; *pmp-3*, Ce02485188; and *Y45F10D.4*, Ce0246751. The expression of test genes was normalized to the geometric mean of the expression of *cdc-42*, *pmp-3*, and *Y45F10D.4*, which were previously determined to be stable endogenous controls for *C. elegans* (13). Samples were run in quadruplicate and averaged for subsequent calculation of gene expression by previously described methods (14).

Accession Numbers. GenBank protein sequences for HARS2 orthologs are as follows: *Homo sapiens* HARS2, NP_036340; *H. sapiens* HARS, NP_002100; *Mus musculus* HARS2, NP_542367; *M. musculus* HARS, NP_032240; *Xenopus laevis* HARS2, AAH76748; *Drosophila melanogaster* Aats-his, NP_728180; *C. elegans* HARS-1A, CAA93416; *S. cerevisiae* Hts1p, NC_001148; *T. thermophilus* HisRS, YP_004335; *E. coli* HisRS, NP_289067.

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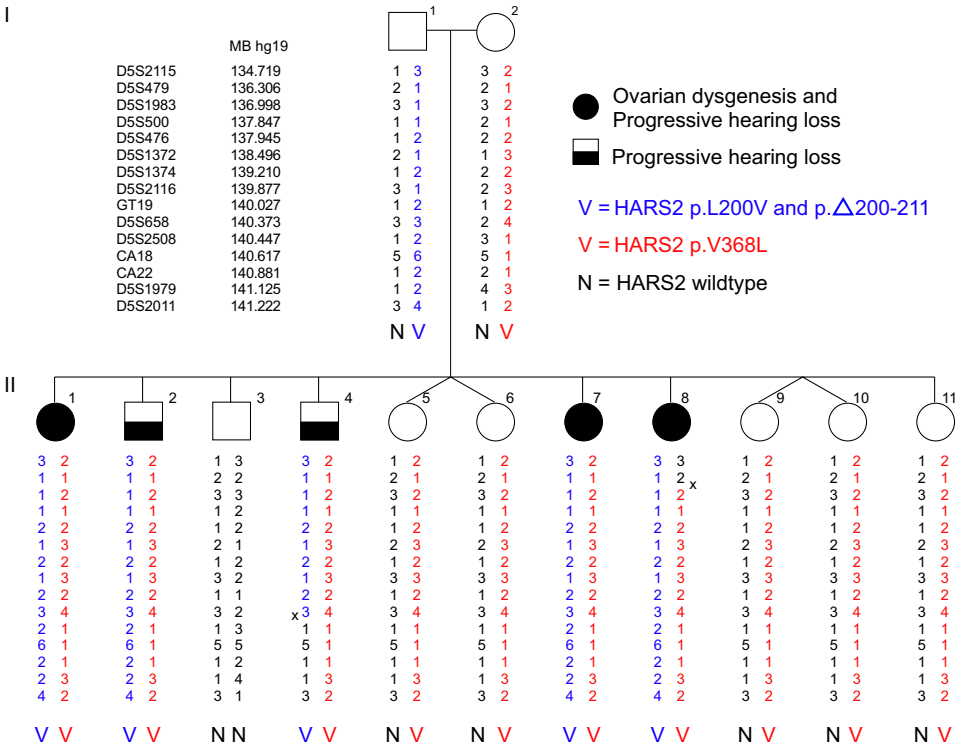

Fig. S1. Identification of mutations in HARS2 in a family with Perrault syndrome. A nonconsanguineous family of French, Irish, and Scottish ancestry, with Perrault syndrome in 5 of 11 siblings is shown. Linkage analysis mapped the critical gene to a 4.142-Mb region of chromosome 5q31, bounded by D5S479 and D5S2508. Affected siblings are compound heterozygotes for mutations in HARS2.

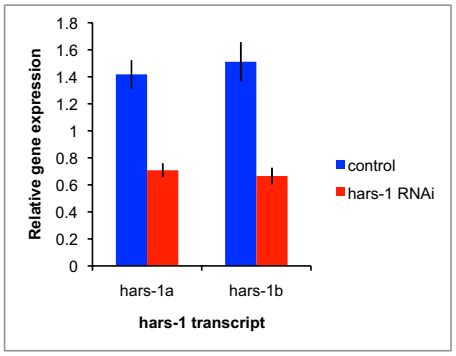

Fig. S2. Expression of *hars-1* after RNAi. Real-time qPCR analysis of RNA from *C. elegans* treated with *hars-1* RNAi indicates reduction of the cytoplasmic (*hars-1a*) and mitochondrial (*hars-1b*) *hars-1* transcripts. Values represent mean ± SD of data from three independent experiments.

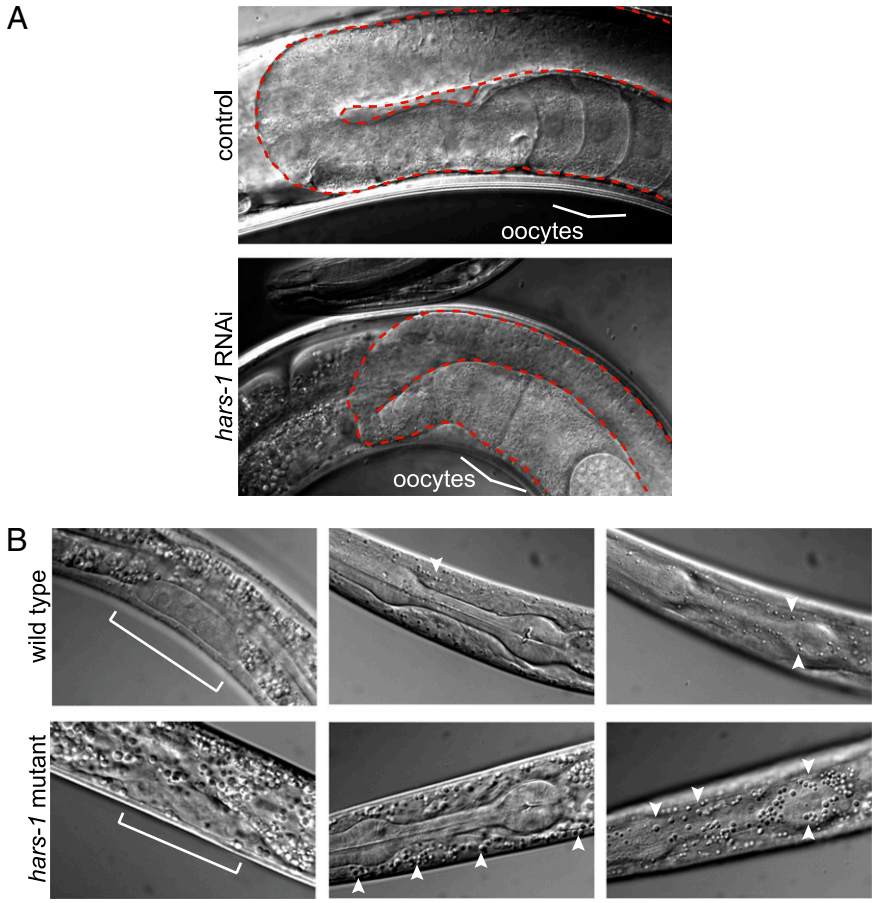

Fig. S3. Effects of *hars-1* RNAi and null mutation. (*A*) Eggs from wild-type N2 worms were laid onto control or *hars-1* RNAi bacteria and larvae were photographed after 5 d at 15 °C. Dashed lines outline the gonad. (*B*) *hars-1(tm4074)* homozygous mutant progeny of heterozygous mothers arrest development when the gonad is similar in size to the gonad of a wild-type L2 larva (*Left*, white brackets). The pseudocoelom of *hars-1(tm4074)* homozygous mutant larvae contains many more granules (arrowheads) than are present in wild-type L2 larvae (Center and Right, two planes of focus).

Table S1. Progeny of *hars-1(tm4074)*/+ parents

	Progeny of single adult, %				
Sample	L4 larvae/adults	L2 larvae	Dead on side	*N*	Inferred parental genotype
1	100.0	0.0	0.0	141	WT
2	100.0	0.0	0.0	121	WT
3	100.0	0.0	0.0	116	WT
4	100.0	0.0	0.0	113	WT
5	100.0	0.0	0.0	94	WT
6	98.6	0.7	0.7	143	WT
7	84.9	9.2	5.9	119	hars-1(tm4074)/+
8	84.6	14.1	1.3	78	hars-1(tm4074)/+
9	76.5	20.9	2.6	115	hars-1(tm4074)/+
10	75.7	19.1	5.2	115	hars-1(tm4074)/+
11	74.5	19.9	5.7	141	hars-1(tm4074)/+
12	73.6	19.8	6.6	121	hars-1(tm4074)/+
13	71.1	20.7	8.1	135	hars-1(tm4074)/+
14	70.4	22.4	7.1	98	hars-1(tm4074)/+

Single adult F_1 progeny of *hars-1(tm4074)*/+ parents were allowed to lay eggs overnight. F_2 progeny phenotype was scored 48 h after removal of adults. Over 99.9% of eggs hatched. Animals scored as "dead on side" were similar in size to arrested L2 larvae and both were presumed to be *hars-1(tm4074)* homozygotes.

Table S2. HARS2 primers

Exon	Forward primer (5′–3′)	Reverse primer (5′–3′)
1	CTGGCTACTAAGGGAACTTG	AAATCAAAACTCCAACCTCTC
2	TTGTGTGGTGAAGACCTGAC	AAAGCAGCCATAGTGAAAAC
3 and 4	AGGACTGACCTCTGCCTTGC	CCAAATGTCTGTGCTTCTGC
5 and 6	TGCTTTCTGCTGAACTTTTAG	AAACATCCCATCCACAATCC
7	ATTTCCATCCTTTTTGTGTG	AGTATGCCTCCTTACCTTCC
8	TTGGGGTGGAAGGTAAGGAG	GCTTTGCCATCTGAAGACAC
9 and 10	AAAGAAAATGAGGAAGACTAGC	TCACCTTTGGTCTAATAAGAGAG
11	GCAGAGGATGAAGGTAGGTC	AGAAAGCCAGAGATAAGTGG
12	TTATGTCTGGGGTGGAACTC	CGGATTTCATTTGTCTTTCTTC
13	ACAAATGAAAATCCGAATGGG	GGAGTCCTCAGGGTCTTCTAC