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Supplemental Data

Mutations in *KARS*, Encoding Lysyl-tRNA

Synthetase, Cause Autosomal-Recessive

Nonsyndromic Hearing Impairment DFNB89

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	p.Tyr173His	p.Asp377Asn		p.Tyr173His	p.Asp377Asn
Mammal	SGGNLIFYDLRGEGV	MAAYDYNELMEITEK	Nematostella vectensis	SGSKLLFYDLRGEGV	MAAYDYNELMEITEK
	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Strongylocentrotus purpuratus	SGAKLIFYDLRGEGV	MAAYDYNELMDIAER
Gorilla gorilla gorilla	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Oikopleura dioica	SGAKLIFYDLRGEGV	MAAYDADLMKTEE
Pongo abelii	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Amphimedon queenslandica	SGSKLIFYDVRGEGV	MAAYDYNELMITEQ
Nomascus leucogenys	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Trichoplax adhaerens	SGKLILIFYDLRADGV	MAFDYDLMITEQ
Callithrix jacchus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Hordeum vulgare var. distichum	MGQKLIFYDLRSQ	MAAYDYNELMITEQ
Macaca mulatta	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Volvic carteri	SGSKLIFYDLRADGV	QAYADYDLMGITES
Macaca fascicularis	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Chlamydomonas reinhardtii	SGSKLIFYDLNGDG3	QAYADYDLMITEQ
Otolemur garnettii	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Vitis vinifera	SSSKLIFYDLNGDGA	MAFDYDLMELTER
Ailuropoda melanoleuca	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Populus trichocarpa	SSSKLIFYDLNGLGA	MAAYDYNELMELTEN
Canis familiaris	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Oryza glaberrima	QSSKLIFYDLYGGGE	MAAYDYNELMELTET
Heterocephalus glaber	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Oryza sativa subsp. indica	QSSKLIFYDLYGGGE	MAAYDYNELMELTET
Myotis lucifugus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Oryza sativa subsp. japonica	QSSKLIFYDLYGGGE	MAAYDYNELMELTET
Cavia porcellus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Ricinus communis	AAAKLIFYDLGGGGV	MAFDYDLMELTER
Cricetulus griseus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Brachypodium distachyon	SSSKLIFYDLYGGDV	MAAYDYNELMELTEA
Loxodonta africana	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	SOYBN Uncharacterized protein	SGSKLIFYDLBGGF	MAIYDYNELMDITEQ
Oryctolagus cuniculus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Sorghum bicolor	SSSKLIFYDLGGGM	MAAYDYNELMELTET
Bos taurus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Micromonas sp.	SGTKLMFYDLRGDG	MAAYDVEDLMQITEE
Mus musculus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Micromonas pusilla	SGTKLIFYDLRGDG	MAAYDVEDLMQITEE
Rattus norvegicus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Zea mays	SSSKLIFYDLGGGM	MAAYDYNELMELTET
Equus caballus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Physcomitrella patens subsp. patens	SGGKLMFYDLHADGM	WAAYDVEDLMKVTEE
Sus scrofa	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Coccomyxa subtilispoidea	QG-KLKFVYDLDAGE	QAYADYDLMVATEE
Mustela putorius furo	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Arabidopsis lyrata subsp. lyrata	QSSKLIFYDLNGDDF	MAFDYDLMQITEE
Ornithorhynchus anatinus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Medicago truncatula	SGAKLIFYDLHDDGF	MAIYDYDLMITEEN
Monodelphis domestica	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Arabidopsis thaliana	SSSKLIFYDLNGDDF	MAFDYDLMEMITEV
Sarcophilus harrisii	NNPVEFTVTTISIHN--	MAAYDYNELMEITEK	Ostreococcus lucimarinus	SGKKLIFYDLIAADGK	QAYADYDLMQITEE
Galus gallus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Ostreococcus tauri	SGKKLIFYDLVADGK	QAYADYDLMQITEE
Taeniopygia guttata	AGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Batrachochytrium dendrobatidis	QSSKLIFYDLQAEGC	MAAYDVEDLMNTTEA
Meleagris gallopavo	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Schizosaccharomyces japonicus	AGAKLRFVYIASEKG	QAYADYDLMQITEE
Gecko japonicus	SGGLILIFYDLRGEGV	MAAYDYNELMEITEK	Schizosaccharomyces pombe	AGNNKLIFYEIADGK	QAYADYDLMQITEE
Anolis carolinensis	SGGLILIFYDLRGEGV	MAAYDYNELMEITEQ	Blastocystis hominis	SGNKLFFNTLQGDGK	MAAYDVEDLMITEQ
Xenopus laevis	SGAKLIFYDLRGEGT	MAAYDYNELMEITEK	Phytophthora infestans	SGSKLIFYDLADAGE	MAAYDVEDLMITEE
Xenopus tropicalis	SGAKLIFYDLRGEGT	MAAYDYNELMEITEK	Phytophthora sojae	SGAKLIFYDLADAGE	MAAYDVEDLMITEE
Luttmelia chalumnae	SGAKLIFYDLRGEQM	MAAYDYNELMEITEK	Phytophthora ramorum	SGAKLIFYDLRADGK	MAAYDVEDLMITEE
Danio rerio	SGAKLIFYDLRGEGV	MAAYDYNELMEITEK	Albugo labachii	SGSKLIFYDLRGDG	MAAYDVEDLMITEE
Oryzias latipes	SGAKLIFYDLRGEGV	MAAYDYNELMEITEK	Ectocarpus siliculosus	KG-KLFLFVYGMTEQ	QAYADYDLMQITEE
Salmo salar	SGAKLIFYDLRGEGV	MAAYDYNELMDITEK	Neosporon caninum	SGGKLFVYDADGK	WAAYDVEDLMITEE
Takifugu rubripes	SGAKLIFYDLRGEGV	MAAYDYNELMEITEK	Perkinsus marinus	SGKEMPFVYDLEDGE	MAIYDVEDLMQITEE
Tetraodon nigroviridis	SGAKLIFYDLRGEGV	MAAYDYNELMEITEK	Capsaspora owczarzaki	SGAKLIFYDLRGDGK	WAAYDVEDLMQITEE
Gasterosteus aculeatus	SGAKLIFYDLRGEGV	MAAYDYNELMEITEK	Salpingoeca sp.	SGKRLIVFYDIDHGET	MAFDYDLMQITEE
Vertebrate	SGAKLIFYDLRGEGV	MAAYDYNELMSITED	Monosiga brevicollis	SGQKLIFYDLSSEGE	MAAYDVEDLMITEQ
Apis mellifera	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Serpula lacrymans var. lacrymans	SGQKLIFYDLSSEKG	MAIYDMDIMDLTEE
Drosophila sechellia	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Coprinopsis cinerea	SGSKLIFYDLHEDGE	MAIYDQDELMITEE
Drosophila simulans	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Schizophyllum commune	SSSKLRFYDHSSEQQ	MAIYDMDIMDLTEA
Drosophila melanogaster	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Perfomyscara indica	ASKLIFYDLSSEGV	MAIYDMDIMDLTEV
Drosophila erecta	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Pichia pastoris	SGSKLIFYFVLHGGGV	QAYADYDLMETTEL
Drosophila yakuba	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Pichia angusta	SGSKLIFYFVLAGEGT	EAYADYDLMOTTEL
Drosophila ananassae	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Pichia sorbitophila	SGSKLIFYFVLGGDG	QAYADYDLMQITEE
D. pseudoobscura pseudoobscura	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Meyeromyxa guillemondi	ASGKLFVYVLRGEQV	QAYADYDLMQITEE
Drosophila willistoni	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Schefersomyces stiptis	SGAKLIFYFVLGGDG	QAYADYDLMQITEE
Drosophila grimshawi	SGAKLIFYDLRGEGV	MAAYDYNELMDITEQ	Candida orthopsis	SGSKLIFYFVLGGDG	QAYADYDLMQITEE
Drosophila virilis	SGAKLIFYDLRGEGV	MAAYDYNELLITEEQ	Candida parapilosus	SGSKLIFYFVLNGDG	QAYADYDLMQITEE
Drosophila mojavensis	SGAKLIFYDLRGEGV	MAAYDYNELLITEEQ	Candida albicans	SGSKLIFYFVLGGDG	QAYADYDLMQITEE
Pediculus humanus subsp. corporis	SGSKLIFYDLRGEGV	MAAYDYNELMEITEK	Candida dubliniensis	SGSKLIFYFVLNGDG	QAYADYDLMQITEE
Aedes aegypti	SGGLILIFYDLRGEGV	MAAYDYNELDITEK	Candida tropicalis	SGSKLIFYFVLNGDG	QAYADYDLMQITEE
Solenopsis invicta	SGAKLIFYDLRGEGV	MAAYDYNELMITES	Candida tenuis	AGSKLIFYFVLNGDG	QAYADYDLMQITEE
Acromyrmex echinatior	SGAKLIFYDLRGEGV	MAAYDYNELMITEK	Debaromyces hansenii	SGSKLIFYFVLNGDG	QAYADYDLMQITEE
Harpagophathus saltator	ASAKLIFYDLRGEGV	MAAYDYNELMSITEK	Aspergillus oryzae	SGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Danous plexippus	SGAKLIFYDLRGEGV	MAAYDYNELMSITET	Aspergillus flavus	SGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Atta cephalotes	SGAKLIFYDLRGEGV	MAAYDYNELMSITET	Aspergillus niger	SGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Culex quinquefasciatus	SGGLILIFYDLRGEGV	MAAYDYNELMITEK	Aspergillus kawachii	SGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Tribolium castaneum	SGAKLIFYDLRGEGT	MAAYDYNELMITEA	Aspergillus clavatus	SGSKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Anopheles gambiae	SGVKLIFYDLRGEGV	MAAYDYNELMITEQ	Aspergillus terreus	SGAKLIFYFIDRAEGA	WAAYDVEDLMQITEE
Anopheles darlingi	SGSKLIFYDLRGEGL	MAAYDYNELMITEQ	Ajellomyces dermatitidis	AGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Antherea pernyi	SGAKLIFYDLRGEGV	MAAYDYNELMITEQ	Ajellomyces capsulata	AGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Glossina morsitans morsitans	SGAKLIFYDLRGEGV	MAAYDYNELMITEQ	Emericella nidulans	SGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Bombyx mori	SGAKLIFYDLRGEA	MAAYDYNELMITES	Paracoccidioides brasiliensis	AGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Camponotus floridanus	SGSKLIFYDLRGEGV	IAAYDYNELMITEK	Arthrobotrys oligospira	FGSKLIFYFIDRISHE	WAAYDVEDLMQITEE
Ixodes scapularis	SGTLILIFYDLRGEGT	MAAYDYNELMDITEV	Saccharomyces cerevisiae	SGSKLIFYFVLNGDG	QAYADYDLMQITEE
Amblyomma maculatum	SGAKLIFYDLRGEKT	MAAYDYNELMTITES	S. cerevisiae x S. kudriavzevi VIN7	SGSKLIFYFVLNGDG	QAYADYDLMQITEE
Ascaris suum	SGGLILIFYDLRGEGT	MAAYDYNELMKTED	Spathaspora passalidarum	AGSKLIFYFVLNGDG	QAYADYDLMQITEE
Loa loa	SGGLILIFYDLRGEGT	MAAYDYNELMKTED	Kazachstanian africana	SGSKLIFYFVLNGDG	QAYADYDLMQITEE
Brugia malayi	SGGLILIFYDLRGEGT	MAAYDYNELMKTED	Arthrobotrys gigyeum	SGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Caenorhabditis brenneri	SGSKLIFYDLRGEGT	MAAYDYNELMKTED	Arthrobotrys ottei	AGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Caenorhabditis briggsae	SGSKLIFYDLINGEQT	MAAYDYNELMKTED	Coccidioides posadasii	SGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Caenorhabditis remanei	SGSKLIFYDLINGEQT	MAAYDYNELMKTED	Lachancea thermotolerans	SGSKLIFYFVLHGGGV	QAYADYDLMQITEE
Caenorhabditis elegans	SGSKLIFYDLINGEQT	MAAYDYNELMKTED	Naumovozyma dairensensis	SGSKLIFYFVLNGDG	QAYADYDLMQITEE
Caenorhabditis japonica	SGSKLIFYDLINGEQT	MAAYDYNELMKTED	Trichophyton tonsurans	SGAKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Trichinella spiralis	AGQKLIFYDLRAEGT	MAAYDYNELMKTIED	Neosartorya fumigata	SGSKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Clonorchis sinensis	AGSKLIFYV18DEK	MAAYDYNELMKTIED	Neosartorya Fischeri	SGSKLIFYFIDRAEGV	WAAYDVEDLMQITEE
Schistosoma mansoni	AGSKLIFYD18EDC	MAAYDYNELMKTIED	Clavicipitaria lusitaniae	SGSKLIFYFVLHGGGV	QAYADYDLMQITEE
Branchiostoma floridae	SGQLIFYD18RAEAM	MAAYDYNELMKTIES	Kluyveromyces lactis	SGSKLIFYFVLHGGDG	QAYADYDLMQITEE
Clona savignyi	AGPNLIFYV1VRGDG	MAAYDYNELMKTIES	Athyreus gossypii	SGSKLIFYFVLHGGDG	QAYADYDLMQITEE
Clona intestinalis	AGSKLIFYV1VRGDG	MAAYDYNELMKTSET		SGSKLIFYFVLHGGDG	QAYADYDLMQITEE

Figure S1. Multiple-Sequence Alignment for KARS and Similar Proteins

Aligned sequences are from 165 non-human species, including 8 primates, 14 placental mammals, 3 non-placental mammals, 3 avian, 2 reptiles, 2 amphibians, 7 bony fish, 27 insects, 2 arachnids, 9 roundworms, 2 flatworms, 8 water animals, 22 plants, 44 fungi and 12 other eukaryotes. Protein sequences were derived from the UniProtKB database using blastp and aligned using ClustalW2. For the human sequence, residues 166-180 and 370-384 from KARS (NP_001123561) are shown. The tyrosine residue at position 173 is highly conserved, and is replaced by a phenylalanine in only 3 of 165 proteins from non-human species (highlighted in cyan), namely *Harpegnathos saltator* (Jerdon's jumping ant), *Apis mellifera* (honeybee) and *Anopheles gambiae* (mosquito). The aspartic acid residue at position 377 (blue letters highlighted in yellow) is identical in all aligned sequences.

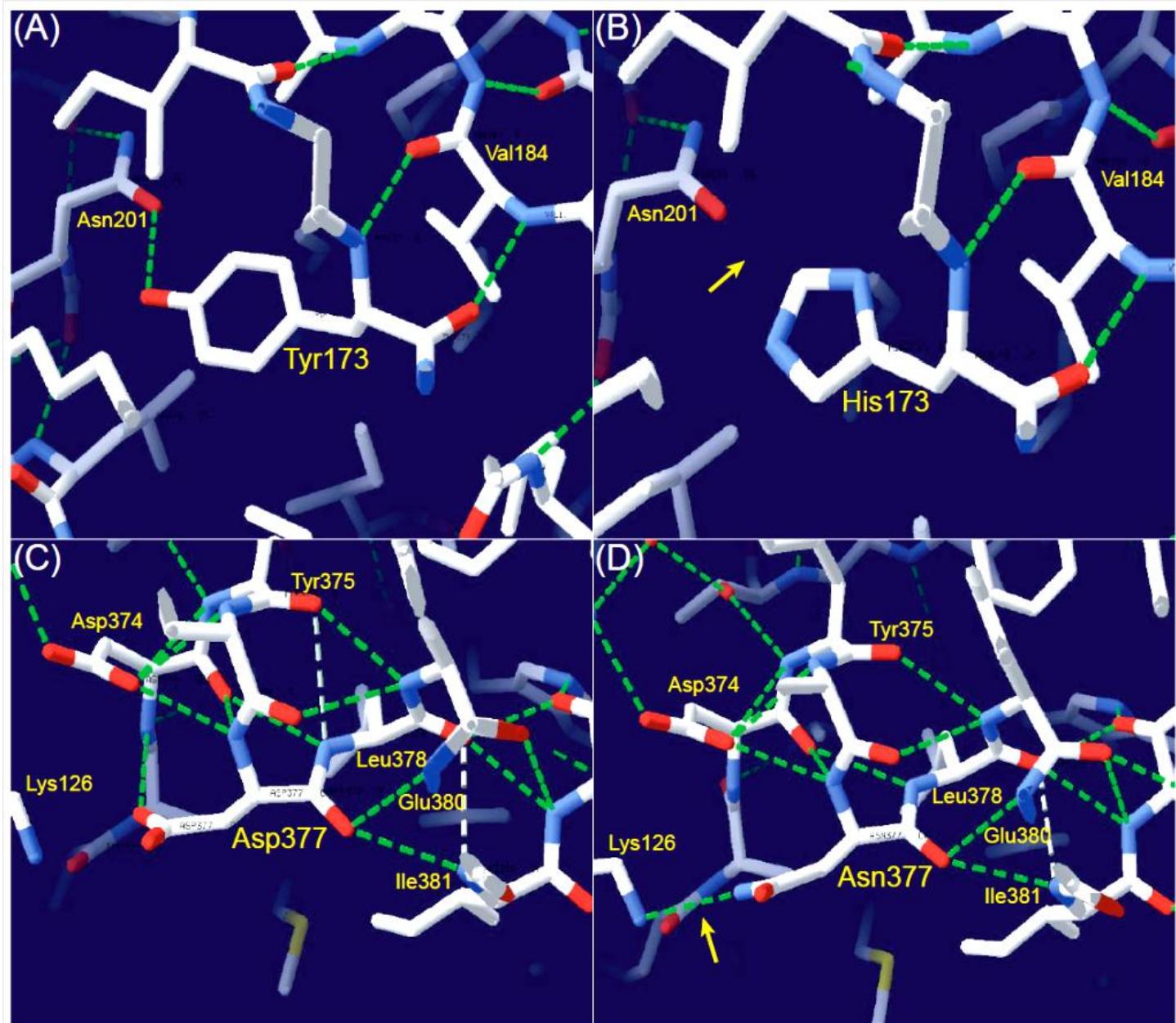


Figure S2. Structural Modeling for Altered KARS

H-bonds are shown as green dotted lines.

(A) Wild-type KARS with tyrosine at position 173.

(B) Altered KARS with histidine at position 173. Yellow arrow indicates loss of an H-bond between β-strand 2 and α-helix 3.

(C) Wild-type KARS with aspartic acid at position 377.

(D) Altered KARS with asparagine at position 377. Yellow arrow points to a gained H-bond between interacting KARS monomers. Additionally an H-bond between asparagine residues at positions 374 and 377 is lost, possibly resulting in loss of α-helix 9.

Table S1. ABR Findings in Individuals with *KARS* Mutations

Individual ID	dB	Side	Latency Wave I (ms)	Latency Wave III (ms)	Latency Wave V (ms)	Inter-peak I-III	Inter-peak III-V	Inter-peak I-V
4406 IV-2	100	Right	1.30	3.65	5.55	2.35	1.90	4.25
		Left	1.10	3.55	5.05	2.45	1.50	3.95
	90	Right	2.85	4.95	7.00	2.10	2.05	4.15
		Left	1.90	4.00	6.05	2.10	2.05	4.15
	80	Right	--	3.35	--	--	--	--
		Left	--	--	5.95	--	--	--
4406 IV-7 ^a	100	Right	1.00	3.60	5.65	2.60	2.05	4.65
		Left	1.31	4.65	6.85	3.34	2.20	5.54
	90	Right	--	3.40	5.05	--	1.65	--
		Left	--	3.10	5.90	--	2.80	--
	80	Right	--	5.23	--	--	--	--
		Left	--	--	--	--	--	--

^aFor individual IV-7 the compliance peak for the tympanogram on the left ear was absent (type B ear), indicating possible middle ear effusion or otitis media at the time of testing.

Table S2. Interexonic Primer Pairs for Zebrafish and Mouse *Kars* Gene

Exon	Forward	Reverse
zf_Kars_1	CAGAATATGCCAACTGCAAGCGC	CCATGACCTGCAGCTTGACTCC
zf_Kars_2	CCTGATCCCTGGTGGCGCAGT	AGCTCTGGGTATGCTGATTG
zf_Kars_3	GGAGGTGAAGTGCATCAACCAA	CCCTCTGCAGGTGCAGCTGGT
Mus_Kars_1	TCAGAGGGAACCTCGACTGGC	GTTGGCCATGACTTGTAAC
Mus_Kars_2	GAGACATAATTGGAGTTGAGGGCA	TGCATAGGCCATGTAGAA
Mus_Kars_3	TCAGCATGGTAGAACAGAGCTTGAGA	ATCGGTGAGAACATGGTGAGCC