The American Journal of Human Genetics, Volume 93

Supplemental Data

Mutations in KARS, Encoding Lysyl-tRNA

Synthetase, Cause Autosomal-Recessive

Nonsyndromic Hearing Impairment DFNB89

Regie Lyn Santos-Cortez, Kwanghyuk Lee, Zahid Azeem, Patrick J. Antonellis, Lana M. Pollock, Saadullah Khan, Irfanullah, Paula B. Andrade-Elizondo, Ilene Chiu, Mark D. Adams, Sulman Basit, Joshua D. Smith, University of Washington Center for Mendelian Genomics, Deborah A. Nickerson, Brian M. McDermott Jr., Wasim Ahmad, and Suzanne M. Leal

		p.Tyr173His	p.Asp377Asn			p.Tyr173His	p.Asp377Ash
	Home cooless	LOCUL TRUNCTOR			No sector de Marco de Cale		
	nomo suprens	SGGRLIFTELRGEGV	MATADINELHEITER		Nematostella vectensis	SGSKILFTDLRGEGS	NATADINBLMETTE.
	ran traglodytes	SCONTINUEDA	MATADYHOLMEITEK		Strongylocentrotus purpuratus	BGARLIFYDLRGEGV	NATADYHDLMDIAE)
	Gorilla gorilla gorilla	SGGKLIFYDLRGEGV	MAYADYHOLMEITER		Oikopleura dioica	3GARLVFYDLRSEGE	MAYADAD DLMKITE
1	Pongo abelii	SGGKLIFYDLRGEGV	MAYADYRDLMEITER		Amphimedon gueenslandica	SGSKLIFYDVRGEGV	MAYADYMDLINITE
1	Nomascus leucogenys	SGGKLIFYDLRGEGV	MAYADYHOLMEITEK		Trichoplax adhaerens	3GQKLIFYDLRADSV	MAFADYTDLMSITE
	Callithrix locchus	SCORLIPYDLRGEGY	MAYADYND METTER	1.0	 Hordeum vulgare var. distichum 	MGORLRFYDLRSESO	MAYADYMDLIEITE
	Macana mulatta	BOOM TRUTT BORD			Voluov corteri	BOBRTTERVOLWADOW	OB VAD VODE LOWTE
	wococo monuto	Deerall Proveder	MAIADIREVALITEA		Chinese damage saich saith	BOOM TO BE AND A REAL	AT US BANKET BA BE
1	Macaca fascicularis	SGGKLIFYDLRGEGV	MAYADYHDVMEITEK		Chiamydomonas reinharatii	SGSKLVFYDLKGDGS	GATAD TRUELDE TE
1	Otolemur garnettii	SGGKLIFYDLRGEGV	MAYADYHDLMEITEK		Vitis vinifera	353KLFFYDL8GDGA	MAFADYNUVMELTER
	Ailuropoda melanoleuca	SGGKLIFYDLRGEGV	MAYADYHOLMEITER		Populus trichocarpa	355KLFFYDLHGLGA	MAYADYHDLMELTES
1	Canis familiaris	SGGKLIFYDLRGEGV	MAYADYHDLMEITER		Oryza glaberrima	QSSHILFYDLYGGGE	MAYADYNDLMELTET
1	Heterocephalus glaber	SGGKLIFYDLRGEGV	MAYADYHOLMEITEK		Oryza sativa subsp. indica	QSSKLLFYDLYGGGE	MAYADYNDLMELTET
	Muntis lucifianus	BOOKLEPVEL BOROV	MANADVADCHETTER		Onvza sativa subsp. japonica	OBSKILFYDLYGGGE	MAYADYMDLIELTET
- 2	Caula norrellus	SCOULTENEL DOROUT		-	Ricinus communis	BAAKI FFYDI BOOCU	WARADYNEY MPT TPT
	cuviu porceilus	COOLING DEFODO	MAIADIRDLAEITEA	E	Brachunodium dictochuon	SECUL PENDINGDON	VENE DAVID VET TEL
	Cricetulus griseus	BEGELIFTELREEGV	MAYADYHELMEITER	0	Brachypoundm aistachyon	SSSHELE EDDIGDOV	INTIN INCIDE IS
1	Loxodonta africana	SGGKLIFYDLRGEGV	MAYADYHDLMEITEK	- TO 1	SUTEN Uncharacterized protein	Seskive Thiabee	MAINDINGLMDITE
	Oryctolagus cuniculus	3GGHLIFYDLRGEGV	MAYADYHOLMEITEK		Sorghum bicolor	333KLFFYDLYGGGM	MATADYNDLMELTE
1	Bos taurus	SGGKLIFYDLRGEGV	MAYADYHOLMEITEK		Micromonas sp.	SGTKLMFTDLRGDGV	NAYADYEDLMEMTER
1	Mus musculus	3GGKLIFYDLRGEGV	MAYADYHDLMEITEK		Micromonas pusilla	BGTKLYFYDLRGDGV	MAYADYEDLMEMTEI
	Rattus norvegicus	SGGKLIFYDLRGEGY	MAYADYHDLMEITEK		Zea mays	333KLFFYDLYGGGM	MAYADYNDLMELTET
	Fauus caballus	SGGKLTEVELBGERY	MANA DUNIE WETTEN		Physcomitrella patens subsp. patens	BGGKLMFTDLHADGH	WAYADYDDLMKVTES
	Suc comfa	SCOTT TENDI DOPOT			Coccomvaa subelliosoidea	OG-KLUFYDLKADGE	OAYADYYEMAT TE
	sus scroju	Sectors	MAIADIBOLNEITEN		Annhidonesis hunta suben hunta	REALTERANT MONTE	VEPERVIEW WEVER
	Mustera putorius Juro	366KLIFTDLRGE6V	MATADYNDLMEITER		Anabiaopois iprana sabop. Iprana	SOULL FEDERODE	nar av invensaries
1	umithomynchus anathrus	BOGICLIFYDLRGEGV	MAYADYNDLMEITEK		weakago truncatula	SWARLVET DLHDDGF	RAINDITULNEITEN
1	Monodelphis domestica	SGGKLIFYDLRGEGV	MAYADYHDLMEITEK		Arabidopsis thaliana	353XLFFYDL#GDDF	MAPADYNDLMENTEN
L :	Sarcophilus harrisii	MNPPEFNYTISIN	MAYADYHOLMEITER		Ostreococcus lucimarinus	SGREATENDLIADGE	OAYADYDDLMQMTES
-	Gallus gallus	SGGKLIFYDLRGEGV	MAYADYRDLMEITEK	L	 Ostreococcus touri 	SGERLYFYDLVADGE.	QAYADYNDLMTHTES
	Taeniopygia guttata	AGGELIFYDLRGEGU	MATADYROLNETTER	E.	 Batrachachytrium dendrobatidis 	QSKGLIFYDLQAEGC	MAYADVHDLMSHTER
	Melegaris gallongun	SGGKLIFYDI DAPCY	MAYADVHOLVETTEN		Schizosaccharomyces iaponicus	AGAKLEFYEIHSEGE	OAYADYYD MEMTER
	Gecko ianonicus	ROCKLEPHOLOGECH	VI VI NY NY NY NY TON		Schizosaccharomyces pombe	AGNELREVETRADOR	OAYADYYD, MDTTE
	Anolis carolin and	00000 0000000000	MATADING MALITER		Binchoustic hominic	SCART FERRET OCTO	NE VE DUIVETUET
	nnunis curunmensis	SWORLIS TULRGEGV	NATADIRECHEITEQ		Division point manufing	BORNAL TATAGOLOGI	MANADY SPIRESTE,
1 3	Kenopus laevis	3GARLIFYDLRGEGT	MAYADYNELMEITEK		Phytophthora injestans	SGSKLVFTDLRADGE	MATADIBULEIRIES
	Kenopus tropicalis	SGARLIFYDLRGEGT	MAYADYYDLMEITER		Phytophthora sojae	SGARLVFYDLRADGE.	MAYADYNDLMTL TEI
1	Latimeria chalumnae	SGAKLIFYDLRGEGM	MAYADYNELMEITEK		Phytophthora ramorum	5GAKLVFYDLRADGK	MAYADYNDLMSLTEI
1	Danio rerio	SGARLLFYDLRGEGV	MAYADYHOLMEITEK		Albugo laibachii	BGSKLLFYDLRGDGA	MAYADYNDLMINTER
1 1	Orvzias latipes	SGAKLLFYDLRGEGV	MAYADYTELMEITEK		Ectocarpus siliculosus	QG-KLLFYGMRADGA	QAYADYEDLMSMTE(
	Salmo salar	SGARLIFYDLRGEGV	MAYADYND MOTTEX		Neospora caninum	SGORLRFYDIRGDGE	WAYADYMDLMDLTER
	Takifumu nubrines	SCAVIT FUTT DOPON	VA US DUDRY UP TOPY		Perkinsus marinus	SEXEMVEYDLWEDGH	MAYADYNDLMIMTER
	Fateradan aineniaidir	SGANLLE TULKGEGV	MATADIADIADITEA		Contractore ourranteli	REAVETPUNCERCY	VENERAL VETTER
	retracidon higrovinais	SOADLIE TULKOEGV	MAYADYNDIMEITER		Capital Sport Owczarzaw	DOWNELL PROPOSED	NATING TRANSPORT
L (Gasterosteus aculeatus	SGARLIFYDLRGEGV	MAYADYHDLMEITEK		Saipingoeca sp.	Several Lorden	RAIADINULRUHILI
	Apis mellifera	SGARLIFECLRGEGV	MAYADYNDLMSITED		Monosiga brevicallis	BGQRLRFYDLHBEGE	MAYADYEDLMEVTE
	Drosophila sechellia	SGARLIFYDLRGEGV	MAYADYADIMDITEQ		Serpula lacrymans var. lacrymans	SGQKLIFYDLHGEGK	MATADNTDIMDLTE
1	Drosophila simulans	SGARLIFYDLRGEGV	MAYADYADIMDITEQ		Coprinopsis cinerea	3GSKLRFYDLHGEGV	MAYADQEBLMEITE:
	Drosophila melanogaster	SGARLIFYDLRGEGV	MAYADYADIMDITEO		Schizophyllum commune	353KLRFYDIH3EGQ	MATADNYDLMDI TEA
	Drosophila erecta	SGARLIFYDLRGEGU	WAYADVADINDITEO		Piriformospora indica	AGEHLIFYDLRGEGV	MATADMEDLMETTEN
	Droroehila uskuba	SCAUT TRUNC DOPOUT	NA VA DVA DTWDTTEO		Pichia pastoris	BGSKLEFYVLHGEGV	CAYADVYDLMETTEL
	Drosophilo panator	CONSTRUCTOR	YID LOW LOW LOW LAW		Dickia onqueta	BCSPT PPUT LCPCT	PAVA DUVER UDTTP
	orosopnila ananassae	SGANGIFIDLRGDGV	MAYADYADIMDITEQ		Pichia angusta	South an and so	ALLA PLANT LINE AND A
	D.pseudoobscura pseudoobscura	3GARLIFYDLRGEGV	MATADYADVMDITEQ		Picnia sorbrophila	DAGUTUS LATURDAA	UNINUVINUM (D
	Drosophila willistoni	SGAKLIFYDLRGEGV	MAYADYADVMDITEQ		Meyerozyma guilliermanaii	ABSKLEPTVLKGDGV	QAYADVYDLMDMTE
1	Drosophila grimshawi	BGARLIFYDLRGEGV	MAYADYHDVIELTEQ		Scheffersomyces stipitis	BGARLRFYVLRGDGV	QAYADVYDLMDMTE:
1	Drosophila virilis	SGAKLIFYDLRGEGV	MAYADYYDLIEITEQ		Candida orthopsilosis	SGSKLHFYVLRGDGV	QAYADVYDLMEMTEN
	Drosophila mojavensis	SGARLIFYDLRGEGV	MAYADYYDLIEITEO		Candida parapsilosis	SGSKLEFYVLKGDGV	QAYADVYDLMDMTES
1	Pediculus humanus subsp. corporis	SGSKLIFYDLRSEGV	MAYADYNDE METTER		Candida albicans	SGSKLEFYVLEGDGV	QAYADVYDLMEMTEL
	ledes nerventi	ACCHI TRVDI DORICI	US VI DUNET TRI PPV		Candida dubliniensis	BOSRI NEWLINGTON	CA YADVYOLMENTES
	Coleonorir invicto	BOX VI TENET DODOUT	MAINDINDLIDLIDA	-	Condida trapicalis	SCONTERNITEDET	OA VA DUVEL MENTET
	Autempoly invited	Sennant Thursday	MATADIMULALIES	2°	Condida tanuir	LOUTVOUTVOLCT	OR VE DUPUT UTUTE
	Acromyrmex echinatior	SGAKLIFYDLRGEGV	MAYADYNDLMEITEN	2	Culturu teriuto	Abontini iv Loopor	CUTURA INCURATES
1	Harpegnathos saltator	ASAKLIFTOLRGEGV	MAYADYNDLHSLTED	_	Debaryomyces nansenii	BOSKLAR AVLAGDOV	UNIADVIDLMDNTE:
1	Danaus plexippus	3GAKLIFYOVRGEGV	MAYADYNDLITITET		Aspergillus oryzae	3GAKLIFYDIRAEGV	WAYADVYDVMRLTER
1	Atta cephalotes	SGARLIFYDLRGEGV	MAYADYNDLHEITEN		Aspergillus flavus	SGARLIFYDIRAEGV	WAYADVYDVMNLTER
1	Culex quinquefasciatus	SGGKLIFYDLRGEG7	MAYADYNDLIEMTEK		Aspergillus niger	SGAKLIFYDIRAEGV	WAYADVYDVMNLTES
	Tribolium castaneum	BEAKLIFYDLRGEET	MAYADYNDLIAITEA		Aspergillus kawachii	SGAKLIFYDIRAEGV	WAYADVYDVHNLTE:
	Anopheles aambioe	SEVELIEFCLAGECT	MAYADYNDITDITOO		Aspergillus clavatus	SGSKLFFYDIRAEGV	WAYADVYDVMNL TER
	Anopheles darlingi	SAGELTEVIT POPOT	MAYADVARTTETRA		Aspergillus terreus	SGARLIFYDIRAEGA	WAYADVYDIMOL TEL
	Anthernan naroui	SCIVI TENER OF BEEN	MANA DANKET TOTAL		Aiellomures dermotifidie	AGAKI IFYTODARCT	NA VA DUVET MUTTER
	filostico pernyi	SGALLIS PULKAEGY	ALADIADLITLIET		Alallamuras conculate	LOANT TRUCTOL POT	PRIME VIDINI IDI
	alosana morsitans morsitans	SGARLIETOLRGEGV	MATADYNDINEITEQ		Emocicella cidulata	SCATTER STRALLY	NATADVIDINAVIEI
	вотрук топ	SWARLIFTDLRAEGA	MATADYNDLMTITES		Emericena maulans	SGALLITTUIRAEGV	BALADVIDVENLTEI
L (Camponotus floridanus	BGBRLVFYDLRGEGV	IAYADYN <mark>ULMEITEN</mark>		Paracoccidioides brasiliensis	ASAKLIFYDVRAEGV	WATADVYD/MWYTES
1	wodes scapularis	SGTKLIFYDLRGEGT	MAYADYNDLMDMTEV		Arthrobotrys oligospora	FGDKLRFYEIRSNGH	MAYADVMDVHDHTE
1	Amblyomma maculatum	SGAKLIFYDLRGENT	MAYADYNDLMTITES		Saccharomyces cerevisiae	SGSKLRFYVLEGDGV	QAYADVYDLMDHTE:
-	Ascoris suum	SGGELIFYDINGEGT	MAYADYEDT THITED		S. cerevisiae x S. kudriavzevii VIN7	SGSKLXFYVLEGDGV	GATADVYDLMDMTE:
B	Loa loa	SGGKLIFYDLHGEGT	MAYADYEDINGTTED		Spathaspora passalidarum	AGSKLEFYSLEGDGV	QAYADVYDIMENTE:
1 1	Revoia molevi	REGULTENET HORSE	MAYA DVERY LINE OF		Kazachstania africana	SGSKLEPPUT HODOU	OA VADUCTOR MENTE
	Cooperhability because	SCONT NEW COLOCOT	MATADILBUNATIED		Arthroderma autocum	SCANTERVICARCE	WE VE DOWN HUMBER
1	cuenomuounos prennen	Seguration Herei	MAYADYEDVIQUTED		Arthrodorma atra	LALUI TRUNALOV	WALKDVIDLAW/IE
1 1	Laenorhabditts briggsae	363KLVEYDINGEGT	MAYADYEDVIQUTED		Animoderma olde	AWARTISTOLEAEOU	WAINDVIDLENVIEL
1	Caenorhabditis remanei	SGSKLVFYDIHGEGT	MAYADYEDVIQUTED		Coccidioides posadasii	SGAKLIFYDIRAEGV	WAYADVYDLMWVTE
1	Caenorhabditis elegans	SGSELVEYDINGEST	MAYADYEBVIQLTED		Lachancea thermotolerans	SGSKLRFYVLSGEGV	QAYADVYDLMDMTE:
	Caenorhabditis japonica	SGSKLVEYDINGEGT	MAYADYEDUTOTTED		Naumovazyma dairenensis	SGSKIKFYVLSGDGV	CAYADVYDLMDMTE:
	Trichinella spiralis	AGOKLIFYDIANECT	MAYADVNOT HUTTED		Trichophyton tonsurans	SGARLIFYDLPAEGY	WAYADVY DL MOUTE
10.00	Clonorthic sinensis	ACOUT TRUNCTER	MA VA DVE TO LOTTER D		Neosartorya fuminata	BESKLEFTUDIDARCH	NA VADUVITANT TO
	Contracting smerists	AGONALTINISDER	HATADIEDLERAVIED		Necessarian in Juliyo Juliyo to	SCOULD BE STANDER	CLUB DINGER CO
	scrissosoma mansoni	AGSELIFYDIRSEDC	MAYADYEBLMTITED		Neosartorya Jischen	BESKLETTDIRAEGV	WATADVIDVMNLTER
F	aranchiostoma floridae	BOOKLIPTOLRAEAM	MAYADYNDLMKITES		Clavispora lusitaniae	BGSKLRFYVLKGDGV	QAYADVYDLMDMTE:
	Ciona cavianui	AGPELIFYDVRGDGV	MAYADYEDLMKITET		Kluwennmuces loctis	BGSKLEFYVLHGDGV	QAYADVYDLMEMTE:
- 1	cione savignin		Contraction of the second s		may recompeter methy		

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
	100	Right	1.30	3.65	5.55	2.35	1.90	4.25
	100	Left	1.10	3.55	5.05	2.45	1.50	3.95
4406	00	Right	2.85	4.95	7.00	2.10	2.05	4.15
IV-2	90	Left	1.90	4.00	6.05	2.10	2.05	4.15
	90	Right		3.35				
	80	Left			5.95			
	100	Right	1.00	3.60	5.65	2.60	2.05	4.65
	100	Left	1.31	4.65	6.85	3.34	2.20	5.54
4406	00	Right		3.40	5.05		1.65	
IV-7 ^a	90	Left		3.10	5.90		2.80	
	90	Right		5.23				
	00	Left						

^a For 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	AGCTCTTGGGTCATGCTGATTCG				
zf_Kars_3	GGAGGTGAAGTGCATCAACCCAA	CCCTCTGCAGGTGCAGCTGGT				
Mus_Kars_1	TCAGAGGGAACTTCGACTGGGC	GTTGGCCATGACTTGTAACTTGAC				
Mus_Kars_2	GAGACATAATTGGAGTTGAGGGCA	TGCATAGGCCATGTAGAACTCACA				
Mus_Kars_3	TCAGCATGGTAGAAGAGCTTGAGA	ATCGGTGAGAAACATGGTGAGCC				