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Supplemental Data

Recessive Mutations in SLC38A8 Cause Foveal Hypoplasia

and Optic Nerve Misrouting without Albinism

James A. Poulter, Musallam Al-Araimi, Ivan Conte, Maria M. van Genderen, Eamonn Sheridan, Ian M. Carr, David A. Parry, Mike Shires, Sabrina Carrella, John Bradbury, Kamron Khan, Phillis Lakeman, Panagiotis I. Sergouniotis, Andrew R. Webster, Anthony T. Moore, Bishwanath Pal, Moin D. Mohamed, Anandula Venkataramana, Vedam Ramprasad, Rohit Shetty, Murugan Saktivel, Govindasamy Kumaramanickavel, Alex Tan, David A. Mackey, Alex W. Hewitt, Sandro Banfi, Manir Ali, Chris F. Inglehearn, and Carmel Toomes

Met34Arg

Glu233Lys Val236Asp

		\downarrow				$\downarrow \downarrow$	
Human	23	TLSSMGAVFILMKSALGAGLLNFPWAF	49	Human	219	FSVFPTIC-FGFQCHEAAVSIYCSMRKRSLS	248
Chimp	23	TLSSMGAVFILMKSALGAGLLNFPWAF	49	Chimp	219	FSVFPTIC-FGFQCHEAAVSIYCSMSKRSLS	248
Gorilla	23	TLSSMGAVFILMKSALGAGLLNFPWAF	49	Gorilla	219	FSVFPTIC-FGFQCHEAAVSIYCSMRKRSLS	248
Marmoset	23	TLSSMGAVFILLKSALGAGLLNFPWAF	49	Marmoset	219	FSVFPTIC-FGFQCHEAAVSIYCSMRKRSLS	248
Squ Monkey	23	TLSSMGAVFILLKSALGAGLLNFPWAF	49	Squ Monkey	219	FSIFPTIC-FGFQCHEAAVSIYCSMRKRSLS	248
Galago	22	TLSSMGAVFILLKSALGAGLLNFTWAF	48	Galago	218	FSVFPTIC-FGFQCHEAAVSVYCSMRDQSLS	247
Mouse	23	TLSSLGAVFILLKSALGAGLLNFPWAF	49	Mouse	216	FSVFPTIC-FGFQCHEAAVSIYCSMWNQSLS	245
Rat	23	PLSSLGAVFILLKSALGAGLLNFPWAF	49	Rat	216	FSVFPTIC-FGFQCHEAAVSIYCSMQNQSLP	245
Hamster	23	TLSSLGAVFILLKSALGAGLLNFPWAF	49	Hamster	216	FSVFPTIC-FGFQSHEAAVSIYCSLRNQSLS	245
Guinea Pig	23	TLSSLGAVFILLKSTLGAGLLNFPWAF	49	Guinea Pig	215	FSVFPTIC-FGFQCHEAAVSIYCSLRPQSLF	244
Dog	63	TLSSLGAVFILLKSALGAGLLNFPWAF	89	Dog	259	FSVFPTIC-FGFQCHEAAVSIYRSMRNQSLS	288
Horse	23	GLSSLGAVFILLKSALGAGLLNFPWAF	49	Horse	219	FSVFPTIC-FGFQCHEAAVSIYCSMRNQRLS	248
Cow	23	GLSSLGAVFILLKSALGAGLLNFPWAF	49	Cow	219	FSVFPTIC-FGFQCHEAAVSIYCSMRHQSLG	248
Sheep	23	GLSSLGAVFILLKSALGAGLLNFPWAF	49	Sheep	219	FSVFPTIC-FGFQCHEAAVSIYCSMRHQSLG	248
Cat	23	SLSSLGAVFILLKSALGAGLLNFPWAF	49	Cat	269	FSVFPTIC-FGFQCHEAGVSIYCSMTHRSLS	298
Elephant	23	SLSSLGAVFILMKSSLGAGLLNFPWAF	49	Elephant	219	FSVFPTIC-FGFQCHEAAVSIYCSMRNQRLS	248
Tas Devil	23	SLSSIGAVFILLKSALGAGLLNFSWAF	49	Tas Devil	225	FNVFPTIC-FGFQCHEASISIYSSMYNQNLS	254
Zebrafinch	25	GLSSAGAVFILLKSALGAGLLSFPWAF	51	Zebrafinch	227	LHGAPAPSPLALQCHEACVAIYSSMRNQSFS	257
Chicken	74	TGSAGLSSAGAVFIMLKSALGAGLLSFPWAF	104	Chicken	276	FSVIPTIC-FGFQCHEACVAIYSSMRNQSFS	305
Frog	23	SLSSMGAIFIMLKSALGAGLLNFPWAF	49	Frog	224	FSVVPTIC-FGFQCHEACVTIYSSMKNKCLS	253
Zebrafish	23	RLGSLGAVFIMLKSALGAGLLIFPWAF	49	Zebrafish	225	FSVIPTIC-FGFQCHEACVAVYSSMENRRLS	254

Ala282del

1

Glv412Arg 1

		\downarrow				\downarrow
Human	267	LTGVYGFLTFGTEVSADVLMSYPGNDMVIIV	297	Human	404	VKCCLEVWGVVSVLVGTFIFGQ 425
Chimp	267	LTGVYGFLTFGTEVSADVLMSYPGNDMVIIV	297	Chimp	404	VKCCLEVWGVVSVLVGTFIFGQ 425
Gorilla	267	LTGVYGFLTFGTEVSADVLMSYPGNDMVIIV	297	Gorilla	404	VKCKGTGPETRPCSPRPSLQVLKGLPM 430
Marmoset	267	LTGVYGFLTFRTEVSADVLMSYPGNNTVIIV	297	Marmoset	404	VKCCLEVWGVVSVLVGTFIFGQ 425
Squ Monkey	267	LTGVYGFLTFGTEVSADVLMSYPGNNTVIIV	297	Squ Monkey	404	VKCCLEVWGVVSVLVGTFIFGQ 425
Galago	266	LTGVYGFLTFGTEVSADVLMSYPGNDVVIVV	296	Galago	432	TFWLSRCCLEVWGVVSVLVGTFIFGQ 457
Mouse	264	LTGVYGFLTFGPEVSADILMSYPGNDTAIIV	294	Mouse	401	VKCCLEAWGILSVLVGTFIFGQ 422
Rat	264	LTGVYGFLTFGTEVSADILMSYPGNDTAIIV	294	Rat	401	VKCCLEAWGTLSVLLGTFVFGQ 422
Hamster	264	LTGVYGFLTFGTEVSADVLMSYPGNDTAIIV	294	Hamster	401	VKCCLEAWGTLSVLVGTFIFGQ 422
Guinea Pig	263	LTGVYGFLTFRTEVSADILMSYPGNNMAIVV	293	Guinea Pig	400	AKCGLEAWGAVSVLVGTFIFGQ 421
Dog	307	LTGVYGFLTFGTEVSAD <mark>I</mark> LMSYPGNDVVVIV	337	Dog	444	VKCCLEVWGVVSVLVGTFIFGQ 465
Horse	267	LTGVYGFLTFGTEVSAD <mark>I</mark> LMSYPGNDVVIIV	297	Horse	404	VKCCLELWGVVSVLVGTFIFGQ 425
Cow	267	LTGVYGFLTFGTDVSADILMSYPGSDGVVIV	297	Cow	404	VKCCLEAWGVVSVLVGAFIFGQ 425
Sheep	267	LTGVYGFLTFGTDVSADILMSYPGSDGVVIV	297	Sheep	404	VKCCLEAWGVVSVLVGAFIFGQ 425
Cat	317	LTGVYGFLTFGAEVSADILMSYPGDDVVIIV	347	Cat	454	VRCCLEAWGVVSVLVGTFIFGQ 475
Elephant	267	LTGAYGFLTFGAGVSADILMSYPGNDVVVIV	297	Elephant	399	LKCCLELWGVASVLVGTFIFGQ 420
Tas Devil	273	LTGLYGFLTFGDNVSADVLMSYPGNDTMVIV	303	Tas Devil	409	IRSCLIFWGVVSILAGAFIFGQ 430
Zebrafinch	276	LTGLYGYLTFGEAVASDVLMSYPGNDPLVIV	306	Zebrafinch	410	KKAALIAWGVLSVLGGAFVCGQ 431
Chicken	324	LTGLYGYLTFGEDVAPDVLMSYPGNDPVVIT	354	Chicken	458	KKAVLITWGVLSVLGGTFVCGQ 479
Frog	272	FTGIYGSLTFGEAVAADILMSYPGNDVAVII	302	Frog	406	AKSCLTAWGAISVVCGAFVFGQ 427
Zebrafish	273	LTGVFGYLTFGKNVAPDILMSYSGGDVTMIV	303	Zebrafish	407	MRYLLVTWGMITLLCGTLIFGQ 428

Figure S1. Protein sequence alignment of human SLC38A8 with all orthologues. Multiple protein alignments were calculated using ClustalW (<u>http://www.ebi.ac.uk/clustalw2/</u>) [Larkin, M.A. et al. (2007). Bioinformatics 23, 2947-8]. Thirty amino acid residues surrounding each mutation are shown. Conserved amino acid residues are shaded. The positions of the missense mutations p.Met34Arg, p.Glu233Lys, p.Val236Asp and p.Gly412Arg are indicated along with the single deleted amino acid residue p.Ala282. Protein sequences were obtained from NCBI (http://www.ncbi.nlm.nih.gov/protein). Accession numbers: Human NP 001073911.1; Cow NP 001179423.1; Mouse NP 001009950.1; Hamster XP 003495171.1; Elephant XP 003418131.1; Marmoset XP 002761268.1; Frog NP 001037900.1; Tasmanian Devil XP 003758500.1; Galago XP 003791463.1; Chimpanzee XP 003315260.1; Squirrel Monkey dbsXP 003922850.1; Rat NP 001178938.1; Horse XP 003364689.1; Dog XP 546805.3; Gorilla XP 004058132.1; Cat XP 003998360.1; Chicken XP 003641956.1; Zebrafinch XP 004175047.1; Sheep XP 004014985.1; Zebrafish NP 001138287.1; Guinea Pig XP 003462017.1.



Figure S2. Paternity testing in family F3. Results are shown for 10 microsatellites markers, from chromosomes 7, 4 and 15, used to genotype DNA from individuals I:3, I:4 and II:4 from family F3 (Figure 2). Haplotypes are represented by coloured bars (each colour representing a different haplotype). The numbers alongside each haplotype bar correspond to the size of each allele amplified by the microsatellite markers, in base pairs. Mendelian inheritance is observed for all markers indicating no sample mix up or non-paternity.



Figure S3. Knockdown of the *Slc38a8* gene does not cause melanogenesis defects in medaka. (A, B) Bright-field stereomicroscopy images of dorsal views of medaka embryos at St40. No differences were observed in tegument melanophores between control (A) and Mo-5'UTR-Slc38a8-c3 and Mo-5'UTR-Slc38a8-c6 co-injected (B) medaka embryos. Dotted red lines indicate the microphthalmic phenotype present in Mo-5'UTR-Slc38a8-c3 and Mo-5'UTR-Slc38a8-c6 co-injected embryos. (C, D) Cryostat frontal sections of the retina from St40 control (C) and Mo-5'UTR-Slc38a8-c3 and Mo-5'UTR-Slc38a8-c6 coinjected (D) medaka embryos. No significant alterations of retinal pigment epithelium (RPE) pigmentation were observed in Mo-5'UTR-Slc38a8-c3 and Mo-5'UTR-Slc38a8-c6 coinjected retina with respect to controls. Other abbreviations: R, retina; L, lens.



Figure S4. Analysis of the optic chiasm in medaka *Slc38a8* **morpholino knockdown morphants shows no abnormalities.** Confocal images of the optic chiasm from frontal cryostat sections of St38 control and Mo-Slc38a8 injected Ath5::GFP transgenic embryos. Retinal ganglion cell (RGC) axons are EGFP stained (A, B, C, D). Sections are counterstained with DAPI (blue, C, D, E, F). No major alterations in the distribution of RGC axons at the optic chiasm were observed in Mo-5'UTR-Slc38a8-c3 and Mo-5'UTR-Slc38a8-c6 co-injected morphant fish as compared with control embryos. oc, optic chiasm; on, optic nerve. Scale bars: 20µm.



Figure S5. Role of common single nucleotide polymorphisms (SNPs) at the *SLC38A8* locus in determining foveal thickness in the general population. Plots were generated using LocusZoom [Pruim, R.J. et al. (2010). Bioinformatics 26, 2336-7] and genomic references refer to hg.18. Results from analysis of the foveal thickness (A), the fovea:parafovea thickness ratio (B), and the fovea:perifovea thickness ratio (C) are presented. SNPs are plotted as the –log₁₀ of the p-value.







Figure S6. Human RNA and protein localization data for SLC38A8. (A) RNA expression results.

SLC38A8 expression is found predominantly in neuronal tissue (brain, fetal brain and spinal cord) with very weak expression also present in the kidney, thymus and testis. Human adult and fetal tissue total RNA was purchased from Clontech and converted to cDNA using standard protcols. A 188-bp fragment of *SLC38A8* spanning intron 7 was amplified from the cDNAs using primers cDNA(7-8)-F 5'-

AATGATATGGTCATCATTGTGG-3' and cDNA(7-8)-R 5'-AGGATGGTCAGCGGCATC-3' (genomic product size was 600 bp). *GAPDH* control primers used were GAPDH-F 5'-

CGACCACTTTGTCAAGCTC-3' and GAPDH-R 5'-CAAGGGTCTACATGGCAAC-3' (cDNA product size was 229 bp and genomic product size was 330 bp). (B) and (C) Protein localization of SLC38A8. Tissues were formalin fixed and paraffin embedded according to standard methods. 4um serial sections were blocked for endogenous peroxidase activity using 3% hydrogen peroxide in methanol for 10 minutes. Blocked sections were subsequently incubated with a custom rabbit polyclonal antibody (GenScript, USA), raised against 14 amino acids located at the N-terminus of SLC38A8 (QTPGSRGLPEKPHP). Primary antibody was applied at a 1/200-250 dilution for eye tissue and 1/400-450 dilution for brain tissue in Zymed diluent (Invitrogen) and incubated overnight at 4°C. To confirm specificity of the antibody, serial sections were incubated with the pre-immune serum of the host rabbit and the custom antibody pre-incubated with the peptide (at a 1:10 ratio). Bound primary antibody was detected with the Rabbit Envision Detection System (Dako, UK) and slides counter-stained in CuSO₄ and Mayers haematoxylin. Finally, sections were dehydrated in increasing concentrations of ethanol (25-100%) and cleared in two changes of xylene before being mounted in DPX mounting media (Sigma). (B) Protein localization in adult human retina. SLC38A8 staining is found throughout the neuronal retina with particularly strong staining evident in the inner and outer plexiform layers and the photoreceptor cell layer. NFL - nerve fiber layer, GCL - ganglion cell layer, IPL – inner plexiform layer, INL – inner nuclear layer, OPL – outer plexiform layer, ONL – outer nuclear layer, POS – photoreceptor outer segments, RPE – retinal pigment epithelium. The scale bars represent 40 μm. (C) Protein localization in human adult brain. A section of grey matter from the occipital lobe is shown and is representative of all brain sections examined (pre-frontal cortex, mid-frontal cortex, primary motor cortex and hippocampus). The majority of the neuronal cells are stained along with the neuropil. However, many glial cells remain unstained. The scale bar represents 100 µm.

Table S1. SLC38A8 primers for PCR/sequencing

Exon	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)
1	GAGCTCTCACTGAGTTGGGG	CCTCATTAGCTGAGCGGG	437
2	TGTGGAGAGTGTGCCCG	GGAGCCTCCCTTCCCTAC	449
3	GTGGATGTCCAACCCCAG	CAAATGTGAAGGCCAATTCC	350
4	ACCTCATTGTCAAGGGCG	CTCTGCAGTGAGCCACGAGT	312
5	TTGCAGCCATGCTCTGTTAC	CCCTGACAGAGAAACCAAGG	266
6	TCAGGCTATTTCAAGAGAAACCTC	TGAGGCCTTTTCCTATGCAC	398
7	AAGGAAAATCTTAGGTGGTAAAGC	AGTCCCACAGCCGCGTAG	352
8	TGTGGAACAGGAGCACTGAC	TCCTACCCATATGTGGCTCC	442
9	CCTTCCAGCTCCACTGTGTT	CAGATGCCCTCATACTGGGT	380
10	CCTACAGGTAACTGGAAATATTCTGA	GGCATCAGTCTCTCCAGCAT	364

Primer sequences for all other genes screened are available on request.

Mutation	PolyPhen2	MutationTaster	SIFT	Blosum62*	PROVEAN	MutPred
p.Met34Arg	Benign (score	Disease causing	Tolerated (score	Score -1	Deleterious	Deleterious mutation
	0.225)	(prediction probability	0.07)		(score	(prediction probability 0.893)
		0.8385)			3.611)	
p.Glu233Lys	Probably	Disease causing	Tolerated (score	Score +1	Neutral	Deleterious mutation
	damaging (score	(prediction probability	0.34)		(score -	(prediction probability 0.752)
	1.000)	1.0000)			2.267)	
p.Val236Asp	Probably	Disease causing	Damaging (score	Score -3	Deleterious	Deleterious mutation
	damaging (score	(prediction probability	0.03)		(score	(prediction probability 0.846)
	1.000)	0.9999)			6.274)	
p.Gly412Arg	Probably	Disease causing	Damaging (score	Score -2	Deleterious	Deleterious mutation
	damaging (score	(prediction probability	0.01)		(score	(prediction probability 0.857)
	1.000)	0.9999)			5.317)	

Table S2. Summary of bioinformatics analyses undertaken to predict the pathogenic nature of the missense mutations

URLs: PolyPhen2, http://genetics.bwh.harvard.edu/pph2/ [Adzhubei, I.A. et al. (2010). Nat. Methods 7, 248-9]; Mutationtaster,

http://www.mutationtaster.org/ [Schwarz, J.M. et al. (2010). Nat. Methods 7, 575-6]; SIFT, http://sift.jcvi.org/ [Ng, P.C. et al. (2003). Nucleic Acids Res. 31, 3812-4]; Blosum62 [Henikoff, S. et al. (1993). Proteins 17, 49-61]; PROVEAN, http://provean.jcvi.org/ [Choi, Y. et al. (2012). PLoS ONE 7, e46688]; MutPred, http://mutpred.mutdb.org/ [Li, B. et al. (2009). Bioinformatics 25, 2744-50]. *Blosum62 scores range from +3 to -3 and negative scores are more likely to be damaging substitutions.

Table S3. Medaka Slc38a8 morpholino sequences

Morpholino name	Sequence (5'-3')	Concentration (mM)
Mo-5'UTR-Slc38a8-c3	GGCAGGGTTTGCAAAGAGTTGACAA	0.06
mmMo-5'UTR-Slc38a8-c3	GGtAGcGTTTcCAAAcAGTTtACAA	0.06
Mo-spl-Slc38a8-c3-ex4	GTAACTCACCCCTGGATGTGTTCTG	0.06
mmMo-spl-Slc38a8-c3-ex4	GTAAcTCACgCCTGcATtTGTTgTG	0.06
Mo-5'UTR-Slc38a8-c6	TCAGAGGGAAGATGCTCGAGGCAGC	0.06
mmMo-5'UTR-Slc38a8-c6	TCAcAGGcAAGATcCTCtAGGtAGC	0.06
Mo-spl-Slc38a8-c6-ex4	AAAACATACCCAGTGGAGTGGATCG	0.06
mmMo-spl-Slc38a8-c6-ex4	AAAAgATACgCAGTaGAGTcGATTg	0.06
Mo-p53 control	CGGGAATCGCACCGACAACAATACG	0.09

Mo = morpholino, mmMo = mismatch morpholino. c3 and c6 = chromosome 3 and 6 respectively.

Family	Ethnicity	Foveal hypoplasia	Optic nerve	Anterior segment	Iris	Other notes
			decussation defect	abnormalities	transillumination	
F1	Pakistani	Present in all affected	Present in all	Posterior embryotoxon in	Absent in all	
		members (IV:1, IV:3,	affected members	all affected members	affected members	
		IV:4, IV:6, IV:7)	tested (IV:1, IV:3)	(IV:1, IV:3, IV:4, IV:6,	(IV:1, IV:3, IV:4,	
				IV:7) and Axenfeld's	IV:6, IV:7)	
				anomaly also present in		
				IV:1 and IV:7		
F2	Afghan	Present in both	Present in both	Posterior embryotoxon	Absent in both	
		affected members	affected members	present in both members	affected members	
		(V:2 and V:5)	(V:2 and V:5)	(V:2 and V:5)	(V:2 and V:5)	
F3	Northern	Present in single	Present in single	None	Absent in single	Kartagener syndrome also
	European	affected case (II:4)	affected case (II:4)		affected case (II:4)	present in II:4
F4	Pakistani	Present in single	Test inconclusive	None	Absent in single	
		affected case (II:1)	in single affected		affected case (II:1)	
			case (II:1)			
F5	Turkish	Present in single	Present in single	None	Absent in single	
		affected case (IV:1)	affected case (IV:1)		affected case (IV:1)	
F6	Indian	Present in all	Not tested	None	Absent in all	IV:7 and VI:1 have bilateral
		examined affected			examined affected	microphthalmia and IV:7 has
		members (IV:7, VI:1,			members (IV:7,	a unilateral retinochoroidal
		VI:2)			VI:1, VI:2)	coloboma (right eye).
F7	Northern	Present in both	Present in both	Axenfeld's anomaly	Absent in both	
	European	affected members	affected members	present in only II:2	affected members	
		(II:1, II:2)	(II:1, II:2)		(II:1, II:2)	

Table S4. Summary of clinical phenotypes of families with SLC38A8 mutations

Detailed descriptions of members of F1, F2, F3 and F6 have been reported previously.^{4, 5, 6, 8}

Table S5. Top associated variants at the *SLC38A8* locus with foveal thickness parameters in the general population, using data from the Raine Eye Health Study

				Effect						
Trait	SNP	CHR	BP	Allele	MAF	Beta	SE	Р	HWE	IxScore
Fovea Thickness	rs7200988	16	82605474	А	0.051	-0.456	0.132	5.77E-04	0.41	0.83
Fovea:Parafovea Thickness Ratio	rs13336065	16	82604407	А	0.045	-0.596	0.143	3.44E-05	1.00	0.84
Fovea:Perifovea Thickness Ratio	rs9673448	16	82601632	С	0.056	-0.467	0.130	3.42E-04	0.72	0.84

SNP, single nucleotide polymorphism; CHR, chromosome; BP, base position on hg.18; MAF, minor allele frequency; SE, standard error; HWE, Hardy-Weinberg

Equilibrium; IxScore, imputation QC score.