

Supplementary Table 1. Primers used for confirmatory and functional studies

Region of Interest	Primer Name	Primer Sequence 5' - 3'	Product Size
COL4A6 Exons 21	Hu COL4A6 Ex21 F	TTCCATTGCTTCCTCAGGGTCA	317 bp
	Hu COL4A6 Ex21 R	GCAAGCCTTCAGATTCCCTCTATGG	
COL4A6 Exons 22-23	Hu COL4A6 Ex22 F	ATAGGGGTGAGGAGACAGGG	774 bp
	Hu COL4A6 Ex22 R	CAATGTGCTGGCCCATGAAG	
DYM Exon 10	Hu DYM Ex10 F	GCGAACTATCCCAAGGACAA	485 bp
	Hu DYM Ex10 R	CAGTCCTTTCCCCTCATCAA	
pSPL3 exons A and B	SD6 F	TCTGAGTCACCTGGACAACC	437 bp/ 257 bp
	SA2 R	ATCTCAGTGGTATTTGTGAGC	
COL4A6 Exons 22-23	Hu COL4A6 Ex22-23 XhoI F	aattctcgagGTGCCAATATGTACCCAAGG	471 bp
	Hu COL4A6 Ex22-23 BamHI R	attggatccTTTCTGGATAGGGGTGAGGA	
COL4A6-specific WISH primers	COL4A6-specific WISH F	gaattgaattaaccctcactaaagggCCACTTGGATTGAGCAACAG	858 bp
	COL4A6-specific WISH R	gaattgtaatacgactcactatagggCCCATCAAAGCCTTTAGCTCC	
COL4A6-specific RT-qPCR primers	COL4A6-RT-qPCR F	GTTGGTCCACAAGGAGTCAGG	255 bp
	COL4A6-RT-qPCR R	GCCTGGATAACGAAATGGGTTTACT	

Transcripts used: COL4A6: NM_033641.4, DYM: NM_001353214.3. Abbreviations: WISH, whole mount in situ hybridization

Supplementary Table 2. Current and previously reported *COL4A6* variants

	Database/ tool	Family 1 Proband	Family 2 Proband	Rost et al., 2014	O'Brien et al., 2022	O'Brien et al., 2022	Feng et al, 2024
Position (GRCh37) NM_001287758.2	g. position	g.107431854C>T	g.108187848C>T	g.108187279C>T	g.108195081C>A	g.108171395C>G	g.107431878C>T
	c. position	c.1480G>A	c.1767G>A	c.1768G>A	c.948+1G>T	c.3320G>C	c.1456G>A
	p. position	p.(Gly494Arg)	p.(Pro589=)	p.(Gly590Ser)	p.(Pro303Glnfs*11)	p.(Gly1107Ala)	p.(Gly486Ser)
	Exon	21	22	23	15	34	21
Reference Databases	dbSNP	rs1341885508	rs781038243	rs779748859	rs1235090929	rs769241359	rs769966341
Population frequency databases	gnomAD v4.1	Female: 0.000002473 Male: 0.000007758 Overall MAF: 0.000004182	Female: 0.00002570 Male: 0.00002985 Overall MAF: 0.00002695	Female: 0.00003857 Male: 0 Overall MAF: 0.00002684	Female: 0.000008689 Male: 0 Overall MAF: 0.000005638	Female: 0.0002338 Male: 0.0001367 Overall MAF: 0.0001985	Female: 0.000002493 Male: 0.000005345 Overall MAF: 0.000003400
Pathogenicity prediction tools	CADD Phred	23.4	No result	31	No result	23.2	23.3
	Mutation Taster	Benign	No result	Benign	No result	Benign	Benign
Splicing Predictions	Total	0.0%	-11.1%	-17.2%	-100.0%	0.0%	22.4
	MaxEntScan	0.0%	-16.7%	-27.8%	-100.0%	0.0%	Benign
	NNSPLICE	0.0%	-3.3%	-19.1%	-100.0%	0.0%	0.0%
	SSF	0.0%	-13.3%	-4.9%	-100.0%	0.0%	0.0%

Note: a possible discrepancy in the c. and p. position of previously published variants is due to different transcripts that were used for reporting. All variants were annotated using the transcript NM_001287758.2 that is also used in the Deafness Variation Database v9.