Supplemental Data

TMEM126A, Encoding a Mitochondrial Protein, Is Mutated in Autosomal-Recessive Nonsyndromic Optic Atrophy

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Figure S1. Full parametric linkage analysis of Family 1 using a combination of Affymetrix GeneChip Human Mapping 10K 2.0 Arrays and microsatellite markers.
This approach points to a unique candidate region on chromosome 11 with a maximum lod-score >+3. Parametric LOD scores were calculated using the MERLIN[1] software program.

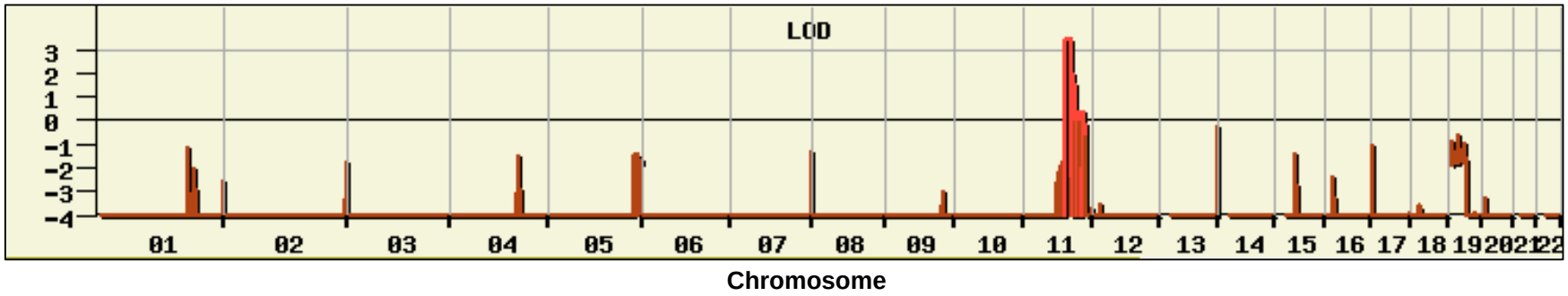
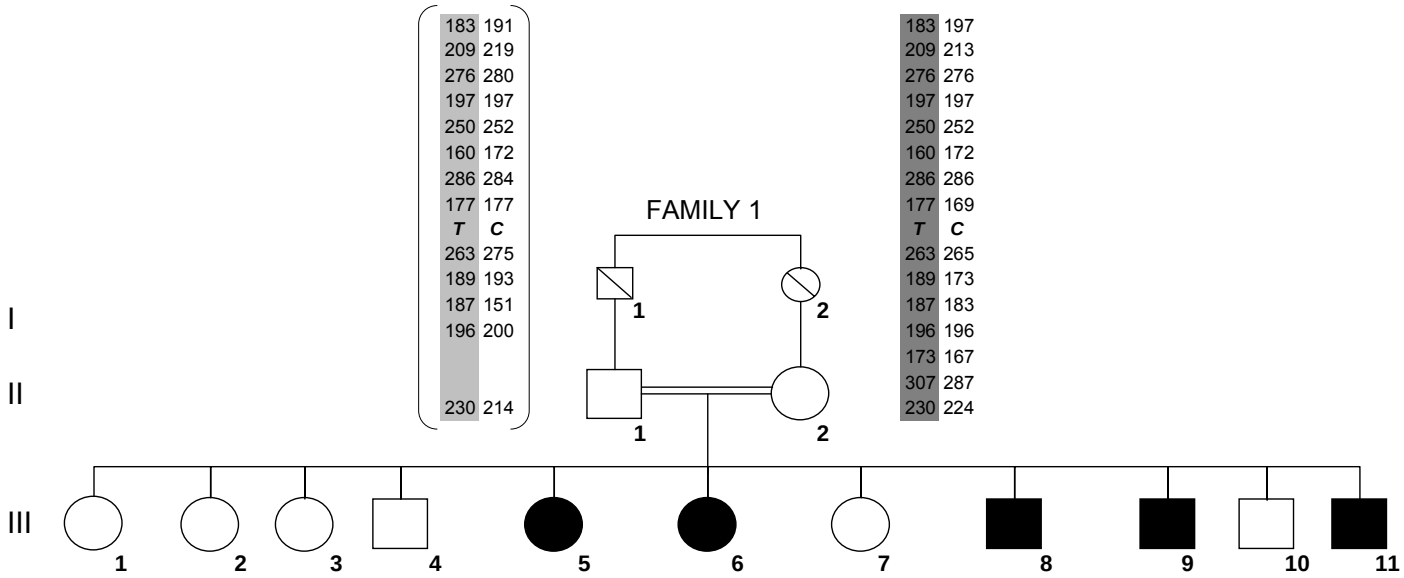
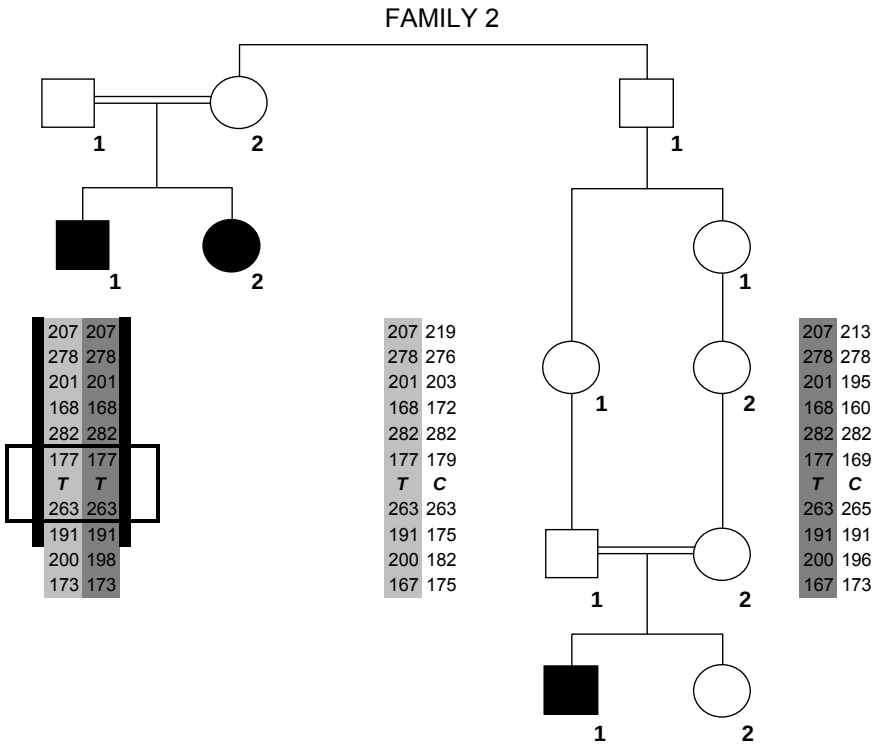


Figure S2. Localization of the *ROA2* locus and segregation of disease-causing mutations.
Pedigree structure of families 1 to 4 with haplotypes reconstruction for informative markers on chromosome 11q14.1-q21. Black circles (women) and squares (men) indicate affected members. The code numbers of all sampled individuals are given below the symbols. Chromosomal positions of SNP and microsatellite markers are indicated in Mega base pairs (Mb) according to the draft of the human genome sequence (UCSC and Ensembl databases). VNTR: variable number of tandem repeats chosen from the UCSC database (primers in the Supplementary Table). Brackets show the deduced haplotype of subject II-1. Hashes point multiallelic markers used to refine the interval indicated by the "Affimetrix 10k SNP" genome scan in individuals III-2, III-5, III-6, III-8 and III-9 (Family 1). The homozygous haplotype in which the mutated gene is most likely located in affected patients is flanked by black boxes. Arrows indicate the position of key recombination events that were used to restrict the candidate *ROA2* interval: obligatory recombination events between loci *D11S4143* and *VNTR19CA* (Family 1, patient III-9), and loci *rs2020351* and *VNTR26AT* (family 1, individual III-8), respectively, defined a 14.4 Mb interval between loci *D11S4143* and *VNTR26(AT)*. The ancestral haplotype shared by the four families is framed.
Segregation of disease-causing mutations: ''C'' indicates wild-type allele and "T" indicates the nonsense mutation c.163C>T, p.Arg55X. Mutations were detected after PCR of the five *TMEM126A* exons (primers in the Supplementary Table), sequencing of the purified fragments using the Big Dye chemistry version 3.1 (Applied Biosystems) and analysis using an ABI-3130 sequencer (Applied Biosystems). We have chosen to number the A of the start codon (ATG) of the cDNA sequence of the *TMEM126A* (Genbank accession numbers NM_ 032273) as nucleotide 1.

MARKERS	Mb	Band
centromere		
D11S918 (AFM203vg1) #	78.4	11q14.1
D11S4143 (AFMb055yd1) #	79.03	
VNTR19CA #	79.28	
D11S1362 (AFMa132xh9) #	79.47	
VNTR23TG #	79.67	
D11S901 (AFM063yg1) #	81.52	
D11S4187 (AFM311wh5) #	83.23	
D11S1354 (AFM338xe1) #	84.35	
TMEM126A - c.163C	**85.04**	11q14.1
D11S1887 (AFMa049wa5) #	86.06	11q14.2
D11S1780 (AFMa082wb9) #	87.32	
D11S4175 (AFM269yg9) #	89.89	11q14.3
D11S1332 (AFM281wf9) #	91.74	
VNTR29TA #	93.41	11q21
VNTR26AT #	93.48	
D11S4176 (AFMb354xa5) #	93.71	
telomere		



MARKERS	Mb	Band	1	2	3	4	5	6	7	8	9	10	11
D11S918 (AFM203vg1) #	78.4	11q14.1	191 197	191 197	183 183	183 183	183 197	183 183	191 183	183 197
D11S4143 (AFMb055yd1) #	79.03		219 213	219 213	209 209	209 209	209 213	209 209	219 209	209 209
VNTR19CA #	**79.28**		280 276	280 276	276 276	276 276	276 276	276 276	276 276	276 276
D11S1362 (AFMa132xh9) #	**79.47**		197 197	197 197	197 197	197 197	197 197	197 197	197 197	197 197
VNTR23TG #	**79.67**		252 252	250 252	250 250	250 250	250 252	250 250	250 250	250 250
rs1479311	**79.7**		A A		B B	B B		B B	B B	
D11S901 (AFM063yg1) #	**81.52**		172 172	160 172	160 160	160 160	160 172	160 160	160 160	160 160
rs1318423	**82.34**		A B		B B	B B		B B	B B	
D11S4187 (AFM311wh5) #	**83.23**		284 286	286 286	286 286	286 286	286 286	286 286	286 286	286 286
D11S1354 (AFM338xe1) #	**84.35**		177 169	177 169	177 177	177 177	177 169	177 177	177 177	177 177
rs1073985	**84.36**		A B		B B	B B		B B	B B	
TMEM126A - c.163C	**85.04**	11q14.1	***C C***	***T C***	***T T***	***T T***	***T C***	***T T***	***T T***	***T T***
D11S1887 (AFMa049wa5) #	**86.06**	11q14.2	275 265	263 265	263 263	263 263	263 265	263 263	263 263	263 263
rs1378879	**86.47**		A A		B B	B B		B B	B B	
D11S1780 (AFMa082wb9) #	**87.32**		193 173	189 173	189 189	189 189	189 173	189 189	189 189	189 189
rs649529	**87.69**		B B		A A	A A		A A	A A	
D11S4175 (AFM269yg9) #	**89.89**	11q14.3	151 183	187 183	187 187	187 187	187 183	187 187	187 187	187 187
rs1404527	**90.29**		A A		B B	B B		B B	B B	
D11S1332 (AFM281wf9) #	**91.74**		200 196	196 196	196 196	196 196	196 196	196 196	196 196	196 196
rs2045462	**92.91**	11q21	A B		A A	A A		A A	A A	
VNTR29TA #	**93.41**		173 167	173 167	173 173	173 173	173 167	173 173	173 173	173 173
rs2020351	**93.43**		A B		B B	B B		B B	B B	
VNTR26AT #	93.48		307 287	307 287	307 307	307 307	307 287	307 287	307 307	307 307
D11S4176 (AFMb354xa5) #	93.71		230 224	230 224	214 230	230 230	230 224	230 224	230 230	230 230


FAMILY 2
I
II
III
IV
V
1
2
1
2
1
1
2
1
2
1
2
1
2
D11S4143 (AFMb055yd1) # 79.03 11q14.1
VNTR19CA # 79.28
D11S1362 (AFMa132xh9) # 79.47
D11S901 (AFM063yg1) # 81.52
D11S4187 (AFM311wh5) # 83.23
D11S1354 (AFM338xe1) # 84.35
TMEM126A - c.163C 85.04 11q14.1
D11S1887 (AFMa049wa5) # 86.06 11q14.2
D11S1780 (AFMa082wb9) # 87.32
D11S1332 (AFM281wf9) # 91.74
VNTR29TA # 93.41 11q21
207 207
278 278
201 201
168 168
282 282
177 177
T T
263 263
191 191
200 198
173 173
207 219
278 276
201 203
168 172
282 282
177 179
T C
263 263
191 175
200 182
167 175
207 213
278 278
201 195
168 160
282 282
177 169
T C
263 263
191 191
200 196
167 173
D11S4143 (AFMb055yd1) # 79.03 11q14.1
VNTR19CA # 79.28
D11S1362 (AFMa132xh9) # 79.47
D11S901 (AFM063yg1) # 81.52
D11S4187 (AFM311wh5) # 83.23
D11S1354 (AFM338xe1) # 84.35
TMEM126A - c.163C 85.04 11q14.1
D11S1887 (AFMa049wa5) # 86.06 11q14.2
D11S1780 (AFMa082wb9) # 87.32
D11S1332 (AFM281wf9) # 91.74
VNTR29TA # 93.41 11q21
207 207
278 278
201 201
168 168
282 282
177 177
T T
263 263
191 191
200 200
167 167
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278 278
201 195
168 160
282 282
177 169
T C
263 265
191 191
200 196
167 173

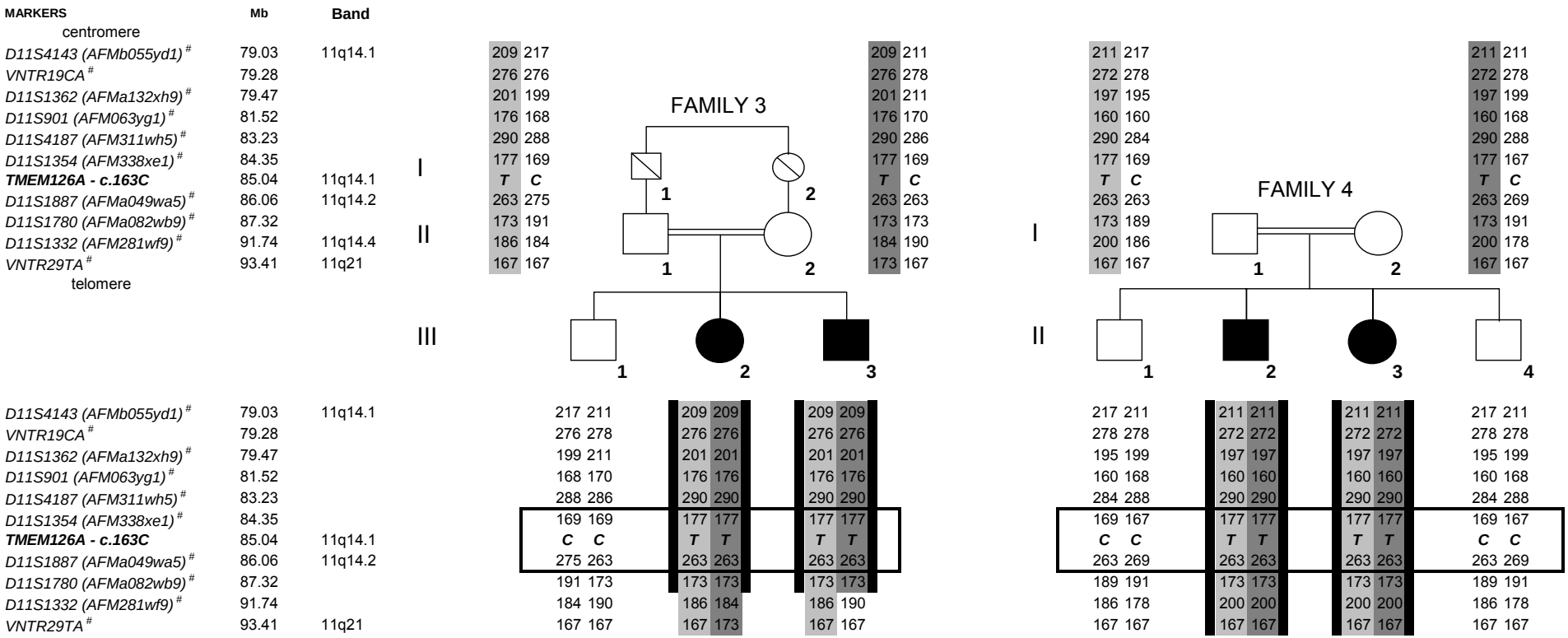

MARKERS
Mb
Band
centromere
D11S4143 (AFMb055yd1) #
79.03
11q14.1
VNTR19CA #
79.28
D11S1362 (AFMa132xh9) #
79.47
D11S901 (AFM063yg1) #
81.52
D11S4187 (AFM311wh5) #
83.23
D11S1354 (AFM338xe1) #
84.35
TMEM126A - c.163C
85.04
11q14.1
D11S1887 (AFMa049wa5) #
86.06
11q14.2
D11S1780 (AFMa082wb9) #
87.32
D11S1332 (AFM281wf9) #
91.74
11q14.4
VNTR29TA #
93.41
11q21
telomere
FAMILY 3
I
II
III
1
2
1
2
1
2
3
209 217
276 276
201 199
176 168
290 288
177 169
T C
263 275
173 191
186 184
167 167
209 211
276 278
201 211
176 170
290 286
177 169
T C
263 263
173 173
184 190
173 167
FAMILY 4
I
II
1
2
1
2
3
4
211 217
272 278
197 195
160 160
290 284
177 169
T C
263 263
173 189
200 186
167 167
211 211
272 278
197 199
160 168
290 288
177 167
T C
263 269
173 191
200 178
167 167

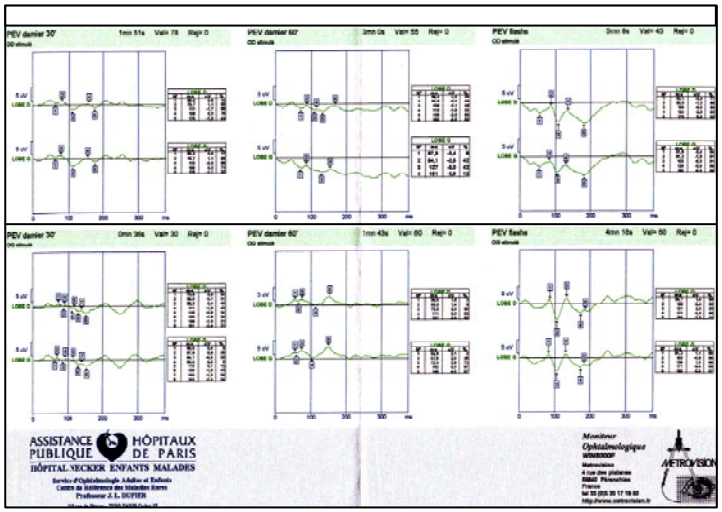
Figure S3. Ophthalmological data in 37-year-old patient III6, Family 1 harboring the *TMEM126* p.Arg55X mutation.
(**A**) The fundus photograph of left eye shows optic disc pallor with normal aspect of the retina including the macular region. (**B**) Flash visual evoked potentials (VEP) show flattened waves, prolonged latencies and highly reduced amplitudes for right eye (RE) and left eye (LE). Electroretinographic recordings were strictly normal. Pattern reversal VEP display hardly recordable traces. (**C**, **D**) Goldman Dynamic perimetry show severe bilateral visual field loss.

A

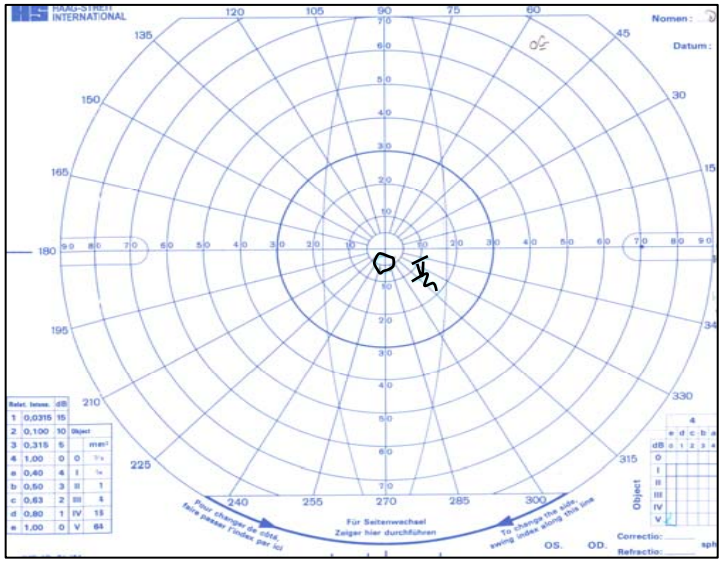
B

RE

LE


ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS
HÔPITAL NECKER ENFANTS MALADES
METROVISION

C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Nomen :
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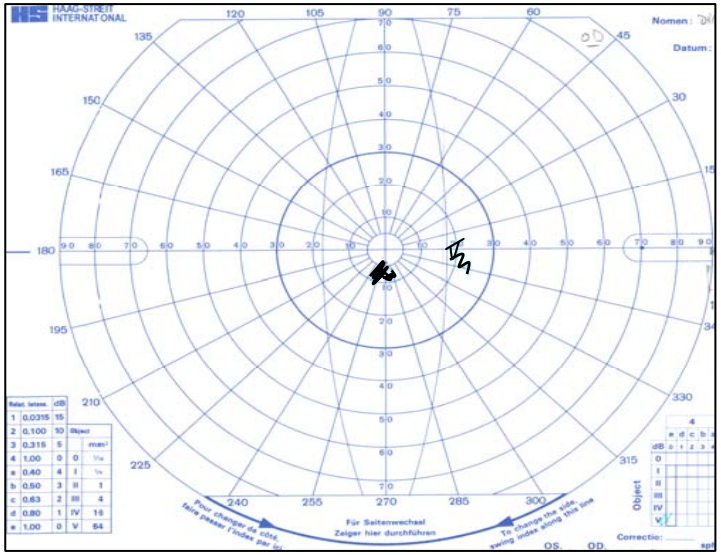
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Figure S4. Brain Magnetic Resonance Imaging (MRI) of 37-year-old patient III6, Family 1 harboring the *TMEM126* p.Arg55X mutation.
(**A**) Axial FLAIR weighted images show asymmetric and homogeneous punctate hyperintensities with no evidence for necrosis (white arrows). (**B**) Coronal weighted FSE T2 and axial FLAIR images show hyperintensities in the bilateral stratum subependymale (left > right side) near the head and corpus of the caudate nuclei (black arrow).

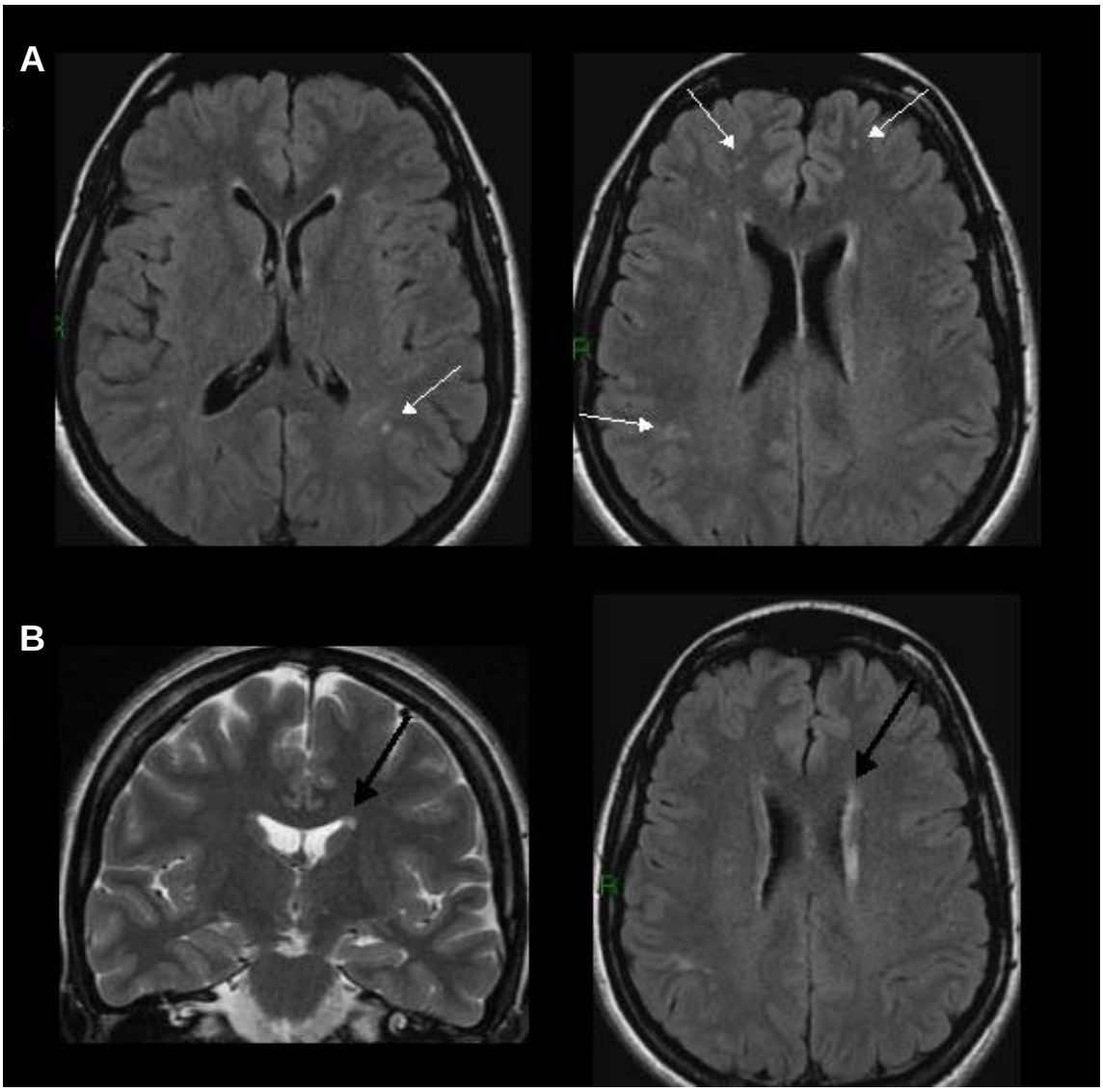


Figure S5. Expression of the TMEM126-myc fusion protein in COS-7 cells, 48 hours after transient transfection.

COS-7 cells were transfected with 1 µg plasmid DNA (OmicsLink ORF Expression Clone-GeneCopoeiaTM clone EX-V0975-M09; Ressourcenzentrumfuer Genomforschung imaGenes) with FuGENE®6 transfection reagent, according to the manufacturer's instructions (Roche). The cells were fixed for 15 min in 4% formaldehyde, 48 hr post-transfection. Immunocytochemistry was performed via classical procedures. Colocalization experiments were performed using specific or myc antibodies (mouse monoclonal anti-myc and rabbit polyclonal anti-myc, 1/200, Santa Cruz Biotechnology), respectively. Secondary antibodies were Alexa Fluor®488 conjugated goat anti-rabbit (1/1000, Molecular Probes) and ZyMax™ goat anti-mouse CY™ 3 conjugate (1/100, Invitrogen). No significant colocalization of Transmembrane 126A protein was noted with Golgi apparatus (mouse monoclonal anti-Giantin, 1/200, Abcam), lysosomes (mouse monoclonal anti-Lamp2, 1/200, Abcam), endoplasmic-reticulum (mouse monoclonal anti-Calreticulin, 1/400, Stressgen), early endosomes (rabbit polyclonal anti-EEA1, 1/200, Abcam), cytoskeleton (mouse monoclonal anti-βActin, 1/100, AbCys) and microtubule (mouse monoclonal anti-γTubulin, 1/200, Sigma), respectively. Cells were counterstained and mounted with ProLong®Gold antifade reagent with DAPI (Invitrogen). Images were recorded using a Leica SP5 confocal microscope (objective 63x oil immersion objective, scale bar = 20 µm) and Leica Application Suite Advanced Fluorescence Lite software.

Expression of a myc-tagged fusion protein of the expected size was verified on Western blots of cell extracts with a myc antibody (mouse anti myc, 1/1000, Santa Cruz Biotechnology, data not shown).

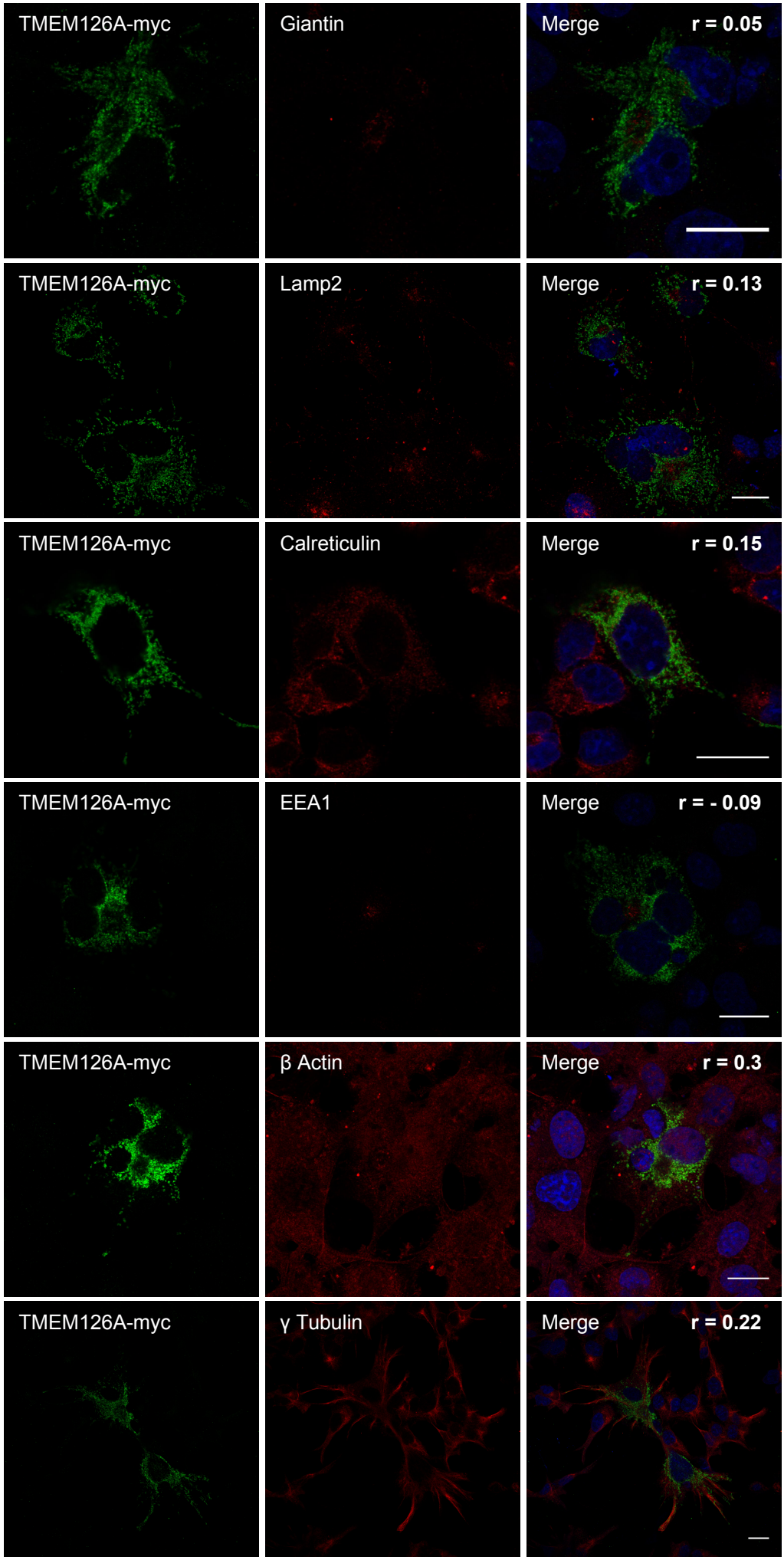
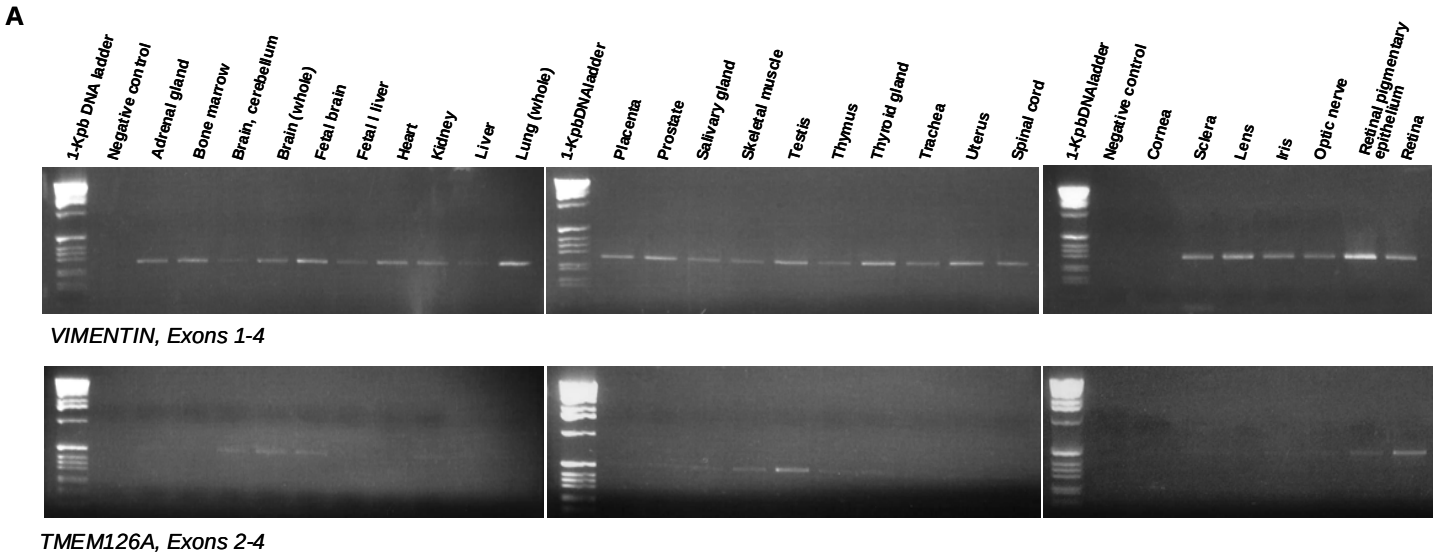

TMEM126A-myc
Giantin
Merge
r = 0.05
TMEM126A-myc
Lamp2
Merge
r = 0.13
TMEM126A-myc
Calreticulin
Merge
r = 0.15
TMEM126A-myc
EEA1
Merge
r = - 0.09
TMEM126A-myc
β Actin
Merge
r = 0.3
TMEM126A-myc
γ Tubulin
Merge
r = 0.22

Figure S6. RT-PCR quantification of *TMEM126A* mRNA in human tissues.
TMEM126A mRNA expression was analyzed by RT-PCR in adult human tissues, normalized with respect to *vimentin* expression, (**A**) semi-quantitatively (at 25 PCR cycles, in the exponential phase) and (**B**) at end point PCR (et 35 cycles, in the plateau phase). One microgram of total RNAs from adrenal gland, bone marrow, brain (whole), cerebellum, fetal brain, fetal liver, lung (whole), placenta, prostate, salivary gland, skeletal muscle, testis, thymus, thyroid gland, trachea, uterus and spinal cord (Human Total RNA Master panel II, Clontech) were reverse transcribed with the High Capacity cDNA Archive Kit (Applied Biosystems) primed with Oligo d(T)16 (Applied Biosystems) in accordance with the supplier's recommendations. 0.5 microgram of total RNAs from retina, optic nerve, retinal pigmentary epithelium (RPE), cornea, iris, sclera and lens extracted from a twenty-week-old fetal human ocular globe were also reverse transcribed. A 523 pb cDNA fragment of *TMEM126A* from exon 2 to exon 5 was amplified using primer: (forward) 5'-taaccagcttccagaagcag-3', (reverse) 5'-gtgaatttctttgccaggttca-3' (Genbank accession numbers NM_032273). A 274 bp cDNA fragment of *VIMENTIN* containing exons 1 to 4 was amplified using primers: (forward) 5'-accagctaaccaacgacaaa-3', (reverse) 5'-tgctgttcctgaatctgagc-3' (GenBank accession number NM_003380), as a reference. Semi-quantitative condition showed a predominantly expression in brain (whole), cerebellum, fetal brain, skeletal muscle, testis, fetal retinal pigmentary epithelium (RPE) and retina (**A**) while a widespread expression of *TMEM126A* mRNA in all adult tissues was noted (**B**).


A
1-Kpb DNA ladder
Negative control
Adrenal gland
Bone marrow
Brain, cerebellum
Brain (whole)
Fetal brain
Fetal liver
Heart
Kidney
Liver
Lung (whole)
1-KpbDNAladder
Placenta
Prostate
Salivary gland
Skeletal muscle
Testis
Thymus
Thyroid gland
Trachea
Uterus
Spinal cord
1-KpbDNAladder
Negative control
Cornea
Sclera
Lens
Iris
Optic nerve
Retinal pigmentary epithelium
Retina
VIMENTIN, Exons 1-4
TMEM126A, Exons 2-4

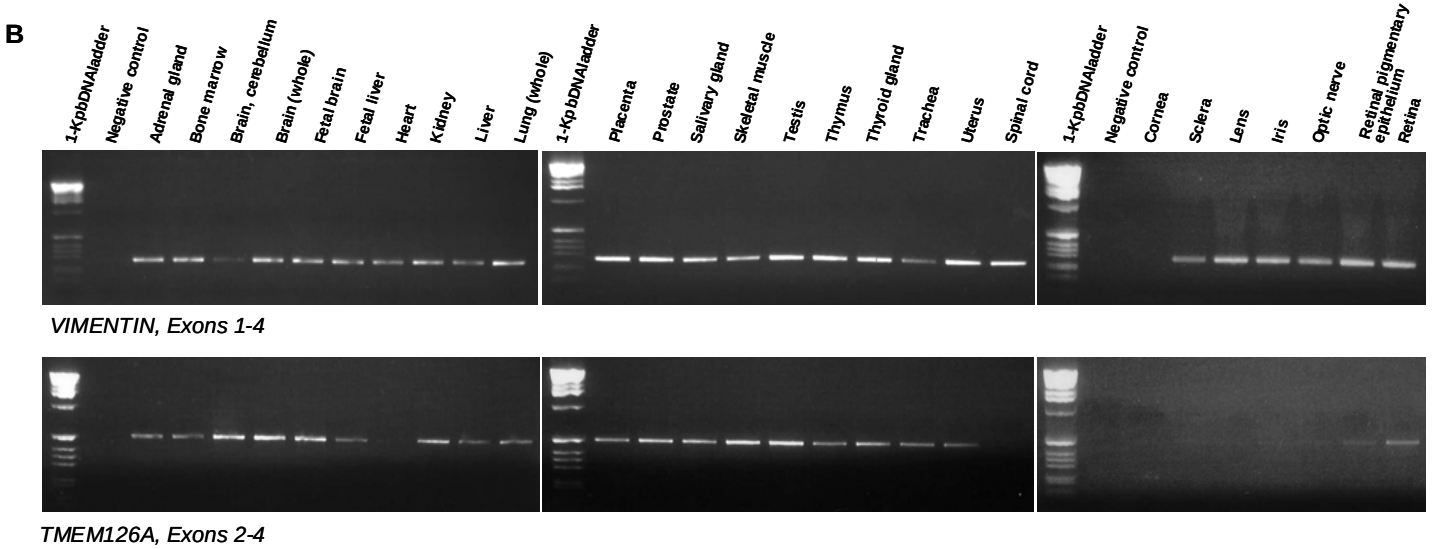

B
1-KpbDNAladder
Negative control
Adrenal gland
Bone marrow
Brain, cerebellum
Brain (whole)
Fetal brain
Fetal liver
Heart
Kidney
Liver
Lung (whole)
1-KpbDNAladder
Placenta
Prostate
Salivary gland
Skeletal muscle
Testis
Thymus
Thyroid gland
Trachea
Uterus
Spinal cord
1-KpbDNAladder
Negative control
Cornea
Sclera
Lens
Iris
Optic nerve
Retinal pigmentary epithelium
Retina
VIMENTIN, Exons 1-4
TMEM126A, Exons 2-4

Table S1. Sequences of primers used for amplification of (**A**) four polymorphic markers developed from the UCSC Draft of the Human Sequence; and (**B**) the five exons of the *TMEM126* gene (Genbank accession number NM_032273).

A) Polymorphic markers (VNTR = variable number of tandem repeat).

VNTRs / chromosomal position	Forward sequence (5'-3')	Reverse sequence (5'-3')
VNTR19CA (79.28Mb)	tgccgggagcctaaaat	actcaccctgcagatcttag
VNTR23TG (79.67Mb)	gatacatgtatacattgtgt	gttaatcattttgacatgtt
VNTR29TA (93.41Mb)	tcactcaactcctgaacctc	aagatctaccccagtgcg
VNTR26AT (93.48Mb)	gcttcctcattccctctctg	tagcctattgtgggaccc

B) *TMEM126*

Exon	Forward sequence (5'-3')	Reverse sequence (5'-3')
1	cttctcagcccaaagccgct	cgtcgtgcttctcctgacac
2	cagattagctctacagtattat	ccttctcacattccatgttg
3	gggatagatgtcgtatccag	attacagcatacagtacttgg
4	caattacatttgatatattacctg	gggtaacattgcctttggtc
5	tttatcttaagacttctaggac	ccttttgttgtaggtcccag

Supplemental References

1. Abecasis, G.R., Cherny, S.S., Cookson, W.O., Cardon, LR. (2002). Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. Nat. Genet. 30, 97-101.