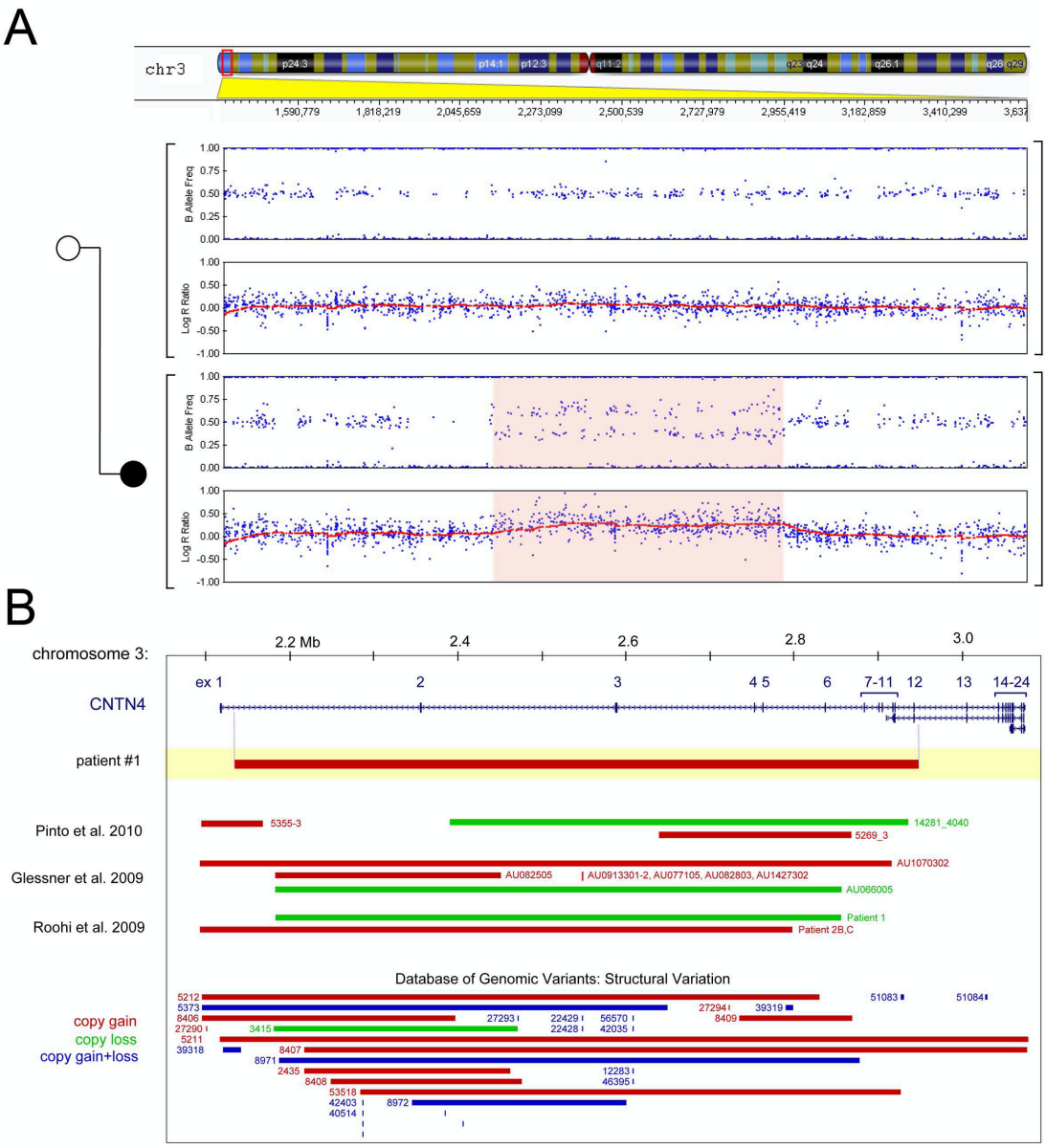
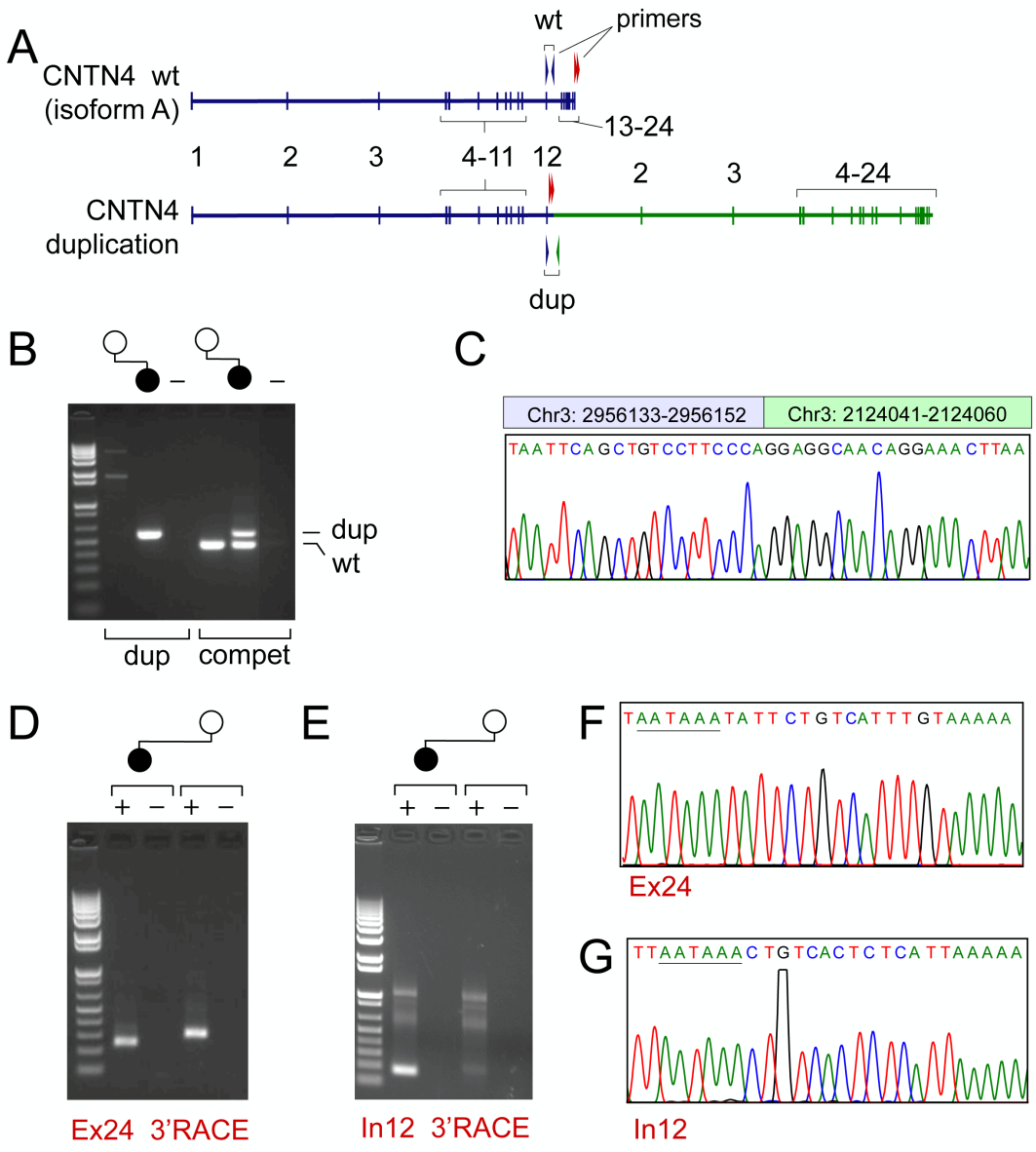
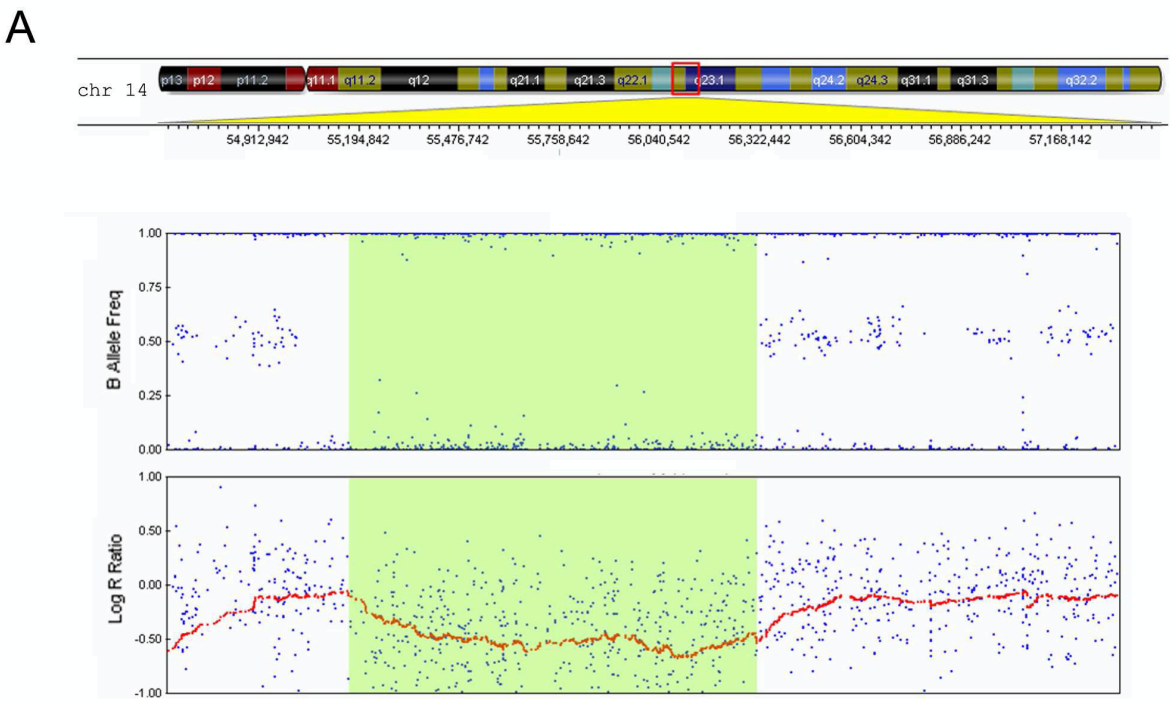
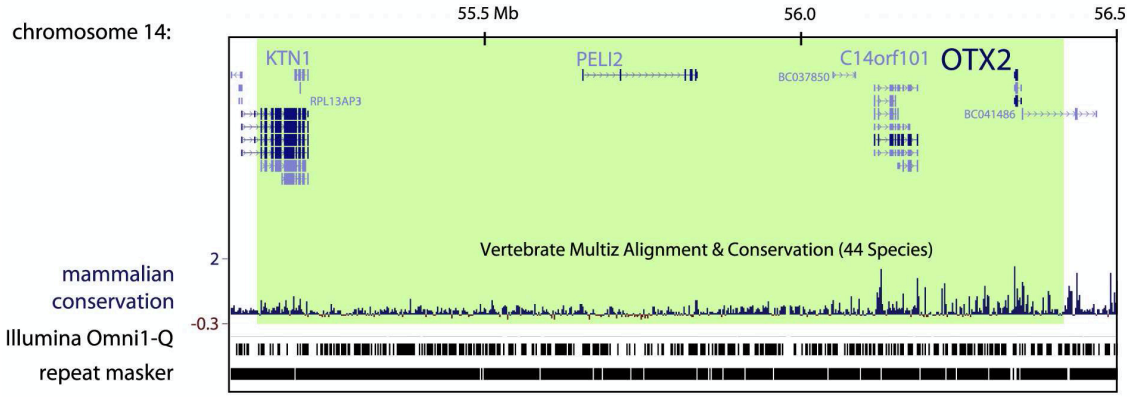


Supplemental Figure 1. ONA Patient 1 carries a duplication that disrupts *CNTN4*. (A) LogR ratio and B allele frequency plots show Illumina Omni1-Quad SNP genotyping for the patient and her unaffected mother. The 0.83 Mb duplication on chromosome 3p26 is shaded in red. The B-allele frequency splitting and logR ratio increase are characteristic of a 3-to-2 copy gain, which was not maternally inherited. (B) *CNTN4* (contactin-4) genomic region. The duplication in patient 1 (thick red line) spans exons 2-12 and is not present in the database of normal genomic structural variants. However, other duplications (red) and deletions (green) overlap *CNTN4* in patients with autism spectrum disorder, as reported in the indicated publications. Human genome coordinates are based on NCBI build 36 (hg18). Mb, megabase.

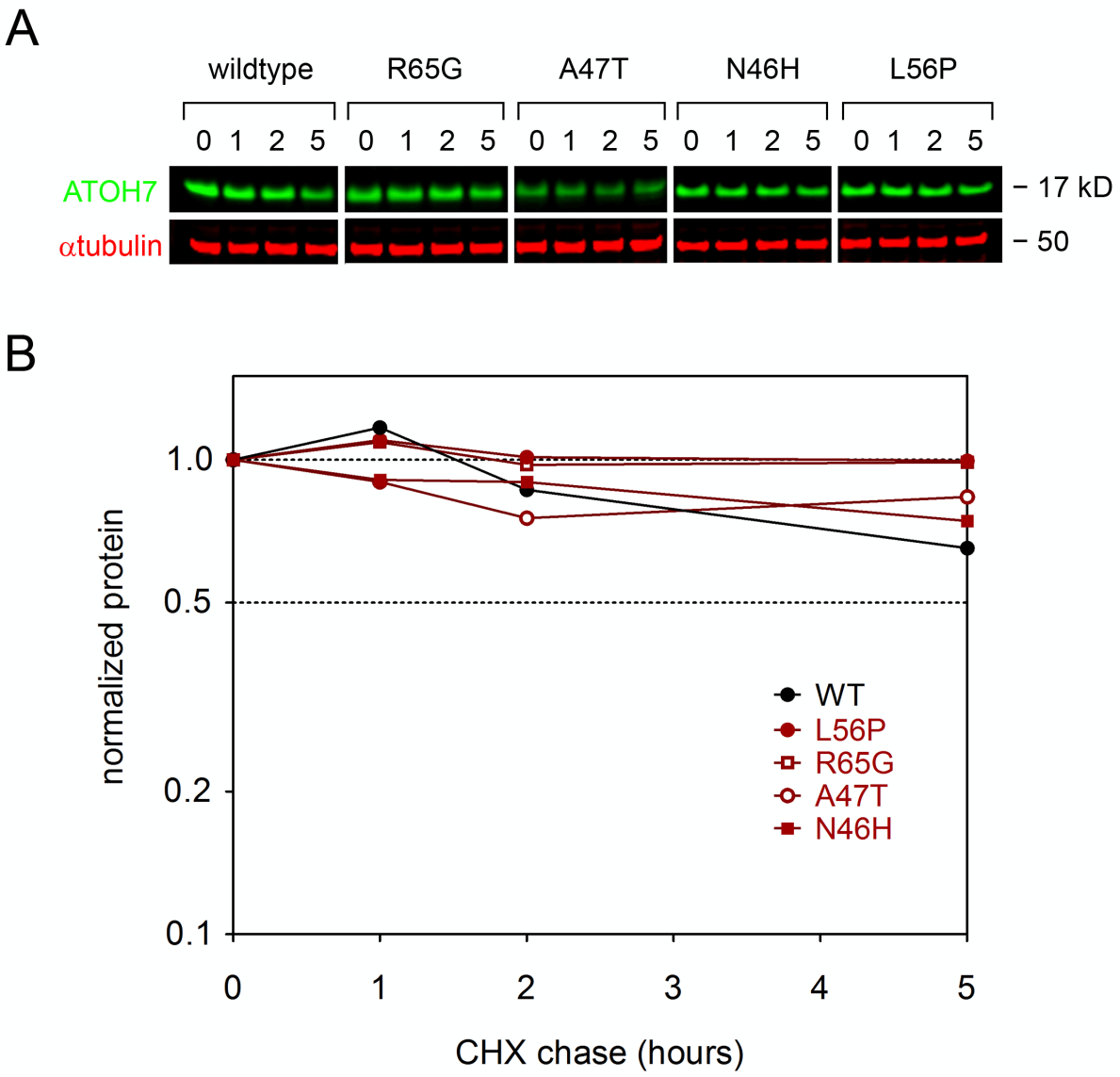


Supplemental Figure 2. The chromosome 3p26 duplication in Patient 1 is capable of producing a truncated *CNTN4* mRNA. (A) Diagram of the *CNTN4* rearrangement, which tandemly duplicates a 832 kb segment encompassing exons 2-12. Arrows mark the PCR primers used to define the molecular breakpoint between 5' (blue) and 3' (green) copies and to test the mRNA terminal structure by 3' RACE (red). (B) Standard duplex (dup) and 3-primer competitive (compet) PCRs amplify a 579 bp product (dup) that spans the novel junction between *CNTN4* intron 12 and intron 1. Only the wildtype (wt) allele was amplified in the mother (465 bp). (C) Sequence chromatogram of PCR products in (B) containing the breakpoint junction. (D,E) 3' RACE analysis of *CNTN4* mRNA in lymphoblastoid cell lines, using nested primers in exon 24 (full length) or exon 12 (truncated). (D) 3' RACE products corresponding to full-length *CNTN4* isoforms were amplified from the patient and her mother. These differ in size due to variable cDNA priming from the polyA tail and the low abundance of *CNTN4* transcripts in lymphoblastoid RNA. (E) The truncated *CNTN4* isoform, which prematurely terminates in intron 12, was only detected in the patient. (F,G) Sequence chromatograms of the wild-type (F) and truncated (G) 3' RACE products. The polyA signal is underlined. In, intron. Ex, exon.

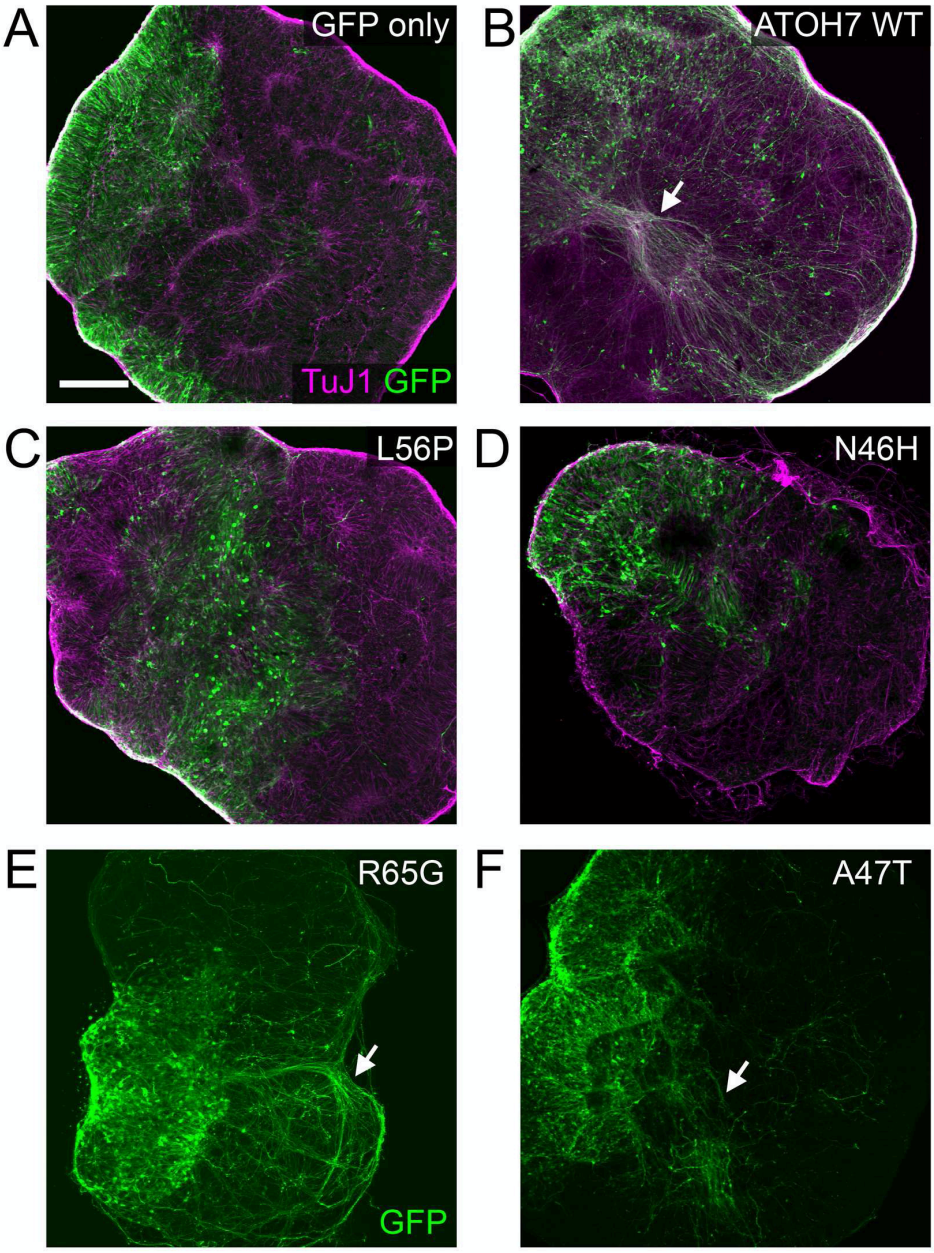




Supplemental Figure 3. Chromosome 14q23 deletion in optic nerve aplasia Patient 2 encompasses the *OTX2* gene. (A) LogR ratio and B allele frequency plots show Illumina Omni1-Quad SNP genotyping data. The 1.2 Mb deletion is shaded in green. The hemizygous region was identified by the pattern of homozygosity in the B-allele frequency plot and decreased intensity in logR ratio plot. (B) Expanded view of the critical region. The deletion spans four genes, including *OTX2*. The endpoints are located in repetitive DNA, so the exact coordinates and junctional sequence could not be determined. Parental DNA was not available for analysis.



Supplemental Figure 4. ATOH7 variants have similar protein stability. (A) LICOR dual fluorescence Western blots of HEK293T cells transfected with wildtype or variant *ATOH7* expression plasmids. Lysates were harvested after 0-5 hours treatment with cycloheximide (CHX), which blocks synthesis of new proteins. The level of ATOH7 polypeptide was normalized to endogenous α-tubulin, which has a long half-life. (B) Quantitative analysis of ATOH7 levels in (A) based on LICOR antibody fluorescence (semilog plot). The variant (red) and wildtype (black) ATOH7 proteins have equivalent decay kinetics.



Supplemental Figure 5. Low power views of retinal explant rescue experiments. (A-F) Retinal explants (Fig. VI-5) coimmunostained for GFP and/or TuJ1 to mark axons. In explants transfected with wild-type *ATOH7* (B), or R65G (E) or A47T (F) variants, ganglion cell axon bundles (arrows) are abundant. However, in explants expressing GFP only (A), or *ATOH7* L56P (C) or N46H (D) variants, very few axon bundles are evident. Scale bar, 200 µm.

Supplemental Table 1. Clinical Features of optic nerve aplasia cases.

Patient Number	Sex	Other ocular abnormalities	Septo-optic dysplasia	Endocrine or pituitary defects	Neurological or brain defects	Genetic findings	Reference
1	F	none	no	failure to thrive	developmental delay; delayed vocalization; auditory processing defects	ATOH7 p.R65>G (het); 828kb tandem dup CNTN4 ex2-12	this study
2	M	bilateral microphthalmia; retinochoroid depigmentation, absence of retinal vasculature, abnormal vessels at area of optic disc	yes	posterior pituitary ectopia; absence of pituitary infundibulum; hypothyroid, pituitary insufficiency	hypoplastic corpus callosum	1.2 MB hemizygous deletion at 14q23 (includes OTX2)	Brodsky et al. 2004
3	F	bilateral microphthalmia, bilateral iris coloboma	no	none	none		Scott et al. 1997
4	M	bilateral micropthalmia, iris coloboma (OD), pigmented epithelium mottling, choroidal neovascularization	yes	none	none		Lee et al. 1996
5	F	unilateral microopthalmia and microcornea(OS); chorioretinal atrophy; bilateral iris colobomas	no	none	none		this study

Supplemental Table 2. Oligonucleotide primers and PCR conditions used in this study

Experiment	Forward (sense primer 5'→3')	Reverse (antisense primer)	Cycling conditions	Notes
ATOH7 p.N46>H mut **ATOH7 p.A47>T mut** **ATOH7 p.L56>P mut**	CCTGGCGGCCCACGCGCGCGAGCGCCGCCGCATGCAG GCCTGGCGGCCAACACGCGCGAGCGCCGCCGCATGC CGCATGCAGGGGCCCAACACTGCCTTCGACCGCTTAC	GCGGCGGCGCTCGCGCGCGTGGGCCGCCAGGCGCCTG ATGCGGCGGCGCTCGCGCGTGTTGGCCGCCAGGCGCCT GCGGTCGAAGGCAGTGTTGGGCCCCTGCATGCGGCGG	94°C for 5 min; 20 cycles of [94°C for 1 min, 57°C for 1 min, 68°C 6 min]; 68°C 10 min	0.2 v/v Masteramp
ATOH7 p.R65>G mut	CTTCGACCGCTTACGCGGGGTGGTTCCCCAGTGG	CCACTGGGGAACCACCCCGCGTAAGCGGTCGAAG	95°C for 1 min; 30 cycles of [95°C for 1 min, 55°C for 1 min, 65°C 14 min]; 68°C for 10 min	0.2 v/v Masteramp
CNTN4 duplication **CNTN4 WT**	TCCAGGTGGTGGGTAAAAGA	CCAGTGTCACAGGAATGTGG ACCAACACTGAACTCTTCACCT	94°C for 3 min; 40 cycles of [94°C for 30 sec, 57°C for 1 min, 72°C 1 min]; 72°C 7 min	0.1 v/v Masteramp
3'RACE RT	N/A	GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT	50°C for 1 hr	
3'RACE CNTN4 ex24 upstream	TTTGCTATAGTTTGTCATTTTTGCTT	GGCCACGCGTCGACTAGTAC	94°C for 3 min; 26 cycles of [94°C for 30 sec, 57°C for 1 min, 72°C 1 min]; 72°C 7 min	
nested	TGTGTTCCTTTCTTAGTTTGATATGGT		same as above, except 33 cycles	
3'RACE CNTN4 in12 upstream	AGACAGCGTTGTTTGGCATC	GGCCACGCGTCGACTAGTAC	94°C for 3 min; 25 cycles of [94°C for 30 sec, 58°C for 1 min, 72°C 1 min]; 72°C 7 min	
nested	AGACCACTCCCTGCCAATGT		same as above, except 33 cycles and 60°C annealing	