**SUPPLEMENTARY INFORMATION**

# Mutations in the RNA exosome component gene *EXOSC3* cause pontocerebellar hypoplasia and spinal motor neuron degeneration

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**Supplementary Note**

***Ascertainment of biallelic mutations in families 5 and 8 by long-range PCR***

Since parental DNA was not available for these two families, we determined that the mutations were in trans by long range sequencing. In Family 5, we amplified in the patient the genomic sequence spanning exons 1 and 2 (**Supplementary Table 2**) with Phusion High-Fidelity DNA Polymerase (NEB) and cloned the gel-extracted 1344 bp product into pCR-Blunt II-TOPO (Invitrogen) for transformation of competent bacterial cells. The two mutations were found in separate clones, as confirmed by direct sequencing of purified plasmids.

For Family 8, we PCR amplified the entire gene genomic DNA with Phusion High-Fidelity DNA Polymerase (NEB) and primers hEXOSC3-ATGf and hEXOSC3-Rcr (**Supplementary Table 2**). The gel extracted amplicon was cloned into pcDNA3.1/Zeo(+) (Invitrogen) with In-Fusion® HD Cloning System (Clonetech) and transformed into competent bacterial cells. Plasmids from multiple colonies were sequenced to ascertain that the two mutations resided in different clones.

**Supplementary Table 1. Nerve conduction studies.** Nerve conduction studies in the **a.** oldest (18-year-old) and **b.** youngest (9-year-old) surviving patients in Family 1 showed motor responses with severely reduced amplitudes but normal sensory responses. The velocities were calculated based on onset latencies.

**a.** Nerve conduction study on the oldest surviving patient in Family 1

| **Sensory Studies** | | | |
|---|---|---|---|
| | Peak Latency | Amplitude | Velocity |
| Left Radial | 1.2 msec | 28.3 µV | 58 m/s |
| Left Sural | 2.0 msec | 11.7 µV | 50 m/s |
| **Motor Studies** | | | |
| | Distal Latency | Amplitude | Velocity |
| Left Ulnar | 3.0 msec | 1.0 mV | 47 m/s |
| Left Tibial | 4.8 msec | 0.3 mV | 37 m/s |

**b.** Nerve conduction study on the youngest surviving patient in Family 1

| **Sensory Studies** | | | |
|---|---|---|---|
| | Peak Latency | Amplitude | Velocity |
| Left Median | 1.8 msec | 48.7 µV | 61 m/s |
| **Motor Studies** | | | |
| | Distal Latency | Amplitude | Velocity |
| Left Median | 3.1 msec | 1.5 mV | 42 m/s |

## Supplementary Table 2. Oligonucleotides and PCR conditions for molecular genetic studies.

| Oligonucleotide Name | Direction | Oligonucleotide Sequence 5'-->3' | Tm | Anneal Temp | Amplicon Size (bp) |
|---|---|---|---|---|---|
| *Primers and PCR conditions for EXOSC3 mutation screening (lower case M13 sequence)* | | | | | |
| Exon 1 | Forward | tgtaaaacgacggccagtACGGCCATCAAGCTTCATAAAC | 54.9 | 67-60 | 539 |
| | Reverse | caggaaacagctatgaccCTCTTCTTTTGGGAGGTCTTCT | 50.4 | | |
| Exon 2 | Forward | tgtaaaacgacggccagtGGGGTGCCTAAGAGATAATGGAG | 55 | 68-55 | 441 |
| | Reverse | caggaaacagctatgaccGATAGCCTTCTGGATATGTGAGTGTTC | 55.7 | | |
| Exon 3 | Forward | tgtaaaacgacggccagtTCCCCAAGACTCAACTCCAAAG | 54.8 | 67-60 | 539 |
| | Reverse | caggaaacagctatgaccATCAGCCCACCAGAAACTACACAG | 56.2 | | |
| Exon 4 | Forward | tgtaaaacgacggccagtTGGAAGAAAGGAGGCAGCAAATG | 59.3 | 67-60 | 515 |
| | Reverse | caggaaacagctatgaccCACAAAAGCGTGGGTGAAAAC | 54.6 | | |
| *Primers and PCR conditions for In-Fusion HD cloning (lower case sequence required for cloning)* | | | | | |
| hEXOSC3-ATGf | Forward | taccgagctcggatccATGGCCGAACCTGCGTCTGTC | 61 | 72-67 | 4708 |
| hEXOSC3-Rcr | Reverse | gccctctagactcgagTTCCTCTGGTGAACCTGGCTTACTG | 60 | | |
| *Primers and PCR conditions for RT-PCR* | | | | | |
| hExosc3-c.423f | Forward | AGCCAGCTTCTTTGTCTTACTTGTC | 54.5 | 67-60 | |
| hExosc3-c.670r | Reverse | GTTTTCCCACTTCCTGTATGATTTC | 54.2 | | |
| *Primers for mutagenesis of zebrafish exosc3* | | | | | |
| zfExosc3-D102A | Forward | CTGGAGACGTCTTCAAAGTGGCCGTTGGAGGAAGTGAGC | 77.6 | | |
| | Reverse | GCTCACTTCCTCCAACGGCCACTTTGAAGACGTCTCCAG | 77.6 | | |
| zfExosc3-W208R | Forward | GCATGAACGGCAGAGTGCGGGTGAAGGCCAGAACCGTC | 82.5 | | |
| | Reverse | GACGGTTCTGGCCTTCACCCGCACTCTGCCGTTCATGC | 82.5 | | |
| zfExosc3-G20A | Forward | GGAGATGTGGTTCTTCCAGCCGACCTGCTGTTCTCCTTCAG | 78.4 | | |
| | Reverse | CTGAAGGAGAACAGCAGGTCGGCTGGAAGAACCACATCTCC | 78.4 | | |
| *Primers for mutagenesis of human EXOSC3* | | | | | |
| hEXOSC3-G31A | Forward | GTCAGGTGGTGCTCCCGGCTGAGGAGCTGCTCCTGCCG | 84.5 | | |
| | Reverse | CGGCAGGAGCAGCTCCTCAGCCGGGAGCACCACCTGAC | 84.5 | | |
| hEXOSC3-D132A | Forward | GAGATATATTCAAAGTTGCTGTTGGAGGGAGTGAG | 80 | | |
| | Reverse | CTCACTCCCTCCAACAGCAACTTTGAATATATCTC | 80 | | |
| hEXOSC3-W238R | Forward | TTGGAATGAATGGAAGAATACGGGTTAAGGCAAAAACCATC | 73.1 | | |
| | Reverse | GATGGTTTTTGCCTTAACCCGTATTCTTCCATTCATTCCAA | 73.1 | | |
| *Morpholino oligonucleotides for zebrafish exosc3* | | | | | |
| AUG MO | | TCCATGATGGAGGAGCGGAAAACAC | | | |
| SPL MO | | CCTCTTACCTCAGTTACAATTTATA | | | |

**Supplementary Table 3. Shared haplotypes spanning *EXOSC3* in Families 1-3.** Affected individuals from Families 1-3 with homozygous D132A mutations share approximately 1 cM of homozygosity with identical haplotypes around D132A (chr9:37751044-38395492 hg18 coordinates). This haplotype was not observed in 126 controls genotyped on the same platform. The findings are consistent with source of the mutation being a remote common ancestor.

| chr | pos_b36 | rsID | cM | FAM1 | FAM2 | FAM3 |
|---|---|---|---|---|---|---|
| 9 | 37751044 | rs1409145 | 62.0880 | BB | BB | BB |
| 9 | 37771455 | rs3827515 | 62.1171 | BB | BB | BB |
| 9 | 37772561 | rs7029518 | 62.1187 | BB | BB | BB |
| 9 | 37772708 | rs13294227 | 62.1189 | AA | AA | AA |
| 9 | 37772967 | rs10973542 | 62.1193 | BB | BB | BB |
| 9 | 37773990 | | 62.1270 | 11 | 11 | 11 |
| 9 | 37779033 | rs10814621 | 62.1279 | BB | BB | BB |
| 9 | 37804051 | rs10814625 | 62.1636 | BB | BB | BB |
| 9 | 37831248 | rs10973580 | 62.2035 | AA | AA | AA |
| 9 | 37847067 | rs12000384 | 62.2267 | BB | BB | BB |
| 9 | 37848861 | rs16934508 | 62.2293 | AA | AA | AA |
| 9 | 37879753 | rs41436845 | 62.2749 | AA | AA | AA |
| 9 | 37882000 | rs16934574 | 62.2783 | BB | BB | BB |
| 9 | 37888768 | rs16934581 | 62.2885 | BB | BB | BB |
| 9 | 37891883 | rs4878724 | 62.2932 | AA | AA | AA |
| 9 | 37926503 | rs12002323 | 62.3456 | AA | AA | AA |
| 9 | 37947229 | rs7048063 | 62.3769 | AA | AA | AA |
| 9 | 37979893 | rs2243893 | 62.4270 | AA | AA | AA |
| 9 | 38014458 | rs2890783 | 62.4800 | BB | BB | BB |
| 9 | 38069516 | rs1999095 | 62.5736 | AA | AA | AA |
| 9 | 38080221 | rs4878183 | 62.5919 | BB | BB | BB |
| 9 | 38094144 | rs10973666 | 62.6150 | BB | BB | BB |
| 9 | 38109900 | rs7033592 | 62.6407 | BB | BB | BB |
| 9 | 38131337 | rs2890776 | 62.6757 | AA | AA | AA |
| 9 | 38132145 | rs1928239 | 62.6770 | AA | AA | AA |
| 9 | 38141190 | rs1001959 | 62.6918 | BB | BB | BB |
| 9 | 38141268 | rs10973683 | 62.6919 | BB | BB | BB |
| 9 | 38177709 | rs10973695 | 62.7516 | AA | AA | AA |
| 9 | 38178524 | rs7034598 | 62.7529 | AA | AA | AA |
| 9 | 38192630 | rs2585669 | 62.7760 | BB | BB | BB |
| 9 | 38193508 | rs2810740 | 62.7775 | AA | AA | AA |
| 9 | 38196362 | rs2053556 | 62.7822 | BB | BB | BB |
| 9 | 38283902 | rs341474 | 62.9442 | AA | AA | AA |
| 9 | 38299503 | rs1885491 | 62.9740 | AA | AA | AA |
| 9 | 38320114 | rs16935064 | 63.0096 | BB | BB | -- |
| 9 | 38352713 | rs1022770 | 63.0659 | BB | BB | BB |
| 9 | 38364977 | rs2181139 | 63.0870 | AA | AA | AA |
| 9 | 38373549 | rs17451412 | 63.1025 | BB | BB | -- |
| 9 | 38394292 | rs12336048 | 63.1406 | AA | AA | AA |
| 9 | 38395492 | rs4878203 | 63.1428 | BB | BB | BB |

**Supplementary Table 4. *In silico* predictions of pathogenicity.** Missense *EXOSC3* mutations were assessed by various algorithms of phylogenetic conservation and functional impact. PhastCons (PHylogenetic Analysis with Space/Time models; evolutionary conservation) http://compgen.bscb.cornell.edu/phast/ spans between 0 and 1, with 1 being the most highly conserved.

GERP (Genomic Evolutionary Rate Profiling) http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html is designated between -11.6 to maximum conservation at 5.82.

Grantham scores, which categorize codon replacements into classes of increasing chemical dissimilarity, are designated conservative (0-50), moderately conservative (51-100), moderately radical (101-150), or radical (≥151).[1]

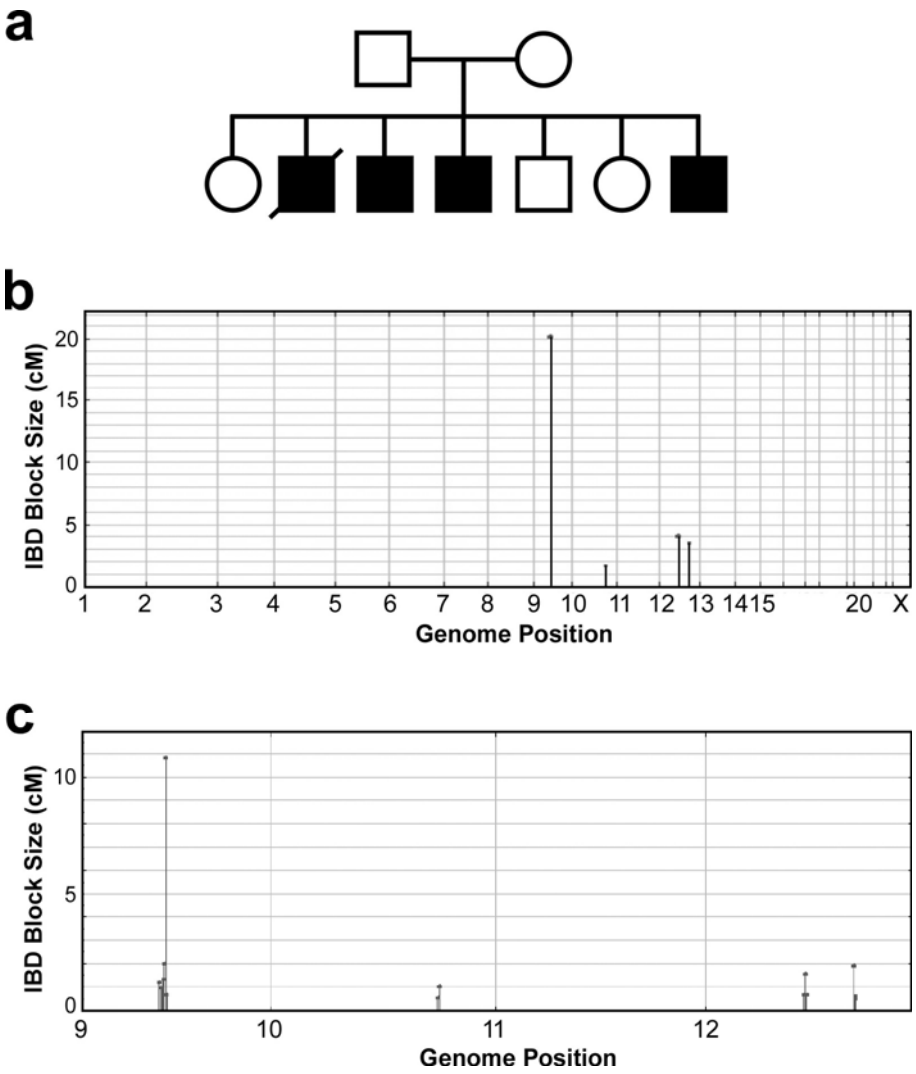
PolyPhen (Polymorphism Phenotype) scores are designated probably damaging (≥2.00), possibly damaging (1.50-1.99), potentially damaging (1.25-1.49), borderline (1.00-1.24), or benign (0.00-0.99).[2]

SIFT (Sorting Intolerant from Tolerant; http://sift.jcvi.org/ ) scores are designated damaging (<0.05) or not.

| Mutation | PhastCons | GERP | Grantham | PolyPhen | SIFT |
|---|---|---|---|---|---|
| G31A | 1 | 5.27 | 60 | 1.35 (potentially damaging) | 0.01 (damaging) |
| D132A | 1 | 5.34 | 126 | 2.493 (probably damaging) | 0.02 (damaging) |
| A139P | 1 | 5.34 | 27 | 1.985 (possibly damaging) | 0 (damaging) |
| W238R | 1 | 5.66 | 101 | 4.142 (probably damaging) | 0 (damaging) |

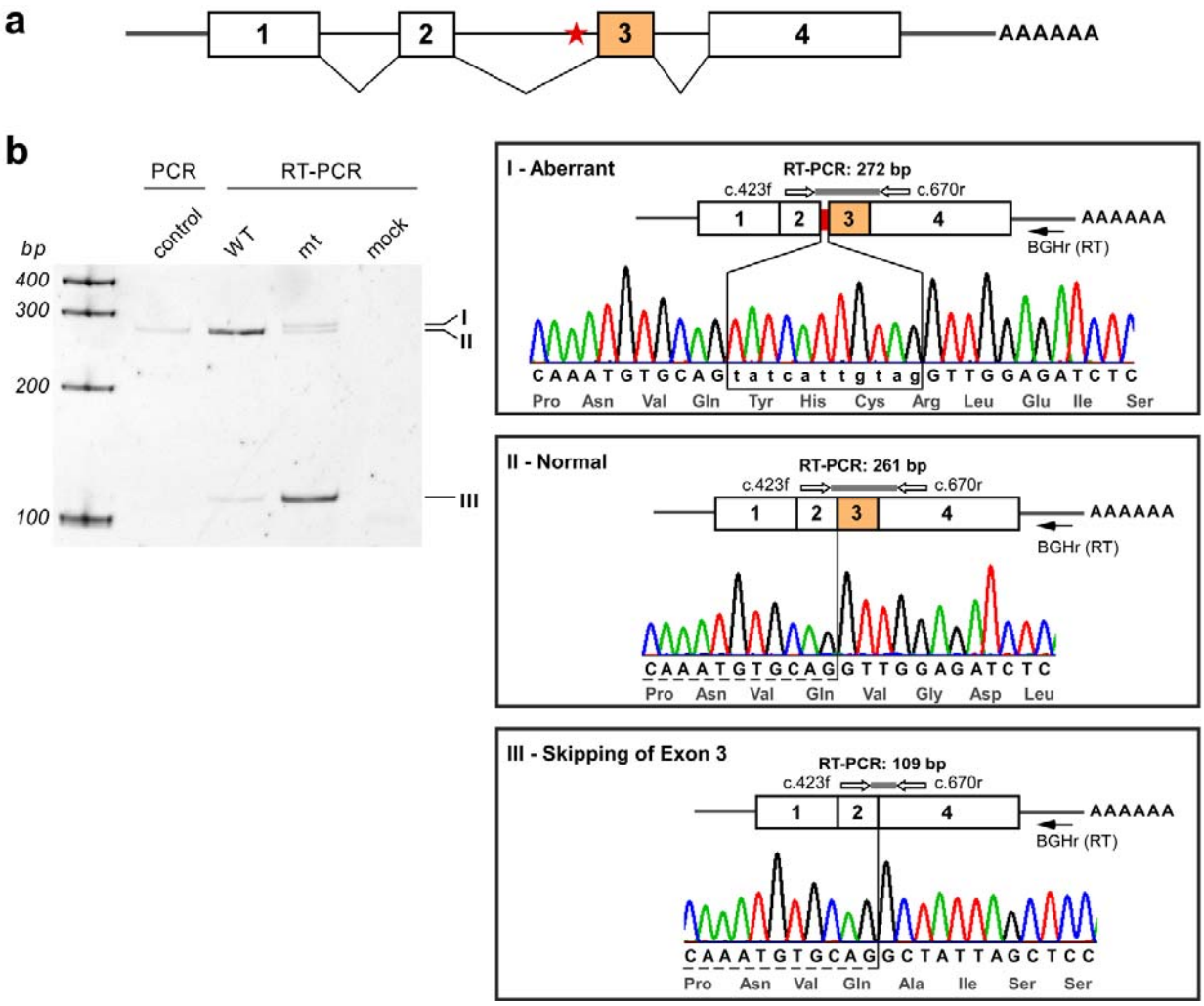
**Supplementary Table 5. Survival assays in zebrafish embryos.** Survival assays were performed in antisense *exosc3*-specific splice morpholino-injected zebrafish embryos that were co-injected with *in vitro* transcribed wildtype or mutant *exosc3* mRNA, with GFP as control. Embryos were scored as normal or abnormal (mildly abnormal, severely abnormal, and dead), as stratified in **Supplementary Figure 4** for embryos 1 dpf. For embryos 2 or 3 dpf (shown in **Figure 3**), those with straight spine of normal length and normal brain were scored as "normal"; those with curved spine and small brain but still mobile were scored as "mildly abnormal"; and those that were severely malformed without movement were scored as "severely abnormal".

| | dpf | normal | mildly abnormal | severely abnormal | dead |
|---|---|---|---|---|---|
| SPL MO + zWT mRNA | 1 | 248 | 5 | 3 | 33 |
| | 2 | 240 | 9 | 1 | 39 |
| | 3 | 206 | 39 | 5 | 39 |
| SPL MO + zDA mRNA | 1 | 56 | 37 | 25 | 33 |
| | 2 | 50 | 32 | 32 | 37 |
| | 3 | 35 | 38 | 21 | 57 |
| SPL MO + zGA mRNA | 1 | 69 | 110 | 27 | 27 |
| | 2 | 65 | 48 | 84 | 36 |
| | 3 | 32 | 57 | 72 | 72 |
| SPL MO + zWR mRNA | 1 | 68 | 50 | 45 | 39 |
| | 2 | 73 | 30 | 46 | 53 |
| | 3 | 44 | 45 | 37 | 76 |
| SPL MO + hWT mRNA | 1 | 129 | 24 | 27 | 29 |
| | 2 | 112 | 35 | 17 | 45 |
| | 3 | 94 | 31 | 18 | 66 |
| SPL MO + hDA mRNA | 1 | 14 | 12 | 29 | 20 |
| | 2 | 18 | 6 | 22 | 29 |
| | 3 | 11 | 6 | 11 | 47 |
| SPL MO + hGA mRNA | 1 | 38 | 43 | 53 | 40 |
| | 2 | 36 | 23 | 65 | 50 |
| | 3 | 23 | 20 | 37 | 94 |
| SPL MO + hWR mRNA | 1 | 12 | 32 | 49 | 56 |
| | 2 | 11 | 22 | 56 | 60 |
| | 3 | 7 | 13 | 30 | 99 |
| SPL MO + GFP mRNA | 1 | 13 | 55 | 3 | 27 |
| | 2 | 6 | 55 | 5 | 32 |
| | 3 | 6 | 47 | 6 | 39 |

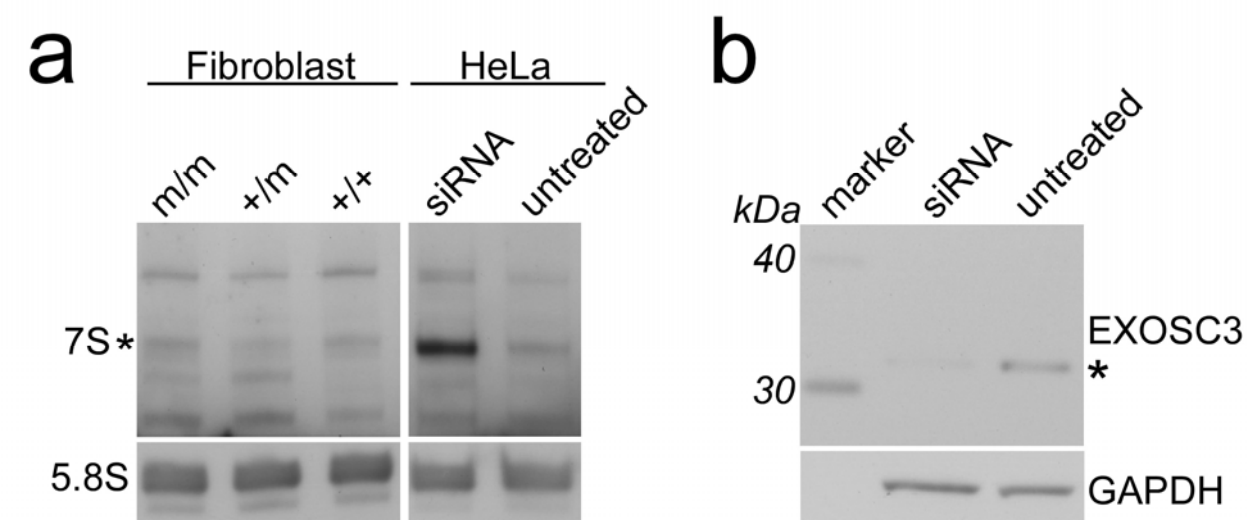



**Supplementary Figure 1. Genome-wide SNP Genotyping & Linkage Analysis. a.** Pedigree of Family 1 with four affected siblings, three unaffected siblings, and their parents. Genotyping on Affymetrix 250K NspI mapping array was performed on DNA from the four affected siblings, three unaffected siblings, and their parents. Copy number analysis was used to look for large deletions or duplications shared by the affected individuals; none were found. Pedigree-free IBD mapping was performed to search for intervals that were compatible with a common extended haplotype among all the affected individuals, and not shared by unaffected family members. **b.** For the most general compound recessive model with exclusion of the unaffecteds, we searched for identical inheritance blocks to find an aggregate of 15 Mb candidate region in 4 loci on three chromosomes 1) where all four affecteds inherited the same allele from the mother and the father and 2) that these

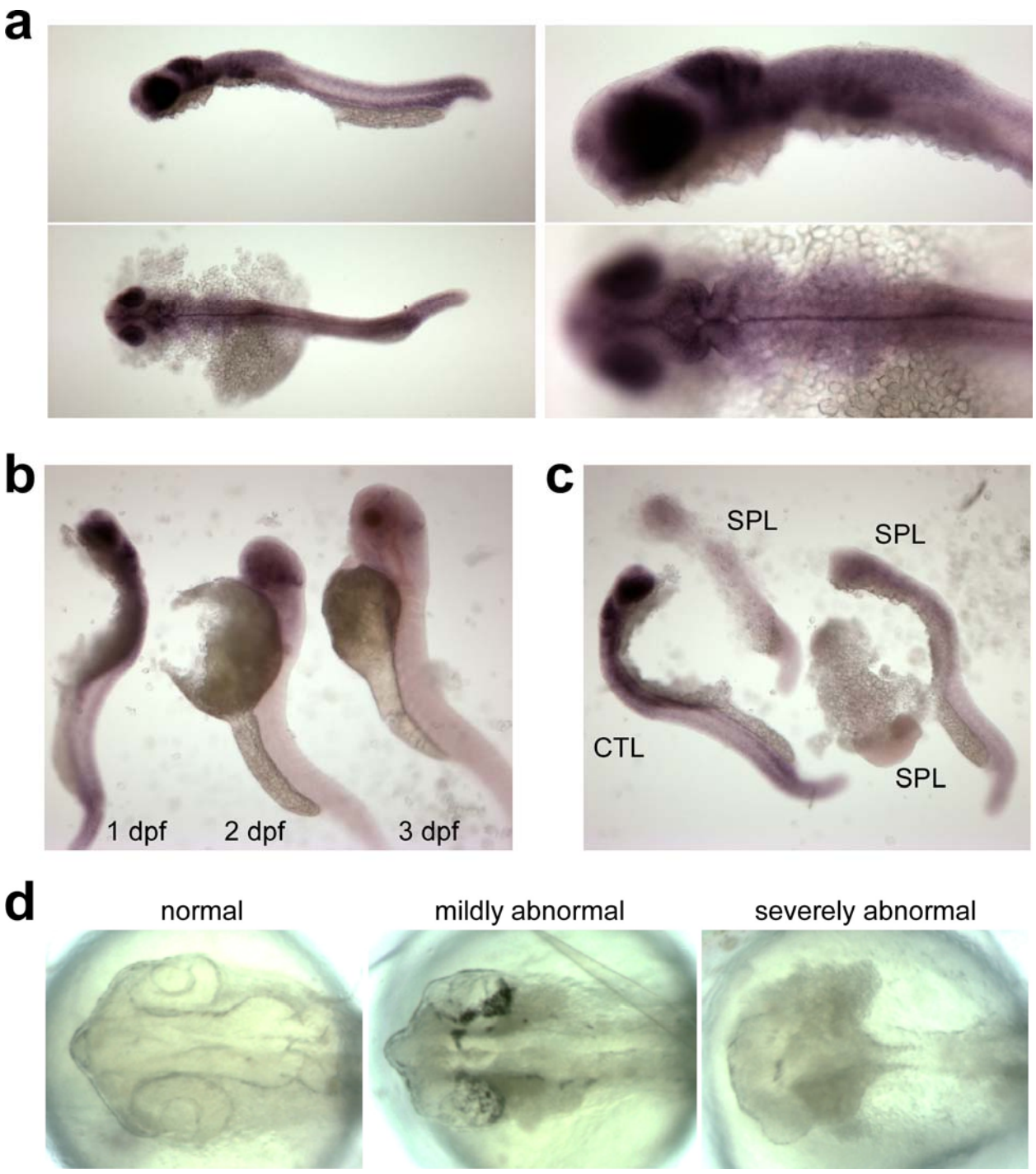
inheritance blocks were shared by the affected but not by unaffected siblings. **c.** The most limited recessive model under this analysis, requiring a single founder allele and exclusion of unaffected, identified 15 blocks >0.5 cM that were identical and homozygous in all four affected and not identical in any affected and unaffected. These loci fit the model of an old founder ~10-20 generations back, with no recent inbreeding. This analysis excluded the possibility of an X-linked disorder.




**Supplementary Figure 2. Aberrant splicing from the intronic mutation c.475-12A>G. a.** Diagrammatic representation of the four exons and the location of the intronic mutation (represented by the star in red). **b.** RT-PCR products run on a 12% polyacrylamide gel. Transcript-specific RT-PCR yielded two products from the cells transfected with the wild type construct: a major product **II** (88% by densitometry)**,** which was of the same size as the PCR product from a full-length EXOSC3 cDNA clone (control), and a minor band of smaller size **III** (12% by densitometry). Sanger sequencing confirmed normal splicing of intron 2 (**II-Normal**) and skipping of exon 3 (**III-Skipping of Exon 3**) and shifting the open reading frame. Transcript-specific RT-PCR yielded three products from cells transfected with the mutant clone harboring the intronic variant. The major band (**III**) was missing exon 3 (85% by densitometry), as confirmed by sequencing. There were two minor bands of larger sizes. The lower band (**II**) was of the same size as the control (6% by densitometry) and confirmed by sequencing. The upper band (**I**) was the product of aberrant splicing using the newly introduced splice acceptor site (9% by densitometry), with the incorporation of 11 additional nucleotides upstream from the normal splice site to shift the open reading frame, as demonstrated by sequencing (**I-Aberrant**).




**Supplementary Figure 3. rRNA processing. a.** Northern blots demonstrating no marked accumulation of unprocessed 5.8S rRNA (*7S, as seen in siRNA-treated HeLa cells) in a patient or his parent compared to an unaffected control. 4μg RNA extracted from patient-derived fibroblasts or HeLa was loaded to each lane on 6% denaturing PAGE and detected with DIG-labeled probe for 5.8S rRNA. m/m-homozygous for mutant D132A; +/m-heterozygous for D132A; +/+-normal control homozygous wildtype; siRNA- HeLa cells treated with EXOSC3-specific siRNA; untreated- HeLa cells not exposed to siRNA. **b.** Western blot demonstrating diminished EXOSC3 in HeLa cells treated with EXOSC3-specific siRNA. 10μg protein extracted from cells treated or untreated with siRNA probed first with EXOSC3-specific antibodies (Santa Cruz Biotechnology), stripped, then probed with GAPDH-specific antibodies (Millipore).




**Supplementary Figure 4. *Exosc3* expression in zebrafish embryos by whole-mount *in situ* hybridization. a.** Control morpholino-injected zebrafish embryos 1 dpf probed with *Exosc3*-specific antisense riboprobes demonstrating diffuse expression with especially strong signal intensity in the brain and eyes, in lateral and dorsal views under different magnifications. **b.** There is a progressive decrease in the expression of exosc3 in zebrafish embryos 1 dpf compared to those at 2 dpf and 3 dpf. The yolk sacs were partially removed to not obscure the visualization of the

embryos. **c.** Zebrafish embryos 1 dpf injected with antisense morpholinos targeting the splice site of exosc3 (SPL) led to a marked but variable decrease in the expression of exosc3 compared to those injected with control morpholinos (CTL). There was higher expression of exosc3 in the embryo on the far right compared to the other two SPL-injected embryos. **d.** Light microscopic examination in dorsal view of unstained live chorion-enveloped zebrafish embryos 1 dpf revealed variable phenotypes. Embryos with smaller brain and eyes are scored as mildly abnormal. Those embryos with very small brain and malformed eyes as well as a very small hindbrain and thin spinal cord are scored as severely abnormal.

**References**


1. Li WH, Wu CI, Luo CC. Nonrandomness of point mutation as reflected in nucleotide substitutions in pseudogenes and its evolutionary implications. *J Mol Evol* **21**, 58–71(1984).
2. Xi T, Jones IM, Mohrenweiser HW. Many amino acid substitution variants identified in DNA repair genes during human population screenings are predicted to impact protein function. *Genomics* **83**, 970–979 (2004).