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Supplemental Data

Recurrent De Novo and Biallelic Variation of *ATAD3A*, Encoding a Mitochondrial Membrane Protein, Results in Distinct Neurological Syndromes

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CLINICAL DESCRIPTIONS

Family 1, II-2

We report a 9-year-old female (BH5923_1, designated Family 1, II-2 in **Figure 1A**) who presented with delayed motor development and hypotonia. She was born to a 28 -year old gravida 2, para 1->2 mother and a 30 -year old father at 42 weeks gestation after an overall uncomplicated pregnancy, via vaginal delivery at a birthweight of 3005 grams (~10th percentile). Motor development was delayed, with crawling at 12 months and walking with a walker at 2 years. Language development was also delayed, with her first words between 18 and 24 months and sentences at about 30 months. She had difficulty feeding since early infancy; a swallow function study revealed oropharyngeal dysphagia and poor clearing of the oropharynx. Medical history was further significant for mild sleep apnea, a history of sleep disturbance, recurrent otitis media, recurrent urinary tract infections, nosebleeds at an early age and hip dysplasia s/p surgery. At 9 years of age, her head circumference was 51.4 cm (40th percentile), height 116.4 cm (Z score = -3.1), and weight 19.6 kg (Z score = -2.94). She was nondysmorphic. Physical examination revealed general hypotonia, muscle wasting most pronounced in the distal extremities with a 'stork leg' deformity of both calves, and poor development of the hypothenar and thenar eminences. She exhibited an abnormal, spastic crouched gait when walking with crutches. Patellar deep tendon reflexes (DTRs) were brisk, but ankle reflexes could not be elicited. DTRs of the upper extremities were normal. No clonus was elicited. Brain magnetic resonance imaging (MRI) showed a Chiari malformation, and MR spectroscopy and spine MRI were normal. Ophthalmology exam revealed temporal optic atrophy and

myopia. EMG and NCV studies were consistent with axonal neuropathy (**Table S1**). Muscle biopsy was not obtained. Previous laboratory studies included chromosome analysis, plasma acylcarnitine profile, plasma amino acids, urine organic acids, lactate, creatine kinase (CK), and thyroid function studies all of which were reported normal. Array comparative genome hybridization (CGH) detected a paternally inherited 0.288 Mb gain on Xq24 including *UPF3B*; mutations in the latter have been associated with X-linked syndromic intellectual disability in males, and this finding is of unclear significance in females. Targeted testing for *MPZ* and *EGR2* was negative. Thus, the overall clinical diagnosis was of possible upper motor neuron involvement and axonal neuropathy with a negative work-up. Whole exome sequencing of the proband and both parents revealed a heterozygous variant in *ATAD3A* (g. chr1:1464679 C>T [hg19]; GenBank:NM_001170535.1; c.1582C>T; p.Arg528Trp) which was not present in either parent. Sanger sequencing confirmed that the variant likely arose *de novo* in the affected individual (**Figure 1B**). The variant arose in a CpG dinucleotide within a CpG island of 298 bp, with a 15.4% percent CpG (ratio of observed to expected CpG: 0.67) (UCSC Genome Browser CpG track; Santa Cruz, CA). The variant was not seen in any publically available database nor in our internal database.

Family 2, II-4

The second individual (designated Family 2, II-4 in **Figure 1A**) was a 5-year-old female with developmental delay, truncal hypotonia and peripheral spasticity. Motor development was delayed, with no independent walking at 5 years of age. Speech was delayed, and she had borderline normal hearing. Cognitive development

was moderately delayed. She had difficulty feeding and required G-tube feeding. In addition, she had sleep apnea, slight acetabular dysplasia and a dislocated hip. At 5 years of age, her head circumference was at the 59th percentile, height Z score was -2.5, and weight was 25th percentile. She was otherwise nondysmorphic. Physical exam revealed truncal hypotonia, peripheral spasticity, and bilateral talipes equinovalgus (**Figure 2**). Nystagmus, esotropia, myopia, and astigmatism were evident on ophthalmologic exam. Brain MRI, echocardiogram and renal ultrasound were normal. EMG and NCV studies were consistent with axonal neuropathy (**Table S1**). Lactate and pyruvate were mildly elevated; creatine kinase (CK) was normal. Muscle biopsy showed no diagnostic abnormalities, and she had normal sequencing and deletion analysis of the mitochondrial genome. Mitochondrial respiratory chain enzyme analysis in muscle showed a deficiency of complex I plus III activity. Chromosome analysis and array CGH were normal.

Whole exome sequencing was undertaken on the proband and her parents. Genomic DNA was extracted from whole blood, and exome sequencing at GeneDx was performed on exon targets isolated by capture with the Agilent SureSelect Human All Exon V4 (50 Mb) kit or the Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA). Sequencing technology and variant interpretation protocol were done as previously described.[1] The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/). A heterozygous variant was identified in *ATAD3A* (g. chr1:1464679 C>T [hg19]; GenBank:NM_001170535.1; c.1582C>T; p.Arg528Trp) which was not present in either parent, and Sanger sequencing confirmed that this variant likely arose *de novo* in the affected individual.

Family 3, II-1

The third individual (Family 3, II-1 in **Figure 1A**) was a 3-year-old male with global developmental delay, borderline microcephaly (~3rd %ile), hypotonia with peripheral spasticity, feeding difficulties that resolved, and sleep abnormalities. He was non-ambulatory at age 3-years. Dysmorphic features included absent eyebrows, deep set eyes with esotropia, a triangular face, small mandibular region, beaked nose, and a high arched palate. Skeletal abnormalities included pectus carinatum and severe scoliosis. Neurological exam showed axial hypotonia with peripheral spasticity, and ophthalmology exam showed esotropia, optic nerve pallor, and macular hypoplasia. An echocardiogram revealed hypertrophic cardiomyopathy, initially detected at age 3 months. NCV studies showed peripheral neuropathy. Additionally, he had growth hormone deficiency, hypoplasia of optic nerves on MRI and possible cone-rod dysplasia. Lactate was intermittently elevated, and a mitochondrial condition was suspected.

Whole exome sequencing of the proband and both parents at GeneDx (see above) revealed a heterozygous variant in *ATAD3A* (g. chr1:1464679 C>T [hg19]; GenBank:NM_001170535.1; c.1582C>T; p.Arg528Trp) which was not present in either parent, and Sanger sequencing confirmed that this variant likely arose *de novo* in the affected individual.

Family 4, II-1

This individual (Family 4, II-1 in **Figure 1A**) was born to non-consanguineous Italian parents at term, after normal pregnancy and delivery (weight 2580 grams). Family history was positive for polyarthritis and congenital bicuspid aortic valve in the mother. No other disorders were reported. The proband presented with growth failure and emesis at 12 days of age. ECG and echocardiography revealed severe hypertrophic cardiomyopathy. At 4 months of age, she was admitted to the hospital for evaluation of severe developmental delay and failure to thrive. Height (54 cm), weight (3960 g) and head circumference (38.5 cm) were all below the 3rd centile. Facial features included low set ears, a triangular facies and micrognathia. Upon neurological evaluation she presented with truncal hypotonia and increased muscular tone in the extremities associated with dystonic movements and buccal dyskinesia. Neither eye contact nor head control were present. Metabolic workup revealed an increase in plasma alanine (394 micromol/l; normal 239-345) and lactic acid (39 mg/dl; normal 5-22), reduction of plasma citrulline (11.2 micromol/l; normal 17-53), and slight excretion of methylglutaconic acid (11UM/37UM; normal <9) and lactic acid (60UM, normal <25). Brain MRI showed enlargement of periencephalic spaces. Muscle biopsy showed lipid droplets, increased sarcolemmal COX activity, and reduction of succinate dehydrogenase (SDH) activity. Respiratory chain activities in muscle homogenate showed reduction of succinate dehydrogenase (SDH, II), citrate synthase (CS) and succinate cytochrome C reductase (II+III) (data not shown). Normal respiratory chain activities were found in skin derived fibroblasts of Family 4, II-1 (**Table S4)**. aCGH did not identify genomic imbalances. Treatments with β-blockers for cardiomyopathy and anti-oxidant/vitamin supplementation for mitochondrial abnormalities (thiamine, idebedenone, L-carnitine) were initiated. Feeding difficulties required nasogastric tube followed by gastrostomy placement.

During the clinical course, she experienced episodes of painful muscular rigidity (hypertonus) associated with vegetative symptoms. Neurophysiological studies (EEG) excluded epileptic seizures. In addition, she developed esotropia, optic nerve pallor, myopia, and oculo-motor incoordination confirmed by ophthalmologic evaluation at 18 months of age. At the last evaluation (5 years of age), height (85.5 cm, Z score -5.2), weight (10 kg, Z score -6.2), and head circumference (45.8 cm, Z score -3.3) were maintained below the 3rd centile. Few progresses in motor development were noticed: eye contact and social interaction, partial head control and intentional movement of the upper limbs. She could not yet sit unaided. Severe axial hypotonia and spasticity persisted.

Whole exome sequencing of the proband and both parents was performed at Columbia University Medical Center, Genome Facility. Samples were prepared using the Agilent SureSelectXT kit, captured with the Agilent All Exon v5 + UTRs library and sequenced on the Illumina HiSeq2500 instrument using a 2x100bp paired end run. NextGENe software was used to map reads to the whole genome sequence (version hg19) and generate variant call files (vcf files) with minimum coverage 10, minimum mutant rate 10% and homozygosis in >3 alleles. Variants in the coding and plus/minus 5p regions were filtered. Common SNVs with minor allele frequencies of >1% were filtered with the 1000 genome database and exome variant server, and when present in more than 20 cases from an in-house database (~1000 cases). A heterozygous variant in *ATAD3A* (g. chr1:1464679 C>T [hg19]; GenBank:NM_001170535.1; c.1582C>T; p.Arg528Trp) was identified in proband DNA and confirmed by Sanger sequencing, while excluded from parental samples. Interestingly, Sanger sequencing of the mother (Famisly 4, I-2) suggested possible low-level mosaicism in the maternal sample (**Figure S4**). The mother was unaffected

aside from cardiac arrhythmia diagnosed in childhood, for which she had pacemaker placement. Nerve conduction velocities in the mother were within normal limits.

Family 5, II-1

The fifth individual (Family 5, II-1 in **Figure 1A**) was a 23-month-old male born to a 27-year-old G3P3 female and a 44-year-old father. Pregnancy was complicated by oligohydramnios and decreased fetal movement, and vaginal delivery was induced at 37 weeks. Birthweight was 2800 grams, and there were no immediate complications. The individual was hypotonic since birth and had global developmental delay. At 23 months, he could roll, commando crawl and sit if placed in sitting position. He could not pull to stand nor cruise. He had language and speech delay, saying only two words at 23 months. Anthropometric measurements were as follows: height 82.9 cm (Z score=-1.38), weight 10.09 kg (Z score=-1.51), head circumference 48.1 cm (~50th %ile). Dysmorphic features included a high forehead, frontal bossing, deep set eyes, upslanting palpebral fissures, and micrognathia. Neurological exam was significant for axial hypotonia, head lag that improved over time, normal patellar DTRs (2+), and reduced ankle DTRs. Ophthalmology exam revealed high myopia (right sphere -7.50; left sphere -6.00) with normal optic nerves. Brain MRI showed mildly prominent extra-axial spaces and a persistent fetal left trigeminal artery. EMC/NCV studies were interpreted as essentially normal with the exception of somewhat small amplitudes for the lower extremity motor responses. Creatine kinase (CK) and echocardiography were normal. Array CGH followed by karyotyping revealed 47,XXY in all observed cells, indicative of Klinefelter syndrome. The hypotonia and developmental delay, which seemed far beyond what

would be expected with Klinefelter, prompted further genetic evaluation with trio whole exome sequencing. Whole exome sequencing of the proband and both parents revealed a heterozygous variant in *ATAD3A* (g. chr1:1464679 C>T [hg19]; GenBank:NM_001170535.1; c.1582C>T; p.Arg528Trp) which was not present in either parent, and Sanger sequencing confirmed that this variant likely arose *de novo* in the affected individual. An additional intronic variant in *ATAD3A* (chr1:1459214 G>A [hg19]; GenBank:NM_001170535.1; c.964-5G>A) was inherited from the father. RNA was not available to study effects on splicing, nor could the phase of the *de novo* variant with respect to the intronic variant be determined.

Family 6, II-1 and II-2

Family 6 included two affected siblings, a 26-year-old female (Family 6, II-1) and her 24-year-old brother (Family 6, II-2 in **Figure 1A**) of Italian descent. There was no known consanguinity, and family history was reportedly negative for ID, congenital cataracts, epilepsy or other early onset neurological conditions. The girl was born at term after an uneventful pregnancy. Measurements at birth were 3150g and 49cm, head circumference was 35cm. She showed developmental delay, with crawling at 10 months and unsupported walking at 22 months. She pronounced her first words at 24 months. Congenital nuclear cataract was diagnosed at three years (surgery performed at 6 years for the right eye, and at 7 years for the left eye). At four years, she started to show an ataxic gait and a neurological examination confirmed a mild limb ataxia with normal muscular tone and normal reflexes. At 6 years, she developed an absence seizure disorder with atonic crises managed with valproic acid. She had a mild delay in pubertal development, with menarche at 16 years. At a

subsequent neurological examination at 19 years, she showed hypotonia, reduced strength (more pronounced in lower limbs), intentional tremors of the upper limbs, and an ataxic gait. By 26 years, she had developed pes cavus deformity in her feet. An EKG and EEG at age 12 months were normal. EMG at age 5 years was normal, and bone age was delayed (3 years at chronological age 5 years). Echocardiogram at age 6 years was normal. Brain imaging performed at the age of 7 and 14 years showed a mildly progressive cerebellar atrophy. It was repeated again in her 20's (see below). IQ tests were performed at 9 years: WISC 61, Leiter 77.

The proband's affected brother was born at term after an uneventful pregnancy. Measurements at birth were 3500g and 50cm, head circumference was 35cm. He showed developmental delay, with crawling at 11 months and unsupported walking at 18 months. He pronounced his first words at 30 months. Zonular congenital cataract was diagnosed at three years (surgery performed at 4 for the right eye, at 5 for the left). At 7 years, he developed an absence seizure disorder managed initially with valproic acid and later with a combination of valproic acid and oxcarbazepine; the neurological examination revealed a normal muscular tone, normal reflexes and no ataxia, but the boy showed locomotor incoordination. At 10 years he underwent surgery for cryptorchidism. Stature at 12 years was 127.0cm (-3DS) and a mild GH deficiency was diagnosed. Somatotropin therapy was carried on from age 15 to age 19, with a final height of 169cm (target height 171cm). Puberty was delayed with onset of pubarche at age 16 years. Thyroid function, cortisol, prolactin and gonadotropins were normal. At a new neurological examination at 17 years, he showed hypotonia, reduced strength in upper and lower limbs, and an ataxic gait. EMG at age 3 years was normal, and an EKG at age 6 years resulted normal. Bone age was delayed (3.5 years at chronological age of 6 years). Brain MRI was

performed at the age of 6 years (normal) and at 13 and 24 years, when a mild cerebellar atrophy was reported. IQ tests were performed at 7 years: 102, although a language delay was present. Muscle histology and histochemistry showed lipid droplets.

At the age of 26 and 24 years respectively, individuals II-1 and II-2 from Family 6 underwent a standardized brain MR protocol on a 1.5 T scanner including axial FLAIR T2-weighted images, FSE coronal T_2-weighted images, volumetric T1-weighted fast spoiled gradient-echo (FSPGR) images (1 mm isotropic voxels), and axial DTI images of contiguous 3 mm slices using a single-shot SE-EPI sequence (25 diffusion gradient directions; b-value=900 s/mm2). Suppressed-water proton MR spectra (^{1}H-MRS) were acquired using the PRESS single-voxel localization sequence (PROBE).[2] Two Volumes of Interest (VOIs) were selected: left cerebellar hemisphere (6.0 mL; TR= 4000 ms, TE = 35 ms, and averaging 64 FIDs) and lateral ventricles for lactate evaluation [8.8 mL in Individual II-1 and 6.7 mL in Individual II-2 (Family 6); TR= 1500 ms, TE = 288 ms, and averaging 384 FIDs for each acquisition]. Metabolite's content was calculated using the fitting program LCModel v. 6.3[3,4] and expressed relatively to creatine.

On conventional brain MR, Individual II-1 (Family 6) presented a slight enlargement of the lateral and of the cerebral subarachnoid spaces, hypoplastic optic nerves and chiasma, a moderate enlargement of the fourth ventricle and of cerebellar hemispheres and vermis subarachnoid spaces (**Figure 2**), and a reduction of sagittal middle cerebellar peduncle diameter and coronal superior cerebellar peduncle diameter [7.0 mm and 2.1 mm, respectively; normal values (n.v.) > 8.10 mm and > 3.1 mm, respectively]. In addition, a pathological increase in mean diffusivity (MD) was detected in the dentate nucleus (0.90 x 10-3mm2/s; n.v. < 0.79 x 10-3mm2/s),

pons (0.95 x 10-3mm2/s; n.v. < 0.93 x 10-3mm2/s), superior cerebellar peduncles (1.05 x 10-3mm2/s; n.v. < 0.90 x 10-3mm2/s), vermis (1.44 x 10-3mm2/s; n.v. < 1.18 x 10-3mm2/s) and cerebellar hemispheres (1.22 x 10-3mm2/s; n.v. < 0.85 x 10-3mm2/s), indicating microstructural changes secondary to neurodegeneration. ^{1}H-MRS revealed a moderate reduction of NAA/Cr ratio in the left cerebellar hemisphere [0.90, -29% compared to mean n.v. = 1.27 (range: 0.91-1.75)], a sign of neuronal-axonal degeneration, while no CSF lactate was detected (data not shown).

As concerns Individual II-2 (Family 6), conventional brain MR sequences showed a mild cerebellar atrophy with hypoplastic optic nerves and chiasma, while ^{1}H-MRS metabolites' ratios in both VOIs were within the normal ranges. Diffusion images analysis demonstrated increased MD in the dentate nucleus (0.93 x 10-3mm2/s), pons (0.98 x 10-3mm2/s), superior cerebellar peduncles (1.03 x 10-3mm2/s) and cerebellar hemispheres (1.01 x 10-3mm2/s). Microstructural changes were milder compared to Family 6, Individual I-1.

Microsatellite genotyping at the *SIL1* [MIM 608005] locus, mutated in Marinesco-Sjogren syndrome [MIM 248800], did not reveal evidence of linkage with the disease. Therefore, the entire family quartet underwent WES, performed through target enrichment with the SeqCap EZ Human Exome Kit v2.0 by Roche Nimblegen (Roche Sequencing, Basel, Switzerland) and next-generation sequencing on an Illumina HiSeq2000 platform (Illumina, San Diego, CA, U.S.). The WES reads were aligned (reference human genome hg19) and processed as previously described,[5] obtaining mean depth of coverage (DoC) between 56.8X and 66.7X with 87.3-90.3% of targeted bases covered at >20X).

Assuming an autosomal recessive mode of inheritance, we examined homozygous or compound heterozygous coding variants (splice-sites, nonsynonymous changes and indels) with minor allele frequency (MAF) <0.01 or absent in public variant databases (1000 genomes, Exome Aggregate Consortium). WES confirmed the exclusion of *SIL1* mutations. No compound heterozygous variants of interest were identified. Homozygous variants were intersected with runs of homozygosity (ROH) longer than 500 kb detected by the H3M2 (Tanaka et al., 2015) software.[6] Previous SNP-array chip analysis on the four subjects using the ERSA software[7] revealed that the parents were likely 5th-degree relatives.

Detection of runs of homozygosity (ROHs) spanning several megabases in the two siblings is in line with the finding of moderately recent parental relatedness, further supporting implication of a variant inherited by descent. Only two homozygous variants, in *SAMD11* and *ATAD3A*, were identified in the same ROH of about 700 kb on distal chromosome 1p shared by the two siblings. Both variants were found to be in heterozygous state in the parents.

The homozygous *ATAD3A* variant (g.chr1:1447806C>T [hg19]; NM_001170535.1; c.158C>T; p.Thr53Ile) showed several features supporting its putative pathogenic role, including novelty, evolutionary conservation of the involved residue and high predicted pathogenicity score (**Table S3**). The *ATAD3A* variant and its segregation were confirmed by Sanger sequencing. Briefly, *ATAD3A* exon 1 was amplified from 20ng of siblings and parents' DNA by using KAPA2G Fast PCR Kit (Resnova) with 5% of dimethyl sulfoxide (DMSO) and 1.5 mM $MgCl_2$, with an annealing temperature of 60°C for 30 cycles. Forward and reverse primer sequences were 5'- AGA CTC TTC TCT GCG TCC TG -3' and 5'- AAA CCC ATC TAC CCA TCT GG -3', respectively. Sanger sequencing was performed through the BigDye

terminator v1.1 cycle sequencing kit (ThermoFisher Scientific) following the manufacturer's protocol.

Family 7, II-1

(Family 7, II-1 **(Figure 1A**) was a female born to a 35 yo G1P1 Indian mother and 32 yo Caucasian (western European ancestry) father at 34 5/7 wks, via emergent C-section for decreased fetal movement and breech position, and a nonreassuring nonstress test. The mother was treated for hypothyroidism during the pregnancy. Pregnancy was unremarkable until 32 wks, when mother noted some concern about decreased fetal movement. Fetal monitor demonstrated persistent minimal variability. C-section was undertaken at 34 5/7 due to the above, and the infant had low Apgar scores (2,4,8) and no respiratory effort, requiring intubation in the delivery room. Birthweight was recorded as 2130 grams, length 47.5 cm and head circumference 31 cm, with repeat measurements at 5 days as follows: weight 2110 grams, length 43.5 cm, HC 28.5 cm. Physical exam revealed bitemporal narrowing, short palpebral fissures, bilateral cloudy corneas but clear lenses, hypotonia and seizures. The infant had no spontaneous movement, and responded to painful stimulation by withdrawal of limbs. She was hypotonic with hypertonicity in distal hands and feet. She had bilateral clenched hands with flexion contractures of digits 3-5 and overlapping thumb and index finger. The thumb was straight and long, and she had bilateral rocker bottom feet deformities with curled in toes. Range of motion was decreased at the elbows and knees.

On day of life 2 she had episodes of jerking, and routine EEG after episodes showed long periods of discontinuity and epileptiform discharges with clinically

correlated seizures. Levetiracetam treatment was started. Head ultrasound revealed a large germinal hemorrhage, renal ultrasound was normal, and echo in the first days of life showed a large PDA, PFO, and mild right ventricular hypertrophy with septal hypertrophy. The proband had hypoglycemia after birth, hypernatremia, increased BUN, mild transaminitis and elevated triglycerides. Work-up included cell free DNA testing for aneuploidy during the 1st trimester of pregnancy which resulted normal. A fetal level II ultrasound at 17 weeks was normal. Infectious serologies were negative, and GBS status was unknown. Chromosome analysis after birth was normal, 46XX. Muscle biopsy showed nonspecific abnormalities, including increased fiber size variability, mildly increased internal nuclei, mildly increased myofiber lipid content/ droplet size, and probably type I fiber type predominance. COX staining did not reveal any abnormal mitochondria; however, these were not examined at higher resolution. CK was normal. Brain MRI demonstrated a smooth sulcal/gyral pattern, and significant hypoplasia of posterior fossa structures including cerebellum and brainstem.

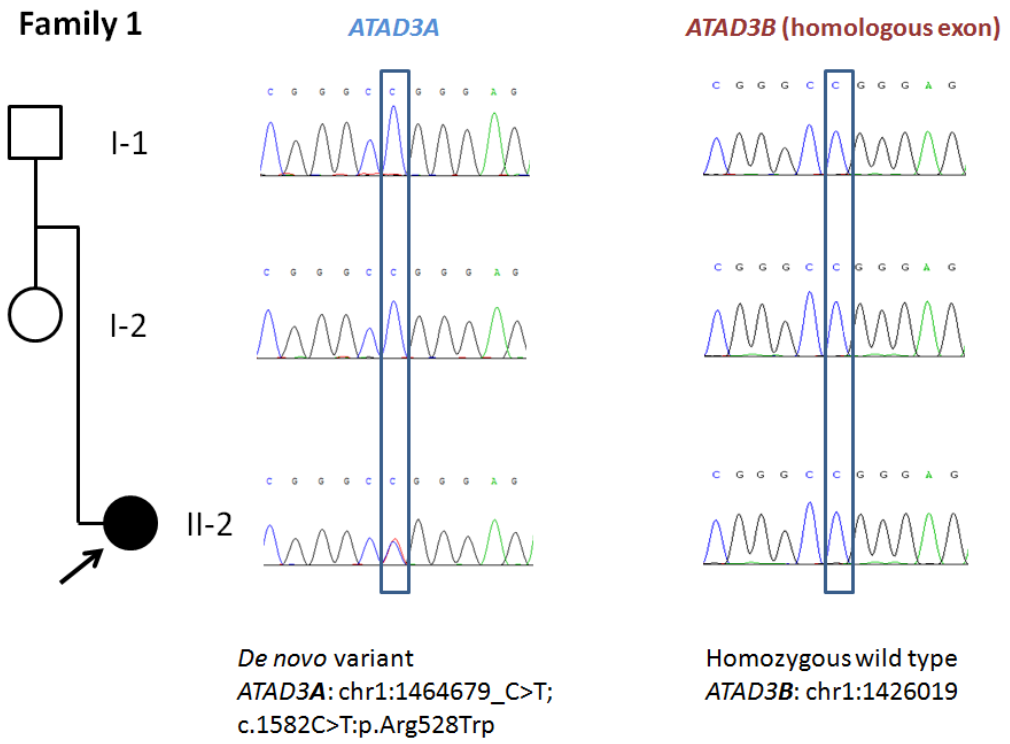

Figure S1. *ATAD3A* c.1582C>T variant is specific to *ATAD3A* and did not arise by gene conversion. Primer design utilizing specific intronic regions allowed for differentiation of the *ATAD3A* locus from the highly homologous *ATAD3B* locus. No variation was seen at the homologous *ATAD3B* locus, ruling out gene conversion as a mechanism of mutation. The paralogous exon in *ATAD3C* shares only 91.8% homology with this exon of *ATAD3A*, and is thus readily distinguishable. Additional single nucleotide variants (SNVs) would be expected to be seen if gene conversion occurred with *ATAD3C* as the donor paralog.

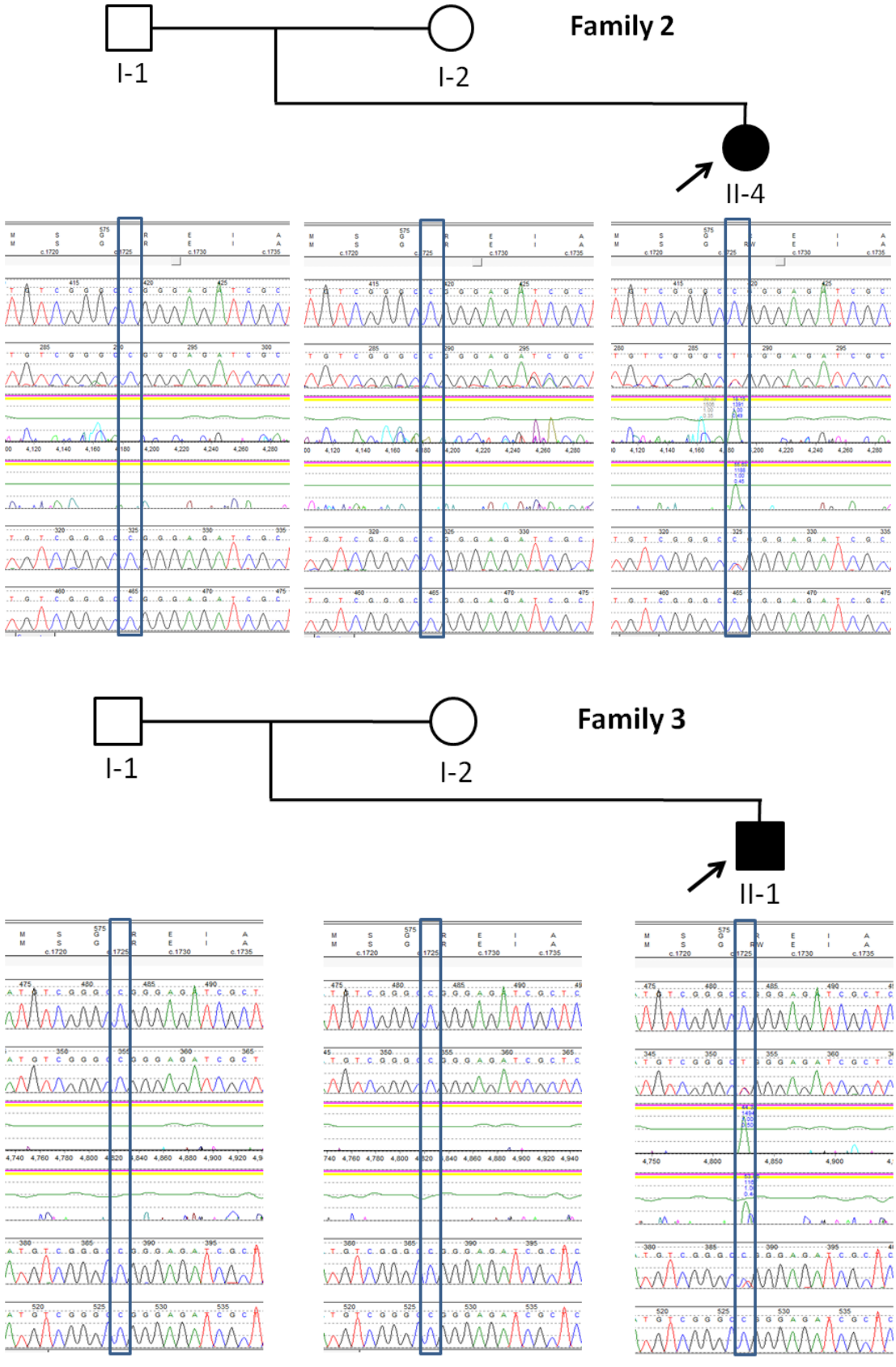

Figure S2. Sanger sequencing in Families 2 and 3 demonstrating the *de novo* c.1582C>T variant.

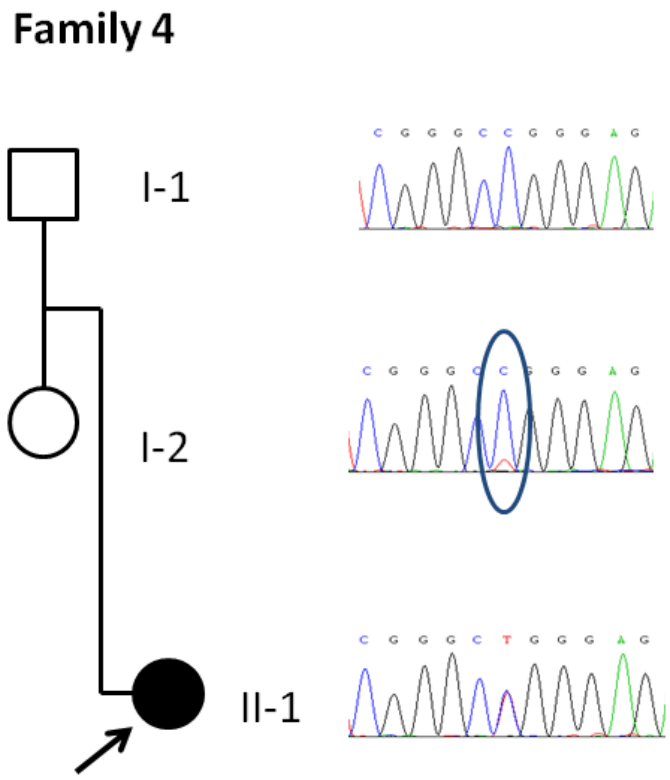

Figure S3. Sanger sequencing in Family 4 suggests low-level mosaicism in the mother.

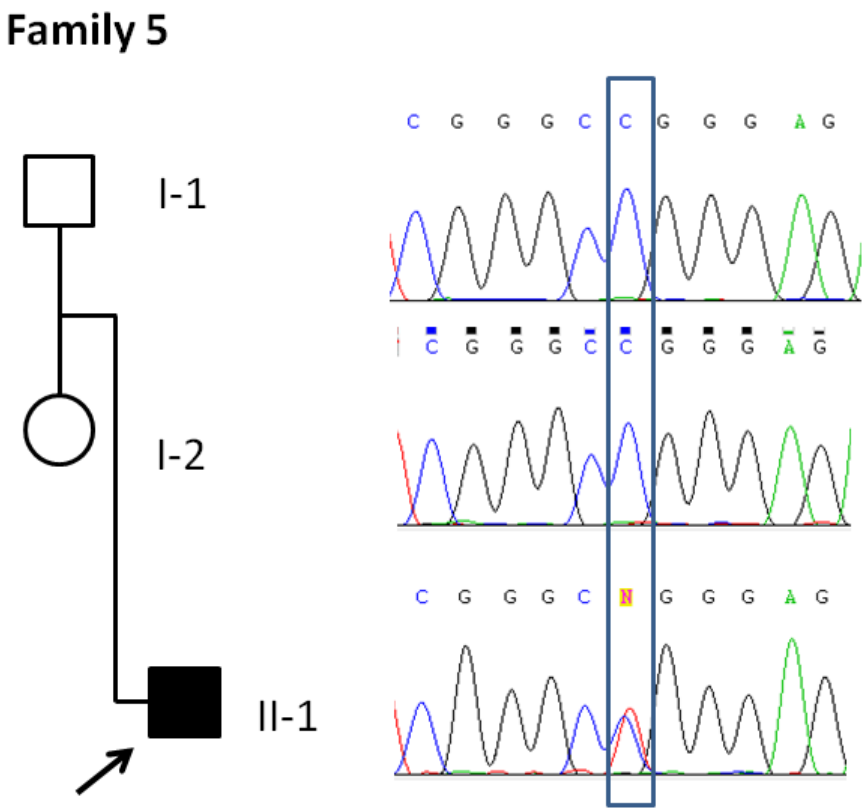

Figure S4. Sanger sequencing in Family 5 demonstrating the *de novo* c.1582C>T variant.

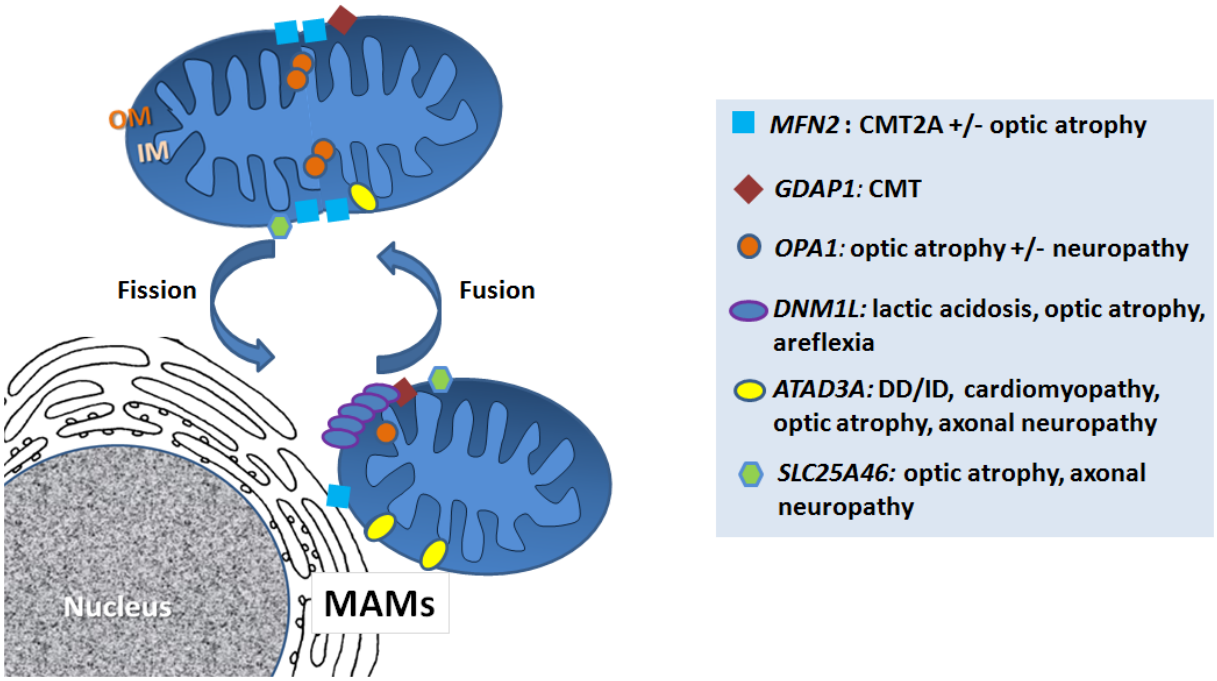

Figure S5. Fusion and fission proteins lead to neurological disease. Schematic diagram of select proteins involved in fusion and fission of mitochondria, and associated human disease.

abbreviations: OM – outer membrane; IM – inner membrane

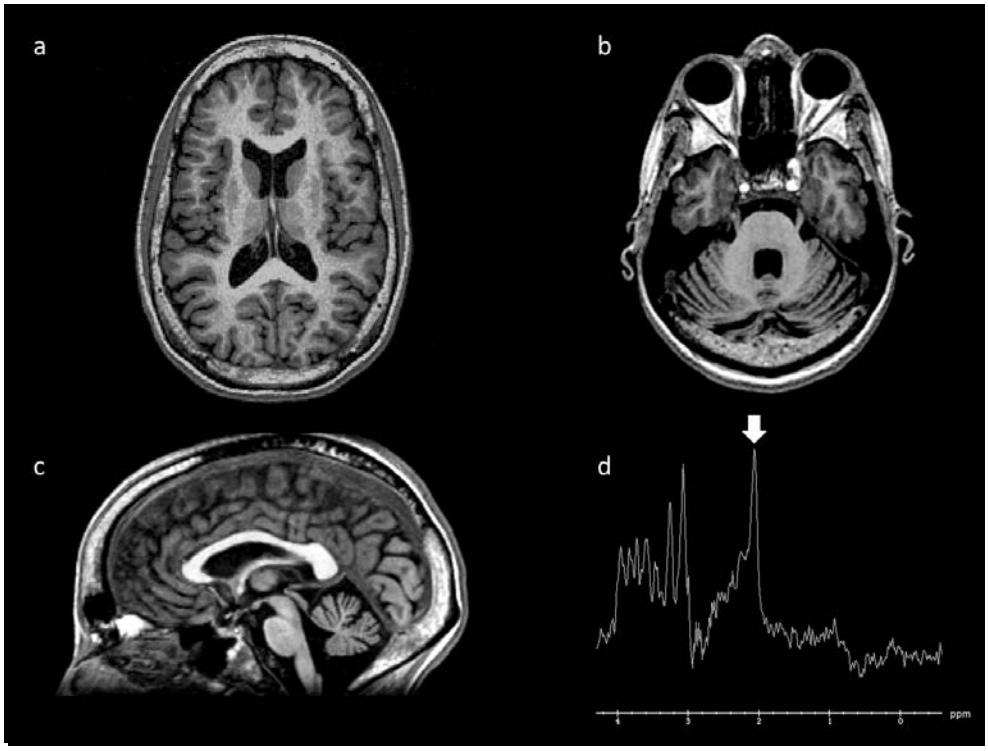

Figure S6. Brain imaging of Individual II-1 in Family 6.

Axial and sagittal brain high-resolution 3D T1 FSPGR images from Individual II-1 in Family 6. Images show an slight increase in the volume of the lateral and fourth ventricles, and atrophy of the cerebellar hemispheres and vermis. ^{1}H-MRS spectra from the left cerebellar hemisphere shows a reduction of the neuro-axonal marker N-Acetyl Aspartate content (arrow).

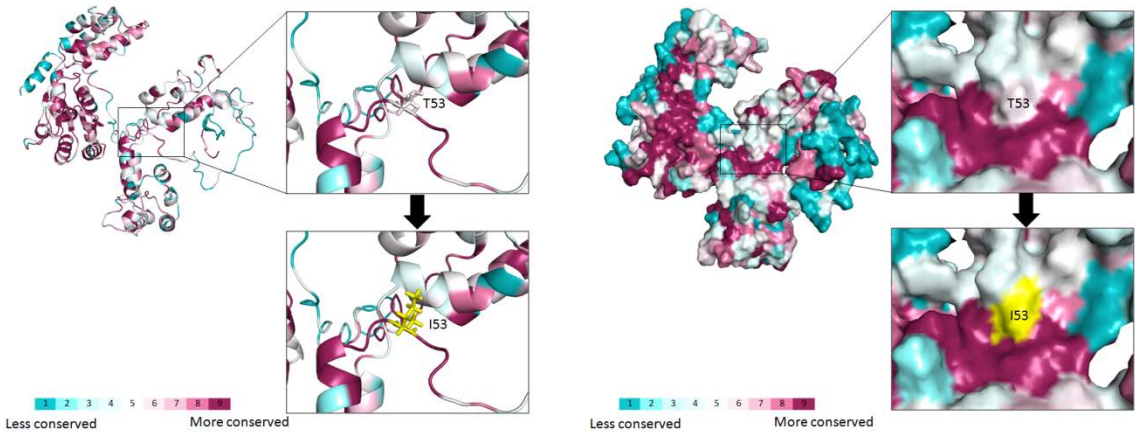

Figure S7. *In silico* modeling of p.Thr53Ile

Protein structure prediction shows that T53 is predicted to reside in coil and is solvent accessible (on the protein surface). The flanking amino acids 50-52 and 54-59 are highly conserved. Surface structure modeling (right) shows that T53 resides at the edge of a pocket, possibly a functional site, which consists of highly conserved residues. T53 may have a structural supporting role in this putative functional site, since T53 seems to protrude outward near the pocket.

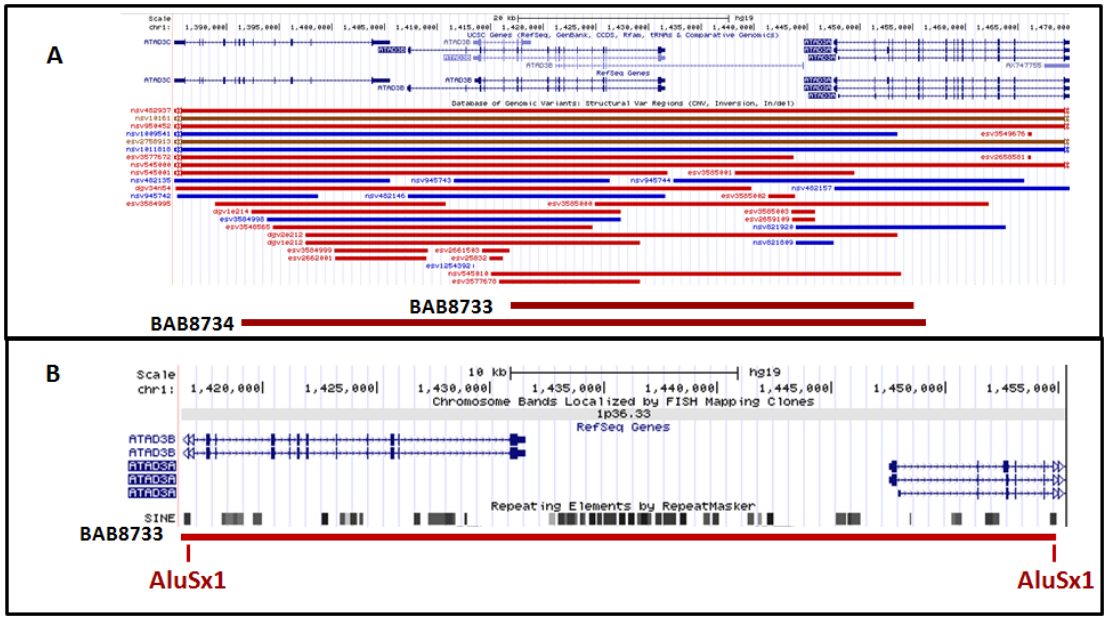

Figure S8. CNVs at the *ATAD3* locus. (A) Multiple CNVs have been reported in the *ATAD3* locus in the Database of Genomic Variants (DGV). Note rearrangements between paralogs. All tracks were derived from the UCSC genome browser. (B) The deletion breakpoints of BAB8733 include identical *Alu*Sx1 elements.

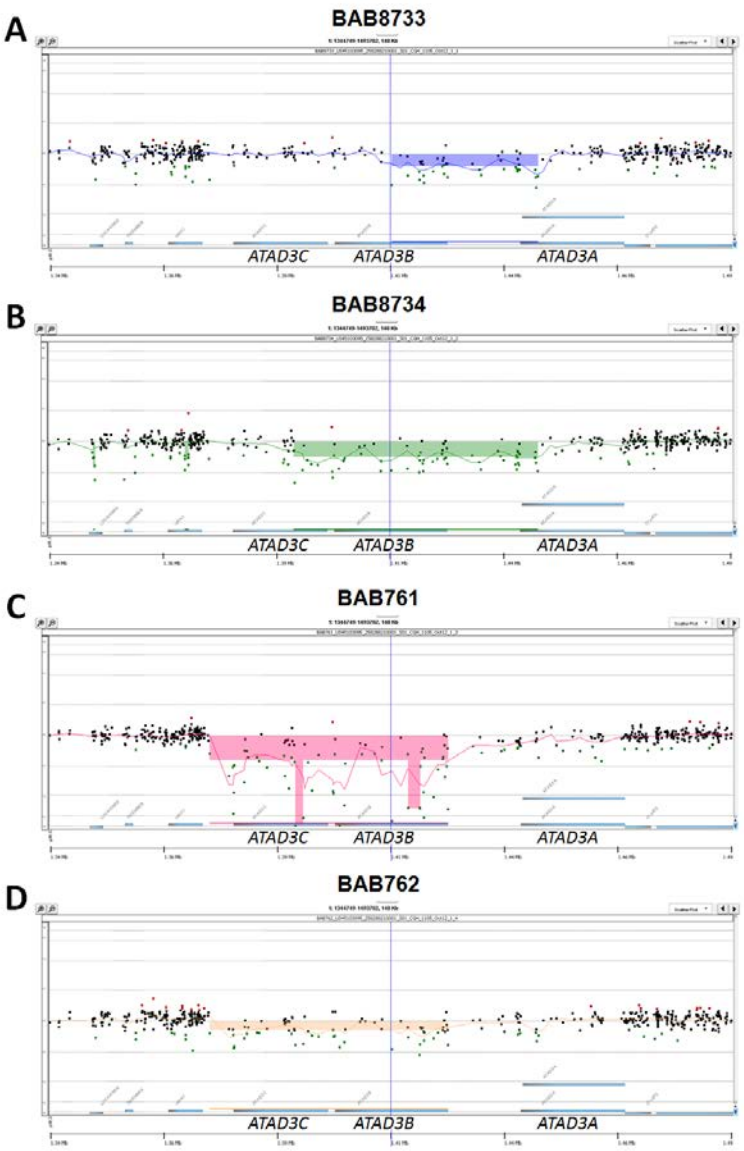

Figure S9. Targeted aCGH data. (A) Array of BAB8733 (Family 7, I-2) indicates a heterozygous deletion spanning from *ATAD3B* to *ATAD3A*. (B) Array of BAB8734 (Family 7, I-1) indicates a heterozygous deletion spanning from *ATAD3C* to *ATAD3A*. (C) Array of BAB761 indicates a homozygous deletion of *ATAD3C* and *ATAD3B*, without involvement of *ATAD3A*. (D) Array of BAB762 (mother of BAB761) indicates a heterozygous deletion of *ATAD3C* and *ATAD3B*.

A

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CLUSTAL O(1.2.1) multiple sequence alignment

ATAD3B          tggattggtgttgagcatttttctggttttaaaggcttttctctttttctgcggcttctt 1416241
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ATAD3A          cccgcacacatgggcacagtcacaggttttaaaggcttttctctttttctgcggcttctt 1454295
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B

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CLUSTAL O(1.2.1) multiple sequence alignment


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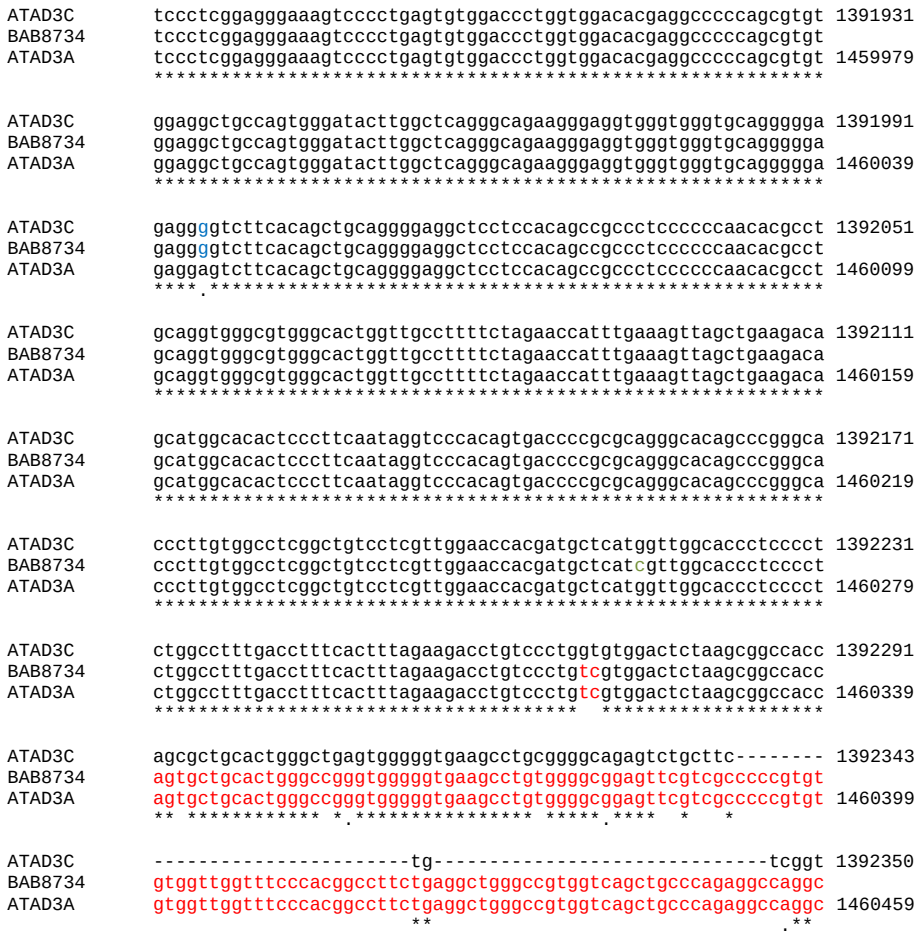

Figure S10. Breakpoint junction analyses in Family 7. (A) Nonallelic homologous recombination (NAHR) in BAB8733 (Family 7, I-2) is mediated by 754 bp of 100% homology in intron 5 of *ATAD3B* and *ATAD3A.* Blue font indicates sequence specific to *ATAD3B*, while red font indicates sequence specific to *ATAD3A. Alu*Sx1 element is shaded in grey. (B) NAHR in BAB8734 (Family 7, I-1) is mediated by 960 bp of 99.7% homology between the regions encompassing exon 7 of *ATAD3C* and exon 11 of *ATAD3A.*

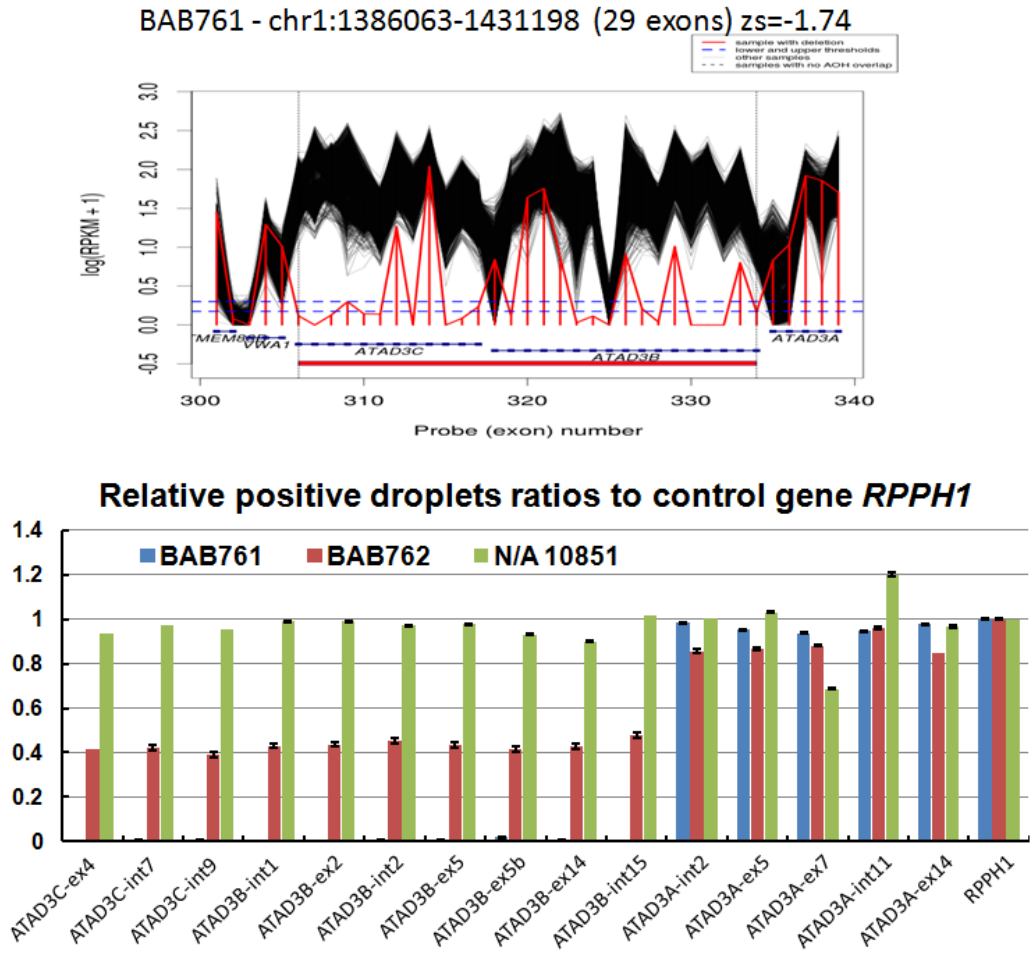

Figure S11. Homozygous CNV in BAB761 involving *ATAD3C* and *ATAD3B* narrows critical region to *ATAD3A*. Analysis of WES read depth in individual BAB761, with a clinical and molecular diagnosis of Smith Magenis syndrome, suggested a homozygous deletion of *ATAD3C* and *ATAD3B* (top). This was confirmed by droplet digital PCR (bottom). BAB761 showed no amplification of select exonic and intronic amplicons of *ATAD3C* and *ATAD3B*, and the proband's mother (BAB762) was heterozygous for these amplicons. Amplification of select *ATAD3A* exons and introns was comparable to controls.

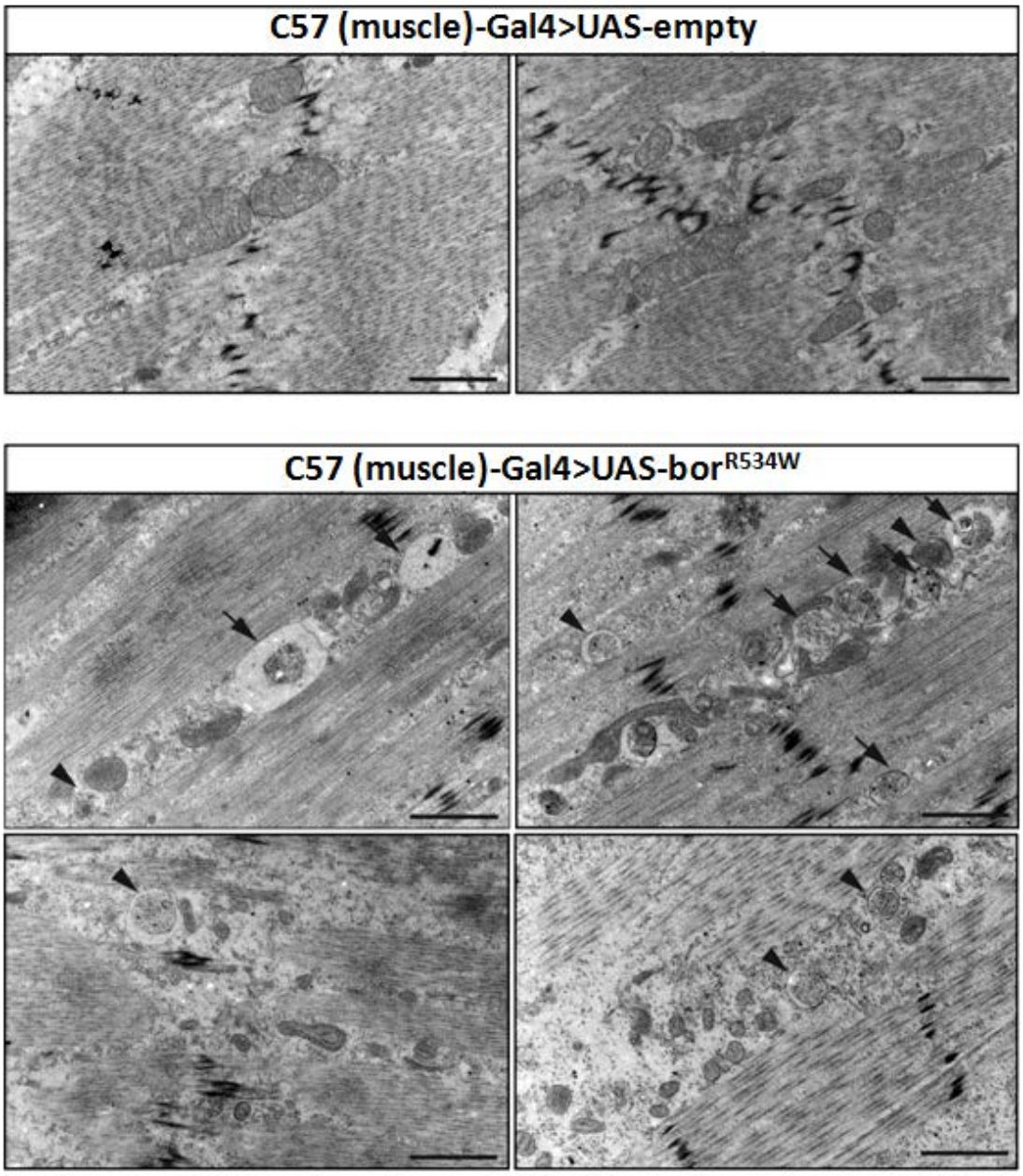

Figure S12. TEM sections of fly larvae muscle expressing *bor*^{R534W}

Top panels represent sections from fly larvae muscles carrying *C57 (muscle)-Gal4* together with *UAS*-empty (control) for comparison. Bottom panels represent sections from larvae muscles carrying *C57 (muscle)-Gal4* together with *UAS-bor*^{R534W}. Arrow heads indicate double-membraned autophagosomes. Arrows indicate autophagic vacuoles. Scale bar, 1 μm.

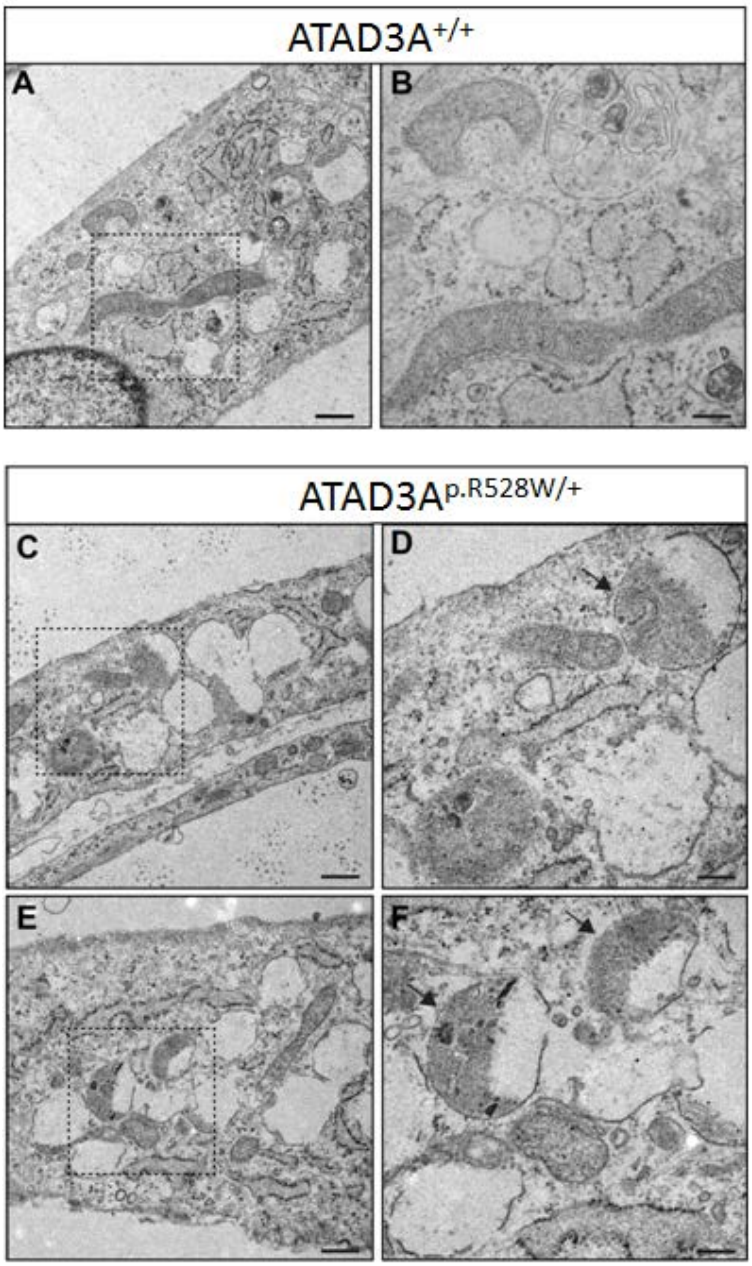

Figure S13. Fibroblasts from proband in Family 1 (*ATAD3A*$^{p.R528W/+}$) exhibit mitophagic events.

(A-B) TEM sections of control fibroblasts (*ATAD3A*$^{+/+}$). (C-F) TEM of fibroblasts from affected individual (Family 1, II-2, *ATAD3A*$^{p.R528W/+}$). (B), (D), and (F) are high magnification images of inserts in (A), (C), and (E), respectively. Arrows indicate mitophagic vesicles. (A,C,E) Scale bars 500 nm. (B,D,F) Scale bars 200 nm.

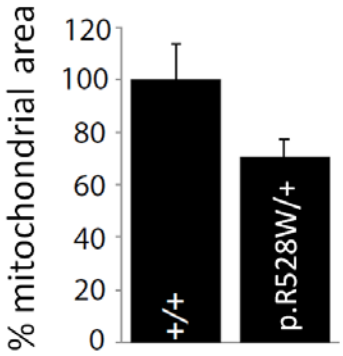

Figure S14. Mitochondria are reduced in fibroblasts from Family 1, II-2.

Percentage of area of mitochondria divided by area of cell in affected (*ATAD3A*$^{p.R528W/+}$) and control (*ATAD3A*$^{+/+}$) fibroblasts. Error bars indicate s.e.m. P values were calculated using Student's t-test. P=0.067

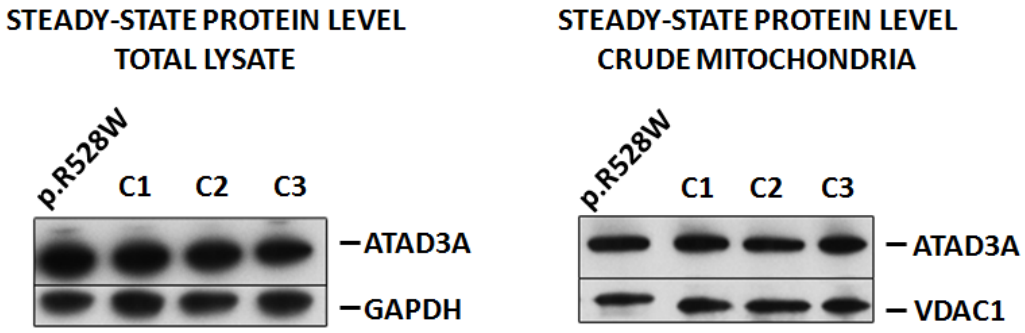

Figure S15. Steady-state levels of ATAD3A protein in fibroblasts.

Western blot analysis of steady-state levels of ATAD3A protein in total lysate (left) and crude mitochondria (right) in the proband from Family 4. Protein levels are not reduced in fibroblasts carrying the p.R528W mutation. Abbreviations: C1 - control 1, C2 - control 2, C3 - control 3.

Table S2. Primer locations and results of droplet digital PCR (ddPCR).

Gene	Forward primer start [chr1:hg19]	Reverse primer end [chr1:hg19]	ddPCR result mother (concentration)	ddPCR result father (concentration)	ddPCR result control (concentration)	ddPCR result mother (# alleles)	ddPCR result father (# alleles)
ATAD3C	1389781	1389985	278	308	278	2	2
ATAD3C	1392335	1392489	292	148	281	2	1
ATAD3C	1395761	1395980	275	146	275	2	1
ATAD3B	1409276	1409458	290	156	290	2	1
ATAD3B	1412616	1412791	288	150	293	2	1
ATAD3B	1413765	1413934	305	158	236	2	1
ATAD3B	1416142	1416386	288	153	250	2	1
ATAD3B	1425606	1425810	157	154	268	1	1
ATAD3B	1426134	1426358	149	146	224	1	1
ATAD3A	1452433	1452604	151	151	270	1	1
ATAD3A	1454246	1454438	146	151	251	1	1
ATAD3A	1455969	1456171	272	149	286	2	1
ATAD3A	1460392	1460617	295	330	344	2	2
ATAD3A	1462949	1463118	273	297	281	2	2
RPPH1	chr14:20811230	chr14:20811309	298	306	296	2	2

Table S3. *ATAD3A* variants, frequency and bioinformatic predictions (NM_001170535.1)

Gene	Chromosome position	Sequence alteration	Protein alteration	Zygosity	ExAC frequency	CADD score	Phylop	SIFT	Mutation taster
ATAD3A	Chr1: 1464679 C>T	c.1582C>T	p.Arg528Trp	Het	0	27.6	1.207	0	0.9999
ATAD3A	Chr1: 1447806 C>T	c.158C>T	p.Thr53Ile	Hom	0	34	4.956	0	0.9999

Table S4. Mitochondrial respiratory chain enzyme analysis in muscle homogenate and in skin fibroblasts

	Muscle						***Fibroblasts***					
	CI	**CII**	**CIII**	**CIV**	**SDH**	**CS**	**CI**	**CII**	**CII**	**CIV**	**SDH**	**CS**
Family 6, II-2	23.1	41.2	210	250	25.1	136	16.7	16.8	134	121	12.1	166
Family 6, II-1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	18.2	19.9	139	134	16.8	133
mean ± sd	25,2 ± 4,4	19,9 ± 4,4	100 ± 15	189 ± 33	13,6 ± 2,6	133 ± 39	23,7 ± 4,6	19,2 ± 3,4	119 ± 15	150 ± 30	13,6 ± 2,7	113 ± 18
range	16,6 – 33.8	11,4 - 28,4	70 - 130	124 - 253	8,5 - 18,7	56 - 209	14,7 - 32,7	12,5 - 26,0	90 - 148	91 - 209	8,3 - 18,8	78 - 149
(m ± sd)												
	Muscle						***Fibroblasts***					
Family 4, II-1	-	-	-	-	-	-	20.07	13.13	19.28	33.08	8.52	61.17
mean ± sd	-	-	-	-	-	-	15.66 ±5.5	11.51 ±1.45	20.83 ±4.62	35.56 ±5.83	7.79 ±2.34	64.29 ±31.6

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