

1 **Biallelic Mutations in *ADPRHL2*, Encoding ADP-Ribosylhydrolase 3,**  
2 **Lead to a Degenerative Pediatric Stress-induced Epileptic Ataxia**  
3 **Syndrome**  
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62 **Abstract**

63 ADP-ribosylation, the addition of poly-ADP ribose (PAR onto proteins), is a response signal to  
64 cellular challenges, such as excitotoxicity or oxidative stress. This process is catalyzed by a  
65 group of enzymes, referred to as Poly(ADP-ribose) polymerases (PARPs). As accumulation of  
66 proteins with this modification results in cell death, its negative regulation is for cellular  
67 homeostasis: a process that is mediated by poly-ADP ribose glycohydrolases (PARGs) and  
68 ADP-ribosylhydrolase proteins (ARHs). Using genome-wide linkage analysis or exome  
69 sequencing, we identified recessive inactivating mutations in *ADPRHL2* in six families. Affected  
70 individuals exhibited a pediatric-onset neurodegenerative disorder with progressive brain  
71 atrophy, developmental regression, and seizures that correlated with periods of stress such as  
72 infections. Loss of the *Drosophila* paralogue *parg* showed lethality in response to oxidative  
73 challenge that was rescued by human *ADPRHL2*, suggesting functional conservation.  
74 Pharmacological inhibition of PARP also rescued the phenotype, suggesting the possibility of  
75 postnatal treatment for this genetic condition.

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96 ADP-ribosylation is a tightly regulated posttranslational modification of proteins involved  
97 in various essential physiological and pathological processes, including DNA repair,  
98 transcription, telomere function, and apoptosis<sup>1-3</sup>. Addition of poly-ADP-ribose (PAR) is  
99 mediated by a group of enzymes, referred to as Poly(ADP-ribose) polymerases (PARPs), in  
100 response to cellular stressors, such as excitotoxicity or reactive oxygen species. PARylated  
101 proteins can subsequently initiate cellular stress response pathways. Following resolution of the  
102 original insult, ADP-ribose polymers are rapidly removed<sup>4,5</sup>. While PAR modification can protect  
103 the cell from death in the setting of cellular stress, excessive PAR accumulation or failure to  
104 reverse PAR modification can trigger a cell death response cascade<sup>6,7</sup>.

105 Humans have two genes encoding specific PAR-degrading enzymes: *ADPRHL2* (MIM:  
106 610624) and *PARG* (MIM:603501). Both are capable of hydrolyzing the glycosidic bond  
107 between ADP-ribose moieties and are ubiquitously expressed<sup>8,9</sup>. *ADPRH* (MIM:603081), and  
108 putatively *ADPRHL1* (MIM:610620), encode proteins that can cleave only mono-ADP  
109 ribosylated residues and thus are not functionally redundant with *ADPRHL2* and *PARG*<sup>8</sup>. *In situ*  
110 hybridization studies showed high levels of *Adprhl2* expression in the developing mouse  
111 forebrain, remaining high in cerebellum, cortex, hippocampus, and olfactory bulb in early  
112 postnatal ages, and persisting into adulthood<sup>10</sup>. *Parg* knockout mice die embryonically due to  
113 PAR accumulation and cellular apoptosis<sup>11</sup>. There are no reports of *Adprhl2* knockout animals,  
114 however, *Adprhl2*<sup>-/-</sup> mouse embryonic fibroblasts (MEFs) engineered to express the catalytic  
115 domain of nuclear PARP1 in mitochondria, show PAR accumulation, as well as increased length  
116 of mitochondrial PAR polymers<sup>12,13</sup>.

117 *Drosophila melanogaster* has a single *parg*-like gene, and null flies are lethal in the larval  
118 stage, but when grown at a permissive temperature a few can survive. The surviving flies  
119 display PAR accumulation, neurodegeneration, reduced locomotion, and premature death<sup>14</sup>,  
120 suggesting increased neuronal vulnerability to PAR accumulation. Although mutations in *PARG*  
121 and *PARP* enzymes have not been reported in human disease, other members of this pathway

122 have been implicated in human phenotypes<sup>15</sup>. For example, mutations in *XRCC1* (MIM:194360),  
123 a molecular scaffold protein involved in complex assembly during DNA-strand break repair,  
124 leads to PARP-1 overactivation and is associated with cerebellar ataxia, ocular motor apraxia,  
125 and axonal neuropathy<sup>16</sup>.

126 In this study, we show that mutations in *ADPRHL2* underlie a novel, age-dependent  
127 recessive epilepsy-ataxia syndrome, initiating with sudden severe seizures in otherwise healthy  
128 individuals, followed by progressive loss of milestones, brain atrophy, and death in childhood.  
129 We describe six independent families with mutations in *ADPRHL2*, leading to a nearly-identical  
130 epilepsy-ataxia syndrome (Figure 1A). Only 1 of the 6 families lacked documentation of parental  
131 consanguinity (Family 2), and the parents from this family were from the same small village. The  
132 clinical details of subjects from all included families are shown in Table 1, and detailed clinical  
133 history is narrated in Document S1 in Supplemental Data. The emerging clinical picture is one of  
134 a stress-induced neurodegenerative disease of variable progression with developmental delay,  
135 intellectual disability, mild cerebellar atrophy (Figure 1B), and recurring seizures.

136 Genome-wide linkage analysis of 14 members of Family 1 mapped the disease locus to  
137 an 11 Mb region on chromosome 1p36 with a genome-wide significant multipoint LOD  
138 (logarithm of odds) score of 3.4 (Figure S1A). Exome sequencing of individual II-IV-6 at >30x  
139 read depth for 96.9% of the exome revealed only a single rare ( $AF < 1:1000$ ) potentially  
140 deleterious variant within the linkage interval: a frameshift mutation in *ADPRHL2*, which  
141 segregated with the phenotype according to a recessive mode of inheritance.

142 Using Genematcher, further pathogenic alleles in *ADPRHL2* were identified by this  
143 international collaborative group of authors. By whole exome sequencing (see Supplemental  
144 Data), we have identified a total of 6 distinct mutations in ADP-ribosylhydrolase like 2  
145 (*ADPRHL2*, RefSeq: 54936) from the 6 families. All variants were prioritized by allele frequency,  
146 conservation, blocks of homozygosity, and predicted effect on protein function (see  
147 Supplemental Data), and in all families the homozygous variant in *ADPRHL2* was the top

148 candidate. Variants were confirmed by Sanger sequencing and segregated with the phenotype  
149 according to a recessive mode of inheritance. All variants were predicted to be disease-causing  
150 by the online program Mutation Taster <sup>17</sup>. These variants were not encountered in dbGaP,  
151 ExAC, 1000 Genomes databases, genomeAD or the Middle Eastern Variome.

152 *ADPRHL2* contains 6 coding exons, yielding a single protein-coding transcript, ADP-  
153 ribosylhydrolase 3 (ARH3) (Figure 2A). The encoded 363 amino acid ARH3 protein predicted a  
154 mitochondrial localization sequence (MLS) and single enzymatic ADP-ribosyl-glycohydrolase  
155 domain (Figure 2B). Family 1 carried a homozygous mutation (*c.1000C>T*) in exon 6,  
156 introducing a premature stop codon (p.Gln334Ter), predicting truncation of the highly conserved  
157 last 30 amino acids of the protein, which includes part of the ADP-ribosylhydrolase domain.  
158 Family 2 harbored a homozygous mutation in exon 3 (*c.316C>T*), also introducing a premature  
159 stop codon (p.Gln106Ter) in the ADP-ribosylhydrolase domain. Family 3 revealed a  
160 homozygous missense mutation (*c.235A>C*) in exon 2, leading to an amino acid change  
161 (p.Thr97Pro) in a residue which is highly conserved among vertebrates (Figure S2A). Using a  
162 previously published crystal structure of ARH3, we localized this residue to an alpha-helical loop  
163 within the ADP-ribosylhydrolase domain and the substrate binding site, which is defined by the  
164 position of 2 Mg<sup>2+</sup> ions located in adjacent binding sites, thus predicted to affect protein structure  
165 and enzymatic activity (Figure S2B) <sup>18</sup>. Family 4 carried a homozygous 5 basepair (bp) deletion  
166 (*c.414-418TGCCC*) in exon 3, resulting in a frameshift in the ADP-ribosylhydrolase domain  
167 (p.Ala139GlyfsTer5). Family 5 carried a homozygous missense mutation (*c.530C>T*) in exon 4,  
168 leading to an amino acid change (p.Ser177Leu), which was also highly conserved among  
169 vertebrates. It is localized in a critical alpha-helical loop within the ADP-ribosylhydrolase  
170 domain, also suggesting an effect on protein structure and activity. Family 6 carried a  
171 homozygous missense mutation (*c.100G>A*) in exon 1, leading to an amino acid change  
172 (p.Asp34Asn), which was highly conserved among vertebrates. This change is also localized in

173 a critical alpha-helical loop within the ADP-ribosylhydrolase domain, suggesting a potential  
174 impact on protein structure and activity.

175         The emerging phenotype of recessive *ADPRHL2* mutations is a degenerative pediatric-  
176 onset stress-induced epileptic-ataxia syndrome. Individuals with mutations in this gene are  
177 asymptomatic early after birth, but gradually develop a cyclic pattern of illness-related  
178 spontaneous epileptic seizures; or may manifest with a neurodegenerative course with  
179 weakness, ataxia and loss of milestones, followed by clinical deterioration in all individuals that  
180 ultimately may lead to premature death. Most of the subjects showed a sudden unexpected  
181 death in epilepsy or by apnoic attacks (SUDEP)-like clinical presentation, suggesting a  
182 hyperacute presentation prior to the family's recognition of a predisposition. We could not  
183 establish an obvious genotype-phenotype correlation, however, as we show below that the  
184 missense mutation also leads to a severe loss-of-function. Thus the clinical variability in the age  
185 of onset might be due to the genetic background or environmental challenges leading to  
186 variable susceptibility to illness-related cellular stress.

187         The differential diagnosis for this condition was based upon the presentation of a  
188 recessive condition with recurrent exacerbations showing predominant features of global  
189 developmental delay, intellectual disability, seizures, neurogenic changes on EMG, hearing  
190 impairment, regression and mild cerebellar atrophy, without microcephaly and cataracts. The  
191 differential diagnosis in our families included mitochondrial disorders, spastic ataxia, and  
192 primary peripheral neuropathy.

193         To determine the impact of these mutations on protein folding and binding activity, we  
194 generated recombinant proteins in *E.coli*, and purified by His-tag affinity chromatography. Our  
195 results showed that the p.Gln334Ter was not evident in the soluble fraction, whereas the  
196 Wildtype (WT) was recovered with good purity (Figure S3A). The p.Thr79Pro protein was  
197 expressed and soluble, possibly slightly reduced in recovery compared with the WT ARH3  
198 protein. The deleterious impact of the p.Thr79Pro variant was studied using Circular Dichroism

199 (CD) spectroscopy (Figure S3B-C). Compared to WT, this mutant exhibited a reduction in alpha-  
200 helical content and altered secondary structure, which agreed with the fact that the p.Thr79Pro  
201 substitution occurred within an alpha-helical domain. Further, the melting temperature ( $T_m$ ) of  
202 the mutant p.Thr79Pro was reduced by more than 10°C, confirming destabilization of the mutant  
203 (Figure S3D-F). We also found that, in contrast to the WT ARH3 protein, the p.Thr79Pro mutant  
204 was not stabilized by ligands such as adenosine diphosphate ribose (ADPr) (Figure S3G-I). We  
205 confirmed the specificity of this assay by using adenosine triphosphate (ATP) and ribose-5-  
206 phosphate as negative controls, which were not predicted to bind or stabilize ARH3. Together,  
207 this data suggests that both disease-causing-truncating and amino-acid-substituting mutants  
208 should be destabilized when expressed in cells.

209         Because the p.Gln334ter mutation of Family 1 was in the last exon, we first excluded  
210 nonsense mediated decay (NMD) of the mutant mRNA. We collected skin biopsies from the  
211 father (III-II) and two affecteds (II-IV-6 and II-IV-7) of Family 1, generated primary fibroblasts,  
212 then performed RT-PCR using primers designed to amplify the last 3 exons of *ADPRHL2*  
213 (Figure S1B). The father's and affected individuals' cells revealed a band of the expected size,  
214 and similar intensity to that of a healthy control, arguing against NMD. However, lysates derived  
215 from the affected individuals showed no detectable ARH3 protein (Figure 2C), using an antibody  
216 that recognizes amino acids 231-245 (see Supplemental Data), consistent with a null effect of  
217 the truncating mutation. Further, western blot analysis of individual II-2 from Family 2 shows an  
218 absence of the protein as predicted by the early stop codon; and fibroblasts from individual II-1  
219 from Family 3 showed a severe reduction of ARH3 levels (Figure 2C), consistent with the  
220 thermal instability of this mutant protein (Figure S3D-F) and the severe alteration of its  
221 secondary structure (Figure S3B-C).

222         While humans have two known genes with specific PARG activity (*PARG* and  
223 *ADPRHL2*; Figure 3A), *Drosophila* have a single gene that regulates this process: *parg*. Using  
224 the Gal4-UAS system to drive RNAi expression, we found that *parg* knockdown led to a 60%



225 decrease in total *parg* mRNA for flies with the ubiquitous *da* promoter and a 50% decrease with  
226 the neuron-specific promoter, *elav* (*embryonic lethal abnormal visual system*) (Figure S4A).  
227 While the *da*-Gal4 and *parg*<sup>RNAi</sup> lines showed normal survival, crossing the two together led to  
228 *da*-mediated expression of *parg*<sup>RNAi</sup>, which reduced survival substantially (Figure S4B).  
229 Ubiquitous knockdown of *parg* also led to decreased survival when animals were exposed to  
230 stress with either Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) in their water or environmental hypoxia (2% O<sub>2</sub>)  
231 (Figure S4C-D). Furthermore, knockdown of *parg* specifically in neurons largely recapitulated  
232 this phenotype using the same two environmental stressors (Figure S4E-F). These data provide  
233 evidence that stress leads to premature death in the absence of *parg*, and neurons play an  
234 important role in this phenotype.

235         However, lethality of these flies was not as severe as in the *parg*<sup>27.1</sup> line, which carries a  
236 p-element insertion that deletes two-thirds of the open-reading frame (nucleotides 34,622-  
237 36,079 of Genbank Z98254)<sup>14</sup>, suggesting that *parg*<sup>RNAi</sup> is only partially inactivating. These  
238 *parg* loss-of-function mutant flies lack the *parg* protein and show elevated levels of PAR,  
239 especially in nervous tissue<sup>14</sup>. Mutant flies die in larval stages, but ¼ of the animals survive  
240 when grown at the permissive 29°C temperature. These adult flies display progressive  
241 neurodegeneration, reduced locomotion, and reduced lifespan<sup>14</sup>, not inconsistent with the  
242 individuals' phenotypes in our families. We confirmed lethality of the *parg*<sup>27.1</sup> line and found that  
243 forced expression of *Drosophila parg* under the ubiquitous *daughterless* (*da*) promoter in the  
244 mutant background increased both survival and motor activity as measured by an established  
245 'climbing index'<sup>19</sup> (Figure 3B-C). Likewise, expression of the human *ADPRHL2* under the same  
246 *da* promoter showed a nearly identical degree of rescue of both survival and locomotor activity  
247 (Figure 3B-C). These results suggest that human *ADPRHL2* is a functional paralogue of  
248 *Drosophila parg*.

249         We next tested whether this phenotype might be ameliorated by inhibition of protein  
250 PARylation. We reasoned that the requirement for dePARylation should be reduced by blocking

251 stress-induced PARylation. Minocycline displays PARP inhibitory activity, with an IC<sub>50</sub> of 42nM  
252 in humans<sup>20</sup> and is well tolerated in flies <sup>21</sup>. We fed flies with a range of concentrations from 0-1  
253 mg/mL Minocycline for 24 hours prior to stress and measured survival rates at 96 hours post  
254 stress induction. Drug treatment of flies with ubiquitous knockdown of *parg* revealed a dose-  
255 dependent partial rescue of the lethality (Figure S4G). This rescue was also seen when drug  
256 was given to flies with neuron-specific knockdown of *parg* (Figure S4H), providing evidence that  
257 PARP inhibition can rescue lethality *in vivo*. While we expect that the effect of Minocycline on  
258 survival in this assay was due to its effect on PARP, we cannot exclude off-target or non-  
259 specific effects <sup>21</sup>.

260         Given that PARP inhibitors are currently in trials for various types of cancer, it is possible  
261 that these drugs could be tested for clinical effectiveness in this orphan disease, where they  
262 may have a positive effect. Potentially clinically relevant PARP inhibitors include: (1)  
263 Minocycline – an FDA-approved tetracycline-derivative that displays PARP inhibitory activity, (2)  
264 Dihydroisoquinoline (DPQ) – a non-FDA-approved potent PARP-1 inhibitor used in experimental  
265 research, (3) Veliparib (ABT – 888) – a potent inhibitor of PARP-1 and PARP-2 currently in  
266 clinical trials for treatment of various type of cancers (IC<sub>50</sub> 42 nM, 37 nM, 4.4 nM, respectively)  
267 <sup>20,22</sup>.

268         The extent to which *ADPRHL2* and *PARG* functionally diverge or converge is not well  
269 understood, based partly on a lack of detailed comparative expression analysis and biochemical  
270 function. *PARG* demonstrates greater specific activity than *ARH3* for removing PAR from  
271 proteins <sup>8</sup>, and loss of *Parg* in mice is embryonic lethal <sup>13</sup>. Taken together, these data suggest  
272 that *PARG* is likely the major contributor to PAR removal in cells that express both genes under  
273 basal conditions. One possibility is that *ADPRHL2* acts as a back-up to *PARG* to remove  
274 excessive PAR moieties under stress conditions. This would be consistent with the clinical  
275 presentation of individuals with loss of *ADPRHL2*, where phenotypes appear to be induced by  
276 environmental stress. Recent studies have shown shown that *ARH3* acts on a recently

277 discovered, new form of Serine-ADP Ribosylation <sup>23</sup>. For example studies illustrate an excessive  
278 accumulation of Ser-poly-ADP ribosylated enzymes in ARH3<sup>-/-</sup> cell lines, and that ARH3 acts  
279 mainly on the Ser-ADPr removal <sup>24</sup>. This would be consistent with the phenotype we see in  
280 subjects with loss of ARH3, where phenotypes do not emerge until environmental stress insults  
281 are encountered. Finally, only ARH3 contains a mitochondrial localization signal, and thus,  
282 another possibility is that ARH3 functions as a mitochondrial-specific glycohydrolase that is  
283 required after oxidative stress induction <sup>25</sup>.

284 PAR signaling has been shown to play a role in a number of cellular processes in  
285 addition to Apoptosis-inducing-factor (AIF)-mediated apoptosis, including regulation of  
286 transcription, telomere function, mitotic spindle formation, intracellular trafficking, and energy  
287 metabolism <sup>2,3</sup>. While we hypothesize that the mechanism of disease is through cell death, it is  
288 possible that PAR accumulation may affect other cellular processes prior to this. Further work is  
289 needed to characterize these effects in the context of this disease.

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## 292 **Supplemental Data**

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294 Supplemental data includes 4 figures, 1 table and Materials and Methods.

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313 **Web Resources**

314 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

315 dbSNP, <http://www.ncbi.nlm.nih.gov/projects/SNP>

316 1000 Genomes, <http://browser.1000genomes.org>

317 Exome Aggregation Consortium [ExAC], <http://exac.broadinstitute.org/>

318 NHLBI Exome Sequencing Project Exome Variant Server, <http://evs.gs.washington.edu/EVS/>

319 PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>

320 SIFT, <http://sift.jcvi.org/>

321 Mutation Assessor, <http://mutationassessor.org/>

322 Provean, <http://provean.jcvi.org>

323 UniProt, <http://www.uniprot.org>

324 HaplotypeCaller and GATK, <https://www.broadinstitute.org/gatk/>

325 Mutation Taster, <http://mutationtaster.org/>

326 GenBank, <https://www.ncbi.nlm.nih.gov/genbank/>

327 Genematcher, <https://genematcher.org>

328 FlyBase, <http://flybase.org>

329 Iranome Database, <http://www.iranome.ir/>

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417 **Figure Legends**

418

419 **Figure 1. Pedigrees of consanguineous families with mutations in *ADPRHL2* and their**  
420 **clinical presentation.**

421 (A) Pedigrees of families 1 to 6 showing consanguineous unions (double bar) and a total of 16  
422 affected individuals. Slash represents deceased individuals. Black shading indicates affected  
423 individuals. Grey shading indicates individuals who passed away from SUDEP, however no  
424 DNA is available. (B) Panels show midline sagittal MRIs for one affected individual for each of  
425 the 6 families. White arrows: cerebellar atrophy, evidenced by widely-spaced cerebellar folia.

426

427 **Figure 2. Truncating and missense mutations in *ADPRHL2* in five independent families**  
428 **predicted to be inactivating.**

429 (A) Schematic of *ADPRHL2* depicting the coding sequence spanning 6 exons and the 5' and 3'  
430 UTRs. Black arrows indicate the positions of the five identified mutations and their coordinates  
431 within the cDNA (Refseq: 54936). (B) Schematic of ARH3 protein depicting the mitochondrial  
432 localization sequence (MLS) and the ADP-ribosyl-glycohydrolase domain. Black arrows:  
433 position and coordinates of the impact of the described mutations. (C) Western blot of  
434 fibroblasts from unrelated control (C), unaffected carrier Father (U), and affected individuals (IV-  
435 II-6 and IV-II-7) from Family 1 shows absent ARH3 protein in affected fibroblasts. Alpha-tubulin:  
436 loading control. Western blot of fibroblasts from unrelated control (C) and affected individual (II-  
437 1) from Family 3 and unaffected carrier mother (U) and affected individual (II-3) from Family 2  
438 shows significant reduction in ARH3 protein levels. Alpha-tubulin: loading control.

439

440 **Figure 3. Premature death and locomotor defects in *Drosophila parg* mutants rescued by**  
441 **human *ADPRHL2*.**

442 (A) Schematic of a poly-ADP-ribosylated protein and the location of cleavage. *PARG* and  
443 *ADPRHL2* both remove poly-ADP-ribose (PAR) from proteins and cleave the same site.  
444 *Drosophila melanogaster* has only one PAR-removing enzyme, *Parg*. (B) *parg*<sup>27.1</sup> mutant flies  
445 (black) show a severe climbing defect, which was rescued by ubiquitous forced expression of  
446 *parg* (red), or in two different transgenic lines mis-expressing human *ADPRHL2* (green and  
447 blue). (C) *parg*<sup>27.1</sup> mutant flies (black) displayed decreased survival, which was rescued with  
448 ubiquitous forced expression of *parg* (red) and two different transgenic lines expressing human  
449 *ADPRHL2* (green and blue).

450

451 **Table Legend**

452

453 **Table 1. Clinical table.**

454 Clinical presentation for affected subjects from families 1 to 6. GTCS: generalized tonic-clonic  
455 seizures. EEG: electroencephalography. MRI: magnetic resonance image. SNHL: sensorineural  
456 hearing loss.

457

	Family 1									Family 2	Family 3	Family 4		Family 5		Family 6
Individual	I-IV-1	I-IV-2	I-IV-3	I-IV-5	I-IV-11	II-IV-2	II-IV-5	II-IV-6 (A1)	II-IV-7 (A2)	II-2	II-1	II-1	II-3	IV-1	IV-2	II-3
Gender	M	F	M	M	M	F	F	M	F	M	F	F	F	M	F	F
Country of Origin	UAE									Italy	Turkey	Pakistan		Iran		Turkey
Parental Consanguinity	+									same village	+	+	+	+		+
Current age (if alive)					4 yrs	3 yrs				16 yrs	15 yrs	13 yrs	2 yrs	-	3 yrs	10 yrs
Age of death	4 yrs	2 yrs	7 yrs	15 yrs	4 yrs	2 yrs	2 yrs	9 yrs	3 yrs	-	-	-	-	6 yrs	-	-
Circumstances of death	Died in sleep	Died in sleep	Had a seizure and died	Respiratory failure	-	Died in sleep 1 week after flu-like illness	Playing, had seizure, then died	Died after long trip by airplane of respiratory failure	-	-	-	-	-	Died in sleep	-	-
Mutation																
Genomic (hg19)	g.36558995C>T									g.36557226C>T	g.36556868A>C	g.36557324_36557328delTGCCC		g.36557524C>T		g.36554605G>A
cDNA	c.1000C>T									c.316C>T	c.235A>C	c.414_418delTGCCC		c.530C>T		c.100G>A
Protein	p.Q334*									p.Q106*	p.T79P	p.A139Gfs*4		p.S177L		p.D34N
Zygosity	homozygous									homozygous	homozygous	homozygous	homozygous	homozygous		Homozygous
Perinatal History																
Normal birth	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Normal early development	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Mild developmental delay	Yes	Yes	Yes
Psychomotor development																
Speech development	Spoke in sentences, then deteriorated	Few words at age 2 yrs	Normal until age 2.5 yrs, then no further development	Normal till 3.5 yrs, then deteriorated	Speaks only few words	Normal speech until death	Normal speech until death	Normal until 25 yrs, then deteriorated	Normal speech, then deteriorated	Slow speech	Normal	Normal	Delayed	Normal until 1.5 yrs, then deteriorated with difficulty speaking	Speaks only a few words	Delayed
Motor development	Normal, then deteriorated	Normal until death	Normal, then deteriorated	Normal, then deteriorated	Normal, then deteriorated	Normal, then deteriorated	Normal until death	Normal, then deteriorated by age 2 yrs	Normal, walked 14mos, then at 19mos poor balance/ataxia	Normal, then deteriorated by age 2 yrs	Normal	Normal, then deteriorated by age 2 yrs	Mildly delayed	Normal until 1 yr then deteriorated	Normal, then deteriorated by age 1.5 yrs	Normal
Seizures																
Seizure Onset	18 mos	19 mos	19 mos	24 mos	15 mos	24 mos	15 mos	18 mos	16 mos	-	-	N/A	age 9 mos	24 mos	36 mos	-
Seizure types	GTCS	GTCS	GTCS	GTCS	Absence, GTCS	GTCS	GTCS	Absence, GTCS	Absence, GTCS	-	-	GTCS with illness	GTCS with illness	Multifocal, GTCS	Multifocal, GTCS	-
Neurological Examination																
Intellect	Normal, then delayed	Normal until death	Normal, then delayed	Normal, then delayed	Normal, then delayed	Normal until death	Normal until death	Normal, then delayed	Normal, then delayed	Normal, then started deteriorating at age 11	Normal	Mild ID (IQ 60)	Mild global developmental delay	Normal, then stagnated	Normal, then stagnated	Mild ID
EEG	-	-	-	-	-	-	-	Generalized epileptiform activity; slow background	Generalized epileptiform activity; slow background	-	-	Mild slowing background activity (3 yrs)	Normal	Generalized epileptiform activity; slow background	Normal	Normal
MRI (age performed)	-	-	-	Normal (5 yrs)	-	-	-	Mild cerebellar atrophy (7 yrs)	Mild cerebellar atrophy (7 yrs)	Cerebellar vermis atrophy (11 yrs)	Mild cerebellar atrophy and spinal cord atrophy (12 yrs)	Mild cerebellar atrophy (4 yrs)	Normal (11mo)	-	Normal (3 yrs)	Mild cerebellar vermis atrophy and spinal cord atrophy (15yrs)
EMG/Biopsy	-	-	-	-	-	-	-	Nerve biopsy with severe axonal loss	-	-	Axonal polyneuropathy (4yr)	Normal muscle biopsy (4 yrs)	-	Normal at age 4 yrs	Axonal polyneuropathy at 4yrs	Axonal polyneuropathy
Onset of unsteady gait	-	-	2.5 yrs	3 yrs	2.5 yrs	-	-	2.5 yrs	20 mos	11 yrs	4 yrs	2.5 yrs	Not yet	1.5 yrs	1.5 yrs	10 yrs
Other Clinical Features																
Exacerbated by illness/stress	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Other Clinical Features	-	-	-	Hypotonia with repeated pneumonia	Can walk, but very unsteady	Progressive weakness	Progressive weakness	Repeated pneumonia, repeated cardiac	Normal hearing, then developed severe SNHL	Myopathic changes on muscle biopsy (11 yrs)	Claw hand and pes cavus deformities, scoliosis, SNHL	Asthma	-	Progressive weakness, tremors, frequent	Progressive weakness and progressive external	Distal muscle atrophy, pes cavus deformity, toe abnormality,



			Ventilator dependent at time of death			arrest Profound type II muscle fiber atrophy,	Severe kyphoscoliosis, one episode of cardiac arrest		at 10 yrs, Tracheotomy, ventilated		falling	ophthalmoplegia	scoliosis, brisk DTRs, positive Babinski's reflex, intentional tremor, ataxia
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