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

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Abstract

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

decrease in total *parg* mRNA for flies with the ubiquitous *da* promoter and a 50% decrease with the neuron-specific promoter, *elav* (*embryonic lethal abnormal visual system*) (Figure S4A). While the *da*-Gal4 and *parg*^{RNAi} lines showed normal survival, crossing the two together led to *da*-mediated expression of *parg*^{RNAi}, which reduced survival substantially (Figure S4B). Ubiquitous knockdown of *parg* also led to decreased survival when animals were exposed to stress with either Hydrogen Peroxide (H_2O_2) in their water or environmental hypoxia (2% O_2) (Figure S4C-D). Furthermore, knockdown of *parg* specifically in neurons largely recapitulated this phenotype using the same two environmental stressors (Figure S4E-F). These data provide evidence that stress leads to premature death in the absence of *parg*, and neurons play an important role in this phenotype.

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

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

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

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

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

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

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

Supplemental Data

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

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Web Resources

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP
1000 Genomes, http://browser.1000genomes.org
Exome Aggregation Consortium [ExAC], http://exac.broadinstitute.org/
NHLBI Exome Sequencing Project Exome Variant Server, http://evs.gs.washington.edu/EVS/
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
SIFT, http://sift.jcvi.org/
Mutation Assessor, http://mutationassessor.org/
Provean, http://provean.jcvi.org
UniProt, http://www.uniprot.org
HaplotypeCaller and GATK, https://www.broadinstitute.org/gatk/
Mutation Taster, htp://mutationtaster.org/
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
Genematcher, https://genematcher.org
FlyBase, http://flybase.org
Iranome Database, http://www.iranome.ir/

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Figure Legends

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

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

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

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

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

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

Table Legend

Table 1. Clinical table.

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

	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		2 yrs	2 yrs	9 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.36558895C>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