# Bi-allelic *ADPRHL2* Mutations Cause Neurodegeneration with Developmental Delay, Ataxia, and Axonal Neuropathy

Katharina Danhauser,[1,2,3] Bader Alhaddad,[1,2] Christine Makowski,[4] Dorota Piekutowska-Abramczuk,[5] Steffen Syrbe,[6] Natalia Gomez-Ospina,[7,8] Melanie A. Manning,[7,8] Anna Kostera-Pruszczyk,[9] Claudia Krahn-Peper,[10] Riccardo Berutti,[2] Reka Kovács-Nagy,[1] Mirjana Gusic,[1,2] Elisabeth Graf,[2] Lucia Laugwitz,[11,12] Michaela Röblitz,[13] Andreas Wroblewski,[13] Hans Hartmann,[14] Anibh M. Das,[14] Eva Bültmann,[15] Fang Fang,[16] Manting Xu,[16] Ulrich A. Schatz,[1,17] Daniela Karall,[18] Herta Zellner,[18] Edda Haberlandt,[19] René G. Feichtinger,[20] Johannes A. Mayr,[20] Thomas Meitinger,[1,2,21] Holger Prokisch,[1,2,16] Tim M. Strom,[1,2] Rafał Płoski,[22] Georg F. Hoffmann,[6] Maciej Pronicki,[23] Penelope E. Bonnen,[24] Susanne Morlot,[25] and Tobias B. Haack[1,12,26,*]

ADP-ribosylation is a reversible posttranslational modification used to regulate protein function. ADP-ribosyltransferases transfer ADP-ribose from $NAD^+$ to the target protein, and ADP-ribosylhydrolases, such as ADPRHL2, reverse the reaction. We used exome sequencing to identify five different bi-allelic pathogenic *ADPRHL2* variants in 12 individuals from 8 families affected by a neurodegenerative disorder manifesting in childhood or adolescence with key clinical features including developmental delay or regression, seizures, ataxia, and axonal (sensori-)motor neuropathy. ADPRHL2 was virtually absent in available affected individuals' fibroblasts, and cell viability was reduced upon hydrogen peroxide exposure, although it was rescued by expression of wild-type *ADPRHL2* mRNA as well as treatment with a PARP1 inhibitor. Our findings suggest impaired protein ribosylation as another pathway that, if disturbed, causes neurodegenerative diseases.

Pediatric neurodegenerative diseases are progressive conditions typically associated with severe disability or even death in early infancy. Identification of the underlying genetic defects is a prerequisite for a better understanding and eventually prevention of the pathophysiological cascades causing pathology. Despite the implementation of unbiased genome-wide sequencing in clinical routine, it is estimated that at least half of the affected individuals and their families remain without a definitive molecular diagnosis. One explanation among many others is that for many loci, a putative disease association remains to be established, and although a specific gene defect might be an obvious candidate in a single affected individual, it could take years to collectively observe enough additional individuals with the same rare condition.

Here, we report on the results of an exome sequencing study in eight families with individuals clinically diagnosed with a neurodegenerative syndrome but without a molecular diagnosis. Informed consent was obtained from all affected individuals or their guardians. The study was approved by the local ethics committees.

Individual F1:II.3, a female, was born after an uneventful pregnancy. Her early development was normal. At 1 year, 5 months of age, she was able to walk independently, but a delay of speech development was noticed. From the age of 3 years on, she had recurrent episodes of diplopia and right-sided ataxic-dystonic posturing possibly triggered by exercise. These signs progressed over several years, and she developed tics such as facial grimacing and throat clearing. Initial brain MRI at age 3 years was without

[1]Institute of Human Genetics, Technische Universität München, 81675 Munich, Germany; [2]Institute of Human Genetics, Helmholtz Zentrum München, 85764 Neuherberg, Germany; [3]Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, Ludwig Maximilian University of Munich, 80337 Munich, Germany; [4]Department of Pediatrics, Technische Universität München, 80804 Munich, Germany; [5]Department of Medical Genetics, Children's Memorial Health Institute, 04-730 Warsaw, Poland; [6]Division of Neuropediatrics and Metabolic Medicine, University Children's Hospital, 69120 Heidelberg, Germany; [7]Department of Pediatrics, Division of Medical Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA; [8]Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA; [9]Department of Neurology, Medical University of Warsaw, 02-097 Warsaw, Poland; [10]Sozialpädiatrisches Zentrum, Heilpädagogisch Therapeutisches Zentrum gGmbH, 56564 Neuwied, Germany; [11]Division of Pediatric Neurology and Developmental Medicine, University Children's Hospital, 72072 Tübingen, Germany; [12]Institute of Medical Genetics and Applied Genomics, University of Tuebingen, 72076 Tübingen, Germany; [13]St. Joseph Krankenhaus, Zentrum Kinder- und Jugendmedizin, Wüsthoffstr. 15, 12101 Berlin, Germany; [14]Clinic for Pediatric Kidney, Liver, and Metabolic Diseases, Hannover Medical School, 30625 Hannover, Germany; [15]Institute of Diagnostic and Interventional Neuroradiology, Hannover Medical School, 30625 Hannover, Germany; [16]Department of Pediatric Neurology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, 100045 Beijing, China; [17]Division of Human Genetics, Medical University of Innsbruck, 6020 Innsbruck, Austria; [18]Clinic for Pediatrics, Division of Inherited Metabolic Disorders, Medical University of Innsbruck, 6020 Innsbruck, Austria; [19]Clinic for Pediatrics, Krankenhaus Stadt Dornbirn, 6850 Dornbirn, Austria; [20]Department of Pediatrics, Paracelsus Medical University Salzburg, 5020 Salzburg, Austria; [21]Munich Cluster for Systems Neurology (SyNergy), 81377 Munich, German; [22]Department of Medical Genetics, Medical University of Warsaw, 02-106 Warsaw, Poland; [23]Department of Pathology, Children's Memorial Health Institute, 04-730 Warsaw, Poland; [24]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA; [25]Institute of Human Genetics, Hannover Medical School, 30625 Hannover, Germany; [26]Centre for Rare Diseases, University of Tuebingen, 72076 Tübingen, Germany
*Correspondence: tobias.haack@med.uni-tuebingen.de
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obvious pathological findings. At the age of 12 years, her condition worsened with more frequent episodes of ataxic-dystonic posturing, ataxia, hypokinetic movements, intermittent hypesthesia, pain, and fatigue. Although cognitive function was reported to be unaffected, brain MRI showed bilateral hippocampal diffusion restriction. Laboratory investigations of blood and cerebrospinal fluid, as well as extensive metabolic investigations, were noncontributory. At the age of 12 years, 2 months, she experienced a drowning event necessitating resuscitation and hypothermia therapy. She completely recovered, but brain MRI at age 12 years, 4 months showed novel pathologies (putatively secondary to hypoxia) involving bilateral basal ganglia, cerebellar, and parietal white matter, as well as the central cortex. Disease progressed with onset of pain in the lumbar area, increasing trunk inclination, ataxia, inability to climb stairs, unsteady gait, and impaired cognition followed by unprovoked pain in the upper limbs and tetraplegia. Respiratory insufficiency necessitated mechanically assisted ventilation from the age of 12 years, 11 months on. Her clinical state further deteriorated, and she deceased at the age of 14 years.

Individual F2:II.2, a female, was born to healthy consanguineous parents from Lebanon. Her two siblings are healthy. Pregnancy, delivery, and postnatal adaption were reportedly normal. Cognitive and motor developmental milestones were reached during infancy. However, from the age of 4 years on, febrile convulsions occurred, and she had mild developmental delay in fine motor skills. At the age of 8 years, muscular weakness, slowed movements, and a progressive nodding of the head triggered by, e.g., cold water were noticed. She developed a progressive ataxia and weakness leading to gait abnormalities and loss of independent ambulation. Brain and spinal MRI at the age of 12 years revealed atrophy of the cerebellum and spinal cord. At the age of 13 years, an acute life-threatening event occurred with unclear acute respiratory insufficiency possibly in the context of a seizure. She was subsequently resuscitated twice because of suspected neurogenic asystole. Prolonged episodes of respiratory insufficiency required mechanical ventilation. A tracheostomy was performed, and a percutaneous endoscopic gastrostomy tube was placed because of dysphagia. Her condition slightly improved to intermediate usage of ventilator support and independent eating. Over the course of the disease, she developed a neurogenic bladder-voiding disorder. At the age of 14 years, 9 months, her condition deteriorated during an infectious episode with respiratory insufficiency requiring mechanical ventilation. Feeding problems increased, and she developed a paralytic ileus. Furthermore, increasing sleep disturbances were reported, and she displayed extensive facial myoclonia. A clinically suspected axonal sensorimotor peripheral neuropathy was supported by absent responses in nerve conduction studies. There was no reliable response to acoustic or optical stimuli, and she lost her ability to speak. Follow-up brain MRI showed progressive atrophy of the cerebellum, edema of the cortex with especially right-sided volume increase, and signal alterations in putamina, caudate nuclei, and the white matter of the corpus callosum. We assume that the neuroimaging findings that evolved in addition to the cerebellar atrophy are likely to represent secondary findings resulting from episodes of respiratory insufficiency with hypoxia. Extensive laboratory testing was noncontributory. Electroencephalography showed a burst suppression pattern, and she died at 17 years of age.

Clinical and genetic findings are summarized in Table 1, pedigrees are shown in Figure 1, and neuroimaging findings are shown in Figure 2. Clinical descriptions of the remaining individuals are provided in the Supplemental Note.

Pregnancy, postnatal adaption, and perinatal development were reportedly normal in all individuals, and all but individual F1:II.2 were born at term. Neurodevelopmental problems involving a delay in speech and psychomotor development were noted in 10 of 11 individuals within the first years of life, and five individuals presented with infection-associated episodes of ataxia or dystonic posturing. Over the course of the disease, gait abnormalities were present in all individuals. 10 of 11 developed ataxia, and individual F1:II.2 showed a spastic diplegia, which could have resulted from perinatal hypoxic brain damage. Seizures and corresponding electroencephalography abnormalities were documented in six individuals. Peripheral axonal isolated motor or sensorimotor neuropathy, as shown by decreased amplitudes with normal latencies in nerve conduction studies, was present in six of eight individuals. Facial myoclonia, a possible sign of developing bulbar palsy, was present in two of the affected individuals. Visual impairment manifesting as diplopia (1/5), nystagmus (3/5), strabismus (2/5), and impaired upward gaze and saccadic movements and ptosis (1/5) was reported in 5 of 11 affected individuals. Additional findings included acquired microcephaly in individuals F5:II.2, F3:II.2, and F8:II.3, and F3:II.2 also showed sensorineural hearing loss. Disease progress was variable but frequently associated with periods of increased stress, such as infections. Three individuals died in childhood, whereas in another five individuals, disease progressed into their teens, and two had life-threatening events requiring resuscitation and mechanically assisted ventilation for respiratory insufficiency.

Extensive laboratory testing and metabolic investigations were not contributory in any affected individuals. Biochemical analysis was performed on skeletal muscle specimen of four individuals and showed normal activity of mitochondrial respiratory-chain enzymes. Histological examinations showed evidence of neurogenic muscle atrophy.

Neuroimaging data were available for all but one individual and were considered unremarkable at an early disease stage. However, over the course of disease, eight of ten individuals developed cerebellar atrophy (Figure 2). At a late stage of the disease, putatively secondary additional

**Table 1. Clinical and Genetic Findings in Individuals with Confirmed Bi-allelic *ADPRHL2* Variants**

| | F1:II.2 | F1:II.3 | F2:II.2 | F3:II.1 | F4:II.3 | F4:II.4 | F5:II.2 | F6:II.1 | F7:II.1 | F7:II.2 | F8:II.1 | F8:II.3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | male | female | female | female | male | female | male | female | female | female | male | female |
| Ethnicity | German | German | Lebanese | ND | ND | ND | Kosovan | Polish | Chinese | Chinese | Turkish | Turkish |
| Identified homozygous changes (cDNA [GenBank: NM_017825.2] and protein GenBank: NP_060295) | c.1004T>G (p.Val335Gly) | c.1004T>G (p.Val335Gly) | c.744_746del p.(Lys248_Ile249delinsAsn) | c.1038C>G (p.Tyr346*) | c.1004T>G (p.Val335Gly) | c.1004T>G (p.Val335Gly) | c.1004T>G (p.Val335Gly) | c.1004T>G (p.Val335Gly) | c.309−1G>T (p.?) | c.309−1G>T (p.?) | c.292delG (p.Val98Trpfs*23) | c.292delG (p.Val98Trpfs*23) |
| **Phenotypic Features** | | | | | | | | | | | | |
| Age of onset (current age or age of death) | 1 y (27 y) | 17 m († 14 y) | 4 y († 17 y) | 2 y (12 y) | 13 y (32 y) | 11 y († 30 y) | 3 y (7 y) | 2 y († 11 y) | 1 y († 12 y, 10 m) | 2 y († 5 y) | 15 m († 4 y, 4 m) | 14 m (22 m) |
| Developmental delay or intellectual impairment | + | + | + | + | − | − | + | + | + | + | + | + |
| Gait abnormalities | + | + | + | + | + | + | + | + | + | + | + | + |
| Ataxia | ND | + | + | + | + | + | + | + | + | + | + | + |
| Seizures | ND | − | + | + | − | − | − | + | + | − | + | + |
| Neuropathy | ND | + | + | + | ND | ND | + | + | + | + | − | ND |
| Facial myoclonia | − | + | + | − | − | − | − | − | − | − | − | − |
| Sensorineural hearing loss | − | − | − | + | − | − | − | − | ND | − | ND | ND |
| Ophthalmologic features | − | diplopia | nystagmus | strabismus | nystagmus, strabismus | − | putative external ophthalmoplegia with ptosis, impaired saccades and upward gaze, and nystagmus; putative retinal pigment epithelium anomalies | − | ND | − | − | − |
| Microcephaly | ND | ND | ND | + | ND | ND | + | − | ND | ND | − | + |
| Respiratory insufficiency | − | + | + | − | − | + | − | − | + | + | + | − |

(*Continued on next page*)

**Table 1.** *Continued*

| | F1:II.2 | F1:II.3 | F2:II.2 | F3:II.1 | F4:II.3 | F4:II.4 | F5:II.2 | F6:II.1 | F7:II.1 | F7:II.2 | F8:II.1 | F8:II.3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **cMRI Findings (Affected Regions)** | | | | | | | | | | | | |
| Basal ganglia | ND | + | + | − | − | − | − | − | − | − | + | − |
| Cortex | ND | + | + | − | − | − | − | − | − | − | − | − |
| Corpus callosum | ND | − | + | + | − | − | − | − | − | − | − | − |
| Cerebellum | ND | + | + | + | + | + | + | − | + | + | − | − |
| **Other** | | | | | | | | | | | | |
| Nerve conduction studies (biopsy) | ND | axonal sensorimotor neuropathy | no signal | axonal sensorimotor neuropathy | ND | ND | axonal motor neuropathy | axonal motor neuropathy | ND | axonal sensorimotor neuropathy (decreased number of nerve fibers) | reportedly normal | ND |
| Respiratory chain activities (muscle) | ND | unremarkable | unremarkable | ND | ND | unremarkable | ND | unremarkable | ND | unremarkable | unremarkable | unremarkable |

Abbreviations are as follows: y, years; m, months; ND, not determined; +, present; −, absent, and ǂ, individual deceased.

abnormalities affecting the central cortical region (2/10), basal ganglia (3/10), and corpus callosum (2/10) were observed.

Exome sequencing was performed at four centers—Munich (families 1, 2, 4, 5, and 8), Baylor Genetics (family 3), Warsaw (family 6), and Beijing (family 7)—on genomic DNA from affected individuals F1:II.3, F2:II.2, F3:II.1, F4:II.3, F5:II.2, F6:II.1, F7:II.2, and F8:II.3, as well as the parents from families 4 and 5, as described previously.[1–3] In individual F1:II.3, prioritization of potentially pathogenic variants included a search for recessive-type nonsynonymous variants with a minor allele frequency (MAF) < 0.1% in both an in-house database containing ~3,000 control exomes and the Exome Aggregation Consortium (ExAC) Browser (accessed January 2018). This search failed to identify pathogenic or likely pathogenic variants in established disease-related genes associated with clinical features of the affected individuals. Given the proposed role of the encoded protein in posttranslational protein modification and *in silico* prediction, the homozygous missense change c.1004T>G (p.Val335Gly) (GenBank: NM_017825.2) in *ADPRHL2* (MIM: 610624), coding for ADP-ribosylhydrolase like 2, was initially considered a promising candidate in individual F1:II.3. The same homozygous missense variant was subsequently identified in the similarly affected individuals of families F4–F6. In families F1, F4, and F5, a comparison of the sequence variation observed in the ~2 Mb region surrounding the variant identified ten homozygous rare (MAF < 1% in public databases) variants. This finding is in line with shared ancestry. Furthermore, an additional four different homozygous *ADPRHL2* variants—an in-frame deletion (c.744_746del [p.Lys248_Ile259delinsAsn]), a predicted truncating variant (c.1038C>G [p.Tyr346*]), a canonical splice-site variant (c.309−1G>T [p.?]), and a frameshift variant (c.292del [p.Val98Trpfs*23])—were identified in four additional unrelated individuals with a similar clinical phenotype. The results of carrier testing performed on available family members were in line with autosomal-recessive inheritance. The change c.1004T>G (p.Val335Gly) (rs201735454) is observed 27 times in a heterozygous state in 277,240 alleles of the gnomAD browser, the change c.1038C>G (p.Tyr346*) (rs531916765) is present three times in 246,234 alleles, and the variants c.744_746del (p.Lys248_Ile259delinsAsn), c.292del (p.Val98Trpfs*23), and c.309−1G>T (p.?) have not been observed in at least 227,988 alleles. None of the variants have been reported in a homozygous state in public databases (ExAC Brower or gnomAD) or an in-house database containing >3,000 exome datasets of individuals with unrelated phenotypes. No bi-allelic loss-of-function variants were observed in ~120,000 alleles of the ExAC Browser. Immunoblot studies in primary fibroblasts available from individuals F1:II.3 and F2:II.2 showed an absence of ADPRHL2 (Figure 1). In summary, the identification of five different bi-allelic functionally relevant *ADPRHL2* variants in eight unrelated families establishes

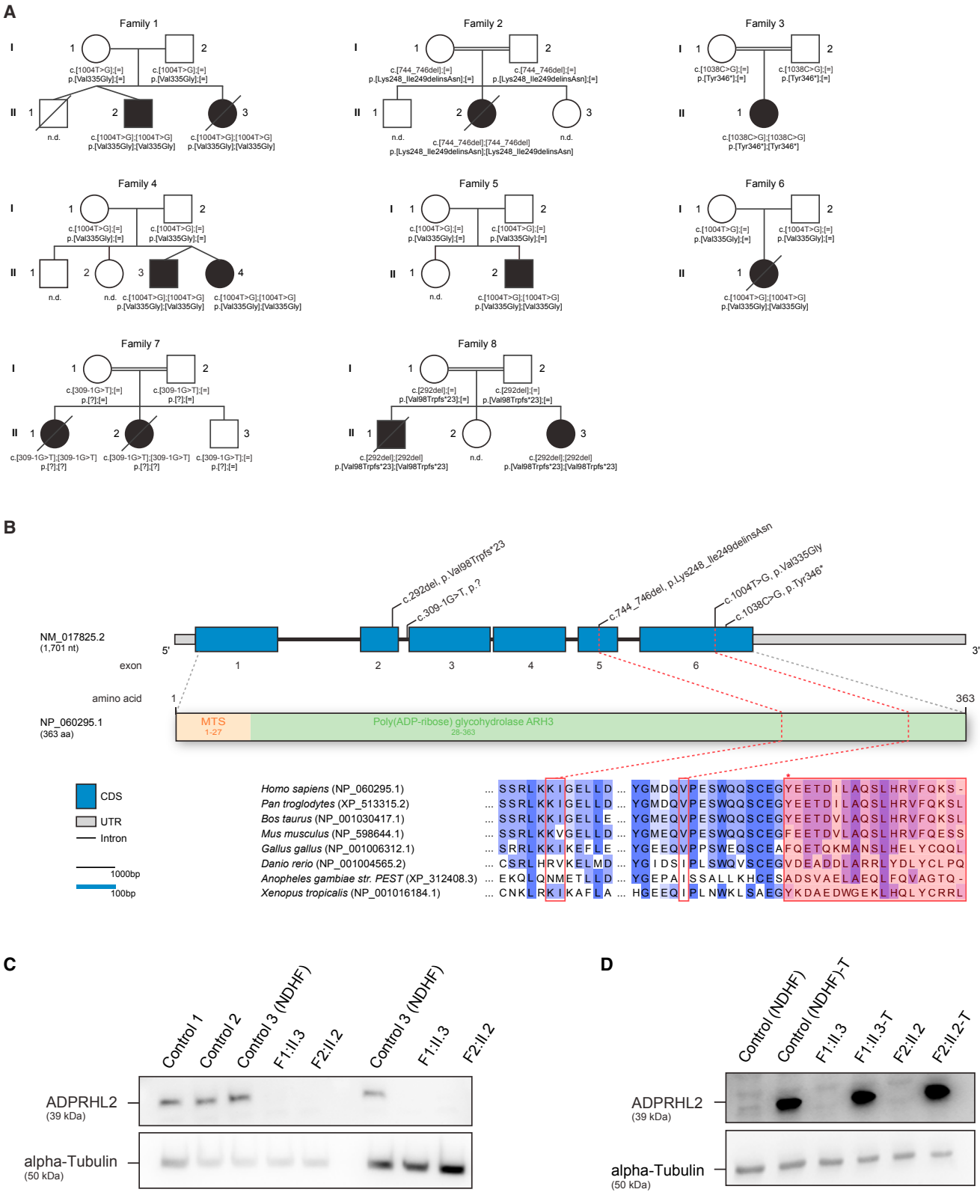


**Figure 1. Pedigrees of Investigated Families, Structure of *ADPRHL2*, and Investigation of ADPRHL2 Amounts**
(A) Pedigrees of eight families carrying pathogenic variants in *ADPRHL2* illustrate the mutation-carrier status of affected (closed symbols) and healthy (open symbols) family members. ND, not determined.
(B) Structure of *ADPRHL2* (top) and ADPRHL2 (bottom) with known protein domains and motifs of the gene product and position of the identified variants. Intronic regions are not drawn to scale.

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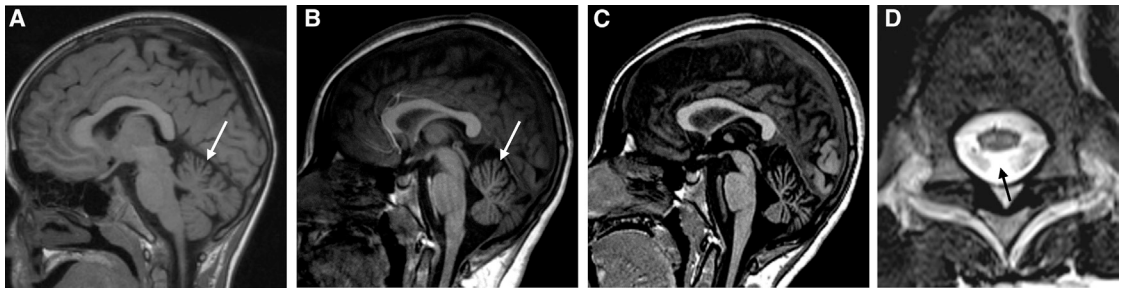


**Figure 2. Neuroimaging Findings in Individuals with ADPRHL2 Variants**
(A) Brain MRI (T1-weighted image, sagittal view) of individual F5:II.2 at the age of 7 years demonstrates mild upper vermian atrophy (arrow).
(B and C) Brain MRI (T1-weighted image, sagittal view) of individual F2:II.2 at the ages of 12 years (B) and 14 years (C) demonstrates progressive cerebellar atrophy (arrow).
(D) MRI of the myelon (T2-weighted image, axial view) of individual F2:II.2 at the age of 12 years shows atrophy of the spinal cord and bilateral cord T2 hyperintensities (arrow).

*ADPRHL2* as a gene confidently implicated in this neurodegenerative disease that includes developmental delay or regression, seizures, ataxia, and neuropathy as key clinical features.

ADPRHL2 is thought to function in the pathway of ADP-ribosylation, which is a reversible posttranslational modification used to regulate key cellular processes such as transcription, DNA repair, translation, and apoptosis.[4] In humans, several ADP-ribose transferases transfer ADP-ribose from nicotinamide adenine dinucleotide ($NAD^+$) to target proteins. Among different protein families putatively catalyzing this reaction, the probably best-characterized enzymes are so-called poly(ADP-ribose) polymerases (PARPs).[5] Some members of the PARP family are able to transfer only ADP-ribose monomers, whereas others produce poly(ADP-ribose) chains.[5] A well-characterized member is PARP1. Once activated by genotoxic stress-induced single-strand DNA breaks, it produces poly(ADP)-ribosylation associated with depletion of cellular $NAD^+$ and translocation of mitochondrial proapoptotic factors to the nucleus.[6] Given that the ultimate consequence of persistent ADP-ribosylation is parthanatos, these dynamic processes require precise regulation, and ADP-ribosylation has to be reversible.[7,8] Poly(ADP-ribose) (PAR) hydrolysis is catalyzed by several enzymes. The function of PAR glycohydrolase (PARG) is well studied: it cleaves the ribose-ribose bonds between ADP-ribose subunits of the poly(ADP-ribose) chains.[9] Another enzyme able to reverse protein poly(ADP)-ribosylation is ADPRHL2. It hydrolyses PAR chains on proteins, albeit less efficiently than PARG.[10] So far, ADPRHL2 is the only known poly (ADP-ribose)-hydrolyzing enzyme in mitochondria.[11] Given that PARG and ADPRHL2 exclusively hydrolyze poly(ADP-ribose) chains, a number of additional factors—including OARD1 (also called TARG1), MACROD1, MACROD2, and ARH—are necessary to remove the last ADP-ribose subunit attached to a protein.[12–14]

We used fibroblast cell lines of individuals F1:II.3 and F2:II.2 to study the cellular consequences of ADPRHL2 deficiency. PARP1 is the main polymerase contributing to the intracellular PAR pool.[15,16] Hydrogen peroxide ($H_2O_2$) stimulates PARP1 via oxidative DNA damage and thereby leads to an increase in intracellular PAR.[17] Given the proposed role of ADPRHL2 in reversing poly(ADP)-ribosylation by hydrolyzing PAR into mono(ADP-ribose), we postulated ADPRHL2 deficiency to promote $H_2O_2$-mediated accumulation of PAR. To test this hypothesis, we performed immunohistochemical staining of ADP-ribosylation in fibroblasts of affected and control individuals. Treatment of fibroblasts with 2 mM $H_2O_2$ for 20 min resulted in a marked ring-shaped accumulation of ADP-ribose in the perinuclear and nuclear region in both control and affected individuals' cell lines (Figure 3). Amounts of ADP-ribose normalized 2 hr after $H_2O_2$ removal in control fibroblasts, whereas a ring-shaped signal remained in ADPRHL2-mutant fibroblasts (Figure 3). This observation is in line with impaired cellular removal of ADP-ribose as a consequence of ADPRHL2 deficiency. PAR accumulation in ADPRHL2 fibroblasts was also documented by immunoblotting (Figure 3).

PAR accumulation has been associated with increased cell death.[7] To assess this hypothesis, we exposed ADPRHL2-mutant and control fibroblast cell lines cultured in high-glucose DMEM (4.5 g/L; Thermo Fisher Scientific) to $H_2O_2$ concentrations ranging from 0 to 1,000 μM for 48 hr. We assessed cell viability by quantification with alamarBlue Cell Viability Reagent (Thermo Fisher Scientific) as a readout. We did not detect a significant difference in cell viability between affected individuals' fibroblasts and control cells

(C and D) Immunoblot studies on ADPRHL2-mutant fibroblast cell lines (C) and transduced cell lines (D) indicated that the homozygous variants c.1004T>G (p.Val335Gly) and c.744_746del (p.Lys248_Ile249delinsAsn) demonstrate a severe reduction of ADPRHL2. Transduction with wild-type *ADPRHL2* led to increased amounts of the protein. Immunoblotting was performed on whole-cell lysates with anti-ADPRHL2 antibody (Sigma-Aldrich, HPA027141, dilution 1:200). An anti-alpha-tubulin antibody (Sigma-Aldrich, T5168, dilution 1:20,000) was used as a loading control.

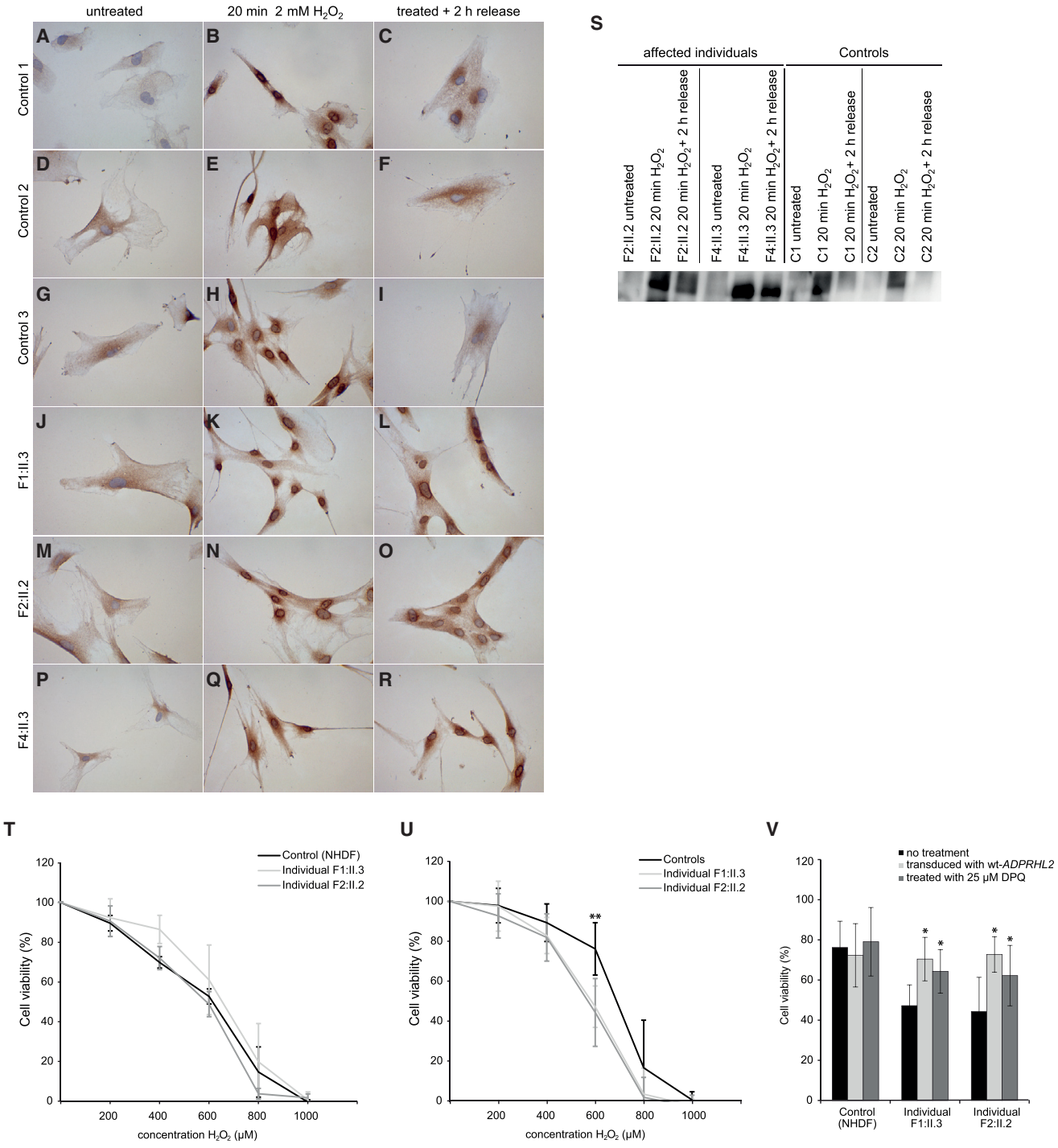


**Figure 3. Investigation of ADP-Ribosylation and Cell Viability upon $H_2O_2$ Treatment**

(A–R) Control (A–I) and mutant (J–R) primary fibroblasts were seeded on chamber slides and allowed to attach overnight. Treatment with 2 mM $H_2O_2$ for 20 min increased ADP ribose in both control (B, E, and H) and mutant (K, N, and Q) cell lines, as shown by immunohistochemistry using the anti-pan-ADP-ribose binding reagent (MABE1016, Merck Millipore, diluted 1:1,000 in 1× blocking solution [Roche] in PBS and 0.5% Tween-20). Subsequent incubation for 2 hr in normal cell-culture medium led to a marked decrease in staining intensity in control cell lines (C, F, and I), whereas the signal intensity remained high in fibroblasts from ADPRHL2-deficient individuals (L, O, and R).

(S) Immunoblot analysis of ADP ribose in fibroblasts of affected and control individuals. Fibroblasts were untreated, treated with 2 mM $H_2O_2$ for 20 min, or treated with $H_2O_2$ for 20 min and allowed to recover for 2 hr (release). Staining was performed with anti-pan-ADP-ribose binding reagent (MABE1016, Merck-Millipore).

(T and U) Cell-viability analysis using alamarBlue Cell Viability Reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. 2,500–3,000 cells were plated per well on 96-well plates 24 hr before treatment. Cells were cultured either in high-glucose (T) or low-glucose (U) DMEM before and during treatment with $H_2O_2$ at concentrations from 0 to 1,000 μM for 48 hr (T) or 24 hr (U).

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(Figure 3). A potential explanation for this observation could be that in this setting, alternative cytosolic PAR-hydrolyzing enzymes can compensate for defective ADPRHL2 function.

As mentioned previously, ADPRHL2 is the only characterized PAR-hydrolyzing enzyme to date in mitochondria.[11] Furthermore, an increase in PARP1 activity has been linked to impaired mitochondrial metabolism.[18] In order to more specifically challenge stress ADPRHL2-dependent PAR hydrolysis, we next cultured the cell lines in low-glucose DMEM (1 g/L; Thermo Fisher Scientific) to promote mitochondrial respiration as a source of cellular energy supply. Cell viability was determined after 24 hr treatment with $H_2O_2$ at concentrations of 0 to 1,000 μM. At a concentration of 600 μM, we observed significantly lower cell viability in ADPRHL2-mutant cell lines than in control cells (Figure 3).

To confirm that this effect was indeed caused by a deficiency of ADPHRL2, we performed a rescue experiment. We transduced ADPRHL2-deficient and control fibroblasts with wild-type *ADPRHL2* cDNA by using a feline-immunodeficiency-virus-based lentiviral expression vector (Gene Copoeia) as described previously.[19] Increased amounts of ADPHRL2 were confirmed by immunoblot analysis (Figure 3). Transduced fibroblasts grown on low-glucose DMEM with 600 μM $H_2O_2$ showed a significant higher cell viability than naive ADPRHL2-mutant cell lines (Figure 3).

To further corroborate the hypothesis that PAR accumulation is indeed the mechanism mediating increased $H_2O_2$ sensitivity in ADPRHL2-deficient cell lines, we treated the cells with the PARP1 inhibitor 3,4-dihydro-5-[4-(1-piperidinyl)butoxyl]-1(2H)-isoquinolinone (DPQ, Sigma-Aldrich). Viability of ADPRHL2-mutant fibroblasts cultured in low-glucose DMEM with 600 μM $H_2O_2$ for 24 hr was significantly increased upon treatment with 25 μM DPQ. Our findings provide additional evidence for the functional relevance of the investigated *ADPRHL2* variants, supporting PAR accumulation as a pathomechanism mediating cell death from increased oxidative stress.

In conclusion, we identified bi-allelic *ADPRHL2* variants as the disease-causing molecular defects underlying a progressive neurodegenerative disorder in nine affected individuals from seven families. Although larger studies are needed to fully define the phenotypic spectrum associated with ADPRHL2 deficiency, overlapping findings in several individuals suggest that disease manifestation with episodic movement disorders at the beginning with periods of partial recovery might constitute clinical hallmarks that deserve special notion in this disease. Additional suggestive features are development of ataxia and peripheral (sensori-)motor axonal neuropathy over the course of the disease. Similar to murine fibroblasts deficient of the mouse ortholog (*Arh3*$^{-/-}$), affected individuals' fibroblasts grown on respiratory medium were less viable upon $H_2O_2$ stress than control cells.[20] This cellular phenotype was rescued by expression of wild-type *ADPRHL2* mRNA as well as treatment with a PARP1 inhibitor, the latter of which supports the hypothesis that PAR accumulation is an important factor in the pathophysiology of this disease. PAR accumulation by PARP1 hyperactivation has been suggested as a mechanism involved in the pathogenesis of the autosomal-recessive disorders spinocerebellar ataxia 26 (SCAR26 [MIM: 617633]) and ataxia oculomotor apraxia 4 (AOA4 [MIM: 616267]), caused by mutations in *XRCC1* and its' interaction partner *PNKP*, respectively. Notably, the phenotypic spectrum associated with SCAR26 and especially AOA4 dysfunction shows remarkable overlap with ADPRHL2 deficiency, including progressive ataxia, oculomotor abnormalities, and peripheral sensorimotor neuropathy.[21] More generally, an increase in intracellular PAR has been associated with more common neurological disorders, such as Alzheimer disease,[22] Parkinson disease, and amyotrophic lateral sclerosis.[23] Although the above-mentioned changes are likely to be secondary phenomena, in addition to our report, currently only one report directly implicates impaired recycling of ADP-ribosylation in neurodegeneration. In 2013, Sharifi et al. described several affected individuals from a single large family affected by childhood-onset neurodegeneration manifesting as severe neurodevelopmental delay, seizures, and peripheral neuropathy leading to death by the age of 10–11 years. Functional studies showed that the identified mutations in the candidate gene *OARD1* (MIM: 614393; also known as *TARG1* or *C6orf130*) result in defective degradation of ADP-ribosylation.[14] Our findings further support the concept of disturbed posttranslational protein-modification pathways, such as ADP-ribosylation, in neurodegenerative diseases.

In both *C. elegans* and mouse neurons, inhibition of poly (ADP-ribosylation) leads to improved neuronal regeneration after axonal injury.[24] Speculatively, the prevention of excessive PAR accumulation with its detrimental downstream effects culminating in cell death could evolve as a potential therapeutic target in selected neurodegenerative disease entities in humans.

## Supplemental Data

Supplemental Data include a Supplemental Note and can be found with this article online at https://doi.org/10.1016/j.ajhg.2018.10.005.

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(V) Cell-viability analysis using alamarBlue Cell Viability Reagent after treatment with 600 μM $H_2O_2$ for 24 hr in low-glucose DMEM after transduction and treatment with 25 μM DPQ.
The data represent at least five experiments for each cell line grown and treated in parallel. Error bars indicate 1 SD from the mean. *$p < 0.01$, **$p < 0.001$, two-tailed unpaired t test.

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## Declaration of Interests

The authors declare no competing interests.

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## Web Resources

ExAC Browser, http://exac.broadinstitute.org
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
OMIM, http://www.omim.org
Combined Annotation Dependent Depletion (CADD), http://cadd.gs.washington.edu/

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## Supplemental Data

# Bi-allelic *ADPRHL2* Mutations Cause Neurodegeneration with Developmental Delay, Ataxia, and Axonal Neuropathy

**Katharina Danhauser, Bader Alhaddad, Christine Makowski, Dorota Piekutowska-Abramczuk, Steffen Syrbe, Natalia Gomez-Ospina, Melanie A. Manning, Anna Kostera-Pruszczyk, Claudia Krahn-Peper, Riccardo Berutti, Reka Kovács-Nagy, Mirjana Gusic, Elisabeth Graf, Lucia Laugwitz, Michaela Röblitz, Andreas Wroblewski, Hans Hartmann, Anibh M. Das, Eva Bültmann, Fang Fang, Manting Xu, Ulrich A. Schatz, Daniela Karall, Herta Zellner, Edda Haberlandt, René G. Feichtinger, Johannes A. Mayr, Thomas Meitinger, Holger Prokisch, Tim M. Strom, Rafał Płoski, Georg F. Hoffmann, Maciej Pronicki, Penelope E. Bonnen, Susanne Morlot, and Tobias B. Haack**

**SUPPLEMENTAL NOTE: CLINICAL CASE REPORTS**

The proband of Family F1, F1:II.3 is described in full in the manuscript. This proband has two affected siblings; two older brothers are preterm fraternal twins both affected with spastic diplegia which has been attributed to perinatal brain damage. One of them (F1:II.1) deceased at the age of 23 years while the other one was still alive the age of 27 years (F1:II.2). No material was available to test the carrier status of individual F1:II.1 and we were unable to receive detailed clinical reports to evaluate clinical signs suggestive of ADPRHL2 deficiency.

Likewise the clinical information available for F1:II.2 was limited. According to the mother individual F1:II.2 had a spastic diplegia and learning disability. He was born as the second of dizygotic twins at 31 weeks of gestation. He showed a motor developmental delay with spasticity and hypertonia since the 6$^{th}$ month of life which has been attributed to a history of perinatal hypoxia. He started to walk at 3 years of age. At the age of 6 years an Achilles tendon extension was performed and he was subsequently able to walk without support. Nevertheless, due to kyphosis his independent ambulation was severely impaired and further declined. From the age of 26 years on he suffered from cramping in the area of the thighs upon exposure to cold. To our knowledge no further neuroimaging or electrophysiological investigations have been performed and the proband was not available for clinical follow-up. It thus remains currently unclear to which extend his clinical presentation can be explained by presumed perinatal hypoxia or might represent a manifestation of ADPRHL2 deficiency and whether he has any additional signs specific for his genetic disease.

Individual F3:II.1, a female, is a currently 12 year old girl born to healthy consanguineous parents. She presented with global developmental delay, a history of seizures, hypertonia, decreased muscle bulk, ataxia, axonal sensorimotor neuropathy, and bilateral sensorineural hearing loss. Seizures were controlled with valproic acid. She never did not have spoken language, but used signs. Brain MRI revealed small corpus callosum and cerebellar volume loss. Sural nerve biopsy at age 2 years revealed axonal neuropathy with Wallerian degeneration. The subject has astigmatism and intermittent esotropia. Dysmorphism included plagiocephaly, hypertelorism, somewhat coarse hypotonic facies. Growth defects

manifested in acquired microcephaly with OFC < 2 % (Z < -2.05), acquired short stature, and weight in the 3rd centile.

Individual F4:II.3, a male, was born as the first of dizygotic twins to healthy unrelated parents by caesarean section. His birth weight was 2400 g, Apgar score 8 at five minutes. His older brother and sister are healthy; his twin sister is similarly affected. His neonatal period, infancy, and childhood were uneventful. At the age of 13 years he first presented with three episodes of ataxia triggered by an infection. At the age of 14 years first sings of muscular weakness became evident. He had walking difficulties, memory problems, nystagmus, and developed a squint. Neurological examination showed hypotonia, high tendon reflexes, positive Babinski's sign, and he developed a pes cavus. Brain MRI at age X years was normal. Plasma lactate concentration was at the borderline or normal (2.01, 16.6, ref. <2.0 mmol/L). He is now aged 32 years and in quite stable condition with dysarthria and balance disturbances.

Similar clinical features were seen in his twin sister (individual F4:II.4) including signs of weakness from the age of 11 years on. Her muscular atrophy was more severe and she developed respiratory insufficiency requiring continuous mechanical ventilation from the age of 14 years on. A brain MRI showed atrophy of the cerebellum and she was diagnosed with an ataxic pyramidal / extrapyramidal syndrome. Extensive laboratory and genetic testing excluded various differential diagnoses (e.g. borreliosis, Friedreich ataxia, Wilson disease, hypobetalipoproteinemia, Refsum disease). Due to suspicion of mitochondrial disorder a muscle biopsy was performed at the age of 16 years. There were features of neurogenic impairment with regeneration signs (muscle fibers grouping). Activities of mitochondrial respiratory chain complexes were normal in muscle and fibroblasts homogenates. She remained dependent on mechanical ventilation until she died aged 30 years.

Individual F5:II.2, a male, was born after normal pregnancy to healthy unrelated parents from Kosovo. He is the second child of his parents with one healthy older sister. He has three healthy older maternal half-brothers. One older maternal half-sister suffered from epileptic seizures starting at 6 months of age, she

had global developmental delay and visual impairment; she eventually died at 8 years of age. No further information was available for this half-sister.

The proband's early motor development was normal. At the age of 3 years, single short episodes of dystonic posturing were noted by the parents, followed by the development of gait abnormalities with weakness, ataxic-dystonic posturing in a bended trunk and head position and choreatic arm movements in association to a prior febrile illness two days before. Weakness and movement disorder slowly improved over several months; however, parents noted general weakness during infections and unprovoked episodes of ataxia lasting for one to two days. Additionally, evolving clinical features included a putative external ophthalmoplegia with ptosis, impaired saccades and bulbar elevation as well as nystagmus. Ophthalmologic examination was indicative of retinal pigment epithelium anomalies. Electrophysiological testing revealed an isolated axonal motor neuropathy at 7 years of age. Cognitive development was delayed from infancy, but showed continuous progress and was not adversely affected during periods of motor weakness or episodic ataxia, He currently shows mild cognitive impairment. Initial brain and spinal MRI and an MR spectroscopy at age 3 years were without pathological findings; at 7 years mild cerebellar atrophy was observed on MRI. At last examination at 7 years and 7 months he displayed acquired microcephaly (OFC 49.5cm, < $1^{st}$ percentile, SDS -2.7) with his other anthropometric measures being within the lower range. His gait was mildly weak and ataxic, with normal deep tendon reflexes.

Individual F6:II.1, a female, was born after normal pregnancy to healthy unrelated parents with body weight of 3,380 g and Apgar score of 9. She is the only child of her parents. Her cognitive and motor development was normal. In the $2^{nd}$ year of life she presented with febrile seizures (upper respiratory tract infection with $39.5^{o}$ C) followed by several afebrile episodes of ataxia and abnormal bended head position. Cranial MRI at that time was normal. Her condition returned to normal. At the age of 4 years episodes of ataxia with abnormal vocalization and head posture recurred. Treatment with anticonvulsants resulted in partial improvement. Significant gait problems developed when she was 9 years old. She had position and intention tremor, weakness and atrophy of her lower and upper extremity muscles, with foot drop. Deep tendon reflexes were preserved. There were no oculomotor abnormalities. Electrophysiological testing revealed an axonal motor neuropathy. Her cranial MRI at age nine years did not show any abnormalities. Extensive laboratory workup was not contributory. She died at the age of 11 years.

Individual F7:II.2, a female, was born after normal pregnancy to healthy consanguineous parents from China. Her early development was normal. At the age of 1 year and 6 months she was able to walk independently and to speak single words. From the age of 2 years on, she had recurrent episodes of one-sided dystonia, triggered by fever or infection. At the age of 3 years, she manifested with episodes of limb jitter during sleep, especially shortly after falling asleep. At the age of 4 years and 7 months, her condition worsened with new episodes of dystonic posturing after asleep and during sleep, progressive muscular weakness, fatigue, and ataxia, leading to gait abnormalities and loss of independent walking and standing. She had a high-arched palate. Brain MRI showed cerebellar atrophy. Spinal cord MRI showed no specific changes. Cerebrospinal fluid analysis showed normal cell count, glucose, chloride, and lactate, but mild elevation of protein (468 mg/L, normal range: 250-450 mg/L). Electrophysiological testing was suggestive of axonal sensorimotor peripheral neuropathy. Nerve biopsy showed a decrease in the number of nerve fibers, with significant axonal degeneration and atrophy. Histopathological studies and testing of activities of mitochondrial respiratory chain complexes performed on a skeletal muscle specimen were normal. Extensive laboratory workup was not contributory. Abdominal ultrasound showed polycystic kidneys. Chronic inflammatory demyelinating polyneuropathy (CIDP) could not be excluded, therefore treatment with methylprednisolone was started with slight improvement of symptoms. At the age of 4 years and 11 months she manifested intermittent fevers, renal dysfunction, and a suspicion of paralytic ileus. Her condition deteriorated with progressive muscular weakness, speech abnormalities, and respiratory insufficiency requiring mechanical ventilation. She received intravenous immunglobulin and plasma exchange treatment without effect and died at the age of 5 years. Her elder sister (F7:II.1) had a similar clinical presentation of abnormal gait, progressive muscular weakness, and seizures 4-5 times when she was around 1 year of age. She died at the age of 12 years and 10 months. A younger brother (F7:II.3) was reportedly healthy.

Patient F8:II.1, a male, was the first child of healthy first cousin parents of Turkish origin. He was born at term after normal pregnancy with birth measures within normal range. He developed normally up to 15 months, when cognitive and motor delay and ataxia became evident. As the disease progressed, he lost walking ability and due to muscular hypotonia also head control until the age of 23 months. Comprehensive metabolic, endocrine, and infectiological investigations were unremarkable. Thoracic X-

Ray, ECG, abdominal and muscular ultrasound showed normal findings. Cerebral MRI showed normal structures and myelinization and an arachnoid cyst (3 cm in diameter) on the right temporal lobe. After admission for further investigations he came down with chickenpox, fell into a comatose state, and had an asystolic episode requiring ICU surveillance. Extensive neurometabolic work-up including muscle biopsy and investigations for neuronal ceroid lipofuscinosis type 1 and 2 were inconspicuous. Brain MRI showed bilateral signs of limbic encephalitis in the hippocampal area. After exclusion of a paraneoplastic cause, further investigations revealed antibodies against neuropil of hippocampus and other cerebral areas. Cortisone treatment (two periods of 5 days each) stabilized his neurological state, and he finally showed limited communication, was able to sit without help, and regained crawling.

At the age of 4 years he deteriorated again in the context of two generalized febrile seizures, losing especially his ability to sit and his social skills. Severe EEG changes and further generalized epileptic seizures were treated with antiepileptic medication (valproic acid) without improvement. Brain MRI now showed extensive hippocampal sclerosis. Few weeks later he was found apneic at home and needed resuscitation for 60 minutes. For 18 days he had no spontaneous breathing, lack of pupillary reflexes with fixed pupils, and lack of all brainstem reflexes and deep tendon reflexes. He recovered poorly and died 90 days after the apneic episode in multiorgan failure.

As limbic encephalitis was postulated, the patient was included in the following manuscript: E Haberlandt, *et al.* Limbic encephalitis in children and adolescents. Arch Dis Child 2011;96:186–191. PMID: 20959359.

Individual F8:II.3 is a currently 22 months-old female, third child of her parents, and has one healthy 12 years-old sister. Individual F8:II.1 was her elder brother. She was delivered spontaneously after regular pregnancy at gestational age of 41 weeks. Her birth weight was 3.224 g (centile 10-25), length 52 cm (centile 25-50), and head circumference 33 cm (centile 3-10). The neonatal period and early infancy were initially unremarkable. At the age of 14 months recurrent episodes of sudden loss of head control were noticed. She started walking at age 15 months, but soon thereafter she presented regression in her motor functions after an episode of pneumonia. Subsequently, ataxia became evident and she was unable to walk and stand alone. Neurological exam displayed muscular hypotonia with normal tendon reflexes and no pyramidal signs. In the clinical investigations a failure to thrive and acquired microcephaly were seen

(head circumference 43.5 cm, 1 cm below centile 3). No indicative dysmorphisms were present. Ophthalmologic investigations, EEG, and abdominal ultrasound were without pathological results. Brain MRI at the age of 15 months displayed spotted white matter lesions in biparietal areas showing no progression during the next 5 months. At the age of 18 months she presented with status epilepticus. Screening for metabolic disorders including serological as well as neurooncological antibodies in serum and cerebrospinal fluid investigations at the age of 20 months were within reference ranges. Neurotransmitters were not analyzed. Electron microscopy of a skin biopsy did not show mitochondrial degeneration or pathological lysosomal storage. Currently, seizures are well-controlled with levetiracetame, but motor functions continue to deteriorate and mood swings are frequent.