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

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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

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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

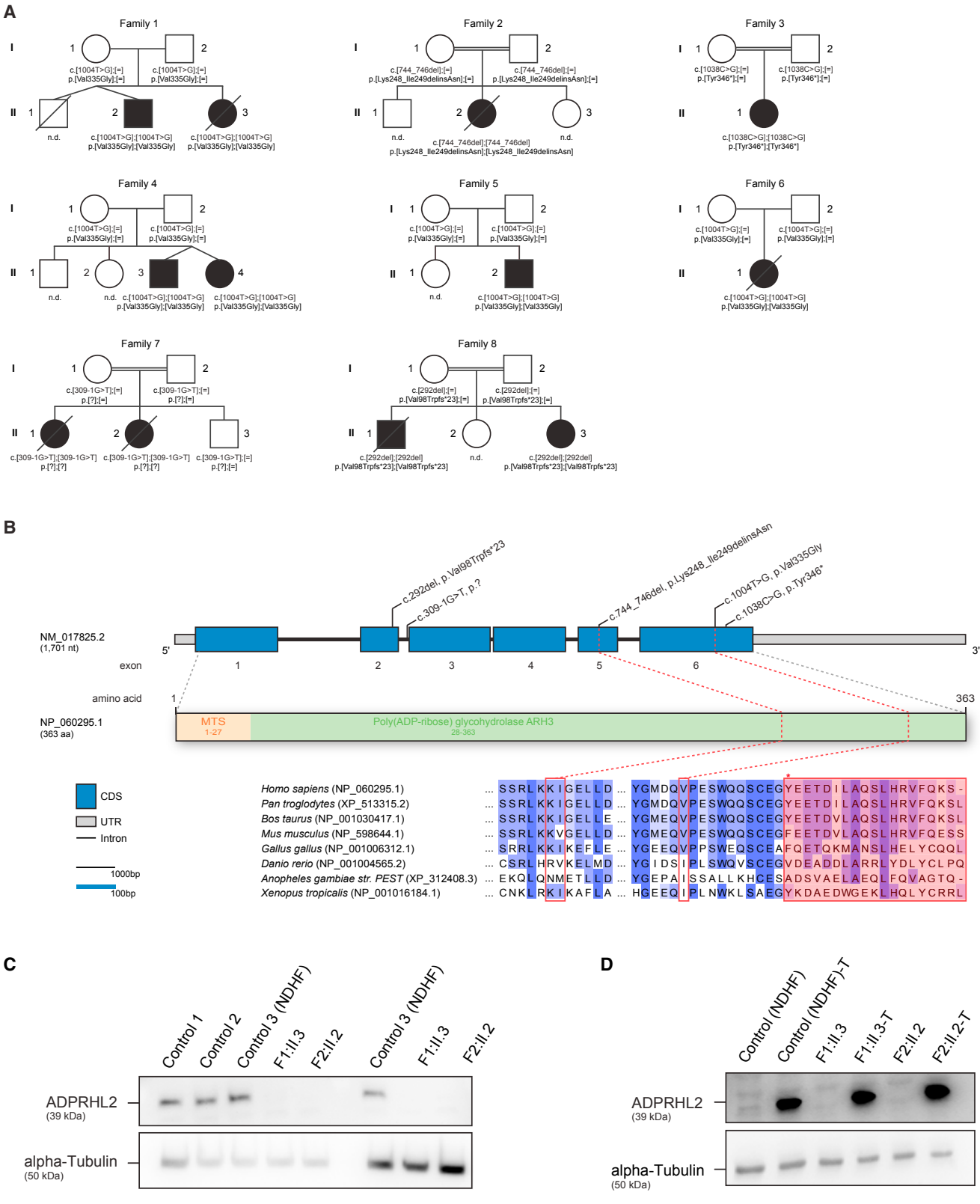

Figure 1. Pedigrees of Investigated Families, Structure of *ADPRHL2*, and Investigation of ADPRHL2 Amounts
(A) Pedigrees of eight families carrying pathogenic variants in *ADPRHL2* illustrate the mutation-carrier status of affected (closed symbols) and healthy (open symbols) family members. ND, not determined.
(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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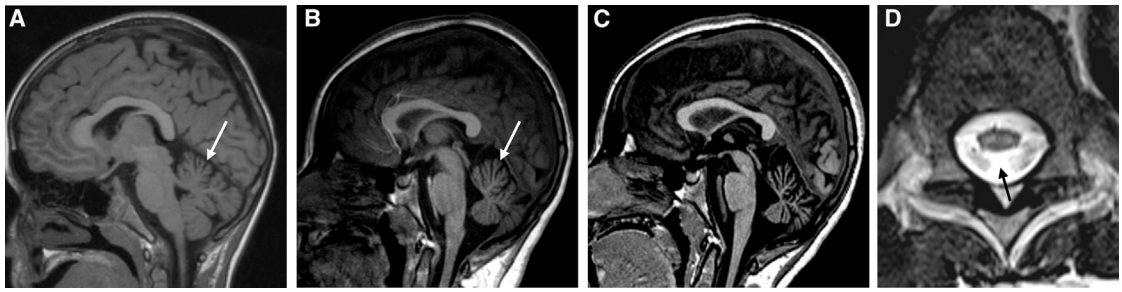

Figure 2. Neuroimaging Findings in Individuals with ADPRHL2 Variants
(A) Brain MRI (T1-weighted image, sagittal view) of individual F5:II.2 at the age of 7 years demonstrates mild upper vermian atrophy (arrow).
(B and C) Brain MRI (T1-weighted image, sagittal view) of individual F2:II.2 at the ages of 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.

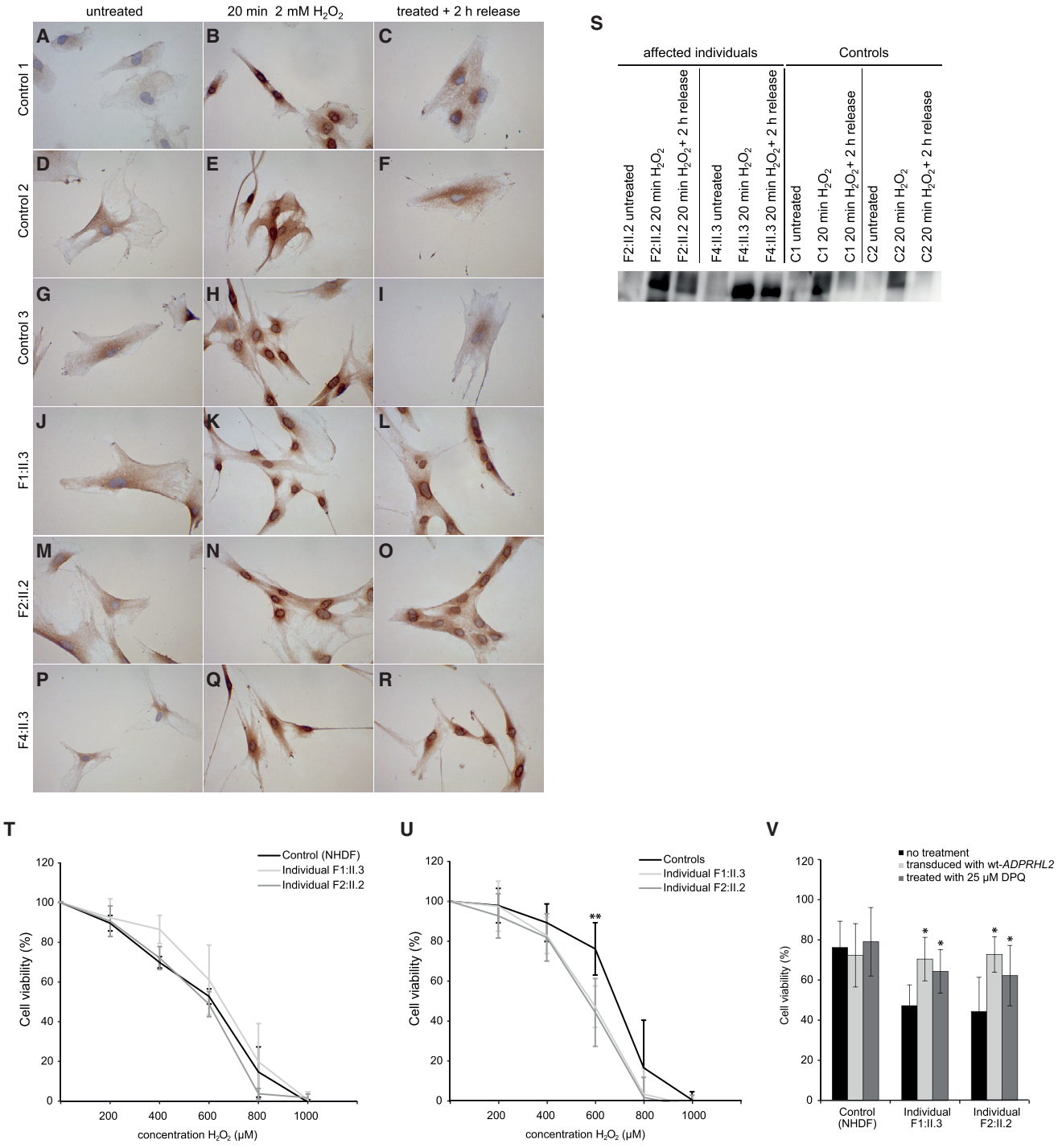

Figure 3. Investigation of ADP-Ribosylation and Cell Viability upon H_2O_2 Treatment

(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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