RESEARCH ARTICLE

Natural History of Polymerase Gamma–Related Ataxia

Friedemann Bender, MD,[1,2] Dagmar Timmann, MD,[3] Bart P. van de Warrenburg, MD, PhD,[4] Astrid D. Adarmes-Gómez, MD,[5,6] Benjamin Bender, MD,[7] Andreas Thieme, MD,[3] Matthis Synofzik, MD,[1,2] and Ludger Schöls, MD[1,2*]

[1]*Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research & Center of Neurology, University of Tuebingen, Tuebingen, Germany*
[2]*German Center for Neurodegenerative Diseases (DZNE), Tuebingen, Germany*
[3]*Department of Neurology and Center for Translational Neuro- and Behavioral Sciences (C-TNBS), University Hospital Essen, University of Duisburg-Essen, Essen, Germany*
[4]*Department of Neurology, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands*
[5]*Unidad de Trastornos del Movimiento, Servicio de Neurología y Neurofisiología Clínica, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, Spain*
[6]*Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain*
[7]*Department of Diagnostics and Interventional Neuroradiology, University of Tuebingen, Tuebingen, Germany*

ABSTRACT: Background: Mutations in the mitochondrial DNA polymerase gamma are causing a wide phenotypic spectrum including ataxia as one of the most common presentations.

Objective: The objective of this study was to determine the course of disease of polymerase gamma–related ataxia.

Methods: In a prospective natural history study, we assessed 24 adult ataxia patients with biallelic polymerase gamma mutations for (1) severity of cerebellar dysfunction using the Scale for the Assessment and Rating of Ataxia score, (2) presence of nonataxia signs using the Inventory of Non-Ataxia Symptoms, (3) gray- and white-matter changes in brain MRI, and (4) findings in nerve conduction studies.

Results: Assessment included follow-up visits up to 11.6 years. The Scale for the Assessment and Rating of Ataxia showed a mean annual increase of 1.02 ± 0.78 points/year. Disease progression was faster in patients with age at onset ≤ 30 years (1.5 Scale for the Assessment and Rating of Ataxia points/year) than with later onset (0.5 points/year); $P = 0.008$. The Inventory of Non-Ataxia Symptoms count increased by 0.30 ± 0.4 points/year. External ophthalmoplegia, brain stem oculomotor signs, areflexia, and sensory deficits were the most common nonataxic features. On MRI cerebellar atrophy was mild. T2 signal alterations affected mostly cerebellar white matter, middle cerebellar peduncles, thalamus, brain stem, and occipital and frontal white matter. Within 4 years, progression was primarily observed in the context of repeated epileptic seizures. Nerve conduction studies revealed axonal sensory peripheral neuropathy with mild motor nerve involvement. Exploratory sample size calculation implied 38 patients per arm as sufficient to detect a reduction of progression by 50% in hypothetical interventions within a 1-year trial.

Conclusion: The results recommend the Scale for the Assessment and Rating of Ataxia as a primary outcome measure for future interventional trials in polymerase gamma–related ataxia. © 2021 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: POLG; polymerase gamma; ataxia; natural history

***Correspondence to:** Prof. Ludger Schöls, Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research & Center of Neurology, University of Tuebingen, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany; E-mail: ludger.schoels@med.uni-tuebingen.de

Relevant conflicts of interest/financial disclosures: All authors report no disclosures relevant to the article.

Funding agencies: This work was supported, in part, via the European Union's Horizon 2020 research and innovation program under the ERA-NET Cofund action no. 643578. It was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) no. 441409627, as part of the PROSPAX consortium under the frame of EJP RD, the European Joint Programme on Rare Diseases, under the EJP RD COFUND-EJP no. 825575 (to M.S. and B.v.d.W.) and to the PREPARE consortium (01GM1607; to M.S. and B.v.d.W.).

Received: 5 April 2021; **Revised:** 31 May 2021; **Accepted:** 16 June 2021

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28713

Mutations in mitochondrial DNA polymerase gamma (POLG) are causing a wide and overlapping phenotypic spectrum of neurological diseases.[1] Neonatal and infant onset leads to myocerebrohepatopathy spectrum disorder (MCHS) and Alpers-Huttenlocher syndrome (AHS), both leading to early death.[2,3] Later onset is associated with chronic progressive external ophthalmoplegia (CPEO), spinocerebellar ataxia with epilepsy (SCAE), mitochondrial recessive ataxia syndrome, and sensory ataxic neuropathy, dysarthria, and ophthalmoparesis (SANDO) involving various combinations of progressive external ophthalmoplegia, epilepsy, ataxia, parkinsonism, chorea, myoclonus, dystonia, peripheral neuropathy, myopathy, and sensorineural hearing loss as well as neuropsychiatric manifestations like depression or cognitive impairment and nonneurological findings such as cataracts, premature ovarian failure, gastrointestinal dysmotility, hepatic failure, diabetes, and cardiomyopathy.[4-16]

As POLG constitutes the only polymerase expressed in mitochondria, the replication of mitochondrial DNA is critically dependent on its proper function.[17,18] *POLG* mutations causing MCHS or AHS are often associated with mtDNA depletion, whereas CPEO, SCAE, and SANDO go along with mtDNA deletions.[19] Although POLG is a mitochondrial protein, it is nuclear coded. Most *POLG* mutations show autosomal-recessive inheritance, but some mutations causing CPEO follow an autosomal-dominant trait. *POLG* mutations are supposed to cause 2%–5% of autosomal-recessive ataxia[11,20] with some regional differences because of founder mutations.[21,22]

Whereas several reports have described the phenotypic spectrum and neurogenetic aspects, less is known about the course of disease in POLG-related ataxia (POLG-A), which is, however, key for trial readiness. In this study, we examined the phenotypic spectrum and prospectively assessed disease progression in 24 POLG-A patients.

Methods

Cohort

Patients with *POLG* mutations were included in this study who attended the ataxia clinics in Tübingen (Germany), Essen (Germany), Nijmegen (Netherlands), and Sevilla (Spain) between 2004 and 2019. We identified 28 individuals. Included were all ataxic patients with 2 *POLG* mutations. Accordingly, 4 patients were excluded. One patient carried a heterozygous G517V variant that is considered most likely not pathogenic.[23] Another patient had 2 recessive variants (P587L and T251I) that have been described several times to occur in cis,[24,25] and compound heterozygosity could not be proven in our participant because the patient was adopted and living relatives were unknown. Furthermore, 2 affected relatives of a POLG-A patient were excluded as they did not present ataxia. Detailed genetic and demographic data of all included patients (n = 24) are given in Table 1.[26,27] Cross-sectional phenotypic data of 13 of 24 patients were reported earlier.[6] The study was approved by the Institutional Review Board of the University of Tübingen (598/2011BO1), and all patients gave their written informed consent.

Clinical Measures of Disease Severity

Severity of ataxia was assessed using the Scale for the Assessment and Rating of Ataxia (SARA), from 0 points indicating no ataxia to 40 points indicating most severe ataxia.[28] The annual prospective progression rate in SARA was calculated with a linear regression model, whereas SARA score was used as the variable depending on the duration of follow-up in years. All participants with follow-up data of at least 1 year (n = 13) were evaluated. To prevent bias from different numbers of assessments between study participants, we calculated the linear regression of data points from each single patient as the individual progression rate. The mean progression rate was calculated as the average of individual progression rates. All participants with SARA data (22 of 24) presented manifest ataxia, defined as a SARA score of 3 points or more.

Nonataxia symptoms and signs were screened using the Inventory of Non-Ataxia Symptoms (INAS), which lists 16 potential nonataxic manifestations of disease including areflexia, hyperreflexia, extensor plantar response, spasticity, paresis, amyotrophy, fasciculations, myoclonus, rigidity, chorea, dystonia, resting tremor, sensory symptoms, brain stem oculomotor signs (defined as ophthalmoparesis and/or slowing of saccades in the INAS count), urinary dysfunction, and cognitive impairment.[29] INAS yields the INAS count, a semiquantitative measure that scores the presence or absence of nonataxia features but does not rate their severity.[30] Annual progression of INAS count was calculated in the same manner as SARA progression including all participants with INAS follow-up data of at least 1 year (n = 11). Further POLG-related symptoms like epilepsy, cataract, elevated transaminases, diabetes, and neuropsychiatric symptoms are not considered by the INAS but were assessed by medical history and clinical examination. Progression of gait disorder was estimated revealing the 3 categories (1) onset of gait disturbance, (2) loss of independent walking, and (3) use of wheelchair with the Kaplan–Meier method.

Brain Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) data were available for 18 POLG patients. MR scans were acquired in

TABLE 1 Study cohort

Subject	Family no.	Sex	Age at onset (y)				Death (y)	Disease duration (y)	First POLG sign	Age at onset gait disorder (y)	*POLG* mutation	Inheritance	MRI scans	Neurography
			First POLG sign	Gait disorder	Loss of independent walking	Wheelchair dependence								
P01	1	F	29	29	49	/	/	27	Gait	29	W748S + E1143G/ W748S + E1143G	aut rec	3	Yes
P02[a]	2	F	39	39	45	/	/	15	Gait	39	A467T/W748S	aut rec[b]	1	Yes
P03[a]	3	M	18	18	/	/	/	9	Gait	18	A467T/A467T	aut rec		Yes
P04[a]	3	F	12	12	27	31	/	23	Gait	12	A467T/A467T	aut rec	1	
P05[a]	3	F	17	22	24	33	34	17	Epilepsy	22	A467T/A467T	aut rec	1	
P06[a]	4	F	29	29	36	43	/	19	Gait	29	R627Q/G848S	aut rec[b]	3	
P07	5	F	14	14	31	33	/	19	Gait	14	R627Q/R1096H	aut rec[b]		
P08	6	F	36	36	Unknown	47	/	11	Gait	36	R627Q/Ins A c.3594[c]	aut rec[b]		
P09	7	F	25	25	42	44	/	25	Gait	25	A467T/W748S	aut rec[b]		
P10[a]	8	M	39	39	55	57	57	18	Gait	39	A467T/W748S	aut rec[b]	1	
P11	8	F	49	49	56	/	/	8	Gait	49	A467T/W748S	aut rec[b]	1	Yes
P12[a]	8	F	41	41	52	58	59	18	Gait	41	A467T/W748S	aut rec[b]	1	
P13[a]	9	F	22	22	41	48	/	28	Gait	22	W748S/W748S	aut rec		Yes
P14[a]	10	F	45	52	/	/	/	9	Ptosis	52	Y955C/WT	aut dom	1	Yes
P17[a]	11	M	16	37	52	57	/	42	Hypacusis	37	A467T/A467T	aut rec	1	
P18[a]	12	F	32	35	36	41	/	13	Chorea	35	W748S/W748S	aut rec	3	Yes
P19[a]	12	F	12	12	25	25	44	32	Epilepsy	12	W748S/W748S	aut rec	3	Yes
P20[a]	13	M	27	27	48	53	/	29	Gait	27	A467T/A467T	aut rec	1	Yes
P22	14	M	60	60	65	/	/	13	Gait	60	R627Q/R627Q	aut rec	1	Yes
P23	15	F	42	42	48	/	/	6	Gait	42	W748S/W748S	aut rec	3	Yes
P24	16	F	22	22	/	/	/	6	Gait	22	R627Q/P1174R[d]	aut rec[b]	1	
P26	17	M	5	22	/	/	/	22	Epilepsy	22	A467T/A467T	aut rec	1	
P27	18	M	63	63	49	/	/	17	Gait	63	P587L/T251I	aut rec[b]		
P28	19	M	48	48	55	/	/	8	Gait	48	A467T/W748S + E1143G	aut rec[b]	1	
n	19	16 F, 8 M											18/24	11/24
Mean ± SD			30.9 ± 15.5					18.1 ± 9.2		33.1 ± 14.5				
Range			5–63					6–42		12–63				

[a]Published before in cross-sectional study.[6]
[b]Compound heterozygosity confirmed.
[c]Insertion mutation with premature stop codon (insA c.3594; p.T1199fs1215X).[26]
[d]Variant of uncertain significance in highly conserved region, once submitted to the National Center for Biotechnology Information.[27]
Overview of the study cohort.
M, male; F, female; aut rec, autosomal recessive; aut dom, autosomal dominant; WT, wild type.

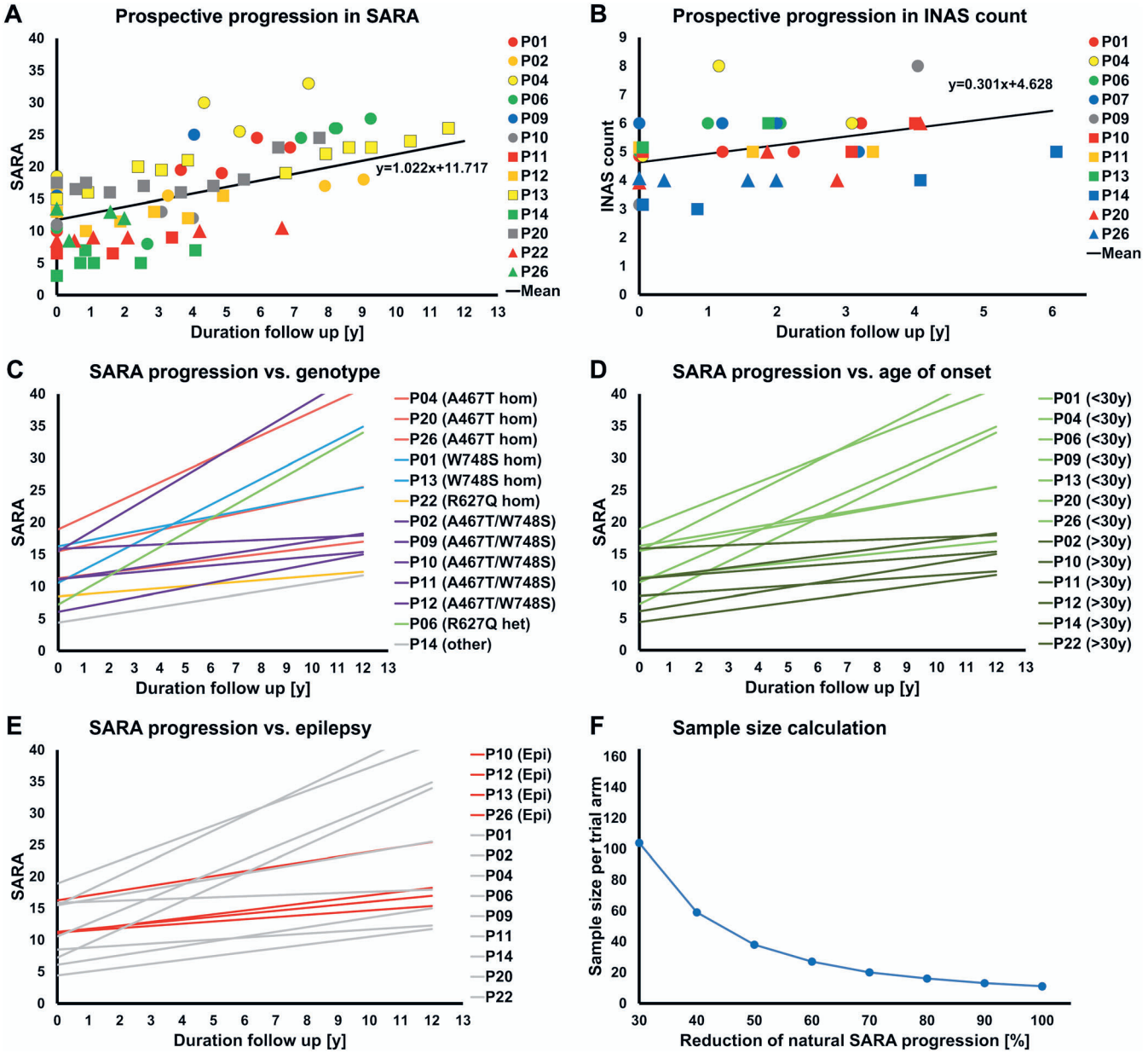

FIG 1. Progression in POLG-related ataxia and sample size calculation. (**A, B**) Progression of SARA total score and INAS count in 13 (SARA) and 11 (INAS) POLG patients with follow-up data > 1 year. Black line, mean of individual progression rates as calculated by linear regression of individual follow-up data indicating an average progression rate of 1.02 SARA points/year. (**C–E**) Individual SARA progression (patients with follow-up > 1 year) color-coded for genotype, age at onset, and presence of epilepsy. (**F**) Sample size calculation for an interventional trial with SARA as outcome measure (1 year, 2 groups, 1:1; power, 0.8; significance level, 0.05).

clinical routine with different scanners and at different sites, but all images were available in a digital format and analyzed in a uniform manner. Referring to earlier MRI studies in POLG patients,[6,31,32] cerebellar and cerebral atrophy was assessed in a semiquantitative way (no, mild, moderate, severe atrophy) by a neurologist experienced in MRI (F.B.) and a neuroradiologist (B.B.). T2/FLAIR hyperintensity in the cerebrum, thalamus, cerebellum, and brain stem was rated as present or absent.

For 5 participants, repeated MRI data were available covering follow-up intervals of 1 month to 4 years, resulting in a total of 28 MRIs. All data sets included T1 and T2 sequences; 26 of 28 data sets had additional fluid-attenuated inverse recovery (FLAIR) sequences, and 20 of 28 data sets contained diffusion-weighted images.

Nerve Conduction Studies

Twelve participants underwent nerve conduction studies, according to standard assessment for peripheral neuropathy including motor nerve conduction studies and F-wave analysis of the right tibial and ulnar nerves and sensory nerve conduction studies of the sural and

radial nerves (in one case, P03, only sensory nerves were examined). To ensure comparability of nerve conduction velocities, a minimum skin temperature of 30°C has been assured by the use of a heat lamp when needed.

Statistics

Quantitative features are given as mean and standard deviation for normally distributed data and as median and interquartile range for not normally distributed data. Normal distribution was tested using the Shapiro–Wilk test. Differences in age at onset between the most frequent genotypes were evaluated with independent *t* tests with a Bonferroni-corrected threshold for multiple testing of $P = 0.005$. We used the Kaplan–Meier method for descriptive data concerning onset of gait disorder, use of walking aids, progression to wheelchair dependence, and death. Further linear regression was used to determine progression in SARA and INAS. To correct for the different amounts of test for each participant, the regression rates of each subject were used to determine the mean progression rate in SARA and INAS. All calculations were performed using SPSS (version 26.0.0.0; IBM Corp., Chicago, Illinois).

Sample size calculation was computed using G*Power 3.1.9.6.[33] We performed a 2-tailed *t* test (1:1, $\alpha = 0.05$, power = 0.8), using mean and standard deviation of the annualized change in SARA after confirming normality by the Shapiro–Wilk test. Because of the assumable slow progression, we excluded patients with a SARA follow-up of less than 1 year from calculations of the progression rate and sample size estimation.

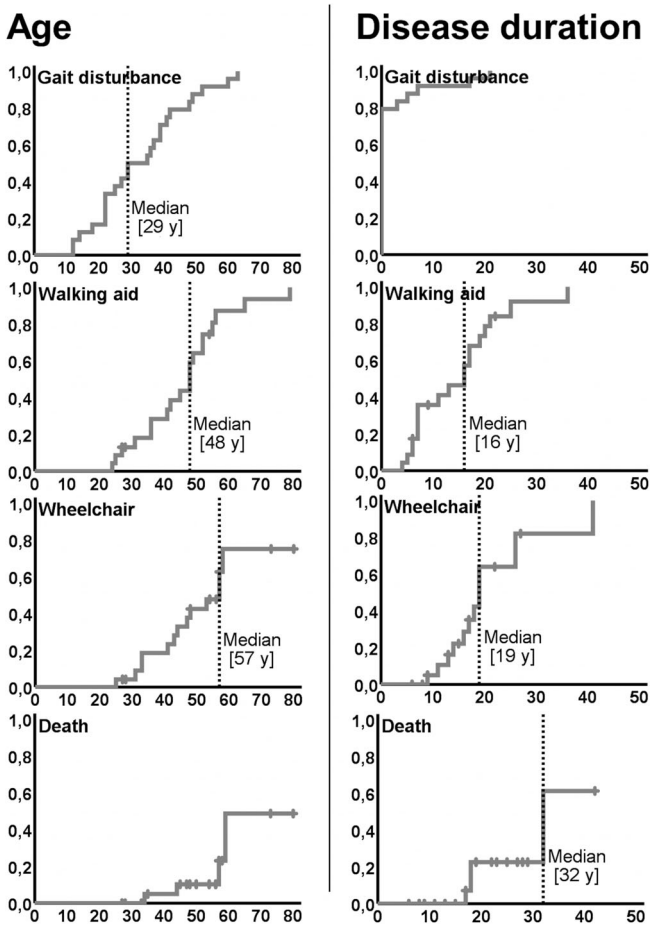

FIG 2. Progression of gait disturbance. Survival functions of Kaplan–Meier calculations for onset of gait disturbance, loss of independent walking, wheelchair use, and death in relation to age and disease duration on the *X* axis (in years).

Results

Age at Onset

Disease onset was defined as the occurrence of the first symptom related to *POLG* mutations based on medical history. Unsteadiness of gait was the most frequent initial symptom defining onset of disease in 18 of 24 patients. In 3 of 24 cases epileptic seizures manifested at onset of disease. Ptosis, hypacusis, and chorea were seen in 1 patient each as the initial POLG-related symptom. Age at onset showed great variability, ranging from 5 to 63 years, with a mean of 30.9 ± 15.5 years. Age at onset was significantly earlier in patients with homozygous A467T mutations compared with patients with heterozygous A467T/W748S mutations ($P = 0.0004$); see details in Table S1. We did not find an effect of sex on age at onset ($P = 0.966$, *t* test).

Progression of Ataxia

SARA scores for the assessment of ataxia were available in all but 2 participants (P07 and P08). Severity in SARA at the first visit ranged from 3.0 to 39.0 points (median, 13.25 points; IQR, 8.25 points) after a disease duration between 1 and 33 years (mean, 12.8 ± 8.3 years). For prospective determination of disease progression, longitudinal data with a follow-up of at least 1 year were available for 13 participants, with a range of 2.0–11.6 years (mean, 6.2 ± 2.8 years) between the first and last ratings. SARA scores indicated a linear progression over time, with a mean annual increase of 1.02 ± 0.78 SARA points per year, as shown in Figure 1A. To explore potential effects of genotypes, age at onset or the occurrence of epilepsy on disease progression we color-coded respective groups and plotted individual progression rates, as shown in Figure 1C–E. There was a clear tendency toward more rapid progression with earlier onset of disease (Fig. 1D). Correlation analysis showed a correlation of $r = -0.455$ but without statistical significance, probably because of small numbers ($P = 0.119$; Spearman correlation for not normally distributed data). We then compared progression rates in patients with early onset (≤30 years of age) with patients with later onset (>30 years of age) and found

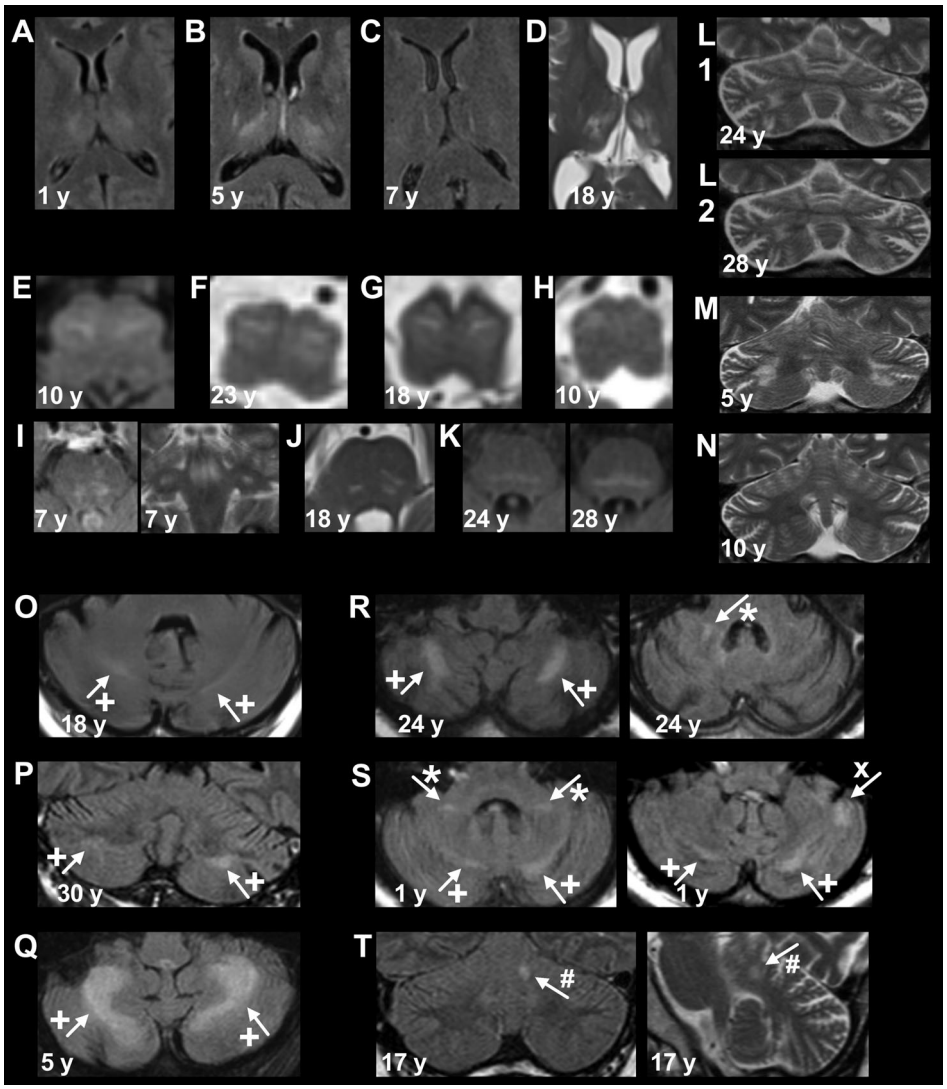

FIG 3. Infratentorial and thalamic MRI findings in POLG-related ataxia. Thalamic lesions in (**A**) P18; (**B**) P23; (**C**) P05;(**D**) P06. Brain stem inferior olivary lesions in (**E**) P12; (**F**) P01; (**G**) P06; (**H**) P04. Brain stem pontine lesions in (**I**) P05; (**J**) P06; (**K**) P19. Cerebellar atrophy in (**L**) P19 with mild progression within 4 years; (**M**) P23; (**N**) P12. **O–T**: Cerebellar lesions (+, bihemispheric deep white matter lesions; *, medial cerebellar peduncle lesions; x, cerebellar gray-matter lesion; #, nodular white-matter lesion) in (**O**) P06; (**P**) P17; (**Q**) P23; (**R**) P19; (**S**) P18; (**T**) P10. Disease duration is indicated in the bottom left of each image.

significant differences, with a progression of 1.5 $\pm$ 0.8 SARA points/year (n = 7) in the early-onset group and 0.5 $\pm$ 0.2 SARA points (n = 6) in the late-onset group ($P = 0.008$; Mann–Whitney U test) with no obvious differences in relation to these factors.

Nonataxia Symptoms

Nonataxic manifestation of POLG-related disease was assessed by INAS and medical history. We observed a wide range of nonataxia symptoms and signs including (in decreasing frequency) brain stem oculomotor signs, areflexia, sensory symptoms, urinary dysfunction, cognitive impairment, myoclonus, chorea/dyskinesia, dystonia, muscle atrophy, paresis, rigidity, hyperreflexia, and spasticity. In addition to nonataxia features covered by the INAS, 23 of 24 patients showed CPEO with gaze palsy and ptosis or only ptosis. Less frequent were hypacusis, depression, anxiety, cataract, elevation of transaminases, diabetes, migraine with aura, and myopathy. Eight of 24 patients had epilepsy, including 3 of 8 cases with focal and generalized seizures, 2 of 8 cases with only generalized seizures, and 2 patients with a single convulsive epileptic state with no previous history of epileptic seizures. In 1 case, the semiology remained unclear. Cardiomyopathy was not observed. The detailed prevalence of nonataxic manifestations is given in Table S2. INAS count at the first visit was available for all 24 patients and ranged from 1 to 9 points (median, 5.0 points; IQR, 1.75 points) after a disease duration of 1 to 33 years (mean, 14.7 $\pm$ 8.5 years). For prospective assessment of the progression of

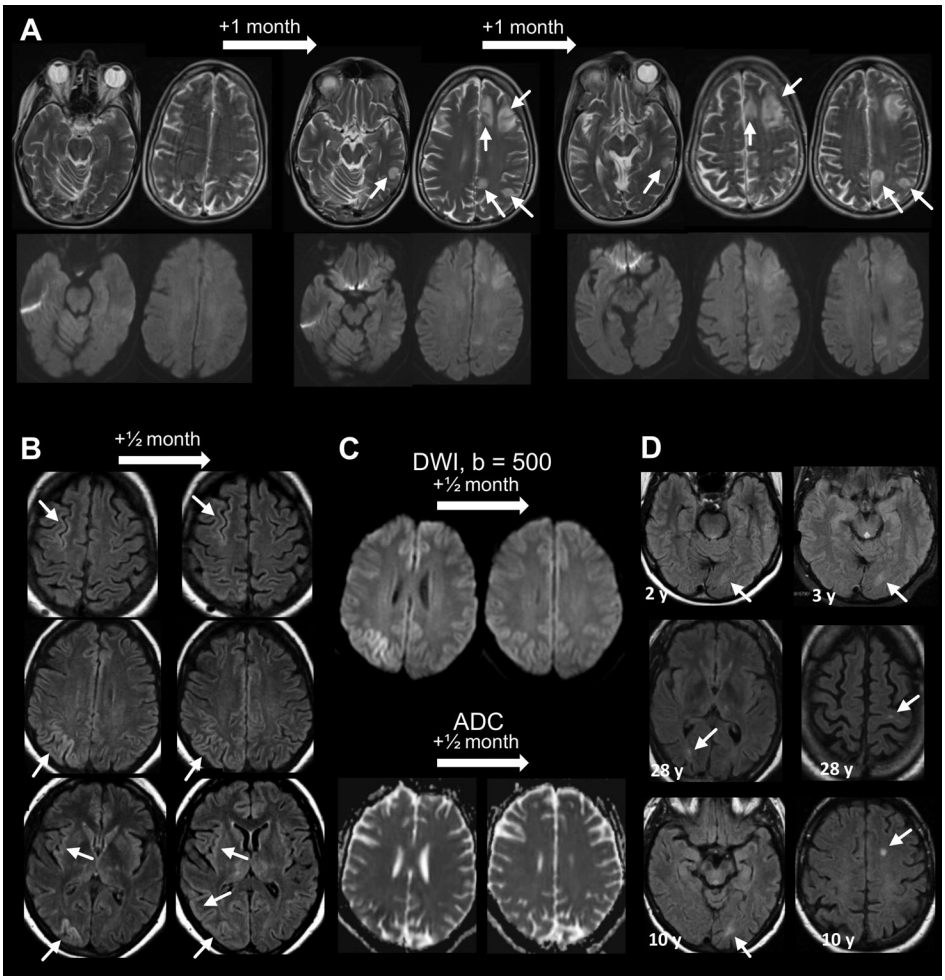

FIG 4. Cerebral MRI findings in POLG-related ataxia. (**A**) Stroke-like cortical and adjacent subcortical lesions (P06); on the left 2 days after an epileptic seizure, in the middle after 1 month, and on the right after another month. (**B, C**) FLAIR lesions (P18, immediately after a series of 4 epileptic seizures and 16 days later). (**B**) Arrows, decreasing signal in the parietal and occipital lobes, increasing signal in the temporal lobe. (**D**) White-matter lesions on FLAIR images. Upper row: P23, slightly increasing within 1 year (arrows); middle row: blurry WM lesions in P19 (arrows) and additional hyperintense thalamus lesion; lower row: blurry lesions in P12 (arrows). Disease duration is given at the bottom left of each image.

extracerebellar involvement, longitudinal INAS data over a period of at least 1 year were available for 11 of 24 subjects with a follow-up period of 1.9 to 6.1 years (mean, 3.4 ± 1.2 years). INAS count increased by 0.30 ± 0.39 points per year but with major variability including decrease of INAS count in some visits in 5 patients (Fig. 1B). Comparing the individual INAS progression rates between patients with early disease onset (≤30 years of age) and patients with later onset (>30 years of age), we found a nonsignificant tendency toward faster progression in the early-onset group (0.4 ± 0.5 points/year, $n = 8$, vs 0.2 ± 0.2 points/year, $n = 3$; $P = 0.536$, t test).

Progression of Gait Disturbance

Clinical gait assessment data were available for all participants (only for P08 was the beginning of use of walking aids unclear) and revealed a predominantly ataxic gait in all but 1 patient (P14). P14 initially presented with a hypokinetic-rigid gait disturbance followed by ataxia only later in the disease. Until the end of the study, 5 of 24 patients had gait difficulties but walked independently, 7 used walking aids, 9 were dependent on a wheelchair, and 4 participants had died. Details are shown in Figure 2.

Gait disturbance occurred at a median age of 29 years (IQR, 19 years). Walking aids were used at a median age of 48 years (IQR, 19 years), and wheelchair use was reached at a median age of 57 years (IQR, 15 years). Four patients died during the study, aged 34, 44, 54, and 59 years. Cause of death in all 4 cases was status epilepticus or its direct sequelae.

Related to the onset of gait difficulties, walking aids were used after a median of 15 years (IQR, 10 years), and wheelchair use occurred after a median of 19 years (IQR, 6 years).

Referring to the first POLG-related symptom, walking aids were required after a median of 16 years (IQR, 13 years), and wheelchair dependence was reached after a median of 19 years (IQR, 10 years).

MRI

MRI data of 18 patients were available for the assessment of atrophy, white-matter changes, and diffusion deficits. Cerebellar atrophy equally involving the vermis and cerebellar hemispheres was found in 10 of 18 patients in T1 images and appeared to be mild in all cases (examples in Fig. 3L–N). Longitudinal follow-up data over 4 years were available for 3 participants. In 2 of them mild progression was seen, whereas cerebellar atrophy did not change in the third case.

Cerebellar white matter was affected in 9 of 18 patients. Eight of them showed lesions in the deep cerebellar white matter (Fig. 3O–S), whereas 1 patient (P10) had a more nodular-shaped white-matter lesion (Fig. 3T). On follow-up scans only 1 patient (P18) presented with a novel hyperintensity in cerebellar gray matter after a series of epileptic seizures (Fig. 3S).

Thalamic T2/FLAIR lesions were seen in 9 of 18 patients, with the predominant effect in the central parts of the thalamus (Fig. 3A–D). These did not change over 4 years of observation.

In the brain stem, 2 types of lesions were observed: (1) T2/FLAIR hyperintensities in the inferior olivary nucleus occurred in 4 patients, with slight progression within 1 year in P01 (Fig. 3E–H); (2) pontine T2/FLAIR hyperintensities in the area of the medial lemniscus ventral to the locus coeruleus or between pontocerebellar fibers were seen in 3 of 18 patients, with progression in 2 patients (P19 and P23) within 3 and 4 years, respectively (Fig. 3I–K). Cerebral lesions in T2 and FLAIR imaging were disclosed in 6 of 18 patients (P06, P12, P18, P19, P22, P23). Isolated subcortical lesions were seen in 4 of them (P12, P19, P22, and P23) and cortical involvement in 2 (P06 and P18). Examples of cerebral lesions are given in Figure 4. Cortical FLAIR hyperintense stroke-like lesions appeared in P06 and P18 within 1 month after several epileptic seizures (Fig. 4A–C). These affected widespread cortical and subcortical areas in P06, but showed an isolated effect in the cortical band in P18. The latter was the only lesion with a decreased ADC signal in our series.

Nerve Conduction Studies

Nerve conduction studies (NCS) were performed in 12 of 24 POLG patients. All 12 cases showed axonal sensory neuropathy with reduced or absent sensory nerve action potentials in the lower and/or upper limbs. In 7 cases, and in addition, compound muscle action potentials were reduced. Nerve conduction velocity was normal in 6 patients and mildly slowed (1–7 m/s below threshold) in the other 6 cases. Detailed results are given in Table S3. In summary, 5 participants presented with isolated sensory axonal neuropathy, and 7 cases showed sensory-motor axonal neuropathy with sensory accentuation.

Sample Size Calculation

For the calculation of the minimally required sample size for future treatment studies, we used the mean SARA progression und standard deviation calculated with the 13 participants who had SARA follow-up data of at least 1 year. For capturing a 50% reduction of SARA progression with 80% power and a significance of 0.05 in a placebo-controlled trial with 1-year duration and 1:1 randomization, at least 38 patients would be required per arm (Fig. 1F).

Discussion

This study provides the first data on the natural history of POLG-related ataxia (POLG-A) in adults. In our prospective assessment, severity of ataxia as measured by the SARA score[28] progressed linearly over more than a decade by 1.02 points/year. This makes the progression rate of POLG-A faster than in Friedreich's ataxia (FA, 0.77 points/year[34]), COQ8A ataxia (COQ8A, 0.45 points/year[35]) or spinocerebellar ataxia type 6 (SCA6, 0.80 points/year[36]), but slower than in spinocerebellar ataxia types 1, 2, and 3 (SCA1, 2.11 points/year; SCA2, 1.49 points/year; SCA3, 1.56 points/year[36]), or multiple system atrophy (MSA, 3.3 points/year[37]).

The identification of factors that influence progression is of major importance for the planning of upcoming interventional trials. We found earlier age at onset to be associated with more rapid progression with substantial differences, 1.5 versus 0.5 points/year with onset before versus after 30 years of age. Confirmation of this age-at-onset factor in a larger cohort is definitely needed, but a similar correlation of earlier onset and more rapid progression was also found in pediatric manifestations of POLG-related disease including predominantly in patients with Alpers syndrome and MCHS[3] and in a large cohort covering many different phenotypes.[38]

We did not find a relevant correlation of the genotype with progression rate in SARA, but the A467T genotype did go along with an earlier onset of disease, as described previously.[16] We could not confirm a correlation of earlier disease onset in compound heterozygous POLG-A patients as reported earlier for POLG-related disease.[38] This could be because of the more homogeneous cohort of POLG-A patients in our study compared with largely heterogeneous phenotypes studied by Hikmat et al (2020).[38] There was also no significant difference in disease onset and progression rate between male and female participants, as observed in a previous study analyzing a broader phenotypic spectrum.[39] Comparing our data with the course of disease in other POLG phenotypes like Alpers syndrome[3] clearly shows that natural history studies as well as clinical trials need to consider stratification of mitochondrial diseases not

only for the underlying gene but also the presenting phenotype.

POLG-A patients required walking aids after a median disease duration of 16 years and became wheelchair dependent after 19 years. This constitutes a slightly faster progression of gait ataxia to wheelchair dependence compared with SCA2 and SCA3 (21 years[40]) and SCA6 (24 years[41]) and a slower progression than in SCA1 (15 years[42]) and FA (approximately 12 years[43]).

Four POLG-A patients were deceased at the end of the study. In all cases death was related to status epilepticus. Drug-resistant epileptic seizures and a significant influence on survival have been described previously in patients with POLG mutations.[8,38] An important point in the antiepileptic treatment of POLG patients is the avoidance of valproate as its mitochondrial toxicity frequently causes hepatic failure in this mitochondrial disease.[44,45]

The most frequent noncerebellar finding in POLG-A was CPEO, which was observed in all but 1 patient. In addition, INAS revealed brain stem oculomotor signs, areflexia, sensory deficits, urinary dysfunction, and cognitive impairment in more than 50% of patients with POLG-A. Cognitive impairment may be underestimated, as it was only assessed by impression of the examiner without further cognitive testing. The INAS category of "brainstem oculomotor signs" may be confounded by the high rate of CPEO. On the single-item level, ophthalmoparesis was seen in 92% of patients and slowing of saccades in 63%. Differentiation from external ophthalmoplegia can be difficult and may lead to overestimation of the effect on the brain stem in our study. However, frequent signal abnormalities of the brain stem on MRI support a high frequency of brain stem involvement.

INAS count in POLG-A increased annually by 0.30 points/year, which is similar to SCA2 and SCA3 (both 0.30 points/year[46]), but slower than in SCA1 (0.56 points/year[46]) and faster than in SCA6 (no progression within 2 years[46]) and FA (0.10 points/year[34]). INAS count is most likely inferior to SARA as a progression marker because INAS showed more variability with a higher standard deviation (0.39 points/year) than its medial progression (0.30 points/year). In addition, we observed some cases with apparently decreasing INAS count potentially because of different examiners. On MRI, white-matter lesions revealed a pattern with predominant effect on the inferior olive, pons, middle cerebellar peduncles, cerebellar white matter, thalamus, and occipital white matter. Occurrence and frequency of lesions in these regions fit well with previous reports.[31,47,48] Although no quantitative MRI data were available, this observational study provides evidence that cerebellar atrophy is only mild in POLG-A with minor progression, even after 4 years of follow-up. This makes it rather unlikely that volumetric MRI is a better progression marker for interventional trials in POLG-A than clinical scores. However, further longitudinal imaging studies with thoroughly conducted quantitative analyses are needed to evaluate the potential of MRI as a biomarker in POLG-related ataxia, as minor changes might escape visual evaluation.

Electrophysiological examination revealed predominantly sensory and axonal neuropathy in all patients who underwent nerve conduction studies in our series. Motor fibers were affected in only 58% of patients and to a most mild degree. These results resemble findings in FA with similar axonal sensory neuropathy and at most mild effects on motor nerves in a few cases.[6,49,50] As amplitude of sensory nerve action potentials and compound muscle action potentials shows limited replicability,[51] nerve conduction studies are unlikely to be useful as progression markers in interventional trials. However, because of the high number of POLG-A patients with effects in the peripheral nervous system, quantitative scores like the Charcot–Marie-Tooth neuropathy score[52] might be a useful outcome parameter to capture progression of peripheral neuropathy but require longitudinal assessment in larger cohorts.

As sufficiently large cohorts may not be available in a rare disorder like POLG-A, development of biomarkers like the amount of mtDNA deletions that are related to POLG pathophysiology should be stimulated, and this may directly indicate successful interference of drug candidates with pathogenesis.

In summary, we found a rather linear progression of ataxia as assessed by SARA in this prospective natural history study. Nonataxia symptoms, MR imaging and nerve conduction studies showed more variability or less sensitivity to change. This suggests SARA as a primary outcome measure for future interventional trials in POLG-A. To provide first evidence on the feasibility of interventional trials, we estimated sample sizes of cohorts dependent on the expected efficacy of the intervention. Our computations revealed a cohort size of at least 38 patients per trial arm to detect a 50% reduction in ataxia progression in POLG-A within 1 year of trial duration. Stratification for age at onset is required, as earlier onset was associated with a more rapid progression of POLG-related ataxia. As variability of progression is a crucial factor in sample size calculations, the rather small number of 13 patients in this analysis may have provided only a preliminary estimate, and future studies with larger sample sizes are necessary to confirm these results. ■

Acknowledgments: We are grateful to all patients for participation in this study. F.B., B.v.d.W., M.S., and L.S. are members of the European Reference Network for Rare Neurological Diseases, Project ID No. 739510. F.B. and L.S. are affiliated to the German Network for Mitochondrial Diseases (mitoNET; https://www.mitonet.org/).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Blok MJ, van den Bosch BJ, Jongen E, et al. The unfolding clinical spectrum of POLG mutations. J Med Genet 2009;46(11):776–785.
2. Wong LJ, Naviaux RK, Brunetti-Pierri N, et al. Molecular and clinical genetics of mitochondrial diseases due to POLG mutations. Hum Mutat 2008;29(9):E150–E172.
3. Hikmat O, Tzoulis C, Chong WK, et al. The clinical spectrum and natural history of early-onset diseases due to DNA polymerase gamma mutations. Genet Med 2017;19(11):1217.
4. Synofzik M, Schüle R, Schulte C, et al. Complex hyperkinetic movement disorders associated with POLG mutations. Mov Disord 2010; 25(14):2472–2475.
5. Synofzik M, Asmus F, Reimold M, Schols L, Berg D. Sustained dopaminergic response of parkinsonism and depression in POLG-associated parkinsonism. Mov Disord 2010;25(2):243–245.
6. Synofzik M, Srulijes K, Godau J, Berg D, Schols L. Characterizing POLG ataxia: clinics, electrophysiology and imaging. Cerebellum 2012;11(4):1002–1011.
7. Whittaker RG, Devine HE, Gorman GS, et al. Epilepsy in adults with mitochondrial disease: a cohort study. Ann Neurol 2015;78(6): 949–957.
8. Anagnostou ME, Ng YS, Taylor RW, McFarland R. Epilepsy due to mutations in the mitochondrial polymerase gamma (POLG) gene: a clinical and molecular genetic review. Epilepsia 2016;57(10):1531–1545.
9. Orsucci D, Angelini C, Bertini E, et al. Revisiting mitochondrial ocular myopathies: a study from the Italian network. J Neurol 2017; 264(8):1777–1784.
10. Luoma P, Melberg A, Rinne JO, et al. Parkinsonism, premature menopause, and mitochondrial DNA polymerase γ mutations: clinical and molecular genetic study. Lancet 2004;364(9437):875–882.
11. Schicks J, Synofzik M, Schulte C, Schols L. POLG, but not PEO1, is a frequent cause of cerebellar ataxia in Central Europe. Mov Disord 2010;25(15):2678–2682.
12. Pitceathly RD, Tomlinson SE, Hargreaves I, et al. Distal myopathy with cachexia: an unrecognised phenotype caused by dominantly-inherited mitochondrial polymerase gamma mutations. J Neurol Neurosurg Psychiatry 2013;84(1):107–110.
13. Mancuso M, Orsucci D, Angelini C, et al. "Mitochondrial neuropathies": a survey from the large cohort of the Italian network. Neuromuscul Disord 2016;26(4–5):272–276.
14. Prasun P, Koeberl DD. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)-like phenotype in a patient with a novel heterozygous POLG mutation. J Neurol 2014;261(9):1818–1819.
15. Tang S, Dimberg EL, Milone M, Wong LJ. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE)-like phenotype: an expanded clinical spectrum of POLG1 mutations. J Neurol 2012; 259(5):862–868.
16. Horvath R, Hudson G, Ferrari G, et al. Phenotypic spectrum associated with mutations of the mitochondrial polymerase gamma gene. Brain 2006;129(Pt 7):1674–1684.
17. Lamantea E, Tiranti V, Bordoni A, et al. Mutations of mitochondrial DNA polymerase gammaA are a frequent cause of autosomal dominant or recessive progressive external ophthalmoplegia. Ann Neurol 2002;52(2):211–219.
18. Nissanka N, Bacman SR, Plastini MJ, Moraes CT. The mitochondrial DNA polymerase gamma degrades linear DNA fragments precluding the formation of deletions. Nat Commun 2018;9(1):2491.
19. Rahman S, Copeland WC. POLG-related disorders and their neurological manifestations. Nat Rev Neurol 2019;15(1):40–52.
20. Synofzik M, Nemeth AH. Recessive ataxias. Handb Clin Neurol 2018;155:73–89.
21. Hakonen AH, Davidzon G, Salemi R, et al. Abundance of the POLG disease mutations in Europe, Australia, New Zealand, and the United States explained by single ancient European founders. Eur J Hum Genet 2007;15(7):779–783.
22. Hakonen AH, Heiskanen S, Juvonen V, et al. Mitochondrial DNA polymerase W748S mutation: a common cause of autosomal recessive ataxia with ancient European origin. Am J Hum Genet 2005;77(3): 430–441.
23. Kasiviswanathan R, Copeland WC. Biochemical analysis of the G517V POLG variant reveals wild-type like activity. Mitochondrion 2011;11(6):929–934.
24. Lamantea E, Zeviani M. Sequence analysis of familial PEO shows additional mutations associated with the 752C→T and 3527C→T changes in the POLG1 gene. Ann Neurol 2004;56(3):454–455.
25. van Goethem G, Schwartz M, Lofgren A, Dermaut B, van Broeckhoven C, Vissing J. Novel POLG mutations in progressive external ophthalmoplegia mimicking mitochondrial neurogastrointestinal encephalomyopathy. Eur J Hum Genet 2003;11(7):547–549.
26. Schulte C, Synofzik M, Gasser T, Schols L. Ataxia with ophthalmoplegia or sensory neuropathy is frequently caused by POLG mutations. Neurology 2009;73(11):898–900.
27. National Center for Biotechnology Information. ClinVar [VCV000493164.1]. 2017. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000493164.1/
28. Schmitz-Hubsch T, du Montcel ST, Baliko L, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 2006;66(11):1717–1720.
29. Schmitz-Hubsch T, Coudert M, Bauer P, et al. Spinocerebellar ataxia types 1, 2, 3, and 6: disease severity and nonataxia symptoms. Neurology 2008;71(13):982–989.
30. Jacobi H, Rakowicz M, Rola R, et al. Inventory of non-Ataxia signs (INAS): validation of a new clinical assessment instrument. Cerebellum 2013;12(3):418–428.
31. Tzoulis C, Neckelmann G, Mørk SJ, et al. Localized cerebral energy failure in DNA polymerase gamma-associated encephalopathy syndromes. Brain 2010;133(5):1428–1437.
32. Uusimaa J, Gowda V, McShane A, et al. Prospective study of POLG mutations presenting in children with intractable epilepsy: prevalence and clinical features. Epilepsia 2013;54(6):1002–1011.
33. Faul F, Erdfelder E, Lang AG, Buchner A. G*power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39(2):175–191.
34. Reetz K, Dogan I, Hilgers R-D, et al. Progression characteristics of the European Friedreich's ataxia consortium for translational studies (EFACTS): a 2 year cohort study. Lancet Neurol 2016;15(13):1346–1354.
35. Traschutz A, Schirinzi T, Laugwitz L, et al. Clinico-genetic, imaging and molecular delineation of COQ8A-ataxia: a multicenter study of 59 patients. Ann Neurol 2020;88(2):251–263.
36. Jacobi H, du Montcel ST, Bauer P, et al. Long-term disease progression in spinocerebellar ataxia types 1, 2, 3, and 6: a longitudinal cohort study. Lancet Neurol 2015;14(11):1101–1108.
37. Giordano I, Harmuth F, Jacobi H, et al. Clinical and genetic characteristics of sporadic adult-onset degenerative ataxia. Neurology 2017;89(10):1043–1049.
38. Hikmat O, Naess K, Engvall M, et al. Simplifying the clinical classification of polymerase gamma (POLG) disease based on age of onset; studies using a cohort of 155 cases. J Inherit Metab Dis 2020;43(4):726–736.
39. Hikmat O, Naess K, Engvall M, et al. The impact of gender, puberty, and pregnancy in patients with POLG disease. Ann Clin Transl Neurol 2020;7(10):2019–2025.
40. Yasui K, Yabe I, Yoshida K, et al. A 3-year cohort study of the natural history of spinocerebellar ataxia type 6 in Japan. Orphanet J Rare Dis 2014;9:118.
41. Antenora A, Bruzzese D, Lieto M, et al. Predictors of survival in spinocerebellar ataxia type 2 population from southern Italy. Neurol Sci 2018;39(11):1857–1860.

42. Klockgether T, Ludtke R, Kramer B, et al. The natural history of degenerative ataxia: a retrospective study in 466 patients. Brain 1998;121(Pt 4): 589–600.

43. Lecocq C, Charles P, Azulay JP, et al. Delayed-onset Friedreich's ataxia revisited. Mov Disord 2016;31(1):62–69.

44. Saneto RP, Lee IC, Koenig MK, et al. POLG DNA testing as an emerging standard of care before instituting valproic acid therapy for pediatric seizure disorders. Seizure 2010;19(3):140–146.

45. Stewart JD, Horvath R, Baruffini E, et al. Polymerase gamma gene POLG determines the risk of sodium valproate-induced liver toxicity. Hepatology 2010;52(5):1791–1796.

46. Jacobi H, Bauer P, Giunti P, et al. The natural history of spinocerebellar ataxia type 1, 2, 3, and 6: a 2-year follow-up study. Neurology 2011;77(11):1035–1041.

47. Tzoulis C, Engelsen BA, Telstad W, et al. The spectrum of clinical disease caused by the A467T and W748S POLG mutations: a study of 26 cases. Brain 2006;129(Pt 7):1685–1692.

48. Tchikviladzé M, Gilleron M, Maisonobe T, et al. A diagnostic flow chart forPOLG-related diseases based on signs sensitivity and specificity. J Neurol Neurosurg Psychiatry 2015;86(6):646–654.

49. Morral JA, Davis AN, Qian J, Gelman BB, Koeppen AH. Pathology and pathogenesis of sensory neuropathy in Friedreich's ataxia. Acta Neuropathol 2010;120(1):97–108.

50. Ouvrier RA, McLeod JG, Conchin TE. Friedreich's ataxia. Early detection and progression of peripheral nerve abnormalities. J Neurol Sci 1982;55(2):137–145.

51. Buchner H, Schönlau L. Reproduzierbarkeit der Neurografie – explorative Untersuchung. Klin Neurophysiol 2019;50(02): 83–88.

52. Murphy SM, Herrmann DN, McDermott MP, et al. Reliability of the CMT neuropathy score (second version) in Charcot-Marie-tooth disease. J Peripher Nerv Syst 2011;16(3):191–198.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

SGML and CITI Use Only
DO NOT PRINT

Author Roles

Friedemann Bender, MD: organization and exuection of research project; design, execution, and review and critique of statistical and data analysis; writing of the first draft and review and critique of the manuscript.

Dagmar Timmann, MD: execution of research project; review and critique of manuscript.

Bart van de Warrenburg, MD, PhD: execution of research project; review and critique of manuscript.

Astrid Daniela Adarnes-Gòmez, MD: execution of research project; review and critique of manuscript.

Benjamin Bender, MD: review and critique of data analysis; review and critique of manuscript.

Andreas Thieme, MD: execution of research project; review and critique of manuscript.

Matthis Synofzik, MD: execution of research project; review and critique of manuscript.

Ludger Schöls, MD: conception, organization, and execution of research project; design and review and critique of statistical and data analysis; writing of the first draft and review and critique of the manuscript.

Full Financial Disclosures (for the Preceding 12 Months)

F. Bender reports no disclosures. D. Timmann has received funding from the German Research Foundation (DFG), Bernd Fink-Foundation, and German Heredoataxia Foundation (DHAG) in the last 12 months unrelated to this work. B. van de Warrenburg received funding from Radboundumc, ZonMW, Hersenstichting, Gossweiler Foundation, and uniQure and is a member of the scientific advisory board of uniQure. A.D. Adarmes-Gómez received honoraria from AbbVie, Italfarmaco, Bial, VCB, Zambon, and TEVA. B. Bender is cofounder of and employed as CTO AIRAmed GmbH, unrelated to this work. A. Thieme reports no disclosures. M. Synofzik reports consultancy honoraria from Orphayzme Pharmaceuticals and Janssen Pharmaceuticals, unrelated to this work. L. Schöls received unrelated support of the European Union (grant 947588), the German Ministry of Education and Research (BMBF; grants 01GM1905A and 01GM1907A), and the German Ministry of Health (BMG; grant ZMVI1-2520DAT94E) as well as from the Innovation Fonds of the GBA (grants 01NVF16024 and 01NVF17031).