BRAIN
ORIGINAL ARTICLE

Phenotype and natural history of mitochondrial membrane protein-associated neurodegeneration

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Mitochondrial membrane protein-associated neurodegeneration (MPAN) is an ultraorphan neurogenetic disease from the group of neurodegeneration with brain iron accumulation (NBIA) disorders. Here we report cross-sectional and longitudinal data to define the phenotype, to assess disease progression and to estimate sample sizes for clinical trials.

We enrolled patients with genetically confirmed MPAN from the Treat Iron-Related Childhood-Onset Neurodegeneration (TIRCON) registry and cohort study, and from additional sites. Linear mixed-effect modelling (LMEM) was used to calculate annual progression rates for the Unified Parkinson's Disease Rating Scale (UPDRS), Barry–Albright Dystonia (BAD) scale, Schwab and England Activities of Daily Living (SE-ADL) scale and the Pediatric Quality of Life Inventory (PedsQL).

We investigated 85 MPAN patients cross-sectionally, with functional outcome data collected in 45. Median age at onset was 9 years and the median diagnostic delay was 5 years. The most common findings were gait disturbance (99%), pyramidal involvement (95%), dysarthria (90%), vision disturbances (82%), with all but dysarthria presenting early in the disease course. After 16 years with the disease, 50% of patients were wheelchair dependent. LMEM showed an annual progression rate of 4.5 points in total UPDRS. The total BAD scale score showed no significant progression over time. The SE-ADL scale and the patient- and parent-reported PedsQL showed a decline of 3.9%, 2.14 and 2.05 points, respectively. No patient subpopulations were identified based on longitudinal trajectories.

Our cross-sectional results define the order of onset and frequency of symptoms in MPAN, which will inform the diagnostic process, help to shorten diagnostic delay and aid in counselling patients, parents and caregivers. Our longitudinal findings define the natural history of MPAN, reveal the most responsive outcomes and highlight the need for an MPAN-specific rating approach. Our sample size estimations inform the design of upcoming clinical trials.

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Introduction

Mitochondrial membrane protein-associated neurodegeneration (MPAN) is a rare monogenic disease from the group of neurodegeneration with brain iron accumulation (NBIA) disorders.[1] It was found in 2011 to be caused by pathogenic variants in the *C19orf12* gene on chromosome 19,[2] which encodes a membrane protein of unknown function, located in mitochondria, endoplasmic reticulum (ER) and mitochondria-ER associated membranes (MAM). With a lifetime risk in the worldwide population of 0.01 per 100 000, MPAN is an ultraorphan disorder.[3] While mostly inherited as an autosomal recessive trait, clinically indistinguishable cases with an autosomal dominant inheritance pattern have been described.[4,5]

Since the first publication in 2011, the clinical phenotype has been described in single case reports and short case series. In most patients, MPAN begins in childhood and manifests with a pallido-pyramidal syndrome, cognitive decline and psychiatric and neuro-ophthalmological abnormalities.[6] Despite single reports of patients with 'atypical' presentations, no clinical subgroups have been defined until now.[7]

There are currently no disease-modifying therapies for MPAN.[8] The lack of understanding of the pathomechanisms of the disease and its progression as well as small patient populations are among the challenges in the development of orphan drugs and subsequent clinical trials.

Patient registries and natural history studies are important pillars in the field of rare diseases enabling a systematic analysis of patient cohorts. They improve the understanding of the disease course and aid the identification of suitable primary and secondary outcome measures for clinical trials. In 2011, international NBIA researchers founded a research consortium—TIRCON (Treat Iron-Related Childhood-Onset Neurodegeneration).[9] Part of this collaborative project was the development of an international NBIA registry and cohort study allowing for prospective longitudinal observation and examination of patients with NBIA disorders. Here we present the first analysis of the MPAN patient cohort from this resource.

Materials and methods

Study design and participants

The current analysis includes data from patients with genetically confirmed MPAN from the TIRCON cohort. All patients or their legal guardians signed a written informed consent upon enrollment in the TIRCON registry. The TIRCON study protocol was approved by local ethics committees. It is registered with https://clinicaltrials.gov (NCT05522374).

For the cross-sectional analysis, additional retrospective data on MPAN patients from two TIRCON-associated sites in Moscow, Russia and Tehran, Iran were included. These patients or their legal guardians signed a written informed consent allowing the pseudonymized usage of their data for scientific purposes.

Procedures

In the framework of the TIRCON registry, structured and comprehensive patient interviews were performed at every visit. Data on demographic and genetic characteristics, family history and disease history were obtained and annually updated. A detailed neurological examination was conducted by trained neurologists at all participating centres according to the same study protocol. The cardinal symptoms of the disease were documented with year of onset.

In our prospective longitudinal analysis, the clinical outcome measures were the Unified Parkinson's Disease Rating Scale (UPDRS) Parts I–III, the Barry–Albright Dystonia (BAD) scale, the Schwab and England Activities of Daily Living (SE-ADL) scale and the Pediatric Quality of Life Inventory (PedsQL).

Each question item of the UPDRS can be rated between 0 and 4 points. A higher score implies increased severity. Part I contains four questions with a possible total score between 0 and 16 points and provides an assessment of mentation, behaviour and mood. Part II, with a range between 0 and 52 points, presents the patient's self-evaluation of activities of daily living (ADLs), such as speech, swallowing, handwriting etc. Part III presents a scale based on the detailed neurological examination of the motor system, including evaluation of *inter alia* fine movements, gait, rigidity and tremor, and reflects the physicians' assessment of patients' motor abilities, with a total score between 0 and 108 points. The sum of the scores from the three parts yields what we are going to regard as the UPDRS total score in this paper, with a range between 0 and 176 points.

The BAD scale is an instrument for the assessment of dystonia and consists of eight sub-items, each representing a separate body region. Each item receives a score between 0 and 4 points, with 0 points corresponding to no and 4 points to severe dystonia. The total score ranges from 0 to 32 points.

The SE-ADL scale measures the independence of patients during their everyday activities and provides a percentage score between 0% (fully dependent) and 100% (completely independent).

The PedsQL is a patient- and parent-reported health-related quality of life outcome measure for a paediatric, adolescent and adult patient population with a total score ranging between 0 and 100, a higher score indicating better quality of life.

Statistical analysis

Demographic features, genetic data and body examination subitems are presented with total number and frequency or percentages. Age at onset, age at diagnosis and diagnostic delay are described by median and interquartile range (IQR). For each

cardinal symptom category of the disease, we calculated the median symptom onset and IQR. We compared the symptom categories using pairwise permutation tests for skewed data, allowing for dependency between the different categories, and created a symptom onset timeline. We used a Kaplan–Meier survival analysis to quantify the probability of remaining free of wheel-chair dependency.

We estimated the yearly progression rate for each outcome using linear mixed-effect models (LMEM) with restricted-maximum-likelihood estimation with years into disease as a time variable, allowing us to include patients with single entries in the analysis. Heteroscedasticity was handled by estimating robust standard errors using the Huber–White Sandwich Estimator. We examined the impact of demographic and disease-related variables (e.g. age at onset, sex, genotype constellation) on predicting the outcome measures and their progression. Using a piecewise analysis, we allowed for a change in the slope of the linear annual progression at one point in the disease course, tested for an optimal break-point and compared the piecewise versus the non-piecewise model. Model selection was performed considering the Bayesian information criterion (BIC). Tobit regression analysis was used to compensate for floor or ceiling effects in the UPDRS and BAD subitem analysis.

For the functional outcome measures, we subset information from patients with follow-up after 1 and 2 years and calculated a standardized response means (SRM) by dividing the mean change in scores from baseline to follow-up by the standard deviation of the change and used this as a measure of the responsiveness of the outcome.

Using latent growth curve modelling (LGCM) and growth mixture modelling (GMM), we tested for unobserved patient subpopulations based on their individual trajectories. Latent growth curve and growth mixture models with different numbers of classes (one to three) were built and compared with a zero-class model, assuming no patient subpopulations. Based on its ability to fit the data, the best model was selected.

Sample size estimation was performed for a 1:1 allocated placebo-controlled randomized clinical trial (RCT) with a duration of 18 months. Based on our natural history findings, we simulated longitudinal patient data for two at baseline identical groups (placebo and treatment) and modelled the targeted changes using an LMEM-based approach. We calculated the sample size needed to detect a 25%, 50%, 75% and 100% reduction of UPDRS, SE-ADL and PedsQL progression at 80% power. Data processing and statistical analysis were carried out using R version 4.2.0.

Results

The first prospective baseline visit by the first patient took place on 11 February 2010, and the last follow-up visit by the last patient took place on 05 May 2022. Within the TIRCON registry, 60 patients with genetically confirmed MPAN disease were recruited in five centres: Warsaw, Poland ($n = 40$); Munich, Germany ($n = 16$); Prague, the Czech Republic ($n = 2$); Ottawa, Canada ($n = 1$); and Groningen, the Netherlands ($n = 1$).

For the cross-sectional analysis, data from an additional 38 patients from associated clinical centres were submitted, but 10 of these patients had to be excluded due to missing genetic information. Accordingly, 28 additional patients were analysed, and these subjects were from Moscow, Russia ($n = 17$) and Tehran, Iran ($n = 11$).

Cross-sectional data on the patients from the centre in Moscow and 24 of the patients from the centre in Warsaw were published in parts prior to this analysis.[2,10,11] Three patients from the Iranian cohort had already been seen and examined at one of the TIRCON centres. Accordingly, the respective data for each of these three patients were merged.

Cross-sectional analysis

In total, we obtained and assessed cross-sectional data from 85 patients (53% male) with molecularly diagnosed MPAN. Sixty-nine patients were of Caucasian and 16 of Asian ethnicity (Table 1).

All patients presented an autosomal recessive inheritance pattern, with 79% being homozygous and 21% compound heterozygous for pathogenic variants (Supplementary Table 1). The most

Table 1 Demographic characteristics

	Cross-sectional analysis ($N = 85$)	Longitudinal analysis ($N = 45$)[a]
Sex, male, *n* (%)	45 (53%)	24 (53%)
Age at onset, years, median (IQR)	9 (4)	8·5 (4)
Age at diagnosis, years, median (IQR)	14 (5)	12 (2–5)
Ethnic background, *n* (%):		
White European	69 (81%)	38 (84%)
Asian	16 (19%)	7 (16%)
Consanguinity of parents[b], *n* (%)	21 (25%)	10 (22%)

IQR = interquartile range.
[a]Number of patients with at least one completed functional outcome measure.
[b]Data were missing for one patient.

Table 2 Clinical phenotype based on neurological bedside examination and patient history for all the patients included in the analysis ($n = 85$)

Findings	*n* (*N*)	%
Gait abnormality	79 (80)	99
Pyramidal involvement	78 (82)	95
Dysarthria	70 (78)	90
Vision abnormalities	67 (82)	82
Neurocognitive abnormalities	60 (82)	73
Skeletal abnormalities	57 (79)	72
Dystonia	58 (83)	70
Psychiatric abnormalities[a]	53 (85)	62
Muscle weakness	44 (71)	62
Muscle atrophy	42 (83)	51
Dysphonia	24 (76)	32
Parkinsonism	24 (79)	30
Ataxia	19 (67)	28
Visual field defects	14 (56)	25
Dyskinesias	16 (82)	20
Sensory abnormalities	14 (78)	18
Aphasia	7 (75)	9
Internal organ abnormalities	6 (81)	7
Myoclonus	3 (63)	5
Fasciculations	1 (63)	2
Hearing abnormality	1 (66)	2
Olfaction	0 (83)	0

N = number of patients with completed information for the corresponding finding.
n = number of patients where the finding is present.
[a]Itemized in Supplementary Table 2.

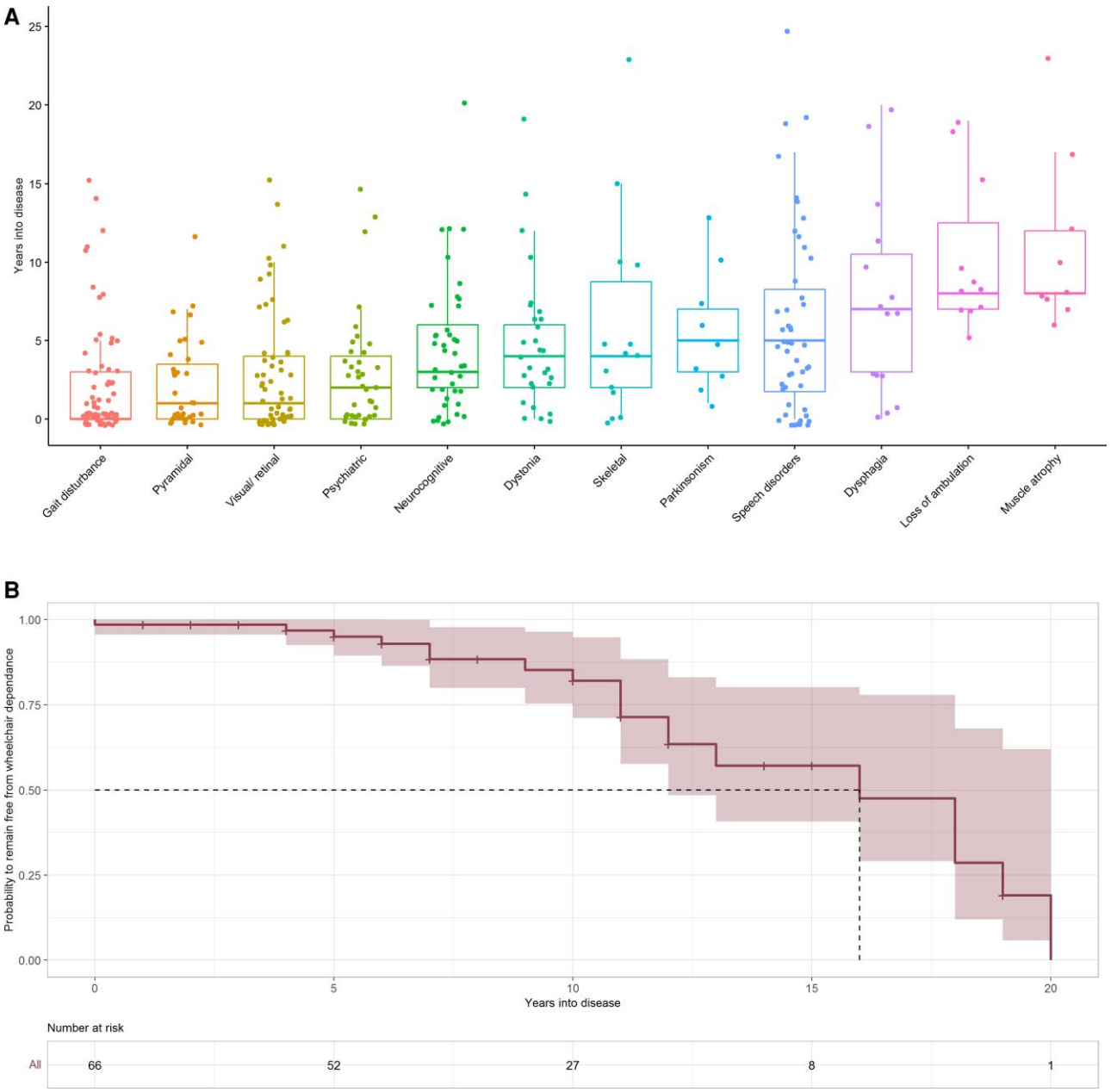

Figure 1 Order of occurrence of symptoms and Kaplan–Meier curve showing probability to remain free from wheelchair dependence during the course of the disease. (**A**) A timeline of symptom occurrence based on their median onset is shown using a combination of a box plot and a scatter plot. (**B**) The Kaplan–Meier curve with 95% confidence interval is shown. *Bottom*: The numbers at risk after 0, 5, 10, 15 and 20 years of the disease are presented.

commonly observed variant was the Polish founder frameshift deletion c.204_214del11 (Gly69ArgfsX10), which was found in 66% of patients in either a homozygous or compound heterozygous state.

Age at onset ranged from 3 to 18 years with a median of 9 years (IQR: 4 years). The median age at diagnosis was 14 years (IQR: 5 years) and the median diagnostic delay, calculated for each patient, was 5 years (IQR: 4 years).

Analysis of the data from the patients' clinical examinations revealed the most common findings to be: gait disturbance (99%); pyramidal involvement (95%); dysarthria (90%); and vision disturbances (82%) (Table 2). Neurocognitive symptoms were common, documented in 73% of patients. Extrapyramidal movement disorders such as dystonia and parkinsonism were seen in 70% and 30% of patients, respectively. Ataxia was present in only 28% of patients. Olfaction abnormalities, fasciculations, hearing impairment and myoclonus were not or barely seen in our cohort (Table 2).

The most frequently observed neuroophthalmological abnormality was pale optic head (62% of all patients). Additionally, 63% of the patients presented with psychiatric abnormalities. Of these, more than 50% experienced anxiety. Attention deficit hyperactivity disorder (ADHD)-like phenotype and pathological laughing/crying were the next most common psychiatric manifestations (Supplementary Table 2).

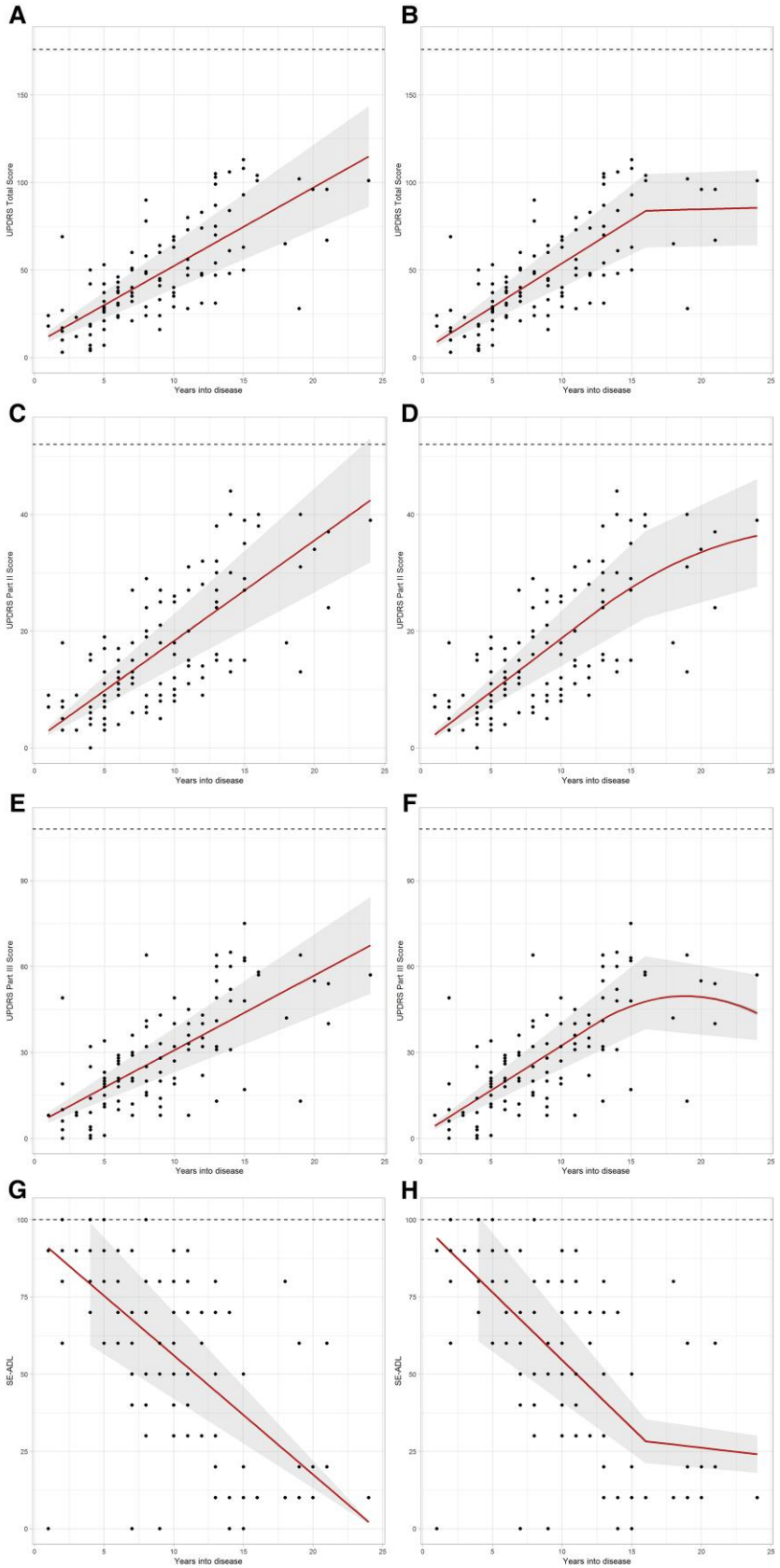

Figure 2 Progression rate of the UPDRS total score, UPDRS Parts II and III, and SE-ADL. (**A–H**) In this paper, the Unified Parkinson's Disease Rating Scale (UPDRS) total score is the sum of the scores from Parts I–III. Each point represents a single observation. One patient can have multiple observations. (**A**) UPDRS total score progression fitting a linear mixed-effect model using years into the disease as a time variable. (**B**) UPDRS total score progression fitting a piecewise linear mixed-effect model with a breakpoint at 16 years into the disease. (**C**) UPDRS Part II progression fitting a linear

(Continued)

Symptom order based on onset

The cardinal symptoms were grouped into categories and arranged from earliest to latest based on the median symptom onset (Fig. 1A). We performed pairwise permutation testing to identify which symptoms differed significantly from one another (Supplementary Table 3). The most frequent early symptoms were gait disturbance, pyramidal involvement, psychiatric abnormalities and ophthalmological disorders, including visual disturbances and retinal pathologies. In contrast, speech disorders, as well as dysphagia, presented later in the disease course, with median symptom onset ≥5 years into the disease course.

Wheelchair dependency

Kaplan–Meier survival analysis based on data from 67 patients, using wheelchair dependency as the outcome measure, revealed that 50% of patients are wheelchair-dependent 16 years after disease onset (95% confidence interval: 12, 20) (Fig. 1B).

Natural history analysis

Annual progression rates

Forty-five patients had at least one observation of a functional outcome measure. Median disease duration of the patients included in the analysis was 9 years (minimum 1; maximum 24; IQR: 7.25). Five patients were treated with deferiprone during the observation period. Three patients had taken deferiprone previously, but the treatment had been discontinued before inclusion in the registry. Because of the small number of patients, varying visit numbers and medication dosages, the effect of deferiprone on disease progression could not be assessed.

Unified Parkinson's Disease Rating Scale

Using 108 observations from 38 patients, we estimated an average annual progression rate (APR) of 4.5 points [standard error (SE) 0.57] for the UPDRS total score (Fig. 2A). We found no difference in the progression rate between males and females. There was also no significant effect for genotype (homozygous versus compound heterozygous variants), ethnicity or age at onset. A piecewise LMEM, allowing for a change in the progression, showed an average annual progression rate of 5.1 points up to the 16th year into the disease and a subsequent plateau with no further significant change (Fig. 2B). The UPDRS subscore for Part I (mentation) did not show any significant progression over time (APR 0.12, SE 0.06). Age at onset, genotype and sex did not affect the score. The annual progression rates for Parts II and III were 1.72 points (SE 0.19, $P < 0.001$) and 2.61 points (SE 0.35, $P < 0.001$), respectively (Fig. 2C–F). No relevant significant predictors (including age at onset, ambulation or genotype) were found.

We estimated the annual progression rates for all UPDRS subitems (Supplementary Table 4). In Part I, a borderline significant annual progression rate was found for two of the four sub-items —mentation and motivation. In Part II, annual progression rates were highest for freezing while walking and turning in bed. For these, we found strong ceiling effects which we accounted for by using Tobit analysis. In Part III, arising from chair, leg agility and hand grips showed the highest annual progression rate. In contrast, sub-items associated with rigidity of the lower limbs and tremor did not show significant progression over time.

Barry–Albright Dystonia scale

We estimated the progression of the BAD scale total score to be 0.17 points (SE 0.13, $P = 0.20$) per year which is not significant and presumably not clinically relevant. The BAD sub-items for eyes, mouth, neck and trunk presented with a median of 0 points (IQR: 0) across the disease course, showing no relevant dystonia in these regions. Although dystonia of the upper extremities was observed in some patients, no significant change over time was found. Significant annual progression was found only for the BAD subitems representing the lower extremities, with an annual progression rate of 0.10 points (SE 0.03, $P < 0.01$) for the right side and 0.12 points (SE 0.03, $P < 0.01$) for the left side. This indicated slowly progressive symmetrical involvement of the legs.

Schwab and England Activities of Daily Living scale

The SE-ADL scale showed significant decline over time. The only predictor of the annual progression was ambulation. In a piecewise model with an interaction term for ambulation, we estimated an annual decline of 4.14 points for ambulatory patients in the first 16 years of disease (Fig. 2G and H). With loss of ambulation, a plateau was reached, and no subsequent significant decrease over time was found.

Pediatric Quality of Life Inventory

We found an annual decrease of the total patient-reported PedsQL score by 2.14 points (SE 0.45, $P < 0.001$) (Fig. 3A). The only significant predictor of the total score was ambulation with ambulatory patients exhibiting higher PedsQL scores by 13.6 points (SE 4.46, $P < 0.005$). The parent-reported PedsQL score showed an annual decrease of 2.05 points (SE 0.47, $P < 0.001$; Fig. 3B). There were no significant predictors. Ambulation showed a borderline significant effect with ambulatory patients exhibiting higher scores by 9.76 points (SE 4.99, P 0.054). The Pearson correlation coefficient between the patient- and parent-reported PedsQL was 0.77, showing high linear correlation between the two scores.

Responsiveness

The highest responsiveness at 2-year-follow-up was found for the patient-reported outcome measures SE-ADL (–1.01) and parent-reported PedsQL (–0.81) (Table 3). Despite no significant annual progression in the LMEM, the BAD scale showed a high index of change, explained by the low standard deviation of the calculated change. The patient-reported UPDRS Part II subscore revealed an SRM of 0.68 higher responsiveness than the total score and the other subscores (Table 3). In the sub-item level analysis, the highest SRM values after a follow-up of 2 years were found for freezing

Figure 2 Continued
mixed-effect model using years into the disease as a time variable. (**D**) UPDRS Part II progression fitting a piecewise linear mixed-effect model with a breakpoint at 16 years into the disease. For UPDRS Part II, the general model in **C** was superior to the piecewise model in **D**. (**E**) UPDRS Part III progression fitting a linear mixed-effect model using years into the disease as a time variable. (**F**) UPDRS Part III progression fitting a piecewise linear mixed-effect model with a breakpoint at 16 years into the disease. For UPDRS Part III, the piecewise model in **F** was superior to the general model in **E**. (**G**) Schwab and England Activities of Daily Living (SE-ADL) progression fitting a linear mixed-effect model using years into the disease as a time variable. (**H**) SE-ADL progression fitting a piecewise linear mixed-effect model with a breakpoint at 16 years into disease and an interaction term for ambulation. For SE-ADL, the piecewise model in **H** was superior to the general model in **G**.

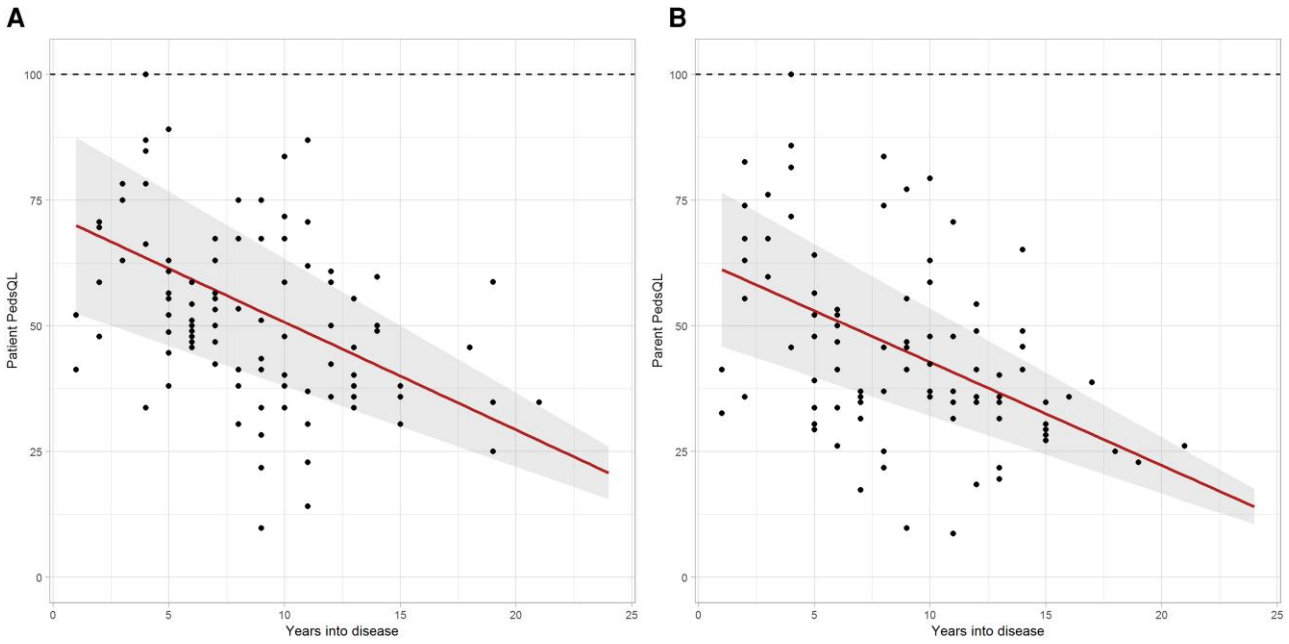

Figure 3 Progression rate of patient- and parent-reported Pediatric Quality of Life Inventory (PedsQL). (**A** and **B**) Piecewise models were found to be inferior to the general linear mixed-effect model and were thus not included in the figure. Each point represents a single observation. One patient can have multiple observations. (**A**) Patient-reported PedsQL progression fitting a linear mixed-effect model using years into disease as a time variable. (**B**) Parent-reported PedsQL progression fitting a linear mixed-effect model using years into disease as a time variable.

Table 3 Standardized response means

Scale	SRM 1-year follow-up	SRM 2-year follow-up
UPDRS subscore 1	−0.14	0.12
UPDRS subscore 2	0.48	0.68
UPDRS subscore 3	0.17	0.32
UPDRS total score	0.26	0.51
BAD scale	0.32	0.80
SE-ADL	−0.22	−1.01
Patient-reported PedsQL	−0.28	−0.64
Parent-reported PedsQL	−0.22	−0.81

BAD scale = Barry–Albright Dystonia scale; PedsQL = Pediatric Quality of Life Inventory; SE-ADL = Schwab and England Activities of Daily Living; SRM = standardized response mean (i.e. mean change compared to baseline divided by the standard deviation of the mean change); UPDRS = Unified Parkinson's Disease Rating Scale. UPDRS Total Score in this paper is the sum of scores from Parts I–III.

when walking and walking for the UPDRS Part II and hand grips and gait for the UPDRS Part III (Supplementary Table 5).

Growth analysis

To investigate whether we could identify unobserved patient subpopulations in our MPAN cohort based on the growth trajectories of their UPDRS total scores, and UPDRS Parts II and III subscores, we built and compared latent growth curve models and growth mixture models with one, two and three classes. Models with more than one patient class were found to be inferior to the model assuming no patient subpopulations.

Power calculations

In an 18-month parallel-group trial, 900 (450 per group), 1600 (800 per group) and 2600 (1300 per group) patients would be needed for the detection of a 50% progression reduction of the UPDRS total score, the patient-derived PedsQL and the SE-ADL, respectively (Table 4 and Fig. 4).

Discussion

Our cross-sectional analysis defines the phenotype of MPAN in the largest cohort to date. It provides novel insight into non-neurological symptoms, e.g. psychiatric abnormalities, specifying anxiety and ADHD to be the most common psychiatric symptoms. Furthermore, our findings substantiate previously reported findings[12] that optic atrophy is the predominant retinal manifestation in MPAN.

The previously described leading role of pyramidal symptoms in the clinical syndrome was confirmed, while movement disorders such as dystonia and parkinsonism were less common. Furthermore, we examined symptom onset and were able to create a timeline of symptom occurrence, differentiating between early symptoms (e.g. gait disturbance, psychiatric symptoms, visual disturbances) and late symptoms (e.g. speech disorders, dysphagia, muscle atrophy). A significant limitation of our analysis was the number of missing data on symptom onset, which may have affected the accuracy and completeness of our results.

Our study provides the first prospective, longitudinal natural history study of MPAN patients. It is also the first study to examine changes in clinical outcome measures over time and their ability to capture disease progression. We quantified the annual progression rate of the UPDRS total score and estimated a progression of 4.5 points. Although psychiatric features are seen in more than 60% of patients with MPAN, as shown in our cross-sectional analysis, Part I of the UPDRS failed to capture these and showed no overall progression as well as no significant responsiveness over time. The questions target symptoms (e.g. depression, thought disorders

Table 4 Sample size estimation for a 1:1 allocated placebo-controlled randomized clinical trial with a duration of 18 months at power 80%

	Reduction of the annual progression rate by:			
	25% N (n)	50% N (n)	75% N (n)	100% N (n)
UPDRS total	3400 (1700)	900 (450)	440 (220)	250 (125)
SE-ADL	10 000 (5000)	2600 (1300)	1100 (550)	600 (300)
Patient-reported PedsQL	5400 (2700)	1600 (800)	600 (300)	340 (170)
Parent-reported PedsQL	7600 (3800)	1800 (950)	800 (400)	400 (200)

N = total sample size; n = sample size per group. PedsQL = Pediatric Quality of Life Inventory; SE-ADL = Schwab and England Activities of Daily Living; UPDRS = Unified Parkinson's Disease Rating Scale. UPDRS total score in this paper is the sum of scores from Parts I–III.

such as vivid dreaming and hallucinations) that are not common in MPAN patients. In turn, anxiety and ADHD-like behaviours, which are common in MPAN, are not assessed by the UPDRS. Furthermore, cognitive impairment, which was seen in our patient population, is defined in the UPDRS mainly by forgetfulness and memory loss, which is more suited to assessing cognitive impairment in an elderly population, e.g. with Parkinson's disease (for which the scale was originally created), than in a patient population consisting of children, adolescents and young adults as in MPAN.

Interestingly, UPDRS Part II, which represents the patient-reported assessment of activities of daily living, showed the highest responsiveness over a follow-up period of 2 years among the UPDRS subscores. Sub-items such as gait freezing and turning in bed were found to be important in the evaluation of MPAN patients. Furthermore, cutting food and dressing showed higher annual progression rates in comparison to activities such as handwriting, which should be considered in future questionnaires.

Our analysis of the detailed motor examination in the UPDRS Part III highlighted the importance of sub-items, testing motor functions of the extremities (e.g. leg agility, hand grips) as well as overall mobility. The lack of significant progression of items such as tremor and rigidity is consistent with the observation in our cross-sectional analysis, where we found parkinsonism in less than one-third of patients.

Furthermore, we explored the progression of the BAD scale, an instrument for the assessment of dystonia. Despite dystonia being present in more than two-thirds of patients in our wider cross-sectional analysis, the BAD scale did not reflect a significant progression over time. In our cohort, only dystonia of the lower extremities showed a slow progression over time. Most of the patients did not present with any axial (trunk and neck), oromandibular or periorbital dystonia, which account for half of the BAD total score. This highlights why the scale is not suitable for the assessment of MPAN patients as opposed to patients with disorders such as panthotenate kinase-associated neurodegeneration (PKAN), where generalized dystonia defines the clinical phenotype.

The patient- and parent-reported outcome measures SE-ADL and PedsQL showed a progressive decline over time. A significant predictor was ambulation, highlighting the importance of therapies to improve or sustain walking ability in patients.

Using a growth curve analysis, we were unable to identify different patient subpopulations based on the longitudinal progression of motor deterioration. A limitation of our study was the predominant inclusion of patients of European origin in the TIRCON registry. In the literature, potential 'atypical' cases with late-onset (in adulthood) and subacute rapid motor deterioration have been described mainly in patients with the mutation c.32C>T (p.Thr11Met)7. In our natural history cohort, such patients were underrepresented, with only one patient being homozygous and two patients heterozygous for this mutation. For future studies, a prospective longitudinal analysis of MPAN patients from countries where this mutation is more prevalent (e.g. Turkey[13]) is needed. Likewise, the number of patients and distribution of genotypes did not allow for robust genotype-phenotype correlations in this cohort. A further limitation of our study was the lack of autosomal dominant MPAN cases.

Our analysis of the above-described outcome measures highlights the need to develop an MPAN-specific rating approach. Based on our cross-sectional results, sub-items suitable for capturing cognitive impairment and psychiatric symptoms in a young patient population are needed. Our longitudinal analysis highlighted the importance of patient- and parent-derived assessment of activities of daily living. Sub-items capturing the patient's mobility (e.g. gait) and motor skills of the upper and lower extremities are of importance. Because of their low prevalence, rigidity and tremor should not be included.

Because of the observed high frequency and early start of pyramidal involvement, the inclusion of an assessment of spasticity and hyperreflexia in an MPAN-specific scale seems reasonable.

We provide the first sample size estimations in an MPAN cohort for future clinical trials. Our results highlight the challenges in the planning of a placebo-controlled RCT in an ultraorphan disease. We found high inter-individual variability in the patient cohort of our natural history study, which impedes the set-up of trials compared with more homogenous patient cohorts. Our calculations display the high number of patients needed to detect an effect of 25% or 50% slowing of progression and highlight the importance of alternative, more specific, outcome measures. In this context, for other disorders (e.g. Alzheimer's disease), drugs have been approved by the US Food and Drug Administration through the accelerated approval pathway based on their effect on a surrogate end point, which is anticipated, but not proven, to predict clinical benefit.[14] This showcases the necessity of research aimed at identifying biomarkers and surrogate end points in MPAN.

In conclusion, we provide the first quantitative estimations of disease progression in MPAN, reveal the most responsive outcome measures, highlight the importance of an MPAN-specific approach and build the basis for the planning of future clinical trials.

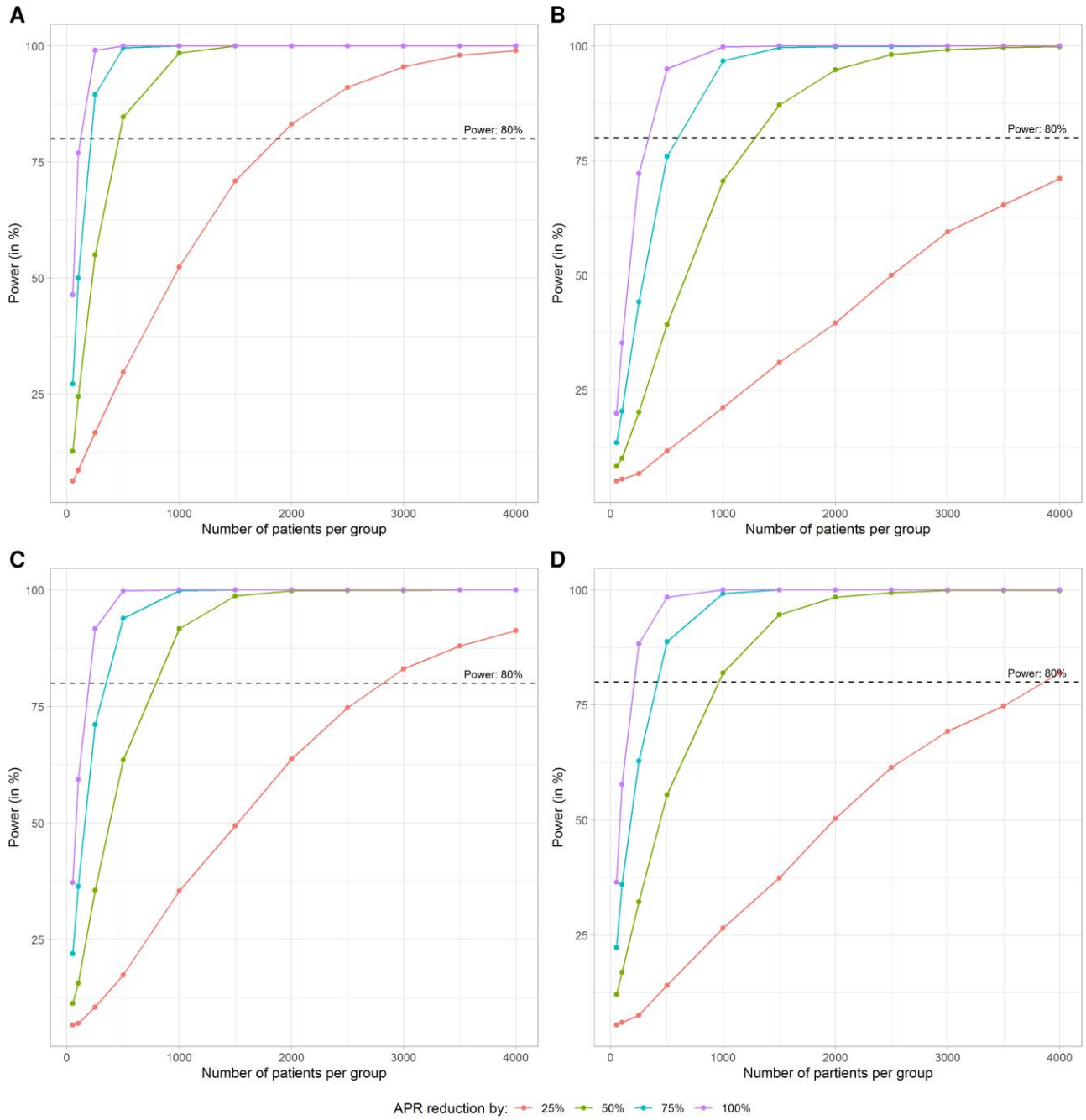

Figure 4 Sample size estimates for a for a 1:1 allocated placebo-controlled randomized clinical trial with a duration of 18 months. (**A–D**) On the *x*-axis are depicted the number of patients per treatment group, not the total number of patients. (**A**) Sample size estimates with corresponding power for the Unified Parkinson's Disease Rating Scale (UPDRS) total score (sum of scores from Parts I–III). (**B**) Sample size estimates with corresponding power for Schwab and England Activities of Daily Living (SE-ADL). (**C**) Sample size estimates with corresponding power for the patient-reported Pediatric Quality of Life Inventory (PedsQL). (**D**) Sample size estimates with corresponding power for the parent-reported PedsQL. APR = annual progression rate.

Data availability

Individual de-identified participant data that underlies the results reported in this article (text, tables, figures and Supplementary material) and the study protocol will be shared with qualified external researchers upon reasonable request according to the criteria and process described by www.clinicalstudydatarequest.com. Researchers who provide a methodologically sound proposal can request de-identified data from the TIRCON steering committee by contacting the corresponding author.

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CoA Therapeutics (San Francisco, CA, USA) and Retrophin, Inc. (San Diego, CA, USA). The funding sources were not involved in the study design; collection, analysis and interpretation of data; writing the report; or the decision to submit the paper for publication.

Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

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