RESEARCH ARTICLE

Progression of Clinical and Eye Movement Markers in Preataxic Carriers of Machado-Joseph Disease

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ABSTRACT: Background: Little is known about preclinical stages of Machado-Joseph disease, a polyglutamine disorder characterized by progressive adult-onset ataxia.
Objective: We aimed to describe the longitudinal progression of clinical and oculomotor variables in the preataxic phase of disease.
Methods: Carriers and noncarriers were assessed at three visits. Preataxic carriers (Scale for Assessment and Rating of Ataxia score < 3) expected to start ataxia in ≤4 years were considered near onset (PAN). Progressions of ataxic and preataxic carriers, considering status at the end of the study, were described according to the start (or its prediction) of gait ataxia (TimeToAfterOnset) and according to the study time.
Results: A total of 35 ataxics, 38 preataxics, and 22 noncarriers were included. The "TimeToAfterOnset" timeline showed that Neurological Examination Scale for Spinocerebellar Ataxias (NESSCA; effect size, 0.09), Inventory of Non-Ataxia Symptoms (INAS0.07), and the vestibulo-ocular reflex gain (0.12) progressed in preataxic carriers, and that most slopes accelerate in PAN, turning similar to those of ataxics. In the study time, NESSCA (1.36) and vertical pursuit gain (1.17) significantly worsened in PAN, and 6 of 11 PANs converted to ataxia. For a clinical trial with 80% power and 2-year duration, 57 PANs are needed in each study arm to detect a 50% reduction in the conversion rate.
Conclusions: NESSCA, INAS, vestibulo-ocular reflex, and vertical pursuit gains significantly worsened in the preataxic phase. The "TimeToAfterOnset" timeline unveiled that slopes of most variables are small in preataxics but increase and reach the ataxic slopes from 4 years before the onset of ataxia. For future trials in preataxic carriers, we recommend recruiting PANs and using the conversion rate as the primary outcome. © 2022 International Parkinson and Movement Disorder Society.

Key Words: clinical scales; Machado-Joseph disease; preataxic period; spinocerebellar ataxia type 3; video-oculography

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Spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD) is an autosomal dominant disorder caused by a CAG repeat expansion at *ATXN3*,[1] related to a progressive ataxia, with no modifying or preventive therapy to date.[2,3] Model studies, as well as clinical and anatomopathological observations, suggest that the disease process starts years before onset of symptoms, and it is reasonable to assume that disease-modifying treatments might be more effective if initiated in preclinical stages.[3]

Clinical scales were developed to ataxic carriers of SCA3/MJD and other similar conditions. The change in time of these scales reflects the nature of gradually progressive disorders, with small effect sizes (ESs), requiring large sample sizes for future clinical trials (CT).[4-6] Little is known regarding the usefulness of these scales in the preclinical period.[7,8]

Outcomes other than clinical scales might help overcome ES issues. Eye movements are clearly dysfunctional,[9-12] may be good outcomes, and might start before ataxia onset.[8,13,14] However, longitudinal progressions of quantitative eye movement parameters are still unknown in SCA3/MJD.

The BIGPRO ("Biomarkers and genetic modifiers in a study of presymptomatic and symptomatic SCA3/MJD carriers") study was launched in 2017 (ClinicalTrials.gov: NCT04229823) to improve knowledge on clinical scales, video-oculography, and other potential biomarkers for disease progression in SCA3/MJD since premanifest disease. This report describes longitudinal results of clinical scales and video-oculography parameters, aiming to determine which variables have the highest sensitivities to change and which may act as biomarkers in preataxic stages.

Subjects and Methods

Population and Procedures

This was a single-center prospective study. Recruitment and procedures have been extensively detailed elsewhere.[8] In summary, ataxic subjects with a confirmed molecular diagnosis of SCA3/MJD followed up at Hospital de Clínicas de Porto Alegre, Brazil, were invited to participate. Healthy individuals at 50% risk of inheriting the mutation were also recruited. The genetic status was established for all participants: those at 50% risk were genotyped in a double-blind manner so that participants and direct examiners did not know their genetic status. DNA was isolated from peripheral blood leukocytes using standard methods. The CAG repeat length analysis was performed by polymerase chain reaction using fluorescent-labeled primers flanking the CAG repeat tract at *ATXN3*, followed by capillary electrophoresis into the genetic analyzer ABI3130xl (Applied Biosystems, Foster City, CA, USA). All investigators but M.-L.S.-P. and L.B.J. were kept blind to genotype results.

Each subject was planned to be assessed in three annual visits with clinical scales and eye movement recording using video-oculography. Evaluations included the Scale for Assessment and Rating of Ataxia (SARA),[15] Neurological Examination Scale for Spinocerebellar Ataxias (NESSCA),[16] the International Cooperative Ataxia Rating Scale (ICARS),[17] SCA Functional Index,[18] Composite Cerebellar Functional Score,[19] and Inventory of Non-Ataxia Symptoms (INAScount).[20] Investigators trained in the application of these clinical scales (C.M.d.O., A.H.C., A.G.R., G.E., G.B.) performed them.

One single investigator (C.M.d.O.) assessed oculomotor neurophysiology using video-oculography (EyeSeeCam, Interacoustics) with a monocular recording of the left eye in all subjects. Each evaluation consisted of two moments: video head-impulse test and oculomotor protocol. Calibration was performed before each one of these parts. Video head-impulse test was done with the subject sitting at 1.5 m from a fixed target on a wall with lights turned on. At least 10 head impulses for each side were performed in the horizontal plane, with amplitude of 10°–20° and velocity of 150°/s to 300°/s, peaking at around 80 ms from the beginning of the head movement. Afterward, the oculomotor protocol was done with the subject sitting with the eyes at 60 cm from monitor, the head resting on a fixator to avoid movement, and the lights turned off. Visual targets were 5.2-mm-diameter white circles displayed on the screen. After calibration, the protocol under study evaluated: (1) vertical reflexive saccades, elicited by random stimuli of 10° and 20° amplitude ranging ±10° from the central position; (2) vertical pursuit, with the target moving in a smooth pendular manner between ±10° of eccentricity; (3) vertical self-paced volitional saccades, a cognitive task, with subject instructed to alternate fixation between two fixed targets in a 20° vertical amplitude as fast as possible; and (4) gaze holding, initially with the subject fixating a target in a central position and then in eccentricities of ±20° horizontally.

Vestibulo-ocular reflex (VOR) gain (the ratio between eye and head velocity) was chosen as the main outcome to measure vestibular function. The mean regression slope between VOR gains (VORr) in the time interval of 10 ms before to 100 ms after the onset of the impulse for both sides was chosen as the main measurement. Outcomes for eye movement abnormalities were chosen as described elsewhere[8]: (1) vertical pursuit gain, calculated by the mean of regression slopes of the eye and target velocities of upward and downward pursuit; the main sequence[21] of (2) reflexive (reflex vertical saccade slope) and (3) volitional vertical saccades (slope), estimated by the mean regression slope of saccadic peak duration versus amplitude; (4) slow-phase velocity of gaze-evoked nystagmus (SPV-GE), considered the mean velocity on the horizontal plane during lateral fixation on both sides; and (5) slow-phase velocity of central fixation nystagmus (SPV-C) in the horizontal plane during central fixation.

During visits 2 and 3, subjects completed the Patient Global Impression of Change (PGI-C), in which they were asked to describe their symptoms as follows: very much improved (1), much improved (2), minimally improved (3), no change (4), worse (5), much worse (6), and very much worse (7).

This study was approved by the Institutional Ethics Committee (Comissão de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre) and by the Brazilian Board (Comissão Nacional de Pesquisa) by the number CAAE 59297316.8.0000.5327. The present protocol was registered at ClinicalTrials.gov under the number NCT04229823. Written informed consent was obtained from all study participants.

Analyses

After data were recorded in protected electronic files, the principal investigator included the genotypes in the spreadsheet, and pseudonymized and deleted all information that could identify the individuals.

Mutation carriers with SARA scores ≥3 were classified as ataxic and the others as preataxic carriers. Subjects were divided into three groups: ataxic carriers, preataxic carriers, and related control subjects. For preataxic carriers, the time left to the onset of gait ataxia (TimeTo) was estimated as the difference between the predicted age of ataxia onset (PAO) based on the length of the CAG expanded repeat[22] and corrected for current age. PAO at birth was used to divide preataxic subjects at baseline: those at ≤4 years of PAO were considered preataxic carriers near (PAN) and the others preataxic carriers far from (PAFF) ataxia onset.

Progression was assessed using two strategies. The first one was designed to describe the natural history of all carriers, gathered in a single timeline called TimeToAfterOnset, where zero means the time of ataxia onset, negative values the time predicted to onset for preataxic subjects, and positive values the disease duration after ataxia onset for ataxic carriers. At first, progressions were analyzed separately according to PAFF, PAN, and ataxic groups at baseline (data not shown). However, the phenomenon of the conversion of preataxic to ataxic subjects needed to be considered in the analysis of natural history. Thus, progressions were then analyzed according to another three groups: preataxic subjects who did not convert (nonconverters) and who converted (converters) to ataxia at the end of the study, and ataxic carriers since the start of the study. The resulting progression curves showed that converters were more similar in rate of progression to ataxic carriers than to nonconverters. Due to that, we chose to pool all carriers who were ataxic at the end of the study into a single group, so that final analysis covered progression curves for two groups considering the status at final visit: preataxic carriers and ataxic carriers. Preataxic carriers who did not convert to ataxia during follow-up had their PAO estimated according to their age at baseline. Preataxic carriers who converted to ataxia during follow-up had their age at ataxia onset (AO) considered as the one at the visit when SARA score became ≥3. Ataxic carriers had their AO considered the age at which the subject or their relatives first noticed gait ataxia.

The second strategy used the study time as timescale. Four different groups were included in this analysis, according to their status at baseline: ataxic carriers, PAN, PAFF, and control subjects.

Statistical analysis was performed using mixed models for both strategies, including random effects for intercept and slope for each subject. Models were adjusted in R software 4.1.1 using the lme4 package. Comparisons between progressions of more than two groups, as in the second strategy, were performed with the Tukey method using the emmeans package. Package ggplot2 was used to produce graphs. The threshold for statistical significance was $P < 0.05$.

ESs were determined using the eff_size function of the emmeans package.[23] They can be interpreted as a standardized change associated with a 1-year variation in the timescale. These ESs were compared to identify which outcomes were more sensitive to change in time, per timeline and per study group.

Responsiveness, or the ability to detect a minimal clinically important difference (MCID), was described as the area under the curve for the receiver operating characteristics curve of each variable change against worse (PGI-C 5–7) or not worse (PGI-C 1–4) groups according to PGI-C as the external criterion, including all carriers. Optimal cutoff values to distinguish between worse and stable groups were determined by Youden's procedure and were considered the MCID for each variable.[24]

For those preataxic individuals who converted to ataxia during follow-up, comparisons were made between the actual AO and both the PAO at birth and corrected for age at baseline. Median (interquartile range [IQR]) differences were given, with minimum and maximum differences, and comparison was done with related-samples Wilcoxon signed rank test.

To calculate sample size using conversion rate as the primary outcome, the control group conversion rate was considered the percentage of PANs who completed at least one follow-up visit and converted to ataxia during the whole follow-up period. The "Power and Sample Size for Health Researchers"[25] tool online version was used, and sample size calculation was done for a 2-year follow-up CT, with two balanced arms (1:1), considering a 5% level of significance and for both 80% and 90% power. The sample size needed to decrease in 50% the progression of some continuous outcomes for PAN individuals was also calculated, considering again a 2-year follow-up CT with annual visits, two balanced arms (1:1), 5% level of significance, and 80% power. This was done using the results of the mixed model with study time as timescale and the longpower package.[26]

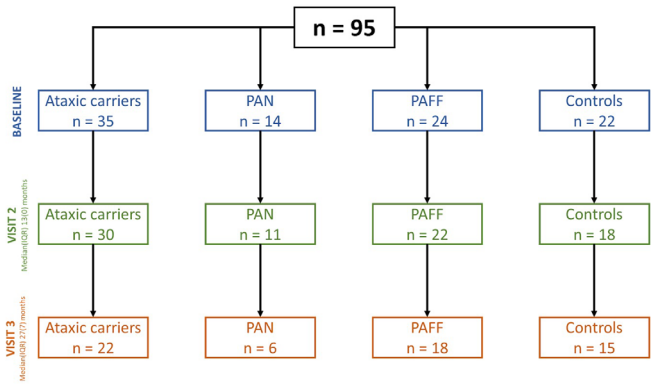

FIG. 1. Diagram showing follow-up visit dropout rate according to subgroups at baseline. IQR, interquartile range; PAFF, preataxic carrier far from the predicted age at onset of gait ataxia; PAN, preataxic carrier near the predicted age at onset of gait ataxia. [Color figure can be viewed at wileyonlinelibrary.com]

Results

Between August 2017 and November 2018, 95 subjects were recruited: 35 ataxic carriers, 38 preataxic carriers, and 22 noncarriers (control subjects). Baseline results were described elsewhere.[8] A total of 81 and 61 subjects completed one and two follow-up visits, respectively (Fig. 1). A total of 237 evaluations were performed: 57/81 and 43/61 subjects were seen by the same examiner in their second and third visits, respectively. Visit 2 happened in median (IQR) 13 (0) months and visit 3 in median (IQR) 27 (7) months from baseline. The maximum follow-up time was 39 months.

In the approach with TimeToAfterOnset, NESSCA, INAScount, and VORr significantly progressed in the preataxic subjects at the end of the study. Among these variables, VORr (−0.12) showed the highest ES. In the ataxic subjects at the end of the study, all clinical scales, VORr, vertical pursuit gain, SPV-GE, and SPV-C showed significant progression, and the highest ESs were that of SARA (0.80) (Table 1, Fig. 2). Notably, the progression rate of all clinical scales was significantly faster in ataxic carriers than in preataxic carriers. In contrast, for the eye movement parameters, only VORr and SPV-GE progressed faster in the ataxic when compared with the preataxic subjects at the end of the study.

In the study time approach, the PAFF group did not show changes in any of the studied variables. In PANs, NESSCA and vertical pursuit gain showed significant worsening (Table 2, Fig. 3). While all clinical scales deteriorated in ataxic carriers, all the oculomotor variables remained stable in this group. ESs for PAN and ataxic groups were also summarized in Table 2.

Changes in all clinical scales were noticed by the overall group of carriers according to PGI-C assessment. Higher areas under the curve were those of SARA and ICARS: worsening of 0.41 point in SARA and 1.08 points in

TABLE 1 *Annual progression of clinical scales and oculomotor variables divided by group at final visit with TimeToAfterOnset as timeline*

	Preataxic carriers at final visit		Ataxic carriers at final visit	
	Annual progression, mean (SE)	**Effect size absolute value**	**Annual progression, mean (SE)**	**Effect size absolute value**
SARA	0.03 (0.04)[a]	–	1.09 (0.08)**[b]	0.80
NESSCA	0.19 (0.07)*[a]	0.09	1.06 (0.14)**[b]	0.51
ICARS	0.16 (0.13)[a]	–	2.69 (0.23)**[b]	0.65
INAScount	0.10 (0.03)*[a]	0.07	0.33 (0.06)**[b]	0.22
SCAFI	−0.017 (0.010)[a]	–	−0.134 (0.018)**[b]	0.47
CCFS	0.001 (0.001)[a]	–	0.011 (0.002)**[b]	0.35
VORr	−0.007 (0.003)*[a]	0.12	−0.021 (0.005)*[b]	0.34
VVS slope	0.029 (0.024)[a]	–	0.005 (0.033)[a]	–
RVS slope	0.011 (0.032)[a]	–	0.033 (0.046)[a]	–
Vertical pursuit gain	−0.009 (0.004)[a]	–	−0.018 (0.006)*[a]	0.19
SPV-GE	0.012 (0.014)[a]	–	0.124 (0.028)**[b]	0.24
SPV-C	0.003 (0.007)[a]	–	0.027 (0.012)*[a]	0.10

Effect sizes are shown for variables that showed progression different from zero.
When progression slope different from zero: *$P < 0.05$, **$P < 0.0001$.
[ab]Different letters mean different progression slopes when comparing preataxic with ataxic carriers for each variable.
SE, standard error; SARA, Scale for the Assessment and Rating of Ataxia; NESSCA, Neurological Examination Score for Spinocerebellar Ataxias; ICARS, International Cooperative Ataxia Rating Scale; INAS, Inventory of Non-ataxia Signs; SCAFI, SCA Functional Index; CCFS, Composite Cerebellar Functional Score; VOR, vestibulo-ocular reflex; VVS, volitional vertical saccade; RVS, reflex vertical saccade; SPV, slow-phase velocity; GE, gaze-evoked nystagmus; C, central nystagmus.

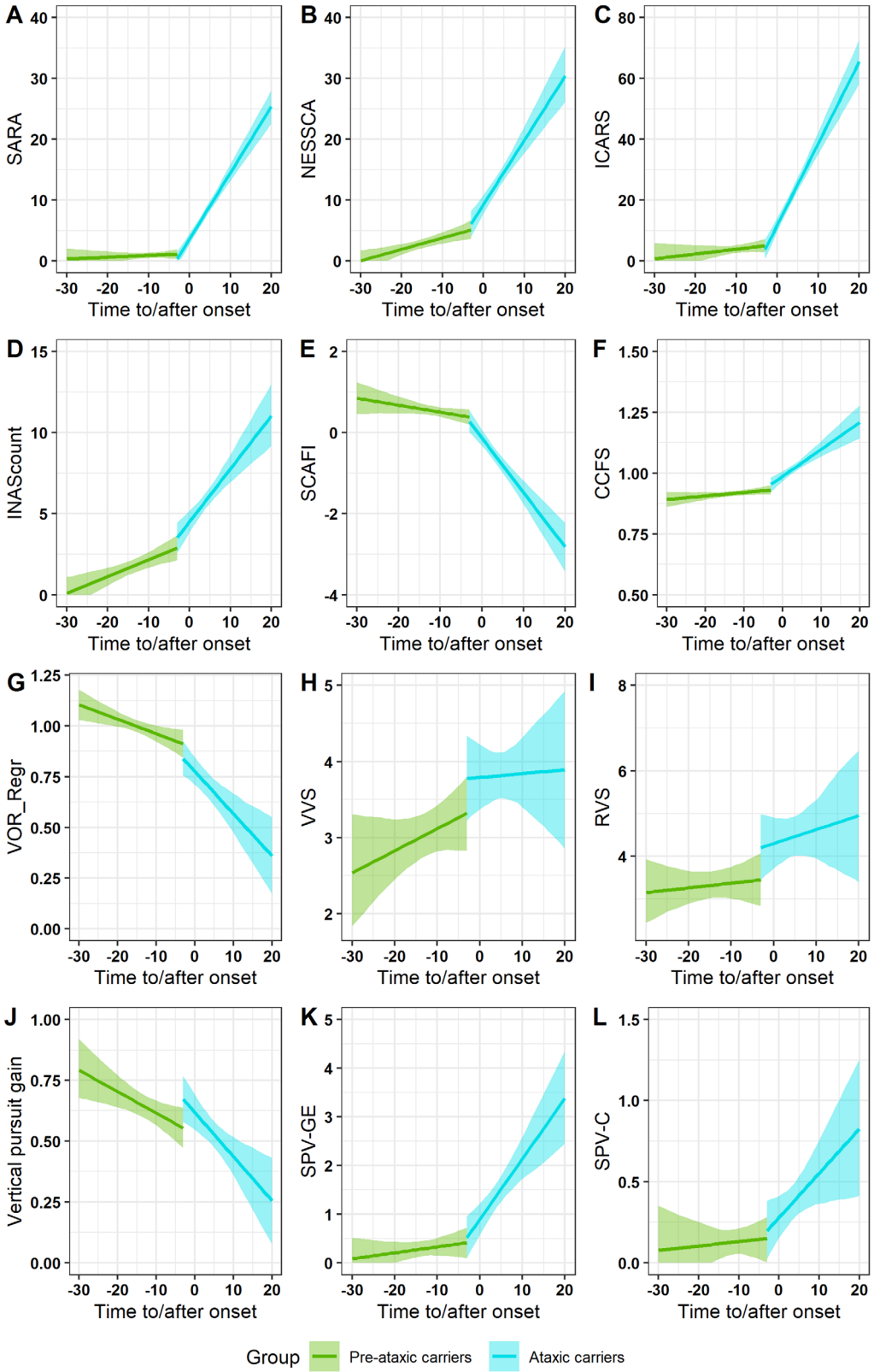

FIG. 2. Longitudinal progression curves of the variables under study in TimeToAfterOnset timescale. Subjects were grouped as ataxic or preataxic in their final visit. Values for each scale as predicted by the mixed model and 95% confidence interval (CI). (**A**) Scale of Assessment and Rating of Ataxia (SARA). (**B**) Neurologic Examination Score of Spinocerebellar Ataxias (NESSCA). (**C**) International Cooperative Ataxia Rating Scale (ICARS). (**D**) Inventory of Non-ataxia Signs (INAScount). (**E**) SCA Functional Index (SCAFI). (**F**) Composite Cerebellar Functional Score (CCFS). (**G**) Vestibulo-ocular reflex (VORr). (**H**) Volitional vertical saccade (VVS). (**I**) Reflex vertical saccade (RVS). (**J**) Vertical pursuit gain. (**K**) Slow-phase velocity of gaze-evoked nystagmus (SPV-GE). (**L**) Slow-phase velocity of central nystagmus (SPV-C). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 *Annual progression of clinical scales and oculomotor variables divided by groups at baseline with study time as timeline*

			PANs		Ataxic carriers	
	Control annual progression (SE)	PAFF annual progression (SE)	Annual progression (SE)	Effect size absolute value	Annual progression (SE)	Effect size absolute value
SARA	−0.12 (0.24)[a]	0.30 (0.21)[a]	1.25 (0.48)[ab]		**1.52 (0.20)[b]**	**1.56**
NESSCA	−0.04 (0.34)[a]	0.46 (0.29)[ac]	**2.04 (0.68)[bc]**	**1.36**	**1.73 (0.29)[b]**	**1.16**
ICARS	−0.34 (0.73)[ac]	0.83 (0.61)[ac]	2.60 (1.45)[bc]		**4.82 (0.59)[b]**	**1.78**
INAScount	−0.16 (0.20)[a]	0.16 (0.18)[ab]	0.72 (0.41)[ab]		**0.77 (0.18)[b]**	**0.62**
SCAFI	0.009 (0.048)[a]	0.049 (0.042)[a]	−0.050 (0.096)[ab]		**−0.173 (0.042)[b]**	**0.62**
CCFS	−0.008 (0.006)[a]	−0.003 (0.005)[a]	0.007 (0.011)[ab]		**0.015 (0.005)[b]**	**0.50**
VORr	−0.004 (0.009)[ab]	−0.010 (0.007)[ab]	0.027 (0.017)[b]		−0.032 (0.008)[a]	
VVS slope	0.018 (0.109)[a]	0.155 (0.091)[a]	−0.406 (0.211)[a]		0.062 (0.091)[a]	
RVS slope	0.050 (0.098)[ac]	0.004 (0.083)[ac]	−0.867 (0.187)[a]		0.163 (0.085)[bc]	
Vertical pursuit gain	−0.003 (0.014)[a]	−0.011 (0.011)[a]	**−0.101 (0.025)[b]**	**1.17**	−0.001 (0.012)[ac]	
SPV-GE	−0.015 (0.080)[a]	−0.008 (0.066)[a]	0.071 (0.154)[a]		0.078 (0.069)[a]	
SPV-C	0.007 (0.056)[a]	0.009 (0.046)[a]	−0.007 (0.108)[a]		0.109 (0.046)[a]	

Effect sizes are shown for those variables that showed different progression from controls. Progressions that differ from controls are shown in bold.
[a–c]Different letters mean different progression for each variable when comparing groups.
SE, standard error; PAFF, preataxic carrier far from disease onset; PAN, preataxic carrier near disease onset; SARA, Scale for the Assessment and Rating of Ataxia; NESSCA, Neurological Examination Score for Spinocerebellar Ataxias; ICARS, International Cooperative Ataxia Rating Scale; INAS, Inventory of Non-ataxia Signs; SCAFI, SCA Functional-Index; CCFS, Composite Cerebellar Functional Score; VOR, vestibulo-ocular reflex; VVS, volitional vertical saccade; RVS, reflex vertical saccade; SPV, slow-phase velocity; GE, gaze-evoked nystagmus; C, central nystagmus.

ICARS were the threshold for the MCID (Supporting Information Material S1). Notably, however, 28/33 and 3/20 of ataxic and preataxic carriers at the end of the study noticed a worsening according to their PGI. In contrast, video-oculography parameters did not present responsiveness to detect MCID in SCA3/MJD.

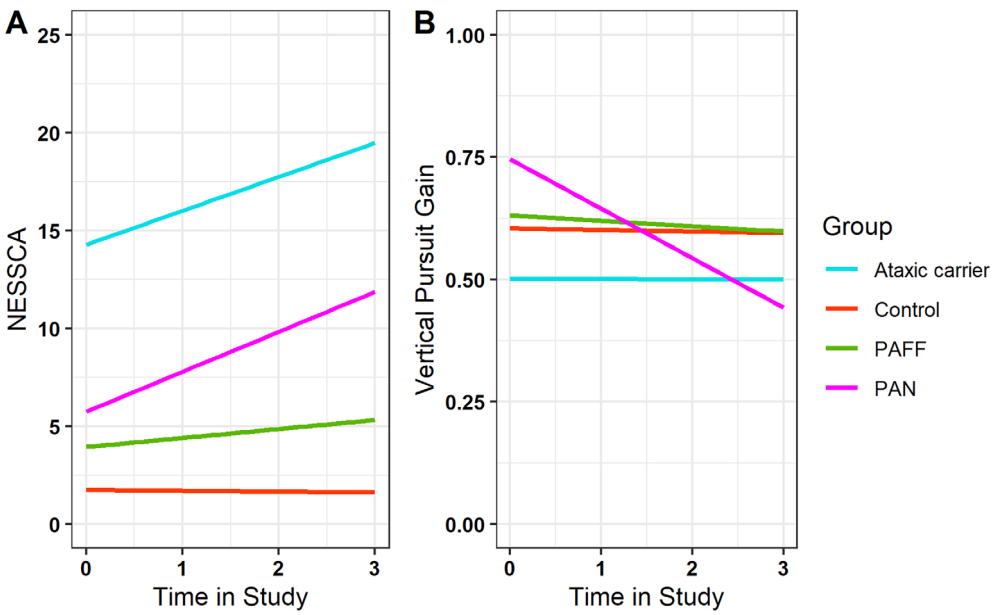

FIG. 3. Progression curves of variables that showed significant progression on study duration in preataxic carriers near the predicted age at onset of gait ataxia (PANs). (**A**) Neurologic Examination Score of Spinocerebellar Ataxias (NESSCA). (**B**) Vertical pursuit gain. PAFF, preataxic carrier far from the predicted age at onset of gait ataxia. [Color figure can be viewed at wileyonlinelibrary.com]

Eight preataxic individuals converted to ataxia during follow-up: six were PANs at baseline (Supporting Information Material S2). The median (IQR) difference between PAO at birth and the actual AO of them was 0.53 (7.12) year with a minimum and maximum difference of −4.95 and 4.43 years, respectively, while the difference between PAO corrected for age at baseline and the actual AO was −3.46 (3.56) with minimum and maximum differences ranging from −6.95 to −1.49. PAO at birth did not statistically differ from the actual AO, whereas PAO corrected for age at baseline did ($P = 0.012$).

From the 11 PANs who completed at least one follow-up visit, six converted to ataxia by 2 years of follow-up, with a conversion rate of 54.5%. Considering a placebo-controlled CT with 80% power, 2-year follow-up duration, and 5% significance level, 57 preataxic subjects within 4 years of PAO calculated at birth would be needed in each study arm to detect a 50% reduction in the conversion rate to ataxia for the treated group (Supporting Information Material S3). Considering NESSCA and vertical pursuit gain slopes in study time as outcomes (Table 2), a CT aiming to decrease in 50% their progression in PAN individuals would need 57 and 23 individuals per trial arm, respectively.

Discussion

Clinical scales and video-oculography parameters were already demonstrated to be altered in premanifest SCA3/MJD carriers. The novelties we brought here were that some biomarkers not only differentiate preataxic carriers from control subjects but also detect worsening over a time compatible with a CT and have sensitivity to change. NESSCA, INAScount, and VORr progressed in the preataxic group when looking at natural history along time to ataxia onset. Furthermore, during the study time, NESSCA and vertical pursuit gain progressed in PANs. Finally, the rate of conversion of PANs into ataxia phase and the slopes of progression of NESSCA and vertical pursuit gain in subjects who were PANs at baseline were all related to relatively satisfactory sample sizes for future CTs.

Different patterns of progression emerged from our observation of the preataxic and ataxic phases of SCA3/MJD. Preataxics who converted to ataxia during the study were mainly PANs; during the years that antecede conversion, all clinical scales plus VORr and SPV-GE showed progression velocities more like those of ataxic carriers than those of the nonconverters, depicting that a shift in the velocity of neurological deterioration occurred around 4 years before ataxia onset. This pattern was similar to that found in a previous longitudinal study that represented these progressions by quadratic curves.[7] Those results, as well as ours, together suggest that neuronal dysfunction appears to start being significant a few years before the onset of ataxia and is not continuous or uniform over the lifetime of carriers. Contradicting this, progression rates of other eye movement variables during preataxic and ataxic periods were more alike (Table 1, Fig. 2E–I). Notably, deterioration of these oculomotor variables cannot be attributed to age.[8] Of course, differences found between speeds of progression may be because of psychometric properties of clinical instruments when applied in different phases of life, such as floor effects. The doubt might persist about the reasons why velocity of neuronal dysfunction changes from preataxic to ataxic periods. However, inflection points distant from the PAN period were not detected by different markers. This similarity between so many clinical and VOG markers suggests a common acceleration of processes, near the onset of ataxia.

Ataxia was the most relevant symptom subjectively during the ataxic phase, because the MCIDs for SARA and ICARS were lower than for the other clinical scales, in this period, results that are in accordance with a previous report.[27] Noteworthy was the fact that SCA Functional Index and Composite Cerebellar Functional Score, originally intended to detect small clinical changes over short periods of time,[28] again did not have greater sensitivity and responsiveness than SARA and other clinical scales.[27,29] SARA was undoubtedly the clinical scale with the greatest sensitivity to change, although MRI markers had even better ESs during ataxic stage.[30] Whereas all clinical scales showed responsiveness, the oculomotor variables did not, suggesting that they might be used, at best, as secondary endpoints in this period.

That said, let us move on to our most relevant results, those related to preataxic phases. NESSCA, INAScount, and VORr progressed significantly in the natural history approach during the preataxic phase (Table 1). In the study time approach, NESSCA and vertical pursuit gain worsened in PANs (Table 2): NESSCA ES in PANs was even higher than in ataxic subjects. These results show that nonataxic manifestations progressed significantly in preataxic SCA3/MJD carriers. NESSCA was originally designed to measure the overall neurological burden, including ataxic, pyramidal, extrapyramidal, oculomotor, and sensory items evaluated as discrete variables.[16] INAScount assesses several nonataxic items as categorical variables.[20] Also, VOR depends on lateral and medial vestibular nuclei and the medial longitudinal fasciculus.[8] These findings could be useful when designing CTs for the preataxic stage.

Responsiveness is a very important characteristic to consider in CTs, but it is difficult to assess in presymptomatic or preataxic subjects. We evaluated responsiveness here by including the whole cohort of

carriers, both before and after ataxia onset. If we assessed this property in the preataxic group only, the absence of recognition of symptoms would make all instruments unresponsive. We have already seen that ataxia was the most relevant manifestation subjectively during the symptomatic phase. However, it does not appear to be useful for tracking changes in the preataxic stage in SCA3/MJD. In this phase, SARA varies between 0 and 2.5 points and, in fact, has not significantly progressed in this cohort (Tables 1, 2). ICARS had a wider variation in preataxic individuals and was found to differentiate PANs from control subjects[8]; however, ICARS also did not show a significant progression in this phase of the carriers' lives. Thus, we think that responsiveness might be replaced by another measure of the subject's perspective for future studies done exclusively in preataxic phases, for instance, conversion to ataxia or to symptomatic stages.

Considering that the ESs of our outcomes were small in the preataxic group, looking for other outcomes and building a recruitment strategy that bypasses this obstacle for future clinical trials is essential. A significant measure of efficacy might be the conversion rate to ataxia. Jacobi et al.[7] calculated a sample size of 136 subjects per arm necessary to detect a 50% reduction in the conversion rate, 2-year follow-up, and 80% power. We showed here that a strategy to decrease this sample is to recruit individuals based on their predicted time to ataxia onset. Our results related to the preataxic carriers who converted to ataxia during follow-up suggest that the PAO at birth is probably the best alternative to select participants for CTs because it did not statistically differ from the actual AO. Our calculated sample size was 57 PANs in each arm, for the same CT scenario described earlier. The reduction of at least 50% in the progression rate of NESSCA and vertical pursuit gain could be added as secondary outcomes, because in PANs a 2-year CT would need 57 and 23 individuals per trial arm, respectively. The difficulty would be to recruit exclusively PAN subjects: they should be rare in isolated sites, but not that rare for multicentric studies.

Another possible way to decrease sample size requirements without restricting the inclusion criteria to PANs would be to use a different model to measure outcome in CTs, as previously proposed for other neurodegenerative conditions.[31-35] Classically, outcomes are determined by measuring the absolute variable change from baseline to a fixed postbaseline time point.[36-39] These so-called study-time analyses, however, may not deal well with high variabilities on disease stage and rate of decline between subjects.[31] Slope analyses can help. For these, instead, using the TimeToAfterOnset as the timeline could be promising. However, instruments able to estimate sample sizes from a TimeToAfterOnset model and the possibility to include subjects at any year from ataxia onset, if using this model, are still lacking.

Our study faced some drawbacks that could have decreased our study power. Although unicentric studies such as ours can guarantee greater standardization, they might be seen as less representative. However, we think the main issue faced by this study was actually the coronavirus disease 2019 pandemic. Restrictions imposed by lockdown occurred during the period of the third visits, so that not all subjects could be assessed in the predicted time, generating variability between intervals that each subject was seen. Statistical modeling was adjusted to account for that. In addition, many individuals refused to come back for the final visit after restrictions subsided because of longstanding apprehension regarding coronavirus disease 2019 transmission. This created significant dropout in the final visit. Despite that, several significant results were obtained, as discussed earlier.

In conclusion, clinical scales and video-oculography variables were already altered in preataxic SCA3/MJD carriers, and several of them deteriorated longitudinally in this period. For the ataxic period, validated clinical scales remained more sensitive to change and responsive when compared with video-oculography variables using both timescale strategies. VORr, NESSCA, and INAScount were the best candidates to measure the natural history of the preataxic stage in SCA3/MJD. Multicenter studies on VOG should standardize it by using the same equipment and following the same instructions in different sites. From the studied candidates, when looking for changes during the study time in PANs, NESSCA, vertical pursuit gain, and the conversion rate were the best possible outcome for future CTs in this disease stage. ■

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Data Availability Statement

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

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Supporting Data

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