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Clinical, Imaging, and Laboratory Markers of Premanifest Spinocerebellar Ataxia 1, 2, 3, and 6: A Systematic Review

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Background and Purpose Premanifest mutation carriers with spinocerebellar ataxia (SCA) can exhibit subtle abnormalities before developing ataxia. We summarized the preataxic manifestations of SCA1, -2, -3, and -6, and their associations with ataxia onset.

Methods We included studies of the premanifest carriers of SCA published between January 1998 and December 2019 identified in Scopus and PubMed by searching for terms including 'spinocerebellar ataxia' and several synonyms of 'preataxic manifestation'. We systematically reviewed the results obtained in studies categorized based on clinical, imaging, and laboratory markers.

Results We finally performed a qualitative analysis of 48 papers. Common preataxic manifestations appearing in multiple SCA subtypes were muscle cramps, abnormal muscle reflexes, instability in gait and posture, lower Composite Cerebellar Functional Severity scores, abnormalities in video-oculography and transcranial magnetic stimulation, and gray-matter loss and volume reduction in the brainstem and cerebellar structures. Also, decreased sensory amplitudes in nerve conduction studies were observed in SCA2. Eotaxin and neurofilament light-chain levels were revealed as sensitive blood biomarkers in SCA3. Concerning potential predictive markers, hyporeflexia and abnormalities of somatosensory evoked potentials showed correlations with the time to ataxia onset in SCA2 carriers. However, no longitudinal data were found for the other SCA gene carriers.

Conclusions Our results suggest that preataxic manifestations vary among SCA1, -2, -3, and -6, with some subtypes sharing specific features. Combining various markers into a standardized index for premanifest carriers may be useful for early screening and assessing the risk of disease progression in SCA carriers.

Key Words spinocerebellar ataxia, premanifest, presymptomatic, marker, systematic review.

INTRODUCTION

Spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders inherited in an autosomal dominant pattern, consisting of more than 40 subtypes.[1] A systematic review of epidemiological studies found that the most common subtypes both worldwide and within Korea are SCA2 and -3, followed by SCA1, -6, and -7,[2-8] which are known as polyglutamine SCAs. The main symptoms of polyglutamine SCAs include impairment of balance and coordination with slurred speech.[5] The polyglutamine SCAs mainly affect the cerebellum and brainstem,[9-13] and several pathophysiological mechanisms have been identified recently; for example, CAG repeats cause protein aggregation that leads to altered protein interactions and intranuclear inclusions. Moreover, DNA damage and transcriptional dysregulation can affect neurons in SCA patients. Ion-channel and mitochondrial dysfunctions are also known to be associated with the mechanism.[14]

Ataxia onset in polyglutamine SCAs most commonly occurs in the third or fourth decade of life.[7,15,16] The premanifest stage of SCAs refers to an individual carrying a genetic mutation but not yet exhibiting overt cerebellar ataxia (Fig. 1), which has been recently defined by a SARA (Scale for the Assessment and Rating of Ataxia) score of <3.[17,18] Reports on the premanifest stage have used various terms such as 'presymptomatic', 'preclinical', and 'prodromal'. In this article we opted to use 'premanifest' to represent the conditions described by these terminologies, and described all clinical signs, laboratory, and image findings in this stage as 'presymptomatic manifestations'.

Premanifest mutation carriers have been reported to exhibit certain abnormal clinical signs such as frequent muscle spasms, subtle gait, speech, and oculomotor signs, as well as abnormalities in brain imaging, blood-derived markers, and neurophysiological tests compared with healthy controls.[17,19,20] Clinical scales for evaluating ataxia and neurological deficit in manifest SCA patients have been validated to show disease progression, which include the SARA and INAS (Inventory of Non-Ataxia Signs).[18,21] However, no scales reflecting the disease severity in the premanifest stage have been established. This demonstrates the need to developing clinical assessment scales using presymptomatic manifestations in order to detect SCA patients in their premanifest stage.

Since polyglutamine SCAs show genetic anticipation,[22,23] individuals with more CAG repeats will have earlier ataxia onset.[24-31] The number of CAG repeats can be used for predicting ataxia onset in SCA patients, but it explains only a subset of the features of symptom development in SCA.[32] Markers of premanifest SCA correlating with ataxia development and clinical progression would be particularly useful for prognostication and the future development of neuroprotective therapeutics applicable at the premanifest stage of SCA. More researches are investigating the clinical, laboratory, and neuroimaging markers of the prodromal condition in other neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease.[33-41] However, only a few studies have investigated polyglutamine SCAs.

In this article we systematically review the clinical, laboratory, and imaging markers investigated in premanifest mutation carriers of SCA1, -2, -3, and -6. We also review features associated with ataxia onset in premanifest SCAs.

METHODS

Information sources and search procedure

We used two search databases for this study: Scopus and PubMed. The searches were conducted on January 2020 and the results were limited to publication dates from January 1998 to December 2019. We applied the following search to the two databases: ('spinocerebellar ataxia' OR 'SCA') AND ('premanifest' OR 'presymptomatic' OR 'asymptomatic' OR 'preclinical' OR 'subclinical' OR 'prodromal' OR 'preataxic').

Eligibility criteria

We comprehensively reviewed published works by applying the following criteria. Eligible subjects were restricted to individuals who were genetically confirmed to have abnormal CAG repeats in SCA1, -2, -3, or -6 genes, and were matched
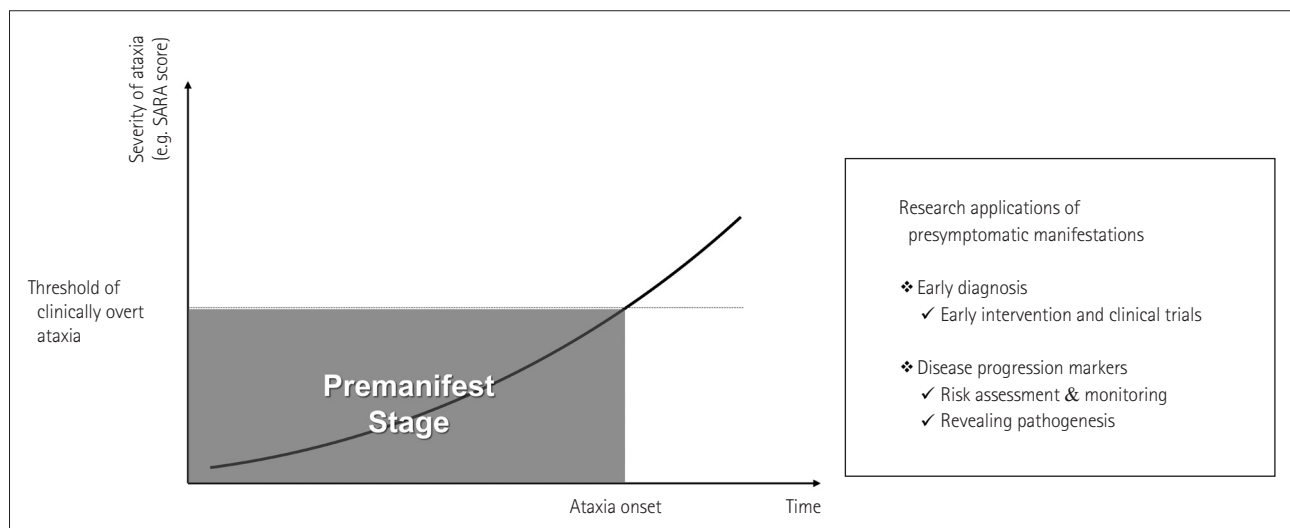
Fig. 1. Schematic diagram of the premanifest stage of SCA and its further implications. Ataxia development in SCA patients can be assessed and classified into several stages using the SARA score. Subtle changes can develop in the premanifest stage with the absence of ataxia signs, and the ability to identify these changes can lead to future applications, as shown in the diagram. There is currently no consensus on the exact criteria for the 'premanifest' stage (see the Discussion section). SARA: Scale for the Assessment and Rating of Ataxia, SCA: spinocerebellar ataxia.

for the premanifest stage as defined below. Although many previous studies have used SARA scores to define premanifest carriers, they have used different cutoffs.[42-44] We adopted a SARA score cutoff of 3 (or 4 in some studies) to identify the premanifest stage. Nonataxic phenotypes of SCA that present with parkinsonism or dementia[45,46] were not considered in this review. In addition, we only included studies that compared a specific symptom or test result between premanifest carriers and normal controls. Regarding study designs, we only included original researches, and excluded case series and case reports. The results of original researches reported in systematic reviews and review articles were also included.

Study selection

After removing duplicates, we screened papers to filter out

irrelevant studies; studies involving animal models, ethical issues, and subtypes other than SCA1, -2, -3, and -6 were excluded by examining each Abstract. Efforts to exclude duplicated reports on the same cohorts were also made by comparing the author names, study size, and main evaluation method of each study. After performing this screening, the full texts for the remaining studies were assessed by applying eligibility criteria as explained above. The results from the finally included studies were then systematically reviewed.

RESULTS

There were 269 and 209 studies identified in Scopus and PubMed, respectively. Removing duplicates resulted in 273 studies remaining, of which 146 passed the screening process. Applying the eligibility criteria yielded 48 studies that were
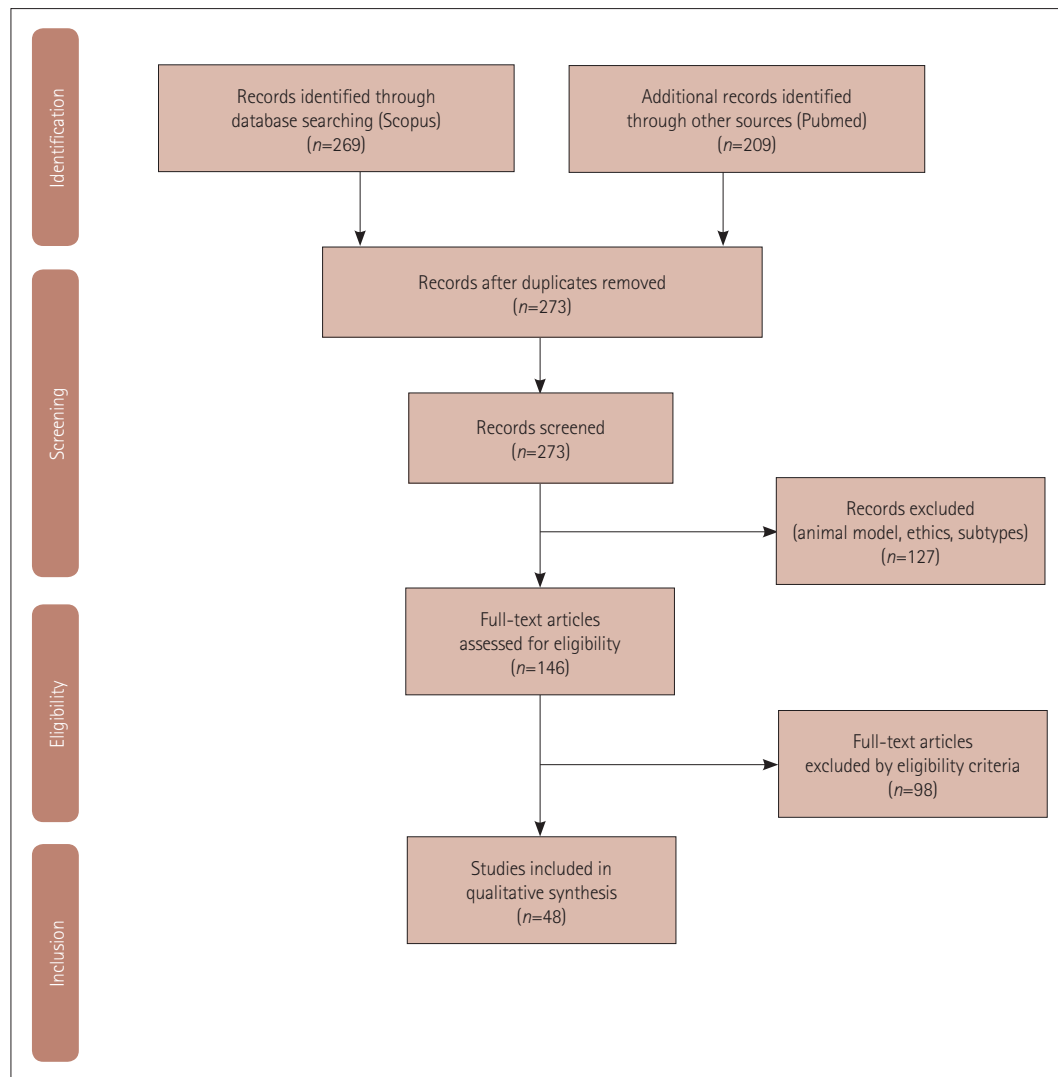

Fig. 2. Flow diagram of study selection. Reproduced with permission from the PRISMA Statement.[93]

finally included in the present analysis (Fig. 2).

Presymptomatic manifestations of SCA mutation carriers

Most of the studies involved SCA2 and -3, with few investigating presymptomatic manifestations of SCA1 and -6. Furthermore, some studies included fewer than five patients, and so the sample sizes were presented for those studies.

Electrophysiological assessments

Video-oculography

Oculomotor deficits can be evaluated using several parameters that are measured in video-oculography (VOG), and some of them exhibited significant changes in premanifest carriers. Increased error and latency in antisaccadic tasks and decreased maximum velocities of horizontal saccadic movements were the main features that presented in premanifest SCA2 carriers.[43,47] Wu et al.[48] showed that premanifest SCA3 carriers also shared these VOG features, although saccadic slowing occurred in vertical rather than horizontal movements. Also, these carriers showed additional VOG features such as impaired smooth pursuit and increased frequency and average amplitude of square-wave jerks during gaze fixation. In SCA6, gaze-evoked nystagmus, impaired smooth pursuit, and saccadic slowing as assessed by VOG separated the premanifest group (n=4) from controls.[49]

Transcranial magnetic stimulation

The following parameters from transcranial magnetic stimulation (TMS) of the motor cortex were increased in premanifest SCA2 carriers: resting motor thresholds, active motor thresholds (measured during weak contraction), central motor conduction time (CMCT) using the F-wave method, and cortical silent periods.[44] Farrar et al.[50] found that CMCT was increased in two premanifest SCA3 carriers, who exhibited reduced cortical responses to stimuli, showing reduced threshold increases in motor evoked potentials immediately after (interval <7 ms) a conditioning stimulus compared with normal controls.

Other methods

Other electrophysiological assessments that were found to be effective were nerve conduction studies, evoked potentials, and coherence analysis by combining electroencephalography (EEG) and electromyography (EMG) data, although these investigations only involved premanifest SCA2. Sensory amplitudes in nerve conduction studies were decreased in the median and sural nerves.[26,51,52] Abnormal morphology in somatosensory evoked potentials (SSEPs) and brainstem auditory evoked potentials were found in premanifest SCA2.[52] Data on P40 latency alterations of SSEPs were inconsistent, with some studies showing increased P40 latency in premanifest SCA2 carriers and others showing no change.[26,51,52] Additionally, statistical corticomuscular coherence (between EEG and EMG) and intermuscular coherence (between upper and lower limb EMG) were decreased in premanifest SCA2, showing reduced excitability and axonal integrity along the corticospinal tract.[53]

Neuroimaging assessments

Magnetic resonance imaging (MRI) volumetry and diffusion-tensor imaging (DTI) are currently the most effective imaging modalities for distinguishing premanifest carriers of SCA1, -2, -3, and -6. In structural MRI, the volumes of brainstem structures (especially the pons) were reduced in premanifest SCA2 and -3, whilst volume reduction of the cerebellum was observed in SCA2 but not in SCA3 carriers.[42,43,54,55] Gray-matter loss in the cerebellum was also observed in both premanifest SCA1 and -2, and gray-matter losses in the pons and medulla were additionally found in premanifest SCA1.[42] Other structures found to have MRI volumetry abnormalities was the cervical spinal cord in SCA3 carriers.[54]

DTI microstructural alterations in the cerebellar peduncles can be useful for detecting premanifest SCA3. Fractional anisotropy (FA) was decreased and diffusivity measures (axial, radial, and mean) were increased in premanifest SCA3.[54,56] For structures outside the cerebellum, axial diffusivity in the midbrain and mean diffusivity in the cerebral peduncles were increased.[54,56] It was particularly interesting that one study showed a tendency for the alteration of these measures to be reversed in SCA6,[57] with increased FA and decreased radial diffusivity observed in superior and middle cerebellar peduncles, suggesting compensatory changes against degeneration. However, the insufficient sample size (n=2) of that study indicates that further large-sample studies are necessary.

Other imaging modalities have also revealed certain changes in premanifest carriers. Magnetic resonance (MR) spectroscopy showed decreases in the N-acetylaspartate/creatine and choline/creatine ratios in the basilar part of the pons in two premanifest SCA1 carriers.[58] Falcon et al.[57] suggested a concept of cortical connectivity alteration in premanifest SCA6. Connectivity in the cerebral cortex in functional magnetic resonance imaging (fMRI) was decreased between parietal regions and increased between occipital lobe and frontal eye fields in two premanifest carriers.

Molecular biomarkers

Several serum biomarkers related to oxidative stress and mitochondrial dysfunction were found in premanifest SCA3,

with higher levels of serum eotaxin reflecting resistance to oxidative stress, and higher levels of serum neurofilament light chains, reflecting the progression of neurodegeneration.[20,59] Raposo et al.[60] found that premanifest SCA3 carriers had increased frequencies of a common deletion (del4977) in mitochondrial DNA and a decreased BCL2/BAX mRNA ratio, implying mitochondrial dysfunction. One study suggested serum reactive oxygen species and glutathione peroxidase as candidates of oxidative stress biomarkers in premanifest SCA, but the results were not statistically significant.[61]

Clinical evaluations and other ancillary tests

Gait and posture
Alterations in gait and posture are the cardinal symptoms of SCA,[3] and studies have shown that they were detectable in premanifest subjects, although they are subtle compared with the symptomatic stage. Several studies showed instabilities in gait and posture in premanifest SCA1, -2, and -6 carriers, although they used different quantification methods: body oscillation frequencies, body sway distance, and variabilities of step width and step time.[62-64] Ilg et al.[65,66] showed that a concept of 'spatiotemporal variability,' a method quantifying gait instabilities using both time and spatial movements, can be effective in distinguishing premanifest carriers of SCA1, -2, -3, and -6 from normal controls, although the subtypes were not separated in that study.

Sleep and autonomic measures
Sleep abnormalities have been found in patients with premanifest SCA2 using polysomnography. Premanifest SCA2 patients had a lower density of sleep spindles in non-REM (rapid eye movement) sleep, and the proportion of time spent in REM sleep was also reduced.[67,68] However, assessing sleep disturbances using subjective assessments tools such as the Pittsburgh Sleep Quality Index and simple interviews have produced results that were inconsistent among several studies.[24,42,43,67,68] The SCOPA-AUT (Scales for Outcomes in Parkinson's Disease–Autonomic Dysfunction)[69] questionnaire was effective in detecting subtle autonomic dysfunction in premanifest SCA2. The SCOPA-AUT questions revealed that constipation and pollakiuria were the most common symptoms.[43,70] Among other measures associated with autonomic dysfunction, several indices related to heart-rate variability were significantly changed in premanifest SCA2.[71]

Cognitive and behavioral symptoms
The scores for Composite Cerebellar Functional Severity (CCFS),[72] a system consisting of tests evaluating cerebellar function such as nine-hole pegboard test, were lower in pre-

manifest carriers of SCA1, -2, and -3 than in normal controls.[42] The SCA Functional Index,[73] consisting of an 8-min walking test, nine-hole pegboard test, and verbal repeat test, was also lower in premanifest SCA2 than in normal controls.[42] Abnormalities in neuropsychological tests of executive function were also found, with premanifest SCA2 carriers showing increased times in Stroop interference tests,[68] lower scores in the Institute of Cognitive Neurology (INECO) frontal screening tests, and poor results in verbal fluency tests and intradimensional/extradimensional set shift tests.[43] Visual memory impairment was also observed in patients with premanifest SCA2, as they showed poor results in delayed-matching-to-sample tests.[43]

Other neurological signs
Consistent with VOG results, premanifest SCA2 patients showed oculomotor disturbances such as horizontal saccadic slowing and gaze-evoked nystagmus in neurological examinations.[43] In premanifest SCA3, horizontal gaze-evoked nystagmus was frequent but diplopia was inconsistent among studies.[24,28,42] Painful, disturbing muscle cramps were more frequent in premanifest SCA1, -2, and -3 than in normal controls.[26,42,43,74] Sensory neuropathic signs such as abnormal vibratory and algesic sensation were more frequent in premanifest SCA2 and -3.[26,43,74] Deep tendon reflexes in lower limbs showed that both hyperreflexia and hyporeflexia were frequent in premanifest SCA2 and -3.[26,43,48,74] Extrapyramidal signs were also found more frequently in premanifest SCA3.[74] van Gaalen et al.[75] showed that patients with premanifest SCA3 acquired less-conditioned eyeblink responses after auditory stimuli in classical conditioning, implying that motor learning is impaired in premanifest SCA. Motor performance such as when throwing objects at a target was also slightly impaired in premanifest SCA2.[76] Findings of handwriting problems in SCA2, positive Babinski reflex in SCA3, and episodic vertigo in SCA6 were inconsistent in patients in the premanifest stage.[24,42,48,74] Dysarthria was also inconsistent in both premanifest SCA2 and -6.[24,42]

Markers associated with the time to ataxia onset
Several longitudinal studies have investigated the relationship of ataxia onset with clinical and laboratory markers. In premanifest SCA2, hyporeflexia, lower sensory amplitudes in median and sural nerves, and prolonged P40 latencies in tibial nerve SSEPs were associated with a shorter time to ataxia onset.[26]

Cross-sectional studies have shown several presymptomatic manifestations to be associated with the predicted time to ataxia onset, which can be calculated using the patient's age and number of CAG repeats.[32] Applying several methods

evaluating postural and gait abnormalities revealed that displacement in body sway during stance (SCA1), body oscillation frequency during stance (SCA2), and spatiotemporal variability during tandem gait (SCA1, -2, -3, and -6; subtypes not separated) were correlated with the predicted time to ataxia onset.[63,65] The rate of errors during throwing motions was also correlated in premanifest SCA2.[76] Among electrophysiological assessments in premanifest SCA2, latency of antisaccadic movements during antisaccadic tasks in VOG,[43,47] CMCT of tibialis anterior muscles in TMS studies,[44] coherence between EEG signals at the CP3 electrode and EMG signals of the first dorsal interosseous muscle, and coherence between EMG signals of the first dorsal interosseous and flexor digitorum superficialis muscles were correlated with the predicted time to ataxia onset.[53] Other ancillary tests in premanifest SCA2 showed that scores on CCFS,[42] SCOPA-AUT,[70] neuropsychological tests such as INECO frontal screening tests, intradimensional/extradimensional set shift tests, and delayed-matching-to-sample tests were associated with the predicted time to ataxia onset.[43] In premanifest SCA1, the SCA Functional Index was related to the predicted time to ataxia onset.[42]

DISCUSSION

We have presented a systematic review of the presymptomatic manifestations in SCA1, -2, -3, and -6 by investigating previous investigations involving premanifest carriers. The presymptomatic manifestations can be divided into the following categories: clinical symptoms, electrophysiological findings, neuroimaging results, molecular biomarkers, and ancillary tests. As expected, most of the presymptomatic manifestations were shared among different SCA subtypes. Painful muscle cramps, abnormal muscle reflexes, instabilities in gait and posture, VOG abnormalities, TMS measures, volume reduction and gray-matter loss in structural MRI, microstructural changes in DTI measures, and abnormal CCFS scores were the presymptomatic manifestations evident in multiple SCA subtypes (Figs. 3 and 4). These results indicate that although different genetic etiologies and pathologies distinguish the SCA subtypes,[3,5] there exist common features in the symptomatic development of polyglutamine SCAs in the premanifest stage.

Some presymptomatic manifestations were detected using different methods specifically in a single SCA subtype, such as when using MR spectroscopy in SCA1, neuropsychological tests and certain electrophysiological assessments (horizontal saccadic slowing, evoked potentials, and coherence between EEG and EMG) in SCA2, oxidative stress biomarkers in SCA3, and cortical connectivity shifting as measured by fMRI in SCA6. These findings suggest the presence of

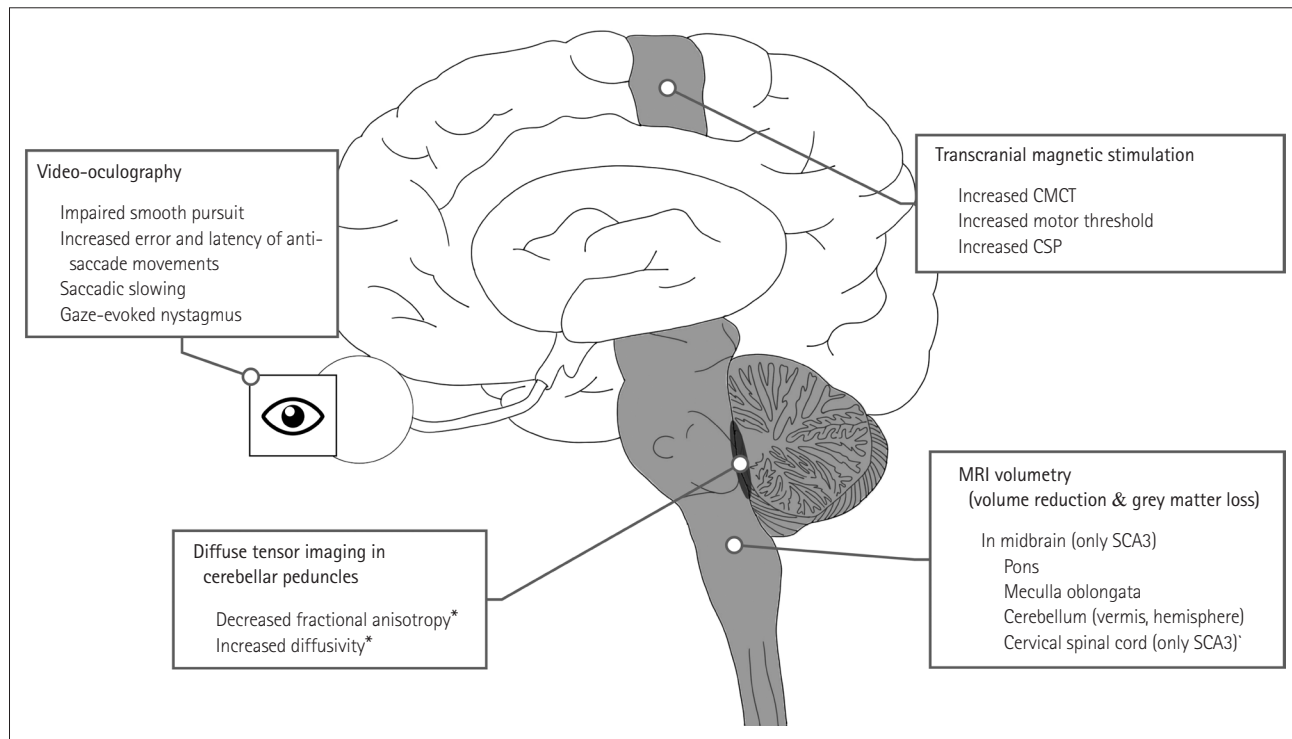**Fig. 3.** Electrophysiological and neuroimaging findings in premanifest carriers of SCA1, -2, -3, and -6. Presymptomatic manifestations exhibited in multiple SCA subtypes are shown based on the data listed in Table 1 and Table 2. The involved regions are shaded in the figure. *Reversed tendency observed in SCA6. CMCT: central motor conduction time, CSP: cortical silent period, SCA: spinocerebellar ataxia.
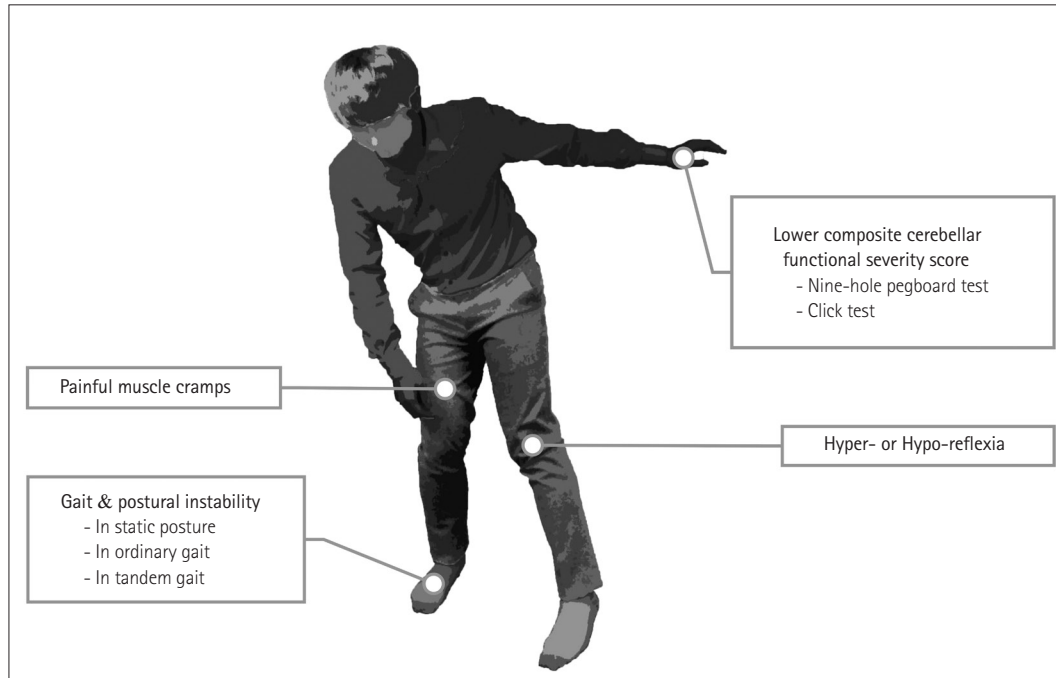
Fig. 4. Clinical symptoms and other ancillary test findings in premanifest carriers of SCA1, -2, -3, and -6. Presymptomatic manifestations exhibited in multiple SCA subtypes based on the data listed in Table 1 and Table 2. SCA: spinocerebellar ataxia.

SCA-subtype-specific pathophysiological mechanisms; however, further studies are required to confirm them.

Some changes in premanifest SCA showed opposite tendencies to those in manifest SCA. For example, DTI studies have shown increased radial diffusivity and decreased FA in both premanifest carriers and manifest patients with SCA3.[54,56] In contrast, SCA6 patients showed decreased radial diffusivity and increased FA in the premanifest stage (asterisks in Fig. 3), whereas there was an increase in radial diffusivity and a decrease in FA in the manifest stage of SCA6.[57] In SCA3, premanifest patients showed decreased resting motor thresholds and cortical silent periods in TMS, while these TMS measures were restored to the normal ranges in manifest patients.[50] For serum biomarkers, premanifest SCA3 carriers showed more-pronounced mitochondrial DNA common deletions and serum eotaxin levels than patients with fully manifested SCA3.[20,60] We suppose that such presymptomatic manifestations reflect adaptive changes for compensating neurodegeneration, which subsequently reverse during disease progression in the manifest stage of SCA. However, the numbers of studies and participants have not been sufficient, and so further comparative studies with large samples and including other polyglutamine SCA subtypes are required to confirm whether these manifestations are unique features in each subtype of SCA.

Positive Babinski reflex, dysarthria, handwriting problems, diplopia, episodic vertigo, sleep disturbances, and P40 latency in SSEPs were inconsistent across independent studies. These symptoms may require more-sensitive evaluation tools because they are mostly qualitative symptoms evaluated by interviews and questionnaires. Further developments should focus on quantifying these symptoms that explicitly appear at the premanifest stage.

The nature of this polyglutamine disorder means that the number of CAG repeats is the strongest predictor of disease progression.[32] However, besides CAG repeats, some markers can be candidates for predicting the time to ataxia onset. Longitudinal studies have found that in premanifest SCA2, sensory amplitudes in nerve conduction studies, P40 latencies in tibial nerve SSEPs, CCFS scores, and abnormal muscle reflexes were correlated with the time to ataxia onset.

Limitations of current literature

There are notable limitations in the current knowledge of the premanifest stage of polyglutamine SCAs. First, there have been insufficient studies, with some presymptomatic manifestations only being evaluated in a single SCA subtype. Also, only a few premanifest patients were enrolled in most studies because they have instead focused on the manifest stage in the same project. It is difficult to find evidence of predictive markers for disease progression besides the number of CAG repeats in premanifest SCA, since only a few longitudinal studies have been conducted in the premanifest stage, and those studies were limited to SCA2.

Table 1. Summary of findings in premanifest carriers of SCA1, -3, and -6

Predictive symptoms and tests	Presymptomatic manifestations*	Sample size: carriers (vs. controls)	Reference
SCA1			
Neuroimaging			
MRI volumetry	Gray-matter loss in pons, medulla oblongata, and cerebellum	39 (vs. 33)	42
MR spectroscopy	Reduced N-acetylaspartate/creatine and choline/creatine in basilar pons	2 (vs. 10)	58
Clinical symptoms and other ancillary tests			
Muscle cramps	Painful muscle cramps (carriers: 40%, controls: 19%)	50 (vs. 32)	42
Gait and posture	Increased instability (body sway) in static posture	9 (vs. 17[†])	62
Functional scoring system	Lower CCFS score	50 (vs. 32)	42
SCA3 (Machado-Joseph Disease)			
Electrophysiological assessments			
VOG	Impaired smooth pursuit (vertical), increased error of antisaccadic movements, saccadic slowing (vertical), more frequent and large gaze-evoked nystagmus and square-wave jerks during fixation	12 (vs. 26[†])	48
TMS	Increased CMCT and decreased resting motor threshold, cortical silent period, and cortical adaptation	2 (vs. 62[†])	50
Neuroimaging			
MRI volumetry	Reduced volumes of midbrain, pons, and cervical spinal cord	12 (vs. 91[†])	54
DTI	Increased diusivity and decreased FA in cerebellar peduncles	12 (vs. 91[†]) 16 (vs. 16[†])	54 56
Molecular biomarkers			
Nucleic acid	Decreased BCL2/BAX mRNA ratio	16 (vs. 16[†])	60
	Increased frequency of common deletion (del4977) in mitochondrial DNA	16 (vs. 16[†])	60
Other serum biomarkers	Higher levels of serum eotaxin	13 (vs. 43)	20
	Higher levels of serum neurofilament light chains	26 (vs. 21[†])	59
Clinical symptoms and other ancillary tests			
Muscle cramps	Painful muscle cramps (carriers: 81%, controls: 16%)	13 (vs. 15[†])	74
Motor signs	Hyperreflexia in lower limbs (carriers: 8.3%, controls: 0%)	12 (vs. 26[†])	48
	(carriers: 23.1%, controls: 0%)	13 (vs. 15[†])	74
	Hyporeflexia in lower limbs (carriers: 8.3%, controls: 0%)	12 (vs. 26[†])	48
	(carriers: 15.4%, controls: 0%)	13 (vs. 15[†])	74
	Extrapyramidal signs (carriers: 15.4%, controls: 0%)	13 (vs. 15[†])	74
	Positive Babinski reflex (inconsistency)		48,74
Oculomotor symptoms	Gaze-evoked nystagmus (carriers: 39%, controls: 5%)	26 (vs. 20)	42
	(carriers: 17%, controls: 0%)	48 (vs. 42)	28
	Diplopia (inconsistency)		24,42
Sensory symptoms	Sensory neuropathic signs (carriers: 15.4%, controls: 0%)	13 (vs. 15[†])	74
Classical conditioning test	Impaired eyeblink conditioning after auditory stimuli	18 (vs. 16[†])	75
Functional scoring system	Lower CCFS score	26 (vs. 20)	42
SCA6			
Electrophysiological assessments			
VOG	Multivariate analysis including gaze-evoked nystagmus, smooth pursuit, and saccadic slowing as variables	4 (vs. 7[†])	49
Neuroimaging			
DTI	Decreased diusivity and increased FA in cerebellar peduncles	2 (vs. 20[†])	57
fMRI	Alterations in cortical connectivity	2 (vs. 20[†])	57
Clinical symptoms and other ancillary tests			
Gait and posture	Increased variabilities in step time and step width	6 (vs. 24)	64
Dysarthria and others	Speech abnormalities and episodic vertigo (inconsistency)		24,42

*'Inconsistency' indicates that different studies showed both significant (*p*<0.05) and not-significant (*p*>0.05) changes in carriers, [†]Age-matched controls, [†]Age- and sex-matched controls.

CCFS: Composite Cerebellar Functional Severity, CMCT: central motor conduction time, DTI: diffusion-tensor imaging, FA: fractional anisotropy, fMRI: functional magnetic resonance imaging, MR: magnetic resonance, MRI: magnetic resonance imaging, SCA: spinocerebellar ataxia, TMS: transcranial magnetic stimulation, VOG: video-oculography.

Table 2. Summary of findings in premanifest carriers of SCA2

Predictive symptoms and tests	Presymptomatic manifestations*	Sample size: carrier (vs. controls)	Reference
SCA2			
Electrophysiological assessments			
VOG	Increased error and latency of antisaccadic movements	37 (vs. 37[†])	43
	Saccadic slowing (horizontal)	54 (vs. 56[†])	47
Nerve conduction study	Decreased sensory amplitudes (median and sural nerves)	21 (vs. 19[†])	26
		62 (vs. 80)	52
		59 (vs. 108)	51
Evoked potentials	Abnormal morphology in BAEP	62 (vs. 80)	52
	Abnormal morphology in SSEP	62 (vs. 80)	52
	Increased P40 latency in SSEP (inconsistency)		26, 51, 52
TMS	Increased CMCT, resting motor threshold, active motor threshold, and cortical silent period	37 (vs. 37[†])	44
EEG and EMG coherence	Decreased corticomuscular EEG–EMG coherence and intermuscular EMG–EMG coherence between upper and lower limbs	15 (vs. 25[†])	53
Neuroimaging			
MRI volumetry	Gray-matter loss in cerebellum	12 (vs. 33)	42
	Reduced volume of brainstem (especially pons) and cerebellum	16 (vs. 18[†])	55
		12 (vs. 33)	42
		37 (vs. 37[†])	43
Clinical symptoms and other ancillary tests			
Muscle cramps	Painful muscle cramps (carriers: 81%, controls: 16%)	21 (vs. 19[†])	26
	(carriers: 81%, controls: 27%)	37 (vs. 37[†])	43
	(carriers: 35%, controls: 13%)	31 (vs. 78)	42
Motor signs	Hyperreflexia in lower limbs (carriers: 33%, controls: 0%)	21 (vs. 19[†])	26
	Hyporeflexia in lower limbs (carriers: 43%, controls: 0%)	37 (vs. 37[†])	43
	(carriers: 38%, controls: 5%)	21 (vs. 19[†])	26
Oculomotor symptoms	Oculomotor disturbance such as saccadic slowing and gaze-evoked nystagmus (carriers: 22%, controls: 0%)	37 (vs. 37[†])	43
Dysarthria	Speech abnormalities (inconsistency)		24, 42
Gait and posture	Increased instability (body oscillation frequency) in static posture	30 (vs. 30[†])	63
Motor performance (upper limbs)	Increased errors when throwing objects at a target	28 (vs. 28[†])	76
	Handwriting problems (inconsistency)		24, 42
Sensory symptoms	Sensory neuropathic signs (carriers: 81%, controls: 21%)	21 (vs. 19[†])	26
	(carriers: 62%, controls: 19%)	37 (vs. 37[†])	43
Sleep problems	Lower density of sleep spindles in non-REM sleep	20 (vs. 20[†])	68
	Reduced proportion of REM sleep	20 (vs. 20[†])	68
		36 (vs. 36[†])	67
	Higher Pittsburgh Sleep Quality Index (inconsistency)		68, 67, 42
	Subjective sleep disturbance (inconsistency)		24, 43
Autonomic dysfunction	Higher SCOPA-AUT score	37 (vs. 37[†])	43
		48 (vs. 97[†])	70
	Heart-rate variability index abnormalities	48 (vs. 48[†])	71
Neuropsychological test	Impaired executive function and visual memory	20 (vs. 20[†])	68
		37 (vs. 37[†])	43
Functional scoring system	Lower CCFS score and SCA Functional Index	31 (vs. 78)	42

*'Iinconsistency' indicates that different studies showed both significant (*p*<0.05) and not-significant (*p*>0.05) changes in carriers, [†]Age-matched controls, [‡]Age- and sex-matched controls.

BAEP: brainstem auditory evoked potential, EEG: electroencephalography, EMG: electromyography, REM: rapid eye movement, SCOPA-AUT: Scales for Outcomes in Parkinson's Disease–Autonomic Dysfunction, SCA: spinocerebellar ataxia, SSEP: somatosensory evoked potential.

Second, review papers (including the present work) cannot avoid bias caused by the dominant publication by one author in a specific subtype. For example, publications in the premanifest stage of SCA2 are dominated by one primary author and his colleagues.[25,26,43,44,47,51-53,63,76-81] Bias caused by overlapping individual participants (e.g., one premanifest carrier being included in multiple studies) and data from a single hospital may also exist, and so global multicenter studies are required to resolve this limitation.

Third, different evaluation methods used in different studies need to be standardized for each presymptomatic manifestation. For example, various methods were used to evaluate instabilities in gait and posture, with some studies calculating the distance of body sway and others measuring the frequency of body oscillation.[63,65]

Fourth, there is no consensus on the criteria for 'premanifest' SCA. The subjects were assessed using the SARA score in some studies, while in others they were simply defined as not having overt cerebellar ataxia. The lack of standardized criteria for the definition of premanifest SCA limits the ability to interpret our findings.

Further implications
The present findings have several important implications. First, there is a need to establish a standardized measurement tool for premanifest carriers of SCA. VOG, MRI, measurement of serum biomarkers, and evaluations of muscle cramps, gait, and postural reflexes can be applied in clinical practice to sensitively detect early changes in premanifest SCA patients. The early diagnosis of SCA at the premanifest stage would lead to early intervention and provide patients with opportunities to participate in future clinical trials. Many attempts have been made to develop therapeutics for SCAs,[82-91] but disease-modifying therapies are yet to be established.

Second, SCA-specific presymptomatic manifestations need to be defined. Some of the presymptomatic manifestations reviewed in this article may also develop in nonataxic SCA or in other neurodegenerative diseases involving the cerebellum. Clinical symptoms such as postural reflex changes and dysarthria may be nonspecific premanifest signs of SCA, whereas serum biomarkers and certain neuroimaging signs may act as specific markers of premanifest SCA. Future studies should investigate these SCA-specific presymptomatic manifestations distinguished from other neurodegenerative diseases involving the cerebellum. In addition, the progressively worsening of presymptomatic markers in manifest SCA is particularly important because these markers may reflect the pathophysiology of the progressive nature of SCA; many of the patients included in the cross-sectional studies reviewed in the present study showed progression in the manifest stage, such as volumetric reductions and DTI changes in premanifest SCAs being more severe in manifest patients. Longitudinal studies measuring the rate of change from the presymptomatic stage to the clinical manifestation of cerebellar ataxia could provide useful information for developing the markers needed for future disease-modifying trials in premanifest SCA.

Presymptomatic markers correlated with the time to ataxia onset could also be predictive markers for use in risk assessments and the monitoring of high-risk individuals. These markers also may shed light on the pathogenesis of SCA, because they are closely related to disease manifestation. The probabilities of developing SCA and the predicted time to ataxia onset are currently calculated only based on the number of CAG repeats and by performing survival analysis in population studies.[24,32,92] However, further studies focused on accurate predictions of disease development may make it possible to redefine the premanifest stage based on laboratory-based biomarkers rather than ataxic symptoms, and also stratify the risk and progression during the early stage of SCAs.

Author Contributions
Conceptualization: Jee-Young Lee. Data curation: Dong-Hoi Kim. Formal analysis: Dong-Hoi Kim. Investigation: Dong-Hoi Kim, Ryul Kim. Visualization: Dong-Hoi Kim. Writing—original draft: Dong-Hoi Kim, Ryul Kim. Writing—review & editing: Jee-Young Lee, Kyoung-Min Lee.

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Conflicts of Interest
The authors have no potential conflicts of interest to disclose.

REFERENCES
1. Parodi L, Coarelli G, Stevanin G, Brice A, Durr A. Hereditary ataxias and paraparesias: clinical and genetic update. *Curr Opin Neurol* 2018; 31:462-471.
2. Thomas EA. Involvement of HDAC1 and HDAC3 in the pathology of polyglutamine disorders: therapeutic implications for selective HDAC1/HDAC3 inhibitors. *Pharmaceuticals (Basel)* 2014;7:634-661.
3. Schöls L, Bauer P, Schmidt T, Schulte T, Riess O. Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. *Lancet Neurol* 2004;3:291-304.
4. Paulson H, Ammache Z. Ataxia and hereditary disorders. *Neurol Clin* 2001;19:759-782.
5. Rüb U, Schöls L, Paulson H, Auburger G, Kermer P, Jen JC, et al. Clinical features, neurogenetics and neuropathology of the polyglutamine

spinocerebellar ataxias type 1, 2, 3, 6 and 7. *Prog Neurobiol* 2013; 104:38-66.

6. Kim HJ, Jeon BS, Lee WY, Chung SJ, Yong SW, Kang JH, et al. SCA in Korea and its regional distribution: a multicenter analysis. *Parkinsonism Relat Disord* 2011;17:72-75.

7. Lee WY, Jin DK, Oh MR, Lee JE, Song SM, Lee EA, et al. Frequency analysis and clinical characterization of spinocerebellar ataxia types 1, 2, 3, 6, and 7 in Korean patients. *Arch Neurol* 2003;60:858-863.

8. Jin DK, Oh MR, Song SM, Koh SW, Lee M, Kim GM, et al. Frequency of spinocerebellar ataxia types 1,2,3,6,7 and dentatorubral pallidoluysian atrophy mutations in Korean patients with spinocerebellar ataxia. *J Neurol* 1999;246:207-210.

9. Sullivan R, Yau WY, O'Connor E, Houlden H. Spinocerebellar ataxia: an update. *J Neurol* 2019;266:533-544.

10. Klockgether T, Paulson H. Milestones in ataxia. *Mov Disord* 2011;26: 1134-1141.

11. Okazawa H, Rich T, Chang A, Lin X, Waragai M, Kajikawa M, et al. Interaction between mutant ataxin-1 and PQBP-1 affects transcription and cell death. *Neuron* 2002;34:701-713.

12. La Spada AR, Fu YH, Sopher BL, Libby RT, Wang X, Li LY, et al. Polyglutamine-expanded ataxin-7 antagonizes CRX function and induces cone-rod dystrophy in a mouse model of SCA7. *Neuron* 2001;31:913-927.

13. Matilla A, Koshy BT, Cummings CJ, Isobe T, Orr HT, Zoghbi HY. The cerebellar leucine-rich acidic nuclear protein interacts with ataxin-1. *Nature* 1997;389:974-978.

14. Klockgether T, Mariotti C, Paulson HL. Spinocerebellar ataxia. *Nat Rev Dis Primers* 2019;5:24.

15. van de Warrenburg BP, Hendriks H, Dürr A, van Zuijlen MC, Stevanin G, Camuzat A, et al. Age at onset variance analysis in spinocerebellar ataxias: a study in a Dutch-French cohort. *Ann Neurol* 2005;57:505-512.

16. Kim JY, Park SS, Joo SI, Kim JM, Jeon BS. Molecular analysis of spinocerebellar ataxias in Koreans: frequencies and reference ranges of SCA1, SCA2, SCA3, SCA6, and SCA7. *Mol Cells* 2001;12:336-341.

17. Maas RP, van Gaalen J, Klockgether T, van de Warrenburg BP. The preclinical stage of spinocerebellar ataxias. *Neurology* 2015;85:96-103.

18. Schmitz-Hübsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. *Neurology* 2006;66:1717-1720.

19. Lima M, Raposo M. Towards the identification of molecular biomarkers of spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease (MJD). *Adv Exp Med Biol* 2018;1049:309-319.

20. da Silva Carvalho G, Saute JA, Haas CB, Torrez VR, Brochier AW, Souza GN, et al. Cytokines in Machado Joseph disease/spinocerebellar ataxia 3. *Cerebellum* 2016;15:518-525.

21. Jacobi H, Rakowicz M, Rola R, Fancellu R, Mariotti C, Charles P, et al. Inventory of Non-Ataxia Signs (INAS): validation of a new clinical assessment instrument. *Cerebellum* 2013;12:418-428.

22. Pearson CE, Nichol Edamura K, Cleary JD. Repeat instability: mechanisms of dynamic mutations. *Nat Rev Genet* 2005;6:729-742.

23. Stevanin G, Dürr A, Brice A. Clinical and molecular advances in autosomal dominant cerebellar ataxias: from genotype to phenotype and physiopathology. *Eur J Hum Genet* 2000;8:4-18.

24. Globas C, du Montcel ST, Baliko L, Boesch S, Depondt C, DiDonato S, et al. Early symptoms in spinocerebellar ataxia type 1, 2, 3, and 6. *Mov Disord* 2008;23:2232-2238.

25. Velázquez-Pérez LC, Rodríguez-Labrada R, Fernandez-Ruiz J. Spinocerebellar ataxia type 2: clinicogenetic aspects, mechanistic insights, and management approaches. *Front Neurol* 2017;8:472.

26. Velázquez-Pérez L, Rodríguez-Labrada R, Canales-Ochoa N, Montero JM, Sánchez-Cruz G, Aguilera-Rodríguez R, et al. Progression of early features of spinocerebellar ataxia type 2 in individuals at risk: a longitudinal study. *Lancet Neurol* 2014;13:482-489.

27. Chen JW, Zhao L, Zhang F, Li L, Gu YH, Zhou JY, et al. Clinical char-

acteristics, radiological features and gene mutation in 10 Chinese families with spinocerebellar ataxias. *Chin Med J (Engl)* 2015;128:1714-1723.

28. Raposo M, Vasconcelos J, Bettencourt C, Kay T, Coutinho P, Lima M. Nystagmus as an early ocular alteration in Machado-Joseph disease (MJD/SCA3). *BMC Neurol* 2014;14:17.

29. Mizushima K, Watanabe M, Abe K, Aoki M, Itoyama Y, Shizuka M, et al. Analysis of spinocerebellar ataxia type 2 in Gunma Prefecture in Japan: CAG trinucleotide expansion and clinical characteristics. *J Neurol Sci* 1998;156:180-185.

30. Martin J, Van Regemorter N, Del-Favero J, Löfgren A, Van Broeckhoven C. Spinocerebellar ataxia type 7 (SCA7) - correlations between phenotype and genotype in one large Belgian family. *J Neurol Sci* 1999; 168:37-46.

31. Cancel G, Dürr A, Didierjean O, Imbert G, Bürk K, Lezin A, et al. Molecular and clinical correlations in spinocerebellar ataxia 2: a study of 32 families. *Hum Mol Genet* 1997;6:709-715.

32. Tezenas du Montcel S, Durr A, Rakowicz M, Nanetti L, Charles P, Sulek A, et al. Prediction of the age at onset in spinocerebellar ataxia type 1, 2, 3 and 6. *J Med Genet* 2014;51:479-486.

33. Dziedzic T, Pera J, Klimkowicz-Mrowiec A, Mroczko B, Slowik A. Biochemical and radiological markers of Alzheimer's disease progression. *J Alzheimers Dis* 2016;50:623-644.

34. Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003;24:197-211.

35. Burciu RG, Ofori E, Archer DB, Wu SS, Pasternak O, McFarland NR, et al. Progression marker of Parkinson's disease: a 4-year multi-site imaging study. *Brain* 2017;140:2183-2192.

36. Yang J, Burciu RG, Vaillancourt DE. Longitudinal progression markers of Parkinson's disease: current view on structural imaging. *Curr Neurol Neurosci Rep* 2018;18:83.

37. Odish OFF, Johnsen K, van Someren P, Roos RAC, van Dijk JG. EEG may serve as a biomarker in Huntington's disease using machine learning automatic classification. *Sci Rep* 2018;8:16090.

38. Scarabino D, Veneziano L, Peconi M, Frontali M, Mantuano E, Corbo RM. Leukocyte telomere shortening in Huntington's disease. *J Neurol Sci* 2019;396:25-29.

39. Wijeratne PA, Young AL, Oxtoby NP, Marinescu RV, Firth NC, Johnson EB, et al. An image-based model of brain volume biomarker changes in Huntington's disease. *Ann Clin Transl Neurol* 2018;5:570-582.

40. Jędrak P, Mozolewski P, Węgrzyn G, Więckowski MR. Mitochondrial alterations accompanied by oxidative stress conditions in skin fibroblasts of Huntington's disease patients. *Metab Brain Dis* 2018;33:2005-2017.

41. Fazio P, Paucar M, Svenningsson P, Varrone A. Novel imaging biomarkers for Huntington's disease and other hereditary choreas. *Curr Neurol Neurosci* Rep 2018;18:85.

42. Jacobi H, Reetz K, du Montcel ST, Bauer P, Mariotti C, Nanetti L, et al. Biological and clinical characteristics of individuals at risk for spinocerebellar ataxia types 1, 2, 3, and 6 in the longitudinal RISCA study: analysis of baseline data. *Lancet Neurol* 2013;12:650-658.

43. Velázquez-Pérez L, Rodríguez-Labrada R, Cruz-Rivas EM, Fernández-Ruiz J, Vaca-Palomares I, Lilia-Campins J, et al. Comprehensive study of early features in spinocerebellar ataxia 2: delineating the prodromal stage of the disease. *Cerebellum* 2014;13:568-579.

44. Velázquez-Pérez L, Rodríguez-Labrada R, Torres-Vega R, Medrano Montero J, Vázquez-Mojena Y, Auburger G, et al. Abnormal corticospinal tract function and motor cortex excitability in non-ataxic SCA2 mutation carriers: a TMS study. *Clin Neurophysiol* 2016;127:2713-2719.

45. Park H, Kim HJ, Jeon BS. Parkinsonism in spinocerebellar ataxia. *Biomed Res Int* 2015;2015:125273.

46. Lindsay E, Storey E. Cognitive changes in the spinocerebellar ataxias due to expanded polyglutamine tracts: a survey of the literature. *Brain*

Sci 2017;7:83.

47. Velázquez-Pérez L, Seifried C, Abele M, Wirjatijasa F, Rodríguez-Labrada R, Santos-Falcón N, et al. Saccade velocity is reduced in presymptomatic spinocerebellar ataxia type 2. *Clin Neurophysiol* 2009;120:632-635.

48. Wu C, Chen DB, Feng L, Zhou XX, Zhang JW, You HJ, et al. Oculomotor deficits in spinocerebellar ataxia type 3: potential biomarkers of preclinical detection and disease progression. *CNS Neurosci Ther* 2017;23:321-328.

49. Christova P, Anderson JH, Gomez CM. Impaired eye movements in presymptomatic spinocerebellar ataxia type 6. *Arch Neurol* 2008;65:530-536.

50. Farrar MA, Vucic S, Nicholson G, Kiernan MC. Motor cortical dysfunction develops in spinocerebellar ataxia type 3. *Clin Neurophysiol* 2016;127:3418-3424.

51. Velázquez L, Medina EE. [Electrophysiological characteristics of asymptomatic relatives of patients with type 2 spinocerebellar ataxia]. *Rev Neurol* 1998;27:955-963.

52. Velázquez Pérez L, Sánchez Cruz G, Canales Ochoa N, Rodríguez Labrada R, Rodríguez Díaz J, Almaguer Mederos L, et al. Electrophysiological features in patients and presymptomatic relatives with spinocerebellar ataxia type 2. *J Neurol Sci* 2007;263:158-164.

53. Velázquez-Pérez L, Tünnerhoff J, Rodríguez-Labrada R, Torres-Vega R, Ruiz-Gonzalez Y, Belardinelli P, et al. Early corticospinal tract damage in prodromal SCA2 revealed by EEG-EMG and EMG-EMG coherence. *Clin Neurophysiol* 2017;128:2493-2502.

54. Rezende TJR, de Paiva JLR, Martinez ARM, Lopes-Cendes I, Pedroso JL, Barsottini OGP, et al. Structural signature of SCA3: from presymptomatic to late disease stages. *Ann Neurol* 2018;84:401-408.

55. Reetz K, Rodríguez-Labrada R, Dogan I, Mirzazade S, Romanzetti S, Schulz JB, et al. Brain atrophy measures in preclinical and manifest spinocerebellar ataxia type 2. *Ann Clin Transl Neurol* 2018;5:128-137.

56. Wu X, Liao X, Zhan Y, Cheng C, Shen W, Huang M, et al. Microstructural alterations in asymptomatic and symptomatic patients with spinocerebellar ataxia type 3: a tract-based spatial statistics study. *Front Neurol* 2017;8:714.

57. Falcon MI, Gomez CM, Chen EE, Shereen A, Solodkin A. Early cerebellar network shifting in spinocerebellar ataxia type 6. *Cereb Cortex* 2016;26:3205-3218.

58. Mascalchi M, Tosetti M, Plasmati R, Bianchi MC, Tessa C, Salvi F, et al. Proton magnetic resonance spectroscopy in an Italian family with spinocerebellar ataxia type 1. *Ann Neurol* 1998;43:244-252.

59. Li QF, Dong Y, Yang L, Xie JJ, Ma Y, Du YC, et al. Neurofilament light chain is a promising serum biomarker in spinocerebellar ataxia type 3. *Mol Neurodegener* 2019;14:39.

60. Raposo M, Ramos A, Santos C, Kazachkova N, Teixeira B, Bettencourt C, et al. Accumulation of mitochondrial DNA common deletion since the preataxic stage of Machado-Joseph disease. *Mol Neurobiol* 2019;56:119-124.

61. de Assis AM, Saute JAM, Longoni A, Haas CB, Torrez VR, Brochier AW, et al. Peripheral oxidative stress biomarkers in spinocerebellar ataxia type 3/Machado-Joseph disease. *Front Neurol* 2017;8:485.

62. Nanetti L, Alpini D, Mattei V, Castaldo A, Mongelli A, Brenna G, et al. Stance instability in preclinical SCA1 mutation carriers: a 4-year prospective posturography study. *Gait Posture* 2017;57:11-14.

63. Velázquez-Pérez L, Sánchez-Cruz G, Rodríguez-Labrada R, Velázquez-Manresa M, Hechavarría-Pupo R, Almaguer-Mederos LE. Postural instability in prodromal spinocerebellar ataxia type 2: insights into cerebellar involvement before onset of permanent ataxia. *Cerebellum* 2017;16:279-281.

64. Rochester L, Galna B, Lord S, Mhiripiri D, Eglon G, Chinnery PF. Gait impairment precedes clinical symptoms in spinocerebellar ataxia type 6. *Mov Disord* 2014;29:252-255.

65. Ilg W, Fleszar Z, Schatton C, Hengel H, Harmuth F, Bauer P, et al. Individual changes in preclinical spinocerebellar ataxia identified via

increased motor complexity. *Mov Disord* 2016;31:1891-1900.

66. Ilg W, Golla H, Thier P, Giese MA. Specific influences of cerebellar dysfunctions on gait. *Brain* 2007;130(Pt 3):786-798.

67. Rodríguez-Labrada R, Velázquez-Perez L, Ochoa NC, Polo LG, Valencia RH, Cruz GS, et al. Subtle rapid eye movement sleep abnormalities in presymptomatic spinocerebellar ataxia type 2 gene carriers. *Mov Disord* 2011;26:347-350.

68. Rodríguez-Labrada R, Galicia-Polo L, Canales-Ochoa N, Voss U, Tuin I, Peña-Acosta A, et al. Sleep spindles and K-complex activities are decreased in spinocerebellar ataxia type 2: relationship to memory and motor performances. *Sleep Med* 2019;60:188-196.

69. Visser M, Marinus J, Stiggelbout AM, Van Hilten JJ. Assessment of autonomic dysfunction in Parkinson's disease: the SCOPA-AUT. *Mov Disord* 2004;19:1306-1312.

70. Montes BJ, Estévez BM, Almaguer MLE. Disautonomic features in presyntomatic subjects and patients with type 2 spinocerebellar ataxia. *Rev Mex Neuroci* 2011;12:76-81.

71. Montes-Brown J, Machado A, Estévez M, Carricarte C, Velázquez-Pérez L. Autonomic dysfunction in presymptomatic spinocerebellar ataxia type-2. *Acta Neurol Scand* 2012;125:24-29.

72. du Montcel ST, Charles P, Ribai P, Goizet C, Le Bayon A, Labauge P, et al. Composite cerebellar functional severity score: validation of a quantitative score of cerebellar impairment. *Brain* 2008;131(Pt 5):1352-1361.

73. Schmitz-Hübsch T, Giunti P, Stephenson DA, Globas C, Baliko L, Saccà F, et al. SCA Functional Index: a useful compound performance measure for spinocerebellar ataxia. *Neurology* 2008;71:486-492.

74. Yang ZH, Shi CH, Zhou LN, Li YS, Yang J, Liu YT, et al. Metabolic profiling reveals biochemical pathways and potential biomarkers of spinocerebellar ataxia 3. *Front Mol Neurosci* 2019;12:159.

75. van Gaalen J, Maas RPPWM, Ippel EF, Elting MW, van Spaendonck-Zwarts KY, Vermeer S, et al. Abnormal eyeblink conditioning is an early marker of cerebellar dysfunction in preclinical SCA3 mutation carriers. *Exp Brain Res* 2019;237:427-433.

76. Velázquez-Perez L, Díaz R, Pérez-González R, Canales N, Rodríguez-Labrada R, Medrano J, et al. Motor decline in clinically presymptomatic spinocerebellar ataxia type 2 gene carriers. *PLoS One* 2009;4: e5398.

77. Velázquez-Perez L, Rodríguez-Labrada R, Canales-Ochoa N, Sanchez-Cruz G, Fernandez-Ruiz J, Montero JM, et al. Progression markers of Spinocerebellar ataxia 2. A twenty years neurophysiological follow up study. *J Neurol Sci* 20105;290:22-26.

78. Velázquez-Pérez L, Rodríguez-Labrada R, García-Rodríguez JC, Almaguer-Mederos LE, Cruz-Mariño T, Laffita-Mesa JM. A comprehensive review of spinocerebellar ataxia type 2 in Cuba. *Cerebellum* 2011;10:184-198.

79. Velázquez-Pérez L, Rodríguez-Labrada R, Laffita-Mesa JM. Prodromal spinocerebellar ataxia type 2: prospects for early interventions and ethical challenges. *Mov Disord* 2017;32:708-718.

80. Velázquez-Pérez L, Rodríguez-Labrada R, Torres-Vega R, Montero JM, Vazquez-Mojena Y, Auburger G, et al. Central motor conduction time as prodromal biomarker in spinocerebellar ataxia type 2. *Mov Disord* 2016;31:603-604.

81. Velázquez-Pérez L, Rodríguez-Labrada R, Torres-Vega R, Ortega-Sánchez R, Medrano-Montero J, González-Piña R, et al. Progression of corticospinal tract dysfunction in pre-ataxic spinocerebellar ataxia type 2: a two-years follow-up TMS study. *Clin Neurophysiol* 2018;129: 895-900.

82. Rodríguez-Díaz JC, Velázquez-Pérez L, Rodríguez Labrada R, Aguilera Rodríguez R, Laffita Pérez D, Canales Ochoa N, et al. Neurorehabilitation therapy in spinocerebellar ataxia type 2: a 24-week, rater-blinded, randomized, controlled trial. *Mov Disord* 2018;33:1481-1487.

83. Morlet É, Hozer F, Costemale-Lacoste JF. Neuroprotective effects of lithium: what are the implications in humans with neurodegenerative disorders? *Geriatr Psychol Neuropsychiatr Vieil* 2018;16:78-86.

84. Duarte-Silva S, Maciel P. Pharmacological therapies for Machado-Joseph disease. *Adv Exp Med Biol* 2018;1049:369-394.

85. Saute JAM, Jardim LB. Planning future clinical trials for Machado-Joseph disease. *Adv Exp Med Biol* 2018;1049:321-348.

86. Rubinsztein DC, Orr HT. Diminishing return for mechanistic therapeutics with neurodegenerative disease duration?: there may be a point in the course of a neurodegenerative condition where therapeutics targeting disease-causing mechanisms are futile. *Bioessays* 2016;38:977-980.

87. Keiser MS, Kordasiewicz HB, McBride JL. Gene suppression strategies for dominantly inherited neurodegenerative diseases: lessons from Huntington's disease and spinocerebellar ataxia. *Hum Mol Genet* 2016; 25(R1):R53-R64.

88. Li X, Liu H, Fischhaber PL, Tang TS. Toward therapeutic targets for SCA3: insight into the role of Machado-Joseph disease protein ataxin-3 in misfolded proteins clearance. *Prog Neurobiol* 2015;132:34-58.

89. Yi J, Zhang L, Tang B, Han W, Zhou Y, Chen Z, et al. Sodium valproate alleviates neurodegeneration in SCA3/MJD via suppressing apoptosis and rescuing the hypoacetylation levels of histone H3 and H4. *PLoS One* 2013;8:e54792.

90. Matilla-Dueñas A, Serrano C, Ivánovic Y, Alvarez R, Latorre P, Genís D. Novel therapeutic challenges in cerebellar diseases. In: Manto M, Schmahmann JD, Rossi F, Gruol DL, Koibuchi N, editors. *Handbook of the cerebellum and cerebellar disorders.* Dordrecht: Springer, 2013; 2370-2394.

91. Watson LM, Scholefield J, Greenberg LJ, Wood MJ. Polyglutamine disease: from pathogenesis to therapy. *S Afr Med J* 2012;102:481-484.

92. Almaguer-Mederos LE, Falcón NS, Almira YR, Zaldivar YG, Almarales DC, Góngora EM, et al. Estimation of the age at onset in spinocerebellar ataxia type 2 Cuban patients by survival analysis. *Clin Genet* 2010;78:169-174.

93. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009;339:b2700.