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Original Article

Sleep spindles and K-complex activities are decreased in spinocerebellar ataxia type 2: relationship to memory and motor performances

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ABSTRACT

Background: Sleep spindles and K-complexes are electroencephalographic hallmarks of non-rapid eye movement (non-REM) sleep that provide valuable information into brain functioning, plasticity and sleep functions in normal and pathological conditions. However, they have not been systematically investigated in spinocerebellar ataxias (SCA). To close this gap, the current study was carried out to quantify sleep spindles and K-complexes in SCA2 and to assess their relationship with clinical and molecular measures, as well as with memory and attention/executive functioning.

Methods: In this study, 20 SCA2 patients, 20 preclinical carriers and 20 healthy controls underwent whole-night polysomnographic (PSG) recordings as well as sleep interviews, ataxia scoring and neuropsychological assessments. Sleep spindles and K-complexes were automatically detected during non-REM sleep stage 2 (N2). Their densities were evaluated as events/minute.

Results: Compared to controls, sleep spindle density was significantly reduced in SCA2 patients and preclinical subjects. By contrast, K-complex density was specifically and significantly decreased only in SCA2 patients. Reduced spindle activity correlated with measures of verbal memory, whereas reduced K-complex activity correlated with age, ataxia severity and N3 sleep percentage in SCA2 patients.

Conclusions: Findings document an impairment of N2 sleep microstructure in SCA2 already in prodromal stages, suggesting an early involvement of thalamo-cortical and/or cortical circuits underlying the generation of sleep spindles and K-complexes. Thus, sleep spindle density may serve as useful biomarker for deficits of neural plasticity mechanisms underlying verbal memory alterations in patients. It may also serve as promising outcome measure in further therapeutical trials targeting memory decline in SCA2. With regard to K-complexes, they have potential usefulness as marker of sleep protection.

Abbreviations: SCA, Spinocerebellar ataxia; CAG, Cytosine-Adenine-Guanine; SARA, Scale for the Assessment and Rating of Ataxia; ANOVA, Analyses of variance; PolyQ, Polyglutamine; TRN, Thalamic reticular nucleus; TST, Total sleep time.

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1. Introduction

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant cerebellar ataxia caused by polyglutamine-coding Cytosine-Adenine-Guanine (CAG) repeat expansions in the *ATXN2* gene [1] which in turn confer toxic functions to the homonymous protein and alter its physiological functions [2].

The disease is clinically characterized by a progressive cerebellar syndrome accompanied by a wide spectrum of neurological symptoms such as slowing of horizontal saccades, peripheral neuropathy, muscle cramps, pyramidal signs, and cognitive disorders [3,4]. The onset of the cerebellar syndrome is significantly influenced by the expanded CAG repeat size and is preceded by a well-known prodromal stage [5].

Until now, no disease-modifying treatments for SCA2 have been established [6]. Nevertheless, the recently proven effectiveness of antisense oligonucleotide therapy in mouse models [7] opens the path for a promising therapeutical approach in humans alongside physical rehabilitation [8] and few established symptomatic treatments [4].

Sleep disorders are also common features of SCA2 even some years prior to the ataxia onset [9]. Clinical assessments frequently recognize insomnia, nocturnal cramps and restless legs syndrome (RLS). Moreover, polysomnographic studies identify periodic legs movements and loss of REM sleep percentage as main features [10–13]. Investigations of the microstructure of sleep in SCA2 have, so far, only focused on the stage of REM sleep, revealing a significant reduction of rapid eye movement density and increase of phasic EMG activity. However, the non-REM (NREM) sleep microstructure of patients with SCAs has not been systematically studied. Only a single short study is available at present [14]. This limits our understanding of some important dynamic characteristics of normal and pathological sleep processes not reflected by macrostructural evaluation of sleep stages.

Microstructural aspects of NREM sleep include recurring phasic EEG events such as K-complexes, sleep spindles, slow–wave activity and a cyclic alternating pattern [15–17]. Among these phasic events, K-complexes and sleep spindles have received increased attention from sleep researchers and physicians given that they are hallmarks of N2 sleep and provide valuable insights into sleep functions [18,19].

K-complexes are high-voltage biphasic (large negative–positive) sharp waves with frequencies within the delta band (1–4 Hz) that are symmetrically distributed over the scalp with a frontal midline maximum. They can be spontaneously elicited or externally evoked by sensory stimulation and are generated by a sequence of depolarizing and hyperpolarizing slow oscillations (<1 Hz) of pyramidal neurons in the cerebral cortex [18]. The functional role of K-complexes is still under debate [20], but some evidence suggests that they reflect a sleep-protecting mechanism and are involved in the processing of information during sleep [18,21].

Sleep spindles are spontaneous rhythmic bursts of waxing and waning waves composed by 5–20 successive deflections occurring within the sigma band at a frequency between 11 and 16 Hz, with durations between 0.5 and 3 s. Sleep spindles are considered global events occurring synchronously across widespread cortical territories but with higher amplitudes in central derivations. The rhythmic burst activity characterizing the sleep spindles is generated by synchronized interactions between the thalamic reticular nucleus (TRN) and thalamocortical neurons, which make up the thalamocortical loop [19,22,23]. Although the functional role of sleep spindles remains under debate, several lines of observational and experimental evidence point to their involvement in the sleep quality regulation and in various sleep-dependent physiological and cognitive processes, such as memory consolidation, neuronal plasticity and neuronal development [23].

Thus, considering the substantial value of K-complexes and sleep spindles for several aspects of brain function and the limited study of these graphoelements in SCAs, in the present paper we assess the density of K-complexes and sleep spindles in SCA2 patients and preclinical carriers, emphasizing their relationship with molecular, clinical and neuropsychological features of the disease.

2. Patients and methods

2.1. Participants

Twenty SCA2 patients and 20 preclinical carriers were admitted to the Centre for Research and Rehabilitation of Hereditary Ataxias in Holguín (CIRAH) for this study. Twenty non-SCA2 mutation carriers from Holguin province were admitted as age and gender matched controls. The main demographical, clinical and molecular data of each group is shown in Table 1. All procedures were in accordance with the declaration of Helsinki and the standards of the institutional ethics committee. All participants gave their written informed consent prior to the experiments.

2.2. Clinical and neuropsychological assessments

All subjects underwent standardized neurological examination. The Scale for the Assessment and Rating of Ataxia (SARA) was used to assess the severity of the cerebellar syndrome [25].

All subjects also underwent a brief neuropsychological assessment including the Mini-Mental State Examination (MMSE)

Table 1
Demographical, clinical and molecular features of included individuals.

	SCA2 patients	Preclinical carriers	Controls
Gender (f/m)	10/10	10/10	10/10
Age (years)	37.80 ± 11.17 (18–60)	36.50 ± 10.31 (20–59)	36.75 ± 10.33 (20–63)
Age at onset (years)	26.10 ± 9.79 (13–49)	NA	NA
Disease duration (years)	11.7 ± 5.22 (2–20)	NA	NA
Time to ataxia onset (years)[a]	NA	18.13 ± 13.60 (−7 to 36)	NA
Expanded CAG repeats (units)	40.05 ± 2.11 (36–44)	35.6 ± 2.33 (32–39)	NA

For quantitative variables the mean ± standard deviation and range (in parenthesis) are shown. NA: Not applicable; N=Number of subjects.

[a] Calculated by subtracting the chronological age from the predicted age at onset, which was estimated using the cumulative probability curves for disease manifestation at a particular age for any CAG repeat length [24]. Negative values of time to ataxia onset correspond to subjects that overdue the predicted onset, who were excluded from correlation analyses.

[26], the evoked verbal memory test [27], the Stroop interference test [28] and the Phonemic verbal fluency test [29]. For measures of verbal memory, all subjects were tested for the immediate and delayed recall of a 10-item uncategorized word list. Subjects were asked to recall each list immediately after presentation and after a delay of 20 min. For immediate recall, a maximum of 10 trials were conducted. The analyzed parameters were the number of trials needed to recall the entire list and the recalled words in the delayed trial [27].

In the Stroop Color-Word Interference task, subjects were first asked to read names of colors printed in black ink ("color naming condition"). In the following "interference condition", subjects were shown color names printed in inconsistent colored inks (eg, the word "blue" written in red ink) and they were required to name the color of the ink (red) instead of reading the word (blue). The analyzed parameter was the adjusted interference time, obtained by subtracting the time needed for the color naming condition from the time needed for the interference condition [28].

In the verbal fluency test all subjects were asked to name as many items as possible from a certain phonemic category (nouns starting with the letters F, A, and S) during 1 min for each letter. Mean successful nouns were analyzed [29].

2.3. Sleep quality assessments

Immediately before the first video-polysomnogram all individuals completed the Pittsburgh Sleep Quality Inventory (PSQI) [30] and the Epworth Sleepiness Scale (ESS) [31] to assess the sleep quality and the daytime sleepiness, respectively. PSQI scores $\geq$5 were indicative of reduced subjective sleep quality, whereas ESS scores $\geq$10 were indicative of abnormal daytime somnolence.

In addition, the subjects and/or their bed partners were interviewed with regard to symptoms of insomnia, RLS, REM sleep behavior disorder (RBD), nightmares, bruxism and other sleep disorders.

2.4. Polysomnographic assessments

All subjects underwent video-polysomnographic recordings for two consecutive nights during their routine sleeping hours between 22.00 h (lights out) and 6.00 h (lights on). A digital polygraph (Brain Lab, Schwarzer, Germany) was used following the standard montage recommended by the American Academy of Sleep Medicine (AASM) [32], which included electroencephalographic (EEG) recordings from F3, F4, C3, C4, O1, and O2 electrode sites, vertical and horizontal electrooculography, electrocardiography, electromyography (EMG) of submental and both tibialis anterior muscles and respiration monitoring. To avoid first-night effects, only results of the second night were analyzed.

All sleep recordings were visually analyzed according to the AASM guidelines [32] by a trained rater who paid special attention to the REM sleep scoring, given that both SCA2 patients and preclinical carriers show documented saccade slowing [33,34] that influences the rapid eye movement density in the polysomnogram [35].

2.4.1. Sleep spindle and K-complex analyses

The sleep spindles and K-complexes were averaged across the scalp electrodes. They were automatically detected using the algorithms implemented in the OSG-BRAINLAB 4 software, followed by a visual inspection performed by a trained rater obliging to the AASM criteria.

Sleep spindles were automatically detected in a two-step algorithm. First, the spindle candidates were obtained using an amplitude threshold (minimal candidate amplitude) after an 11.5–16 Hz band filtering [36]. In the second step, the candidates were reanalyzed in order to select the spindles out of them by looking at the candidate characteristics such as amplitudes, durations and frequencies. In the case of K-complexes, a two-step algorithm was also used. First, the K-complex candidates were detected by identification of the K-complex's half waves after a 0.5–3 Hz band filtering. The actual K-complexes were subsequently distinguished using thresholds for the candidate half wave's duration, amplitude and temporal isolation of other delta waves. Both NREM graphoelements were counted in all artifact-free 30s-epochs of the N2 sleep stage. The minimal number of sleep spindles or K-complexes for the subjects to be included in the analysis was not limited. The density of sleep spindles and K-complexes was expressed as the number of these graphoelements per minute.

2.5. Molecular studies

DNA extracts from peripheral venous blood underwent amplification of the CAG-rich region in the *ATXN2* gene by polymerase chain reactions and determination of CAG repeat length by polyacrylamide gel electrophoresis on an ALF Express II apparatus (Amersham Biosciences, Sweden).

2.6. Statistical analyses

For descriptive statistics of quantitative variables, means, median, standard deviations, 95% confidence intervals and ranges were obtained. For categorical variables the frequencies (percentages) were used. The normality of the distribution of quantitative variables was assessed by the Kolmogorov–Smirnov test. Multiple comparisons were performed using one-way analyses of variance (ANOVA) followed by the Tukey's HSD post-hoc test for variables with normal distribution and with the Kruskal–Wallis test followed by the post-hoc Dunn test for variables with non- normal distribution. Analyses of covariance (ANCOVAs) were conducted to assess whether the total sleep time and the number of arousals (covariates) influence the intergroup differences of the sleep stages and the K-complex and sleep spindles activities. Frequencies of sleep disorders were compared using a Chi-square test. Partial correlations followed by Bonferroni correction were performed between variables with normal distributions, whereas the Spearman Rank Order correlation coefficients were calculated for variables with non-normal distributions. Correlation analyses between continuous variables including the percentage of sleep stages and the K-complex and sleep spindles activities were also performed regressing out total sleep time (TST) and arousal index. In all cases, correlation analyses were performed in each individual group and in the overall sample. Significance level was set to $p < 0.05$. All analyses were performed using the commercially available STATISTICA software package (StatSoft, Inc., 2003 STATISTICA data analysis software system, version 6. www.statsoft.com). For graphs, the GraphPad Prism software v5.01 (La Jolla, CA 92037 USA) was used.

3. Results

3.1. Clinical and neuropsychological features

Findings of clinical and neuropsychological characterization in SCA2 patients and preclinical carriers were consistent with previous reports in distinct Cuban cohorts [37–39]. All SCA2 patients exhibited a cerebellar syndrome accompanied with the slowing of horizontal saccades. Seventeen of 20 patients (85%) exhibited sensory abnormalities whereas 14 (70%) reported painful muscle cramps. Age at disease onset was significantly correlated with

expanded CAG repeats in this group ($r = -0.67$; $p = 0.0007$). Mean SARA score of SCA2 patients was 19.2 (SD: 4.41; range: 11–26). Within the preclinical carriers group, the most frequent clinical features were the painful muscle cramps (85%), sensory abnormalities (75%) and hyperreflexia (50%). SARA scores ranged between 0 and 1.5 points with a mean of 0.28 (SD: 0.47).

Data of neuropsychological measures are listed in the Supplemental Material 1. The MMSE revealed that all control subjects were cognitively unimpaired. The Kruskal–Wallis test followed by the Dunn test revealed that only SCA2 patients showed a significant reduction of MMSE scores as compared with controls ($H = 12.41$; $p = 0.002$). In addition, SCA2 patients required significantly more trials to recall the entire word list in the verbal memory test ($H = 33.65$; $p < 0.001$). Also, patients recalled fewer words in the delayed trial ($H = 7.03$; $p = 0.029$). The adjusted time to complete the Stroop interference task was significantly increased both in SCA2 patients and preclinical carriers ($H = 43.49$; $p < 0.0001$), whereas the mean score of the phonemic verbal fluency test was reduced only in the patients ($H = 16.07$; $p = 0.0003$).

3.2. Sleep quality findings

Measures of sleep quality are shown in Table 2. Fourteen SCA2 patients (70%), 10 preclinical carriers (50%) and only three controls (15%) had abnormal PSQI scores. The three controls had only marginally elevated scores. A one-way ANOVA followed by Tukey's post-hoc test revealed significantly higher PSQI scores for SCA2 patients, but not for preclinical carriers, compared to controls, indicating a subjective reduction of sleep quality in the symptomatic cohort. No subject showed abnormal scores of ESS and consequently the ANOVA yielded no significant intergroup differences. The frequencies of sleep insomnia (35%) and RLS (40%) were significantly higher in SCA2 patients compared to controls. One quarter of preclinical carriers reported sleep insomnia. However, frequency analyses revealed no significant differences between preclinical carriers and controls. Other sleep disorders such as RBD and bruxism were less common in these cohorts.

3.3. Sleep architecture findings

Findings on sleep architecture are shown in Table 3. As shown, the one-way ANOVAs yielded a significant group effect for TST and sleep efficiency as well as for the percentages of N2, N3 and for REM sleep. In patients, the post-hoc analyses revealed a significant reduction in TST, sleep efficiency, N2 and REM sleep percentages. In addition, SCA2 patients showed a significant increase in the percentage of N3 sleep. Within the preclinical carrier group, only a reduction of the REM sleep percentage was observed.

ANCOVAs disclosed a significant effect of TST on the percentage of N2 ($F = 4.77$; $p = 0.033$) and REM sleep ($F = 11.5$; $p = 0.001$) stages, nevertheless the current group effects computed for this covariate were still significant for both sleep stages (N2 sleep: $F = 4.66$; $p = 0.013$; REM sleep: $F = 4.18$; $p = 0.020$). Similar analyses using the arousal index as covariate only disclosed significant effect on the percentage of N3 sleep stage ($F = 5.36$; $p = 0.024$) and the current group effect remained significant too ($F = 7.67$; $p = 0.001$).

Correlation analyses followed by Bonferroni correction within the SCA2 group revealed no significant associations between sleep architecture measures and demographical, clinical and molecular features of the disease, even when the percentages of sleep measures were regressed out TST and arousal index.

Similar analyses in the preclinical carriers yielded a significant association between age and sleep efficiency ($r = -0.65$; $p = 0.002$) as well as the percentage of N2 sleep ($r = -0.60$; $p = 0.005$). Nevertheless, this former correlation became marginal when the N2 sleep was regressed out TST ($r = -0.46$; $p = 0.041$), but remained significant when it was regressed out arousal index ($r = -0.62$; $p = 0.003$). Further, the predicted time to ataxia onset was correlated with sleep efficiency ($r = 0.64$; $p = 0.007$) in the same group. Moreover, significant correlations were observed neither in the control group, nor in the overall sample, even when the percentages of sleep measures were regressed out TST and arousal index.

3.4. N2 sleep stage microstructure

The Kruskal–Wallis test revealed a significant group effect for sleep spindle density, with a post-hoc Dunn test showing significant lower sleep spindle density in SCA2 patients and preclinical carriers as compared to controls (Fig. 1A). Similarly, a significant group effect was observed for the K-complex density but post-hoc comparisons only revealed a significant decrease of K-complex density in the SCA2 patient group (Fig. 1B). ANCOVAs did not revealed significant effects of TST and arousal index on the intergroup differences observed for sleep spindles (TST: $F = 0.001$; $p = 0.984$; arousal index: $F = 0.274$; $p = 0.602$) nor for K-complexes (TST: $F = 0.003$; $p = 0.956$; arousal index: $F = 2.24$; $p = 0.116$).

Spearman Rank Order Correlations among the SCA2 patients revealed significant associations of the sleep spindle density with

Table 2
Sleep symptomatology in the studied groups.

		SCA2 patients	Preclinical carriers	Controls	
PSQI score	Abnormal scores N (%)	14 (70%) **	10 (50%)*	3 (15%)	$X^2 = 10.2$; $p = 0.001$[a] $X^2 = 4.1$; $p = 0.048$[b]
	Mean ± SD (95% CI)	6.55 ± 3.09* (5.10–7.99)	4.80 ± 3.41 (3.20–6.40)	3.20 ± 1.40 (2.54–3.85)	$F = 7.28$; $p = 0.001$
ESS score	Abnormal scores N (%)	0 (0)	0 (0)	0 (0)	ns
	Mean ± SD (95% CI)	4.00 ± 1.91 (3.10–4.90)	3.70 ± 1.34 (3.07–4.33)	3.75 ± 1.21 (3.18–4.32)	$F = 0.22$; $p = 0.801$
Insomnia	N (%)	7 (35%)*	5 (25%)	1 (5%)	$X^2 = 3.91$; $p = 0.048$
RLS	N (%)	8 (40%)*	0 (0%)	0 (0%)	$X^2 = 7.66$; $p = 0.006$
RBD	N (%)	3 (15%)	0 (0%)	0 (0%)	ns
Bruxism	N (%)	1 (5%)	0 (0%)	1 (5%)	ns

PSQI: Pittsburg sleep quality index; ESS: Epworth sleepiness scale; RLS: Restless legs syndrome; RBD: REM sleep behavior disorder; ns: not significant; SD: standard deviation; *: $p < 0.05$; **: $p < 0.001$; 95% CI: 95% confidence limits for means.
[a] Yates corrected Chi-square analysis between SCA2 patients and controls.
[b] Yates corrected Chi-square analysis between preclinical carriers and controls.

Table 3
Analysis of sleep macrostructure in the studied groups.

Variable	SCA2 patients	Preclinical carriers	Controls	ANOVA findings
Total sleep time [TST] (hr)	6.41 ± 0.28* (5.83–6.99)	6.50 ± 0.17 (6.14–6.86)	7.18 ± 0.15 (6.86–7.50)	F = 5.22, p = 0.008
Wake after sleep onset (hr)	0.81 ± 0.16 (0.48–1.13)	0.59 ± 0.17 (0.20–0.97)	0.57 ± 0.12 (0.30–0.82)	F = 0.74, p = 0,484
Sleep efficiency (%)	80.2 ± 3.0* (73.8–86.6)	85.3 ± 2,3 (80.5–90.0)	89.0 ± 1,5 (85.9–92.0)	F = 3.53, p = 0.036
Sleep latency (min)	15.9 ± 2.14 (11.4–20.4)	16.2 ± 2.80 (10.3–22.0)	11.8 ± 1.63 (8.33–15.2)	F = 1.22, p = 0.304
N1 stage (%)	11.0 ± 1.33 (8.26–13.8)	9.48 ± 0.84 (7.72–11.2)	7.64 ± 1.04 (5.45–9.82)	F = 2.45, p = 0.095
N2 stage (%)	36.0 ± 2.05** (31.7–40.3)	44.6 ± 2.48 (39.4–49.8)	46.9 ± 1.88 (42.9–50.8)	F = 7.09, p = 0.002
N3 stage (%)	21.9 ± 1.63* (18.6–25.4)	15.7 ± 1.30 (13.0–18.5)	17.6 ± 1.52 (14.4–20.8)	F = 4.63, p = 0.014
REM stage (%)	16.0 ± 1.56** (12.7–19.3)	15.0 ± 0.90** (13.1–16.9)	20.9 ± 0.77 (19.2–22.5)	F = 7.72, p = 0.002

*:p < 0.05; **p < 0.005; ***:p < 0.0005.

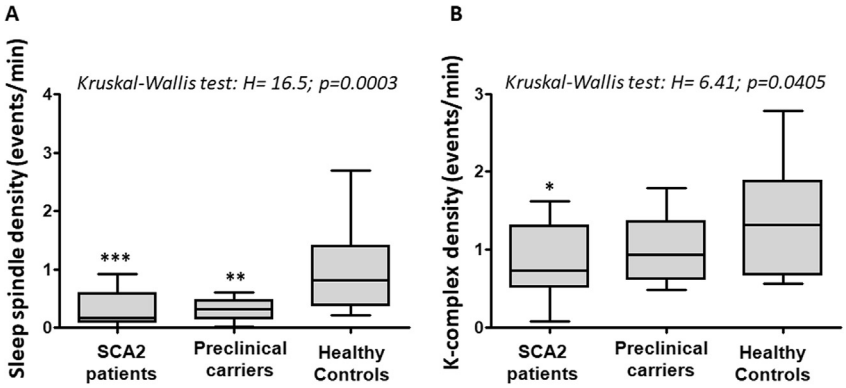

Fig. 1. Analysis of sleep spindle density (A) and K-complex density (B) in SCA2 patients, preclinical carriers and controls. *:p < 0.05; **:p < 0.001; ***:p < 0.0001.

the number of trials required to recall the complete list of words in the verbal memory test (Fig. 2A), as well as with the number of words remembered in the delayed trial (Fig. 2B). These correlations remained significant when the sleep spindle density was regressed out TST (number of trials: r = −0.60; p = 0.005; number of words: r = 0.55; p = 0.010) and arousal index (number of trials: r = −0.76; p < 0.001; number of words: r = 0.62; p = 0.003).

Within the remaining groups, the sleep spindle density was not significantly correlated with the number of trials required to recall the word list (preclinical carriers: r = −0.31, p = 0.179; controls: r = 0.07 p = 0.781), or the number of words remembered in the delayed trial (preclinical carriers: r = −0.10, p = 0.687; controls: r = −0.10 p = 0.707). In addition, sleep spindle density was not correlated with the remaining parameters, ie, demographical, clinical, sleep quality, neuropsychological and molecular measures in SCA2 patients, preclinical carriers and controls.

Regarding K-complex density, inverse correlations were only observed for age (Fig. 2C) and SARA score (Fig. 2D) in SCA2 patients. Moreover, the K-complex density was correlated with the percentage of N3 sleep (Fig. 2E) in this group. Nevertheless, when the K-complex density was regressed out arousal index only the correlations with SARA score (r = −0.65; p = 0.002) were significant.

No significant correlations were found within preclinical carriers, whereas in the control group inverse correlations were observed between age and K-complex density (raw data; r = −0.62; p = 0.003; regressing out TST: r = −0.64; p = 0.002; regressing out arousal index: r = −0.61; p = 0.004).

The correlation between the sleep spindle density and verbal memory performance was also observed when the data from all groups were pooled (sleep spindle density and the number of trials required to recall the list: r = −0.51; p < 0.0001; sleep spindle density and the number of words remembered in the delayed trial: r = 0.35; p = 0.005). Furthermore, the relationships between the K-complex density with age (r = −0.49; p = 0.0001) and the SARA score (r = −0.28; p = 0.028) were noted in the overall sample. All these correlations remained significant when we regressed out TST and arousal index except for the correlation between the number of words remembered in the delayed trial and the TST-regressed sleep spindle density.

4. Discussion

The present paper reports on the largest study of N2 sleep microstructure in SCA2 or any other hereditary ataxia. It is also the first to describe these measures in the prodromal stage of the disease. Our findings revealed a significant reduction of sleep spindle density in SCA2 patients and preclinical carriers as well as an attenuated K-complex density in patients but not carriers. Of note, this study demonstrated a closed relationship between sleep spindle density and memory dysfunction in SCA2 patients as well as between K-complex density and disease severity.

In general, data on sleep quality measures and sleep architecture were in accordance with previous work [9–13], although ESS scores seem to be low considering the known sleep disturbances in SCA2. Most likely, this apparent contradiction is

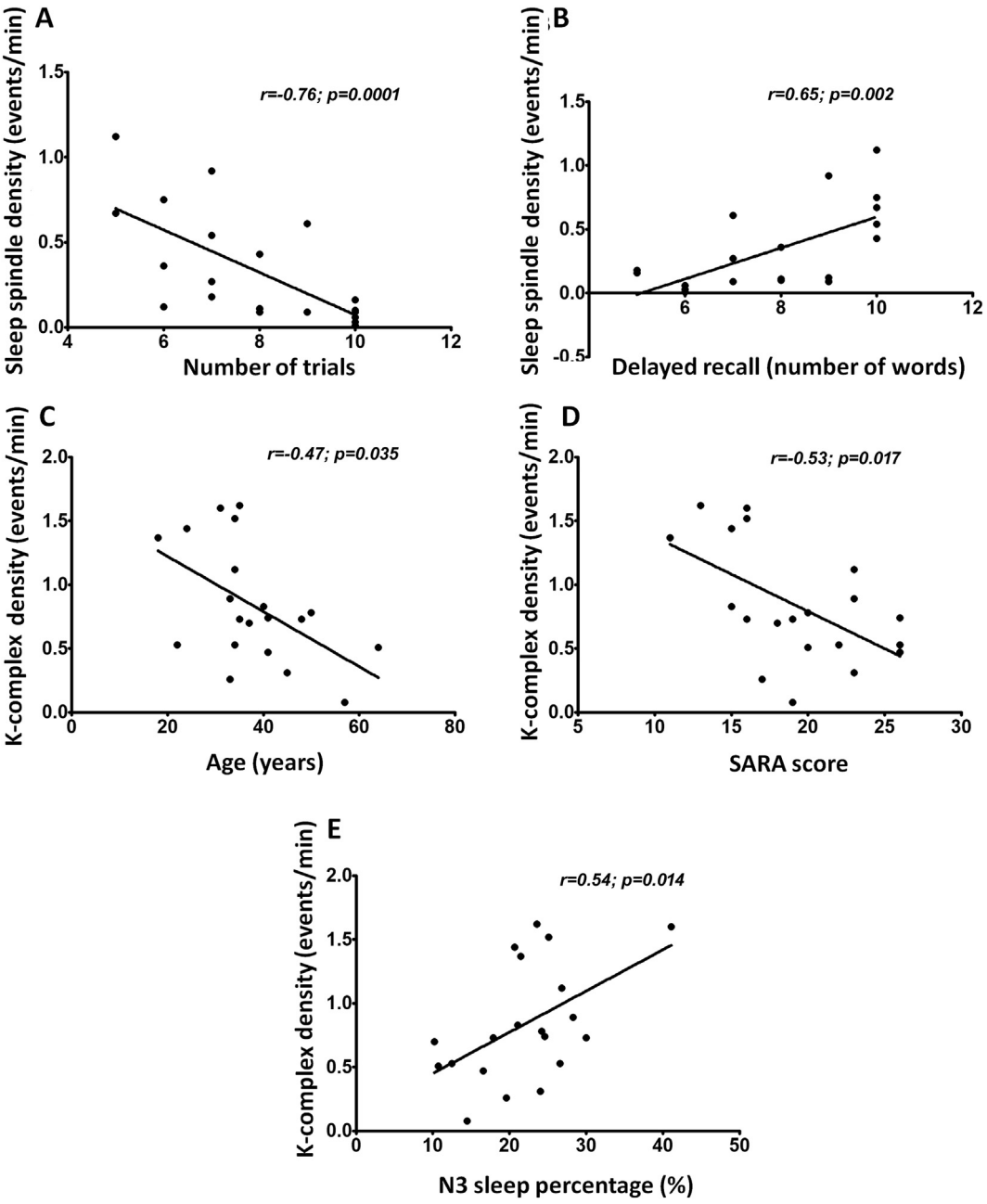

Fig. 2. Correlation analyses of sleep spindle density versus number of trials (A) and delayed recall (B) in the verbal memory test, as well as of K-complex density versus age (C), SARA score (D), and N3 sleep stage percentage (E) in the SCA2 patients' group.

neurobiologically attributable to the homeostatic control of sleep, to the occurrence of compensatory mechanisms and/or to the distortion of self-perception of health status caused by the cerebellum-related mood disturbances. Methodologically, this paradox may be related to the low contextualization of Cuban SCA2 patients to some of the imaginary circumstances included in the ESS questionnaire.

Regarding the N2 sleep microstructure in SCA2 patients and preclinical carriers, the reduction of sleep spindles suggests the involvement of thalamocortical circuits in SCA2 even before the onset of the cerebellar syndrome. As is widely understood, this oscillatory activity is initiated in the TRN -the sleep spindle pacemaker- and then synchronized by a reciprocal synaptic interplay between mutually interconnected GABAergic inhibitory neurons of the TRN, the TC neurons and cortical pyramidal neurons [23]. In fact, our findings are supported by previous neuroanatomical evidence obtained in post-mortem samples of SCA2 patients, which have demonstrated a notable degeneration of TRN cells [40,41].

The reduction of sleep spindles in SCA2 can be also explained at the neuronal level. It is known that the generation of rhythmic burst discharges underlying the sleep spindles depends of strictly controlled mechanisms for intracellular Ca^{2+} handling [19,23]. Thus, considering the established role of Ataxin-2 in intracellular calcium homeostasis [42,43], we hypothesize that the PolyQ related gain of toxic function and/or the loss of physiological function of mutated Ataxin-2 cause the dysfunction of the sleep spindle generating circuit via the impairment of normal intracellular Ca^{2+} handling in the TRN.

Regarding K-complexes, our findings are indicative of the involvement of cortical circuits underlying these depolarizing-hyperpolarizing brain oscillations, which is neuroanatomically supported by the loss of giant Betz pyramidal cells [41]. Additionally, the thalamic involvement in SCA2 could contribute to the decrease in K-complexes, given that once they are generated, propagation occurs through the cortex and the thalamus, synchronizing thalamo-cortical rhythms [44]. Moreover, the decrease in K-complexes in SCA2 patients can be explained by the neurodegenerative changes in the cholinergic basal forebrain and the brainstem [45], two structures implicated in K-complex generation.

Accordingly, with the plethora of non-motor functions attributed to the cerebellum and the co-occurrence of cerebellar fMRI signals with K-complexes [46] and sleep spindles [47], one could hypothesize that the cerebellar degeneration contributes to the impairments of N2 sleep microstructure in SCA2. However, the role of this organ in the generation of EEG oscillations during sleep is still an enigma [48] that requires further experimental studies, before we understand how the cerebellar degeneration modulates sleep in SCA2.

Recent papers have suggested that sleep spindles appear in spindle-rich intervals and sleep-poor intervals which define the ultraslow 0.02-Hz oscillations of the sleep spindle band and divides NREM sleep into environmental alertness and internal memory processing phases [49,50]. Thus, the significant decrease of sleep spindles in SCA2 patients and preclinical carriers could result from the specific reduction of spindle-rich intervals or the general reduction of both time intervals. Although we do not have data available to rigorously discern between both possibilities, an inspection of the individual histograms of the number of spindles within each 30s intervals suggests that the second possibility seems to be more probable, whereby spindles are just less likely to occur in these populations. Representative sleep spindle histograms for each study population are shown in the supplemental material 2. Further meta-frequency studies assessing whether power in the infraslow or interspindle bands differ between these populations could help to elucidate this issue.

Several studies in humans have reported significant correlations between sleep spindles and sleep-dependent consolidation of declarative memory and consequently with learning ability and IQ [51–54], but a recent meta-analysis revealed that these associations are more tenuous than previously assumed [55]. In line with this relationship, in the present paper we observed significant correlations between the sleep spindle density and verbal memory performance in SCA2 patients, which provides new evidence supporting the role of these graphoelements in mnemonic processes. SCA2 patients had greater difficulty to learn verbal material resulting in a weaker memory performance. Here we did not assess the overnight changes in sleep spindles density after the presentation of the word list, so we cannot interpret the correlations between sleep spindle density and verbal memory parameters as specific to declarative memory consolidation, but to memory performance more generally. However, considering that all patients were able to actually acquire memory traces of the presented verbal lists, an impact of sleep spindles on memory consolidation processes seems likely.

The correlation between sleep spindle activity and verbal memory was specific for SCA2 patients, but was not observed in preclinical carriers or healthy controls. This could, of course, be merely related to the small sample size and the higher homogeneity shown by the data of verbal memory test in these groups. Alternately, it is possible that sleep spindles and verbal memory are related in a non-linear fashion, eg, such that verbal memory is only affected when sleep spindle frequency lies below a certain threshold.

Animal and experimental studies suggest that this functional role of sleep spindles is expressed via the facilitation of the synaptic plasticity involved in memory. Specifically, sleep spindles are thought to induce massive influxes of calcium ions into cortical pyramidal cells, where they prompt long-term synaptic changes [56,57]. Thus, sleep spindle density could be considered an objective biomarker of neuroplasticity mechanisms, but further studies measuring spindle activity following memory and/or motor learning tasks are mandatory to confirm the practical usefulness of sleep spindles in SCA2.

The correlation between K-complex density and SARA score in patients suggests that the rate of the neurodegenerative process underlying this polysomnographic feature is relatively similar to the rate of cerebellar degeneration. Nevertheless, this notion will be clarified with an ongoing follow-up study of NREM sleep microstructure in SCA2 patients. Increased age was associated to reduced K-complex density in SCA2 patients, controls and in the overall sample which is consistent with previous works and is interpreted as an age-related dysfunction of cortical-thalamocortical regulatory mechanisms [58,59]. Similarly, the relationship between K-complex density and slow wave sleep (SWS) percentage in patients is in line with previous studies demonstrating the role of K-complex as sleep-protective element and a building stone of SWS [60].

In general, the group differences for the sleep stage percentages, sleep spindle density and K-complex density as well as most of correlations involving these measures were not significantly influenced by the covariate effects of TST and arousal index. These findings suggested that the main macrostructural and microstructural PSG alterations are driven by disease-specific mechanisms rather than the reduced TST and/or increased sleep arousals.

So far, only one study assessed sleep spindles in spinocerebellar ataxias [14]. The authors included a heterogeneous sample encompassing 18 SCA patients (SCA1:6; SCA2:5 and SCA3:7) and six healthy controls. They reported a significant decrease of sleep spindle density in SCA2 and SCA3, but not in SCA1 patients. No correlation was reported between this PSG measure and age, disease duration, disease severity, nor CAG repeat length. Notably, the authors did not include subjects at prodromal disease stages, they did not measure K-complexes, nor did they assess the relationship between sleep spindles and memory performance.

Among other neurodegenerative disorders, reduced sleep spindle activity is observed in patients with Parkinson disease [61], Alzheimer's disease [62] and Creutzfeldt-Jakob disease [63]; whereas in patients with Huntington disease these NREM graphoelements are increased [64]. In the case of K-complexes, a significant reduction is noticed in Alzheimer disease [65].

Although the relationship between sleep spindles and memory has not been previously observed in SCAs, it has been documented in several neurological diseases. For example, the reduction of sleep spindles is found to be a predictive biomarker of dementia in Parkinson disease [66], whereas in Alzheimer disease, the decrease of fast spindles is associated with abnormal immediate recall performance [67]. Furthermore, the reduction of sleep spindles density in patients with schizophrenia is linked to impaired sleep-dependent memory consolidation [68].

5. Conclusions

In summary, our study demonstrated the impairment of N2 sleep microstructure in SCA2 since prodromal stages as evidence of the early involvement of thalamo-cortical and/or cortical circuits underlying the generation of sleep spindles and K-complexes. In particular, the decrease of sleep spindles density offered new insights into the affection of neural plasticity mechanisms underlying mnemonic processes in SCA2, whereas K-complex density has

potential usefulness as marker of the sleep protection mechanisms in SCA2. Thus, both polysomnographic measures can contribute to the understanding of disease physiopathology and sleep disturbances in SCA2 and they could be used as outcome measures in further therapeutical trials targeting sleep disturbances and/or memory dysfunctions in SCA2.

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Conflict of interest

Authors received funding from the Cuban Ministry of Public Health and disclose no conflicts in relation to the submitted work.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2019.04.005.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2019.04.005.

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