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Early cranial nerve dysfunction is correlated to altered facial morphology in spinocerebellar ataxia type 2.

Cranial nerves and facial morphology in SCA2

Disfunción temprana de nervios craneales correlaciona con alteraciones de la morfología facial en la ataxia espinocerebelosa tipo 2.

Los nervios craneales y la morfología facial en SCA2

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Abstract

The aim of our cross-sectional study was to quantify trigeminal and facial nerve electrophysiological alterations and to determine its correlation with facial morphology abnormalities and expanded CAG repeats in Spinocerebellar ataxia type 2 (SCA2). 90 SCA2 patients and 41 preclinical mutation carriers together with 100 sex-, age- and facial type- matched healthy subjects as controls were assessed by facial motor nerve conduction, blink reflex (BR) and mandibular reflex (jaw jerk. Facial morphology features were analyzed by the determination of the facial type using a standardized morphometric facial index and the measurement of three distinct planes over pictures. Patients exhibited a significant prolongation of latency and duration and decreased amplitude in the facial motor potentials. The mandibular reflex revealed prolonged latency and decreased amplitude. Moreover, the bilateral R2 component of the blink reflex was prolonged. Preclinical carriers showed prolonged duration for facial nerve potentials and mandibular reflex, as well as increased latency of bilateral R2 BR component. Facial morphology measures revealed periorbital, perioral and masseter alterations in patient and preclinical groups, and some of them were correlated to the electrophysiological features and expanded CAG repeats.
These electrophysiological and morphological features widen the prodromal phenotype of SCA2, and offer new clues about the role of ATXN2 mutations for muscle atrophy, neuronal energy balance and lipid metabolism.

Resumen:

Se realizó un estudio transversal con el objetivo de cuantificar las alteraciones electrofisiológicas de los nervios facial y trigémino y determinar su correlación con las anomalías de la morfología facial y el número de repeticiones de CAG en la Ataxia Espinocerebelosa Tipo 2 (SCA2). Se evaluaron 90 pacientes SCA2 y 41 portadores preclínicos de mutación junto con 100 sujetos sanos como controles, pareados por sexo, edad y tipo facial a través de estudios de conducción nerviosa motora periférica del nervio facial, reflejo de parpadeo (BR) y reflejo T mentoniano (reflejo mandibular). Para el análisis de las características de la morfología facial se determinó el tipo facial mediante el índice facial morfológico estandarizado y mediciones a partir de tres planos distintos sobre fotografías. Los pacientes mostraron una prolongación significativa de la latencia y la duración y una reducción de la amplitud del potencial motor del facial. El reflejo mandibular reveló prolongación de la latencia y disminución de la amplitud así como prolongación del componente R2 bilateral del BR. Los portadores preclínicos mostraron duración prolongada del potencial del nervio facial, del reflejo mandibular y de la latencia del componente R2 bilateral del BR. Se obtuvieron alteraciones morfológicas faciales sobre los músculos periorales, periorbitarios y maseterinos en ambos grupos, y algunas de ellas correlacionaron con las hallazgos electrofisiológicos y el número de repeticiones de CAG.
Estas características electrofisiológicas y morfológicas amplían el fenotipo prodrómico de SCA2 y ofrecen nuevas pistas sobre el papel de la mutación ATXN2 en la atrofia muscular, el equilibrio energético neuronal y el metabolismo lipídic

Introduction

Spinocerebellar Ataxia type 2 (SCA2) is an autosomal dominant cerebellar ataxia caused by CAG repeat expansions in the ATXN2 gene on chromosome 12q [1-6]. It is characterized by several core symptoms including a progressive cerebellar ataxia with dysarthria, slowing of horizontal saccades in more than 90% of cases, peripheral neuropathy, dysphagia, olfactory deterioration, autonomic abnormalities, sleep disturbances and cognitive dysfunction leading to reduced survival [7-16]. In Cuba, there are about 578 living SCA2 patients from 163 families and 7,200 asymptomatic at-risk individuals, representing an estimated ATXN2 mutation prevalence of nearly 28.5 cases per 100 000 inhabitants in the whole country. SCA2 represents 87% of all SCA subtypes in Cuba. In Holguin province, the prevalence rate is about 40.18 cases per 100 000 inhabitants, distributed in ten municipalities of the province. This topographic location gives unique characteristics to the disease in our country and is supposed to be considered a founder gene effect [9,17].

Although most phenotypical features of SCA2 have been characterized thoroughly with clinical, electrophysiological and neuropathological approaches [7, 8,10-12, 14,17-31], the prominent affection of the cranio-cervical area during the preclinical and early disease stages deserve special attention, particularly the morphological alterations of the face. It has been known for some time that SCA2 and SCA3 patients within the first years of disease appear distracted due to their characteristic belated gaze reaction, that they show fasciculation-like movements in a cranio-cervical distribution and seem to be frightened or have a mask-like face. This is due to additional lid retraction, probably a product of subcutaneous fat loss, autonomic pathology and cranial nerve degeneration [3, 19,32-34]. Some parents of affected individuals have reported that they recognize the onset of pathology among their offspring first through the changes of facial morphology. However, there is no objective quantitative characterization of facial alterations and their correlation with clinical and molecular variables so far.

Material and Methods

Here we performed a cross-sectional assessment of facial alterations by neurophysiological studies and morphological measurements and in 90 SCA2 patients (37 female and 53 male subjects), 41 preclinical carriers (28 female and 13 male) and 100 healthy subjects (60 female, 40 male) as controls.

Inclusion criteria for SCA2 patients were: i) age between 15 and 65 years old, ii) positive molecular diagnosis of SCA2 mutation and iii) presence of definite cerebellar syndrome. In the case of preclinical carriers, the absence of definite cerebellar syndrome was assumed as third inclusion criteria. The control group was selected following these criteria: i) absence of cerebellar manifestations by exhaustive neurological examination, ii) absence of ataxia or other related diseases in the family history, and iii) age, gender and facial type matching with patients and preclinical carriers.

Exclusion criteria for all participants were: chronic alcohol abuse or use of CNS- depressant drugs, psychiatric disorders and patients with other diseases affecting the nervous system such as hypothyroidism, diabetes, hypertension and others. Also, subjects with history of facial trauma were not included.

The study protocol was approved by the ethical standards of the committee on human experimentation of the Centre for Research and Rehabilitation of Hereditary Ataxias and was in agreement with the Helsinki declaration. All participants gave their written informed consent prior to the experiments

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Neurological assessments

All subjects underwent a complete neurological examination and the International Cooperative Ataxia Rating Scale (ICARS) to assess the severity of the cerebellar syndrome [35].

Neurophysiological studies

Neurophysiological evaluations were also performed in all enrolled subjects and consisted in facial motor nerve conduction, blink reflex (BR) and mandibular reflex (jaw jerk, JJ) studies, which were conducted using standardized protocols [36] by a researcher blinded to the experimental group of each individual.

Facial morphological measures

As previously described, human faces show considerable variance of height and other features in comparison to facial width being constant [37]. Therefore, facial morphology analyses were conducted in 41 SCA2 patients, 41 preclinical carriers and 41 sex-, age- and facial type- matched controls. In all groups, the gender distribution was 28 females/12 males. Facial measures were conducted to classify the facial type and to quantify the morphological alterations. The determination of the facial type was performed by direct measures with a cranio

meter using a morphologic facial index [38]. Accordingly, three facial types were defined: i) Leptoprosopo or long face (index [length/width. >104), Mesoprosopo or intermediate face (index 97-104) and Euriprosopo or wide face (index < 97).

Frontal and right view photography to each subject were taken, with Kodak DC290 Zoom digital camera (November 1999, Canada), with head orientation in Frankfort plane parallel to the floor and 1m focal length.

Measures to quantify the facial alterations were performed indirectly on frontal and lateral right view pictures using Adobe Photoshop program version 7. Measures were assessed using the following standard landmarks and lines as references: Frankfurt (FP), Mid Sagittal (MSP) and commissural (CP) planes for frontal views and FP, anterior frontal (Izard) and later frontal (Simon) planes for lateral right views (Fig. 1). For the frontal views the measures were assessed bilaterally and consisted in:

(1) Distance from MSP to internal eye angle (MSP-IEA).
(2) Distance from MSP to external eye angle (MSP-EEA)
(3) Distance from MSP to the more depressed point of the cheek (MSP-DPC)
(4) Distance from MSP to the labial commissure of both sides in the CP (MSP-LC)
(5) Distance from FP to labial commissure (perpendicular) (FP-LC)
(6) Distance from FP to internal eye angle (FP-IEA)
(7) Distance from FP to external eye angle (FP-EEA)

In the lateral right views measures were:

(1) Distance from FP to labial commissure (perpendicular) (FP-LC),
(2) Distance from Simon plane to labial commissure (SP-LC),
(3) Distance from Izard plane to the more depressed point of the upper eyelid (IP-DPUE),
(4) Distance from Izard plane to the more depressed point of the lower eyelid (IP-DPLE).

All morphological measures were conducted by a researcher blinded to the experimental group of each individual

CAG repeat quantification

The ATXN2 CAG repeat length was assessed by PCR amplification followed by polyacrylamide gel electrophoresis [39].

Lateral right view pictures, to quantify the facial alterations.

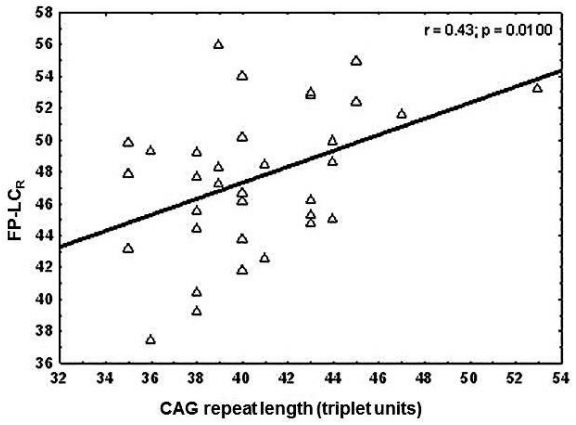

Figure 3. Correlation between CAG repeat length and the morphometric distance FP-LCR.

Statistical Analysis

Collected information from this study was entered into a data file using the Statistic for Windows (v 6.1, 2003, USA) computer software. Scatter plots were obtained to evaluate the data and to confirm the presence of a normal distribution before the application of parametric statistics.Descriptive statistics of the studied variables, analyses of univariant variance, and Spearman´s correlation tests between electrophysiological, morphologic and molecular variables were performed.

Results

General characteristics of study population

All patients had clinical and molecular diagnosis of SCA2, with age ranging between 16 to 60 years (Mean = 39.9 years; SD: 11.41), whereas age at onset varied from 8 to 60 years (Mean = 26.7 years; SD: 9.86). Disease duration was between 1 to 42 years (Mean=13.25; SD: 7.61); ICARS score mean was 50.06 (SD: 17.32) and varied from 5.88 to 92 points. Pathological CAG repeat lengths covered sizes from 34 to 53 (Mean=40.58; SD: 3.37). Normal alleles ranged between 19 and 33 CAG (Mean=22.08; SD: 1.31).

Preclinical carriers group had mean age of 36.7 years (SD: 10.27), ranging from 19 to 62 years. Pathological CAG repeat length varied between sizes 32 and 42 (Mean=36.68; SD: 2.47) and normal alleles mean was at 22.74 (SD: 2.33).

Mean age of the control group was 34.02 years (SD: 8.43; range: 20-60).

Neurophysiological findings

Findings of the mean comparison tests for neurophysiological assessments are shown in Table 1. The study of the facial (F) motor nerve conduction revealed a significant prolongation of latency (p=0.000) and duration (p=0.000) of the potential as well as a significant (p=0.029) decrease of the amplitude in SCA2 patients compared to controls. There was also a significant prolongation of duration (p=0.004) of the potential in preclinical mutation carriers (Table 1).

Table 1. Mean comparison of neurophysiological variables in SCA2 patients, preclinical mutation carriers and control groups.

Variables *Mean ± SD*	Patients	Control	Preclinical carriers	Control
Latency F (ms)	3.06 ± 0.57 ***	2.64 ± 0.49	2.82 ± 0.35 ns	2.64 ± 0.05
Duration F (ms)	12.35 ± 3.55 ***	10.42 ± 3.62	12.87 ± 3.79 **	10.64 ± 3.16
Amplitude F (mV)	1.82 ± 0.78 **	2.12 ± 1.09	1.96 ± 0.62 ns	2.28 ± 1.80
Latency JJ (ms)	10.35 ± 5.60 ***	5.73 ± 1.05	8.14 ± 2.16 ***	5.85 ± 0.97
Amplitude JJ (mV)	0.51 ± 0.36 ***	1.26 ± 1.06	1.51 ± 2.16 ns	1.22 ± 0.41
R2 Ipsi. BR (ms)	37.63 ± 5.58 ***	32.38 ± 3.38	34.31 ± 5.01 **	32.12 ± 3.30
R2 Contra. BR (ms)	39.62 ± 6.63 ***	33.27 ± 3.58	35.21 ± 5.41 **	32.67 ± 3.34

F: facial; JJ: jaw jerk; BR: Blink reflex. Lat.: Latency; Ipsi: Ipsilateral; Contra: Contralateral. ns: next to the mean (SD) value represents no statistical differences (p>0.05); * means statistical difference (p<0.05); ** means higher statistical difference (p<0.005) and *** means highest statistical difference (p<0.0005).

In the jaw jerk (JJ) study, an increment of latency values (p=0.000) with a decrease of the amplitude (p=0.000) was observed in patients, whereas in the preclinical carriers only the prolongation of latency (p=0.000) was observed (Table 1).

Blink reflex (BR) alterations mainly consisted in the prolongation (p=0.000) of the ipsilateral and contralateral latency of the R2 component in SCA2 patients and preclinical mutation carriers compared to the control group (p=0.020 and p=0.011, respectively) (Table 1). These findings confirm the early demyelination of cranial nerves at the preclinical stage and it´s progressing to axonal damage on manifest SCA2 subjects.

Facial morphometric analyses

Given that the early vulnerability of cranial nerves will also result in tissue atrophy over time, quantitative morphological assessments of facial features were attempted by standardized digital procedures. Consistent with the literature, the mesoprosopo facial type was most common among all groups.

Relevant alterations of facial morphology in SCA2 patients were located (see Fig. 2) at linear distances that point to a depression of the cheek (MSP-DPCL; MSP-DPCR) (p=0.000) and eyelids atrophy (IP-DPUE; IP-DPLE) (p=0.022 and p=0.040, respectively). Importantly, the alterations located at distances pointing to a decline of labial commissures (FP-LCL; FP-LCR) were significant not only in SCA2 patients (p=0.016 and p=0.018, respectively), but already in the preclinical carriers (p=0.017 and p=0.013, respectively) (Table 2). These results represent the first morphometric identification of SCA2 facial shape anomalies and demonstrate the preferential affection of lip

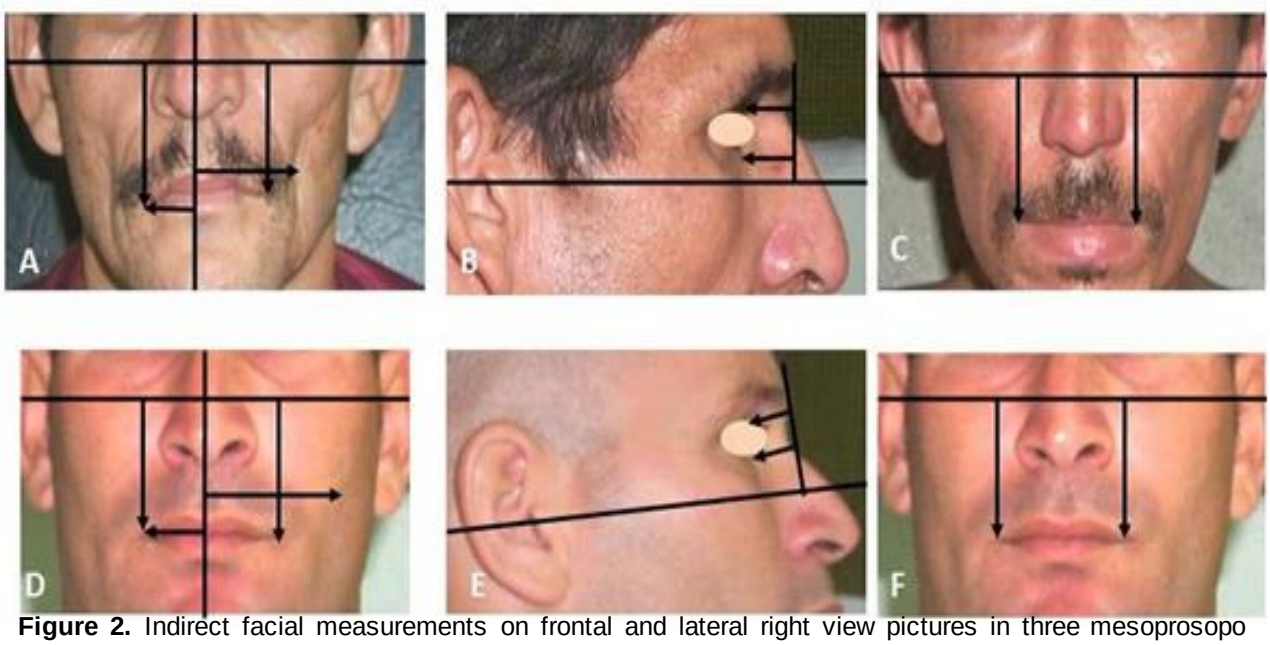

Figure 2. Indirect facial measurements on frontal and lateral right view pictures in three mesoprosopo facial type subjects. A: frontal view of a 39-years-old SCA2 patient; B: lateral right view of same SCA2 patient; C: frontal view of preclinical 34-years-old mutations carrier; D, E and F: frontal and lateral views of a healthy subject aged 34 years, as a comparison to the facial alterations above, which are illustrated by arrows in each case. (All subjects gave the consent to publish their pictures)

Table 2. Mean comparison of facial morphological measurements in SCA2 patients, preclinical carriers and control individuals

Variables (mm) *Mean ± SD*	Patients	Controls	Preclinical carriers	Controls
MSP-DPC_L	41.67 ± 4.67 ***	46.32 ± 5.13	46.51 ± 4.64 ns	46.08 ± 4.86
MSP-DPC_R	41.81 ± 3.89 ***	46.24 ± 4.76	45.42 ± 5.29 ns	45.88± 4.51
MSP-LC_L	22.09 ± 2.73 **	23.41 ± 2.04	23.01 ± 2.76 ns	23.41 ± 2.17
MSP-LC_R	22.46 ± 2.48 **	23.75 ± 2.26	23.01 2.46 ns	24.04 ± 2.27
FP-LC_L	47.59 ± 4.94 **	46.64 ± 5.12	49.41 ± 5.02 *	46.65 ± 5.13
FP-LC_R	47.22± 4.90 **	46.43 ± 4.99	49.23 ± 4.86 *	46.43 ± 4.99
IP-DPUE	14.05 ± 3.80 *	12.21 ± 3.24	11.81 ± 3.61 ns	12.56 ± 3.14
IP-DPUE	16.48 ±4.07 *	15.22 ± 3.32	15.62 ± 3.54 ns	15.27 ± 3.30

*Morphological measurements on soft tissue. ns: next to the mean (SD) value represents no statistical differences (p>0.05); * means statistical difference (p<0.05); ** means higher statistical difference (p<0.005) and *** means highest statistical difference (p<0.0005).*
MSP-DPCL/MSP-DPCR: Midsagittal plane distance to the most depressed left/right cheek point; MSP-LCL/ MSP-LCR: Midsagittal plane distance to left/right labial commissure; FP-LCL/FP-LCR: Frankfurt plane distance to left/right labial commissure and IP-DPUE/IP-DPLE: Izard plane distance to the most depressed upper/lower eyelid point.

commissure measures during the preclinical stage of SCA2.

Factorial ANOVAs using facial measures as dependent variable and the group (patients Vs. preclinical carriers) and facial types (Mesoprosopo, Leptoprosopo and Euriprosopo) as factors, showed no significant interaction between the group and the facial type for none facial measure (Table 3).

Genotype-phenotype correlation analyses

area. The distance pointing to a decline of labial commissures of both sides showed positive correlations with the duration of facial potential (FP-LCL p=0.024; FP-LCR p=0.014) in SCA2 patients (Ta ble 4A), suggesting that the pathology of the facial nerve is mirrored by the position of labial angles. The most depressed point of the superior and inferior eyelid showed significant positive correlations with ipsilateral (IP-DPUE p=0.027; IP-DPLE p=0.005) and contralateral R2 components

Table 3. Factorial ANOVAs findings for the assessment of the group x facial types interaction effect on morphometric variables

Variables (mm)	F	p
MSP-DPC$_L$	0.916	0.405
MSP-DPC$_R$	0.715	0.493
MSP-LC$_L$	0.185	0.831
MSP-LC$_R$	0.206	0.814
FP-LC$_L$	0.121	0.886
FP-LC$_R$	0.004	0.996
IP-DPUE	10.48	0.234
IP-DPLE	10.778	0.176

MSP-DPCL/MSP-DPCR: Midsagittal plane distance to the most depressed left/right cheek point; MSP-LCL/ MSP-LCR: Midsagittal plane distance to left/right labial commissure; FP-LCL/FP-LCR: Frankfurt plane distance to left/right labial commissure and IP-DPUE/IP-DPLE: Izard plane distance to the most depressed upper/lower eyelid point

To evaluate if the electrophysiological and mor phometric findings correlate with disease severity as represented by the ATXN2 CAG repeat length, and to identify the features that are particularly susceptible even at mildest stages of SCA2, we performed genotype-phenotype and electrophysiology-morphology association analyses. In SCA2 patients, Spearman´s correlation tests revealed that the CAG repeat length correlated positively with the electrophysiological latency of the facial motor nerve (r=0.316; p=0.016) and with the morphometric distance between Frankfurt plane to labial commissure (r=0.439; p=0.010) (Fig. 3).The morphometric measures that point to a sinking of the cheek (MSP-DPCL; MSP-DPCR) were inversely correlated to the mandibular reflex latency (p=0.049 and p=0.039 respectively) and directly correlated to the mandibular reflex amplitude (p=0.003 and p=0.017, respectively) in SCA2 patients (Table 4A), suggesting that this morphometric feature might reflect atrophy of the masseteric of the BR (IP-DPUE p=0.045; IP-DPLE p=0.006) in SCA2 patients (Table 4A). In addition, the same morphometric features exhibited significant positive correlations with the facial motor nerve latency (IP-DPUE: p=0.045; IP-DPUE: p=0.034) in SCA2 patients (Table 4A), suggesting that the loss of myelin in the facial motor nerve is mirrored by the enophthalmos.

In the preclinical mutation carriers the distance between mid-sagittal plane to left labial corner was negatively correlated with the JJ reflex latency (MSP-LCL: p=0.024) and latency of the facial motor (MSP-LCR: p=0,008; MSP-LCL: p=0.000). The latency of the facial motor nerve also had a significant direct correlation with the measure that reflected the vertical decline of lip corners (FP-LCL: p=0.045; FP-LCR: p=0.046) (Table 4B). Thus, in preclinical carriers the demyelination of the facial motor nerve as well as lip depression were particularly conspicuous markers of incipient SCA2.

A

Table 4. Spearman´s correlation test between electrophysiological and morphological variables in SCA2 patients (A) and preclinical mutation carriers (B).

Variables	Mean comparison		Spearman's correlation coefficients							
	Patients	Controls	Facial motor nerve conduction study		Blink reflex				Jaw Jerk	
			Latency	Duration	R1 Lat 1	R1 Pico	R2 Ipsi	R2 Contra	Latency	Amplitude
MSP-DPC$_L$	41,67 (±4,67)	46,32 (±5,13)	- 0.055	- 0.090	- 0.069	- 0.079	- 0.005	0.002	- 0.229 *	0.342 **
MSP-DPC$_R$	41,81 (±3,89)	46,24 (±4,76)	- 0.129	- 0.164	- 0.162	- 0.150	- 0.108	- 0.089	- 0.241 *	0.277 **
FP-LC$_L$	47,59 (±4,99)	46,64 (±5,12)	0.156	0.262 *	0.010	0.117	0.192	0.156	0.024	0.181
FP-LC$_R$	47,22 (±4,90)	46,43 (±4,99)	0.139	0.284 *	0.136	0.153	0.227	0.195	0.026	0.161
IP-DPUE	14,05 (±3,80)	12,21 (±3,24)	0.234 *	0.160	0.077	0.123	0.256 *	0.234 *	0.216	- 0.038
IP-DPLE	17,48 (±4,37)	15,22 (±3,32)	0.296 *	0.128	0.141	0.210	0.323 **	0.314 **	0.117	- 0.016

B

Variables	Mean comparison		Spearman's correlation coefficients							
	Preclinical carriers	Controls	Facial motor nerve conduction study		Blink reflex				Jaw Jerk	
			Latency	Duration	R1 Lat 1	R1 Pico	R2 Ipsi	R2 Contra	Latency	Amplitude
MSP-LC$_L$	23,01 (±2,17)	23,41(±2,00)	- 0,420 **	0,074	0,085	- 0,324	- 0,052	- 0,024	- 0,362*	- 0,224
MSP-LC$_R$	23,01 (±2,27)	23,83 (±2,17)	- 0,533 ***	- 0,022	0,182	- 0,043	- 0,183	- 0,146	- 0,278	- 0,112
FP-LC$_L$	49,41 (±5,13)	46,64 (±5,12)	0.312 *	- 0.072	- 0.106	- 0.031	- 0.019	0.004	- 0.002	- 0.028
FP-LC$_R$	49,23 (±4,99)	46,43 (±4,99)	0.321 *	- 0.088	- 0.114	- 0.007	- 0.024	- 0.016	0.034	- 0.020

Legend: $p \leq 0.05$: *; $p \leq 0.005$: ** ; $p \leq 0.0005$:

Discussion

In the present paper, we presented the first study assessing the neurophysiological abnormalities of cranial nerves and their relationship with facial morphological alterations and expanded CAG repeats in SCA2. The main findings were the presence of electrophysiological signs of myelin and axon damage within the trigeminal and facial nerves in patients and myelin lesion, predominantly in small caliber facial´s fibres on preclinical mutation carriers, which correlated well with the facial morphology measures and the mutation size.

Early in the disease course, before ataxia onset, the prolonged latency of facial motor nerve, jaw jerk and blink reflex suggest the facial nerve involvement [28,40.] The shinking of cheek is related to the muscle atrophy and /or loss of fat within the tissue. Axonal damage of cranial nerves appears later during the disease progression.

The strong involvement of cranial nerves in SCA2 has been substantiated previously by histological data. Gierga et al, [28] observed significant cell loss and astrogliosis in all studied cases as well as atrophy and myelin loss of associated fibres in most of them. The preferential affection of peripheral nerves such as the facial and trigeminal nerve during the preclinical period is in excellent agreement with other studies of prodromal SCA2, which showed electrophysiological alterations in a pattern of peripheral neuropathy with central pathway damage and neuronal cell loss to depend directly on the CAG repeat expansion size of the ATXN2 gene [9, 18,40-58.] The physiological function of the Ataxin-2 protein and its highly conserved orthologues until yeast and plants [59-62] appears to be the recruitment of fat stores, amino acid reserves in the muscle mass, and glycogen from liver during times of bioenergetics deficit [31,63-66,] probably through its influence on the endocytosis machinery and on mTOR pathway growth signalling [67-73]. To adapt cells to hunger periods, Ataxin-2 performs RNA processing tasks at stress granules and influences mRNA translation [74-77], selectively for mitochondrial precursor proteins that are involved in the breakdown of fatty acids, amino acids and pyruvate [63,77-79.] Indeed, the preferential effects of Ataxin-2 mutations on RNA processing and on mitochondrial factors may become helpful in the diagnosis and progression analysis of SCA2 patients via blood sample RNA sequencing [80,81], thus explain a loss of fat tissue and muscle mass may occur through the immobility or cranial neuropathy at late stages of SCA2.

The loss of retroorbital soft tissue and the altered facial expression indeed are known as a very early feature of SCA2. This is probably due to a loss of fat volume and of muscle mass, given that facial shape in profile photos is known to reflect total fat proportion and to correlate reasonably well with body mass index, [82] rather than correlating to skeletal parameters [83]. It shows obvious changes with increasing age [84.] Previous investigations of patients with enophthalmos demonstrated that the retro-orbital fat has strong similarities with the buccal fat pad, regarding their admixture with mast cells/endothelial cells/collagen, while truncal adipose tissue is significantly different [85]. This may explain the preferential affection of the face at a SCA2 stage when the body weight is still normal.

The results from this study offer a novel tool for the neurophysiological follow up and evaluation of SCA2 patients, allowing to determine the ideal moment for starting a preventive therapy such as hypercaloric high-carbohydrate diets, which were recently reported as promising in the treatment of ALS patients [86]. Such a neuroprotective approach might delay the age at onset or slowing disease progression, but this would be difficult to demonstrate convincingly, unless objective quantitative measurements of disease endophenotypes and electrophysiological deficits can be assessed as well.

This pioneer study can be expanded to an analysis of 3D stereophotogrammetry and geometric morphometrics [87-90] as well as to a follow-up longitudinal evaluation, in order to assess the progression of these abnormalities over years within individuals.

As conclusion, these electrophysiological and morphological features offer new insights into the prodromal phenotype of SCA2, while at the same time giving new clues about the role of ATXN2 for muscle atrophy, neuronal energy balance and lipid metabolism. In a clinical setting, our findings could help to the early diagnosis and the design of future therapeutic interventions.

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