MRI Evidence of Cerebellar and Extraocular Muscle Atrophy Differently Contributing to Eye Movement Abnormalities in SCA2 and SCA28 Diseases

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Submitted: November 25, 2015
Accepted: March 17, 2016

Citation: Politi LS, Bianchi Marzoli S, Godi C, et al. MRI evidence of cerebellar and extraocular muscle atrophy differently contributing to eye movement abnormalities in SCA2 and SCA28 diseases. *Invest Ophthalmol Vis Sci*. 2016;57:2714–2720. DOI:10.1167/iovs.15-18732

Purpose. Spinocerebellar ataxias type 2 and 28 (SCA2, SCA28) are autosomal dominant disorders characterized by progressive cerebellar and oculomotor abnormalities. We aimed to investigate cerebellar, brainstem, and extraocular muscle involvement in the mitochondrial SCA28 disease compared with SCA2.

Methods. We obtained orbital and brain 1.5 T-magnetic resonance images (MRI) in eight SCA28 subjects, nine SCA2, and nine age-matched healthy subjects. Automated segmentation of cerebellum and frontal lobe was performed using Freesurfer software. Manual segmentations for midbrain, pons, and extraocular muscles were performed using OsiriX.

Results. Eye movement abnormalities in SCA2 subjects were characterized by slow horizontal saccades. Subjects with SCA28 variably presented hypometric saccades, saccadic horizontal pursuit, impaired horizontal gaze holding, and superior eyelid ptosis. Quantitative brain MRI demonstrated that cerebellar and pons volumes were significantly reduced in both SCA2 and SCA28 subjects compared with controls ($P < 0.03$), and in SCA2 subjects compared with SCA28 ($P < 0.01$). Midbrain and frontal lobe volumes were also significantly reduced in SCA2 compared to controls ($P < 0.03$), whereas these volumes did not differ between SCA2 and SCA28 and between SCA28 and control subjects. The extraocular muscle areas were 37% to 48% smaller in SCA28 subjects compared with controls ($P < 0.002$), and 14% to 36% smaller compared with SCA2 subjects ($P < 0.03$). Extraocular muscle areas did not differ between SCA2 and controls.

Conclusions. Our MRI findings support the hypothesis of different cerebellar and extraocular myopathic contributions in the eye movement abnormalities in SCA2 and SCA28 diseases. In SCA28, a myopathic defect selectively involving the extraocular muscles supports a specific impairment of mitochondrial energy metabolism.

Keywords: spinocerebellar ataxia, mitochondrial disorders, cerebellum, volumetric mri, ocular motility

Spinocerebellar ataxias (SCAs) are a heterogeneous group of autosomal dominant neurodegenerative disorders clinically characterized by progressive gait and limb ataxia, dysarthria and oculomotor abnormalities. At present, more than 40 distinct genetic subtypes of SCAs have been described, and the most frequent forms are caused by the presence of a coding CAG repeat expansion in the respective gene (SCA1, SCA2, SCA3, SCA6, SCA7, SCA17). In more recent years, as more families and cases were analyzed, a group of conventional coding mutation or gene deletions were identified.

So far, SCA28 is the only autosomal dominant spinocerebellar ataxia caused by mutations affecting a mitochondrial-gene, the adenosine triphosphatase (ATPase) family gene 3-like 2 (*AFG3L2*); AFG3L2 protein is a component of the mitochondrial adenosine triphosphatase ATPases associated with diverse cellular activities (m-AAA) metallo-protease complex family located in the inner mitochondrial membrane.[1–3]

The most common clinical phenotype associated with heterozygous *AFG3L2* missense mutations is characterized by juvenile-onset slowly progressive cerebellar syndrome with mild pyramidal signs and oculomotor abnormalities including gaze-evoked nystagmus, ophthalmoparesis, and ptosis.[1,4] Though in most SCAs several types of oculomotor abnormalities are invariably present in the phenotypic spectrum due to involvement of cerebellar and brainstem structures, in SCA28 the eye movement abnormalities not only reflect cerebellar and brainstem pathology but also suggest a myopathic component. In fact, SCA28 subjects often have chronic progressive external ophthalmoplegia (CPEO), which is commonly found in diseases associated with mitochondrial DNA (mtDNA) deletions. Recently, multiple mtDNA deletions have been identified in skeletal muscle of subjects with CPEO and *AFG3L2* mutations, supporting the evidence for a specific role of the protein complex in mtDNA maintenance.[5]

We evaluated the neurologic and ophthalmologic characteristics and the cerebellar and extraocular muscle magnetic resonance imaging (MRI) measures in a group of SCA28 subjects and compared the findings with those of SCA2-affected subjects and healthy controls. We chose SCA2 disease among the various group of SCAs, because of the homogeneous and consistent oculomotor phenotype characterized by early slowness of the horizontal saccades up to complete saccade paresis due to brainstem and cerebellar degeneration. The aim of this clinical and MRI study was to investigate whether a myopathic component may contribute in the origin of the oculomotor SCA28 phenotype, even in the absence of mtDNA abnormalities in skeletal muscle

Subjects and Methods

Participants

The study participants included eight SCA28 subjects belonging to four Italian families carrying previously described *AFG3L2* gene mutations[1,4,6] and nine age-matched SCA2 subjects carrying CAG expansions in the ataxin-2 gene ranging from 35 to 43 repeats.

Severity of clinical cerebellar impairment was assessed by the scale for assessment and rating of ataxia (SARA).[7] The age at examination and the score at the SARA ataxia scale were matched in SCA28 and in SCA2 subjects (mean age at onset: 47.8 ± 18 years for SCA28 and 44.1 ± 12 years for SCA2 subjects; SARA score of 11 ± 5 for SCA28, and 15 ± 8 for SCA2 subjects). The neuroradiological measures were compared with those of nine age-matched healthy individuals (mean age: 43.6 ± 9; range, 31–56 years). Consent forms for the clinical tests performed during the study have been approved by the local ethic committee, and written informed consent was obtained from each subject in accordance to the tenets of the Declaration of Helsinki.

Clinical Assessment and Ophthalmologic Examinations

Eye movement qualitative examinations were performed by an ophthalmologist with experience in neurodegenerative disorders to assess the eye position, range of eye movements, presence/absence of nystagmus or square wave jerks on fixation, and presence/absence of gaze-evoked nystagmus or defect in the gaze-holding function. Smooth pursuit eye movements were evaluated by asking the patient to follow visually an object moving in vertical and horizontal directions (10–20°/second). Velocity and accuracy of the reflexive saccades were observed by asking the subject to switch to gaze between two horizontal and two vertical visual targets. The optokinetic nystagmus (OKN) was tested by the use of the optokinetic drum. The vestibulo-ocular reflex (VOR) test and the fixation suppression of the VOR (VORs) were tested by oculocephalic maneuver.[8] Superior eyelid position was evaluated to look for unilateral or bilateral ptosis.

MRI Acquisition

Eight subjects with SCA28, seven subjects with SCA2, and nine sex- and age-matched healthy controls underwent brain and orbit MRI examinations on a 1.5T scanner (Philips Achieva 1.5T; Philips Medical Systems, Best, The Netherlands), using an eight-channel phased-array head coil. Two subjects with SCA2 (A3517, A3518) were not able to complete MRI evaluations due to claustrophobia. Brain MRI included axial and sagittal fast spin echo T2-weighted sequences, axial 3D fast field echo (FFE) T1-weighted sequence, axial fluid attenuated inversion recovery and axial diffusion weighted imaging sequence. For orbital MRI, coronal Spin Echo T1 and Fast Spin Echo T2-weighted images with spectral pre-pulse inversion recovery were acquired.

Brain analyses were performed on the axial 3D FFE T1-weighted sequence acquired with the following parameters: time of repetition/time of echo (TR/TE), 7.3/3.6 ms; flip-angle (FA), 8°; voxel-size, 1 × 1 × 1 mm; matrix, 256 × 256 × 150. The analysis of the extraocular muscle (EOM) area was performed on a coronal spin echo T1-weighted sequence (TR/TE, 568/15 ms; FA, 90°; voxel-size, 0.7 × 0.3 × 3 mm; matrix, 256 × 256 × 22).

Brain Volume Analysis

An automated segmentation for the volumetric brain analysis of cerebellum and frontal lobe was performed using the FreeSurfer software package (in the public domain [http://surfer.nmr.mgh.harvard.edu]). All the volume values from the brain analysis were normalized according to the patient's estimated total intracranial volume.[9] Manual segmentations were performed for midbrain volume, pons volume and cross-sectional EOM area using OsiriX (in the public domain [http://www.osirix-viewer.com]). Manual segmentation of the ROIs was performed by an experienced neuroradiologist.

Extraocular Muscles Cross-Sectional Areas

The extraocular muscles were manually contoured on the first coronal slice behind the eyeball using OsiriX by a neuroradiologist with an expertise in orbital imaging (CG). For each subject, the cross-sectional area from the medial rectus (MR), lateral rectus (LR), inferior rectus (IR), superior rectus (SR), and superior oblique (SO) of both sides was measured. For statistical analyses, the average of the measurements of both eyes was used.

Statistical Analyses

The prevalence of clinical and ophthalmologic characteristics was compared in SCA28 and SCA2 subjects with χ^2 test. Brain MRI volumes and EOM areas in SCA28, SCA2, and healthy controls were compared using ANOVA. The post hoc Tukey honestly significant difference (HSD) test was applied to find the pairs of means that were significantly different from each other. To assess the significance of clinical variables, such as age at examination and disease duration, on EOM areas and brain volumes linear regression analysis was performed. For ordinal variable, logistic regression was used. The values of P were adjusted from multiple comparisons using Bonferroni's method. Values of $P < 0.05$ were considered significant. All statistical analyses were performed with commercial software (JMP 11.2.0; SAS Institute, Inc., Rockville, MD, USA).

Results

Clinical Features

Clinical and genetic data of SCA28 and SCA2 subjects are summarized in Table 1. All subjects were able to walk, except one SCA2 subject who was wheelchair bound. Subjects with SCA28 and SCA2 had progressive gait and limb ataxia, 14/17 had dysarthria, and only one SCA28 subject had mild spasticity at lower limbs. Clinical features, disease duration, and SARA scores did not differ among SCA28 subjects belonging to families with different *AFG3L2* gene mutations. The only obvious difference concerned the age at onset that was in the

Table 1. Clinical and Quantitative MRI EOM Areas in Subjects with SCA28, SCA2, and in Healthy Controls

Group	Patient Code	Gene Mutation*	Sex	SARA Score	Age at Exam, y	EOM Area,† mm²				
						LR	MR	SR	IF	SO
	A1766	p.E691K	F	3	21	10.4	18.65	14.4	10.95	7.75
	A1640	p.E691K	F	4.5	28	14.25	13.9	11.8	10.35	5.7
	A102	p.E691K	M	9	50	15.2	14.5	13.05	11.5	6.25
SCA28	A131	p.E691K	F	18	58	12.05	11.2	10.55	12.8	4.1
	A1948	p.S674L	M	7.5	39	10.6	18.75	11.95	13.3	5.6
	A2123	p.S674L	M	13	66	12.3	15.3	13.25	18.8	6.0
	A0762	p.R702Q	F	11	55	10.9	15.4	9.1	16.85	6.15
	A2274	p.M666V	M	11	74	11.4	10.4	9.35	15.25	5.3
Mean (SD)				9.6 (4.8)	48.9 (18.3)	12.1 (1.7)	14.8 (3.0)	11.7 (1.9)	13.7 (2.9)	5.9 (1.0)
	A2897	22/43	M	10	28	15.25	13.5	13.9	15.45	8.5
	A3516	22/38	M	7	44	16.35	16.8	13.95	17.85	7.3
	A3242	27/42	F	32	38	9.5	17.0	18.4	22.95	10.2
SCA2	A1543	22/39	M	17	48	16.35	21.45	21.3	21.4	10.15
	A3269	22/39	F	15	49	12.8	18.4	20.35	13.9	9.8
	A2360	22/39	M	15	60	11.15	16.15	16.75	20.0	6.3
	A1381	22/35	M	11.5	63	17.45	20.15	24.15	19.3	8.45
Mean (SD)				14.2 (7.4)	44.2 (12.0)	14.1 (3.0)	17.6 (2.6)	18.4 (3.8)	18.7 (3.2)	8.7 (1.5)
	1	–	F	0	52	16.9	16.35	18.9	15.45	10.55
	2	–	M	0	54	10.6	27.9	23.95	25.05	12.45
	3	–	F	0	42	25.25	25.05	20.25	29.9	14.3
	4	–	M	0	31	19.45	21.2	19.4	20.35	7.3
CNT	5	–	F	0	45	19.8	24.7	16.9	23.25	10.9
	6	–	F	0	31	23.75	16.8	18.65	25.25	8.05
	7	–	M	0	56	13.6	28	26.05	27.35	9.0
	8	–	M	0	41	23.65	25.85	31.3	28.25	12.7
	9	–	M	0	40	21.5	24.85	26.5	15.95	6.5
Mean (SD)			–	–	43.6 (9.2)	19.4 (4.9)	23.4 (4.4)	22.4 (4.8)	23.4 (5.2)	10.2 (2.7)
ANOVA *P* values			–	–	0.685	**0.0012**	**0.0002**	**0.0001**	**0.0003**	**0.0005**
Comparison between groups' *P* values‡										
SCA28 vs. CNT						**0.0012**	**0.0001**	**0.0001**	**0.0002**	**0.0004**
SCA28 vs. SCA2						**0.0213**	**0.01**	**0.006**	0.066	**0.026**
CNT vs. SCA2						0.541	0.275	0.107	0.073	0.279

* Gene mutations: for SCA28 subjects carryin missense mutations in the *AFG3L2* gene, the amino acid change in the protein is indicated. For SCA2 subjects, the number of the CAG triplet in the expanded allele is indicated.

† Measurements represent an average of both eyes for each subject.

‡ Post hoc HSD–Tukey-Kramer test. Statistically significant *P* values (<0.05) are in bold.

second decade of life in all cases, except for subjects A0762 who presented the symptoms at the age of 38. Age at onset and disease duration significantly differed between SCA2 and SCA28 subjects due to the different rate of disease progression. Age of onset was earlier in SCA28 (mean, 21 ± 7; range, 16–38 years) compared with SCA2 subjects (mean, 34 ± 10; range, 22–50 years; $P = 0.009$). In SCA2, the age at onset significantly correlated with the number of CAG triplet expansion ($r^2 = 0.57$; $P = 0.018$).

Clinical Assessment of Ocular Movements

Subjects with SCA2 showed slow horizontal saccades (8/9); impaired OKN (9/9); and abnormal VORs (6/9). Subjects with SCA28 had hypometric saccades (5/8); broken up by saccades horizontal pursuit (5/8); mild limitation of horizontal gaze (5/8); centripetal drift due to unsustained maintenance of an eccentric position (7/8); impaired OKN (5/8); and VOR (3/8).

Vestibulo-ocular reflex was impaired in 67% SCA2 subjects while it was normal in all SCA28 subjects. None of the subjects in both SCA groups had impaired vertical-ocular movements, downbeat nystagmus, or square wave jerks on fixation. Superior eyelid ptosis occurred only in SCA28 subjects (3/8).

MRI Brain Volumes

Brain volumes were segmented on 5 of 7 subjects with SCA2, 7 of 8 subjects with SCA28, and on 9 healthy voluntary controls. Images that were 3D-T1-weighted of two SCA2 subjects and one SCA28 patient were excluded due to excessive motion artefacts. The measures of the normalized brain volumes significantly differed between the three groups of subjects (ANOVA $P < 0.03$).

The post hoc HSD–Tukey-Kramer test showed that SCA2 subjects had significantly lower volumes for all the examined brain regions in comparison with the controls subjects ($P < 0.03$). Subjects with SCA2 had the lowest mean values for all volume areas. The normalized volumes for the cerebellum, pons, midbrain, and frontal lobe in SCA2 subjects were found to be smaller (57%, 68%, 33%, and 15%, respectively) than in control subjects.

Subjects with SCA2 had significant lower volumes for the cerebellum (39%) and pons (63%) in comparison with SCA28 subjects (*P* values <0.005). Subjects with SCA2 and SCA28 did not differ in the midbrain and frontal lobe volumes (Table 2).

The subjects with SCA28 had significant lower volumes of cerebellum (26%) and pons (30%) compared with control subjects ($P < 0.01$ for both). A reduced volume of the midbrain

Table 2. MRI-Based Measures of Selected Brain Regions in SCA28 and SCA2 Subjects and in Healthy Controls

	Normalize MRI Volume Ratios*			
	Cerebellum (IQR)	**Pons (IQR)**	**Midbrain (IQR)**	**Frontal lobe GM (IQR)†**
SCA28 (n.7)	61.25 (55.0–70.63)	7.67 (6.92–8.59)	4.81 (4.29–5.17)	105.56 (94.5–112.3)
SCA2 (n. 5)	36.53 (24.78–47.06)	3.21 (2.74–3.84)	4.18 (3.54–4.86)	92.15 (85.92–97.88)
CNT (n. 9)	83.98 (75.29–93.41)	10.08 (8.99–10.99)	5.54 (4.74–6.35)	107.52 (100.6–113.7)
ANOVA P values	**0.0001**	**0.0001**	**0.019**	**0.0281**
Comparison between groups' *P* values‡				
SCA28 vs. CNT	**0.0022**	**0.0002**	0.188	0.914
SCA28 vs. SCA2	**0.0038**	**0.0001**	0.380	0.071
CNT vs. SCA2	**0.0001**	**0.0001**	**0.016**	**0.027**

IQR, interquartile range values (25°–75° percentiles).
* Normalized volume ratios were calculated by dividing the structure volume (mm^3) by the estimated total intracranial volume (mm^3).
† Frontal lobe values depict only the grey matter (GM) volume ratio.
‡ Post hoc HSD-Tukey-Kramer test. Statistically significant *P* values (<0.05) are in bold.

was suggested by the data, but they were not significantly different. Frontal lobe volumes were similar in SCA28 and in controls subjects (Fig.1, Table 2).

Extraocular Muscle Areas

Analysis of variance and the post hoc HSD–Tukey-Kramer test indicate that SCA28 subjects had significant smaller areas of EOMs in comparison with both the areas in healthy control subjects and in SCA2 subjects (Fig. 2, Table 1). The average areas for the lateral, medial, superior, and inferior rectus muscles and of the superior oblique muscle were 37% to 48% smaller in SCA28 subjects in comparison with the respective areas in control subjects (all *P* values < 0.002; Fig. 3, Table 1). The areas of EOM in SCA28 subjects were also significantly reduced (14%–36%) in comparison with those measured in SCA2 subjects (*P* values < 0.03), except for inferior rectus muscle that was similar in the two groups (Fig. 3). The areas of EOM in the SCA2 subjects were similar to those measured in healthy control subjects (Table 1).

No significant correlations were found between clinical variables and quantitative MRI measurements of brain regions and EOM areas.

Discussion

In autosomal dominant spinocerebellar ataxias, the presence of oculomotor deficits is almost invariably recognized as part of the typical clinical picture, and it is due to dysfunctions of cerebellar and brainstem structures. Though no specific eye movement defect is pathognomonic to any SCA, some ocular abnormalities have been more frequently recognized in particular genotypes. Gaze-evoked nystagmus is frequent in SCA1, SCA3, and SCA6 genotypes, saccade velocity is normal in SCA3 and mildly reduced in SCA1, severe saccade slowing is a characteristic feature of SCA2.[10–13]

The characteristic eye movement abnormalities are represented by gaze-evoked nystagmus in SCA28 subjects with short disease duration, and progressive ophthalmoparesis, and ptosis in those with longer duration.[14] These oculomotor features partially overlap those described in subjects with cerebellar ataxia and those described in subjects with CPEO, that is a common clinical manifestation of mitochondrial diseases.[15]

It is well known that the SCA28 disease is caused by mutations in a nuclear gene encoding a mitochondrial protein.[1–3] The mutated SCA28 protein, AFG3L2, takes part in the proteolytic quality control and chaperon-like activities in mitochondria by degrading misfolded proteins and promoting the assembly of respiratory chain complexes.[1–3] This protein forms either a homo-oligomeric isoenzyme or a hetero-oligomeric complex with paraplegin, a homologous protein mutated in a recessive form of inherited spastic paraplegia (SPG7).[1,2,16] Recently, subjects diagnosed with CPEO have been shown to carry mutations either in the *SPG7* or in the *AFG3L2* genes and to have multiple mtDNA deletions in skeletal muscle, suggesting a common downstream causative

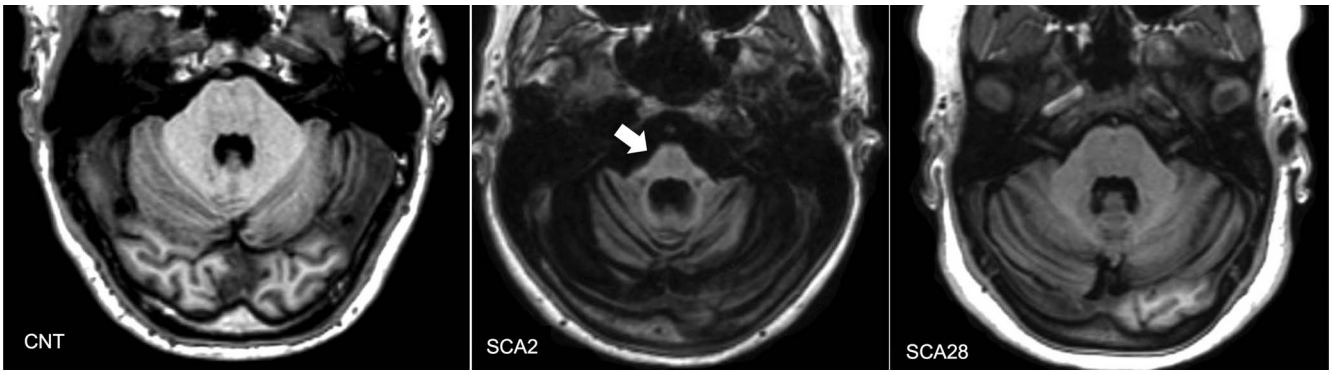

Figure 1. Axial T1-weighted brain images showing a severe atrophy in the pons and cerebellar peduncles (*white arrow*) in a representative image of a SCA2 affected subject (*central image*) in comparison with the images obtained in healthy control (*left image*) and in SCA28 subject (*right image*). Statistical analyses showed a significant volume reduction in these brain areas in SCA2 in comparison with both control and SCA28 subjects, whereas in SCA28 pons and cerebellar volumes were within the range of normal controls (see Table 2).

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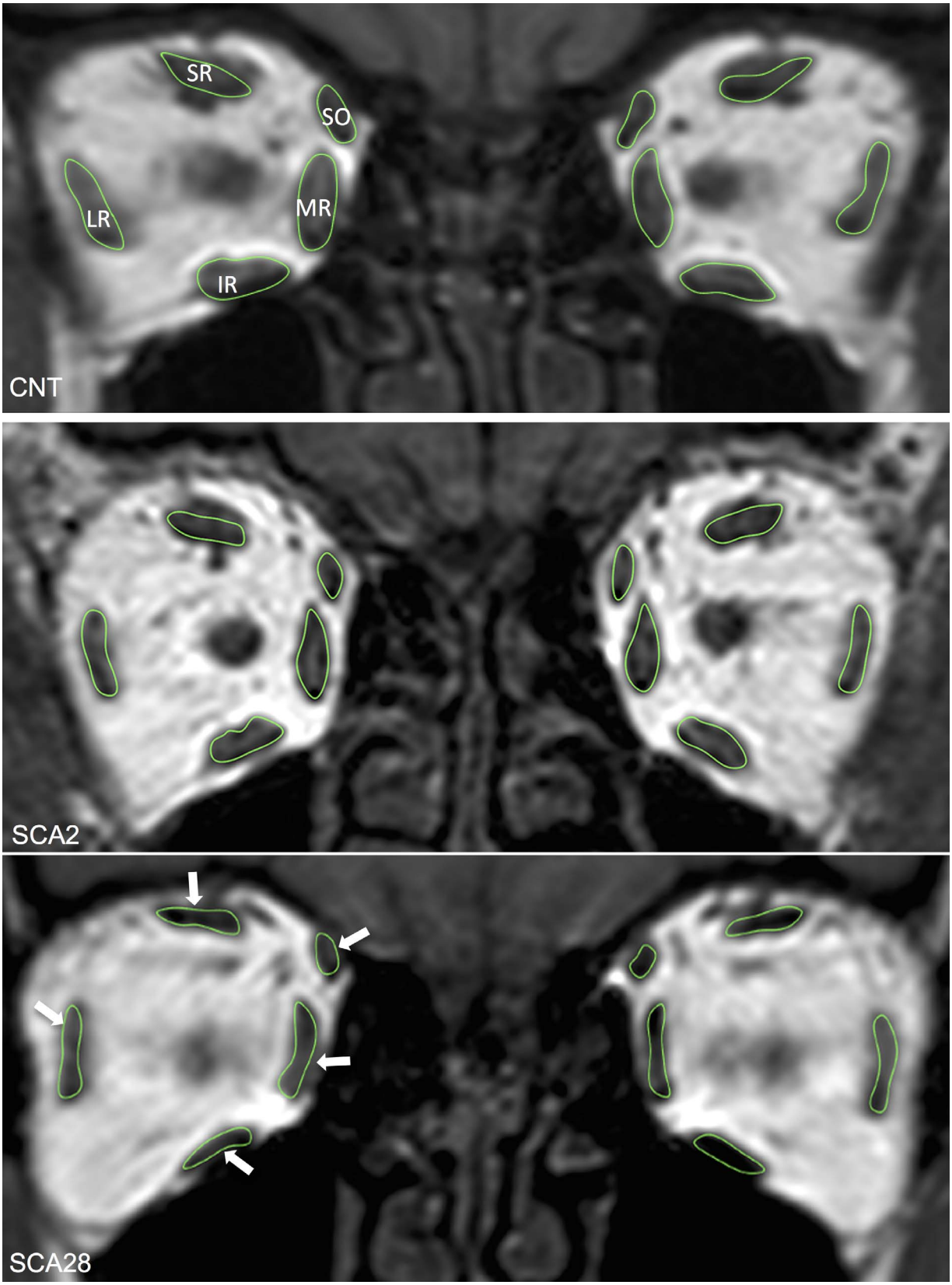

FIGURE 2. Representative coronal T1-weighted images of the right and left orbit from a healthy control subject (CNT, *upper panel*), SCA2 subject (*central panel*), and SCA28 subject (*lower panel*). The cross-sectional area of extraocular MR, LR, IR, SR, and SO muscle of both sides were manually contoured and measured. Subjects with SCA28 showed a diffuse and severe atrophy of all extraocular muscles (*white arrows*). The medial rectus, LR, SP, and SO muscle areas were significantly reduced in comparison with the areas measured in control and in SCA2 subjects, whereas extraocular muscle areas did not differ between SCA2 and control subjects ($P < 0.03$; see Table 1). For statistical analyses, the average of the measurements of both eyes was used.

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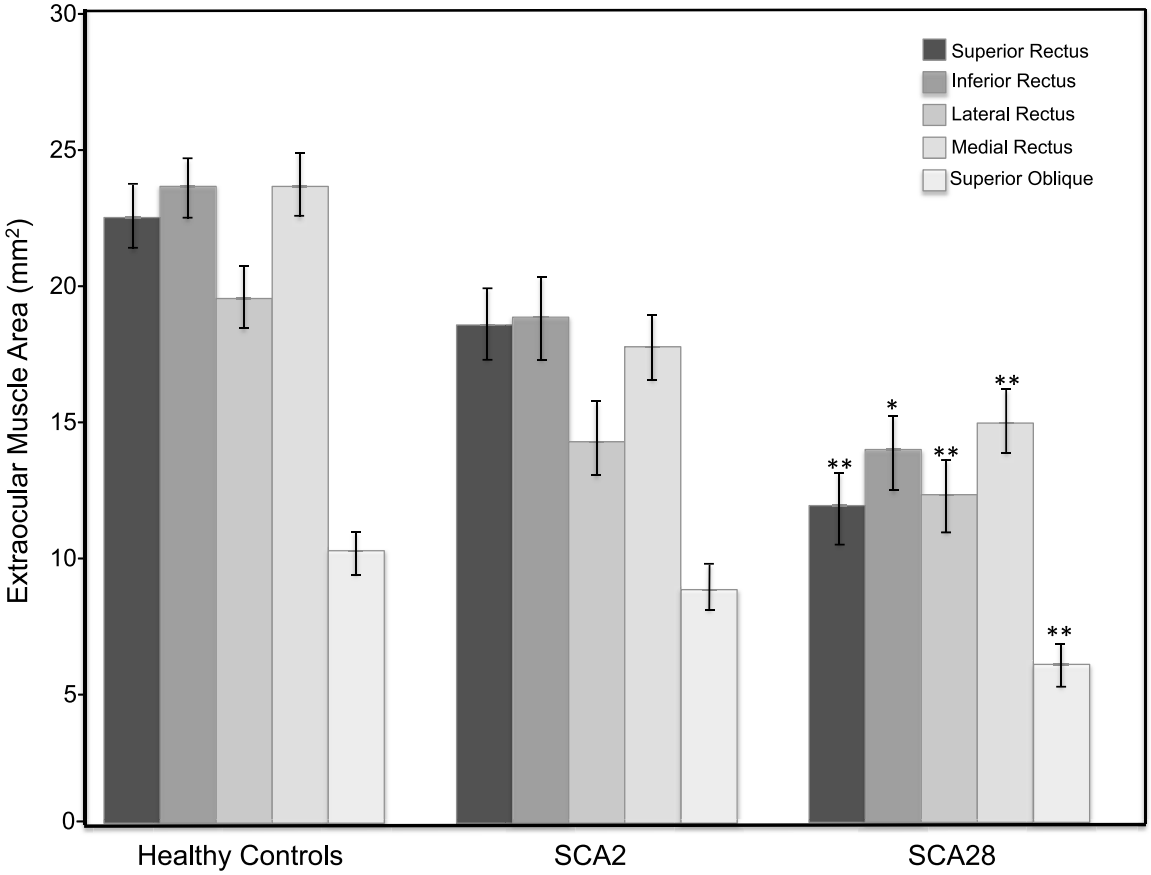

FIGURE 3. Graph illustrating the mean cross sectional areas (mm^2) of EOM in SCA2, SCA28, and healthy control subjects. Average measurements of both sides for each study group are indicated. *Bars* represent SEM. Analysis of variance and the post hoc HSD–Tukey-Kramer test indicate that SCA28 subjects had significant lower EOM areas in comparison with both the control and the SCA2 subjects for all EOMs ($^{**}P < 0.03$) except for the IR. The mean muscle area of the IR was significantly reduced in SCA28 compared with controls ($^{*}P = 0.0002$) but did not differ from the area measured in SCA2 subjects (see also Table 1).

mechanism for external muscle myopathy in these two disorders.[5,17,18]

In our SCA28 subjects, we did not find mtDNA deletions in skeletal muscle biopsies (data previously reported).[1,4,14] However, we speculated that the presence of a SCA28 mutation could be responsible for energy metabolism and cell maintenance impairment in the ocular muscles even in the absence of detectable respiratory chain defects or mtDNA deletion in skeletal muscle. Extraocular muscles, in fact, have been shown to have distinct properties in respect to skeletal muscles that make them selectively vulnerable to mitochondrial disorders.[19,20]

In our study we aimed at determining the relative contribution of the neurologic and the myopathic components in the SCA28 ocular phenotype using both clinical and MRI-based EOM measurements. In subjects with CPEO, the presence of diffuse EOM atrophy at MRI investigation has already been demonstrated.[14,19–20]

We chose, as disease controls, a group of SCA2 subjects with the typical ocular motor changes characterized by early slowness of saccades evolving to complete horizontal and vertical paresis. The subjects with SCA2 and the healthy controls were age-matched with SCA28 subjects at examination in order to have a comparable stage of eye movement abnormalities, and to correct neuroimaging results for a possible role of normal aging. Subjects with SCA2 also had comparable SARA scores in respect to the SCA28 group; however, SARA scale does not include eye movement evaluations and the comparison of the oculomotor phenotype between the two disease groups was possible only on the basis of clinical impression.

Our results indicate that in SCA28, the eye movement abnormalities may be consistent with both supranuclear involvement and myopathic defects. From the clinical point of view, a high percentage of SCA28 subjects had, broken-up by saccadic pursuit, impaired OKN and VOR, which are the most common supranuclear ocular defects observed in other neurodegenerative ataxias, such as SCA1, SCA2, and SCA3.[10,11] Subjects with SCA28 also had hypometric saccades, and progressive ophthalmoparesis similarly to that observed in SCA2 disease, in which a dysfunction and/or a loss of excitatory burst neurons in the paramedian pontine reticular formation has been described.[10–13,15] In subjects with SCA28, a clear myopathic component can also be recognized for the presence of: gaze-evoked centripetal drift in maintaining eccentric position, gaze palsy or single extraocular muscle defect, and eyelid ptosis. In addition, VOR and OKN changes may also indicate an effect of muscle weakness, as similar defects were observed in CPEO patients.[15]

Magnetic resonance image–based measures of EOM also supported a myogenic pathologic substrate for SCA28, as it occurs in CPEO subjects, in whom a significant EOM atrophy has been demonstrated.[19,20]

No previous studies on EOM measurements are currently available for SCA28 and SCA2 diseases. We demonstrated that total mean cross-sectional area of EOM in SCA28 is significantly

reduced in comparison not only with healthy age-matched controls, but also in comparison to SCA2 subjects (Table 2). We hypothesized that the relatively mild EOM atrophy measured in SCA2 subjects could be attributed to a reduced muscle activity, whereas the more severe and diffuse SCA28 muscle atrophy could suggest an additional myopathic component. Though the degree of EOM atrophy and of the signal changes within the muscles in subjects with mitochondrial diseases may be highly variable, the observation of severe EOM atrophy is suggestive of a mitochondrial muscular defect. In our study, we also performed volumetric measurements of brain regions, such as cerebellum, pons, midbrain nuclei, and the frontal area, to assess the extent of central nervous system involvement and the possible correlation with eye movement abnormalities. We did not observe significant correlation between EOM areas and age at examination, disease duration, and SARA score. Interpretation of these negative findings may be controversial; however, the limited sample size suggests a prudent approach and does not allow conclusive evaluations.

While extensive MRI data are reported in the literature with respect to SCA2 subjects, few volumetric MRI findings have been previously reported for SCA28 subjects.[21] Our data confirmed that SCA2 subjects had a more severe degree of cerebellar and pontine atrophy in comparison to SCA28 subjects, despite a milder degree of extraocular muscle atrophy.

In conclusion, we collected clinical and neuroimaging evidences in favor of a combined effect of supranuclear degeneration and mitochondrial myopathy contributing in the development of the characteristic oculomotor phenotype of SCA28 disease.

Acknowledgments

We thank all the subjects and family members enrolled in the study and the Italian Association for Ataxic Syndromes for covering the travel costs for the participants.

Supported in part by a grant from the Italian Ministry of Health (RF-2011-02347420 to C. Mariotti).

Disclosure: **L.S. Politi**, None; **S. Bianchi Marzoli**, None; **C. Godi**, None; **M. Panzeri**, None; **P. Ciasca**, None; **G. Brugnara**, None; **A. Castaldo**, None; **D. Di Bella**, None; **F. Taroni**, None; **L. Nanetti**, None; **C. Mariotti**, None

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