Imaging alterations in skeletal muscle channelopathies: a study in 15 patients

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Skeletal muscle channelopathies (SMC), including non dystrophic myotonias (NDM) and periodic paralyses (PP), are characterized by considerable clinical overlap and clinical features not always allow addressing molecular diagnosis. Muscle imaging has been shown to be useful for differential diagnosis in neuromuscular disorders, however it has been relatively poorly investigated in SMC.
We studied 15 patients affected by genetically confirmed SMC (NDM = 9, PP = 6) through muscle MRI or CT of thighs and legs, including 11 patients mutated in *SCN4A* gene, 2 in *CACNA1S* and 2 in *CLCN1*. Mean age at muscle imaging was 45.2 ± 18 years (range 22-70).
Overall, fatty infiltration was found in thigh muscles in 8 (53%) patients and in leg muscles in 10 (60%). All patients mutated in *CLCN1* and *CACNA1S* had abnormal thigh and/or leg muscle MRI, regardless the disease duration. On the contrary normal thigh and leg muscle MRI or CT scans were observed in 4/15 (27%) patients, all mutated in *SCN4A*. Variable degrees of fatty changes were found in patients mutated in *SCN4A*, *CACNA1S* and *CLCN1*. No differences on overall score of fatty infiltration were detected between NDM and PP (p-value = 0.953) neither between presence or absence of permanent weakness (p-value = 0.951).
Our data confirm the presence of muscle fatty changes in the majority of SMC patients, although without any specific pattern of involvement. However muscle MRI may be a useful tool for longitudinal follow-up of SMC patients, in particular to evaluate the occurrence and the progression of fixed myopathy.

Key words: muscle MRI, periodic paralyses, non dystrophic myotonias, SCN4A, CACNA1S, CLCN1

Introduction

Skeletal muscle channelopathies (SMC) are rare (prevalence in UK: 1.12/100000) neuromuscular disorders clinically characterized by paralytic attacks and/or myotonia (1); on the basis of the predominant clinical feature SMC are classified as non dystrophic myotonias (NDM) or periodic paralyses (PP) (1). NDM include myotonia congenita (MC), paramyotonia congenita (PMC) and sodium channel myotonia (SCM) (1, 2). PP comprise hypokaliemic periodic paralysis (HypoPP), hyperkaliemic periodic paralysis (HyperPP) and Andersen-Tawil syndrome (ATS) (1, 2). SMC are dominantly inherited diseases, except for recessive MC, and are caused by mutations in genes encoding skeletal muscle ion channels that affect muscle excitability. In particular, MC are due to mutations in *CLCN1* gene, encoding the voltage-gated chloride channel (CLC-1), PMC, SCM, HypoPP type II and HyperPP to mutations in *SCN4A*, encoding the alpha subunit of the voltage-gated sodium channel (NaV1.4), HypoPP type I to mutations in *CACNA1S*, encoding the voltage-gated calcium channel (CaV1.1), and ATS to mutations in *KCNJ2*, encoding the inward-rectifying potassium channel (Kir2.1).

Considerable overlap exists among different SMC entities, being myotonia also present in HyperPP and paralytic attacks described even in PMC, hence clinical features not always allow to address molecular diagnosis (2).

In the last decade muscle MRI has been proven to be a highly reliable, non-invasive tool for the differential diagnosis and the evaluation of progression of muscular diseases, revealing specific patterns of muscle involvement associated with different myopathies (3-8). However muscle MRI has been relatively poorly investigated in SMC (9-13).

Aim of our study was to investigate the presence and severity of fatty infiltration in lower limb muscles through MRI or CT scan in a cohort of patients with genetically determined SMC.

Methods

Subjects

We investigated retrospectively 15 patients (11 males, 4 females) affected by genetically characterized SMC (NDM = 9, PP = 6) and undergone muscle MRI or CT scan in our Institute during the period ranging from January 2008 and December 2013. Eleven patients were mutated in *SCN4A*, 2 in *CLCN1* and 2 in *CACNA1S*. Among patients with mutations in *SCN4A*, 4 had PMC, 2 SCM, 2 HypoPP type II and 2 HyperPP. Mean age at onset was 10.2 ± 8.7 years (range 1-30); mean age at muscle imaging was 45.2 ± 18 years (range 22-70). Mean time from symptom onset to muscle imaging was 36.6 ± 14.9 years (range 8-62).

Muscle imaging protocol

Nine (*SCN4A* = 5, *CLCN1* = 2, *CACNA1S* = 2) out of 15 patients underwent muscle MRI and 6 (*SCN4A* = 6) CT scan. In first subgroup patients were scanned with MRI at 1.5T (Philips Achieva, Eindhoven, Holland) in a supine position with surface array coils. Patients were scanned with a clinical protocol comprising T1w axial and coronal images for the simultaneous bilateral evaluation of leg and thigh (representative parameters: matrix for coronal images 504 x 298 in the thigh, 452 x 262 in the leg, for axial images 448 x 214 both in thigh and leg; number of signal averages (NEX): 1 for coronal, 2 for axial images; leg coronal: repetition time (TR): 572 mms, echo time (TE): 10 mms, thickness: 3 mm, 20 slices; leg axial: TR: 467 mms, TE: 4.4 mms, thickness: 10 mm, 40 slices; thigh coronal: TR: 570 mms, TE: 10 mms, thickness: 4 mm, 30 slices; thigh axial: TR: 467 mms, TE: 4.4 mms, thickness: 10 mm, 40 slices) with 1 mm slice gap.

CT scan (Philips Brilliance™ CT, Eindhoven, Holland) comprised axial images (representative parameters: 512 x 512 matrix, 120 kV, 400mAs, FOV-field of view-500) with 2 slides for thigh and leg, respectively, with 4.5 mm thickness and 2 cm slice gap. Scanning time was about 20 minutes for MRI and 5 minutes for CT.

Muscle imaging analysis

Lower limb muscles were analyzed for fatty infiltration and then the overall degree of involvement at thigh and leg level was categorized. The following muscles were assessed on both sides: rectus femoris, vastus lateralis, vastus intermedius, vastus medialis, adductor magnus, gracilis, sartorius, biceps femoris, semimembranosus and semitendinosus in the thigh; tibialis anterior, peroneus longus, tibialis posterior, soleus, medial and gastrocnemius in the leg. These 32 muscles were assessed on T1w sequences for the presence of fatty infiltration using Fischer's semi-quantitative scale (5): 0 – normal appearance, 1 – occasional scattered T1 hyperintensity, 2 – confluent areas of T1 hyperintensity < 50% of muscle involved, 3 – confluent areas of T1 hyperintensity > 50%, 4 – complete replacement of muscle with fat. Finally muscle involvement of thigh and leg, respectively, was categorized as already reported by Morrow and colleagues (12):

- Normal: all muscles grade 0.
- Mild limited changes: grade 1 changes in £50% of the muscles (10/20 in thighs; 6/12 in legs).
- Mild extensive changes: grade 1 changes in > 50% of the muscles.
- Marked changes: any muscle with grade 2 changes.

The same analysis, although initially set up for MRI images, was performed also for muscle CT scan.

Statistical analysis

Overall score was defined as the mean fatty infiltration detected by muscle imaging for all investigated muscles in each patient. Spearman's rank correlation coefficient was used to assess correlation between overall score and age. Differences on the overall score between independent groups (phenotypes and presence/absence of permanent weakness) were investigated with Mann-Whitney test. P-value less than 0.05 was considered as significant. Statistical analyses were performed using SPSS 20.0.

Results

Overall, normal thigh and leg muscle MRI or CT scans were observed in 4 out of 15 (26.7%) patients, all mutated in *SCN4A* (PMC = 2, SCM = 1, HypoPP type II = 1); among them 1 had muscle MRI and 3 only CT scan. All patients mutated in *CLCN1* and *CACNA1S* had abnormal thigh and/or leg muscle MRI, regardless the disease duration. Patients clinical, genetic and muscle imaging data are shown in Table 1.

Fatty infiltration was found in thigh muscles in 8 (53.3%) patients and in leg muscles in 10 (60%). In particular abnormal findings were observed in 94 out of 300 (31.3%) evaluated thigh muscles and in 65 out of 180 (36.1%) leg muscles. Of note, none of the investigated muscles resulted normal in all patients. Muscles displaying more commonly fatty infiltration were soleus (60%) and medial gastrocnemius (50%); on the other hand muscles more commonly showing no fatty changes were tibialis anterior and rectus femoris (86.7%). Distribution and severity of fatty infiltration in individual thigh and leg muscles of investigated patients are shown in Figure 1.

Patients with abnormal muscle imaging had variable degree of fatty infiltration. In the thigh we observed grade 1 in 55 out of 300 muscles (8 patients), grade 2 in 21

Table 1. Patients clinical, genetic and muscle imaging data.

Pt, Sex	Gene	Mutation	Phenotype	Age at onset (y)	Paralysis	Myotonia	Fixed weakness	Treatment	Age at MRI/CT (y)	Thigh imaging	Leg imaging	Overall score
1,F	SCN4A	R1448C	PMC	5.5	yes	C,H,LL	no	Mex	40 (MRI)	-	-	0.00
2,M	SCN4A	T1313M	PMC	17	yes	C,H	no	Mex	48 (MRI)	+	±	0.56
3,M	SCN4A	T1313M	PMC	2	yes	C,H,LL	A,P,D,F	Mex	64 (MRI)	++	++	0.81
4,F	SCN4A	T1313M	PMC	2	yes	C,H,LL	F	Mex	33 (MRI)	±	±	0.16
5,M	SCN4A	R1448C	PMC	1.5	yes	C,H,LL	P,D	none	30 (CT)	-	-	0
6,F	SCN4A	N275K	SCM	13.5	no	C,LL	A,P	none	63 (CT)	-	-	0
7,M	SCN4A	V445L	SCM	17	no	C,LL	P	Mex	68 (CT)	-	++	0.31
8,M	SCN4A	R675G	HyperPP	1	yes	no	P,D	Acz	45 (CT)	++	++	1.87
9,M	SCN4A	I692M	HyperPP	6	yes	C,H,LL	A,P,D	Hyct	53 (MRI)	++	++	0.94
10,F	SCN4A	T704M	HypoPP II	5	yes	no	no	Hyct	22 (CT)	±	-	0.19
11,M	SCN4A	R672C	HypoPP II	14	yes	no	no	Acz	30 (CT)	-	-	0
12,M	CLCN1	homo R496S	Becker	3	no	C,H,LL	P,D	none	50 (MRI)	++	++	1.25
13,M	CLCN1	c.IVS13+5-11delGTTCTGA + F167L	Thomsen*	30	no	H,LL	P,D	none	70 (MRI)	-	+	0.06
14,M	CACNA1S	R528H	HypoPP I	20	yes	no	A,P,D	No	63 (MRI)	++	++	2.40
15,M	CACNA1S	R528H	HypoPP I	16	yes	no	no	Acz	24 (MRI)	-	+	0.31

Pt: patient; F: female; M: male; homo: homozygous; PMC: paramyotonia congenita; SCM: sodium channel myotonia; HyperPP: hyperkaliemic periodic paralysis; HypoPP: hypoka-liemic periodic paralysis; y: years; C: cranial; H: handgrip; LL: lower limb; A: axial; P: proximal; D:distal; F: facial; Mex: mexiletine; Acz: acetazolamide; Hyct: hydrochlorothiazide; -: all muscles normal; ±: mild limited changes; +: mild extensive changes; ++: marked changes (as defined in the methods section).

* Patient 13 was clinically considered as Thomsen myotonia, despite the 2 recessive mutations, due to relative mild phenotype. Compound muscle action potential after short exer-cise test with and without cooling revealed a pattern compatible with dominant myotonia congenita.
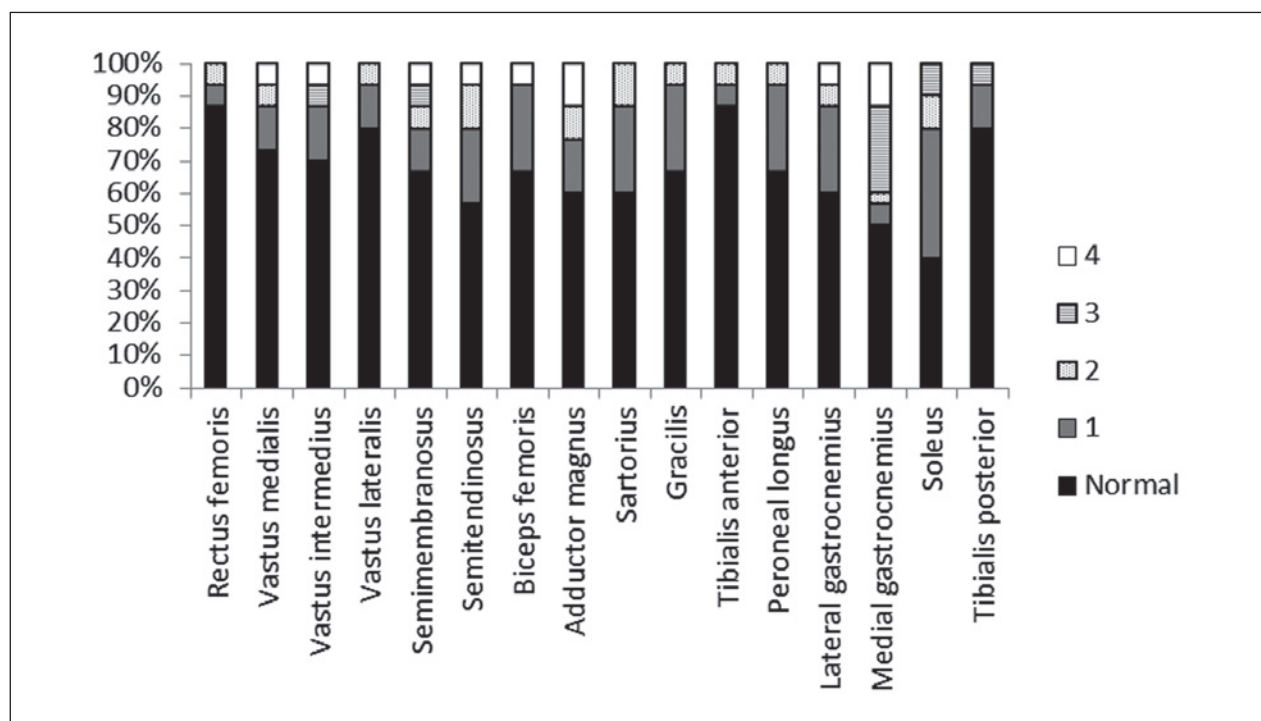
Figure 1. Distribution and severity of fatty infiltration in thigh and leg muscles of the investigated patients. Severity of fatty infiltration was categorized for each muscle using Fischer's semi-quantitative scale as described in the methods section.

muscles (5 patients), grade 3 in 4 muscles (2 patients) and grade 4 in 14 muscles (2 patients); in the leg: grade 1 in 36 out of 180 muscles (7 patients), grade 2 in 10 muscles (3 patients), grade 3 in 13 muscles (5 patients) and grade 4 in 6 muscles (2 patients). Grade 4 was found in thigh muscles in 2 patients and in leg muscles in 2 other patients and muscles more frequently affected were adductor magnus and medial gastrocnemius. Interestingly, patients displaying grade 4 fatty infiltration in thigh had also a consistent involvement (grade 2-3) in leg muscles, contrary to patients with grade 4 in leg muscles in which fatty changes were limited to the calves. Fatty infiltration was almost invariably symmetrical; among 240 muscles investigated on both sides in thighs and legs only 7 (2.9%) muscles from 6 different patients showed asymmetrical involvement, usually differing for 1-grade severity in Fischer's semiquantitative scale, mostly with right side worse than the left.

No specific pattern of muscle involvement was observed in different clinical phenotypes which were associated with variable degrees of fatty changes, as revealed by overall score (see Table 1 for details and figure 2 for images from patients); this value was higher in both 2 patients affected by HyperPP, in 1 patient with recessive MD and in 1 with HypoPP type I. To this purpose the highest value of overall score was reached in patient 14

with HypoPP type I, showing severe progressive myopathy since the age of 54, after 2 years from the cessation of paralytic attacks. His son (patient 15) had only initial minimal changes at muscle MRI performed at the age of 24, without any detectable muscle weakness at neurological examination, although with relatively frequent paralytic attacks.

Fixed muscle weakness was evident at neurological examination in 10 out of 15 (66.7%) patients (*SCN4A* = 7, *CLCN1* = 2 and *CACNA1S* = 1), mainly in proximal lower limbs (9/10). All these 10 patients had variable degree of muscle involvement at muscle imaging, except for patient 5 and 6 (1 PMC and 1 SCM), showing normal lower limb CT scan.

No Spearman's rank correlation was found between overall score and age at muscle imaging (r = 0.288, p-value = 0.298) or disease duration (r = 0.394, p-value = 0.146). No differences on overall score between NDM and PP (p-value = 0.953) neither between presence or absence of fixed weakness (p-value = 0.951) were found.

Discussion

In recent years muscle imaging has been widely used to support clinical diagnosis of neuromuscular disorders
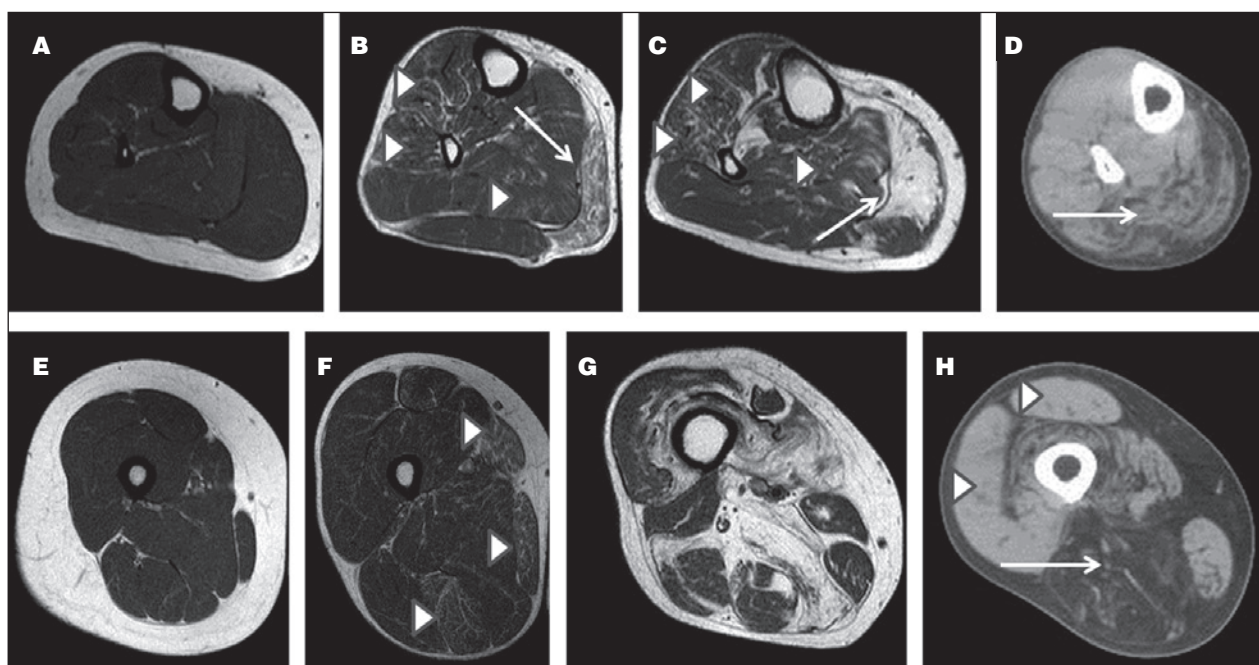
Figure 2. Normal leg (A) and thigh (E) muscle MRI in a patient affected by PMC. Marked T1w changes in medial gastrocnemius (arrows) in a patient with recessive MC (B) and in a patient with HypoPP type I (C) in association with lower severity involvement of soleus, peroneal longus and tibial anterior (arrowheads). Both patients had also thigh involvement, limited to sartorius, gracilis and semitendinosus (arrowheads) in recessive MC (F), more marked and diffuse in HypoPP type I (G). Muscle CT scan hypodensity (arrows) limited to posterior leg (D) and predominant in posterior thigh (H) in a patient with HyperPP. To be noticed the sparing of rectus and vastus lateralis (arrowheads).

and for their differential diagnosis through the identification of specific patterns of muscle involvement. However, few data are available in literature on muscle imaging in SMC (9-13).

Here, we report our experience in a cohort of patients affected by SMC investigated with muscle MRI or CT scan to evaluate the frequency and severity of muscle fatty infiltration. A recent study including a slightly wider cohort of patients was focused only on NDM (12); on the contrary in our study we investigated also PP due to *SCN4A* and *CACNA1S* gene mutations.

Our findings revealed that fatty infiltration was found in thigh muscles in about 50% of the patients and in leg muscles in about 60%. In particular abnormal findings were observed in about one third of evaluated thigh and leg muscles, in agreement with the aforementioned study (12). On the contrary, a study investigating 3 patients with recessive MC through whole body MRI did not demonstrate any abnormality (10).

Grade 1 fatty infiltration was detected in more than a half of abnormal muscles, supporting the hypothesis that severe fatty infiltration is not a predominant finding in SMC. Unfortunately, our study did not include comparative data in age-matched normal individuals, making difficult to evaluate the pathological meaning of the

mild changes detected by muscle imaging in our patients. However the study by Morrow and colleagues revealed mild limited T1w changes also in healthy volunteers, in particular in leg muscles (12); hence the slight and limited muscle fatty infiltration should be considered as a negligible and unspecific finding. Higher severity of fatty infiltration (grade 3 and 4) were slightly more frequent in our cohort than in the aforementioned study (12), probably because we included also patients affected by PP, which may be associated with the development of progressive myopathy (14). Of note, higher severity of fatty infiltration was observed more frequently in medial gastrocnemius, although this should be considered an unspecific finding, being reported also in other myopathies (15).

Within the limitation of a small cohort of patients, we did not confirm the correlation between age at muscle imaging and overall score detected by Morrow and colleagues (12), probably due to the lower rate of patients with recessive MC, for which the correlation was stronger; moreover we did not find any correlation between disease duration and overall score.

Contrary to other muscle diseases (3-8), no specific pattern of muscle involvement was observed, in agreement with Morrow and colleagues (12). Overall score was very variable among different phenotypes and no

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significant differences were found between NDM and PP. However, among PP patients overall score was higher in both HyperPP cases, which had also fixed weakness, than in those affected by HypoPP type II, without any detectable weakness. Considering also that the highest overall score was observed in a patient mutated in *CACNA1S,* our data support the hypothesis that patients affected by HypoPP type I or HyperPP develop more frequently a progressive myopathy than those with HypoPP type II (14). Of note, fatty infiltration in PP has been investigated previously only in one patient affected by HypoPP type I, revealing diffuse degeneration of calf muscles, except the tibialis anterior (16); no data on thigh muscle were provided. Among 9 NDM patients we detected fatty infiltration in thigh and leg muscles about in one and two third of the patients, respectively, similarly to findings already reported (12).

Our study had some limitations. First, MRI STIR sequences have not been performed; STIR hyperintensity reflecting oedema has been found in patients with NDM, in particular in medial gastrocnemius ("central stripe") (12); the central stripe has not been reported in healthy volunteers or in other conditions, suggesting a possible specific feature of NDM. Second, we used a 1.5T and not 3T MRI as in the aforementioned stud (12). Third, we included 6 patients with muscle CT scan, which is less sensitive than MRI, in particular for the detection of oedema (15), hence some subtle changes in the muscle might be missed. Despite the last two limitations, our results in terms of detection of fatty infiltration are similar to those reported by Morrow and colleagues (12).

In recent years innovative muscle imaging approaches have been carried out in SMC. 3T muscle MRI showed muscle oedema in HypoPP type I and II similarly to healthy subjects after exercise, although with higher severity and more frequent involvement of the calf muscles (13). Of note, HypoPP patients were investigated during the interval period between the episodes of paralysis, suggesting the presence of muscle oedema also when the patient was asymptomatic. Thus muscle MRI may be useful for monitoring treatment effects in between paralytic attacks. Similarly, 3-T sodium ^{23}Na MRI has been studied in HyperPP revealing Na$^+$ accumulation during weakness episodes after provocation with specific triggers and showed increased myoplasmic Na+ content in HyperPP patients with permanent weakness compared to those with only episodic weakness (9, 11); this suggests that Na+ overload may cause muscle degeneration developing with age, thus ^{23}Na MRI may evaluate the possible efficacy of treatments that reduces this overload. In this regard remarkable benefit of acetazolamide on permanent weakness in a patient affected by HyperPP has been documented clinically and through MRI, which revealed

a significant increase in muscle bulk (17). Sodium accumulation has been also documented by ^{23}Na MRI in a HypoPP patient mutated in *CACNA1S* (16). A further study focused on in vivo imaging of chloride and sodium in patients with Hypo PP type I through ^{35}Cl and ^{23}NA MRI, showing increased muscle concentrations of both sodium and chloride compared to healthy volunteers (18). At last, orbital MRI revealed extraocular muscle hypertrophy in 2 NDM patients mutated in *SCN4A* (19) At present most of the imaging techniques are limited to research context and further studies are needed to clarify whether these imaging approaches are useful in clinical routine practice; however new MRI techniques appear promising as possible outcome measure in pharmacological clinical trials in SMC.

In conclusion our data confirm the presence of muscle fatty changes in the majority of the patients affected by SMC, although often characterized by minimal severity and without any specific pattern of involvement. However, muscle MRI may be a useful tool for longitudinal follow-up of SMC patients, in particular to evaluate the occurrence and the progression of fixed myopathy.

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