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(Myopathies, Cardiomyopathies and Neuromyopathies)

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Founders: Giovanni Nigro and Lucia Ines Comi

Three-monthly

G. CONTE ACADEMY - NAPOLI

PACINI EDITORE MEDICINA

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EDITORIAL

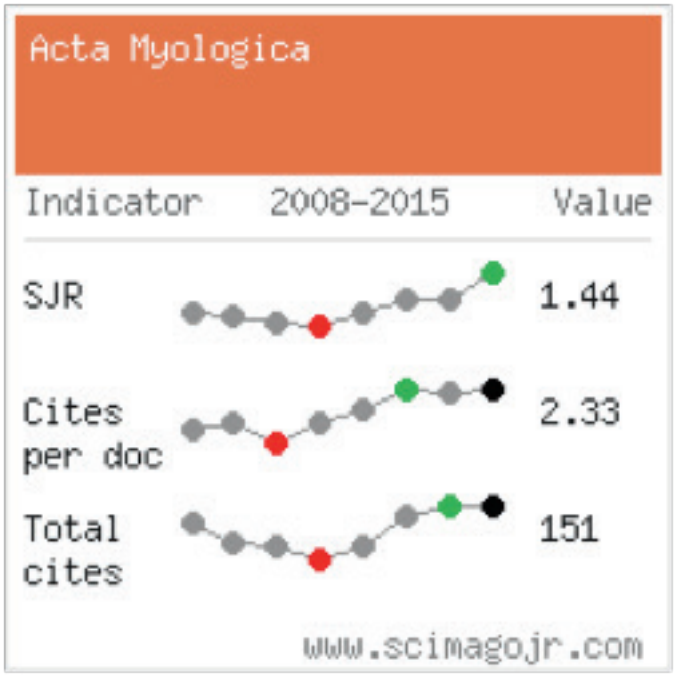
AMO (*Acta Myologica Online*) – the official journal of the Mediterranean Society of Myology and of the Associazione Italiana di Miologia (AIM) – celebrates its first year with the new process of electronic submission, fast reviewing and a completely free web-based access.

During the last years the Journal has increased the number of its citations (see Figure 1) and according to the rating of the *Scimago Journal & Country Rank* it should have a H Index of 22.

So the Board of the Journal decided to increase the number of issues per year passing from the current three issues per year to four issues to be published in March, June, September and December respectively.

Guest Editors for special issues focusing on the most recent development on the field of neuromuscular disorders are welcome. We invite the members of both scientific Societies and the followers to support us in this initiative.

Giovanni Nigro
Editor-in-Chief of Acta Myologica



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ORIGINAL ARTICLES

^{23}Na MRI and myometry to compare eplerenone vs. glucocorticoid treatment in Duchenne dystrophy

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In this pilot study we tested whether a low dose application of a mild diuretic substance such as eplerenone is beneficial in early stages of Duchenne muscular dystrophy using ^{23}Na und ^{1}H imaging, myometry, and clinical testing versus the glucocorticoid gold standard.

Two 7-years old patients with DMD were examined on a 3T MRI system. ^{1}H MRI and ^{23}Na density-adapted 3-dimensional radial MRI sequences were performed both before and 1, 3 and 6 months after therapy with eplerenone respectively cortisone. We quantified fatty infiltration on T1-weighted images using subcutaneous fat as reference and fat fraction with a two-point DIXON sequence. Muscle oedema was quantified on STIR images and DIXON water maps with background noise as reference. We quantified Na^+ by a muscular tissue concentration sequence with a 51.3mM Na^+ with 5% agarose reference tube. A Na^+ IR-sequence was used for determination of mainly myoplasmic Na^+. Correspondingly myometry of muscles and tendons were assessed. Clinical tests (i.e. 4-steps-test) and blood counts (i.e. K^+) were done by a pediatrician.

Under eplerenone therapy we detected a reduction of muscular oedema, intracellular-weighted sodium IR signal and muscular sodium concentration. The oedema reduction in the DMD patient receiving eplerenone was more pronounced to the patient with cortisone. Myometric-measured tissue parameters such as muscle stiffness had a more pronounced effect in the child treated with eplerenone after a first increase in muscle stiffness both after eplerenone and cortisone treatment. Clinical abilities during both therapies were mostly constant.

Eplerenone might be a possible new therapy option in DMD patients.

Key words: Duchenne, eplerenone, ^{23}Na MRI

Introduction

The progressive Duchenne muscular dystrophy is the most frequent and a severe muscular disease. Due to recessive x-linked inheritance boys are nearly solely affected. At birth symptoms are typically not present. At the time of school enrollment they present motor awkwardness. Subsequently, precarious walk, frequent falls, the phenomenon of GOWERS (this means that a patient has to use his hands and even arms to erect his own body from a squatting position) and the shamble walk indicate a muscular dysfunction. The loss of ability to walk usually appears at the age of 10. In the following wheelchair period muscular contractures of hip and knee muscles and scolioses evolve. A restrictive ventilation disorder as a result of muscular weakness and scoliosis is today treated by constant artificial ventilation and operative erection of the spine. Later on, cardiomyopathy will appear.

Present-day drug therapy, according to the current guidelines, consists of glucocorticoids which effect a light delay of muscular dystrophy. As glucocorticoids in the long-term cause grave adverse effects as osteoporosis, weight increase and even cortisone-induced (cardio-)myopathy, we attempt to test whether mineralocorticoids (without the previous named adverse effects) are more adequate in treatment of Duchenne muscular dystrophy. We have used eplerenone in this study, which is a specific antagonist of aldosterone and promote leachate of sodium and water out of muscle cells (1). That refers especially to the fact that

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the cytoplasm of DMD cells accumulate sodium and water even before the degeneration of the cell (1-3).

The missing dystrophin as a linker protein between cell membrane and contractile proteins causes major disturbance of the cellular mechanotransduction and overall gene expression of components of the extracellular matrix. Proteomic approaches demonstrate drastic increase of extracellular matrix proteins and cytoskeletal proteins leading to fibrosis (4). It has been demonstrated that eplerenone attenuates fibrosis of heart, vessels and liver (5).

Recently, Raman et al. (6) showed that low dose application of eplerenone is beneficial for early cardiomyopathy in Duchenne muscular dystrophy by preservation of ejection fraction and sustain left ventricular systolic function. A recent observation of a 22-years old Duchenne patient reported that eplerenone application had improved muscle strength and mobility (1).

Thus, we want to assess in a pilot study whether low dose application of eplerenone can be beneficial in early stages of Duchenne muscular dystrophy using recent imaging, clinical and tissue measurement techniques.

Patients and methods

Patients

This individual drug treatment was conducted according to the Declaration of Helsinki in the present form. Written consent to treatment with eplerenone was obtained from all study subjects and their parents. Two boys with genetically proven DMD (one with eplerenone treatment (25 mg/day), one with glucocorticoid treatment (deflazacort, 0.9 mg/kg/day), all 7-years old were included. As control, two boys with DMD but without any treatment (same age, both 7-years old) who have already been examined with MRI twice before [data shown in (3) are mentioned in Figure 1; they underwent in addition to the data shown in (3) subsequent additional MRI examinations in a 3-year time (first patient) and 7-month time (second patient)].

Patient examination protocol

The two boys of the first study arm (i.e. following glucocorticoid or eplerenone treatment underwent 3 Tesla ^{1}H and ^{23}Na imaging of both lower legs, myometry and clinical examination at 4 different time points (before therapy as well as 1, 3 and 6 months after respective therapy).

MRI protocol

^{1}H and ^{23}Na MRI of both calves were performed on a 3 Tesla clinical MR system (MAGNETOM Trio, Siemens, Erlangen, Germany) using a CE certified double-resonant birdcage coil (32.6 MHz/123.2 MHz, Rapid Biomed Inc., Würzburg, Germany). All patients tolerated the entire MRI examination well. The imaging protocol included axial T1-weighted turbo spin echo (for the dectection of fatty muscular degeneration) followed by axial short-tau inversion recovery (STIR) ^{1}H MR sequences (for the identifaction of muscular oedema). Additionally, muscular fat fraction was measured using a two-point DIXON sequence. Sodium (^{23}Na) imaging was performed using two ^{23}Na pulse sequences based on a density-adapted three dimensional (3D) radial sequence (7). The first sequence (spin-density image contrast TE/TR = 0.3/100 ms, $\alpha = 90°$; voxel size of 5 x 5 x 5 mm^3; acquisition time (TA) = 8min 20s) assessed a spin density image contrast and was used to quantify the muscular tissue Na^+ concentration; the second one (TE/TR = 0.3/124ms; TI = 34ms, voxel size 6 x 6 x 6 mm^3; TA = 10min 20s) – an inversion recovery sequence - suppressed the Na^+ signal of free Na^+ ions (e.g. Na^+ in saline solution) and, therefore, shifting the weighing towards the intracellular compartment (8). At follow-up, the calves were positioned at exactly the same position using the knee joint space and the coil borders as reference and by using additional skin markers. The calves were well fixed in this position (in the coil) using foam plastic. Also, all examinations were performed by the same technician.

Analysis of the ^{23}Na and ^{1}H MR imaging data

A radiologist with 3 years of experience in musculoskeletal imaging set in consensus with a senior musculoskeletal radiologist the positioning of the regions of interest (ROIs) on 4 different muscle compartments (anterior muscle compartment, peronaeus compartment, soleus compartment, deep posterior compartment) in the ^{23}Na images on the lower legs of the examined patients (Fig. 2). The radiologists were blinded to the final treatment of the patients. The ^{1}H imaging data served as reference. Additionally, two reference tubes were assessed with ROIs as described before (8). The first control phantom was filled with 51.3 mM NaCl solution to counterfeit unrestricted Na^+, the other phantom used 51.3 mM NaCl in 5% agarose to mimic Na^+ with restricted mobility. ^{23}Na signals were normalized by dividing Na^+ values of different muscles with signal intensity in the existing Na^+ agarose control phantoms. Due to the fact that the reference tube with free NaCl solution was suppressed in the IR sequence, the tube containg NaCl in 5% agarose gel was used for normalization.

Fatty infiltration was measured using a ^{1}H T1w Ratio ($ROI_{muscle}/ROI_{subcutaneus\ fat}$) of the soleus muscle (Fig.2), fat fraction was measured using ^{1}H DIXON of the soleus muscle (ROIs were set on water (w) and fat (f) images and followed by the calculation of the fat fraction ff = f/

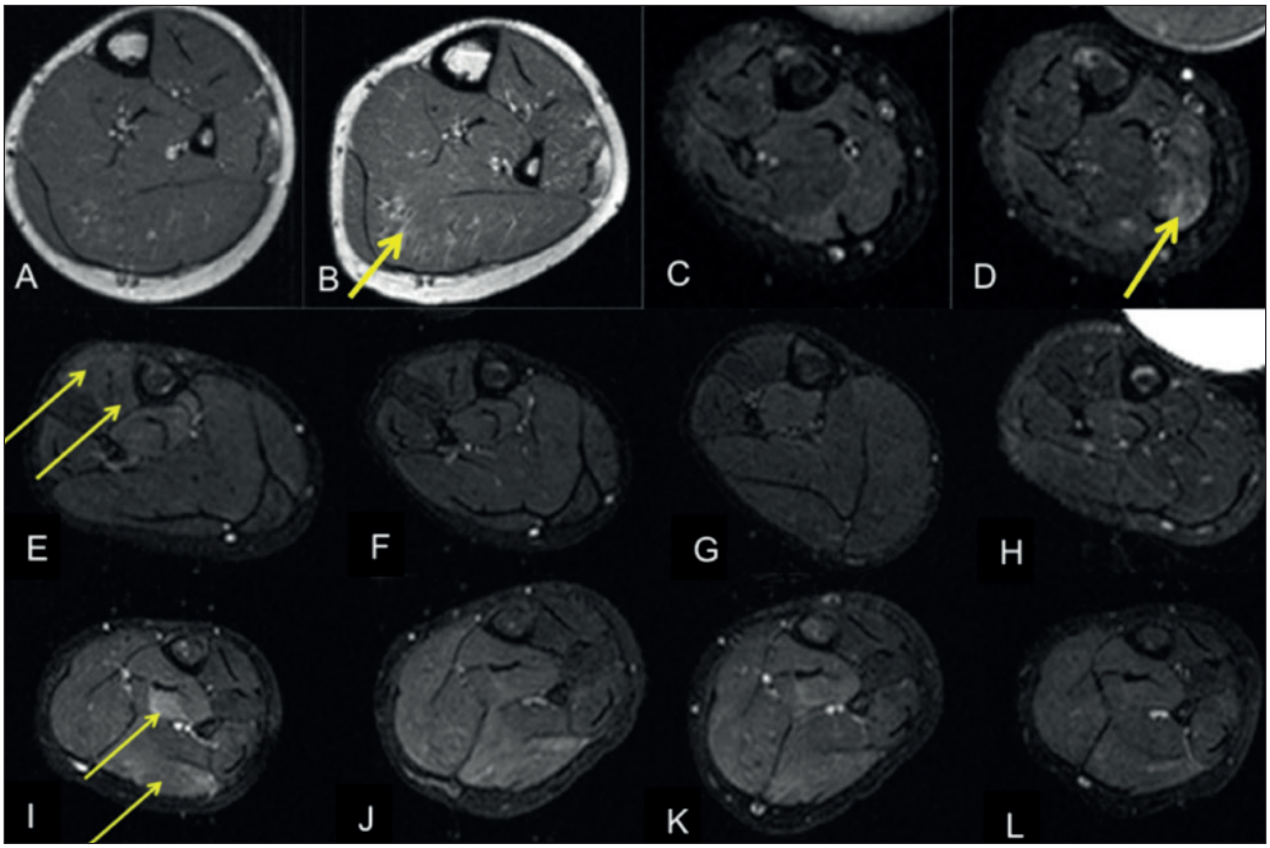

Figure 1. MRI of calves of four 7-years old boys with DMD. T1-w (A-B), short tau inversion recovery (STIR, C-L). Development of fatty muscular infiltration (T1-w) and muscular oedema (STIR). Two DMD patients (A-D) without therapy at any time in 3 years' (first patient without therapy, A-B) and 7 months' time period (second patient without therapy, C-D). DMD patient before (E) and 1,3 and 6 months (F-H) after therapy with glucocorticoids (0,9mg Deflazacort/kg/day). DMD patient before (I) and 1,3 and 6 months (J-L) after therapy with eplerenone (25mg/day). Increasing fatty degeneration of the soleus muscle (A-B) and increasing oedema-like changes in the medial gastrocnemius muscle (C-D) in two patients without therapy. Prior to therapy, oedema-like changes especially in the anterior compartment (E; afterwards glucocorticoid treatment) respectively deep posterior compartment and medial gastrocnemius muscle (I, afterwards treatment with eplerenone). Following therapy with glucocorticoids (standard) and eplerenone, there are decreasing oedema-like changes.

(f+w)). Additionally, ^{1}H STIR imaging and ^{1}H DIXON water maps were used for evaluation of oedematous changes. Therefore the extent of muscular oedema could be normalized each by using the backround signal ($ROI_{muscle}/ROI_{backround\ noise}$).

Myometry/ Assessment of muscle viscoelastic properties

In addition to imaging data, biomechanical and viscoelastic parameters of muscles and tendons were assessed by a recently introduced handheld indentometer called MyotonPRO® (Myoton Ltd, London). This device measures the damping of a mechanical impulse of 0.5 N at the surface of the skin in the first 400 ms after impulse. From these oscillation curves, the following tissue parameters were calculated: the tissue tone (frequency (Hz)), the tissue stiffness (N/m), decrement as parameter for the elastic stiffness of the tissue and viscoelastic parameters like relaxation time (in ms) and creep as nonelastic tissue strain. Children were measured in a relaxed laying position. Three measurement sets of 10 measurements were performed on the left and right side for each position. Three measurements (mean of 10 taps each) were taken consecutively on the same position. For the skeletal muscles, the lateral M. gastrocnemius and upper M. trapezius (pars transversa) muscle belly were chosen as measurement position. The thoracolumbar fascia was measured at the level of the iliac crest, 3 cm lateral to the posterior median line at both sides. The Achilles tendon was assessed 4 cm proximal to the insertion of the Achilles tendon into the calcaneus. All aforementioned points were measured in prone position at

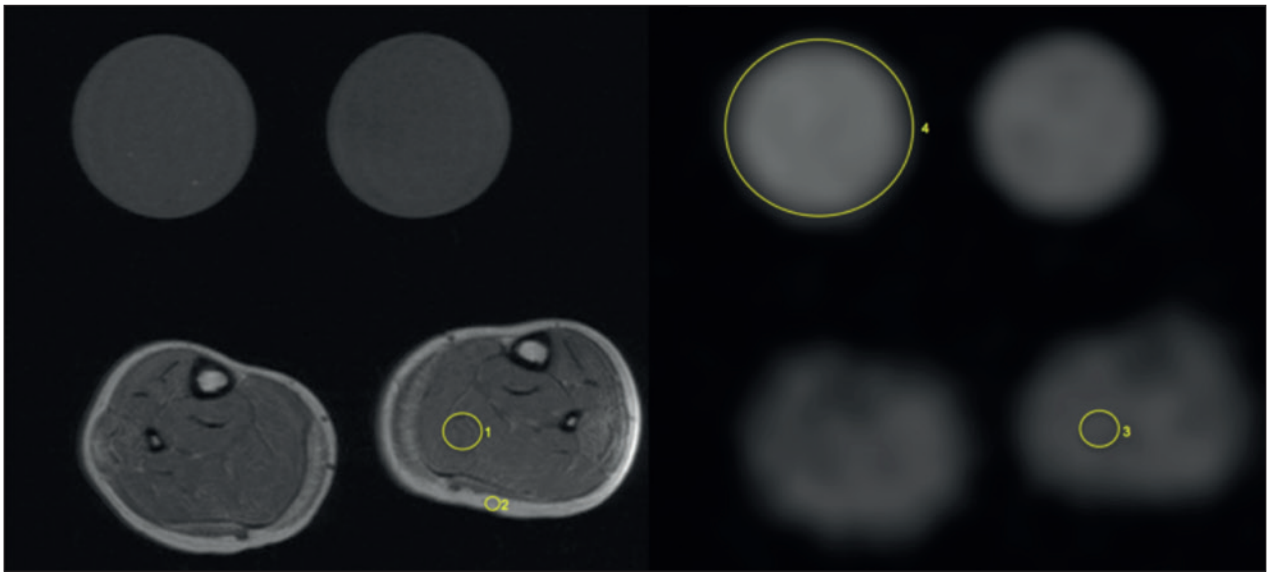

Figure 2. Analysis of ^{1}H MR and ^{23}Na image data, DMD patient, exemplary measurements. ^{1}H-T1w (left) and ^{23}Na-MR image (spin density image contrast, no inversion recovery); reference tubes (in each image): right-hand side of the patient (51.3 mM NaCl in 5% agarose, mentioned as 4) and left-hand side (51.3mM NaCl solution) reference tube. Exemplary measurement of fatty infiltration using a ^{1}H T1w Ratio ($ROI_{muscle}/ROI_{subcutaneus\ fat}$) of the left soleus muscle (ROI 1/ROI 2 in left image); accordingly measurement of muscular sodium concentration using a ^{23}Na-MR sequence (no IR, ROI of Na^+ value of respective muscle / ROI of signal intensity in the existing Na^+ agarose control phantom) within the left soleus muscle (ROI 3/ROI 4 in right image).

both sides, except the M. trapezius that was measured in upright sitting position. A total of 30 measurements per position were recorded over 4 testing sessions before therapy and after 1, 3, and 6 months of drug administration, respectively, one year before the eplerenone administration. For the boy receiving eplerenone, the measurements were not performed directly before eplerenone intake.

Clinical examination

The examination comprised the measurement of blood pressure, body weight and several blood parameters (Na^+, K^+, Ca^{2+}, TSH, CK, creatinine, carbamide, phosphate, cholesterine, triglycerids, LDL- and HDL-cholesterine, lipoprotein A and vitamin D). Muscle endurance and functional abilities were assessed by an pediatrician with a 10 meter walking and a 4-steps-test followed by tests for the functional evaluation of going up (from sitting on chair, lying and sitting on floor). The examiner was blinded to the treatment scheme of the patients.

Results

Patients without therapy

Two seven-year-old boys with genetically proven DMD underwent no therapy in our observation period. The first boy showed increasing fatty degeneration of the left soleus muscle on ^{1}H T1w images in a time-period of 3 years, while the second boy presented with increased muscular oedema in the the right medial gastrocnemius muscle during the 7 months' period.

Effect of therapy with eplerenone

^{23}Na sodium and ^{1}H MR imaging

Table 1 shows that ^{23}Na IR was reduced in every muscle compartment after six months of therapy with eplerenone. Correspondingly, the muscular sodium concentration was reduced in every measured compartment, too. The reduction ranged between -17% (soleus muscle compartment) and -23% (anterior muscle compartment) in correlation with the first value before treatment (Fig. 3). The fatty infiltration remained constant with a mean ratio of 0.44 before and after six months of therapy with eplerenone. Equally, the fat fraction measured by the DIXON sequence showed no distinct change with a mean fat fraction of 0.14 and 0.14 six months later. The highest decrease of the STIR ratio was detected in the deep posterior compartment (25.9 before therapy, 13.3 after six months of therapy resulting in a reduction of muscular water content of 48%) followed by the soleus compartment (22.5 before therapy, 15.1 six months after therapy, oedema reduction of 33%). Both the anterior muscle and

			Before treatment	1 month	3 months	6 months	% change[1]
Eplerenone intake	^{23}Na IR signal	Soleus muscle comp.	0.60	0.61	0.59	0.58	-3%
		Deep posterior muscle comp.	0.58	0.57	0.54	0.49	-16%
		Anterior muscle comp.	0.54	0.49	0.50	0.43	-20%
		Peroneus muscle comp.	0.54	0.50	0.48	0.45	-17%
	Muscular Na+ concentration (mmol/l)	Soleus muscle comp.	37.0	35.5	34.3	30.6	-17%
		Deep posterior muscle comp.	37.7	33.2	32.4	30.6	-19%
		Anterior muscle comp.	32.0	29.1	26.4	24.6	-23%
		Peroneus muscle comp.	33.1	29.1	28.2	26.1	-21%
	Normalized DIXON water mapping	Soleus muscle comp.	38.4	37.2	37.0	34.4	-10%
		Deep posterior muscle comp.	38.1	34.4	35.0	29.6	-22%
		Anterior muscle comp.	35.1	34.7	34.4	31.6	-10%
		Peroneus muscle comp.	30.8	27.8	27.2	26.3	-15%
	Fatty infiltration[2]	Soleus muscle comp.	0.44	0.44	0.44	0.44	~constant
	Fat fraction[3]	Soleus muscle comp.	0.14	0.15	0.14	0.14	~constant
Gluco-corticoid intake	^{23}Na IR signal	Soleus muscle comp.	0.71	0.66	0.65	0.66	-7%
		Deep posterior muscle comp.	0.67	0.55	0.61	0.51	-24%
		Anterior muscle comp.	0.68	0.51	0.61	0.47	-31%
		Peroneus muscle comp.	0.62	0.53	0.61	0.52	-16%
	Muscular Na+ concentration (mmol/l)	Soleus muscle comp.	43.2	40.5	36.5	34.1	-21%
		Deep posterior muscle comp.	36.5	39.8	36.7	31.6	-13%
		Anterior muscle comp.	37.9	30.7	29.0	28.5	-25%
		Peroneus muscle comp.	37.3	26.9	31.3	26.0	-30%
	Normalized DIXON water mapping	Soleus muscle comp.	39.9	39.7	38.7	36.8	-8%
		Deep posterior muscle comp.	35.2	38.7	33.7	35.2	~constant
		Anterior muscle comp.	38.4	37.1	33.9	31.8	-17%
		Peroneus muscle comp.	32.8	33.3	31.3	36.8	+12%
	Fatty infiltration[2]	Soleus muscle comp.	0.43	0.44	0.44	0.44	~constant
	Fat fraction[3]	Soleus muscle comp.	0.10	0.10	0.10	0.11	~constant

1 Calculated as % change before treatment to 6 months
2 (T1w Ratio)
3 F/(F+W)) DIXON

Table 1. Treatment effects of two 7-years old DMD boys under eplerenone and glucocorticoid treatment, respectively. There is a decrease of the 23Na IR signal and the muscular sodium concentration following both eplerenone and glucocorticoid treatment. The reduction of muscular water content (measured by the DIXON water mapping) was more pronounced in the boy with eplerenone treatment (especially in deep posterior muscle compartment and peroneus muscle compartment). A slighter change has been found for glucocorticoid treatment. Both the degree of fatty degeneration and the fat fraction (measured within the soleus muscle) remains constant over the 6 months' period.

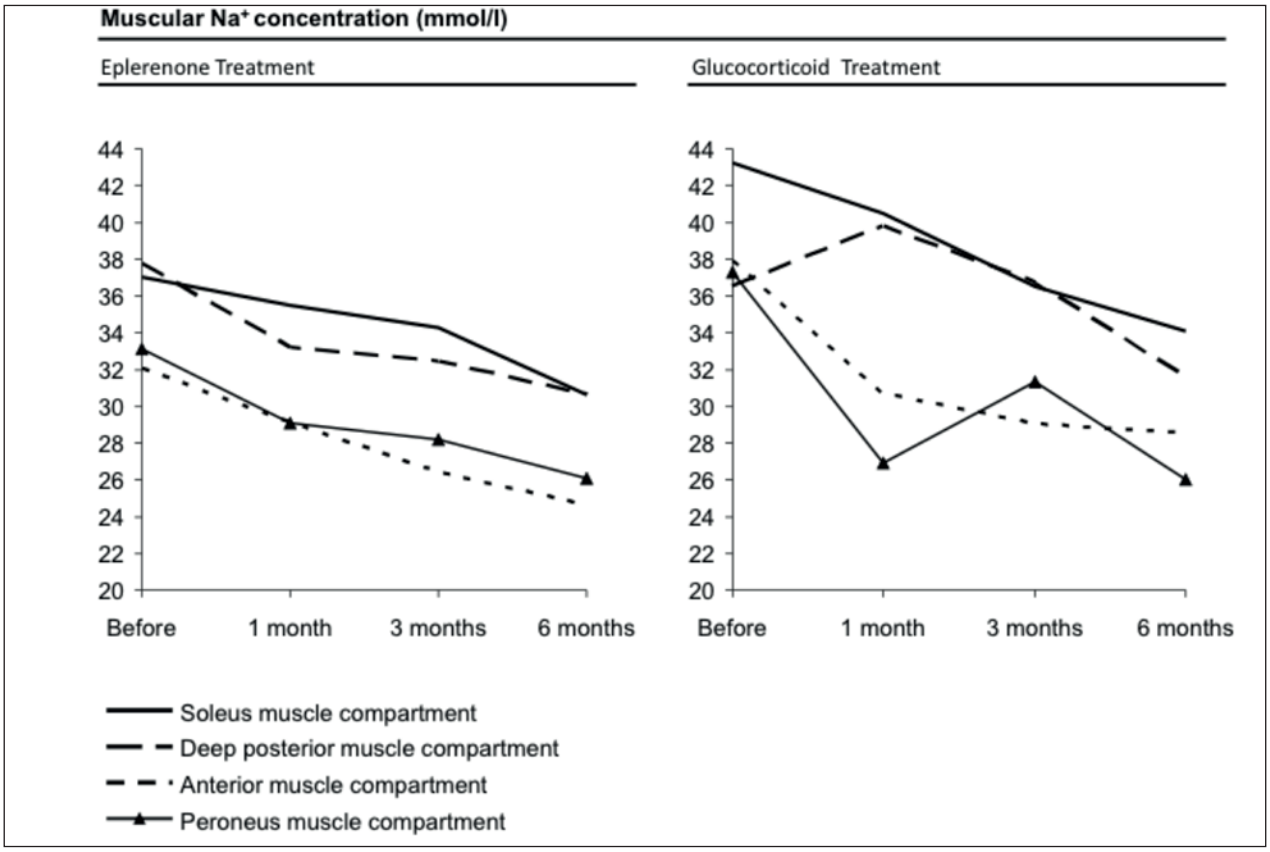

Figure 3. Illustration of declining muscular sodium concentration of four different muscle compartments comparing eplerenone vs. cortisone treatment in a graph according to Table 1. Both therapies showed a distinct effect.

peronaeus muscle compartment showed both an oedema reduction of 21% (decrease of the STIR ratio from 11.4 to 9 six months after therapy) as mentioned in Figure 4. As depicted in Table 1, the highest decrease of the normalized DIXON water mapping was detected in the deep posterior compartment (38.1 before therapy, 29.6 after six months of therapy resulting in a reduction of muscular water content of 22%) followed by the peronaeus compartment (30.8 before therapy, 26.3 after six months of therapy, reduction of muscular water content of 15%). Both the anterior and the soleus muscle compartment showed an reduction of muscular water content of 10% after six months of therapy.

Myometry

Table 2 illustrates the stiffness measurements at different time points. The boy who received eplerenone medication was first measured before drug administration (approximately one year before drug treatment) at the age of 6, and presented with 273 N/m within the gastrocnemius muscle (left) and 180 N/m within the trapezius muscle (left) an age-typical muscle stiffness at rest. In the following first measurement after eplerenone intake, the stiffness increased drastically to 355 N/m within the gastrocnemius muscle (left) and 426 N/m of the trapezius muscle (left) to values equivalent to those of healthy adults. Three month after eplerenone intake, the muscle stiffness was much lower measured with 288 N/m for the gastrocnemius muscle (left) and 287 N/m for the trapezius muscle (left). Other muscle groups showed the same effect; the deltoideus and the femoralis muscle, for example, showed a reduced stiffness of about 10% (data not shown). After 6 months (at the age of 8 years) of eplerenone intake, the muscle stiffness consolidated. Concerning the stiffness parameters under eplerenone treatment, the viscoelastic properties of the muscles were in a similar range compared to two years before at the age of 6. It is likely that this resembles a reduction of tissue fibrosis after an initial increase of stiffness after starting therapy. For the Achilles tendon and the lumbar fascia on both sides, there was a reduction of stiffness of about 16-25% measurable (Table 2, change 1 month to six month after therapy). Regarding tendon tissue, we observed a decrease of tissue stiffness corresponding to the results observed within the skeletal muscles.

		Stiffness (N/m)							
		M. gastrocnemius		M. trapezius		Thoracolumbar fascia		Achilles tendon	
		Left	*Right*	*Left*	*Right*	*Left*	*Right*	*Left*	*Right*
Eplerenone intake	Before treatment	273	n.d.	180	n.d.	304	n.d.	n.d.	n.d.
	1 month	355	340	426	487	307	329	510	531
	3 months	288	248	287	344	294	288	602	n.d.
	6 months	273	271	321	240	259	263	382	401
	% change *(1 month to 6 months)*	-23%	-20%	-25%	-51%	-16%	-20%	-25%	-24%
Glucocorticoid intake	Before treatment	261	238	478	289	354	341	498	621
	1 month	329	357	411	380	287	249	310	412
	3 months	223	243	483	339	310	350	426	579
	6 months	263	209	371	355	275	297	544	541
	% change *(1 month to 6 months)*	-20%	-41%	-10%	-7%	-4%	+19%	+75%	+31%

Table 2. Illustration of myometry: Myometric changes in stiffness of muscles and tendons were quantitatively assessed as described in the methods' section.

Clinical examination

The clinical tests that were part of the clinical protocol for every visit before and under therapy showed no obvious change under therapy (Fig. 5). It can be summarized that no negative clinical effect was observed by the eplerenone treatment, especially no hyperkalaemia. Also, no distinct change in the blood counts or the blood pressure was noted and no abnormal weight increase over time occurred.

Effect of therapy with glucocorticoids

^{23}Na sodium and ^{1}H MR imaging

Table 1 shows that the ^{23}Na IR signal is reduced in every muscle compartment following six months of therapy; the same effect has been also observed with eplerenone. Correspondingly, muscular sodium concentrations decreased between -13% (deep posterior muscle compartment) and -30% (peroneus muscle compartment) in comparison with the first value before treatment (Fig. 3). The fatty infiltration remains, as also observed with eplerenone treatment, constant with 0.43 before and 0.44 after six months after therapy with glucocorticoids. Moreover, the fat fraction measured by the DIXON sequence showed no distinct change with 0.10 and 0.11 six months later. The STIR ratio was calculated for four different muscle compartments. The highest decrease of ^{1}H STIR ratio was detected in the anterior muscle compartment (9.5 before therapy, 7.3 six months after therapy with glucocorticoids, oedema reduction: 23%) followed by the soleus compartment (9.3 before therapy, 7.7 after six months of therapy, oedema reduction: 17%). Whereas the deep posterior muscle compartment showed only a slight decrease with 2% oedema reduction (9.8 versus 9.6 six months later), the STIR ratio of the peroneus muscle compartment was slightly increased under cortisone treatment (increase: 6%) as mentioned in Figure 4.

As depicted in Table 1, the highest decrease of the normalized DIXON water mapping was detected in the anterior compartment (38.4 before therapy, 31.8 after six months of therapy resulting in a reduction of muscular water content of 17%) followed by the soleus compartment (39.9 before therapy, 36.8 after therapy, reduction of muscular water content of 8%). Whereas the deep posterior muscle compartment showed an undulant pattern with no measurable change after six months of therapy. The normalized DIXON water mapping of the peroneus muscle compartment was slightly increased under cortisone treatment (muscular water content plus 12%).

Myometry

The tissue measurements for the child with glucocorticoid intake varied to some extent between the measur-

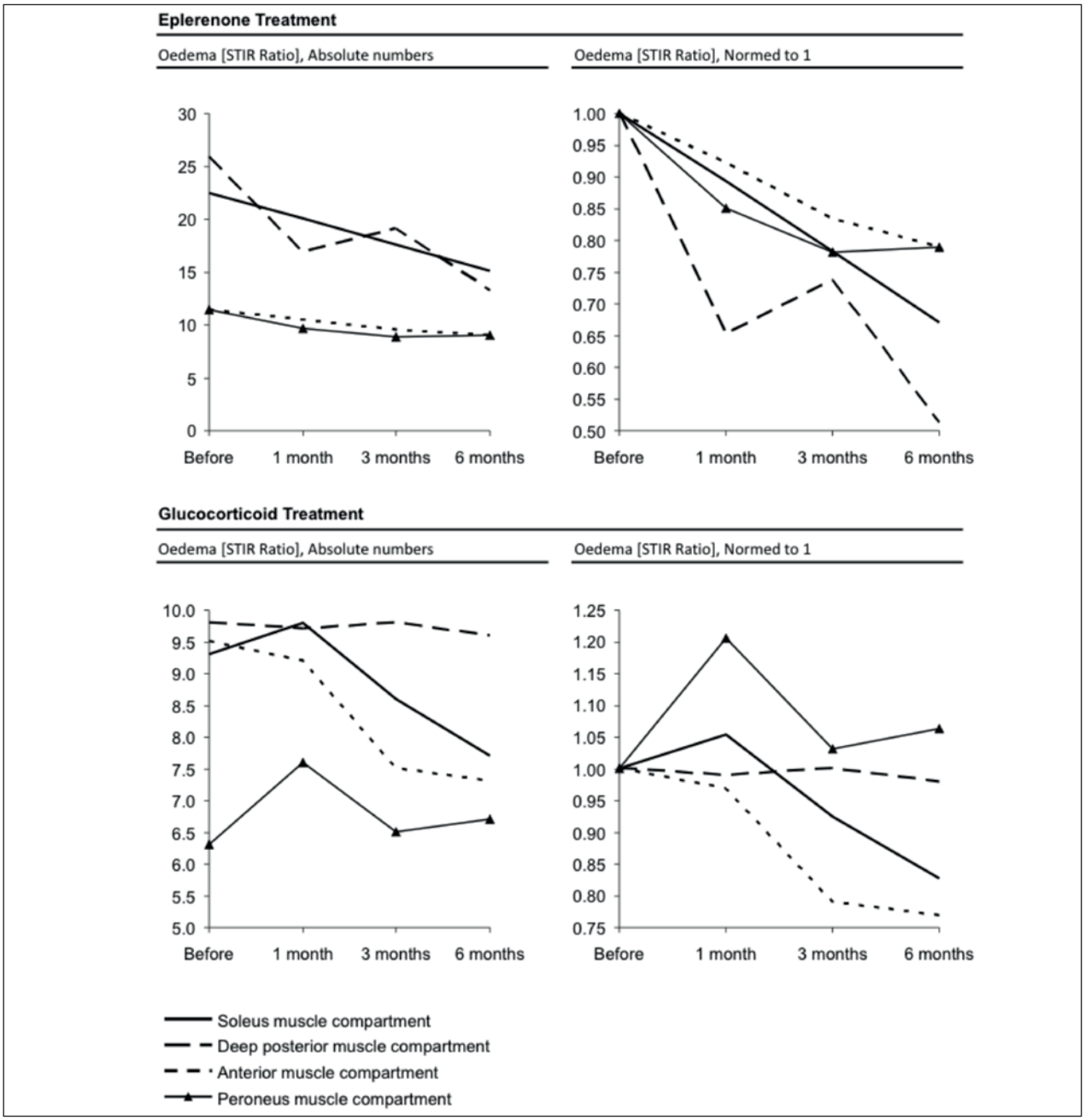

Figure 4. ^{1}H STIR Imaging: Illustration of the STIR ratio of four different muscle compartments comparing eplerenone vs. cortisone treatment. Symbols for the respective compartment as given in the legend of the figure. The oedema reduction for the patient following eplerenone treatment showed a higher oedema reduction especially in deep posterior and soleus muscle compartment. A slighter decrease is also shown for the patient receiving cortisone treatment (as showed in figures normed to 1).

ing intervals. The gastrocnemius muscle had a stiffness of 261 N/m on the left and 238N/m on the right side before glucocorticoid intake. After an initial increase of muscle stiffness after starting therapy, the right gastrocnemius muscle showed a stiffness decrease after six months of corticoid therapy (reduction of 20%; change 1 month to six months as mentioned in table 2), the left side showed even a more distinct effect (reduction of 41%, change 1 month to six months) after initial increase. The reduction in stiffness of both trapezius muscles was smaller (10%

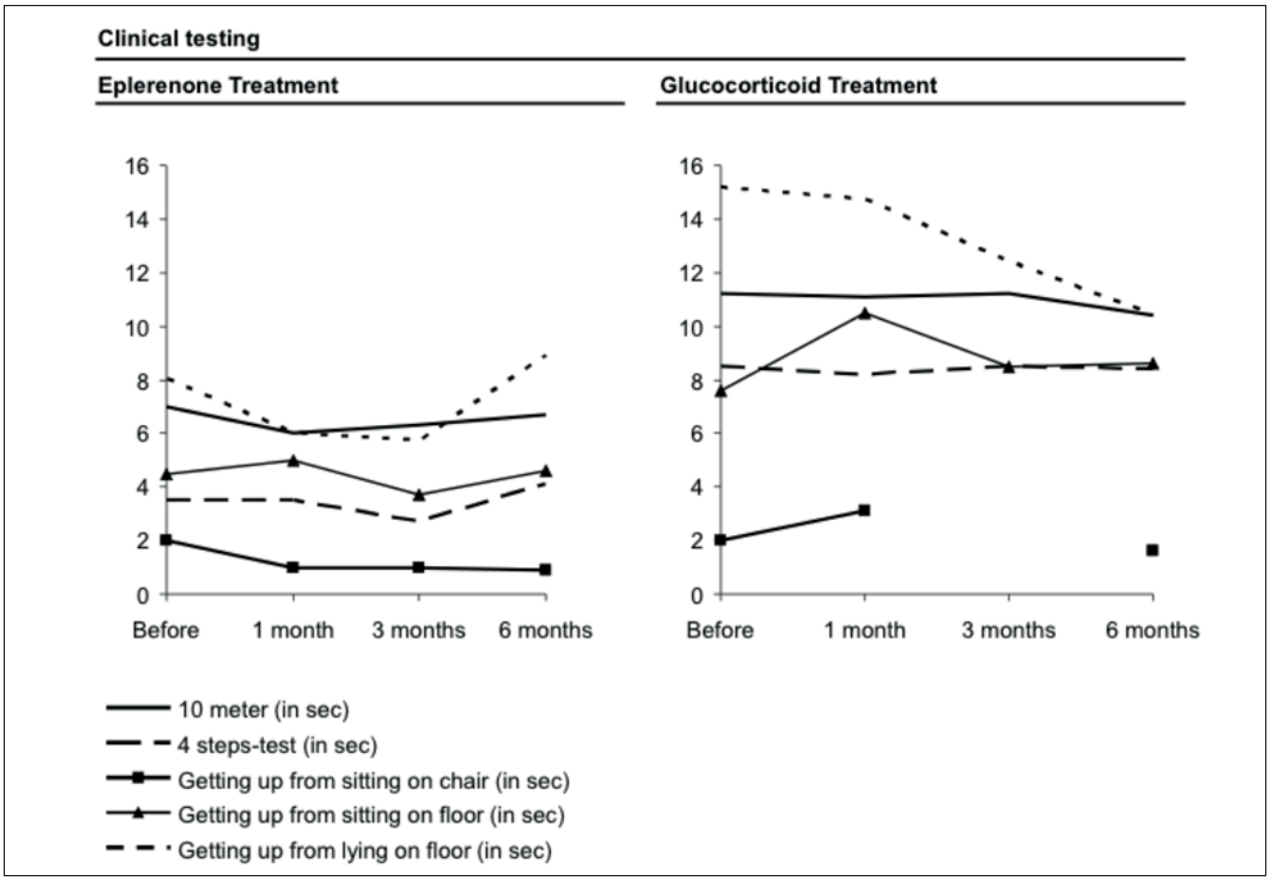

Figure 5. Clinical testing (i.e. walking of 10 meters, 4-steps-test) for evaluation of muscle endurance and muscular abilities following the respective therapy. Both therapies (eplerenone and cortisone) showed no distinct changes in test results over a time-period of six months.

left and 7% right, change 1 month to six months) after initial increase. For the trapezius muscle we noted at the beginning a remarkable asymmetric stiffness 478 N/m and 289 N/m between the left and the right side probably as a consequence of the progression of the muscle dystrophy. Tendon stiffness showed no initial increase in thoracolumbar fascia and Achilles tendon after starting therapy but varied between a slight decrease (left thoracolumbar fascia, change 1 month to six months -4%) and stiffness increase between +19% and +75% (right thoracolumbar fascia, left Achilles tendon). Of note, the reduced stiffness is still untypically high for children of this age. In summary, we found considerably more heterogeneous data for the boy with glucocorticoid intake.

Clinical examination

No changes of the clinical abilities under cortisone treatment were observed.

Discussion

In our pilot study, two 7-years old children were monitored (^{23}Na and ^{1}H MRI, myometry, clinical tests, blood counts for the child treated with eplerenone) under treatment with eplerenone, respectively, cortisone for a time period of six months after starting therapy. In both patients we detected a reduction of muscular oedema, intracellular-weighted sodium IR signal and muscular sodium concentration (Fig. 3). The oedema reduction in the DMD patient under eplerenone treatment was more pronounced than the reduction observed in the DMD patient under glucocorticoid treatment (in due consideration of oedema reduction, Figs. 2 and 4 and Table 1). Similarly, myometric-measured tissue parameters such as muscle stiffness had a more pronounced effect in the child treated with eplerenone. Clinical abilities of both children during therapy were mostly constant.

It is common practice to start a drug therapy (the current gold standard is cortisone despite adverse effects like osteoporosis, weight increase and corticoid myopa-

thy) when first clinical and motoric limitations appear. Unfortunately, at this timepoint, oedema-induced cytotoxic and consecutive fibrotic tissue remodelling of DMD muscle or brain cells already have taken place. Hirn et al. showed that deregulation of voltage gated $Na_{V1.4}$ channels in dystrophic muscle cells of mdx mice led to massive influx of sodium in the cytoplasmatic compartment and consequently cell death. The effect could be reversed by a specific blocking of $Na_{V1.4}$ channels with tetrodotoxin (9). Even more, the nNOS (neuronal nitric oxide synthases, catalyze the production of NO from L-Arginin) signalling pathway as a modifier of dystrophic pathology seems to play a major role in Duchenne muscular dystrophy. Froehner et al. showed that in DMD nNOS&#946 and nNOS&#956 are mislocalized, less expressed and, thus, less functional (10). Impaired NO-triggered vasodilation of affected muscles could lead to a worse blood perfusion and, finally, to degeneration of muscle (11). Zhou et al. (12) demonstrated that a lack of NOS in cardiac muscle cells didn't interrupt the protein expression of Na^+/K^+-ATPase and Ca^{2+}-ATPase, but significantly altered activity and, therefore, function of both above-mentioned ATPases leading to a cellular sodium disequilibrium.

In this context, a new paradigm for pathogenetic mechanisms in Duchenne was shaped in focus of a osmotic cytoplasmic sodium elevation causing intracellular and mainly osmotic muscular oedema (2, 3). Therefore, lowering the overall sodium concentration in the body by a mild diuretic substance could be a reasonable approach to diminish the pathogenic effect. An anti-oedematous effect of cortisone was detected and described previously (2). In a severe case of Duchenne, a 22-years old woman benefits remarkably from eplerenone treatment and gained mobility again (1). Regarding the heart, eplerenone application showed positive effects in preservation of ejection fraction and left ventricular systolic function (6). Requisite for the development of early starting therapy concepts is an adequate measurement technique to quantify tissue quality. In our preliminary study, this was already possible at the age of 7 years. We could verify tissue improvements in our pilot study under drug therapy (eplerenone and cortisone). DMD children without treatment showed increased muscular oedema and fatty degeneration over time. Fatty degeneration using ^{1}H T1-weighted and DIXON sequences capped equal in a 6 month's period under drug treatment. On the cellular level the lack of dystrophin leads to a reduced mechanotransduction and major signalling pathways (nNOS) and causes an increase of oxidative stress levels. Under mechanical stress cations mainly Ca^{2+} and Na^+ can enter the cell. This intracellular increase of sodium leads to an osmotic pressure that results in oedema formation that we detected in ^{1}H STIR imaging and reconfirmed in the DIXON water mapping. Ca^{2+} influx, even to a small extent, does change calcium modulated several signalling pathways leading for example to apoptotic degeneration (13) (Fig. 6). In the end, massive oxidative stress leads to cell necrosis (14). Therefore, antiinflammatory nutrition may have an beneficial antidegenerative effect in DMD (15). In this context, the role of utrophin as a dystrophin homologue and as a scaffolding protein that stabilizes lipid microdomains and clusters channel subunits may be considered. Compensatory upregulation of utrophin in mdx mice only leads to mild forms of Duchenne muscular dystrophy (16). Fibrosis goes along with stiffening of tissue (muscle, tendon, connective tissue) and is characterized by massive deposition of collagen in the extracellular matrix (17). Transforming growth factor ß1 is one component that induces the differentiation of fibroblasts into collagen-producing myofibroblasts, the major collagen-producing cells of the extracellular matrix (18). We assume that the measured stiffness reduction upon eplerenone treatment is due to reduction of fibrosis. Concerning the stiffness parameters under eplerenone treatment, the viscoelastic properties of the muscles were in a similar range as two years before at the age of 6. It is likely that this resembles a reduction of tissue fibrosis after an initial increase in stiffness after starting therapy. The tissue measurements for the child with glucocorticoid intake vary to some extent between the measuring intervals and showed slightly more heterogeneous data (change 1 month to six months after starting therapy) between decrease (muscles, left thoracolumbar fascia) and increase (right thoracolumbar fascia, Achilles tendon). This can be due to a different degree of Duchenne pathology, medication or even tissue type susceptibility, like it is found for hypermobile individuals. For the child under eplerenone application, we noted a reduction in tissue stiffness between 20% and 51% for skeletal muscles and 16% and 25% for tendons (change 1 month to six months after therapy). Van den Hoorn et al. showed pain effects on gait stability underlining the biomechanical importance of reducing hypertrophy of calf muscles in early stages of DMD patients (19).

The clinical testing showed no clear tendency in our pilot study (Fig. 5). This could be due to the fact that a six month's period was too short to show clinical manifestations or would have needed more extensive testing, for instance testing for changes of endurance at this stage of DMD manifestation. The low level eplerenone medication had no influence of the blood potassium level. For preclinical and clinical studies, sodium (^{23}Na) MRI and myometry may have the potential to serve as adequate techniques for evaluating especially early stages of Duchenne muscular dystrophy. In clinical practice, it may be helpful in case clinicians could assess a tissue fibrotic

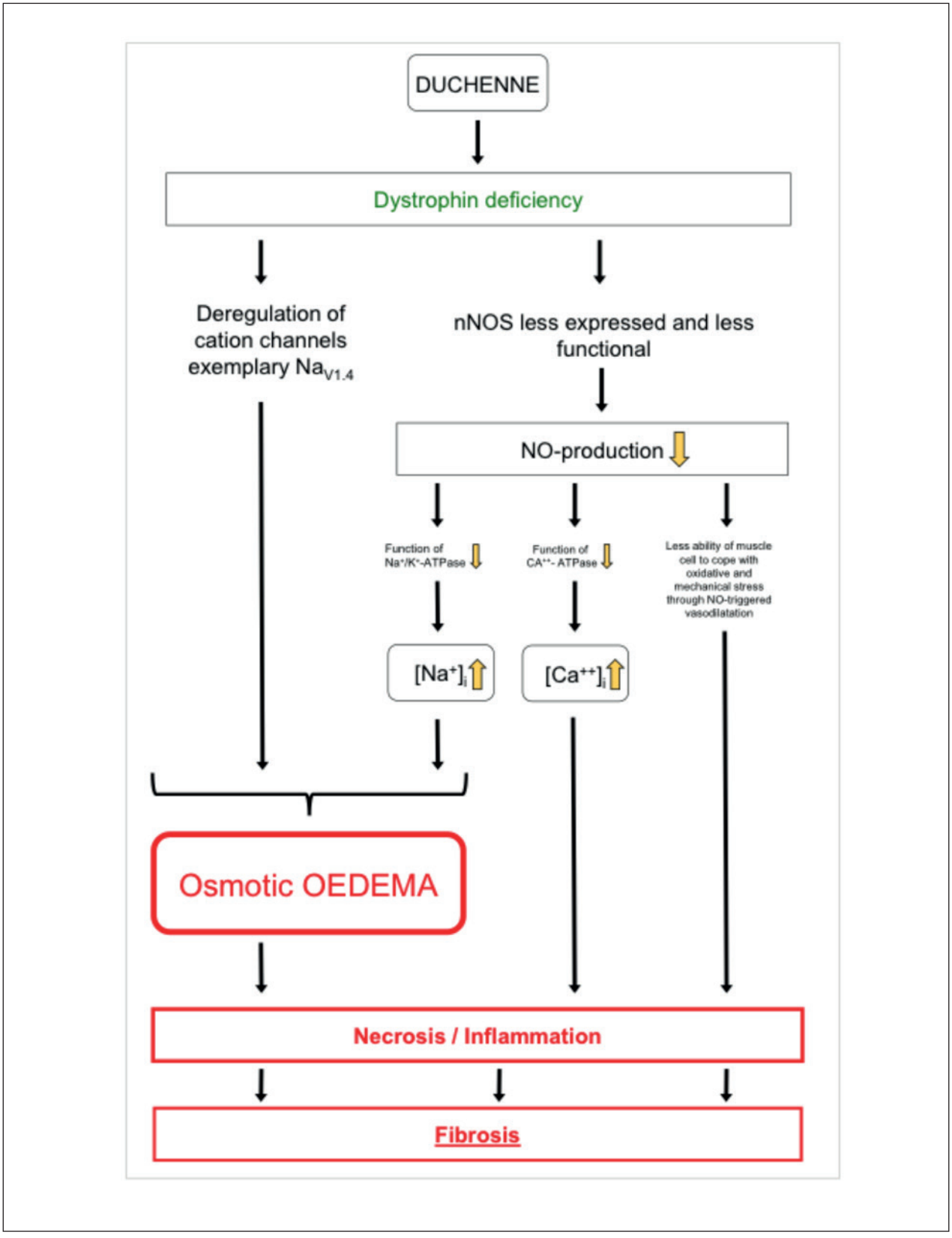

Figure 6. Model illustrating the presumed pathophysiology in Duchenne muscular dystrophy. Dystrophin deficiency, nNOS and consecutive inaccurate sodium homeostasis as a major contributor in Duchenne muscular dystrophy.

status in different parts of the body without having to perform time-consuming measurements such as blood analysis or tissue histology or without the option for MRI analysis.

In conclusion, according to our data we observed in our patient treated with eplerenone a cortisone-comparable tissue effect over 6 months of medication on MRI and myometry. Therefore, further trials with larger patient numbers need to demonstrate whether (in the long term) eplerenone should be regarded as a possible new therapy option in DMD patients.

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Personality traits in patients with myotonic dystrophy type 2

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Myotonic dystrophy type 2 (DM2) is a multisystem disorder that affects many organs and systems, including the brain. The objective is to analyze personality patterns in myotonic dystrophy type 2 (DM2) compared to DM1 control group. The study comprised 27 consecutive genetically confirmed DM2 patients and control group of 44 DM1 patients. Personality traits were assessed with the Millon Multiaxial Clinical Inventory III (MMCI III). In DM2 group there were no scale with pathological scores, although compulsive and paranoid traits were the most prominent. DM2 patients had lower scores compared to DM1 patients in almost all scales. Pathological scores on clinical symptom scales were not observed, although anxiety scale almost approached this value. Patients with higher compulsive score had higher level of education (rho = +0.53, p < 0.01). On the other hand, higher paranoid score correlated with younger age at onset (rho = -0.34, p < 0.01) and lower educational level (rho = -0.26, p < 0.05). Our results did not show significant personality impairments in patients with DM2. However, following personality traits were predominant: compulsive (in patients with higher education) and paranoid (in patients with lower education and earlier age at onset). The most common clinical symptoms were anxiety and somatization.

Key words: myotonic dystrophy type 2, personality, quality of life, compulsive, paranoid

Introduction

Myotonic dystrophy type 2 (DM2) is a multisystemic disorder that affects many organs and systems, including the brain (1).

One study showed similar histopathological findings in the brain of patients with DM2 and myotonic dystrophy type 1 (DM1) (2), magnetic resonance imaging revealed similar white matter impairments in both diseases (3-5), and in both diseases dysexecutive impairment has been described, although less severe in DM2 (4-7). On the other hand, previous studies showed less pronounced grey matter loss in DM2 brain (3, 8).

Reported frequencies of personality disorders in DM1 patients are between 20% and 64%, but some authors did not find significant personality changes (6, 9-12) which is far above the prevalence in the general population. However, there is a single previous study that specifically assessed personality pattern in DM2 subjects (6). Although none of the patients in this study fulfilled the DSM-IV criteria for the diagnosis of personality disorder, significant avoidant behavioural trait was observed in both DM2 and DM1 compared to controls.

The aim of this study was to analyze personality patterns in a cohort of DM2 patients compared to DM1 subjects.

Materials and methods

The cross-sectional study comprised 25 DM2 patients consecutively recruited during their first hospitalization at the Inpatient Unit of the Neurology Clinic, Clinical Centre of Serbia in the period from March 2013 until January 2014. Genetic diagnosis of CCTG repeats expansion using repeat primed polymerase chain reaction (RP-PCR) was obtained for all patients in addition to typical clinical and electromyographic data (13). Patients with any other associated somatic and neurological diseases not related to DM2 were excluded. Control

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group consisted of 44 genetically confirmed DM1 patients examined at the Clinic in the same period. Patients with congenital and childhood-onset DM1 were excluded from the study. During this period no DM1 patients with mild, late-onset phenotype were hospitalized, thus they also were not included in the research. All participants gave informed consent to participate in the study and the study was approved by the Ethical Board of the School of Medicine, University of Belgrade.

Manual muscle testing (0 to 5 scale according to Medical Research Council (MRC) scale) was performed in DM2 patients by experienced clinicians (VRS, SP). We added strength of the weakest muscle of the proximal arms, distal arms, proximal legs and distal legs, with maximum score being 20. Global cognitive status of DM2 and DM1 subjects was assessed using the Addenbrooke's Cognitive Examination - Revised (ACE-R) (14). Values below 82 were considered indicative of cognitive impairment and these patients were excluded from the study.

Personality traits and psychopathology in our subjects were assessed with the Millon Multiaxial Clinical Inventory (MMCI III) (15). The scores were converted directly into base rate (BR) scores, which take into account the prevalence of a particular characteristic. According to Millon's criteria, BR punctuations > 75 signify the presence of a trait and BR punctuations > 85 are considered as an impairment.

Student t test and Mann Whitney U test were used for group comparisons, as appropriate. Spearman's coefficient was applied for correlation analyses. Significant testing was two-sided, with alpha set at 0.05 for statistical significance and 0.01 for high statistical significance.

Results

Main sociodemographic, clinical and cognitive features of DM2 and control DM1 patients are presented in Table 1.

Mean scores on personality scales are presented in Table II. In DM2 group there were no scale with mean score above 75 although compulsive and paranoid traits were the most prominent. DM2 patients had lower scores compared to DM1 patients in all scales, except for narcissistic and antisocial ones where significant differences were not registered between groups (scores were normal in both groups). Mean score above 75 was not observed in DM2 patients on clinical symptom scales, although anxiety scale almost approached this value. The second highest score was somatization. All scores were lower in DM2 compared to DM1 control group.

We further correlated personality scales with the highest score (compulsive and paranoid) with sociodemographic, clinical and cognitive findings (ACE-R subscores and total score). Patients with higher compul-

Table 1. Sociodemographic, clinical and cognitive features of investigated patients.

Features	DM2 patients	DM1 patients
Gender (% of males)	32	43
Age (mean years ± SD) *	52.1 ± 10.5	46.2 ± 8.6
Education (mean years ± SD)	11.9 ± 3.1	10.7 ± 2.1
Profession (%)		
Physical work	24	34
Intellectual work	24	20
Unemployed	12	14
Retired	40	32
Marital status (%) **		
Married	84	61
Never married	8	39
Divorced	8	0
Age at onset (mean years ± SD) **	37.2 ± 10.5	27.8 ± 6.7
Duration of disease (mean years ± SD)	15.3 ± 13.5	18.4 ± 8.2
MRC sum score (mean ± SD) *	17.4 ± 2.0	15.1 ± 1.7
ACE-R (mean ± SD)	86.4 ± 11.6	78.6 ± 11.0
ACE-R Attention and Orientation **	17.3 ± 1.3	16.0 ± 1.8
ACE-R Memory	22.6 ± 4.3	22.3 ± 3.0
ACE-R Fluency **	9.4 ± 2.6	6.6 ± 4.4
ACE-R Language	23.0 ± 3.2	21.9 ± 2.9
ACE-R Visuospatial	14.0 ± 2.1	13.3 ± 2.2

MRC - Medical Research Council Scale; ACE-R - Addenbrooke's Cognitive Examination Revised; N.A. - not applicable; * $p < 0.05$, ** $p < 0.01$

Table 2. Results on MMCI scales.

Scales	DM2	DM1
Number of patients	25	44
Personality traits		
Schizoid ** % *	49.2 ± 24.5 0.0%	70.2 ± 20.4 22.7%
Avoidant ** % *	40.7 ± 25.1 0.0%	63.2 ± 25.6 18.2%
Dependent ** % **	47.0 ± 27.3 12.0%	83.2 ± 20.7 52.3%
Histrionic * %	48.3 ± 21.4 0.0%	59.7 ± 22.2 4.5%
Narcissistic %	59.8 ± 19.6 8.2%	66.4 ± 17.2 9.1%
Antisocial %	61.4 ± 15.7 0.0%	63.5 ± 15.9 6.8%
Compulsive ** %	68.8 ± 16.8 4.0%	55.7 ± 17.0 0.0%
Negativistic ** %	33.2 ± 28.2 8.0%	66.0 ± 28.5 27.3%
Schizotypal ** %	49.6 ± 15.3 0.0%	60.8 ± 11.7 2.3%
Borderline ** %	46.0 ± 21.4 0.0%	66.3 ± 17.6 13.6%
Paranoid ** % **	64.2 ± 15.5 0.0%	82.2 ± 17.3 40.9%
Clinical syndromes		
Anxiety ** % *	71.4 ± 21.7 32.0%	86.6 ± 22.7 61.4%
Somatization ** %	64.9 ± 17.0 16.0%	76.4 ± 17.0 27.3%
Hypomania ** %	27.6 ± 27.6 0.0%	59.3 ± 26.8 6.8%
Dysthymia * %	57.9 ± 23.6 12.0%	71.1 ± 26.7 27.3%
Drug abuse ** % *	44.6 ± 24.6 0.0%	73.1 ± 18.0 18.2%
Alcohol abuse ** %	40.2 ± 21.0 0.0%	62.6 ± 18.4 2.3%
Psychotic thoughts ** % *	51.9 ± 18.0 0.0%	70.5 ± 16.7 15.9%
Major depression ** %	42.0 ± 17.4 0.0%	60.2 ± 13.1 2.3%
Psychotic delusions ** %	59.6 ± 21.0 12.0%	75.1 ± 18.9 29.5

* $p < 0.05$, ** $p < 0.01$

sive score had higher level of education (rho = +0.53, $p < 0.01$). On the other hand, higher paranoid score correlated with younger age at onset (rho = -0.34, $p < 0.01$) and lower educational level (rho = -0.26, $p < 0.05$). Other correlations were not established.

Discussion

Our results did not show significant personality impairments in patients with DM2 and these patients scored better on personality scales compared to DM1 patients. Our DM1 and DM2 groups were well matched regarding gender and education, while difference in age of six years seems to be not relevant regarding personal-

ity since they all were in the mature adulthood period. Our results are in line with the study by Meola et al. who reported that although significant avoidant behavioural trait was observed in DM2 patients, no one fulfilled the criteria for the diagnosis of personality disorder (6). On the other hand, reported frequencies of personality disorders in DM1 patients are between 20% and 64% (9-12) which is far above the prevalence in the general population. Bertrand et al reported that 30% of the adult onset DM1 patients were at risk of developing a psychiatric disorder, had high score in paranoid ideation, delusional ideation, psychoticism and phobic-anxiety, lower score in self-esteem, as well as higher score in avoidant behaviour and social withdrawal (16). Moreover, low self-esteem and anxiety found in the more severe phenotype correlated with a low cognitive profile and with difficulties in executive tasks'Although DM2 affects the brain and although reaction on such a chronic disease is expected, it seems that neither of these two factors is strong enough to cause personality impairment. Possible explanations are that brain seems to be less affected in DM2 compared to DM1 (3-8). Our results also suggest that DM2 patients may adapt and realize themselves better in relation with partners since they were married more frequently compared to DM1 subjects.

In our DM2 group the highest score was observed on compulsive scale, although not reaching a pathological level. This finding is in accordance with Meola's study that also showed the highest score for the same scale but without compulsive personality disorder (6). Compulsive personality is characterized by a general pattern of concern with orderliness, organization, preoccupation with details, mental and interpersonal control and the control of one's environment (17-19). They have a tendency to keep control on everything in order to compensate for unpredictable consequences of a chronic disease. These symptoms may cause distress and interfere with a person's occupational and social functioning, but they also might have positive consequences since workaholism is often seen in those with this personality disorder (17). Accordingly, our results showed higher compulsive score in patients with higher level of education. Conscientiousness, conformation to rules, and wish to confirm himself/herself are the main features of compulsive personality that might explain its association with educational achievement (20).

Second highest score was observed on paranoid scale. Similarly, this was the highest personality score in our DM1 cohort (11). Fear of being abandoned is the core feature of paranoid personality. It is expressed as an attachment anxiety, so these patients are suspicious and mistrustful of others, think they are in danger and may be hypersensitive and hostile in relations to other people (18). It is also of note that our DM2 patients and also DM1 patients from our previous study had higher paranoid scores if they were less educated (11). Lower educational level means less ability to consider all life circumstances and less flexibility of thoughts. Association between paranoid traits and earlier age at onset of DM2 might be explained with the fact that younger people are more hypersensitive and more vulnerable (21).

Previous studies showed correlation between personality disorders and cognitive findings in general population (22). Also, Meola et al. reported low scores on cognitive tests of frontal lobe function in parallel with avoidant trait personality in both DM1 and DM2 patients. We have also previously reported significant dysexecutive syndrome and certain impairment of episodic verbal memory, while dysexecutive and visuospatial/visuoconstructional deficits predominated in DM1 (7). However, we did not confirm association between cognitive deficit and personality traits when directly comparing neuropsychological and psychological results in the temporary study.

The most prominent clinical symptoms in our DM2 patients was anxiety. Similar was observed in our research on DM1 subjects (11). This comorbidity worsens all aspects of patients' life-biological, psychological and mental (23). This was previously confirmed in DM1 patients (24, 25). Thus, adequate psychiatric and psychological treatment of anxiety should be considered in these patients.

This study has few limitations. Number of investigated patients is small, but this is a rare disease. Furthermore, correlation with neuroimaging findings would be of interest.

Conclusions

Our results did not show significant personality impairments in patients with DM2. However, following personality traits were predominant: compulsive (in patients with higher education) and paranoid (in patients with lower education and earlier age at onset). The most common clinical symptoms were anxiety and somatization.

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Integrated care of muscular dystrophies in Italy. Part 1. Pharmacological treatment and rehabilitative interventions

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This paper describes the pharmacological therapies and rehabilitative interventions received by 502 patients with Muscular Dystrophies, evaluated in relation to patient's socio-demographic and clinical variables, and geographical areas.

Data were collected by the MD-Socio-Demographic and Clinical Schedule (MD-SC-CS) and by the Family Problems Questionnaire (FPQ).

The most part of the enrolled patients were in drug treatment. The number of the medications increased in relation to patient's age, disability degree and duration of illness and was higher among patients with Duchenne Muscular Dystrophy (DMD) compared with Becker (BMD) or Limb-Girdle Muscular Dystrophies (LGMD). Steroids (deflazacort or prednisone) were the drug most frequently used, followed by cardiologic and bone metabolism drugs. In general, patients using steroids were younger and had a shorter duration of illness; patients using cardiac drugs and dietary supplements were older and had a longer duration of illness.

Rehabilitative interventions were provided to about 70% (351/502) of patients, mainly DMD. Of these, physiotherapy was the more frequent treatment (96.6%) and was prevalently performed in rehabilitative centres (about 70% of patients) and at home in only 30%. Hydrokinetic-therapy was practiced by 6.8% of patients. Respiratory rehabilitation was provided to 47.0% of patients (165/351) and assisted mechanical ventilation to 13.1% (46). The amount of rehabilitative interventions increased in relation to the patient's age, level of disability and duration of illness.

Compared to Central and Northern Italy, in Southern Italy there was a higher attention to cardiological impairment as shown by a higher number of patients receiving heart drugs.

No statistically significant differences concerning the possibility to have access to rehabilitative interventions were noted among the three geographical areas. However, patient living in Southern Italy tend to receive rehabilitation more often at home.

Key words: muscular dystrophies, integrated care, pharmacological treatment, rehabilitative intervention

Introduction

Muscular dystrophies (MDs) include a group of inherited disorders characterized by progressive muscle weakness and wasting, and classified according to pattern of inheritance, age of onset, and involvement of specific skeletal muscles (1, 2). The identification of

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dystrophin (3, 4) and the subsequent characterization of the dystrophin-glycoprotein complex (DGC) was the first step towards the clarification of the molecular pathogenesis of MDs (5, 6). Several forms of MD arise from primary mutations in genes encoding the components of DGC complex (7). The most common forms – affecting both children and young adults – are Duchenne (DMD), Becker (BMD) and Limb-Girdle Muscular Dystrophies (LGMDs). Due to the multi-systemic involvement, the management of MDs requires a multifaceted approach and a multidisciplinary expertise (8, 9). The clinical management is mainly based on the use of drugs [steroids (10-12), ace-inhibitors (13, 14) or beta-blockers (15) followed by other cardiological and/or respiratory medication when appropriate (15, 16)] and rehabilitative treatments (9). This integrated approach was able to improve quality and prolong life expectancy even in patients affected by the most severe forms (17, 18). As a consequence, DMD should now be considered as an "adulthood" disease (17, 18) requiring long term family assistance which may be very demanding (family burden) when professional and social supports are poor or lacking (19, 20).

In 2012, a national study on the families of patients with muscular dystrophies was carried out in Italy with the aim to describe the difficulties of the care-giver experience as well as the professional and social supports the relatives may rely on (21, 22). We found that relatives whose children had higher degree of disability, spent more daily hours in caregiving and/or had poor social support experienced a higher burden. Nevertheless, 88% of them reported something positive out of the situation (21, 22).

Based on the same data set, in this paper, we report data on the pharmacological and rehabilitative treatments provided to the 502 patients, and investigate differences in relation to demographic and clinical variables, and geographical areas.

Patients and methods

Design of the study

The study was carried out in 8 specialized centres for MDs, located in Northern (3 centres), Central (3 centres), and Southern Italy (2 centres). The patients' selection criteria were the following: diagnosis of DMD, BMD, or LGMD confirmed by molecular analysis or muscle biopsy; age between 4 and 25 years; in charge to the participating centres for at least 6 months; living with at least one adult relative. For each patient the key-relative was interviewed if he/she was aged between 18 and 80 years and not suffering from illness requiring long-term intensive care (21, 22).

Data were collected concerning: a) family socio-demographic characteristics and patient's clinical variables through the Muscular Dystrophy-Socio-Demographic and Clinical Schedule (MD-SD-CS); b) patient's level of functional autonomy according to the Barthel Index (BI); c) therapies provided to patients and support received by the families, through the MD Care Schedule (MD-CS); d) family burden through the Family Problems Questionnaire (FPQ).

The protocol of the study was approved by the Ethic Committee of the Second University of Naples (coordinating centre), and by the Ethical Committee of each participating Centre.

Instruments description

MD-SD-CS collects information on the main socio-demographic characteristics of the patients and their families, and on patients' clinical variables. Barthel Index (BI) assesses the patient's degree of independence in daily activities. It provides a global 1-100 score (0 "totally dependent"; 100 "totally independent"). Questions ad hoc developed by the researchers for the present study were used to interview the key-relative on patient's functional autonomy in the previous month. The inter-rater reliability in BI scoring was tested preliminary (Cohen's kappa coefficient ranging from 1 to 0.90 for 9 BI items and equal to 0.67 for the lasting BI item).

MD-CS collects information on pharmacological therapies received by the patient in the two months preceding the interview and on psycho-educational interventions and social/welfare support provided to patients and their families in the past six months. The schedule also collects information on where each treatment was provided.

FPQ explores relative's burden, attitudes toward the patient, and professional and social network support in emergencies concerning the patient (23). It contains additional items on expenses sustained by the family in the previous 12 months for care.

The psychometric properties of the FPQ was previously tested in this study sample (21).

Statistical analysis

Differences in pharmacological therapies and rehabilitative interventions related to patients' socio-demographic, clinical and geographic variables were explored by the analysis of variance and χ^2, as appropriate. Correlations between the number of drugs or rehabilitative interventions and patients' age, duration of illness and levels of functional abilities (BI global score) were explored by Spearman's r coefficient. Multiple regression analyses were performed to explore the simultaneous ef-

Table 1. Characteristics of the 502 patients and their key-relatives.

	Patients (N = 502)	**Key-relatives (N = 502)**
Sex, N (%)		
Males	484 (96.4)	74 (14.7)
Females	18 (3.6)	428 (85.2)
Age, mean (SD) years	12.8 (5.6)	43.4 (7.4)
Marital status, N (%)		
Single	502 (100)	61 (12,1)
Cohabitant/spouse	0	441 (87.8)
	Attendance	Degree
Education, N (%) yes	430 (85.6)	502 (100)
Pre-school	50 (11.6)	-
Primary school	148 (34.4)	35 (6.9)
Secondary school	90 (20.9)	184 (36.6)
High school	127 (29.1)	219 (43.6)
University	17 (4.0)	64 (12.7)
Currently employed (adults) N (%) yes	7 (7.4)	264 (52.6)
Relationship with the patient, %		
Mother	-	424 (84.6)
Father	-	70 (14.0)
Others	-	7 (1.4)
Duration of symptoms, mean (SD) years	8.9 (5.5)	-

fects on drugs and rehabilitative interventions (dependent variables) of patients' socio-demographic and clinical characteristics. Only variables related to drugs or rehabilitative interventions statistically significant in the univariate analysis were included in the multivariate ones. Statistical significance was set at $p < 0.01$.

Results

Of the 502 patients consecutively recruited, the most part was male, young, and school attending (Table 1). Three-hundred-thirty-three (66%) of them were DMD, 129 (26%) BMD, and 40 (8%) LGMDs. The mean level of independence in daily activities, measured by the BI, was 68.3 (31.3sd). One-hundred-ninety-four patients (39%) were in wheelchair.

Most of the 502 key-relatives were mothers and married or cohabiting. Almost half of them had received higher education and were employed (Table 1). They spent on average 5.7 (4.6sd) daily hours in patient's care-giving in the previous two months.

Pharmacological treatment

As reported in Table 2, most patients (73.5%) were in drug treatment. The number of the medications increased in relation to patient's age ($r = .32$, $p < .0001$), disability degree (BI global score $r = -.39$, $p < .0001$), and duration of illness ($r = .38$, $p < .0001$). Moreover, it was higher among patients affected by DMD compared with BMD or LGMDs (2.5 ± 1.8 *vs* 1.3 ± 1.8 vs. 1.5 ± 2.4, $F = 23.0$, df 2,499; $p < .0001$).

Steroids were the drug most frequently used, followed by cardiologic and bone metabolism drugs. Patients using corticosteroids were younger (11.6 (5.1) *vs* 13.6 (5.9), $F = 16.0$; df 1,500; $p < .0001$) and had a

Table 2. Pharmacological treatment received by patients with MDs in the past six months (N = 502).

N = 369 (73.5%)				
	DMD	**BMD**	**LGMDs**	**Total sample**
Type of drugs, N (%)				
Corticosteroids	205 (90.3)	14 (6.2)	8 (3.5)c	227 (61.5)
Cardiologic	144 (74.6)	41 (21.2)	8 (4.1) a	193 (52.3)
Bone metabolism	127 (86.4)	13 (8.8)	7 (4.8) c	147 (39.8)
Gastric	63 (84.0)	7 (9.3)	5 (6.7) b	75 (20.3)
Mean number of drugs/patient (sd)	2.5 (1.8)	1.3 (1.8)	1.5 (2.4) c	2.9 (1.7)
Differences among the three groups, a $p < .005$; b $p < .001$; c $p < .0001$				

shorter duration of illness (8.0 (4.9) *vs* 9.6 (5.9), F = 9.9, 1, 460, <. 0001), while patients using cardiac drugs and dietary supplements were older (16.9 (4.4) *vs* 10.0 (4.6), F = 259,6; df 1,500; p < .0001; 14.0 (5.9) *vs* 12.3 (5.0), F = 8.4, df 1,500; .01), and had a longer duration of illness (12.7 (4.8) *vs* 6.3 (4.3), F = 227.9, df 1,460; p < .0001; 10.3 (5.9) *vs* 8.4 (5.3), F = 10.1, df 1,460, p < 0.05).

Patients assuming cardiologic, bone metabolism and/or gastric protective drugs had lower levels of functional abilities (50.5 (31.6) *vs* 79.4 (25.4), F = 126.5, df 1,500, p < .0001; 58.2 (30.2) *vs* 72.5 (30.8), F = 22.5, df 1,500; p < .0001; 59.8 (29.3) *vs* 69.8 (31.4), F = 6.6; 1,500; p < .01).

A higher number of pharmacological prescriptions, particularly those concerning cardiological treatment, was found in centres located in Southern Italy compared with those in Central or Northern Italy (3.0 ± 2.1, vs 1.4 ± 1.5 vs 1.8 ± 1.6, F = 38.1, df 2,499; p < .0001).

Rehabilitative treatments

Three-hundred-fifty-one patients (70%) benefited of rehabilitative interventions. Physiotherapy was the most frequent treatment provided to MD patients, followed by respiratory rehabilitation and assisted mechanical ventilation (Table 3). Hydrokinetic-therapy was performed in only 21/502 (4.2%) patients. Rehabilitation was provided at home in about one-third (107/351, 30.5%) of cases. Although the percentage of rehabilitative interventions received by patients did not differ among the three geographical areas, however the home care rehabilitative treatments were more frequently performed in Southern Italy (24 in North, 31 in Central and 52 in South Italy (p < 0.003).

The complexity of rehabilitation treatment, intended as a number of rehabilitative interventions, increased in relation to patient's age (r = .33, p < .0001), level of disability (BI global score r = -.63, p < .0001), and duration of illness (r = .38, p < .0001).

Multiple regression analyses

Socio-demographic and clinical variables accounted for 23% of variance in pharmacological therapies provided to patients in the previous two months (Table 4). As shown by the standardized beta weights, number of drugs was significantly higher among patients' with longer duration of illness, and suffering from DMD.

Patient's clinical variables accounted for 42% of variance observed in rehabilitative interventions (Table 4) received by the patients in the previous six months, confirming that the number of the interventions was higher among patients with more severe disabilities, and in those suffering from DMD or LGMDs.

Discussion

The study reveals that about 75% of patients, independently from the type of muscular dystrophy, receive a drug treatment. This finding outlines a shift from past views of MDs as "incurable diseases" toward a clinical approach based on effective pharmacotherapy. In line with the current clinical guide-lines, the steroids (deflazacort or prednisone) were the drug more frequently administered in DMD (8, 9); they were more frequently used by patients still ambulant (119/205) compared with those wheelchair-bound (86/205) (X^2 = 55.7 df 1, p < .0001). This result can be explained by the current debate on the use of corticosteroids in the wheelchair stage, although recent studies have shown that the long-term steroid administration is useful to a) preserve upper limb strength (24), b) reduce the progression of scoliosis and the decline of respiratory function (25, 26) and c) delay the onset of heart dysfunction (27).

The higher number of cardiac drugs prescribed in Centres located in Southern Italy, may be related to the long-term expertise in cardiological monitoring of these Centres (28-30) and by the recent adoption of *Treat-NMD and National Council for Rare Diseases guide-lines* (31, 32).

The study also shows that the majority of MD patients in Italy receive rehabilitative interventions, whose complexity increased as the illness progresses. However some differences exist in the modality of provision, as in Southern Italy a higher number of patients receive domiciliary treatment. This condition, probably due to the poor availability of rehabilitative centers in Southern Italy, leads to an indirect benefit, both for patients and families, in terms of comfort of care, time saving and transfer costs.

Table 3. Rehabilitative treatment received by patients with MDs in the past six months (N = 502).

	DMD	BMD	LGMDs	Total sample
Rehabilitation, N (%)				351 (69.9%)
Type of rehabilitation, N (%)				
Physiotherapy	278/339 (82)	35/339 (10.3)	26/339 (7.7)[a]	339 (96.6)
Respiratory rehabilitation	138/165 (83.6)	17/165 (10.3)	10/165 (6.1)[a]	165 (47.0)
Assisted mechanical ventilation	45/46 (97.8)	0	1/ (2.2)[a]	46 (13.1)
Differences among the three groups, [a] p < .0001				

Table 4. Multiple regression analyses: effects of socio-demographic and clinical variables on pharmacological and rehabilitative treatments provided to patients with MDs.

	Drug treatments	Rehabilitative treatments
	Standardized Beta	
Variables		
Patient's age	.08	.05
Barthel Index	-. 04	-.38c
Duration of illness	.33c	.14
Type of MD- BMD	- .26c	-.26c
Type of MD-LGMD	- .15b	- .07
Model's F, df , p<	27.13; 5, 456; .0001	66.4; 5, 456; .0001
R2	.23	.42
[a] = p < .05; [b] = p < .001; [c]= p < .0001		

This study also reveals that in Italy – although with the known different regional shortages – an integrated pharmacological/rehabilitative care is guaranteed to the majority of patients with muscular dystrophies. Hopefully the recent changes in the Italian health care policy will further facilitate the patient's access to evidence based treatment.

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CASE REPORTS

Mitochondrial ANT-1 related adPEO leading to cognitive impairment: is there a link?

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ANT1 is one of the nuclear genes responsible of autosomal dominant progressive external ophthalmoplegia (adPEO) with mitochondrial DNA multiple deletions. The course of ANT1-related adPEO is relatively benign, symptoms being generally restricted to skeletal muscle.
Here we report the case of an Italian 74 years old woman with ANT1-related adPEO and dementia.
Further studies are needed to assess the prevalence of central neurological manifestations in ANT1 mitochondrial disease.

Key words: mitochondrial disease, adPEO, ANT1, cognitive impairment, mitochondrial dementia

Introduction

ANT1, encoded by the *SLC25A4 gene*, is a member of a family of integral membrane transport molecules widely expressed in the inner mitochondrial membrane. ANT1 forms a channel which moves ADP into the mitochondrion and ATP out of the mitochondrion to be used as energy for the cell. This protein seems also to be a component of the mitochondrial permeability transition pore, the formation of which appears to be an important step of apoptosis. ANT1 is highly expressed in heart and skeletal muscle, but also in the brain (1, 2). *SLC25A4* gene mutation can be responsible of three main phenotype: autosomal dominant progressive external ophthalmoplegia (adPEO) with mitochondrial DNA (mtDNA) multiple deletions, first described in 2000 (3); Autosomal Dominant Mitochondrial DNA Depletion Syndrome 12A (4) characterized by hypotonia, hypertrophyc cardiomyophaty and pulmonary involvement; Autosomal Recessive Mitochondrial DNA Depletion Syndrome 12B described later in the text (5).

The course of ANT1-related adPEO is relatively benign, symptoms being generally restricted to skeletal muscle. This is explained by the observation that ANT1 is the main isoform of the ADP/ATP carrier in skeletal muscle mitochondria (1, 2). Afterwards, Palmieri et al. described a 368C-A transversion homozygous mutation of ANT1 gene associated with hypertrophic cardiomyopathy, mild myopathy with exercise intolerance and lactic acidosis, but no ophthalmoplegia (5). Despite a well-established knowledge of this metabolic dysfunction in skeletal muscle, other aspects of adPEO caused by ANT1 mutation are less known. In particular, there are no data showing an association between ANT1 mutation and cognitive impairment. Here we describe a case of cognitive impairment in a woman harboring a mutation in *SLC25A4 gene*.

Case report

A 74 years old Italian woman arrived to our attention complaining about a ten years history of eyelid ptosis. The patient also presented weakness and fatigue. Patient's parents were not consanguineous. She had family history of eyelid ptosis (mother, maternal grandmother, maternal aunt), ischaemic heart disease (mother and brother), and sudden death (father). Her personal history was significant for autoimmune thyroid disease and heart palpitations. She underwent echocardiography revealing hypertrophic cardiomyopathy with mild diastolic dysfunction. Neurological examination showed bilateral eyelid ptosis, ophtalmoplegia and mild weakness of lower limbs (MRC 4/5). Blood tests, including lactate, were normal. Electromyography showed a myopathic pattern. MtDNA and POLG analyses were negative. Sequencing of the *SLC25A4* gene revealed the heterozygous mutations (c.340G > C) in the exon 2, resulting in an Ala114-to-Pro (A114P) substitution, previously described in five

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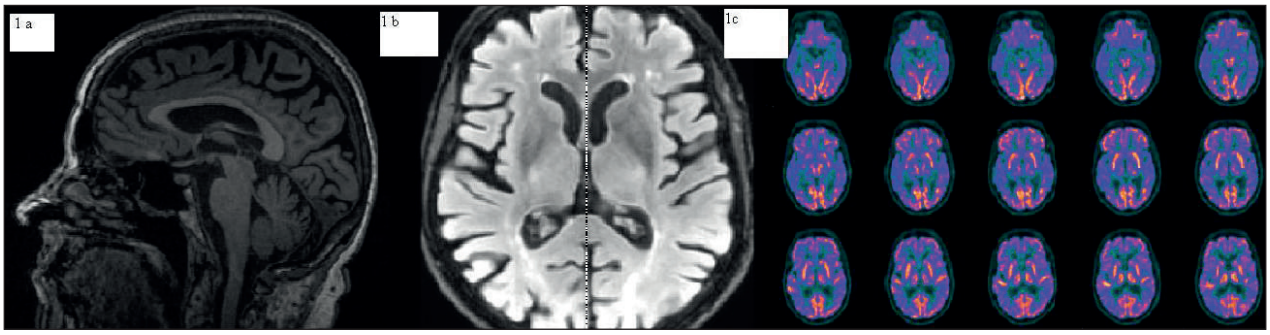

Figure 1. 1a and 1b Brain MRI demonstrates cortical atrophy. 1c positron emission tomography (PET) revealed bilaterally reduced metabolism in the temporal poles, lateral temporal and posterior parietal areas.

families with PEO (3). At age 75, the patient started to report disorders of short-term memory; the neuropsychological evaluation demonstrated a mild impairment of verbal memory and the attentive functions.

Magnetic resonance of brain revealed widespread moderate cortical atrophy and mild leukoaraiosis.

(Figs. 1a,1b). Magnetic resonance spectroscopy of brain showed mild elevation of lactate in posterior intermispheric region and in ventricular region. The brain fluorodeoxyglucose positron emission tomography (PET) revealed bilaterally reduced metabolism in the temporal poles, lateral temporal and posterior parietal areas (Fig. 1c). Cerebrospinal fluid (CSF) sample was obtained by lumbar puncture and concentrations of βeta amyloid protein (Aβ1-42), tau protein (Ttau) and phosphorylate tau protein (P-tau) were determined. Aβ1-42 was slightly reduced (575 pg/mL -normal values > 600), T-tau was increased (1131 pg/ml - v.n. < 275) as well as P-tau (141 pg/ml, n.v. < 60), whereas lactate was 14 md\dl. Apolipoprotein E genotype was E3E3.

Discussion

Dementia is a chronic condition characterized by a gradual decrease in the ability to remember, associated with a decrease of other cognitive functions which it translates into a reduced ability to judge or to reflect; interfering with activities of daily living (6). Cognitive domains affected include memory, language, orientation, constructional abilities, abstract thinking, problem solving, or praxis. Most evident is impairment in learning new information (6). Mitochondrial diseases are multisystem disorders that also affect central nervous system. One of the clinical features of these diseases is the so called "mitochondrial dementia" which is often characterized by specific cognitive deficits, particularly in abstract reasoning, verbal and visual memory, language (naming and fluency), executive or constructive functions, calculation, attention (attention deficit disorder and decreased attention span), or visuo-spatial function (7-9). It's not well defined the role of mitochondrial dysfunction in the development of cognitive impairment (10). The differential diagnosis of mitochondrial dementia and symptomatic dementia is critical and the timing of mitochondrial disease diagnosis is important because the onset of cognitive dysfunction as only or first symptoms of mitochondrial disease is rare; vice versa, if a patient develops mitochondrial phenotype before the development of dementia, the cognitive involvement can be more easily suspected as mitochondrial dementia. Other important elements that can orient towards a diagnosis of mitochondrial dementia are the elevation of lactate at cerebrospinal fluid and\or a peak of lactate at magnetic resonance spectroscopy.

ANT1 mutations commonly lead to a "pure PEO", characterized by eyelid ptosis, ophthalmoplegia and mild muscle symptoms. This is because ANT1 is mainly expressed in the mitochondria of skeletal muscle tissue and heart (2). Nevertheless, ANT1 expression is not skeletal muscle-restricted, and a low expression is present also in the brain. While few literature data reported an association between ANT1 mutation and hypertrophic cardiomyopathy (5), the link between ANT1 mutation and cognitive impairment is a new finding.

We are aware that the association of adPEO and cognitive dysfunction observed in our patient may be a casual coincidence. However, previous literature data support our hypothesis of mitochondrial dementia (11). Features that arguments in favor of mitochondrial dementia are the presence of a mild lactate peak in brain spectroscopy, the diffuse cortical atrophy associated with mild leukoaraiosis, the timing of mitochondrial disease progression (PEO onset before cognitive impairment), and the reduced metabolism in PET whit a pattern non typical of Alzheimer disease. Moreover, the Aβ1-42 values in our patient are only mildly decreased, differently on what we commonly see in Alzheimer disease and are more similar to what we observe in normal aging (12). Conversely, the dosage of T-tau and P-tau in the patient CSF is not strongly

indicative of mitochondrial dementia. Taken together, the above-mentioned findings suggest that in some patients with *ANT1* mutation, a multisystem involvement may be observed, including cognitive impairment. Further studies in large groups of ANT1 patients are needed in order to assess the prevalence of CNS involvement in ANT1 patients and to better characterize the clinical heterogeneity (if any) of this genotype.

Acknowledgments

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Congenital myasthenic syndrome: phenotypic variability in patients harbouring p.T159P mutation in *CHRNE* gene

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Congenital myasthenic syndromes (CMS) are rare and heterogeneous genetic diseases characterized by compromised neuromuscular transmission and clinical features of fatigable weakness; age at onset, presenting symptoms, distribution of weakness, and response to treatment differ depending on the underlying molecular defect. Mutations in one of the multiple genes, encoding proteins expressed at the neuromuscular junction, are currently known to be associated with subtypes of CMS. The most common CMS syndrome identified is associated with mutation in the CHRNE gene, causing principally muscle nicotinic acetylcholine receptor deficiency, that results in reduced receptor density on the postsynaptic membrane.
We describe the clinical, neurophysiological and molecular features of two unrelated CMS Italian families with marked phenotypic variability, carrying the already reported p.T159P mutation in the *CHRNE* gene. Our report highlights clinical heterogeneity, intrafamily variability in spite of the same genotype and a possible gender effect; it confirms the efficacy and safety of salbutamol in patients who harbor mutations in the epsilon subunit of acetylcholine receptor.

Key words: Congenital myasthenic syndromes, *CHRNE* gene, phenotype variability

Introduction

Congenital myasthenic syndromes (CMS) comprise heterogeneous genetic diseases characterized by compromised neuromuscular transmission. CMS can be classified as presynaptic, synaptic or postsynaptic, depending on the location of the primary defect within the neuromuscular junction (1, 2). Some patients present signs from birth, or shortly after, especially those with mild presentations, who remain undiagnosed until adolescence. To date, 31 causative genes in SMC have been identified including genes that code for the AChR subunits (CHRNE, CHRNA1, CHRNB1, CHRND and CHRNG), molecules expressed in the neuromuscular junction and, recently, proteins involved in abnormal glycosylation of AChR subunits (1-9). The most common CMS identified is associated with mutations in the *CHRNE* gene, encoding the epsilon subunit of the acetylcholine receptor (AChR).

We describe the clinical, neurophysiological and molecular features of two unrelated CMS Italian families with marked phenotypic variability, carrying the already reported p.T159P mutation in the *CHRNE* gene: in one family this mutation is present in homozygous state, whereas in the second family it is compound heterozygous associated with the known p.S235L mutation (10, 11).

Case reports

Family 1

Patient 1 is a female, 4 years old, second child of third cousins healthy parents. Since first months she presented bilateral ptosis, difficulties in sucking and dysphagia, leading to ab ingestis pulmonitis at 8 months of age. Psychomotor development was normal, but mild weakness, unsteady gait and fatigability since early infancy, and slight fluctuations of symptoms with worsening during the evening, were referred. Neurological examination at 4 years of age, showed bilateral ptosis (Fig. 1a), facial muscles weakness, nasal voice, generalized hypotonia, muscle weakness more marked at lower limbs, positive Gower's sign, anserine ambulation, and running inability. AChR antibodies were absent. Electromyography revealed mild

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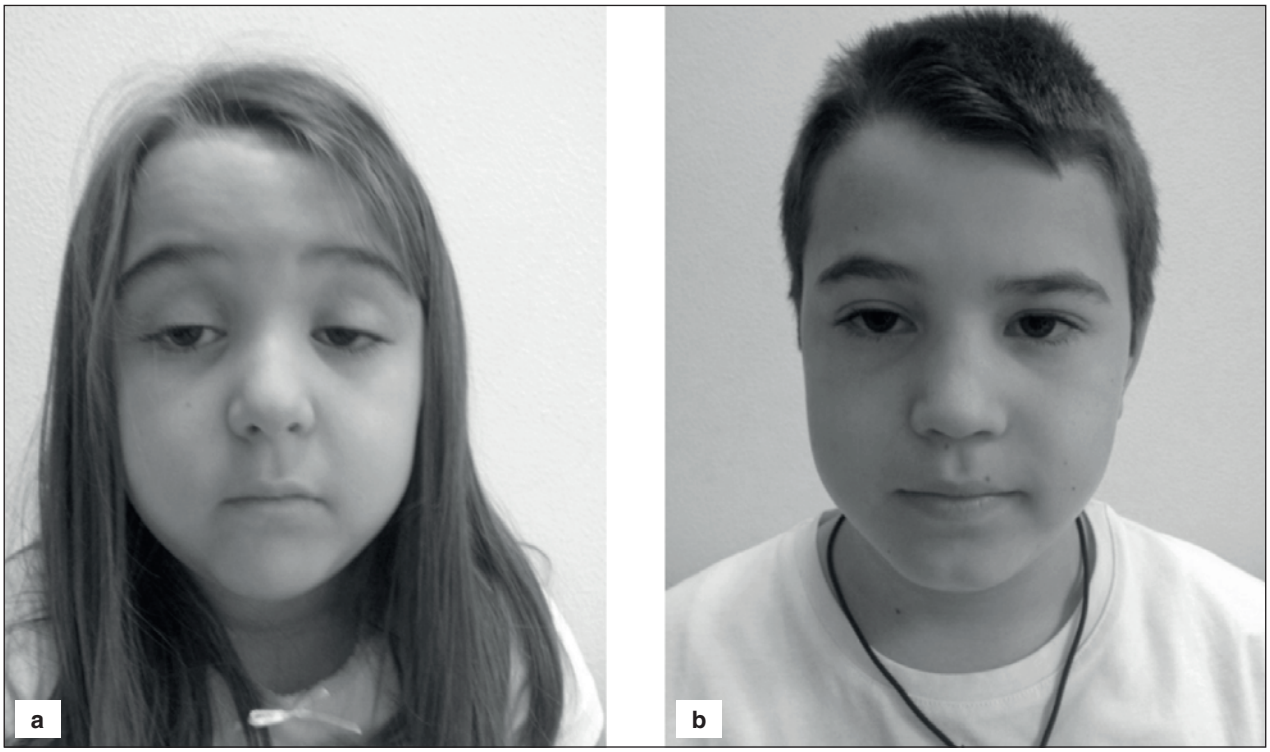

Figure 1. Patient 1 and patient 2 from family 1. The female presents marked bilateral ptosis (a) while the older brother shows slight bilateral ptosis (b).

myopathic alterations; single fibre test was not performed because of patient's poor compliance. Muscle biopsy revealed aspecific myopathic features. At follow up, 6 months later, clinical evolution was stable. Parents noticed substantial improvement during treatment with salbutamol for a trivial respiratory disease; a post synaptic CMS was suspected. Oral treatment with salbutamol was started: a marked improvement of ptosis, weakness and activities of daily living was reported, without side effects.

Patient 2, now 10 years old, is the older brother of Patient 1. After his sister's hospitalization, he underwent neurological examination showing only slight bilateral ptosis (Fig.1b), and very mild lower limb girdle weakness (MRC: 4+).

All 12 exons of the *CHRNE* gene were sequenced following the already reported protocol (12). The analysis in family 1 revealed a previously identified c.475A>C mutation in exon 6 (p.T159P), in homozygous form (10). Genetic analysis in her older brother (Patient 2) revealed the same homozygous p.T159P mutation. The healthy parents carry one mutant allele each (Fig. 2).

Family 2

Patient 3 is a girl, now 20 years old, second child of healthy non consanguineous parents. Since first months of life she presented with bilateral ptosis and axial weakness. Subsequently ophthalmoparesis, diurnal fluctuations of ptosis, facial weakness, fatigability, difficulties in running and climbing stairs were reported. At age 4 years a diagnosis of CMS was reached. Clinical conditions remained stable during adolescence; electromyographic study revealed mild myopathic changes in upper and lower limbs and repetitive nerve stimulation (RNS) of facial nerve showed a pathological decremental response. At last observation, 20 years of age, she presented with marked bilateral ptosis, almost complete ophthalmoparesis, axial weakness, positive Gower's sign, and running inability.

Patient 4 is the younger brother of Patient 3, now 13 years old. He similarly showed since birth presence of ptosis, ophthalmoparesis and mild axial weakness, that remained stable during subsequent years. The electrophysiological findings were similar to those observed in his sister. Treatment with Pyridostigmine was uneffective.

Direct sequencing of the *CHRNE* gene in both siblings revealed the known p.T159P mutation associated with a second already reported mutation c.704C>T (p.S235L) in exon 7, both mutations were present in heterozygous form (10, 11). The mother was the carrier of p.T159P mutation, and the father of the p.S235L mutation (Fig. 2).

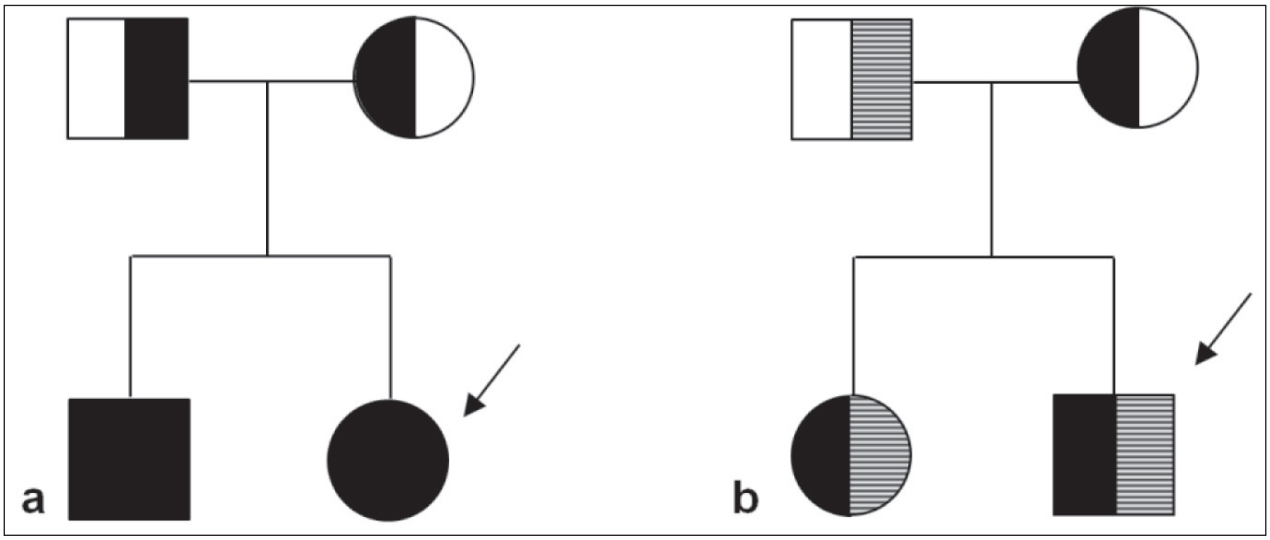

Figure 2. Families 1 and 2: genomic DNA of propositi (arrows) and family members. Closed symbols indicate affected individuals carrying two mutant alleles. Half shaded symbols represent asymptomatic carriers harbouring a single mutant allele. (a) Pedigree of the family 1: the p.T159P mutation is present in homozygous form in the sons, while in heterozygous form in carrier parents. (b) Pedigree of the family 2: the children present compound heterozygous mutations p.T159P and p.S235L, whereas the mother and the father are carrier of p.T159P and p.S235L, respectively.

Table 1 summarizes the clinical aspects of the 4 patients of 2 families described in this study.

Genetic analysis

In patients all 12 exons and the adjacent splice donor and acceptor sequences of the *CHRNE* gene were sequenced, using genomic DNA isolated from blood, following the already reported protocol (5), while in their healthy parents the only mutated exons were analysed. The PCR products were purified by EuroSAP (Euroclone) and sequenced by bidirectional sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific), on an 3130xl Genetic Analyzer (Thermo Fisher Scientific). The obtained sequences were analysed with SeqScape v.3.0 software (Thermo Fisher Scientific) and compared with reference wild-type sequence (GenBank CHRNE accession numbers: NM_000080.3).

Informed consent

Written informed consent for genetic analysis and for photos from children of Family 1 was obtained from probands' relatives and their familial members.

Discussion

All CMS patients share same clinical features, but age at onset, presenting symptoms, distribution of weakness, and response to treatment differ depending on the molecular mechanism that results from the genetic defect (1, 2).

We report four Italian CMS patients harboring *CHRNE* mutations and showing marked clinical variability, ranging from isolated mild ptosis to marked ptosis associated with ophthalmoparesis, facial and lower limb-girdle weakness (Patients 3 and 4) and intrafamily phenotypic variability in both families.

Genotype is different in the two families. In Family 1 the known p.T159P mutation is present in homozygous state whereas in Family 2 the p.T159P mutation is associated with the known p.S235L mutation (10, 11).

The p.T159P mutation is localized on the long cytoplasmatic N-terminal portion of the epsilon protein, which contains several loop regions which are critical for receptor function (6). Expression study showed that this mutation causes principally AChR deficiency (10, 14). The p.T159P mutation was previously identified in one CMS proband in compound heterozygous whit a second one (p.A411P) (10).

The p.S235L mutation is localized at the end of the membrane-spanning M1 domain of the epsilon protein, which joins covalently the four a-helical segments M1-M4 to the extracellular domain, hence this mutation may change this structural link (13). The p.S235L mutation was previously found in one Portuguese CMS patient associated with a second p.70insG mutation, presenting the clinical signs of ptosis, ophthalmoparesis, dysphagia,

Table 1. Clinical characteristics of the patients.

Patient		Gender	Onset age/ symptoms	Evolution	Cinical findings at diagnosis	Treatment/ response
Family 1	1	Female	First months/ bilateral ptosis, difficulties in sucking and dysphagia	Worsened	Bilateral ptosis, facial muscles weakness, nasal voice, generalized hypotonia, limb girdle weakness more marked at lower limbs, positive Gower's sign, anserine ambulation	Salbutamol/effective
	2	Male	Early infancy/ mild ptosis	Stable	Mild bilateral ptosis and mild lower limb girdle weakness	No treatment
Family 2	3	Female	First months/ ptosis	Worsened in infancy/ stable in adolescence	Bilateral ptosis, ophthalmoparesis, axial weakness, positive Gower's sign	Pyridostigmine/ uneffective
	4	Male	First months/ ptosis	Worsened in infancy/ stable in adolescence	Bilateral ptosis, ophthalmoparesis and mild axial weakness	Pyridostigmine/ uneffective

proximal weakness, and electrophysiological studies revealed a RNS decrement (11). Also in our patients the p.S235L mutation in compound heterozygous state seems to aggravate the phenotype.

In siblings of Family 1, harbouring p.T159P mutation in homozygous state, a marked clinical variability is evident. Marked phenotypic variability has been already described in two siblings with CMS due to mutations in *MUSK* gene: the sister was reported to be much more severely affected than the brother and a gender-effect was hypothesized since menstrual periods and fever worsened her symptoms (15). Although Patient 1 was in a prepuberal age, our report confirmed the hypothesis of a gender effect in the phenotypic expression. Our report underlines intrafamily clinical variability in spite of the same genotype and a possible gender effect; confirms the efficacy and safety of salbutamol in patients who harbour mutations in the epsilon of AchR (16).

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Acta Myologica • 2017; XXXVI: p. 33-35

Effect on lung function of mounthpiece ventilation in Steinert disease. A case report

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In patients with muscular dystrophies both muscle length tension relationship changes and muscle elasticity and plasticity are decreased, resulting in impaired inspiratory muscle function and decreased vital capacity. Furthermore, the loss of deep breathing further increases the risk of alveolar collapse, hypoventilation and atelectasias. In this case report, a stable improvement of vital capacity after treatment with mounthpiece ventilation (MPV), was observed, suggesting that not invasive ventilation (NIV) might help to maintai lung and chest wall compliance, prevent hypoventilation and atelectasias which in turn may slow down the development of the restrictive respiratory pattern. The improvement of vital capacity may have a positive impact on alveolar ventilation by reducing the time with SaO2 values below 90%. This case illustrates that MPV is an effective method to improve respiratory function in patients non-tolerant of nasal mask and a valid alternative option for those who need NIV support for the most part of the day. Furthermore, the use of MPV, alone or combined with other interfaces, improves the quality of life of the neuromuscular patients and promotes a greater adherence to mechanical ventilation.

Key words: Steinert disease, lung function, not invasive nasal ventilation, mounthpiece ventilation

Introduction

Type 1 myotonic dystrophy or Steinert's disease is a progressive multisystem disorder mainly, characterized by myotonia, muscular dystrophy, cataracts, hypogonadism, frontal balding, and cardiac and respiratory involvement (1-5). The disease results in progressive weakness and wasting of respiratory muscles. Many patients develop sleep disorders and respiratory failure, and often need noninvasive mechanical ventilation. Sometimes patients refuse the noninvasive mechanical ventilation due to excessive air leakage, claustrophobia, and anxiety of being unable to communicate with family members.

Case description

A 44-year-old woman with Steinert disease was referred to our hospital for evaluation with complaints of daytime fatigue and headaches. The symptoms were assessed with a Epworth sleepiness scale questionnaire. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were measured with a hand held spirometer. Arterial blood gas tension was determined from arterialized ear lobe blood in automated blood gas analyzer. Overnight polysomnography was also performed.

The pulmonary function test revealed a severe reduction of respiratory capacity: FVC was 0,70 L (19%) and FEV1 0,64 (19%). The arterial blood gas analysis showed diurnal hypercapnia ($PaCO_2$ = 47 mmHg), and mild hypoxemia (PaO_2 = 68 mmHg).

The overnight polysomnography demonstrated REM sleep hypopneas with REM sleep hypoventilation and continuous sleep stage-independent hypoventilation with apneas/hypopneas index (AHI) = 4 events/hour, and 31% of the time with arterial oxygen saturation (SaO_2) less than 90% (T90 SaO_2).

A non-invasive positive pressure ventilation (Trilogy; Philips Respironics, Murrysville, PA, USA) with nasal mask was proposed to the patient, but he immediately refused it due to claustrophobia and anxiety of not being able to call a family member, if necessary. Therefore a mouthpiece mask with an exhalation valve circuit was adopted. A pressure control (PC) with support of 8 cm H2O was preferred, which was gradually increased until a suitable tidal volume, an optimal value of peripheral saturation (confirmed by arterial blood gas analysis) and the stabilization of the heart rate were reached. The inspiration time was set at 1.3 sec, the expiratory positive air-

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Table 1. Respiratory parameters observed pre and post mouthpiece treatment.

	pO_2	pCO_2	FVC	FEV1	ESS
pre NIV	68	47	0,71	0,64	21
post NIV	78	44	1,33	1,16	8
after 6 months	81	42	1,39	1,22	6

way pressure (EPAP) at 0 cmH_2O, the rise time amounted to 3 s. The respiratory rate was set at 0 breaths/min. The time of disconnection alarm was set at 15 minutes to allow the patient to speak and make breaks without triggering alarm. Blood gas analysis showed an improvement in oxygen saturation and the normalyzation of capnia ($PaCO_2$ = 44 mmHg).

The patient tolerated well this ventilation mode with a good adaptation to the interface (Fig. 1). She accepted the treatment and continued the non-invasive mechanical ventilation at home.

After two months of treatment, the patient was re-evaluated. The analysis of the device usage showed a good compliance with the therapy, performed on average for 8 hours per day; furthermore an improvement of symptoms was reported.

Also the diurnal respiratory function showed an improvement after MPV, with values of FVC equal to 1.33 L (43%) vs 0,70 L (19%) and FEV1 equal to 1.16 L (43%) vs 0,64 L (19%), accompanied by an improvement of gas exchange (PaO_2 = 78 mmHg and PaCO2 = 44 mmHg vs 68 mmHg and 47 mmHG, respectively.

Figure 1. Steinert patient during MPV.

Six months after the initiation of MPV, the improvement in pulmonary function and gas exchange were still present (FVC = 1.39 L (46%); FEV1 = 1.22 (46%); (PaO_2 = 81 mmHg and $PaCO_2$ = 42 mmHg. The overnight polysomnography showed a reduction (16% vs 31%) of the periods with a T90 SaO_2 (Table 1).

Discussion

The use of MPV has been previously described (6-8) in neuromuscular diseases. However no data exist on the use of MPV in patients with Steinert disease. These patients often require non-invasive mechanical ventilation and the choice of the proper interface can play a decisive role in the management and the compliance to the treatment, often hindered by complications such as skin lesions, excessive pressure and claustrophobia. In fact, it has been reported that the compliance with non-invasive ventilation is usually poor in patients with no subjective symptoms of respiratory failure and that the non-invasive ventilation (NIV) is more frequently abandoned in cases who experienced excessive leaks (9-12).

It has been reported that patients in general are satisfied with MPV, feel it as and efficient and comfortable mode of ventilation and prefer the mouthpiece rather than the nasal mask (10, 11). MPV can be also useful to promote a positive approach and a rapid acceptance of the new condition, taking into account that the patient's perception can have a positive effect on the adherence to NIV. In patients with muscular dystrophy muscle length tension relationship changes and muscle elasticity and plasticity are decreased, which results in impared inspiratory muscle function and decreased vital capacity. In addition, loss of deep breathing further increase the risk of alveolar collapse, hypoventilation and atelectasis (12). In the case described, we observed a stable improvement of vital capacity after treatment. One hypothesis to explain the improvements in lung volume is that application of NIV might help maintain lung and chest wall compliance (13), preventing hypoventilation and atelectasis which in turn may slow down the development of the restrictive respira-

tory pattern. It is possible that the improvement of vital capacity has a positive impact on alveolar ventilation by reducing the time with SaO_2 spent below 90%. Therefore, the mouthpiece approach should always be considered in patients with neuromuscular diseases requiring NIV support. In our experience it can be successfully proposed to patients with Steinert's disease who have previously rejected the application of NIV due to tightness of the interface, or claustrophobia.

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NEWS FROM AROUND THE WORLD

AIM

The 2017 Congress of AIM will take place in the beautiful setting of Syracuse, Sicily from May 31 to June 3, 2017. Topics will include innovations in diagnostic technologies, therapies, disease registries, biobanks, physical activity and muscle diseases and muscle aging; collaborative scientific projects, relations between myology reference centers, institutions and patient organizations will also be discussed. Details for the registration to the Congress, hotel booking and the preliminary program are available on the website of AIM *www.miologia.org*.

On the AIM website it is possible to consult the more recent guidelines in neuromuscular diseases management and a list of the upcoming scientific events sponsored by the Association.

GCA

During the Gala dinner of the 13th Congress of the Mediterranean Society of Myology the 2018 Gaetano Conte Prizes will be assigned for basic and clinical research.

MSM

The 13th Congress of the Mediterranean Society of Myology will be held in Turkey in 2018, organised by Prof. Haluk Topaloglu. The symposium was in the traditional two-days MSM format with selected topics.

WMS

The 22nd International WMS Congress will be held in Saint Malo, France from 3 to 7 October 2017. The symposium will be held in the traditional format with 3 selected topics

1. Excitation-contraction coupling: basic aspects and related disorders
2. Extra-muscular manifestations in NMD
3. Advances in the treatment of neuromuscular disorders

Contributions will also be welcome on new advances across the neuromuscular field.

FORTHCOMING MEETINGS

2017

February 9-10

36th Belgian Society of Cardiology Annual Congress. Brussels, Belgium.
Information: website: *https://www.eiseverywhere.com/ehome/bsc2017registration/home/?eb=309050*

February 24-26

CardioRhythm 2017. Hong Kong, China.
Information: website: *www.cardiorhythm.com*

March 17-19

American College of Cardiology. ACC.17. Washington, DC. USA.
Information: website: *https://accscientificsession.acc.org*

April 2-4

European Cardiac Arrhythmia Society (ECAS). Rome, Italy.
Information: *website: http://ecas-heartrhythm.org/*

May 6-8

Mediterranean Cardiology Meeting 2017. Catania, Italy.
Information: *website: http://www.mcmweb.it/*

May 10-13

Heart Rhythm 38th Annual Scientific Sessions (HRS). Chicago, IL.
Information: website:
https://www.heartrhythminternationalgrouphousing.org/

May 22-24

World Heart Congress 2017. Osaka, Japan.
Information: website:
http://heartcongress.conferenceseries.com/

May 27-30

European Human Genetics Conference. Copenhagen, Denmark.
Information: *conference@eshg.org*

May 31-June 3

XVII Meeting of the Italian Association of Myology. Siracusa, Italy.
Information: website: *www.miologia.it*

June 18-21

European Heart Rhythm Association (EHRA). Vienna, Austria.
Information: website: *http://www.escardio.org/Sub-specialty-communities/European-Heart-Rhythm-Association-(EHRA)*

June 24-27

3rd Congress of the European Academy of Neurology. Amsterdam, The Netherlands.
Information: *www.ean.org/amsterdam2017*

July 13-15

Asian Pacific Society of Cardiology (APSC). Singapore, Singapore.
Information: website: *http://www.apscardio.org/*

July 14-16

20th World Congress on Heart Disease. International Academy of Cardiology. Vancouver BC, Canada.
Information: website: *http://www.cardiologyonline.com/*

July 30-August 1

World Congress on Heart Disease-Boston, Mass.
Information: website: *http://cardiologyonline.com/wchd2016/index.html*

August 26-30

European Society of Cardiology (ESC). Barcelona, Spain.
Information: website: *https://www.escardio.org/*

August 31-September 1

19th Annual Cardiology Congress. Philadelphia, USA.
Information: website: *http://cardiac.conferenceseries.com/*

September 5-9

IDMC-11. San Francisco, CA,USA.
Information: website: *http://www.idmc11.org*

September 13-15

Global Biobank Week. Towards harmony in biobanking. Stockholm, Sweeden.
Information: website: *http://globalbiobankweek.org*

September 14-16

International Academy of Cardiology Scientific Sessions - World Congress on Heart Disease Vancouver, Canada.
Information: website: *http://www.cardiologyonline.com/wchd2017/*

September 14-17

Asia Pacific Heart Rhythm Society (APHRS). Yokohama, Japan.
Information: website: *http://www.aphrs.org/*

October 3-7

22nd Congress of World Muscle Society. St. Malo, France.
Information: website: *www.worldmusclesociety.org*

October 17-21

ASHG Annual Meeting. Orlando, Florida, USA.
Information: *website: www.ashg.org*

October 25-27

15th edition of Venice Arrhythmias. Venice, Italy.
Information: website: *http://www.venicearrhythmias.org/*

November 22-24

Imaging in Neuromuscular Disease 2017. Berlin, Germany.
Information: *www.myo-mri.eu*

2018

June 28-30

XIII Congress of Mediterranean Society of Myology. Uçhisar-Cappadocia, Turkey.
Information: *Haluk Topaloglu htopalog@hacettepe.edu.tr*

August 25-29

European Society of Cardiology (ESC). Munich, Germany.
Information: website: *https://www.escardio.org/*

October 16-20

ASHG Annual Meeting. San Diego, CA,USA.
Information: website: *www.ashg.org*

October 17-21

Asia Pacific Heart Rhythm Society (APHRS). Taipei, Taiwan.
Information: website: *http://www.aphrs.org/*

October 31-November 02

World Congress on Human Genetics. Valencia, Spain.
Information: website:
http://humangenetics.conferenceseries.com/

To be announced

23rd Congress of World Muscle Society. Mendoza, Argentina.
Information: website: *www.worldmusclesociety.org*

2019

May 2019

Heart Rhythm 40th Annual Scientific Sessions (HRS). Chicago, IL.
Information: website: *http://www.hrssessions.org/*

October 22-26

ASHG Annual Meeting. Toronto, Canada.
Information: website: *www.ashg.org*

To be announced

Asia Pacific Heart Rhythm Society (APHRS). Bangkok, Thailand.
Information: website: *http://www.aphrs.org/*

24th Congress of World Muscle Society. Copenhagen, Denmark.
Information: website: *www.worldmusclesociety.org*

2020

October 27-31

ASHG Annual Meeting. San Diego, CA,USA.
Information: website: *www.ashg.org*

To be announced

24th Congress of World Muscle Society. Toronto, Canada.
Information: website: *www.worldmusclesociety.org*

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Case Reports, Scientific Letters (maximum 1500 words, 10 references, 3 figures or tables, maximum 4 authors). A summary of 150 words may be included.
Letters to the Editor (maximum 600 words, 5 references). Letters commenting upon papers published in the journal during the previous year or concerning news in the myologic, cardio-myologic or neuro-myologic field, will be welcome. All Authors must sign the letter.
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