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EDITORIAL

Dear Colleagues,

Welcome to this special issue of Acta Myologica, dedicated to the peculiar clinical items in muscle diseases occurring in the age of transition from adolescence to adulthood. It is not only important as a circumstance to discuss this critical developmental phase of the individual with its consequences on the burden a muscle disease implies, but it is also an attempt to approach, in a more comprehensive perspective, an extraordinary fascinating timing along the individual lifespan, although in a diseased condition.

In the previous number of this Journal (Acta Myol, volume XXXV, October 2016) papers from Astrea et al. and Magliano et al. have anticipated this topic by reporting cognitive disabilities in the contest of a progressive muscular disease and family context in muscular dystrophies with psychosocial aspects of social integration, respectively.

This special issue contains five extended papers, in the form of short reviews, of the main contributors presented at the congress "New and view. Neuromuscular diseases from child to adulthood: new tools and new opportunities", held in Pisa, Italy, in September 23rd-24th 2016, promoted by the Italian Association of Myology (AIM).

In that conference the more relevant aspects in the diagnosis and treatment of muscle diseases were discussed, addressing the sensitive issue of the transition from childhood to adulthood. In particular, the contributors highlighted some common neuromuscular disorders presenting in childhood, such as dystrophinopathies, myothonic dystrophies, metabolic and mitochondrial disorders, pointing out successes in treatment, change in natural history, transitions to adult care and challenges of adult patients with a pediatric disease.

Several difficulties emerged during this phase of transition in a patient with a neuromuscular disease, including change from a family centred care to individual care, challenges in employment, education, and social integration.

We are confident that this issue will be useful to increase awareness on a topic like that. We really want to thank all the contributing authors for their efforts in building up this edition which we hope will be appreciated in our scientific community.

Gabriele Siciliano
Filippo Maria Santorelli
Roberta Battini

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SHORT REVIEWS

Genetic diagnosis as a tool for personalized treatment of Duchenne muscular dystrophy

Luca Bello and Elena Pegoraro

Department of Neurosciences, University of Padova, Italy

Accurate definition of genetic mutations causing Duchenne muscular dystrophy (DMD) has always been relevant in order to provide genetic counseling to patients and families, and helps to establish the prognosis in the case where the distinction between Duchenne, Becker, or intermediate muscular dystrophy is not obvious. As molecular treatments aimed at dystrophin restoration in DMD are increasingly available as commercialized drugs or within clinical trials, genetic diagnosis has become an indispensable tool in order to determine eligibility for these treatments. DMD patients in which multiplex ligation-dependent probe amplification (MLPA) or similar techniques show a deletion suitable to exon skipping of exons 44, 45, 51, or 53, may be currently treated with AONs targeting these exons, in the context of clinical trials, or, as is the case for exon 51 skipping in the United States, with the first commercialized drug (eteplirsen). Patients who test negative at MLPA, but in whom *DMD* gene sequencing shows a nonsense mutation, may be amenable for treatment with stop codon readthrough compounds such as ataluren. Novel molecular approaches such as CRISPR-Cas9 targeting of specific DMD mutations are still in the preclinical stages, but appear promising. In conclusion, an accurate genetic diagnosis represents the entrance into a new scenario of personalized medicine in DMD.

Key words: Duchenne muscular dystrophy, genetic diagnosis, exon skipping, stop codon readthrough, CRISPR-Cas9

Introduction

In 1987, the discovery that Duchenne muscular dystrophy (DMD) is caused by the absence of the protein dystrophin in skeletal muscle fibers (1) first provided a rationale for molecular treatments, aimed at restoring dystrophin expression. Shortly thereafter, the discovery that Becker muscular dystrophy (BMD) is a milder allelic form of dystrophinopathy, in which dystrophin is present but qualitatively altered and quantitatively reduced (2) suggested that clinical benefit may be provided to DMD patients, even with partial restoration of dystrophin expression. Despite the hope stirred by these seminal discoveries, after three decades such molecular treatments are not yet widely available to DMD patients. In fact, formidable obstacles still stand in the way of the translation of dystrophin-restoring treatments from proof-of-concept experiments to clinical trials and everyday clinical practice: for instance, because of several genetic and environmental factors, there is considerable variability in disease progression , which has made the design and interpretation of clinical trials more challenging (3, 4). Nevertheless, several molecular therapies for DMD have reached advanced stages of clinical experimentation, and the first drugs have recently reached the market through accelerated and provisional regulatory approvals (5, 6).

In the complex and swiftly changing scenario of experimental treatments for DMD, the expression "molecular therapy" requires a more precise definition. Dystrophin restoration may be achieved in two different ways: through genetic therapies, i.e. delivery of new genetic material, that is not naturally present in the patient's cells, such as a functioning copy of the DMD gene or transcript; or through molecular therapies, i.e. small or larger molecules (i.e. oligonucleotides, enzymes), that interact with the patients' own genetic material and transcription/translation machinery to restore the expression of viable dystrophin. Here, we intend to focus on molecular therapies, mainly for two reasons: first, molecular therapies have reached more advanced stages in human experimentation than genetic therapies for DMD, and are therefore of more immediate interest to neuromuscular clinicians; and second, they highlight the importance of reaching a well-defined molecular diagnosis, at the genomic level, in DMD. In fact, while genetic therapies would theoretically benefit any DMD patient, molecular therapies are an

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example of personalized therapies, as they are expected to work only in those patients who harbor specific mutations, targeted by the molecule of choice.

We will first briefly review the current standards in molecular diagnosis of DMD, and then the main molecular treatment strategies accessible to patients with different identified mutations: exon skipping through antisense oligonucleotides (AONs), and readthrough of premature termination codons through the small molecule ataluren. Recently, the prospect of editing patients' genomes using the CRISPR-Cas9 technology has opened a new exciting perspective in this field.

Genetic diagnosis of DMD

Typically, the diagnosis of DMD may be established based on the clinical picture and the finding of absent dystrophin expression in muscle by immunohistochemistry and/or immunoblot (7). However, internationally adopted guidelines (8, 9) indicate that an accurate characterization of the causative mutation at the genomic level is of paramount importance in order to provide genetic counseling to the family, establish genotype-phenotype correlations and prognosis, and, as we will show in the next paragraphs, assess eligibility for novel molecular treatments. The first molecular assay to be requested when DMD is suspected should be quantitative, i.e. provide a measure of the copy number of genomic regions corresponding to each of the 79 *DMD* exons. In fact, about 60 to 70% of DMD-causing mutations are large rearrangements (deletions or duplications) including one or more exons (10). These are readily identified in both affected males and female carriers by multiplex ligation-dependent probe amplification (MLPA) (11). In the case of single-exon deletions, results should be confirmed by an independent technique, as some small mutations may prevent probe hybridization and be misinterpreted as deletions of the corresponding exons. Alternative quantitative techniques include multiplex PCR and competitive genomic hybridization (CGH) arrays. Whenever quantitative assays cannot completely characterize the mutation, PCR amplification and sequencing of the coding *DMD* regions and flanking nucleotides allows the identification of single nucleotide substitutions, small sub-exonic rearrangements, and canonic splice-site mutations. While classic Sanger sequencing is still widely used, next generation sequencing (NGS) approaches are becoming increasingly accessible (9). In rare cases in which no mutation can be identified by sequencing of genomic DNA coding regions, *DMD* mRNA may be isolated from skeletal muscle tissue (either archived from a previous diagnostic biopsy, or obtained specifically for this purpose), reverse-transcribed to cDNA, and sequenced. The localization of sequence anomalies in the transcript may point to deep intronic causative mutations that may be identified by targeted Sanger sequencing of intronic gDNA regions, or by NGS. The systematic application of this workflow in DMD patients (illustrated in Fig. 1) leads to the identification of a clear pathogenetic mutation in the vast majority of cases.

Exon skipping with antisense oligonucleotides

The rationale for "exon skipping" is based on several facts: in about two thirds of cases, DMD is caused by large deletions involving one or more exons, and disrupting the *DMD* open reading frame (ORF); on the contrary, deletions that respect the ORF cause the milder allelic disease BMD (12); because of the *DMD* genomic structure, it is often possible to restore the ORF of an out-of-frame deletion by splicing out just one exon, adjacent to the deletion borders at the 5' or 3' side, from the mature mRNA. Therefore, by targeting and inactivating specific exon splicing signals in the pre-mRNA with sequence-specific AONs, it is possible to obtain an internally deleted, yet in-frame *DMD* transcript, which is similar to transcripts naturally observed in BMD patients. These transcripts may then be translated into a viable, albeit internally deleted and quantitatively reduced, dystrophin protein, hopefully shifting the patient's clinical picture towards the milder BMD phenotype. The application of AON treatments to DMD and other genetic conditions became technically possible in the early 2000s, with the invention of chemically modified nucleotide backbones, which resisted to nucleases and had a favourable pharmacokinetic and pharmacodynamic profile in humans. These were 2'-*O*-methyl RNA phosphorothioate AONs (administered subcutaneously) and morpholino AONs (administered intravenously) (13). For both chemistries, first-in-human studies with local intramuscular injections of AONs showed promising dystrophin restoration in biopsies of injected muscles (14 ,15). The leading compounds targeted exon 51, whose skipping is predicted to restore the ORF in the highest portion of DMD patients (around 10-15%). Subsequent phase 2 dose-escalation studies (16, 17) were also promising, as they seemed to provide not only assurances of good tolerability of these compounds, but also biochemical evidence of dystrophin restoration and encouraging stabilization or improvement of some functional outcome measures. Unfortunately, in the years immediately following these exciting breakthroughs, the enthusiasm of the DMD community was thwarted. An international, multi-center phase 3 trial of drisapersen (clinicaltrials.gov NCT01254019), the 2'-*O*-methyl AON for the skipping of exon 51 developed by

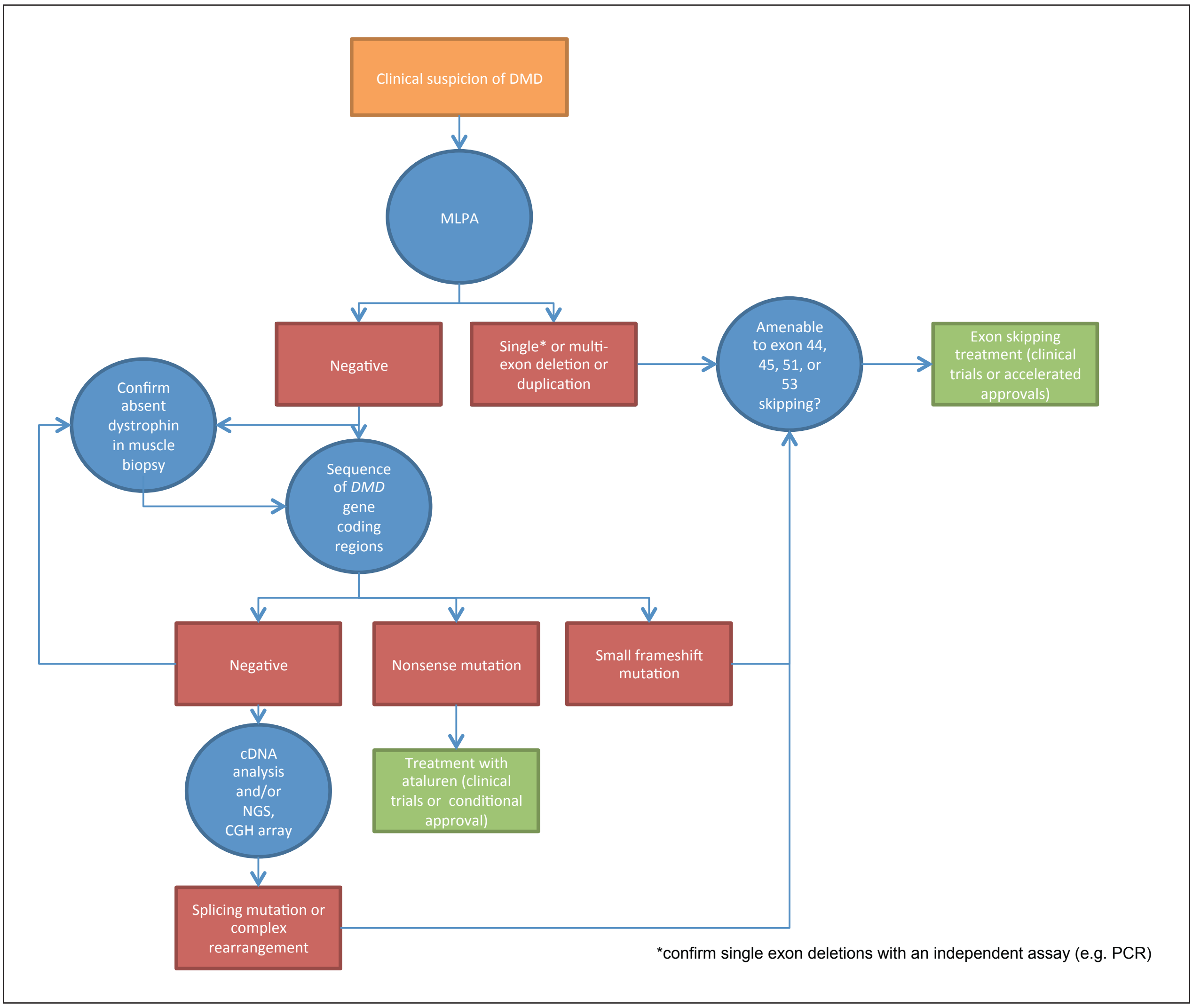
Clinical suspicion of DMD
MLPA
Negative
Single* or multi-exon deletion or duplication
Amenable to exon 44, 45, 51, or 53 skipping?
Exon skipping treatment (clinical trials or accelerated approvals)
Confirm absent dystrophin in muscle biopsy
Sequence of *DMD* gene coding regions
Negative
Nonsense mutation
Small frameshift mutation
cDNA analysis and/or NGS, CGH array
Treatment with ataluren (clinical trials or conditional approval)
Splicing mutation or complex rearrangement
*confirm single exon deletions with an independent assay (e.g. PCR)

Figure 1. Flow diagram of genetic diagnosis in DMD, highlighting indicated molecular assays (blue circles) and corresponding possible findings (red rectangles), leading to further assays, or, in some cases, to established amenability for molecular treatments (green rectangles).

Biomarin/Prosensa, failed to achieve a significant clinical benefit (the results have not yet been published). While more partially encouraging data came from a phase 2b extension study (18), the company decided to discontinue the drisapersen development program. These events have triggered a lively debate (19, 20) about several controversial aspects of exon skipping treatments, such as the difficulty to accurately measure efficacy of treatments both at the molecular (21) and clinical level (22), and the amount of dystrophin needed to actually obtain a clinical benefit (23). As for eteplirsen, the morpholino AON for the skipping of exon 51 developed by Sarepta, phase 3 studies are still underway (NCT02255552), but interesting data have derived from a small but prolonged phase 2b extension study (24). Clinical data from this open-label study, also corroborated by comparison to a mutation-matched external natural history control group (25), seem to support stabilization of ambulatory function in 12 patients. Unfortunately, data regarding dystrophin quantification in several longitudinal muscle biopsies in the same study indicate less abundant protein expression increase than suggested by earlier studies, and have been the object of controversy (26, 27). Despite these uncertainties, the Food and Drugs Administration has recently granted accelerated approval to eteplirsen in the United States of America, recognizing that a demonstrated increase in a biologically relevant biomarker, i.e. dystrophin, however small, may reasonably be expected to benefit DMD patients (6). Hopefully, successful confirmatory trials will allow the clinical efficacy of exon skipping AONs to be established without ambiguity. Clinical trials of AONs targeting exons other than 51 are underway (e.g. NCT02310906 and NCT02500381), and new generations of AONs are being experimented at the preclinical stage (28, 29), so that exon skipping still represents a promise of future effective treatments for a large portion of DMD patients.

Stop codon readthrough compounds

About 15% of the causative mutations in DMD are single nucleotide substitutions introducing a premature termination codon (nonsense mutations) (10). This causes the ribosomial complex to stall during translation, usually resulting in nonsense-mediated decay of the transcript and absence of dystrophin (30). However, nonsense mutations may also be observed in association with BMD or intermediate phenotypes, probably due to naturally occuring alternative splicing of in-frame exons (31). Furthermore, ribosomes may be pharmacologically induced to “read through” premature stop codons, and continue downstream translation, giving rise to normal dystrophin. Aminoglycosides were demonstrated to effectively promote dystrophin expression in the *mdx* mouse model, which carries a nonsense mutation in exon 23 (32), but human trials were hindered by excessive toxicity (33, 34). The pharmaceutical company PTC Therapeutics has developed a small molecule compound, ataluren, which has been shown to maintain the same nonsense readthrough effect as aminoglycosides (although the exact molecular mechanism has not been completely cleared) (35) with a more favorable tolerability profile. After proof-of-concept studies in animal models (36) and phase 1 trials showing no relevant safety issues (37), ataluren was dosed in DMD in a first phase 2a study with further reassurance of safety, and encouraging results (38). The results of a larger phase 2b trial were controversial (39), as the primary endpoint (48-week change in the 6 minute walk test [6MWT]) was not achieved, and better ambulation outcomes were unexpectedly observed in a 10, 10, 20 mg/kg than 20, 20, 40 mg/kg study arm. Unfortunately, quantitative assessments of dystrophin restoration in muscle tissue from participant biopsies were hindered by technical issues in this study. However, indications of efficacy in the phase 2b study were sufficient for the European Medicines Agency to issue a conditional approval for the marketing of ataluren in European Union countries in 2014 (5), with the obligation on the company’s part to conduct a phase 3 confirmatory study, the results of which (NCT01826487) have recently been released online (http://ir.ptcbio.com/releasedetail.cfm?ReleaseID=936905) although not yet published as a peer-reviewed article. While the primary endpoint of change in the 6MWT still remained elusive, pre-specified subgroup analyses and meta-analyses in conjunction with the previous phase 2b study showed a clear, although not dramatic drug effect in delaying the deterioration of ambulatory function in DMD. Currently, ataluren is prescribable to DMD patients with nonsense mutations who are older than 5 years and ambulatory in several European countries. The continued development of the nonsense readthrough strategy will hopefully provide a solid therapeutic option for a relevant subgroup of DMD patients.

“Exon snipping”: mutation-specific gene editing

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems are an adaptive immune defense evolved in bacteria and archaea, which uses short RNAs for the degradation of foreign nucleic acids. In 2013, several independent groups reported the successful reprogramming of CRISPR-Cas9 to cut DNA at any site of choosing in eukaryotic cells, by coupling it with a strand of guide RNA (gRNA) with a custom sequence (40-42). This breakthrough invention has revolutionized molecular biology with its myriad potential applications. Applied to DMD, the CRISPR-Cas9 approach has been aptly named “exon snipping” (43), in analogy with the “exon skipping” obtained by AON targeting of mRNA. “Exon snipping” would primarily consist in excising an in-frame exon containing a small nonsense or frameshifting mutation, as successfully demonstrated with adeno-associated virus (AAV) delivery in the *mdx* murine model (44, 45), or, alternatively, in excising specific exons in order to restore the ORF in the case of large rearrangements (deletions or duplications) (46). The main drawbacks of this approach are represented by the challenges of AAV vector delivery in humans, and the fear of off-target effects. Nevertheless, the versatility and wide applicability of this technology to virtually every DMD causing mutation makes it one of the most exciting and promising novel approaches to molecular therapy of DMD.

Conclusions

The advancements of molecular treatments described above have made reaching a precise genetic diagnosis in DMD more and more important over the last few years. Currently, DMD patients with deletions bordering exon 44, 45, 51 or 53 bordering may be eligible for recruitment in one of several ongoing clinical trials of exon skipping. Patients eligible for exon 51 skipping may be treated with commercialized eteplirsen in the United States (Exondys

51®, Sarepta Therapeutics, Cambridge, MA, USA). Patients in whom MLPA (or other equivalent quantitative assays for large deletions/duplications) tests negative, should be studied with *DMD* gene sequencing in order to be able to provide genetic counseling to the family, and because patients with nonsense mutations may be eligible for treatment with ataluren, commercialized as Translarna® in the European Union (PTC Therapeutics, South Plainfield, NJ, USA), or within future clinical trials. While the advancement of these treatments has been painstakingly slow in the eyes of DMD patients and their families, who struggle every day against the progression of this disabling disease, there are reasons to hope that the experience gathered in designing better clinical trials, as well as an increasing number of novel drugs in the pipeline of preclinical research, will bring on a faster and more effective translation of scientific findings into benefit for patients in the upcoming years.

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Management of cardiac involvement in muscular dystrophies: paediatric versus adult forms

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Muscular dystrophies are a group of genetic disorders characterized by muscle degeneration and consequent substitution by fat and fibrous tissue. Cardiac involvement is an almost constant feature in a great part of these diseases, as both primary myocardial involvement and secondary involvement due to respiratory insufficiency, pulmonary hypertension or reduced mobility. Primary myocardial involvement usually begins more precociously compared to the secondary involvement. In fact the first signs of cardiomyopathy can be observed in the first decade of life in muscular dystrophies with childhood onset and later in adult form of muscular dystrophies as myotonic dystrophy type 1.

At least an annual cardiac follow-up is recommended in these patients including clinical and instrumental examination (ECG, 24h Holter monitoring, ECHO), to detect cardiac involvement.

A more frequent monitoring may be required according to the type of cardiomyopathy and the patient's needs.

In this short review practical guide-lines are shown for physicians routinely involved in the management of these patients.

Key words: muscular dystrophies, cardiomyopathy, DMD, BMD, Steinert Disease, EDMD

Introduction

Muscular dystrophies are a group of genetic disorders characterized by muscle degeneration and consequent substitution by fat and fibrous tissue. Several forms have been described on the basis of pattern of inheritance (autosomal dominant, autosomal recessive, X-linked), age of onset (childhood or adulthood), involvement of specific muscle groups and more recently on protein deficiency.

Cardiac involvement is an almost constant feature in a great part of these diseases, as both primary myocardial involvement and secondary involvement due to respiratory insufficiency, pulmonary hypertension or reduced mobility.

In this short review, we will show few examples of cardiomyopathy occurring in infantile or adult forms of muscular dystrophies, underlying that usually the myocardial disease manifesting as cardiomyopathy and congestive heart failure is characteristic of dystrophinopathies and some types of sarcoglycanopathies - gamma and delta-sarcoglycanopathies in particular. Conversely, abnormalities in the conduction system causing heart blocks, arrhythmias, and sudden cardiac death are more commonly seen in Myotonic Dystrophy type 1 (DM1), Emery-Dreifuss muscular dystrophies (EDMD), Limb-Girdle Muscular Dystrophy 1B (LGMD1B) and less frequently in Facio-Scapulo-Humeral-dystrophy (FSHD).

Heart involvement in infantile forms of muscular dystrophies

Cardiomyopathy in muscular dystrophies is generally the result of the same mutational event that leads to the onset of skeletal muscle involvement (1, 2). A paradigmatic example of this kind of myocardial involvement, is the cardiomyopathy observed in patients with dystrophinopathies, due to mutations in the dystrophin gene (3-10). It usually starts early in life, in an asymptomatic manner detectable by instrumental investigation (the presymptomatic stage) (11). The lack of dystrophin at the myocardium level causes the apoptosis and fibrotic changing of isolated cardiomyocites (stage of focal fibrosis), often

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inducing phenomena of compensatory hypertrophy of the surrounding cardiomyocytes or the onset of arrhythmias (12, 13). The confluence of these areas of fibrosis leads to a picture of diffuse fibrosis (diffuse fibrosis stage) that preludes to the dilation of the heart chambers (dilated cardiomyopathy stage), with a reduced ejection fraction (EF) (14). Dilated cardiomyopathy in turn evolves toward the stage of heart failure (HF) characterized by dyspnoea, peripheral edemas and liver enlargement (15). To be noted that the first episodes of HF are usually responsive to standard pharmacological treatment; however, recurrent episodes of HF lead to the stage of intractable or irreversible heart failure (16, 17).

In patients with Duchenne muscular dystrophy (DMD, OMIM 310200), the age of onset of cardiomyopathy is usually in the first decade of life, and it can be recognized in 25% of DMD patients aged 6 years through instrumental investigations (3, 7, 8, 14-18). With the age the cardiomyopathy progresses from the asymptomatic stage to the overt cardiac involvement involving about 80-85% of patients at the age of 18 (3).

Cardiomyopathy in Becker muscular dystrophy (BMD) is characterized by qualitative and/or quantitative anomalies of dystrophin at the myocardium level, similar to those found at the skeletal muscle level. BMD patients manifest signs and symptoms of cardiomyopathy in a late period of their life, usually in the third- fourth decade, but onset before 20 years is also observed (19). Cardiomyopathy may be the presenting symptom of dystrophinopathy (20). Interestingly patients with BMD seem to pay a more favorable evolution of myopathy with a more severe picture of cardiac involvement, likely due to the an increased request to a heart defective in dystrophin by a prolonged ambulation (4, 5).

Diagnosis

The diagnosis of cardiomyopathy in dystrophinopathic patients is based on cardiological examination and the use of ECG, Holter monitoring and echocardiography (7, 8, 14, 15). The follow-up can benefit of periodical cardiac evaluations – at least once a year – starting from the age of the diagnosis of the underlying myopathy or from the time of the cardiac diagnosis. More frequent examinations and supplemental investigations such as cardiac magnetic resonance imaging (MRI) study, cardioscintiscan, electrophysiological study (EPS) may be necessary according to the stage of cardiomyopathy and the patient's needs (21-28).

Management

The pharmacological treatment of dystrophinopathic cardiomyopathy relies on the use of ACE-inhibitors (29-33), beta-blockers (34-37), combined with the steroid treatment (prednisone in the USA and deflazacort in Europe, Canada and Australia) currently considered as the gold standard for Duchenne muscular dystrophy (38-45). There is an almost unanimous consensus that the treatment with steroids is able to delay the onset of dystrophinopathic cardiomyopathy (17, 46, 47).

In case of dilated cardiomyopathy and/or heart failure, digitalis, diuretics (furosemide) and anticoagulants (48-50) are indicated without forgetting a careful monitoring of electrolytes (K+, Mg++, Na+) balance (Fig. 1).

A similar pattern and evolution of cardiomyopathy can be observed in other infantile forms of muscular dystrophy such as gamma (LGMD2C, OMIM 253700) and delta (LGMD2F, OMIM 601287) sarcoglycanopathies and merosinopathy (CMD1C, OMIM 601493); therefore these pathologies can benefit of the same cardiological protocol of diagnosis, follow-up and treatment (16).

In the final stages of dystrophinopathic cardiomyopathy, where the refractory or intractable heart failure picture dominates, the use of ventricular devices such as Jarvic 2000 may ameliorate the clinical course of cardiomyopathy and prolong the life expectancy of these patients for which cardiac transplantation remains still unaccessible (51-54). Further information can be found in reference 17.

Heart involvement in adult forms of muscular dystrophies

The most frequent forms of adult muscular dystrophies presenting heart involvement are Myotonic Dystrophy type 1 (DM1, OMIM 160900) or Steinert Disease, Emery-Dreifuss Muscular Dystrophies (EDMDs, OMIM 310300; 150330), Desminopathies (DES, OMIM 125660) and Facio-scapulo-humeral dystrophy (FSHD, OMIM 158900).

All these forms share a prevalent involvement of cardiac conduction tissue that leads to the onset of brady or tachy-arrhythmias. These dystrophies are characterized by the presence of increasing degrees of atrio-ventricular (A-V) blocks, ranging from the first degree in which a prolonged PR interval is observed to the complete A-V block and cardiac arrest, caused by the onset of atrial fibrillation/flutter or ventricular tachycardia/fibrillation.

DM1 is the most frequent muscular dystrophy in adults with a prevalence of 1:8000 individuals. Arrhythmias, often manifesting with the dramatic event of the sudden cardiac death (SCD), occur in about 40-60% of cases (55-60). The onset of arrhythmias is unrelated to the age of the patients and the size of the triplets expansion (61-63). Dilated cardiomyopathy is rare and usually limited to the final stages of the disease.

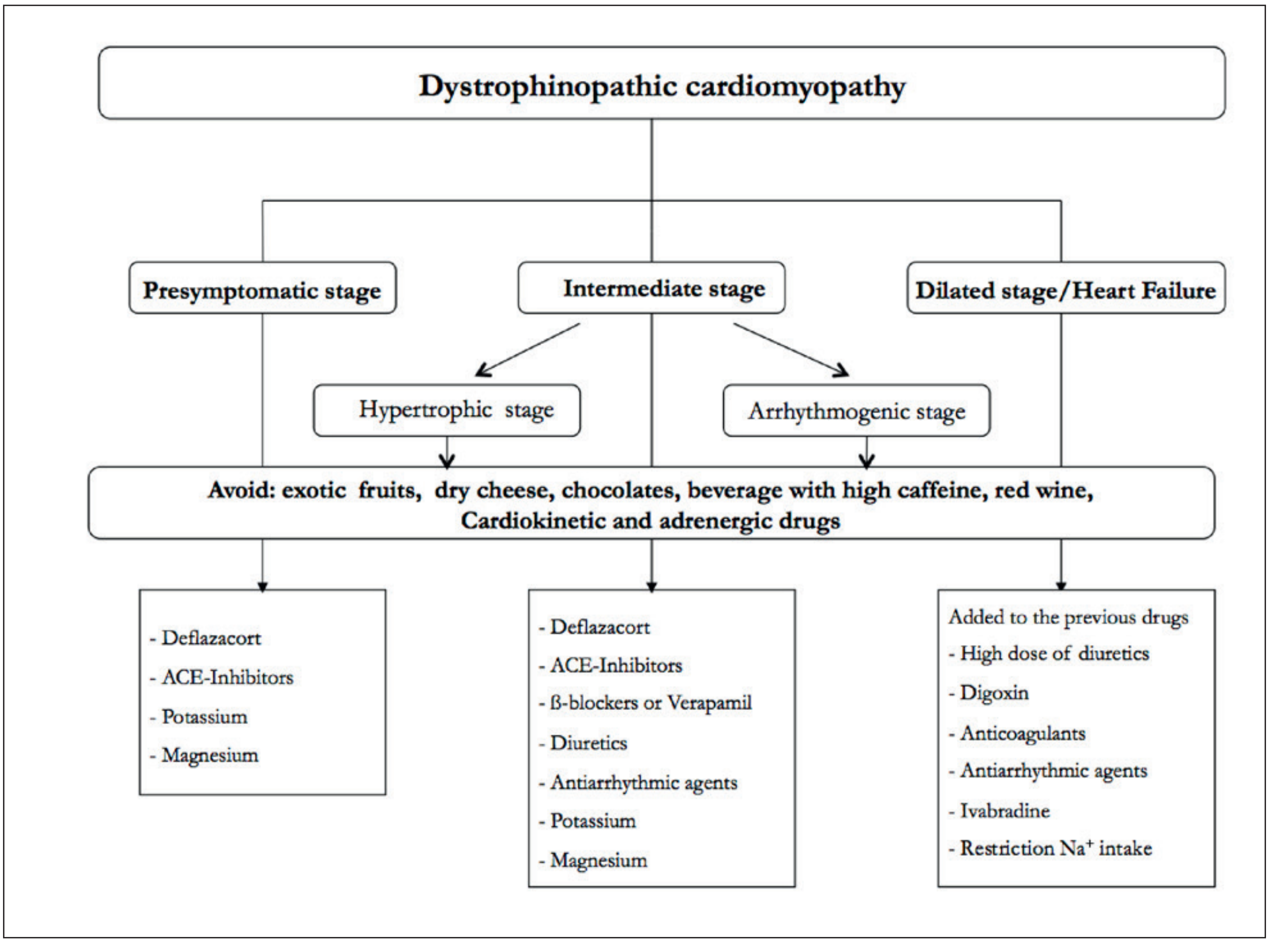

Dystrophinopathic cardiomyopathy
Presymptomatic stage
Intermediate stage
Dilated stage/Heart Failure
Hypertrophic stage
Arrhythmogenic stage
Avoid: exotic fruits, dry cheese, chocolates, beverage with high caffeine, red wine, Cardiokinetic and adrenergic drugs
- Deflazacort
- ACE-Inhibitors
- Potassium
- Magnesium
- Deflazacort
- ACE-Inhibitors
- ß-blockers or Verapamil
- Diuretics
- Antiarrhythmic agents
- Potassium
- Magnesium
Added to the previous drugs
- High dose of diuretics
- Digoxin
- Anticoagulants
- Antiarrhythmic agents
- Ivabradine
- Restriction Na+ intake

Figure 1. Pharmacological treatment of dystrophinopathic cardiomyopathy.

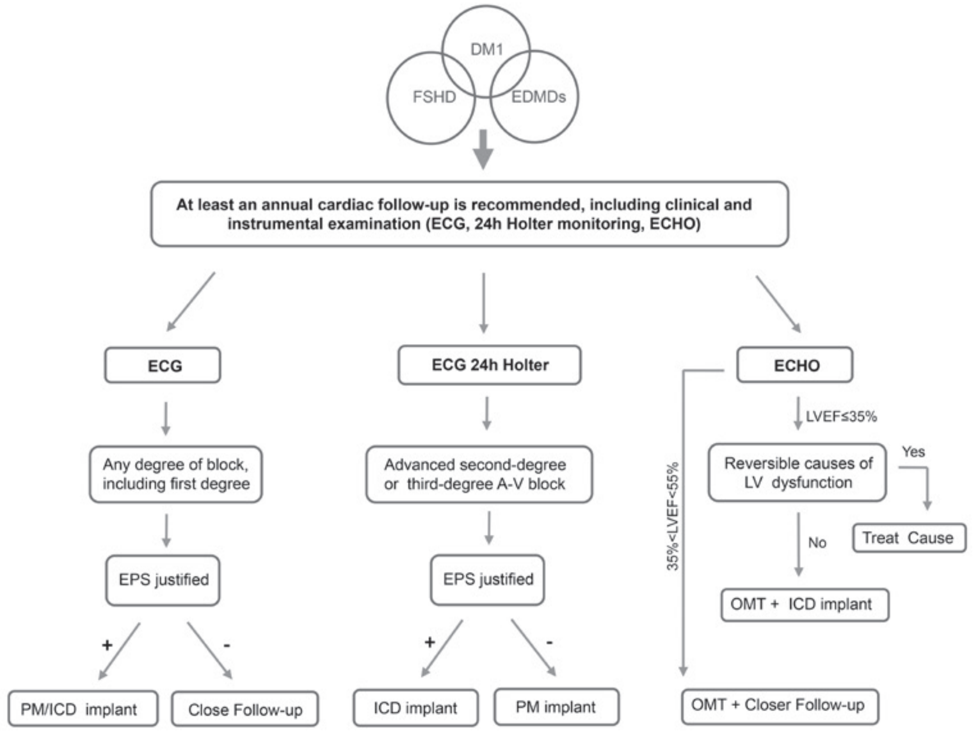
A dedicated cardiac protocol (64) has been developed to prevent the risk of SCD in these patients and includes periodical investigations such as the cardiological examination, ECG, ECG Holter monitoring and echocardiogram to be performed at least every six months (Fig. 2). If during the follow-up increasing degrees of A-V block or pauses > 2,5 sec on Holter monitoring are observed, an EPS is required to evaluate the needs of a device implantation. In our experience, the adoption of this protocol on 247 DM1 patients in the last 15 years resulted in only 2 cases (8 ‰) of SCD, at the beginning of the study. A family history positive for SCD is also a major criterion for a device implantation (65).

It is important to remember that a high number of SCD has been reported in the past in patients with DM1 despite a pacemaker implantation (66). As a consequence, there is an almost unanimous consensus that in these patients the implant of an ICD should be considered as the first choice (67).

A similar protocol can also be applied in patients with Emery-Dreifuss Muscular Dystrophies caused by both emerin (X-linked form) or lamin A/C (Autosomal Dominant or Recessive forms) gene mutations (68-73). However, while in patients with emerinopathy the atrial fibrillation represents one of the triad elements characterizing the phenotype (74), in patients with laminopathy the device implantation may precede of many years the time of the diagnosis. Furthermore in these patients the muscle involvement can be minimal or even absent, further diverting attention from a proper diagnosis. Sudden cardiac death may frequently occur in laminopathic patients (75, 76).

There is no consensus concerning the optimal time to implant a device in these patients, as some clinicians suggest to be aggressive and implant at the time of the molecular diagnosis, while others prefer a periodical follow-up taking into account the criteria adopted for patients with DM1 (66, 76).

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent inherited neuromuscular disorders (77), having significant intra- and interfamilial variability in disease presentation, progression and age


DM1
FSHD
EDMDs
At least an annual cardiac follow-up is recommended, including clinical and instrumental examination (ECG, 24h Holter monitoring, ECHO)
ECG
ECG 24h Holter
ECHO
Any degree of block, including first degree
Advanced second-degree or third-degree A-V block
LVEF≤35%
35%<LVEF<55%
Reversible causes of LV dysfunction
Yes
No
Treat Cause
EPS justified
EPS justified
OMT + ICD implant
+
-
+
-
PM/ICD implant
Close Follow-up
ICD implant
PM implant
OMT + Closer Follow-up

DM1: Myotonic dystrophy type 1; FSHD: Facioscapulohumeral Muscular Dystrophy; EDMDs: Emery-Dreifuss muscular dystrophies, related to Lamin A/C or Emerin gene mutations; ECG: electrocardiogram; ECG 24h Holter dynamic ECG monitoring; ECHO: echocardiography; LVEF: left ventricular ejection fraction; OMT: optimal medical therapy; PM: pacemaker; ICD: implantable cardioverter defibrillator; EPS: electrophysiological study; EPS+: electrophysiological study positive for induction of sustained VT/VF; EPS-: electrophysiological study negative for induction of sustained VT/VF; HV: invasive measure of infrahissian conduction time; VT/VF: ventricular tachycardia/ventricular fibrillation

Figure 2. Cardiac follow-up and management of the most common adult forms of muscular dystrophies.

of onset, with most individuals becoming symptomatic in their second decade (78-81). Patients with childhood onset (i.e. infantile FSHD) have a more severe clinical presentation (78). Juvenile or adult onset male patients are more severely affected than females (80). The disease is characterized by progressive weakness and atrophy of muscles of the face and shoulder girdle, further extending to the proximal arms and legs (77). Non-muscular manifestations of the disease include sensorineural deafness and retinal vasculopathy (82, 83) as well as central nervous system alterations in some severely affected children (84).

Subjects with FSHD generally do not suffer from significant cardiac symptoms (85-87). Studies reported to date indicate that heart alterations unrelated to cardiomyopathy are possible in FSHD, especially in patients older than 45 years (88).

However in the last years some articles appeared showing that a few percentage of FSHD patients may present atrial involvement with episodes of atrial fibrillation/flutter (85, 89). The protocol adopted in these patients includes a cardiological examination, an ECG and a echocardiogram to be performed once at year; more frequently checks – every six month or more – are recommended in the case of arrhythmias.

Management

The pharmacological treatment of patients with a prevalent involvement of the cardiac tissue conduction relies on the use of ACE-inhibitors and appropriate anti-arrhythmic drugs.

In case of atrial arrhythmias it is indicated to prefer drugs as anti-arrhythmics (flecainide, propafenone) and beta-blockers. Amiodarone should be limited to patients not responders to the previous drugs, taking in mind that we are faced with young patients, a long-term therapy, and a high risk of negative effects on the thyroid and pulmonary function (90).

The anti-coagulant therapy – either with the classic warfarin or with the modern class of drugs – is indicated in patients who are implanted to avoid the onset of thrombo-embolic events. In all patients the monitoring of the electrolytes – natrium, potassium and magnesium –

is particularly recommended as the electrolyte imbalance can favor the onset of arrhythmias.

Invasive cardiac treatment should follow the guidelines shown in Figure 1.

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NEWS and VIEWS: mitochondrial encephalomyopathies

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In recent years, there has been a surge of interest in mitochondrial diseases, a group of metabolic conditions caused by impairment of the oxidative phosphorylation system. The ubiquitous presence of mitochondria in all the cells of the body, their role as cell powerhouse and their particular genetic characteristics explain the phenotypic complexity and the diagnostic difficulties, bridging from paediatrician to neurologist.

Key words: mitochondria, mitochondrial DNA, nuclear DNA, encephalopathy, myopathy

Introduction

The mitochondrion is a membrane-enclosed cytoplasmic organelle which has evolved from a primitive aerobic bacteria by means of a symbiotic relationship that started 1.5 billions years ago (1). Mitochondria are described as "cellular power plants" because they produce most of the cell's supply of adenosine triphosphate (ATP), by means of the oxidative phosphorylation (OXPHOS) machinery, which comprises electron transport chain (ETC) and ATP synthase (complex V). The ETC provides the cell with the most efficient energetic outcome in terms of ATP production. It consists of four multimeric protein complexes (complex I to IV) located in the inner mitochondrial membrane together with complex V (2) and also requires two electron carriers, coenzyme Q10 (CoQ10, or ubiquinone) and cytochrome c (cyt c). This metabolic pathway is under control of both nuclear (nDNA) and mitochondrial (mtDNA) genomes (2). Mitochondria, in fact, have their own DNA, the mitochondrial DNA (mtDNA), represented by a circular molecule of 16.5 kb without introns; one mitochondrion can contain two to ten copies of mtDNA (2). Mitochondrial diseases (MD) are the commonest inherited neuromuscular disorders with a prevalence of 1-2 in 10000 (3). They are a heterogeneous group of disorders caused by impairment of the mitochondrial ETC and, although initially considered neuromuscular disorders, MD were soon recognized to be more than just myopathies, when pediatric neurologists placed attention to the frequent occurrence of brain disease in children with mitochondrial alterations in their muscle biopsies and coined the term mitochondrial encephalomyopathies (4, 5), today widely accepted and reserved for defects of the respiratory chain (6).

Molecular and clinical features

The genetic classification of mitochondrial encephalomyopathies distinguishes disorders due to defects in mtDNA, which cause maternally inherited or sporadic disorders, from those due to defects in nDNA, which show a Mendelian inheritance pattern. A more practical way of categorizing mitochondrial encephalomyopathies includes defects of mtDNA maintenance (impairment of intergenomic communication) (7).

Mitochondrial genetics differs from Mendelian genetics in three major aspects: maternal inheritance, heteroplasmy and threshold effect, and mitotic segregation. This genetic complexity explains the great phenotypic variability of mitochondrial disorders and the lack of specific genotype-phenotype correlations. Deleterious mutations of mtDNA usually don't affect all mtDNAs (heteroplasmy) being required a minimum critical mutation load to cause mitochondrial dysfunction in a particular organ or tissue and mitochondrial disease in an individual (threshold effect); moreover, at cell division, the propor-

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tion of mutant mtDNAs in daughter cells may change, varying the phenotype (mitochondrial segregation) (6). The effects of mtDNA defects may have a wide phenotypic variability leading to heterogeneous multisystemic disorders and, although many overlapping syndromes have been described, mitochondrial medicine identified well-defined syndromes: Kearns-Sayre syndrome (KSS), chronic progressive external ophthalmoplegia (CPEO), Pearson syndrome (PS), these first three two syndromes due to large-scale deletions; mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonus epilepsy with ragged-red fibres (MERRF), caused by transfer RNA (tRNA) mutations that globally affect mitochondrial protein synthesis; and Leber hereditary optic neuropathy (LHON), neuropathy, ataxia and retinitis pigmentosa (NARP), maternally inherited Leigh syndrome (MILS) (8), attributable to mutations in a protein-coding gene.

Disorders of nDNA defects refers to genes that encodes approximately 1,700 mitochondrial proteins; pathogenic mutations can directly affect nDNA-encoded respiratory chain subunits, especially in the gigantic complex I and complex II, or indirectly affecting pathways such as proteins expression, translation, import into mitochondria, assembling with their mtDNA encoded counterparts (9, 10). Complex I deficiency is the commonest biochemical defect found in mitochondrial disorders and it is associated with a broad range of clinical phenotypes ranging from lethal neonatal disease to adult onset neurodegenerative disorders (11). Mutations in complex I assembly proteins can manifest as Leigh syndrome (NDUFAF2 and NDUFAF5), encephalopathy (NDUFAF4) and cardioencephalomyopathy (NDUFAF1) with a high level of genetic heterogeneity and weak genotype–phenotype correlations (10). Leigh syndrome, also defined as subacute necrotizing encephalomyelopathy, is a devastating progressive neurodegenerative disorder of infancy or early childhood associated by specific neuropathological features: bilaterally symmetrical foci of cystic cavitation, vascular proliferation, neuronal loss, and demyelination in the basal ganglia, brainstem, and posterior columns of the spinal cord. It is characterized by decompensation during an intercurrent illness and typically associated with psychomotor retardation or regression, often followed by transient or prolonged stabilization or even improvement, but inevitably resulting in eventual stepwise progressive neurologic decline (12). Mutation in complex II proteins are rarely associated with Leigh's syndrome but are a common cause of inherited paragagliomas and phaeochromocytomas (13). Complex III deficiency typically causes a severe multisystem early onset disorder, which is recessively inherited and rare (14). Mutations in complex IV or in complex IV assembly factors result in severe, typically fatal, infantile disease. Complex IV assembly gene disorders include *SURF1* (Surfeit locus protein 1), associated with Leigh Syndrome. Mutations in nDNA-encoded complex V subunit genes also appear very rare (10).

The role of mtDNA maintenance, including replication and integrity, is under control of the nucleus; when the dialogue between the two genomes becomes impaired, the resulting diseases are characterized by mtDNA depletion, multiple mtDNA deletions, and site-specific mtDNA point mutations (15). Mutations in several genes have been associated with defects in mtDNA maintenance, these include *ANT1*, which encodes the adenosine nucleoside translocator; *PEO1*, which encodes a helicase called Twinkle; *TYMP*, which encodes the cytosolic enzymem thymidine phosphorylase (TP); *POLG*, which encodes the mitochondrial polymerase γ catalytic subunit and is by far the commonest cause of mtDNA stability disorders; and *POLG2*, which encodes the dimeric accessory subunit of POLG (16). *POLG* gene example shows how a single mutant gene can cause either mtDNA depletion or multiple mtDNA deletions and result in diverse syndromes from a severe hepatocerebral disorder of infancy or childhood (Alpers syndrome), through to adult-onset autosomal dominant or recessive progressive external ophthalmoplegia (PEO), to parkinsonism and other clinical phenotypes, including sensory ataxic neuropathy, dysarthria and ophthalmoparesis (SANDO), or mitochondrial recessive ataxia syndrome (17-18). MtDNA depletion may be linked to other syndromes: encephalomyopaty (linked to mutations in *SUCLA2*, *SUCLG1* or *RRM2B*), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE; caused by mutations in *TYMP*), myopathy (caused by mutationsin *TK2*), hepatoencephalopathy (associated also to mutations in *DGUOK* and *POLG*).

The concept of multi-system disease is crucial in mitochondrial medicine and makes the molecular diagnosis challenging, as many different medical specialties are involved. Tissues and organs with high energy demands are most severely affected. Accordingly, besides clinical pictures that primarily affect a specific tissue such as primary mitochondrial myopathy, mitochondrial dysfunction typically affects also brain (e.g., seizures, stroke–like episodes, diffuse encephalopathy, ataxia, parkinsonism, dementia), sense organs (deafness, optic atrophy, retinal pigmentary degeneration), extraocular muscle (ptosis, external ophthalmoplegia), skeletal muscle (myopathy, exercise intolerance), heart, liver (hepatopathy), kidney (renal tubular acidosis) and endocrine system (diabetes).

Diagnosis

The diagnosis of MD diseases is complex and requires several investigations including routine and particular laboratory tests, electrophysiological studies, neuroimaging studies, muscle biopsy and genetic test.

The first step in the diagnosis is represented by the knowledge of patient and family history and physical and neurologic examination of the patients (19), in order to research the "mitochondrial red flags" often overlooked (19).

Generally, serum creatine kinase levels are normal or mild elevated; one exception is the myopathic variant of the mtDNA depletion syndrome (19). Elevated lactate and/or elevated lactate to pyruvate ratio can suggest the presence of mitochondrial dysfunction but they can also be caused by other conditions such as organic acidemias, other inborn errors of metabolism, toxins, tissue ischemia, and certain other diseases (20). Simple, non-invasive test for metabolic myopathies are the forearm exercise test and incremental exercise test on a cycle ergometer, which can reveal an exaggerated increased production of lactate with muscle activity in the first case and an anticipation of the anaerobic threshold in the second case (21-24). Several amino acids including alanine, glycine, proline, and threonine found to have high levels in mitochondrial disorders, conversely citrulline, was found to be significantly decreased in the plasma of subjects with mitochondrial disease (25-27). The exact sensitivity and specificity of amino acid elevations in patients with mitochondrial disease are not yet known. Lactic aciduria is often seen in mitochondrial diseases and the major biomarkers of mitochondrial dysfunction are 3-methylglutaconic acid, dicarboxylic aciduria, 2-oxoadipic aciduria, 2-aminoadipic aciduria, and methylmalonic aciduria, malate and fumarate (28-32).

Recently, Lehtonen and coworkers analyzed serum values of FGF21 and GDF15, two new promising biomarkers, from patients with mitochondrial diseases and non-mitochondrial disorders partially overlapping with mitochondrial disorder phenotypes providing Class III evidence that elevated FGF21 accurately distinguishes patients with mitochondrial myopathies from patients with other conditions (33-35), and FGF21 and GDF15 mitochondrial myopathy from other myopathies (33-36). However, the validation of this evidences and the definition of a reliable specific biomarker of mitochondrial disease, also for prognosis and, especially, form monitoring drug-response to treatment is still strongly needed.

Usually electromyography shows specific myopatich pattern but may be also normal (24-37). Electroneurography is abnormal in those forms of mitochondrial myopaties presenting with neuropathy (38).

Neuroimaging may play a significant role in the diagnosis of mitochondrial disorders, especially some patterns of magnetic resonance imaging (MRI) of the brain. MELAS syndrome is characterized by stroke-like lesions that do not respect vascular territories (39), while more florid white matter abnormalities are seen in MNGIE, Leigh syndrome, and mitochondrial disorders due to defects in the aminoacyl-tRNA synthetases. Also Proton magnetic resonance spectroscopy (MRS) is useful and may demonstrate high levels of lactate or succinate (39-41).

The gold standard to demonstrate mitochondrial dysfunction in vivo is muscle biopsy (42, 43). The main pathological features of MD are ragged red fibers (RRF) (obtained trought modified Gomori trichrome stain) or ragged blue fibers (RBF) (when using succinate dehydrogenase staining) and COX negative fibers (44). RRF or RBF consist of a subsarcolemmal accumulation of enlarged, abnormal mitochondria with ultrastructurally dense cristae and paracrystalline inclusions (45), probably an attempt to compensate the respiratory chain dysfunction. Mitochondrial proliferation with RRF is typically found in patients with deletions, depletion or point mutations in tRNA genes (MELAS, MERRF) (46). In contrast, RRF are almost never observed in patients with mtDNA point mutations of structural genes (LHON, NARP, Leigh) (47). On the contrary, RRF may be found in Leigh syndrome (48). Importantly, normal muscle histology does not rule out a MD. Biochemical spectrophotometric investigations can be performed in tissue homogenates to measure the activity of respiratory chain (RC) enzymes. A mutation in a nDNA or mtDNA gene encoding a structural subunit of the RC commonly results in deficiency of the solitary affected enzyme, whereas the impairment of mitochondrial protein synthesis (mutations in tRNA, single or multiple deletions, and mitochondrial depletion) reduces the activity of respiratory complexes I, III, and IV while sparing complex II (SDH) which is entirely encoded by nDNA (19).

Genetic studies can be performed on muscle biopsy, to detect single or multiple deletions of mtDNA and/or mtDNA depletion and to sequence the entire mtDNA for point mutations (49). Molecular studies on peripheral circulating cells or other easily accessible tissues (like urinary sediment, oral mucosa, hair follicles and cultured skin fibroblasts) can be performed. Genetic studies on blood cells are more useful in nDNA than in mtDNA-associated disorders because, as a result of the mitotic segregation, mtDNA mutations (especially mtDNA deletions) are more easily detected in muscle than other tissues (49-19). Interestingly, urine sediment often contains mtDNA mutations at higher levels than blood, buccal swabs, or even fibroblasts so screening urine for mtDNA mutations may be recommended before muscle biopsies (24-37).

Therapy

Currently, there is no available disease-modifying therapy for mitochondrial disorders. Therefore, treatment of mitochondrial disease involves predominantly supportive care, early treatment of organ-specific manifestations with pharmacological therapy (for example, antiepileptic drugs for seizures, dietary or pharmacological therapy for diabetes mellitus) and surgical remedies (such as blepharoplasty, cochlear implants for hearing loss and placement of a cardiac pacemaker or ablation of secondary conduction pathways) which are useful in prolonging and improving the quality of life of patients (50).

The most obvious strategy to treat mitochondrial disorders is to enhance RC function mitigating both energy crisis and oxidative stress, and remove noxious metabolites (like lactate, thymidine). Several agents (mostly nutritional supplements) have been investigated with double-blind, placebo-controlled studies. These include antioxidants (such as CoQ, α-lipoic acid, vitamin C and vitamin E), agents that modulate mitochondrial electron transfer flux (such as riboflavin), ROS scavengers (Coenzyme Q10, MitoQ, glutathione) nitric acid precursors (such as l-arginine), energy buffers (such as creatine and l-carnitine) and drugs involved mitochondrial biogenesis (such as vitamin B3) (51).

None has demonstrated a striking efficacy in clinical trials, although numerous non-blinded studies, anecdotal case reports and small series have suggested modest efficacy (52-54). Coenzyme Q10 is specifically indicated in patients with defects of CoQ10 biosynthesis who show a dramatic improvement following high doses and long-term CoQ10 supplementation (55). Idebenone, a short-chain benzoquinone, is the only disease-specific drug recently approved to treat visual impairment in adolescents and adults with Leber's hereditary optic neuropathy. Early and prolonged idebenone treatment may improve significantly the frequency of visual recovery and possibly change the natural history of the disease, as demonstrated in two clinical trials and retrospective case series (56, 57). Patients with stroke like episodes seem to benefit from oral/intravenous arginine or citrulline (nitric oxide precursors) supplementation with improvements on clinical symptoms associated with stroke-like episodes and decreased severity and frequency of these episodes (58, 59).

Children with liver failure due to hepatocerebral syndromes associated with mtDNA depletion and mutations in *DGUOK* or *POLG* may also benefit from liver transplantion, especially if the brain and other organs are relatively spared (60).

There has been great interest in exercise regimen and their benefit on both biochemical and clinical end-points in mitochondrial disorders. Aerobic, endurance, and resistance training programs have been studied. It is likely the benefits of exercise are due to reversal of deconditioning, which is a common feature of many muscle diseases. Furthermore exercise seems to alter the underlying pathology by promoting mitochondrial biogenesis, increasing antioxidant enzyme activity, muscle mitochondrial enzyme activity, maximal oxygen uptake, and peripheral muscle strength (61, 62).

Other management considerations in mitochondrial disease include the avoidance of agents, which may worsen the patient's condition (51). Statins often cause toxic effects on skeletal muscle, although the precise mechanisms remain unclear. Statin should therefore be used cautiously in mitochondrial disease, with careful monitoring of symptoms and the serum creatine kinase. Antiretroviral agents are known to cause reversible and dose-dependent mitochondrial toxicity. Valproic acid is known to interfere with mitochondrial function and in clinical practice may aggravate symptoms in patients with mitochondrial disease, and valproate-induced hepatotoxicity may be more common. Antibiotics, (specifically minocycline, chloramphenical, and aminoglycosides), can be harmful to the mitochondria because they inhibit mtDNA translation and protein synthesis, causing hearing loss as well as cardiac and renal toxicity. Mitochondrial patients may be at a higher risk for propofol infusion syndrome and propofol use should be avoided or limited to short procedures; narcotics and muscle relaxants can create respiratory depression, and caution must be used in mitochondrial patients who may already have hypotonia, myopathy, or an altered respiratory drive.

Although therapies for specific mitochondrial diseases, such as MNGIE, are emerging, treatment for the vast majority of mitochondrial disorders is limited and relies on symptomatic management, so new treatment approaches are strongly needed. One of the most promising strategy is the use of molecules able to enhance mitochondriogenesis. Biotechnology companies in the US and the Netherlands have already launched early phase I and II studies for drugs targeted at MD patients. In 2014, Cerutti and Coworkers showed here that supplementation with nicotinamide riboside, a natural NAD(+) precursor, or reduction of NAD(+) consumption by inhibiting the poly(ADP-ribose) polymerases, leads to marked improvement of the respiratory chain defect and exercise intolerance of the Sco2 knockout/knockin mouse, a mitochondrial disease model characterized by impaired cytochrome c oxidase biogenesis, highlithing this strategy as potentially translatable into therapy of mitochondrial disorders in humans (63). Future strategies are also expected for MNGIE, such as liver transplantation (64) which has been demonstrated capable of rapidly normalize serum levels of toxic nucleosides, or gene therapy using a liver-targeted AAV vector transferring of the human *TYMP* coding sequence (65).

Future perspective

While enormous progress has been made in diagnostics and pathomechanisms in MD, major advances in treatment have unfortunately not paralleled this so far and the clinical management is mainly focused on symptom control. The heterogeneity of MD, the contribution of two genomes, the little knowledge of their natural history, lack of awareness among general practitioners and sometime specialists and the complexity of the diagnostic approach all contribute to the unsuccessful management of these diseases and limit the correct interpretation, reproducibility and comparability (including the possibility of meta-analyses) of clinical trials. Thus, it is crucial to better define all of the clinical, biochemical, histological and molecular factors involved in MD as well as genotype-phenotype correlations and the natural history of the different syndromes.

In the last several years mitochondrial medicine has been extremely successful in the characterization of the molecular and genetic basis of disease. However, "deep phenotyping" in mitochondrial disorders is a challenging but necessary task as well. Clinical variability is broad even in individuals with the same genotype and the statistical power is low in single-center studies, owing to the rarity of these conditions. Large and comprehensive patient registers may represent the right instruments to fill this lack and put fundamental basis to launch collection of longitudinal clinical data and to build controlled clinical trials. Granted by Telethon-UILMD in 2009, the nation-wide Italian collaborative network has been established a web-based registry of patients with MD harmonized with other European Databases and Networks, collecting and characterizing clinically, histologically and genetically more than 1400 patients so far, with both adulthood and childhood onset of the disease. This Network has been instrumental to redefine the clinical features of common mtDNA mutations (e.g., m.3243A > G (66), m.8344A > G (67), single deletions (68)) and to better elucidate some signs and symptoms of mitochondrial diseases including myoclonus (69) and peripheral neuropathy (38). The register has also allowed the dissemination of new knowledge and highlight MD to public opinion makers.

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New generation emerging technologies for neurorehabilitation and motor assistance

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This paper illustrates the application of emerging technologies and human-machine interfaces to the neurorehabilitation and motor assistance fields. The contribution focuses on wearable technologies and in particular on robotic exoskeleton as tools for increasing freedom to move and performing Activities of Daily Living (ADLs). This would result in a deep improvement in quality of life, also in terms of improved function of internal organs and general health status. Furthermore, the integration of these robotic systems with advanced bio-signal driven human-machine interface can increase the degree of participation of patient in robotic training allowing to recognize user's intention and assisting the patient in rehabilitation tasks, thus representing a fundamental aspect to elicit motor learning

Key words: exoskeletons, EMG, EEG, human-machine interface, ADL, neurorehabilitation, motor assistance, emerging technologies

In the recent years we have assisted to a raising and growing interest in wearable technologies for neuromotor rehabilitation and assistance and in particular upper and lower limb robotic exoskeletons[1] that can provide autonomous walking to paraplegics after cerebrovascular stroke, spinal cord injury, or peripheral neuropathies.

In general (1, 2), a wearable device is defined as an active mechanism that: (i) is essentially anthropomorphic in nature; (ii) can be "worn" by an operator; (iii) fits closely to his\her body; (iv) works in concert with the operator's movements.

Robotic exoskeletons for rehabilitation and assistance

In developed countries, stroke affects 1/500 *every year*, representing the 3rd cause of death (after cardiovascular diseases and tumours), and is the leading cause of long-term disability, with severe limitation in activities of daily living (ADLs). Motor recovery after stroke is still possible to different extents thanks to regain of function promoted by physical therapy and exercise, induced by changes in cortical reorganization according to the residual neuroplasticity. Four main factors are considered as the major determinants of motor recovery: early intervention, task-oriented training, amount and scheduling of practice and degree of participation (3, 4). In particular task oriented training stands for a repetitive training of functional (skill-related) tasks. Task-oriented training has been clinically tested for training locomotion (5), balance (6), arm-hand function recovery, motor control and strength in stroke patients (7). A fundamental principle in motor learning is that the degree of performance improvement increases with the amount of practice. Practice can be accomplished in a number of ways that are more effective than blocked repetition of a single task (massed practice). The degree of participation in patient represents a fundamental aspect to elicit motor learning, as volitional effort and active involvement is required to induce cortical reorganization (8).

In this regards, robotics represents *a key enabling technology* to enhance the recovery process and *minimize functional disability,* with consequent earlier reintegration in ADLs. Conventional neuro-rehabilitation appears to have little impact on impairment over and above that of spontaneous biological recovery (8). Robotic aided therapy has shown to be *more effective* than traditional physical therapy in providing high intensity of exercise, better movement controllability and measurement reliability, which makes robots ideal instruments to help neurologists and therapists in addressing the challenges in neuro-

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[1] From the greek ("exos" outside and "skeleton").

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rehabilitation. In fact, unlike conventional therapy, rehabilitation robots can deliver training at a much higher dosage (i.e., number of practice movements) and/or intensity (i.e., number of movements per unit time) with hundreds if not thousands of repetitions in a single session (8).

Several studies have already demonstrated the efficacy of the robotic treatment. In the article of Lo et al. (9) the authors have shown how robot-assisted therapy in upper limb rehabilitation led to significant improvements in motor capability and motor-task performance, as compared with usual care, and demonstrated how robot-assisted therapy is equivalent to an intensive conventional therapy. Recent available reviews of literature prove that patients who receive electromechanical-assisted arm training after stroke are more likely to improve their generic ADLs and may improve arm function (10).

Upper limb exoskeletons are typically used for rehabilitation of arm and hand function (11).

The ALEX exoskeleton developed at SSSA (12) is an example of a new exoskeleton device with unique properties in terms of kinematics and actuation: due to the adopted design the system can achieve a very light weight for actuating 4 degrees of freedom, thanks to a series elastic tendon transmission that guarantees intrinsic mechanical compliance. This compliance represents an important requirement for granting adaptability to patient movements on one side and high safety standards.

Lower limb exoskeletons, instead, offer patients' the freedom from a wheelchair, resulting in important positive aspects such as increased freedom to move and perform ADLs and hence improved quality of life; but also in terms of improved function of internal organs and general improved health status. The approach that makes use of robotic exoskeleton for gait assistance in disabled persons represents a revolution and a paradigmatic shift beyond traditional wheelchairs. Recent market estimates forecast the global exoskeleton market, in three markets (military, factory and rehab/healthcare), to grow at a 72.5% Compound Annual Growth Rate (CAGR) from 2014 through 2019.

Although robotic exoskeletons for lower limbs have attracted a strong attention from medical and patients' communities, they are still far from addressing the real needs of paraplegic persons. One aspect that needs to be further researched, it is that only a few of them are able to provide the balance of person by the device itself, but rather rely on the person to use crutches to keep the balance.

In 2012, it was estimated that worldwide over 185 million people use a wheelchair daily, and almost 20 per cent of the world's population is now aged over 65 years, and that is forecast to exceed 35 per cent by 2050. Accordingly, there is a growing need for devices that can assist the injured and elderly to enjoy a degree of independence and maintain a more active lifestyle.

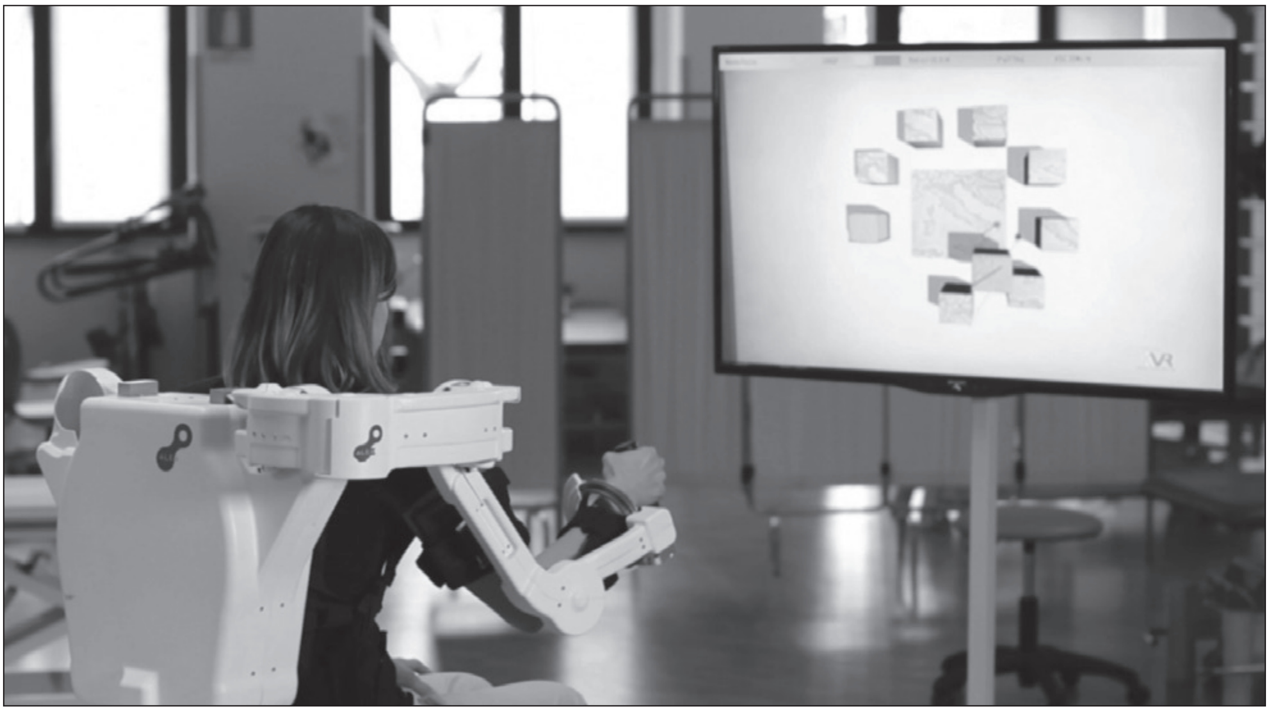

Figure 1. The Alex 2 exoskeleton developed by Scuola Sant'Anna (commercialized by Wearable Robotics srl) integrated with a serious rehabilitation game.

At the state of the art (2, 13), the broad variability in mechatronic design, control and human-robot interface of these devices is due to differences in the targeted end- users and expected usage.

Among all the assistive devices emerging in the last decade, wearable robotic exoskeletons' were proposed as an innovative solution by many research centers active in the field of medical robotics to provide additional power for walking or stair-climbing to people affected by gait disorders. In such different human-robot interaction scenarios, the assistive torques contribute only partly to the body motion and, in the meantime, the exoskeleton must comply with the user's motion. Such a wearable medical exoskeletons require the patient to balance themselves which is in contrast to rehabilitation exoskeleton which are often equipped with a body weight support system.

The most of these devices use electric motors to actuate hip and knee joints and sometimes also the ankle joints.

Exoskeletons used for therapy are not portable and do not stand-alone mechanically (the so called treadmill-based exoskeletons). In fact, the main role of these robotic platforms is to support the patient weight and to generate symmetrical and periodic gait patterns for rehabilitation purpose only.

Finally, the impressive aspect of all described devices and tools is that all the outcomes and the developed technologies resulting from post-stroke research can be translated to neuromuscular rehabilitation and motor assistance since some medical needs and consequences of people suffering from dystrophinopathies are shared with post-stroke patients.

Biosignal-based interface for robotic tool control

One of the major challenges for tools for new generation rehabilitation is to produce devices able to recognize user's intention and assisting him/her in rehabilitation tasks. An effective approach for tool control is based on the use of bio-signals. Indeed, they allows to increase the degree of participation of patient in robotic training which has been demonstrated to represent a fundamental aspect to elicit motor learning, since volitional effort and active involvement are required to induce cortical reorganization, while passive movement driven by the robot along a trajectory does not result in learning (14).

Two increasingly used bio-signals in rehabilitation are Electromyography (EMG) (the electrical manifestation of the neuromuscular activation associated with a contracting muscle) collected from preserved or moderately impaired muscles and Electroencephalography (EEG) (the electrophysiological monitoring method to record electrical activity of the brain).

EMG-based interfaces

The "assistance as needed" rehabilitation paradigm (15) according to which the degree of assistance provided by the robotic tool is no more than the required one, has led to the development of adaptive controllers aimed to provide a rehabilitation protocol tailored to the condition of each patient. In particular, surface EMG-based (sEMG-based) control of robot represents a natural way to implement assistive controllers that provide assistance based on the level of muscle activation.

Most upper limb exoskeleton robots also use regression control approaches to map EMG signal features into torques either proportionally (16), or through a muscle model, or a neural network (17).

In triggered assistance, the subject initiates the movement without assistance, with the robot observing on-going performance and intervening taking full control when the task is not completed. On the other side in adaptive control strategies such as EMG-proportional, the power of the EMG signal is used to directly control the actuators (18). This is the case of probably the simplest case of model-free paradigm, the EMG-proportional approach, in which the assistance proportional to the EMG activation signal is provided through the robot.

To overcome these limitations, in more advanced model-free paradigms, sEMG signals are processed using machine learning techniques (17).

EEG-based interface

A Brain Computer Interface (BCI), or Brain Machine Interface (BMI), is a system that uses brain signals to drive external devices without the use of peripheral physiological activities (19).

Once the users' brain activity is recorded, it is decoded by means of an on-line classification algorithm and the output of the classifier is fed back to users allowing them to modulate their brain activity (20).

Depending on the aim of BCI application, two major approaches can be distinguished: assistive BCI and restorative BCI (21).

Assistive BCI systems aim at high dimensional control of robotic limbs or functional electric stimulation (FES) that specifically activate paralyzed muscles to assist in performing ADLs (22). These systems aim at having a large number of output commands and they often use mental strategy based on external cues such as Evoked Potentials (EP). For instance Ortner et al. (23) proposed the use of a Steady State Visual Evoked Potential (SSVEP) based BCI to control a two-axes electrical hand prosthesis.

The aim of Restorative BCI, instead, is to selective induce use-dependent neuroplasticity to facilitate motor recovery. In neuro-rehabilitation, there are now sufficient evidences that non-invasive BCI (often based on EEG) may provide an advantage compared to traditional rehabilitation methods in patients with severe motor impairment (24).

For instance, BCI based on Motor Imagery (MI-BCI) can provide a valid substitute for active motor training as a mean to activate the motor network (25), thus influencing motor recovery in a positive way.

The combination of MI with a congruent and appropriate bio-feedback originated from the BCI system can provide a twofold advantage: it generates a normal afferent-efferent feedback loop (20), useful for neuro-rehabilitation purposes, and improves consistency of MI features detected by BCI (26). The development of restorative BCI systems is tightly associated with the development and successes of neurofeedback and its use to purposefully up-regulate or down-regulate brain activity.

The use of sensory feedback in BCI could improve the performances of the BCI itself and it allows to close the sensorimotor feedback loop (13) .

Considering that BCI technology is based on feedback and exploits learning mechanisms, a logical step forward would be to design and develop specific BCI-protocols for patients.

Numerous research groups have recently provided evidence that this type of BCI leads to functional improvements in upper limb or hand function.

Motivations and benefits of the MI-BCI initiated rehabilitation systems have been discussed by several researchers so far and, currently, it might be concluded that BCI systems are a promising tool to add to the neuro-motor rehabilitation toolbox.

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Hard ways towards adulthood: the transition phase in young people with myotonic dystrophy

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Myotonic dystrophy type 1 (DM1), also called Steinert's disease, is a genetic multisystem disorder that has raised, in the last years, high interest because of the high variable clinical spectrum and related disability. Children with myotonic dystrophy are affected by behavioural problems and intellectual disability, finally impacting on their degree of engagement in family, work and social activities. The transition phase, representing the process of moving from adolescence to adulthood, can be severely affected by growing up with a neuromuscular disorder, with significant impact on patient's and families' quality of life. Although conceptual models of health assistance for individual with genetic disorders have already been proposed the burden for the patient and his family is still relevant. Therefore to afford this critical condition it would be suitable to plan proper educational and psychosocial programs, identifying areas of unmet needs and targeted health objectives that ensure the right support to DM1 population.

Key words: myotonic dystrophy, transition, cognitive impairment, neuropsychological impairment

Myotonic dystrophy

Myotonic dystrophy type 1 (DM1), also called Steinert's disease, is a multisystem disorder due to a CTG triplet repeat expansion ([CTG]n) within the myotonic dystrophy protein kinase (DMPK [RefSeq NM_001081563.2]) gene located on chromosome 19q13.3. The main pathogenic mechanisms consists in the deposition of an abnormal transcribed but not-translated RNA from the sequence [CTG]n, within the nuclei of affected cells that express such gene.

In the last years, myotonic dystrophies have been the subject of extensive research because of the high variable clinical spectrum and related disability. Tipically, symptoms become evident during mid-life, but signs can be detectable in the first or second decade; different clinical phenotypes can be recognized based on the age of the affected individual when symptoms first appear: congenital, childhood and classic or late-onset (1).

Congenital DM1, a potentially life-threatening condition at birth, present with hypotonia and severe generalized weakness at birth, often with respiratory insufficiency; severe intellectual disability is also frequent in these children.

Individuals with childhood-onset DM1 develop symptoms between early childhood and early adolescence after a relatively normal birth and infancy, while In classic and adult-onset DM1 forms, symptoms can appear from late adolescence through old age. The disease usually worsen over time, affecting many organs and tissues including skeletal muscle as well as eyes, heart, gastrointestinal tract, endocrine system, and central nervous system (CNS).

There is a general correlation between the degree of expansion and the severity of clinical manifestations (2); an increase of the repeat sizes of the CTG expansions is more frequently associated with female than male transmission (3).

Patients affected by DM1 can be subclassified on the basis of the [CTG]n: E1: 37-150 CTG; E2: 150-1000 CTG; E3: over 1000 CTG (4).

Since the clinical spectrum of different forms of DM1 is highly variable, we will focus on this disease in our forthcoming argumentation, as a complex disease-model in which the *transition* phase can be critical.

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Transition in DM1: a bridge from adolescence to young adulthood

In medicine, the *transition* phase indicates the process of changing or moving from one state to another that involves young people aged from 13 to 25; the transition phase usually involves also the families and the professionals living around the patient that *is moving* from being a child to become a young adult.

The definition of transition given actually, grows out of *crisis theory*, the foundations of which were laid by Erich Lindemann's studies (5); according to later investigations by Moos and Tsu (6) "crisis theory asserts that people generally operate in consistent patterns, in equilibrium with their environment, solving problems with minimal delay by habitual mechanisms and reactions. When the usual problem-solving strategies do not work tension arises and feeling of discomfort and strain occur and the individual experiences anxiety, fear, feeling of helplessness, and disorganization". Adolescent psychologist, Erickson (7), explains this crisis is not only the search for a new identity but also a biologically driven physical change. Transition to adulthood for young people, particularly for those affected by a neuromuscular disorder, means to navigate in a vast range of social frameworks (Fig. 1), at a time of life in which an individual is already vulnerable, whether living with muscle disease or not.

Much empirical work has been done on *human adaptation* to life transitions, showing that individuals are different in their ability to adapt to novelty and change, especially if affected by a chronic disease (8).

Neuromuscular disorders have a high symptom burden and are frequently associated with many adverse psychosocial outcomes, particularly reduced quality of life and impairment in adaptive behaviour (9, 10). A recent work assessed that the psychosocial wellbeing of younger adolescents on degenerative disease trajectories is severely affected by growing up with a neuromuscular disorder, with significant impact on mental health and on levels of engagement in social activities; anyway longitudinal studies are still scarce and there is lack of population based recruitment strategies (11).

It is widely recognized that beyond muscle involvement, DM1 patients present with intellectual, behavioural and emotional impairments, in all the four clinical phenotypes, as a result of central nervous system (CNS) involvement. In the last decades the study of DM1 patients' behaviour became systematic and some authors have re-

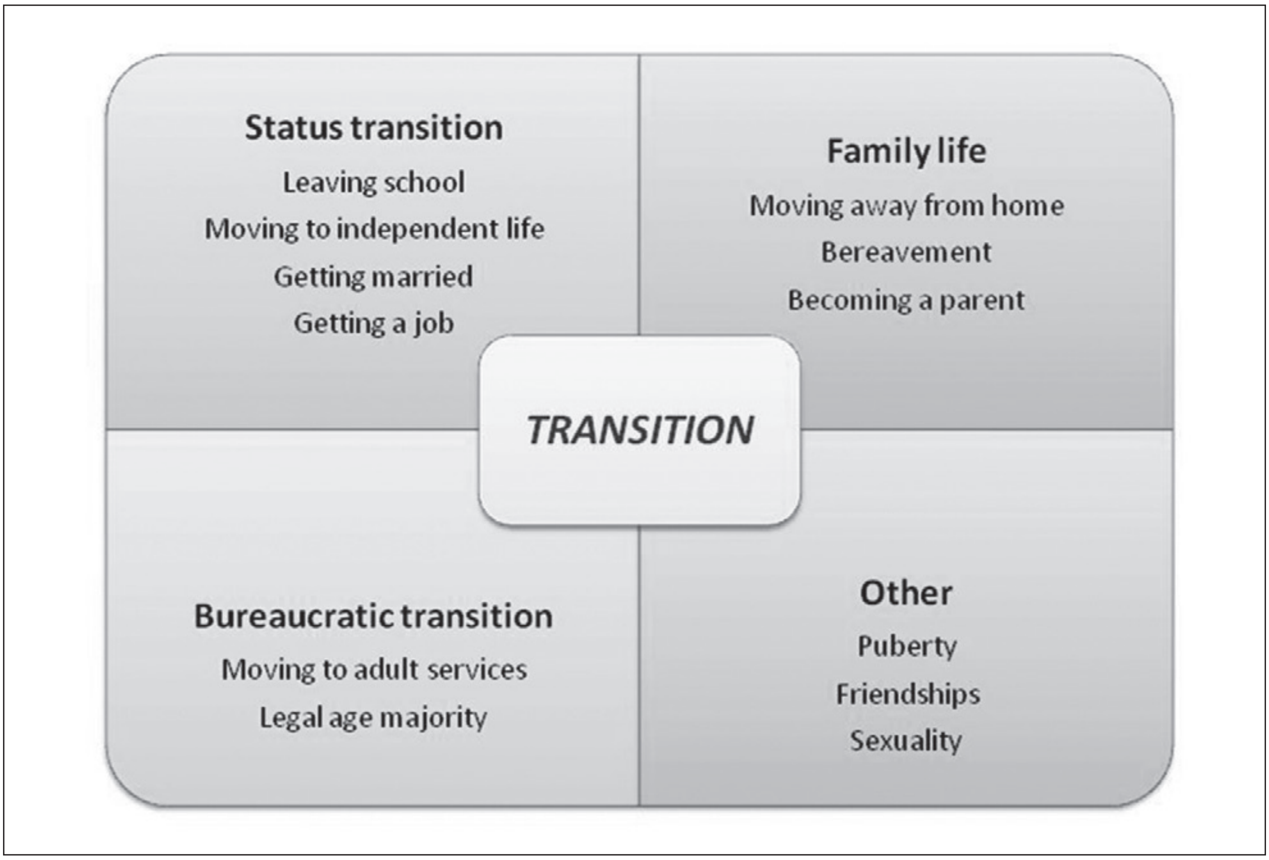

Figure 1. Different social frameworks involved in transition.

ported learning disability to be the most important feature of congenital and childhood DM1 (4, 12, 13), and muscle strength is not pointed out as a major clinical problem, in opposition to the adult-onset phenotype (14); notably, no studies have specifically defined the adult period for the childhood phenotype.

Even if there is no absolute distinction between the different phenotypes the presence and severity of symptoms as well as management issues are quite different from one phenotype to another; in literature while the adult- and late-onset phenotypes have been much studied, publications about the congenital and childhood, especially the long-term outcome, are scarce.

When symptoms develop early in life, this is referred to as congenital or childhood-onset myotonic dystrophy. Currently accepted classifications define congenital myotonic dystrophy patients as having symptoms at birth, whereas childhood-onset myotonic dystrophy patients develop symptoms after birth but before age 10 (4, 15). Children affected by DM1 present learning disability and lower performance in relation to adaptive behaviours, communication and socialization (16); these difficulties in association with lower cognitive functioning hamper participation in playing appropriate social roles while reaching adulthood.

Children with congenital myotonic dystrophy frequently are hypotonic at birth and can have feeding difficulty, respiratory failure, moderate to severe intellectual disability, and autistic features as they age (17). Differently, myotonic dystrophy with childhood-onset can be characterized by development problems, especially intellectual disability and prominent dysarthria (18).

In spite of in the literature the highest prevalence of intellectual impairment is reported in cumulative studies not stratified for the different clinical forms, therefore considering adult and congenital onset of DM1 alltogether, it is commonly accepted that congenital form is that one usually characterized by a critical reduction of intelligence quotients in both verbal and nonverbal abilities, as compared to normal control subjects (19-21).

Young DM1 patients can also present with an autism spectrum disorder (ASD), significantly associated with the DM1 phenotype; moreover ASD and/or other neuropsychiatric disorders such as mental retardation, attention deficit hyperactivity disorder, and Tourette's disorder have been found (17), this rising the importance of be aware of behavioural comorbidities in DM1 patients. Interestingly (22) reported that also adult-onset patients with DM1 are affected by social cognitive impairment – commonly defined *theory of mind* (ToM) – that refers to the ability to understand people's mental states, and to establish good relationships in social situations, this suggesting the hypothesis of a continuum between children and adult disturbances in social interaction.

A comparative study highlighted the impact of DM1 symptoms on quality of life, referred by the patients: communication difficulties, cognitive impairment, and social role limitations were the most frequently identified themes, which play a key role in the disease burden (23). However congenital and childhood-onset myotonic dystrophy patients highlighted more learning/concentration difficulties, whereas adult myotonic dystrophy participants were more likely to identify fatigue, reduced motivation and memory deficits as the issues that most affect their lives (24, 25).

Similarly, both populations reported emotional issues; however the congenital and childhood-onset myotonic dystrophy interviews highlighted an inflexibility and a narrow scope of interest in life, aspects not prominently mentioned by adult-onset myotonic dystrophy patients (23).

In a recent attempt to define the portrait of long-term participation in adult DM1with childhood phenotype (26) the authors showed that behavioural, cognitive, and social stigma problems are related to a guarded prognosis regarding long-term social participation: children affected by DM1 are more likely to rely on social security rather than live independently, and to become isolated in regard to friendship, marriage and having children.

Management of DM1 patients facing transition

Despite the impressive advances in medical care, young people with DM1 are at increased risk for psychological imbalance and social integration difficulties in addition to the burden of motor disability and multysistemic comorbidities; recent evidences show also that a range of symptoms contributing to the burden of disease risk to be under-recognized due to patients' reduced ability to be aware of their difficulties and to some possible bias in caregivers' reports (24, 27, 28).

The complexity of clinical manifestations in this disease pose an important challenge for families and clinicians involved, particularly during the transition phase, in which DM1 patients' management can become fragmented or even deficient, due to a lack of an agreement upon standards of care (29).

Young people and their families have to face a number of issues including:

- lack of continuity in funding, information and expertise in clinical services;
- limited access to education and employment opportunities;
- challenges in their social lives, particularly when forming relationships;
- limited access to appropriate equipment and support for independent living.

Even if new models of health assistance for individual with genetic disorders have emerged in the last 20 years within public health and education services, still quite high is the burden for the patient to afford critical conditions along the lifespan such as effective transition without proper educational and psychosocial programs; to fill this gap, it is important to explore what is working well and to identify areas of unmet needs, in order to achieve a series of **targeted health objectives** that ensure the right support to DM1 population.

In DM1 **targeted health objectives** can be summarized as follows:

Intellectual disability and cognitive difficulties in domains of attention, memory, visuo-spatial abilities and processing speed, have to be managed integrating specific teaching and supporting strategies into the school setting; the possibility of successful planning relies on the careful planning of a multidisciplinary team (including medical experts in DM1, school personnel and the child's parents).

Communication problems are important in children with DM1; treating clinicians who recognize this issue in patients with DM1 may consider an early referral for speech therapy to reduce dysarthria.

Disease burden: an anticipatory guidance would be suitable to evaluate the possible association between the disease onset and the related disease severity later in life. In this view clinicians should try to alleviate the commitment of follow-up visits, basically boring for young people but still essential to ensure patients well-being as long as possible. Moreover physicians should be constantly aware for the risk ASD and Attention-Deficit/Hyperactivity Disorder (ADHD) comorbidities, that require tailorized therapeutic approaches.

Apathy: a child will suffer from a lack of motivation in academic and school-related activities, this suggesting that schools can best help children with chronic illnesses by targeting the mediating variables in the child's environment that affect *motivation* with support of a trained psychotherapist.

Fatigue, irritability, daytime sleepiness may reduce academic and work patients' engagement in hobbies and leisure activities, especially those performed in groups; a step-by-step guidance should be advised to improve patients' ability to be actively involved in groups and emotional control.

In order to achieve the developmental milestones of transition properly, children affected by DM1 need the continuous availability of two main "*educational poles*", the family and the school. According to the Alberta Benchmark Survey (30) parents and teachers have a good understanding of physical milestones, such as when children learn to walk, but are less familiar with important intellectual and social stages. Therefore it could be useful to refer to "psychology of anticipation", a management strategy that has already been postulated to compensate for the potentially harmful effects of any genetic disorder (31). In line with this a medical multidisciplinary team in charge of these patients could be proposed as a "*third educational pole*" in connection with the family and the teaching staff at school, making it suitable that school personnel observe the child's academic functioning and report back to the medical team in a timely fashion. Further assessment by the medical team, in coordination with the school multidisciplinary team, may be necessary to adjust medications, modify treatment schedules, or alter the school day so that the child's academic performance is maximized.

Traditionally the medical care model focuses on treatment of impairments; however, the healthcare process of DM1 patient in transition must be improved not only in terms of assessment of disability but also promoting an action on environmental factors that can be crucial to modify the disability outcome. This can be realized only through an integrated organization of care involving all members of the patients' environment. When properly identified and included in a continuum of care, social and family environmental factors can partly counterbalance existing impairments and disabilities, and avoid the development of handicap situations in life habits.

Conclusions

Transition, indicates the process of changing from one state to another that involves young people aged from 13 to 25; this transition phase, that usually involves also the families and the professionals living around the patient, is a bridge to adulthood that many young individuals find fraught with great difficulty, confusion and profound loneliness. The rapid and numerous changes often characterizing this period may become overwhelming when a young people is affected by myotonic dystrophy.

It would be suitable to define cognitive training programs that may help to potentiate DM1 patients' ability to adapt to novelty and change, focusing on three main endpoints: enhancement of neuropsychological skills, support for mood/personality alterations, self-awareness improvement. The merge of competencies between family, medical and school staff may be beneficial, as a continuous support to cope with disease-related psychological disturbances and motor disability by providing social guidance.

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NEWS FROM AROUND THE WORLD

MSM

The 13th Congress of the Mediterranean Society of Myology will be held in Turkey in 2018, organised by Prof. Haluk Topaloglu.

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.

AIM

The 16th AIM Congress was held in Lecce from 8 to 11 June 2016. Over 100 scientific contributions were selected by the AIM Board and presented as Oral Communications or Posters.

The Congress was attended by over 200 members and it has been characterized by a high scientific profile and a variety of the topics ranging from the latest knowledge on pathophysiology to molecular pathogenic mechanisms, novel diagnostic methods and advanced therapeutic perspectives.

An interesting joint meeting between AIM and Associations of Italian Dieticians (ADI) also took place. It has allowed to start a cooperation between the two scientific Associations for a more effective and standardized approach to the nutritional problems of patients with neuromuscular diseases.

Great interest was raised by the talks of internationally renowned experts as Frédérique Magdiniér (Marseille), Antoine Muchir (Paris) and Massimo Zeviani (Cambridge) who shared with the participants their experience in their respective fields of research.

The usual meeting with the Associations of patients has been very useful to better define the demands and the needs of care of patients. AIM has always had special attention to the cultural exchange with the associations of patients. AIM members attended the Congresses of Parent Project, Italian Association of Glycogenoses (AIG), Mitocon and UILDM (Italian Musculr Dystrophy Association); collaborative working relationship with Famiglie SMA, ASAMSI, Laminopathy and sarcoglycanopathy Associations, Myotonic Dystrophy Association and the novel Coordination of Associations for Neuromuscular Diseases (CAMN) have been activated.

Collaborations have also been made with various scientific societies such as the Italian Society of Rheumatology (joint event on inflammatory myopathies at the Congress SIR 2016), the Societies of Physical and Rehabilitative Medicine, SIRN and SIMFER, the Italian Association of Neuropsychology (SINP) and the Forum Risk Management-Tuscany Region with the aim of drafting guidelines / recommendations in patients with neuromuscular diseases.

AIM has worked as a scientific hub for maintaining and developing collaborative networks involving different neuromuscular centers in Italy. AIM affiliated collaborative networks include *Registry for Mitochondrial diseases, Registry for Myotonic Dystrophy, Registry for Muscle Glycogenoses, Group of Study for Pompe Disease, Laminopathies Network, LGMD networks* and others.

AIM had its own space within the Italian Neurology Society Congress which was held in Venice 22 to 25 October 2016. A workshop entitled "Myopathies and Multisystem Disease" and the training course "BRAIN DM1: Neuroimaging and rehabilitation " were held during the congress. As part of the training course "Emergencies in Neurology", AIM speakers discussed about neuromuscular diseases.

On 4th March 2017 AIM will organize the first National Day for Neuromuscular Diseases that will take place throughout the national territory and will involve many AIM centers. You will find more info on the AIM website at www.miologia.org.

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; yet will be discussed collaborative scientific projects, relations between myology reference centers, institutions and patient organizations. Details for the registration to the Congress and hotel booking will be available shortly on the website of AIM www.miologia.org as well as the preliminary program and deadline for submission of abstracts.

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

WMS

The 21st International WMS Congress was held in Granada, Spain from 4th to 8th October, 2016.

The Congress was held in the traditional WMS format with three selected topics. One day of the symposium was dedicated to each of the selected topics addressing emerging discoveries in the field of: 1) Structural myopathies and diseases of the sarcomere; 2) Adult onset myopathies: hereditary and acquired; 3) Advances in the treatment of neuromuscular disorders.

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

2016

1 December

Annual Congress of the Lithuanian Society of Cardiology 2016. Kaunas, Lithuania.
Information: website: *http://www.lcs.lt*

December 2-4

International Society of Cardiomyopathies and Heart Failure 2016 (ISCHF2016). Kyoto, Japan.
website: *www.npo-apacvd.jp/ischf2016/*

December 8-10

68th Annual Conference of the Cardiological Society of India (CSI). Kochi, India.
Information: website: *www.csikochi2016.org*

December 8-10

16th World Cardiology Congress. Dubai, UAE. Information: website: *http://worldcardiology.conferenceseries.com/*

December 16-19

Italian Society of Cardiology; 77th annual Congress. Rome, Italy.
Information: *website: http://www.sicardiologia.it/*

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 12-17

2017 ISBER Annual Meeting. Toronto, Canada.
Information: website: *http://meetings.isber.org/2017*

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 14-17

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

September 14-16

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

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

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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