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Mediterranean Society of Myology
and
Associazione Italiana di Miologia

Founders: Giovanni Nigro and Lucia Ines Comi

Three-monthly

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**Official Journal of
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ORIGINAL ARTICLE

Integrated care of muscular dystrophies in Italy. Part 2. Psychological treatments, social and welfare support, and financial costs

LORENZA MAGLIANO¹, MARIANNA SCUTIFERO², MELANIA PATALANO¹, ALESSANDRA SAGLIOCCHI¹, ANTONELLA ZACCARO¹, FEDERICA CIVATI³, ERIKA BRIGHINA³, GIANLUCA VITA⁴, SONIA MESSINA⁴, MARIA SFRAMELI⁴, MARIA ELENA LOMBARDO⁵, ROBERTA SCALISE⁵, GIULIA COLIA⁶, MARIA CATTERUCCIA⁶, ANGELA BERARDINELLI⁷, MARIA CHIARA MOTTA⁷, ALESSANDRA GAIANI⁸, CLAUDIO SEMPLICINI⁸, LUCA BELLO⁸, GUJA ASTREA⁹, GIULIA RICCI⁹, MARIA GRAZIA D'ANGELO³, GIUSEPPE VITA⁴, MARIKA PANE⁵, ADELE D'AMICO⁶, UMBERTO BALOTTIN⁷, CORRADO ANGELINI⁸, ROBERTA BATTINI⁹ AND LUISA POLITANO²

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This paper describes the psycho-social treatments received by 502 patients with MDs and their relatives, and the costs for care sustained by the families in the previous six month period.

Data were collected by the MD-Care Schedule (MD-CS) and the Family Problems Questionnaire (FPQ).

Psycho-educational interventions were provided to 72 patients (14.3%), and social/welfare support to 331 patients (65.9%). Social/welfare support was higher in patients with DMD or LGMD, in those showing more severe disability, and in patients who were in contact with centres located in Northern Italy.

Psycho-educational interventions were received by 156 (31%) relatives, and social/welfare support by 55 (10.9%) and mainly provided by Family/Patients Associations (83.6%).

Relatives with higher educational levels, who spent more daily hours in the assistance of patients with DMD, and in contact with centres in Central Italy more frequently benefited from psycho-educational interventions.

In the previous year, costs for care were sustained by 314 (63.9%) relatives. Financial difficulties related to patient's condition, were higher in families of patients who needed more intensive rehabilitation and daily hours of caregiving,

and in families who lived further away from the reference's centre.

These results showed that psycho-social aspects of MDs care are only partially met in Italy, and that ad hoc supportive interventions for these patients and their families should be potentiated.

Key words: muscular dystrophies, psycho-social treatments, costs for care

Introduction

We have recently shown that the clinical management of MDs in Italy is complex and varies in relation to functional disabilities and type of MD, pattern of care and geographical area (1).

This paper focuses on the psycho-social management of MDs and on the financial costs for care sustained by the families.

Patients and methods

Details on participating centers, study design, and assessment instruments are reported by Magliano et al. (2).

The sample consisted of 502 key-relatives, of which the majority were mothers (84.6%) and cohabiting (87.8%) and had a mean age of 43.4 (7.4) years. Almost half of them had received higher education (56.3%) and were employed (52.6%). They spent on average 5.7 (4.6sd) daily hours in patient's care-giving in the previous two months. Of the 502 patients, most of them were male (96.4%), and school attending (85.6%). Three-hundred-thirty-three (66.3%) of them suffered from DMD, 129 (25.7%) from BMD, and 40 (8.0%) from LGMDs. The mean level of independence in daily activities, measured by the BI, was 68.3 (31.3sd). One-hundred-ninety-four patients (38.6%) were in wheelchair. Three-hundred-sixty-nine (73.5%) patients assumed drugs, and 351 (69.9%) attended rehabilitation treatment. Patients lived, on average, 183.9 (255.7 sd) kilometres far away from the reference centre.

Statistical analysis

Differences in psychosocial treatments, social/welfare support and financial difficulties in relation to patients' socio-demographic, clinical, and geographic variables were explored by analysis of variance and χ^2 , as appropriate. Correlations between the above-mentioned variables and patient' socio-demographic and clinical variables were explored by Spearman's r coefficient.

Multiple regression analyses were performed to explore the simultaneous effects on psychosocial treatments, social/welfare support, and financial difficulties (dependent variables) of patients' socio-demographic characteristics and clinical variables (first block), and geographic variables and relatives' perception of support received by social network and professionals (second block). Only variables statistically significant related to the dependent variables in univariate analyses were included in the multivariate ones. Statistical significance was set at $p < 0.01$.

Results

Psycho-educational interventions

Seventy-two patients (14.3%) received a psycho-educational intervention in the six months preceding the interview. Of these, 38 (52.8%) received a psychological support and 28 (38.9%) information on MD treatments.

Of the 156 relatives receiving psycho-educational interventions, 107 (68.5%) were informed on clinical and rehabilitative procedures and 84 (53.8%) on MD treatments; 34 relatives (21.7%) received psychological support (Table 1).

Psycho-educational interventions were more often provided to relatives who spent more daily hours in caregiving ($r = .28$, $p < .0001$), and to relatives of patients younger ($r = -.15$, $p < .001$), with a shorter duration of illness ($r = -.21$, $p < .0001$), and suffering from DMD

Table 1. Psycho-educational interventions, and social/welfare support received by patients with md and their relatives in the past six months ($n = 502$).

	Patients	Relatives
Psycho-educational intervention Yes, N (%)	72 (14.3)	156 (31.1)
Type of intervention N (%)		
Information on patient's illness	24 (33.4)	64 (41.0)
Information on MD treatments	28 (38.9)	84 (53.8)
Education on clinical and rehabilitative procedures	-	107 (68.5)
Psychological support	38 (52.8)	34 (21.7)
Genetic counselling	10 (13.9)	22 (14.1)
Mean (SD)	0.2 (0.5)	0.6 (1.1)
Social/welfare support Yes, N (%)	331 (65.9)	55 (10.9)
Type of support N (%)		
School support	52 (15.7)	-
Health volunteers' help	15 (4.5)	-
Self-help groups	-	12 (21.8)
Family and users associations help	-	46 (83.6)
School transport	51 (15.4)	-
Medical care and rehabilitation transports	21 (6.3)	-
Economic benefits	319 (96.4)	-
Mean (SD)	0.9 (0.9)	0.1 (0.3)

(0.8 (1.2) vs. 0.3 (0.7) vs. 0.2 (0.7), $F = 14.8$, $df\ 2$, 499, $p < .0001$). Psycho-educational interventions were more frequently available at centres located in central Italy [0.4 (0.8) vs. 1.2 (1.3) vs. 0.3 (0.9), $F = 42.8$, $df\ 2$, 499, $p < .0001$; 0.3 (0.7) vs. 1.4 (1.3) vs. 0.2 (0.6), $F = 79.6$, $df\ 2$, 499, $p < .0001$].

Social/welfare support

In the previous six months, 331 patients received social/welfare support, mainly consisting (96.4%) in economic benefits. Other supports, such as school support, were provided to 15.7% of patients only (Table 1).

Patients who were older ($r = .18$, $p < .0001$), had a poorer functional autonomy ($r = -.51$, $p < .0001$), a longer duration of illness ($r = .24$, $p < .0001$), and/or suffering from DMD received more social/welfare support (DMD 1.1 (0.9), BMD 0.4 (0.6), LGMD 0.7 (0.8), $F = 41.7$, $df\ 2$, 499, $p < .0001$). Furthermore, patients who were in contact with centres located in Northern Italy received more social/welfare support (North 1.2 (1.0), Central 0.7 (0.6), South 0.8 (0.8), $F = 14.9$, $df\ 2$, 499, $p < .0001$).

Only 55 (10.9%) relatives received social/welfare support in the previous six month, mainly provided (83.6%) by Family/Patients Associations. Furthermore, the support was more often provided to relatives of patients who had lower functional autonomy ($r = -.14$, $p < .01$) and/or suffered from DMD or LGMD (DMD 0.1 (0.4), BMD 0.03 (0.2), LGMD 0.1 (0.3), $F = 6.5$, $df\ 2$, 499, $p < .01$).

Costs and economic difficulties

Three-hundred-fourteen (63.9%) out of 406 relatives reported costs for patient's care in the previous year. Of these, 207 (65.9%) listed costs for medical/nursing care and 201 (64.0%) for drugs. Moreover, 88 (28.1%) relatives reported "other costs" as transfer (36.4%), rehabilitation (26.1%), medical devices (25%), adaptive devices (15.9%), and psychological support (6.8%).

Costs for care ($N = 53.5\%$, $C = 54.0\%$, $S = 75.8\%$, $\chi^2 = 26.6$, $df\ 2$, $p < .001$; $N = 59.2\%$, $C = 56.7\%$, $S = 75.3\%$, $\chi^2 = 14.7$, $df\ 2$, $p < .001$), drugs ($N = 26.7\%$, $C = 34.6\%$, $S = 54.4\%$, $\chi^2 = 29.0$, $df\ 2$, $p < .0001$; and $N = 31.2\%$, $C = 30.1\%$, $S = 55.9\%$, $\chi^2 = 31.8$, $df\ 2$, $p < .0001$, respectively), doctors and nurses ($N = 30.4\%$, $C = 35.0\%$, $S = 58.6\%$, $\chi^2 = 30.6$, $df\ 2$, $p < .0001$), $N = 39.4\%$, $C = 31.3\%$, $S = 55.9\%$, $\chi^2 = 21.5$, $df\ 2$, $p < .0001$, respectively) resulted higher in families of patients living in Southern Italy.

Difficulties related to patient's condition were reported as moderate by 24.7% of relatives and marked by 9.6% in the previous 12 months. These difficulties were higher among relatives of patients who were older, had a longer duration of illness ($r = .18$, $p < .0001$), less au-

tonomy ($r = -.32$, $p < .0001$), requiring more drug therapies ($r = .27$, $p < .0001$) and/or rehabilitative interventions ($r = .32$, $p < .0001$), and in DMD group (1.6 (0.8) vs. 1.2 (0.5), vs. 1.3 (0.6), $F = 15.3$, $df\ 2$, 497, $p < .0001$). Finally, economic difficulties were higher among relatives who were unemployed (1.6 (0.8) vs. 1.4 (0.7), $df\ 1$, 498, $p < .01$); who dedicated more daily hours to the patient's assistance ($r = .13$, $p < .01$), had lower level of support by social network ($r = -.16$, $p < .0001$) and professionals ($r = -.14$, $p < .01$) and living further away from the clinical centre of reference ($r = .12$, $p < .01$).

Multivariate analyses

Socio-demographic and clinical variables (block 1) explained 15% of variance in family psycho-educational intervention, while geographical areas and relative's perceived support explained a further 16% of variance (Table 2). In particular, psycho-educational interventions were more frequently reported by relatives of DMD patients, with higher educational levels, who spent more daily hours in the patient daily assistance or lived in central Italy. Thirty-two percent of variance in patient social welfare support was explained by level of disability, type of disease and geographical location. Family financial difficulties were higher in relation to the daily hours for care, poor social network support and longer distance from the reference centre.

Discussion

The results of this study suggest that psycho-social management of MDs is scarcely available in Italy. In particular, only 14% of patients and 31% of relatives received psycho-educational interventions in the reference period. This condition is likely due to the low integration of medical, psychological and social expertise in services for rare diseases (5-8), in line with the poor psychological support available for patients with other severe diseases (3, 4).

The low percentage of patients receiving information could be related to several factors, such as: a) the duration of illness, whereas information is mainly provided in the phase of communication of the diagnosis; b) the fact that most patients were adolescent, an age in which health issues are managed throughout parents (9). The poor availability of professionals trained in the psychological care of children with rare diseases, should explain the low percentage of patients (38/502) receiving such a support (5, 10).

In our sample, family interventions mostly consisted in education on medical procedures and treatments for MDs, while psychological support was provided to 34 caregivers out of 502, only. Providing the relatives with information on MDs and their treatments may reinforce

Table 2. Multiple hierarchical analyses: effects of socio-demographic, clinical variables, and geographic variables on psycho-educational interventions received by the families, social/welfare support provided to patients with MD, and relatives' economic problems due to patient's health care.

	Family psycho-educational interventions		Patient social/welfare support		Family financial difficulties	
	1 st step	2 nd step	1 st step	2 nd step	1 st step	2 nd step
Blocks	Standardized Beta					
1 - Socio-demographic and clinical variables						
Patient's age	-.07	-.15	-.28	-.23	.10	.11
Barthel Index	-	-	-.45 ^d	-.40 ^d	-.16 ^a	-.08
Duration of illness	-.24 ^c	-.12	.15	.12	-.13	-.14
Type of MD- BMD	-.18 ^c	-.15 ^b	-.14 ^a	-.15 ^b	-.06	-.04
Type of MD-LGMD	-.12 ^b	-.15 ^b	-.08	-.07	-.04	-.02
Patient's education	.18	.24	-.19	-.16	-	-
Relative's education	.13 ^c	.10 ^a	-	-	-	-
Daily hours in caregiving	.19 ^d	.10 ^a	-.06	-.01	.11 ^a	.11 ^a
Relative's employment	-	-	-	-	-.05	-.01
Number of drugs	-	-	-	-	-.08	-.03
Number of rehabilitative interventions	-	-	-	-	.13 ^a	-.07
2 - Geographical area and relatives' perceived support						
Centre located in Northern Italy		-.14		.17 ^b		-
Centre located in Central Italy		.35 ^d		-		-
Centre located in Southern Italy		-		.05		-
Family living area: North Italy		.09		-		-
Family living area: South Italy		-.11		-		-
Professional support		-		-		.02
Social network support		-		-		-.26 ^d
Distance from the Centre						.22 ^d
Model's F, df, p<	15.6; 11,380; .0001		20.6; 9, 382; p < .0001		10.0; 12,445; .0001	
R²	.15	.31	.30	.32	.11	.21

^a = p < .05; ^b = p < .01; ^c = p < .001; ^d = p < .0001

their abilities to deal with practical aspects of daily assistance (8, 9). However, medical education is unable to address relatives' needs for psychological support, which remains largely unmet (5, 8). This is particularly worrying, given the high level of psychological burden among the caregivers of patients with MDs (2).

Our data reveal that psycho-educational interventions were more frequently provided to relatives of patients with DMD, and to relatives of patients young and with short duration of illness. Psycho-educational support was found more available for families who lived or were in contact with centres located in Central Italy. This finding is probably related to the greater presence of a Family Association in this geographical area, and a closer collaboration with the NMD Centres (16).

Undoubtedly, the psychological support is crucial in the early phase of these disorders (9, 10), when the diagnosis of

DMD can be associated with feelings of despair and deep worries for the future (10). However, the psychological support is equally important in the subsequent phases of patient's and family adaptation to the disease (10, 11), when clinical conditions of the patient get worse (10, 12) and daily assistance become more demanding for caregivers (2, 5, 6).

As far as social/welfare support to patients, we found that it mainly consisted in economic benefits, while other support such as school support was available only for a minority of patients. The impossibility for the patient to attend school is a critical event for him/her and his/her relatives, both psychologically and practically (11, 12, 14, 17). Therefore, it is important to provide patients with supports facilitating school attendance as longer as possible. These supports should include dedicated school transfer as well as dedicated helper at school, both available for only 15% in our sample.

The possibility to receive more social/welfare supports by patients who live in Northern Italy reflects the differences in health care policies at regional level (18). In fact, in Southern Italy, the health care provision is poorer due to cutting investment in health services, and regional financial deficit (19). This situation leads to inequalities in accessibility and availability of public health services, as supported by the lower levels of perceived professional support among relatives living in this geographical area ($N = 3.2$ (0.6), $C = 3.1$ (0.7), $S = 2.9$ (0.7), $F = 7.6$, df 2, 497, $p < .0001$).

This situation may also explain why about 25% of patients living in Southern Italy are followed by extra-regional centres. Furthermore, inequalities in the Italian health services resources justify the higher costs sustained by families in Southern Italy (19).

Despite its strengths, the study has some methodological limitations, such as the cross-sectional design, and the cases age limited to 4-25 years.

The results of the present study represent a first step to define the needs for care for patients and relatives, and to develop appropriate psycho-social interventions.

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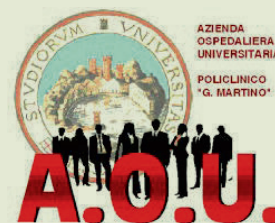


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Cari amici,

L'Associazione Italiana di Miologia (AIM) organizza quest'anno il 17° incontro riguardante le Malattie Muscolari, che appartengono in vasta maggioranza al grande capitolo delle Malattie Rare.

La rarità può infatti condizionare l'acquisizione della diagnosi in tempi rapidi, rendendo così difficoltoso il tempestivo accesso ad una terapia.

Le Malattie Muscolari mostrano una grande eterogeneità clinica con un esordio che può variare da un'età precoce all'età adulta.

Le Miopatie possono anche avere un coinvolgimento multisistemico con interessamento di vari organi ed apparati quali, ad esempio, cuore, fegato, rene, apparato respiratorio, sistema nervoso centrale, occhio, orecchio e sistema endocrino.

A tal proposito, i medici che abitualmente hanno il primo impatto con le suddette patologie sono prevalentemente pediatri, neurologi, neuropsichiatri infantili, fisiatristi, cardiologi e pneumologi.

Il riconoscimento in tempi ragionevoli di una malattia muscolare può consentire, mediante opportuni screening e, secondo quanto suggerito da specifiche linee guida diagnostico-terapeutiche, una migliore gestione del paziente consentendo un miglioramento dei livelli di assistenza e prevenzione dell'handicap.

Questo Congresso si pone l'obiettivo di presentare, a tutti coloro che fanno parte di gruppi Italiani e stranieri che svolgono assistenza e ricerca nel campo delle Malattie Muscolari, le più recenti innovazioni in ambito di aspetti diagnostici, terapeutici, riabilitativi e gestionali dei pazienti con patologie muscolari.

Durante il Convegno si alterneranno vari relatori italiani e stranieri che presenteranno i risultati delle ricerche più recenti.

È inoltre previsto ampio spazio per le tavole rotonde con le Associazioni dei pazienti ed i dibattiti tra specialisti delle varie patologie muscolari al fine di migliorare la collaborazione tra ricercatori, pazienti e loro familiari.

Prof. Antonio Toscano
Responsabile Scientifico

COMITATO SCIENTIFICO

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Scientific Programme

WEDNESDAY, 31st MAY

13.30 Registration of participants

14.00 Welcome and meeting introduction: **A. Toscano (Messina), G. Siciliano (Pisa),
L. Provinciali (Ancona)**

14.30 - 15.50 WORKSHOP-1: “Distal myopathies: a clinical and therapeutic update”

Chairpersons: **G.P. Comi, C. Fiorillo**

14.30 Emerging clinical aspects. **E. Pegoraro**

14.45 Pathogenesis and myopathology. **M. Mora**

15.00 Muscle imaging. **G. Tasca**

15.15 GNE Myopathy: update and therapeutic perspectives. **M. Mirabella**

15.30 *Discussion*

15.50 LECTURE-1

Chairperson: **M. Filosto**

“An overview of EURO-NMD a European Reference Network for Rare Neuromuscular Diseases”

T. Evangelista (Newcastle)

16.30 - 16.50 Coffee break

16:50 - 18:50 Round Table: “Clinicians and patients associations roles: state of the art and new collaborative perspectives”. The goal of this round table is to facilitate the relationships between clinicians and patients associations as regard a better management of patients with dystrophies, myopathies, motoneuron disorders and genetic and disimmune neuropathies.

Chairpersons and discussants:

**L. Politano, P. Santantonio (Mitocon),
M. Marra (CIDP Onlus ITALIA), A. Toscano,
D. Lauro (Famiglie SMA), G. De MARTINO (M.i.A.)**

- CAMN (Coordinamento Associazioni Malattie Neuromuscolari): a year later. **M.L. Solinas - CAMN**
- New LEA (Livelli Essenziali di Assistenza): “pros and cons” **M. Rasconi-UILDM**
- The age transition: an emerging problem. **G. Siciliano**
- Data sharing and biobanks: how can we improve clinical and scientific outcomes. **M. Moggio**
- Giornata per le Malattie Neuromuscolari (GMN): first outcomes and future proposals. **A. Schenone**
- Discussion

18.50 - 20.10 WORKSHOP-2: “From clinical research into clinical practice in Duchenne Muscular Dystrophy”

Chairperson: **G. Vita**

18.50 Introduction and welcome. **G. Vita**

19.05 From Genetic diagnosis to personalized medicine in DMD. **E. Pegoraro**

19.20 New standard treatment in DMD. **M. Pane**

19.35 Observational registries: how they evolve after drugs approval. **E. Mercuri**

19.50 Discussion and conclusions. **G. Vita**

20:30 Welcome cocktail

THURSDAY, 1st JUNE

07.30 - 08.30 BREAKFAST SEMINAR: “A multidisciplinary approach for the DMD patients management”

Chairperson: **G. D’Angelo**

07.30 Guidelines for an early diagnosis and its advantages. **S. Messina**

07.45 Management of cardiac complications. **R. Adorisio**

08.00 Respiratory urgencies and complications. **A. Vianello**

08.15 Discussion

08.30 - 09.30 Oral Communications 1 - Metabolic myopathies

Chairpersons: **S. Ravaglia, P. Tonin**

- Polymorphisms of the GAA gene causing amino-acid changes and analysis of the impact on protein structure in a cohort of 50 Late Onset Pompe Disease (LOPD). **Danesino C., Ravaglia S., Scotti C., De Filippi P. and The Italian GSDII Group (Pavia)**
- IgG anti rh-GAA assessment in an Italian cohort of patients with late-onset Pompe Disease. **Filosto M., Cotti Piccinelli S., Marchesi M., Di Muzio A., Donati M.A., Galvagni A., Lerario A., Marrosu G., Moggio M., Mongini T., Musumeci O., Pegoraro E., Piras R., Ravaglia S., Sacchini M., Sancricca C., Semplicini C., Servidei S., Siciliano G., Telese R., Tonin P., Caria F., Rota S., Padovani A., Toscano A. (Brescia, Chieti, Firenze, Milano, Cagliari, Torino, Messina, Padova, Pavia, Roma, Pisa, Verona)**
- Registries for muscle glycogenoses: the Italian side of EUROMAC. **Martinuzzi A., Vavla M., Musumeci O., Siciliano G., Toscano A., Bruno C., Marti Seves R. (Conegliano, Messina, Pisa, Genova, Barcelona)**
- Report on Nationwide Italian collaborative network for Muscle Glycogen Storage Disorders. **Musumeci O., Mongini T., Angelini C., Bruno C., Moggio M., Siciliano G., Tonin P., Maggi L., Martinuzzi A., Filosto M., Servidei S., Donati A., Bembi B., Marrosu G., Di Iorio G., Ravaglia S., Pegoraro E., Bertini E., Di Muzio A., Fiumara A., Massa R., Toscano A. (Messina, Torino, Venezia, Genova, Milano, Pisa, Verona, Conegliano, Brescia, Roma, Firenze, Udine, Cagliari, Napoli, Pavia, Padova, Chieti, Catania)**

09.30 - 10.10 LECTURE-2

Chairperson: **A. Toscano**

“How diagnostic are serological antibodies in myopathies?” **B. Schoser (Munich, Germany)**

10.10 - 10.30 Coffee break

10.30 - 11.30 WORKSHOP-3: “Neuromuscular junction disorders”

Chairpersons: **G. Antonini, M. Grandis**

10.30 MUSK (Muscle Specific Kinase)-positive myasthenia. **A. Evoli**

10.45 Thymectomy today: indications and limits of different surgical procedures. **M. Lucchi**

11.00 Eaton-Lambert syndrome: clinical variability and treatment efficacy. **C. Rodolico**

11:15 *Discussion*

11.30 - 13.00 Oral Communications 2 - SMA/Myopathies

Chairpersons: **L. Bello, L. Politano**

- Correlation between IGHMBP2 protein levels in human motor neuron and non-neuronal somatic cells and phenotype of 5 patients affected with spinal muscular atrophy with respiratory distress type 1. **Govoni A., Magri F., Salani S., Del Bo R., Taiana M., Forotti G., Bresolin N., Comi G.1, Nizzardo M., Corti S. (Milano)**
- POPDC1 Gene mutations screening in Laminopathies: possible role as a modifier. **Rossi R., Scotton C., Maggi L., D'Amico A., Ricci G., Vercelli L., Benedetti S., Bertini E., Mercuri E., Rodolico C., Mongini T., Carboni N., Marrosu G., Chico L., Santoro L., Merlini L., Lattanzi G., Morandi L., Barton P.J.R., Buchan R., Walsh R., Cook S.A., Milting H., Bonne G., Brand T., Ferlini A. (Ferrara, London, Bad Oeynhausen, Paris)**
- Glycosilation of Alpha-Dystroglycan: one pathway, several phenotypes. **Iucc G., D'Amico A., Battini R., Berardinelli A., Bertini E., Bruno C., Cassandrini D., Catteruccia M., Comi G.P., Fattori F., Fiorillo C., Giannotta M., Gorni K., Magri F., Mercuri E., Messina S., Mongini T., Mora M., Morani F., Moro F., Pane M., Pegoraro E., Pini A., Politano L., Ricci F., Sframeli M., Santorelli F.M. (Pisa, Roma, Pavia, Genova, Milano, Bologna, Messina, Torino)**
- Next-generation sequencing approach for the diagnosis of congenital myopathies: a 3-yr experience. **Cassandrini D., Trovato R., Rubegni A., Lenzi S., Baldacci J., Fiorillo C., Savarese M., Nigro V., Bruno C., Astrea G., Santorelli F., and Italian Network on Congenital Myopathies (Pisa, Genova, Napoli)**
- Autosomal recessive myopathy associated with cataracts caused by mutations in the gene encoding INPP5K, an inositol phosphatase. **Roos A., Wiessner M., Cox D., Hathazi D., Marini-Bettolo C., Straub V., Barresi R., Senderek J., Lochmüller H. (Newcastle upon Tyne, Dortmund, Munich)**
- Clinical expression of facio-scapulo-humeral muscular dystrophy in carriers of 33-35 kb D4Z4 reduced alleles: experience of the Italian National Registry for FSHD. **Ruggiero L., Mele F., Ricci G., Vercelli L., Govi M., Nikolic A., Louise M., Sera F., Berardinelli A., Angelini C., Antonini G., Bucci E., Filosto M., Cao M., Giardina E., Pegoraro E., Di Muzio A., D'Amico M.C., Maggi L., Portaro S., Rodolico C., Villa L., Mongini T., Siciliano G., Tomelleri G., D'Angelo G., Maioli M.A., Moggio M., Santoro L., Tupler R. (Napoli, Pisa, Modena, Torino, Worcester, London, Pavia, Venezia, Roma, Brescia, Padova, Milano, Messina, Verona, Bosisio Parini, Cagliari)**

13.00 - 14.00 Lunch

14.00 - 15.30 Poster Session 1

Chairpersons: **M. Garibaldi, R. Piras, G. Primiano, M. Scarlato, M. Scarpelli**

P1-1 MYASTHENIA

P1-2 CONGENITAL MYOPATHIES

P1-3 INFLAMMATORY MYOPATHIES

P1-4 METABOLIC MYOPATHIES

P1-5 MITOCHONDRIAL MYOPATHIES

P1-6 OTHER MYOPATHIES

15.30 - 16.30 WORKSHOP-4: “Recent molecular diagnostic techniques: shall we get earlier diagnosis?”

Chairpersons: **G. Ricci, S. Servidei**

15:30 Limb-Girdle Muscular Dystrophies. **V. Nigro**

15.45 Mitochondrial disorders. **M. Zeviani**

16.00 Facio-Scapulo-Humeral Dystrophy. **R. Tupler**

16.15 *Discussion*

16.30 - 17.30 Oral communications 3 - Duchenne muscular dystrophy

Chairpersons: **A. Ardisson, G. Di Iorio**

- Observational study on the nutritional and metabolic features in Duchenne muscular dystrophy: the Italian “N&M_Duchenne study”. **Bertoli S., Baranello G., Foppiani A., Giaquinto E., De Amicis R., Leone A., Battezzati A. (Milano, Cesena)**
- Respiratory function in ataluren-treated, non-ambulatory patients with nonsense mutation Duchenne (NMDMD) muscular dystrophy from a long-term extension trial versus untreated patients from a natural history study. **Comi G.P., Bertini E., Magri F., Luo X., McIntosh J., Ong T., Riebling P., Triffilis P., Souza M., Peltz S.W., Mercuri E. (Milano, Roma, South Plainfield)**
- Vision DMD: a drug development program for Vamorolone in Duchenne muscular dystrophy. **Guglieri, M., Clemens P., Cnaan A., Damsker J., Gordish-Dressman H., Morgenroth, L., Davis R., Smith A., Storch K., Head R., Demotes-Mainard J., Nagaraju K., Hathout Y., Athanasiou D., Vroom E., Bushby K., Hoffman E. (New Castle upon Tyne, Pittsburgh, Washington)**
- Long-Term treatment with Eteplirsen in non-ambulatory patients: a case study in identical twins. **Lowes L.P., Alfano L., Dracker R.A., Duda P., Mendell J.R. (Columbus, Liverpool, Cambridge)**

Social programme

a) Guided walking tour of Ortigia

b) Evening at the Greek Theater for Classical Plays (Greek Tragedies)

FRIDAY, 2nd JUNE

07.30 - 08.30 BREAKFAST SEMINAR: “ERT (Enzyme Replacement Therapy) in Pompe disease: 10 years of experience looking at the future”

Chairperson: **C. Angelini**

07.30 A clinical and laboratory update. **A. Toscano**

07.45 Classic and non-classic forms: treatment and management. **A. Fiumara**

08.00 Therapy in Late Onset forms (LOPD - Late Onset Pompe Disease): clinical assessment and future views. **M. Moggio**

08.15 *Discussion*

08.30 - 09.30 Muscle Club: discussion of peculiar diagnostic pathways

Chairpersons: **L. Maggi, E. Pennisi**

- Expanding clinical and histological spectrum of DNM2 mutations **Barcellona C., Musumeci O., Savarese M., Nigro V., Toscano A., Rodolico C. (Messina, Napoli)**
- Novel compound heterozygous mutations of AGRN resulting in a complex muscular phenotype. **Gemelli C., Cassandrini D., Fabbri S., Lamp M., Santorelli F.M., Bruno C., Reni L., Broda P., Fiorillo C., Mandich P., Grandis M. (Genova, Pisa)**
- Myotonia in Filamin-C-related myopathies. **Lerario A., Peverelli L., Villa L., Lucchiari S., Tironi R., Conca E., Fagiolari G., Grimoldi N., Sciacco M., Comi G.P., Moggio M. (Milano)**
- Core myopathy with early respiratory failure and titin gene mutation. **Petrucci A., Costanzi-Porrini S., Giacanelli M., Lispi L., Santorelli F.M., Cassandrini D., Rastelli E., Nigro V., Massa R., Savarese M. (Roma, Pisa, Napoli)**
- Muscle pathological features of a hyperkalemic paralysis/dermatomyositis “double trouble”. **Rota S., Galvagni A., Caria F., Marchesi M., Cotti Piccinelli S., Baronchelli C., Padovani A., Filosto M. (Brescia)**
- Castleman disease and inflammatory myopathy: is there a link? **Simoncini C., Montano V., Ricci G., Calì L., Giorgetti M., Alì G., Rousseau M., Tavoni A., Siciliano G. (Pisa)**

09.30 - 10.10 LECTURE-3

Chairperson: **R. Liguori**

“How still relevant is in muscle disorders the neurophysiological support for the diagnosis?” **P. Girlanda (Messina)**

10.10 - 10.30 Coffee break

10.30 - 11.30 WORKSHOP-5: “Spinal muscular atrophy: new clinical and therapeutic aspects”

Chairpersons: **A. Berardinelli, G. Marrosu**

10.30 Emergent phenotype and new causative genes. **E. Bertini**

10.45 EAP (Expanded Access Program) Nusinersen experience. **S. Messina**

11.00 A clinical trial overview. **E. Mercuri**

11.15 Discussion

11.30 - 13.00 Oral Communications 4 - New results from large populations studies

Chairpersons: **G.R. Barresi, A. Di Muzio**

- Myositis-specific antibodies (MSA): high prevalence in biopsy-proven myositis population. **Lucchini M., De Fino C., Tasca G., Primiano G., Ricci E., Silvestri G., Servidei S., Mirabella M. (Roma)**
- NGS target re-sequencing analysis in patient with persistent asymptomatic or mildly symptomatic hyperCKemia. **Fiorillo C., Madia F., Robbiano A., Pozzolini G., Trucco F., Pedemonte M., Diana M.C., Grandis M., Gemelli C., Fabbri S., Schenone A., Nobili F.M., Foadelli T., Trabatti C., Savasta S., Schiaffino M.C., Picco P., Morcaldi G., Celle M.E., Mancuso M., Tonin P., Mandich P., Bruno C., Zara F. (Genova, Pavia, Pisa, Verona)**
- NGS in neuromuscular disorders: an update. **Gibertini S., Saredi S., Pasanisi M.B., Ardisson A., Moroni I., Baranello G., Peverelli L., Tonin P., Previtali S.C., Grandis M., Gemelli C., Canioni E., Bernasconi P., Mantegazza R., Morandi L., Maggi L., Mora M., Ruggieri A. (Milano, Verona, Genova)**

- Comprehensive CNVS assessment in 234 diagnosis-resistant myopathic patients. **Giugliano T., Garofalo A., Savarese M., Torella A., Musumeci O., Maggi L., Ruggiero L., Vercelli L., D'Amico A., Fiorillo C., Magri F., Piluso G., De Bleecker J., Toscano A., Mora M., Santoro L., Mongini T., Bertini E., Bruno C., Comi G.P., Santorelli F.M., Angelini C., Politano L., Nigro V. (Napoli, Pozzuoli, Helsinki, Messina, Milano, Torino, Roma, Genova, Pisa, Padova, Belgium)**
- Phenotypical and molecular definition of a multi-center cohort of 49 patients with limb girdle muscular dystrophy Type 2A. **Bello L., Magri F., Lazzarotto A., Semplicini C., Fanin M., Gandossini S., Diella E., D'Angelo M.G., Comi G.P., Pegoraro E. (Padova, Milano, Bosisio Parini)**
- Big Data in genetic research: the example of titin gene and titinopathies. **Savarese M., Johari M., Johnson K., Torella A., Topf A., Evilä A., Arumilli M., Rubegni A., Kuhn M., Fattori F., Santorelli F.M., Schoser B., Straub V., Nigro V., Hackman P., Udd B. (Helsinki, Napoli, Pozzuoli, New Castle upon Tyne, Pisa, Neu-Ulm, Roma, Munich, Tampere)**

13.00 - 14.00 Lunch

14.00 - 15.30 Posters Session 2

Chairpersons: **G. Baranello, F. Biasini, A. D'Amico, J.F. Desaphy, F. Santorelli**

P2-1 DYSTROPHIC AND NON-DYSTROPHIC MYOTONIAS

P2-2 DYSTROPHINOPATHY/LGMD/CONGENITAL MUSCULAR DYSTROPHIES/FSHD

P2-3 SMA

15.30 - 16.10 LECTURE- 4

Chairperson: **C. Bruno**

“Early onset metabolic myopathies: from diagnosis to therapy” **A. Donati (Firenze)**

16.10 - 16.30 Coffee break

16.30 - 18.15 Oral communications 5 - Myopathies/Dystrophic and non-dystrophic myotonias

Chairpersons: **R. Massa, L. Ruggiero**

- The importance of muscle biopsy for muscle diseases experts: a review of Sardinian data. **Piras R., Maioli M.A., Murru M.R., Costa G., Solla E., Mancosu C., Mammoliti R., Marrosu G. (Cagliari)**
- Very late-onset non-thymomatous and thymomatous myasthenia gravis (MG) are associated with different HLA class II alleles. **Massa R., Greco G., Testi M., Antonini G., Marfia G.A., Rastelli E., Terracciano C., Pompeo E., Andreani M. (Roma)**
- 20 years clinical follow-up in patients with oculopharyngeal muscle disease (OPMD). **Brizzi T., Barca E., Biasini F., Lupica A., Vita G., Mazzei R.L., Musumeci O., Rodolico C., Toscano A. (Messina, Palermo, Cosenza)**
- Assessing the impact of gender on the phenotype of Myotonic dystrophy type 2: a cohort of 307 patients. **Montagnese F., Mondello S., Wenninger S., Schoser B. (Munich, Messina)**
- A respiratory snapshot of a cohort of DM1 patients: clinically stable does not mean normal. **Falcier E., De Mattia E., Sannicolò G., Lizio A., Iatomasi M., Lupone S., Mollar E., Castino V., Roma E., Conti C., Rao F., Sansone V.A. (Milano)**

- Precision medicine to address therapy in myotonia caused by sodium channel mutations. **Farinato A., Altamura C., Imbrici P., Maggi L., Mantegazza R., Filosto M., Siciliano G., Sansone V.A., Lo Monaco M., the Italian Network for Muscle Channelopathies, Conte Camerino D., Desaphy J-F (Bari, Milano, Brescia, Pisa, Roma)**
- Novel zebrafish models of sarcoglycanopathy. **Soardi M., Carotti M., Fecchio C., Sacchetto R., Sandonà D. (Padova)**

18.15 - 19.45 Annual meeting of AIM members

20.45 Social dinner

SATURDAY, 3rd JUNE

08.30 - 09.30 Discussion on AIM projects in progress

Chairpersons: **A. Martinuzzi, G. Siciliano**

- The nation-wide Italian collaborative network of mitochondrial diseases: from 2009 to today. **Mancuso M., Caldarazzo Ienco E., Angelini C., Bertini E., Pegoraro E., Carelli V., Comi G.P., Minetti C., Bruno C., Moggio M., Mongini T., Vercelli L., Primiano G., Servidei S., Orsucci D., Tonin P., Toscano A., Musumeci O., Moroni I., Santorelli F.M., Filosto M., Lamperti C., Zeviani M., Siciliano G. (Pisa, Venezia, Roma, Bologna, Milano, Genova, Torino, Verona, Messina, Brescia)**
- TREAT-CDM, an international observational longitudinal study on congenital myotonic dystrophy (CDM): a spin-off for the creation of the Italian CDM network. **Sansone V., Albamonte E., Casiraghi J., Pini A., Berardinelli A., D'Angelo G., D'Amico A., Bertini E., Ricci F., Ardisson A., Filippini M., Conti C., Moscardi M., Morettini V., Iatomasi M., De Biaggi M.L., Maestri E., Zanolini A., Pane M., Battini R., Astrea G., Baranello G., Moroni I., Messina S., Mercuri E., Bruno C., Fiorillo C., Siciliano G., Baldanzi S., Fossati B., Mongini T., Vita G., Meola G., Rodolico C., Toscano A., Campbell C., Johnson N. (Milano, Bologna, Pavia, Bosisio parini, Roma, Torino, Pisa, Messina, Genova, Ontario, Utah)**
- Muscle pain in mitochondrial diseases: a survey on a large cohort from the Italian Network. **Filosto M., Cotti Piccinelli S., Marchesi M., Angelini C., Bertini E., Bruno C., Caldarazzo Ienco E., Carelli V., Comi G.P., Lamperti C., Minetti C., Moggio M., Mongini T., Moroni E., Musumeci O., Orsucci D., Pegoraro E., Primiano G., Santorelli F.M., Servidei S., Simoncini C., Tonin P., Toscano A., Vercelli L., Zeviani M., Galvagni A., Caria F., Rota S., Padovani A., Siciliano G., Mancuso M. (Brescia, Venezia, Roma, Genova, Pisa, Bologna, Milano, Torino, Messina, Padova, Verona, Cambridge)**
- CARE-DMD, A LONGITUDINAL STUDY ON DMD CARE: Energy expenditure and multidisciplinary involvement at transitional age and towards adult life in DMD. **Messina S., Berardinelli A., D'Angelo G., D'Amico A., Pane M., Bruno C., Sansone V.A., Mongini T., Pegoraro E., Comi G.P., Moggio M., Previtali S., Mercuri E., Vita G. (Messina, Bosisio Parini, Roma, Genova, Milano, Torino, Padova)**

09.30 - 10.10 LECTURE-5

Chairperson: **O. Musumeci**

"Therapy and diagnostic functional testing in metabolic myopathies" **J. Vissing (Copenhagen, Denmark)**

10.10 - 10.30 Coffee break

10.30 - 11.15 WORKSHOP-6: “Recent therapeutic approaches in Muscle disorders”

Chairpersons: **C. Minetti, V. Sansone**

10.30 Innovative treatments in Muscular Dystrophies. **T. Mongini**

10.45 Experimental approach in Merosin-Deficient Congenital Muscular Dystrophy. **S. Previtali**

11.00 Therapeutic perspectives in dystrophic and non-dystrophic myotonias. **G. Meola**

11.15 Mitochondrial medicine: what's new? **M. Mancuso**

11.30 *Discussion*

11.45 - 13.30 Oral Communications 6 - Clinical and experimental DMD features

Chairpersons: **A. Ferlini, M. Sciacco**

- The mildest end of the dystroglycanopathy phenotypic spectrum: from asymptomatic hyperCKemia to limb girdle muscular dystrophy. **Brisca G., Pedemonte M., Trucco F., Ferretti M., Diana M.C., Magnano G.M., Valle M., Broda P., Minetti C., Fiorillo C., Bruno C. (Genova)**
- Tele-monitoring in paediatric ventilated neuromuscular patients: results of an Italian multicentric study. **Trucco F., Pedemonte M., Romano C., Wenzel A., Tacchetti P., Bella C., Falsaperla R., Racca F., Bruno C., Minetti C. (Genova, Alessandria, Catania)**
- Insights into bone mineral density and bone metabolism in Duchenne muscular dystrophy. **Vita GL., Catalano A., Sframeli M., Distefano MG., La Rosa M., Barcellona C., Bonanno C., Nicocia G., Profazio C., Morabito N., Lunetta C., Vita G., Messina S. (Messina)**
- Development of a human cardiac organoid to study heart dysfunctions in Duchenne and Becker muscular dystrophies. **Piga D., Rinchetti P., Salani S., Calandriello L., Forotti G., Nizzardo M., Magri F., Comi G.P., Corti S. (Milano)**
- Multilevel molecular analysis identifies all dystrophin gene mutations pointing out that DMD is a genetically homogenous disease: repercussions on diagnosis, prevention and therapy. **Neri M., Selvatici R., Falzarano M.S., Trabanelli C., Ravani A., Rimessi P., Fabris M., Scotton C., Mauro A., Fortunato F., Osman H., Rossi R., Armaroli A., Buldrini B., Merlini L., Mora M., Gorni K., Sansone V., Mongini T., Pegoraro E., Tonin P., Fiorillo C., Pini A., Filosto M., D'Amico A., Bertini E., Messina S., Vita G., Pane M., Mercuri E., Muntoni F., Fini S., Gualandi F., Ferlini A. (Ferrara, Milano, Torino, Padova, Verona, Genova, Bologna, Brescia, Roma, Messina, London)**
- Fibroblasts-derived exosomes: potential role in the fibrotic process of Duchenne muscle dystrophy. **Zanotti S., Gibertini S., Blasevich F., Saredi S., Maggi L., Bragato C., Ruggieri A., Mantegazza R., Mora M. (Milano)**
- Chromatin configuration RNA and protein studies identified novel DNA elements that influence the dystrophin transcription dynamics. **Falzarano M.S., Gherardi S., Bovolenta M., Passarelli C., Erriquez D., Scotton C., Armaroli A., Rossi R., Osman H., Mora M., Bernasconi P., Maggi L., Morandi L., Perini G., Ferlini A. (Ferrara, Bologna, Roma, Milano, London)**

13.30 - 13.45 Closing remarks

13.45 - 14.00 ECM Questionnaire

POSTER SESSION - 1st June 2017, 14.00 - 15.30

Chairpersons: **M. Garibaldi, R. Piras, G. Primiano, M. Scarlato, M. Scarpelli**

P1-1 MYASTHENIA

P.1 Thymoma-associated Myasthenia Gravis: clinical and serological features of Pisa's Cohort
De Rosa A., Ricciardi R., Maestri M., Guida M., Rizzo S., Lucchi M., Mussi A., Bonuccelli U. (Pisa)

P.2 Clinical, morphological and immunological findings in myasthenia - myositis association
Garibaldi M., Fionda L., Bucci E., Vizzaccaro E., Di Pasquale A., Morino S., Pennisi E.M., Antonini G. (Roma)

P.3 A case with chronic inflammatory demyelinating polyneuropathy and ocular myasthenia gravis
Montano V., Maestri M., Ricci G., Ali G., Mancuso M., Ricciardi R., Siciliano G. (Pisa)

P.4 Impact of myasthenia gravis on quality of life
Lupidi F., Carlini G., Provinciali L., Logullo F. (Ancona, Macerata)

P1-2 CONGENITAL MYOPATHIES

P.5 A Novel ACTA1 mutation in a patient affected by congenital myopathy with histopathologic progression
Cuccagna C., Fattori F., Primiano G., Sancricca C., Bernardo D., Sauchelli D., Verardo M., Bertini E., Servidei S. (Roma)

P.6 Pseudo-dominant inheritance of a novel homozygous HADC1 mutation associated with congenital myopathy: the first Caucasian family
Emmanuele V., Savarese M., Musumeci O., Brizzi T., Torella A., Conca E., Nigro V., Moggio M., Toscano A., Rodolico C. (Messina, Pozzuoli, Milano)

P.7 Fetal akinesia deformation sequence and recessive central core disease: a rare presentation of mutations in RYR1 gene
Emmanuele V., Torella A., Sframeli M., Musumeci O., Messina S., Nigro V., Rodolico C., Toscano A. (Messina, Pozzuoli)

P.8 Congenital myopathy with protein aggregates and nemaline bodies related to CFL2 mutations
Fattori F., Fiorillo C., Rodolico C., Tasca G., Verardo M., Bellacchio E., Fagiolarì G., Lupica A., Broda P., Moggio M., Bruno C., Tartaglia M., Bertini E., D'Amico A. (Roma, Genova, Messina, Milano)

P.9 Neuropsychological pattern in centronuclear myopathy due to DNM2 gene mutations
La Rosa C., Sframeli M., Vita G.L., Di Stefano M.G., La Rosa M., La Foresta S., Faraone C., Russo M., Fattori F., D'Amico A., Bertini E., Rodolico C., Lunetta C., Messina S., Vita G. (Messina, Pisa, Roma)

P1-3 INFLAMMATORY MYOPATHIES

P.10 Atypical clinical pictures in inflammatory myopathies: a case series
Caria F., Galvagni A., Baronchelli C., Rota S., Gallo Cassarino S., Marchesi M., Cotti Piccinelli S., Padovani A., Filosto M. (Brescia)

P.11 Statin-induced necrotizing autoimmune myopathy: clinical, histopathological and radiological characterization of five patients
Villa L., Peverelli L., Lerario A., Tironi R., Ciscato P., Woods A.M., Sciacco M., Moggio M. (Milano, Oxford)

P.12 Muscle biopsy findings and outcome in necrotizing autoimmune myopathy
Amati A., Lia A., Girolamo F., Serlenga L., Giannini M., Iannone F., Trojano M. (Bari)

P1-4 METABOLIC MYOPATHIES

P.13 Asymptomatic primary carnitine deficiency unmasked in a mother by newborn screening
Cotti Piccinelli S., Marchesi M., Carducci C., Angeloni A., Rota S., Caria F., Galvagni A., Baronchelli C., Padovani A., Filosto M. (Brescia, Roma)

P.14 Atypical features in multiple ACYL-CoA dehydrogenase deficiency: report of two cases
Lupica A., Musumeci O., Barca E., Mazzeo A., Rodolico C., Toscano A. (Messina)

P.15 Dilative Arterial malformations in patients with Late Onset Pompe Disease (LOPD)
Musumeci O., Granata F., Rodolico C., Arrigo R., Mosca V., Brizzi T., Ciranni A., Longo M., Toscano A. (Messina)

P.16 Riboflavin transporter deficiency (BVFL): transient expanded newborn screening (NBS) positivity for beta-oxidation abnormalities
Pasquini E., Sacchini M., Cavicchi C., Malvagia S., Funghini S., Donati M.A. (Firenze)

P.17 Newborn screening for Pompe disease in Tuscany and Umbria: current overview and first preliminary results after two years
Pasquini E., La Marca G., Morrone A., Daniotti M., Forni G., Catarzi S., Scolamiero M., Sacchini M., Donati M.A. (Firenze)

P.18 Development of a mobile app conceptually designed for patients with Pompe disease
Peviani S., Carlini F., Proietti C., Ricci G., Vianello A., Baldanzi S., Ubaldi U., Seidita F., Antonini G., Siciliano G. (Pisa, Milano, Padova, Roma)

P.19 Polymorphisms in exercise genes and respiratory outcome after ERT in a cohort of Late Onset Pompe Disease (LOPD)
Ravaglia S., Carlucci A., Malovini A., Danesino C., De Filippi P. and the Italian GSDII Group (Pavia)

P.20 Functional assessment tools in infantile Pompe disease. A critical analysis and pilot study
Ricci F., Brusa C., Berardinelli A., Rolle E., Rossi F., Placentino V., Spada M., Pagliardini V., Vitiello B., Mongini T. (Torino, Pavia)

P.21 Three Dimensional Gait Analysis in Late Onset Pompe Disease (LOPD)
Sancricca C., Rossellini G., Denza G., Pelliccioni M., Primiano G., Cuccagna C., Bernardo D., Sauchelli D., Servidei S. (Roma)

P.22 Young girl complaining of fatigue and muscle contractures
Agazzi E., Colombo O., Gardinetti M., Rottoli M.R. (Bergamo)

P.23 The importance of a non-invasive screening in proximal myopathies
Sampaolo S., Allegorico L., Bruno G., Lombardi L., Di Iorio G. (Napoli)

P1-5 MITOCHONDRIAL MYOPATHIES

P.24 Familial ALS, clinical heterogeneity and mitochondrial disorders: description of a family
Bisordi C., LoGerfo A., Caldarazzo Ienco E., Mancuso M., Siciliano G. (Pisa)

P.25 Mitochondrial Involvement in Patients with Autism spectrum disorders
Borgione E., Lo Giudice M., Castello F., Santa Paola S., Musumeci S.A., Giusto S., Di Vita G., Vitello G.A., Di Blasi F., Savio M., Scuderi C. (Troina)

P.26 Mitochondrial Giant Crystals in muscle biopsy

Costa R., Papa V., D'Angelo R., Rinaldi R., Tonon C., Lodi R., Cenacchi G. (Bologna)

P.27 Liver transplantation reverses biochemical imbalance and improves clinical conditions in mitochondrial neurogastrointestinal encephalomyopathy

Rinaldi R., D'Angelo R., Pironi L., Pinna A.D., Caporali L., Boschetti E., Cenacchi G., Lodi R., Carelli V., De Giorgio R. (Bologna)

P.28 Growth Differentiation Factor 15 as a useful biomarker for mitochondrial Disorder

Salvatore S., Formichi P., Taglia I., Bracalente I., Battisti C., Malandrini A., Federico A. (Siena)

P1-6 OTHER MYOPATHIES

P.29 Discordant manifestations in two Italian brothers with GNE myopathy

Dotti M.T., Lornage X., Malandrini A., Bohm J., Romero N.B., Laporte J., Malfatti E. (Siena, Illich, Paris)

P.30 A PGM NGS protocol in a single center cohort for patients with undiagnosed myopathy

Marchesi M., Lanzi G., Galvagni A., Cotti Piccinelli S., Mori L., Caria F., Rota S., Gallo Cassarino S., Facchetti F., Padovani A., Giliani S., Filosto M. (Brescia)

P.31 Severe muscle involvement caused by A193T mutation in FILAMIN-C

Monforte M., Ricci E., Udd B., Tasca G. (Roma, Helsinki, Tampere, Vaasa)

P.32 Clinical next generation sequencing gene Panel in patients orphan of genetic diagnosis

Neri M., Selvatici R., Scotton C., Storbeck M., Vezyroglou K., Heller R., Tugnoli V., Bigoni S., Timmerman V., Wirth B., De Grandis D., Gualandi F., Ferlini A. (Ferrara, Cologne, Antwerpen, Verona)

P.33 The success of whole exome sequencing analysis in neuromuscular diseases patients: the unife experience within neuromics project

Neri M., Scotton C., Selvatici R., Gualandi F., Wirth B., Schols L., Klockgether T., Lochmuller H., Muntoni F., D'Amico A., Bertini E., Pane M., Mercuri E., Ferlini A. (Ferrara, Cologne, Tübingen, Bonni, Newcastle upon Tyne, London, Roma)

P.34 Systemic AL amyloidosis revealed by a muscle biopsy: a case report

Papa V., Costa R., Bellanova M.F., Capozzi A.R., Sacconi E., Verga L., Capello G.L., Accardi F., Giuliani N., Pietrini V., Cenacchi G. (Bologna, Parma, Pavia)

P.35 GNE myopathy functional activity scale (GNEM-FAS): a four year follow up in 10 HIBM patients

Parisi D., Portaro S., Brizzi T., Biasini F., Cavallaro F., Vita G., Toscano A., Rodolico C. (Messina, Palermo)

P.36 McLeod syndrome: an Italian family with a novel mutation in the XK gene.

Piccolo G., Tartara E., Terzaghi M., Cortese A., Cittadella R., Benna P., Cavallaro S., Galimberti C.A. (Pavia, Catania, Torino)

P.37 Detection and multidisciplinary care of myopathic patients in Ogliastro

Piras R., Maioli M.A., Murru M.R., Costa G., Solla E., Mancosu C., Mammoliti R., Marrosu G. (Cagliari)

P.38 PLEC gene mutations cause familial disto-proximal myopathy and long QT syndrome mimicking mitochondrial disease

Primiano G., Tartaglia M., Cuccagna C., Sauchelli D., Bernardo D., Sancricca C., Lucchini M., Mirabella M., Servadei S. (Roma)

P.39 Myalgias, cramps and muscle rippling: a case report

Ricci G., Ali G., Chico L., Logerfo A., Cassandrini D., Comi G., Sorrentino V., Costa R., Cenacchi G., Siciliano G. (Pisa, Milano, Siena, Bologna)

P.40 Encephalocardiomyopathy with severe recurrent rhabdomyolysis due to TANGO2 mutations: a case report
Ricci F., Brusa C., Ferraris S., Ferrero G.B., Bertini E., Di Rocco M., Moroni I., Larosa P., Zoppo M., Manole A., Scalco R., Vitiello B., Mongini T. (Torino, Roma, Genova, Milano, London)

P.41 Next-generation sequencing approach for the diagnosis of genetic basis of hyperckemia: results from 25 patients
Rubegni A., Astrea G., Battisti C., Cassandrini D., Donati M.A., Dotti M.T., Lenzi S., Maioli M.A., Malandrini A., Melani F., Pasquini E., Trovato R., Santorelli F.M. (Pisa, Siena, Firenze, Cagliari)

P.42 Expression of Aquaporin 4 in normal human muscle is independent from myosin heavy chain isoform
Vizzaccaro E., Terracciano C., Rastelli E., Massa R. (Roma)

P.43 Benign monomelic amyotrophy of lower limb: report of 15 Italian cases
Di Muzio A., Barbone F., Telese R. (Chieti)

P.44 Quantitative muscle ultrasound analysis in neuromuscular disorders
Di Pasquale A., Fragiotta G., Vanoli F., De Santis T., Fionda L., Garibaldi M., Bucci E., Vizzaccaro E., Morino S., Antonini G. (Roma)

P.45 Neurorehabilitation in ALS: consequences at micrnas level
Giaretta L., Pegoraro V., Merico A., Angelini C. (Venezia)

P.46 The Questionnaire GNAMM: the eating habits of 436 people with neuromuscular disease in Italy.
Pini A., Zoni L., Poli C., Giannotta M., Di Pisa V., Siciliano G., Santorelli F., Mongini T., Filosto M., Politano L. (Bologna, Pisa Torino, Brescia, Napoli)

P.47 BAG3 mutation: from cardiomyopathy to a complex severe neuromuscular disorder with myofibrillar myopathy in a pediatric case
Scarpini G., Di Pisa V., Giannotta M., Nigro V., Ragni L., Valentino L., Pini A. (Bologna, Napoli)

P.48 A singular case of Rhabdomyolysis with reversible paralysis
Rastelli E., Vizzaccaro E., Frezza E., Massa R. (Roma)

POSTER SESSION - 2nd June 2017, 14.00 - 15.30

Chairpersons: **G. Baranello, F. Biasini, A. D'Amico, J.F. Desaphy, F. Santorelli**

P2-1 DYSTROPHIC AND NON-DYSTROPHIC MYOTONIAS

P.49 Functional study of myotonia congenita mutations in the C-terminus of CLC-1 and proof of concept study for chaperone-mediated rescue of trafficking-defective CLC-1 mutant
Altamura C., Imbrici P., Lucchiari S., Sahbani D., Comi G.P., Dotti M.T., Meola G., Politano L., Lo Monaco M., Desaphy J.F., Conte Camerino D. (Bari, Milano, Siena, Napoli)

P.50 Myotonic dystrophy type 2 in a Sicilian cohort: a challenging diagnosis by biomolecular tests
Biasini F., Rodolico C., Brizzi T., Cardani R., Valaperta R., Toscano A., Meola G., (Messina, Milano)

P.51 Cardiac troponin T in skeletal muscle from myotonic dystrophies patients: a possible biomarker of cardiac dysfunctions
Bose' F., Renna L.V., Ferrari N., Arpa G., Fossati B., Meola G., Cardani R. (Milano)

P.52 Skeletal muscle CLC-1 channel: from gene to protein, from birth to aging
Conte E., Fonzino A., Cibelli A., De Luca A., Pierno S., Nicchia G.P., Camerino G.M. (Bari)

P.53 Mild phenotype in DM1 young boy due to interrupted repeat of the DMPK expanded tract
Fossati B., Cardani R., Valaperta R., Cavalli M., Brignonzi E., Meola G. (Milano)

P.54 Monitoring motor function and disease progression in DM1
Gualandris M., Maestri E., Lizio A., Gatti V., Paci E., Beshiri F., Zanolini A., Sansone V. (Milano)

P.55 Gender-related characteristics of myotonic dystrophy type 1 in a large Italian database
Lozzi F., Rastelli E., Antonini G., Bianchi M.L.E., Botta A., Bucci E., Casali C., Costanzi Porrini S., Frezza E., Giacanelli M., Greco G., Inghilleri M., Novelli G., Pennisi E.M., Petrucci A., Piantadosi C., Silvestri G., Terracciano C., Vanacore N., Massa R. (Roma)

P.56 Non dystrophic myotonias: review of our cases with focus on genotype-phenotype correlations and therapeutic effects of Mexiletine
Montano V., Ricci G., Simoncini C., Chico L., Bernasconi P., Lehmann-Horn F., Siciliano G. (Pisa, Milano, Ulm)

P.57 Thomsen disease with central core features at muscle biopsy; a new morphological pattern or an unusual double trouble?
Peverelli L., Villa L., Lerario A., Lucchiari S., Pagliarani S., Tironi R., Conca E., Fagiolari G., Grimoldi N., Sgiacco M., Comi G.P., Moggio M. (Milano)

P.58 Post-receptor abnormalities contribute to insulin resistance in myotonic dystrophy type 1 and type 2 skeletal muscle
Renna L.V., Iachettini S., Fossati B., Saraceno L., Colombo R., Meola G., Cardani R. (Milano)

P.59 Non invasive ventilation in DM1: evaluation of compliance in a cohort of patients followed at the Nemo Center Milan
Sannicolò G., De Mattia E., Falcier E., Lizio A., Iatomasi M., Lupone S., Mollar E., Castino V., Conti C., Roma E., Rao F., Sansone V. (Milano)

P.60 Expanded [CCTG]_n repetitions are not associated with abnormal methylation at the CNBP locus in myotonic dystrophy type 2 (DM2) patients
Santoro M., Maiorca M., Fontana L., Centofanti F., Russo S., Massa R., Silvestri G., Novelli G., Botta A. (Milano, Roma)

P.61 Clinical variability in myotonic dystrophy type 1: a five-categories disease classification fits clinical but not brain complexity
Simoncini C., Baldanzi S., Ricci G., Cecchi P., Migaleddu G., Cosottini M., Siciliano G. (Pisa)

P.62 Evidence of mitochondrial dysfunction delays the diagnosis of myotonic dystrophy type 2
Valentino M.L., La Morgia C., Pellegrini C., Caporali L., Lodi R., Liguori R., Carelli V. (Bologna)

P.63 TP-PCR as a secondary analytical level in Myotonic Dystrophies diagnostic pathway
Lucchiari S., Conti B., Pagliarani S., Brusa R., Magri F., Govoni A., Peverelli L., Comi G.P. (Milano)

P.64 Cardiological assessment in a cohort of patients affected by congenital Myotonic Dystrophy type 1
Petillo R., D'Ambrosio P., Scutifero M., Orsini C., Palladino A., Politano L. (Napoli)

P.65 Mutational variety in patients with Myotonia Congenita from Campania Region
D'Ambrosio P., Petillo R., Scutifero M., De Luca C., Picillo E., Ergoli M., Passamano L., De Luca A., Politano L. (Napoli, Roma)

P.66 A novel mutation in KV1.1 channels in a patient with paroxysmal ataxia, myokymia, painful contractures and metabolic dysfunctions

Imbrici P., Altamura C., Gualandi F., Mangiatordi G.F., Neri M., De Maria G., Ferlini A., Padovani A., Sahbani D., D'Adamo M.C., Nicolotti O., Pessia M., Conte D., Filosto M., Desaphy J.F. (Bari, Ferrara, Brescia, Msida, Perugia)

P.67 Periodic paralysis: an emergency department presentation

Agazzi E., Pavanelli D., Riva R., Rottoli M.R. (Bergamo)

P2-2 DYSTROPHINOPATHY/LGMD/CONGENITAL MUSCULAR DYSTROPHIES/FSHD

P.68 Hippo signaling pathway in muscular dystrophies

Aguennouz M., Polito F., Rodolico C., Vita G.L., Migliorato A., Oteri R., Ciranni A.M., Messina S., Toscano A., Di Giorgio R.M., Vita G. (Messina)

P.69 Circadian rhythm genes in Duchenne muscular dystrophy

Armaroli A., Osman H., Scotton C., Falzarano M.S., Rossi R., Capogrosso R.F., Cozzoli A., Camerino G.M., Schwartz E., De Luca A., Ferlini A. (Ferrara, Bari, Rockville, London)

P.70 LGMD2B with high dysferlin retention: two case reports

Barresi R., Hudson J., Hilton-Jones D., Petty R., Longman C., Farrugia M.E., Marini-Bettolo C. (Newcastle upon Tyne, Oxford, Glasgow)

P.71 Plectin mutation without skin involvement as a possible cause of CMD

Berardinelli A., Rossi M., Ciscato P., Tironi R., Pichiecchio A., Cassandrini D., Santorelli F.M. (Pavia, Milano, Pisa)

P.72 A rare case of myopathy with Pipestem capillaries in a female carrier of Becker muscular dystrophy

Cosentino G., Fierro B., Brighina F., Mirabella M., Rodolico C. (Palermo, Roma, Messina)

P.73 Effects of Long-Term Treatment with Eteplirsen on Cardiac Function: Left Ventricular Ejection Fraction in Eteplirsen-Treated Patients vs Disease Natural History

Duda P.W., Moody S., Colan S., Dworzak J., Mendell J.R. (Cambridge, Durham, Boston, Columbus)

P.74 Management of adult DMD patients: the experience of neuromuscular unit IRCCS e Medea

Gandossini S., Diella E., Scarpazza P., Marchi E., Russo A., Sala S., Lo Mauro A., Comi G.P., Aliverti A., D'Angelo M.G. (Bosisio Parini, Vimercate, Milano)

P.75 A case of limb-girdle muscular dystrophy type 2L mimicking Dermatomyositis

Gemelli C., Fiorillo C., Fabbri S., Cabona C., Zara F., Madia F., Mandich P., Grandis M. (Genova)

P.76 Custom micro-fluidic exome array to detect transcript mutations in undiagnosed patients with Collagen VI myopathies

Osman H., Rossi R., Falzarano M.S., Scotton C., Armaroli A., Selvatici R., Gualandi F., Ferlini A. (Ferrara, Khartoum, London)

P.77 Muscle ultrasound elastography and MRI in preschool children with Duchenne muscular dystrophy: a pilot study

Pichiecchio A., Alessandrino F., Bortolotto C., Cerica A., Rosti C., Raciti M.V., Rossi M., Baranello G., Bastianello S., Berardinelli A., Calliada F. (Pavia, Milano)

P.78 Cognitive and psychiatric alterations in facioscapulohumeral muscular dystrophy: a case report

Pizzamiglio C., Solara V., Cantello R., Mazzini L. (Novara)

P.79 Facioscapulohumeral muscular dystrophy and 18p syndrome

Capet N., Renard D., Lagha N., Garibaldi M., Khau Van Kien P., Feasson L., Manel V., Cristofari G., Sacconi S. (Nizza, Nimes, Lione, Saint Etienne)

P.80 Respiratory pattern in FSHD patients as possible outcome measure

Scarlato M., Pozzi M., Velardo D., D'Angelo M.G., Pasanisi B., Maggi L., Previtali S.C., Tettamanti A. (Milano, Bosisio Parini)

P.81 International-DMD (IDMD): a PTC therapeutics-supported diagnostic project to widely identify dystrophin mutations by NGS technologies

Selvatici R., Rossi R., Trabanelli C., Rimessi P., Fini S., Gualandi F., Ferlini A. (Ferrara)

P.82 Epidemiology of facioscapulohumeral muscular dystrophy in Abruzzo

Telese R., Tupler R., Di Muzio A. (Chieti, Modena)

P.83 Next-generation sequencing analysis for the diagnosis of Duchenne/Becker muscular dystrophies

Trabanelli C., Selvatici R., Rimessi P., Venturoli A., Fini S., Fabris M., Neri M., Gualandi F., Ferlini A. (Ferrara)

P.84 Clinical and molecular consequences of EXON 78 deletion in DMD gene

Traverso M., Assereto S., Baratto S., Iacomino M., Pedemonte M., Diana M.C., Ferretti M., Bruno C., Zara F., Broda P., Minetti C., Gazzero E., Madia F., Fiorillo C. (Genova)

P.85 Multi-parametric characterization of highly fat infiltrated Limb Girdle muscular dystrophy patients: results of a multi-variate analysis

Velardo D., De Luca A., Bertoldo A., Froeling M., Gandossini S., Russo A., Magri F., Arrigoni F., D'Angelo M.G. (Bosisio Parini, Padova, Utrecht, Milano)

P.86 A diagnostic anoctamin-5 western blot

Vihola A., Luque H., Savarese M., Penttilä S., Lindfors M., Leturcq F., Eymard B., Tasca G., Brais B., Conte T., Charton K., Richard I., Udd B. (Helsinki, Tampere, Paris, Roma, Montreal, Evry, Vaasa)

P.87 Muscle magnetic resonance imaging as a prognostic biomarker in Becker muscular dystrophy

Bello L., Barp A., Caumo L., Campadello P., Semplicini C., Lazzarotto A., Zanato R., Stramare R., Pegoraro E. (Messina, Roma)

P.88 Effectiveness of treatment with ivabradine on clinical and instrumental endpoints in patients with Duchenne Muscular Dystrophy

Distefano M.G., Vita G.L., Sframeli M., Di Bella G., Pugliatti P., Recupero A., Barcellona C., La Rosa M., Nicocia G., Carerj S., Lunetta C., Messina S., Vita G. (Messina)

P.89 Cardiac involvement in a patient with congenital-muscular-dystrophy related to POMT2 gene mutation.

La Rosa M., Sframeli M., Distefano M.G., Barcellona C., Vita G.L., Nicocia G., Astrea G., D'Amico A., Bertini E., Santorelli F., Vita G., Lunetta C., Messina S. (Messina, Pisa, Roma)

P.90 Ten years (2006-2016) of molecular diagnosis in Collagen-VI related myopathies: are intragenic coding SNPS of COL6A GENES modifiers of disease severity?

Rossi R., Trabanelli C., Venturoli A., Scotton C., Armaroli A., D'Amico A., Berardinelli A., Filosto M., Fiorillo C., Bruno C., Marrosu G., Pane M., Rodolico C., Mongini T., Moroni I., Baranello G., Santoro L., Pegoraro E., Politano L., Pini A., Fusco C., Merlini L., Morandi L., Bertini E., Messina S., Mercuri E., Fini S., Ferlini A., Gualandi F. (Ferrara, Roma, Pavia, Brescia, Genova, Cagliari, Messina, Torino, Milano, Napoli, Padova, Caserta, Bologna, Reggio Emilia)

P.91 Congenital muscular dystrophy and epilepsy: a prospective observational study on 16 pediatric patients
Vitaliti G., Romano C., Sciuto C., Ruggieri M., Falsaperla R. (Catania)

P.92 Pivotal role of the clinical geneticist in diagnosing rare diseases. The index case of laminopathies
Passamano L., D'Ambrosio P., Petillo R., Della Pepa C., De Luca C., Torella A., Papa A.A., Palladino A., Novelli A., Nigro V., Politano L. (Napoli, Roma)

P2-3 SMA

P.93 Segmental body composition in young children with SMA type 2: correlation with motor function abilities
Baranello G., Arnoldi M.T., Zanin R., Masson R., Mastella C., De Amicis R., Battezzati A., Bertoli S. (Milano)

P.94 Fasting glucose in children with spinal muscular atrophy type I and II
Bertoli S., De Amicis R., Foppiani A., Leone A., Battezzati A. (Milano)

P.95 Scoliosis is an inescapable comorbidity in SMA type II. A single center experience
Catteruccia M., Colia G., Bonetti A.M., Carlesi A., Oggiano L., La Rosa G., Turturro F., Bertini E., D'Amico A. (Roma)

P.96 Longitudinal assessments in discordant twins with SMA
Palermo C., Pane M., Abiusi E., Lapenta L., De Sanctis R., Luigetti M., Ranalli D., Fiori S., Tiziano F., Mercuri E. (Roma)

P.97 Improved distal spinal muscular atrophy genetic diagnosis by targeted NGS sequencing
Moro F., Rubegni A., Lenzi S., Trovato R., Astrea G., Battini R., Battisti C., Bruno C., DiFabio R., Fiorillo C., Gallone S., Malandrini A., Mari F., Massa R., Pegoraro E., Petrucci A., Pini A., Santorelli F.M. (Roma, Siena, Genova, Torino, Firenze, Padova, Bologna)

P.98 Spinal muscular atrophy type 2 and 3: evaluation of autonomic nervous system function
Sframeli M., Stancanelli C., Vita G.L., Terranova C., Rizzo E., Cavallaro F., Lunetta C., Vita G., Messina S. (Messina, Palermo)

P.99 Improving SMARD1 long-term outcome and quality of life: the role of a multidisciplinary setting
Bonanno C., Sframeli M., Vita G.L., Distefano M.G., La Rosa M., Barcellona C., Profazio C., Versaci A., Mercurio L., Gitto E., Romeo C., Vita G., Lunetta C., Messina S. (Messina)

ABSTRACTS OF ORAL COMMUNICATIONS

(in alphabetical order of the first Author)

Glycosilation of alpha-dystroglycan: one pathway several phenotypes

Astrea G.¹, D'Amico A.², Battini R.¹, Berardinelli A.³, Bertini E.², Bruno C.⁴, Cassandrini D.¹, Catteruccia M.², Comi G.P.⁵, Fattori F.², Fiorillo C.⁴, Giannotta M.⁶, Gorni K.⁷, Magri F.⁵, Mercuri E.⁸, Messina S.⁹, Mongini T.¹⁰, Mora M.¹¹, Morani F.¹, Moro F.¹, Pane M.⁸, Pegoraro E.¹², Pini A.⁶, Politano L.¹³, Ricci F.⁹, Sframeli M.⁸, Santorelli F.M.¹

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Alpha-dystroglycanopathy is associated with a wide spectrum of muscle disorders and is the results of mutations in at least 20 genes directly or putatively involved in the glycosylation pathway.

We present the phenotypic breakdown in a large Italian cohort of patients presenting with hypoglycosylation of alpha-dystroglycan (a-DG) in muscle biopsy and a molecular definition.

Using standardized immunohistochemical staining methods in muscle biopsies, 81 patients with a possible low a-DG glycosylation were referred to us for genetic analyses as part of a multicenter study supported by the Telethon Foundation. For genetic studies we used a targeted resequencing method in NGS that included 95 genes associated with CMD, LGMD or related diseases. Detailed clinical, morphological, and neuroradiological data were collected.

A diagnosis was genetically confirmed in 47/81 patients. According to the clinical classification proposed by Godfrey et al (2011) 17 patients were MEB/FCMD, CMD-MR or CMD-CRB, and 17 had a diagnosis of CMD-NOMR. Moreover, 2 cases had a LGMD-MR and 9 a LGMD-NOMR. All the cases but 7 had elevated blood CK levels. Asymptomatic high serum CK level was the only manifestation in 2 patients.

Two major findings emerge from this study: 1) not all the patients harbour mutations in genes directly related to glycosylation of a-DG and secondary glycosylation defects can be attributed to several CMD-related genes; 2) no genotype-phenotype correlations seem evident in our cohort.

The wide spectrum of phenotypes related to alpha-dystroglycanopathy impose the use of high throughput technologies in clinical practice.

Phenotypical and molecular definition of a multi-center cohort of 49 patients with limb girdle muscular dystrophy type 2a

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Limb Girdle Muscular Dystrophy 2A (LGMD2A) is a rare disorder characterized by progressive muscular weakness due to mutations in the CAPN3 gene. We evaluated a population of 49 patients affected with LGMD2A with validated, quantitative outcome measures (OMs). We recruited 22 males and 27 females, aged 8–68 years. Inclusion criteria were 2 mutated CAPN3 alleles (43 patients), or 1 mutation and reduced calpain-3 protein at Western Blot (WB) (6 patients). Onset of symptoms and loss of independent ambulation (LoA) were evaluated retrospectively. We cross-sectionally evaluated patients with North Star Ambulatory Assessment (NSAA), Six Minute Walk Test (6MWT), timed function tests (TFTs), and Performance Upper Limb (PUL). Immunoblot showed absent calpain-3 in 17 patients (35%), a partial defect in 16 (33%), and a normal amount in 9 (18%) (WB unavailable in 7 patients). Age at disease milestones did not differ significantly between genders, while complete calpain-3 deficiency was associated with earlier onset ($p = 0.026$), earlier LoA ($p = 0.017$), lower average NSAA score ($p = 0.007$), shorter 6MWT distance ($p = 0.001$), slower 10 m walk/run ($p = 0.005$), slower stand from floor ($p = 0.009$), and slower climb 4 standard steps velocities ($p = 0.019$). Male patients showed significantly lower proximal PUL scores ($p = 0.021$). Age was correlated with NSAA, TFTs, and proximal PUL items, but not with 6MWT and distal PUL items. Protein amount by WB showed no linear correlation with OMs. In conclusion, the presence of reduced or normal calpain-3 protein at WB predicted a milder phenotype. NSAA, 6MWT, TFTs, and shoulder/elbow PUL items are clinically meaningful OMs for calpainopathy.

Observational study on the nutritional and metabolic features in Duchenne muscular dystrophy: the Italian "N&M Duchenne Study"

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In the management of Duchenne Muscular Dystrophy (DMD), nutrition and metabolism are important issues because they can be adversely affected by range of complicating features since early childhood. Boys with DMD are at risk of overweight and obesity due to reduction of physical activity, reduction of basal metabolic rate and use of corticosteroids. On the other hand, delayed gastric emptying, dysphagia and gastroesophageal reflux poses them at risk of underweight. Finally, a critical

role body composition on neurofunctional impairment has been suggested.

In order to improve the dietary management in DMD, we are starting a two-year collaborative study (founded by Parent Project foundation) aimed to (1) start a national survey on dietary habits, eating behaviours and gastrointestinal symptoms in Italian DMD patients, (2) map nutritional status and body composition with a standardized measurement procedure tailored to DMD patients' characteristics, (3) study energy expenditure, nutrients and energy needs, insulin resistance, metabolic syndrome, dyslipidaemia and adipokines secretion. The main outcomes of this study will be estimates the prevalence of poor dietary quality, gastrointestinal symptoms and malnutrition and the definition of the the most urgent dietary interventions to improve the nutritional management. Furthermore, we will produce basal metabolic rate and anthropometric predictive equations specific to DMD patients and we will investigate the relationship between body composition and neuromuscular function.

The mildest end of the dystroglycanopathy phenotypic spectrum: from asymptomatic hyperckemia to limb girdle muscular dystrophy

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Dystroglycanopathies are a heterogeneous group of diseases caused by mutations in 15 different genes, all leading to defect in alpha-dystroglycan (alpha-DG) function. Most dystroglycanopathies were originally described in patients with congenital muscular dystrophies, affecting not only muscle but also the eye and the brain, with onset by age 6 months. Subsequently mutations in some of these genes have been associated with milder phenotypes presenting in adult life with limb girdle muscular dystrophy (LGMD) or with isolated rhabdomyolysis. We describe clinical, genetic, morphological and imaging features of 11 patients (age-range 4-52 yrs) with genetic defect of alpha-DG, presenting at the mildest end of this clinical spectrum. Nine were diagnosed because of asymptomatic hyperckemia but four developed LGMD during follow-up; two patients presented with myalgia and rhabdomyolysis episodes. Three patients showed intellectual disability with normal brain MRI; two patients had cardiac involvement and one had respiratory impairment. Mutations in the following genes were identified: *FKRP* (6 patients), *POMT2* (3 patients) and *GMPPB* (2 patients). Muscle biopsies showed mild to moderate signs of necrosis, degeneration-regeneration in most severely affected cases. Immunolabelling of glycosylated alpha-DG was variably reduced. Muscle MRI performed in 9 patients suggest a common pattern of muscle involvement with predominant alteration in posterior compartment of thigh and leg. Our data underline that a defect of alpha-DG can result in a very mild phenotype of muscle involvement from subclinical hyperckemia of children, passing through rhabdomyolysis, to adult onset LGMD, stressing the importance of alpha-DG testing in histological analysis of these patients.

20 years clinical follow-up in patients with oculopharyngeal muscle disease (OPMD)

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Oculopharyngeal muscle dystrophy (OPMD) is an autosomal dominant muscle disease. OPMD is clinically characterized by ptosis, eye movement abnormalities, dysphonia and dysphagia. It is caused by an abnormal (GCN) triplet expansion within the PABPN1 gene located on chromosome 14 (14q11.2-q13).

We present a cohort of 19 patients (13 F and 6 M) with OPMD. We performed quantitative EMG in all patients and muscle biopsy in 12 out of 18. We also applied MRC score for muscle strength evaluation as well as the EAT-10 (Eating Assessment Tool) which is a self-administered scale for dysphagia evaluation. All patients were genetically defined for PABPN1 gene variants.

From the clinical point of view, dysphagia and dysphonia worsened during the course of the disease as well as orbicularis oculi muscle strength. In addition, we observed that either axial muscles or posterior thigh muscles were progressively affected. 10 patients were evaluated with EAT-10 showing a worsening of dysphagia.

Our data confirms that, at disease onset, the orbicularis oculi are the weaker mimic muscles. However, during the clinical progression, OPMD also shows a worsening of dysphonia and dysphagia with a progressive involvement of proximal limb and axial weakness.

Next-generation sequencing approach for the diagnosis of congenital myopathies: a 3-yr experience

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Congenital myopathies (CM) are a group of genetic muscle disorders characterized clinically by hypotonia and weakness, usually from birth, and a static or slowly progressive clinical course. Historically, CM have been classified on the basis of the major morphological features seen on muscle biopsy and different genes have been identified. In recent years, next-generation sequencing has increasingly been used for the diagnosis of CM because of their larger than expected genetic and clinical heterogeneity. Between 2014 and 2016 we analyzed 107 molecularly undefined patients meeting clinical and histological criteria for a diagnosis of CM. We used in clinical settings, the MiSeq Illumina methodology and HaloPlex and SureSelect-designed (Agilent, Santa Clara, California, USA) multigene panels containing a total of 57 genes. Of 107 patients, 66% (n = 71) received a molecular diagnosis, including 13% who had mutations in *RYR1* and three cases with myofibrillar myopathy harboring new mutations in the *LDB3* gene. Moreover 15 patients were found to carry mutations in *TTN*. In the latter cases, only combination of muscle biopsy studies, segregation in relatives and functional

studies can confirm or confute the disease-related role of the mutations identified. Our results offer the perspective of a single center experience in congenital myopathies and could help to draw more firm diagnosis and better counseling in CM families.

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Respiratory function in ataluren-treated, non-ambulatory patients with nonsense mutation Duchenne (nmDMD) muscular dystrophy from a long-term extension trial versus untreated patients from a natural history study

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nmDMD is a rare, X-linked disorder, caused by a nonsense mutation in the dystrophin gene, which leads to muscle function decline and loss of pulmonary function.

The effects of ataluren on lung function in non-ambulatory nmDMD boys was assessed as part of an open-label, long-term, safety trial (NCT01557400-study 019).

Data from patients in study 019, receiving ataluren 40 mg/kg/day, were compared with data from patients ("untreated") from a long-term DMD natural history study (NCT00468832;CINRG). Patient populations were matched using the following criteria: non-ambulatory (requiring wheelchair use), ≤ 25 years old, with ≥ 24 months of corticosteroid use. To reflect the standard of care available during study 019, only CINRG data from 2012 onward were included. Data were analyzed using piecewise linear regression (absolute FVC).

Overall, 114 patients from the CINRG study and 53 pa-

tients from study 019 were included (mean age: 15.9 years, 14.1 years; mean baseline absolute FVC: 1.74 L, 2.03 L [CINRG, 019, respectively]). As expected, absolute FVC increased with age in untreated patients until 12.5 years; after this breakpoint, they tended to decrease with increasing age. In comparison, breakpoint in ataluren-treated patients was 16.5 years. Using the geometric mean log FVC, adjusted for within-patient correlation using a repeated-measures analysis of variance, a difference of 13.8% in predicted absolute FVC was seen in favour of ataluren patients compared with untreated patients ($p = 0.005$).

This historically-controlled, matched analysis shows a clinically important preservation of respiratory function in ataluren-treated versus untreated patients with nmDMD.

Study financed by: PTC Therapeutics Inc.

Polymorphisms of the GAA gene causing amino-acid changes and analysis of the impact on protein structure in a cohort of 50 late onset Pompe disease (LOPD)

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In addition to the identification of the pathogenic mutation, mutation analysis by sequencing of the entire GAA gene for LOPD diagnosis will often reveal the presence of several polymorphisms. We classify them in three main types: polymorphisms localized in introns; in exons, without amino-acid (aa) changes; in exons, with aa changes. While polymorphisms in introns and those without aa change do not modify protein structure, those polymorphisms causing aa changes will modify the primary structure of the protein, and eventually its secondary or tertiary structure also. We will report our data on three of these polymorphisms occurring in exons and changing the aa, namely: c.596G > A; c.668A > G; c.2338A > G. We were able to collect these data thanks to the collaboration of the Italian GSDII Group, that is gratefully acknowledged. For each of the polymorphisms we controlled their status by using prediction tools (Mutation Taster or Sift); we analyzed their evolutionary conservation; we reported their frequency in control population and we assessed their effect on protein structure. We studied a group of 50 LOPD patients, in which the three polymorphism are consistently present on the same chromosome, and are present both as heterozygous and homozygous haplotypes. For patients carrying the polymorphisms, when data are available, we also considered the effect of the pathogenic mutations (missense vs nonsense, severe vs mild) to evaluate whether and in which cases the aa change introduced by the polymorphisms may interact with the effect of the pathogenic mutations.

A respiratory snapshot of a cohort of DM1 patients: clinically stable doesn't mean normal

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Cardiorespiratory failure is the main cause of death in DM1. However, data on the prevalence and respiratory pattern of involvement are limited.

The purpose of this study was to investigate the physiological variables of lung function and sleep disordered breathing in clinically stable DM1 patients.

Retrospective data on sitting and supine spirometry, nocturnal oxymetry and arterial blood gas analysis were obtained from 71 DM1 patients. Epworth sleepiness scale (ESS) scores and hypoventilation symptoms were also analyzed.

Only 31% patients (22/71) had normal respiratory function, 12.5% (9) were already adapted to Non Invasive Ventilation (NIV) and 56% (40/71) had indications for NIV adaptation. Of these 40 (19 females and 21 males), 32.5% reported daytime sleepiness and 61% obtained an ESS score > 10 points. Women were more symptomatic than men, with 52.6% of them complaining of daytime sleepiness ($p = 0.0097$), while instrumental data were worst in males, with higher values of paCO_2 (46.72 ± 3.62 versus 44.67 ± 4.19) and HCO_3^- (31.48 ± 2.47 versus 30.18 ± 2.6). Patients with a Body Mass Index (BMI) > 26 had significantly higher hypoventilation symptoms and worse respiratory sleep parameters (ODI/h 21.54 ± 18.39 vs 9.58 ± 7 , $p = 0.0143$).

Clinically stable DM1 patients exhibit a high prevalence of sleep-disordered breathing and a significant incidence of daytime sleepiness and hypoventilation symptoms, especially in patients with BMI > 26. It is therefore highly recommended to screen patients with DM1 for respiratory muscle involvement and for sleep disorders for a better management of care.

Chromatin configuration, RNA and protein studies identified novel dna elements that influence the dystrophin transcription dynamics

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Despite of the several reports describing dystrophin (DMD) gene expression abnormalities, the DMD transcriptional process remains poorly understood. We therefore investigated the transcriptional capacity of the dystrophin locus. We designed a DMD locus ChIP-chip array to identify open chromatin regions, and a Chromosome Conformation Capture assay was used for interac-

tion studies. We selected muscle biopsies from 9 BMD patients to evaluate the different amount of dystrophin and its link to the novel regulatory regions identified; dystrophin transcript and protein were quantified by FluidDMD card and Western blot. We identified two novel DMD regions which bind the RNA Pol II, one in intron 52 (DMEi52) and another one in exon 62 (DMEe62). The DMEi52 contains a genuine RNA pol II pausing site used during DMD locus transcription. We also showed that two DMD regions, one in intron 34 (DMEi34) and one in exon 45 (DMEe45), share histone marks of open chromatin, but are unrelated to RNA Pol II activity. The DMEi34 bi-directionally stimulates the Dp427m promoter transcription. Supporting the DMEi34 transcription enhancing function we found lower dystrophin RNA and higher protein levels in BMD patients carrying genomic deletions encompassing the intron 34 compared to other differently deleted BMDs.

We identified the first DMD pausing site which is located in exon 62 and a novel dystrophin muscle enhancer in intron 34. These findings have important repercussions on the understanding of the transcriptional regulation of the DMD locus, as well as for the designing of splicing therapies.

Precision medicine to address therapy in myotonia caused by sodium channel mutations

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Mexiletine is an orphan drug for the treatment of myotonic syndromes. By blocking sodium channels, mexiletine reduces action potential firing, thereby counteracting myotonia. Yet it is estimated that about 20 % of myotonic patients do not benefit from mexiletine due to contraindications, side effects, or unsatisfactory response. Alternative therapies are thus required to improve patients' quality of life. In previous studies, we wondered whether mutations in hNav1.4 muscle sodium channel causing the disease might alter channel sensitivity to mexiletine. We showed, from bench to patient, that two mutations in hNav1.4, G1306E and P1158L, causing a severe phenotype impair mexiletine block in vitro but respond well to flecainide, another sodium channel blocker. The patients carrying such mutations obtained great benefits by shifting from mexiletine to flecainide [Desaphy et al, Eur J Clin Pharmacol 2013; Desaphy et al., Neurology 2016]. Encouraged by this success, in collaboration with the Italian Network for Muscle Channelopathies, we functionally and pharmacologically characterized other five hNav1.4 myotonic mutants, located close to G1306E in the molecular machinery for channel fast inactivation, using patch-clamp technique in transfected cells. The results highlight the molecular mechanisms by which the mutations cause myotonia and demonstrate how mexiletine sensitivity of the channels depends on the mutation-induced shift in channel fast inactivation volt-

age dependence. In contrast, flecainide effects were not significantly affected by mutations. This study paves the way toward a bench-to-patient pharmacogenetics approach to address therapy in myotonic syndromes caused by sodium channel mutations. (Supported by Italian Telethon GGP14096)

IgG anti rh-GAA assessment in an Italian cohort of patients with late-onset Pompe disease

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The role of IgG anti rh-GAA antibodies in modulating efficacy of enzyme replacement therapy (ERT) in late-onset Pompe disease (LOPD) has been evaluated only in a limited number of patients. We report clinical findings and serial measurements of IgG anti rh-GAA antibodies from 30 LOPD patients treated with ERT for a time ranging between 12 and 124 months, in order to investigate the possible impact of antibodies on treatment efficacy. Twenty two patients showed positive low or medium antibody titer (AT) of anti rh-GAA while 8 patients were persistently negative. Patients treated between 12 months and 35 months present an overall improvement in 6MWT, MRC and FVC. Among them, those with TA zero show a higher improvement than patients with positive TA, although no statistical significance was found. Patients treated between 36 months and 120 months show a substantial stability or slight worsening regardless the presence and the levels of TA. Our results confirm ERT long-term effectiveness, though it appears to be reduced over time compared to the first years of therapy. Correlation between functional deterioration and anti rh-GAA antibodies needs to be assessed on multiple patient cohorts.

NGS target re-sequencing analysis in patient with persistent asymptomatic or mildly symptomatic hyperckemia

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Persistent asymptomatic or mildly symptomatic hyperckemia, albeit extremely common, represents a diagnostic challenge for being an unspecific manifestation of different muscular dystrophies, metabolic conditions and several other neuromuscular disorders. The diagnostic work up of hyperckemia is complex and requires a growing list of investigations, gene testing and accurate analysis of muscle biopsy. Still in a great percentage of patients the diagnosis remains pending. In this study, we applied a NGS target resequencing of 20 genes, known to cause hyperckemia associate with muscular dystrophy or with other genetically determined metabolic conditions, all of which are not easily spotted at muscle biopsy. Ampliseq/Ion Torrent PGM technology was used. Analysis of variants was performed using IonReporter and CLC softwares setting a minimum coverage of 20X. Validation of selected rare variants was obtained by Sanger. 80 patients have been fully analyzed. 13 cases (16%) reached a definite genetic diagnosis including: 5 patients with compound heterozygous mutations in ANO5, 2 patients with homozygous mutation in CPT2 and 6 patients with previously reported dominant mutations in RYR1. In one case a second biopsy confirmed the presence of cores, the hallmark of this disorder. In 4 cases molecular tests allowed to identified other affected family members. In addition, we acknowledged 18 variants of unknown significance and 14 cases carrying a single variant in autosomal recessive genes (CPT2, GAA, PYGM, CAPN3, ANO5, FKTN) which need further investigations to recon possible clinical relevance. In conclusion mutations in ANO5 and RYR1 genes are the most common form of asymptomatic or mildly symptomatic hyperckemia, in our cohort. NGS analysis provide clinicians with helpful data, however interpretation of results can be challenging.

NGS in neuromuscular disorders: an update

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The use of Next-Generation Sequencing (NGS) in muscle disorders, because of the heterogeneity of conditions and the

overlap of clinical and histopathological features, has become essential in laboratory diagnostics and is a robust tool for discovery of new causative genes. Our experience with NGS panels in muscular disorders continued from last year, with a change in the grouping of genes selected. While previously genes were arranged in the panels based on age of onset of the associated disorders (childhood vs adulthood), at present we have three different panels with genes grouped by clinical and histopathological presentation of the related disorders, notably dystrophies of childhood and adulthood, congenital myopathies and myofibrillar myopathies, for a total of 161 genes. Our patients' cohort comprises as well patients recruited within the Myofibrillar Myopathies Network. Our rate of uncovered causative mutations is consistent with our previous data. In particular we solved 17% of the cases for the dystrophies panel, 11% for the myopathies panel and 30% for the myofibrillar myopathies one. Remarkably, the myofibrillar myopathies panel has a higher rate of mutations identified compared to our other panels. This is likely due to careful analysis of the histopathological (in particular altered expression of desmin and myotilin proteins) and clinical features of the patients, which allows an accurate pre-selection of the cases. Once more, NGS technology combined with a precise clinical definition and the use of muscle biopsy, can be a powerful mean for a faster, cheaper and accurate genetic diagnosis of muscular diseases.

Comprehensive CNVs assessment in 234 diagnosis-resistant myopathic patients

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An extensive NGS molecular study of 504 genetically undiagnosed patients with clinical diagnosis of muscular dystrophies, congenital myopathies or other conditions affecting muscles, has allowed to identify putative causative mutations in 218 out of 504 cases. After this screening, 286 cases remained undiagnosed. Elusive genetic changes, such as deletions, duplications or deep intronic mutations or novel genes may be responsible for these diagnosis-resistant patients. We recruited 234 out of 286 undiagnosed patients: in 127 NGS previously revealed a single mutation in a recessive disease gene, while in

107 NGS was totally negative. All patients were analyzed by Motor Chip, a custom CGH-array for the identification of deletions and duplications in neuromuscular disorders. Motor Chip allowed the identification of a causative deletion or duplication in 17 out of 234 patients. In more details, CNVs clearly involved in the observed phenotype were found in 10 out of 17 patients; in 7 cases deletions or duplications included genes different from those of clinical suspicion. Surprisingly only 5 out of 17 were heterozygous for a causative mutation and in 2 cases deletion occurred on the other allele of the same gene. Additional 5 out of 234 probably pathogenic rearrangements were found. Although they might be responsible for the observed phenotype, a further clinical and molecular evaluation is required. Finally, 3 deletions of uncertain significance (VUS) were revealed. Our results indicate that CNVs contribute for 7-9% of elusive mutations in undiagnosed myopathic patients and suggest they occur with lower frequency than expected in heterozygous patients.

Correlation between IGHMBP2 protein levels in human motor neuron and non-neuronal somatic cells and phenotype of 5 patients affected with spinal muscular atrophy with respiratory distress type 1

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Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is an autosomal recessive neuromuscular disorder characterized by degeneration of lower motor neurons clinically manifesting with distal muscle weakness and respiratory failure before 13 months of age. SMARD1 is caused by mutations in the *IGHMBP2* gene that encodes a ubiquitous protein with a putative RNA/DNA helicase function. The reasons of selective motor neurons vulnerability are still unknown and currently there is no therapy. Recent reports described milder phenotype of Charcot-Marie-Tooth type 2S (CMT2S) without respiratory failure associated to mutations in *IGHMBP2* gene, expanding the phenotypic spectrum. Genotype of patients with SMARD1 and CMT2S are similar, hence it has been hypothesized that severity of the phenotype is correlated to the amount of residual protein. We report on five patients with classical SMARD1 phenotype who developed severe distal muscle weakness, and respiratory failure that required permanent ventilation before six months of age. All of them carried mutations in *IGHMBP2* gene, in four patients we identified novel recessive *IGHMBP2* variants. To thoroughly elucidate the molecular profile present in SMARD1, we examined the expression of IGHMBP2 protein level in different patients' cells (fibroblasts, lymphoblastoid cell lines, and induced pluripotent stem cells and their derived motor neurons) both from controls and SMARD1 patients. We detected a profound reduction of the corresponding protein level in patients' cells. These results provide complementary data useful for the diagnosis as well as supportive for the development of gene therapy aimed at obtain the re-expression of *IGHMBP2*.

Vision DMD: a drug development program for vamorolone in Duchenne muscular dystrophy

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Vamorolone (VBP15) is an innovative dissociative steroid that has shown improved safety and efficacy in mouse models of Duchenne muscular dystrophy (DMD) and Phase I pharmacodynamics biomarker studies in healthy volunteers compared to glucocorticoids. Vamorolone has been designed to retain or improve glucocorticoid efficacy and increase membrane stabilization with reduced side effects, potentially increasing the therapeutic window of glucocorticoid, slowing disease progression and causing less side effects. The ongoing Phase IIa study, assessing safety, tolerability, pharmacokinetics and pharmacodynamics of ascending doses of vamorolone (0,25/0,75/2,0/6,0 mg/kg) will be followed by a Phase IIb pivotal safety and efficacy study in 30 international sites (3 in Italy: Milan, Padova, Rome). The Phase IIb study is a 24-week randomized, placebo- and glucocorticoid-controlled, multiple-dose study. It will enroll 100 steroid naïve and ambulant DMD boys age 4- < 7 years. The primary efficacy outcome is measured by the time to stand test and the primary safety outcome by the change in BMI.

These studies have innovative design, including use of serum biomarkers (both safety and efficacy), a new muscle MRI protocol aiming to increase the feasibility of MRI in large multicentre studies involving young children and new radiology biomarkers for bone health. Preliminary results of the Phase IIa study in summer 2017 will prompt the selection of the vamorolone doses for the Phase IIb and initiate the start of the study. The Phase IIa and IIb are followed by a long-term extension study. Further studies in additional age groups (younger boys and older patients) are also planned.

Long-term treatment with eteplirsen in non-ambulatory patients: a case study in identical twins

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We describe identical twins with Duchenne muscular dystrophy (DMD) enrolled in 2 phase 2 studies of eteplirsen who lost ambulation early in the trial, continued to receive eteplirsen, and were monitored for nonambulatory outcomes through 240 weeks.

In study 201, a double-blind placebo-controlled trial, patients aged 7–13 y with DMD amenable to exon 51 skipping were randomized to intravenous eteplirsen 30 or 50 mg/kg or placebo once weekly for 24 weeks. At week 25, all patients received open-label eteplirsen through week 28. In the open-label extension (study 202), patients continued once-weekly eteplirsen 30 or 50 mg/kg. Functional efficacy assessments through week 240 included the 6-minute walk test (6MWT), loss of ambulation, NSAA, and cardiac, pulmonary, and upper limb function.

Twelve patients were enrolled. Identical twin boys aged 9.9 y (eteplirsen 30 mg/kg) had greater baseline disease severity (6MWT distance 330 m and 256 m) vs other patients (6MWT range 341–429 m) and were taller (138 cm and 136 cm) vs other patients (range 116–133 cm). At study 201 week 24, the twins had a rapid decline in walking ability, losing ambulation near week 36 (study 202). No other patient lost ambulation. Dystrophin was analyzed at baseline and at weeks 24, 48, and 180. Dystrophin was increased at week 24. Functional data analysis from week 24 on showed that the twins maintained cardiac, pulmonary, and upper limb function comparable to study means for these measures through week 240.

In both studies, identical twins with more severe baseline DMD experienced rapid decline before the observed increase in dystrophin expression and lost ambulation shortly thereafter. Following observed posttreatment increases in dystrophin production, measures of disease progression were similar for these patients vs study means.

Myositis-specific Antibodies (MSA): high prevalence in biopsy-proven myositis population

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Myositis-specific Antibodies (MSA) are a group of autoantibodies often present in serum of patient with idiopathic inflammatory myopathies (IIM). Each MSA is usually associated with characteristic muscle biopsy findings and extramuscular involvement. On serum samples of 32 patients with clinical and histologically confirmed IIM, we performed a dot-blot (13 antibodies) analysis to investigate the presence of MSA. Twenty-three patients (71,8%) presented at least one MSA. Anti-SRP was the most frequent antibody in our population with 8 positive patients. Other positive antibodies were: 4 anti-HMGCR, 4 anti-NXP2, 3 anti-tRNA synthetase (1 PL7, 1 PL12, 1 Jo1), 2 anti-TIF1g, 2 anti-Mi2, 1 anti-MDA and 1 anti-Ku. Two different MSA were detected in two patients. Five patients with paraneoplastic myositis presented at least one MSA: two patients with poor prognosis had anti-TIF1-g, one anti-SRP, one anti-MDA5, one anti-NXP2/Mi2 antibodies. Necrotizing autoimmune myopathy was the most frequent form with 13 patients and only 1 seronegative. Anti-SRP and anti-synthetase patients presented a more aggressive and relapsing course with significant extramuscular involvement (myocarditis in anti-SRP patient, ILD in Jo1) with suboptimal response to standard therapy. In conclusion, by examining our IIM population, well characterized by clinical, laboratory and immunopathological findings, we found a higher rate of MSA positivity than previously reported, emphasizing the importance of their detection for a better phenotypic characterization. Our data confirm MSA testing as a very helpful tool in patient management, advising a more extensive search for malignancy and extramuscular involvement and providing prognostic clues for personalized therapy selection.

Registries for muscle glycogenoses: the Italian side of EUROMAC

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Rarity and clinical heterogeneity of muscle glycogen storage disorders (mGSD) are critical barriers towards the development of better care and effective treatment. Registries are an asset providing better knowledge of rare conditions. EUROMAC is a EU supported initiative for a European Registry for rare mGSD by a consortium representing 7+2 European countries including Italy. We evaluated the Italian registered population within EUROMAC in comparison with the other European cohorts. 34 of the 261 records in the registry were Italians (12,9%). 61% of Italians were males compared to 48% in the non-Italian cohort. The Italians were younger ($39,1 \pm 16,9$ y, versus $45,7 \pm 18,21$ y for non-Italians). Age at diagnosis was also younger in the Italian ($25,1 \pm 14,93$) versus the non-Italian cohort ($32,37 \pm 17,27$). The disease duration (measured as a difference between the age at the visit and age at diagnosis) was similar in both cohorts (Italian $13,97 \pm 12,86$; non-Italian $13,33 \pm 12,63$). The BMI showed a mild overweight in all patients (Italian cohort $26 \pm 3,46$; non-Italian cohort $26,08 \pm 7,89$). The prevalent diagnosis was GSD5 (90% in the Italian cohort and 96,6% in the non-Italian cohort). Other diagnosis were GSD3, 7, 9, 10 and 15. 32% of Italian patients and 59% of the non-Italian patients were homozygous for the p.R50X allele. Myoglobinuria was reported 52% of all patients. Fixed weakness was reported in 55% of Italians and 47% of non-Italians. Distribution of severity score showed a skewed distribution in the Italian cohort towards less severe phenotypes.

Very late-onset non-thymomatous and thymomatous myasthenia gravis (MG) are associated with different HLA class II alleles

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A variable association of several HLA alleles with MG subtypes has been reported in different ethnic groups. Among subtypes, non-thymomatous MG with late onset is reported to be increasingly frequent. Aim of this work was to define specificities and prevalence of MG subtypes in a large series of Italian patients according to clinical (gender, age at onset, severity of symptoms, autoantibody profile and thymic abnormalities, n = 230) and immunogenetic (HLA-II alleles, n = 179) characteristics. Two main peaks of incidence emerged: one with onset between 21 and 40 years of age, with overwhelming female preponderance, the second between 61 and 80 years, with higher male prevalence. This Very Late Onset subtype was largely composed by subjects with non-thymomatous, anti-AChR antibody-positive MG (VLOMG), and showed an increasing incidence. Thymoma (prevalently grade B2) was present in 23% of cases. Patients with MGFA grade V had higher anti-AChR antibody titers compared to milder cases. HLA-II allelic fre-

quencies were compared with those of 315 healthy controls, applying the Fisher exact test. P values were corrected (Pc) for multiple comparison, according to Bonferroni. A highly significant association was observed between alleles DQB1*05:02 (Pc < 0.01) and DRB1*16 (Pc = 0.01) and VLOMG. Conversely, the DQB1*05:01 allele showed a significant association with thymomatous MG (Pc < 0.05). Our results suggest that, among Italian patients, VLOMG is by large the most frequent MG subtype and differs from thymomatous MG in terms of association with HLA-II susceptibility markers.

Assessing the impact of gender on the phenotype of myotonic dystrophy type 2: a cohort of 307 patients

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Myotonic dystrophies are autosomal dominant diseases characterized by a combination of muscular and multisystemic involvement. Gender has been recently found to influence the phenotype of myotonic dystrophy type 1. Our aim was to study the impact of gender on the phenotype of myotonic dystrophy type 2 (DM2).

We retrospectively studied 307 patients with DM2 analysing the following data: i) demographics (age, gender), ii) clinical features (first symptom, diagnostic delay, presence of myotonia, weakness and/or pain, comorbidities), and iii) diagnostic assessments (serological tests, electromyography, muscle biopsy). Statistical analyses were performed.

Our cohort comprised 186 females (61%) and 121 males. Muscle weakness was more common in women than men (64.9% vs. 43.8%, p = 0.0006), while pain was a more frequent presentation in men (49.5% vs 29.9%, p = 0.001). Patients with weakness at onset were older than those with pain and myotonia (median 49, vs. 39 and 30 years, p < 0.0001). A multinomial regression model revealed that age at onset and sex were significantly and independently associated with specific types of symptoms. Cataract and thyroid diseases occurred more frequently in women (p = 0,002 and p < 0,001). CK and GGT were more frequently abnormal in men (p < 0,001 and p = 0,019) whereas no differences were found for electromyography and biopsy results.

In conclusion it seems that, as in DM1, gender influences, independently from age, the phenotype of DM2. These gender-specific manifestations should be considered in the diagnosis and management of patients.

Report on nationwide Italian collaborative network for muscle glycogen storage disorders

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“Muscle Glycogen Storage Disorders (MGSD)” indicate a group of rare diseases due to defects of glycogen metabolism. In the past 15 years, there has been an increasing interest in MGSD but often awareness of these conditions as well as ability to diagnose and manage the patients is still limited. When dealing with rare diseases, a small population of patients represents the major obstacle to progress in research and management. A development of a web-based registry for MGSD patients, became necessary to better understand frequency, different phenotypes and natural history of these patients.

Natural history and epidemiological data were collected from patients from 21 Italian Centers with specific expertise on MGSDs.

To date we have already collected 187 patients with both adulthood and childhood onset of MGSDs. The most common disorders are represented by GSD II or Pompe disease, followed by GSDV or McArdle disease. There are also other more rare MGSD in the registry as GSDIII, GSD IV, VII, X, XIII, GSD0. Diagnostic delay was 5,2 yrs in GSD II whereas 15,2 yrs in GSDV. Hyperckemia was elevated in about 50% of patients at onset. The most common symptoms in GSDII were muscle weakness and fatigue whereas in GSDV were exercise intolerance, myalgia and fatigue. Myoglobinuria was reported only in 30% of GSDV patients. Muscle weakness at onset was reported in 15/72 GSDV patients in the hip muscles. The diagnoses were based on a biochemical and molecular confirmatory tests.

Analysis of this large cohort of patients has revealed several important informations on natural history of these disorders. CK abnormal in about 50% of patients suggested that not always it increases in such disorders. Muscle weakness in GSDV was highly present rather than reported in smaller cohorts. Considerable diagnostic delay was evident, mainly in GSD V, highlighting the need for better awareness and more attention to signs and symptoms characterizing these disorders.

Multilevel molecular analysis identifies all dystrophin gene mutations pointing out that DMD is a genetically homogenous disease: repercussions on diagnosis, prevention and therapy

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Dystrophinopathies are a group of allelic diseases caused by mutation in the dystrophin (DMD) gene: deletions (~ 65%), duplications (~ 10%), small mutations (25%) and deep intronic mutations and complex rearrangements (~ 1%).

Many exceptions do exist to the reading frame rule and a fine genetic characterization is mandatory for providing genetic diagnosis and making patients eligible for novel personalized trials. We analyzed the dystrophin gene in a large heterogeneous cohort of 891 individuals; reasons for testing were: clinical diagnosis of DMD (485), clinical diagnosis of BMD (226), symptomatic female carriers (43), asymptomatic patient with high CK (38), limb girdle muscle dystrophy (24) and at risk females tested for known familial mutation (78). We identified the dystrophin causative mutation in all the probands except for two boys with a clinical diagnosis of DMD and absence of dystrophin in the biopsy; in both the transcript analysis pinpointed the presence of a dystrophin gene mutation. Our data demonstrate that dystrophinopathies are genetically homogenous and a multilevel diagnostic approach is able to identify all DMD mutations; therefore a monogenic NGS approach should be endorsed in this disease. These findings carry some consequences in the address and interpretation of dystrophin genetic tests in clinical genetic counselling, in the definition of the role of modifiers, in the design of new therapies and in the plan of clinical care flowcharts.

Development of a human cardiac organoid to study heart dysfunctions in Duchenne and Becker muscular dystrophies

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An impeding factor in the research advancement on heart dysfunctions in Duchenne (DMD) and Becker (BMD) Muscular Dystrophies is the lack of human heart samples. Progresses in pluripotent stem cell differentiation and tissue engineering have facilitated the development of human cardiac organoids (hCO), which resemble fetal heart tissue. In addition, human pluripotent stem cells (hPSCs) can be propagated indefinitely and retain the capacity to differentiate into somatic cells, offering the potential to generate large numbers of functional patient-specific cardiomyocytes. The canonical Wnt signaling pathway induces differentiation of hPSCs into mesodermal and endodermal progenitor

cells, that can further differentiate into endothelial, cardiac and vascular smooth muscle lineages. The temporal modulation, via small molecule inhibitors of Wnt signaling, is essential and sufficient to drive an efficient cardiac induction. Starting from control hPSCs we generated embryoid bodies (EB) and subsequently cardiomyocyte differentiation was performed according to an EB-based protocol. Phenotypically heart organoids presented autonomous rhythmic contractile activity. hCOs were characterized by immunostaining to assess the expression of cardiac transcription factors and myofilament proteins, such as Ki67, cardiac myosin, cardiac troponin T, alpha-actinin and smooth muscle actin. Recently we produced the first EBs of BMD and DMD to develop functional hCOs, for study heart dysfunctions in these pathologies. They were originated from cells derived from patients harbouring deletions of exons 50-51 and 54 of dystrophin gene respectively. Therefore, the ability to generate functional cardiomyocytes by manipulation of a single developmental pathway facilitated the production of 3D cardiac models useful for research and drug screening.

The importance of muscle biopsy for muscle disease experts: a review of Sardinian data

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Muscle biopsy is a common and minimally invasive procedure for the evaluation of suspected neuromuscular disorders (NMD). In Sardinia this is performed only at Multiple Sclerosis Centre (MSC) in Cagliari as a second level diagnostic exam requested by specialists of hospital wards. Since 2015 the MSC team began with a supervision activity in order to improve level of appropriateness of the exams and reduce useless procedures. The needle-biopsies performed in Sardinia from 2012 to 2016 were evaluated. Requisition forms (sending wards, suspected disease and age of proband) and biopsy medical reports were used as source data. Follow up data are obtained by medical records or by interview to the ordering physicians. 283 biopsies were analyzed. 194 biopsies were made in 2012-2014: 46% were normal, 16.9% inflammatory myopathies, and 30.8% hereditary NMD. In 2015-2016, 89 exams were performed: 31% normal (85% requested by non-neuromuscular-expert physicians), 40.5% inflammatory myopathies, 23% hereditary NMD. We noticed a decrease in number of exams performed and a significant difference in diagnostic relevance of biopsies ordered by the MSC and other requesting physicians (89% Versus 22%), except for rheumatologists (95%). Our data underline the importance of the NMD expert clinicians to reduce useless and inconclusive procedures. To lead to diagnosis is necessary to be familiar with the procedure and add the results obtained from a biopsy to the clinical examination, electrodiagnostic and laboratory findings. For these reasons, in author's opinion, muscle biopsy must be ordered just in NMD Centers to get the full benefit of the procedure.

Autosomal recessive myopathy associated with cataracts caused by mutations in the gene encoding INPP5K, an inositol phosphatase

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INPP5K encodes the type II inositol polyphosphate 5-phosphatase K, an endoplasmic reticulum (ER) resident skeletal muscle-enriched inositol phosphatase. Here, we report on recessive missense and in-frame deletions affecting the functional INPP5K protein in six families with bilateral early childhood cataracts and myopathy. Learning difficulties were also reported occasionally. The muscle biopsy specimens showed features suggestive of congenital muscular dystrophy. Pathogenicity of loss of functional Inpp5k was confirmed in zebrafish via injection of respective morpholinos. Knock-down of Inpp5K expression was in accordance with disturbed architecture of skeletal muscle and narrowed eyes 48 hours post transfection. Proteomic-studies on patient derived fibroblasts allowed further insights into the molecular nature of the disorder. Identification of INPP5K mutations implicate the combined myopathy-cataract phenotype as a disease of ER- and inositol polyphosphate phosphatase-dysfunction and thus put INPP5K on the growing list of multisystemic disorders associated with this organelle.

POPDC1 gene mutations screening in laminopathies: possible role as a modifier

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POPDC1 is the best-studied member of the Popeye domain-containing gene family. The members of this family encode transmembrane proteins, which are abundantly expressed in cardiac and skeletal muscle. The evolutionary conserved Popeye domain functions as a high-affinity 3'-5'-cyclic adenosine monophosphate (cAMP) binding domain. Recently POPDC1 has been identified as causative gene in a family affected by Limb-girdle Muscular Dystrophy and atrioventricular (AV) block (OMIM LGMD2X).

In order to evaluate if POPDC1 may contribute to pheno-

type variability in other LGMDs with or without cardiac involvement were screened by Sanger sequencing 240 patients with different phenotypes either orphan of known gene mutations or with known gene mutations. We identified 9 different heterozygous POPDC1 mutations in 11 patients, 6 of them do also carry LMNA mutations. Phenotypes vary from classical LGMD phenotype to pure cardiomyopathy with rhythm disturbances. We have demonstrated that POPDC1 mutations do cause rare recessive phenotypes, and we suggest that POPDC1 gene may also act as disease modifiers. Considering that laminopathies are characterized by high clinical heterogeneity and by intra- and interfamilial phenotypic variability, we speculate that POPDC1 may participate to LMNA phenotype modulation.

Clinical expression of facioscapulohumeral muscular dystrophy in carriers of 33-35 kb D4Z4 reduced alleles: experience of the Italian National Registry for FSHD

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Objective: to reevaluate the clinical spectrum of proband carriers of 33-35 kb D4Z4 reduced alleles (DRA) and their relatives through the use of new clinical tool: the Comprehensive Clinical Evaluation Form (CCEF).

We performed an observational study of 154 probands (**P**) and 223 relatives (**R**) from a consecutive group of 252 probands and 306 relatives from the Italian National Registry for FSHD collected between xxx and xxx. In this large cohort we applied the new CCEF. Based on the distribution of muscle weakness (FSHD score) and the combination of clinical features suggestive or not of FSHD we assigned patients to different phenotypic

new categories. (A1-A2-A3: typical phenotype; B1-B2: incomplete phenotype; C1-C2: asymptomatic; D1-D2: atypical FSHD/different disease)

We observed an average FSHD score of 5,6/15 for probands and 1,7/15 for relatives. The clinical categories were distributed as follow: A1 (P 2%; R0%); A2(P 29%; R7%); A3(P 24%; R8%); B1(P 20%; R9%); B2(P 1%; R12%); C1(P 1%; R15%); C2(P 0%; R40%); D1(P 20%; R6%); D2(P 3%; R3%).

This study shows two main aspects. First, the majority of probands carrying 33-35 DRA (53%) had moderate phenotype with partial or mild facial involvement in of them (A2-A3); 40% showed a prevalent shoulder girdle impairment (B1) or atypical phenotype (D1); 3% displayed clinical features inconsistent with the clinical diagnosis of FSHD (D2). Second, the majority of relatives was non penetrant carriers (55%) and only few of them showed typical phenotype (17%) while the remaining had atypical phenotype. Overall among 377 carriers of 33-35 DRA 119 (31.6%) presented a classical FSHD phenotype (categories A), 124 (32.9%) were asymptomatic (categories C), 78 (20.6%) had incomplete phenotypes (categories B), 56 (14.9%) showed atypical phenotypes (categories D).

These results highlight the necessity of additional parameters to evaluate the risk of developing disease in particular for relatives and point at the difficulty of genetic counseling.

Big data in genetic research: the example of titin gene and titinopathies

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The introduction of high throughput sequencing tools has revolutionized the approach to complex and genetically heterogeneous disorders, and made sequencing of very large genes possible in individual patients. Because of its sheer size, variants in titin gene (*TTN*) are detected frequently in any sequencing approach. An accurate resequencing of the coding portion of *TTN* results in almost one rare variant per allele.

The interpretation of these variants is the most significant challenge related to NGS investigation in our field and it often requires additional investigations such as segregation studies, protein and cDNA assays. In order to respond to the challenges arising from the use of NGS, we set up a large collaborative network which may take advantage of the power of shared resources and expertise and which may benefit of combining cohorts of patients into larger groups. We performed a systematic analysis of rare *TTN* variants identified in over 3000 patients affected by a skeletal muscle disorder as well as of variants listed

in publicly available databases (ExAC, 1000G and LOVD). Our approach allowed the identification of harmless rare variants as well as of potentially causative missense variants, significantly enriched in our cohort, which can be prioritized for further functional assays. A comprehensive analysis of all reported, novel detected and rare *TTN* variants from patients all over the world, listed into a single accessible database, would help all the researchers and clinicians to assess the pathogenicity of *TTN* variants and would promote significant advances in the understanding of titinopathies.

Novel zebrafish models of sarcoglycanopathy

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Sarcoglycanopathy is a rare genetic diseases caused by defects in the genes coding for alpha-, beta-, gamma- or delta-SG, which form a key complex that assures sarcolemma stability during muscle contraction. Most of the reported cases of sarcoglycanopathy are due to missense mutations that originate a folding-defective SG that is eliminated, although potentially functional, by the quality control of the cell. The loss/strong reduction of the mutated subunit results in the secondary loss of the wild-type partners. Many missense mutants retain their function as the entire complex can be properly rescued by skipping the degradation of the mutant. These findings opened new perspectives for the therapy of this neglected disease, allowing the design of small-molecule-based approaches aimed to inhibit degradation of SG mutants, or to help their folding. We verified that several compounds, known as CFTR correctors are effective in recovering different mutants of alpha-SG and the whole complex in both cellular models and, notably, primary myotubes from a patient with sarcoglycanopathy. To confirm *in vivo* this successful strategy, we are now generating novel sarcoglycanopathy animal models expressing different folding-defective SG. We have chosen zebrafish as it is emerging as an excellent vertebrate model for muscular disorders and it is relatively easy to introduce any desired mutation by genome-editing technologies. Here we report data showing that knock-down of delta-SG in zebrafish leads to severe muscular abnormalities, well mimicking the human disease, and preliminary results concerning the generation, by the CRISPR/Cas9 technique, of beta-SG and delta-SG knock-in and knock-out zebrafish lines.

Tele-monitoring in paediatric ventilated neuromuscular patients, results of an Italian multicentric study

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Tele-monitoring (TM) has largely been proved to be effective in adult ventilator-dependent neuromuscular patients (Ambrosino et al EurRespirJ 2016). We aimed to evaluate the effectiveness of a two-years longitudinal observational multicentric TM trial, specifically designed for paediatric ventilated neuromuscular (NMD) patients, in terms of feasibility, patients'

satisfaction, hospitalisation rate reduction. TM included weekly scheduled physiotherapist calls after an overnight SaO₂, heart rate and ventilation telemetric monitoring. A baseline clinical score (Vitacca et al EurRespirJ 2009) was given to patients; variations > 3 were considered exacerbations and managed by physicians. Hospitalisations were compared with those of an age-disease-severity-matched control population. Patients' satisfaction has been assessed by questionnaires. Forty-eight patients were enrolled, 30 males. Median age was 16.4 years (8.9-22.1), median ventilation/day was 10.5 hours (8-6). Total exacerbations matched in TM patients and controls (59 vs 53), however the total hospitalisation rate was reduced in TM patients (11 vs 21, *p* 0.0286). Although the stratification in number of ward and ICU admissions (9vs15 and 2vs6, *p* 0.52) did not show differences, the median of admissions length in days was significantly reduced both considering the total (108 vs 219, *p* 0.0282), the ward (*p* 0.0038), and the ICU admissions (*p* 0.0492). All patients reported improvement in the items involving "communication".

The present trial has shown for the first time in ventilated paediatric patients, the efficacy of TM in the management of respiratory exacerbations.

Insights into bone mineral density and bone metabolism in Duchenne muscular dystrophy

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Low bone mineral density (BMD) and increased fracture risk are frequently observed in Duchenne muscular dystrophy (DMD). Our aim was to explore BMD and bone turn over and to evaluate their main determinants in a cohort of DMD subjects. BMD at lumbar spine, detected by DXA and expressed as Z-score values, C-terminal telopeptide of procollagen type I (CTX) and osteocalcin (BGP), as bone resorption and formation markers respectively, and sclerostin were assessed. Prevalent fragility fractures were recorded. Left Ventricular Ejection fraction (LVEF%) and Forced Vital Capacity (FVC%) were evaluated. Thirty-one patients [median age 14 (12 to 21.5) yr.] were studied. Ambulant subjects showed significantly higher Z-score values in comparison with not ambulant ones, and subjects with prevalent clinical fractures had significantly lower Z-score values in comparison to subjects without fractures. Z-score values were positively correlated with FVC (*r* = 0.50; *p* = 0.01), but not with GCs use, and FVC was positively associated with BGP (*r* = 0.55; *p* = 0.02). In not ambulant subjects, Z-score values were associated with BMI (*r* = 0.54; *p* = 0.02) and sclerostin was associated with age (*r* = 0.44; *p* = 0.05). At a stepwise multiple regression analysis, age, BMI, FVC and sclerostin levels were retained in the model as independent predictors of BMD.

Our data confirm low BMD values in DMD subjects, especially in not ambulant ones, irrespective of the use of GCs, and identify, for the first time, FVC and sclerostin as main determinants of BMD in this cohort. Therefore, a multidisciplinary setting, focusing on rehabilitative and respiratory care, is warranted to reduce bone complications in DMD.

Fibroblasts-derived exosomes: potential role in the fibrotic process of Duchenne Muscle Dystrophy

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Fibrosis, the excessive connective tissue proliferation progressively replacing muscle fibres in severe muscle diseases, involves cellular and circulating microRNAs (miRNAs). Our previous results showed increased expression of the pro-fibrotic miR-21 in Duchenne muscle dystrophy (DMD) fibroblasts and significant reduction of the anti-fibrotic miR-29 in DMD myoblasts. Exosomes, small vesicles secreted by several cell types, contain proteins, mRNAs and miRNAs, and are actively involved in cell-cell communication. We have now characterized exosome release from DMD muscle-derived fibroblasts and exosome up-take by control muscle-derived fibroblasts, and investigated the role of exosomes and of miRNAs transported by exosomes. In DMD fibroblast-derived exosomes the anal-

ysis with a focused miRNA array showed, among others, high levels of miR-199a, similarly to what occurs in tissue fibrosis of different organs. DMD fibroblast-derived exosomes added to control fibroblasts induced increased expression of the fibrotic markers α -SMA, collagen 1, fibronectin, TIMP-1 and TGF- β -1, increased production of soluble collagens, and increased cell proliferation. To confirm that this change is at least partially related to miR-199a and to a secondary reduction in caveolin-1, a known target gene of miR-199a, control fibroblasts were transfected with miR-199a mimic. An increased expression of the fibrotic markers was observed as consequence. Our results suggest that exposure to DMD fibroblast-derived exosomes induces an "activated" phenotype in control fibroblasts. Our findings also indicate that exosomes produced by DMD fibroblasts contribute to the phenotypic conversion, typical of fibrosis, of fibroblasts to myofibroblasts, and point to miR-199a as potential therapeutic target for reducing fibrosis.

MUSCLE CLUB SESSION

(in alphabetical order of the first Author)

Expanding clinical and histological spectrum of DNM2 mutations: a case report

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DNM2 gene encodes an ubiquitously expressed large GTPase dynamin 2 which is involved in endocytosis and intracellular membrane trafficking.

Mutations in DNM2 gene are associated with Autosomal Dominant (AD) Centronuclear Myopathy (CNM), as well as with Dominant Intermediate Charcot-Marie-Tooth Disease (CMTDIB), and in its axonal variant (CMT2M).

We report a 58-years old woman with a 13-years history of bilateral ptosis and dysphonia; she also developed later dysphagia and asthenia. In the following years the disease severely progressed, mainly with worsening of ptosis and dysphagia. At neurological examination bilateral ptosis with mild ophthalmoparesis was present as well as velopharyngeal dysfunction with nasal voice, difficulty walking on heels and later, moderate neck flexor muscles weakness. Electromyography evidenced a myopathic pattern. Muscle biopsy showed a minicore-myopathy. Next Generation Sequencing revealed a heterozygous mutation in the DNM2 gene as c.2264 C > A p.Thr755Asn (exon 19).

This mutation has not been previously described, is located in a known pathogenetic locus and it was not found in 1200 controls. This is the first report of DNM2 mutation in a patient with a minicore myopathy and atypical clinical features involving cranio-facial, bulbar and neck muscles.

This case contribute to expand the clinical and histological features of patients with DNM2 mutations.

Novel compound heterozygous mutations of AGRN resulting in a complex muscular phenotype

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We describe a 41 years old adopted woman with a complex muscle phenotype carrying two compound heterozygous mutations in *AGRN* gene.

Patient was firstly admitted to hospital at age 7 months for paroxysmal sinus tachycardia and generalized weakness. Her motor development was within normal ranges, but relevant fatigability associated to fatigability associated to horizontal gaze limitation with double vision appeared over the years. At the age of 20 years, she underwent surgical repair of atrial septal defect, several catheter ablation treatments and a muscular biopsy showing unspecific myopathic changes with predominance of type I fibers. Current neurological examination shows hor-

izontal gaze limitation with double vision, generalized muscle weakness and wasting prevalent in distal compartment with joint laxity and severe myalgias. Onychodystrophy and diffuse lipodystrophy further expand the clinical spectrum. Electrophysiological tests revealed a diffuse myopathic pattern, while repetitive nerve stimulation was normal. Muscle MRI showed a selective pattern of muscle involvement with biceps femuri and bilateral gastrocnemii being more affected.

We performed a target gene panel testing for congenital myopathies and myasthenic syndromes by NGS approach which identified two novel variants in *AGRN*: *exon28:c.4976 + 18delA* and *exon23:c.C3964T/p.R1322W* in the C-terminus domain. A subsequent re-examination of muscle biopsy with ENS confirms disorganization of neuromuscular junctions including dispersion and fragmentation of endplate areas.

This case widens the spectrum of disorders associated to *AGRN* mutation, adding cardiac involvement and severe ophthalmoplegia. It also underlines the difficulty in the diagnosis of CMS without extensive genetic analysis and deep histological studies.

Myotonia in filamin-C-related myopathies

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Filamin-C-related myopathies are autosomal dominant inherited myopathies caused by mutations in *FLNC* gene. Two distinct phenotypes have been described: myofibrillar myopathy (MFM) and distal myopathies. MFM-filaminopathy is characterized by adult-onset predominantly proximal weakness with involvement of respiratory muscles and cardiac abnormalities.

A 40 years-old male began to suffer from limb and axial muscle weakness and hands, eyes myotonia early in his teen. No cardiac involvement was reported. His father presented similar symptoms. CK levels were elevated (5000U/L) and EMG evidenced the presence of a myopathic signs with myotonic discharges. Mutations in *DM1*, *DM2* and *CLCN1* genes were excluded.

Patient's muscle biopsy showed presence of core-like areas and numerous vacuoles filled with amorphous material. Some of them had the appearance of rimmed vacuoles. Electron microscopy showed Z-line disruption and fibrillar material accumulation in scattered areas, associated with mitochondrial structural alterations.

Molecular analysis discovered an eteherozygous dominant paternally inherited mutation in *FLNC* (c.C5765T) leading to aminoacidic change in exon 35 (p.A1922V) indicated as pathogenic in several predictor programs.

This case shows that the phenotype of filaminC-related myopathy is more variable than previously known. The diagnosis of an MFM might be taken into consideration also in patients presenting clinical/electrical myotonia, when *DM1* and *DM2* have been excluded by genetic blood testing. We also underline the usefulness of muscle biopsy in this case allowing to discover

a further, peculiar, morphological-phenotype leading to schedule genetic testing in myopathy patients.

Core myopathy with early respiratory failure and titin gene mutation

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Core myopathies are related to different genetic mutations and respiratory failure may be part of the clinical spectrum.

We describe a woman who developed respiratory insufficiency at age 49 years; symptoms were progressive, requiring non-invasive ventilation one year later; she also complained of generalized fatigue. Her medical history included scoliosis since childhood and family history was negative for neurological diseases. Neurological examination showed mild neck flexor and proximal lower limb weakness, with diffuse muscle hypotrophy. Routine laboratory analyses and thyroid hormones were in normal range except for a mild hyperCKemia. Cardiac function was normal. Standard nerve conduction studies were normal, while EMG showed a diffuse myopathic pattern.

A biopsy of the *biceps brachialis* muscle showed diffuse core-like lesions, with preferential eccentric distribution within muscle fibers, along with type 2 fiber atrophy. Cytoplasmic bodies and rimmed vacuoles were absent.

DNA analysis failed to show mutations in the *RYR1* gene. A targeted next generation sequencing platform, MotorPlex, revealed a previously reported mutation (c.95195C > T p.P31732L), in heterozygosity, in the exon 344 of titin gene (*TTN*), associated with an hereditary myopathy with early respiratory failure (HMERF).

The clinical picture slowly deteriorated until 55 years of age, when the patient died of pneumonia complications.

At variance with the two previously described families with the same mutation, defined as semi-dominant, our patient presents a severe phenotype in the heterozygous condition. She also displays a unique histopathological pattern, not observed in other patients with HMERF linked to *TTN* mutations.

These findings expand the genotype-phenotype correlation in HMERF.

Muscle pathological features of a hyperkalemic paralysis/dermatomyositis "double trouble"

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Here we report muscle pathological findings of a 66-year-old woman which presented with hand and face erythematous rashes, progressive proximal limb weakness and dysphagia with a subacute onset.

Serum CK levels were elevated 3-4 times the normal values and EMG analysis showed a diffuse chronic myogenic pattern. About six months after symptom onset a cholangiocarcinoma was diagnosed.

In the past, she received a genetically defined diagnosis of hyperkalemic periodic paralysis.

Deltoid muscle biopsy showed variability of fiber size, many fibers with internal nuclei, some fiber splitting, rare cell necrosis with phagocytosis, some small perivascular and perimysial inflammatory infiltrates, multifocal perifascicular atrophy and few COX-negative fibers. Several fibers presented pseudo-vacuolations that appeared optically empty or containing some amorphous material on routine histological staining. They usually had curving or undulating borders and a clearly defined limiting membrane. Some of them were connected with the extracellular space as an intra-cytoplasmic sarcoplasmic invagination. Diffuse MHC I sarcolemmal positivity was observed also involving the pseudo-vacuolation limiting membranes.

Muscle biopsy confirms a singular "double trouble" in this patient. On one hand, such pathological pattern is suggestive for proliferation, regeneration and dilation of components of the T tubular system and the sarcoplasmic reticulum as often observed in dyskalemic periodic paralysis; on the other hand, presence of perifascicular atrophy, cell necrosis and inflammatory cells support the clinical diagnosis of dermatomyositis.

Castleman disease and inflammatory myopathy: is there a link? A case report

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A 57 year old man arrived to our attention for one-year history of myalgia, progressive pelvic girdle muscle weakness and hyperCKemia (700 U/L). In anamnesis, he had been diagnosed as having Castleman disease in the past, at the age of 32, and then regressed at the follow-up investigations; his daughter reported the diagnosis of infantile dermatomyositis. The electromyography showed a myopathic pattern; the nuclear magnetic resonance of lower limb revealed a mild edema and a diffuse fatty infiltration of thighs muscles. The quadriceps biopsy showed necrotic fibers, some of them with vacuoles, rare ragged red and COX-negative fibers and an inflammatory infiltrate in multiple foci within the endomysium. At immunohistochemical analysis several muscle fibers resulted MHC-I and HLA positive. The diagnosis of inflammatory myopathy was performed.

Castleman's disease, also named angiofollicular lymph node hyperplasia or giant lymph node hyperplasia, is an uncommon reactive lymphadenopathy with unknown causes. We will discuss the possible association with the inflammatory myopathy, focusing on the intersection of Castleman disease and autoimmunity with shared pathology and mutually beneficial treatments.

ABSTRACTS OF POSTER COMMUNICATIONS

(in alphabetical order of the first Author)

Periodic paralysis: an emergency department presentationAgazzi E., Pavanello D., Riva R., Rottoli M.R.
ASST-PG23, Bergamo

A 43 years old man reached the Emergency Department complaining impossibility to move when waking up. Neurological examination could not evidence any sign a part from four limbs hyposthenia, mainly distal at upper arms and mainly at lower limbs. He had negative medical history, not taking any drugs. Brain CT scan and blood pressure were normal as Doppler Carotid ecography. He was complaining other three-four hyposthenic episodes, happened during last year, one waking up and the other two after lunch. Blood samples showed hypokalemia (2,9 mg/dl) and correcting this value, the patient started feeling better, although the morning after he still presented the same symptoms with recurrent hypokalemia. We found out hyperthyroidism and then decided not to perform analysis of CACNA1S (1q32) e GABRA3 (Xq28) polymorphisms. Still, it is mandatory thinking about channelopathies also at Emergency Department, although they're rare diseases and could induce to classify these symptoms as psychogenic.

Young girl complaining of fatigue and muscle contracturesAgazzi E., Colombo O., Gardinetti M., Rottoli M.R.
ASST-PG23, Bergamo

A 26 years old girl went to our Out-patients Clinic complaining weakness after mild exercise, with stiffness in her arms and hands, difficult climbing stairs, which were present since few years before getting progressively worse. She had negative family history and had performed a previous muscle biopsy which was normal. At neurological examination she had a myopathic facies, MRC 4/5 orbicularis oculis, 4+/5 upper and lower limbs. DMPK gene was negative for mutation and EMG showed only myopathic potentials. She was having persistent CK elevation (around 1200-1500), normal thyroid function and normal cardiac evaluation. She was forcing herself to keep on with normal daily activity noticing that at the beginning of this effort she was getting worse, but after a rest she was much better performing. Actually, she also noticed few episodes of dark urines. We finally performed PYGM analysis which gave us the confirmation of McArdle disease. Now this patient has a much better quality of life because she's following prescription for a correct muscle exercise, she has never had myoglobinuria during this last year and she's also receiving dietetic prescription.

Hippo signaling pathway in muscular dystrophies

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The Hippo signaling pathway is considered as a key regulator of tissue homeostasis, cell proliferation and apoptosis; alterations of this pathway seem to contribute to tumorigenesis.

Yes-associated protein 1 (YAP1) is a downstream target of the Hippo pathway, acting as a transcription co-activator, and its role has been suggested in various types of cancer. Furthermore, YAP1 has emerged as an important player in mechanotransduction, transmitting mechanical cues into a transcriptional cell response. At the same time, YAP1 has been shown to be involved in skeletal muscle development and regeneration, contributing to the regulation of activation, proliferation and differentiation of satellite cells. Beyond that, YAP1 signaling is also important in adult skeletal muscle homeostasis as misregulation can lead to atrophy or hypertrophy, and aberrant YAP1 activities have been observed in disease states, including muscular dystrophies. We have investigated the expression of YAP1 in twenty different human muscle dystrophies samples (5 DMD, 5 BMD, 5 LGMD2A and 5 LGMD2B) using immune-histochemical and western blot analysis. We also assessed in muscle tissue gene expression of miR-21 that targets the YAP1, and the expression of Survivin as one of the downstream product of the Hippo pathway. Our results showed an overexpression of the phosphorylated YAP1 in DMD versus other muscular dystrophies and normal controls. In addition, the gene expression of miR-21 was slightly expressed in the DMD vs other dystrophies. These preliminary data suggest a crucial role of Hippo pathway in muscle homeostasis, suggesting that it could be a potential therapeutic target in muscle dystrophies.

Functional study of myotonia congenita mutations in the c-terminus of CIC-1 and proof of concept study for chaperone-mediated rescue of trafficking-defective CIC-1 mutantAltamura C.¹, Imbrici P.¹, Lucchiari S.², Sahbani D.¹, Comi G.P.², Dotti M.T.³, Meola G.⁴, Politano L.⁵, Lo Monaco M.⁶, Desaphy J.-F.⁷, Conte Camerino D.¹

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Myotonia congenita is a skeletal muscle hyperexcitability disorder, caused by loss-of-function mutations in CIC-1 chloride channel, affecting its gating or membrane density. Today no CIC-1 channel opener is available and therapy is only symptomatic. In collaboration with clinicians, we have already characterized sixteen CIC-1 mutations identified in Italy (Desaphy et al., 2013; Portaro et al., 2015; Imbrici et al., 2015; 2016). Here we report the functional analysis of eight novel mutations located in the intracellular C-terminal region of the protein, which molecular function is little known. Wild-type and mutant CIC-1 channels were expressed in HEK293 cells and whole-cell chloride currents were recorded with patch-clamp.

The V851M and G859V mutants did not generate any chloride current; T832I and V947E showed current reduction at every tested potential; and V829M and P883T induced a positive shift of activation voltage-dependence, thus confirming their guilty in determining myotonia. Conversely, L861P generated chloride currents very similar to WT. As this mutation was found in compound heterozygosis with G190S mutation, ongoing coexpression experiments would allow elucidating the pathomechanism of L861P.

In parallel, we tested the ability of niflumic acid (NFA), a reversible CIC-1 blocker, to act as a pharmacological chaperone on the trafficking-defective A531V mutant. Incubation with 50 microM NFA for 24 hours increased A531V currents by about two fold, restoring chloride currents similar to wild-type.

This study improves our understanding of C-terminal region of CIC-1 and provides a proof of concept for small molecule chaperones able to rescue trafficking-defective CIC-1 mutants. (Supported by Italian Telethon GGP14096)

Muscle biopsy findings and outcome in necrotizing autoimmune myopathy

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Necrotizing autoimmune myopathy (NAM) is a rare and severe idiopathic inflammatory myopathy. Several NAM patients do not respond to standard treatment. This study has been carried out to investigate whether histopatologic findings and quantitative analyses on immunolabelled sections with ubiquitin, autophagy marker LC3b, SMI31, p62, TDP-43, membrane attack complex, MHC I, CD31, CD68, CD4, CD8, CD20, desmin and vimentin correlate with clinical parameters as disability scale, rate of progression and therapeutic response. Percentage of myofiber necrosis, MHC I-, SMI31-, TDP43-, p62-, ubiquitin-, LC3b- positive non-necrotic myofibers together with density of CD68 + macrophages correlated with increased clinical severity and longer treatment. Distinct pattern of expression of autophagy-lysosome and ubiquitin-proteasome pathways could be predictive of the risk of remaining on treatment in patients with NAM and may be useful for identification of patients at greater risk of severe disease.

Circadian rhythm genes in Duchenne muscular dystrophy

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Muscular dystrophies are genetic conditions that cause progressive weakness and loss of muscle mass. Mutations in structural proteins, such as dystrophin, cause muscle fibers' instability with muscle damage. Circadian rhythm coordinates biological processes with the 24h cycle; its role in maintaining muscle functions is known, both in animal models and in humans. Recently, we disclosed a link between CollagenVI myopathy and circadian genes. To define the involvement of circadian circuit in muscle damage, we designed a Fluidic-Card-

TaqMan based assay, including 30 genes related to circadian rhythms and muscle regeneration. We tested gastrocnemius and tibialis anterior muscles from unexercised and exercised mdx mice. We subsequently selected 7 most deregulated genes and performed expression analysis by Real-time PCR in 10 DMD patients with different mutations. We demonstrated the profoundly de-regulation of circadian genes in mdx mice, both exercised and unexercised. Such deregulation was confirmed in DMD patients muscle biopsies. Genes mostly deregulated were CSNK1E, SIRT1, MYOG. In order to explore if the transcript deregulation reflects on plasma, we designed an ELISA assay for the profound up-regulated CSNK1E protein and tested 16 DMD and 5 male control plasma samples. The CSNK1E showed a variable expression profile however, the sample's cohort proves to be rather limited and an enlargement of samples number is needed. Our preliminary data demonstrate that circadian genes are affected in both DMD patients and mdx mice supporting a correlation between circadian circuit and DMD, open the way to new biomarkers and interesting therapeutic options.

Segmental body composition in young children with SMA type 2: correlation with motor function abilities

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The aim of the present study was to investigate the correlation between fat mass (FM) and fat-free mass (FFM), measured by total and segmental body dual energy x-ray absorptiometry (DEXA), and the Hammersmith Functional Motor Scale Expanded (HFMSE) and the Upper Limb Module (ULM). 40 children with SMA type 2 (mean age 4.07 ± 1.90 years) were included in the study. Total FFM and FM were not correlated with HFMSE scores. However, when we investigated the correlation of segmental FFM and FM with motor function scores, we found that lower limbs FFM was significantly correlated with HFMSE scores ($r = 0.375$; $p = 0.017$), while no correlations were found between upper limbs FFM and HFMSE ($r = 0.050$; $p = 0.756$) or ULM ($r = 0.154$; $p = 0.392$). When we divided the whole cohort into Low-Functioning (HFMSE < 12) and High-Functioning (HFMSE > 12) children, we found that the Low-Functioning group had lower upper and lower limbs FFM, and higher upper and lower limbs FM, compared to the High-Functioning group. To our knowledge this is the first study investigating the correlation between total and segmental body composition and motor function in young children with SMA 2. The present study demonstrated that segmental lean mass at the lower limbs was correlated with global motor function abilities, as assessed by the HFMSE, and that segmental lean and fat mass was significantly different when children were stratified by level of functional abilities. These data further highlight the validity of HFMSE as an outcome measure in SMA, and may be helpful in monitoring the effects of therapeutic strategies.

Neuropsychological pattern in centronuclear myopathy due to *DNM2* gene mutations

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Mutations in *DNM2* gene are associated with autosomal dominant centronuclear myopathy (ADCNM) as well as with Charcot-Marie-Tooth neuropathy. So far, few patients with centronuclear myopathy (CNM) and concomitant central nervous system impairment have been reported. Cognitive involvement was described in two families and in one additional patient carrying mutations in the middle domain of the *DNM2* gene. We describe four patients belonging to the same pedigree, in whom, in addition to a complete diagnostic work-out and multidisciplinary care, we evaluated the cognitive and the neuropsychological function. The proband, a 64-year-old man, firstly experienced symptoms in the second decade of life, with difficulty in running and climbing stairs. Neurological examination showed: ptosis, hyperlordosis and proximal weakness with distal involvement particularly in lower limbs. A diagnosis of CNM was confirmed on the basis of the muscle biopsy and the molecular genetic analysis: heterozygous mutation in *DNM2* gene (c.1105C>T, p.R369W). His sister, 57-year-old, had a similar phenotype with a slowly progressive childhood-onset myopathy. In both patients, we found a specific involvement of verbal memory function, with particular selective involvement of short-term memory. The neuropsychological evaluation performed one year later, ruled out a cognitive decline in the two sibilings. The son of the proband, 35 year-old, showed at neurological examination: bilateral foot drop without cognitive impairment and genetic testing is ongoing. The 21 year-old son of the sister, was referred to our attention due to concerns regarding his cognitive function. Although he showed the same neuropsychological pattern of his mother, the genetic analysis did not confirm the mutation in the *DNM2* gene. Further studies to better define the segregation, enlarging the pedigree or involving different families, are needed to elucidate the genotype-phenotype correlations of the cognitive status and the CNM in patients carrying *DNM2* gene mutations.

LGMD2B with high dysferlin retention: two case reports

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Dysferlinopathies are a clinically heterogeneous group of autosomal recessive muscular dystrophies caused by mutations

in the dysferlin gene (*DYSF*). The large size of the gene had so far discouraged direct genetic testing. Western blot analysis of muscle biopsies or isolated monocytes is a reliable method for diagnosis of dysferlinopathy since all cases with a level of dysferlin $\leq 20\%$ are subsequently found to carry pathogenic mutations. In addition, rare cases with higher, but still reduced, levels of dysferlin have been reported. Here we describe two LGMD2B patients where routine diagnostic tests have detected normal expression of dysferlin on Western blot. Analysis of the *DYSF* gene was prompted by strong diagnostic clues and genetic exclusion of diseases with similar phenotype (e.g. LGMD2L) and both patients were diagnosed as compound heterozygous for known or likely pathogenic changes. Lower costs and easier access to Next Generation Sequencing technologies are changing the diagnostic approach to genetically heterogeneous disorders. It is therefore possible that the biochemical paradigm for dysferlinopathies will be invalidated if a larger number of patients with normal or near-normal dysferlin expression are found to carry mutations in *DYSF*. This will provide important clues for studies of epigenetic factors and for possible correlations between phenotype and protein expression levels.

Muscle magnetic resonance imaging as a prognostic biomarker in Becker muscular dystrophy

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We aimed to evaluate patterns of fatty replacement and myoedema in the skeletal muscle of patients with Becker Muscular Dystrophy (BMD) with Magnetic Resonance Imaging (MRI), and correlate it with DMD gene mutations and the functional outcome measures, North Star Ambulatory Assessment (NSAA) and 6 minute walk test (6MWT). Fifty-one molecularly confirmed BMD patients (aged 7-69 yrs) underwent muscle MRI. The degree of fat infiltration and myoedema in 3 upper limb and 22 lower limb muscles (T1w sequences) was evaluated by expert radiologists according to the Mercuri scale. All patients were evaluated with functional scales, i.e. North Star Ambulatory Assessment (NSAA) and 6 minute walk test (6MWT), both at the time of the MRI and longitudinally after one year. Fat infiltration and edema mainly affected hip adductors (70.6%), semimembranosus (67%), semitendinosus (67%), biceps femoris (69%) and gastrocnemius medialis (69%) muscles. The severity of muscle involvement was significantly correlated with DMD mutation: patients carrying the isolated deletion of exon 48 or with deletions including exon 51 showed a milder muscular involvement at MRI, when compared with other deletions or mutation classes. Fat infiltration scores of affected muscles correlated significantly at baseline with 6MWT ($p < 0.0001$) and NSAA ($p < 0.001$) and predicted functional changes after 1 year (6MWT, $p = 0.004$; NSAA, $p < 0.001$). In conclusion, severity of muscle involvement as visualized by MRI is strongly correlated to specific DMD mutations and with clinically meaningful outcome measures; furthermore, it is a prognostic biomarker of functional deterioration in a 12-month time frame.

Plectin mutation without skin involvement as a possible cause of CMD

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Plectin is a giant multifunctional cytoskeletal linker protein abundantly expressed in a wide variety of mammalian cells and tissues. There is growing evidence for an essential role of plectin for cytoskeleton stability, integrity of cell and tissue as well as regulating the signal complexes. Mutations in plectin gene (PLEC1) have been described in autosomal recessive forms of epidermolysis bullosa simplex (EBS) associated with muscular dystrophy. As far as we know, the absence of dermatologic involvement in patients affected mutation in PLEC1 gene has been described only in two recent case reports. We describe the case of a young Italian male patient presenting from birth with features of a congenital muscular dystrophy and a progressive proximal, moderate limb muscle weakness. CK have always been around 1000. He also showed bilateral congenital cataract, psychomotor development and a subsequent mild intellectual disability and failure to thrive. Muscular biopsy showed aspecific features. Whole Exome Sequencing technique led to identification of a compound heterozygous mutation in PLEC gene, but it also detected a heterozygous mutation in ALG6 gene, responsible for a specific congenital disorder of glycosylation Plectin3 was proved to be absent in his muscle biopsy. This observation suggest that PLEC1 should be considered in the differential diagnosis of congenital muscular dystrophies or limb-girdle muscular dystrophies, even in the absence of prominent skin involvement.

Fasting glucose in children with spinal muscular atrophy type I and II

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Spinal muscular atrophy (SMA) is a rare autosomal recessive disease due to mutation of the SMN1 gene causing degeneration of spinal cord motor neurons, characterized by severe muscular atrophy, particularly in the most common and severe forms, SMA type I and II. Two studies reported hypoglycemia during fasting and another one showed insulin resistance (IR) in 6 of 7 children. Severe changes in body composition (BC) could be the cause of glucose metabolism impairment: decreased lean body mass can cause hypoglycemia by reducing lipolysis, glycogen stores and protein catabolism, whereas increased fat mass can lead to insulin resistance (IR). In 42 SMAI and II (2-10 yrs) we measured glucose and insulin plasma level, calculating HOMA IR and BC by anthropometry and DXA. Hypoglycemia (blood glucose < 100 mg/dL) occurred in one patient, whereas HOMA-IR above cutoff value (> 2.67) were found in 2 SMAI. SMAI compared to SMAII showed both a worse nutritional sta-

tus with lower Lean Body Mass (6344 1223 g vs 9030 1841 g, $p < 0,001$), and glucose profile with higher insulin (8,51 6,7 $\mu\text{U/ml}$ vs 4,24 2,58 $\mu\text{U/ml}$ $p < 0,001$), and HOMA-IR (1,81 1,45 0,81 0,57 $p < 0,001$) values. Glucose, insulin plasma level, HOMA IR were not related to BC. In conclusion, impairment of fasting glucose occurred in 10% of patient. The lack of association with BC suggests that other mechanisms could be involved in the deregulation of glucose metabolism in SMA disease. Further insights could be gain by glucose tolerance by OGTT associated with insulin and C-peptide profile.

Myotonic dystrophy type 2 in a sicilian cohort: a challenging diagnosis by biomolecular tests

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Myotonic dystrophy type 2 (DM2) is an autosomal dominant multisystem disorder caused by expansion of CCTG tetranucleotide repeats in the first intron of the *nucleic acid-binding protein (CNBP)* gene on chromosome 3q21. Typically, disease onset is in the adulthood, with proximal muscular weakness and myalgia as the main muscular features. However, clinical symptoms are variable and aspecific, thus complicating the diagnosis. We retrospectively evaluated a cohort of 26 Sicilian patients with suspected DM2 at clinical and/or histopathological level, who come to our department since 2005. Biomolecular diagnosis was performed by genetic test on blood DNA on all patients and fluorescence in situ hybridization (FISH) on muscle sections to verify the presence of nuclear toxic RNA accumulation. 12 out of 26 patients (46,1%) were genetically confirmed. 15 out of 26 patients underwent FISH analysis, resulting abnormal in 9 cases (60 %); 2 of these patients were negative at genetic test. RT-PCR analysis was also performed on muscle biopsies to study the splicing pattern of several genes commonly involved in DM2 pathogenetic mechanisms and resulted abnormal in all the affected subjects. Our study underlines the limits and pitfalls of current diagnostic methods to diagnose the disease. These results strongly suggest performing a combination of these methods, namely fluorescence in situ hybridization together with muscle histopathology and genetic test, to increase the diagnostic sensitivity.

Familial ALS, clinical heterogeneity and mitochondrial disorders: description of a family

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It is quite accepted that mitochondrial dysfunctions may have, directly or indirectly, a key role in the etiopathogenesis of both sporadic and familial ALS. We present here a peculiar familial form of ALS that showed high clinical heterogeneity together which evidence of mitochondrial dysfunction. Mother's

disease onset was at the age of 61, with proximal hyposthenia, cramps, increased CK blood values (400 U/L). Electromyography showed a mixed damage, neurogenic and myopathic, with fasciculations at four limbs. Muscular biopsy showed a pattern of COX negative fibers, reduced activity of II+III complexes of mitochondrial respiratory chain with multiple deletions of mitochondrial DNA. She now has a 11 year disease history with slow clinical progression, her neurological examination showing a moderate tetraparesis (especially proximal), absence of dysphagia, absence of respiratory failure, cognitive-motor slowing. Son's disease onset was at the age of 44, with right hand hyposthenia and intrinsic muscles hypotrophy. Electromyography showed evident lower motor neuron damage with denervation and fasciculations. Muscle biopsy showed a pattern of COX fiber hyporeactivity, but no deletions in mitochondrial DNA. He rapidly developed tetraplegy and respiratory failure with necessity of tracheotomy one year after the diagnosis and death 3 years after the diagnosis. Genetic investigations for *SOD1*, *FUS*, *TDP43*, *POLG1*, *ANT1*, *Twinkle* mutations were negative in both mother and son. Further deepening are needful.

Improving SMARD1 long-term outcome and quality of life: the role of a multidisciplinary setting

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Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is an inherited neuromuscular disorder resulting from recessive mutations in the *immunoglobulin mu-binding protein* (IGHMBP2) gene. SMARD1 is characterised by diaphragmatic palsy, symmetrical distal muscular weakness, muscle atrophy, peripheral sensory neuropathy and autonomic nerve dysfunction. It is considered a fatal infantile motor neuron disease and most of the patients survive until the first year of life. We report an 8-year-old girl with genetically confirmed SMARD1, who showed a relatively mild phenotype and an atypical long-term outcome. Despite a diaphragmatic palsy diagnosed at 6 months of age, she had no need of ventilation support until 48 months of age when non invasive ventilation (NIV) was started. To avoid weight loss and pulmonary complications due to dysphagia, the child underwent Nissen fundoplication with gastrostomy. Two hours after surgery the girl was successfully extubated with a prompt pulmonary response and placed on NIV with facial mask. After 24 hours, she was placed back on nasal mask interface with her usual parameters. Ten days after surgery the child was discharged with a weight gain of 2 kilos (15% gain of initial weight). A multidisciplinary setting, focusing on intensive and rehabilitative care, may surprisingly improve SMARD1 long-term outcome and quality of life.

Mitochondrial involvement in patients with autism spectrum disorders

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Mitochondrial encephalomyopathies are inherited disorders of oxidative metabolism, with a wide clinical, biochemical and genetic spectrum of expression, requiring a complex diagnostic flow-chart. Infantile forms often occur with defects in development, intellectual disabilities (ID) and dysmorphisms.

Autism spectrum disorders (ASD) are neurodevelopmental disorders, with heterogeneous aetiology, characterized by deficiencies in social interaction and communication and by repetitive and stereotyped behaviours. ASD has been associated with mitochondrial respiratory chain deficiency and/or mitochondrial DNA (mtDNA) mutations; however reports in literature are based on case reports or limited samples and regard specific aspects. Our study pointed at the identification of mitochondrial dysfunction in 19 subjects (16 males and 3 females) presenting ASD and clinical signs of neuromuscular involvement. For these patients we carried out muscle biopsy for histological, biochemical and genetic investigations. On histological examination we found myogenic or neurogenic changes in 16 patients and mitochondrial abnormalities, such as lipid accumulation, and/or mitochondrial proliferation, and/or COX deficient fibers, in 12 patients. The biochemical investigations showed one or more respiratory chain complexes deficiency in 3 patients. Finally, genetic studies revealed multiple deletions of mtDNA in 1 patient with normal histological and biochemical findings, four different mtDNA mutations in 4 patients, and *POLG1* pathogenic mutations in 1 patient with respiratory chain complex IV deficiency. The present study confirms the hypothesis of an aetiological link between autism and mitochondrial dysfunction. However additional studies in a larger group of subjects are needed.

Cardiac troponin T in skeletal muscle from myotonic dystrophies patients: a possible biomarker of cardiac dysfunctions

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Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are autosomal dominant neuromuscular disorders caused by expanded CTG or CCTG repeats in two different genes. Nuclear accumulation of mutant RNA leads to aberrant alternative splicing of different genes that have been linked to the multiorgan involvement. Perturbation of cardiac Troponin T (cTnT) splicing leads to the co-expression of both adult (excluding-exon 5) and fetal (including-exon 5) isoform of the protein in cardiac tissue of DM patients and is considered one cause of conduction abnormalities and cardiac dysfunctions in these patients. Recently, it has been observed that cTnT splicing is also altered in skeletal muscle of DM patients. The aim of this work is to verify if skeletal muscle expression of cTnT fetal isoform might

be considered a biomarker of heart dysfunctions in patients with myotonic dystrophy. RT-PCR analysis was performed on RNA samples from muscle biopsies of 12 DM1, 9 DM2 and 6 healthy subjects using primers flanking *cTNT* exon 5. DM1 and DM2 patients were divided in two subgroups on the basis of cardiac evaluation performed by ECG, echocardiogram and ECG-Holter. A significantly higher expression of cTnT fetal isoform was observed in DM1 and DM2 patients presenting cardiac involvement. On the contrary, no correlation was found between fetal cTnT expression detected in DM patients and histopathological abnormalities. In conclusion, cTnT fetal isoform expression in skeletal muscle of DM patients seems to be related to cardiac dysfunctions but not to skeletal muscle histopathological alterations.

FSHD and 18p deletion syndrome

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by genetic and epigenetic derepression of *DUX4*, a gene located within a repeat array of D4Z4 sequences of polymorphic length and located in the subtelomeric region of chromosome 4. FSHD type 1 (FSHD1) is associated with the pathogenic contraction of the D4Z4 repeat array. In contrast, FSHD type 2 (FSHD2) is linked to mutations in *SMCHD1*, a chromatin modifier gene located in chromosome 18. Both types of FSHD require the presence of a permissive polyadenylation signal (4qA) downstream of the D4Z4 array. Recently, 18p microdeletion encompassing the *SMCHD1* gene and causing haploinsufficiency have been associated with an FSHD clinical phenotype in individuals carrying a permissive 4qA allele with a borderline number of D4Z4 repeats, raising the possibility that patients with 18p deletion (18p-) syndrome may also develop FSHD when carrying a relatively short D4Z4 array and a 4qA allele. Here we report clinical, radiological, genetic and epigenetic data on four families confirming this hypothesis. We conclude that clinical signs of FSHD have to be searched in patients with 18p- syndrome and, when present, FSHD diagnosis should be suspected. On the other hand, patients with atypical FSHD may present additional 18p deletion syndrome.

Atypical clinical pictures in inflammatory myopathies: a case series

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Accepted criteria for diagnosing idiopathic inflammatory myopathies (IIM) include subacute and insidious development

(usually over a period of 3-6 months) of limb symmetrical muscle weakness, most prominent in proximal muscles. Here we report five patients with history and clinical picture that are not typical for an IIM but with muscle biopsy features that meet the criteria for pathological diagnosis. A personal and family history of neuromuscular disease, endocrinopathy, rheumatological diseases or exposure to myotoxic drugs or toxins was absent. The first patient was a 67-year-old male complaining of exercise intolerance and proximal lower limb weakness since about ten years with no clinical progression. The second patient was a 73-year-old female who complained of "food bolus block" in the pharynx/esophagus since 7 years. A 66-year-old male patient complained of dysphagia, hypophonia and exercise-related dyspnea since two years without developing any significant limb and face muscle weakness. The fourth patient was a 65-year-old male who noted a progressive focal atrophy of the right thigh in the past five years; in the last year, proximal lower limb weakness and difficulty in rising from a squatting position and climbing stairs appeared. The fifth patient was a 72-year-old female which was submitted to muscle biopsy for the occasional evidence of increased levels of CK (2000 U/L). She did not complain of neurological symptoms. All these cases suggest that atypical clinical pictures can be found in IIM and muscle biopsy can be critical for obtaining diagnosis and ensuring the proper treatment.

Scoliosis is an inescapable comorbidity in SMA type II. A single center experience

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Scoliosis is the most debilitating and unresolved problem in SMA type II patients.

We present data of progression of scoliosis and treatment in a cohort of 20 SMA type II patients followed in the last fifteen years (2002-2017) in our Neuromuscular Unit. Medical records were collected prospectively. In all patients brace was prescribed since Cobb angle in sitting position was $\geq 20^\circ$ or in case of marked kyphosis. All patients underwent systematical X-Ray, functional and pulmonary assessments.

Seventeen of 20 patients (age range 2-20 y) have scoliotic curve. Mean age at scoliosis development was 3,4 y (range 2-7 y) and Cobb angle at diagnosis varied from 5° to 35° . Nine patients underwent scoliosis surgery. Six patients had Growing-rods or VEPTR at mean age of 8,1 y (range 6,2- 10y). Five patients (two of them received at first growing-rods) underwent definitive spine fusion at a mean age of 12,2 y (range 10-14y). Postoperative complications were observed in 3 patients: urinary infection, difficulty in extubation and weaning from mechanical ventilation and infective complication of the surgical wound.

Despite the use of brace, scoliosis progressed in all cases and, as observed in our series, since the age of 6 years patients develop severe rotoscoliosis that, following the current

standards of care, requires surgery. These findings have to be carefully considered in the developing of potentially effective therapy intrathecally administered.

Skeletal muscle CIC-1 channel: from gene to protein, from birth to aging

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CIC-1 chloride channels play a critical role in sarcolemma excitability. Loss of function mutations of CIC-1 gene cause myotonia with different severity of symptoms according to effect of the mutation on channel structure (Imbrici et al. FASEB J. 2016). Studies on extensor digitorum longus (EDL) rat muscle showed that at 7-days after birth, the resting CIC-1 conductance (gCl) is very low, it increases rapidly with age and decreases during aging with respect to adulthood (Conte et al. Pflügers Arch. 1989). Here we performed a systematic study of CIC-1 protein and mRNA expression, in SOL and EDL rat muscles from birth to old age. Our data show that CIC-1 expression, evaluated by western-blot and qPCR, increases 10-fold from 1-day to 8-months and decreases by 50% with aging (27-months) both in SOL and EDL muscles. Since protein kinase C (PKC) negatively controls CIC-1 activity, we measured by ELISA total PKC activity, showing that it decreases from birth until 8-months and slightly increases in aged muscles. To determine CIC-1 fiber localization we performed double-immunofluorescence analysis by using the sarcolemmal beta-dystroglycan as reference. CIC-1 channel was found to be present either in sarcolemma or in cytoplasm from birth to 12-days while it was present only in sarcolemma of old rats in both muscles. Our findings provide the evidence that CIC-1 expression and localization varies during muscle development offering a point of comparison for all the situations in which CIC-1 protein is altered due to mutations as occur in myotonia or in consequence of drug treatment such as statins.

A rare case of myopathy with pipestem capillaries in a female carrier of Becker muscular dystrophy

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Myopathy with pipestem capillaries represents a very rare entity of uncertain classification, characterized by necrosis of muscle fibers, minimal cellular infiltration, and vessel wall thickening with luminal narrowing. We describe the case of a carrier woman of Becker muscular dystrophy (BMD), who presented at the age of 61 yr with acute onset of neck and shoulder pain followed by proximal weakness of the upper limbs and the neck. Over the next 3 years, weakness progressively worsened also involving the distal muscles of the upper limbs and the pelvic girdle muscles. The electromyographic evaluation showed myopathic signs with minimal denervation in the affected muscles. Serum levels of creatine kinase were slightly

increased, and a myositis-specific antibody panel was negative. A muscle biopsy of the right tibialis anterior muscle showed abnormal variation in fiber size with thin atrophic fibers, central nuclei, presence of numerous pipestem capillaries, and minimal necrosis of muscular fibers. Some cytochrome-oxidase (COX) negative muscle fibers compatible with slight mitochondrial abnormalities were also observed. As autoimmunity is considered to play a role in the development of myopathy with pipestem capillaries, patient was treated with oral corticosteroids, which determined only a slight improvement (especially as regards pain), and subsequently with methotrexate, which resulted in a more significant clinical benefit. In conclusion, this case suggests that, albeit rarely, a necrotizing myopathy with pipestem capillaries could occur in previously asymptomatic patients with dystrophin gene mutations. The early recognition and treatment of this unusual clinical condition is fundamental for a favorable outcome.

Mitochondrial giant crystals in muscle biopsy

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The occurrence of highly ordered crystalline inclusions is the most characteristic mitochondrial abnormality, frequently found in mitochondrial myopathies. These are a heterogeneous group of neuromuscular disorders in which an altered mitochondrial metabolic function may or not be associated with mitochondria structural changes. In this report we describe a 52-year-old man, with no family history of neuromuscular disease, that developed a mild myopathy. He complained of proximal muscles fatigability, light muscle pain and difficulty at climbing stairs. His previous clinical history also included a HBV infection and a non-alcoholic steatohepatitis. Laboratory tests revealed hyperCKemia (> 1000 U/L, normal value < 175) without elevation of inflammatory markers. Lower limbs electromyography showed a myopathic pattern correlated with the non uniform distribution of fat and fibrosis muscle replacement at MRI whereas a mild oxidative phosphorylation deficit was detected at phosphorus MR spectroscopy. Muscle biopsy revealed the presence of inclusions with polygonal profile, eosinophil with H&E, red at modified Gomori Trichrome and unstained with NADH-TR. Combined cytochrome C oxidase and succinate dehydrogenase staining showed a high percentage of fibres negative for cytochrome C oxidase activity. Transmission electron microscopy revealed the presence of intramitochondrial crystals, made of closely packed parallel lines with the typical parking lot appearance, and the presence of bigger crystalline inclusions aligned along the axis of the fiber and spanning length of several micrometers. At higher magnification the typical crystal structure became more obvious, with parallel lines regularly packed. Further analyses will be necessary to clarify the etio-pathogenesis of morphological and functional mitochondrial abnormalities described.

Asymptomatic primary carnitine deficiency unmasked in a mother by newborn screening

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Primary carnitine deficiency is a rare condition caused by recessive mutations in the *SLC22A5* gene which encodes for the plasma membrane carnitine transporter OCTN2 (organic cation transporter type 2). Systemic carnitine depletion causes impairment of the entry of long-chain fatty acids into the mitochondrial matrix and unavailability of long-chain fatty acids for beta-oxidation and production of ketone bodies. Signs and symptoms of primary carnitine deficiency can include encephalopathy caused by hypoketotic hypoglycemia, cardiomyopathy and progressive proximal weakness. However the severity of the disease varies among affected individuals, and usually it becomes evident when carnitine levels are less than 20% of normal values. We here report a 32-year-old otherwise healthy woman having a 28% of normal values of plasmatic carnitine levels (2.64 mg/l, normal levels 3.69-8.58). The test was performed because of a carnitine deficiency at the newborn screening in her daughter. The patient did not complain of any neurological or neuromuscular symptoms. Vastus lateralis muscle biopsy was normal while the molecular analysis of the *SLC22A5* gene showed the well-known variant c.136C > T (p.Pro46Ser) and a still not described c.391G > A (p.Glu131Lys) variant. Bioinformatic analysis for this second variant showed a probably mild deleterious effect. Our report expands the allelic heterogeneity of primary carnitine deficiency and emphasizes that reduced levels of carnitine in newborns may unmask a systemic primary carnitine deficiency in their mothers. In our case, the novel mildly pathogenic c.391G > A variant likely causes a not severe dysfunction of carnitine transport which prevents the appearance of symptoms.

A novel ACTA1 mutation in a patient affected by congenital myopathy with histopathologic progression

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A 36-year old woman had a history of fetal hypokinesia, severe neonatal hypotonia, motor developmental delay and generalized muscle weakness, but stable course. She had received at age 27 a diagnosis of multi-minicore myopathy, but genetic analysis for *SEPN1*, *RYR1* and *DNM2* were negative. Neurological exam revealed moderate proximal and distal muscle weakness, multiple contractures and myopathic facies. Muscle MRI demonstrated diffuse fatty infiltration, in particular of adductors, gracilis and anterior tibialis bilaterally. Muscle biopsy documented severe fatty replacement and core-like areas, and modified Gomori trichrome staining revealed dark red aggregates positive for alpha-actinin and myotilin at immunohis-

tochemistry. Moreover the aggregates and some intranuclear inclusions in a few fibers were also positive with phalloidin. Altogether these findings suggested a new diagnosis of nemaline myopathy with intranuclear rods. Accordingly, genetic analysis documented a novel and de novo heterozygous mutation (p.Phe92del) in a highly conserved residue of *ACTA1* gene. In our patient the second biopsy demonstrated the nemaline bodies that were absent in the first one, although a restaining with phalloidin showed scattered positive foci, suggesting a progressive process of sarcoplasmic actin filament accumulation, not accompanied, however by the worsening of muscle weakness. The diagnosis of congenital myopathies can be difficult, particularly when muscle biopsy is aspecific or ambiguous, and this report demonstrates that a second biopsy may reveal new aspects useful for the diagnosis. Intranuclear rods, i.e., may be a hint for searching *ACTA1* changes. The mutation reported here is novel and pathogenic, and is associated with severe congenital manifestation, but relatively mild course.

Mutational variety in patients with Myotonia Congenita from Campania Region

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Myotonia congenita (MC) is a rare disease characterized by muscle stiffness of striated muscle groups including facial and extrinsic eye muscles and tongue. Muscles are usually hypertrophic. Symptoms are relieved by repeated contractions of the muscle (the "warm-up" phenomenon). According to the pattern of inheritance, two forms have been described: the autosomal recessive form (Becker type) often associated with more severe symptoms, and the autosomal dominant form (Thomsen type). Age of onset is variable, ranging from infancy or early childhood in dominant forms to a slight older age in recessive forms. However older age of onset has been reported in both forms. The diagnosis is made by the presence of typical symptoms, electromyography revealing myotonic bursts, elevated serum CK concentration and family history. *CLCN1*, encoding the chloride channel CIC-1, is the only gene known to be associated with myotonia congenita. Sequence analysis of *CLCN1* detects more than 95% of pathogenic variants causing both recessive and dominant forms. We report our cohort of 25 patients affected by MC, 11 isolated and 14 familial cases, in which the *CLCN1* gene was sequenced. The following variants were identified: c. 86A > C; c.180 + 3A > T; c.302-1G > A; c.313C > T; F468S; c.501C > G; c.817G > A; c.826G > A; c.899G > A; c.1167-10T > C; c.1403T > C; c.1785G > A; c.2551G > A, alone or more often in compound heterozygosity. Interestingly in two families, we found 3 mutations, 2 of them in cis on the same chromosome (c.86A > C + c.2551G > A and c.313C > T + 501C > G), whose meaning is under investigation. At our knowledge, these variants have never been reported in patients from Italian origin.

Thymoma-associated Myasthenia Gravis: clinical and serological features of Pisa's Cohort

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TAMG (Thymoma-associated MG) represents one of the subtypes of MG associated with autoantibodies directed against the acetylcholine receptor (AChR-Ab). Approximately 10–20% of MG patients have a thymoma and about 30% of thymoma patients have thymoma-associated MG. The recurrence of thymoma is rare and the rate ranges according to different reports. Our aim was to analyze our large cohort of MG patients with thymoma and relapsed thymoma according to: age of MG onset, age of thymectomy, surgical approach, oncological features (according to histological classifications: WHO and Masao-ka-Koga), post-thymectomy status, current MG clinical status and AChR-Ab serum titres. We selected 388 MG patients with AChR-Ab and thymoma: 360 with TAMG and 28 (7.2%) who experienced one or more recurrences of thymoma. Patients with recurrent neoplastic disease were usually younger than those with thymoma, with a mean age of MG onset of 39.5 ± 12.1 years. There was not a significant sex prevalence and bulbar symptoms were relevant in this subtype of MG; compared to the thymoma group, the current MG clinical status was not related to the severity of neurological disease before thymectomy. The average neoplastic disease-free time was 3.7 years. Antibody-titres usually declined after thymectomy and chemo/radiotherapy whilst recurrences of thymoma were not associated with significant variations in AChR-Ab serum titres or clinical status of the patients. Compared to non-thymomatous MG, TAMG clinical presentations tended to be severe at onset and immediately after thymectomy but, in the long-term, pharmacological and complete stable remission was achieved in a high percentage of patients.

Benign monomelic amyotrophy of lower limb: report of 15 Italian cases

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Benign Monomelic Amyotrophy of Lower Limb (BMALL) is a very rare sporadic disease more common in the oriental countries. Few western cases have been reported. BMALL is characterized by leg and thigh asymmetric muscular atrophy with insidious onset, very slow progression and poor disability. The involvement is exclusively motor and there are no clinical or laboratory pathognomonic findings.

We reviewed data of 15 BMALL patients, afferent to the Neuromuscular Center of Chieti, in the last 10 years. We have collected a detailed history and evaluated them by using electrophysiology, muscle and spine MRI and, in some cases, muscle biopsy. The follow-up was from one to ten years. They have been ruled out all other possible disorders.

Twelve patients originate of the Abruzzo and 10/15 are male: The left leg is more frequently interested (10/15) and MRI shows the steady involvement of the medial head of the gastrocnemius. We did not notice clear clinical progression of the disease during the follow-up.

Our data suggest that BMALL in our country is more frequent than expected. They do not show correlations with physical activity or other, but it seems more common the non-dominant limb involvement. The course of the disease is always benign.

Quantitative muscle ultrasound analysis in neuromuscular disorders

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Muscular imaging is an important diagnostic tool for the detection and quantification of muscular changes during the clinical workup of patients with muscular disorders. Although largely superseded by MRI, ultrasound (US) remains a valuable additional tool in this field. Diminished muscle thickness and increased echo-intensity due to increased fibrous and fatty tissue are the US hallmarks of muscle damage. However, considering the qualitative modality of the evaluation of these parameters, the high operator-dependency, the influence of machine- and patient-related variables and signal differences among different muscles, the US diagnostic value for identifying neuromuscular disorders is still debated. Aim of our research is to evaluate the applicability of quantitative muscle ultrasound (QMUS) in the study of neuromuscular diseases. We perform QMUS by using computer-aided gray-scale analysis and calculate the histogram of grey values after selection of a region of interest within the muscle. The main points to achieve a repeatable QMUS were: constant settings (depth, compression, focus and time gain compensation); gray-scale values acquisition form muscle type; age, sex and weight normalization. QMUS was not able to distinguish among fatty infiltration or fibrosis, or inflammation. Opposite to deeper muscles, superficial muscles could be easily imaged. For its accessibility, relative inexpensiveness and dynamicity QMUS could play a role for a routinary detection of muscle involvement and a valuable tool for follow-up studies.

Effectiveness of treatment with ivabradine on clinical and instrumental endpoints in patients with Duchenne Muscular Dystrophy

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Increased survival in Duchenne Muscular Dystrophy (DMD) is due to an improvement in clinical care of the musculoskeletal and respiratory systems and has led to an increased incidence of dilated cardiomyopathy (DCM). Cardiac-related deaths are now seen in approximately 20% of DMD patients. The treatment for the cardiovascular complications contemplates the association of ACE inhibitors and Beta-blockers, which showed to improve the survival rate. The effects of ivabradine treatment in this population has not yet been investigated. Ivabradine inhibits the hyperpolarization-activated pacemaker (If) current in the sino-atrial node and provides pure heart rate (HR) reduction

without effects on other hemodynamic parameters. HR reduction mediated by ivabradine demonstrated also an improvement of total arterial compliance, a reduction of the arterial elastance and, as a consequence, an increasing of the stroke volume in patient with heart failure (HF). Our study aims to demonstrate the effect of ivabradine on clinical (dyspnea, palpitations, edema, fatigue, quality of life, NYHA Class), echocardiographic (diastolic function, left ventricular ejection fraction, ventricular volumes and echocardiogram-Color Doppler analysis with two-dimensional trans-thoracic strain and strain rate), electrocardiographic and serological (NT-proBNP) endpoints in DMD patients. We included 30 patients (20 cases and 10 controls, who met the inclusion criteria (age 14-35 years; FE < 50%), Ivabradine was administered at a dose of 5 mg/day in addition to optimized medical therapy. After at least 6 months of observation period, we found reduced heart rate, improved diastolic function and furthermore a positive effect about ejection fraction. Treatment was well tolerated in all patients, without side effect. Further studies with more robust methodology are needed to confirm these encouraging preliminary results.

Discordant manifestations in two Italian brothers with GNE myopathy

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GNE myopathy is a rare, recessively inherited, adult-onset distal myopathy. Patients first show weakness and atrophy of distal muscles, later proximal muscles are affected, with sparing of quadriceps. We describe clinical, histopathological, and genetic findings from two Italian brothers. P1 reported lower limbs distal weakness from 32 years. Examination at 37 years showed bilateral foot-drop, waddling gait, distal lower limbs (MRC 3) and milder proximal upper limb weakness (MRC 4). Muscle MRI revealed fibro-fatty substitution of legs, and posterior thigh compartment. Examination at 44 yrs revealed severe leg weakness (2 at MRC), and milder arm weakness (3-4 MRC). P2, the younger brother of P1, presented wasting and weakness of lower limbs from age 25, leading to foot-drop. Examination at 26 years revealed pronounced leg weakness (MRC 2) and milder scapular weakness (MRC 4). Muscle MRI showed fibro-fatty substitution of legs. Examination at 36 years showed a wheelchair bound patient with severe and diffuse muscle weakness quoting 1-2 MRC, with relative quadriceps sparing (3-4 MRC). Muscle biopsies from both patients disclosed the presence of rimmed vacuoles and unspecific myopathic findings. Exome sequencing revealed two heterozygous mutations in the *GNE* gene on chromosome 9p13.3. Here we describe prominent clinical discordance in two brother with GNE myopathy. Clinical heterogeneity is quite common in GNE myopathy. However great interfamilial variability is unreported. Exome sequencing reanalysis in P2 failed to show the presence of genetic alterations in other myopathy genes. Unknown epigenetic or environmental factors could be responsible for the clinical discordance in this family.

Effects of long-term treatment with eteplirsen on cardiac function: left ventricular ejection fraction in eteplirsen-treated patients vs disease natural history

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Duchenne muscular dystrophy (DMD) is an X-linked, recessive genetic disease characterized by dystrophin deficiency or absence. Eteplirsen, a phosphorodiamidate morpholino oligomer, targets DMD pre-mRNA to trigger skipping of exon 51 in order to restore the reading frame, thereby enabling production of internally truncated dystrophin protein. Herein we report effects of long-term eteplirsen treatment on cardiac function in patients with DMD in comparison to reports from disease natural history and a clinical study of perindopril.

Eteplirsen patients were treated in two consecutive studies. Study 201 was a randomized, double-blind, 28-week trial; patients aged 7-13 years with DMD amenable to exon 51 skipping were randomized to receive 1 of 2 once-weekly intravenous (IV) eteplirsen infusions (30 mg/kg, n = 4; 50 mg/kg, n = 4) or placebo (n = 4). At week 25, all patients received open-label eteplirsen through week 28. Study 202 was an open-label extension of study 1; all patients received once-weekly eteplirsen at the same dose (30 mg/kg or 50 mg/kg) for up to 240 weeks. Both trials included longitudinal evaluation of cardiac function using echocardiographic left ventricular ejection fraction (LVEF).

The mean annual rate of decline in ejection fraction for eteplirsen-treated patients over the 240-week trial was 0.18%. A study of perindopril (N = 57; mean age at baseline, 10.6 years) reported a 1.28% annual decline in perindopril-treated patients (n = 28) and a 1.88% annual decline in placebo-to-perindopril patients (n = 29; placebo treatment, 3 years; perindopril treatment, 2 years) over the 5-year study period, as measured by radionuclide ventriculography. A disease natural history study of 98 DMD patients aged 7-29 years reported a 0.58% annual decline in LVEF, as measured by magnetic resonance.

Eteplirsen may slow deterioration of cardiac function, as reflected by slower decline in LVEF, in comparison to disease natural history.

Pseudo-dominant inheritance of a novel homozygous *HACD1* mutation associated with congenital myopathy: the first Caucasian family

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Congenital myopathies are a clinical and genetic heterogeneous group of early onset muscle diseases. Mutations in *HACD1* gene cause Congenital Centronuclear Myopathy in dogs and have also been recently described in one consanguineous Bedouin family with congenital myopathy that improved

with age. We report herein the second family with congenital myopathy due to *HACD1* mutations.

Clinical data were collected from the family members. Skeletal muscle biopsies were performed in two patients. The *HACD1* mutation was identified by next-generation sequencing.

The proband is a 28-year-old woman with facial and limb-girdle muscle weakness, who was born from consanguineous Caucasian parents. At birth, she presented with severe hypotonia that gradually improved. Muscle biopsy at 8 years of age revealed myopathic features with increased variation in myofiber size, type-1 fibers predominance, and slightly increased internal nuclei. The younger sister had similar clinical and histopathological findings. The mother and maternal grandmother had a slowly progressive proximal muscle weakness since childhood. After neurological evaluation, surprisingly, also the father showed the same clinical picture (there was a double consanguinity). A novel homozygous variant in *HACD1* gene (p.G213A) was detected in all the affected members.

To our knowledge, this is the second report on human mutations in *HACD1*. Our data highlight the implication of *HACD1* in human pathology. Moreover, the long term follow-up of the affected individuals, revealed a mild and slowly progressive course, even at advanced age, which constitutes an important finding for patients' counseling.

Fetal akinesia deformation sequence and recessive central core disease: a rare presentation of mutations in *RYR1* gene

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RYR1 gene encodes a ryanodine receptor localized to the sarcoplasmic reticulum of skeletal muscle, an important mediator of excitation-contraction. *RYR1*-mutations have been associated with a broad spectrum of phenotypes, ranging from severe neonatal-onset myopathy with or without cores, to malignant hyperthermia without muscle weakness. Fetal akinesia is a rare severe neonatal syndrome characterized by developmental abnormalities due to lack of intrauterine fetal movements. This syndrome has been associated with several congenital myopathies, and described in few patients with autosomal recessive and dominant *RYR1*-myopathies. We describe herein a family with a homozygous *RYR1* mutation, central core disease (CCD), and fetal akinesia.

Clinical data were collected from the parents. Skeletal muscle biopsy was performed. DNA was extracted and the mutation identified by next-generation sequencing.

A 3-year-old boy was diagnosed with severe growth retardation, hypotonia, dysmorphism, skeletal abnormalities, and motor-developmental delay. He was the second son of non-consanguineous parents. Family history was unremarkable. The boy was born at 37 weeks of gestation complicated by polyhydramnios, growth retardation, and fetal akinesia. He presented hypotonia, multiple arthrogryposis, and scoliosis at birth. Muscle biopsy performed at 4 years revealed eccentric cores, fibrosis, increased fiber size variability, internal nuclei, and type 1-fiber predominance. Patient died at 4 years of age

of respiratory insufficiency. *RYR1* gene sequencing revealed a homozygous mutation (p.Phe4976Leu), not previously reported in association with fetal akinesia.

RYR1 mutations have been rarely associated with presence of severe fetal akinesia. The association of fetal akinesia and CCD with eccentric cores should prompt evaluation of *RYR1* gene.

Congenital myopathy with protein aggregates and nemaline bodies related to *CFL2* mutations

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Congenital myopathy (CM) caused by mutations in cofilin-2 gene (*CFL2*) is a rare neuromuscular disorder. The few reported cases show phenotypic heterogeneity ranging from early onset and rapid progressive form to milder myopathy characterized by slow progressive limb girdle and axial muscles weakness. Muscle histology is also heterogeneous showing features of nemaline or myofibrillar myopathy or the coexistence of both histopathological changes. *CFL2*-null mutations result in more severe disease variants than those related to missense mutations although the precise mechanism through which cofilin-2 abnormality results in nemaline myopathy remains elusive. We report on three new cases, from two unrelated families, of severe CMs related to novel homozygous (p.D86H) or compound heterozygous (p.D79Y and p.S94LfsX6) loss-of-function mutations in *CFL2*. All babies presented severe neonatal generalized muscle weakness needing continuous respiratory and nutritional support since birth. One baby died at the age of 3 months; his sister and the other proband are still alive at the age of 1 year and 7 years respectively. Muscle biopsies showed severe myopathic changes, with fiber splitting, internal nuclei, many rod bodies and sarcoplasmic protein aggregates that mainly look like actin filaments. In the older baby dystrophic features such as fatty infiltration and fibrosis were also observed and *CFL2* protein is reduced thus suggesting that the p.D86H mutation results in a misfolding or destabilization of the protein's tertiary structure, which leads to cofilin-2 degradation.

Mild phenotype in DM1 young boy due to interrupted repeat of the DMPK expanded tract

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Myotonic Dystrophy type 1 (DM1) is a multisystem autosomal dominant disorder caused by the expansion of (CTG)_n in the 3'UTR of the DMPK gene, on chromosome 19q13.3. DM1 patients with different patterns of CCG/CTC/CGG interruptions at the 3' or 5' end of the DMPK expanded tract have been described. However, the role of these interruptions in DM1 pathogenesis is still unclear. Recently a boy 14 years old came to our Neuromuscular Center with a positive genetic test for DM1 paternally transmitted. The patient was clinically asymptomatic and he did not have delayed development nor history of intellectual or learning disabilities. EMG was also negative. The genetic test performed in our laboratory revealed the presence of expanded allele only in his father but neither in the proband nor in his mother. Electropherogram results of TP-PCR indicated the presence of an expanded allele in both directions (Forward and Reverse) in the father and of two normal alleles (12/15 CTG repeats) in the mother. A pathological allele with a 3' interruption in the TP-PCR Forward primer was evident in the proband. Proband's skeletal muscle analysis did not show histological alterations and FISH in combination with MBNL1 immunofluorescence on muscle sections did not show nuclear accumulation of mutant RNA or MBNL1 protein. No alterations of splicing pattern of several genes commonly involved in DM1 pathology were observed by RT-PCR analysis on proband's muscle biopsy. Our results support the hypothesis that interrupted repeat within the DMPK expanded alleles can modulate the phenotype in DM1 patients.

Management of adult DMD patients: the experience of Neuromuscular Unit IRCCS E Medea

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Improvement of respiratory support and pharmacotherapy for heart has brought an extension of lifespan in Duchenne muscular dystrophy (DMD) patients. Therefore, improved survival led to an increased incidence of cardiomyopathy as a major cause of patients' morbidity.

Between 2000 and 2016, 169 genetically defined DMD patients have been assessed yearly or twice a year in Neuro-muscular Unit, IRCCS E Medea. At this moment 80 patients an

age range 4-39 yrs (50 patients over 18 yrs) have regular follow up visits including neuromuscular, cardio-respiratory and nutritional evaluations.

Along these years 25 patients died, 40% of these deaths due to cardiac causes (mean age of death 18 yrs) and 60% to respiratory causes combined with nutrition deficit (mean age of death 22 yrs). At the moment 41 adult patients (82%) receive nocturnal non-invasive ventilation, NIV (mean age at onset $19,1 \pm 3$ yrs) and coughing aids (mean age at onset $13,2 \pm 3,8$ yrs). 48 patients (96%) receive cardiac therapy, 7 among them present severe cardiomyopathy. In 3 patients implantable cardioverter-defibrillators (ICD) was placed. 14 patients (28%) underwent spinal surgery (mean age at surgery $14,4 \pm 1,4$ yrs). Six patients have gastrostomy for enteral feeding (mean age at placement $23,6 \pm 5,3$ yrs).

We support survival prolongation by NIV concomitantly with cough assistive devices. When respiratory function is stable, life expectancy is limited by cardiac deficit, but also by nutritional deficit. Further research is required to improve cardiac support and outcome in particularly the benefit of ICD has not been established for the DMD population.

Clinical, morphological and immunological findings in Myasthenia - Myositis Association

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We present clinical, histological, radiological and immunological features of a cohort of six patients with Myasthenia (MG) and Inflammatory Myopathy (IM). Median age at disease onset was 62 years (range 48-72). In five patients the diagnosis of both MG and IM was made since the disease onset. All these patients had a thymic neoplasm. In one more patient IM and thymic tumor were diagnosed 10 years after MG. The clinical discrimination of myasthenic and myopathic symptoms was very difficult, so that in most patients the suspicion of an association of MG and IM derived from electrophysiological data and the detection of high AChR-antibodies and CK levels. Other myasthenia and myositis-associated antibodies were also tested. Muscular weakness was generalized in all patients and associated to respiratory involvement in four. Ocular symptoms were mildly present in few subjects. One patient had very strong muscle pain. Muscle MRI showed STIR positive images consistent with inflammation in several muscles. IM was confirmed by muscle biopsy in all patients. All patients underwent mediastinal surgery and required the association of two or more immunosuppressive treatments to achieve an improvement of neuromuscular symptoms. Two patients died during follow-up because of infectious complication. In conclusion, we present six patients presenting both myasthenia and inflammatory myopathy with common clinical, serological and histological findings. Myasthenia associated myositis could represent a particular paraneoplastic phenotype related to thymic neoplasms. Larger cohorts of patients are needed to characterize this clinical association and to better understand its immunological profile.

A case of limb-girdle muscular dystrophy type 2L mimicking Dermatomyositis

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A 68-year-old female was referred to our neuromuscular centre for progressive proximal muscle weakness, hyperCKemia and periocular heliotrope rash, starting when she was 50 years old. Her medical history was notable for autoimmune diseases, i.e type 1 diabetes, thyroiditis and alopecia. In the past the patient had received a diagnosis of possible dermatomyositis and has been treated with immunosuppressive and immunomodulatory therapies (cyclosporine, methotrexate, steroid and intravenous immunoglobulin), without any clinical response. Therefore, her diagnosis was reconsidered. In particular, appropriate blood testing for autoimmune and myositis-specific or associated autoantibodies were negative; electromyography showed a slight myogenic pattern, muscular MRI highlighted a diffuse severe fatty infiltration of posterior muscles of the thighs and a muscle biopsy showed a predominance of type I fibers with occasional inflammatory infiltrates, along with rare necrotic fibers. Considering that the exams did not support an inflammatory myopathy and the relentless clinical course, we proposed to perform a target gene panel testing for IperCKemia by NGS approach which identified two pathogenic variants in *ANO5* (c.172C > T and c.2141C > G), already described.

This is the description of a woman affected by limb-girdle muscular dystrophy type 2L, who was for long time misdiagnosed with an inflammatory myopathy within a predisposition to autoimmune disorders. We suggest that the complete lack of response to immunosuppressive therapies should raise suspicion of a genetic origin of the myopathy.

Neurorehabilitation in ALS: consequences at micrornas level

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MicroRNAs(miRNAs) are small non-codingRNAs that have been shown to modulate a wide range of biological functions under various pathophysiological conditions. MiRNAs regulate mRNA expression at post-transcriptional level, resulting in translational repression and gene silencing. Circulating miRNAs have recently gained attention for their potential as minimally invasive and cost-effective disease biomarkers and their stability in serum. MiR-206,miR-133a,miR-133b,miR-1 are called "myo-miRNA" and are considered as markers of muscle regeneration, myogenesis and fiber type differentiation and might represent indicators of residual muscle mass consequent to a chronic atrophy of muscle. Amyotrophic Lateral Sclerosis (ALS) is a rare, progressive, neurodegenerative disorder caused by degeneration of upper and lower motoneurons. A moderate and regular exercise is useful in the treatment of many neuromuscular diseases included ALS. In this study we analysed the role of circulating myomiRNAs after physical rehabilitation and we analysed the clinical features correlated to

rehabilitation scale. We measured muscle specific microRNAs (miR-1,miR-206,miR-133a,miR-133b) by Real Time PCR in 17 ALS patients (10 male,7 female) in serum collected before(T0) and after (T1) a period of 6-8 weeks of physical rehabilitation. We observed a general down-regulation of all miRNAs studied after rehabilitation. In our group myomiRNAs decreased in a similar manner in male and female patients and we didn't detect any significative difference in the levels of microRNAs in patients over and under 55 years old. We have found that microRNAs are an important tool to monitor rehabilitation in ALS patients and suggest a positive effect of the treatment. Further studies are needed to correlate circulating microRNAs with muscle atrophy.

Monitoring motor function and disease progression in DM1

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In myotonic dystrophy type 1 (DM1) muscle weakness is progressive and responsible for increasing motor disability over time. To date there is no standardized physical assessment to monitor disease progression.

Aim: to identify which physical test best correlates with disease severity and disability.

Ten Meter Walk Test (10MWT), Six Minute Walk Test (6MWT), 30 Second Chair Stand Test, Jamar Handgrip and 9 Hole Peg Test (NHPT) were administered to 46 consecutive patients with DM1 (44,41 ± 15,60 years) who were able to perform these functional tests regardless of age, phenotype or CTG expansion. Severity of disability was evaluated by the Muscular Impairment Rating Scale (MIRS) and the Rivermead Mobility Index (RMI). Correlations among the tests of lower and upper limb were assessed, adjusting for age at evaluation, sex and CTG expansion. A p-value < 0.05 was considered statistically significant.

10MWT (seconds) significantly correlates with 6MWT (meters) and 30 second chair-stand test (number of repeats). 10MWT also significantly correlates with upper limbs tests (NHPT dx: $r = 0,41556$; Hand grip dx: $r = -0,57587$), while 6MWT and 30 seconds chair-stand test does not.

The correlation between lower limbs tests is significant, as expected; however we detected that only 10MWT is correlated with upper limbs tests too. Further studies are needed to confirm this data. Preliminarily we can speculate that this test could give a summary of patient's global motor function and could be used as a rapid and easy-to-perform test to monitor disease progression.

A novel mutation in kv1.1 channels in a patient with paroxysmal ataxia, myokymia, painful contractures and metabolic dysfunctions

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Episodic ataxia type 1 (EA1) is a rare dominant neurological syndrome characterized by episodic attacks of ataxic gait and myokymia that can be triggered by emotional stress and fatigue (D'Adamo et al., 2015). It is caused by missense mutations in the *KCNA1A* gene coding for the voltage-gated potassium channel Kv1.1, which contributes to nerve cell excitability in the brain but is also expressed in the heart, vascular smooth muscle, retina and pancreatic b-cells. Some patients respond well to acetazolamide and carbamazepine. We identified a novel *KCNA1A* mutation, E283K, in an Italian proband presenting with paroxysmal ataxia and myokymia aggravated by painful contractures and unusual metabolic dysfunctions. The E283K mutation is located in the S3-S4 extracellular linker belonging to the voltage sensor domain of Kv channels. To test whether the E283K mutation affects Kv1.1 biophysical properties and causes EA1 symptoms, we transfected HEK293 cells with WT or mutant cDNAs alone or in a 1:1 combination, and recorded potassium currents through whole-cell patch-clamp. Mutant E283K channels shifted the voltage-dependent activation by 10 mV towards positive potentials and slowed kinetics of activation by ~ 2 fold compared with WT channels. Heteromeric WT/E283K channels showed voltage-dependent gating and kinetics of activation intermediate between WT and mutant homomeric channels. Homology modeling studies suggest that the replacement of a negatively charged residue with a positively charged lysine at position 283 reduces voltage sensitivity and slows channel opening. Overall, the biophysical changes of E283K channels cause a drop of potassium current that likely accounts for EA-1 features and possibly for the additional symptoms in the carrier.

Cardiac involvement in a patient with congenital-muscular-dystrophy related to *POMT2* gene mutation

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Dystroglycanopathies are a genetically heterogeneous group of congenital muscular dystrophies (CMD) with autosomal recessive inheritance, presenting with a broad spectrum of phenotypes. Cardiac involvement appears to frequently occur in patients with *FKRP* and *FKTN* gene mutations. To date, only two unrelated patients with CMD due to *POMT2* mutations and cardiac involvement have been reported. We describe a boy with

clinical history of delayed motor milestones, proximal weakness, intellectual disability and cardiac involvement. His maximum motor ability was walking with support at the age of 4 years. At the age of 9 years, a muscle biopsy was performed and he received the diagnosis of CMD with depletion of merosin and alpha-dystroglycan glycosylated complex. He was referred to our attention at the age of 14 years. He was wheelchair-bound and his neurological examination showed: generalised hypotonia, muscle weakness more proximal than distal, absent tendon reflexes. The creatine kinase value was elevated, 2000 U/L. Brain MRI showed a mild brain atrophy. The ophthalmologic evaluation was normal. The cardiological evaluation showed: mild left ventricular dysfunction with ejection fraction (EF) of 49% (mildly decreased), mitral regurgitation and electrocardiographic features of incomplete right bundle branch block. Given the depressed EF systolic function, the patient was started with Enalapril 2.5mg/day and is on regular cardiological follow-up. Genetic analysis showed the homozygous missense mutation R72H in *POMT2* gene. Our data show that cardiac evaluation and follow-up are warranted in this form. Moreover, further cohort studies might help to define possible genotype-phenotype correlations of the cardiac involvement.

Gender-related characteristics of myotonic dystrophy type 1 in a large Italian database

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Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy among adult Caucasians. We have recently ascertained its prevalence in the Rome province to be different among females and males (8.35 and 11.07/100,000, respectively). The aim of this work was to assess comparatively the prevalence of the main genetic and clinical characteristics of DM1 in male and female patients from a large collaborative database in order to detect possible gender-related specificities. In a sample of 943 DM1 patients, the male/female ratio was 1.2, the mean age was 50 years for both sexes and the mean age at death was 60 and 58 years for females and males, respectively. Transmission of the mutation was paternal in 63% and maternal in 37% of patients. The mean size of CTG_n expansion was 605 for females and 496 for males and females were more numerous in the (E3) expansion class with more severe mutations. The degree of muscle impairment was more severe in females than in males (MIRS grade > 3 = 37% vs 31%, respectively). Important differences were observed in the prevalence of some major clinical features, including: cardiac conduction defects

and seborrheic dermatitis (M > F) and cataract, hypothyroidism and cutaneous xerosis (F > M). Other features, such as cardiac arrhythmias, daytime somnolence and dysphagia were equally prevalent in the two sexes. These data reveal that DM1 affects asymmetrically patients with regard to gender. Indeed, both genotype and phenotype seem to be influenced by gender-related factors, although the mechanisms that produce these effects are largely undetermined.

TP-PCR as a secondary analytical level in Myotonic Dystrophies' diagnostic pathway

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Myotonic Dystrophies type 1 and 2 are dominantly inherited multisystemic disorders due to microsatellite expansion, CTG and CCTG motif respectively. For both, a multistep genetic diagnosis is required to correctly characterize the instable repeats. Starting from white blood cell gDNA, we perform a three-step pathway including: a) conventional PCR to detect normal-sized alleles b) Triplet Primer-PCR (TP-PCR) for samples with unresolved pattern c) Southern blot of XL-PCR to detect and size pathologic expansions. We developed a TP-PCR assay relied on a single fluorescent primer (P1-FAM), corresponding to forward primer used in the conventional PCR, and we perform the same amplification protocol, protracting extension to 3 minutes. Five microliters of PCR product mixed with deionized formamide was run for capillary electrophoresis on ABI 3100 automatic sequencer, and fragments resolution was displayed by GeneMapper 3.0. Specificity was assured by the complete absence of DM2 cross reaction in DM1 TP-PCR assay and vice versa. Over the years, we collected 142 DM1 patients and 70 DM2 patients coming from several Italian neurological centers, and we are performing TP-PCR on all samples. Preliminary data collected until abstract submission indicated good sensitivity and specificity: 30/140 DM1 samples and 30/70 DM2 samples analyzed presented a characteristic peak pattern from expanded fragments, clearly different from negative samples. Whether this retrospective analysis will be successful we will adopt the low-cost and step-by-step method presented as diagnostic *iter* for DM1 and DM2.

Atypical features in multiple acyl-CoA dehydrogenase deficiency: report of two cases

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Multiple acyl CoA dehydrogenase deficiency (MADD) is a rare metabolic lipid disorder characterized by exercise intolerance and muscle weakness with excellent response to riboflavin treatment. Recently in six cases with MADD a severe sensory neuropathy has been reported. We describe two cases with MADD and uncommon clinical features. In both CK and LDH serum levels were increased. Multiple acylcarnitine profiles revealed an elevation of medium and long chains acylcarnitines. The first one is 50 years old male with dropped head at onset, and ataxic neuropathy. The second case come to our department

for ataxic gait, progressive muscle weakness and dysesthesias. Electrophysiological studies show a mainly sensory neuropathy. In both cases muscle biopsy showed a lipid storage. Riboflavin therapy resolved fatigability and muscle weakness and normalized CK and LDH levels. Dysesthesias and ataxic disorder remain unmodified after therapy. Our cases confirm that a severe sensory neuropathy is a feature of MADD and that is unresponsive to riboflavin treatment.

Impact of Myasthenia Gravis on quality of life

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Myasthenia gravis (MG) produces long term disability and affects health-related quality of life (HRQoL). In 2000 a Task Force of the Myasthenia Gravis Foundation of America recommended development of a quality of life measure specific for myasthenia gravis. We present the relationship between specific indicators of HRQoL and disability in a group of patients with MG. Adult patients with MG were consecutively enrolled at the Neurological Clinic of Polytechnic University of Marche from January to April 2016. Seventy consecutive adult patients with MG (33 female; mean age 48.03, length of disease 9.16 years) were recruited and divided according therapeutic, clinical and demographics features. The MG quality of life-15 (MGQoL-15), Myasthenia Gravis-specific Activities of Daily Living scale (MG-ADL) and the Myasthenia Gravis Questionnaire (MGQ) were employed. Scores obtained by the individual groups in each questionnaire were compared. Among the characteristics studied those that have proven to be most important in influencing the quality of life were found to be: over 50 years of age at diagnosis, more than 50 years old at the time of administration of the questionnaire, absence of complete disease remission, presence of dual therapy and occurrence of myasthenic crisis. HRQoL is able to different clinical profiles' features and we recommend employing QoL specific assessments. MG has an extensive impact on physical, psychological and social well-being which also affects doctor-patient relationship in a lifelong process and treatment disease. As more advanced therapy becomes available for MG, it is relevant to use HRQoL as an outcome for choice of treatment strategies.

A PGM NGS protocol in a single center cohort for patients with undiagnosed myopathy

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Next Generation Sequencing (NGS) has largely increased the diagnostic accuracy in patients with myopathy and several NGS studies have conducted by using large panels of genes. We have developed three inexpensive next generation sequencing gene-panels including only key genes involved in adult metabolic and dystrophic myopathy and have evaluated the improvement in diagnostic accuracy. The panels group 63 genes

involved in muscular dystrophies (MD), metabolic myopathies (MM) and some other different myopathies i.e. myofibrillar myopathies. The coverage was 99.13% for the first panel, 99.93% for the second one and 99.20% for the third one. A total number of 61 undiagnosed myopathic patients has been analyzed and a definitive diagnosis has been reached in 12 patients (19.67% of the total). In 20 patients (32.79 %), the presence of mutation of unknown pathogenicity needs further functional studies. In 29 patients (47.54 %) no significant mutations have been found and a Sanger analysis of the single regions not covered by NGS is needed. By these panels, pathogenic variants in *DMD*, *EMD*, *PGM*, *CAV3*, *MYOT*, *ANO5*, *SGCA*, *ISPD*, *CAPN3* and *PLEC* were found. These relatively small panels have allowed to reach a correct diagnosis in a significant number of patients and may represent a cheap and rapid screening protocol to be integrated to the traditional diagnostic myopathological process, before going on to more complex larger panels NGS or whole exome sequencing studies.

Severe muscle involvement caused by A193T mutation in Filamin-c

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Filamin-C gene (*FLNC*) defects cause different types of autosomal dominant myopathies. Mutations in the rod domain are associated with a proximal myofibrillar myopathy (MFM), while distal involvement at onset without predominant MFM pathology is described with frameshift mutations causing haploinsufficiency and with missense mutations in the actin-binding domain (ABD) of *FLNC*. The ABD A193T mutation has been described in one family and in three other patients so far. Here we present three members of one family harboring this aforementioned mutation with different severity of muscle involvement.

We report clinical, pathology and muscle imaging data of these new patients. Results: Two patients reported late-onset (5th decade) lower limb proximal weakness, that rapidly progressed leading them to the need for walking support after 10 years from onset. One patient, the youngest, presented with early onset (2nd decade) isolated calf hypotrophy and inability to walk on tip toes. Creatin-kinase ranged from normal to 4x. Muscle pathology showed the presence of myopathic changes with minor myofibrillar abnormalities in one patient. Muscle MRI showed almost exclusive involvement of gastrocnemii and soleus muscles at leg level, with subsequent fatty replacement of thigh and pelvic muscles in correlation with disease severity.

The phenotype associated with the A193T ABD-filaminopathy mutation appears to be variable, with the possibility of a more diffuse (proximal and distal) muscle involvement at later stages of the disease. Calf hypotrophy and weakness, which is likely the presenting sign in all our patients, could have been ignored until more proximal weakness appeared.

A case with chronic inflammatory demyelinating polyneuropathy and ocular myasthenia gravis

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We describe the case of a Caucasian female that since the age of 31 years, during the second pregnancy, has started to complain asthenia and progressive paresthesias at upper and lower limb. Electromyography showed neurogenic changes, with nerve conduction velocities reduced, increased distal motor latency and dispersed compound muscle action potentials. Cerebrospinal fluid protein was raised. Serum creatine kinase resulted mildly increased (2 x normal). The diagnosis of chronic inflammatory demyelinating polyneuropathy (CIDP) was performed. She received therapy with intravenous immunoglobulin, with a first significant clinical benefit. After 8 months, the clinical picture was further worsened with the occurrence of alternating eyelid ptosis, diplopia and precocious fatigability, in association with diffuse paresthesias. Anti-acetylcholine receptors and anti-MUSK antibodies were negative; thoracic computed tomography was inconsistent. However, single-fiber EMG was suggestive of neuromuscular transmission defect. A diagnosis of a concomitant seronegative ocular myasthenia gravis (MG) was hypothesized. The patient started therapy with pyridostigmine bromide, and then stopped it for side effects. To date, after four years of follow-up, intravenous immunoglobulin preparations are the current treatment, with a medium infusion frequency of two months and a positive therapeutic response.

We will discuss the association with CIDP and MG focusing on shared pathophysiology and treatments.

Non dystrophic myotonias: review of our cases with focus on genotype-phenotype correlations and therapeutic effects of mexiletine

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Non dystrophic myotonias are an heterogeneous group of genetically determined diseases caused by mutations in skeletal muscle ion channels; the main forms are myotonia congenita (a chloride channel myotonia) and paramyotonia congenita (a sodium channelopathy). The recessive form, Becker's myotonia, is believed to be caused by two loss-of-function mutations, whereas the dominant form, Thomsen's myotonia, is assumed to be a consequence of a dominant-negative effect. However, a subset of *CLCN1* mutations can cause both recessive and dominant myotonia congenita. Mexiletine, a non-selective voltage-gated sodium channel blocker which belongs to the Class IB anti-arrhythmic group, is an effective antimyotonia drugs and ameliorates myotonia in sodium channelopathies. Here, we review our case series of non dystrophic myotonic myopathy with focus on genotype phenotype correlation with special regard on mutations with incomplete penetrance. We currently follow 20

patients (12 M, 8 F), 12 subjects with myotonia congenita and 8 with paramyotonia congenita. All patients had a molecular diagnosis (12 CLCN1 mutations and 8 SCN4A mutations). Some families in which CLCN1 mutation segregates report an incomplete penetrance, maybe due to different allelic expression or different genetic background. Heterozygous family members for the same pathogenic variant exhibit variable phenotypes ranging from absence of myotonia to severe myotonia. Our data confirm that mutations in the CLCN1 can show reduced penetrance or incomplete dominance. Thirteen patients of them are treated with mexiletine. Mexiletine significantly improved self-reported stiffness in 9 patients, while in 2 subjects only a partial subjective improvement of myotonia is observed and in one the therapy is discontinuously performed because of side effects.

Improved distal spinal muscular atrophy genetic diagnosis by targeted NGS sequencing

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Distal spinal muscular atrophy (SMA) and distal hereditary motor neuropathies (HMN) cover a broad spectrum of clinically and genetically heterogeneous diseases characterized by the selective involvement of motor neurons in the peripheral nervous system. In recent years, next-generation sequencing (NGS) has increasingly been used for the diagnosis distal SMA because of their larger than expected genetic and clinical heterogeneity. Between 2015 and 2016 we evaluated 46 patients (21F/25M) with childhood or adult onset clinical symptoms and signs of distal motor neuropathy. We tested for mutations in the coding regions and flanking intronic sequences of 91 genes examined in a multiple gene panel designed with the Haloplex technology. Twelve patients (26%) presented known disease-associated variants, or variants predicted to be deleterious, confirmed by Sanger sequencing, tested for segregation and ultimately classified as likely pathogenic variants, pending their functional validation in all but one case with SMA-LED. Fifteen cases tested negative for the presence of causative variants (33%), whereas variants of uncertain pathogenic definition were identified in 19 cases (41%). This study offers the perspective of a single center experience in distal SMA and highlights the advantage of using NGS for genetic diagnosis in highly heterogeneous neuromuscular diseases.

Dilative arterial malformations in patients with Late Onset Pompe Disease (LOPD)

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Pompe disease is a rare inherited multisystemic metabolic disorder with involvement of several tissues and organs including smooth cells. Some reports have documented cerebrovascular malformations as aneurysms or vertebra basilar dolichoectasia in patients with Pompe disease whereas the occurrence of arterial malformations is quite rare in different sites. We describe the presence and type of arterial abnormalities in 4 Late Onset Pompe Disease (LOPD) patients. A cerebral, thoracic and abdominal CT angiography, was used to study these patients. Results – 3 unruptured intracranial aneurysms were found (respectively 2 mm, 4 mm and 4.7 mm) in three LOPD patients (M/54, M/ 50, F/63) whereas in a 48 yrs old man a large thoracic aortic aneurysm was detected. The 4.7 mm aneurysm of anterior communicating artery was immediately treated with success with endovascular procedures. Our data confirm that dilative arteriopathy, not only in the cerebrovascular system, is a phenotypic feature in LOPD. Early recognition of ectasias and aneurysms as well as an appropriate intervention by stenting, coiling or other surgical procedure may prevent mild or severe cerebrovascular complications or even death in LOPD patients. Consequently, performing a CT angiography or a MR angiography in all LOPD patients is recommended for early detection of this kind of vascular malformations.

Clinical next generation sequencing gene panel in patients orphan of genetic diagnosis

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Within the European Neuromics Project we have studied with a clinical gene-panel approach 6 families orphan of genetic definition: we identified the causative mutation in 3 out of 5 families. Two brothers with a distal axonal motor neuropathy and autonomic dysfunction were studied using a lower motor neuron diseases gene panel; the analysis identified in both the affected an hemizygous missense mutation (p.A991D) in the X-linked ATP7A gene (SMAX3). The phenotype we describe in our family is novel since includes autonomic dysfunction, expanding the ATP7A clinical spectrum. NGS analysis of a targeted genes panel (183 genes) for SCA was carried in the families with recurrence of autosomal dominant cerebellar ataxia. In the first family the analysis identified a heterozygous missense mutation in AFG3L2 gene associated to SCA28. The progression of the disease is very slow but there is an anticipation of the onset in the proband (at 35 years) compared to the mother (at 70 years) despite the identified mutation is not a trinucleotide expansion. In the second family the proband started experiencing progressive ataxia since childhood, slowly progressive. The

analysis identified a missense mutation in KCNC3 gene (Arg423His) known to be causative of SCA13. In the remaining two families and in the family with recurrence of arthrogryposis the analysis did not identify any mutations in the causative genes; a clinical redefinition of the phenotype has been requested in the patients with ataxia in order to address any further analysis. Whole genome sequencing is ongoing in the arthrogryposis family.

The success of whole exome sequencing analysis in neuromuscular diseases patients: the unife experience within neuromics project

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The heterogeneous genetic landscape of NMDs raises challenges regarding the definition of a molecular diagnosis, now becoming mandatory for the inclusion in emerging therapeutic trials. To improve the diagnostic definition in our NMDs patients we used a Next Generation Sequencing approach: clinical gene panel analysis for the screening of known genes, WES (whole exome sequencing) and WGS (whole genome sequencing) analysis aimed at the identification of novel causative genes. WES analysis was performed in 6 “families of four” and 2 “trios”: 3 of them with congenital myopathy/dystrophy, 1 with spastic paraplegia, 2 with ataxia, 1 with Myofibrillar Myopathy, and 1 with AV block and LGMD. WES analysis unraveled the genetic cause of 5 out of 8 families. We identified 3 mutations in known genes (RYR1, ISPD and STIM1) and 2 novel causative genes POPDC1 and MSTO1, functionally validated. In the remaining 3 families, 2 candidate genes SARS2 and MMP8 were identified however not similar phenotypes were observed within the project making difficult the variation clinical validation. The WES in the last family identified a compound heterozygosis in the CPSF3L gene and transcript analysis is ongoing. Moreover, we performed WES analysis in 2 families with Bethlehem myopathy and in 2 patients affected by Ullrich muscular dystrophy with no identified mutation in COL6 genes. The WES output data were prioritized on the basis of a list of 115 candidate genes involved in Collagen VI myopathy (clinical exome). All the prioritized variants were validated by Sanger and functional validation by pathway analysis is ongoing.

Custom micro-fluidic exome array to detect transcript mutations in undiagnosed patients with collagen vi myopathies

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Collagen VI-related myopathy is a form of muscular dystrophy caused by mutations in the genes encoding three different Collagen VI (ColVI) chains $\alpha 1$, $\alpha 2$ and $\alpha 3$. About 40% of Col VI patients are however negative for ColVI genes mutations. In these cases analysis of ColVI transcript might be valuable in identifying mutations which escape routine DNA testing. We designed and validated a customized micro-fluidic exome array (FluiColVI) for the analysis of ColVI transcripts to identify mutations missing the DNA diagnosis. We designed a custom TaqMan gene expression assay to profile the entire 115 exons of ColVI genes covering all the exon-exon junctions of the three ColVI transcripts $\alpha 1$, $\alpha 2$ and $\alpha 3$. RNAs were extracted from both muscles biopsies and native/myogenic transformed (MyoD) urine stem cells (USCs). RNA from patients with a known mutations was used to validate the FluiColVI method. FluiColVI identified the three ColVI transcripts with the detection of all junction-junction systems in both muscle and native USCs of controls. The analysis of MyoD-USCs did not detect any ColVI transcripts, as expected to be absent in myogenic cells. In the patients, the FluiColVI confirmed the presence of the mutations in ColVI $\alpha 1$ identified at the DNA level. This study demonstrated that FluiColVI is a validated diagnostic tool for molecular profiling of ColVI transcripts in skeletal muscles and can be used in undiagnosed patients. Interestingly, native urine stem cells represent a noninvasive and cost effective human cell model to study ColVI transcripts in myopathies.

Longitudinal assessments in discordant twins with SMA

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We report longitudinal clinical and neurophysiological assessments in twins affected by spinal muscular atrophy (SMA) with discordant phenotypes. The boy had the homozygous deletion of *SMN1*, a typical type 1 SMA course, and died at the age of eight months. His twin sister, asymptomatic at the time of the diagnosis in her brother, had the same genetic defect; she developed clinical and electrophysiological signs of type 2 SMA. The reduction of tendon reflexes was the first clinical sign at 4 months followed, within few weeks, by a mild decrement in the amplitude of the compound motor action potentials. After the age of 9 months she showed a sudden clinical and electrophysiological deterioration. Among molecular tests, we determined *SMN2* copy number, *SMN2* and *Plastin 3* transcript levels in peripheral blood, and observed no differences between twins. Our findings highlight the need of identifying genetic/epige-

netic modifiers of the SMA phenotype, since the molecular and/or clinical predictors available so far are not enough sensitive to distinguish the different phenotypes in individual patients, and even more in siblings. This issue is particularly relevant in the age of experimental treatments adopted in pre-symptomatic subjects. As neonatal screening is still only present in very few countries, most asymptomatic patients are still identified on the basis of previous familial cases. Although the chances of having discordant sibling phenotypes is very low, a better understanding of these factors would strengthen the possibility that the possible improvement observed is related to the treatment.

Systemic al amyloidosis revealed by a muscle biopsy: a case report

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Amyloidoses, diseases resulting from extracellular deposition of amyloid, with diverse and rarely specific clinical features, lead to difficulties and delays in diagnosis. A stepwise approach to diagnosis and staging is critical and involves, primarily, confirmation of amyloid deposition and identification of fibril type. We propose a peculiar case of a 74 years old man with muscular weakness proximal pains and dysphagia. Laboratory data showed normal blood count, renal and liver function. A monoclonal component on lambda-region on serum protein electrophoresis was 2 gr/dl. Serum immunofixation was IgG-lambda positive and Bence Jones proteinuria was 244 mg/24h. Bone marrow aspirate (with 30% kappa chain restriction) resulted compatible with diagnosis of smoldering multiple myeloma. Inflammatory index and CK were normal but a clarifying muscle biopsy was taken. It showed alterations of nonspecific type with a mixed myopathic and neurogenic involvement. Because of myeloma, systemic light chain deposition was supposed; the diagnostic turning point was the demonstration of characteristic Congo red staining positivity around perymysial vessels. Electron microscopy confirmed amyloid fibrils around them associated with collagen fibrils. Umbilical and salivary glands biopsies were obtained but Congo red was positive only for the latter. Immunoelectronmicroscopy showed a strong staining by polyclonal anti-kappa light chains antibody on both muscle and salivary gland biopsies identifying the fibril type. A diagnosis of AL amyloidosis with muscle involvement was made and patient began a cyclic treatment (Melphalan, Dexamethasone, Bortezomib). This report suggests that a multidisciplinary approach is the cornerstone of the diagnostic work-up to recognize the rare amyloid myopathy.

GNE myopathy functional activity scale (GNEM-FAS): a four year follow up in 10 HIBM patients

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Hereditary inclusion body myopathy (HIBM) is an autosomal recessive myopathy presenting with distal leg weakness in early adulthood. It is caused by a mutation in the GNE gene blocking the biosynthesis of sialic acid. A disease-specific measurement of functional activity is needed to better understand the burden of illness, inform the design of clinical studies and optimize care.

We present a cohort of 10 patients (7 Female and 3 Male) with HIBM. All patients are genetically defined for GNE gene variants. Two of these patients are enrolled in UX001-CL301 trial with Aceneuramic Acid. We evaluated patients with GNE myopathy functional activity scale (GNEM-FAS) in order to assess ability and independence in three domains: mobility, upper extremity use and self-care.

We found that at the first visit the medium value of the GNE-FAS was 67 point out of 100 and at the last visit (performed within last year) was 56 point out of 100. 80% of our patients lost deambulation within 10 years since the onset of the first symptoms, in fact the most involved domain was the mobility one.

GNEM-FAS revealed to be a useful tool to assess evolution of mobility and self-care limitations in HIBM patients. The scale is simple and it takes less than 30 minutes to apply.

Riboflavin transporter deficiency (BVVL): transient expanded newborn screening (NBS) positivity for beta-oxidation abnormalities

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Brown-Vialetto-Van Laere (BVVL) syndrome is a rare autosomal recessive disorder characterized by generalized hypotonia, bulbar palsy, respiratory failure, sensorineural deafness due to Riboflavin metabolism defect. We describe a case of BVVL, NBS positive for high medium chain acylcarnitines not typical for medium chain beta oxidation defect (MCADD). The newborn was recalled for confirmatory test: clinical evaluation, plasma acylcarnitines and urinary organic acids (UOA) were normal. Because parental consanguinity, we alerted the pediatrician for a close monitoring although acylcarnitines were normalized. The pediatrician sent him to our attention at 9 months because of hypotonia and psychomotor retardation. He showed severe generalized hypotonia, diaphragmatic breathing, tongue fasciculations, swallowing difficulties, absent deep tendon reflexes. Cardiac evaluation, EEG, brain MRI, electromyography were normal. Mutations analysis of SMN1 gene, NAIP and IGHMBP2 excluded spinal muscular atrophy (SMA1 and SMARD). Plasma acylcarnitines profile and UOA with high ethylmalonic acid were evocative of multiple beta oxidation defect (MADD). Clinical history and biochemical findings were suggestive of BVVL and treatment with riboflavin was started.

Visual evoked potential, auditory evoked potential and audiometric examination were normal. The new homozygous mutation c.505C > T (p.Arg169Cys) in SLC52A2 gene confirmed the diagnosis. Plasma acylcarinitines and UOA normalized with riboflavin treatment. Muscular tone improved after 6 months and the child was able to sit alone at 16 months and to walk at 3 years. Because oral riboflavin supplementation (10-50 mg/kg/day) is effective and life saving, it should begin as soon as a riboflavin transporter deficiency is suspected. This is the first report of transient NBS positivity for beta-oxidation abnormalities in BVVL.

Newborn screening for Pompe Disease in Tuscany and Umbria: current overview and first preliminary results after two years

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Lysosomal storage disorders (LSDs) are a group of more than 50 different genetic disorders with a reported prevalence of 1 in 7,000 live births. New treatments and improved strategies for screening test on dried blood spot (DBS) have led to the development of several pilot newborn screening program for some LSDs. PD is an autosomal recessive disorder, caused by acid alpha-glucosidase (GAA) deficiency with a prevalence of 1:40,000. Infantile onset Pompe disease (IOPD) is the most severe form and is characterized by cardiomyopathy, respiratory distress, hypotonia. Enzyme Replacement Therapy is available. Early detection avoids diagnostic odyssey and allows higher treatment efficacy. In November 2014, after Ethics Committee approval, a prospective pilot project for Pompe (PD), Fabry (FD) and Mucopolysaccharidosis type I (MPS I) has been introduced into the routine newborn screening (NBS) program of Tuscany and Umbria. Newborn samples with informed consent for LSD pilot project were analysed for enzyme activities on the same DBS collected at 48-72 hours of life used for expanded NBS, without any additional sample. 49469 newborns were screened for GAA enzyme activity: 30 samples resulted positive at NBS. 17/52 were confirmed by GAA enzymatic assay on leukocytes. Molecular analysis showed 7 true positives for PD and 5 heterozygotes, 2 pseudo-deficiencies + heterozygotes, 3 pseudo-deficiencies. The incidence of PD in Tuscany/Umbria is 1:7100. One patient affected by IOPD with hypertrophic cardiomyopathy is currently on ERT. All patients identified by newborn screening are in long-term follow-up. The detection of new variant of uncertain significance represents a criticism.

Pivotal role of the clinical geneticist in diagnosing rare diseases. The index case of laminopathies

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LMNA-related disorders are rare genetic diseases caused by mutations in LMNA A/C gene, which encodes for lamins A and C, structural proteins of the nuclear envelope. Mutations in the gene are associated with a wide spectrum of disease phenotypes, ranging from neuromuscular, cardiac and metabolic disorders to premature aging syndromes. Skeletal muscle involvement may present as autosomal dominant/recessive Emery-Dreifuss muscular dystrophy, LGMD type 1B or LMNA-related dystrophy and congenital muscular dystrophy. We present the case of two families in which the diagnosis of laminopathy was reached thanks the intuition of a clinical geneticist. In the first family, the proband was a young boy who underwent heart surgery for aortic coarctation and familial bicuspidia. During the follow-up, an interview with a clinical geneticist leads to the molecular analysis for a cardiomyopathy-associated genes panel. It revealed the c.673C > T (p.Arg225Ter) mutation in LMNA gene, inherited by his mother, affected by dilated cardiomyopathy. In the second family the index case – a female aged 64y – was referred to our attention for an apparently asymptomatic hyperCKemia (2x). An accurate reconstruction of the family history revealed a high prevalence of PMK implantation (4 sibling) and sudden cardiac death (2). We suspected a laminopathy, diagnosis confirmed by the LMNA gene analysis that showed the mutation c.207delG (p.Val70Ser fs X26). Both cases show the pivotal role of the clinical geneticist for both an easy access to NGS panels in the diagnostic process, and directing an appropriate single-gene analysis.

Cardiological assessment in a cohort of patients affected by congenital Myotonic Dystrophy type 1

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Myotonic dystrophy (DM1) is a multisystemic disorder affecting skeletal muscle as well as eyes, heart, endocrine, respiratory and central nervous system. It is caused by a CTG trinucleotide repeat expansion in the non-coding region of DMPK gene. Clinical findings, spanning from mild to severe forms have been categorized into three phenotypes, mild, classic, and congenital. Congenital DM1 is characterized by the presence of repeats usually > 1000, leading to an earlier onset and more severe disease. Usually the affected newborns present hypotonia, severe generalized weakness, respiratory failure and early death; whether they survive present intellectual disability. Cardiac abnormalities, particularly conduction defects leading to an increased risk of sudden cardiac death are commonly reported in DM1. However, few studies focused on cardiac involvement in patients with congenital form (CDM1). To determine the

prevalence of cardiac abnormalities and their progression over time in patients with CDM1, we retrospectively evaluated data related to standard and dynamic ECG and echocardiography of 14 subjects (6M/8F), followed at our Service. The mean age at the first examination was 9y6m (range 1 month-24 years); the mean period of follow-up (FU) was 6y3m (range 1m-19y). At birth 2 of them – both males – presented with atrial septal defects. During the FU, 1 patient died from respiratory failure at 1m, while 2 patients (14.2%) presented conduction defects. In particular one female developed BAV I° and sinus pauses at 21y, and one male ventricular tachy-arrhythmia, at 14y. These data suggest to include cardiological examination in the follow-up of patients with CDM1.

Thomsen disease with central core features at muscle biopsy; a new morphological pattern or an unusual double trouble?

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Thomsen Myothonia is a rare disease due to dysfunction in muscle chloride membrane channel. Distinctive signs of the disease are clinical and electric myotonia and an almost all normal muscle biopsy. A 26 years-old male, born of non consanguineous healthy parents, began to suffer from limb and axial muscle stiffness, hand and eyes myotonia early in his teen. No cardiac involvement was ever reported. CK levels were elevated (400-600U/L) and EMG shown myotonic discharges. He underwent muscle biopsy which showed diffuse central nuclei and multiple minicores areas, sometimes filled with lysosomal activity. Electronic microscopy shown diffuse Z-line disruption, cores and scattered accumulation of fibrillar material. Molecular analysis discovered an eteherozygous mutation in CLCN1 (c.G950C) leading to an aminoacidic change in exon 8 (p.Arg317Pro) indicated as pathogenetic in several predictor programs. Neither CLCN1 mutation were detected in both parents nor mutations in genes involved in myofibrillar myopathy were detected so far in the patient. In conclusion we report the case of a patient carrying a homozygous CLCN1 mutation associated with peculiar muscle biopsy alterations typical of others kind of muscle diseases such as myofibrillar myopathy or congenital myopathy. We are going to perform a broad exome sequence's analysis to exclude the unlikely possibility of a second mutation in another gene. We think that this case could be of interest increasing the knowledge of myotonic myopathy spectrum; it also underline the usefulness of muscle biopsy in neuromuscular disorders because it has allowed us to discover a further morphological phenotype starting from a "plain" case.

Development of a mobile app conceptually designed for patients with Pompe disease

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The potential of *e-health* to provide innovative solutions for disease management has raised high expectations by patients' and healthcare professionals community. We are developing the second phase project of a mobile app specifically designed for patients with Pompe disease, with the following purposes: to help patients affected by Pompe disease to manage illness related issues and to reduce its burden, being continuously aware of health and quality of life; to provide clinicians with continuous and timely tracking of disease in each patient at real-time and ecological conditions of everyday life; to collect data useful to improve knowledge on Pompe disease natural history. To set up a fast acquisition/visualization of clinical parameters with the possibility to be constantly connected with referred medical staff and to improve research activities, we released a revised version of the mobile app to allow patients to connect directly with their hospital. This web platform could be useful to support clinicians to store patients records and follow up management data (neurological, psychological and pneumological nowadays). All collected data can be stored in a data base and can be analysed using different algorithms and protocols. Clinicians could have the possibility to continuously access each patient file via web portal, and for research purposes, data could be easily displayed in different ways (graphs or tables) to grant the best and the quickest interpretation.

McLeod syndrome: an Italian family with a novel mutation in the XK gene

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McLeod syndrome (MLS) is a rare X-linked recessive neuroacanthocytosis caused by mutations in the XK gene, which manifests with multisystem haematological, neuromuscular and CNS involvement and wide phenotypic variability. We report three male cousins on the maternal side, presenting with clinical and laboratory features supporting MLS diagnosis, which was confirmed in one of them by molecular genetic analysis. **Case 1.** This 41-yo patient was initially investigated for myalgia and persistent hyperCKemia (up to 2000 UI/L) with normal EMG/ENG and slight myopathic changes on muscle biopsy; acanthocytes were absent in his blood smear. At age 38 he developed focal epileptic seizures responding to levetiracetam. **Case 2.** This 46-yo patient presented at age 38 with pharmacoresistant epileptic seizures and persistent hyperCKemia up to 3000 UI/L; EMG/ENG showed axonal motor/sensor neuropathy; quadriceps muscle biopsy resulted normal. He recently developed mild dyskinetic involuntary movements; acanthocytosis and the expression pattern of Kell antigens supported MLS phenotype. Finally, molecular genetic analysis of the three exons of the XK

gene detected a hemizygous 17 base-pair frameshift deletion at axon 1 (c.248_264delGGCCGAGACAACGGCGG - 378616.3 ref. seq. ENST00000378616.3). **Case 3.** This 38-yo patient at age 32 presented with focal pharmacoresistant epileptic seizures with normal neurological examination and CK serum level; acanthocytosis was found on blood smear. To the best of our knowledge, the genetic variant we found in case 2 has not been previously reported and is not present in public databases. MLS diagnosis should always be taken in mind in patients with persistent hyperCKemia and symptoms of CNS involvement.

Muscle ultrasound elastography and MRI in preschool children with Duchenne muscular dystrophy: a pilot study

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The aim of this study was to determine muscle tissue elasticity at rest, measured with shear-wave elastography (SWE), in selected lower limb muscles of patients affected by Duchenne muscular dystrophy (DMD) and to correlate the values obtained with those recorded in healthy children (HC) and with muscle magnetic resonance imaging (MRI) data from the same DMD children. Five preschool DMD children (median age = 48 months) and five age-matched healthy children (HC) were studied. Six muscles of the inferior limbs and pelvic girdle at specific anatomic landmarks were measured with conventional B-Mode ultrasound and SWE, specifically rectus femoralis (RF), vastus medialis, vastus lateralis (VL), adductor magnus (AM), anterior tibialis, medial gastrocnemius, gluteus maximus (GM). Inferior limb muscle MRI was also performed using axial T1-weighted(w) and short-tau inversion recovery (STIR) sequences. In the DMD children, muscle stiffness was significantly increased in the RF ($p = 0.001$), VL ($p = 0.001$), AM ($p = 0.02$) and GM ($p = 0.02$) muscles. On muscle MRI T1-w images showed fatty replacement in 3/5 patients at the level of the GM, while thigh and leg muscles were affected in 2/5; hyperintensity on STIR images was identified in 4/5 patients. No significant correlation was observed between stiffness values and MRI scoring. Our study demonstrated that lower limb muscles of preschool DMD patients show fatty replacement and patchy edema on muscle MRI and increased stiffness on SWE, findings which are not related to each other. Further studies in larger cohorts are needed to establish whether these changes might be used as follow-up markers.

The Questionnaire GNAMM: the eating habits of 436 people with neuromuscular disease in Italy

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Recent studies indicate that an increase of oxidative stress and inflammation play a role in the pathogenesis of many neuromuscular diseases (NMD). These mechanisms may be contrasted by drugs but also by the diet. In order to obtaine informations on the eating habits of people with NMD in Italy, the questionnaire GNAMM (Gruppo Neurologia Alimentazione Malattie Neuromuscolari) was created, with the collaboration of Associations in particular UILDM.

GNAMM is a computerized questionnaire including disease diagnosis, severity of motor and respiratory impairment, groupings of foods and intake frequencies. These groupings were compared to WHO and Med-diet guidelines, rich in vegetables (which polyphenols) and essential fatty acids and balanced to saturated fatty acids (precursors of inflammatory molecules).

436 questionnaires were completed. Subjects were divided into homogeneous subgroups in relation to the degree of motor and respiratory impairment. In the group of ambulatory patients, 92 subjects aged > 16 years completed GNAMM, in the group of non-ambulatory patients there were 97 subjects, in the non-ambulatory group with non-invasive ventilation 48 subjects completed GNAMM. In all groups emerged an occasional consumption of fruits and vegetables, few grains and legumes, low frequency of fish intake. People with FSH reported better eating habits than other disease groups.

The questionnaire GNAMM documented that the majority of NMD individuals in the Italian population follow a power line that deviates unfavorably by WHO and the Med-diet. The collected informations will be used to raise awareness among dieticians and nutritionists of the multidisciplinary team. A nutritional approach prompting antioxidants food sources and minor sources of inflammatory precursors should be considered in NMD.

Detection and multidisciplinary care of myopathic patients in Ogliastro

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Incidence and prevalence of neuromuscular disease in Sardinia are not known. The Ogliastro is a province in eastern Sardinia that is suitable for genetic studies because of the homogeneity of his population. In this area there isn't a Neuromuscular disease center (NMDC) but there are many neurorhabilitation centers (NRC). The aim of this study is the identification of NM patients in this area, in order to obtain epidemiological data and improve level of care by the multidisciplinary team of the

NMDC of Cagliari. The study population comprised all patients leading to the in the NRC from 2014 to 2015. Data are collected in retrospective and prospective manner. 652 cases were evaluated. We identified 10 NM patients: 9 adults and 1 pediatric. For each patient clinical, demographical and familial data were collected. The results were matched with the NMDC data base. 5 patients head to the NMDC, 2 patients were known to the Center but were not in follow-up, 3 patients were unknown. All patients were included in the multidisciplinary follow up. A network with the physicians of the area was created in order to improve the local management. Further observation are scheduled and this pilot study is going to be extended to the whole island in order to create a regional NM patient register. Our data provide epidemiological data of NMD in a particular area of Sardinia and underlines the central role of the NMDC in the identification and in the establishment of an appropriate diagnostic and follow up tools.

Cognitive and psychiatric alterations in facioscapulohumeral muscular dystrophy: a case report

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Central nervous system disturbances in facioscapulohumeral muscular dystrophy (FSHD) were thought to be minimal in contrast with other muscular disorders. However, recent studies detected brain atrophy, alterations of neuronal networks, reduced cognitive functions in patients with large gene deletions. In addition, it is becoming increasingly evident that non-muscular symptoms do not depend from the contraction size of D4Z4 allele and they are not proportional to muscle impairment. Few articles studied the association between psychiatric disorders and FSHD: personality, behavioural disturbances, depression, phobias were detected; a single association was made with schizophrenia.

We describe a 30 years-old woman with a one-year history of facial and shoulder girdle muscles weakness, exacerbated after the first pregnancy (positive family history for FSHD). Since the first examination, behavioural disturbances and fatuous attitude were evident: the patient was irritable and aggressive against doctors and husband. A routine psychological interview pointed out compulsive and obsessive rituals focused on cleanliness and avoiding illness towards herself and her child.

The genetic testing confirmed the diagnosis of FSHD1 (fragment of 25 kb). The neuropsychological evaluation showed impairment of executive functions, reasoning and visuo-praxic skills. The psychiatric evaluation concluded for adjustment disorder with obsessive compulsive disorder (OCD). A pharmacological therapy with sertraline was started with improvement of behavioural symptoms, quality of life and disease disability perception.

To the best of our knowledge, this is the first report of association between FSHD and OCD. Furthermore, this case confirms that additional factors should be analysed to explain extra-muscular involvement in FSHD.

Plec gene mutations cause familial disto-proximal myopathy and long QT syndrome mimicking mitochondrial disease

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Plectin, a giant multifunctional cytolinker protein, plays a crucial role in orchestrating intermediate filament networks in a wide variety of tissues, such as epithelia, skeletal muscle and heart. Mutations of the human plectin gene (PLEC) on chromosome 8q24 cause autosomal recessive epidermolysis bullosa simplex with muscular dystrophy (EBS-MD), EBS-MD with myasthenic features (EBS-MD-MyS), EBS with pyloric atresia (EBS-PA), EBS-Ogna, and LGMD2Q. Here we report the clinical, myopathological and genetic findings in a Italian family with a distinctive muscle phenotype and autosomal recessive transmission in which Whole Exome Sequencing identified homozygous mutations in *PLEC* gene. Three brothers (2 female and one male) age 52, 51 and 46 were affected by adult-onset myopathy characterized by disto-proximal symmetrical muscle weakness starting in the upper limb muscles and later affecting lower limbs, mild facial weakness, ptosis and strabismus. There were no skin abnormalities. Cardiac investigation showed arrhythmia and long QT syndrome that required implantation of ICD in one patient. Myopathological analyses showed typical hallmarks of mitochondrial pathology, desmin accumulation in some fibers and markedly reduced plectin staining. Muscle respiratory chain activities were normal. This report expands the phenotypes of plectin-associated disorders and supports the hypothesis that mitochondrial dysfunction contributes to the progression and severity of muscle damage in plectin myopathy. "Plectinopathies" represent a considerable diagnostic challenge for their chameleon presentation and this study support the value of next generation technologies to improve our abilities to diagnose complex neuromuscular disorders. Analysis of *PLEC* should be included in the workout of mitochondrial disorders and distal myopathies.

A singular case of rhabdomyolysis with reversible paralysis

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We report the case of a 38 year-old woman hospitalized for the occurrence of acute and generalized muscle weakness and pain. Her medical history was characterized by anorexia nervosa and strenuous physical exercise with concurrent and continuous abuse of laxatives and diuretics (Senna pods and Furosemide, respectively). On admission, she presented a profound and mainly proximal weakness. Laboratory workup revealed a severe hypokalaemia (1.6 mEq/L) and hyperCKemia (up to 9821 UI/L). During hospitalization she developed a severe heart conduction abnormality associated with an increase of cardiac biomarkers, which required continuous EKG monitoring and parenteral nutrition in order to correct fluid and electrolyte imbalances. EMG showed diffuse myopathic changes. Muscle biopsy showed myonecrosis and regeneration of myofibers, as well as diffuse atrophy and basophilic aggregates within atrophic fib-

ers. Upon correction of potassium values, she rapidly recovered motility; she fully recovered from the cardiac conduction defect within few days and the muscular impairment in less than two weeks. In this case we recognize a combination of hypokalaemic paralysis with rapid reversal and rhabdomyolysis. The dramatic potassium loss has been certainly induced by the synergic effect of toxic doses of Furosemide and Senna. However, we believe that a chronic muscle damage may have been induced by Senna with a direct myotoxic effect. Indeed, it is known that anthraquinone derivatives ingested in large amounts can exert a disturbance in mitochondrial homeostasis resulting in myopathy. Although this effect is well known in cattle, there are few reports concerning human cases.

Polymorphisms in exercise genes and respiratory outcome after ERT in a cohort of Late Onset Pompe Disease (LOPD)

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Respiratory dysfunction is usually proportional to the degree of skeletal muscle weakness in LOPD, and is attributed primarily to diaphragm weakness. Compared to other skeletal muscles, diaphragm has peculiar features: continuous low-intensity contraction, usual recruitment of type I only fibers, distinct micro-vascular supply. We assessed the role of genes known to affect muscle properties on the respiratory outcome after ERT. To address diaphragm function, we analyzed FCV in supine and sitting position in 43 pts (25 males) aged 50.8 ± 13.6 years, all treated with ERT. We defined 25% as the cut-off for significant postural drop, and annotated the time in months to this event during ERT. We then analyzed the relationship between postural drop and polymorphisms in the following genes: Angiotensin Converting Enzyme (ACE I/D), Alpha-Actinin 3 (ACTN3 R/X), Peroxisome Proliferator Activated Receptor Alpha (PPRalpha C/G), Glycogen Synthase (GYS1 XbaI), Angiotensin Gene (AGT G/A), Sirtuin 1 (SIRT1 G/C), Glycogen Synthase Kinase 3 Beta (GSK3b G/A), Autophagy Related Gene (ATG7 T/C). The stronger association was with the ACE polymorphism: individuals carrying two copies of the D-allele shared a 24 folds increase in the risk of significant postural drop despite ERT. ACTN3-XX polymorphism also was associated with poorer respiratory outcome. In line with previous observations on skeletal muscles, both ACE I/D and ACTN3 R/X polymorphisms are likely to influence diaphragm function also, while the others analyzed polymorphism did not.

Post-receptor abnormalities contribute to insulin resistance in myotonic dystrophy type 1 and type 2 skeletal muscle

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Myotonic dystrophies (DMs) are autosomal dominant multisystemic disorders caused by a nuclear accumulation of toxic

RNA that lead to aberrant alternative splicing of different genes. Splicing alteration of insulin receptor (IR) gene is considered one of the causes of metabolic dysfunctions, such as insulin resistance, hyperinsulinemia and a fourfold higher risk of developing Diabetes mellitus type 2 (T2DM). The aim of this work is to investigate if post receptor molecular defects in insulin signal can contribute to peripheral insulin resistance in DMs. The basal activation of several proteins of insulin pathway has been analysed by western blot in proximal and distal muscles from 6 DM1, 3 DM2 and 7 healthy subjects. The analysis of insulin pathway activation was performed on myotubes at 5 days (T5) of differentiation from 5 DM1, 5 DM2 and 5 healthy subjects. As control for *in vitro* study, 2 patients affected by T2DM were also used. Our results indicate that DMs skeletal muscle exhibits high basal expression and activation of some proteins involved in the insulin pathway. These alterations have been confirmed on control and DM myotubes both expressing fetal IR isoform. Insulin action appears to be impaired in DM myotubes in terms of protein activation, glucose uptake and GLUT4 translocation. Our data indicate that post receptor signalling abnormalities might contribute to DM insulin resistance, however, further investigations will be necessary to identify novel biomarkers that could be target for therapeutic intervention to improve the quality of life of DM patients.

Myalgias, cramps and muscle rippling: a case report

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We describe the case of a 67-year-old Caucasian male that came to our attention for ten months history of muscle cramps, pain, stiffness induced by exercise and occurrence of localized mounding and rapid contractions at upper and lower limb muscles. Neurological examination did not show muscle weakness, but the mechanical percussion of muscle bellies induced a transient local mounding phenomenon, resembling rippling. His family history was inconsistent for neuromuscular diseases and his clinical history was unremarkable. Serum creatine kinase resulted mildly increased (2x normal). Needle electromyography recorded a myopathic pattern; the involuntary rolling muscle contractions were electrically silent. Anti-acetylcholine receptor-antibodies were negative. Thigh and leg muscle magnetic resonance imaging was normal. Muscle biopsy from quadriceps femoris revealed several fibers contained granular material that stained red with modified Gomori trichrome stain, intense blue with NADH, but was non-reactive to SDH. The ultrastructural study confirmed that the abnormal areas observed by light microscopy contained tubular aggregates, sharply demarcated from the adjacent myofibrils and closely packed. Genetic screening for tubular aggregate myopathy was then performed. Further details will be added on in site discussion.

Encephalocardiomyopathy with severe recurrent rhabdomyolysis due to TANGO2 mutations: a case report

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To present a case of TANGO2 mutation in a 3-year-old child with severe recurrent rhabdomyolysis, psychomotor development delay, hypothyroidism and heart failure.

We report the case of a boy referred to diagnostic investigation following two extremely severe rhabdomyolysis episodes. The first episode occurred at 13 months, triggered by throat infection, with a CK raise from 1.400 to 210.000 IU/L within three days. Complications included hyponatraemia with comatose state and heart failure not related to electrolytes alterations. Brain MRI showed mild hypomyelination and reduced thickness of corpus callosum. During the admission he was diagnosed with mild hypothyroidism, and treated. Normal investigation included respiratory chain enzymes, urine organic acids, acylcarnitine profile, plasma aminoacidogram, ab anti-myositis and spectroscopy. Muscle biopsy showed myopathic changes. In the period between hospitalizations the child slowly regained muscle tone but psychomotor development delay persisted. The second episode occurred at 27 months following viral infection (CK picked at 225.000 IU/L), when he also presented severe heart failure. He was extensively investigated for genetic causes of rhabdomyolysis. WES confirmed compound heterozygous TANGO2 mutations in the proband and in his asymptomatic sister (16 months), which were inherited from both parents. The child is regularly monitored by a multidisciplinary team (child neurologist, cardiologist, metabolist, physiotherapist, speech therapist). An increment of sugar intake during infectious episodes and integration with coenzyme Q10, riboflavin and carnitine are prescribed. Loop recorder implant is under evaluation.

TANGO2 mutation is a rare genetic cause of severe recurrent rhabdomyolysis associated with encephalocardiomyopathy.

Functional assessment tools in infantile Pompe disease. A critical analysis and pilot study

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Study objective: to establish which motor functional scales and which cognitive tests can measure outcome in children and adolescents with Pompe disease.

This is a monocentric prospective pilot study. Motor functions were investigated using the CHOP INTEND (CI), the Motor Function Measure 20 and 32 (MFM-20/32), the Hammersmith Motor Functional Scale (HMFS), the Expanded Hammersmith Motor Functional Scale (MFSE), the North Star Ambulatory Assessment (NSAA) and the Gait, Stairs, Gowers, Chair, Arms Functional Test (GSGCA). Muscle strength

(Medical Research Council scale, MRC), range of movement (ROM) and Six-Minute Walk Test (6MWT) were included. Developmental/Intelligence Quotient was evaluated by Bayley III, WPPSI III (Wechsler Preschool and Primary Scale of Intelligence III) and WISC IV (Wechsler Intelligence Scale for Children IV). Inclusion criteria were: age range from 1 month-old to 16 years-old, established diagnosis of Pompe disease, attendance to treatment and clinical evaluations to the Regina Margherita Children Hospital of Turin. Eight patients were included: 4 Classical Infantile Pompe patients (all with ERT) and 4 Non-Classical Infantile Pompe patients (two with ERT); mean age 5.9 years. Patients were evaluated at T0 and, when possible, T12.

Motor functions in children with Pompe disease, both Classical and Non-Classical form, are better assessed by CHOP INTEND, MFM-20 and NSAA according to age and functional level. About cognitive functions, additional tests could be useful to better describe specific neuropsychological aspects. Swallowing and feeding functions, sensory functions, communication, psychosocial and neuroradiological domains still need to be addressed.

Liver transplantation reverses biochemical imbalance and improves clinical conditions in mitochondrial neurogastrointestinal encephalomyopathy

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Thymidine phosphorylase (TP) deficiency in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) leads to toxic nucleoside (thymidine/deoxyuridine) accumulation. Based on the demonstration that normal liver contains elevated TP levels and on the absence of safe, effective, and stable TP replacement therapy, we performed orthotopic liver transplantation (OLT) in two MNGIE patients. Patient 1 is a 25-year-old male with end-stage disease (homozygosis c.1160-1G > A) showing prominent gastrointestinal phenotype (daily vomiting, abdominal pain, abdominal distension, severe malnutrition; mild imbalance and ophthalmoparesis and, subsequently, lower-limb hyposthenia with inability to walk). Patient 2 is a 21-year-old female (compound heterozygosis, c.977G > A and c.215-11_223del20) with prominent neurological involvement (bilateral ptosis, marked ophthalmoparesis and distal lower limb hyposthenia). Both patients had electromyography, brain MRI, and muscle biopsy consistent with MNGIE and their biochemical profile showed a reduced plasma TP activity (4 and 0 nmol/h*mg, respectively; n.v. > 253) with increased thymidine/deoxyuridine levels (16-16 and 8-17 microM, respectively, n.v. absent). OLT and standard post-transplant immunosuppressive treatment were performed with rapid toxic nucleoside clearance and without major complications. At 2 years of follow-up, patient 1 has permanent normalization of nucleoside levels and relevant clinical improvement (restored oral feeding, fully mobile, and improved quality of life). At 6 months of follow-up patient 2

has permanent normalization of nucleoside levels and clinical stabilization with improvement of quality of life. These cases indicate that OLT is feasible and tolerated in MNGIE, reverses biochemical imbalance and improves clinical conditions. However, long-term clinical outcome needs to be defined.

Ten years (2006-2016) of molecular diagnosis in collagen-vi related myopathies: are intragenic coding snps of COL6a genes modifiers of disease severity?

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Collagen VI myopathies range phenotypically from the severe Ullrich congenital muscular dystrophy (UCMD) to the milder Bethlem Myopathy (BM). Mutations in the COL6A1, COL6A2 and COL6A3 genes, encoding for a key component of the extracellular matrix, underlie a phenotypic continuum with unclear genotype-phenotype correlations. COL6A genes show a great allelic heterogeneity, both in term of pathogenic mutations and frequent polymorphisms. As a national referral center, in 10 years (2006-2016) we have analyzed by Sanger Sequencing a total of 221 families with UCMD/BM/intermediate phenotypes and a causative mutation was identified in 141 affected subjects (DR of 64%). The mutations were distributed equally in the three genes, and were mainly represented by missense changes (60%), followed by mutations affecting splicing (25%). Some recurrent missense mutations were identified, each occurring in more than 5 subjects within the cohort. The same mutation was found to associate to the entire phenotypic spectrum, from mild BM to UCMD. We collected and compared the frequency and type of intragenic SNPs in the three genes among phenotypically discordant patients carrying the same pathogenic change. Preliminary results from

this analysis suggest differences in SNPs number and representation. We recently set up a gene-specific TSCA Low Input NGS panel, based on an Illumina sequencing technology, composed of 188 amplicons covering the 100% of the three COL6 genes coding region. We are applying this novel tool in order to expand genotypic analysis and more rapidly collect data from the full sequencing of the three COL6A genes.

Next-generation sequencing approach for the diagnosis of genetic basis of hyperckemia: results from 25 patients

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Elevations in serum creatine kinase (CK) are most commonly associated with several genetically well-defined myopathies. Next-generation sequencing (NGS) has recently been proposed as a cost-effective strategy for the molecular diagnosis of genetically heterogeneous disorders such as inherited neuromuscular disorders. We questioned if NGS might help in the early detection of the molecular basis of repeatedly elevated serum CK levels. We evaluated 25 patients (aged 6 to 62) presenting with hyperckemia with or without muscle weakness at first examination. Serum CK levels ranged from 400 U/l to 16000 U/l. Most patients were sporadic, while familial recurrence was found in three patients, including one with a possible autosomal recessive form and two with an autosomal dominant condition. Nine patients presented asymptomatic hyperckemia, and three subjects referred exercise intolerance and myalgia. Neurological examination revealed limb-girdle muscle weakness in 13 patients. We tested for mutations in the coding regions and flanking intronic sequences of 78 genes involved in limb-girdle muscular dystrophies, distal myopathies and metabolic myopathies examined in a multiple gene panel designed with SureSelect technology (Agilent, Santa Clara, CA). Ten cases (40%) showed known disease-associated variants, or variants of a likely pathogenicity significance, including four who harbored heterozygous mutations in *RYR1*, and two patients carrying biallelic mutations in *ANO5*. Variants of uncertain pathogenic definition were identified in nine cases (36%) whereas six cases (24%) resulted negative for the presence of causative variants. Our study confirms the importance of NGS screening as a powerful tool in the diagnostic workflow for inherited neuromuscular disorders.

Growth differentiation factor 15 as a useful biomarker for mitochondrial disorder

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Growth differentiation factor 15 (GDF-15) is a member of transforming growth factor beta superfamily. Expression of GDF-15 in almost all tissues suggests the general importance in cellular functions. GDF-15 has been proposed as a biomarker for heart

disease, kidney disease, and cancer; however, its exact biological functions remain poorly understood. Recent studies have proposed GDF-15 as a biomarker of mitochondrial diseases (MID). The main objective of this study is to confirm the diagnostic efficacy of serum dosage of GDF-15 in patients with MIDs and compare the diagnostic utility with a differentiated growth factor-21 (FGF-21) results in the same MID adult cohort.

We used ELISA (Enzyme-Linked Immunosorbent Assay) to analyze GDF-15 and FGF-21 serum concentration in patients recruited in our neuromuscular center since 2012, included in 4 groups: MIDs without muscles involvement, mitochondrial myopathies (MMs), nonmitochondrial neuromuscular disease, healthy controls.

GDF-15 serum concentration is significantly elevated ($p < 0.0001$) in patients with MM with respect to the other groups. This study confirms serum GDF-15 levels as a more reliable biomarker of muscle-related MIDs, showing a higher sensitivity and specificity of classical serum markers (creatinine kinase, lactate, pyruvate, lactate / pyruvate ratio) and FGF-21. Our data suggest that GDF-15 is generally indicative of MIDs inherited independently from the clinical phenotype, while FGF-21 appears to be more indicative of MIDs when muscles manifestations are present.

The importance of a non-invasive screening in proximal miopathies

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The Late-Onset Pompe Disease (LOPD) is caused by compound mutations of the acid alpha-glucosidase gene (GAA) which lead to deficiency of GAA enzyme activity and accumulation of glycogen within autophagic vacuoles. LOPD affects primarily proximal muscle, as in a limb-girdle muscular dystrophy and polymyositis.

We describe unusual cases of three patients presenting hyperCKemia, proximal muscle weakness, autoimmunity nonspecific indices slightly above the reference range, that received inflammatory miopathy diagnosis. So they underwent to steroid and immunosuppressor therapy, that worsened the clinical picture. Therapeutic failure imposed clinical and instrumental reevaluation.

EMG extended to paraspinal muscles revealed a myopathic pattern with *complex repetitive discharges*. Lymphocytes on blood smears disclosed PAS positive glycogen granula in their cytoplasm. A new muscle biopsy revealed a myopathy pattern characterized by consistent glycogen storage at intermyofibrillary and sub-sarcolemmal levels and numerous PAS and acid phosphatase positive vacuoles in many fibres. In suspect of LOPD, patients also performed an ANGIO-MRI that disclosed dolicho-ectasis of basilar trunk, and Dried Blood Spot (DBS) that revealed reduced GAA enzyme activity. Genetic investigations confirmed LOPD. **Conclusions.** Proximal muscle weakness and hyperCKemia should be screened at first, with rapid and non-invasive methods like peripheral blood smear and DBS to avoid, despite a rise in aspecific inflammatory markers, immunomodulatory therapies able to worsen, potentially, the clinical features of a metabolic myopathy.

Three dimensional gait analysis in Late Onset Pompe Disease (LOPD)

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Due to the strong clinical impact of skeletal muscle dysfunction in LOPD and to the high variability among different patients, we analyzed gait pattern in a population of 10 patients. We performed gait analysis by BTS Smart DX-700, a stereophotogrammetric and optoelectronic system with eight infrared cameras, four force digital platforms and an eight wireless channel system for surface EMG. To the best of our knowledge, this is the first study of gait pattern in LOPD by 3D dynamic and kinematic analysis, while a single study is reported on temporospatial parameters only, acquired by GAITrite system. Temporospatial evaluation demonstrated increased time in support and in stance phase, reduction of swing phase, average speed and cadence, consistently with the previous report. Three dimensional analysis revealed increased abduction of hip (kinematic analysis), and abnormalities in ankle dorsiflexion/rotation, in knee moment and in ankle generated power (kinetic analysis). The entity of this abnormalities well correlated with the severity of the gait impairment and could be detected even in subclinical manifestations. Moreover, the contemporary assessment of EMG in various muscle groups showed, differently from normal people, persistent activation of rectus femoris and biceps femoris during the whole gait cycle, maybe related to compensatory mechanisms. A standardized characterization of gait in LOPD could represent a useful "patient-targeted" tool in monitoring disease progression and response to treatment and, given the significant variability among different patients, in working out personalized neurorehabilitation programs and/or orthotic interventions.

Non invasive ventilation in DM1: evaluation of compliance in a cohort of patients followed at the Nemo Center Milan

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Myotonic dystrophy type 1 (DM1) is a hereditary autosomal dominant disorder characterized by muscle dysfunction and involvement of several other systems. The main cause of death is cardiopulmonary failure. Non-invasive ventilation (NIV) can improve symptoms of chronic respiratory failure, but effectiveness of NIV highly depends on compliance.

The aim of this study was to determine NIV compliance in our DM1 patients based on ventilator SD card download analysis.

Data from 16 patients with DM1 were analyzed in order to determine the daily time of ventilation (VD) and the percent of use (%use, days of use/total days). Ventilator card data were downloaded every 6 months, for a total of 3 measurements. Clinically, efficacy was considered if NIV use was > 240 minutes/day.

There was no improvement over time in neither median %

use nor median VD ($p = 0.7263$ and $p = 0.3800$, respectively), these ranging from 20.3% to 25.45% for % use, and from 26 to 44 minutes/day for VD.

Both % use and VD underline that compliance with NIV is very poor. This suggests that respiratory DM1 dedicated education pathways of care, adequate psychological support to patients and families and closer follow-up may be of help to improve compliance to NIV in DM1. Ongoing studies on telemonitoring of NIV at the NEMO Center, will provide additional data on NIV usage and will help to identify non compliant patients early during the ventilatory support, and will possibly allow to address the causes of NIV failure.

Expanded [CCTG]_n repetitions are not associated with abnormal methylation at the CNBP locus in myotonic dystrophy type 2 (DM2) patients

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Myotonic Dystrophy type 2 (DM2) is a multisystemic disorder associated with an expanded [CCTG]_n repeat in intron 1 of *CNBP* gene. Epigenetic modifications have been reported in many repeat expansion disorders, including myotonic dystrophy type 1 (DM1), either as a mechanism to explain somatic repeat instability or transcriptional alterations in the disease genes. The purpose of our work was to determine the effect of DM2 mutation on the methylation status of *CNBP* gene. By MS-HRM, we characterized the methylation profile of three different CpG islands within these regions in whole blood and skeletal muscle tissues of DM2 patients ($n = 72$ and $n = 7$, respectively) and controls ($n = 50$ and $n = 7$, respectively). MS-HRM results have been further confirmed by direct sequencing of the MS-HRM products. Finally, relative mRNA transcript levels of *CNBP* gene were evaluated in leukocytes and in skeletal muscle tissues from controls ($n = 10$ and $n = 7$, respectively) and DM2 patients ($n = 16$ and $n = 7$, respectively) by qRT-PCR analysis. We found different pattern of CpG methylation in *CNBP* gene: CGs-1 located in the promoter region showed hypomethylation, whereas CGs-2 and CGs-3, flanking the CCTG array, are hypermethylated. Statistical analyses did not demonstrate any significant differences in the methylation profile between DM2 patients and controls in all tissues analyzed. Accordingly, with the methylation analysis, *CNBP* gene expression levels are not significantly altered in DM2 patients. These results show that [CCTG]_n repeat expansions do not influence the methylation status of the *CNBP* gene and suggest that other molecular mechanisms are involved in the pathogenesis of DM2.

Respiratory pattern in FSHD patients as possible outcome measure

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Finding a suitable outcome measure to evaluate the disease progression is the challenging aim for all muscular dystrophies. In particular, it is crucial for facioscapulohumeral muscular dystrophy (FSHD) due to the extreme phenotypic variability and the slow progression. The widely used FSHD clinical score is a clinical evaluation of the most involved districts but it is not so sensitive to pick changes over a one year time frame. In FSHD, the respiratory muscles are not primarily affected and in particular diaphragm is spared whereas the abdominal muscles represent one of the disease target. Nevertheless, it is already been reported that expiratory muscle function is weaker than inspiratory muscle function due to abdominal weakness. Aim of this prospective study is to measure the maximum inspiratory and expiratory pressures (MIP and MEP) and other lung functional test values in a cohort of 45 FSHD patients (M:F = 2:1) at different disease stage (61% with Beever sign) in order to establish a correlation with the FSHD clinical score ($R = 0.41$; $p = 0.006$). Inclusion criteria are: length of D4Z4 fragment ≤ 38 KDa, FSHD score between 0 and 15, category subtypes A, age between 18-80. Exclusion criteria are: pneumothorax, primary cardiac or respiratory diseases, recent (< 1 year) abdominal surgery and severe scoliosis. This study will further consider a one year follow up to establish if the MIP and MEP measurements are more sensitive than FSHD scale to pick disease progression. It will be proposed also as collaborative study for other neuromuscular centers in Italy.

BAG3 mutation: from cardiomyopathy to a complex severe neuromuscular disorder with myofibrillar myopathy in a pediatric case

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Myofibrillar myopathies are an heterogeneous group of neuromuscular diseases characterized by degeneration of Z discs, followed by the disintegration of myofibrils with ectopic accumulation of various proteins. They may be caused by mutations in different genes, among these, the BAG 3 gene (Bcl-2 associated-athanogene-3), more expressed in heart and skeletal muscle, encodes a multidomain protein that playing an important role in many cellular processes. We report a case of a 12 year old patient with myofibrillar myopathy (MFM) caused by BAG3 mutation.

Symptoms started at 4 years with hypertrophic obstructive cardiomyopathy. At the age of 8 there was a progression of cardiomyopathy, a moderate CPK rise and gait abnormalities.

Neurological and neuromuscular examination showed elbows, ankle and spine contractures, pes cavus, absent osteotendinous reflexes in upper and lower limbs, mild pelvic girdle weakness and, more recently, restrictive respiratory failure requiring non invasive ventilation. The boy is progressively worsening because of severe respiratory and cardiac involvement. Diagnostic investigations showed peripheral sensory motor axonal neuropathy and normal brain MRI. Muscle biopsy showed MFM with desmin, aB-cristallin and myotilin deposits and acid phosphatase positive vacuoles. Genetic investigation found out a *de novo* heterozygous mutation of BAG 3 c.626C > T (p.Pro209Leu) on exon 3 on chromosome 10. Mutations in the BAG3 gene are very rare, in most cases *de novo*, and cause a severe condition with rapidly progressive muscle, nerve, respiratory and heart involvement, with poor prognosis usually within the second to third decade of life. An early diagnosis is critical both for prognostic implications and for cardiomyopathy and restrictive respiratory failure treatment.

International-DMD (IDMD): a PTC therapeutics-supported diagnostic project to widely identify dystrophin mutations by NGS technologies

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Extensive molecular diagnosis in genetic diseases is vital to confirm clinical diagnosis and to enable genetic counseling and personalized management. Duchenne muscular dystrophy (DMD) is a rare genetic neuromuscular disease affecting 1/5000 males, due to a variety of dystrophin gene mutations. The first signs and symptoms of DMD include delayed milestones such as walking and talking, and enlarged calves. PTC Therapeutics International Ltd. and the University of Ferrara, Italy, have established a collaboration focused on identifying patients affected by rare genetic disorders through increased genetic testing activities, with an initial focus on DMD. Genetic testing is available to patients throughout European countries, potentially expanding to other regions.

Diagnostic settings include MLPA (MRC Holland) and NGS dystrophin gene sequencing (Multiplicom).

Currently DNAs from 57 DMD boys were collected. Patients were from Poland (34), Hungary (10), Lithuania (5), Romania (3), Russia (1) and Bosnia (4). Among the 30 samples analyzed, 7 deletions, 4 duplications, 11 small mutations (8 nonsense) were identified.

The early identification of the underlying genetic mutation is critical to potentially affecting the course of a disease such as DMD as well as the choice of treatment and aids in the setup of appropriate and effective care and follow up as well as eligibility for clinical trials. Genetic counselling can also be offered to patients and families with important repercussions on reproductive choices and lifestyle planning (full details and contacts at www.ospfe.it/medicalgenetics).

Spinal muscular atrophy type 2 and 3: evaluation of autonomic nervous system function

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Spinal muscular atrophy (SMA) is a selective lower motor neuron disease due to reduced level of Survival Motor Neuron protein, which is ubiquitously expressed. There are emerging data indicating the involvement of additional organs in patients with the most severe type of SMA. Autonomic nervous system (ANS) involvement has been mainly reported in patients with SMA type 1, suggesting a correlation with clinical severity. We evaluated the possible ANS involvement in patients with a confirmed diagnosis of SMA type 2 and 3 in regular follow-up. Symptoms of autonomic dysfunction were assessed using the Composite Autonomic Symptom Scale (COMPASS 31) and specific autonomic tests. They comprise of: Head-up tilting (HUT), Valsalva manoeuvre, deep breathing and cold pressure tests and skin sympathetic reflex. Moreover, we tested the plasma and urinary levels of catecholamines and sudomotor dysfunction with the novel Sudoscan technique. Twenty patients were included, 10 type 2 and 10 type 3 SMA, with a broad age range and age- and sex-matched healthy controls. A significant number of patients (16/20) reported symptoms of ANS dysfunction, mainly with gastrointestinal involvement. Eleven patients were able to perform a complete HUT test, the other patients were evaluated on reaching the sitting position from supine due to severe lower limbs contractures. None experienced orthostatic intolerance symptoms during tilt test, although we showed high supine level of epinephrine at baseline and no significant rising of norepinephrine after tilt compared to controls. The plasmatic dosage of catecholamines on the 24-hour urine protein test did not confirm differences with controls. Valsalva manoeuvre, deep breathing and cold pressure tests, skin sympathetic reflex and Sudoscan test were normal. Our results suggest the novel hypothesis that the reported symptoms are due to an adrenergic hyperactivity and not to an ANS impairment. Further studies on larger cohorts are needed to confirm these interesting results.

Clinical variability in myotonic dystrophy type 1: a five-categories disease classification fits clinical but not brain complexity

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Myotonic dystrophy type 1 (DM1), is an autosomal dominant multisystem disorder, caused by an unstable cytosine-thymine-guanine (CTG) triplet repeat expansions: although patients can be subclassified on this basis, the clinical presentation is characterized by high phenotypical variability. In literature (De Antonio et al. 2016), it has been proposed to classify this complexity into five main clinical categories (congenital, infantile,-

juvenile, adult-onset, late-onset forms), according to age at onset, but these classification is still under debate. We enrolled 73 patients affected by DM1 and included in the Italian Registry of myotonic dystrophies project. Each patient was evaluated on the basis of a comprehensive clinical protocol. Considering the high burden of brain involvement on overall health status, a subgroup of 31 patients with cognitive impairment underwent brain MRI imaging. Our sample (N = 73) was subgrouped in the abovementioned 5 clinical categories with large majority of adult-onset cases (N = 30, 41.1%). Motor impairment assessment revealed high prevalence of cases with moderate disability (MIRS between 2 and 3, 74% cases). Multisystem involvement was characterized mainly by cardiac, respiratory and central nervous system involvement with 36.9% of patients carrying cardiac conduction defects, 38.4% practicing mechanical ventilation (VMNI) and 46.4% showing cognitive impairment. MRI imaging evaluation failed to reveal distinctive features of the brain for each clinical form, thus strengthening the statement that multisystem and brain involvement do not fit the same disease severity time-elapsing trajectory in DM1. The achievement of peculiar phenotypic features in each clinical form may be a key step for better comprehension of DM1 natural history as well as for patient stratification readiness for therapeutic trials.

Epidemiology of facioscapulohumeral muscular dystrophy in Abruzzo

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Facioscapulohumeral muscular dystrophy (FSHD), the third most common muscular dystrophy, is an autosomal dominant disease with an insidious onset and a progressive wasting of a highly selective set of muscle groups. The chronology of disease progression is unpredictable and there is a wide variability in clinical spectrum among patients. Clinical severity of FSHD can be evaluated by the FSHD score, a validated tool to define its clinical expression. The estimated prevalence of the disease is 1:20,000 in the general population. The aim of our study is to evaluate FSHD prevalence in our region.

We reviewed clinical data of FSHD patients afferent to the Neuromuscular Center of Chieti, the only reference for the entire region, in the last 8 years. We evaluated patients by using the FSHD score and included those with a score > 3. In order to calculate prevalence we used ISTAT data on Abruzzo population.

By March 2017, 75 patients were diagnosed by FSHD in our center. On an estimated population of 1322760 people in Abruzzo, we report a disease prevalence of 1:17636.

Our data suggest a prevalence of FSHD higher than previously reported. The current calculated prevalence probably underestimates real impact of the disease. New epidemiological studies about FSHD are needed.

Next-generation sequencing analysis for the diagnosis of Duchenne/Becker muscular dystrophies

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Duchenne/Becker muscular dystrophies are the most fre-

quent inherited neuromuscular diseases caused by mutations in dystrophin gene. Molecular diagnosis is difficult because of the complex mutational spectrum and the large size of the gene (79 exons). Next-generation sequencing (NGS) is a suitable method for the detection of various types of mutations in the dystrophin gene. Genomic DNAs were analysed by NGS technology using the DMD MASTR assay (Multiplicom), covering the coding region of the DMD gene. Samples were run on MiSeq (Illumina) and data analysed using Sophia Genetics pipeline. All variants detected were confirmed by Sanger sequencing. Multiplicom Kit includes control amplicons for detection of copy number variations in 4 multiplex PCR reactions, that unfortunately does not work as well as expected. So we perform the multiplex ligation-dependent probe amplification (MLPA) analysis to detect DMD gene duplications/deletions and only the negative samples are screened by NGS technology. NGS analysis was performed in 57 patients: 15 females and 42 males. We identified small mutations in 4 females and 25 males: 17 nonsense, 7 frame shift, 2 splicing, 3 missense. The sequencing data covered almost 100% of the exonic region of the dystrophin gene by ≥ 50 reads with a mean read depth of 150. In conclusion, NGS technology may offer several advantages over Sanger sequencing technology: higher throughput, faster turnaround time, small amounts of starting material and low cost introducing a method for the genetic analysis of DMD patients and female carriers.

Clinical and molecular consequences of exon 78 deletion in DMD gene

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We present a 13 years old patient who came to our unit for persistent increase of serum CK (from 285 to 2465 U/L), associated with myalgia after exertion. The patient played agonist boating, and cardiological visit with ECG never detected alterations. Neurological examination revealed only mild hypotrophy of shoulder girdle and pectoral muscles. Skeletal muscle biopsy showed slight fiber size variation, absence of degeneration/necrosis, and minimal increase of connective tissue. When tested by Immunofluorescence on muscle sections, DYS-COOH terminus expression was markedly reduced, whereas other DGC-complex proteins, including beta-dystroglycan, showed normal staining. Genetic analysis by Multiplex-Ligation-dependent-Probe-Amplification detected a single deletion of exon 78 in DMD gene. This mutation was also found in heterozygous in his mother and older sister. Since exon 78 deletion was never described in literature but, according to prediction, should lead to loss of reading frame in the dystrophin gene, we analysed cDNA from muscle mRNA. This analysis confirmed the absence of 78 fragment and a bioinformatic study in ExPASy showed the loss of the correct aminoacid sequence of the very last part of the protein. Albeit loss of reading frame usually lead to protein degradation and severe phenotype, in this case we demonstrated that deletion of DMD exon 78 can be associated with a functional protein able to binding DGC-complex and a very mild phenotype. This study adds a novel deletion in DMD gene in human and

helps to define the compliance between maintaining/disrupting the reading frame and clinical form of the disease.

Evidence of mitochondrial dysfunction delays the diagnosis of myotonic dystrophy type 2

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Myotonic Dystrophy type 2 (DM2) is an autosomal dominant disorder clinically characterized by a heterogeneous phenotype ranging from multisystem involvement to asymptomatic individuals. We observed a 73-year-old woman with a positive family history for asymptomatic hyperCKemia and muscle weakness, respectively, in her son and in a maternal aunt. She suffered of a long term history of cervico brachial and oro mandibular dystonia and, at 72y, complained proximal muscle weakness. EMG disclosed myopathic signs, ECG a right bundle branch block and audiometry a neurosensorial hearing loss. Cerebral proton MR spectroscopy revealed the presence of lactate and neurodegenerative changes of cortex, basal ganglia and cerebellum. Her 50-year-old son showed a negative neurological examination but EMG revealed multisegmental denervation signs. Both patients showed lactic acidosis after aerobic effort, a muscular deficit of ATP synthesis at 31P- MR spectroscopy and myopathic changes with COX negative fibers at the muscle biopsy. Multiple mitochondrial DNA (mtDNA) deletions were evident in the muscle but extensive examination of mitochondrial nuclear genes by NGS was negative, as well as the full mtDNA sequence. The presence of type II atrophic fibers and a significant increase of central nuclei in their biopsies redirected the genetic analyses toward DM2 and a (CCTG)n expansion in CNBP gene was identified in both patients. The evidence of mitochondrial dysfunction in this family suggests a possible role of mitochondria in the pathogenesis of DM2. Further studies are in progress.

Multi-parametric characterization of highly fat infiltrated limb girdle muscular dystrophy patients: results of a multi-variate analysis

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Limb Girdle Muscular Dystrophy (LGMD) is a heterogeneous group of rare diseases characterized by progressive muscle wasting. Previous studies underlined the effects of fat on diffusion MRI (dMRI) parameters, and suggested to discard voxels with high fat infiltration. However, such approach is difficult to pursue in highly fat infiltrated patients. In the context of a multi-parametric MRI study that includes Dixon, T₂ quantification and dMRI sequences, we aimed to address the effect of fat infiltration in LGMDs.

11 healthy controls (HC, 6 males, 44 ± 10 years), 7 LGM-

D2A (Calpain-3 deficit, 2 males, 39 ± 7 years) and 7 LGMD2B (Dysferlin deficit, 3 males, 47 ± 7 years) patients underwent 3T MRI of the thigh. Regions of interest (ROIs) of the posterior muscles, anterior muscles, gracilis and sartorius were manually delineated, and then median values of the parametric maps were computed for each ROI. MRI metrics were individually corrected for fat effect; boxplots of the corrected MRI metrics were computed and differences among ROIs and patient groups tested. Finally, the correlations between corrected MRI parameters and clinical measures were evaluated.

Volumetric analysis of ROIs shows significant reduction of muscle volume in both patient groups compared to controls. Fat fraction (FF) is the most effective measure to split the groups. Fat fraction inversely correlates with muscular strength in LGMD2A but not in LGMD2B, particularly in the adductor muscles.

FF correlates with muscular strength in LGMD2A but not in LGMD2B. Further characterization of this approach should be pursued in future studies.

A diagnostic anoctamin-5 western blot

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Detection of ANO5 protein has been complicated by un-specific antibodies, most of which have not identified the correct protein. A sample preparation method compatible with membrane proteins, combined with tissue fractionation was used to determine ANO5 expression in cell cultures expressing ANO5, in normal muscles and eight patient biopsies with six different ANO5 mutations in homozygous or compound heterozygous states, and in other dystrophies. A specific monoclonal N-terminal ANO5 antibody was efficient in detecting the protein, showing that ANO5 is expressed as a single 107 kD polypeptide in human skeletal muscle. The truncating mutations c.191dupA and c.1261C > T were found to abolish ANO5 expression. All the other studied ANO5 mutations resulted in clearly reduced ANO5 expression in the patient muscle membrane fraction. ANO5 protein expression is decreased in ANO5-mutated muscular dystrophy and most of the non-truncating pathogenic ANO5 mutations likely destabilize the protein and cause its degradation. The method described here allows direct analysis of human ANO5 protein, which can be used in diagnostics, for evaluating the pathogenicity of the potentially harmful ANO5 variants of uncertain significance. Moreover, a western blotting method to study ANO5 protein expression is a valuable second tier test to chase the presence of elusive variants in patients with a single heterozygous ANO5 mutation identified by a genetic analysis.

Statin-induced necrotizing autoimmune myopathy: clinical, histopathological and radiological characterization of five patients

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Statin associated myotoxicity can be classified into toxic and autoimmune forms. Statin induced necrotizing autoimmune myopathy is characterized by significant proximal muscle weakness, elevated serum creatine kinase levels and persistent symptoms despite statin discontinuation. This statin-induced myopathy improve with immunosuppressive treatment, suggesting an immune-mediated pathophysiological mechanism, related to anti 3-hydroxy-3-methylglutaryl-coenzyme A reductase antibodies (anti HMGCR). We examined a cohort of five patients with statin exposure who developed a necrotizing autoimmune myopathy associated with HMGCR antibodies. Herein we present their clinical, serological, histopathological, radiological characterization and immunosuppressive therapy used. Prednisone monotherapy was insufficient to control the disease and all patients required treatment with combined immunosuppressive regimen to achieve clinical and radiological remission. We think that muscle biopsy should be performed in all statin-exposed patients with both muscle symptoms and CK elevation that do not resolve with discontinuation of the drugs. Furthermore, diagnostic confirmation required a positive anti HMGCR antibodies test. Early identification of the diagnosis, aggressive immunotherapy and close clinical monitoring may improve the long-term outcome of these patients.

Congenital muscular dystrophy and epilepsy: a prospective observational study on 16 pediatric patients

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Congenital Muscular Dystrophies (CMDs) can be considered a heterogeneous group of diseases characterized by marked weakness, generalized hypotonia, joint contractures. They are divided into pure and classical forms, without eye and cerebral involvement and complex forms, which are usually associated with cerebral abnormalities. Seizures have rarely been described in the pure forms while seem to be more frequent in those complex.

The aim of our study was to evaluate the incidence of seizure in patients with CMD.

Herein authors describe 16 cases of Congenital Muscular Dystrophy (CMD) associated with different kind of epileptic events, in order to study the pathogenic connection between the two clinical manifestations. In all described patients we have reviewed clinical, neurophysiologic, and neuroimaging data to determine any association with epilepsy. The patients were di-

vided in two groups: the first one included 14 cases with merosin-positive CMD and the second included 2 patient with WWS.

In our merosin-positive CMD patients we found the following epileptic patterns: 1 benign myoclonic epilepsy (BME), 1 benign febrile convulsions and in one patient without seizures, the EEG study revealed a moderately high voltage slow background with diffuse sharp waves, reaching 300mV in amplitude; these were not associated with clinical signs.

In our merosin-positive CMD patients (first group) the presence of two different epileptic diseases, BME in one and ESE, may represent a new expression of merosin-positive CMD (PCMD) in which the deficiency of an undiscovered muscular protein with a cerebral isoform may be the cause of epileptic events.

Expression of Aquaporin 4 in normal human muscle is independent from myosin heavy chain isoform

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Aquaporin 4 (AQP4) is member of a family of proteins that allow trans-cellular and trans-epithelial water and glycerol movement and appear involved in maintenance of osmotic gradients. The AQP4 is abundant in the plasma membrane of glial cells; it is also expressed in skeletal muscle, mostly at the sarcolemma. Its expression pattern in normal muscle has been explored only in laboratory animals and is reportedly selective of fast-twitch fibers. A similar distribution has been inferred for humans, although specific studies are lacking. The physiological role of AQP4 in skeletal muscle is unknown. In neuromyelitis optica, AQP4 may be targeted by specific autoantibodies, not only in glial cells but also in myofibers, resulting in sarcolemmal damage and hyperCKemia. Aim of this work was to ascertain whether the expression of AQP4 in human muscle is fiber type-specific or not. We performed, therefore, a double immunofluorescence study of AQP4 expression in parallel with slow and fast myosin heavy chain (MHC) expression. Six diagnostic biopsies from two different muscles (deltoid and vastus) and from patients of both sexes with no overt histopathological alterations were analyzed. The pattern of distribution of AQP4 among fast-twitch fibers was highly consistent in all biopsies, with positivity ranging between 64% and 99%. However, AQP4 expression was also present in slow-twitch fibers, although with a high variability, ranging between 5% and 72% in different samples. These data demonstrate that AQP4 is present in the cell membrane of normal human myofibers irrespective of their type, as determined by MHC isoform expression.

ABSTRACTS OF STUDY GROUPS

(in alphabetical order of the first Author)

Muscle pain in mitochondrial diseases: a survey from the large cohort of the Italian Network

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Based on the large database of the "Nation-wide Italian Collaborative Network of Mitochondrial Diseases", we reviewed the clinical data of 1398 patients with special regard to muscle pain. Muscle pain was present in 164 patients (11,7%) being one of the ten most common signs and symptoms. To date, we have collected data from 43 out of these patients. Twenty two patients (51,1%) present pure mitochondrial myopathy, 11 (25,6%) cPEO, 3 (7%) MELAS syndrome, 2 (4,4%) Leigh syndrome, 1 (2,2%) Kearns Sayre syndrome while 4 patients (8,8%) have different non specific phenotypes. Twenty seven out of 43 patients (60,5%) have a molecular diagnosis. The majority of them (81%) harbored a mtDNA change while only 19% has nuclear DNA changes. Twenty three patients (54,6%) complained of diffuse muscle pain, while 20 (45,4%) of focal muscle pain (lower limbs, upper limbs or muscle trunk). Sixteen patients (37,2%) have myalgia only at rest, 10 patients (23,2%) during and/or after exercise and the remaining 17 patients (39,5%) both at rest and exercise-related. Interestingly, only 7 patients (16,3%) showed a satisfactory response following drug therapy. Forty two per cent of them present muscle pain at rest, 42% both at rest and exercise-related myalgia and 14% only exercise-related myalgia. Patients with nDNA mutations tend to have a better response than patients with mtDNA mutations

($p < 0.05$). In conclusion, from these preliminary results, patients complaining of myalgia result to have mainly muscle pain at rest, mutations in mitochondrial DNA and poor response to specific therapy (better in the nDNA mutated patients). An analysis on a larger number of patients is mandatory.

The nation-wide italian collaborative network of mitochondrial diseases: from 2009 today

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Mitochondrial disorders (MD) are among the most common genetic disorders. While enormous progress has been made in diagnostics and elucidation of pathomechanisms in MD, major advances in treatment have unfortunately not paralleled this so far, limited by the small patient populations. Supported by Telethon (GUP09004), eleven Italian center with specific expertise developed an Italian network and a web-based register of mitochondrial patients, to better understand the phenotypes and the natural history of these diseases. From 2009 to date, we have collected 1478 patients, with both adulthood and childhood onset, harmonizing the database with other European databases and networks. We performed two type of approach, both phenotype-based, for example demonstrating that exercise intolerance was more strongly associated with specific mutations (i.e., 3243A > G) and that CK levels were increased in $\approx 34\%$ of the patients, not confirming the notion that CK are normal in mitochondrial patients, but also genotype-based approach showing for instance that MERRF syndrome caused by 8344A > G mutation could be better defined as a myoclonic ataxia rather than a myoclonic epilepsy. Besides a low prevalence, also the lack of shared outcome measures, of useful biomarkers and the incomplete understanding of the natural history represent other major impediment, limiting the "clinical readiness" for the de-

velopment of forthcoming clinical trials. We are going to start a project for these proposal in Primary Mitochondrial Myopathies of the adulthood (Telethon GSP16001), with specific aims: • Development and validation of shared functional outcome measures; • evaluation of two promising biomarkers in vivo (FGF21 and GDF15); • characterization of the natural history of selected PMM. These steps are fundamental to monitor in vivo the evolution of the disease, and, finally, to have a good armamentarium for future clinical trials.

CARE-DMD, a longitudinal study on DMD care: energy expenditure and multidisciplinary involvement at transitional age and towards adult life in DMD

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Collaborative study held by Centres of the Italian DMD Network to be further defined

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Increased survival in Duchenne Muscular Dystrophy (DMD) is due to an improvement in multidisciplinary care and has led to an increased incidence of life-threatening cardiac events in adult life. Reducing energy expenditure has been suggested as a central and crucial point of standards of care to prolong survival. The international community feels the burning need to empower this area of expertise. CARE-DMD is an observational collaborative study held by the Centers of Italian DMD network following patients towards adult age.

Aims: to ameliorate quality of life of people with DMD in transitional age and towards adult life by: (i) empowering the infrastructure of the Italian DMD network; (ii) harmonizing protocols and procedures in this age range; (iii) validating new technologies and protocols in the multidisciplinary management.

Methods: (i) select patients at transitional and adult age with confirmed diagnosis of DMD and harmonize the administration of the selected protocol among Centers; (ii) apply and validate new technologies to test energy expenditure (e.g. accelerometers) and correlate the outputs with nutritional and swallowing status at baseline and over a year follow-up. Ancillary studies will also correlate those with data on other aspects of the multidisciplinary care (e.g. surgical policies and procedures, cognitive, respiratory and cardiac rehabilitation protocols).

We expect to perform an observational study on around 200 individuals with DMD followed in Italian tertiary referral Centres, on the basis of feasibility; to finally improve multidisciplinary care and ameliorate quality of life of people with DMD in transitional age and towards adult life.

TREAT-CDM, an international observational longitudinal study on congenital myotonic dystrophy (CDM): a spin-off for the creation of the Italian CDM network

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Congenital Myotonic Dystrophy (CDM) is the most severe form of Myotonic Dystrophy type 1, mortality ranging from 20-40% in most series. Although very different from the adult type, drugs potentially designed to address the adult form of DM1 may become applicable for CDM. Trial readiness is limited by the lack of natural history data. TREAT-CDM is an observational collaborative study between the University of UTAH, London Ontario and the Nemo Center in Milan to define Trial Readiness and Endpoint Assessment in Congenital Myotonic Dystrophy.

Aims: To create the infrastructure for the Italian CDM network by: (i) including CDM children all over Italy in the TREAT-CDM protocol; (ii) harmonizing protocols and procedures for these patients; (iii) applying ancillary studies which are not covered by the TREAT-CDM protocol (eg nutrition, additional cognitive studies, swallowing studies etc); (iv) Validating tests and procedures which are part of the TREAT-CDM protocol and which are not yet validated in Italy.

Methods: (i) identify centers in Italy where CDM patients are studied and determine the number of potentially eligible patients; (ii) discuss and apply ancillary studies to be coordinated by local Italian centers for defined patient cohorts; (iii) apply validation procedures to specific scales (eg Congenital and Childhood Myotonic Dystrophy Health Index, CCMdHI).

We expect to enrol 30 CDM patients in Italy; to acquire data for natural history studies including nutrition, breathing, sleep etc; to improve care by harmonizing protocols and procedures; and to create the Italian framework for clinical research studies in CDM.

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NEWS FROM AROUND THE WORLD

AIM

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

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

GCA

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

MSM

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

WMS

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

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

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

FORTHCOMING MEETINGS

2017

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-\(EHRA\)](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.
Information: [website: http://www.apscardio.org/](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-Aug 01

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

August 26-30

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

August 31-September 1

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

September 5-9

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

September 13-15

Global Biobank Week. Towards harmony in biobanking. Stockholm, Sweden.
Information: website: <http://globalbiobankweek.org>

September 14-16

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

September 14-17

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

October 3-7

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

October 17-21

ASHG Annual Meeting. Orlando, Florida, USA.
Information: [website: www.ashg.org](http://www.ashg.org)

October 25-27

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

November 22-24

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

2018

June 28-30

XIII Congress of Mediterranean Society of Myology. Uçhisar - Cappadocia, Turkey.
Information: Haluk [Topaloglu htopalog@hacettepe.edu.tr](mailto:Topaloglu.htopalog@hacettepe.edu.tr)

August 25-29

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

For application or renewal to MSM

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APPLICATION/RENEWAL FORM

Application/Renewal for 1yr 2 yrs
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INSTRUCTIONS FOR AUTHORS

Acta Myologica publishes articles related to research in and the practice of primary myopathies, cardiomyopathies and neuromyopathies, including observational studies, clinical trials, epidemiology, health services and outcomes studies, and advances in applied (translational) and basic research.

Manuscripts are examined by the editorial staff and usually evaluated by expert reviewers assigned by the editors. Both clinical and basic articles will also be subject to statistical review, when appropriate. Provisional or final acceptance is based on originality, scientific content, and topical balance of the journal. Decisions are communicated by email, generally within eight weeks. All rebuttals must be submitted in writing to the editorial office.

On-line submission

Manuscript submission must be effected on line: **www.actamyologica.it** according to the following categories:

Original articles (maximum 5000 words, 8 figures or tables). A structured abstract of no more than 250 words should be included.

Reviews, Editorials (maximum 4000 words for Reviews and 1600 words for Editorials). These are usually commissioned by the Editors. Before spontaneously writing an Editorial or Review, it is advisable to contact the Editor to make sure that an article on the same or similar topic is not already in preparation.

Case Reports, Scientific Letters (maximum 1500 words, 10 references, 3 figures or tables, maximum 4 authors). A summary of 150 words may be included.

Letters to the Editor (maximum 600 words, 5 references). Letters commenting upon papers published in the journal during the previous year or concerning news in the myologic, cardio-myologic or neuro-myologic field, will be welcome. All Authors must sign the letter.

Rapid Reports (maximum 400 words, 5 references, 2 figures or tables). A letter should be included explaining why the author considers the paper justifies rapid processing.

Lectura. Invited formal discourse as a method of instruction. The structure will be suggested by the Editor.

Congress Proceedings either in the form of Selected Abstracts or Proceedings will be taken into consideration.

Information concerning new books, congresses and symposia, will be published if conforming to the policy of the Journal.

The manuscripts should be arranged as follows: 1) Title, authors, address institution, address for correspondence; 2) Repeat title, abstract, key words; 3) Text; 4) References; 5) Legends; 6) Figures or tables. Pages should be numbered (title page as page 1). **Title page.** Check that it represents the content of the paper and is not misleading. Also suggest a short running title.

Key words. Supply up to three key words. Wherever possible, use terms from Index Medicus – Medical Subject Headings.

Text. Only international SI units and symbols must be used in the text. Tables and figures should be cited in numerical order as first mentioned in the text. Patients must be identified by numbers not initials.

Illustrations. Figures should be sent in .jpeg or .tiff format. Legends should be typed double-spaced and numbered with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, each should be explained clearly in the legend. For photomicrographs, the internal scale markers should be defined and the methods of staining should be given.

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Books and other monographs: Dubowitz V. Muscle disorders in childhood. London: WB Saunders Company Ltd; 1978.

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