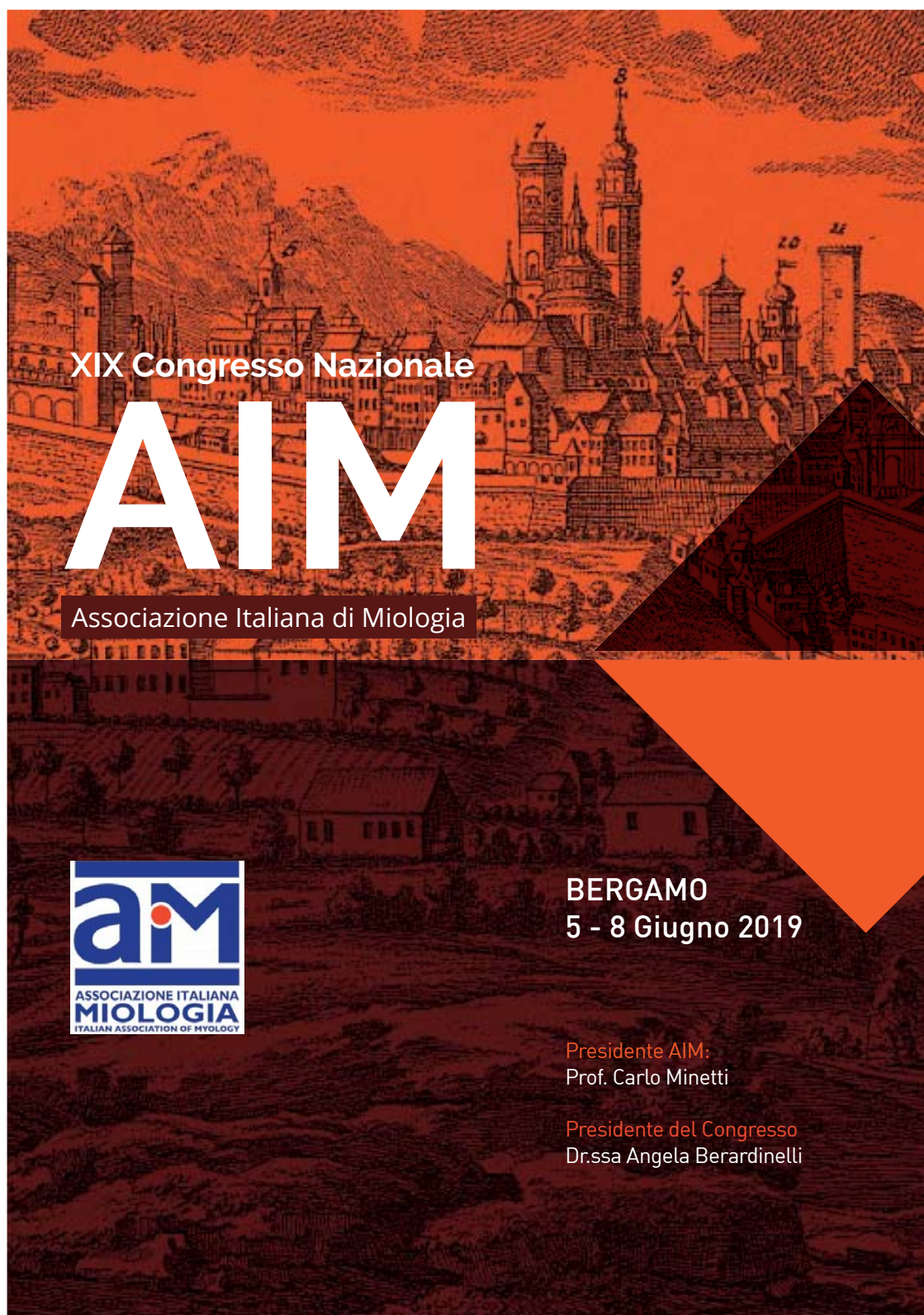


**PROCEEDINGS OF THE XIX CONGRESS
OF THE ITALIAN SOCIETY OF MYOLOGY**

Bergamo, Italy

June 05-08, 2019



XIX Congresso Nazionale

AIM

Associazione Italiana di Miologia

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5 - 8 GIUGNO 2019 BERGAMO

XIX CONGRESSO NAZIONALE
AIM Associazione Italiana di Miologia





XIX CONGRESSO NAZIONALE

AIM Associazione Italiana di Miologia



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Prof. Carlo Minetti

Presidente del Congresso

Dr.ssa Angela Berardinelli

Consiglio Direttivo

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BERGAMO 5 - 8 GIUGNO 2019

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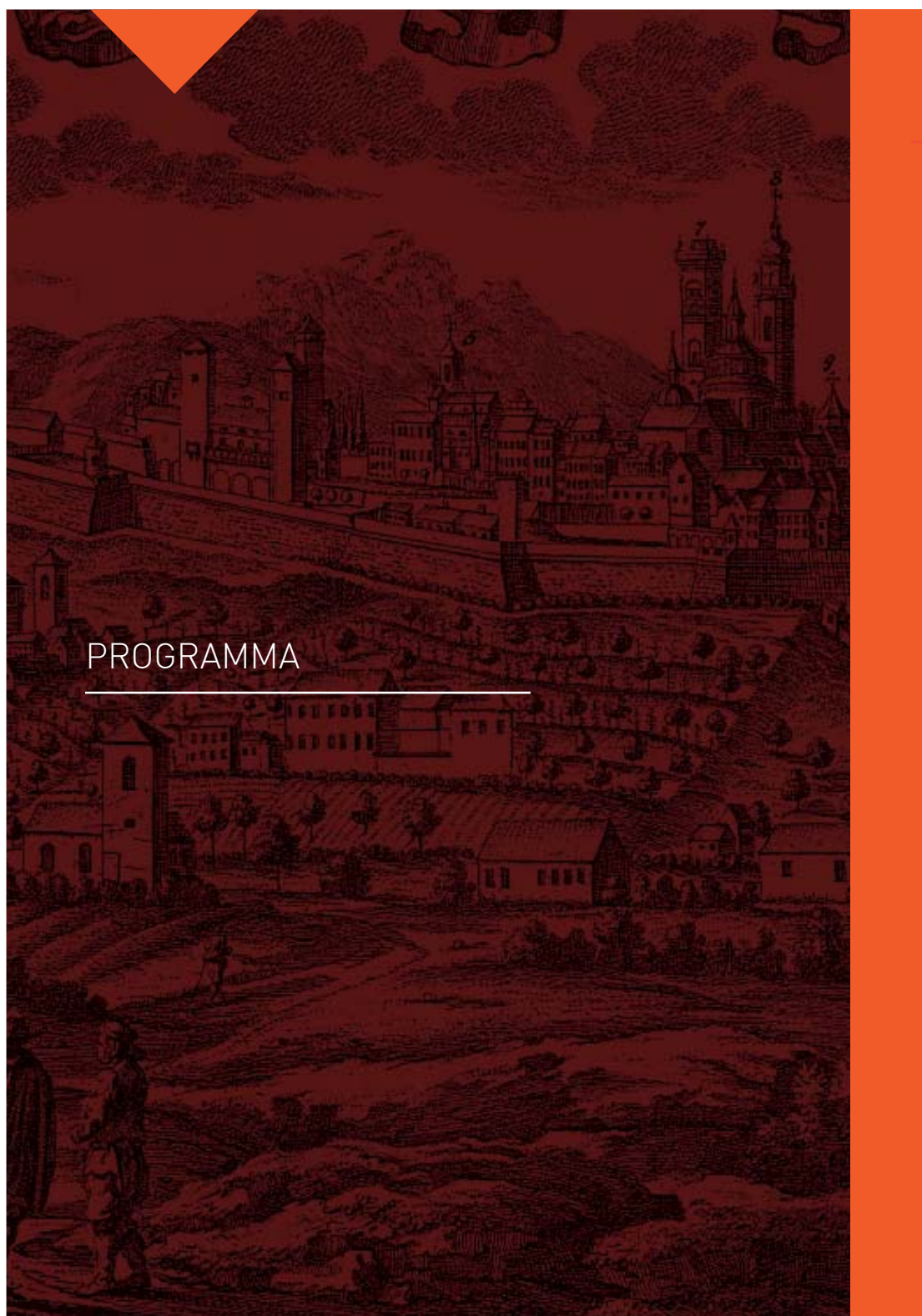
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 John Vissing, *Copenhagen*
 Giuseppe Vita, *Messina*
 Massimo Zeviani, *Cambridge, UK*





Physical exercise: pros and cons for taking care of myopathic patients



Pavia, 5 Giugno 2019

Simposio
satellite
del
XIX Congresso
Nazionale AIM



PROGRAMME	
08.30	Welcome: Dr.ssa A. Berardinelli, Prof. G. D'Antona Greetings from the Authorities Chairpersons: Prof. G. Siciliano, Prof. A. Toscano
09.00	<u>Physiological aspects translated to disease</u> Prof. G. D'Antona <i>Sport Medicine Centre Voghera, Italy University of Pavia</i>
09.15	<u>Pros:</u> Prof. N. Voet <i>Departments of Rehabilitation and Neurology, Donders Center for Neuroscience, Radboud University Medical Center, Nijmegen, Netherlands</i>
10.00	<u>Cons:</u> Prof. J. Vissing <i>Copenhagen Neuromuscular Center, Department of Neurology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark</i>
10.45	Coffee break

Chairpersons: **Dr.ssa A. Berardinelli, Prof. G. Vita**

11.00 Technical Issues

Prof. L. Féasson, Prof. G. Millet

University of Lyon - University Jean Monnet Saint-Etienne, Laboratoire Interuniversitaire de Biologie de la Motricité, France

11.45 Open issues and future perspectives

Prof. G. Siciliano

Departments of Neurology and Neurophysiopathology, Departments of Clinical and Experimental Medicine, University Hospital "Santa Chiara", University of Pisa

Prof. G. Vita

*Unit of Neurology and Neuromuscular Diseases
University Hospital "G. Martino", University of Messina*

12.15 **Discussion**

12.30 The experience from local associations
meeting local wheel chair-hockey team
at lunch-time

F. Pirastu

President UILDM Pavia





Presidente AIM
Prof. Carlo Minetti

Organizing Committee:

Prof. G. D'Antona

Department of Public Health, Experimental and Forensic Medicine and Sport Medicine Centre, Voghera - University of Pavia (Italy)

Dr.ssa A. Berardinelli

IRCCS Mondino Foundation, Pavia

Sede Congressuale:

Fondazione Mondino

Via G. Mondino, 2 - 27100 Pavia

Modalità di Iscrizione:

Per effettuare l'iscrizione si prega di inviare una email di richiesta alla Segreteria Organizzativa:

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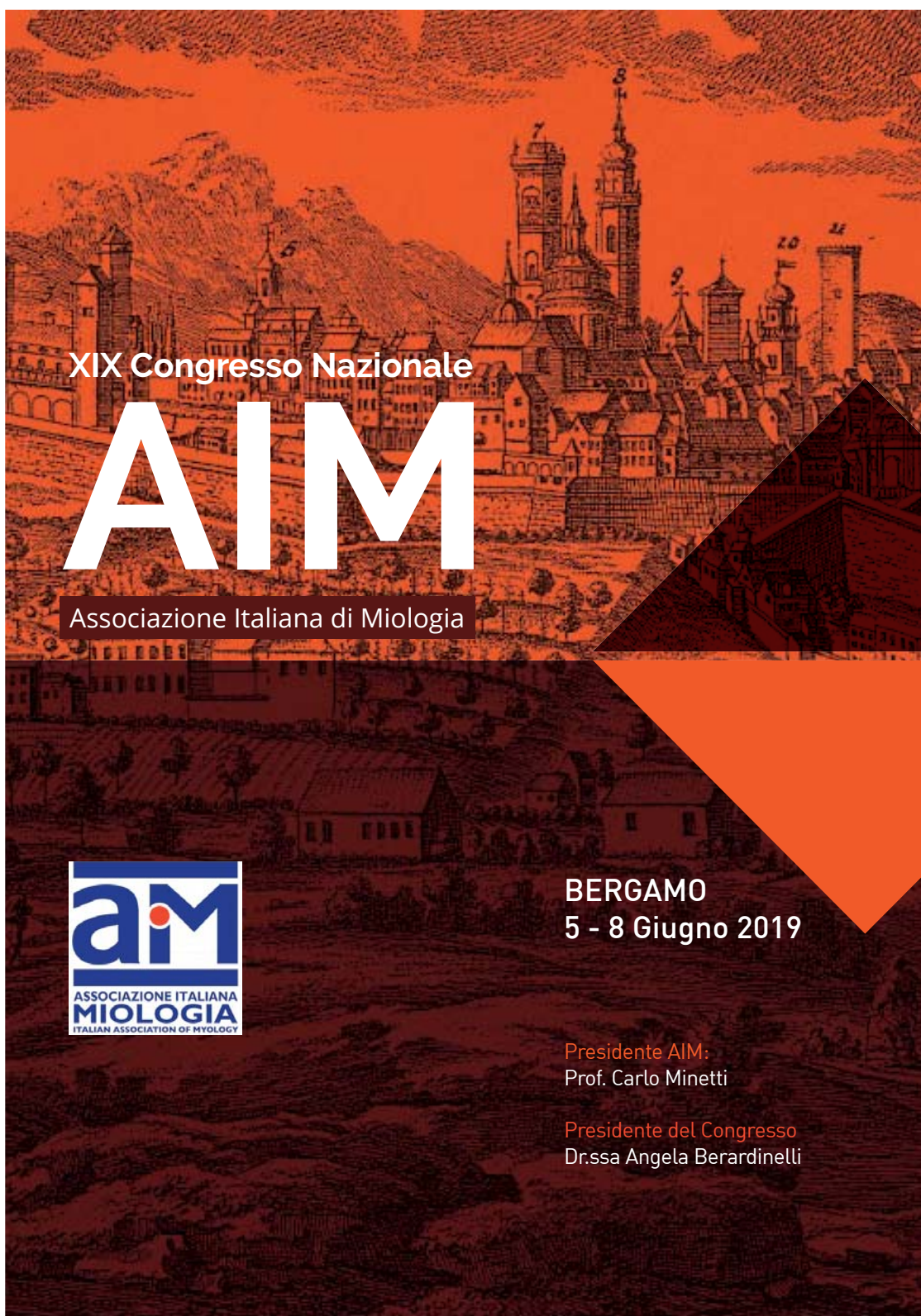
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MERCOLEDÌ 5 GIUGNO



15.00 - 15.30 | **Saluti autorità e inizio lavori** | *C. Minetti, A. Berardinelli*

15.30 - 16.50 | **Workshop 1. Treatment opportunities in DMD and SMA: what's on**
Chairs: C. Bruno, G. Siciliano

- Nusinersen open access: what did we learn? | *M. Pane*
- What else about SMA Therapy? An overview | *E. Mercuri*
- Pharmacological therapies in DMD: what's on? | *S. Messina*
- Italian Consensus on rehabilitation in MDs | *M. Lombardo*
- Gene therapy and iPS-derived molecular targets in SMA | *S. Corti*

16.50 - 17.10 | **Coffee Break**

17.10 - 18.40 | **Round Table: MEETING WITH PATIENTS ASSOCIATIONS:
Sustainability of the new therapies for
Neuromuscular Disorders(NMDs): which problems and which
possible strategies to overcome the problems?**
Chairs: L. Politano, A. Pini

- Innovative therapies in NMD:
(the cost and) the perspective of the hospital | *L. Tronconi*
- Possible synergies between scientific associations and lay associations | *C. Giacobini*
- The work of the Associations; examples: the association and its territory
*D. Lauro (Famiglie SMA), D. Bettani (UILDM di Bergamo), A. Ambrosini (Teletthon),
S. Mazzariol (Parent Project), F. Ieva (AltroDomani), more associations involved*
- Discussion

18.40 - 19.40 | **COCKTAIL SEMINAR:
Cardiac and respiratory involvement in NMD**
Chairs: A. Vianello, G. D'Angelo

- Heart involvement in NMDs: different patterns and new therapeutical approaches
L. Politano
- Update on respiratory treatments in NMDs | *A. Vianello*
- Sleep respiratory disorders in NMDs: new issues | *F. Fanfulla*
- Respiratory decline and pharmacological treatment in DMD | *A. Aliverti*

20.00 | **Cocktail di Benvenuto**

GIOVEDÌ 6 GIUGNO

**7.30 - 8.30 | BREAKFAST SEMINAR (non accreditato ECM)****The relevance of early diagnosis and proper management of SMA in the light of forthcoming treatments***Chairs: E. Mercuri, G. Comi*

- The relevance of early diagnosis. | *F.D. Tiziano*
- New protagonists in SMA scenario: reciprocal relevance of the clinical management, clinical trajectories and of the new therapeutic options in the approach to the “new” SMA patients | *E. Mercuri*
- Systemic involvement in spinal muscular atrophy: the evidence | *G. Comi*

8.30 - 9.30 | Oral Communications 1**Extramuscular manifestations and muscle Phenotypes in NMD: clinical and laboratory studies of cohort of patients***Chair: A. Ardisson*European muscle MRI study in Limb Girdle Muscular Dystrophy Type 2A (LGMD2A)

A. Barp, P. Laforet, L. Bello, G. Tasca, J. Vissing, M. Monforte, E. Ricci, A. Choumert, T. Stojkovic, E. Malfatti, E. Pegoraro, C. Semplicini, R. Stramare, O. Scheidegger, J. Haberlova, V. Straub, C. Marini Bettolo, N. Løkken, J. Diaz-Manera, J. A. Urtizberea, E. Mercuri, M. Kyncl, M. C. Walter, Robert Y Carlier
(Padova; Garches, France; Roma; Copenhagen, Denmark; La Réunion, France; Paris, France; Bern, Switzerland; Prague, Czech Republic; Newcastle Upon Tyne, United Kingdom; Barcelona, Spain; Hendaye, France; Prague, Czech Republic)

Motor performances in exon-2 duplication of the dystrophin gene

A.A. Zambon, A. Ferlini, E. Albamonte, G. Baranello, E. Bertini, A. D'Amico, G. D'Angelo, C. Fiorillo, S. Gandossini, F. Gualandi, F. Magri, S. Messina, M. Moggio, T. Mongini, M.G. Natali Sora, M. Pane, A. Pini, L. Politano, F. Ricci, V. Sansone, G.L. Vita, E. Mercuri, G. P. Comi, S.C. Previtali
(Milano; Ferrara; Roma; Lecco; Genova; Messina; Torino; Bologna; Napoli)

Modifiers of respiratory and cardiac function in the Italian Duchenne muscular dystrophy Network and CINRG Duchenne Natural History Study

L. Bello, G. D'Angelo, M. Villa, A. Fusto, S. Vianello, B. Merlo, A. Barp, S. Gandossini, F. Magri, G.P. Comi, M. Pedemonte, P. Tacchetti, V. Lanzillotta, F. Trucco, A. D'Amico, E. Bertini, G. Astrea, R. Battini, L. Politano, G. Baranello, E. Albamonte, E. De Mattia, F. Rao, V. Sansone, S.C. Previtali, G.L. Vita, S. Messina, A. Berardinelli, T. Mongini, A. Pini, M. Pane, E. Mercuri, A. Vianello, C. Calore, C. Bruno, E. P. Hoffman, L. Morgenroth, H. Gordish-Dressman, C. M. McDonald, CINRG-DNHS Investigators, E. Pegoraro
(Padova; Bosio Parini (LC); Milano; Genova; Roma; Pisa; Napoli; Messina; Pavia; Torino; Bologna; Binghamton, NY, USA; Washington, DC, USA; Sacramento, CA, USA)

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Morphofunctional evaluation of TNPO3 and related proteins in LGMD1F/D2 patients: a confocal microscopy and in silico study

*R. Costa, M.T. Rodia, S. Santi, G. Lattanzi, V. Papa, V. Pegoraro, S. Vianello, E. Pegoraro, C. Angelini, G. Cenacchi
(Bologna; Venezia; Padova)*

9.30 - 10.30 | Workshop 2: FSH

Chairs: R. Tupler, T. Mongini,

- The FSHD diagnostic challenges | *G. Ricci*
- Outcome measures and trial readiness | *L. Vercelli*
- The role of muscle MRI in FSHD | *G. Tasca*
- New insights in FSHD pathogenesis and possible therapies | *R. Tupler*

10.30 - 11.00 | Coffee Break

11.00 - 11.30 | INVITED LECTURE 1:

Titin Role in NMDs: overview and new phenotypes | *B. Udd*

Chair: A. Toscano

11.30 - 12.30 | Oral Communications 2

Outcome measures and natural history: a look at SMA story

Chair: L. Bello

CSF biomarkers in patients affected by Spinal Muscular Atrophy type 1 treated with nusinersen

M. Sframeli, G. Vita, A. Ciranni, A. Versaci, V. Di Bella, V. Ferlazzo, E. Gitto, M. Aguenmouz, G. Vita, S. Messina (Messina)

AVXS-101 Gene-Replacement Therapy in Spinal Muscular Atrophy Type 1: Long-Term Follow-Up From the Phase 1 Clinical Trial

*J. R. Mendell, S. Al-Zaidy, K. J. Lehman, M. McColly, L. P. Lowes, L. N. Alfano, N. F. Miller, M. A. Iannarino, K. Church, M. Bernat Fuertes, F. G. Ogrinc, J. L'Italien, E. Kernbauer, S. Shab, D. M. Sproule, D. E. Feltner
(Columbus, OH, United States; Bannockburn, IL, United States)*

AVXS-101 Gene-Replacement Therapy for Spinal Muscular Atrophy Type 1: Pivotal Phase 3 Study (STRIVE) Update

*J. W. Day, C. A. Chiriboga, T. O. Crauford, B. T. Darras, R. S. Finkel, A. M. Connolly, S. T. Iannaccone, N. L. Kuntz, L. D. M. Peña, M. Schultz, P. B. Shieh, E. C. Smith, M. Bernat Fuertes, D. E. Feltner, F. G. Ogrinc, A. Shab, H. Ouyang, T. A. Macek, E. Kernbauer, L. M. Muebring, J. L'Italien, D. M. Sproule, B. K. Kaspar, J. R. Mendell
(Stanford, CA, United States; New York, NY, United States; Baltimore, MD, United States; Boston, MA, United States; Orlando, FL, United States; St. Louis, MO, United States; Dallas,*

GIOVEDÌ 6 GIUGNO



TX, United States; Chicago, IL, United States; Cincinnati, OH, United States; Bannockburn, IL, United States; Los Angeles, CA, United States; Durham, NC, United States; Columbus, OH, United States)

FIREFISH Part 1: 1-year results on motor function in infants with Type 1 spinal muscular atrophy (SMA) receiving risdiplam (RG7916)

R. Masson, G. Baranello, L. Servais, J.W. Day, N. Deconinck, E. Mercuri, A. Klein, B. Darras, H. Kletzl, Y. Cleary, M. El-Khairi, T. Seabrook, C. Czech, M. Gerber, C. Nguyen, K. Gelblin, K. Gorni (Milano; London, UK; Liège, Belgium; Palo Alto, CA, USA; Brussels, Belgium; Ghent, Belgium; Roma; Basel, Switzerland; Bern, Switzerland; Boston, MA, USA; Welwyn Garden City, UK)

12.30 - 13.15 | Workshop 3: Update on Adult Myopathies

Chairs: M. Moggio, C. Fiorillo

- LGMD: phenotypes, genes and pathogenesis: up-to date | *G. Comi*
- Inclusion body myopathy: up to date | *M. Mirabella*
- Oculopharyngeal Muscular Dystrophy | *G. Siciliano*

13.15 - 14.15 | Lunch

14.30 - 15.30 | POSTER SESSION 1 | (Sessione non accreditata ECM)

P. 1-1 Muscular dystrophies

P. 1-2 SMA, Congenital myopathies and CMD

15.30 - 16.00 | INVITED LECTURE 2:

Therapeutical perspectives for DMD: where are we going?

E. Pegoraro, Chair: G. Comi

16.00 - 17.15 | Workshop 4: Diagnosis in NMDs: what's new?

Chairs: E. Bertini, A. Di Muzio

- Neonatal screening | *A. Donati*
- New insights and tools in molecular Diagnosis of DMD | *A. Ferlini*
- NGS approach: beyond Exome | *V. Nigro*
- Role of SMN2 copy number: do we know enough about it? | *F. D. Tiziano*
- New imaging techniques and their possible role in planning muscle clinical trials
M. Paoletti

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17.15 - 17.30 | Coffee Break

**17.30 - 18.30 | Oral Communications 3:
Muscular Dystrophies: LGMDs and dystrophinopathies**
Chair: G. Marrosu

Plasma screening of dystrophin protein fragments using novel dystrophin-specific immunoassays

R. Rossi, M. S. Falzarano, A. Margutti, N. Spedicato, R. El Dani, M. Fabris, M. Neri, Fr. Gualandi, S. Fini, P. Rimessi, Ca. Jobansson, C. Al-Khalili Szogyarto, A. Ferlini (Ferrara; Stockholm, Sweden)

Transcriptome analysis (RNAseq) is a powerful diagnostic and research strategy in inherited skeletal muscle diseases

M. Savarese, M. Jobari, P. H. Jonson, S. Koivunen, T. Quareshi, A. Vibola, B. Udd, P. Hackman (Helsinki, Finland; Tampere, Finland; Vaasa, Finland)

Diagnostic algorithm of hyperckemia and proximal weakness in the era of next generation sequencing

C. Gemelli, L. Trevisan, S. Fabbri, L. Pisciotto, G. Meo, M. Traverso, F. Zara, C. Minetti, C. Bruno, A. Schenone, P. Mandich, C. Fiorillo, M. Grandis (Genova)

Interpreting genetic variants in ryanodine receptor type 1-related muscle disorders: results from 71 patients

C. Dosi, D. Cassandrini, A. Rubegni, D. Tolomeo, G. Astrea, A. Berardinelli, C. Bruno, G.P. Comi, M.A. Donati, M. Filosto, C. Fiorillo, F. Giannini, M. Grandis, F. Magri, M.A. Maioli, A. Malandrini, R. Massa, M. Moggio, E. Pegoraro, G. Ricci, A. Schenone, G. Siciliano, P. Tonin, N. Volpi, F.M. Santorelli (Pisa; Pavia; Genova; Milano; Firenze; Brescia; Siena, Cagliari; Roma; Padova; Verona)

18.30 - 19.00 | Workshop 5: New Emerging Phenotypes in Early and Adult onset Myopathies | *Chairs: M. Sciacco, S. Servidei*

- GMPPB related early onset myopathies: broad phenotypic spectrum | *G. Astrea*
- Dusty-Core Myopathies | *M. Garibaldi*
- Triadopathies: up to date | *C. Semplicini*

20.30 | Cena Faculty

VENERDÌ 7 GIUGNO



8.30 - 9.00 | INVITED LECTURE 3:
The role of neuromuscular junction in the physiopathology of NMDs
E. Palma - Chair: A. Evoli

9.00 - 9.45 | Workshop 6: Metabolic myopathies and mitochondriopathies
Chairs: M. Filosto, M. Mancuso

- Update on Skeletal Muscle Glycogenosis | *A. Toscano*
- Update on Lipid Myopathies | *E. Pennisi*
- Update on mitochondrial diseases | *M. Zeviani*

9.45 - 10.15 | INVITED LECTURE 4:
Genome Editing, disease modelling and therapeutics
F. Santorelli, Chair: V. Nigro

10.15 - 10.30 | Coffee break

10.30 - 11.30 | Oral Communications 4
Disease mechanisms and possible therapeutic approaches
Chair: S. Ravaglia

Functional and Histological Improvements Comparing 4 Micro-dystrophin Constructs in the mdx Mouse Model of DMD

Rachael A. Potter, Danielle A. Griffin, Kristin N. Heller, Jerry R. Mendell, Louise R. Rodino-Klapac (Columbus, Ohio, USA; Cambridge, Massachusetts, USA)

D4Z4 elements constitute a drug responsive chromatin structure affecting gene expression in FSHD

V. Salsi, M. Salani, P.D. Kaufman, M.R. Green, R. Tupler (Modena e Reggio Emilia; Worcester, MA, USA)

The RNA binding protein FRG1 controls transcription landscape regulating muscle maturation and metabolism

Vallarola, A. Termanini, M. Cortini, V. Ghiaroni, M. Forcato, E. Germinario, G. D'Antona, B. Blaauw, R. Tupler (Modena and Reggio Emilia; Padova; Voghera (PV))

Finding treatments for Tubular Aggregate Myopathy

A.A. Genazzani, N. Filigheddu, M. Garibaldi (Roma)

11.30 - 12.30 | Focused Workshop 7: NMJ disorders. | *Chairs: G. Antonini, R. Liguori*

- Myasthenia Gravis: an update | *A. Evoli*

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- Myasthenic crisis and respiratory failure | *F. Racca*
- Eaton Lambert Myasthenic Syndrome: treatment and open issues | *C. Rodolico*
- New genes: how to tailor treatment for Congenital Myasthenias | *L. Maggi*

12.30 - 13.30 | Lunch Symposium:

Evolving perspectives in the treatment of SMA

Chairs: E. Mercuri, A. Berardinelli

- The importance of early treatment: new NURTURE data | *E. Bertini*
- Round table: real world experience in adult patients
E. Pegoraro, T. Mongini, A. K. Patanella, V. Sansone, M. Sframeli

13.30 - 14.15 | Lunch

14.30 - 16.00 | POSTER SESSION 2 | (Sessione non accreditata ECM)

P. 2-1 Mitochondrial and metabolic myopathies

P. 2-2 Myotonias, Channelopathies, Neuromuscular Junction Disorders and inflammatory myopathies

P. 2-3 Miscellanea

16.00 - 16.30 | INVITED LECTURE 5:

Extracellular Matrix and NMDs | *P. Bonaldo - Chair: M. Mora*

16.30 - 17.30 | Clinical Networks: Chairs | *C. Minetti, C. Rodolico*

17.30 - 18.00 | INVITED LECTURE 6:

MRI in muscle disorders | *J. Vissing - Chair: A. Pichiecchio*

18.00 - 19.30 | Assemblea dei soci

20.45 | Cena sociale

SABATO 8 GIUGNO

**8.30 - 9.30 | Muscle club | Chairs: P. Tonin, R. Massa****A 9 years-old-girl with hypoglycemic coma and lactic acidosis**

G. Brigati, C. Fiorillo, M. Pedemonte, C. Diana, C. Panicucci, P. Broda, C. Nesti, D. Cassandrini, F. Santorelli, C. Minetti, C. Bruno
(Genova; Pisa)

A 48-year-old man with slowly progressive asymmetric weakness and hypotrophy of the scapulohumeral girdle

S. Gallo Cassarino, S. Cotti Piccinelli, A. Galvagni, F. Caria, E. Baldelli, N. Necchini, C. Baroncelli, A. Padovani, M. Filosto
(Brescia)

Gastroparesis in two neuromuscular patients: a not-to-underestimate clue

G. Grecoa, C. Nestib, E. Frezzaa, E. Rastellia, C. Terraccianoa, F. M. Santorellib, R. Massa
(Roma; Pisa)

A 66-years-old man with subacute ophthalmoplegia and bilateral eyelid ptosis

M. Garibaldi, G. Merlonghi, S. Pugliese, T. Tartaglione, F. Calabrò, G. Antonini, A. Petrucci (Roma)

9.30 - 10.45 | Workshop 8: CNS involvement in NMDs

Chairs: S. Previtali, A. Martinuzzi

- CNS in Duchenne Muscular Dystrophy | *R. Battini*
- CNS in Myotonic Dystrophies | *V. Sansone*
- CNS in Pompe Disease | *O. Musumeci*
- CNS in Infantile Pompe Disease | *F. Ricci*
- CNS in Mitochondriopathies | *C. Lamperti*

10.45 - 11.15 | Coffee break**11.15 - 12.15 | Workshop 9: What is coming now? More therapies on the horizon**

Chairs: I. Moroni, M. Savarese

- Channelopathies | *G. Meola*
- Myotubular myopathy: a new hope | *A. D'Amico*
- Upcoming therapies in Autosomal Recessive LGMDs | *L. Bello*
- Discussion

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12.15 - 13.15 | Oral communications 5

Clinical and Laboratory studies of patients cohorts

Chair: M. Mirabella

Large scale genotype-phenotype correlation study in 1703 carriers of D4Z4 reduced alleles from the Italian National Register for FSHD

F. Mele, L. Ruggiero, G. Ricci, L. Vercelli, C. Bettio, S. Tripodi, M. Govi, E. Bucci, A. Di Muzio, L. Maggi, M. Scarlato, L. Villa, G. D'Angelo, G. Antonini, M. Filosto, C. Rodolico, R. Piras, M.A. Maioli, R. Massa, S. Previtali, C. Angelini, A. Berardinelli, E. Pegoraro, M. Moggio, L. Santoro, G. Siciliano, T. Mongini, G. Tomelleri, R. Tupler (Modena e Reggio Emilia; Napoli; Pisa; Torino; Padova; Roma; Chieti; Milano; Bosisio Parini; Brescia; Messina; Cagliari; Venezia; Pavia; Worcester, USA)

Muscle pain in mitochondrial diseases: the final data from italian network

S. Cotti Piccinelli, C. Lamperti, T. Mongini, S. Servidei, O. Musumeci, P. Tonin, F. M. Santorelli, C. Simoncini, G. Primiano, L. Vercelli, A. Rubegni, A. Galvagni, F. Caria, S. Gallo Cassarino, E. Baldelli, N. Necchini, M. Moggio, G. Pietro Comi, V. Carelli, A. Toscano, A. Padovani, G. Siciliano, M. Mancuso, M. Filosto (Brescia; Milano; Torino; Roma; Messina; Verona; Pisa; Bologna)

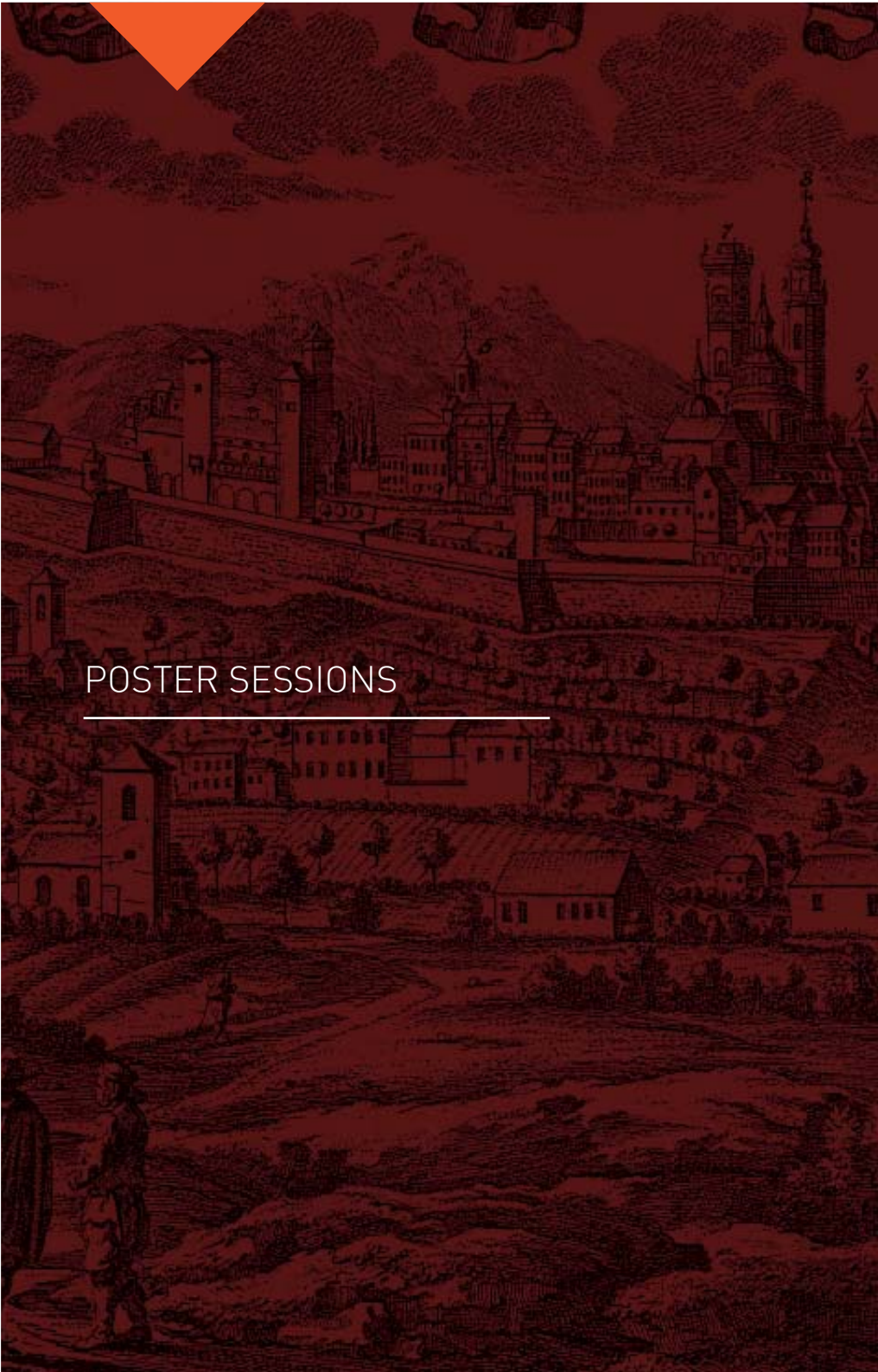
Clinical, morphological and genetic data in Italian patients with fiber-type-disproportion

L. Maggi, M. Verardo, A. Rubegni, C. Bruno, A. Lupica, A. Berardinelli, M. Ripolone, G. Vattemi, L. Ruggiero, A. D'Amico, C. Rodolico, M. Sciacco, V. Nigro, F.M. Santorelli, P. Tonin, C. Fiorillo, M. Mora (Milano; Roma; Pisa; Genova; Messina; Pavia; Verona; Napoli)

Prevalence of anti-CN1A antibodies in a large Italian cohort of s-IBM

M. Lucchini, L. Maggi, E. Pegoraro, M. Filosto, C. Rodolico, G. Antonini, M. Garibaldi, G. Siciliano, V. De Arcangelis, C. De Fino, L. Santovito, S. Cotti Piccinelli, M. Mirabella (Roma; Milano; Padova; Brescia; Messina; Pisa)

Poster premiati e conclusione lavori entro le 14.30



POSTER SESSIONS

Poster Session 1



Sessione 1 – 6 giugno dalle ore 14.30 – 15.3

P 1-1 Muscular dystrophies

P.1 Clinical course of two brothers with LGMD2D under steroids therapy

*M. Catteruccia, G. Colia, A.M. Bonetti, A. Carlesi, E. Bertini, A. D'Amico
(Roma)*

P.2 Burden of Duchenne Muscular Dystrophy (DMD) in Italy: a systematic review

*M. Pane, E. Mercuri, G. Bruno, S. Di Matteo, M. Valentino, M. Oselin, C. Martinotti,
X. Entela, G.L. Colombo
(Roma; Milano; Pavia)*

P.3 A new tool to evaluate multidisciplinary clinical outcomes in Duchenne Muscular Dystrophy: a pilot study

*A. Russo, A. LoMauro, S. Gandossini, D. Velardo, G.P. Comi, A.C. Turconi, N. Bresolin,
A. Aliverti, M. D'Angelo
(Lecco; Milano)*

P.4 Becker muscular dystrophy: analysis of an Italian sample with childhood onset or diagnosis in developmental age

*A. Ferrero, M. Rossi, C. Palermo, L. Bello, M. Giannotta, E. Rolle, M. Derchi, A. Gardani,
U. Balottin, F. Ricci, T. Mongini, C. Fiorillo, A. Pini, E. Pegoraro, M. Pane, E. Mercuri, A.
Berardinelli
(Pavia; Roma; Padova; Bologna; Torino; Genova)*

P.5 DMD nonsense variants position may be accurate to predict phenotype.

*T. Giugliano, A. Torella, A. Garofalo, M. E. Onore, B.F. Del Vecchio, G. Piluso,
L. Politano, V. Nigro
(Napoli; Pozzuoli)*

P.6 DMD gene molecular genetic characterization in eastern Europe and non European countries.

*R. Selvatici, C. Trabanelli, B. Buldrini, S. Fini, P. Rimessi, A. Venturoli, M. Neri,
F. Fortunato, A. Potulska, A. Chirita Emandi, I. Lebman, A. Herczegfalvi,
V. Guergueltcheva, T. Kyriakides, Y. Sifi, M. J. Molnar, B. Burnyte, A. Shatillo,
D. Vlodavets, F. Gualandi, A. Ferlini
(Ferrara; Warsaw, Poland; Timisoara, Romania; Zagreb, Croatia; Budapest, Hungary;
Sofia, Bulgaria; Cyprus; Algeria; Vilnius, Lithuania; Ukraina; Moscow, Russia)*

P.7 A case of DMD and Autism: double trouble or complex disease entity?

*M. Scali, M. Ripolone, P. Piscato, F. Menni, G. D'Angelo, E. Mani, F. Magri, M. Moggio,
G.P. Comi, M. Sciacco
(Milano; Bosisio Parini (LC))*

Poster Session 1



P.8 Urinary stem cells are a non-invasive model for the identification of dystrophin mutations by DMD transcript profiling.

M.S. Falzarano, M. Fabris, P. Rimessi, N. Spedicato, A. Margutti, R. El Dani, R. Rossi, F. Gualandi, M. Neri, R. Selvatici, T. Mongini, A. Ferlini
(Ferrara; Torino)

P.9 Nonsense and single nucleotide frameshift mutations in Becker Muscular Dystrophy

M. Traverso, C. Panicucci, M. Catteruccia, S. Baratto, V. Nesich, M. Iacomino, P. Broda, A. Torella, C. Bruno, V. Nigro, F. Zara, C. Minetti, E. Bertini, A. D'Amico, C. Fiorillo
(Genova; Roma; Napoli)

P.10 Cardiac involvement in Becker muscular dystrophy: bridging the gap between perihelal muscles impairment and myocardial damage

V. Castiglione, A. Giannoni, G. Ricci, F. Florio, G. Astrea, R. Battini, A. Rocchi, G. Siciliano, M. Emdin
(Pisa)

P.11 Eteplirsen Is Well Tolerated in Men With Mild or Moderate Renal Impairment

C. Fratazzi, E. Naughton, H. Krenz
(Cambridge, MA, USA)

P.12 Limb Girdle Muscular Dystrophies (LGMD): clinical and genetic characterization of a wide cohort of patients from a single center.

M.G. Distefano, C. Rodolico, O. Musumeci, A. Lupica, S. Messina, G. Vita, A. Toscano
(Messina)

P.13 The known R818Q missense mutation of TPNO3 gene in a further unrelated patient with early onset LGMD phenotype

C. Panicucci, C. Fiorillo, V. Nesich, P. Broda, F. Madia, F. Zara, M. Traverso, C. Minetti, C. Bruno (Genova)

P.14 Multiplex Ligation-Dependent Probe Amplification usefulness in improving Limb Girdle Muscular Dystrophies molecular diagnosis.

F. Magri, E. Mauri, D. Ronchi, A. Govoni, R. Brusa, F. Fortunato, M.G. D'Angelo, M. Moggio, N. Bresolin, S. Corti, G.P. Comi
(Milano; Lecco)

P.15 Longitudinal functional outcomes in Limb Girdle Muscular Dystrophy type 2A (Calpainopathy)

V. Zangaro, L. Bello, C. Semplicini, A. Lazzarotto, M. Fanin, E. Pegoraro
(Padova)

P.16 Role of autophagy in the pathogenesis of muscular dystrophies

E. Picillo, M. Ergoli, L. Politano
(Napoli)

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P.17 The role of inflammation in pediatric sarcoglycanopathies: novel therapeutic perspectives.

S. Baratto, E. Principi, G. Del Zotto, F. Antonini, C. Panicucci, M. Ognio, S. Bruzzone, E. Gazzero, C. Minetti, C. Bruno, L. Raffaghello (Genova; Berlin, Germany)

P.18 Study of cognitive and psychological profiles in FSHD patients

E. Lai, F. Torri, L. Chico, G. Ricci, G. Siciliano (Pisa)

P.19 Otoacoustic emissions are a sensitive tool for detecting cochlear damage in facioscapulohumeral muscular dystrophy type1 (FSHD1)

E. Frezza, E. Fuccillo, G. Nucera, A. Petrucci, G. Greco, R. Di Mauro, S. Di Girolamo, R. Massa (Roma)

P.20 Facio-Scapulo-Humeral Muscular Dystrophy and infantile onset epilepsy with uncommon trend: a case report

S. Siliquini (Ancona)

P 1-2 SMA, Congenital myopathies and CMD

P.21 Clinical and muscle MRI profile of a cohort of SMA patients being treated with Nusinersen

A. Govoni, F. Magri, M. Meneri, D. Velardo, D. Ronchi, C. Cinnante, F. Triulzi, M. Vergari, F. Cogiamanian, N. Stocchetti, E. Calderini, E. Albamonte, V.A. Sansone, G. D'Angelo, S.P. Corti, G.P. Comi (Milano; Lecco)

P.22 Update from SUNFISH Part 1: Safety, tolerability and PK/PD from the dose-finding study, including exploratory efficacy data in patients with Type 2 or 3 spinal muscular atrophy (SMA) receiving risdiplam (RG7916)

M.C. Pera, E. Mercuri, G. Baranello, J. Kirschner, L. Servais, N. Goemans, M. Tichy, W.Y. Yeung, H. Kletzl, M. Gerber, C. Czech, M. Annoussamy, Y. Cleary, Ks. Gorni (Roma; London, UK; Freiburg, Germany; Liège, Belgium; Leuven, Belgium; Basel, Switzerland; Welwyn Garden City, UK)

P.23 The c.859G>C variant in SMN2 modulates clinical severity in SMA: a case report

A. Barp, E. Carraro, E. Albamonte, F. Salmin, C. Lunetta, E. Mercuri, G. Comi, L.M. Sconfienza, V. Sansone (Milano; Roma)

P.24 SMN1 intragenic mutations in a cohort of Italian SMA patients.

M. Meneri, A. Govoni, F. Magri, D. Ronchi, G.F. Manenti, R. Brusa, D. Velardo, E. Albamonte, V. Sansone, S.C. Previtali, A. Berardinelli, S.P. Corti, G.P. Comi (Milano; Pavia)

Poster Session 1



P.25 Nusinersen and skin necrosis in an infant with spinal muscular atrophy type 1: effects over time.

*F. Salmin, E. Carraro, E. Albamonte, V. Morettini, N. Gagliano, E. Mercuri, A. Barp, V.A. Sansone
(Milano; Roma)*

P.26 SMN genes molecular testing in a cohort of 1546 subjects tested for genetic diagnosis and trial enrolment

*Margutti, A. Venturoli, M. Neri, F. Fortunato, F. Gualandi, P. Rimessi, A. Ferlini,
(Ferrara)*

P.27 Circulating microRNAs as potential biomarkers to monitor response to nusinersen in SMA patients

*S. Bonanno, S. Marcuzzo, C. Malacarne, E. Giagnorio, L. Maggia, R. Masson, R. Zaninc, F. Andreetta, O. Simoncinia, P. Bernasconia, R. Mantegazza, G. Baranello
(Milano; London, UK)*

P.28 Nusinersen treatment in Spinal muscular atrophy: the experience of Bambino Gesù Hospital

*A. D'Amico, M. Catteruccia, C. Cherchi, MB Chiarini Testa, G. Colia, A. Bonetti, A. Carlesi, M. Rollo, R. Bianchi, A. Longo, F. Nicita, T. Corsetti, R. Cutrera, E. Bertini
(Roma)*

P.29 Central core disease and facioscapulohumeral dystrophy-like phenotype in a family of South Italy carrying a novel heterozygous mutation in hnRNPA1 gene and a D4Z4 partial deletion: clinical features of an overlapping syndrome and genotype correlation

*G. Bruno, L. Allegorico, L. Lombardi, F. Napolitano, S. Sampaolo
(Napoli)*

P.30 Severe congenital RYR1-associated myopathy with multiple fractures

*M. Catteruccia, F. Fattori, A.M. Bonetti, E. Bertini, A. D'Amico
(Roma)*

P.31 STIM1 mutations: new mutations and different phenotypes

*D. Cassandrini, A. Rubegni, G. Bertocci, D. Tolomeo, V. Guglielmi, G. Vattemi, P. Tonin, S. Matà, G. Ricci, A. Malandrini, G. Siciliano, A. Federico, F.M. Santorelli, L. Merlini
(Pisa; Verona; Firenze; Siena; Bologna)*

P.32 Congenital myopathy with fiber type disproportion related to novel STAC3 mutation

*F. Fattori, M. Verardo, M. Catteruccia, F. Nicita, E. Bertini, A. D'Amico
(Roma)*

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P.33 Phenotypic spectrum of RYR1 recessive myopathy with early onset

C. Matucci-Cerinic, C. Fiorillo, D. Cassandrini, G. Astrea, M. Catteruccia, A. D'Amico, F. Fattori, M. Garibaldi, M. Giannotta, L. Maggi, P. Bernasconi, E. Mercuri, R. Battini, M. Sframeli, S. Messina, M. Mora, M. Pane, L. Bello, E. Pegoraro, A. Pini, F. Ricci, T. Mongini, G. Tasca, M. Traverso, P. Broda, E. Bertini, F. Santorelli, C. Minetti, C. Bruno (Genova; Pisa; Roma; Bologna; Milano; Messina; Padova; Torino)

P.34 Novel ACTA1 mutation causes late-onset nemaline myopathy with fuzzy-dark cores

M. Garibaldi, E.M. Pennisi, G. Merlonghi, L. Fionda, F. Vanoli, L. Leonardi, S. Loreti, E. Bucci, S. Morino, A. Micaloni, S. Raffa, G. Antonini (Roma)

P.35 Severe Neonatal Congenital Myopathy and Hypotonia: a new perspective through NGS.

A. Torella, F. Del Vecchio Blanco, G. Blasio, M. Savarese, A. Varone, G. Piluso, V. Nigro (Napoli)

P.36 Genome and transcriptome analysis of COLVI genes and characterization of a new promising cellular model

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

P.37 Mutations in ACTN2 gene cause a novel form of adult-onset distal myopathy

M. Savarese, J. Palmio, J. J. Poza, J. Weinberg, M. Olive, A. M. Cobo, A. Vibola, P. H. Jonson, J. Sarparanta, F. García-Bragado, J. A. Urtizbarea, P. Hackman, B. Udd (Helsinki, Finland; Tampere, Finland; San Sebastián, Spain; Stockholm, Sweden; Barcelona, Spain; Hendaye, France; Vaasa, Finland)

P.38 Compound heterozygous nonsense LAMA2 mutations detected by exome sequencing in two siblings with atypical phenotype and normal brain MRI

S. Gibertinia, S. Saredia, L. Matalongab, L. Farinac, Ardissoned, I. Moronid, M. Mora (Milano; Barcelona, Spain)

P.39 Longitudinal functional changes in a cohort of adult nusinersen- treated spinal muscular atrophy patients at the Padova Neuromuscular Center

L. Caumo, V. Bozzoni, L. Bello, C. Semplicini, G. Cester, J. Gabrieli, F. Causin, G. Sorarù, E. Pegoraro (Padova)

Poster Session 2



Sessione 2 - 7 giugno dalle ore 14.30 - 15.30

P. 2-1 Mitochondrial and metabolic myopathies

P.40 Identification of maternal uniparental disomy of chromosome 10 in a patient with PITRM1 mutation and mitochondrial dysfunction

D. Tolomeo, A. Rubegni, R. Battini, D. Galatolo, F. D'Amore, C. Nesti, G. Astrea, S. Doccini, S. Giglio, M. Pantaleo, S. Guarducci, F.M. Santorelli
(Pisa; Firenze)

P.41 Cognitive impairment precipitated by head trauma in MELAS syndrome

R. Telese, A. De Rosa, L. Napoli, G. Fagiolari, M. Scali, M. Moggio, M. Sciacco
(Chieti; Pisa; Milano)

P.42 Novel NARS2 mutations in two children with early onset epileptic encephalopathy and mitochondrial dysfunction

A. Rubegni, C. Nesti, D. Tolomeo, M. Montomoli, F. D'Amore, M.M. Mancardi, F. Mari, L. Pisciotta, D. Galatolo, G. Morana, C. Fiorillo, R. Guerrini, F.M. Santorelli
(Pisa; Firenze; Genova)

P.43 Effectiveness of different non-anti-arrhythmic sodium-channel blockers in two patients with paramyotonia congenital

S. Arceri, S. Ravaglia, G. Cosentino, L. Maggi, P. Bernasconi, E. Alfonsi
(Pavia; Milano)

P.44 A less severe phenotype of glycogen synthase deficiency myopathy in two unrelated cases

A. Pugliese, C. Rodolico, S. Volta, R. Oteri, A. Ciranni, A. Lupica, G. Vita, A. Toscano, O. Musumeci
(Messina)

P.45 Sleep Disordered Breathing in Mitochondrial Diseases: epidemiological and clinical characterization

G. Primiano, G. Della Marca, V. Brunetti, C. Sancricca, C. Vollono, S. Servidei
(Roma)

P.46 Lipid composition of cellular membranes in ATGL deficit

A. Maccone, M. Garibaldi, S. Missaglia, N.I. Noguera, E. Palma, D. Tavian, E.M. Pennisi
(Roma; Milano)

P.47 Long-term follow up in presymptomatic LOPD patients

O. Musumeci, G. Tavilla, M.G. DiSfetano, A. Pugliese, S. Volta, A. Toscano
(Messina)

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P.48 A new case of autophagic vacuolar myopathy presenting LOPD features

F. Napolitano, C. Terracciano, G. Bruno, G. Di Iorio, M.A.B. Melone, T. Esposito, S. Sampaolo
(Napoli; Philadelphia, PA, USA; Pozzilli (IS))

P.49 Bioimpedance Phase Angle as a prognostic tool in a population of late-onset Pompe disease

A. Tartara, S. Ravaglia, S. Arceri, A. Picchiecchio, H. Cena
(Pavia)

P.50 MRI evidence of structural muscle damage in McArdle Disease: clinico pathological implications

C. Stefan, G. Brondani, E. Trevisi, A. Martinuzzi
(Pieve di Soligo-Conegliano; Latisana - Friuli Venezia Giulia)

P.51 Adult form of Multiple acyl-CoA dehydrogenases deficiency triggered by statin treatment

A. Lupica, C. Rodolico, T. Brizzi, M.G. Distefano, A. Pugliese, A. Ciranni, S. Volta, G. Vita, A. Toscano, O. Musumeci
(Messina)

P.52 Case report: New missense variants of NDUFA11 associated with late onset myopathy

L. Peverelli, A. Legati, E. Lamantea, A. Nasca, S. Marchet, A. Lerario, V. Galimberti, D. Ghezzi, C. Lamperti
(Milano)

P.53 Glycogenosis VII worsened by cyclosporine and amiodarone: a clinical and muscle MRI report

F. Caria, A. Picchiecchio, S. Cotti Piccinelli, O. Musumeci, E. Baldelli, A. Galvagni, S. Gallo Cassarino, R. Vitale, A. Padovani, A. Toscano, M. Filosto
(Brescia; Pavia; Messina)

P.54 The Role of anti rh-GAA in modulating response to ERT in late-onset pompe disease: the final data form the IgERT study

S. Cotti Piccinelli, S. Ravaglia, S. Servidei, M. Moggio, O. Musumeci, M. A. Donati, E. Pegoraro, A. Di Muzio, L. Maggi, P. Tonin, G. Marrosu, C. Sancricca, A. Lerario, M. Sacchini, C. Semplicini, V. Bozzoni, R. Teles, S. Bonanno, R. Piras, M. A. Maioli, G. Ricci, L. Vercelli, A. Galvagni, S. Gallo Cassarino, F. Caria, E. Baldelli, N. Necchini, T. Mongini, G. Siciliano, A. Padovani, A. Toscano, M. Filosto
(Brescia; Pavia; Roma; Milano; Messina; Firenze; Padova; Chieti; Verona; Cagliari; Pisa; Torino)

Poster Session 2



P.55 Liver transplantation in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): update from Bologna case series

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

P.56 Mitochondrial diseases related to mtDNA in childhood: novel mutation in mtCO3 associated to familial mitochondrial leukoencephalopathy expanding repertoire of mtDNA mutations in human diseases

A. Ardisson, E. Fernandez-Vizarra, S. Marchet, L. Farina, V. Tiranti, M. Zeviani, I. Moroni, E. Lamantea, C. Lamperti
(Milano; Cambridge, UK)

P. 2-2. Myotonias, Channelopathies, Neuromuscular Junction Disorders and inflammatory myopathies

P.57 Longitudinal cognitive changes in DM1 patients in a 5 years follow up study

E. Pinzan, V. Pegoraro, R. Marozzo, G. Siciliano, C. Angelini
(Venezia; Pisa)

P.58 Characterization of brain involvement to study the phenotypic complexity of Myotonic Dystrophy Type 1

C. Simoncini, G. Ricci, M. Cosottini, C. Angelini, P. Cecchi, G. Migaleddu, G. Siciliano
(Pisa; Lido di Venezia)

P.59 An Italian patient with autosomal dominant hypomagnesemia associated by KCNA1 mutation

C. Simoncini, G. Ricci, V. Montano, P. Bernasconi, L. Calì Cassi, G. Siciliano
(Pisa; Milano)

P.60 Efficacy of metformin on mobility and strength in myotonic dystrophy type 1 (METMYD): study protocol outline.

R. Massa, A. Botta, E. Frezza, E. Rastelli, G. Greco, A. Petrucci, G. Silvestri, G. Antonini
(Roma)

P.61 Bioelectric impedance analysis (BIA), anthropometric and nutritional characteristics in myotonic dystrophy type 2 (DM2) patients.

E. Frezza, M. Cintoni, G. Pulcini, G. Greco, C. Palombi, M. Salvia, G. Tammam, A. De Lorenzo, R. Massa
(Roma)

P.62 Longitudinal, quantitative assessment of hand muscle strength decay in myotonic dystrophy type 1 (DM1)

E. Frezza, E. Rastelli, G. Greco, G. Tammam, M. Salvia, C. Palombi, R. Massa
(Roma)

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P.63 Genetic and phenotypical characterization of a pediatric cohort with myotonic dystrophy type 1 (DM1)

*M. Catteruccia, G. Colia, A.M. Bonetti, D. Carlesi, D. Diodato, F. Nicita, A. D'Amico, E. Bertini
(Roma)*

P.64 Functional tests in Myotonic Dystrophy type 1: a three-year longitudinal study

*V. Bozzoni, L. Bello, S. Tripodi, L. Caumo, G. Sorarù, E. Pegoraro
(Padova)*

P.65 Congenital myasthenic syndromes due to CHRND mutation: a report of two lethal phenotypes

*C. Bonanno, A. Lupica, G. Nicocia, F.M. Foti, A. Toscano, C. Rodolico
(Messina; Reggio Calabria)*

P.66 Clinical features in a cohort of Italian MuSK-MG patients in a long-term follow-up

*C. Bonanno, S. Messina, G. Nicocia, A. Lupica, A. Toscano, C. Rodolico
(Messina)*

P.67 Electrophysiological tests in patients with anti-MuSK Myasthenia Gravis: a retrospective study

*A. De Rosa, R. Ricciardi, T. Bocci, M. Maestri, M. Guida, M. Sciacco, M. Moggio, U. Bonuccelli, G. Siciliano
(Pisa; Milano)*

P.68 Muscle involvement in myasthenia gravis: expanding the clinico-pathological spectrum of myasthenia-myositis association from a large cohort of patients

*M. Garibaldi, L. Fionda, F. Vanoli, L. Leonardi, S. Loreti, E. Bucci, A. Di Pasquale, S. Morino, E. Vizzaccaro, G. Merlonghi, M. Lucchini, M. Mirabella, F. Andreetta, E.M. Pennisi, A. Petrucci, G. Antonini
(Roma; Milano)*

P.69 Clinical features of an Italian cohort of patients with very late-onset myasthenia gravis

*A. Lupica, S. Messina, C. Bonanno, G. Nicocia, T. Brizzi, S. Sinicropi, G. Vita, A. Toscano, C. Rodolico
(Messina)*

P.70 Myasthenia gravis after Etanercept and Ustekinumab treatment for Psoriatic Arthritis: a case report

*G. Nicocia, T. Brizzi, C. Bonanno, A. Lupica, A. Toscano, C. Rodolico
(Messina)*

Poster Session 2



P.71 Anti-AChR Myasthenia Gravis presenting with early atrophy and nonfluctuating weakness of proximal limb muscles

E. Pancheri, G. Sajeve, F. Goffi, M. Zanoni, L. Bertolasi, P. Tonin, G. Vattermi (Verona)

P.72 Pediatric anti-HMGCR necrotizing myopathy resembling limb-girdle muscular dystrophy

D. Velardo, E. Abati, R. Brusa, F. Magri, A. Govoni, M. Meneri, M. Sciacco, L. Peverelli, C. Cinnante, F. Triulzi, S. Corti, N. Bresolin, M. Moggio, G. P. Comi (Milano)

P.73 Altered aquaporin-4 immunolocalization in human idiopathic inflammatory myopathies : a common feature?

F. Girolamo, A. Lia, M. Giannini, M. Fornaro, A. Amati, D. D'Abbicco, M. Tampoia, L. Serlenga, F. Iannone, M. Trojano, A. Frigeri (Bari)

P.74 Congenital myasthenic syndromes: improved diagnostic yield based on combined use of targeted NGS sequencing and deep phenotyping

C. Ticci, D. Cassandrini, A. Rubegni, D. Tolomeo, G. Astrea, C. Battisti, C. Bruno, G.P. Comi, M.A. Donati, C. Fiorillo, M. Grandis, L. Maggi, F. Magri, M.A. Maioli, A. Malandrini, F. Mari, F. Melani, M. Moggio, E. Pegoraro, G. Ricci, C. Rodolico, A. Schenone, G. Siciliano, P. Tonin, F.M. Santorelli (Pisa; Siena; Genova; Milano; Firenze; Cagliari; Padova; Messina; Verona)

P.75 Congenital myasthenic syndrome: clinical and genetic features of five unrelated patients

G. Ricci, M. Maestri, A. De Rosa, V. Montano, G. Ali, F. Sartucci, G. Cenacchi, D. Cassandrini, G. Astrea, A. Torella, R. Ricciardi, G. Siciliano (Pisa; Bologna)

P. 2-3 Miscellanea

P.76 Clinical features and muscle MRI imaging in two trasportinopathy families with different mutations

C. Angelini, R. Marozzo, V. Pegoraro, E. Pinzan (Venezia)

P.77 InGene: a novel approach for gene analysis and cluster definition in patients with hyperckemia

G. Bertocci, I. Ceppa, A. Rubegni, C. Dosi, D. Tolomeo, J. Baldacci, G. Astrea, E. Dati, S. Frosini, D. Cassandrini, R. Conte, M. Calderisi, F.M. Santorelli (Pisa)

P.78 InGene: an integrated tool for data collection in neuromuscular diseases

R. Conte, F. Sansone, G. Diodato, A. P. Pala, M. C. Scudellari, A. Tonacci, M. Calderisi, I. Ceppa, F. Giorgolo, D. Marfisi, G. Astrea, S. Frosini, F. M. Santorelli (Pisa)

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P.79 Founder mutation in Eastern Europe patients with GNE myopathy

R. Brusa, R. Del Bo, D. Ronchi, L. Peverelli, D. Velardo, M. Meneri, F. Magri, A. Govoni, I. Faravelli, D. Gagliardi, E. Mauri, E. Abati, G. Costamagna, C. Cinnante, M. Sciacco, M. Moggio, S. Corti, G.P. Comi, Y. Torrente (Milano)

P.80 Multifunctional evaluation of neuromotor performance in a CMT pediatric population: a pilot study

S. Malcontenti, M. Coluccini, S. Frosini, S. Perazza, F.M. Santorelli, R. Battini, G. Astrea (Pisa)

P.81 MRI-index: an automatic tool for early quantitative evaluation of fat infiltration at muscle MRI in neuromuscular diseases

C. Amador, D. Marfisi, G. Astrea, G. Ricci, F. Florio, S. Frosini, E. Dati, A. Rubegni, G. Bertocci, R. Conte, A. Tonacci, F. Sansone, M.C. Scudellari, A.P. Pala, A. Grande, G. Diodato, S. Roccella, A. Spezzaneve, M. Calderisi, F. Giorgolo, I. Ceppa, G. Siciliano, R. Battini, M.E. Fantacci, F.M. Santorelli (Pisa; Pontedera)

P.82 Axial myopathy: an overlooked cause of late onset camptocormia

E. Iori, A. Ariatti, M. Mazzoli, N. Fini, M. Genovese, G. Galassi (Modena; Reggio Emilia)

P.83 Genetic heterogeneity of axial myopathy: report of three cases presenting with camptocormia

M. Neri, F. Fortunato, R. Selvatici, L. Merlini, E. Sette, V. Tugnoli, F. Fattori, E. Bertini, V. Nigro, A. Ferlini, F. Gualandi (Ferrara; Bologna; Roma; Napoli)

P.84 Autosomal dominant Distal Spinal Muscular Atrophy (DSMA) is associated with MYH14 and MME genes mutations in an Italian family

M.G. Rispoli, F. Moro, S. Mero, M. Vitale, F. Barbone, V. Di Stefano, M.V. De Angelis, F.M. Santorelli, A. Di Muzio (Chieti; Pisa)

P.85 Myopathy, psychomotor delay and seizures due to a novel homozygous TBCK mutation in two sisters

A. Ruggieri, S. Saredi, E.S. Cauley, T. Spivey, A. Ardisson, M. Mora, I. Moroni, M.C. Manzini (Milano; Washington DC, USA)

P.86 Amyloid myopathy: an intriguing diagnosis

E. Pancheri, P. Tonin, G. Vattemi, R. Orlandi, A. Gajofatto, R. Rinaldi, R. D'Angelo, V. Papa, G. Cenacchi (Verona; Bologna)

XIX CONGRESSO NAZIONALE

AIM Associazione Italiana di Miologia



Informazioni Scientifiche

SESSIONI POSTER

I poster – dimensioni cm 65 di larghezza e cm 90 di altezza- potranno essere affissi a partire da Giovedì 6 giugno ore 08:00 e rimanere esposti per tutta la durata del congresso.

I poster dovranno essere ritirati entro le ore 14.00 di Sabato 8 Giugno.

I poster che non saranno ritirati a fine evento non saranno restituiti.

Un pannello numerato sarà riservato ad ogni poster ed il materiale per l'affissione sarà disponibile presso la segreteria del congresso.

Gli autori sono pregati di essere presenti nell'area poster per la presentazione dei loro contributi nelle fasce orarie dedicate.

La presentazione dei contributi scientifici è subordinata all'iscrizione del congresso.

CENTRO SLIDE

I relatori potranno caricare le proprie presentazioni (salvate su penna USB) presso il Centro Slide, dopo aver concluso le pratiche di registrazione e comunque almeno un'ora prima del proprio intervento. Il formato ottimale per le presentazioni è la videoproiezione di presentazioni in Power Point in formato 16:9. Se vi sono presenti filmati di grandi dimensioni è preferibile averli fuori dalla presentazione in formato mp4, viceversa all'interno della presentazione in riproduzione automatica.

Le presentazioni saranno trasferite direttamente dal Centro Slide. I PC utilizzano Microsoft Office 2016.

Non sono previsti altri formati.

Non è previsto l'uso di pc personali per la presentazione nella sala.

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ACCREDITAMENTO ECM



Numero di crediti assegnati: 6

L'evento sar  accreditato ECM per le seguenti professioni e discipline

Professione	Discipline
Farmacista:	<i>Farmacia Ospedaliera; Farmacia Territoriale;</i>
Biologo:	<i>Biologo;</i>
Logopedista:	<i>Logopedista;</i>
Terapista Occupazionale:	<i>Terapista Occupazionale;</i>
Psicologo:	<i>Psicoterapia; Psicologia;</i>
Medico Chirurgo:	<i>Cardiologia; Genetica Medica; Malattie Metaboliche e Diabetologia; Malattie Dell'apparato Respiratorio; Medicina Fisica e Riabilitazione; Neonatologia; Neurologia; Neuropsichiatria Infantile; Pediatria; Chirurgia Pediatrica; Neurochirurgia; Ortopedia e Traumatologia; Anestesia e Rianimazione; Laboratorio di Genetica Medica; Neurofisiopatologia; Neuroradiologia; Pediatria (pediatri di libera scelta); Scienza dell'alimentazione e dietetica;</i>
Infermiere Pediatrico:	<i>Infermiere Pediatrico</i>
Fisioterapista:	<i>Fisioterapista</i>
Infermiere:	<i>Infermiere</i>
Dietista:	<i>Dietista;</i>
Tecnico di Neurofisiopatologia	<i>Tecnico di Neurofisiopatologia</i>
Terapista della Neuro e Psicomotricit� dell'et� Evolutiva	<i>Terapista della Neuro e Psicomotricit� dell'et� Evolutiva</i>

Avranno diritto ai crediti ECM solo coloro che saranno presenti almeno al 90% delle ore totali previste dal congresso ed avranno compilato il questionario di apprendimento e di valutazione nonch  la scheda anagrafica. La rilevazione delle presenze avverr  attraverso la firma di presenza, da apporre ad inizio e fine giornata. Il questionario dovr  essere riconsegnato al personale addetto all'uscita della sala al termine del Congresso. Sar  cura del partecipante ritirare il materiale ECM al desk registrazioni. Informiamo che le sessioni poster non sono accreditate ECM.

XIX CONGRESSO NAZIONALE

AIM Associazione Italiana di Miologia



Informazioni generali

Sito web del Congresso:

www.fclassevents.com/it/19-congresso-nazionale-aim-associazione-italiana-miologia**ISCRIZIONI:**

È possibile iscriversi online entro il 19 Maggio 2019 con pagamento tramite bonifico bancario o con carta di credito. Dopo il 19 Maggio sarà possibile iscriversi solo in sede congressuale, con pagamento in contanti.

	Early Registration <i>(entro il 16 Aprile, incluso IVA)</i>	Late Registration <i>(oltre il 16 Aprile, incluso IVA)</i>
Socio AIM	€ 305,00	€ 355,00
Non Socio AIM	€ 340,00	€ 385,00
Under 35 anni	€ 200,00	€ 230,00
Accompagnatore	€ 215,00	€ 215,00

*Socio AIM in regola con le quote associative

La quota di iscrizione include:

Ammissione alle sessioni scientifiche
Coffee break e colazioni di lavoro
Cena Sociale, Venerdì 7 Giugno
Attestato di partecipazione, kit congressuale, badge nominale, programma

La quota di iscrizione per gli Accompagnatori include:

Accesso all'area espositiva ed aree pubbliche
Coffee break e colazioni di lavoro
1 biglietto per la Cena Sociale di Venerdì 7 Giugno

Segreteria Organizzativa e Provider ECM 362**First Class srl**

Viale Italia, 173 - 57128 Livorno
Tel. 0586.849811 - Fax 0586.349920
email: elena.falciola@fclassevents.com



La Segreteria Organizzativa First Class sarà a disposizione dei partecipanti in Sede Congressuale nei seguenti orari:

Mercoledì, 5 Giugno | h.14.00 - h.19.00 **Venerdì, 7 Giugno** | h. 08.00 - h. 19.00
Giovedì, 6 Giugno | h. 07.00 - h. 19.00 **Sabato, 8 Giugno** | h. 08.00 - 14.00

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BADGE

Il badge nominativo è incluso nella documentazione congressuale da ritirare presso il desk della Segreteria Organizzativa, all'atto della registrazione.

I congressisti sono pregati di indossarlo durante tutti i lavori scientifici perché è prova della regolare iscrizione al congresso e dà accesso a tutti gli spazi della sede congressuale ed ai servizi catering.

ATTESTATI DI PARTECIPAZIONE

Gli attestati di partecipazione saranno disponibili per tutti i partecipanti a partire dalle ore 10.00 di sabato 8 giugno

CENA SOCIALE

La Cena Sociale si terrà venerdì 7 giugno alle ore 20,30 presso il Ristorante "Il Pianone" (Via al Pianone, 21, 24129 Bergamo)

La partecipazione alla serata è riservata agli iscritti al congresso.

Si prega di ritirare l'invito presso il desk della Segreteria Congressuale entro **Mercoledì 5 giugno, alle ore 19.00.**

L'ingresso sarà consentito solo dietro presentazione del relativo invito.



Vista della città di Bergamo nel seicento.



XIX CONGRESSO NAZIONALE

AIM Associazione Italiana di Miologia

Sede Congressuale

Mercoledì 5 Giugno:

**Aula Magna dell'Università
degli Studi di Bergamo
(Ex-Monastero di Sant'Agostino)**

Piazzale S. Agostino, 1
24129 Bergamo Alta

**Giovedì 6, Venerdì 7
e Sabato 8 Giugno:**

Teatro Sociale

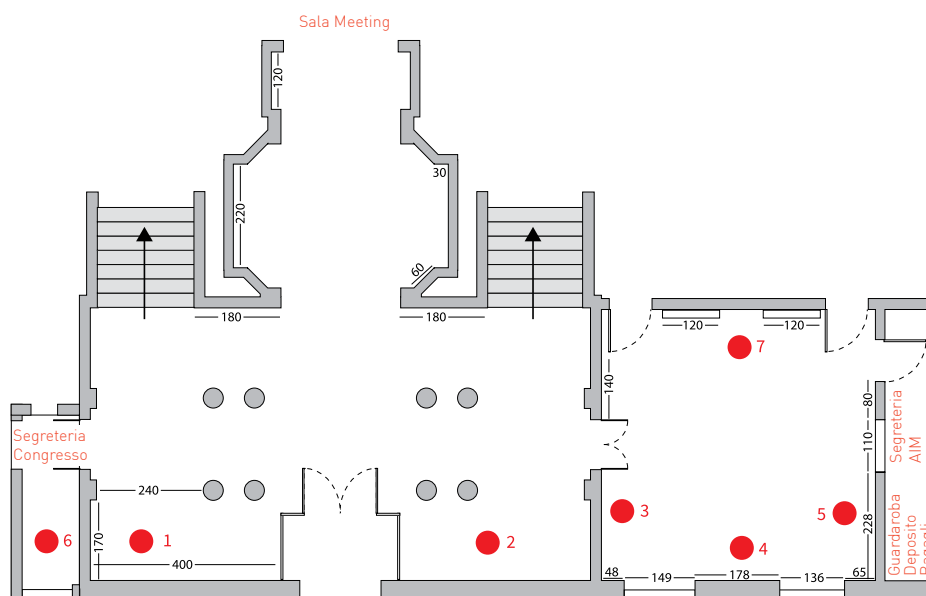
Via Bartolomeo Colleoni, 4
24129 Bergamo Alta



Bergamo 5 - 8 Giugno 2019 | Teatro Sociale



Area Congressuale



Area catering - locali del ridotto - piano 2°



Legenda

- | | |
|--------------------|--------------------|
| 1 BIOGEN | 6 AVEXIS |
| 2 SAREPTA | 7 DIAMETRA |
| 3 PTC Therapeutics | 8 SANOFI - GENZYME |
| 4/5 ROCHE | |

Come raggiungere Bergamo

In auto

Bergamo è raggiungibile attraverso l'Autostrada A4 Milano-Venezia (Uscita Bergamo). Si consiglia di parcheggiare nella Città Bassa e di raggiungere Bergamo Alta con mezzi pubblici, essendo per gran parte zona pedonale.

In treno

Bergamo è collegata via treno con Milano, Lecco e Brescia. La durata del viaggio verso Milano e Brescia è di circa 50 minuti, circa 40 invece per Lecco. La Stazione FS della città si trova in piazzale Guglielmo Marconi in pieno centro.

In aereo

L'Aeroporto internazionale Milano Bergamo, a soli 5 chilometri dalla città, è servito da diverse linee nazionali ed internazionali ed è collegato alla città da un servizio di bus navetta molto frequente. L'autobus ATB linea 1 parte dal piazzale arrivi dell'aeroporto ogni 20 minuti dal lunedì al sabato, ogni 30 minuti la domenica e i giorni festivi.

Info ATB Point 035 236026 - atbpoint@atb.bergamo.it - ATB

Aeroporto Milano Malpensa (90 km) - Aeroporto Milano Linate (40 km)

Come raggiungere Città Alta con i mezzi pubblici

La Città Alta è raggiungibile con il taxi ed è servita anche dalla linea 1 ATB, dalla linea 3 ATB e dalla funicolare.

Linea 1

La linea 1 parte da diversi comuni dell'hinterland (Torre de' Roveri, Grassobbio, Scanzo) e, a partire dalla Fiera, ha un tragitto comune: dopo aver toccato la Malpensata, gira in via Bonomelli, arriva in Stazione FS. Nel cuore della Città Bassa ha una fermata fuori dalla Chiesa delle Grazie (Porta Nuova) e prosegue il suo tragitto in Viale Vittorio Emanuele. Entra in Città Alta dalla Porta Sant'Agostino e, dopo aver percorso tutte le Mura, arriva in Colle Aperto (Marianna).

Per maggior informazioni si invita a visitare il sito:
www.atb.bergamo.it/it/viaggia-con-noi/linee-e-orari

Linea 3

La linea 3 collega l'Ostello in zona Monterosso (Via Ferraris) a Piazza Mercato delle Scarpe (quella dove c'è la stazione alta della funicolare).

Il tragitto passa per Viale Giulio Cesare, lo Stadio, Via Pescaria, Via Ruggeri da Stabello, Via Maironi da Ponte e accede in Città Alta da Porta San Lorenzo (Garibaldi).

Per maggior informazioni si invita a visitare il sito:
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XIX CONGRESSO NAZIONALE

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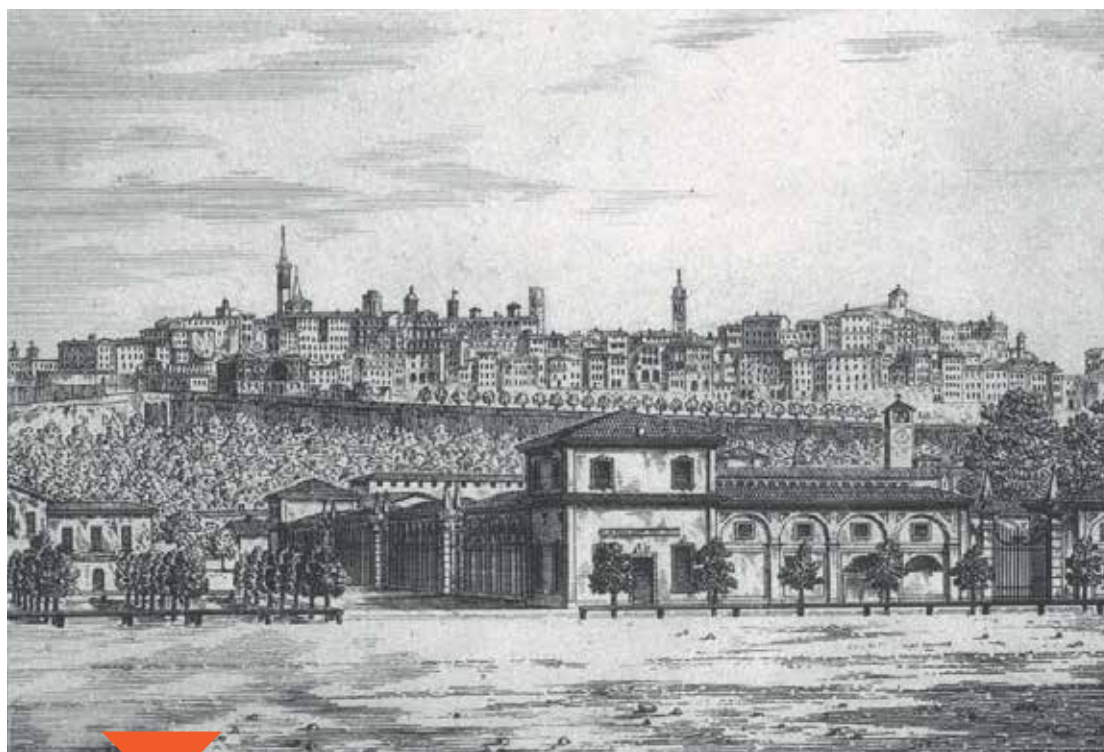


AIRPORT BUS

Airport Bus è l'autobus che collega l'Aeroporto Internazionale di Orio al Serio con la città di Bergamo, tutti i giorni, con corse ogni 20 minuti e porta alla stazione di Bergamo, a due passi dal centro cittadino.

FUNICOLARE

Situata a metà di Viale Vittorio Emanuele, la funicolare per Città Alta è il mezzo più veloce (ma non meno suggestivo) per trovarsi in Piazza Mercato delle Scarpe: la salita è rapida e panoramica.



Stampa raffigurante il "Prato S. Alessandro", dominato dall'imponente struttura della Fiera di Bergamo

ABSTRACTS OF INVITED LECTURES

(in alphabetical order of the first Author)

GMPPB related early onset myopathies: broad phenotypic spectrum

Astrea G.

IRCCS Fondazione Stella Maris, Pisa, Italia

and the CMD-Italian network: Battini R., Berardinelli A., Bertini E., Bruno C., Catteruccia M., Comi G.P., D'Amico A., Fattori F., Fiorillo C., 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., Toscano A., Santorelli F.M.

GMPPB, encoding the guanosine-diphosphate-mannose (GDP-mannose) pyrophosphorylase B protein, has recently been associated with a wide clinical spectrum ranging from severe Walker-Warburg syndrome to pseudo-metabolic myopathy and even congenital myasthenic syndromes.

In our previous research, we identified 13 additional cases from 12 families and defined seven novel mutations.

In line with data from the literature, patients displayed variable phenotypes and mutations in **GMPPB** are more common in relatively milder forms of neuromuscular disorders.

However, what our study adds to this already broad clinical spectrum is the possible presence of arthrogryposis and congenital clubfoot, particularly in patients with very severe, generalized involvement, as well as nystagmus and upgaze palsy. Ataxia could be part of the clinical picture, in line with possible evidence of cerebellar atrophy. Intellectual disability was evident in all the congenital forms, predominantly affecting the language domain.

The onset of motor manifestations in the LGMD group occurred at different ages and the extent of weakness was unrelated to the timing of onset of the disease. Less severe phenotypes are also observed, such as exercise intolerance and myoglobinuria or easy fatigability or asymptomatic hyperCKemia with subtle weakness, evident only on expert clinical examination.

We also demonstrated that few mutations recur in the Italian **GMPPB**-mutated population.

Our findings, combined with literature data, show that there are at least three forms of **GMPPB**-related myopathy: i) CMD, ii) early onset LGMD, and iii) adult onset LGMD, often with evidence of neuromuscular junction involvement.

Neurocognitive profile and executive functioning in Duchenne Muscular DystrophyBattini R.^{1,2} Lenzi S.², Astrea G.²

¹ Department of Clinical and Experimental Medicine, University of Pisa; ² Department of Developmental Neuroscience, IRCCS Stella Maris, Calambrone (Pisa), Italy

Duchenne muscular dystrophy (DMD) is not only the most common single gene disorder leading to muscle wasting, but several studies have reported cognitive difficulties and neuropsychological alterations in these patients: in fact, a possible non-progressive cognitive impairment with worse performances in verbal than in nonverbal domain has been described both in older and younger patients. Furthermore, DMD children often show a variable language involvement and suffer from deficits in executive functions, with a possible negative impact on academic skills. The lack of specific dystrophin isoforms in the brain could be related to this impairment, taking account of the involvement of cerebellum as part of a more general involvement of the cerebellar-thalamo-cortical network. Recently, this hypothesis has been supported by the results obtained in a multicenter study on a cohort of DMD boys without intellectual disability during school age. The specific selection of the DMD sample has allowed a targeted neurocognitive detection free from potential bias, characterized by an impairment of multitasking, problem solving, inhibition and working memory and an implicit learning deficit. (Battini R et al, 2018; Vicari S. et al, 2018).

In the era in which the life expectancy of DMD has increased, the DMD boys, especially those without intellectual disability, are a particularly vulnerable population and a prompt recognition of neuropsychological impairments is important in order to plan early specific treatments and to avoid an high impact on daily living with results both on academic and adaptive functioning.

Upcoming therapies in Autosomal Recessive LGMDs

Bello L., Pegoraro E.

Department of Neurosciences DNS, University of Padua, Italy

Limb Girdle Muscular Dystrophies (LGMDs) are a genetically and clinically heterogeneous group of rare neuromuscular conditions, comprising more than 30 causative autosomal loci and a multiplicity of complex and overlapping muscular and extra-muscular phenotypes. The “greatest common denominator” of this nosographic

group is the association of some degree of proximal muscle weakness, with myopathic or overtly dystrophic muscle histology. Advances in genomics and muscle imaging have increased our abilities to diagnose these conditions; but effective therapies are still lagging behind.

As the first molecular and gene therapies are being approved for more common neuromuscular diseases, such as dystrophinopathies and spinal muscular atrophy, several of these conditions emerge as potential, sometimes even ideal candidates for homologous approaches. The main hurdles, along the path to effectively bringing these treatments to approval and to the clinic, lie in scarce clinical trial readiness because of partial knowledge about natural history, lack of validated and standardized outcome measures, and difficulty in recruiting large and homogeneous groups of patients. We will review chances and challenges currently faced by clinicians, researchers, and patients. In particular, we will focus on autosomal recessive forms, which are usually determined by the lack of a specific protein which future treatments will aim at re-expressing in muscle fibers.

The importance of early treatment: new NURTURE data

Bertini E.

Unità Malattie Neuromuscolari, Ospedale Bambino Gesù IRCCS, Roma, Italia

Nusinersen is the first approved treatment for spinal muscular atrophy (SMA). Interim results from the ongoing NURTURE study (NCT02386553) examining efficacy/safety of intrathecal nusinersen, initiated prior to symptom onset, in infants with 2 or 3 *SMN2* copies will be presented. The study enrolled 25 infants with age ≤ 6 weeks at first dose, clinically presymptomatic, and genetically diagnosed with SMA. Primary endpoint was time to death or respiratory intervention (≥ 6 hours/day continuously for ≥ 7 days or tracheostomy). As of 15 May 2018, infants (2 copies *SMN2*, $n = 15$; 3 copies, $n = 10$) were enrolled. Median age at last visit was 26.0 (range 14.0-34.3) months. All infants were alive and none required permanent ventilation. Four infants (all with 2 *SMN2* copies) required respiratory intervention for ≥ 6 hours/day continuously for ≥ 7 days, with all cases initiated during acute, reversible illness. All infants achieved the WHO motor milestone sitting without support and 22/25 (88%) achieved walking with assistance; 17/22 (77%) were walking alone. Phosphorylated neurofilament heavy chain levels rapidly declined during the nusinersen loading phase and then stabilized. AEs occurred in all infants; 20/25 had AEs mild/moderate in severity; 9 had SAEs. No new safety concerns were identified. Results from 15 May 2018 interim analysis, will be presented. These findings demonstrate there was continued benefit to infants

who initiated nusinersen before symptom onset, emphasizing the value of early treatment and newborn screening. Updated analyses will provide further information.

Systemic involvement in spinal muscular atrophy: the evidence

Comi G.P.

Dino Ferrari Centre, Department of Pathophysiology and Transplantation, Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Italy

Spinal muscular atrophy (SMA) is a motor neuron (MN) disorder caused by mutations in Survival Motor Neuron 1 (*SMN1*) gene. The role of MNs vulnerability is clearly established. The involvement of other cell types in the Central Nervous System (CNS), such as astrocytes, microglia and sensory neurons, as well as outside the CNS, such as Schwann cells, muscle cells or heart, may play a role in disease initiation and/or progression, as well as in the emergence of clinical symptoms. Heart alterations have been reported in the most severe forms of SMA; caused either by congenital anomalies manifesting during cardiogenesis, or secondary to autonomic nervous system defects, and/or to respiratory dysfunction. Metabolic defects such as fasting hyperglycemia, glucose intolerance, hypersensitivity to insulin, and hyperglucagonemia have been reported. The body of evidence derived from experimental models and patients' experience will be analyzed to weight its relevance to the current therapeutic scenario.

Gene therapy and iPS derived molecular targets in SMA

Corti S.

Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Spinal muscular atrophy (SMA) is a neuromuscular disease characterized by the degeneration of motor neurons leading to progressive muscle weakness and atrophy. It is caused by mutations in the *SMN1* gene. Recently, the first SMA therapy based on antisense oligonucleotides, namely nusinersen, that modulates the splicing of the paralogous gene *SMN2*, has been approved. Moreover, the first gene therapy clinical trial using adeno-associated virus (AAV) vectors encoding SMN showed positive results in patients' survival and motor milestones achievement. Based on these data, phase III gene therapy trials are ongoing in USA and Europe. Nevertheless, it is likely that in SMA patients the elevated SMN level may still be insufficient to restore motor neuron function lifelong, in particular if the patients are treated in symptomatic stages. To identify novel SMN independent therapeutic tar-

gets we recently analyzed SMA patient specific induced pluripotent stem cells (iPSC) 2/3D disease models. RNA sequencing on human SMA iPSC derived motor neurons revealed many deregulated genes, such as the neurexin and synaptotagmin families, that are implicated in critical motor neuron functions. Motif-enrichment analyses of differentially expressed/spliced genes pointed out a common motif, motif 7, which is a target of SYNCIP. Remarkably, SYNCIP binds only with full-length SMN, modulating several downstream motor neuron transcripts, including SMN itself. We demonstrated that SYNCIP overexpression rescued SMA motor neurons, due to the subsequent increase in SMN and their downstream target NRXN2 and ameliorated SMN-loss-related pathological phenotypes in animal models. Thus SMN/SYNCIP complex may represent a novel potential therapeutic target for SMA.

Newborn Screening in neuromuscular disease

Donati M.A.¹, Tubili F.¹, Morrone A.², la Marca G.³, Daniotti M.¹

¹ Metabolic and Muscular Unit, Meyer Children's Hospital, Italy; ² Molecular and Cell Biology Laboratory, Paediatric Neurology Unit and Laboratories, Meyer Children's Hospital, Italy; ³ Newborn Screening, Biochemistry and Pharmacology Laboratory, Pediatric Neurology Unit and Laboratories, Meyer Children's University Hospital, Florence, Italy

Expanded newborn screening (ENBS) by LC-MS/MS can detect inborn errors of metabolism life-threatening in a presymptomatic phase. In 2004, with a regional law, Tuscany was the first Italian Region to screen for 40 inborn errors of metabolism including fatty acid beta-oxidation defects (FAOD). With the law 167/2016 in Italy the ENBS is part of essential levels of assistance. In Tuscany, after a 3-years pilot project, lysosomal storage disease (MPSI, Pompe and Fabry disease) are included in the panel with a regional law.

FAOD may occur with neuromuscular involvement both in early and late onset. Through ENBS we have diagnosed carnitine primary, VLCAD and LCHAD defects. In some case newborns showed iperCKemia or cardiomyopathy at the recall; in one case SIDS occurred before the ENBS results. It is important the chronic management of these patients with a low-fat diet and avoidance fasting. CPT2 deficiency usually presents with rhabdomyolysis starting in adolescents and young adults; in ENBS, the (C16 + C18:1)/C2 ratio is altered and the enzymatic assays and molecular analysis confirmed the diagnosis, but we keep in mind that ENBS can be negative.

Since 2014 we screened 80.000 newborns for Pompe disease with a diagnosis of one newborn affected by cardiomyopathy in which ERT was started a one month of

age. We have diagnosed 9 patients with late onset form, actually in follow up.

In Tuscany and Lazio a pilot project for spinal muscular atrophy NBS will start, since the recent availability of new treatment strategies can change the disease course.

Myasthenia Gravis: an update

Evoli A.

Istituto di Neurologia Università Cattolica del S. Cuore, Policlinico A. Gemelli IRCCS, Roma, Italia

In the past few years, there have been several advances in the management of myasthenia gravis (MG). In patient with generalized anti-acetylcholine receptor positive MG (AChR-MG), thymectomy not only proved superior to conservative therapy, but appears to confer prolonged benefits. Increased availability of biologics provides targeted immunotherapies. Eculizumab, that inhibits complement activation, proved effective in refractory AChR-MG in a phase III trial. B cell depletion with rituximab is thought to be the most effective therapy for MG with anti-muscle specific tyrosine kinase antibodies, although a phase II trial in AChR-MG failed to meet the primary outcome. Increase of IgG clearance through blocking the neonatal Fc receptor (FcRn), is a new therapeutic tool in MG. Efgartigimod, an IgG1-derived Fc fragment, significantly improved AChR-MG and depleted specific antibodies; the anti-FcRn rozanolixizumab is currently under study. Belimumab, which targets B-cell activating factor (BAFF), was not superior to placebo in a phase II trial. Tolicizumab (IL-6 receptor antagonist), ruxolitinib (janus kinase inhibitor) and bortezomib improved MG in single case reports. A more extensive usage of new immunotherapies will require a careful patient selection, surveillance of potential side effects and cost/benefit appraisal.

New insights/tools in molecular diagnosis of DMD

Ferlini A.^{1,2}

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Neuromuscular diseases (NMDs) have been privileged by intense genetic characterization via pioneering and fruitful collaboration between neurologists and geneticists; genetic diagnosis of NMDs is now an integral part of the diagnostic flowchart, mandatory for eligibility to treatments.

Next-generation sequencing (NGS) is increasingly being applied to NMD testing, leading to a remarkable amelioration of genetic diagnosis, via new genes or new phenotypes discovery; nevertheless its clinical translation

is incomplete and standard molecular genetics tests are still needed.

Indeed, NGS cannot always ensure an exhaustive mutation detection, since still some mutation types (as copy number variations and dynamic mutations) escape its identification. Since NGS allows high parallelism and throughput runs, several samples can (and should) be contemporarily analyzed to cover the run costs. For some ultra-rare NMDs this might not be ideal and these conditions may have consequently a delayed diagnosis. Prenatal diagnosis is still mainly based on classical molecular testing, due to the pregnancy timing.

DMD (or dystrophinopathies, including Duchenne and Becker muscular dystrophies, X-linked dilated cardiomyopathy, and other milder phenotypes, as quadriceps myopathy or isolated high CK) can benefit of standard diagnostic genetic tests based on CNV detection (typically MLPA) and exon sequencing (typically by Sanger or NGS methods).

Using these two strategies a genetic diagnosis of dystrophinopathies is achieved in the vast majority of patients, with a detection rate close to 99%.

A few atypical mutations or unsolved cases do exist requiring a deep characterization based on RNA or genome profiling. Ideally, it would be greatly desirable to have a single genetic method able to detect all possible occurring mutation types in DMD (and more generally NMD) gene.

Finally, a reflection can be made about the powerfulness of the new non-invasive prenatal diagnostic testing (NIPT), from one side, and the new personalized therapies now available, from the other. Finding synergic strategies for these two interventions in DMD/NMDs would be greatly beneficial for patients and families.

Dusty Core Myopathies

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Several morphological phenotypes have been associated to *RYR1*-recessive myopathies. We recharacterized the *RYR1*-recessive morphological spectrum by a large monocentric study performed on 54 muscle biopsies from a large cohort of 48 genetically confirmed patients, using histoenzymology, immunohistochemistry, and ultrastructural studies. We also analysed the level of RyR1 expression in patients' muscle biopsies. We defined "dusty cores" the irregular areas of myofibrillar disorganisation characterised by a reddish-purple granular material deposition with uneven oxidative stain and devoid of ATPase activity, which represent the characteristic lesion in muscle biopsy in 54% of patients. We named Dusty Core Disease (DuCD) the corresponding entity of congenital myopathy. Dusty cores had peculiar histological and ultrastructural characteristics compared to the other core diseases. DuCD muscle biopsies also showed nuclear centralization and type1 fibre predominance. Dusty cores were not observed in other core myopathies and centronuclear myopathies. The other morphological groups in our cohort of patients were: Central Core (CCD: 21%), Core-Rod (C&R:15%) and Type1 predominance "plus" (T1P+:10%). DuCD group was associated to an earlier disease onset, a more severe clinical phenotype and a lowest level of RyR1 expression in muscle, compared to the other groups. Variants located in the bridge solenoid and the pore domains were more frequent in DuCD patients. In conclusion, DuCD is the most frequent histopathological presentation of *RYR1*-recessive myopathies. Dusty cores represent the unifying morphological lesion among the DuCD pathology spectrum and are the morphological hallmark for the recessive form of disease.

Italian Consensus on rehabilitation in MDs

Lombardo M.L.* and Italian Consensus Group
Neuropsychiatra Infantile presso Centro di riabilitazione UILDM Lazio Onlus

The clinical demand for appropriate and precise

clinical recommendations in a rehabilitative program is a daily request in management of patients with muscular dystrophies (MD) and related disorders. Internationally validated guidelines are only available for Duchenne muscular dystrophy and few additional congenital forms but it is unclear if these can easily be applied to other forms of MD.

Based on these issues we revised pertinent literature contributions related to neuromuscular rehabilitation in MDs, with the final goal to draw up a largely agreed document of practical recommendations through a consensus conference methodology. This is critical for both clinicians, offering a rapid tool to counsel patients, and patients themselves, needing assurance they are receiving the best care at the best time of their disease story. The document was committed by Unione Italiana Lotta alla Distrofia Muscolare (UILDM), an Italian association of patients suffering from neuromuscular diseases, and has the purpose to provide technical, updated and detailed directions to clinicians, patients and caregivers for rehabilitation in MDs both in childhood and in adult age. It will be based on the major literatures evidences and expert opinions, will include specific definition of rules and responsibilities of the professional figures involved in the rehabilitation program, and will offer specific information on frequency, intensity, Time and Type (F.I.T.T) of physical activity. Furthermore, it will detail practical measures to manage contractures, mobility and daily activity life. We anticipate that the Italian consensus will represent a base for official standardized guidelines and a new scenario of the patient-doctor alliance.

New genes: how to tailor treatment for congenital myasthenias

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Congenital myasthenic syndromes (CMS) are a heterogeneous group of genetic disorders affecting the neuromuscular junction (NMJ) and clinically characterized by non-progressive fluctuating muscle weakness and fatigability. CMS diagnosis requires a high clinical suspicion, because they are rare diseases and myopathic features are often more evident than myasthenic signs. To date, more than 30 genes, encoding for pre-synaptic, synaptic and post-synaptic NMJ proteins, have been implicated in CMS, which are recessively inherited in most of the cases. CMS usually present at birth or within the first 3 years of life, although around 20% of the patients display later onset, including adult age. Mutations are identified in only about 50% of CMS patients, but many novel genes have been recently discovered, most of them codifying for proteins localized at presynaptic level, as synaptobrevin-1,

Munc 13-1, synaptogamin-2, laminin $\alpha 5$, vesicular acetylcholine transporter, synaptosomal-associated protein 25, high affinity choline transporter solute carrier family 5 member 7, myosin 9a, and rabphilin 3A. Mutations in these genes cause CMS usually presenting at birth and often in association with involvement of central nervous system, mainly showing intellectual disability, seizures or brain malformations/atrophy. CMS treatment is based on different symptomatic therapies, which could markedly improve symptoms over the time. Interestingly, drugs with positive effects on specific CMS, may worsen other CMS forms. Hence, definite genetic diagnosis is highly recommended to optimize pharmacologic treatment.

Pharmacological therapies in DMD: what's on?

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Duchenne muscular dystrophy (DMD) is a progressive X-linked degenerative muscle disease due to mutations in the DMD gene. Improvements in the standard of care for DMD have led to improved survival. Nowadays the only treatments obtainable in the clinical arena for DMD are corticosteroids and the drug Ataluren in EU and also Exondys 51 in US. Nevertheless, several other therapeutic options are currently tested in clinical trials. Novel treatments for DMD have focused on reducing the dystrophic mechanism of the muscle disease, modulating utrophin protein expression, and restoring dystrophin protein expression. Among the strategies to reduce the dystrophic mechanisms are: 1) inhibiting inflammation, 2) promoting muscle growth and regeneration, 3) reducing fibrosis, and 4) facilitating mitochondrial function. The agents under investigation include a novel steroid, myostatin inhibitors, idebenone, an anti-CTGF antibody, a histone deacetylase inhibitor, and cardiosphere-derived cells. For utrophin modulation, AAV-mediated gene therapy with GALGT2 is currently being investigated to upregulate utrophin expression. Finally, the strategies for dystrophin protein restoration include 1) nonsense readthrough, 2) synthetic antisense oligonucleotides for exon skipping, and 3) AAV-mediated micro/minidystrophin gene delivery. With newer agents, we are witnessing the use of more advanced biotechnological methods. These potential breakthroughs provide significant promise.

Inclusion-body myopathy: an up to date

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The most common form of hereditary IBM is due to mutations of the *GNE* gene that is involved in sialic acid (SA) biosynthesis. More than 170 different *GNE* mutation have been characterized to date. The cellular pathogenic mechanism/s of *GNE* myopathy are mostly undefined but abnormal sialylation and processing of proteins may have a critical role. Most of the evidence point out to *GNE* myopathy being a disease of deranged proteostasis with increased protein transcription, abnormal post-translational modifications and inadequate disposal rather than based solely on the impairment of a specific molecular player.

The identification of *GNE* gene defect has allowed recognition of phenotypic variants although only about 20% of variability of clinical features can be explained by *GNE* mutations (p.D207V mutation associated with later onset of symptoms, p.V603L with an earlier onset). Therefore other epigenetic factors may modulate the clinical phenotype accounting also for heterogeneous rate of progression. Since early restoration of a normal sialylation status within cellular environment may help to recover muscle fibers homeostasis, an extended release formulation of SA to achieve a stably elevated serum level with significant muscle uptake was developed to be used in a phase 3 clinical trial. This was the largest clinical trial in *GNE* myopathy patients and demonstrated that SA is not superior to placebo in improving muscle strength. The study provided novel viewpoint on conducting trials in patients with distal myopathies as understanding differences between patients with *GNE* myopathy will help to develop better outcome measures sensitive for early detection of therapy efficacy. Oral treatment with ManNAc, a precursor in the SA biosynthetic pathway that prevented muscle weakness in a murine model of *GNE* myopathy, is now being studied in an open-label phase 2 study. Gene therapy is also being explored to treat *GNE* myopathy.

Central Nervous System involvement in LOPD

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Although Pompe disease has been considered for a long time a primary muscle disorder, it is becoming evident that the pathophysiological mechanism underlying the disease involves several organs and tissues such as central nervous system.

Similarly to other lysosomal storage disorders (i.e. Fabry disease), in the late onset form of Pompe disease (LOPD), vascular abnormalities have been reported with a prominent involvement of the cerebrovascular system

presenting with aneurysms, vertebrobasilar dolichoectasia (VBD) or dilative arteriopathy. The recurrence of these vascular anomalies is higher than in the general population. In a previous study we demonstrated the presence of unruptured intracranial aneurysms in 9.5% of examined patients as well as an elevated frequency (47%) of VBD. In a recent study we evaluated CNS involvement performing a comprehensive investigation on morphological and functional brain areas, demonstrating characteristic signs of a small vessels disease (lacunar encephalopathy) in a substantial number of LOPD patients

The impact of brain damage on cognitive functions in LOPD has been evaluate in few studies revealing a moderate involvement of cognitive ability with a prevalent impairment in visual-constructive activities and executive functions.

A possible physiopathological explanation is that glycogen accumulation in the vessel walls induced a dilative arteriopathy with dolichoectasia that could be responsible for cerebrovascular alterations leading to multiple ischemic insults.

Nusinersen open access: what did we learn?

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Spinal muscular atrophy (SMA), is an autosomal recessive disorder caused by mutations in the *survival motor neuron (SMN1)* gene. In the last few years a number of therapeutic approaches have been proposed for SMA and some of them have completed phase 2 and 3 clinical trials

The clinical efficacy of Nusinersen, an antisense oligonucleotide designed to increase full-length SMN protein levels, had been initially suggested by phase 2 trials in infants with infantile-onset SMA and subsequently by a phase 3 sham-controlled studies showing increased survival and significant improvements on motor functional scales in infants receiving Nusinersen compared to sham controls.

The aim of EAP was to report twelve month changes after treatment with Nusinersen in a cohort of 85 type I Spinal Muscular Atrophy patients.

There was a difference between baseline and the 12 month scores on both the CHOP INTEND and the HINE for the whole group ($p < 0.001$) as well as for the subgroups with 2 *SMN2* copies ($p < 0.001$), and for those with 3 *SMN2* copies ($p < 0.001$). The difference was found not only in patients younger than 210 days at baseline ($p < 0.001$) but also in those younger than 5 years on the CHOP INTEND and younger than 2 years on the HINE.

Our results, expanding the age range and the severity of type I patients treated with Nusinersen over one year, provide additional data on the range of efficacy of the drug that

will be helpful to make an informed decision on whether to start treatment in patients of different age and severity.

New imaging techniques and their possible role in planning muscle clinical trials

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Magnetic resonance imaging (MRI) has progressively become an important tool for the diagnosis and monitoring of neuromuscular diseases, with the ability to display the severity and distribution of pathology, to identify involvement patterns and, sometimes, to even suggest the diagnosis in certain difficult cases where other data are unequivocal. The advances in imaging techniques have importantly expanded the potential to assess the ongoing pathology in the skeletal muscle, going beyond the knowledge provided by conventional imaging. With the so called quantitative MRI (qMRI), in fact, a number of specific characteristics can today be studied and quantified, ranging from tissue composition, architecture, mechanical properties and perfusion, only to cite a few. The application in clinical practice of such advanced quantitative techniques is already leading to a further evolution in the approach to neuromuscular diseases, even considering a number of technical difficulties. Above all, the possibility to precisely track the changes of specific features of the underlying pathology in the muscle (i.e. edema, fat replacement, alteration of metabolism, diffusivity properties etc.) appears of particular interest to define and project clinical trials in this field. The presentation will particularly focus on what qMRI can effectively provide to the clinician and how it can be implemented not only as a diagnostic tool but also as a promising tracker of disease progression and of response to therapy, when available.

Therapeutical perspectives for DMD: where are we going?

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Duchenne muscular dystrophy (DMD) is devastating lethal neuromuscular disease due to loss-of-function mutations in the *DMD* gene leading to a complete dystrophin deficiency in skeletal muscle. Recent years have seen a renaissance of therapeutical approaches, including the partial correction of the underlying gene defect or the treatment of its downstream consequences.

The first molecular treatment for DMD approved in Italy is a small molecule (ataluren) able to read through

stop codons, allowing the production of functional dystrophin in nonsense mutation DMD. This treatment has been shown to be safe and results in a significant functional improvement in patients. Antisense oligonucleotides (AO) targeted to restore the *DMD* frame in single or multiple exon deletion are in advanced clinical phase. Deletions amenable to exon 51 skipping are treated with eteplirsén, a morpholino antisense oligomer, which triggers excision of exon 51 during splicing, allowing for the synthesis of an internally deleted, but partially functional dystrophin. Eteplirsén has been approved by FDA but not yet by the European Medicines Agency. Other chemistries for AOs are in advanced developmental phase with the goal to enhance delivery and efficiency.

Systemic adeno-associated virus (AAV)-based gene therapy for DMD is currently into phase I-II clinical trial. A rAAVrh74.MHCK7-micro-dystrophin to achieve targeted skeletal and cardiac muscle expression of a shortened functional dystrophin protein has been administered to 4 DMD boys. Preliminary results showed lack of serious adverse events and good dystrophin re-expression.

The therapeutical scenario for DMD is rapidly changing including disease-modifying therapies and personalized genetic therapies.

Update on lipid myopathies

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Lipids are essential for the structural and functional maintenance of cell. Some disorders of lipid metabolism may produce prevalent damage in skeletal and cardiac muscle. The spectrum of lipid myopathies (LM) is expanding with the knowledge of new molecules involved in fatty acid metabolism. Most of the LM are caused by a gene defect, rarely by a toxic cause or a deficiency in the diet. Several classifications of the LM have been proposed based on clinical or morphological features, but a classification based on genetic defect seems more complete.

Clinically, LM result in acute or indolent clinical pictures, in all ages, sometime life-threatening. The muscular symptoms consist in weakness and fatigability, they can be fluctuant or fixed, rarely with muscle atrophy. When muscular symptoms are acute, occur with massive muscle necrosis (rhabdomyolysis and myoglobinuria) and are triggered by metabolic stress, as fasting, maximal exercise, infections. Muscle biopsy can show excess of lipid, but normal findings do not rule out a lipid myopathy, because some of these diseases do not cause excessive lipid storage. Acylcarnitines profile and urinary organic acids dosage can help in the diagnostic work-up of lipid myopathies before going to the genetic confirmation. Prevention of triggers should be recommended in all LM regardless of the gene involved. Basic life-saving

procedures must be adopted in case of suspected LM with rhabdomyolysis. In other condition administration of deficient substances is sufficient to resolve symptoms. New molecules are been tested for the treatment in other cases.

Heart involvement in NMDs: different patterns and new therapeutic approaches

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Cardiac disease is a common clinical manifestation of several neuromuscular disorders (NMDs), most notably the muscular dystrophies (MD). In recent years, cardiac involvement has been observed in a growing number of genetic muscle diseases, and considerable progress has been made in understanding the relationships between disease skeletal muscle and cardiac muscle disease. In several forms of MD, cardiac disease may even be the predominant manifestation of the underlying genetic myopathy and precede of many years the onset of skeletal muscle involvement. Heart involvement may result from pathologic changes in the myocardium and/or the cardiac conduction system. Involvement of the myocardium manifests most frequently as a cardiomyopathy evolving to the final stage of dilation and systolic dysfunction. Cardiac conduction defects, supraventricular and ventricular arrhythmias are also common cardiac manifestations of NMDs. Arrhythmias may evolve into life-threatening ventricular tachycardias, asystole, or even sudden cardiac death. Cardiac involvement carries great prognostic significance on the outcome of dystrophinopathies, laminopathies, desminopathies, nemaline myopathy, myotonic dystrophies, metabolic myopathies, Danon disease, and Barth-syndrome. However an increasing number of genes that cause muscle diseases, are continuously reported as capable of causing even cardiac involvement. The spectrum of cardiac dysfunction in these inherited muscle disorders will be presented and practical recommendations for their monitoring and management proposed. An early detection of MD-associated cardiomyopathy is of considerable importance, as a prompt institution of cardio-protective medical or supportive therapies may slow adverse cardiac remodeling and attenuate heart failure symptoms or avoid the occurrence of sudden cardiac death in these patients.

CNS in Infantile Pompe Disease

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As Enzyme Replacement Therapy (ERT) enables patients with classic infantile onset Pompe disease (IOPD) to reach adulthood, white-matter abnormalities are becoming increasingly evident at neuroimaging, affecting the neuropsychological development. Previously published studies in children with IOPD showed IQ (Intelligence Quotient) scores in the lower normal range without any evidence of cognitive decay over time, but recent studies in ERT treated children showed wider cognitive development range from stable and normal to declines that lead to intellectual disabilities. In our cohort, we tested 6 patients, all of them in the normal range (IQ between 85 and 121 at baseline). In two of them, a slight decline of IQ was observed after two years, but still in the normal range. These important observations need programs to capture central nervous system (CNS) involvement in larger patient cohorts, including late-surviving IOPD patients.

A longitudinal multicentric study for comprehensive CNS functions evaluation in Italian children affected by Pompe disease is proposed, to collect longitudinal data with a standardized protocol, exploring cognitive features, speech and hearing functions, behavioural data, and neuroradiological features, that will be compared to motor functions; as an innovative, relevant observation, this protocol will also investigate the children adaptive behaviour, assistance needs and quality of life. Current knowledge on CNS involvement in IOPD should be included in the counselling of parents before the start of treatment, and the brain should be considered as an additional target in the development of next-generation therapeutic strategies.

The FSHD diagnostic challenges |

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At present molecular diagnostics in genetic diseases is facing several challenges. Genomic investigations in human diseases are easily accessible, but the relationship between observed phenotypes and their underlying genotypes, modes of transmission and frequencies of diseases and variants maybe of difficult interpretation. Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common neuromuscular diseases. The clinical and molecular complexity has complicated FSHD diagnosis. The disease first presents with weakness of the facial and shoulder girdle muscles, followed by the ankle dorsiflexors and finally the proximal leg muscles. However, there are many patients who do not fit this well-known classical FSHD phenotype. Infantile and late-onset cases are not uncommon; clinical severity and sequence of involvement in different muscle groups can vary. Presently,

two genetically distinct disease subtypes, FSHD1 and FSHD2, have been described. FSHD1 is associated with contractions of a polymorphic macrosatellite repeat on chromosome 4q35.2. FSHD2 defines a smaller number of affected individuals carrying two D4Z4 arrays in the healthy range. FSHD1 and FSHD2, considered clinically undistinguishable, are characterized by DNA hypomethylation of the 4q35 D4Z4 array. The phenotypic and genetic classification of patients and families will be crucial to define the natural history of disease, to propose suitable measure of outcome and to identify new susceptibility/causative factors contributing to FSHD.

Lambert-Eaton Myasthenic Syndrome: treatment and open issue

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Lambert-Eaton myasthenic syndrome (LEMS) is a very rare pre-synaptic disorder of the neuromuscular and autonomic transmission. It is caused by antibodies interacting with the voltage gated calcium channels (VGCCs) of P/Q-, N- and R-type. Over 50% of LEMS patients have an underlying tumour, most often small-cell lung cancer (SCLC). VGCC antibodies are more frequently detected in LEMS patients with SCLC, being present in up to 100% when compared to LEMS patients without underlying cancer. However the molecular mechanisms of LEMS remains largely unknown a careful screening for the early detection of the possible associated cancer is a crucial step. Almost all patients will benefit initially from symptomatic treatment with 3,4-diaminopyridine (3,4-DAP), a potassium channel blocking agent. Often, additional treatment is required in the form of immunosuppression. Prednisolone has been shown to be effective from observational studies; to date there are no data to suggest that intravenous immunoglobulin (IVIg) infusion is an effective long-term treatment for LEMS symptoms. Plasma exchange has been used effectively as acute treatment of severe LEMS symptoms. In patients with symptoms refractory to oral immunosuppression, novel treatment approaches may be beneficial, such as the anti-CD20 monoclonal antibody agent, Rituximab.

Genome editing, disease modelling and therapeutics

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Muscle weakness and floppiness in infancy are the most common symptoms of neuromuscular disease and may result from a large set of genetic aetiologies lead-

ing to muscle dysfunction or neuronal and neuromuscular junction abnormalities. To date, more than 780 monogenic neuromuscular diseases, linked to over 400 different genes, have been identified in humans and therapeutic opportunities have been proposed for few. Genome-editing methods, especially the CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated protein 9) system, hold clinical potential for curing many monogenic disorders, including NMD such as Duchenne muscular dystrophy, and myotonic dystrophy type 1. Importantly, lack of curative treatments available for most neuromuscular disorders (NMD) is in part due to the lack of in vivo models that can be utilized in high-throughput approaches for discovery of effective and personalized therapeutic options.

We will review current bottlenecks in making CRISPR-Cas9-mediated gene editing a therapeutic reality, show new success obtained in Duchenne muscular dystrophy, and we will outline recent strategies that implement disease modelling and open a new path to personalized therapies.

Oculopharyngeal muscular dystrophy

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Oculopharyngeal muscular dystrophy (OPMD) is a late-onset degenerative disorder clinically characterized by ptosis, dysphagia, axial and proximal limb muscles weakness. Swallowing difficulties mainly determine bad prognosis, increasing the risk for aspiration pneumonia and poor nutrition. In the majority of cases the disease shows an autosomal dominant inheritance. OPMD is caused by mutations in the poly(A)-binding protein nuclear-1 (PABPN1) gene, resulting in a short GCG expansion in the polyalanine tract of PABPN1 protein. Expression of PABPN1 appears to be ubiquitous but symptoms in OPMD are limited to skeletal muscles. At skeletal muscle level, the pathological hallmark of OPMD is the accumulation of filamentous intranuclear inclusions detecting by electron microscopy, as well as the presence of muscle fibers with rimmed vacuoles. Symptomatic treatment is gradually introduced during the progression of disease, while no pharmacological treatment is presently available. In vitro and in vivo disease models are described and novel gene and cell therapies are currently under study.

The role of muscle MRI in FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is

caused by a unique, non-conventional genetic mechanism, whose downstream pathophysiology is still largely elusive.

Using MRI to characterize muscle involvement and follow patients up, a picture has emerged in which sequential bursts of degeneration involve individual muscles in an asynchronous manner. This peculiar radiological progression is in line with results obtained with multidisciplinary approaches, thus configuring FSHD as a “muscle by muscle” disease.

In this context, a common feature of FSHD is the presence of areas of hypersignal on STIR (short-tau inversion recovery) sequences, which represent areas of muscle edema/inflammation. Imaging and molecular evidences point toward the fact that these STIR+ lesions mark a different phase of disease at single muscle level. Consequently, the detection of these abnormalities is important to monitor disease evolution.

Even in the frame of this highly peculiar disease progression, which contributes to the pronounced clinical variability and atypical presentations, still muscle imaging is able to identify muscles that are more likely to be involved, and others that are selectively spared. Reasons of this different susceptibility are still unknown and definitely represent an intriguing field of research.

Relevant for clinical trials, muscle imaging can be therefore useful for choosing the patients who are in an “active” phase of the disease, as well as for correctly stratifying patients, and accurately following muscle involvement over time. Longitudinal, large cohort imaging studies using both standard and quantitative MRI are definitely needed to move forward in our understanding of FSHD natural history and pathophysiology.

Update on skeletal muscle glycogenoses

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Muscle glycogenoses are a heterogeneous group of rare disorders where skeletal muscle is primarily compromised but even other organs are often involved.

These disorders are relatively rare and may show a wide range of symptoms at infancy, childhood or adulthood.

More recently, increased awareness, detailed characterization of the clinical spectrum and improved diagnostic workup have made easier to recognize these clinical entities although this is still a challenge either in the infantile or in the adult cases

Innovative diagnostic techniques such as use of newborn screening, Dried Blood Spot (DBS), different biochemical approaches or molecular genetic methods as NGS (Next Generation Sequencing) or whole exome/genome sequencing, are currently considered to better

evaluate either known or emerging clinical entities in the field of muscle glycogenoses

Nowadays, it is important to update the evaluation of these disorders, also taking into consideration the main pathogenic mechanisms, mainly involving skeletal muscle dysfunction but also other organs or apparatuses.

In fact, reaching as early as possible the diagnosis, will allow physicians to early apply a specific therapy where it is already available in an attempt to limit progressive degeneration of organs.

Large scale genotype-phenotype correlation study in 1703 carriers of D4Z4 reduced alleles from the Italian National Register for FSHD

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Clinical reports show high phenotypic heterogeneity in carriers of D4Z4 reduced alleles (DRA) ranging from healthy carriers to typical FSHD to complex muscular phenotypes. 3% of healthy people carry one DRA.

The Italian Clinical Network for FSHD applied the CCEF, describing nine clinical categories, on 1703 individuals, 846 index cases and 857 relatives carrying one contracted alleles with 1-10 D4Z4 repeats.

Subjects' stratification based on D4Z4 allele size shows that clinical categories are distributed in all groups. Disease penetrance is reduced with 44.3% of healthy relatives. The age at evaluation of healthy relatives is not significantly different from that of relatives who developed the full disease. The facial-sparing FSHD phenotype represents 10.2% of index cases regardless the size of the D4Z4 contracted allele.

Our study shows that standardized clinical evaluation can facilitate the comprehension of phenotypic diversity among carriers of D4Z4 contracted alleles and guide clinical studies and research.

Titin role in NMDs: overview and new phenotypes

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The first titinopathy was reported by clinical delineation long before it was known to be a titinopathy. This dominant late onset distal myopathy in Finland was reported as Tibial muscular dystrophy and after the Titin (TTN) gene mutation identification in 2002, together with the childhood onset LGMD R10 (2J) in the homozygotes, only hereditary myopathy with early respiratory failure (HMERF) was associated with Titin mutations before the era of NGS. After now 7 years of using exome sequencing and NGS gene panels the variety of the phenotypes caused by TTN mutations has exploded covering currently more than 10 different disease phenotypes. The easier part of confirming TTN variants as pathogenic are the deleterious truncating variants: nonsense, frameshifts and clarified splice site mutations. Thus congenital and severe childhood diseases with biallelic deleterious mutations have been extensively reported and covered. However, there are also congenital forms without progressive loss of muscle tissue due to mutations in exons expressed in fetal development but not after birth. The big unsolved problem with variants is still how to categorize missense variants because predictive tools for pathogenicity are misleading and the gene cannot be expressed in model systems. Thus only few missense mutations have confirmed pathogenicity, above all the missenses in exon 344 causing HMERF disease, but also missenses in the last exon 364 causing distal myopathy and some missenses with confirmed pathogenicity in compound heterozygosity with known mutations. The spectrum of titinopathies now covers almost everything from fetal lethality to almost asymptomatic very late onset distal ankle weakness after age 65.

Five-year follow-up study and new predictors in disease progression for facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is a genetic disorder in which the classical phenotype is characterized by a progressive muscular weakness, involving facial, shoulder and upper arms muscle; the molecular feature is a contraction in D4Z4 repeat element on chromosome 4.

Currently, approved treatments are unknown and disease natural history is missing and it is crucial defining modifying outcome measure in prevision of future therapeutic clinical trials. The main aim of our study is evaluating disease progression within a 5-years time period in a cohort of 246 subjects from the Italian National Registry for FSHD. Patients, all genetically confirmed, were underwent to the same protocol including clinical evaluation using FSHD Clinical Score for functional impairment, Medical Research Council (MRC) for muscle strength examination and in the second evaluation the CCEF (Comprehensive Clinical Evaluation Form), a recent published tool in which FSHD patients are divided in different subcategories according to the affected districts. Study population was divided in index case and relatives

and we extrapolated the Delta FSHD Score (Δ FSHD score) between first and second control.

In our follow-up study 141 index cases and 105 relatives were admitted. 38.2% of subjects did not show progression of disability; 64.9% with mild or no disability (FSHD scores 0-2) at baseline maintained the same FSHD score; a baseline FSHD score ≥ 3 was associated with a more rapid progression, with 80% of patients increasing the FSHD score (Δ FSHD score ≥ 1). Disease progression is rapidly in index cases compared to relatives (Δ FSHD score 2.3 *versus* 1.2) while gender effect did not observed.

According MRC examination, tibialis anterior muscle was significantly more affected muscle with difference between probands and relatives (35.5% of index cases *versus* 4.8% relatives with MRC $\leq 3/5$).

A category (which consists in the complete phenotype with affected facial and shoulder girdle districts) is associated with higher FSHD score at baseline and more deterioration at follow-up; we observed a slower progression in subjects with incomplete clinical phenotype (B category, $P < 0.0001$).

In therapeutic era for neuromuscular diseases, our study provides clues for the understanding of the pathophysiology of FSHD disorder. Differences between familiar and index cases underline importance of genetic background.

Update on respiratory treatments in Neuromuscular Disorders

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Individuals with Neuromuscular Disorders (NMDs) not rarely develop primarily ventilatory impairment, although the probability of its occurrence is different, according to baseline disease. Once respiratory muscle impairment has become pronounced, patients can use both inspiratory and expiratory muscle aids to prevent the onset of acute and/or chronic progressive respiratory failure, have excellent prognoses with long-term home Non-Invasive Mechanical Ventilation (NIV) and usually do not require tracheostomy for ventilatory reasons.

By releasing effective mechanical ventilation via a nasal mask or a mouthpiece, NIV is characterized by ease of administration, preservation of upper airway function and low cost. Its long-term application is indicated when spontaneous respiratory muscle efforts are unable to sustain adequate alveolar ventilation, causing chronic stable or slowly progressive ventilatory failure. Administration of NIV to NMD patients with chronic hypoventilation may be expected to improve physiologic function and quality of life as well as decrease the frequency of episodes requiring

acute care facilities (1). If NIV is not well-tolerated or unsuccessful, a decision to electively perform a tracheostomy can be taken before the patient has developed major complications of chronic ventilatory insufficiency (2).

The onset of Acute Respiratory Failure (ARF) may be caused by airway encumbrance with mucous, as a result of weakened respiratory muscles and an inability to cough effectively. A non-invasive approach to the management of tracheo-bronchial secretions, based on the combination of expiratory muscle aid and NIV may result in a reduced need of nasal suctioning and conventional intubation, and/or tracheostomy (3).

MRI in muscle disorders

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Multiple treatment options are emerging for muscle diseases, but conduction of clinical trials in the field is challenged by the rarity and slow progression of the diseases. There is therefore a great unmet need to define biomarkers and outcome measures that are sensitive to change in rare, slowly progressive patient populations. MRI of muscle has attracted increasing attention in recent years as a possible outcome measure. It has the advantage that it is non-invasive, almost independent of patient-cooperation, and unlike functional tests, is independent of losing skills during a study, and can therefore be applied to the whole phenotypic spectrum of a disease.

Muscle disease activity and progression is reflected by increased muscle water T2 and muscle fat replacement. If therapeutic interventions are efficacious, they should reduce muscle water T2 and replacement of muscle by fat on MRI, which suggests that skeletal muscle MRI could be a suitable biomarker for treatment efficacy.

The muscle fat content correlates with muscle function in most muscle diseases. Changes in muscle fat content have been detected after a year in many muscular dystrophies, in many cases before changes in muscle function can be detected. As the body of evidence favoring MRI as a sensitive outcome for change in muscle pathology is growing, it will likely play an important role as an outcome measure in future clinical trials.

From a diagnostic viewpoint, muscle MRI is also helpful as individual diseases often show particular patterns of muscle involvement. Even so, there is quite a bit of overlap in several diseases, which makes MR-guided diagnosis difficult even for experts in the field. New developments in artificial intelligence tools aimed at analyzing muscle MRI show promise in providing quick and precise diagnosis for these diseases. Current status of muscle MRI as a diagnostic and biomarker tool in longitudinal studies will be reviewed.

Mitochondrial disorders: from gene discovery to pathomechanism and experimental therapy

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Mitochondria are the major source of ATP that is synthesized by the respiratory chain through the process of oxidative phosphorylation (OXPHOS), a complex biochemical process carried out through the dual control of physically separated, but functionally interrelated, genomes, nuclear and mitochondrial DNAs. The genetic and biochemical intricacy of mitochondrial bioenergetics explains the extreme heterogeneity of mitochondrial disorders, a group of highly invalidating human conditions, for which no effective treatment is nowadays available. In addition to bioenergetic failure, other mechanisms are probably predominant in the pathogenesis of specific

syndromes, such as alterations of cellular redox status, the production of reactive oxygen species, compromised Ca^{2+} homeostasis, mitochondrial protein and organelle quality control, and mitochondrial pathways of apoptosis. By investigating selected families and patients, we have identified several new disease genes, each responsible of distinct defects of the respiratory chain, mtDNA metabolism, or both, associated with paediatric or adult-onset clinical presentations. Recently published and still unpublished findings will be presented and discussed. Structural analysis and the creation of ad hoc recombinant lines in yeast, flies, and mice have allowed us to dissect out the molecular consequences of the ablation or defects of some of these proteins, and their physical status in normal and disease conditions. These models have also been exploited to implement experimental therapeutic strategies, based on gene and cell replacement, or pharmacological control of mitochondrial biogenesis.

ABSTRACTS OF ORAL COMMUNICATIONS

(in alphabetical order of the first Author)

European muscle MRI study in Limb Girdle Muscular Dystrophy Type 2A (LGMD2A)

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Background. Limb Girdle Muscular Dystrophy type 2A (LGMD2A) is a progressive myopathy caused by deficiency of calpain 3, a calcium-dependent cysteine protease of skeletal muscle, and it represents the most frequent type of LGMD worldwide. In the last few years, muscle magnetic resonance imaging (MRI) has been proposed as a tool for identifying patterns of muscular involvement in genetically disorders, and as a biomarker of disease progression in muscle diseases.

Methods. In this study, 57 molecularly confirmed

LGMD2A patients from a European cohort (age range 7-78 years) underwent muscle MRI and a global evaluation of functional status (Gardner-Medwin & Walton score and ability to raise the arms).

Results. We confirmed a specific pattern of fatty substitution involving predominantly the hip adductors and hamstrings in lower limbs. Spine extensors were more severely affected than spine rotators, in agreement with higher incidence of lordosis than scoliosis in LGMD2A. Hierarchical clustering of lower limb MRI scores showed that involvement of anterior thigh muscles discriminate between classes of disease progression. Severity of muscle fatty substitution was significantly correlated with CAPN3 mutations: in particular patients with no or one "null" alleles showed a milder involvement, compared to patients with two null alleles (predicting absence of calpain-3 protein). Expectedly, fat infiltration scores strongly correlated with functional measures. The "pseudocollagen" sign (central areas of sparing) was associated with longer and more severe disease course.

Conclusions. We conclude that skeletal muscle MRI represents a useful tool in the diagnosis and clinical management of LGMD2A.

Motor performances in exon-2 duplication of the dystrophin gene

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Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder characterized by progressive weakening and wasting of skeletal muscles, which usually leads to loss of ambulation between 12-14 years. DMD is caused by mutations in the dystrophin gene: 70% deletions, 15-30% point mutations and 10% duplications that induce a frameshift in the protein-coding sequence.

Here we report on a 18-year-old DMD patient harboring exon-2 duplication and presenting with a milder-than-expected phenotype. When he was 6-year-old, a muscle biopsy was performed because of incidental detection of elevated CK. Complete absence of dystrophin was observed and the diagnosis of DMD was genetically confirmed by exon-2 duplication. At present, the patient is able to walk > 400 meters at the 6MWT and still run. Muscle biopsy was repeated when he was 15 and faint dystrophin was detected at immunostaining and western blotting.

The finding of minor motor impairment after age 16 is exceptional in DMD. To investigate the influence of exon-2 duplication on the disease phenotype, we performed an explorative survey to assess the outcome of these patients. We collected data from 27 patient, whose 16 above age 14. At age 14 and 16, the percentage of ambulant patients was 50%, and still the 30% of patients could walk at age 20.

In conclusion, it is important to consider such variability as a confounding factor when analyzing outcomes of newly available treatments. Moreover, a better understanding of the mechanisms that protect these patients may provide new avenues for treatment.

Modifiers of respiratory and cardiac function in the Italian Duchenne muscular dystrophy Network and CINRG Duchenne Natural History Study

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We tested the effects of glucocorticoids (GCs) and genetic factors on respiratory and cardiac function in a large Italian DMD cohort followed by the Italian DMD Network, with validation in the CINRG Duchenne Natural History Study (DNHS).

The Italian cohort comprised 327 patients with spirometric data and 374 with echocardiographic data. We used generalized estimating equations to evaluate effects of DMD mutation types and SNP modifiers: rs28357094 (*SPP1*), rs10880 (*LTBP4*), rs1883832 (*CD40*), and rs1815739 (*ACTN3*).

In the Italian cohort, we estimated annual decreases of forced vital capacity (FVC, -4.2%), peak expiratory flow (PEF, -2.9%), and left ventricular ejection fraction (EF, -0.7%). GCs significantly improved respiratory (+14.5% FVC, $p < 0.001$) but not cardiac function. DMD mutations involving Dp140 showed a negative effect on FVC (-6.1%, $p = 0.008$) but not EF. Patients amenable to skipping of exon 8 had dramatically higher PEF (+23.0%). *LTBP4* rs10880 was associated with preserved EF (+4.5%, $p < 0.01$). In the CINRG-DNHS ($n = 277$ with genetic data) GC treatment significantly improved not only FVC, but also EF (+2.1%, $p = 0.028$). The effect of DMD mutations involving Dp140 on FVC (-5.9%, $p = 0.015$) and that of rs10880 on EF (+2.6%, $p = 0.027$)

were independently validated. A meta-analysis of the two cohorts showed that the minor alleles at *SPP1* and *CD40* SNPs were associated with reduced FVC (-4.5%, $p = 0.02$, and -4.8%, $p = 0.006$ respectively) and PEF (-6.3%, $p = 0.009$ and -4.1%, $p = 0.024$ respectively).

In conclusion, GCs preserve respiratory, and probably also cardiac function; distal *DMD* and *SPP1/CD40* SNPs affect respiratory function. *LTBP4* haplotype is protective against dilated cardiomyopathy.

Morphofunctional evaluation of TNPO3 and related proteins in LGMD1F/D2 patients: a confocal microscopy and in silico study

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Limb-girdle Muscular Dystrophy 1F/D2 (LGMD1F/D2) is a rare neuromuscular disorder with autosomal dominant inheritance firstly identified in an Italo-Spanish family. Clinical investigation revealed high variability in clinical symptoms and disease progression with generalized muscle atrophy as common feature. Histopathology revealed atrophy of muscle fibers, alteration in nuclear localization, aggregates of material positive for p62, desmin and myotilin. Genetic investigation identified a causative mutation in the *TNPO3* gene which normally encodes for Transportin 3, a shuttle protein that transports, from cytoplasm to the nucleus, proteins involved in alternative splicing and RNA metabolism among which the splicing factor SRSF1. Even if the genetic cause of LGMD1F/D2 has been clarified, the role of TNPO3 in skeletal muscle and its participation in the pathological mechanism are still unknown. We analysed, in muscle biopsies of two LGMD1F/D2 patients, the expression of TNPO3 itself and of a selection of nuclear (SRSF1, Lamin A/C, Emerin) and sarcomeric (alpha-actinin) proteins that are related to or that could be influenced by mutated TNPO3. We observed by confocal microscopy that some of the selected proteins showed an altered expression or localization in patients' biopsies in comparison to control muscle. Moreover, we performed an *in silico* study based on the analyses of databases that collect data on biological processes, protein-protein interactions and transcriptome maps; in this way we aim to identify or make predictions on the possible relationship between some selected markers of atrophy and autophagy, increased in LGMD1F/D2 patients (p62 and MuRF-1), and TNPO3 and its preferential cargo SRSF1.

CSF biomarkers in patients affected by Spinal Muscular Atrophy type 1 treated with nusinersen

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The recent advent of new therapies, such as the antisense oligonucleotide nusinersen, has significantly improved the natural course of spinal muscular atrophy (SMA) type 1. Tau proteins and neurofilaments are well known markers of axonal degeneration.

We measured the cerebrospinal fluid (CSF) concentration of total tau (ttau), phosphorylated tau (ptau), neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH) proteins in 14 patients (age range: 2-156 months) with a diagnosis of SMA type 1, at baseline and after six months of treatment with nusinersen.

Patients were assessed using the functional scale "Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders" (CHOP-INTEND). Eight out of 14 patients had a functional improvement of more than 3 points at the CHOP-INTEND. At baseline CSF ttau ($p = 0.0002$) and ptau ($p = 0.0054$) concentration showed a significant negative correlation with the age of patients and a positive correlation with the CHOP INTEND score ($p = 0.0075$ and $p = 0.0342$, respectively).

After treatment the level of the tau biomarkers did not show any change, whereas NfL and pNfH concentration significantly decreased ($p = 0.0001$), being NfL related to age at baseline ($p < 0.05$). We also found a significant correlation between the decrease of NfL and the amelioration of the CHOP INTEND score in the subgroup of patients with a functional improvement above 3 points ($p < 0.05$). Further studies on longer treatment time-frame and larger cohorts of patients are needed to confirm our promising results.

AVXS-101 gene-replacement therapy in spinal muscular atrophy type 1: long-term follow-up from the phase 1 clinical trial

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Rationale and objective. Spinal muscular atrophy type 1 (SMA1) is a rapidly progressing neurodegenerative disease caused by biallelic survival motor neuron 1 gene (*SMN1*) deletion/mutation. Onasemnogene abeparvovec (AVXS-101), a one-time gene-replacement therapy, treats the genetic root cause of SMA, and is designed for immediate, sustained expression of SMN protein, allowing rapid onset and durable effect. In the phase 1/2a trial (NCT02122952), 15 SMA1 patients received a one-time intravenous dose of AVXS-101 (lower dose [cohort 1]: n = 3; proposed therapeutic dose [cohort 2]: n = 12). There was dramatic event-free survival and developmental motor milestones.¹ Here we report long-term follow-up study design and data.

Methods. Patients in the phase 1/2a study could roll-over into a long-term follow-up study (NCT03421977). The primary objective is long-term safety (incidence of serious adverse events, hospitalizations, adverse events of special interest). Patients will have annual visits for 5 years followed by annual phone contact for 10 years. Additionally, patient record transfers from their local physician and/or neurologist will be requested. Safety assessments include medical history and record review, physical examination, clinical laboratory evaluation, and pulmonary assessments. Efficacy assessments include developmental milestones (physical examination).

Results. As of September 27, 2018, the oldest patients in cohort 1 and 2 were 59.2 and 52.1 months old, respectively, and free of permanent ventilation. Preliminary data (survival, developmental milestones) will be presented.

Discussion and conclusions. Patients treated with a one-time dose of AVXS-101 continue to gain strength, develop, and achieve new milestones, demonstrating a long-term, durable response.

AVXS-101 gene-replacement therapy for spinal muscular atrophy type 1: pivotal phase 3 study (STRIVE) Update

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Rationale and objective. Spinal muscular atrophy type 1 (SMA1) is a rapidly progressing neurodegenerative disease caused by biallelic survival motor neuron 1 gene (*SMN1*) deletion/mutation. Onasemnogene abeparvovec (AVXS-101) is a gene-replacement therapy treating the genetic root cause of SMA1. In the phase 1/2a study, AVXS-101 dramatically improved outcomes in SMA1 patients. We report study design and preliminary data of STRIVE, a pivotal phase 3 study (NCT03306277) of AVXS-101.

Methods. STRIVE is a phase 3, open-label, one-time-infusion study in SMA1 patients < 6 months old (biallelic *SMN1* mutation/deletion, 2 x *SMN2*). Primary outcomes are independent sitting for ≥ 30 seconds at 18 months old, and survival (avoidance of death/permanent ventilation) at 14 months. Secondary outcomes include ability to thrive and ventilatory support at 18 months. Exploratory outcomes include CHOP INTEND and Bayley score.

Results. Enrollment is complete (22 patients). Mean age at symptom onset, genetic diagnosis, and enrollment was 1.9 (0-4.0), 2.1 (0.5-4.0), and 3.7 (0.5-5.9) months. At baseline, no patient required ventilatory/nutritional support; all exclusively fed by mouth. Mean baseline CHOP INTEND score was 32.6 (17.0-52.0). As of June 29, 2018, mean CHOP INTEND increase from baseline was 6.9 (-4.0-16.0, n = 20), 10.4 (2.0-18.0, n = 12), and 11.6 (-3.0-23.0, n = 9) points at 1, 2, and 3 months.

Discussion and conclusions. Data from STRIVE show rapid motor function improvements (CHOP INTEND) in patients with SMA1 that parallel phase 1/2a study findings and may correlate with survival benefit, motor milestone achievement, and bulbar function improvements. Updated data will be presented.

FIREFISH Part 1: 1-year results on motor function in infants with Type 1

spinal muscular atrophy (SMA) receiving risdiplam (RG7916)

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Background. Type 1 SMA is a severe neuromuscular disease caused by reduced levels of the survival of motor neuron (SMN) protein due to deletions and/or mutations of the SMN1 gene. A second SMN gene, SMN2, produces low levels of functional SMN protein. Risdiplam (RG7916/RO7034067) is an orally administered, centrally and peripherally distributed small molecule that modulates SMN2 pre-mRNA splicing to increase the levels of functional SMN protein.

Methods. FIREFISH (NCT02913482) is an ongoing, multicenter, open-label, study of risdiplam in infants aged 1-7 months at enrollment with Type 1 SMA and two SMN2 gene copies. Dose-finding Part 1 (n = 21) assesses the safety, tolerability, and PK/PD of different risdiplam dose levels. Pivotal Part 2 (n = 41) is assessing the safety and efficacy of risdiplam.

Results. In a Part 1 interim analysis (data-cut, 07 September 2018), 13/14 (93%) of infants had ≥ 4-point improvement in CHOP-INTEND total score from baseline at 8 months (median change: 16 points). Infants met the following HINE-2 motor milestones: full head control 6/14 (43%), horizontal or upward kicking 7/14 (50%), rolling to side or from prone to supine 4/14 (29%) and sitting with or without support 6/14 (43%). To date (data-cut, 07 September 2018), no drug-related safety findings have led to withdrawal of any infant from the study. One-year motor milestone data will be presented from FIREFISH Part 1.

Conclusions. Despite not being designed to detect efficacy, risdiplam improved motor function in infants with Type 1 SMA in FIREFISH Part 1. FIREFISH Part 2 is ongoing worldwide.

Plasma screening of dystrophin protein fragments using novel dystrophin-specific immunoassays

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Duchenne Muscular Dystrophy (DMD) is a progressive, lethal X-linked neuromuscular disorder with a point prevalence of 1:5000 male newborns. The clinical diagnosis must be confirmed by dystrophin gene testing and it is achieved at the average age of 5 years, with remarkable differences among countries. Early diagnosis gives broader advantages to the patient and his family, considering the recent availability of new therapies acting at the protein restoration. Based on preliminary data obtained within the BIO-NMD EU project, we tested 82 plasma samples (3 BMD, 16 DMD, 16 female carriers, 14 controls, and 33 other neuromuscular non-DMD diseases) in 5 replicates of a multiplexed antibody suspension bead array. The array consists of magnetic beads coupled to 2 different anti-dystrophin antibodies, which recognize the N- and the C-terminus respectively. Preliminary studies show that fragments of dystrophin protein can be detected in plasma. Moreover the dystrophin abundance varies across the subjects analyzed, with a decreased level in DMD patients compared to controls. This immunoassay test is not expensive and can be easily applied for a huge number of samples. For these reasons, this approach, if validated, might represent a very appealing protein testing for disease screening to allow an early diagnosis and monitoring drug efficiency.

Transcriptome analysis (RNAseq) is a powerful diagnostic and research strategy in inherited skeletal muscle diseases

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Targeted resequencing of genes of interest is an effective strategy for the routine diagnostics of skeletal muscle diseases. However, the current diagnostic rate is, at best, 40-50%, resulting in a large number of unsolved cases.

At the same time, the massive use of large resequencing panels has increased the number of potentially

causative variants and expanded the clinical phenotypes associated with the already known disease genes. Consequently, we have a limited understanding of the genotype-phenotype correlation although this is crucial for patients in order to receive optimal care and a correct prognosis.

We have successfully introduced RNA sequencing in our molecular diagnostic pipeline. By analyzing RNA extracted from a skeletal muscle biopsy of myopathy patients, we identify single nucleotide variants in the coding regions; we detect and characterize splicing defects; we can observe an altered expression due to a monoallelic expression or due to variants in regulatory elements.

Simultaneously, we are using transcriptome analysis to study the differential gene expression in different stages of muscle development. An interesting hypothesis, supported by our preliminary data, is that a differential exon usage in different muscles and in different developmental stages explains some of the clinical heterogeneity observed in patients. A form of titin-related arthrogryposis multiplex congenita, for example, is specifically caused by variants in exons that have a higher expression during the embryonic development of skeletal muscles. The same mechanism of alternative splicing can explain the selective muscle involvement and the specific age of onset in other genetic Mendelian and complex myopathies.

Diagnostic algorithm of hyperckemia and mild proximal weakness in the era of next generation sequencing

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Objective. HyperCKemia and proximal “Limb-Girdle” weakness represent a frequent and unspecific presentation of muscle diseases without straightforward guidelines for the clinical workout. Method: the outpatient clinic for neuromuscular diseases takes care of 800 adult patients with different neuromuscular disorders. We retrospectively analysed the clinical features and the diagnostic algorithm in 34 patients that presented with hyperCKemia and/or mild proximal “Limb-Girdle” weakness. Patients with facial involvement, distal or congenital myopathies have been excluded. The first step of analysis was the DBS for Pompe disease and nerve con-

duction and electromyography studies. In all patients we analysed DM2-related gene and further excluded copy number variations in DMD gene. Undiagnosed patients were investigated by target gene panels including genes associated with benign hyperCKemia or genes causing LGMDs, based on clinical presentation. For the remaining unsolved patients we proposed a muscle biopsy.

Results. After the first step we identified 1 neuropathy. A confirmed genetic diagnosis was achieved in 10/33 (30.3%) patients. The diagnoses included: 4 LGMD (3 LGMD2L, 1 LGMD1C), 1 RYR1 mutation, 2 type DM2, 1 female carriers of Duchenne muscular dystrophy, 1 type V glycogenosis and 1 metabolic myopathy due to CPT2 mutation. 14 of undiagnosed patients underwent muscle biopsy, 6 of them required further investigation, while 8 evidenced only minimal changes.

Conclusion. based on our experience, we propose a diagnostic approach with target gene panels as first-line tools for patients with mild and unspecific clinical presentation of muscle diseases.

Interpreting genetic variants in ryanodine receptor type 1-related muscle disorders: results from 71 patients

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Mutations in the *RYR1* gene are associated with a clinically heterogeneous group of neuromuscular disorders that can be challenging to diagnose because of overlapping clinical features and nonspecific muscle pathology. Moreover, recently, the *RYR1*-associated phenotypic spectrum has further expanded.

Next-generation sequencing (NGS) is rapidly being implemented into routine clinical practice, improving the molecular diagnosis of genetically heterogeneous disorders such as inherited neuromuscular disease. By using a customized, targeted sequencing panel able to investigate the coding exons and flanking intronic regions of 241 genes we screened *RYR1* and the other muscle disease genes in 400 patients with skeletal muscle disorders.

We identified 71 patients carrying “probable” or “likely pathogenetic” disease-related mutations in *RYR1*. Fourteen patients underwent NGS analysis because of a clinical and histological suspicion of central-multimicromyopathy, and 14 because a clinical diagnosis of congenital myopathy. Also, 18 patients presented hyperckemia whereas 42 subjects presented with unclassified myopathy. Whilst 39 cases were sporadic, positive family history of muscle weakness was found in 32 patients, with autosomal dominant inheritance in 17 and autosomal recessive in 15.

Detailed description of muscle weakness distribution in symptomatic patients used the Human Phenotype Ontology (HPO) nomenclature and coding system.

Our study confirms that the interpretation of *RYR1* variants is particularly challenging and often requires further analyses, including a comprehensive evaluation of the clinical phenotype (deep phenotyping).

Functional and histological improvements comparing 4 micro-dystrophin constructs in the mdx mouse model of DMD

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Introduction. Duchenne muscular dystrophy (DMD) is the most common severe childhood form of muscular dystrophy. More than 2000 mutations of the DMD gene are responsible for progressive loss of muscle strength, and ultimately respiratory and cardiac failure. Through head-to-head comparison, functional and histological benefit across different micro-dystrophin constructs was evaluated.

Methods. We designed 4 unique constructs of AAVrh74 vector, including use of the MHCK7 promoter in comparison to a less active MCK promoter with the same micro-dystrophin transgene that contains the N-terminus and spectrin repeats R1, R2, and R3, respectively (AAVrh74.MHCK7.micro-dystrophin; AAVrh74.MCK.micro-dystrophin), a mini-dystrophin construct that contains the nNOS binding site (AAVrh74.DV.minidystrophin), and a micro-dystrophin containing the C-terminus (AAVrh74.MHCK7.microdys.Cterm). To test the efficacy of the 4 constructs of AAVrh74.micro-dystrophin, we evaluated both functional and histological benefit 4 weeks post intramuscular vector delivery in the mdx mouse model.

Results. Delivery of the AAVrh74.MHCK7.micro-dystrophin construct is the most advantageous in normalizing histologic and functional outcome measures among these constructs. Specific force output significantly increased in the tibialis anterior muscle compared with the other 3 constructs and there was no difference from wild-type levels. Muscle environment was normalized, as demonstrated by reductions in centralized nucleation and normalized myofiber diameters. Transgene expression through immunofluorescent staining and Western blot was significantly increased compared with the other constructs, indicating functional and histological advantages of the AAVrh74.MHCK7.micro-dystrophin construct.

Conclusions. Findings from this preclinical study provided proof-of-principle for safety and efficacy of systemic delivery of AAVrh74.MHCK7.micro-dystrophin in a dose-escalation study in the mdx mouse model for DMD.

D4Z4 elements constitute a drug responsive chromatin structure affecting gene expression in FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary myopathy with autosomal mode of inheritance. FSHD has not been associated with a classical mutation within a protein-coding gene. Instead, the majority of FSHD cases (> 95%) carry a monoallelic partial deletion of tandemly arrayed D4Z4 repeats at the 4q subtelomere.

Genotype-phenotype studies reveals wide clinical variability among the affected individuals and reduced penetrance in FSHD families. Moreover, alleles with reduced number of D4Z4 elements (DRA) are found in 3% of healthy subjects and not all individuals with a DRA develop FSHD.

D4Z4 is a 3.3 kb CpG-rich (73%) DNA element with multiple repeat sequences normally associated with heterochromatin. A pervasive idea is that epigenetic changes in FSHD following the D4Z4 deletion lead to disease through changes in chromatin organization and consequent inappropriate expression of nearby genes.

Indeed, using a permissive cell line carrying DRA and primary cells from FSHD individuals, we found that the 4q subtelomere is subdivided into discrete domains characterized by different histone modifications. These specific epigenetic signatures lead to a different expression of 4q35 genes that inversely correlates with D4Z4 size. We observed that one of these genes, FRG2, is subjected to reversible silencing and that a general de-repression in 4q35 region occurs in FSHD cells after various drug treatments. This point at the D4Z4 array as a sensitive locus, dynamically regulated, capable of responding to external stimuli, whose de-repression could be strongly correlated with the observed differences in penetrance among patients and/or with the risk to develop the disease.

The RNA binding protein FRG1 controls transcription landscape regulating muscle maturation and metabolism

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Among animal models for FSHD, mice overexpressing FRG1 present a progressive myopathy with features of human disease. To investigate the molecular changes occurring during disease development, we analysed gene expression profiles of skeletal muscles of mice overexpressing increasing levels of FRG1 at 28d (dystrophy onset) and at 96d (full dystrophy). We found a profound transcriptional deregulation correlating the severity of the muscle phenotype and FRG1 expression. GSEA and GO revealed alterations in pathways related to myogenesis, energy metabolism and inflammation. Indeed, genes related to adult and normal myogenesis were downregulated with a significant enrichment of genes specifically expressed during embryogenesis. In FRG1 mice at 7d and 14d the embryonic isoforms of myosin remain high instead of following the physiological downregulation occurring in WT mice, meanwhile the expression of the mature isoforms is reduced. Starting from 14d we observed the deceleration of growth curve and a reduction of muscle cross-sectional area. Moreover, FRG1 muscles displayed the significant reduction of ATP and the phosphocreatine in association

with the transcriptional downregulation of Glut4, HK2 and AldoA. Our results indicate that FRG1 overexpression induce the impairment of muscle maturation and energy metabolism that precedes dystrophy. Our study opens new perspectives on the molecular mechanisms at the basis of muscular dystrophies.

Finding treatments for tubular aggregate myopathy

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Stim1 and Orai1 are the key proteins involved in store-operated Ca^{2+} -entry, i.e. the process that allows intracellular stores to be refilled upon depletion. Gain of function mutations of these two proteins lead to ultra-rare syndromes (tubular aggregate myopathy, Stormorken, York) mainly characterized by muscle and platelet dysfunctions.

We have recently developed a mouse model bearing the I118F mutation on Stim1, one of the most frequent mutations found in patients. Animals are smaller in size, have a normal life-span and breed normally. Heterozygous animals display a dystrophic muscle phenotype (although no aggregates are observed), perform poorly in the rotarod and treadmill but not in the hanging test and also show haematological defects, mainly referring to platelets and myeloid cells, thereby conferring face-validity to the model. Myotubes from knock-in animals show an increased Ca^{2+} -entry upon store depletion that parallels what has been previously observed in myotubes from patients.

Recently, two animal models bearing the R304W mutation, associated with Stormorken syndrome, have also been reported showing similar, yet non-superimposable phenotypes. More, importantly, medicinal chemistry and repurposing programs are undergoing to identify modulators of store-operated Ca^{2+} -entry. These modulators are being developed for disorders with a higher prevalence but it is highly likely that they would mitigate the progression and symptoms of tubular aggregate myopathy patients. The time therefore appears mature to make headway in developing translational programs to provide pharmacological answers for tubular aggregate myopathy patients.

Large scale genotype-phenotype correlation study in 1703 carriers of D4Z4 reduced alleles from the Italian National Register for FSHD

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Clinical reports show high phenotypic heterogeneity in carriers of D4Z4 reduced alleles (DRA) ranging from healthy carriers to typical FSHD to complex muscular phenotypes. 3% of healthy people carry one DRA.

The Italian Clinical Network for FSHD applied the CCEF, describing nine clinical categories, on 1703 individuals, 846 index cases and 857 relatives carrying one contracted alleles with 1-10 D4Z4 repeats.

Subjects' stratification based on D4Z4 allele size shows that clinical categories are distributed in all groups. Disease penetrance is reduced with 44.3% of healthy relatives. The age at evaluation of healthy relatives is not significantly different from that of relatives who developed the full disease. The facial-sparing FSHD phenotype represents 10.2% of index cases regardless the size of the D4Z4 contracted allele.

Our study show that standardized clinical evaluation can facilitate the comprehension of phenotypic diversity among carriers of D4Z4 contracted alleles and guide clinical studies and research.

Muscle pain in mitochondrial diseases: the final data from the italian network

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Although muscle pain has been already reported in mitochondrial diseases (MD), no extensive studies have been performed so far in order to quantify and clinically characterize this symptom in MD patients.

We reviewed clinical findings of 1398 MD patients which were collected from the database of the "Nation-wide Italian Collaborative Network of Mitochondrial Diseases".

Muscle pain was present in 11.7% of the patients listed in the database. It was usually observed in subjects affected with chronic progressive external ophthalmoplegia (cPEO) and primary myopathy without cPEO. Less frequently, it has been reported also in multisystem phenotypes such as Kearns Sayre syndrome, MERFF, MELAS, MNGIE, NARP and Leigh syndrome.

Main complain was diffuse exercise-related muscle pain, but focal/multifocal and at rest myalgia were also described. No significant correlation between muscle biopsy/genotype findings and muscle pain was found, although patients with mtDNA mutations more frequently complained of muscle pain than nDNA mutated patients (67.8% vs 32.2%).

Pharmacological control of pain was obtained in only 34% of them by using many different analgesic and modulating drugs. Interestingly, a higher prevalence of responder patients among the nDNA-mutated subjects respect to mtDNA-mutated ones was observed, suggesting a possible role of genotype in influencing the response to therapy.

Our study demonstrated that muscle pain is a common symptom in MD patients.

Subjects with a myopathic phenotype are more prone to develop muscle pain, but this is also observed in patients with a multi system involvement, representing an important and disabling symptom having poor response to current therapy.

Clinical, morphological and genetic data in Italian patients with fiber-type-disproportion

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Introduction. Although found in different muscle diseases in association with other features, fiber type disproportion (FTD) represents the unique histological abnormality of a specific form of congenital myopathy. However, poor data on this disease have been reported in the literature. We aimed at clinically and histologically characterize a large cohort of Italian patients with FTD.

Methods. We collected clinical, histological, molecular and imaging data from patients followed in 9 Italian tertiary referral centers for neuromuscular diseases. Inclusion criteria were: infantile and adult patients with at least 25% difference between type I and II fiber diameters, on average, and, as adjunct anomalies, fiber type predominance, central nuclei or cores. Other histological abnormalities were considered as exclusion criteria.

Results. We included in this study 47 patients, 12 females and 35 males. Mean age at muscle biopsy was 6 years; onset at birth was observed in 23 (48.9%) patients, whereas late-onset (> 5 years) was reported in 13

(27.7%) patients. Genetic characterization was achieved in 22 (46.8%) patients, with TPM3 (n = 8) the most frequent gene harboring pathogenic mutations, followed by MYH7 (4), RYR1 (3), ACTA1 (2), TPM2, TNT, LMNA, DOK7 and DNM2 (1 each). About one fourth of the patients could not walk independently at the end of the follow-up period.

Conclusions. The diagnosis of FTD is difficult due to lack of uniformity in clinical presentation. Diagnosis is made after excluding other causes of myopathy and on the basis of histological evidence of type 1 muscle fiber hypotrophy. Genetic analysis shows also a great heterogeneity. Our data shows that presentation at birth accounts only for half of the cases. An integrated multidisciplinary approach of neuromuscular experts, geneticists, neuropathologists, will improve and optimize the diagnosis in this group of congenital myopathies.

Prevalence of anti-CN1A antibodies in a large Italian cohort of s-IBM.

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Background. In the last few years an autoantibody directed against the 5'-citosolic nucleotidase 1A (CN1A) was identified in sera of sporadic inclusion body myopathy (s-IBM) patients. Sensitivity of anti-CN1A antibodies in s-IBM significantly varies in different studies ranging from 33 to 76% with a specificity between 87 to 100%. We evaluated anti-CN1A antibodies sensitivity and specificity in a large Italian cohort of s-IBM and looked for potential phenotypic differences between anti-cN1A positive and negative patients.

Methods. We collected clinical data and serum from 55 consecutive s-IBM patients attending seven Italian neuromuscular center and 62 patients with other inflammatory myopathies. Testing for anti-cN1A autoantibodies was performed using a well characterized commercially available ELISA.

Results. Anti-CN1A antibody was detected in 20 out of 55 s-IBM resulting in a sensitivity of 36.4% with a

specificity of 96,8% (only two other myositis were positive). We did not find differences in terms of age at onset or disease duration. We found a significant difference regarding associated swallowing problems (item 1 of IBMFRS with lower scores in Ab-positive patients). Anti-cN1A Ab-positive patients were significantly more likely to have more severe swallowing problems, expressed as IBMFRS item 1 score ≤ 2 (55.6% vs 7.4%).

Discussion. We confirm the low sensitivity and high specificity of anti-CN1A Ab in s-IBM patients with high positive predictive value. Anti-CN1A test could be helpful in diagnostic work-up to reduce delay to a definite s-IBM diagnosis. In our cohort the presence of anti-CN1A antibodies identified patients with greater risk of clinically significant dysphagia.

MUSCLE CLUB SESSION

(in alphabetical order of the first Author)

A 9 years-old-girl with hypoglycemic coma and lactic acidosis

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The *YARS2* gene encodes the mitochondrial tyrosyl tRNA synthetase, a key enzyme in mitochondrial protein synthesis. Pathogenic mutations in the *YARS2* gene causes a clinical triad of Myopathy, Lactic Acidosis and Sideroblastic Anemia (MLASA) and have been described so far in less than 30 cases. Patients manifest multiple mitochondrial respiratory chain defects in skeletal muscle, often demonstrated by the severe loss of cytochrome c oxidase (COX) activity.

Our patient is a 9 years old girl who presented soon after birth with hypoglycemic coma and lactic acidosis. This metabolic crisis was treated with bicarbonate and glucose and did not recur over time. At 2 years of age during respiratory infection she was diagnosed with sideroblastic anaemia requiring only a single blood transfusion. Thereafter she became transfusion independent spontaneously. At 4 years of age she was admitted to paediatric neurology clinic for exercise intolerance and mild hypotonia/joint laxity. In the suspect of a myopathy a muscle biopsy was performed showing severe reduction of COX activity. Mitochondrial activity dosage in muscle revealed multiple defects, while mtDNA sequencing was normal. Targeted NGS panel allowed the identification of homozygous c.933A > G, p.Asp311Glu variant in *YARS2* gene. This variant has been previously reported in patients with transfusion-dependent sideroblastic anaemia and lactic acidosis without overt myopathy. Clinical picture at age 9 years shows scoliosis and mild limb girdle weakness with minimal increase of CK and lactate, and the patient is constantly treatment with bicarbonate and vitamins.

A 48-year-old man with slowly progressive asymmetric weakness and hypotrophy of the scapulohumeral girdle

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Fibrous myopathy is a poorly known side-effect of chronic intramuscular drug administration.

It is characterized by induration of subcutaneous and muscle tissue in the injected sites associated with muscle contractures, EMG myopathic findings and histological demonstration of increased connective tissue and fiber degeneration in muscle biopsy.

We here describe a 48-year-old man with a history of intramuscular drug abuse who presented with slowly progressive asymmetric weakness and hypotrophy of the scapulohumeral girdle for about three years. A scleroderma-like skin pattern was evident especially in the forearms and thighs.

Endocrine, renal and liver dysfunctions, inflammatory or rheumatological disorders, electrolyte imbalance, vitamine deficiencies, hepatitis B and HIV infection were ruled out by appropriate laboratory investigations. HCV positivity has been known for many years.

Deltoid muscle biopsy showed the presence of diffuse endomysial fibrosis, muscle fiber degeneration, adipose infiltration and widespread anti MHC-I antibody membrane positivity. Immunohistochemical studies for Dystrophin, Alpha-sarcoglycan, Gamma-sarcoglycan, Beta-sarcoglycan, Delta-sarcoglycan, Caveolin-3, Dysferlin, Alpha-dextrglycan, Merosine, Collagen IV, Collagen VI and Telethonin were normal.

Skin biopsy showed a reticular dermal thickening as per a sclerodermal process.

Final diagnosis was a fibrous myopathy related to drug abuse.

Prognosis of this rare disorder is currently not well established and treatment are limited to discontinuation of injections and physical therapy.

Fibrous myopathy should be considered in patients using intramuscular drugs because timely recognition may prevent further muscle damage.

Gastroparesis in two neuromuscular patients: a not-to-underestimate clue

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A 55 year-old woman developed visual disorders characterized by diplopia, ophtalmoparesis and bilateral eyelid ptosis. Since the age of 53 she had been suffering of several episodes of melena which, in the following years, associated to early satiety, vomiting and bowel dysmotility. A myopathic pattern emerged from electromyography; furthermore, alterations at visual evoked potentials (VEP) and bilateral hearing loss at audiometric test were found. At the age of 63 she underwent surgical laparotomy due to abdominal pain and distension and a perforation of a small bowel diverticulum was found. Due to this finding a small bowel resection was performed.

A 54 year-old woman developed progressive sensory loss in both of her feet, which evolved during the following months in a balance disorder associated to many episodes of falling. Electroneuromyography showed a pattern of sensory-motor axonal neuropathy, associated to alterations of motor, sensory, brainstem and VEP, as a sign of central nervous system impairment. At the age of 59 she developed dyspepsia and early postprandial sense of fullness, associated with several episodes of vomiting. A CT scan of the abdomen showed a huge stomach, which extended to the left iliac fossa. Explorative laparotomy was performed in the hypothesis of bowel obstruction, but no mechanical occlusion was found.

Both these patients showed a progressive evolution of the gastrointestinal symptoms together with a worsening of the neurological status. A complete radiological and instrumental workup was performed. Finally, biochemical and genetic tests led to the diagnosis in both cases.

A 66-years-old man with subacute ophthalmoplegia and bilateral eyelid ptosis"

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A 66 years old man was referred to our attention because affected by a subacute and painless ophthalmoplegia, with bilateral eyelid ptosis. He was receiving Pembrolizumab-Lenvatinib therapy for a renal carcinoma. Routine laboratory analysis were in normal range, except for hyperCKemia, elevated up to more than 5000 UI/L. No facial, bulbar, proximal, distal or axial muscular weakness was detected. Electromyography (EMG) showed myopathic pattern, with spontaneous activity, in orbicularis oculi and proximal upper limbs muscles; repetitive nerve stimulation and single-fiber EMG documented normal neuromuscular transmission function, confirmed by negative acetylcholine and Musk antibodies. Full panel of myositis specific and myositis associated antibodies were normal. Cardiac and respiratory functions were preserved. Whole body muscle MRI revealed normal images, whereas orbital MRI disclosed bilateral hyperintensities in inferior rectus, medial rectus and superior oblique muscles in both T1 and STIR sequences, with mild muscle atrophy. Deltoid muscle biopsy documented mixed lymphocytic/macrophagic endomysial inflammatory infiltrates, with prevalent CD8 and CD68 cells, sometimes expressing PD-1 or PD-L1 antigens; sarcolemmal and cytoplasmic MHC-1 overexpression was observed in clusters of non necrotic cells. CD56 positive cells were observed in perifascicular regions. Patient discontinued Pembrolizumab and received corticosteroid treatment with progressive clinical improvement and CK normalization. Immune check point inhibitors are a novel class of anti-tumor agents, which have been linked to several neurological adverse events, including myositis and myasthenia; only few cases have been reported with isolated ocular myositis. Our findings support this clinical entity, suggesting that isolated ocular myositis represents a subgroup of generalised myositis with predominant ocular symptoms.

ABSTRACTS OF POSTER COMMUNICATIONS

(listed in order of presentation)

Sessione 1.

6 giugno dalle ore 14.30-15.30

P 1-1 Muscular dystrophies

Clinical course of two brothers with LGMD2D under steroids therapy

Catteruccia M.¹, Colia G.¹, Bonetti A.M.², Carlesi A.², Bertini E.¹, D'Amico A.¹

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Sarcoglycanopathies are a subgroup of LGMD, caused by mutations in sarcoglycan genes. They usually have childhood onset and rapidly progressive course with loss of ambulation over 12-16 years. We describe the clinical and neuroradiological course of two brothers with LGMD2D and on steroids therapy. The patients are on regular clinical, functional, cardiological, pulmonary and neuroradiological follow-up. Physiotherapy and the use of nocturnal ankle-foot orthoses were started at the age of 5y. Deflazacort was started at the age of 7y. Both children regularly attained motor skills. Diagnosis was made after incidental detection of high CK in the youngest boy. Muscle biopsy showed an almost complete absence of the sarcoglycans immunosignal. Genetic analysis detected the c.308 T > C in exon 3 and c.dupC L161L fs X28 in exon 5 of SGCA gene. The brothers are currently 12 (Pt1) and 10 (Pt2) years old. Major motor difficulties were observed at the age of 7y. In Pt1 the disease rapidly progressed and he lost walking ability at the age of 10y. Pt 2 is still ambulant. Muscle MRI confirmed the progression of the disease. No cardiological or pulmonary impairment have been observed. There are only few reports in the literature on the use of steroids in LGMD. In our patients, steroid therapy seems not to determine a clinical improvement or a delay in the course of the disease. Potentially trajectories appear unpredictable in sarcoglycanopathies in relation to DMD and more studies including a large number of patients are necessary to define a possible effect of steroids.

Burden of Duchenne Muscular Dystrophy (DMD) in Italy: a systematic review

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Background. Duchenne Muscular Dystrophy (DMD) is a rapidly progressive, lethal neuromuscular disorder, present from birth, which occurs almost exclusively in males. However, to date, little is known of the burden of DMD, including cost of illness and impact on health-related quality of life (HRQoL).

Material and method. A systematic review on evidence of burden and illness costs of DMD has been carried out from the interrogation of Electronic database (Pubmed and Cochrane library). Time period was October 2018 to January 2019. Keywords used were: Duchenne Muscular Dystrophy, burden of disease, economic evaluation, cost of illness.

Results. 78 records were identified through database searching, 15 were duplicates and deleted from the search, records screened were 63. Records excluded were 53; exclusion criteria were: publication status, not in accordance with keywords and research objective. 10 studies were examined as results of the review: 7 Cost of Illness studies, 2 Systematic Review on Cost of Illness, 1 Cost Effectiveness Analysis. Main outcomes considered were: medical direct costs, not medical direct costs, indirect costs, health related quality of life. Only three studies were focusing on Italy. In all studies a correlation between disease progression and cost emerges with a delta cost between € 34,500 and € 63,500.

Conclusions. This is the first systematic review of burden and cost in DMD in Italy. It has emerged that the economic cost of DMD climbs dramatically with disease progression. Future research is needed to investigate the quality of life impact linked to the condition progresses.

A new tool to evaluate multidisciplinary clinical outcomes in Duchenne muscular dystrophy: a pilot study

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Duchenne muscular dystrophy patients, due to more widespread prolonged survival, progressively experience multisystem complications. We retrospectively reviewed the charts of 132 Duchenne patients (112 alive/20 dead, age 3.5-32.3 years) referred to an Italian tertiary care center, with the aim to identify a scoring tool useful for benchmarking between national and international specialized centers for treatment of DMD. Four items were analyzed: cardiac function, respiratory function, nutrition and scoliosis. For each item, different functional parameters were considered and classified, according to clinical severity, and an incremental scoring was assigned. In addition, a global score incorporating all items was defined. The scoring system tested confirmed a documented knowledge that, despite the significant protective role of steroids, all functions deteriorate with age. The value of the global score became significantly higher since the age of 13 years. Comparing alive and dead patients at the same age range, the latter were characterized by significantly worse cardiac function, no steroid therapy and later

use of respiratory assistive devices. In conclusion, the scoring system showed that cardiac dysfunction seems to be associated to premature death, while respiratory care to prolonged life. The proposed index allows aggregating and correlating different parameters, items and functions, and giving either an individual prognostic indicator of decline, either a global score to evaluate changes in clinical trials.

Becker muscular dystrophy: analysis of an Italian sample with childhood onset or diagnosis in developmental age

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Becker Muscular Dystrophy (BMD; OMIM #300376) represents an X-linked inherited neuromuscular disorder due to mutations in the dystrophin gene, which cause partial lack of Dystrophin in muscle fibers. Clinical phenotype is generally significantly less severe than his allelic Duchenne variant and much more heterogeneous. The most frequent presenting symptom at the onset is the presence of cramps or exercise-induced myalgias in childhood (Bushby, 1993; Magri, 2011), but can also be episodes of isolated myoglobinuria or motor difficulties such as frequent falls, slowness, difficulty in climbing stairs, waddling gait or toe-walking. Subjects who receive an earlier diagnosis often have a slight psychomotor delay. In literature (Angelini, 1994; Van den Bergen, 2013; Bello, 2016) a wide phenotypic variability was also identified.

We describe an Italian multicenter case series of 163 patients with BMD characterized by the onset of symptoms and/or diagnosis (even in the absence of clear symptoms - due to hyperckemia or familiarity-) in developmental age. We propose a phenotypic analysis based on the first signs of disease, early neurodevelopmental milestones, the presence and the degree of muscular, cardiac and respiratory impairment and brain involvement (described by the type and the severity of cognitive impairment) or other neuropsychiatric comorbidities such as neurodevelopmental disorders or behavioural disturbances.

Aim of the work is to identify the presence of different clinical phenotypes and disease trajectories in this population. We will pay particular attention to the possible involvement of the CNS and studying, where possible, the evolution over time even in adulthood.

DMD nonsense variants position may be accurate to predict phenotype

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Variants in *DMD* gene may cause Duchenne, Becker muscular dystrophies or additional phenotypes comprising cardiomyopathy, hyperCKemia and/or limb-girdle muscular weakness. It depends on the modified dystrophin amount, function and distribution.

Nonsense mutations are expected to result in premature termination of protein translation, and therefore be associated with severe *DMD* phenotype. However, it is well known that the effect of a stop codon can be rescued by an alternative splicing. In addition, a very early 5' stop can be rescued by re-initiation of translation as well as a 3' stop can produce a functional shortened dystrophin resulting in a milder phenotype.

We collected *DMD* nonsense variants reported in HGMD, LOVD and ClinVar databases and included nonsense *DMD* variants found in a cohort of over 200 BMD/*DMD* patients. For each patient, we also extracted clinical data and dystrophin expression. By a careful analysis, we selected 841 unique stop codon variants. Among these, we identified 58 patients harboring nonsense variants with a milder phenotype. Clinical, genetics and protein data were accurate to provide a clinical diagnosis of Becker muscular dystrophy.

As the distribution of nonsense variants along the dystrophin transcript associated with a BMD phenotype is non-random, we may conclude that the position of a stop codon in *DMD* gene can be predictive of the phenotype. This may have important implication for therapeutic use of nonsense suppression drugs able to read through stop signals and produce a functional protein.

Key words: *DMD*, nonsense variants, Duchenne/Becker muscular dystrophy.

DMD gene molecular genetic characterization in eastern Europe and non European countries

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Duchenne Muscular Dystrophy (DMD) is a rare genetic

neuromuscular disease affecting 1 in 3,500 male births worldwide, due to a variety of dystrophin gene mutations.

Diagnostic settings include MLPA (MRC-Holland) and NGS dystrophin gene sequencing (DMD MASTR assay Multiplicom).

Thanks to the International DMD project we have tested 182 patients from Eastern European and non-European countries: Poland (75), Hungary (19), Lithuania (6), Romania (55), Serbia (2), Croatia (8), Bosnia (2) Bulgaria (13) Cyprus (2) and 172 DNAs from Extra-European countries: Russia (1), Ukraina (92) and Algeria (79) were collected. In the European samples we identified 33 large del/dup (33.6%), 33 nonsense (33.6%), 17 small del/dup (18%), 16 splice site (16%) and 3 missense mutations (3%). In non-European patients we identified 73 large del/dup (62%), 20 nonsense (17%), 9 small del/dup (7.7%), 9 splice site (7.6%) and 4 missense mutations (3%).

Sixty-two European patients and fifty-four Extra-European patients remained undiagnosed using routine methods, suggesting the presence of atypical mutations in the DMD gene or other genes involvement.

The early identification of the underlying genetic mutation is critical to potentially affect the course of Duchenne Muscular Dystrophy as well as the choice of treatment, the setup of appropriate and effective care and the eligibility for clinical trials. Genetic counselling can also be offered to patients and families with important repercussions on reproductive choices and lifestyle planning (see details at www.ospfe.it/medicalgenetics).

Acknowledgments

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A case of DMD and autism: double trouble or complex disease entity?

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In addition to muscular involvement, Duchenne Muscular Dystrophy (DMD) children may present cognitive impairment and behavioural disorders especially if the mutation is in the distal portion of dystrophin gene

A 3 years-old DMD boy born from heterologous artificial insemination presents both disease-related neuromuscular symptoms and severe psychomotor delay, namely retardation in achieving motor milestones, severe language delay, repetitive behaviour and lack of social skills (normal audiometry). The parents reported normal neuropsychological development until the age of 12 months, regression being observed after a vaccine. The child carries a splice-site mutation (c.8390+1G > A) with deletion of exon 56 of the dystrophin gene.

He recently underwent thorough neuropsychiatric examination which is suggestive for Autism Spectrum Disorder (ASD). Brain MRI did not show structural abnormalities.

We were recently advised that another child, born from heterologous artificial insemination performed in the same foreign

centre, is affected with DMD caused by the same mutation and is having a normal neuropsychological development.

A higher (10-20%) ASD frequency has been reported in DMD patients compared with aged-matched population.

In our case, the fact that a second child, most probably born from the same donor and, anyway, carrying the same mutation, is having a regular psycho-behavioral development, makes a strict *Dys* mutation-ASD association unlikely, though the contribution of regulatory genes cannot be excluded.

The etiologic role of vaccine is improbable because the child had no acute post-vaccine reactions and brain MRI is normal.

Urinary stem cells are a non-invasive model for the identification of dystrophin mutations by DMD transcript profiling

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Nowadays, there is growing interest in the use of urine specimens as novel non-invasive approach to isolate patient-specific stem cells.

We have already showed that both native urinary stem cells (USCs) and differentiated myogenic USCs are a good model for studying dystrophin gene expression and for prescreening studies of therapeutic molecules.

Based on this previous evidence, we isolated the native USCs from a DMD patient missing the mutation identification by standard genomic methods (MLPA, NGS) in order to profile the dystrophin transcript and find the causative genetic variant. The DMD transcript analysis by FluidDMD card revealed the absence of amplification of the junction between exons 10-11. The DNA sequencing of intron 10 identified the mutation c.1149+250 C > T that generates a donor splice site. This activates two cryptic splice sites causing the creation of two alternative exons, both leading to premature stop codons.

Therefore, USCs represent an extremely valuable alternative source to the muscle biopsy and offer a novel strategy to obtain ideal cells to study the molecular mechanisms of diseases. Considering this, we created a biological repository to preserve USCs from healthy and affected subjects to be used for different purposes such as diagnostic test, disease modeling and drug screening. So far, our Biorepository is composed of 37 samples among which 2 are Becker Muscular Dystrophy (BMD) patients, 17 are DMD patients and 9 samples are derived from individuals affected by other diseases such as mental retardation and dismorphism, Bethlem myopathy, peripheral neuropathy and hereditary spastic paraplegia.

Nonsense and single nucleotide frameshift mutations in becker muscular dystrophy

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We present clinical, molecular and immunohistochemistry study of 9 Italian Becker Muscular Dystrophy (BMD) patients carrying nonsense or single nucleotide mutations in DMD gene, leading to a premature stop codon.

From a clinical point of view, all patients are still ambulant, however 5 presented moderate phenotype and the oldest patient was not able to climb stairs or rise from the floor autonomously; CK was significantly elevated. The 2 oldest patients also displayed signs of heart involvement and were in therapy with ACE inhibitors. No patient was taking corticosteroids.

Muscle biopsies showed variable degree of necrosis and degeneration of muscle fibres, which correlates with age at biopsy and CK level. Dystrophin expression was only partially reduced with immunohistochemistry (IIH) and western blot (WB). Study of the transcript via RT-PCR was also performed.

It is known that nonsense and frameshift mutations in DMD potentially determine the interruption of protein synthesis and degradation of truncated dystrophin molecules, for this reason they are usually associated with Duchenne phenotype. The exact location of the mutation is considered an important factor. Nevertheless other factors, such as presence of splicing regulatory elements, can influence the ultimate outcome of nonsense mutation and they are largely unexplored.

These cases underscore the importance of a complete analysis of DMD gene and its transcript in order to provide better prognostic information for dystrophin patients and genotype-phenotype correlation. Nonsense mutations in BMD patients also raise question on the possible therapeutic approaches such as molecules able to read through premature stop signals.

Cardiac involvement in becker muscular dystrophy: bridging the gap between perihelal muscles impairment and myocardial damage

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Becker muscular dystrophy (BMD) is a genetic disorder of the skeletal muscle resulting from the altered expression of the protein dystrophin. Myocardial disease is common in BMD and usually manifests as a dilated cardiomyopathy phenotype, which is often recognized only when clinically overt. Understanding the pathophysiology of cardiac involvement in BMD could help identify subclinical myocardial damage and direct the appropriate therapeutic choices to counteract disease progression. Both in chronic heart failure and in mitochondrial diseases, it has been demonstrated that muscular damage increases ergoreflex sensitivity, causing a chronic sympatho-vagal imbalance that can negatively affect the heart. We aim to test this so called "muscle hypothesis" in the context of BMD. In our protocol patients with skeletal myopathy but without known cardiac disease will undergo a thorough cardiopulmonary evaluation including two-dimensional echocardiography, cardiac magnetic resonance imaging, 24-hour ECG recording, cardiopulmonary exercise testing, pulmonary function tests and biohumoral characterization comprehensive of high sensitivity

troponin T/I, brain natriuretic peptides, catecholamines, renin, aldosterone, galectin-3 and soluble suppression of tumorigenesis 2. Finally, baro-, chemo- and ergoreflex assessment will provide indexes of the autonomic function. Clinical and instrumental data will be correlated with autonomic function tests in order to investigate the association between peripheral muscles impairment, autonomic imbalance and heart involvement.

Eteplirsen is well tolerated in men with mild or moderate renal impairment

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Introduction. Duchenne muscular dystrophy (DMD) is a rare neuromuscular disease caused by *DMD* gene mutations that prevent production of functional dystrophin protein. Eteplirsen is an antisense oligonucleotide approved in the US for the treatment of DMD in patients who have a confirmed mutation of the *DMD* gene that is amenable to exon 51 skipping. In studies of DMD patients, approximately 64% of total systemic clearance of eteplirsen 30 mg/kg was via renal excretion.

Aim. Determine the pharmacokinetics (PK), safety, and tolerability of eteplirsen in men with mild or moderate renal impairment.

Methods. This single-dose, parallel-group study enrolled male volunteers with mild (estimated glomerular filtration rate [eGFR] ≥ 60 to < 90 mL/min; $n = 8$) or moderate (eGFR ≥ 30 to < 60 mL/min; $n = 8$) renal impairment. Participants received intravenous eteplirsen 30 mg/kg. Postdose PK, safety, and tolerability results were compared with demographically matched men with normal renal function ($n = 9$).

Results. All enrolled participants completed the study. Total plasma clearance decreased by 27.5% (mild group) and 60.6% (moderate group), with proportional reductions in renal clearance (22.7% and 56.6%, respectively), and higher overall exposure versus the normal group. The single intravenous dose was well tolerated by all groups. Three participants, one in each group, reported 6 treatment-related adverse events (AEs): dizziness (normal group); pyrexia (mild group); and pollakiuria, micturition urgency, and incontinence (moderate group). All AEs were mild to moderate, nonserious, and resolved.

Conclusions. Eteplirsen was well tolerated across renally impaired and normal renal function groups. Eteplirsen exposure increased as a function of decrease in renal clearance.

"Study sponsored by Sarepta Therapeutics, Inc."

Limb Girdle Muscular Dystrophies (LGMD): clinical and genetic characterization of a wide cohort of patients from a single center

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Background. Limb Girdle Muscular Dystrophies (LGMD) are a clinically heterogeneous group of disorders presenting with a spectrum of disease severity ranging from severe childhood onset muscular dystrophy to adult-onset myopathy. LGMDs include both dominant and recessive forms.

Methods. We reviewed detailed retrospective data of LGMD patients followed up at the Neuromuscular Centre at the University of Messina in the last 10 years. The cohort included 164 patients

in whom the diagnosis of LGMD has been defined using a combination of clinical, biochemical, morphological and genetic studies.

Results. 85 were females (51.8%) and 79 were males (48.2%). Mean age at last follow-up was 48.2 years ranging from 9 to 82 years. 99 patients of all (60.4%) were genetically confirmed. Among them, 15 patients (15.2%) were LGMD type 1 and 84 patients (84.8%) were LGMD type 2. Considering the LGMD classification, LGMD1B was the most frequent among the autosomal dominant forms, while the most represented among the autosomal recessive forms was LGMD2V, followed by LGMD2B and LGMD2A. Three patients LGMD2L presented a pseudometabolic phenotype. The age at onset, clinical progression and cardiac and respiratory involvements showed a wide variability in each LGMD subtypes.

Conclusions: Given the broad clinical spectrum, the combination of clinical and laboratory tests remains critically important to guide the physician to a final diagnosis of LGMD. Considering that natural history data are still poor, a systematic exploration of different LGMD phenotypes, the search for genotype-phenotype correlation and the identification of phenotypically homogeneous subgroups could be relevant for prognostic purposes and for further therapeutic approaches.

The known R818Q missense mutation of *TPNO3* gene in a further unrelated patient with early onset LGMD phenotype

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TPNO3 encodes a nuclear membrane protein of the importin family which transport serine/arginine-rich proteins into the nucleus. A heterozygous deletion (c.2771del) in the termination codon of *TPNO3* has been recognized as the causative genetic defect in a large autosomal dominant family affected by a form of Limb Girdle Muscular Dystrophy, namely LGMD1F. An additional missense mutation (R818Q) in exon 20 has been recently described in a sporadic male patient presenting an adult form of slowly progressive limb girdle weakness.

We report on a further LGMD patient in which we identified the same R818Q mutation in *TPNO3* gene as possible causative variant. Patient is the only child of healthy unrelated parents and family history is negative for neuromuscular disorders. He was first admitted at age 4 years for global hypotonia and raised CK (6546 U/L). Muscle biopsy showed scattered hypotrophic and necrotic fibres. Mutation in DMD gene were excluded. At age 10 years neurological examination disclosed diffuse muscle hypotrophy and weakness. Axial muscles and neck extensor were particularly affected. Last neurological examination at age 33 showed waddling gait, scapular winging, rigid spine and scoliosis. Weakness and hypotrophy of limb girdle muscles were present with Gowers sign. Muscle MRI showed fatty substitution of most hip and thigh muscles sparing biceps and soleus.

NGS analysis of known LGMD genes disclosed the R818Q change in *TPNO3* as the only variant. This mutation is present in gnomAD with a population frequency of 0.00004215 and is predicted to be damaging. The mutation is inherited from the father.

Multiplex ligation-dependent probe amplification usefulness in improving limb girdle muscular dystrophies molecular diagnosis

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Limb Girdle Muscular Dystrophies (LGMD) type 2A and 2B are autosomal recessive disorders associated with mutations in *CAPN3* and *DYSF* genes, respectively. Conventional analysis of these genes through direct sequencing is generally laborious and time-consuming due to their large size and mutational hot spots absence. Next-Generation Sequencing (NGS) has improved the diagnostic rate, but few LGMD2A/2B cases still remain unsolved.

We applied Multiplex ligation-dependent probe amplification (MLPA) method in a cohort of 30 suspected LGMD patients without molecular confirmation (18 LGMD2A and 12 LGMD2B). In these patients Western blot analysis supported the diagnosis and confirmed the reduction of calpain-3 and dysferlin in muscle. However conventional or NGS resulted in the identification of only one or no candidate/causative mutations in the respective coding genes.

MLPA allowed the molecular diagnosis in 1/18 LGMD2A (5%) and 4/12 LGMD2B (33%) patients. In particular MLPA detected: 1) an heterozygous *CAPN3* deletion including exons 1 to 6 in a LGMD2A patient; 2) the heterozygous *DYSF* deletion of exons 25 to 27 in two patients; 3) the homozygous *DYSF* deletion of exon 55 in two subjects. No duplication was found. Among *DYSF*-deleted patients, protein deficiency degree correlated with the probability of finding mutations.

We confirmed the usefulness of MLPA analysis in selected cases. MLPA should not be used as screening technique because it is tailored for the suspected candidate gene. It is strongly suggested in cases with only one mutation identified and/or protein absence at Western blot analysis. In the latter scenario, MLPA could precede gene sequencing.

Longitudinal functional outcomes in Limb Girdle Muscular Dystrophy type 2A (Calpainopathy)

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Limb Girdle Muscular Dystrophy type 2A (LGMD2A) is an autosomal recessive disease caused by *CAPN3* mutations determining quantitative and/or qualitative defects of calpain-3 protein. We sought to characterize the clinical progression of a single-center cohort of LGMD2A patients followed at our Neuromuscular Clinic at the University of Padova, in a 2-year time frame.

We evaluated 24 genetically defined LGMD2A patients at baseline and after 2 years with Manual Muscle Test (MMT),

North Star Ambulatory Assessment (NSAA), Six Minute Walk Test (6MWT), timed function tests (TFTs), grip/pinch dynamometry, and Performance Upper Limb (PUL). We also considered quality of life with psychometric tests (Individualized Neuromuscular disease Quality of Life, INQoL, and Fatigue Severity Scale, FSS). Cardiac and respiratory function were assessed. Significant changes were detected in hip adductor (right $p = 0.022$ and left $p = 0.030$), total (0.07 ± 0.19 , $p = 0.030$), and lower limb composite (-0.03 ± 0.24 , $p = 0.005$) MRC scores. NSAA and 6MWT show no significant 2-year change. Among TFTs, time to run/walk 10 m was the only one to increase significantly ($-0.15 \pm 0.36 = 0.045$). PUL showed a significant decrease (total score -2.5 ± 3.9 $p = 0.0078$ and elbow subscore -1.7 ± 3.3 , $p = 0.011$).

In conclusion, lower limb MRC scores, PUL, and 10 m walk/run seem the most promising outcome measures for LGMD2A clinical trials. However, sample size calculations suggest that adequately powered studies will need large samples for rare disease standards, in the order of 200-300 participants, even when the most sensitive measures are employed. Alternatively, subpopulations with the risk of fast functional worsening should be selected by inclusion criteria.

Role of autophagy in the pathogenesis of muscular dystrophies

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Autophagy is a critical process for the removal of damaged and dysfunctional organelles, protein aggregates and cellular components from the cell. Autophagy defects have been observed in many infectious and autoimmune diseases, in tumors, in neurodegenerative disorders and in some forms of muscular dystrophies. The aim of the research is study the expression by western blot of autophagy markers such as AMPK, phospho-AMPK (Thr 172), Beclin 1, LC3I, ULK Ser555, pACC (Ser 79) on samples of muscle biopsies from patients with muscle diseases DMD - BMD - LGMD - FSO - Bethlem Myopathy - Pompe disease stored in Naples Human Mutation Gen Biobank, to understand the role of autophagic signaling in the dystrophic muscle and determine the relationship between the degree of impairment and the progression of the disease. AMPK expression was first assessed in muscle samples from patients with different forms of muscular dystrophy comparing with healthy subjects. The expression resulted increased in dystrophic muscles, such as for Beclin 1 and LC3I, however, the expression of the phosphorylated form appears to be almost the same in the studied groups. The expression levels of pULK S 555 and pACC S79 in specimens of muscular dystrophies at different stages of disease were studied. The results obtained showed a decrease in their expression. These results, which to date are preliminary, show how the evaluation of autophagy is important to understand the degree of progression of the disease that could be the basis of pharmacological research able to modulate autophagic signals for a slower progression of disease.

The role of inflammation in pediatric sarcoglycanopathies: novel therapeutic perspectives

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Limb-girdle muscular dystrophies (LGMDs) are primary myopathies of the pelvic and shoulder girdles characterized by progressive muscle degeneration, and aggravated by sterile inflammation. Among them, sarcoglycanopathies (SGCs) are caused by mutation in the genes coding for a-b-g-d sarcoglycans which are crucial components of the dystrophin-glycoprotein complex, a multi-subunit complex that connects the cytoskeleton of a muscle fiber to its extracellular matrix and guarantees the membrane integrity during muscle contraction. In LGMDs the underlying pathophysiological mechanism and the role of inflammation are poorly investigated and no treatment is available for these rare diseases. Aim of this study is to define the immunological signature of patients and mice affected by SGC.

Immunophenotypical analysis by flow cytometry indicates that lymphoid and myeloid infiltrates were significantly higher in limb muscles and diaphragm of SGCa mice *versus* WT. In particular, we observed a pronounced increase of CD3⁺CD4⁺, CD3⁺CD8⁺, CD3⁺CD4⁺CD8⁺ T lymphocytes sub populations. Analogously, the number of CD11b⁺/Ly6G⁺ neutrophils, CD11b⁺/Ly6G⁺/Ly6C⁺ monocytes, F4/80⁺ macrophages, F4/80⁺CD11c⁺ dendritic cells and CD3⁺CD1d⁺ NKT cells was significantly higher in SGCa mice *versus* WT. In contrast, no quantitative differences in lymphoid and myeloid cells were detected in the spleen and PB of SGCa and WT mice. Immunohistochemical analysis of LGMD2 patient muscle biopsies showed a significant increase of CD3⁺CD4⁺, and CD3⁺CD8⁺ cells in comparison to control subjects not affected by muscular dystrophy.

In conclusion, this study defines the immunological signature of patients and mice affected by SGC suggesting a relevant role of inflammatory responses in the pathogenesis of these muscular dystrophies.

Study of cognitive and psychological profiles in FSHD patients

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Introduction. While signs of systemic and cerebral nervous system (CNS)'s abnormalities are occasionally reported in rare paediatric severe form of FSHD, a CNS involvement in the adult-onset classic form of FSHD has not been accurately investigated.

Methods. Here, we present a neuropsychological and psychopathological evaluation protocol aimed to evaluate the cognitive and psychological functioning, also in relation to degree of motor impairment and disability, in a cohort of 20 adult patients clinically and genetically characterized. In particular, the protocol includes the evaluation of visuo-spatial short-term memory span and working memory, verbal learning, visual at-

tention, executive functioning and cognitive processing; the protocol also investigates subclinical occurrence of psychopathological symptoms and psychological status.

Results and conclusions. Patients showed a generally reduced performance in every cognitive functional area. These results are suggestive of a cognitive and psychopathological impairment in adult-onset forms of FSHD, thus encouraging the planning of future investigations to better characterized the cognitive profiles of these patients.

Otoacoustic emissions are a sensitive tool for detecting cochlear damage in facioscapulohumeral muscular dystrophy type 1 (FSHD1)

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Facioscapulohumeral muscular dystrophy type 1 (FSHD1) is characterized, beyond selective muscular weakness and atrophy, by extra-muscular symptoms like sensorineural hearing loss, retinal vasculopathy, epilepsy and cognitive impairment. We assessed the cochlear function in a cohort of 26 FSHD1 patients and in healthy controls matched for age and gender. All subjects underwent a complete neurological and audiological examination, including pure-tone audiometry (PTA) and otoacoustic emissions (OAEs) which explore outer hair cell function. Transient evoked otoacoustic emissions (TEOAEs) and distortion product evoked otoacoustic emissions (DPOAEs), which extend the examination to a wider frequency spectrum, were recorded. PTA results of patients and controls were comparable. However, FSHD1 patients showed significantly reduced responses of both DPOAEs and TEOAEs at all frequencies, even when considering only subjects with a normal PTA or with a mild muscular involvement (FSHD score ≤ 2). No correlation between OAEs and EcoRi fragment length was found. In conclusion, cochlear echoes represent a sensitive tool in detecting subclinical outer hair cell damage in FSHD1, independently from hearing loss and severity of genotype.

Facio-Scapulo-Humeral Muscular Dystrophy and infantile onset epilepsy with uncommon trend: a case report

Silicini S.

Facio-Scapulo-Humeral dystrophy is the third most common form of muscular dystrophy with a prevalence of 1:15000-20000 and represents a disease with multisystemic involvement.

We describe a case of a 16-year-old boy affected by FSHD, inherited from his mother.

Francesco regularly achieved the first stages of psychomotor development, but at primary schools he was diagnosed for specific learning disability.

His physical examination detected facial hypomimia, chewing fatigue, winged scapulae, Beever's sign and "poly-hill" sign; proximal weakness of the limbs, with greater left impairment, difficulty in walking on the heels; FSHD score was 4/15. CCEF (Comprehensive Clinical Evaluation Form) could be classified as A3.

At the age of 3, he presented focal to bilateral tonic-clonic seizures.

The EEG showed paroxysmal multifocal epileptiform abnormalities, over the left parietal and the right frontal areas. Brain MRI was unrevealing. Treatment with carbamazepine (CBZ) was started with complete seizure remission.

Six years after CBZ withdrawal, his school performances worsened, with deficits mainly in attention and memory skills. The EEG showed an increase of the epileptiform abnormalities with left anterior prevalence both during wakefulness and sleep. Thereafter, the patient presented episodes characterized by brief psychomotor arrest with palpebral myoclonias and myoclonic jerks of the upper limbs usually upon awakening without loss of awareness but with falling objects from the hands.

We reported this case for the peculiar association of FSHD and epilepsy, and for the particular course of the epileptic phenotype at long-term follow-up.

P 1-2 SMA, Congenital myopathies and CMD

Clinical and muscle MRI profile of a cohort of SMA patients being treated with Nusinersen

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In recent years the discovery of effective therapies has marked the history of Spinal Muscular Atrophy (SMA). The only currently approved drug (from Agenzia Italiana del Farmaco in September 2017) is Nusinersen which is an antisense oligonucleotide that binds to the SMN2 pre-mRNA downstream of exon 7, leading to the translation of a fully functional SMN protein and which is administered intrathecally by lumbar puncture. The treatment has opened up a new scenario with the creation of a new phenotypic spectrum. To monitor the effects of therapy, serial evaluations have been planned: neurological exam, muscle strength (MRC), motor functional scales, timed tests, muscle MRI, CMAP and MUNE. We report the data of a cohort of 7 patients treated with Nusinersen. Six of them are affected by SMA3 with different degrees of motor impairment and duration of illness, two of them are in wheelchairs since the age of 18 and 29 years. The starting age of treatment is between 9 and 35 years. The only side effect noticed was headache post lumbar puncture in 4 subjects. The seventh patient is a 10 month old child affected by SMA1 who started therapy when he was three months old. All of the patients have received the load-

ing dose, four of them received 6 treatments and two patients 5 treatments. Monitoring long-term outcome measures will allow us to characterize the new phenotypes of the disease and to define the efficacy spectrum of Nusinersen in patients with different ages and degrees of motor impairment.

Update from SUNFISH Part 1: Safety, tolerability and PK/PD from the dose-finding study, including exploratory efficacy data in patients with Type 2 or 3 spinal muscular atrophy (SMA) receiving risdiplam (RG7916)

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Background. SMA is caused by reduced levels of survival of motor neuron (SMN) protein due to deletions and/or mutations of the *SMN1* gene. While *SMN1* produces full-length SMN protein, a second gene, *SMN2*, produces low levels of functional SMN protein. Risdiplam (RG7916/RO7034067) is an orally administered, centrally and peripherally distributed small molecule that modulates *SMN2* pre-mRNA splicing to increase the levels of functional SMN protein.

Methods. SUNFISH (NCT02908685) is an ongoing multicenter, double-blind, placebo-controlled (randomized 2:1, risdiplam:placebo) in patients aged 2-25 years, with Type 2 or 3 SMA. Part 1 (n = 51) assesses safety, tolerability and PK/PD of different risdiplam dose levels. Pivotal Part 2 (n = 180) is assessing the safety and efficacy of the risdiplam dose level that was selected based on results from Part 1.

Results. SUNFISH Part 1 included patients of broad age ranges and clinical characteristics (functional level, scoliosis and contractures). To date (data-cut, 06 July 2018), a sustained, > 2-fold increase in median SMN protein versus baseline was seen after 1 year of risdiplam. Adverse events have been mostly mild, resolved despite ongoing treatment and reflect the underlying disease. No drug-related safety findings have led to withdrawal. Despite not being designed and powered to detect efficacy, patients on risdiplam experienced improvement over 12 months in motor function measures versus natural history. Exploratory efficacy will be presented in patients treated for ≥1 year.

Conclusions. To date, no drug-related safety findings have led to withdrawal. Risdiplam has led to sustained increases in SMN protein. Part 2 is ongoing worldwide.

The c.859G > C variant in *SMN2* modulates clinical severity in SMA: a case report

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Background. The number of copies of *SMN2* is the most important gene modifier of disease severity in SMA. Patients with 2 copies of *SMN2* usually develop type 1 or type 2 SMA and only rarely present with type 3 SMA. Recent reports have demonstrated that single base substitution in *SMN2*, c.859G > C in exon 7 creates a new exonic splicing enhancer element improving the amount of full-length transcripts, thus resulting in the less severe phenotypes, regardless of the number of *SMN2* copies.

Case presentation. We present two siblings of 43 and 45 years-old (yo) bearing 2 copies of *SMN2* but bearing a type 3b phenotype. Despite the identical genetic background, and the apparently similar onset at 10 years old, the progression of disease differed in these 2 siblings. The younger lost ambulation at 30 yo while the brother is still able to walk for a few meters with support. Neither show significant involvement of the upper limbs, complain of dysphagia, or have respiratory impairment. Both started nusinersen treatment at age 42 and 44 respectively. Results of baseline muscle MRI, functional motor tests, respiratory assessments, quality of life and disease burden perception were collected at each nusinersen infusion.

Conclusion. We confirmed the good clinical prognosis of the c.859G > C variant in exon 7 of *SMN2*; however the different clinical progression over time and response to treatment in these genetically identical siblings suggests that other factors playing a role in modifying the phenotype.

***SMN1* intragenic mutations in a cohort of Italian SMA patients**

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Spinal Muscular Atrophy (SMA) is a motor neuron disorder due to recessive mutations in *SMN1*, encoding for the Survival Motor Neuron (SMN) protein. Most of SMA patients harbor homozygous *SMN1* deletions while *SMN2* copy number predicts the clinical subtype (SMA-I, -II, -III). Few (3-5%) SMA patients display small mutations on the second allele.

In the last 15 years, we achieved a molecular diagnosis in a cohort of 127 Italian SMA patients. Homozygous *SMN1* deletion was detected in 120 probands (94.5%).

In 7 patients a heterozygous *SMN1* deletion was in compound with a point mutation. A null allele was observed in 3 SMA-I cases: c.469C > T (p.Q157*), c.888+1G > C, c.511G > T (p.E171*). The missense mutation c.389A > G (p.Y130C) was found in a SMA-III patient while c.815A > G (p.Y272C) substi-

tution occurred in 3 probands (1 SMA-I, 2 SMA-II). The Y130C change has been previously associated with SMA-III presentations, irrespective of SMN2 copies. Conversely, Y272C severely impairs SMN oligomerization and function.

Direct sequencing of SMN2 exon 7 detected the modifier variant c.859G > C in 3 SMA-III subjects (2.5% of our cohort of SMN1-deleted patients) harboring 2 SMN2 copies. This transversion is expected to increase full-length transcript originating from SMN2 alleles. Late onset and mild clinical course of our c.859G > C carriers support this conclusion.

The behavior of point mutations in SMN1/SMN2 is heterogeneous and requires proper validation. The identification of small mutations in SMA patients is important to improve diagnosis and prognosis, to clarify the response variability to available treatments and to design novel therapies addressing these peculiar mutations.

Nusinersen and skin necrosis in an infant with spinal muscular atrophy type 1: effects over time

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Introduction. Spinal muscular atrophy (SMA) is a primary motor neuron disorder, however autonomic system dysfunction has been increasingly observed. Vascular perfusion abnormalities and skin involvement have been reported in few infants with severe SMA1 and are considered a feature of possible multiple organ involvement associated with severe phenotype.

Case presentation. We describe a 9-months old child with diagnosis of SMA 1 (1 copy of SMN2) at birth. At 1 month, tracheostomy was performed followed by gastrostomy at 2 months. At 4 months she presented necrosis of toes followed by extensive necrosis of distal phalanges of her hands in the next month. She lost the distal phalanx of one toe at 5 months. Dystrophic epidermolysis bullosa was initially suspected, and she started treatment with fucidic acid cream with no change. At 7 months and half, when the child was admitted to our institution, she presented an hypotonic tetraplegia and clearly evident skin lesions in upper and lower limbs; she started intrathecal nusinersen treatment. At 4th infusion, the skin lesions were largely improved and replaced by scar tissue. Follow-up confirmed distal and proximal necrosis resolution and an improvement in general health conditions as per parent impression.

Conclusion. Based on previous studies antisense oligonucleotide leakage from the central nervous system does not reach a sufficient concentration in peripheral tissues to affect SMN2 splicing. The timing of improvement in our patient suggests a possible causal relationship to intrathecal nusinersen suggesting a centrally mediated mechanism on peripheral tissues which persists over time with repeated injections.

SMN genes molecular testing in a cohort of 1546 subjects tested for genetic diagnosis and trial enrollment

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder, characterized by symmetrical muscular weakness and atrophy. The incidence is variable from 1 in 6000 live births but the heterozygotes frequency in caucasian population is about 1/64. *SMN1* and *SMN2* highly-homologous genes play a crucial role in SMA etiopathogenesis: *SMN1* mainly homozygous deletions occur in more than 95%, while *SMN2* number of copies may modulate the phenotype. The frequency of variation in *SMN1* copy number per allele also varies and differs among populations. Here we report *SMN* genetic results obtained during the last eight years in our reference center (EURO-NMD). The most frequent reasons for referral are represented by consanguinity, positive family history for *SMN1* deletion or SMA, and carrier preconception screening.

We calculated the frequency of different *SMN1* genotypes in 1546 tested subjects including 60 prenatal tests performed on chorionic villous samples. *SMN1* heterozygous deletion were 16%, homozygous deletion 4.6% and heterozygous duplication 6.5% (3 copies of *SMN1*). Majority of subjects with duplication comes from North and West Africa or from Pakistan, and were tested since of consanguinity.

Among SMA patients, the majority has 3 copies of *SMN2* (45%), the remaining patients have 2 or 4 copies equally frequent. The high rate of *SMN1* duplication occurrence highlights its importance when performing carrier testing and risk assessment. Considering the approved orphan drugs for SMA, genetic testing is now compulsory and determining *SMN1* and *SMN2* copy number cistronic association might be very valuable for therapy outcome interpretation.

Circulating microRNAs as potential biomarkers to monitor response to nusinersen in SMA patients

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in survival motor neuron 1 gene (*SMN1*), resulting in a truncated SMN protein responsible for progressive degeneration of brain stem and spinal motor neurons. The paralogous *SMN2* gene partially compensates the production of full length SMN protein, mitigating the phenotype. Antisense oligonucleotide (ASO) nusinersen (SpinrazaTM), designed to increase the transcription levels of *SMN2* gene, is the only approved treatment for SMA in USA and Europe. SMN protein function in SMA pathogenesis is still not completely under-

stood. Notably, SMN is involved in RNA processing, and it has been linked to microRNAs (miRNAs) biogenesis. MiRNAs are small, non-coding RNAs that regulate post-transcriptional gene expression; they contribute to different biological functions implicated in the pathogenesis of neuromuscular diseases, including SMA. Nonetheless, miRNAs are stable in body fluids, indicating their potential as biomarker. In the present study, we characterized the expression of selected neural and muscular-specific miRNAs in the serum of 19 pediatric SMA patients (15 type 2, 4 type 3) at baseline and after 6 months of nusinersen treatment. Molecular results were correlated with motor function assessed by the Hammersmith Motor Function Scale Expanded (HFMSE). We observed that circulating miRNA expression in SMA patients' serum changed from baseline under nusinersen treatment, and that miRNA level changes predicted response to treatment (HFMSE scores ≥ 3 points from baseline). Our data support the potential of targeting miRNAs as non-invasive biomarkers to monitor disease progression and therapeutic response in SMA.

Nusinersen treatment in Spinal muscular atrophy: the experience of Bambino Gesù Hospital

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Background. In December 2016 and in June 2017, respectively, the FDA and EMA approved Nusinersen as the first treatment for SMA. Bambino Gesù Hospital started to treat patients with the SMA type 1, from november 2016, as part of the Expanded Access Programm (EAP), and from november 2017, after the commercial approval, the later onset forms. Methods: we recruited patients followed in the Neuromuscular Unit. We activated a multidisciplinary team composed of neurologists, pneumologists, anaesthesiologists, radiologists, physiotherapists and pharmacists, in order to prepare the drug, assess the motor functional abilities and monitor the effectiveness, assess the respiratory function and the risk of sedation and perform the injection in severe scoliosis. The intrathecal delivery was performed by pneumologists or neurologists. In ASA3 the intrathecal administration was performed with local, in ASA2 patients anaesthesia was induced in all patients with Propofol and the procedure was performed in

NORA. In patients with complex spine due to severe scoliosis or spinal instrumentation the procedures were performed with fluoroscopic guide.

Results. We treated 57 patients (29 SMAI, 15 SMAII and SMAIII). The age range was 2 m-7.9 ys. 20 patients were in NIV (8 type I, 4 type II); 13 type I patients had tracheostomy; 20 patients had scoliosis (10 SMAI, 6 SMAII, 2 SMAIII) of whom 6 were submitted to spine surgery; 8 patients received the intrathecal injection by fluoroscopic guide. In 8 SMAI patients the treatment was stopped (in 5 for no effectiveness, in 3 for SAE). 24 patients completed 1 year of treatment. In 66% of

patients improvement in muscle strength or in QOL together to stabilization of respiratory and swallowing function was served.

Conclusions. Nusinersen is effective, feasible and safe even in patients with complex spinal anatomies and respiratory insufficiency. To guarantee the quality of the procedure, we recommend establishing an experienced interdisciplinary team.

Central core disease and facioscapulohumeral dystrophy-like phenotype in a family of South Italy carrying a novel heterozygous mutation in hnRNPA1 gene and a D4Z4 partial deletion: clinical features of an overlapping syndrome and genotype correlation.

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We report the first familial case of a novel heterozygous Gly270Val mutation in the exon 8 of the heterogeneous nuclear ribonucleoproteins hnRNPA1 associated with a 41 kb D4Z4 allele on chromosome 4q35 realizing an overlapping phenotype between facio-scapulo-humeral dystrophy and central-core myopathy. The patients, a young girl and his father, showed bilateral winged scapula with severe asymmetric atrophy of upper limb muscles and waddling gait. Muscle biopsy didn't detected any dystrophic changes such as an increase in fibrous and adipose tissue or internal nuclei, but the most relevant alteration were numerous central-core and moth-eaten fibers evident with oxidative enzymes staining. We discuss the hypothesis that the phenotype observed in this family is the result of two genetic mutations that could determine a synergic effect and the possible role of a novel mutation of hnRNPA1 gene determining a myopathic pattern.

Severe congenital RYR1-associated myopathy with multiple fractures

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Clinical features associated with the severe neonatal presentation of RYR1-associated myopathy include decreased fetal movement, hypotonia, poor feeding, respiratory involvement, arthrogryposis, ophthalmoplegia, femur fractures and hip dislocation at birth. We describe the clinical and genetic characteristics of a newborn male, fifth son of healthy consanguineous parents, who presented severe hypotonia, respiratory distress requiring intubation and mechanical ventilation, difficulty in suctioning and swallowing requiring nasogastric tube feeding, dysmorphic features, contractures and multiple congenital fractures of femur, tibia and humerus. Pregnancy was complicated by polyhydramnios. No reduction in fetal movement was reported. An older sister died at 34 days of age with the same clinical phenotype. CK levels were normal. EMG showed myopathic abnormalities. Muscle MRI showed marked fatty infiltration of both upper and lower limbs. Muscle biopsy was not

specific but showed severe myopathic changes. Array CGH and genetic test for SMN1 were normal. Genetic analysis performed with TruSight panel showed the c.6661A > T/p.(Lys2221*) and c.13742A > G/p.(Tyr4581Cys) mutations in RYR1. Clinical course gradually improved. The baby was extubated and placed on NNIV. Dysphagia progressively ameliorated with complete restoring of oral feeding. Spontaneous movements of both upper and lower limbs improved as well as contractures. The last follow up visit was performed when the baby was 7 months old and showed that he had acquired a partial head control. Our report confirms and further expands the clinical and histological variability associated with severe congenital RYR1-associated myopathy.

STIM1 mutations: new mutations and different phenotypes

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STIM1 is a reticular Ca²⁺ sensor composed of a luminal and a cytosolic domain. Dominant mutations in STIM1 are a cause of three allelic conditions: tubular aggregate myopathy, Stormorken syndrome (a complex phenotype including myopathy, hyposplenism, hypocalcaemia and bleeding diathesis), and a platelet dysfunction disorder, York platelet syndrome

In the clinical study and molecular characterization of 332 patients with neuromuscular disorders we used a customized targeted multigene panel in NGS dedicated to muscle diseases and identified pathogenic rare variants in 305 cases. In this study we report on seven patients (age ranged 26-57 years) who harbored mutations in the *STIM1* gene identified in our NGS study. The age of onset of the affected patients ranged from birth to adulthood. Two patients presented clinical characteristic compatible with Stormorken syndrome, three out of seven presented with congenital muscle weakness, one with adult onset of non-specified myopathy and one referred only myalgia. The ability to walk was quite conserved in all patients. In three patients muscle biopsy showed the presence of tubular aggregates, atrophy of type I fibers in two patients and non-specific myopathic signs in two. The mutations we identified were distributed throughout the gene, and five were novel mutations. Of interest, a specific variant (p.Asp84Glu) was associated with of Stormorken syndrome. Our work extends the series of mutations in the *STIM1* gene and associated phenotypes.

Congenital myopathy with fiber type disproportion related to novel STAC3 mutation

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SH3 and cysteine-rich domain-containing protein 3, encoded

by STAC3 gene, is a protein essential for skeletal muscle contraction, involved in excitation-contraction coupling (ECC) through a not completely understood mechanism. Native American myopathy (NAM), characterized by congenital hypotonia and weakness, cleft palate, short stature, ptosis, kyphoscoliosis, talipes deformities, and susceptibility to malignant hyperthermia (MH) has been originally described in 5 Native American families as a result of the single founder p.Trp284Ser variant in STAC3.

Additional 21 families of non-Native American ethnicity, affected by congenital myopathy of variable severity, have been later described, mostly in association to the common p.Trp284Ser variant. Only 5 different pathogenic variants have been reported in *STAC3* so far.

Here we report an Italian baby (aged 7 months) with severe neonatal hypotonia and respiratory impairment. Muscle biopsy, performed at the age of 2 months, showed congenital fiber size disproportion with a marked type I predominance.

Next generation sequencing in this patient revealed a novel homozygous c.685_686delGA variant in exon 8 of *STAC3* converting the Asp²²⁹ to a premature stop codon. The deletion of the C-terminal 135 aminoacids of STAC3 by the p.(Asp229Ter) mutation causes a loss of the two tandem SH3 domains of the protein which are known to be involved in interaction with Ca_v1.1 and disruption of this interaction perturbs skeletal muscle EC coupling.

We describe the first Italian patient with *STAC3*-related congenital myopathy expanding the genotypic spectrum of this gene.

Phenotypic spectrum of ryr1 recessive myopathy with early onset

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Ryanodine receptor 1 (RyR1) is a calcium release channel with a pivotal role in the muscular excitation-contraction coupling. RyR1 is encoded by a 106 exons gene whose mutations have been linked with a variety of myopathies and with malignant hyperthermia susceptibility (MHS). Both dominant and recessive mutations have been reported. Dominant mutations are typically associated to central core disease (CCD) and MHS. Recessive mutations are characterized by a wider histo-

pathological spectrum, comprising CCD, multiminicore disease (MmD), centronuclear myopathy (CNM) and congenital fiber type disproportion (CFTD).

Herein we present clinical, histopathological and genetic features of 50 unrelated patients carrying *RYR1* recessive mutations from 12 Italian Neuromuscular Centres. Patients currently aged 2-63 years, all presented with symptoms at birth or in the very first years of life. 11 patients are not ambulant. 22 patients developed important scoliosis and joint retractions. Only 9 patients presented with ophthalmoplegia but myopathic face was evident in most cases. 10 patients require ventilation support. Three patients required PEG for feeding problems. Muscle biopsies were consistent with core or multiminicore disease in 29 cases. Four had central nuclei and 3 also had increased connective tissue and fibrosis. Predominance of type 1 fibres was another common feature. Genetic analysis revealed compound heterozygous mutations spread over the entire length of the gene in most patients. 11 patients carry homozygous mutation. Most variants were missense. 4 patients carry stop mutations on one allele and resulted to be more severely affected.

Novel ACTA1 mutation causes late-onset nemaline myopathy with fuzzy-dark cores

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ACTA1 gene encodes skeletal muscle alpha-actin, the principal actin isoform in adult skeletal muscle, which forms the core of the thin filament of the sarcomere where it interacts with a variety of proteins to produce the force for muscle contraction. ACTA1 is implicated in several muscle disorders including nemaline, cores, rod-core or actin aggregate myopathies and fiber-type disproportion. We report clinical, muscle imaging and histopathological data from an Italian family harboring a novel ACTA1 mutation with peculiar histopathological findings. Affected members showed a late-onset diffuse muscle weakness (facial, axial, proximal and distal) with muscle hypotrophy. Muscle MRI showed fibro-fat replacement in gluteus minimus, quadriceps, biceps, abductors and gastrocnemius. Muscle biopsy showed the presence of nemaline bodies with several fuzzy-dark areas at Gomori Trichrome in type I muscle fibres, corresponding unstructured cores at electron microscopy, with abundant electrodense material. The molecular analysis revealed a new mutation in exon 3 of the ACTA1 gene in heterozygous state, segregating with affected members in the family. This mutation has never been previously reported and this findings define a peculiar histopathological presentation of nemaline myopathies. Our findings enlarge the genetic and morphological spectrum of ACTA1 related myopathies.

Severe neonatal congenital myopathy and hypotonia: a new perspective through NGS.

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Congenital myopathy is an uncommon neonatal disorder defined by hypotonia and muscle weakness that can manifest in the neonatal period. Presentation with signs of global hypotonia and respiratory insufficiency is among the classic findings.

We here report clinical and genetic characteristics of two newborns, whose phenotype was characterized by severe hypotonia and serious respiratory distress syndrome that required mechanical ventilation.

The diagnostic procedure was completed by NGS analysis using a panel of 5228 genes (Constitutional Panels, Agilent) that identified rare mutations in *RYR1* and *TTN*, respectively.

The first patient, a premature infant male, showed a homozygous variant in exon 104 of *RYR1* (p.Phe4976Leu). This variant was previously reported in affected males with severe neonatal myopathy, dysmorphism and motor-developmental delay, confirming its pathogenetic role.

The second patient, a newborn female, presented two loss of function variants (p.Pro34879Glnfs*36 and p.Arg5308Stop) in exon 54 and 358 of *TTN*.

Prior to NGS, the complete analysis of these genes was routinely impossible due to their giant size and complexity. Due to widespread use of NGS, *TTN* and *RYR1* are emerging as responsible in different cases of human congenital myopathy.

For these neonatal disorders, the early diagnosis and an accurate NGS-based genomic investigation help in defining disease prognosis and patient management and enable a proper genetic counselling of the reproductive risk.

Key words: congenital myopathy, hypotonia, NGS

Genome and transcriptome analysis of COLVI genes and characterization of a new promising cellular model

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Collagen VI-related diseases (COL6-RD) are a group of inherited myopathies with varying degree of clinical severity, caused by mutations in the *COL6A* genes.

As EU reference center for neuromuscular disorders and partner within the EURO-NMD, we aim at providing a nationwide study of COL6-RDs patients and an overview of *COL6A* genes variants. 245 patients were recruited via our Genetic Counselling Service as well as referred to us by other Italian centers (pediatrics, genetics and neurologists) and analyzed over a 12-year period (2006-2018). 222 patients were studied by standard diagnostic tools and 23 by NGS Illumina TruSeq Custom *COL6A* genes panel. 186 disease-causing variants, evenly distributed through the three *COL6A* genes, were identified in 150 patients with a detection rate of 61.3%. Using RNA tools (Custom Fluidigm microfluidic card and RNA-Seq) we characterized the *COL6A* genes transcripts on urine stem cells (USCs) and fibroblasts from patients and healthy controls. The transcripts comparison with the skeletal muscle showed that some splicing choices and isoforms representation are different in USCs. Indeed, the *COL6A3* full-length isoform represents the prevalent transcript in skeletal muscle while is expressed in USCs and fibroblast at very low levels. We also demonstrated that native USCs secrete functional collagen VI proteins, able to organize in the extracellular network.

Our data provide a large COL6-RD patients cohort fully genetically characterized and propose native USCs as a non-invasive in vitro tool for functional studies, drug screening and validation in COL6-RDs.

Mutations in *ACTN2* gene cause a novel form of adult-onset distal myopathy

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A cohort of patients from four different families (three Spanish and one Swedish), presenting with a novel form of adult-onset, asymmetric distal myopathy, was clinically and pathologically characterized. All of patients shared a characteristic pattern of muscle involvement with the atrophy of tibialis anterior and the initial involvement of ankle dorsiflexion later progressing to

proximal limb muscles. In the most severe cases, histological analyses revealed a variation in the fiber size, internal nuclei and a large number of fibers containing rimmed vacuoles.

A missense variant, c.1459T > C (p.C487R), in the alpha-actinin-2 gene (*ACTN2*) was identified in the probands of the three Spanish families. A second *ACTN2* missense variant, c.392T > C (p.L131P), was identified in the affected members of the Swedish family. All the *ACTN2* missense variants identified are fully penetrant and co-segregate with the disease in all the families enrolled.

ACTN2 encodes for alpha actinin2, a protein highly expressed in the Z-disk of cardiac and skeletal muscles, where it interacts with other clinically relevant sarcomeric proteins, including titin and UDPN-acetylglucosamine 2-epimerase (GNE).

Our study demonstrates that *ACTN2* mutations cause a new type of dominant distal myopathy with a late-onset and a slow progression.

At the same time as our study, two different *ACTN2* variants (a missense and an in-frame deletion) have been reported in patients with a congenital myopathy. Further additional studies are needed to clarify the molecular mechanisms of the actinopathies and to improve our understanding of the genotype-phenotype correlation.

Compound heterozygous nonsense *LAMA2* mutations detected by exome sequencing in two siblings with atypical phenotype and normal brain MRI

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LAMA2 mutations cause the most frequent congenital muscular dystrophy subtype MDC1A and a variety of milder phenotypes, characterized by total or partial laminin- α 2 deficiency. Most common presenting symptoms are hypotonia at birth or in the first weeks of life or delayed motor milestones during the first year of age. Rare patients, among those with complete absence of laminin α 2 protein on muscle biopsy, achieve independent ambulation; conversely, most patients, among those with partial laminin- α 2 deficiency, achieve ambulation. In both severe and milder cases brain MRI invariably shows abnormal white matter signal intensity. We report clinical, histopathological, imaging and genetic data on two siblings with very subtle, and at first undetected, reduction in laminin- α 2 expression, and brain MRI showing minor non-specific abnormalities. Clinical features in the female proband were characterized by muscle weakness involving neck and axial muscles, and pelvic girdle and distal lower limb muscles, reduced tendon reflexes and pes cavus. Clinical features in a younger brother were similar, and remained stable in both siblings during the follow up. Whole exome sequencing (WES) detected two heterozygous truncating *LAMA2* mutations. Brain MRI in combination with laminin- α 2 immunohistochemistry might not be sufficient and WES might be the only means to reach a diagnosis.

Longitudinal functional changes in a cohort of adult nusinersen-treated spinal muscular atrophy patients at the Padova Neuromuscular Center

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Objective. To evaluate longitudinal functional changes in clinical outcome measures in a cohort of nusinersen-treated spinal muscular atrophy (SMA) type II and III patients in a single center.

Methods. Thirty-three ambulant and non-ambulant SMA patients (4 type II and 29 type III), aged 15-68 years, were treated with nusinersen, an antisense oligonucleotide modulating pre-mRNA splicing of the survival motor neuron 2 gene (*SMN2*). Patients underwent intrathecal administration of 12 mg of nusinersen on days 1 (L1), 14 (L2), 28 (L3), 63 (L4) (loading doses) and approximately every 4 months thereafter (maintenance doses) (M1, etc). The patients were clinically evaluated at L1, L4 and thereafter every 4 months using Hammersmith Functional Motor Scale-Expanded (HFMSE), Six-Minute Walk Test (6MWT), manual muscle strength evaluation according to Muscle Research Council (MRC) scale, Timed-Function Tests (TFTs) and revised Upper Limb Module (RULM).

Results. All patients reported subjective benefit from the treatment. HFSME (L1-L4 $p = 0.00002$; L1-M1 $p = 0.001$, L1-M2 $p = 0.029$) and MRC (L1-L4 $p = 0.013$; L1-M1 $p = 0.002$, L1-M2 $p = 0.007$) improved significantly. RULM and time to climb 4 stairs were not significant but a positive trend was observed. The 6MWT was significant at L1-L4 ($p = 0.036$) and trended but did not reach significance thereafter.

Conclusions. SMA type II and type III patients treated with nusinersen showed subjective and statistically significant improvement in some meaningful outcome measures.

Sessione 2 7 giugno dalle ore 14.30-15.30

P. 2-1 Mitochondrial and metabolic myopathies

Identification of maternal uniparental disomy of chromosome 10 in a patient with *PITRM1* mutation and mitochondrial dysfunction

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PITRM1 is a mitochondrial metalloproteinase, which digests oligopeptides including the mitochondrial targeting sequences, cleaved from proteins imported across the inner mitochondrial membrane. Mutations in *PITRM1* have recently been associated with early onset autosomal recessive spinocerebellar ataxias.

We describe a six-year-old boy, born from healthy unrelated parents, presenting delayed motor and language development. Since 25 months of age the child showed febrile tonic-clonic seizures with brain MRI showing a severe cerebellar atrophy and moderate atrophy of the pons with a diffuse signal alteration of cerebellar cortical and subcortical areas. At the age of 6 years neurological examination revealed ataxic and distal dyskinesias, severe intellectual disability and absent speech. Brain MRI demonstrated that the cerebellar atrophy was stable but a thalamic involvement was highlighted. Muscle biopsy showed slight mitochondrial proliferation and reduced enzyme activity of the respiratory chain complexes I+III.

Using a targeted multigene panel we identified a new homozygous mutation in *PITRM1*. Segregation analysis showed that the mother was heterozygous whereas the father was wild-type. We then performed high-density SNP-CGH array analyses demonstrating a segmental uniparental disomy (UPD) in chromosome 10, by localizing interspersed regions of heterodisomy and isodisomy with maternal UPD10. In 10p15.3 region is located the mutated *PITRM1*, in homozygosity. In skin fibroblasts, we detected a significant reduction of *PITRM1* protein expression in the patient, with low oxygen consumption and impaired basal respiration.

This report expands the genotype-phenotype correlations of *PITRM1* mutations and corroborates the need of a full molecular examination of apparently homozygous changes in mitochondrial disorders.

Cognitive impairment precipitated by head trauma in MELAS syndrome

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After a traumatic brain injury, mitochondrial dysfunction occurs with an increase in reactive oxygen species and a decrease in ATP production; this can lead to headache and cognitive/behavioral deficits.

Few reports in the literature show the consequences of head trauma in patients affected by mitochondrial disorders.

We report the clinical history of a patient with Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes (MELAS) who started to manifest behavioral changes immediately after a head trauma, to further investigate the assumption that traumatic brain injury can trigger the progression of mitochondrial disorders.

A male patient affected with MELAS syndrome (3242A > G mutation) and followed in our Neuromuscular Unit reported a head trauma at the age of 43. Until that moment, no cognitive decline had been observed nor had he manifested overt MELAS syndrome symptoms. He became unconscious and ended up in a coma for about two weeks.

When he woke up, a marked change in his mental status was noticed i.e. behavioral abnormalities (disinhibition, sexual delusions), personality change and cognitive impairment. His clinical condition remained stable over ten years, then a further, slowly progressive decline set in with episodes of hallucinations, loss of consciousness and severe loss of spontaneous speech.

Mitochondrial dysfunction has a central role in determining cellular damage in head injuries. In our case, the trauma affected a tissue already suffering an oxidative damage and triggered a rapid disease progression causing severe sequelae. We therefore bring further evidence that head trauma is a precipitating factor in the progression of mitochondrial disorders.

Novel NARS2 mutations in two children with early onset epileptic encephalopathy and mitochondrial dysfunction

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The NARS2 gene encodes the mitochondrial asparaginyl-tRNA synthetase, and enzyme critical in mitochondrial protein synthesis where it catalyzes the ligation of asparagine to tRNA molecules. Bi-allelic mutations in NARS2 have recently been linked to heterogeneous phenotypes including intellectual disability, epilepsy, hearing and visual impairment, myopathy, hepatic and renal failure, all combined with features of mitochondrial dysfunction.

Here we describe two unrelated children presenting with developmental regression and infantile epileptic encephalopathy. Both patients presented episodes of failure to thrive and their latest neurological examination revealed apostural tetraparesis and severe intellectual disability. Brain MRI showed the presence of stroke-like lesions in Pt1 whereas in Pt2 there were symmetric lesions involving basal ganglia and the anterior surface of the cerebral peduncle, combined with bilateral cortical atrophy and marked T2 hyperintensity of the white matter. Muscle biopsy in Pt2 showed alterations of oxidative metabolism and abundant lipid droplets in most fibers.

Using a multigene targeted resequencing panel, we identified four novel heterozygous mutations in NARS2: the c.716G > T/p.Gly239Val and c.749G > A/p.Arg250Gln in Pt1, and the c.688G > C/p.Gly230Arg and c.1339A > G/p.Met447Val in Pt2. The mutations segregated in healthy parents and were predictably damaging when examined in silico. In patients' fibroblasts, we observed in both cases reduced expression of NARS2 protein, and a significantly impaired oxygen consumption and low basal ATP levels.

Overall, our data suggest to consider NARS2-related disorder in infants presenting with early onset epileptic encephalopathy and failure to thrive.

Effectiveness of different non-anti-arrhythmic sodium-channel blockers in two patients with paramyotonia congenita

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Paramyotonia congenita is a skeletal muscle sodium channelopathy caused by SCN4A gene mutations. The clinical picture is characterized by paradoxical myotonia, cold sensitivity, and episodes of paralysis, either spontaneous or exercise/cold induced. The classical first-line drug is mexiletine, followed by other sodium-blocker anti-arrhythmics, but this class of drugs requires cardiac monitoring and expose the patients to arrhythmia risks, especially when high dosages are required.

To assess the electrical correlates of myotonia and paralysis, and to evaluate the effect of alternative treatments on membrane excitability, we adopted a standardized EMG protocol (long and short exercise test, with and without cold). We evaluated two male patients that had been previously treated with oral mexiletine, carbamazepine, and propafenone, subsequently discontinued either due cardiac side effects or lack of efficacy. Searching for a non anti-arrhythmic alternatives, we evaluated the effects of three drugs, each administered off-label, and each with a distinct and peculiar mechanism of inactivation of the sodium channel: ranolazine, lacosamide, and buprenorphine. Of these, we found that only buprenorphine produced improvement in patient symptoms at a relatively low-dosage. By the exercise test we could confirm that improvement was not only symptomatic (and possibly related to the analgesic effect of opioids): indeed, we could detect an improvement of both cold-induced and exercise-induced paralysis, as well as improvement in myotonia.

A less severe phenotype of glycogen synthase deficiency myopathy in two unrelated cases

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Background. Glycogenosis type 0 is a very rare metabolic myopathy due to glycogen synthase (GS) deficiency. So far, this condition have been described in few patients with childhood onset and severe cardiac involvement leading to sudden death in the first decade of life.

Cases description. We report herein two unrelated adult subjects, who presented early fatigue, exercise intolerance and diffuse myalgia after brief physical exertion since infancy. Clinical examination revealed in both neck flexors and limb girdle muscles weakness.

Results. Blood routine investigations were normal. Forearm test evidenced no rise of serum lactate in both patients. Electromyography showed a myopathic pattern only in one of them. Cardiac investigations including ECG, cardiac ultrasound and heart MRI were normal. Muscle MRI showed adipose substitution in thigh, gluteus and paraspinal muscles.

Muscle biopsy revealed a marked depletion of glycogen at PAS stain in all fibers and negative stain for myophosphorylase. Muscle glycolytic enzymes assays were normal but glycogen synthase was virtually absent (0.001 and 0.002; n.v. 12.5 ± 1.2

nmol/min/mg). Sequence analysis of *GYS1* revealed homozygous c.630G > C mutation in exon 3 (p.D145H) in a patient and IVS4+1 G > C homozygous mutation in the other one. The intronic mutation created two mRNA variants: 1) mRNA 1 with an insertion of 34 bp of intron 4 and mRNA 2 with skipping of exon 4.

Conclusions. Hence, we described a mild form of GSD0, characterized by myopathy without cardiac involvement. These findings highlight the opportunity to considering this very rare condition in the evaluation of metabolic myopathies.

Sleep disordered breathing in mitochondrial diseases: epidemiological and clinical characterization

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Background and aims. Mitochondrial diseases (MDs) are a heterogeneous group of genetic disorders due to a primary defect in mitochondrial oxidative phosphorylation. The aim of this study was to describe prevalence and characteristics of SDB in a large cohort of patients with genetic confirmed MDs.

Material and methods. A cohort of 103 consecutive patients affected by MDs, recruited in the Neurophysiopathology Unit of Gemelli Hospital in Rome in a period of five years, were enrolled. All patients were investigated by full night polysomnography (PSG).

Results. SDB was demonstrated in 49 patients (47.6%). As regard phenotypes, there were differences in distribution between groups: SDB was more frequently associated with progressive external ophthalmoplegia (PEO, 59%) and myoclonic epilepsy with ragged red fibres (MERRF, 54.5%). The prevalence of SDB was higher in patients with single or multiple mtDNA deletions and in m.8344A > G mutation. Interestingly, as far as the m.3243A > G mutation, patients with a predominant involvement of the central nervous system, as MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) had a lower prevalence of SDB than MIDD (maternally inherited diabetes and deafness), with percentages of 16.6 and 35.3% respectively.

Conclusions. SDB has a higher prevalence in MDs compared to general population-based data. Moreover, SDB is more frequent in MDs characterized by predominantly skeletal muscle involvement. Overall, these data identify SDB as a common manifestation of MDs with predominant skeletal muscle involvement. The early identification of this disorder is crucial in the management of these fragile patients.

Lipid composition of cellular membranes in ATGL deficit

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Lipids represent the major building blocks for the synthesis of neo-generated membranes, besides having their well-known role as energy storage. It is possible to hypothesize that the regulation of lipid metabolism plays a pivotal role in the phospholipid composition and in building cellular membranes. In the regulation of triglycerides (TGs) in the cytoplasm (lipolysis) the adipose triglyceride lipase (ATGL) plays an important role. ATGL initiates the hydrolysis of TGs promoting the release of fatty acids (FAs) from lipid droplets. TG are crucial energy substrates, precursors for the synthesis of membrane lipids, and ligands of nuclear receptors. Patients with mutations in the PNPLA2 gene, that codifies for ATGL, suffer from a defect in TGs catabolism that reduce TGs release in cytoplasm and causes the neutral lipid storage disease type M (NLSD-M) characterized by lipid myopathy with significant disability. The pathogenesis of muscle damage in NLSD-M is not still well defined. We investigated whether cellular dysregulation of lipolysis in NLSD-M could induce a significant modification of cell membrane. Lipid composition of cell membranes of cultured fibroblast has been determined by gas chromatography electron ionisation mass spectrometry.

The composition of cellular membranes is modified with respect to the control fibroblasts. In the membranes of NLSD-M patient there are significantly few short chain FAs, in particular C12, compared to the other FAs. It is possible to speculate that an alteration in the lipid composition of the membranes might leads to a membrane instability in NLSD-M cells. These observations need further investigations to confirm data and to assess their importance in causing muscle damage.

Long-term follow up in presymptomatic LOPD patients

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Late onset Pompe disease (LOPD) is characterized by a wide spectrum of clinical presentations ranging from classical forms with manifested muscle weakness and/or respiratory impairment to isolated hyperckemia. A better awareness of the disease and the diffusion of newborn screening programs increased number of patients diagnosed at presymptomatic stage. The identification of these patients raises the consideration how to follow these patients in the view of an early detection of disease progression to start therapy.

Herein we report on 8 patients with presymptomatic Pompe disease followed at our Neuromuscular Unit since the diagnosis was made.

The patients had a mean age of 29 (range 4-58) years, a median follow-up duration of 10 (range 4-15) years. All patients were diagnosis because of isolated hyperckemia (CK range 400 to 1100 IU) and/or myalgia. Muscle biopsy revealed a vacuolar myopathy with glycogen storage in 4 pts whereas was unspecific in 3 pts, not performed in 1. Muscle GAA residual activity range from 3.8% to 15% of n.v. Patients were followed every 6-12 months with clinical examination including functional tests, pulmonary function tests (PFTs) and muscle MRI. Two patients, after respectively 9 yrs and 6 yrs of follow-up, start

ERT because of detection of signs of muscle weakness and respiratory impairment. 6 pts were stable over the years with only persistent mild hyperckemia but no other signs of progression.

Our data demonstrated that presymptomatic LOPD patients may remain clinically silent for decades but they should be monitored closely for overt signs of the disease to promptly start ERT.

A new case of autophagic vacuolar myopathy presenting lopd features

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Background. Autophagic vacuolar myopathies (AVMs) are an emerging group of heterogeneous inherited muscle diseases linked to genes involved in autophagosomal-lysosomal processes in which glycogen deposition and autophagic vacuoles in muscle tissue are their pathological hallmarks. The best known AVMs are Danon disease, X-MEA and Pompe Disease (PD).

Objectives. We aimed to dissect the mutational profiling of a neuromuscular patient sharing clinical, myopathological and biochemical findings with Late Onset Pompe Disease (LOPD) without GAA pathogenic mutations.

Material and methods. 56 years old woman has been studied with a diagnostic protocol for suspected LOPD comprehensive of blood smears PAS-positive lymphocytes counting, DBS-GAA, muscle biopsy histological and immunofluorescence studies, GAA activity assay and expression studies on muscle homogenate, GAA sequencing, GAA-MLPA and WES.

Results. The patient disclosed a limb girdle like muscular pattern with persistent hyperckemia and defective FVC. PAS-positive lymphocytes, glycogen accumulation and features of impaired autophagy were observed in muscle biopsy. A significant reduction of GAA activity was also measured. While GAA sequencing identified no pathogenic mutations, WES approach allowed to identify a peculiar mutational pattern in genes (*MYOT*, *WDR24*) cooperating in autophagic-lysosomal system.

Discussion. Our data suggest that GAA reduction activity might occur in every condition of impaired autophagy. Therefore, GAA-DBS and PAS-positive lymphocytes counting should be considered as AVM markers in LOPD-like patients, including LOPD.

Conclusions. Our study extend the number of phenotype-genotype compounds which can be listed as AVMs and WES is the tool with the best cost-benefit ratio to define the diagnosis.

Bioimpedance phase angle as a prognostic tool in a population of late-onset pompe disease

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Pompe disease (PD) is an autosomal-recessive metabolic myopathy caused by deficiency of the lysosomal acid alpha-glucosidase, leading to an accumulation of glycogen mainly in muscles. Body Impedance Analysis (BIA) is a validated tool for the measurement of fat-free-mass (FFM), including muscle tissue, and fat mass (FM). Another value obtained by BIA is Phase Angle (PhA), proposed as an index of malnutrition and as a survival factor several diseases. We assessed the usefulness of BIA (BIOSMART, Eupraxia srl, Italy) in 15 pts with PD (7 males), all but two with normal BMI.

We measured PhA, FFM, FM, extracellular fluids, and analysed their relationship with nutritional status (anthropometric parameters, skinfold thickness, laboratory nutritional indices), disease parameters (motor, respiratory function, muscle MRI), and response to enzyme replacement treatment (ERT).

Baseline PhA was below normal range in 7/15 patients and correlated with disease severity and worse ERT response (cut-off PhA separating good vs bad responders: 4.4°). In 2/11 patients in ERT and in 4/4 patients not in ERT, the PhA declined by an average of 0.6° on 3-year mean follow-up assessment. FM correlated with disease severity and MRI findings, and showed a similar trend.

BIA may not be a valuable tool to assess nutritional status in PD, since it is influenced by disease parameters (muscle replaced by fat has a different impedance); however, FM, FM, and PhA correlate with disease severity and predict worse response to ERT, thus providing a cheap, fast, and easy-to-perform method to assess disease severity and ERT response.

MRI evidence of structural muscle damage in McArdle disease: clinico pathological implications

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Background. McArdle disease, or glycogenosis type 5 (GSD5), the most common muscle glycogenosis usually presents as a dynamic disorder of muscle metabolism with exercise intolerance and risk of exercise induced myoglobinuria. In a substantial proportion of patients however the disease evolves into a fixed myopathic phenotype with preferential involvement of specific muscle groups. Muscle magnetic resonance imaging (M-MRI) is a powerful non-invasive tool providing valuable information on structural damage and tissue replacement increasingly used in support of the clinical evaluation.

Methods. We performed whole-body MR examination in 9 adults with molecularly defined GSD5. Degree of myofibrillar edema and fatty substitution in the various muscles were graded semiquantitatively (Poliachick 2012, Fischer 2008). Demographics, laboratory values, clinical grading and objective exercise capacity indicators were assessed in the same patients and checked for correlations with the results of the imaging study.

Results. Significant MRI signs of muscle structural modification were detected in 7 out of 9 subjects. The degree of change

ranged from mild intrafibrillar edema to extensive fibroadipous substitution affecting mostly the posterior compartment. MRI detected changes showed a weak correlation with clinical symptoms severity and a moderate inverse-correlation with muscle power developed during exercise test (W max). No correlation could be found with age, CK levels, and peak VO₂.

Conclusions. Despite its definition as a mainly dynamic myopathy, GSD5 is very frequently associated with muscle structural changes that can be detected by MRI also in young subjects without apparent fixed myopathy. Maximum power expressed during standardized exercise testing seems the most reliable clinical indicator of muscle structural damage. M-MRI is a non-invasive and repeatable diagnostic tool assessing muscle structural changes that could provide efficient follow-up.

Further investigation on larger cohorts of patients and into the relationship between MRI detected muscle alterations, clinical and functional indicators is warranted.

Adult form of multiple acyl-CoA dehydrogenases deficiency triggered by statin treatment

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Multiple Acyl-CoA dehydrogenases deficiency (MADD) is an inherited disorder of fatty acid oxidation. Age at onset is variable, ranging from neonatal to late-onset forms. The latter form is sometimes triggered by stressor agents as fasting, fever, drugs and often is responsive to riboflavin administration. Few reports of metabolic myopathies manifesting after statin administration are described but, to date, no cases of statins induced MADD have been reported. Among our cohort of 18 unrelated patients with late onset MADD, 4 pts (2F and 2M) started to complain of muscular symptoms after being exposed to statins. Symptoms were exercise intolerance, myalgia and proximal muscular weakness, appearing few weeks after treatment with Atorvastatin (2pts) or Pravastatin (1pt) and after increasing dosage of Simvastatin (1pt). Syndrome persisted after drugs withdrawal, halving statin dosage or changing type of statin (1pt). Serum CK was increased (400 to 3000) and LDH was 2 to 3 times nv in 3 patients. Serum acyl-carnitine profile showed an increased of all intermediates. Conventional EMG showed fibrillation potentials and PSW (3pts) and myogenic MUPs (1pt) while nerve conduction studies were normal. Muscle biopsy showed a vacuolar lipid storage myopathy. Patients were treated with riboflavin 400mg/die with a great response: after a month CK normalized and symptoms regressed. Our results suggest that a sudden motor impairment triggered by statin could be due to a MADD. An early recognition of this cases can allow a prompt treatment leading to a full clinical recovery.

Case report: new missense variants of NDUFA11 associated with late onset myopathy

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Human mitochondrial respiratory chain (MRC) complex I (CI) deficiency (MIM 252010) is the most common enzymatic defect in mitochondrial disease, ranging from mild muscle involvement to Leigh syndrome or fatal neonatal multiorgan disease.

Here we describe a 72-year-old male who showed adult onset (65 years of age) of exclusive neuromuscular phenotype characterized by muscle weakness and muscle pain.

Histological and histochemical analyses of a muscle biopsy from the quadriceps showed diffuse mitochondrial alterations. The biochemical assessment of the MRC revealed an isolated and severe deficiency of CI. The whole mtDNA sequence was normal. A genetic panel screening for nuclear genes encoding CI subunits and assembly factors led to the identification of two heterozygous variants in *NDUFA11* (c.317C > T, p.Thr106Ile and c.394G > C, p.Ala132Pro. NGS revealed that the two variants were on different alleles. This case represents the second report of biallelic mutations in *NDUFA11*. So far, only one mutation was detected in this gene and was associated with an extremely severe, early onset phenotype characterized by fatal infantile metabolic acidosis or severe encephalocardiomyopathy. The two variants identified in our patients affect isoform 2 of *NDUFA11*, not isoform 1. This may explain the mild phenotype observed in the present patient. We speculate that the observed tissue specificity of the clinical presentation may be due to a specific or predominant expression of isoform 2 in skeletal muscle tissue. In our patient, idebenone therapy induced a mild improvement in clinically-measured muscle strength (MRC scale) and reported by himself.

Glycogenosis vii worsened by cyclosporine and amiodarone: a clinical and muscle mri report

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Glycogenosis VII (GSD VII) is an autosomal recessive glycogen storage disorder caused by mutations in the *PFKM* gene encoding the phosphofructokinase (PFK) enzyme.

The classical form of GSDVII presents with exercise intolerance, contractures and myoglobinuria while the late-onset form is usually characterized by muscle pain and mild fixed proximal weakness.

We describe a 65-year-old man affected by muscle PFK deficiency who, since the age of 33, presented with a classical form of disease showing exercise intolerance and myoglobinuria.

Muscle biopsy showed a vacuolar myopathy with glycogen storage. Genetic analysis of *PFKM* gene displayed the presence of the heterozygote c.1817A > C (p.Asp543Ala) and c.488 G > A (p.Arg100Gln) pathogenic mutations.

In his fifth decade, he started cyclosporine after liver transplantation and, then, amiodarone because of atrial fibrillation. In the following years, he developed a severe and progressive muscle weakness, mainly involving lower limbs, up to a loss of independent walking.

Muscle MRI showed adipose substitution of both anterior and posterior thigh muscles with selective sparing of the medial ones. A selective replacement of gemelli and peroneus muscles and a relatively milder involvement of the soleus and tibialis anterior muscles were also reported. Face, abdominal and lumbar muscles were spared while minimal adipose involvement of the deltoid muscles were detected.

The temporal relationship between the patient's clinical worsening and starting chronic treatment with cyclosporine and amiodarone suggests an additive toxic damage by these two potentially myotoxic drugs in determining such an unusual phenotype, also confirmed by muscle MRI findings.

The role of anti rh-GAA in modulating response to ert in late-onset pompe disease: the final data from the IgERT study

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The exact role of IgG antibodies against α -glucosidase (anti-rhGAA) in modulating efficacy of ERT in patients with late-onset Pompe disease (LOPD) is still not fully understood.

In order to assess if anti rh-GAA antibodies interfere with treatment efficacy, we analyzed clinical findings and performed serial measurements of IgG anti rh-GAA antibody titers from 64 LOPD patients treated with ERT.

Two examinations (T0 and T1) were performed and the T0-T1 Delta of Six Minute Walking test (6MWT), MRC sum score (MRC), gait, stairs and chair performance (GSGC) and forced vital capacity (FVC) was considered and then related to the antibody titers.

Seventy eight per cent of patients had an anti rhGAA positive titers (31% patients developed a low titer, 44% a medium titer and 3% a high titer) while almost 22% of the patients never developed antibodies.

In a subgroup of patients treated less than 36 months, those with null antibody titers showed higher MRC sum score values than patients with positive titers. Differently, no statistical sig-

nificance was found in relating the T0-T1 Delta differences and antibody titers for the other studied variables.

Our results confirm that anti rh-GAA antibody generation did not significantly affect ERT efficacy. However, in the first 36 months of treatment, low-medium antibody titers might interfere with the clinical outcome. Therefore, a regular and careful evaluation of antibody titers, especially in cases with evidence of clinical decline despite ERT, should be conducted.

Liver transplantation in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): update from Bologna case series

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We recently reported the first 2 MNGIE patients treated with orthotopic liver transplantation (OLT).

We update their clinical and biochemical results, adding the findings of another transplanted MNGIE patient. At OLT patient-1 and patient-3 were at end-stage of disease (bed-restricted) while patient-2 had mild neurological symptoms and signs.

OLT promptly and permanently normalized serum nucleosides in all patients. We didn't observe substantial weight gain.

Patient-1 recovered full ambulation and 2-years-after-OLT EMG showed nerve conduction improvement. He continued to complain of episodes of recurrent vomiting and pseudobstruction. Brain MRI remained unchanged. Unfortunately he died at 960 days after OLT for gastrointestinal hemorrhage.

Patient-2 recovered from bilateral foot drop and at 900 days after OLT she has no major neuropathic and any gastrointestinal symptoms. 2-years-after-OLT EMG showed nerve conduction improvement. Brain MRI remained unchanged.

Patient-3 is at 210 days after OLT. He suffered from pulmonary infection after surgery, needing of mechanical ventilation and tracheostomy. Currently he recovered ambulation, spontaneous ventilation and enteral nutrition.

In MNGIE, OLT shows strong biochemical efficacy and the procedure and subsequent immunosuppressive therapy seem well tolerated. Plasma nucleoside clearance makes a long-term improvement of some aspects (in particular nerve functions and conduction, possibly related to mitochondrial DNA damage) but doesn't influence other features (in particular gastrointestinal functions and leucoencephalopathy, possibly related to other thymidine phosphorylase actions)

Mitochondrial diseases related to mtDNA in childhood: novel mutation in mtCO3 associated to familial mitochondrial leucoencephalopathy expanding repertoire of mtDNA mutations in human diseases

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Cytochrome c oxidase subunit III (COIII or MTCO3) is 1 of 3 mitochondrial DNA (mtDNA) encoded subunits of respiratory Complex IV. To date MTCO3 mutations have been associated to Leber optic atrophy, myopathy with exercise intolerance or infantile encephalopathy Leigh like. Cerebral white matter involvement has been reported to mtDNA-related diseases with later onset as MELAS, MERRF and KSS. To date only few cases have been reported in childhood. Infantile mitochondrial leukoencephalopathy (ML) are related to nDNA genes mutations and isolated complex deficiency.

We report a 6 years-old boy affected by mild psychomotor delay, hypotonia, retinopathy, neurosensorial hypoacusia; MRI performed at 30 months of age disclosed sylvian fissure cavities leukoencephalopathy. Isolated complex IV defect was detected both in muscle and fibroblasts. Clinical evolution was stable.

Screening of nuclear gene related to ML and defect of complex IV were negative while mtDNA sequence identified a novel mutation in COIII.

In muscle tissue, blood, fibroblast and urinary sediment of patient nearly 100% of the mtDNA was mutated. The mutation was present in 69% of urinary sediment and 2% of peripheral leukocytes from the referred healthy patient's mother. Nevertheless further investigations revealed peripheral sensory neuropathy and focal gliosis area a brain MRI while multielevated potential and plasmatic lactate level were normal.

In the mutated cybrids from the patient, the amounts of fully assembled CIV are reduced and there is an accumulation of CIV assembly intermediates.

Our report expands phenotype related to COIII mutations, lends further evidence to the expanding repertoire of mtDNA mutations in human diseases and suggests to look for variants in the mitochondrial genome when dealing with otherwise undetermined leukodystrophies of childhood.

Longitudinal cognitive changes in DM1 patients in a 5 years follow up study

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disorder characterized by clinical variability, affecting CNS skeletal and heart muscles. Cerebral involvement in DM1 is variable in cognitive impairment (i.e. executive, memory and visuo-constructive functions) and often associated with personality and behavioural dysfunctions. We observed during a 5 years follow up study 9 patients affected by DM1, that were periodically examined in our centre. All DM1 patients were genetically confirmed (age range was 18-75 years) and were assessed by psychological and neuropsychological tests. Brain MRI and serum myomiRNA were performed. At the baseline were memory, executive and visuo-constructive abilities the most compromised functions. Agnosognosia was present in more than 50% of patients both at baseline and follow up assessments. We observed that patients improved their performance in different tests, such as verbal abilities (verbal fluencies and verbal memory) and non verbal fluid intelligence, at the follow up when compared to the baseline assessment,

although not statistically significant. We observed a worsening of performance in the Rey-Osterrieth complex figure test ($p < 0.05$) and the working memory task (digit span memory test). Our study demonstrates that in the follow up in DM1 patients there is a slight improvement of cognitive abilities that could be useful to investigate in further studies. This suggests some differences and disease evolution is compared to other types of dementia and advance several possibly strategies to treat patients such as social intervention and CBT training.

P. 2-2. Myotonias, Channelopathies, Neuromuscular Junction Disorders and inflammatory myopathies

Characterization of oxidative stress during exercise in the study of the phenotypic complexity of Myotonic Dystrophy Type 1

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Aim of the study has been to identify phenotypic subgroups in patients with juvenile, adulthood and late-onset forms of DM1 with particular attention to exercise-related oxidative stress as epistatic phenomenon.

40 patients with DM1 were included. The different clinical forms were compared on the basis of the CTG-expansion class and the frequency of the main disease manifestations. The genetic features was also compared to muscular impairment (MIRS scale) and cardiac score. An incremental forearm exercise was used to assess biochemical markers of oxidative stress in the serum.

There was no significant correlation between the CTG expansion class and the clinical form. However, when considered all together, an inverse relationship between CTG expansion class and clinical muscle scores. Increased serum levels of oxidized proteins and reduced serum levels of reduced glutathione were detected in patients compared to controls, however without any significant correlation with the size of the CTG expansion.

Our study underlines the need to identify further and possibly multidimensional prognostic factors to better characterize the disease trajectory in DM1 in order to follow up and predict evolution as well as response to eventual therapies along the natural history of this disease.

An Italian patient with autosomal dominant hypomagnesemia associated by KCNA1 mutation

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Case. A 31 year old woman came to our attention for a symptomatology, started from infancy, characterized by recurrent tetanic episodes and tremor especially in distal limbs and face and with a diagnosis of spasmodophilia. Her father presented

episodes of muscle spasms. Neurological examination was normal. Genetic test revealed a heterozygous mutation A763G in *KCNA1* gene.

Discussion. In 2009, a study conducted on a large Brazilian family with symptomatology similar to our patient, heterozygous mutation A763G in *KCNA1* gene was found and associated with hypomagnesemia.

Generally *KCNA1* mutations are associated with different phenotypes, ranging from, classically, episodic ataxias, a group of diseases characterized by recurrent, discrete episodes of vertigo and ataxia, to new recently described phenotype (delay in motor development, choreoathetosis, cognitive dysfunction, transient postural abnormalities in infancy, shortening of the Achilles tendon in children, epileptic seizures, migraine, isolated neuromyotonia and myokimia).

Diagnosis is primarily based on clinical and laboratory findings of hypomagnesemia, electromyographic myokymia (in some cases the myokymic activity only becomes apparent after application of regional ischemia) and genetic testing of *KCNA1* gene. Our case confirms the broad phenotypic variability of this gene.

In conclusion, we report an Italian patient with hypomagnesemia and tetany caused by a *KCNA1* mutation, and confirms the spread of the known A763G mutation, present also in Italy, and stresses the need to perform a *KCNA1* test in patients with spasmodophilia / myokimia and hypomagnesemia.

Efficacy of metformin on mobility and strength in myotonic dystrophy type 1 (METMYD): study protocol outline

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Background. Metformin acts as a modifier of the aberrant alternative splicing of myotonic dystrophy type 1 (DM1) and reduces oxidative stress, which is enhanced in DM1. Preliminary animal and human studies showed its efficacy in improving motor function. This new mechanism of an old drug can bear a therapeutic potential for patients with DM1

Methods. METMYD is a phase III b randomized, double blind, multicenter trial (EudraCT n° 2018-000692-32) aimed at evaluating the superiority of a 24 month-treatment with metformin (500 mg, t.i.d; n = 97) over placebo (n = 97) in improving mobility and strength in DM1 patients with motor disability.

The primary endpoint is a 40-meter difference in the 6-minute walk test. Secondary clinical endpoints address: 1) Dexterity and mobility; 2) Muscle quantitative testing; 3) Fatigue; 4) Quality of life. Moreover, circulating splicing products deficient in DM1, and markers of oxidative stress will be assessed before and after treatment.

Expected results. This study will allow to explore the hypothesis that metformin may exert a protective effect through an additional mechanism of action. Circulating alternative splicing products may represent a surrogate outcome to validate the proposed principal action mechanism of this drug on motor functions. The expected positive results of this study will prompt the

undertaking of confirmatory trials aimed at demonstrating the usefulness of metformin.

Conclusions. Confirming the efficacy of metformin through its epigenetic mechanism on clinically meaningful outcome measures can provide an important therapeutic perspective for DM1 patients.

Bioelectric impedance analysis (BIA), anthropometric and nutritional characteristics in myotonic dystrophy type 2 (DM2) patients

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Introduction. Metabolic alterations are an important feature of DM2; therefore, recognition of changes in body composition by BIA and its correlation with other disease features might be useful to assess disease severity.

Methods. We obtained anthropometric measures, nutritional data, and blood tests in 18 DM2 patients. BIA was performed in all cases, recording phase angle (PA), body composition and body cell mass-index (BCMI). Motor function tests, including 30 second chair test (30SCT), functional index-2 (FI-2) for upper extremity, and the quick motor function test (QMFT) were administered and their results correlated with BIA data. Descriptive statistics and Pearson's coefficient were performed.

Results. Waist to hip circumference was above normal values in 67% of patients. Based on body mass index (BMI), 56% of patients were overweight and 28% of them were obese.

By BIA, fat mass (FM) was increased in 56% and fat-free mass (FFM) was reduced in 50% of patients. Total body water (TBW) and PA were reduced in 61% of patients and the latter showed direct correlation with 30SCT, FI-2 flexion and abduction test, and QMFT. A direct correlation of BCMI with FI-2 flexion and of BMI with HOMA index was also found.

Conclusions. This pilot study suggests that an alteration in the relative prevalence of FFM and TBW versus FM, as suggested by reduction of PA is a common feature of DM2 and parallels motor function tests impairment. Therefore, further studies on larger cohorts and with a prospective approach could validate BIA as an outcome measure for DM2.

Longitudinal, quantitative assessment of hand muscle strength decay in myotonic dystrophy type 1 (DM1)

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Introduction. Hand muscle weakness is a core feature of DM1 and outcome measures assessing finger pinch and grip strength should be available for natural history and trial studies.

Methods. We prospectively evaluated self-assessed hand muscle strength with a hand-held dynamometer (HHD) in 57 adult DM1 patients of either gender. Three-finger pinch and handgrip strength were annually recorded in both hands with an up to four-year follow-up. Descriptive statistics, Student's t test and correlation were performed.

Results. Handgrip and pinch median values were markedly reduced at baseline in patients as compared to sex-matched normal values. In particular, P50 values for handgrip and pinch were 86% and 68% less than control values, respectively. Moreover, patient values for handgrip and pinch were significantly decreased by 23% and 29%, respectively, at 4 years, with a mean annual loss rate of 6-7%.

Conclusion. Pinch and handgrip, measured by HHD, are valid instruments for detecting progression of muscle power loss in a short term and seem suitable for being used in clinical trials.

Genetic and phenotypical characterization of a pediatric cohort with myotonic dystrophy type 1 (DM1)

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Myotonic dystrophy type 1 is an autosomal dominant neuromuscular disorder resulting in unstable CTG repeats within the myotonic dystrophy protein kinase (*DMPK*) gene. The pediatric forms of myotonic dystrophy manifest much differently than the adult form of the disease. The aim of this study is to clinically and genetically characterize a cohort with pediatric myotonic dystrophy type 1 (DM1). Ten patients (8 males, 2 females) aged 1 to 30 years and regularly followed in our centre have been included. GTG expansion was confirmed in all the patients. Six patients had congenital onset with mild to severe neonatal symptoms including hypotonia, respiratory distress, sucking or swallowing difficulties, skeletal deformities. In these children musculoskeletal impairment varied from severe to moderate involvement and only three of them acquired independent ambulation. Cognitive deficits varying from severe to mild degree or behavioral disorders were observed in most of the patients. Three patients had respiratory impairment requiring NNIV. Gastrointestinal symptoms have been observed in 4 patients. Endocrine or metabolic disorders have not been detected as well as cataracts. Cardiological alterations have been observed in 4 patients since the first decade. Two patients required PM implantation. The early detection of heart involvement suggests that a regular cardiological follow up has to be started in the first years of life.

Functional tests in myotonic dystrophy type 1: a three-year longitudinal study

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Background. Myotonic Dystrophy Type 1 (DM1) is an autosomal dominant multisystem disease caused by an unstable CTG repeat expansion in the 3' UTR of the *DMPK* gene. DM1 is clinically heterogeneous. Outcome measures in DM1 should be explored to capture patients clinical status, to describe the natural history and to facilitate clinical trial design. Functional tests - including Six-minute walk test (6MWT), 10-meter run and time up and go (TUG) - could represent a reliable method

to quantify disease progression. Longitudinal studies in DM1 patients using functional tests, manual muscle testing (MRC scale) and CTG expansion data are scarce. Here we present an observational 3-year longitudinal study to assess the role of functional tests as clinical progression biomarker in DM1.

Materials and methods. Between 2015 and 2018 we longitudinally evaluated 57 DM1 patients according to a neuromuscular assessment protocol including MRC score from 20 muscle, MIRS scale and functional tests - 6MWT, 10-meter run, TUG. Data were analyzed including as covariate CTG expansion size.

Results. Of the 57 DM1 patients included in the study protocol, longitudinal data were available for 49. 6MWT showed a statistically significant decrease at 3 years (-47.5m, SD +/- 87.8, p = 0.031), while no significant variations were detected on the 10 meter-run and TUG tests. Muscle strength showed a progressive significant decrement on the average MRC values during the 3-year follow-up (-0.33, SD +/- 0.23, p = 0.001).

Conclusions. Our data confirm the well-known slowly progressive course of DM1 and support the use of 6MWT for clinical follow-up.

Congenital myasthenic syndromes due to CHRND mutation: a report of two lethal phenotypes

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Congenital myasthenic syndromes (CMS) are a group of heterogeneous inherited disorders caused by mutations in genes encoding proteins essential for the integrity of neuromuscular transmission. The number of genes known to cause CMS is currently 30. The main proteins involved in the pathogenesis of CMS are: choline acetyltransferase (ChAT), the endplate species of acetylcholinesterase (AChE), β 2-laminin, the acetylcholine receptor subunits (*CHRNE*, *CHRND*), rapsyn, plectin, Na(v)1.4, the muscle specific protein kinase (MuSK), agrin, downstream of tyrosine kinase 7 (Dok-7), and glutamine-fructose-6-phosphate transaminase 1 (GFPT1). CMS are characterized by fatigable muscle weakness (e.g., ocular, bulbar, limb muscles) with onset at birth or in early childhood; rarely, symptoms may present later. Clinical, electrophysiological and morphological studies are essential for molecular studies, counseling and therapy. *CHRND* mutations are a rare cause for CMS but they should be considered in patients with a severe, early onset form, with respiratory distress.

We describe two sisters, not twins, clinically and genetically diagnosed with CMS, carrying two new different mutations in the *CHRND* gene (c.730C > T; p.Arg244Cys; c.1304T > C; p.Leu435Pro).

They both clinically showed at birth generalized hypotonia, sucking and swallowing difficulty, weak cry, severe eyelid ptosis and ophtalmoparesis and, during the first days of life, they experimented an acute respiratory distress requiring invasive ventilation. A therapy with pyridostigmine was started without benefit; in a second time, salbutamol was tried, showing an initial partial response. These two cases expand the clinical spectrum of CMS linked to *CHRND* mutations, suggesting their role in determining a lethal phenotype.

Clinical features in a cohort of Italian MuSK-MG patients in a long-term follow-up

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Background. Muscle specific kinase Myasthenia Gravis (MuSK-MG) is a distinctive, frequently more severe, subtype of MG. The prevalence varies among countries and ethnic groups, 30% to 70% in European anti-AChR antibody negative patients and between 2.5% and 3% in Asian countries. MuSK-MG usually demonstrates an acute bulbar onset with rapid progression within a few weeks. We report clinical features, treatment outcomes and follow-up data of 31 Italian MuSK-MG patients.

Patients and methods. We retrospectively evaluated 31 patients (17 F, 14 M) diagnosed with MuSK-MG who attended our Outpatient Department between January 1995 and January 2019. Ages at presentation ranged from 10 to 70 years old. According to Osserman criteria, the majority of patients at onset showed a type-2 MG (23/31), 4 patients type-1 MG, 3 patients type-3 and only one patient type-5.

Results. Follow-up time ranged from six months to 23 years. Six patients experienced respiratory insufficiency. Five underwent thymectomy without benefit. Each patient assumed prednisolone with a partial initial response. 19/31 were treated with azathioprine, 3 in association with another immunosuppressant. Rituximab was given to 3 patients with severe phenotypes and 1 out of 3 showed a complete stable remission at six months follow up.

Discussion. In our cohort MuSK-MG patients are 4.1% with a female/male ratio of approximately 1:1. A 12.9% of pure ocular form was found. Posterior neck variant was present at the onset in 19.3%. The long-term follow-up allows us to identify osteoporosis as the main long-term complication, frequently being cause of pathological fractures.

Electrophysiological tests in patients with anti-MuSK Myasthenia Gravis: a retrospective study

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Introduction. Myasthenia gravis (MG) with autoantibodies to muscle-specific tyrosine kinase (MuSK) represents a distinct type of disease, as compared MG with antibodies against acetylcholine receptor (AChR-Abs).

The role of electrophysiological tests in these patients is still argued.

Methods. We retrospectively evaluated repetitive nerve stimulation (RNS) and single-fibre electromyography (SF-EMG) reports of 63 MuSK patients and we compared them with 35 non-timatomatous AChR-Abs (ACAB+) patients matched with MuSK subjects according to gender, onset age, age at diagnosis and disease severity.

Results. Fifty-nine MuSK patients and 31 ACAB+ patients underwent RNS, whereas SF-EMG was tested in 17 MuSK pa-

tients and in 7 ACAB+ patients.

RNS showed a significant decrement in a higher percentage of ACAB+ patients when compared to the MuSK group ($p < 0.0008$) whereas no significant SF-EMG difference was found ($p = 0.5$).

In MuSK positive group, proximal muscles showed a more significant decrement ($p < 0.02$) whereas no significant differences were found in ACAB+ group.

In MuSK patients, pathological RNS did not correlate either with MG severity ($p = 0.9$) or with the length of drug-free-period ($p = 0.4$).

Jitter was increased in 14 (82.3%) MuSK and 5 (71.4%) ACAB+ patients, borderline in one MuSK patient and absent in 2 MuSK (11.7%) and 2 ACAB+ patients (28.5%).

Conclusion. RNS-EMG positivity was significantly higher in AChR-Abs positive group, no difference being evident at SF-EMG.

In MusK group, RNS decrement is more evident in proximal muscles and can be absent in distal ones.

Neurophysiological evaluation is important for MG diagnosis but it plays a secondary role after anti-MuSK titration for MuSK MG diagnosis.

Muscle involvement in myasthenia gravis: expanding the clinico-pathological spectrum of myasthenia-myositis association from a large cohort of patients

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Myasthenia Gravis-Inflammatory Myopathy (MG-IM) association has been described in less than 50 cases, as isolated reports or in few case series. Thymic pathology is present in about 50% of cases with muscle biopsy consistent of IM. Muscle specific antibodies (MSA) and muscle MRI involvement have not been systematically investigated. In this study we investigated the clinical, serological, pathological and muscle imaging findings from 13 patients with MG-IM, from a cohort of 441 MG patients (2,9%). Median age at disease onset of 51 year (range 24-73) with clinical follow-up of 88 months (range 31-237). IM was suspected by CK elevation in all patients (range 500-3000 UI/L). Three main categories of muscle involvement, sometimes overlapping, were observed: focal, generalized and mild/asymptomatic, leading to 3 main clinical groups (A,B,C) and 2 overlapping subgroups (A/B and B/C). Muscle biopsy confirmed IM in all patients. MSA were negative in all patients. Antibodies against the acetylcholine receptor were detected in all patients associated with anti-Titin and -RyR1 in patients with thymoma. Thymus pathology was present in 10/13 patients. Antibodies against the acetylcholine receptor were detected in all patients, associated with anti-Titin and -RyR1 in patients with

thymoma. In conclusion we redefined the clinical spectrum of muscle involvement in MG-IM association, manifesting as a continuum among 3 main clinical groups: focal, generalized and mild/subclinical muscle involvement. Minimal muscle involvement and focal myositis could be underestimated among myasthenic patients and increased clinical awareness is warranted to better recognize these clinical entities.

Clinical features of an Italian cohort of patients with very late-onset myasthenia gravis

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Background. Myasthenia gravis is an autoimmune disorder of NMJ; in the last years an unexpected increased incidence of elderly-age MG was found. Very late onset MG can be underdiagnosed because some disturbances can be ascribed to more common chronic diseases. We evaluated the incidence and the features of MG, presenting after the age of 75 years, among our MG population

Patients and methods. 36 patients (17F,19M) with a MG onset > 75 years (age-range 75-89) were identified. All the patients were evaluated at onset with clinical examination, QMG score, SFEMG, RNS, thorax CT, routine blood examinations, Ab AChR and Anti MUSK assays.

Results. According to Osserman criteria, the majority of patients at onset showed a type-2 MG (23/35), 7 patients type-1 MG, 2 patients type-3 and only one patient type-4.

Ab AChR were positive in (32/36); 4 patients were negative for Ab AChR and MuSK. Thymoma was found in 3 patients. The average time before the diagnosis was 11 months.

The most common regimen of therapy was prednisone at low doses (less than 12.5mg/day); Azathioprine (50 to 100mg) was used as steroid sparing agent.

Discussion. Our findings show that the diagnosis and therapy of MG in the elderly can be difficult. Among our population, there were no patients with MuSK related MG.

MG type, comorbidities (hypertension, diabetes, glaucoma, osteoporosis) should be carefully considered for a positive outcome.

Myasthenia gravis after etanercept and ustekinumab treatment for Psoriatic Arthritis: a case report

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A 50 years-old man affected by hypertension, was diagnosed with psoriatic arthritis (PsA) at the age of 35 years. He was followed by the rheumatologists who started methotrexate (15 mg/weekly) and cyclosporine, that was interrupted 3 years later because of hypertension. Subsequently, etanercept (15mg/weekly) was introduced and continued for 10 years with an adequate reduction of pain symptoms related to PsA. Afterwards, etanercept was replaced with ustekinumab (45 mg/monthly, than 45 mg every 3 months) on Rheumatologist's indication and continued for one year. Six months after the beginning of

the ustekinumab treatment, the patient underwent to a chest CT scan for a follow up after pneumonia. The chest CT scan showed a neoplastic anterior mediastinal mass, revealed to be a thymoma at surgery. Three months later the patient was referred to our outpatient department with generalized weakness, fatigue, difficulty in raising arms and transient episodes of diplopia after physical effort. He came to our observation at the age of 50 years; a deep clinical history revealed that these symptoms had begun about 7 years before but they were ascribed to PsA. A diagnosis of anti-acetylcholine receptor antibody-positive (4 nmol/l) myasthenia gravis was than made; methotrexate (20 mg/weekly) and prednisone (12.5 mg/daily) were started with regression of MG symptoms in three months.

Our case increases the number of clinical reports of MG onset in patients with rheumatic disease treated with anti-TNF-alpha drugs and it could cast doubt on a possible role of ustekinumab in MG clinical worsening.

Anti-AChR Myasthenia Gravis presenting with early atrophy and nonfluctuating weakness of proximal limb muscles

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Myasthenia Gravis (MG) is an autoimmune disorder of neuromuscular transmission caused by antibodies targeting postsynaptic muscle end-plate at the neuromuscular junction leading to an impairment of signal transduction. The cardinal symptom is a fluctuating weakness of voluntary muscles. The disease is characterized by a wide spectrum of clinical presentations ranging from purely ocular symptoms to severe generalized forms with respiratory involvement. MG, however, may present at onset with atypical clinical pattern of weakness including dropped head, acute bilateral facial weakness, sudden bilateral lower limb weakness, foot drop, distal weakness without atrophy and limb-girdle muscle weakness.

We describe an anti-AChR MG patient who manifested at onset nonfluctuating weakness and wasting involving shoulder girdle and proximal arm and leg muscles associated with dysphagia and bilateral calf hypertrophy.

This unusual presentation can mimic clinically a primary disorder of muscle but neuromuscular junction studies (repetitive nerve stimulation and single-fiber electromyography) helped us to achieve the correct diagnosis.

Our patient adds to the phenotypic variability of AChR positive MG and highlights that muscle atrophy may occur in the early stages of this condition, not only in patients with longstanding disease, in those who have been in corticosteroids for long periods of time or in patients seropositive for MuSK antibody.

Pediatric anti-HMGCR necrotizing myopathy resembling limb-girdle muscular dystrophy

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A 7-year-old child presented with a 2 months subtle onset, progressive lower limbs weakness. Creatine kinase (CK) level was 10,000. MLPA analysis of DMD gene showed no deletion or duplication. Muscle biopsy showed dystrophic pattern with numerous necrotizing fibers. Western blot analysis highlighted normal signals of dystrophin-associated glycoprotein complex proteins. Limb-girdle muscular dystrophy next generation sequencing panel revealed no definitely pathogenic mutations. Lower limbs muscle magnetic resonance imaging (MRI) showed proximal muscles relative hypotrophy with mild fibroadipose substitution, but without signs of edema. Echocardiography was normal.

In the next six months the patients complained of rapidly progressive fatigue, climbing stairs difficulty and running ability loss. Neurological examination evidenced four limbs muscles weakness, diffuse loss of muscles tone involving axial muscles and complete Gowers' sign.

Re-evaluation of muscle biopsy revealed anti-MHC-I antibodies sarcolemmal positivity in some fibers and complement deposition on capillaries. Autoimmune screening revealed anti-HMGCR antibodies positivity. Four limbs MRI showed the appearance of inflammatory findings. Echocardiography showed interventricular septal hypokinesis.

Patient was treated with intravenous steroid then with slow tapering oral prednisone, with initial progressive improvement of symptoms and normalization of echocardiography. One month later therapy was implemented with administration of intravenous immunoglobulins every two months, with further functional recovery.

Our case report confirms that HMGCR autoantibody testing should be part of the initial evaluation in pediatric patients with suspected muscular dystrophy and unrevealing genetic testing or negative family history. Early diagnosis and treatment could be the right approach, but there is still a substantial need for new data.

Altered aquaporin-4 immunolocalization in human idiopathic inflammatory myopathies: a common feature?

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Aquaporins are integral membrane water channels that regulate the flow of water in various tissues and organs. Aquaporin-4 (AQP4) is abundant in skeletal muscle fast fibers, where it is associated with glycolytic metabolism. AQP4 is also expressed

by astroglial endfeet, where it is the target of a human autoimmune disease, neuromyelitis optica. To date, the involvement of AQP4 in idiopathic inflammatory myopathies (IIMs) has not been studied. We investigated the immunolocalization of AQP4 and inflammatory markers as CD68, CD56, MMP2, CXCL12, CXCR7, and fast myosin heavy chain in 28 human muscle biopsies obtained from diagnostic samples. The studied population was composed of 19 IIM patients diagnosed as 6 immune mediated necrotizing myopathy patients, 2 anti-synthetase myopathy patients, 6 dermatomyositis patients, a polymyositis patient, 2 inclusion body myositis patients, 2 B-cell inflammatory myopathy patients and 9 control subjects diagnosed as a genetically confirmed myoclonic epilepsy with ragged red fibers patient, a oculopharyngeal muscular dystrophy patient, a type 1 fiber predominance patient and 6 patients who underwent muscle biopsy for suspected myopathy, not confirmed after the clinical and diagnostic workout. AQP4 immunolocalization inversely correlated with MMP2 localization, number of CD68⁺ macrophages and CD56⁺ lymphocytes in IIMs. The immunolocalization of AQP4 in normal human myofibers appeared variable among different muscles and patients whereas in IIMs the expression of AQP4 was restricted to the preserved areas of muscle samples where inflammatory cells and molecules were absent. The MMP2 release could be responsible of agrin digestion and disruption of the correct AQP4 sarcolemmal localization.

Congenital myasthenic syndromes: improved diagnostic yield based on combined use of targeted ngs sequencing and deep phenotyping

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Congenital myasthenic syndromes (CMS) are heterogeneous neuromuscular disorders (NMD) due to mutations in over 25 genes coding for proteins involved in the neuromuscular

junction structure and function. Although considered rare, collectively CMS are underestimated due to diagnostic difficulties. In recent years, next-generation sequencing (NGS) has increasingly been used in NMD and might prove to be important in the diagnosis of CMS.

Between 2014 and 2018, among over 400 NMD patients referred to our center we evaluated 45 patients with clinical suspicion of CMS. In all we tested the coding regions of 241 genes associated with NMD using a targeted NGS technology. In each case we also collected clinical and laboratory data as well as the results of familial segregation analyses. The distribution of the muscle weakness was recorded using the Human Phenotype Ontology (HPO) codes and nomenclature.

A molecular diagnosis was reached in 19/45 cases (42%). Variants of unknown significance were found in 19 patients, whereas 7 cases remained molecularly undefined. Pathogenic mutations were identified in genes already associated with CMS in 13 individuals whereas 6 cases showed mutations in other genes not previously linked to defects of neuromuscular junction.

Major features of the diagnosed CMS cases were ptosis (HP:0000508; in 90%) and limb-girdle muscle weakness (HP:0003325; in 60%).

This study highlights the advantages of NGS used as a first-tier diagnostic approach in genetically heterogeneous conditions and underlines the importance of a standardized clinical work-up as a component of the diagnostic pathway.

Congenital myasthenic syndrome: clinical and genetic features of five unrelated patients

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Introduction. Congenital myasthenic syndromes (CMS) are a heterogeneous group of early-onset genetic neuromuscular transmission disorders due to mutations in proteins involved in the organization, maintenance, function, or modification of the motor endplate (endplate myopathies)

CMS are clinically characterized by abnormal fatigability, or transient or permanent weakness.

The field of CMS is steadily expanding.

Cases reports. Here we describe 5 unrelated cases (3 F, 2 M, age of 13, 21, 28, 38, 52 years). Their family history was negative. All patients reported first symptoms in the first-second decade of life. The extra-ocular muscles involvement is a common feature, only in one case also associated with diplopia. Severe bulbar weakness at onset was reported in one patient. Muscle fatigability is the more disabling symptom. Fluctuating and/or persistent limb muscle weakness, with both distal and proximal distribution, is also observed, while respiratory involvement is not detected. Blood CPK is normal. Electrophysiological investigation supported the CMS diagnosis. Muscle biopsy showed mild myopathic changes, but in one case scattered fibers with negative acid phosphatase vacuoles. DNA analysis confirmed

the genetic diagnosis, with detection of heterozygous variants in the following genes: *CHRNA1*, *RAPSN*, *CHRNE*. Therapy with AchEI appear partially effective in these cases, salbutamol is beneficial in one case.

P. 2-3 Miscellanea

Clinical features and muscle mri imaging in two trasportinopathy families with different mutations

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We present two families affected by LGMD D2, an autosomal dominant form of LGMD due to a mutation of *TNPO3* gene. This rare disorder was firstly genetically identified in a large Italo-Spanish family (mother and daughter) and we present another family with a congenital myopathy-LGMD phenotype in a child and his mother of Hungarian origin.

Clinical data, MRI imaging and quality of life were performed and compared in two families with transportinopathy.

The onset of symptoms was often characterized by difficulty in walking or climbing stairs, collected by a standard LGMD questionnaire. Muscle MRI was quantified using Mercuri score. The assessment of Quality of Life(QoL) was done by an Individualized Neuromuscular Quality of Life(INQoL) test.

In the Italo-Spanish family, the mutation was identified in a single nucleotide deletion (c.2771delA, p.X924C, exon22) in the *TNPO3* gene while in Hungarian family the causative mutation of LGMD D2 is due to an heterozygous frameshift deletion c.2767delC. in exon 23.

Mother and daughter of Italian-Spanish family presented marked atrophy of upper girdle muscles. The child of Hungarian family and his mother presented difficulty to stand from the floor. Muscle MRI of daughter and mother of Italo-spanish family showed pronounced atrophy in posterior thigh muscles. In son and mother of Hungarian family we observed generalized muscle atrophy. We monitored with age progression, connective and fat tissue increased, involving mostly posterior thigh, we propose that muscle MRI represents a valuable tool to document disease progression.

InGene: a novel approach for gene analysis and cluster definition in patients with hyperckemia

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Increased serum creatine kinase (CK) can be found in several genetically well-defined myopathies, but it can also be associated with no obvious clinical manifestations. Next-generation sequencing (NGS) has recently been proposed as a cost-effective strategy for the molecular diagnosis of inherited neuromuscular disorders. Over a two-year period, we evaluated 66 patients presenting with hyperCKemia and performed targeted NGS studies using a neuromuscular multigene panel. We identified a definitive molecular diagnosis in 33 patients. Among these patients, mutations in *RYR1* were found in 11, four cases had

mutations in *ANO5* and four in genes encoding sarcoglycans, while three cases harbored mutations in *CAPN3*. Within the Regione Toscana Health project Ingene, genetic data, clinical information (signs/symptoms in the HPO language) and MRC values in 33 positive patients were analyzed using non-metric multidimensional scaling (nMDS), a well-known algorithm for multivariate analysis, and clustering techniques. The algorithm used the information about the specific gene variants (rare and common) found in each patient identifying if specific genes or mutations could cluster and present association with clinical manifestations. With this method we identified three definite clusters characterized by presence/absence of muscle weakness and dystrophic features in muscle but not matching our earlier categorization based on clinical data only. We anticipate that a computer assisted integration of data will help identify more precisely genotype/phenotype correlations and also pinpoint possible genetic modifiers among hyperckemias.

InGene: an integrated tool for data collection in neuromuscular diseases

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Neuromuscular Diseases require a careful study of the genotype-phenotype association to understand the peculiarities of each subject affected by such conditions, and to tailor a personalized treatment, maximizing its outcome. It is therefore pivotal to collect genetic, instrumental, muscular, physiotherapy, cardiovascular data to draw a comprehensive patient picture, defining treatment plans, monitoring the course of the pathology and helping the clinicians in the patient care.

In the InGene project, an integrated tool to collect such data in neuromuscular diseases was developed. It includes several modules, with an integrated user interface.

The PhysioTest module allows administering physiotherapy tests, with customized interfaces for the Performance of the Upper Limb (PUL), the Six-Minute Walk Test (6MWT), the Motor Function Measure (MFM) and the North Star Ambulatory Assessment (NSAA), as well as the Neurological Physical Examination. The GenoStore module archives the patient's genotype and select patients with specific variants.

The MRIIndex module integrates the muscular MRI, providing an index for the muscular involvement, measuring the fat infiltration within the muscle.

Finally, the "InGene" module allows selecting several parameters (also from clinical dataset), applying them exploratory statistical analysis of selected patients, through multivariate techniques, to retrieve patients' clusters based on correlations between genotype and phenotype as a useful research aid tool.

The platform, collecting data within an Electronic Health Record, named Health360, developed as a modular, Cloud-based, Software as a Service (SaaS) platform, represents a diagnostic aid tool for the clinicians in personalizing diagnosis and treatment plans addressed to patients with neuromuscular conditions.

Founder mutation in Eastern Europe patients with GNE myopathy

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GNE myopathy is a rare hereditary inclusion body myopathy (HIBM) due to autosomal recessive mutations in the *GNE* gene, encoding for an enzyme involved in the sialic acid synthesis. Typical clinical presentation, with onset around the third decade of life, is characterized by weakness of the distal muscles of the leg and subsequent slowly progressive involvement of proximal and upper limbs muscles, generally sparing the quadriceps. Rimmed vacuoles are a distinctive finding at muscle biopsy.

We detected the same homozygous *GNE* mutation c.1853T > C (p.Ile618Thr) in two families originating respectively from Kosovo and Albania.

In the first proband, muscle weakness began at 27 years of age with bilateral foot-drop, subtle weakness of iliopsoas and adductors and mild hyperCKemia. Vacuoles and necrosis were reported at the muscle biopsy. Muscle MRI detected atrophy and fibro-fatty substitution of the medial and posterior compartments of the thighs. Inclusion body myopathy was reported in other three members of the family.

The second proband was a 23 years old man with myalgia and proximo-distal muscle involvement in the four limbs, started about 3 years earlier. CK levels were up to 5000 U/L. Muscle MRI revealed selective fibro-fatty substitution in lower limbs. Muscle biopsy showed a severe myopathy with rimmed vacuoles. His 22-year-old brother was affected by mild lower limbs muscle weakness with hyperCKemia and rimmed vacuoles at muscle biopsy. Other three members of his family presented with different degrees of muscular involvement. Cardiomyopathy was detected only in one of them.

Given the possibly common ethnic origin of the two families, allegedly Sinti, we suggested a possible founder *GNE* mutation c.1853T > C in this sub-population of Eastern Europe. Similar founder mutations are described in Japanese and Persian Jewish ethnicity.

Multifunctional evaluation of neuromotor performance in a CMT pediatric population: a pilot study

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Introduction. Charcot-Marie-Tooth disease (CMT) is an inherited peripheral neuropathy with progressive length-dependent weakness and atrophy of the distal muscles of the limbs. In children there is significant phenotypic variability, which can complicate assessment, diagnosis and treatment.

In this study we have examined the upper and lower limbs abnormalities in a group of children with CMT.

Methods. Thirteen children (age range 6-17) were evaluated with CMTPedS, Movement ABC-2 Test, 6MWT and Gait Analysis.

Results. At CMTPedS two cases were mildly affected (scores 0-14), eight cases moderately affected (15-29) and three severe affected (0-44). At Movement ABC-2 two cases showed normal behaviour, two were "at risk" and eight presented severe difficulty in movement. The patients performed a range between 379 to 513m at 6MWT. Using Gait Analysis we observed in some cases an increase of speed compared to standard walking. At the ankle joint level we documented a reduction of power (W/kg) generated during push-off with decreased of the dorsi-flexor moment (N/Kg) during loading response.

Discussion and conclusions. A general age-dependent correlation in the scores of CMTPedS emerges and the Movement ABC-2 revealed early difficulty on movement in upper limbs. 6MWT results were within normal range but the pattern of gait in these patients appeared altered indicating the important role of Gait Analysis will monitoring disease outcome.

Our pilot study reveals the importance of investigating upper limbs impairment and deterioration of gait in children with CMT to structure timely therapeutic interventions.

MRI-index: an automatic tool for early quantitative evaluation of fat infiltration at muscle MRI in neuromuscular diseases

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Introduction. Quantitative skeletal muscle MRI (mMRI) has been widely studied as a reliable outcome measure in patients with Neuromuscular Disorders (NMD). However the standard 3-point Dixon technique scoring system is not widely used in all centers for technical limitations. Conversely, we have developed a MRI-index, a measure extrapolated with an algorithm of image analysis able to derive automatically the fat percentage in mMRI images from NMD patients. Normative values, stratified for ages in healthy subjects, were acquired in our earlier studies.

Methods. Using a standard mMRI protocol in the past two years, we acquired myoimaging results of 46 patients (age range 7-70) with variable disease severity. Of these cases, 39 were genetically characterized and included 27 BMD boys, 4 subjects presenting mutations in *RYR1*, 2 in *MHY7*, 2 in *TPM3*, 1 *DMD* carrier, and 3 patients harboring mutations in other genes. Images of the medial third of the thighs of each subject were taken into account for the analysis of MRI-index. Genotype and phenotype correlations were subsequently made.

Results. Analyzing patients with the same genotype (BMD patients) we observed that disease severity correlates with the degree of fat percentage in muscle (high MRI-index). Consid-

ering patients with milder phenotype and no fat infiltration at qualitative imaging, MRI-index showed higher values when mutations occur in genes encoding structural muscular proteins. In asymptomatic hyperCKemia and mutations in *RYR1*, MRI-index was in the normal range.

Conclusions. The MRI-index can be a valid tool to detect muscle involvement and early genotype-phenotype correlation in NMD.

Axial myopathy: an overlooked cause of late onset camptocormia

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Myopathies are classified according to limb or cranial muscle affection, but the growing use of the imaging (MRI) showed that many myopathies have significant or selective involvement of the axial musculature.

A 83 year-old lady developed weakness and atrophy of neck muscles with dropped head. Electromyography showed myopathic features confirmed by the deltoid muscle biopsy. A genetic panel ruled out several genetic disorders including glycogen storage disease. Case 2 was 74 year-old woman evaluated because of progressing over 5 year weakness of paraspinal muscles and forward flexion of the trunk. There were myopathic features on EMG in limb muscles and neurogenic changes in quadriceps biopsy. Case 3 was 65 year old female evaluated because of spine rigidity, persistent elevation of CK up to 800 UI/L (Normal < 145). Neurological examination showed neck extensor weakness, wasting and weakness of paraspinal muscles. Electromyography suggested chronic myopathy.

Discussion The patients presented a late onset myopathy with indolent course, elevation of serum enzymes, predominant weakness of axial musculature. Such presentation led to extensive differential diagnosis including myasthenia gravis, amyotrophic lateral sclerosis, idiopathic inflammatory myopathies. Clinical presentation and ancillary data excluded these diseases. In accordance with profound weakness of neck extensor muscles and wasting of the paraspinals, MRI showed complete fatty transformation of what should be the paraspinal musculature. The diagnosis of axial myopathy presenting and progressing solely or predominantly either with neck extensor and paraspinal muscle weakness is challenging for clinicians and it might extend the clinical phenotype of myopathies with late onset.

Genetic heterogeneity of axial myopathy: report of three cases presenting with camptocormia

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Camptocormia is characterized by the hyperflexion of the thoracolumbar spine during the upright position. Its etiologies

are heterogeneous, including parkinsonism and neuromuscular disorders. Here, we report three patients with camptocormia due to a late-onset axial myopathy and we describe a genetic heterogeneity of the disease.

The patients are three unrelated women with a late onset axial myopathy (ranging from 50 to 60 years of age) and in one is present bilateral atrophy of intrinsic hand muscles; the family history is negative apart in one case in which the son had CPK elevation.

Next Generation Sequencing analysis of myopathy related genes was performed in the patients and identified variations in two genes: CAPN3 and SQSTM1. In the first patient homozygous p. Arg608Lys in CAPN3 was detected, previously identified in LGMD2A.

In the second case, heterozygous p. Pro392Leu in SQSTM1 was identified in the patient and in her son. The variant was previously reported as cause of distal myopathy in double heterozygosity with a TIA1 gene mutation.

In the last patient a double heterozygosity for p.Pro439Leu in SQSTM1 gene and p.Asp753Asn in CAPN3 gene was identified. The CAPN3 variant was previously reported in heterozygosity in LGMD patients. The SQSTM1 variant is described associated to different clinical phenotypes ranging from SLA to fronto-temporal dementia.

Our findings highlight the genotypic heterogeneity of axial myopathy, speculate a digenic inheritance of the disease and broaden the phenotypic spectrum of recessive calpainopathy.

Autosomal dominant Distal Spinal Muscular Atrophy (DSMA) is associated with MYH14 and MME genes mutations in an Italian family.

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Distal Spinal Muscular Atrophy (DSMA) is a genetically heterogeneous disorder affecting the lower motor neurons. We report clinical and genetic findings of an Italian family with autosomal dominant (AD) DSMA with five affected members in five generations. In all, weakness and atrophy started from leg and foot. Two have sensorineural hearing loss. The younger affected member (V-1), a 5-year-old boy, shows only some difficulties in heel walking. His 27 year-old mother (IV-5) is unable to walk on toe and heel, shows slight atrophy and weakness of the fingers extensor and intrinsic muscles of the hand and bilateral Achilles tendon retraction. His grandmother (III-2) presented a similar weakness and atrophy patterns with slow progression. Motor nerve conduction study showed reduced CMAP amplitude. Sensory conduction was normal and EMG was neurogenic. Biceps brachialis muscle biopsy was neurogenic. Muscle MRI confirmed leg muscles involvement. A mutation screen of 122 genes involved in DSMA revealed three rare heterozygous variants in MYH14 (p.R941L), MME (p.Y347C) and MFN2 (p.R663H) genes. Segregation analysis, performed in IV-5 and V-1, showed that variants in MYH14 and MME genes were present in heterozygosity. MFN2 variant was de novo in III-2. MYH14 is linked to AD distal weakness and deafness. Recently, the p.R941L mutation in MYH14 has been described in a Korean and a North American family with progressive DSMA associated to hearing loss. MME mutations

were found only in homozygosity in a late-onset CMT2 phenotype, so we excluded p.Y347C variant as causative. To our knowledge, this is the first Italian family in which these mutations are reported.

Myopathy, psychomotor delay and seizures due to a novel homozygous TBCK mutation in two sisters

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Mutations in TBC1-domain containing kinase (TBCK) cause a syndrome characterized by hypotonia, global developmental delay with severe cognitive and motor deficits, and variable presentation of dysmorphic facial features and brain malformations. Loss of TBCK was shown to decrease mammalian target of rapamycin (mTOR) signaling, increase autophagy, and decrease mitochondrial function. It remained unclear whether muscle weakness and hypotonia in individuals affected by this disorder were neurogenic or associated with progressive muscle disease. All previous muscle biopsy studies were performed in infants and were normal or inconclusive, but several groups had suspected muscle involvement.

Here, we report two sisters, initially diagnosed with congenital myopathy and severe psychomotor delay, in whom a novel homozygous truncation in TBCK was found by exome sequencing. We show that muscle disease can be associated with TBCK, as muscle biopsies performed on both sisters show myopathic features. Our findings add to the variability of phenotypes associated with TBCK mutations which can be ascribed to the multiple roles of this protein in intracellular signalling and endolysosomal function in different tissues.

Amyloid myopathy: an intriguing diagnosis

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Immunoglobulin light chain (AL) amyloidosis is the most frequent type of acquired amyloidosis due to a monoclonal misfolded insoluble light chain that forms amyloid fibrils with a tendency to tissue deposition leading to organ dysfunction. The most common encountered neurological manifestations are carpal tunnel syndrome, sensory-motor polyneuropathy and autonomic neuropathy.

Muscle involvement in AL amyloidosis is a rare, under diagnosed condition and can be the initial manifestation of the disease. Affected patients usually present with proximal muscle weakness, macroglossia and, sometimes, muscle pseudohypertrophy.

Diagnosis of amyloid myopathy is challenging and can be delayed if Congo red staining is not routinely included in the pathological work-up of muscle biopsy. The prompt recognition of this myopathy as presenting symptom of systemic amyloido-

sis is important in order to begin the treatment and improve the patient outcome.

We report on two patients with multiple myeloma who manifested amyloid myopathy at different stages of their dis-

ease. Both patients underwent a muscle biopsy which allowed to reach the correct diagnosis. Congo red staining plays a crucial role in the pathological diagnosis of this disease and its systematic use can likely increase the frequency of diagnosis.



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