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DOCTORAL THESIS

"Assessment of the central nervous system involvement in neuromuscular disorders: the evolution of outcome measures in childhood rare diseases"

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Table of contents

INTRODUCTION

1.1 Genetic neuromuscular disorders: the new scenarios

Genetic neuromuscular diseases (NMDs) impair the function of lower motor neurons, peripheral nerves, neuromuscular junctions and/or muscles (1). NMDs are rare conditions, with fewer than 200,000 affected people according to the US definition, or fewer than 1 affected in 2,000 people according to the EU definition (2). NMDs are genetically heterogeneous. To date, more than 1170 monogenic NMDs, linked to 658 different genes, have been identified in humans (3).

Some of these diseases, including Duchenne muscular dystrophy (DMD), spinal muscular atrophy (SMA), Glycogen Storage Disease type II (GSD2), and myotonic dystrophy type 1 (DM1), are among the most severe inherited disorders (1). Muscle weakness, hypotonia, and myalgia are common and usually incurable consequences. Many genetic NMDs are usually first diagnosed in childhood, and, as the disease progresses, severe disability with respiratory and cardiac failure can develop, resulting in very poor quality of life and premature death.

Diagnosing NMDs can be challenging as the initial presenting signs and symptoms tend to be non-specific and overlapping with other conditions. The initial step involves gathering information from caregivers or patients through an anamnestic questionnaire. This questionnaire covers patient history, the onset of signs and symptoms, medication usage, family history, and social background. These details provide crucial insights and assist clinicians in making a differential diagnosis. Subsequently, comprehensive physical examinations, including assessments of general health, musculoskeletal function, neurologic status, and overall functionality, should follow (4). During a clinic visit, the following signs warrant attention: hypotonia, psychomotor delay, feeding and/or respiratory difficulties, abnormal posture and gait characteristics such as toe walking, lordosis, or scoliosis, frequent falls, transitions difficulties, difficulty ascending stairs or arising from the floor, fatigue, pain, muscle cramps, or stiffness. While these signs are important, they are not necessarily indicative of a neuromuscular disorder when considered in isolation. For instance, hypotonia alone can also signal a central nervous system disorder. Weakness may manifest in various symptoms, including twitching, cramps, pain, breathing difficulties, contractures, and motor disability. Strength loss can vary, and its presentation can be highly specific depending on the type of neuromuscular disorder. Researchers have made several attempts to characterize patterns of strength loss to assist clinicians in differential diagnosis. When the neuromuscular junction (NMJ) is affected, weakness may manifest alongside symptoms such as droopy eyelids, double vision, and fatigue. Additionally,

NMDs can exhibit specific breathing patterns (such as the diaphragmatic pattern) or bulbar dysfunction (5–7). During the psychomotor examination, clinicians should consider age-appropriate milestones for both gross motor and fine motor development. For gross motor milestones, they should observe head control, independent sitting, crawling, standing (with and without support), and walking (with and without support). Regarding fine motor development, attention should be paid to fine prehension and bimanual skill acquisition. Furthermore, language acquisition and development can serve as an indicator of neuro-psychomotor delay.

Primary additional diagnostic measures include serum creatine kinase (CK) levels, electromyography (EMG), nerve conduction studies (NCS) and muscle or nerve biopsy, which has historically been the gold standard. In the last several decades less invasive testing has become more common such as muscle magnetic resonance imaging (MRI) and genetic testing. The advances in molecular genetics, such as next generation sequencing (NGS) which includes whole-exome sequencing (WES) and whole-genome sequencing (WGS), enable clinicians to pinpoint more accurately exact gene mutations. Among the most frequently performed blood tests is creatine kinase (CK). While a high CK level has traditionally been associated with NMDs and is often considered a diagnostic indicator, it is important to recognize that some NMDs, such as spinal muscular atrophy (SMA), do not consistently exhibit elevated CK levels in the blood. Consequently, laboratory examinations should be regarded as complementary rather than definitive diagnostic tools (8–12).

Over the last decade, improvement in the clinical course of some NMDs, such as SMA and DMD, has been achieved thanks to a proactive approach to functional rehabilitation and the introduction of non-invasive ventilation and enteral feeding (11). With multidisciplinary care, patient survival has improved, although the distal prognosis remains unfavourable. The diagnostic and therapeutic approaches of the medical subspecialties relevant to the care of NMD patients are evolving, and focus is now placed on early diagnosis and anticipatory treatment strategies addressing expected and potentially modifiable disease complications (10). The development of innovative genetic and molecular therapies for NMDs has been the major advance in the last ten years, leading to the approval of the first genetic treatment for DMD in 2014, the first genetic treatment for SMA in 2017, the first gene therapy for SMA in 2019, and the first gene therapy for DMD in 2023. Genetic therapies are mainly based on translational readthrough modifications or antisense oligonucleotide (ASO)-induced exon skipping/retention, without manipulation of host genome. Several clinical trials are ongoing with promising early results, providing the potential to permanently correct the underlying causes of some NMDs. Gene therapy, involving host genome manipulation, is another potential treatment (e.g., recently approved gene replacement

with adeno-associated virus for the treatment of SMA and DMD). However, these technologies are still in their infancy, and many questions and challenges remain to be addressed (1).

The increased understanding of genetic causes and of the natural progression of diseases, along with the availability of advanced treatments and improved multidisciplinary care, significantly influence the clinical phenotypes, life expectancy, and functionality of patients with genetic neuromuscular disorders. Henceforth, clinicians and health care providers dealing with these disorders face novel scenarios and challenges. These include the imperative for early diagnosis, the development of multidisciplinary treatment and rehabilitation plans, the adequate management of social and psychological needs, and the formulation of innovative outcome measures.

For many of these disorders, diagnostic delay is a significant hurdle as the irreversible muscle damage that occurs prior to the diagnosis can significantly limit treatment efficacy (13–17). Even in the absence of a rapid muscle destruction process, the long diagnostic journey can cause decades of limitation in the quality of life before the NMD is diagnosed and the appropriate treatment prescribed (18). In the era of disease modifying treatments (DMTs) for metabolic, genetic, and neuromuscular diseases, with many therapies expected in the coming years, early diagnosis is becoming an unavoidable public health necessity. Specific newborn screening (NBS) programs are the gold standard to identify individuals with some rare treatable conditions (e.g., SMA), but, so far, not all patients with treatable rare diseases are screenable through NBS techniques, and whole exome and genome sequencing as screening tools for all newborns pose many scientific, economic, and ethical concerns.

Now that patients with neuromuscular diseases are living longer, more attention has been paid to their health-related quality of life and psychological care. People living with chronic medical conditions—as most people with a neuromuscular disease—are at higher risk of developing mental disorders like anxiety and depression. To investigate the effect of the burden of the disease on mental health, patient reported outcome measures (PROM) and quality of life (QOL) instruments are available, both general and disease specific ones, e.g., for Spinal Muscular Atrophy (SMA) and Duchenne Muscular Dystrophy (DMD) (19–24). For the pediatric population, questionnaires for parents are available to report information about their child, and age-specific questionnaires using appropriate language for that child's development have been created and validated (25,26). In addition to the mental disorders generally experienced by people living with a chronic disease, specific comorbid neurodevelopmental or other psychiatric disorders have been described as part of the clinical phenotype of some neuromuscular diseases. High rates of intellectual disability—ID (17–27%), learning disabilities (26%), autism spectrum

disorder—ASD (15%), attention-deficit hyperactivity disorder—ADHD (32%), and anxiety (27%) have been reported in people with DMD (27–30). Similarly, ASD, ADHD, alexithymia, and other behavioral problems have been described in patients with Myotonic Dystrophy type 1 (DM1) (31,32). Published data for over nearly half of a century have demonstrated an association between Myasthenia Gravis (MG) and mood disorders (33). Patients with mitochondrial disorders can present with primary psychiatric symptomatology, including anxiety, depression, bipolar disorder, psychosis, and obsessive-compulsive disorder, which are mainly described in those with a diagnosis of mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) (34). Patients with psychiatric or behavioral issues should be referred to a mental health care professional for assessment and management. Milder symptoms may benefit from nonpharmacological interventions. Should these interventions not be effective, e.g., due to the increased severity of symptoms, psychopharmacological treatments might be required. However, very little is known about psychopharmacological management of mental disorders in patients with neuromuscular diseases, as regards to both their efficacy and safety profile. Studies on real world data in large populations are lacking. No guidelines for clinicians dealing with neuromuscular patients are available in terms of recommended medications (type, regimen, drug interactions) and follow-up schedules to monitor clinical outcomes and adverse events.

New knowledge on NMDs' natural history and new phenotypes after the advent of DMTs impact outcome measures. However, clinical trial design and the perspective of regulatory authorities also have an impact on them. For the identification of trial design to satisfy regulatory authorities, endpoint selection is crucial. Clinically meaningful endpoints should have obvious value to the patient and define how the patient feels, functions, or survives. To measure individual aspects of disease progression we have highly accurate instruments (quantitative muscle testing; myometry; several different functional scales) (35). However only limited information is available regarding how these measures correlate with ‘‘life-limiting events” which are relevant for patients with neuromuscular diseases. Moreover, no information is provided regarding the minimum clinical important difference (MCID) for the selected measures, except for the 6 min walking test. While some interventions may show statistical significance, this does not necessarily mean that the observed changes may be clinically important to the patients examined (36). Surrogate measures are a substitute for clinical benefit and may not have an immediate value to the patient. Forced vital capacity (FVC) could be an example of this, as well as muscle MRI. Only very few surrogate outcome measures have been shown to clearly correlate with disease progression; and little is known about the correlation of various biomarkers to events that are clinically meaningful to the patient. While it would be desirable to have biomarkers that track

progression and response to treatment of the disease, these are at the moment of limited use (35). Patient-reported outcome measures (PROMs) are increasingly being used to assess the quality of life, functional status, and disease burden of patients with neuromuscular disorders (21). An ideal pivotal trial will have a mixture of these kinds of endpoints. Finally, during the last few decades, several innovative technologies have been proposed and adopted for the biomechanical assessment and monitoring of human movement disorders in different clinical fields (37,38). Among these technologies, wearable inertial devices have gained prominence in recent years. Inertial Measurement Units (IMUs) are portable, easy to use, low-cost systems that allow continuous monitoring of human movements during outdoor and daily activities (39). In fact, Regulators stipulated the Stride Velocity 95th Centile as the first validated and suitable digital endpoint when continuously measured by a wearable device (40).

1.2 Measures of outcome for neuromuscular disorders: what is known

An outcome measure or endpoint is a measure within medical practice or research (primarily clinical trials) which is used to assess the effects, both positive and negative, of an intervention or treatment. Outcomes measures can be patient-reported or gathered through laboratory tests or through medical examination (41). Outcome measures are generally classified into different categories:

- primary (efficacy measures that address the main research question)
- secondary/tertiary (generally not sufficient to influence decision-making alone, but may support the claim of efficacy by demonstrating additional effects or by supporting a causal mechanism)
- surrogate (a laboratory measurement, radiographic image, physical sign, or other measures that is not itself a measure of clinical benefit but is reasonably likely to predict clinical benefit)

A good outcome measure should be simple, fast, reliable, reproducible, easily standardized (usually clinical trials need to be multicentric due to the rarity of the diseases), sensitive to change, clinically meaningful, with no floor or ceiling effects, with no learning effect, non-invasive, non-painful, inducing minimal risks, and cost-effective. For NMDs, a good outcome measure should also be adapted to possibly very weak patients, non-ambulatory patients, and patients with muscle contractures.

Current outcome measures in clinical trials for NMDs address survival, motor function, and other functions. Surrogate endpoints are frequently used as secondary outcome measures, or rarely as primary outcome measures.

1.2.1 Survival outcome measures in clinical trial for NMDs

Overall survival (OS) is defined as the time from randomization to death or as the average length of time patients are alive after the start of treatment. Any patients lost to follow up or still alive at the time of evaluation are censored (42). Event-free survival (EFS) is defined as the time from randomization to an event which may include disease progression, discontinuation of the treatment for any reason, or death (43). In other words, it is defined as the possibility of having a particular group of defined events (e.g., permanent ventilation, free of non-oral or mechanical feeding) after a treatment that is designed to delay or prevent that group of events. A generic measure of disease burden, including both the quality and the quantity of life lived, is the QALY (quality-adjusted life year). It is a quantitative outcome measure mainly used in economic evaluation to assess the value of medical interventions (44).

1.2.2 Motor function outcome measures in clinical trial for NMDs

The motor function historically has been measured using muscular strength test (MRC, Medical Research Council) in different groups of muscles depending on the specific disease or using instruments for strength quantification (dynamometers). In childhood, the motor development evaluation is used as motor outcome measure (e.g. motor milestones WHO-MGRS (45), or fine motor and gross motor scales from the Bayley Scales (46)). Moreover, some standardized functional assessments have been validated for DMD and SMA, and implemented for other neuromuscular disorders (47). For example: Timed Functional Tests (TFTs) and the Nort Star Ambulatory Assessment (NSSA) (48) have high validity and reliability in DMD patients still able to walk 10 meters independently older than 3 years, the Hammersmith Functional Motor Scale Expanded (HFMSE) (49) is used to evaluate subjects affected by SMA sitters and walkers over the age of 2 years, and the CHOP-INTEND (50) to evaluate infants and very weak young children suffering from non-sitting SMA and other similar neuromuscular diseases.

1.2.3 Other function outcome measures in clinical trial for NMDs

Other outcome measures used in clinical trial for NMDs target other body systems and functions, in particular respiratory function (e.g., Forced Vital Capacity-FVC, which is the total amount of air exhaled during spirometry test), nutrition, growth (e.g., weight WHO percentiles), cognition (e.g., psychometric tests-WPPSI-WISC-WAIS), and Quality of Life-QoL (patient reported).

1.2.4 Surrogate outcome measures in clinical trial for NMDs

Biomarkers are measurable indicators that can be used to diagnose but also to monitor neuromuscular disorders. Muscle biopsy is a common method used to identify biomarkers in neuromuscular disorders. The sample can be analyzed using various techniques such as immunohistochemistry and electrophoresis to identify specific proteins (51). Neurophysiology is another method used to identify biomarkers in neuromuscular disorders. A compound muscle action potential (CMAP) is the summated action potential recorded from muscle during a motor nerve conduction study (NCS) and has been used as secondary outcome measures in clinical trials (52). Also, neurofilaments can be used as a biomarker for neuromuscular disorders. They are found in the axons of neurons and can be measured in the blood or cerebrospinal fluid. Elevated levels of neurofilaments have been associated with disease progression in some neuromuscular disorders (53).

1.3 Innovative outcome measures for neuromuscular disorders

In the last twenty years the field of neuromuscular diseases, much more than other areas of neuroscience, has developed with an exponential increase in the knowledge of basic biological-molecular mechanisms and of clinical aspects, with direct involvement of patients and families in scientific research processes. Therefore, a great effort has been put on the adequacy of the classification of different clinical phenotypes and on improvement of standards of care and multidisciplinary approach, including the implementation of outcome measures evaluating cognitive functioning, the use of digital-based outcome measure in daily life, and the assessment of quality of life, daily activities independency, and care giver burden using patient reported outcome measures.

1.3.1 Cognition, psychological, and neuropsychological functions

In-depth knowledge of neuromuscular clinical phenotypes has become increasingly important, in order to identify validated clinical and functional outcome measures to better define treatment results, to describe long-term clinical evolution of treated patients, including new emerging phenotypes, and to better identify area of interventions and care recommendations. In fact, most neuromuscular diseases are multisystemic diseases, involving, sometimes from onset, organs or systems other than the neuromuscular system. Cardiac involvement (cardiomyopathies and rhythm disturbances in myotonic dystrophy and dystrophinopathies), hepatic involvement (metabolic myopathies) and smooth muscles alterations (gastro-intestinal disorders) are frequent features of NMDs.

One of the less known aspects, especially in the developmental age, concerns the central nervous system involvement, not only for the aspects directly related to the underlying pathology, but also for the impact of the different types of early motor disability on the development of the child's cognitive ability and personality. The involvement of the CNS in children with neuromuscular diseases has been variously studied, depending on the type of pathology. In some rare cases, neuro-imaging data (Magnetic Resonance Imaging, MRI, and advanced functional radiological examinations) have been published that have documented morphological alterations in the brain. In other cases, the central involvement from a functional point of view was explored through the assessment of patients' cognitive profile (54,55). For some types of neuromuscular disease, however, both aspects have been investigated very little, so our knowledge is still very limited.

Very few is known about the neuropsychological aspects of cognition, meaning how the brain processes information and how this affects behavior in NMDs. For example, one of the most important aspects of cognition is executive function. This refers to a set of cognitive processes that are responsible for planning, organizing, initiating, and monitoring goal-directed behavior. Executive functions include attentional control, working memory, inhibition, and problem-solving, among others. These functions are thought to originate in the brain's prefrontal cortex and are essential for everyday life, including coping with disability, and gaining personal and occupational goals.

Moreover, patients with neuromuscular disorders (NMDs) and their family members are at an increased risk of depression and anxiety, particularly during major care transition points in the progression of the disease. In some NMDs, many other psychological, emotional, and behavioral difficulties may arise, but these aspects are rarely formally evaluated and specifically addressed in both clinical practice and clinical trials, despite the exponential availability of psychometric tests (56).

1.3.2 Patient reported outcome measures

Understanding the many impacts of disease is crucial to providing optimal patient care. Valid, responsive, and meaningful outcome measures for the measurement of the impairment, activity limitations, and quality of life in patients with neuromuscular disease are crucial to identify the natural history of disease and benefits of therapy in clinical practice and trials (57).

One of the many tools available for clinicians and researchers to objectively quantify patient experiences are Patient Reported Outcome Measures (PROMs). PROMs are defined by the Canadian Institute for Health Information (CIHI) as measurement instruments that are completed

by patients and obtain information on aspects of the patients' health status relevant to domains such as quality of life, symptoms, function, pain, and physical, mental or social health. PROMs are known to be valuable tools capable of capturing patient changes which may otherwise be missed. They are rapidly being studied and implemented in areas of medicine including oncology, orthopedics, mental health, and chronic disease management. Regulatory agencies are also requiring PROMs in some cases. For NMDs the most important aspects to capture are Health Related Quality of Life (HRQoL), Activities of Daily Living (ADLs), and the caregiver burden (58).

1.3.3 Digital outcome measures

The availability of reliable, valid, and accurate outcome measures for motor function assessment is essential to quantify functional changes and allow clinical trial readiness in neuromuscular disorders (NMDs). Manual muscle testing, functional motor scales, and timed tests are currently the most widespread methods to monitor clinically meaningful changes in motor function of NMD patients. These methods, however, present some limitations, including learning effect, limited reproducibility, influence of motivation and attention (especially in pediatric patients), and lack of sufficient sensitivity to capture relevant changes in slowly progressive muscle diseases.

Over the past 20 years, we have witnessed the birth and development of several technologies and their application to the assessment and monitoring of neurological conditions. A technology outcome measure (TOM) can be defined as the outcome of device-based or instrumented clinical tests either conducted by clinicians in standardized environments to objectively measure specific behaviors or self-administered by patients to detect and monitor impairments in specific or overall function in everyday life. TOMs can potentially be applied in clinical practice for the assessment of several aspects of neurological manifestations, such as motor and sensory impairment, health-related quality of life, and rehabilitation purposes. In the neuromuscular field, muscle strength assessment with isokinetic dynamometers and gait analysis with three-dimensional (3D) motion capture systems are examples of the implementation of TOMs in clinical practice. Portable devices are broadly identified as lightweight instruments that can be carried around, whereas wearable devices specifically encompass active mechanisms that are anthropomorphic, can be "worn" by an operator, fit closely to the body, and work in concert with the operator's movements. Examples of wearable sensors are accelerometers, which measure linear acceleration in the three dimensions, and gyroscopes, which measure angular velocity and record orientation and postural changes. These technologies allow monitoring of patients' physical functions, such as gait and

upper limb function, as well as overall physical activity (PA), both inside and outside the boundaries of a clinical setting. In addition, these technologies can detect subtle changes of motor performances that would otherwise go unnoticed, both between and within patients. Therefore, their potential use in clinical trials is of major interest.

Large and appropriately designed cross-sectional studies investigating both criterion and content validity of these outcome measures in NMD patients should be promoted. At the same time, longitudinal studies should also be encouraged, to explore their responsiveness, to verify whether these technologies are sensitive enough to describe the natural history of specific, slowly progressive, neuromuscular disorders, and to capture clinically significant variations in the limited time frame of a clinical trial (39,59).

1.4 Clinical collaboration groups: the Italian networks

1.4.1 The DMD working group

The Italian network, in collaboration with some foreign institutions, has published over the years short- and long-term follow-up data on the specific functional aspects of patients with DMD. The network has been involved in the design of the upper extremity module to record functional changes in DMD patients, in the validation of several instruments, such as the North Star Ambulatory Assessment, and on exploring trajectories of disease progression also on the respiratory aspects of DMD.

Collaborators: Catholic University, Rome; Gemelli University Hospital Foundation, Rome: University of Messina; Ospedale Bambino Gesù IRCCS, Rome; Istituto Mondino IRCCS, Pavia; Gaslini Institute, Genoa; Besta Institute, Milan; Stella Maris Institute, Pisa; Ospedale Maggiore, Bologna; University of Napoli, Napoli; University of Turin; Turin; University of Padua, Padua; University of Milano, Padova; San Raffaele IRCCS, Milan.

1.4.2 The SMA working group

In 2014 a group of expert Italian researchers and clinicians gathered to build up a national collaboration group for the collection and study of data on SMA patients including 5 Italian sites coordinated by the Gemelli University Hospital Foundation IRCCS, Catholic University of the Sacred Heart), in collaboration with the US and UK network. In 2021 the group was extended to the Italian network of neuromuscular centres. The network has been involved in the creation of an Italian registry of SMA patients and on exploring trajectories of disease progression.

Collaborators: Catholic University, Rome; Gemelli University Hospital Foundation, Rome: University of Messina, Messina; Ospedale Bambino Gesù IRCCS, Rome; Istituto Mondino IRCCS, Pavia; Gaslini Institute, Genoa; Besta Institute, Milan; Stella Maris Institute, Pisa; Ospedale Maggiore, Bologna; University of Napoli, Napoli; University of Turin; Turin; University of Padua, Padua; University of Milan, Milan; San Raffaele IRCCS, Milan; Ospedali Riuniti, Ancona; University of Brescia, Brescia; IRCCS, Burlo Garofolo, Trieste; IRCCS E.Medea, Bosisio Parini, Lecco; IRCCS E.Medea, Brindisi; Metabolic Unit A. Meyer, Firenze; University of Bari, Bari; University Hospital Santa Maria della Misericordia, Udine.

SCOPE OF THE DISSERTATION

The primary objective of the presented projects was to collect quantitative and qualitative longitudinal data on CNS functions and better define motor function and disease trajectory in children affected by neuromuscular disorders, to identify suitable outcome measures for trial readiness.

The secondary aims were to improve the insight into the clinical phenotypes, provide these children and their families with more targeted interventions in terms of rehabilitation programs, and share evaluation protocols with other Neuromuscular Centers to increase data collection and eventually elaborate consensus guidelines for multidisciplinary care of these children.

Furthermore, a better definition of clinical phenotypes could give us new insights into the mechanism of the disease and information about possible new therapeutic approaches.

MYOTONIC DYSTROPHY TYPE 1 (DM1)

3.1 Disease characteristics

Myotonic Dystrophy type 1 (DM1/Steinert disease; OMIM 160900) is a rare genetic neuromuscular disorder with a prevalence of 1/8300–10700 in Europe (60,61). It is the most common type of myotonic dystrophy in adults, but it may occur also in children and neonates (60,61).

The DM1 transmission is autosomal dominant with variable penetrance and is associated with unstable expanded CTG repeats in the 3' untranslated region of the Myotonic Dystrophy Protein Kinase (DMPK) gene on chromosome 19q13.3 (62,63). The anticipation phenomenon is responsible for a more precocious and severe clinical phenotype in the offspring of affected individuals (63). Due to enlarged noncoding RNAs accumulation in nuclei, several regulatory processes are impaired, leading to disruption of key cellular pathways in various tissues (64).

Classically, four clinical phenotypes are recognized according to the age of onset: congenital DM1 (CDM1, with symptoms onset before 12 months of age) or childhood DM1 (ChDM1, with symptom onset between 1 and 10 years), classical DM1 (or adult-onset DM1), and mild DM1 (or late-onset DM1) (60,65). The main features of the disease are myotonia, muscular weakness and atrophy. The multisystemic involvement includes cataract, cardiac conduction blockage and arrhythmias, endocrine and gastrointestinal impairment, cognitive and psychological dysfunction, and excessive fatigue (66–68). Congenital DM1 is characterized by severe hypotonia and weakness at birth, respiratory and nutritional impairment, intellectual disability and an early mortality rate of 30–40% (69). In childhood DM1, mild motor delay with hypotonia is common, although learning difficulties, behavioural and mood alterations can also be the first signs of the disease (69,70).

The diagnostic gold standard for DM1 is represented by the genetic test to identify the number of CTG triplets in the DMPK gene through the application of the following methods: PCR (Polymerase Chain Reaction) and Southern Blot (71). It is possible to carry out prenatal screening for DM1; this is done on fetal cells from amniocentesis (between the 15th and 18th week of gestation). Prenatal screening is generally used in case of a parent affected by DM1 or in case of polyhydramnios or reduced foetal activity ecographically documented in the third trimester of pregnancy.

3.2 Central nervous system functioning characteristics in DM1

There is increasing evidence that central nervous system (CNS) involvement is part of the core manifestations of DM1 (72), and the need for developing standardized neuropsychological

assessments for both adults and children with DM1 has been identified in recent consensus-based care recommendations (31,73).

Recently, two reviews and a meta-analysis of clinical, radiological, and histopathological findings have highlighted heterogeneity of CNS involvement, with different combinations of cognitive and neuropsychological impairment in the disease subtypes (74,75). In congenital DM1, moderate to severe intellectual disability with involvement across different domains, including social cognition, memory, and visuospatial functioning, was reported (76). In childhood DM1, mild to moderate intellectual disability with impairment of visuospatial skills, attention deficit/hyperactivity disorder (ADHD), autism spectrum disorder, communication difficulties, and social anxiety have been described (76–78).

However, how the cognitive and neuropsychological profile changes over time in DM1 is still a matter of debate. Longitudinal studies in adolescents and adults have reported a significant deterioration in linguistic functions, and a tendency toward a decline in visuospatial/visuoconstructive abilities and executive functions (79–81). Few longitudinal studies in DM1 children have been performed so far. A decline of intellectual abilities has been reported in the congenital and the childhood DM1 types (82,83). A recent longitudinal prospective study assessed 51 children, adolescents, and young adults with congenital and childhood forms of DM1 using the Vineland Adaptive Behaviour Scales (VABS) and the Wechsler scales (84). A slower rate of development compared to normative data was found using these scales, but no absolute decline in cognitive and adaptive abilities was demonstrated, except for a tendency towards a decline in both intellectual and adaptive functioning in the congenital DM1 group. The authors hypothesized that children and adolescents with DM1 could present a neurodevelopmental disorder, followed by cognitive decline in adulthood (84).

The reported heterogeneity in the cognitive and neuropsychological profile in DM1 can be an obstacle for planning clinical trials. One of the reasons could lie in the fact that most tests cannot be considered ‘process-pure’ measures of a single cognitive process since they intercept multiple cognitive functions. Therefore, it has been suggested that cognitive batteries able to capture more specific processes rather than broader cognitive domains could be helpful as this approach may allow the identification of specific profiles in relevant subgroups (75).

3.3 Standards of care

No treatment can radically modify the course of the disease. In general, the main therapeutic objectives are as follows: preserving the patient’s independence; preventing cardiopulmonary complications; symptomatically treating disabling clinical manifestations. These objectives can be

pursued through regular patient follow-up, which allows monitoring of the disease's progression and timely identification of new onset problems.

In fact, most of the therapeutic interventions implemented for patients with DM1 are conservative in nature, that is, they aim to preserve functions and prevent complications associated with the disease. The main causes of death associated with DM1 are represented by pulmonary, cardiac, and neoplastic pathologies, so it is very important to monitor, investigate, and support respiratory and pulmonary functionality and to diagnose possible complications early. This is valid not only for adult patients but also for those in pediatric age. Over the years, progress in the management of respiratory and nutritional problems has led to an evolution in the natural history of neuromuscular pathologies. Intubation and ventilation during the neonatal period, assisted non-invasive ventilation (BiPAP or CPAP), respiratory physiotherapy techniques as well as logopedic rehabilitation for dysarthria and dysphagia have had a positive impact on the survival and quality of life of patients. Surveillance and support for cardiac function are fundamental at all ages. Support can be pharmacological or consist of implanting pacemakers or implantable defibrillators in cases of confirmed cardiac anomalies, second- or third-degree atrioventricular blocks, or tachyarrhythmias (31,73).

3.4 Approved and investigational treatments

At present, pharmacotherapy allows a symptomatic approach to the clinical problems of patients with DM1. There are no specific drugs for DM1, and medications used in clinical contexts other than DM1 are used. Therefore, in case of cardiac involvement, antiarrhythmic drugs are useful, and in case of insulin resistance or dyslipidaemia, antidiabetic or hypolipemic drugs can be used. In case of thyroid dysfunction or hypogonadism, replacement therapy is used. To reduce symptoms of intestinal hypomotility, prokinetic drugs such as metoclopramide and erythromycin can be used intermittently. In case of constipation, a high-fibre diet and/or the use of laxatives is indicated. Cholestyramine can improve diarrheal symptoms, anal incontinence, and abdominal pain. When the latter is not effective, norfloxacin can be used to stabilize the intestinal flora. It is important to evaluate the psychological/psychiatric sphere to intervene in case of psychiatric symptoms that interfere with daily activities (tricyclic antidepressants such as imipramine or clomipramine, benzodiazepines, etc.) (73,85).

In some patients, myotonia can be debilitating and can seriously interfere with daily life activities, therefore the introduction of specific pharmacotherapy is considered. Calcium channel blocking drugs such as mexiletine, propafenone, flecainide, carbamazepine, and low doses of steroids improve myotonia. Although there is no specific drug approved by the Food and Drug Administration (FDA)

for myotonia, there is Class 1 evidence supporting the use of mexiletine for myotonia at a dose of 150-200 mg three times a day (86). These drugs can, however, increase the risk of cardiorespiratory complications and exacerbate muscle weakness, so they must be used with caution. In any case, before starting treatment, cardiac activity should always be evaluated with an electrocardiogram. For pain, therapeutic options include mexiletine, gabapentin, and nonsteroidal anti-inflammatory drugs. In adult patients, daytime sleepiness can be very disabling and sometimes lead to the administration of methylphenidate or sometimes modafinil (87).

Recently, metformin, the most widely used antidiabetic drug, has been proved able to ameliorate mobility of DM1 patients, with significant increase in 6 MWT performance in a 52-week monocentric, double-blind, placebo-controlled phase 2 randomized study. Metformin counteracts alternative splicing deregulation of a subset of genes, including DMPK. However, the link with a biological effect on alternate splicing of specific genes was not established, and metformin may induce clinical benefit via other molecular mechanisms, e.g., autophagia, glycogen synthesis or insulin sensitivity (88).

It has been recently confirmed that the reduction of CUGexpRNA improves muscle strength in DM1 mice, suggesting that DM1 patients may benefit from elimination of toxic RNAs (89). A phase 1/2a blinded, placebo-controlled study to assess safety, tolerability, and dose-range finding of ISIS 598769, an ASO drug administered subcutaneously, was conducted in adult patients and showed good safety profile (NCT02312011). It is a gapmer ASO, a DNA–RNA hybrid with target mRNA, that recruits RNase H and promotes degradation of mRNA (90). In preclinical studies, systemic administration of ISIS 486178 induced rapid knockdown of CUG-repeat RNAs and corrected pathogenic features of the disease (91). Unfortunately, after phase 1/2a trial results the company discontinued further development of the drug.

3.5 Research Project

Considering the burden caused by cognitive and neuropsychological impairment, CNS alterations could become an appealing target for future therapeutic strategies for DM1. Therefore, a detailed knowledge of the neuropsychological profile and its progression over time is of utmost importance for evaluating efficacy. This would help identify specific domains of functioning that are worthy of systematic evaluation and inclusion in the standards of care for children with DM1. This, in turn, may help to identify suitable CNS outcome measures for trial readiness in DM1.

The aim of the research project was to examine the feasibility of administering a series of neuropsychological assessments to patients with congenital or childhood DM1, and to identify preliminary trends of changes over a 2-year follow-up.

Material and method

Participants

This was an observational, longitudinal study performed in DM1 children followed at the Centre for Neuromuscular Diseases of the Regina Margherita Children's Hospital in Turin (Italy). All the DM1 patients attending the centre between January and December 2017 who fulfilled the following criteria were included: age at baseline up to 16 years; diagnosis of genetically confirmed congenital DM1 (CDM1, with symptom onset before 12 months of age) or childhood DM1 (ChDM1, with symptom onset between 1 and 10 years); regular attendance at the Centre for clinical evaluations and treatment; and written informed consent to study participation by parents or legal guardians. When possible, patients were re-evaluated prospectively after two years (January–December 2020). According to the institution's research ethics committee, ethical approval was not required as data were collected as part of clinical care.

Procedures and assessments

Patients were assessed at baseline (T0) and, when available, after 2 years (T1). Over this period of time all patients continued attending regular follow-up appointments, as per current standards of care. The neuropsychological evaluation battery included:

A. Cognitive assessment:

Developmental/intelligence quotient (IQ) was evaluated with the Bayley Scales of Infant and Toddler Development- Third Edition for patients younger than 42 months of age; the WPPSI-III (Wechsler Preschool and Primary Scale of Intelligence) for patients aged 2 years 6 months–7 years 3 months; and the WISC-IV (Wechsler Intelligence Scale for Children) for patients older than 6 years of age. Based on the global IQ score, intellectual functioning was categorized as normal (IQ 85 or greater), borderline (IQ 70 to 84), mild intellectual disability (IQ 50 to 69), moderate intellectual disability IQ 36 to 49, severe intellectual disability (IQ 20 to 35), or profound intellectual disability (IQ less than 20) (92).

B. Neuropsychological assessment:

- Visuospatial functions were evaluated with the visual perception and visuo-motor integration (TPV) test, validated for children aged 4 years 5 months–11 years 0 months. The first four subtests of the TPV were selected: "Eye-Hand Coordination", "Position in space", "Copying and Reproduction" and "Figure-Ground". The results were compared to normative data to obtain standard scores, percentile ranges and equivalent ages. We considered abnormal results under the 10th percentile.
- Attentional functions were evaluated with the Bells Test, a cancellation task that investigates attention in children aged 4–8 years. Standard deviations and percentile ranges were calculated

according to the age of the subjects. We considered abnormal results under 2 standard deviations for both the "Rapidity" and the "Accuracy" domain. Although this test is validated for patients younger than 8 years old, according to clinical practice, we used it also for older patients.

- Executive functions were evaluated with the Tower of London (TOL), which measures planning ability in patients aged 5–18 years. Interpretation is based on standard deviations and percentile rank scores for both the main (Planning ability) and the secondary variables. We considered abnormal results for the main variable under 2 standard deviations.

C. Emotional-behavioural assessment: the Child Behaviour Checklist (CBCL), a questionnaire for parents/legal guardians, was used to assess competences and emotional- behavioural problems in children and adolescents aged 18 months to years (Preschool and School-Age versions). The questionnaire provides a "Competence scores" (Total, Activities, Social, School) and multiple scores across different syndrome scales, combined in two main groups: "Internalizing problems" and "Externalizing problems" and a "Total problem" score. These scores are interpreted as falling in the normal (below the 84th percentile), borderline (between the 84th and the 90th percentile) or clinically significant (above the 90th percentile) range. From 2001, additional scores can be obtained across a set of "DSM oriented" scales and, from 2007, the Sluggish Cognitive Tempo scale has been added. The scores are interpreted as falling in the normal (below the 93rd percentile), borderline (between the 93rd and the 97th percentile) or clinically significant (above the 97th percentile) range.

Statistics

For cognitive scores both descriptive statistics (means and standard deviations) and statistical tests were used. In particular, a paired-sample student t-test was performed to evaluate the trend of IQ between T0 and T1, and the Shapiro-Wilk test to verify the normal distribution of the scores. Only descriptive statistics was applied to the data from the neuropsychological and emotional-behavioural assessments. Analyses were performed using IBM SPSS Statistics for Windows, Version 25 software.

Results

A total of 10 patients were enrolled in the study, 5 CMD1 patients and 5 ChDM1 patients, age range 18 months to 16 years, mean age 9 years and 1 month. The details of each participant enrolled in this study are reported in Table 1.

Table 1
Sample demographics and clinical characteristics.

ID	Phenotype	Transmission	Sex	Age at clinical onset	Age at diagnosis	Age at T0 assessment	Myotonia	Hypotonia
ID01	CMD1	Maternal	F	Neonatal	Prenatal	1 y 6 m	No	No
ID02	CMD1	Maternal	M	Neonatal	1 month	1 y 7 m	No	No
ID03	CMD1	Maternal	F	Neonatal	3 months	13 y 3 m	Yes	Yes
ID04	CMD1	Maternal	M	Neonatal	2 months	16 y 1 m	Yes	Yes
ID05	CMD1	Maternal	F	Neonatal	3 months	16 y 4 m	Yes	Yes
ID06	ChMD1	Maternal	F	7 years	Prenatal	4 y	No	No
ID07	ChMD1	Paternal	M	9 years	Prenatal	7 y 1 m	No	No
ID08	ChMD1	Paternal	F	6 years	6 years	8 y	No	No
ID09	ChMD1	Paternal	F	8 years	8 years	10 y 3 m	No	No
ID10	ChMD1	Paternal	M	8 years	Prenatal	12 y 6 m	Yes	No

F: female; M: male; y: years; m: months.

Six patients were re-evaluated after two years (T1, 3 CMD1 patients and 3 ChMD1 patients, age range 3 years and 6 months to 15 years and 8 months, mean age 10 years). Four patients were not re-evaluated due to parental refusal or family organizational problems that prevented compliance with the protocol deadlines. During this 2-year period, no clinically significant decline in motor functions was observed (Table 2).

Table 2

Motor function result at T0 and T1 evaluation.

ID	Age at assessment	CI	MFM20	MFM32	6MWT
ID01 T0 ID01 T1	1 y 6 m 3 y 6 m	64/64 64/64	NE NE	NA NA	NA NA
ID02 T0 ID02 T1	1 y 7 m 3 y 11 m	NE NE	NE NE	NA NA	NA NA
ID03 T0 ID03 T1	13 y 3 m 15 y 8 m	NA NA	NA NA	83/96 85/96	273 m 368 m
ID04 T0	16 y 1 m	NA	NA	65/96	NE
ID05 T0	16 y 4 m	NA	NA	71/96	264 m
ID06 T0	4 y	NA	NA	96/96	529 m
ID07 T0 ID07 T1	7 y 1 m 9 y 4 m	NA NA	NA NA	93/96 96/96	459 m 488 m
ID08 T0	8 y	NA	NA	96/96	NE
ID09 T0 ID09 T1	10 y 3 m 12 y 8 m	NA NA	NA NA	96/96 96/96	NE 550 m
ID10 T0 ID10 T1	12 y 6 m 14 y 10 m	NA NA	NA NA	95/96 96/96	442 m 580 m

All patients were evaluated for motor functioning at baseline and two years later, with the functional motor scales routinely and periodically used at the center for patients with neuromuscular disorders, including DM1. According to age and functional level, the following scales had been used: Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders-CHOP Intend (CI), Motor Function Measure Scale for Neuromuscular Diseases 20 and 32 (MFM20 and MFM32), 6 Minute Walk test (6MWT). Results are reported in the table.

CI: Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders-CHOP Intend; MFM20 and MFM32: Motor Function Measure Scale for Neuromuscular Diseases 20 and 32; 6MWT: 6 Minute Walk test; y: years; m: months; NE: not evaluable; NA: not applicable.

Cognitive assessment

At T0 the developmental/intelligence quotient was assessed, based on age, with the Bayley III scale in two patients, the WPPSI-III in one patient and the WISC-IV in seven patients. The mean IQ was 72.00 (standard deviation SD±21.410, range 38–106). Intellectual disability was detected in 5 out of 10 patients (4 CMD1 and 1 ChMD1) In CMD1 patients the level of intellectual disability ranged from mild (1 patient) to moderate (3 patients). The only ChMD1 patient had mild intellectual disability. At T1 the cognitive/developmental quotient was assessed with the Bayley III scale in one patient, the WPPSI-III in another patient and the WISC-IV in the remaining 4 patients. Only for one CMD1 patient it was necessary to change the test used, due to increase in age. Intellectual disability was confirmed in all CMD1 patients evaluated, ranging from mild to severe. Two out of 3 ChMD1 patients had intellectual disability ranging from mild to moderate range. Notably, all patients showed a decline in their cognitive performances compared to the evaluation at T0.

Comparing results obtained at T0 and T1, all tested patients showed a decrease in their cognitive level: IQ was 72.00 ± 21.410 at T0 versus 53.33 ± 20.156 at T1 (Fig. 1). To evaluate the trend of the IQ scores in the sample between T0 and T1, a paired-sample student t-test was performed (T0-T1). Shapiro-Wilk test indicated probable normal distribution of the IQ data: for IQ at T0, $p = 0.768$; for IQ at T1, $p = 0.515$. The analysis showed that there was a statistically significant reduction ($p = 0.006$) in the IQ between T0 and T1, with a predetermined level of statistical significance alpha = 0.05.

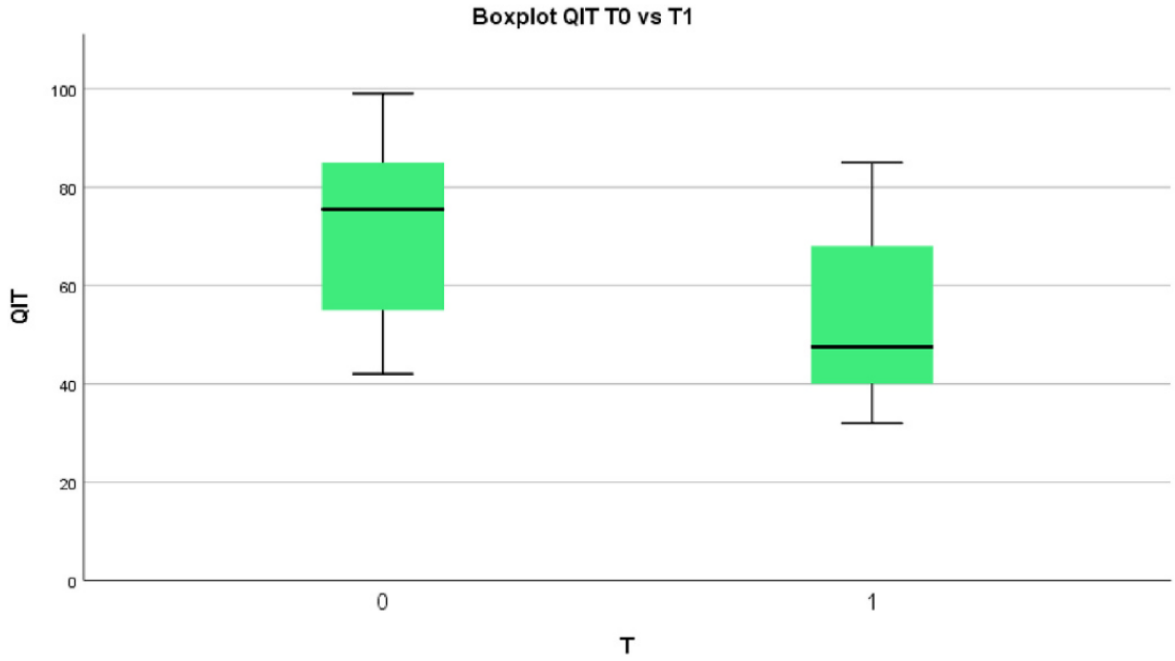

Fig. 1. Boxplot graph representing QIT reduction T0vsT1.

Neuropsychological assessment

At T0 the TPV test was performed in 8 patients (2 CMD1 were excluded for age). The CMD1 patients obtained results under the 10th percentile in all four subtests. The ChMD1 patients obtained results under the 10th percentile in some of the subtests (2/5 in "Eye-Hand Coordination", 3/5 in "Position in Space", 1/5 in "Copying and Reproduction", and 1/5 in "Figure-Ground"). At T1 the TPV could be performed only in 1 ChMD1 patient due to age limit. The patient obtained scores in the normal range except for the "Copying and Reproduction" subtest (9th percentile).

At T0 the Bells test was performed in 8 patients (2 CMD1 were excluded for age). All CMD1 patients showed abnormal results both for the "Rapidity" and the "Accuracy" domain. Among ChMD1 patients, all performed well in "Rapidity" and only two had abnormal results in "Accuracy". At T1 the Bells test could be performed in 4 patients (2 CMD1 were excluded for age). The only CMD1 patient tested obtained normal result for the "Rapidity" domain and abnormal results for the "Accuracy" domain. All the ChMD1 patients scored in the normal range.

At T0 the Tower of London test was performed in 7 patients (2 excluded for age and 1 for poor compliance). All patients obtained normal scores. At T1 the test was performed in 4 patients. One CMD1 patient, who was not evaluable at T0 due to poor compliance, scored below normal range. Two ChMD1 patients obtained results in the normal range and 1 in the pathological range.

The results of the cognitive and neuropsychological evaluations are summarized in Table 3.

Table 3
Cognitive and neuropsychological results at T0 and T1 evaluation.

ID	Age at assessment	TIQ	TPV sub1 Eye-Hand Coordination (centile)	TPV sub2 Position in Space (centile)	TPV sub3 Copying and Reproduction (centile)	TPV sub. 4 Figure-Ground (centile)	Bells test Rapidity (SD)	Bells test Accuracy (SD)	TOL (SD)
ID01 T0 ID01 T1	1 y 6 m 3 y 6 m	**55** (Bayley III) **32** (Bayley III) ↓	NE NE	NE NE	NE NE	NE NE	NE NE	NE NE	NE NE
ID02 T0 ID02 T1	1 y 7 m 3 y 11 m	85 (Bayley III) **55** (WPPSI III) ↓	NE NE	NE NE	NE NE	NE NE	NE NE	NE NE	NE NE
ID03 T0 ID03 T1	13 y 3 m 15 y 8 m	**42** (WISC IV) **40** (WISC IV) ↓	**4.5** NE	**4.5** NE	**4.5** NE	**4.5** NE	**-2** -1.6 ↑	**-3.9** **-4.8** ↓	NE **-4.42**
ID04 T0	16 y 1 m	**38**	**0**	**0.5**	**0.5**	**2**	**-3**	**-6**	-1.42
ID05 T0	16 y 4 m	**38**	**5**	**1**	**2**	**0.5**	**-2.4**	**-7.8**	-1.4
ID06 T0	4 y	106	84	50	50	84	1.2	1	-1.37
ID07 T0 ID07 T1	7 y 1 m 9 y 4 m	85 (WISC IV) **68** (WISC IV) ↓	**9** 37 ↑	**5** 16 ↑	37 **9** ↓	50 37 ↓	-0.4 -0.2 ↑	**-2.7** -0.73 ↑	0.31 -1.45 ↓
ID08 T0	8 y	93	37	**9**	91	63	-1.5	**-3.87**	-0.94
ID09 T0 ID09 T1	10 y 3 m 12 y 8 m	99 (WISC IV) 85 (WISC IV) ↓	25 NE	63 NE	50 NE	63 NE	-1.2 -1.76 ↓	-0.6 -0.3 ↑	0.37 2.16 ↑
ID10 T0 ID10 T1	12 y 6 m 14 y 10 m	**66** (WISC IV) **40** (WISC IV) ↓	**5** NE	**5** NE	**5** NE	**5** NE	-0.3 -0.64 ↓	0.5 0.18 ↑	-0.29 **-2.14** ↓

TIQ: Total Intelligence Quotient (including Composite Score of the Cognitive Scale assessed by Bayley III, and Full Scale Intelligent Quotient assessed by WPPSI III and WISC IV); NE: not evaluable; SD: standard deviation; y: years; m: months. In **bold** type abnormal results.

Emotional-behavioural assessment

At T0 the CBCL was administered to the parents of 9 patients (5 CMD1 and 4 ChMD1); the parents refused to complete it in one case. Only 7 patients had a complete evaluation due to age-range limitations of some subscales of the questionnaire. At T1 the CBCL was administered to the parents of all 6 patients, with a complete evaluation for 4 of them. At T0 the "Total Competence" score was in the clinical range in 6 out of 7 (86%), in particular on the "Activities" scale. Evaluation at T1 confirmed this, with a "Total Competence" score in the clinical range in 2 out of 4 (50%), in particular on the "Social" and the "Activities" scales. On the "Problems" scales at T0 2 patients obtained scores in the clinical range on the "Internalizing Problems" scale and on the "Social Problems" scale, respectively. The same patients at T1 scored in the clinical range on multiple scales, and one more patient scored in the clinical range on the "Attention Problems" scale.

The results of CBCL evaluation are summarized in Table 4.

Table 4
CBCL results at T0 and T1 evaluation.

ID	Total Scale	School Scale	Social Scale	Activities Scale	Internalizing Problems	Externalizing Problems	Social Problems	Thought Problems	Attention Problems	Anxiety Problems	Attention Deficit/Hyperactivity Problems	Sluggish Cognitive Tempo scale
ID01 T0	ne	ne	ne	ne	b	n	ne	ne	b	b	n	ne
ID01 T1	ne	ne	ne	ne	n	n	ne	ne	**C**	n	n	ne
ID02 T0	ne	ne	ne	ne	n	n	ne	ne	n	n	n	ne
ID02 T1	ne	ne	ne	ne	n	n	ne	ne	n	n	n	ne
ID03 T0	**C**	n	b	n	**C**	b	b	n	n	n	b	n
ID03 T1	**C**	n	**C**	b	**C**	**C**	**C**	b	**C**	n	**C**	**C**
ID04 T0	**C**	ne	**C**	**C**	n	n	b	n	n	n	n	n
ID05 T0	**C**	n	n	**C**	n	n	b	n	n	b	n	n
ID06 T0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
ID07 T0	**C**	n	n	**C**	n	n	n	n	n	n	n	n
ID07 T1	**C**	n	n	**C**	n	n	b	n	n	n	n	n
ID08 T0	**C**	n	n	**C**	n	n	n	n	n	n	n	n
ID09 T0	n	n	n	n	b	n	n	n	b	n	n	b
ID09 T1	n	n	n	b	n	n	n	n	n	n	n	n
ID10 T0	**C**	**C**	b	**C**	n	b	**C**	b	b	b	b	n
ID10 T1	n	n	n	n	**C**	n	n	**C**	n	**C**	n	b

n: normal; b: borderline; C: Clinical; ne: not evaluable; nd: not done. In capital letter and **bold** type clinical (abnormal) results.

Discussion

Our baseline cognitive evaluation showed the presence of intellectual disability in both the CMD1 and the ChMD1 group, as already reported in previous studies (74,76,77). The follow-up cognitive evaluation showed a clear-cut reduction of the mean and individual developmental/intelligence quotient after 2 years in all six re-tested patients. This result is partially divergent from the data reported by Lindeblad and colleagues (84). This discrepancy may be explained by patient selection biases in our sample which has a smaller size (10 versus 51 patients). However, these results highlight the importance of IQ evaluation in DM1 paediatric population, and the complexity in evaluating change in cognitive functioning over time in atypically developing patients. In fact, distinguishing between cognitive decline, with loss of previous acquired abilities, and a slower pace of development is challenging and underscores the need of larger, multicentric longitudinal studies.

As regards to the neuropsychological aspects, our baseline evaluation clearly identified impairments in visuospatial skills (TPV test), as already reported, in both CMD1 and ChMD1 patients (74,77). Unfortunately, only one patient could be re-assessed after 2 years, with comparable results; therefore, no informative data on progression couldbe obtained from our study.

Regarding the attentional functions (Bells test), our baseline evaluation showed abnormal results for both the “Rapidity” and the “Accuracy” domain in all CMD1 patients. Among ChMD1 patients, only two had abnormal results in “Accuracy”. No clear trend was observed at the follow-up evaluation.

As per the executive functions (TOL test), no significant impairment was detected at T0 (to note, 3/5 CMD1 patients were not tested due to their very young age). At T1 one CMD1 patient, not evaluated at T0, obtained very low result and one ChMD1 patient with a previously normal score showed results below the normal range. Executive functions evaluation requires a larger cohort of patients.

At the emotional-behavioural assessment (CBCL), the evaluation identified scores in the clinical range on the “Total Competence scale”, in particular on the “Activities” and the “Social” scales, both at baseline and after two years. Abnormal results on “Problems” scales were found, as already reported, but they were heterogeneous, and no trends could be recognized.

In fact, many CNS symptoms responsible for the burden of myotonic dystrophy in children are different when compared to adults (93). An early screening not only for intellectual disability, but also for neuropsychological as well as emotional and behavioural problems is fundamental for timely referral of the patient to a specialist and providing him/her with the appropriate supportive

interventions, as already highlighted by the recent consensus-based recommendations for the paediatric DM1 population (85).

From these data, although limited by the small sample size, it appears that a neuropsychological, emotional and behavioural assessment of paediatric DM1 patients is feasible. Considering the patients' intellectual disability and the dominant inheritance of the disease with an affected parent, whenever possible the assessment should be based on operator-administered tests rather than on parents- or self-reported questionnaires. Tests with a wide range of age-validation should be preferred to explore longitudinal modifications of functions.

This research project has been published in 2022 (see following image).

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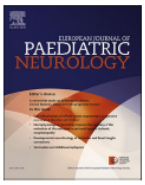

Cognitive, neuropsychological and emotional-behavioural functioning in a sample of children with myotonic dystrophy type 1

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ABSTRACT

Aim: An observational longitudinal study to evaluate the feasibility of assessing cognitive, neuropsychological and emotional-behavioural functioning in children with myotonic dystrophy type 1 (DM1), and to estimate prospectively changes in functioning over time.
Method: Ten DM1 patients, aged 1.5–16 years (mean 9.1), 5 with congenital DM1, and 5 with childhood DM1, were assessed with standardized measures of intellectual, neuropsychological, and emotional-behavioural functioning. For 6 patients, assessments were repeated 2 years later.
Results: At baseline, intellectual disability was found both in the congenital and the childhood group. A clear-cut reduction of the mean and individual developmental/intelligence quotient after 2 years was demonstrated in re-tested patients. As regards to the neuropsychological aspects, the baseline evaluation identified impairments in visuospatial skills and attentional functions, with no clear trend observed after two years. In executive functions, no significant profile was identified even though impairments were detected in a few patients. At the emotional-behavioural assessment, scores in clinical range were found, but they remained heterogeneous and no trends could be recognized.
Conclusion: Several aspects of CNS functions in DM1 children deserve better definition and a longitudinal assessment. A comprehensive protocol should include cognitive, neuropsychological, emotional and behavioural assessment but larger longitudinal studies are needed to better evaluate the trajectories over time and inform practice.

3.6 Future research development

To better characterize the population of children and adolescents affected by DM1, and to identify appropriate outcome measures for upcoming clinical trials, our center had participated in a longitudinal international multicenter study aimed at collecting standardized data on the evolution of motor function as early as 2020. The study aimed to increase the sample size, and the observation period was 12 months, with a focus on motor function.

The study concluded that, overall, children with CDM perform worse than healthy children of the same age. When evaluated over the age of 10 y, the gap between children with CDM and those healthy controls widens. Our observations in the 6MWT, 10 Meter Run, and 4 Stair Climb descent support the trend of improvement in function in younger years, followed by a plateau or decrease in rates of improvement, before a steady decline in participants greater than 10 y of age. This finding is consistent with clinical observations and previous studies (82,93,94). Given that only one subgroup had significant change over 12 mo, and only in the 6MWT, outcome assessments at intervals of 12 mo or longer appears to be reasonable. More frequent assessments likely would not reveal significant changes.

Lip force and grip strength in CDM participants did not mimic this pattern, but were obscured by some CDM subjects performing notably better than peers. It is also worth noting that cognitive impairment influenced feasibility of these two measures more than the functional measures, an important consideration for future clinical trials.

Interestingly, participants with repeat lengths between 500-1000 did not perform differently than those with repeats >1000. Although repeat length is often considered as a disease severity marker, the correlation with many disease characteristics is weak and overall the repeat size should not be used for prognosis. Continued analysis of progression into older age ranges merits further study.

Results from the VABS showed a considerable functional decline in composite score and both motor subscores for those aged less than 3 y, although our sample size was very small. VABS did not demonstrate reliable trends across any of the other age groups. This could be because the scale is parent-reported, or because it does not capture the complexity of CDM comorbidities. Other comprehensive tools considered for evaluation of our CDM population included the Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2), although previous cross-sectional data21 in a CDM population demonstrated such poor results on the BOT-2, that further use of this tool as a potential clinical trial outcome measure would be futile. It appears most likely that a different measure, encompassing multiple functional and behavioral domains specific to myotonic dystrophy, which can be reliably completed by parent proxy, is needed for better evaluation.

Several limitations should be noted, most importantly the difficulty in obtaining reliable outcomes from patients with physical, cognitive, and behavioural impairments. Due to these challenges, it is unclear whether functional and motor testing outcomes are a reflection of true maximum physical effort, or a child's ability to understand and follow instructions. This could be overcome by controlling for cognitive function with either IQ or neuropsychological testing, although reliable data on cognitive measurement selection is not yet available in this population. Oral aversion, which is recognized clinically in CDM and many other congenital muscular dystrophies, although not directly assessed in this study, also contributes to difficulties in obtaining reliable measures of orofacial function and should be further evaluated as a limitation in future investigations (95).

Selecting outcomes appropriate for patients across age groups is also challenging, although the availability of normative data can help to distinguish changes in longitudinal CDM outcome measurements that would be expected to improve in parallel with childhood development, from the relative flattening of the slope compared to healthy controls demonstrated in our cohort. This separation from the normal development curve is distinct from other pediatric neuromuscular diseases in which there is a frank decline in performance (96).

Regardless, 12-mo progression data does not paint the whole picture of CDM disease evolution and progression. More data, over a longer period of time, and in other CDM patient samples are needed to better characterize longitudinal trends in this patient population, as well as more detailed investigation into feasibility of functional outcomes as they directly relate to cognitive impairment. For purposes of clinical trial design and enrolment, it would be reasonable to target children at the plateau point, or the start of the slope of decline, in order to measure clinically meaningful delay or halt of disease progression. Evaluation of outcomes at 12-mo intervals is also a reasonable timeline for assessment in future trials. These data are foundational work in moving the field toward selecting the most feasible, reliable, and valid outcome measures for clinical and research work. From this study, functional measures such as 6MWT, 10 Meter Run, and 4 Stair Climb appear to be the most feasible and consistent with prior cross-sectional data.

The CDM community needs progress in understanding outcome measures beyond motor measures, such as patient reported outcomes and parent proxy questionnaires, as well as cognitive and disease severity biomarkers, to be fully ready for evaluating novel therapeutics.

For data details and abstract see following images and tables.

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CLINICAL RESEARCH ARTICLE

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12-Month progression of motor and functional outcomes in congenital myotonic dystrophy

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Abstract

Background: We aim to describe 12-mo functional and motor outcome performance in a cohort of participants with congenital myotonic dystrophy (CDM).

Methods: CDM participants performed the 6 Minute Walk Test (6MWT), 10 Meter Run, 4 Stair Climb, Grip Strength, and Lip Force at baseline and 12-mo visits. Parents completed the Vineland Adaptive Behavior Scale.

Results: Forty-seven participants, aged 0 to 13 y old, with CDM were enrolled. 6MWT, 10 Meter Run, and 4 Stair Climb were completed in >85% of eligible participants. The only significant difference between mean baseline and 12-mo performance was an improvement in 6MWT in children 3-6 y old (P = .008). This age group also had the largest mean % improvement in performance in all other timed functional testing. In children >7 y, the slope of change on timed functional tests decreased or plateaued, with further reductions in performance in children ≥10 y. Participants with CTG repeat lengths <500 did not perform differently than those with repeat lengths >1000.

Conclusions: The 6MWT, 10 Meter Run, and 4 Stair Climb were the most feasible measures. Our findings are consistent with the clinical profile and prior cross-sectional data, helping to establish reasonable expectations of functional trajectories in this population as well as identifying points in which therapeutic interventions may be best studied. Further study of outcomes in children >10 y old and <3 y is warranted, but this new information will assist planning of clinical trials in the CDM population.

KEYWORDS
congenital myotonic dystrophy, functional outcomes, mobility measures, myotonic dystrophy, Six Minute Walk Test

Abbreviations: 6MWT, 6 Minute Walk Test; ABC Score, Adaptive Behavior Composite Score; BOT-2, Bruininks-Oseretsky Test of Motor Proficiency; CDM, congenital myotonic dystrophy; DM1, myotonic dystrophy type 1; VABS, Vineland Adaptive Behavior Scale; UTR, untranslated region.

TABLE 1 Baseline characteristics

Variable	CDM (n = 47 unless otherwise indicated)
Age, y	
Mean ± SD	6.41 ± 3.64
Age group (n)	
<3 y of age	8
3-6 y of age	22
7-9 y of age	8
≥10 y of age	9
Gender, n (%)	
Male	25 (53%)
Female	22 (47%)
CTG_n repeats (n = 40)	
0-499	4
500-999	10
1000-1499	16
1500-1999	8
2000+	2
Gestational age, wk (n = 45)	
35[a]	12
35-37	10
38-41	22
>41	1
Respiratory assistance, wk (n = 34)	
Mean ± SD	24.71 ± 42.71
Range	0.3-156
ECG (n = 38)	
Abnormal, clinically significant findings	
Yes (n,%)	5 (13%)
No (n,%)	33 (87%)
Age at independent ambulation, mo (n = 30)[b]	
Mean ± SD	25.94 ± 9.99
Range	11-60

[a]Exact gestational age was available for 6 of the 12 participants born before 35 wk. One participant was born at 29 wk, the remainder were born between 30-37 wk.
[b]Two children were below 12 mo of age at time of evaluation and were excluded from calculation.

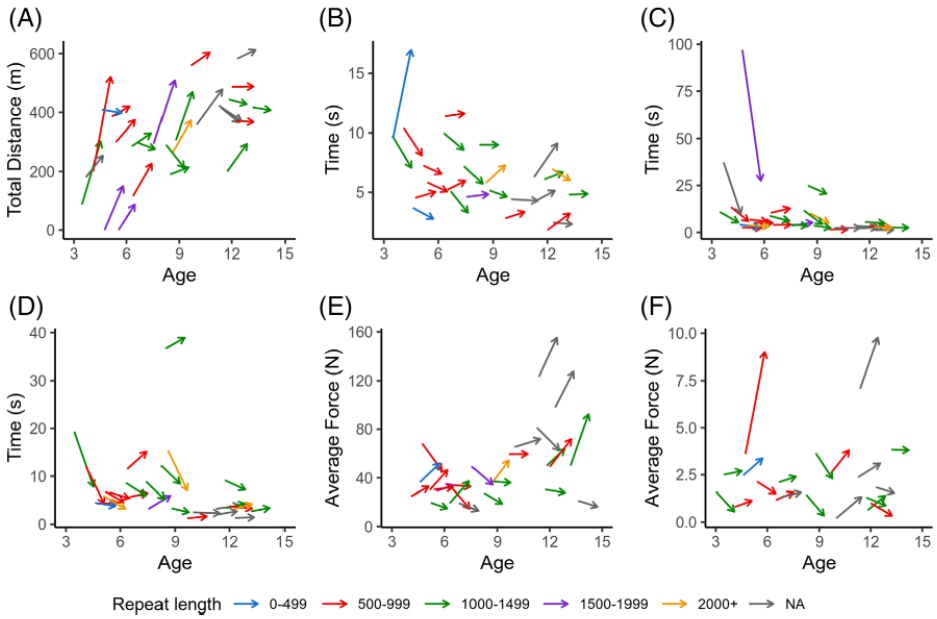

FIGURE 1 Baseline to 12 –mo performance with repeat length. A, 6MWT. B, 10 Meter Run. C, 4 Stair Climb-Ascent. D, 4 Stair Climb-Descent. E, Grip strength. F, Lip Force. Arrows represent individual subject performance. Blunted end of arrow represents baseline performance. Arrowheads correspond with 12-mo visit performance. NA, not available; s, seconds; m, meters; N, Newtons

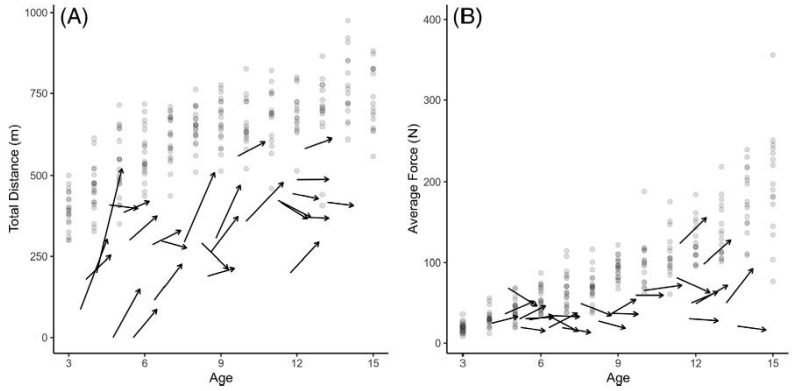

FIGURE 2 CDM participant performance compared to normal controls. A, 6MWT. B, Grip strength. Controls from the 1000 norms study are represented by shaded circles. Increasing densities represent overlapping performance. Solid arrows represent individual subject performance. Blunted end of arrow represents baseline performance. Arrowheads correspond with 12-mo visit performance. m, meters; N, Newtons

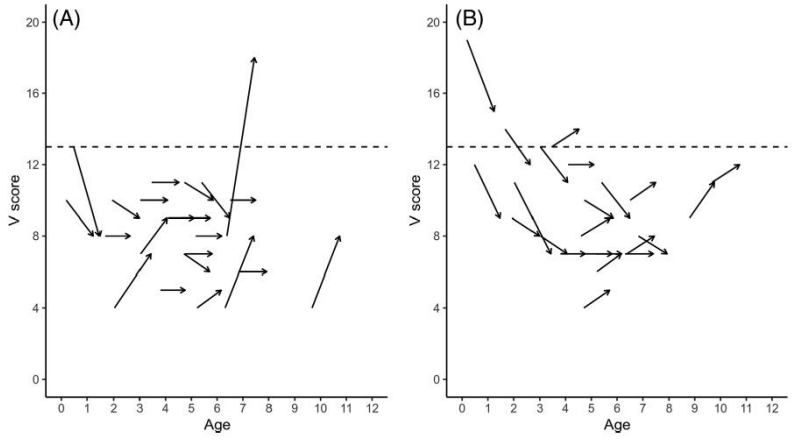

FIGURE 3 VABS gross and fine motor subscales. A, Gross Motor Subscale. B, Fine Motor Subscale. CDM participant performance from baseline to 12 mo visits. Results above the dashed line (V-score = 13) are considered at least an "adequate" level of adaptive functioning

Following this study, and the preliminary data of our monocentric study, both of them underlying the need to progress in understanding outcome measures beyond motor measures, including patient reported outcomes and parent proxy questionnaires, as well as cognitive and disease severity biomarkers, we took part in a longitudinal multicentric study promoted by NEMO Clinical Centre, Milano (Prof. V. Sansone). The study started in 2021 and was founded by Telethon Foundation (Telethon Project GUP19002H "Trial readiness and endpoint assessment in congenital and childhood myotonic dystrophy"). Enrolment closed in May 2023: data analysis is ongoing.

The first poster on baseline clinical data has been presented at the XXI Telethon Scientific Convention (Riva del Garda, 13-15 March 2023): "Trial readiness and endpoint assessment in congenital and childhood myotonic dystrophy: outcome measures and endpoint assessments".

A second poster has been presented at the XXIII National Congress of the Italian Association of Myology (Padua, 8-10 June 2023): "pre-and post-natal outcomes in congenital and childhood onset DM1 - the impact of parental diagnostic delay".

Two more abstracts have been submitted for a poster presentation in the next IDMC Congress (IDMC-14: International Myotonic Dystrophy Consortium, Nijmegen The Netherlands, 9-13 April 2024), one to present the Italian validation of the Congenital and Childhood Myotonic Dystrophy Health Index (CCMDHI), and another one to describe the prevalence of diagnostic delay in women affected by DM1 and the perinatal characteristics and the developmental outcomes of patients with CDM1 and childhood ChDM.

Trial readiness and endpoint assessment in congenital and childhood myotonic dystrophy: outcome measures and endpoint assessments

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INTRODUCTION AND AIMS

The design of therapeutic trials in children with congenital (CDM) or childhood-onset (ChDM) myotonic dystrophy type 1 has been significantly limited by the lack of long-term, appropriate clinical endpoints and disease biomarkers.

The main limitations in CDM and ChDM are represented by thye heterogeneous and often extramuscular clinical phenotype and the severe behavioral issues of these children.

This project is aimed to the establishment of an Italian clinical network for the diagnosis and management of CDM and ChDM across nine Italian Centres.
Specific aims are to prospectively collect functional measures to define disease-specific standardized protocols and procedures and to identify clinically meaningful endpoints in preparation for international therapeutic clinical trials.

METHODS

STUDY DESIGN

Three-year, multicenter observational longitudinal study involving paediatric patients (age <18 years) with with expanded trinucleotide (CTG) repeat (i.e. above 200) in the *DMPK* gene

The study includes patients with CDM in one arm, and patients with ChDM in a second arm.

- **INCLUSION CRITERIA**
  - CDM - onset of symptoms in the newborn period (< 30 days of life)
  - ChDM - children having symptoms of myotonic dystrophy after day 30 from birth
- **EXCLUSION CRITERIA**
  - Any other non-DM1 illness that would interfere with the ability or results of the study
  - Significant trauma within one month
  - Unable to walk more than 50 feet if over the age of 3

PROTOCOL OF ASSESSMENTS at baseline and every 6 months

a. Motor function
- 6 minute walk test
- 10 meters walk
- Grip and pinch strength via Jamar+
- 4 star climb
- Myotonia testing

b. Oro-pharyngeal function
- Lip and tongue strength via Oral Performance Instrument, IOPI
- Test of Mastication and Swallowing Solid, TOMASS-P
- Reading passage (Volume of voice)

c. Respiratory function
- Forced Vital Capacity % pred. via spirometry
- Pediatric Daytime Sleepiness Scale (PDSS)

d. Quality of life and cognitive measures
- Congenital and Childhood Onset Myotonic Dystrophy Health Index (CCMDHI).
- Pediatric Quality of Life (Peds QL).
- Behavior Rating Inventory of Executive Function (BRIEF) and pre-school (BRIEF-p)
- Wechsler Preschool and Primary Scales of Intelligence, (WPPSI-III) 2.6-4 y
- Wechsler Intelligence Scale for Children, (WISC-IV) 6-18 y

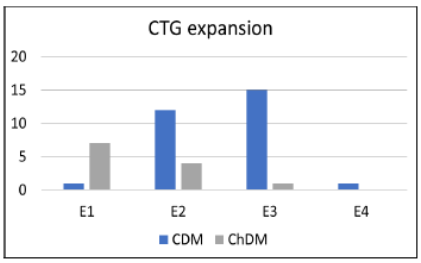
PRELIMINARY RESULTS

STUDY POPULATION

Table 1 - Demographic and anthropometric features of study population

Study population (n=60)	CDM (n=45)	ChDM (n=15)
Age at baseline, median [IQR], y	12.0 [9.1-15.7]	12.7 [9.1-15.7]
Follow-up (n)		
6 months	15	5
12 months	9	4
24 months	7	3
36 months	6	2
Class of CTG expansion*		
E1	1	7
E2	12	4
E3	15	1
E4	1	0

Figure 1 - Distribution of CTG repeats across CDM and ChDM patients



**E1–E4 class of CTG repeat size: E1= 200–500; E2=500–1,000;E3=1,000–1,500; E4>1,500*

MULTISYSTEMIC ASSESSMENTS AT BASELINE

Motor function

- 6MWT

- CDM (tot=42)
  7 non ambulant
  Median (n=20) 442 m [390-506]
- ChDM (tot=14)
  All ambulant
  Median (n=12) 486 m [417- 526]

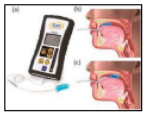
- Grip and pinch strength

Pinch
- Tested via digital pinch dynamometer
- Valid test in 14/ 19 pts

Grip
- Assessed via hand dynamometer
- Valid test in 17/ 19 pts

Oro-bulbar function

- Iowa Oral Performance Instrument (IOPI)



Tongue strength
Feasible in 14 pts (9/9 ChDM, 5/8 CDM)

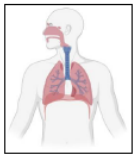
Lip strength
Feasible in 11 pts (7/8 ChDM, 4/7 CDM)

- Test of Mastication and Swallowing Solid, TOMASS

Most impaired skills in both CDM and ChDM:
- swallowing
- prolonged time to eat

- Reading passage
  - Normal voice in 5/11 pts
  - Hypophonia in 5/11 pts

Respiratory function



- FVC % pred. (n=24) – median 68% [49-82]
  - CDM (n=14) 67.5% [51-84]
  - ChDM (n=10) 73% [58-88]
- FVC (L) abs. (n=26) – median 1.9 L [1.3-2.4]
  - CDM (n=14) 1.7 L [1.3-2.9]
  - ChDM (n=12) 1.9 L [1.4-2.4]

Myotonia

- Video Hand Opening Time (VHOT)

- CDM - myotonia in 14/22
- ChDM - myotonia in 7/10

CONCLUSIONS

- This project supported by Telethon is in its final year and results are being finalised.
- The identification of specific outcome measures to assess the multisystemic aspects of the disease and their progression over time is pivotal for the design of clinical trials for CDM and ChDM.

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XXI Scientific Convention
PALAZZO DEI CONGRESSI - RIVA DEL GARDA (TN)
10-11 MARCH 2023
Telethon

Pre- and post-natal outcomes in congenital and childhood onset DM1 - the impact of parental diagnostic delay

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INTRODUCTION AND AIMS

Myotonic dystrophy type 1 (DM1) is often diagnosed during pregnancy in women or after the development of symptoms in one parent. This reflects in missed prenatal diagnosis in children affected. However, the impact of the parental diagnostic delay on the prognosis of their children is not established.

This study aims to describe the perinatal characteristics and the developmental outcomes of patients with congenital (CDM) and childhood (ChDM) forms in relation to the timing of diagnosis of the affected parent.

METHODS

STUDY DESIGN. Retrospective study of children with congenital (CDM) or childhood onset (ChDM) DM1 followed by 13 Italian centres.

Children's disease type and parental timing of diagnosis was recorded. Perinatal features, motor and cognitive development were collected.

CTG expansions were classified as E1=150-500,E2=500–1.000,E3=1.000–1.500,E4>1.500.

INCLUSION CRITERIA.

- **Congenital onset DM1 (CDM)** - Onset of symptoms within the first 30th days of life.
- **Childhood onset DM1 (ChDM)** - Onset of symptoms after the 30th days of life and before 10 years of age.

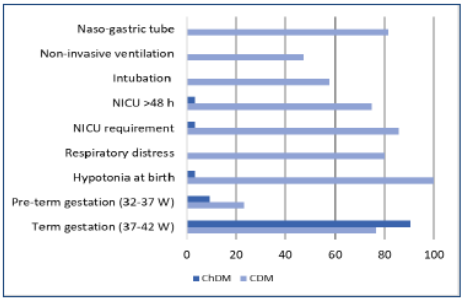
RESULTS

STUDY POPULATION.

N tot = 82	CDM	ChDM
N (%)	48 (59%)	34 (41%)
Gender, n (%)		
Male	23 (48%)	17 (50%)
Class of CTG expansion		
E1	5 (11%).	11 (34%)
E2	16 (34%)	18 (54%)
E3	25 (53%)	3 (9%)
E4	1 (2%)	1 (3%)
Age of onset, mean (±SD), y	0.1 (±0)	3.8 (±4)

PERINATAL COMPLICATIONS

(Data available for 76 children, expressed as percentage)



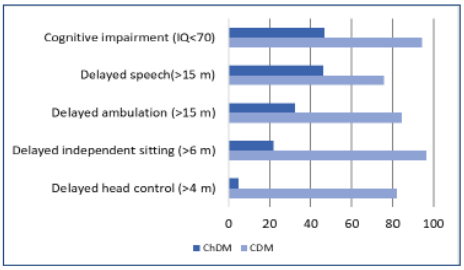
TIMING OF DIAGNOSIS OF CHILDREN AFFECTED AND PARENTAL DIAGNOSIS.

	Overall (n=82)	CDM (n=48)	ChDM (n=34)
Maternal inheritance, n (%)	58/79 (73%)	44/45 (98%)	14/34 (41%)
Class of CTG expansion, n	33	25	8
E1	16	11	5
E2	16	13	3
E3	1	1	0
E4	0	0	0
Paternal inheritance, n (%)	21/79 (27%)	1/45 (2%)	20/34 (59%)
Class of CTG expansion, n	12	1	11
E1	6	0	6
E2	5	0	5
E3	1	1	0
E4	0	0	0
Known parental diagnosis at the time of birth, n	29/80	15/46	14/34
No prenatal testing, n (%)	17 (59%)	11 (73%)	6 (43%)
Prenatal testing, n (%)	12 (41%)	4 (27%)	8 (57%)
Parental diagnosis secondary to child affected, n (%)	51/80	31/46	20/34
Child diagnosis at birth, n	3	3	0
Child diagnosis after birth, n	45	25	20
Age of child diagnosis, mean (±SD), y	5.2 (±5)	4.5 (±4)	6.3 (±6)

- Only 29 of 80 parents had a known diagnosis of DM1 at the time of pregnancy
- Only 12 of those 29 subjects carried out a pre-natal test during pregnancy

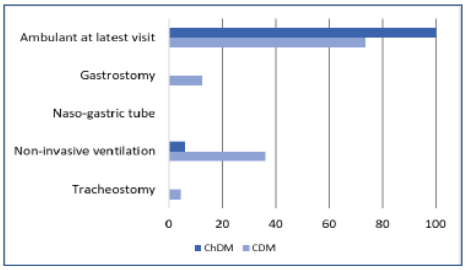
DEVELOPMENTAL MILESTONES

(Data available for 76 children, expressed as percentage)



RESPIRATORY AND NUTRITIONAL SUPPORT at LAST VISIT

(Data available for 76 children, expressed as percentage)



Median age CDM 11.9 [7.8-16.2]
Median age ChDM 12.2 [7.8-16.4]

CONCLUSIONS

Diagnostic delay in patients with DM1, especially in females of child-bearing age, has a significant impact on the pre-and perinatal management of children.

These results highlight the need for an early recognition of the disease and for a pro-active prenatal counseling.

Even when family history is not reported, considering the potential avoidant personality traits and frontal-related reduced awareness which may characterize adults living with DM1.

GLYCOGEN STORAGE DISEASE TYPE 2 (GSD2)

4.1 Disease characteristics

Glycogen storage disease type 2 (GSD2, OMIM # 232300), also known as Pompe disease (PD), is a rare an autosomal recessive lysosomal disorder caused by homozygous or compound heterozygous mutation in the GAA gene, which encodes acid alpha-1,4-glucosidase, also known as acid maltase, on chromosome 17q25 (97). The enzyme has a key role in the degradation of intralysosomal glycogen. GAA deficiency results in an abnormal intracellular accumulation of glycogen, with dysfunction of autophagic processes and degeneration of the cell, in particular muscle fibers (98–100).

The disease has a highly variable clinical spectrum, ranging from very severe infantile forms to adult-onset forms with minor limitations. The most severe form, referred to as infantile-onset PD (IOPD), is characterized by a complete or near-complete GAA deficiency, leading to early massive glycogen storage in cardiac, skeletal, and smooth muscles. Clinically, IOPD presents in early life with hypertrophic cardiomyopathy, profound hypotonia, and progressive respiratory failure generally leading to death within the first year of life (101). Less severe is the late-onset form (LOPD), which manifests usually with symptoms resembling a proximal limb-girdle myopathy with respiratory failure due to diaphragm involvement (102).

Bembi et al. (2008) provided a detailed guide to the diagnosis of GSD2, with emphasis on the importance of early recognition of clinical manifestations (103). Alpha-glucosidase activity can be simply and reliably measured in dried blood spots. Diagnosis is confirmed by biochemical assays showing absent or decreased GAA enzyme and enzyme activity in peripheral blood cells, skin fibroblasts, or muscle biopsy (103).

4.2 Standards of care

Recommendations for multidisciplinary management for patients with PD have been reported in consensus-based standards of care and in evidence-based guidelines, reporting both ERT regimens and proactive and rehabilitation care project.

Infants should be evaluated for swallowing difficulties, and methods to promote sufficient intake to provide adequate growth should be used; they should be on a diet that supplies adequate protein and energy, and vitamin D status should be optimized consistent with recommendations for the general population. They should also be monitored for the development of respiratory complications using pulmonary function tests at regular intervals. Both resistance and cardiovascular exercise to improve general conditioning and quality of life should be encouraged. Interventions

should be tailored to individual abilities. Periodic quality-of-life assessments and motor function tests, which can include questionnaires, should be part of the routine management of patients.

Goals of care should be reviewed on a regular basis and with interval changes in health. When disease control is no longer an objective, discontinuation of enzyme replacement therapy, supportive care and palliative measures should be available to patients (97,104)

4.3 Approved and investigational treatments

Since 2006, the standard care of Pompe disease has been enzyme replacement therapy (ERT). ERT dramatically changed the natural course of the disease in infants, resulting in a much longer survival. The most reliable effect of ERT in infant has been demonstrated on cardiac pathology and motor functions; in adults, motor performances and respiratory parameters, although less impressively, were improved or maintained (105,106).

In treated IOPD patients, a new phenotype, mainly myopathic, is emerging (107). Some factors have been related to treatment ineffectiveness in some cases, including later age at diagnosis, worse clinical picture at the beginning of treatment, cross-reactive immunologic material (CRIM)-negative status, and development of immune response against recombinant human GAA. As for the latter, immune tolerance protocols were proposed to prevent the formation of antibodies against the recombinant enzyme (108,109).

Neo-GAA (GZ402666), a second-generation GAA, was well tolerated with favourable safety and preliminary efficacy profile in a phase 1 study (110) and recently meaningful data has been published from a phase 3 randomized, multicentre, multinational, double-blind study (111).

An emerging strategy for the treatment of PD is chaperone therapy, which promotes folding, stability, and lysosomal trafficking of chaperone-responsive mutant enzymes and improves pharmacokinetics and pharmacodynamics of recombinant enzymes. In an exploratory trial, the chaperone N-butyldeoxynojirimycin was given at the time of the enzyme infusion in 13 patients with different presentations and type of gene mutations (3 infantile-onset, 10 late-onset). In the whole patient population, α-glucosidase activity was significantly increased at 12 h (2.19-fold, $P = 0.002$), 24 h (6.07-fold, $P = 0.001$), and 36 h (3.95-fold, $P = 0.003$) (112). Safety, tolerability, and efficacy of coadministration had been investigated in a phase 2 clinical trial in LOPD (NCT02675465) and some data have been published from a multicenter, randomized, double-blind phase 3 study with new generation GAA + chaperone versus rh-GAA in adult subjects with LOPD is in progress (PROPEL Study, NCT03729362) (113).

A potential alternative to ERT is gene therapy. Systemic and intradiaphragmal delivery of rAAV1-hGAA was shown to improve respiratory function in KO mice, but the first-inhuman trial of

diaphragmatic gene therapy (AAV1-CMV-GAA), conducted in children with IOPD requiring assisted ventilation, showed minimal beneficial effects (114).

4.4 Central nervous system functioning characteristics in GSD2

In 2006, enzyme replacement therapy (ERT) with human recombinant GAA received broad-label marketing approval in Europe and in the USA. This therapy is currently the standard of care to treat Pompe disease, it has changed the natural course of the disease and has significantly extended the lifespan of infants. However, most long-term survivors still carry the burden of the disease. IOPD remains a life-threatening condition: many patients do not survive ventilator free, and respiratory infections can be life-threatening. Even the most optimally treated infants, receiving since birth higher and more frequent dosing of the drug (40 mg/kg/week instead of the currently recommended 20 mg/kg/every other week), tend to develop motor problems (115). Survived children treated with ERT seem to develop a new complex phenotype including gross motor weakness, ptosis, facial muscle weakness, dysphagia with aspiration risk, speech difficulties and hearing loss (116,117). On top of that, recent longitudinal studies on brain magnetic resonance imaging (MRI) and cognitive and neuropsychological tests revealed cerebral white matter abnormalities and different degrees of cognitive decline in long-term survivors (55,118–120) . A very recent publication also reported that among a subset of IOPD patients on long-term ERT, CNS manifestations including hyperreflexia, encephalopathy and seizures had become prominent, with likely an association between these symptoms and significant white matter hyperintensities (WMHI) on MRI (121).

These data highlight another limitation of the ERT: the inability of the recombinant enzyme to cross the blood-brain barrier. Further study is needed to identify risk factors for CNS deterioration among children with IOPD and develop interventions to prevent neurological decline.

4.5 Research Project

In 2018 we reported results from a monocentric pilot comparative study to identify suitable outcome measures for the functional assessment in children with Pompe disease. In this study, disease progression up to three years in eight young patients with PD was monitored. Based on the literature data and the long term personal experience, we selected validated functional scales for neuromuscular disorders and compared the results to identify a simple and reliable protocol for the follow-up of children with PD. Moreover, we evaluated cognitive functions using developmental/cognitive tests.

Based on study results, we suggested that motor functions in children with PD could be better assessed by Chop Intend, MFM20 (Motor Function Measure Scale for Neuromuscular Diseases 20) and NSAA (North Star Ambulatory Assessment), according to age and functional level. Evaluation

should be completed with ROM (Range Of Motion) measurement, MRC (Medical Research Council) evaluation and 6MWT (6 Minute Walk test) when possible. This proposed protocol seems to be reliable and should be done every six months, because of the progressive natural history of the disease, the rapid changes typical of developmental age and the need to document ERT effects. About cognitive functions, additional tests to classical intelligence scales (WISC, WPPSI) should be useful to better describe specific neuropsychological profile (see following images and tables).

EUROPEAN JOURNAL OF PAEDIATRIC NEUROLOGY 22 (2018) 1103–1109

Official Journal of the European Paediatric Neurology Society

Original article

Functional assessment tools in children with Pompe disease: A pilot comparative study to identify suitable outcome measures for the standard of care

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ABSTRACT

Background: Pompe disease (PD) is a rare condition caused by mutations in gene encoding for the enzyme alpha-glucosidase, resulting in an abnormal intracellular accumulation of glycogen. The disease clinical spectrum ranges from severe infantile forms to adult-onset forms with minor limitations. Since 2000 enzyme replacement therapy (ERT) is available and disease natural history has changed, with prolonged survival and evidence of myopathic features.

Methods: In this study, we monitored disease progression up to three years in eight young patients with PD. Based on the literature data and the long term personal experience, we selected validated functional scales for neuromuscular disorders and compared the results to identify a simple and reliable protocol for the follow-up of children with PD. Moreover, we evaluated cognitive functions using developmental/cognitive tests.

Results: Based on study results, we suggest that motor functions in children with PD could be better assessed by Chop Intend, MFM20 (Motor Function Measure Scale for Neuromuscular Diseases 20) and NSAA (North Star Ambulatory Assessment), according to age and functional level. Evaluation should be completed with ROM (Range Of Motion) measurement, MRC (Medical Research Council) evaluation and 6MWT (6 Minute Walk test) when possible.

Conclusions: The proposed protocol seems to be reliable and should be done every six months, because of the progressive natural history of the disease, the rapid changes typical of developmental age and the need to document ERT effects. About cognitive functions, additional tests to classical intelligence scales (WISC, WPPSI) should be useful to better describe specific neuropsychological profile.

Table 1 — Population details.

ID	Form	Age at diagnosis	Age at T0 evaluation	Sex	ERT	CRIM
1	Classical Infantile Pompe	3 m	7 m	M	yes	−
2	Classical Infantile Pompe	4 m	3 y 4 m	F	yes	+
3	Classical Infantile Pompe	3 m	1 y 4 m	M	yes	+
4	Classical Infantile Pompe	5 d	2 m	M	yes	+
5	Non-Classical Infantile Pompe	7 y	11 y 5 m	M	No	+
6	Non-Classical Infantile Pompe	3 m	4 y 3 m	F	No	+
7	Non-Classical Infantile Pompe	9 y	14 y 6 m	M	yes	+
8	Non-Classical Infantile Pompe	2 m	9 y 1 m	F	yes	+

ERT: Enzyme Replacement Therapy; CRIM: Cross-Reactive Immunological Material; d: days; m: months; y: years; M: male; F: female.

Table 2 – Motor functional assessment overview at T0.

ID	Age (months-m, years-y)	Form	MRC	ROM limitations	6MWT	Functional scales						
						CI	MFM-20	HMFS	HMFSE	NSAA	GSGCA	MFM-32
1	7 m	Classical Infantile		Upper limbs: no Lower limbs: Hips: yes knees: yes ankles: yes		46/64						
2	3 y 4 m	Classical Infantile		Upper limbs: no Lower limbs: Hips: yes knees: yes ankles: yes		52/64	26/60	8/40	10/66			
3	1 y 4 m	Classical Infantile		Upper limbs: no Lower limbs: Hips: yes knees: yes ankles: yes		60/64						
4	2 m	Classical Infantile		Upper limbs: no Lower limbs: Hips: no knees: no ankles: no		48/64						
5	11 y 5 m	Late Onset Infantile	100%	Upper limbs: no Lower limbs: no	492 mt	64/64	60/60	40/40	66/66	34/34	5/32	96/96
6	4 y 3 m	Late Onset Infantile		Upper limbs: no Lower limbs: no		64/64	60/60	40/40	66/66	34/34		
7	14 y 6 m	Late Onset Infantile	100%	Upper limbs: no Lower limbs: no	520 mt	64/64	59/60	40/40	66/66	33/34	5/32	95/96
8	9 y 1 m	Late Onset Infantile	70%	Upper limbs: no Lower limbs: Hips: yes knees: yes ankles: yes	233 mt	64/64	48/60	38/40	48/66	18/34	19/32	71/96

MRC is expressed as average percentage for all muscular districts according to the formula: [total MRC score/(number of muscles evaluated x 5)] x 100. About ROM limitations, not degree but joints location of limitation is reported because more useful for the present study. For subject 6, GSGCA and MFM-32 could not be used at T0 because validated for older ages (5 years and 6 years respectively); he could not cooperate for the 6MWT neither.

MRC: Medical research Council; ROM: Range of Motion; CI: CHOP Intend; MFM20 and MFM32: Motor Function Measure Scale for Neuromuscular Diseases 20 and 32; HMFSE: Expanded version of the Hammersmith Functional Motor Scale-HMFSE; NSAA: North Star Ambulatory Assessment, GSGCA: Gait, Stairs, Gowers, Chair, Arms Functional Test; y: years; m: months.

Table 3 – Cognitive/Development evaluation results.

ID	Form	ERT	Age at T0 evaluation (months-m, years-y)	Test	IQ/Composite score of cognitive scale	Re-test (at least after 2 years)
1	Classical Infantile Pompe	yes	7 m	ne	–	–
2	Classical Infantile Pompe	yes	3 y 4 m	ne	–	–
3	Classical Infantile Pompe	yes	1 y 4 m	Bayley-III	95	na
4	Classical Infantile Pompe	yes	2 m	Bayley-III	85	na
5	Non-Classical Infantile Pompe	No	11 y 5 m	WISC-IV	119	107
6	Non-Classical Infantile Pompe	No	4 y 3 m	WPPSI-III	121	107
7	Non-Classical Infantile Pompe	yes	14 y 6 m	WISC-IV	89	96
8	Non-Classical Infantile Pompe	yes	9 y 1 m	WISC-IV	108	110

IQ: intelligence quotient; WPPSI III: Wechsler Preschool and Primary Scale of Intelligence 3rd edition; WISC IV: Wechsler Intelligence Scale for Children 4th edition; CI: Confidence Interval 95%; ne: not evaluable; na: not assessed; m: months; y: years.

Table 4 – Proposed motor functional evaluation scales for children with Pompe disease (y: years).

Age	Functional level	Motor Functional Scale
0-2 y	Any	CI
2-18 y	Not able to sit without support	CI
2-18 Y	Able to sit without support	MFM-20
4-18 y	Able to walk	NSAA

CI: CHOP Intend; MFM20: Motor Function Measure Scale for Neuromuscular Diseases 20; NSAA: North Star Ambulatory Assessment; y: years.

4.6 Future research development

In 2019, we developed a non-profit, longitudinal, multicenter study to comprehensively evaluate the central nervous system (CNS) function in Italian children affected by Pompe disease. This study is supported by Sanofi (formerly known as Sanofi-Genzyme), and the Coordinator Centers are Turin and Pavia (Principal Investigator Federica Ricci). Extract from the protocol is provided below.

As of the most recent update in January 2024, the study has received approval from both the sponsor and the local ethical committee (Del 0068708, 05/06/2023). Currently, the assignment of the Contract Research Organization (CRO) and the activation of the study sites are underway.

STUDY TITLE

Comprehensive central nervous system (CNS) functional evaluation in Italian children affected by Pompe disease: a longitudinal multicentre study.

Study Rationale

Since the approval of ERT in 2006 there has been a growing scientific interest on the emerging phenotype of long-term surviving children with Pompe disease. However, proper longitudinal studies are lacking, and no disease-specific comprehensive protocol for the long-term functional follow-up has been validated so far.

In a pilot study on a small cohort of children with Pompe disease we performed cognitive assessments, and we compared several motor function scales commonly used in patients with neuromuscular disorders in order to identify a subset of tests more suitable to cover the disease-specific phenotypic spectrum in different ages (134).

Here we propose an observational longitudinal prospective study aiming to investigate long-term central nervous system involvement in a cohort of Italian children affected by Pompe disease. The study will help to better characterize the emerging phenotype in this population, and to provide significant insight on the current natural history of the disease. Data collected will facilitate the identification of special needs that must be taken into account when planning appropriate clinical follow-up schedules to prevent complications and comorbidities, thus supporting the development of consensus recommendations for the multidisciplinary care in this population. Finally, data from this study will help to define the most suitable outcome measures to evaluate efficacy of current and new developing drugs for children with Pompe disease.

Study Objectives

The primary objectives of the study are as follow:

- *To define the clinical characteristics of CNS functions in a large cohort of children with Pompe disease up to 18 years of age, affected by both infantile onset (IOPD) and later onset (LOPD) form, through a standardized longitudinal protocol over a two-years period.*
- *To correlate cognitive, neuropsychological, speech and language data with respiratory, bulbar, motor function and hearing data as well as additional disease specific information (GAA enzyme activity level, genotype, ERT, CRIM status)*
- *To investigate, as an innovative and relevant aspect of this protocol, also adaptive behaviour, assistance needs and quality of life in children with Pompe disease.*

The collection of standardized longitudinal data on integrated CNS functions will provide significant insight on the emerging phenotype in this population.

The secondary objective of the study is as follow:

- *To create a comprehensive management and follow-up protocol, based on disease-specific care needs, and to share it with other Italian and European Centres in order to define consensus recommendations for the multidisciplinary care in children with Pompe disease.*

The validation of this protocol will help to identify unmet care needs and, therefore, to provide these children and their families more targeted interventions in terms of rehabilitation programs. Ultimately, the protocol will help to define the most suitable outcome measures to evaluate efficacy of current and new developing drugs for children with Pompe disease.

Overall Study Design

This is a 2 years-long multicentre, observational, longitudinal, prospective study in a large cohort of Italian children with Pompe disease up to 18 years of age. Patients will be assessed every 6 months until the subject and/or the parent/legal guardian decides to withdraw, or until the study is terminated.

This is intended as a natural history study, and data collected may be used as a comparator arm for future clinical trials.

The study involves 2 Italian Coordinating Centre and 7 Italian Partner Centres with expertise in Pompe disease. Each Centre will be identified by a number or code (Co for coordinator and P1 to P7 for partners). Patients will be de-identified, and their ID will be provided as consecutive numbers (Co-01, Co-02, Co-03, Co-0n; P1-01, P1-0n; P2-01, P2-0n; P3-01, P3-0n, …).

Number of Subjects

The potential number of patients to be involved in this study is 40 to 45 with either IOPD or LOPD.

Enrolment

The Principal Investigator or qualified Sub-Investigator at each site will ensure that, before any study-specific procedure is performed, written informed consent is obtained from each subject's parent/legal guardian and appropriate written informed assent is obtained from each subject according to age (> 8 years old) and intellectual abilities.

Patients will be enrolled according to study-specific inclusion and exclusion criteria.

Inclusion Criteria

Patients must meet all the following inclusion criteria in order to be enrolled in this study:

- diagnosis of classic infantile-onset Pompe Disease (IOPD, onset in the first twelve months of life, with cardiomyopathy) or non-classic, juvenile later-onset Pompe Disease (LOPD, early symptoms or diagnosis before 16 years of age, without cardiomyopathy);

- confirmation of diagnosis of Pompe disease: absent or markedly reduced GAA enzyme activity on dried blood spot (DBS), lymphocytes, skin fibroblasts or muscle biopsy, confirmed by the presence of two DNA mutations at genetic analysis;

- age at baseline: 6 months to 16 years, 11 months of age;

- gender: any;

- regular attendance of clinical appointments at the referral centre;

- signed informed consent by parent/legal guardian, and signed informed assent by subject when applicable;

- subject and parent/legal guardian willing and able to comply with study visits and study procedures.

Exclusion Criteria

Patients must not meet any of the following exclusion criteria in order to be enrolled in this study:

- no signed informed consent by parent/legal guardian, and no signed informed assent by subject when applicable;

- any clinically significant medical finding that - in the judgment of the Investigator - would prevent the child from safe assessments;

- any social condition that - in the judgment of the Investigator - will make the patient unsuitable for participation in, and/or unable to complete the study procedures.

Description of Study Assessments

Medical History

A complete medical history will be collected at baseline (T0) including age at onset of symptoms, cardiac involvement at onset of symptoms (yes/no and type), other presenting symptoms, creatinine kinase (CK) at diagnosis, GAA enzyme activity level, specific DNA mutations, ERT (yes/no, age at starting and regimen), CRIM status, concomitant medications (e.g. immunomodulation protocol).

Other medical history data will be collected at each visit and can include, but are not limited to, the following – if available:

- *respiratory function: incidence and duration of respiratory infections in the previous 6 months, number and duration of hospitalizations in the previous 6 months, cough assist (yes/no and start date), ventilation dependence (yes/no), age at start of ventilation support, invasive or non-invasive ventilation support, type of non-invasive ventilation support, number of hours on non-invasive ventilation in a 24-hours period (PRN, nocturnal, >16 hours), FVC litres and % of predicted (upright and supine), cough peak of expiratory flow (L/min), maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), sleep study findings;*
- *scoliosis (yes/no), date of onset, Cobb angle (degrees), brace (yes/no), scoliosis surgery (yes/no, date and type);*
- *feeding: dysphagia, failure to thrive (weight < 2nd centile) without dysphagia, oral fed only, nasal gastric tube and age at insertion, percutaneous endoscopic gastrostomy(–jejunostomy) PEG-(J) and age at insertion.*

Anthropometric measurements

*The following measurements will be performed at each visit: weight (kg and centile), height (cm and centile), head circumference (cm and centile).**Cognitive function***

Cognitive function assessment will be performed at baseline (T0) and after 24 months (T24) (total: 2 evaluations) by a child/adolescent neuropsychiatrist / psychologist depending on staff availability at each site. The test will be administered according to age and individual expressive communication skills as listed below:

- *0 – 30 months of age:*
  The Griffiths Scales of Child Development, Third Edition (Griffiths III), Italian language (135) OR the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley III), Italian language (136) depending on current protocols and availabilities at each site;
- *31 months – 6 years of age:*
  The Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI III), Italian language (137);
- *> 6 years of age (up to 16 years and 11 months):*
  The Wechsler Intelligence Scale for Children, Fourth Edition (WISC IV), Italian language (138);
- *> 2 years of age (up to 20 years) with significant expressive communication difficulties:*
  The Leiter International Performance Scale, Revised (Leiter R), Italian language (139), a non-verbal test suggested in case of hearing disorders, speech disorders, attention deficit and hyperactivity disorder - ADHD, autistic spectrum disorder, severe motor disabilities, foreigners not familiar with the Italian language.

Neuropsychological functions

Neuropsychological functions assessments will be performed at baseline (T0) and after 24 months (T24) (total: 2 evaluations) by a child/adolescent neuropsychiatrist / psychologist / physiotherapist / speech and language therapist depending on staff availability at each site. Tests will be administered according to the age of the patients and will focus on the aspects listed below:

- *Attention: "Test delle Campanelle" (Bells Test) or "Dot Cancellation Test and Inhibition" [NEPSY-II,] (if NEPSY-II could be available for all centers, "Dot Cancellation Test and Inhibition" can be used instead)*
- *Attention: the "Dot cancellation test"; the subtest "Inhibition" of the developmental Neuropsychological-II (NEPSY-II) assessment, 5 to 16 years, Italian language (140).*
- *Memory: subtest "Selective reminding words" e "Face memory" as part of the "Test of Memory and Learning-TEMA" or Rey Auditory-Verbal Learning Test and Memory for Designs [NEPSY-II]*
- *(if NEPSY-II could be available for all centers Rey Auditory-Verbal Learning Test and Memory for Designs can be used instead)*
- *Memory: the "Rey Auditory Verbal Learning Test (AVLT), > 16 years, Italian language; the subtest "Memory for Designs" of the developmental Neuropsychological-II (NEPSY-II) assessment, 3 to 16 years, Italian language.*
- *Visuospatial processing: the "Rey Complex Figure Test", > 6 years; and the subtests "Geometric Puzzles" and "Design Copying" of the developmental Neuropsychological-II (NEPSY-II) assessment, 3 to 16 years, Italian language (if NEPSY-II could be available for all centers Geometric Puzzles and Design Copying can be used instead).*
- *Executive Functions: Tower of London or subtests of the developmental Neuropsychological-II (NEPSY-II) assessment, Italian language****Speech and language***

Speech and language assessment will be performed at baseline (T0), after 12 months (T12) and after 24 months (T24) (total: 3 evaluations) by a dedicated speech and language therapist at each site. The following tests will be performed according to age:

- *The parent-reported questionnaire "MacArthur-Bates Communicative Development Inventories" complete forms: children 8-24 months – Words and Gestures, and children 18-36 months – Words and Sentences, Italian language (141);*
- *The Boston Naming Test (BNT): children older than 3 years, Italian language (142);*
- *The subtest "Comprehension of Instructions" of the developmental Neuropsychological-II (NEPSY-II) assessment, 3 to 16 years, Italian language (140).****Oral motor function***

Oral motor function assessment will be performed at baseline (T0), after 12 months (T12) and after 24 months (T24) (total: 3 evaluations) by a dedicated speech and language therapist at each site. The assessment will vary depending on patient's age as described below:

- *0 – 4 years of age: clinical observation;*
- *> 4 years of age: "test delle prassie oro-facciali di Fabbro", Italian language (143) (see Appendix 2).*

Bulbar function and dysphagia

Bulbar function and dysphagia assessment will be performed at baseline (T0), after 12 months (T12) and after 24 months (T24) (total: 3 evaluations) by a dedicated speech and language therapist at each site. The level of oral feeding will be assesses with the Functional Oral Intake Scale (FOIS) - paediatric version. In orally-fed patients, the assessment will include an interview with parents and the clinical observation of the patient

during meals and liquids intake. A video-fluoroscopy test will be performed to document and characterize dysphagia should clinically significant findings at interview and/or at clinical observation be present.

Signs of dysphagia might include the following aspects:

- *Breathing difficulties when feeding that might be signalled by:*
  - *increased respiratory rate;*
  - *changes in normal heart rate (bradycardia or tachycardia);*
  - *skin colour change such as turning blue around the lips, nose and fingers/toes (cyanosis);*
  - *temporary cessation of breathing (apnea);*
  - *frequent stopping due to uncoordinated suck-swallow-breathe pattern;*
  - *desaturation (decreasing oxygen saturation levels).*
- *Coughing and/or choking during or after swallowing.*
- *Crying during mealtimes.*
- *Decreased responsiveness during feeding.*
- *Difficulty chewing foods that are texturally appropriate for age (may spit out or swallow partially chewed food).*
- *Difficulty initiating swallowing.*
- *Difficulty managing secretions (including non-teething-related drooling of saliva).*
- *Disengagement/refusal shown by facial grimacing, facial flushing, finger splaying, or head turning away from food source.*
- *Frequent congestion, particularly after meals.*
- *Frequent respiratory illnesses.*
- *Gagging.*
- *Loss of food/liquid from the mouth when eating.*
- *Noisy or wet vocal quality during and after eating.*
- *Taking longer to finish meals or snacks (longer than 30 minutes).*
- *Refusing foods of certain textures or types.*
- *Taking only small amounts of food, overpacking the mouth, and/or pocketing foods.*
- *Vomiting (more than typical "spit-up" for infants).*

Gross motor function

Gross motor function assessment will be performed at baseline (T0), and then every 6 months (T6, T12, T18, T24) (total: 5 evaluations) by a trained physiotherapist at each site. The gross motor function scales will be administered according to age and motor function level as described below:

- *Children aged < 2 years or older children with no independent sitting:*
  CHOP INTEND (Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders) (144) (145);
- *Children aged > 2 years and able to sit independently:*
  MFM-20 (Motor Function Measure-20) (146);
- *Children aged > 4 years and able to walk independently for at least 10 metres:*
  NSAA (North Star Ambulatory Assessment) (147) and 6MWT (Six Minutes-Walk Test) (148) (149).

In addition, muscle strength and joint mobility will be evaluated at each visit in all children with the Medical Research Council (MRC) Scale for muscle strength and ROM (Range Of Motion), respectively.

Hearing

Auditory tests will be performed at baseline (T0) and after 24 months (T24 (total: 2 evaluations). The Auditory Brain Evoked Responses will be evaluated in case of clinically significant findings at auditory tests.

Adaptive Behaviour

Adaptive behaviour assessment will be performed at baseline (T0), after 12 months (T12) and after 24 months (T24) (total: 3 evaluations). The study staff will administer the Vineland Adaptive Behaviour Scales – Second Edition (Vineland – II) (150) to parent(s) / legal guardian(s) of children aged > 6 years.

Assistance needs

Assistance needs assessment will be performed at baseline (T0), after 12 months (T12) and after 24 months (T24) (total: 3 evaluations). The study staff will administer the Barthel Index, Italian language, to the subject's parent(s) / legal guardian(s) (151).

Quality Of Life

Quality of life assessment will be performed at baseline (T0), after 12 months (T12) and after 24 months (T24) (total: 3 evaluations). The study staff will administer the Euro-Quality Of Life 5 Dimensions questionnaire - 3 levels (EQ5D-3L) (152), Italian language, to the subject's parent(s) / legal guardian(s).

Schedule of Events

Study visit	**Baseline (T0)**	**Month 6 (T6)**	**Month 12 (T12)**	**Month 18 (T18)**	**Month 24 (T24)**
Visit window	n/a	±2 weeks	±2 weeks	±2 weeks	±2 weeks
Informed consent/assent	X				
Inclusion and Exclusion criteria	X				
Medical History	X	X	X	X	X
Anthropometric measurements	X	X	X	X	X
Cognitive test	X				X
Neuropsychological tests	X				X
Speech and language assessment	X				X
Oral motor assessment	X		X		X
Bulbar motor assessment	X		X		X
Video-fluoroscopy test	(X)		(X)		(X)
Gross motor function scales	X	X	X	X	X
MRC	X	X	X	X	X

ROM	X	X	X	X	X
Adaptive behaviour assessment	X		X		X
Assistance needs assessment	X		X		X
Quality Of Life assessment	X		X		X
Auditory test	X				X
Auditory Brain Evoked Responses	(X)				(X)

(X) = if clinically indicated and/or available according to site-specific equipment and regulations

5Q SPINAL MUSCULAR ATROPHY (5Q SMA)

5.1 Disease characteristics

Spinal muscular atrophy (SMA), including types 1, 2 and 3 (OMIM 253300, 253550, 253400), is one of the most common neuromuscular disorders in childhood, with an incidence of 1:11,000 live births, and the primary genetic cause of infant mortality (153).

SMA is caused by a homozygous deletion or mutation in the survival motor neuron 1 gene (SMN1) on chromosome 5q13.2, leading to insufficient levels of SMN protein, which is crucial for motor neuron survival (154). SMN2, a centromeric gene, differs from the SMN1 gene by five nucleotides, and the substitution of a C with a T in exon 7 alters a splicing modulator, resulting in exon 7 exclusion in 90% of SMN2 mRNA transcripts. A truncated, highly unstable, and non-functional variant of the SMNprotein (SMNΔ7) is therefore produced. There is an inverse correlation between the number of SMN2 gene copies and clinical severity so that SMN2 copy number is considered the major phenotypic modifier of the disease (155).

SMA is classified into three subtypes, according to the age of onset of symptoms and the motor milestones achieved. Type 1 (also known as Werdnig-Hoffman disease) is the infantile and most severe form, with onset before 6 months of age, lack of head movement control, and death usually occurring within the second year of life. Type 2 has onset at 6–18 months of age, and patients can sit without support but cannot walk. Type 3 has onset after 18 months of age, and patients initially achieve the ability to stand and walk, while frequently losing it later in the course of illness (153). Regardless of the type, SMA clinical features include progressive muscle atrophy due to degeneration of the anterior horn cells in the spinal cord, early development of joints limitations, kyphoscoliosis, failure to thrive, and variable bulbar and respiratory weakness (156,157).

5.1 Standards of care

Until 2017, treatment for SMA patients had relied only on multidisciplinary supportive care focused on respiratory, nutritional, physical, and orthopedic interventions. However, in 2018, updated standards of care were introduced, driven by advancements in clinical trials. The approval of the first drug for SMA in December 2016 shifted the perspective of physicians and families, making them more proactive in managing this disorder, particularly in type 1 cases (11,158). Subsequent approvals of additional drugs and the emergence of new phenotypes have prompted ongoing revisions to these recommendations.

A multidisciplinary approach is the key element in the management of SMA patients. SMA is a complex disorder involving different aspects of care and professionals, and each of the aspects should not be dealt in isolation but as part of a multidisciplinary approach. Specifically, regular evaluations should be conducted for rehabilitation, orthopedic, nutritional, swallowing, gastrointestinal, and pulmonary management, with prompt implementation of necessary interventions. Additionally, attention should be directed towards acute care, concurrent medications, and the initiation of palliative care. In the past families had to coordinate all the assessments and visits but it is now recommended that this should be coordinated by one of the physicians, generally the neurologist or pediatric neurologist, who is aware of the disease course and potential issues (11,158).

5.3 Approved and investigational treatments

Three disease-modifying treatments are now clinically available for individuals with SMA after demonstrating efficacy in clinical trial. These treatments increase full-length SMN protein levels, either by optimizing SMN2 pre-mRNA inclusion of exon 7 or by introducing the SMN1 gene copy. Several non-SMN–directed treatments are under development, most to complement the SMN-directed treatments (159).

Nusinersen is a 2′-O-(2-methoxyethyl) ASO consisting of 18 nucleotides with high specificity for the intron downstream of exon 7 in the SMN2 pre-mRNA normally occupied by a specific cisacting splicing regulatory motif, the intronic splicing silencer N1 (ISS-N1). Blocking ISS-N1, nusinersen promotes exon 7 inclusion in the SMN2 pre-messenger RNA and increase the expression of the full-length SMN protein (160). This ASO does not cross the blood–brain barrier, and in animals, intravenous administration results in kidney accumulation and renal proximal tubular degeneration (161). For these reasons, nusinersen must be administered directly into the cerebrospinal fluid through lumbar puncture. The pharmacokinetics of the drug and its bioavailability represent a potential limitation, when considering also the multiorgan nature of SMA and the preclinical data pointing to the importance of peripheral SMN restoration for long-term rescue in animal models of severe spinal disease (162). Nusinersen was approved by the FDA in December 2016 and by the EMA in June 2017. Three pivotal trials demonstrate nusinersen's efficacy. The first was ENDEAR, a sham-controlled triple-blind randomized trial that enrolled symptomatic SMA type 1 infants 7 months or younger who were dosed according to product monograph dosage. Infants with symptoms in the first week of life were excluded (163). The sham procedure arm terminated early at interim analysis as there was a significant difference in survival between the two groups. The study met its primary endpoints of survival and improvement in motor milestones as measured on the Hammersmith Infant Neurological Examination Section 2. The second pivotal trial was CHERISH, a sham-controlled,

triple-blind randomized trial that enrolled children 2 to 12 years old who were able to sit independently but never walked (164). The inclusion and exclusion criteria restricted enrolment to children without significant contractures that would hinder improvement in motor scores, without severe scoliosis, and without ventilatory or nutritional supplementation. The nusinersen dosage was different from the product monograph, with 4 loading doses administered over 2 months followed by a maintenance dose every 6 months. The final study population of 2- to 9-year-old children met the primary outcome. Finally, NURTURE was an open-label pivotal trial of nusinersen initiated in the first 6 weeks of life in presymptomatic infants with two or three copies of SMN2 (165). A historic comparison group of symptomatic infants was used; however, no true natural history of presymptomatic infants with two or three copies of SMA is available. They enrolled a total of 25 infants with a 5-year follow-up. At interim analysis, marked improvements in motor milestones in all infants compared with historical controls were noted. Those with three copies of SMN2 met their motor developmental milestones within the World Health Organization–appropriate age limits. However, a proportion of infants with two copies of SMN2 developed proximal weakness and bulbar symptoms over time. Nusinersen is generally well tolerated, although case reports of communicating hydrocephalus have been reported. Most reactions, such as positional headache, are secondary to the lumbar puncture procedure (159).

Safety and efficacy of a single IV infusion of 1.1x10^14 vector genomes (vg)/kg of onasemnogene abeparvovec (gene therapy) was demonstrated in two open-label, single-arm, single-dose phase III studies using historical control groups (20,166). The two studies that included patients with SMA type 1 with two copies of SMN2 who were younger than 6 months differed not only by the localization of the investigation sites (one in the United States, the other in the EU) but also by the inclusion criteria that were more expansive in the EU study, allowing the inclusion of patients with nutritional or respiratory support at baseline. The co-primary endpoints were event-free survival at 14 months and independent sitting for 30 seconds or longer in the US study and 10 seconds in the EU study at 18 months. Secondary outcomes included the ability to maintain proper growth without nutritional support requirements or swallowing issues and independence of ventilatory support at 18 months. Both studies achieved a remarkable event-free survival at 14 months (20 of 22 in the US study, 31 of 33 in the EU study), and the proportion of patients who achieved independent sitting, despite a more stringent definition of sitting (30 versus 10 seconds), was slightly higher in the US study (13 of 22 versus 14 of 33). In both studies, the drug was well tolerated, and the most common drug-related adverse events were pyrexia, increased hepatic transaminase concentrations, and gastroenteritis. One patient presented with hydrocephalus. Onasemnogene abeparvovec was also studied in an open-label trial (SPR1NT) of patients younger than 42 days with two (n = 14) and three

(n = 15) copies of SMN2 and presenting with no symptoms at treatment initiation (167,168). The objective primary endpoint was the acquisition of an independent sitting position at 18 months of age and standing without support at 24 months of age for patients with two and three copies of SMN2, respectively. All patients with two and three SMN2 copies remained alive, were free of ventilation and nutritional support, and achieved their respective primary efficacy endpoint. Onasemnogene abeparvovec was equally well tolerated as no infant enrolled in SPR1NT experienced a serious adverse event that was considered treatment-related by the investigator, and no new safety signals were identified. These data were broadly reproduced in real-world experience and expanded the eligible population in terms of age and weight, also with the report of cases of thrombotic microangiopathy in post-marketing surveillance within the first 2 weeks following infusion.

Risdiplam is an orally administered, systemically distributed small molecule that alters the SMN2 pre-mRNA splicing to produce more full-length SMN protein. Its efficacy was established in two pivotal trials, obtaining initial FDA and EMA approval in 2020 and 2021 respectively (19,167). FIREFISH was an open-label phase 3 study using an external comparison group in infants with SMA type 1B born at term and treated between 1 and 7 months. The objective primary outcome of sitting without support for at least 5 seconds after 12 months, as assessed by item 22 of the gross motor scale of the Bayley Scales of Infant and Toddler Development, Third Edition, was reached. The second pivotal trial was SUNFISH part 2, a blinded placebo-controlled phase 3 trial in a broad spectrum of children and young adults 2 to 25 years old with SMA type 2 or 3 who were non ambulatory. A third pivotal trial still underway is Rainbowfish, an open-label study in pre-symptomatically treated infants with either two or three copies of SMN2. Interim results were encouraging, showing after 12 months of treatment with risdiplam that most presymptomatic babies met key motor developmental milestones in addition to maintaining the ability to swallow. The most common adverse events from these trials included rash, diarrhea and aphthous ulcers. Data on safety during pregnancy are insufficient as risdiplam does cross the placenta. Preclinical studies suggest potential teratogenicity. Data on lactation are also insufficient, although preclinical studies show transmission in breast milk. There is also a potential effect on spermatogenesis, supported exclusively by preclinical studies.

Although several trials are underway exploring the safety of any of these three treatments in participants previously exposed to another treatment, there is currently no evidence of efficacy for combining therapies aimed at increasing SMN protein production. Finally, for the unmet needs in the population of patients treated after symptom onset, several therapies not dependent on increasing functional SMN are on development: the myostatin pathways-directed drugs, the facilitating neuromuscular junction transmission drugs, and neuroprotectors (159).

5.4 Central nervous system functioning characteristics in 5Q SMA

As the number of long-term survivors with SMA type 1 increases worldwide, new phenotypes are emerging. These phenotypes exhibit varying outcomes in respiratory, bulbar, and motor functions. Additionally, there is emerging data in other areas of functioning, such as cognition, speech, and language development, even if a comprehensive understanding of brain involvement in SMA type 1 is still lacking.

Although the expression of the SMN protein throughout the CNS has been known since the late 1990s, neuropathological studies in SMA type 1 are sparse (168). The study of brain involvement through autopsies of very young infants was probably slowed down by ethical considerations and neuroimaging studies in this population are very limited (169). Available data show that brain structures can be primarily affected in the severe forms of SMA (170,171). Several structures, including thalami, basal ganglia, temporal and frontal cortices, hippocampi, and cerebellum have been reported to be variably affected. Interestingly, a marked progression of initial brain abnormalities has been documented by follow-up brain scanning (171,172). Of note, neuropathological alterations in other nervous system areas such as the primary spinal sensory neurons of dorsal root ganglia were reported as well, both in human and mouse model studies (170,173) and sensory inputs impairment could play a role in the overall brain functioning of these patients.

The clinical correlates of these findings in terms of cognitive and other neuropsychological functions, including speech and language, are still largely unclear, and no study has investigated the correlation between neuropathology/neuroimaging reports and cognitive and neuropsychological functioning. As already mentioned, this was mainly due to the clinical severity of untreated SMA type 1 patients, who were often not able to provide verbal or gestural responses during the assessments. Most of the studies focused on the intellectual abilities in SMA were initially performed in less severely affected patients (SMA types 2 and 3), who could be more easily assessed using the available validated tests and assessments. These studies showed normal to higher than normal IQ and speech/language abilities scores compared with their peers (174). A very recent paper on adult patients with SMA Type 3 found that greater motor difficulties were associated with worse performance in attention and working memory and better performance in language, visuospatial abilities, and memory (175). This partly confirms the hypothesized existence of compensational mechanisms for physical disability to contribute to enhance cognitive abilities in children with SMA, through brain plasticity mechanisms involving reorganization of motor system structures (174). Studies specifically investigating cognitive abilities in patients with SMA type 1, also using adapted assessments, showed that they have poorer performances than their peers, particularly in the attention and executive function domains (176). Speech and language development is also affected, with published data

showing that functional and intelligible speech is rarely achieved in children untreated for SMA type 1 (177). Considering that the limited interaction with the environment owing to poor expressive communication skills has been shown to further impact on cognitive development in several neurodevelopmental disorders, alternative and augmentative ways to communicate are recommended from early in life.

Considering the recently approved DMTs for 5Q SMA, it is still unclear whether other areas of functioning, including cognitive development and the achievement of effective speech and language abilities, which can significantly impact on independence and quality of life, may equally benefit from treatments. Additionally, the role of bulbar function in the development of articulate speech abilities is also unclear. Whether children with SMA type 1 treated with the new SMN-modulating treatments will recover bulbar function and improve speech, having the possibility to experience and develop their pre-verbal and verbal social and communication skills as typically developing children do, or whether residual structural and/or functional brain pathology will affect achievement of mature language abilities, still need to be clarified. Longitudinal multicentric studies with standardized assessments of cognitive and speech/language abilities are required in children with SMA type 1. The use of adapted methodology to perform these assessments should be considered. Moreover, longitudinal neuroimaging studies performed alongside the clinical assessments would provide additional valuable information on the new emerging clinical phenotypes in this population, especially in patients at the most severe end of the spectrum.

5.5 Research Projects

In 2014 a group of expert Italian researchers and clinicians gathered to build up a national collaboration group for the collection and study of data on SMA patients including 5 Italian sites coordinated by the Gemelli University Hospital Foundation IRCCS, Catholic University of the Sacred Heart), in collaboration with the US and UK network.

In 2021 the group was extended to the Italian network of neuromuscular centres. The network has been involved in the creation of an Italian registry of SMA patients and on exploring trajectories of disease progression: ISMAC-ITASMAC registry (MER-SMA-18-003); Coordinator Centre Ospedale Gemelli Roma (Prof. E. Mercuri); Local Principal Investigator for Children Section Federica Ricci; EC approval: protocol 0053778, 14/05/2021. Data entry is ongoing and first data focused on epidemiology and disease trajectories have been published (see following abstracts).

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ORIGINAL ARTICLE

Type I spinal muscular atrophy patients treated with nusinersen: 4-year follow-up of motor, respiratory and bulbar function

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Biogen

Abstract

Background: We report the 4-year follow-up in type I patients treated with nusinersen and the changes in motor, respiratory and bulbar function in relation to subtype, age and *SMN2* copy number.

Methods: The study included SMA 1 patients with at least one assessment after 12, 24 and 48 months from the first dose of nusinersen. The assessments used were Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) and the Hammersmith Infant Neurological Examination (HINE-II).

Marika Pane and Giorgia Coratti are equal first authors.

Results: Forty-eight patients, with ages ranging from 7 days to 12 years (mean 3.3 years, SD 3.6 years) were included in the study. The CHOP INTEND and HINE-II scores significantly increased between baseline and 48 months ($p < 0.001$). When age at starting treatment subgroups (<210 days, <2 years, 2–4 years, 5–11 years, ≥12 years) were considered, the CHOP INTEND increased significantly in patients younger than 4 years at treatment, while the HINE-2 increased significantly in patients younger than 2 years at treatment. In a mixed-model analysis, age, nutritional and respiratory status were predictive of changes on both scales while *SMN2* copy number and decimal classification were not.

Conclusions: Our results confirm the safety profile previously reported and support the durability of the efficacy of nusinersen at 4 years with an overall stability or mild improvement and no evidence of deterioration over a long period of time.

KEYWORDS
longitudinal study, long-term results, motor function, nusinersen, spinal muscular atrophy

CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

Prevalence of Spinal Muscular Atrophy in the Era of Disease-Modifying Therapies

An Italian Nationwide Survey

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Abstract

Objective

Spinal muscular atrophy (SMA) is a neurodegenerative disorder caused by mutations in the SMN1 gene. The aim of this study was to assess the prevalence of SMA and treatment prescription in Italy.

Methods

An online survey was distributed to 36 centers identified by the Italian government as referral centers for SMA. Data on the number of patients with SMA subdivided according to age, type, *SMN2* copy number, and treatment were collected.

Results

One thousand two hundred fifty-five patients with SMA are currently followed in the Italian centers with an estimated prevalence of 2.12/100,000. Of the 1,255, 284 were type I, 470 type II, 467 type III, and 15 type IV with estimated prevalence of 0.48, 0.79, 0.79 and 0.02/100,000, respectively. Three patients with SMA 0 and 16 presymptomatic patients were also included. Approximately 85% were receiving one of the available treatments. The percentage of treated patients decreased with decreasing severity (SMA I: 95.77%, SMA II: 85.11%, SMA III: 79.01%).

Discussion

The results provide for the first time an estimate of the prevalence of SMA at the national level and the current distribution of patients treated with the available therapeutical options. These data provide a baseline to assess future changes in relation to the evolving therapeutical scenario.

Onasemnogene abeparvovec in spinal muscular atrophy: predictors of efficacy and safety in naïve patients with spinal muscular atrophy and following switch from other therapies

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Summary

Background Efficacy and safety of onasemnogene abeparvovec (OA) for Spinal Muscular Atrophy infants under 7 months and <8.5 kg has been reported in clinical trials. This study examines efficacy and safety predictors in a wide age (22 days–72 months) and weight (3.2–17 kg) range, also including patients previously treated with other drugs.

Methods 46 patients were treated for 12 months between January 2020 and March 2022. Safety profile was also available for another 21 patients with at least 6 month follow-up after OA infusion. 19/67 were treatment naïve when treated with OA. Motor function was measured with the CHOP-INTEND.

Findings CHOP-INTEND changes varied among age groups. Baseline score and age at OA treatment best predicted changes. A mixed model post-hoc analysis showed that in patients treated before the age of 24 months the CHOP-INTEND changes were already significant 3 months after OA while in those treated after the age of 24 months the difference was only significant 12 months after OA. Adverse events occurred in 51/67. The risk for elevated transaminases serum levels was higher in older patients. This was also true for weight and for pre-treatment with nusinersen when analysed individually. A binomial negative regression analysis showed that only age at OA treatment had a significant effect on the risk of elevated transaminases.

Interpretation Our paper describes OA 12-month follow-up showing efficacy across various age and weight groups not targeted by clinical trials. The study identifies prognostic factors for safety and efficacy in treatment selection.

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RESEARCH ARTICLE

Clinical Phenotype of Pediatric and Adult Patients With Spinal Muscular Atrophy With Four *SMN2* Copies: Are They Really All Stable?

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Objective: The aim of this study was to provide an overview of the clinical phenotypes associated with 4 *SMN2* copies.
Methods: Clinical phenotypes were analyzed in all the patients with 4 *SMN2* copies as part of a nationwide effort including all the Italian pediatric and adult reference centers for spinal muscular atrophy (SMA).
Results: The cohort includes 169 patients (102 men and 67 women) with confirmed 4 *SMN2* copies (mean age at last follow-up = 36.9 ± 19 years). Six of the 169 patients were presymptomatic, 8 were classified as type II, 145 as type III (38 type IIIA and 107 type IIIB), and 8 as type IV. The remaining 2 patients were asymptomatic adults identified because of a familial case. The cross-sectional functional data showed a reduction of scores with increasing age. Over 35% of the type III and 25% of the type IV lost ambulation (mean age = 26.8 years ± 16.3 SD). The risk of loss of ambulation was significantly associated with SMA type ($p < 0.0001$), with patients with IIIB and IV less likely to lose ambulation compared to type IIIA. There was an overall gender effect with a smaller number of women and a lower risk for women to lose ambulation. This was significant in the adult ($p = 0.009$) but not in the pediatric cohort ($p = 0.43$).
Interpretation: Our results expand the existing literature on natural history of 4 *SMN2* copies confirming the variability of phenotypes in untreated patients, ranging from type II to type IV and an overall reduction of functional scores with increasing age.

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DUCHENNE MUSCULAR DYSTROPHY (DMD)

6.1 Disease characteristics

DMD (OMIM #310200) is a severe progressive X-linked recessive neuromuscular disorder caused by mutations (deletions, duplications, and point mutations) in the dystrophin gene, resulting in the absence or impaired functioning of the cytoskeletal protein dystrophin. Instability of the muscle membrane leads to cell necrosis and inflammatory response, followed by an exhaustible regeneration and consequent impairment in the strength, stability, and functionality of the myofibers (10,178). The prevalence of DMD has been reported to be 15.9 cases per 100,000 live male births in USA and 19.5 cases per 100,000 live male births in UK (10).

Early non-specific signs can be evident since 2 years of age (motor and cognitive/language development delay, weakness, toe walking, calves hypertrophy, very high serum creatine kinase levels) but the average age at diagnosis is around 4–5 years and can vary among countries, with a significant delay between symptoms onset and genetic diagnosis (179). Typically, after loss of ambulation by a median age of 12 years, the clinical course is characterized by respiratory impairment, cardiomyopathy, nutritional complications, osteoporosis with fractures, scoliosis and joint contractures.

Corticosteroids and proactive medical and rehabilitative interventions help to maintain function, and quality of life and improve longevity, so that the children who are diagnosed today have a life expectancy into their fourth decade, with some patients reaching the fifth decade (180). Despite improvements in the clinical course, early death still usually occurs because of cardiac or respiratory failure.

6.2 Standards of care

Experience is growing with existing pharmacotherapies, such as steroids, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, β-adrenergic blockers, bisphosphonates. In particular, steroids are now part of the recommended standard care for DMD (10,181). An international study, FORDMD (NCT01603407), is ongoing to compare the three most frequently prescribed regimens (i.e., prednisone 0.75 mg/kg/day 10 days on and 10 days off, prednisone 0.75 mg/kg/day, and deflazacort 0.9 mg/kg/day) in terms of efficacy and safety [43,44]. In 2022, the results of a randomized clinical trial, in which we participated, were published. The conclusion drawn from these results was that treatment with daily prednisone or daily deflazacort, compared with intermittent prednisone alternating 10 days on and 10 days off, resulted in significant improvement over 3 years in a composite outcome comprising measures of motor function,

pulmonary function, and satisfaction with treatment; there was no significant difference between the 2 daily corticosteroid regimens. The findings support the use of a daily corticosteroid regimen over the intermittent prednisone regimen. Once defined, the steroid treatment should be tailored to each individual patient based on endocrinological and other clinical characteristics and tolerability (182).

Regarding multidisciplinary care, considering the improved patient survival, a shift to more anticipatory diagnostic and therapeutic strategies has occurred, with a renewed focus on patient quality of life. In 2014, a steering committee of experts from a wide range of disciplines was established to update the 2010 DMD care considerations, with the goal of improving patient care. The new care considerations aim to address the needs of patients with prolonged survival, to provide guidance on advances in assessments and interventions, and to consider the implications of emerging genetic and molecular therapies for DMD. The committee suggested care considerations for diagnosis of DMD, neuromuscular rehabilitation, gastrointestinal (including nutrition and dysphagia), respiratory, cardiac, bone health, and orthopaedic management. Three new topics have been added: endocrine management (growth, puberty, and adrenal insufficiency), primary care, emergency management, and transitions of care across the lifespan (27,181,183).

6.3 Approved and investigational treatments

In August 2014, ataluren, a treatment for nonsense mutation, was granted conditional marketing authorization by EMA for use in the EU, in September 2016 the FDA approved the use of eteplirsen, a genetic treatment based on ASO, and in 2023 the FDA approved the first gene therapy for DMD (delandistrogene moxeparvovec). Other dystrophin restoration therapies are in development, and some are under regulatory review. Other drugs target muscular strength and function (myostatin inhibition, utrophin production or mitochondrial function increasing), inflammation (anti-inflammatory and antioxidant molecules) and fibrosis (184).

Ataluren is an oral drug which can be prescribed in DMD patients with premature stop codon mutations (about 10% of overall Xp21 mutated DMD patients) (185). When a point mutation determines a premature stop codon in pre-mRNA, the translational ribosomal complex stops, resulting in a prematurely truncated and non-functional protein. Ataluren acts by binding to ribosomal RNA subunits, thus allowing stop codons readthrough to take place. Interestingly, some nonsense mutations are associated with a more benign phenotype, more similar to Becker Muscula Dystrophy (BMD), possibly due to a point mutation induced exon skipping mechanism, i.e., exclusion of the nonsense mutation-containing exon (186). According to some models, the exon skipping event could be linked critical disruption of exonic splicing enhancer (ESE) or creation of exonic splicing suppressor (ESS) motifs by point mutation (187). On the basis of the subanalysis of the ACT DMD,

a multi-center, randomized, double-blind, placebo-controlled, phase 3 trial, in August 2014, EMA gave ataluren a conditional approval for the treatment of DMD ambulatory patients aged5 years and older with nonsense mutations. In May 2018, based on positive results of a phase 2 study in DMD children aged 2–5 years (NCT02819557), EMA has approved the medication starting at age 2. On February 2016 FDA filed a Refuse to File letter claiming the results of the phase 2b study, and ACT DMD phase III trial did not demonstrate adequate evidence of effectiveness. A randomized, double-blind, placebo-controlled clinical trial assessing the long-term outcomes of ataluren has been conducted (NCT03179631). In 2015 the company had already started an observational study based on a real-world international drug registry for DMD patients receiving ataluren (STRIDE Registry, NCT 02369731). However, in 2023 EMA's human medicines committee (CHMP) has confirmed the 2023 recommendation to not renew the conditional marketing authorisation for Translarna (ataluren).

Exon skipping technology for DMD is based on synthetic nucleic acid analogs called ASOs. ASOs bind target complementary sequences in the pre-messenger RNA (pre-mRNA) and influence splicing machinery to exclude an exon (or exons) from the final transcript, thus restoring the translational reading frame of a gene, which results in the production of shortened functional proteins similar to the ones associated to clinical BMD phenotype (188). Eteplirsen is a 30-nucleotide phosphorodiamidate morpholino oligomer (PMO) type of ASO which targets an exonic splicing enhancer region of exon 51 in DMD gene when delivered intravenously. In September 2016, eteplirsen obtained conditional approval by the FDA to treat DMD patients amenable to exon 51 skipping (about 13% of DMD patients). Following, golodirsen, able to skip exon 53 (about 10% of DMD patients), and casimersen for exon 45 skipping (about 9% of DMD patients) had been approved as well. In 2018, the EMA Committee for Medicinal Products for Human Use (CHMP) found the available evidence insufficient for efficacy and repeatedly gave negative opinion about approval. A number of other drug candidates based on proprietary RNA-based technologynd phosphorodiamidate morpholino oligomer chemistry are currently under development (189).

Delandistrogene moxeparvovec (SRP-9001) is an investigational rAAV vector-based gene therapy, designed to compensate for missing functional dystrophin in Duchenne muscular dystrophy (DMD) by delivering a transgene encoding SRP-9001 dystrophin, an engineered dystrophin protein that retains key functional domains of the wild-type protein. Different clinical trials (Study 101 [SRP-9001-101; NCT03375164], Study 102 [SRP-9001-102; NCT03769116], and ENDEAVOR [Study 103; NCT04626674]; data not published) supported the 2023 FDA authorization to commerce (190).

6.4 Central nervous system functioning characteristics in DMD

The DMD gene contains 79 exons, 78 introns, and at least seven independent, tissue-specific promoters, producing different isoforms, including Dp427p, Dp427c, Dp427m, Dp260, Dp140, Dp116, Dp71, and Dp40 (191). The full-length muscular isoform (Dp427m) is a rod-shaped cytoplasmic protein connecting the dystrophin-glycoprotein complex to the intracellular contraction apparatus and extracellular matrix of cell and serving to stabilize the sarcolemma by transferring forces generated from sarcomere contraction. Dp260 is highly expressed in the retina, and Dpl16 expression occurs in mature peripheral neural cells (Schwann cells). Dp427c, Dp140, and Dp71 are the primary DMD gene products expressed in the brain. Dp427c is highly expressed in neurons in the cerebral cortex and hippocampus and is involved in transmembrane transport and signal transmission (54,192). Dp140, mostly distributed in the cerebral cortex and hippocampus, regulates neuronal differentiation, projection morphogenesis, and chromatin modification (193). Dp71, which is widely distributed in the brain, plays a crucial role in neuronal differentiation, adhesion, cell division, excitatory synaptic organization, and scaffold proteins that stabilize the nuclear envelope (194).

Affected boys generally manifest motor symptoms and enlarged calves in the first few years of life. In recent years, research has shown that boys with DMD show vulnerability for cognitive deficits, such as intellectual disabilities, verbal working memory deficits, learning difficulties, and reading dysfunction (195–198). Growing evidence from earlier studies supports associations between the loss of dystrophin isoforms and intellectual disability (199).

Recently, standardized multidisciplinary comprehensive management of DMD, together with DMTs, have slowed down the progression of the disease and helped extend patients' lifespans. Although glucocorticoids are the first choice for standardized treatment of children with DMD, there is still a lack of studies on the effects of glucocorticoids on cognition.

6.5 Research Projects

The Italian network, in collaboration with some foreign institutions, has published over the years short- and long-term follow-up data on the specific functional aspects of patients with DMD. The network has been involved in the design of the upper extremity module to record functional changes in DMD patients, in the validation of several instruments, such as the North Star Ambulatory Assessment, and on exploring trajectories of disease progression also on the respiratory aspects of DMD.

Collaborators: Catholic University, Rome; Gemelli University Hospital Foundation, Rome: University of Messina; Ospedale Bambino Gesù IRCCS, Rome; Istituto Mondino IRCCS, Pavia; Gaslini Institute, Genoa; Besta Institute, Milan; Stella Maris Institute, Pisa; Ospedale Maggiore,

Bologna; University of Napoli, Napoli; University of Turin; Turin; University of Padua, Padua; University of Milano, Padova; San Raffaele IRCCS, Milan.

At the moment our centre is involved in the following projects:

Project Title: Telethon Project GUP21003H “Characterizing Phenothypes in non-ambulant Duchenne Muscular Dystrophy”; Coordinator Centre: NEMO Clinical Centre, Rome (Dr. M. Pane); Local Principal Investigator: Federica Ricci. Last update: Submission to the local ethics committee in progress.

Project Title: Telethon Project GUP21006E “Characterization of the phenotypic diversity in DupEx2 Duchenne Muscular Dystrophy and identification of predictive/prognostic markers”; Coordinator Centre: San Raffaele Hospital, Milan (Dr. S. Previtali); Local Principal Investigator: Federica Ricci. Last update: approved from central and local ethics committees (Del. 0001010, 29/06/2022). Data collection is ongoing.

From 2021 to

The most recent publications from the network follow:

Upper Limb Changes in DMD Patients Amenable to Skipping Exons 44, 45, 51 and 53: A 24-Month Study. Brogna C, Pane M, Coratti G, D'Amico A, Pegoraro E, Bello L, Sansone VAM, Albamonte E, Messina S, Pini A, D'Angelo MG, Bruno C, Mongini T, Ricci FS, Berardinelli A, Battini R, Masson R, Bertini ES, Politano L, Mercuri E, Italian Dmd Group. Children (Basel). 2023 Apr 19;10(4):746. doi: 10.3390/children10040746.

Longitudinal Analysis of PUL 2.0 Domains in Ambulant and Non-Ambulant Duchenne Muscular Dystrophy Patients: How do they Change in Relation to Functional Ability? Pane M, Coratti G, Brogna C, Bovis F, D'Amico A, Pegoraro E, Bello L, Sansone V, Albamonte E, Ferraroli E, Mazzone ES, Fanelli L, Messina S, Catteruccia M, Cicala G, Ricci M, Frosini S, De Luca G, Rolle E, De Sanctis R, Forcina N, Norcia G, Passamano L, Gardani A, Pini A, Monaco G, D'Angelo MG, Capasso A, Leone D, Zanin R, Vita GL, Panicucci C, Bruno C, Mongini T, Ricci F, Berardinelli A, Battini R, Masson R, Baranello G, Dosi C, Bertini E, Politano L, Mercuri E. J Neuromuscul Dis. 2023 Apr 12. doi: 10.3233/JND-221556.

Age, corticosteroid treatment and site of mutations affect motor functional changes in young boys with Duchenne Muscular Dystrophy. Giorgia Coratti, Jacopo Lenkowicz, Giulia Norcia, Simona Lucibello, Elisabetta Ferraroli, Adele d'Amico, Luca Bello, Elena Pegoraro, Sonia Messina, Federica Ricci, Tiziana Mongini, Angela Berardinelli, Riccardo Masson, Stefano C Previtali, Grazia D'angelo, Francesca Magri, Giacomo P Comi, Luisa Politano, Luigia Passamano, Gianluca Vita, Valeria A Sansone, Emilio Albamonte, Chiara Panicucci,

Claudio Bruno, Antonella Pini, Enrico Bertini, Stefano Patarnello, Marika Pane, Eugenio Mercuri, italian DMD study group. 2022 Jul 29;17(7):e0271681. doi: 10.1371/journal.pone.0271681.

Health related quality of life in young, steroid-naïve boys with Duchenne muscular dystrophy. Neuromuscul Disord . 2021 Jun 10;S0960-8966(21)00155-3. doi: 10.1016/j.nmd.2021.06.001. Online ahead of print. Craig Campbell a, Elaine McColl, Michael P. McDermott, William B. Martens, Michela Guglieri, Robert C. Griggs, The Muscle Study Group, and TREAT-NMD (Volker Straub, Anne-Marie Childs, Emma Ciafaloni, Perry B Shieh, Stefan Spinty, Russell J Butterfield, Iain Horrocks, Helen Roper, Lorenzo Maggi, Giovanni Baranello, Kevin M Flanigan, Nancy L Kuntz, Adnan Y Manzur, Basil T Darras, Peter Kang, Jean K Mah, Tiziana Mongini, Federica Ricci, Leslie Morrison, Monika Krzesniak-Swinarska, Maja von der Hagen, Richard S Finkel, Ashutosh Kumar, Matthew Wicklund, Craig M McDonald, Erik K Henricson, Ulrike Schara-Schmidt, Ekkehard Wilichowski, Richard J Barohn, Jeffrey Statland, Janbernd Kirschner, Giuseppe Vita, Gian Luca Vita, James F Howard Jr, Imelda Hughes, Hugh J McMillan, Elena Pegoraro, Luca Bello, W Bryan Burnette, Mathula Thangarajh, Taeun Chang.

North Star Ambulatory Assessment changes in ambulant Duchenne boys amenable to skip exons 44, 45, 51, and 53: A 3 year follow up. PLoS One. 2021 Jun 25;16(6):e0253882. doi: 10.1371/journal.pone.0253882. eCollection 2021. Giorgia Coratti, Marika Pane, Claudia Brogna, Valeria Ricotti, Sonia Messina, Adele D'Amico, Claudio Bruno, Gianluca Vita, Angela Berardinelli, Elena Mazzone, Francesca Magri, Federica Ricci, Tiziana Mongini, Roberta Battini, Luca Bello, Elena Pegoraro, Giovanni Baranello, Stefano C Previtali, Luisa Politano, Giacomo P Comi, Valeria A Sansone, Alice Donati, Jean Yves Hogrel, Volker Straub, Silvana De Lucia, Erik Niks, Laurent Servais, Imelda De Groot, Mary Chesshyre, Enrico Bertini, Nathalie Goemans, Francesco Muntoni, Eugenio Mercuri, on behalf on the International DMD Group and the iMDEX Consortium.

The nonsense mutation stop+4 model correlates with motor changes in Duchenne muscular dystrophy. Neuromuscul Disord . 2021 Jun;31(6):479-488. doi: 10.1016/j.nmd.2021.02.015. Epub 2021 Feb 21. Claudia Brogna, Giorgia Coratti, Rachele Rossi, Marcella Neri, Sonia Messina, Adele D' Amico, Claudio Bruno, Simona Lucibello, Gianluca Vita, Angela Berardinelli, Francesca Magri, Federica Ricci, Marina Pedemonte, Tiziana Mongini, Roberta Battini, Luca Bello, Elena Pegoraro, Giovanni Baranello, Luisa Politano, Giacomo P Comi, Valeria A Sansone, Emilio Albamonte, Alice Donati, Enrico

Bertini, Nathalie Goemans, Stefano Previtali, Francesca Bovis, Marika Pane, Alessandra Ferlini, Eugenio Mercuri, on behalf on the International DMD group.

Long-term natural history data in Duchenne muscular dystrophy ambulant patients with mutations amenable to skip exons 44, 45, 51 and 53. PLoS One. 2019 Jun 25;14(6):e0218683. Brogna C, Coratti G, Pane M, Ricotti V, Messina S, D'Amico A, Bruno C, Vita G, Berardinelli A, Mazzone E, Magri F, Ricci F, Mongini T, Battini R, Bello L, Pegoraro E, Baranello G, Previtali SC, Politano L, Comi GP, Sansone VA, Donati A, Bertini E, Muntoni F, Goemans N, Mercuri E; on behalf on the International DMD group.

CONCLUSIONS

Neurodevelopmental and mental health disorders often coexist in children, adolescents, and adults with chronic conditions, such as NMDs. Additionally, certain NMDs exhibit specific comorbidities with neurodevelopmental or other psychiatric disorders, which are also considered part of the clinical phenotype. Nonetheless, brain pathology, cognition, speech/language development, and psychological functioning remain underexplored aspects in NMDs. Typically, available data are constrained to parent-reported information or informal assessments.

In 2021 we published a little case series on cognitive evaluation in different NMDs and provided evidence of the feasibility and the importance of a regular and early assessment of cognitive functions in these patients, to enable interventions aimed at preserving functioning and quality of life. Moreover, a specific focus on cognitive deficits in these disorders could help to reduce the global pathology-related family burden (200).

Following our initial focus on DM1, which began with a monocentric study, we subsequently participated in a multicentric international study. In the limited case series conducted within the monocentric setting, a discernible pattern of neurodevelopmental disorder emerged. Our data indicated that a comprehensive assessment protocol should encompass cognitive, neuropsychological, emotional, and behavioral evaluations, ideally administered periodically to pediatric DM1 patients during their developmental stages. However, considering the substantial time investment required for repeated testing, which would add to the multidisciplinary assessments already necessary for standard care, we concluded that larger longitudinal studies are essential for a more thorough evaluation of trajectories over time and to inform clinical practice. Additionally, we proposed that cognitive, behavioral, and selected neuropsychological tests could serve as outcome measures in upcoming therapeutic clinical trials for this multisystemic genetic disorder. In the multicenter study, the emphasis was placed on motor outcome measures and adaptive behavior. The latter was assessed using questionnaires reported by parents. In the conclusions, the importance of evaluating various functional and behavioral domains specific to myotonic dystrophy was highlighted. These assessments could likely be collected either directly by patients or through their caregivers using PROMs. However, the reliability of PROMs completed by caregivers who are also affected by the same disease remains a topic of ongoing debate.

For many other NMDs (e.g. GSD2, DMD, and SMA), further studies with larger samples are needed to characterize trajectories of cognitive, neuropsychological, and adaptive functioning, with a special attention to new phenotypes that are emerging with the availability of innovative therapies. We are actively promoting and participating in research projects centered around this topic.

A better understanding of the characteristics and extent of brain involvement in is crucial for a deeper comprehension of the clinical features of the disease and for personalized patient management.

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