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## Beyond muscle

A multidisciplinary approach to the involvement of the  
Central Nervous System in neuromuscular diseases

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## DECLARATION

I hereby declare that the present thesis is related to the scientific works authored by the PhD candidate and colleagues. Most of the studies have led to scientific articles and will be presented along with the results of ongoing research. The contributions have been adapted for the purposes of this thesis presentation. Still, they will maintain the structure of a scientific manuscript, with sections describing background and rationale, methods, results, and discussion.

All the projects presented in this thesis were carried out at the Pediatric Neuromuscular Diseases Center referred to the Pediatric Neuroscience Unit of the IRCCS Mondino Foundation in Pavia (Italy), a tertiary referral center for neurologic conditions. The Pediatric Neuromuscular Diseases Center is devoted to diagnosing, treating, following up, and rehabilitating children and adolescents with neuromuscular diseases.

Most of the projects were carried out in the context of the department outpatient service of child neuropsychiatry of the IRCCS Mondino Foundation. Some projects were part of a collaboration between our Center and the Italian Pediatric Technological Rehabilitation Network. The FACE-DMD project was conducted as a multicentric study, in collaboration with the IRCCS “Stella Maris” Foundation (Pisa, Italy), the IRCCS “E. Medea” (Bosisio Parini, Italy), the University of Udine (Italy), and the University Hospital “Città della Salute e della Scienza” affiliated to the University of Turin (Italy). The Virtual Park project was conceptualized and declined into a clinical protocol in collaboration with CNR – Stiima (Lecco, Italy) and the IRCCS “E. Medea” (Bosisio Parini, Italy).

## Acronyms and abbreviations

- 6MWT: 6-minute-walk test  
ADHD: Attention Deficit and Hyperactivity Disorder  
AR: augmented reality  
ASD: Autism Spectrum Disorder  
ASL: arterial spin labeling  
BMD: Becker Muscular Dystrophy  
CBCL: Child Behavior Checklist  
CMD: Congenital Muscular Dystrophies  
CNS: Central Nervous System  
DMD: Duchenne Muscular Dystrophy  
DTI: diffusion-tensor imaging  
DWI: diffusion weighted imaging  
EACD: European Academy of Children Disability  
EC: ethics committee  
ENMC: European NeuroMuscular Center  
ER: emotion recognition  
fMRI: functional magnetic resonance imaging  
HC: healthy control  
ICF-CY: International Classification of Functioning, Disability and Health - Children&Youth Version  
ICTs: Internet and Communication Technologies  
IQ: intelligence quotient  
MDs: muscular dystrophies  
MRI: Magnetic Resonance Imaging  
NHMRC: National Health and Medical Research Council  
NMDs: Neuromuscular disorders  
NPS: neuropsychological  
NSAA: North-Star Ambulatory Assessment  
OCD: obsessive-compulsive disorder  
PET: positron emission tomography  
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses  
PUL: Performance of Upper Limb  
RCT: randomized-controlled trialsLD: specific learning disorder  
SC: Social Cognition  
SMA: Spinal Muscular Atrophy  
TCMS:Trunk Control Measurement Scale  
TFT: timed functional tests  
ToM: theory of mind  
VABS: Vineland Adaptive Behavior Scale  
VR: virtual reality  
WISC/WPPSI/WAIS: Wechsler Intelligence Scale for Children/Preschool and Primary Scale of Intelligence/Adult Intelligence Scale  
WP: work package

## Abstract

This PhD project focused on the neuropsychological profile of pediatric patients with Duchenne Muscular Dystrophy and the implementation of technological devices in their rehabilitation. In this disease it is known the relationship between dystrophin deficiency and neurodevelopmental disorders and neuropsychological impairments, which are frequent but often overshadowed by the more apparent motor disabilities. With the advent of novel treatments there is an even more urgent need to address the cognitive and neuropsychological manifestations of the disease. The primary goal of the project was to address some of the unmet needs in DMD by further describing the CNS-related phenotype (particularly exploring unexplored domains like social cognition), contributing to the development of a standardized toolkit for neuropsychological assessment, and investigating the potential of integrating technology into rehabilitation.

The introductory Chapter 1 summarizes the main evidence from the current literature about the CNS-related phenotype of DMD, some of the currently unmet needs, and the possible role of technologies applied to rehabilitation of this pathology.

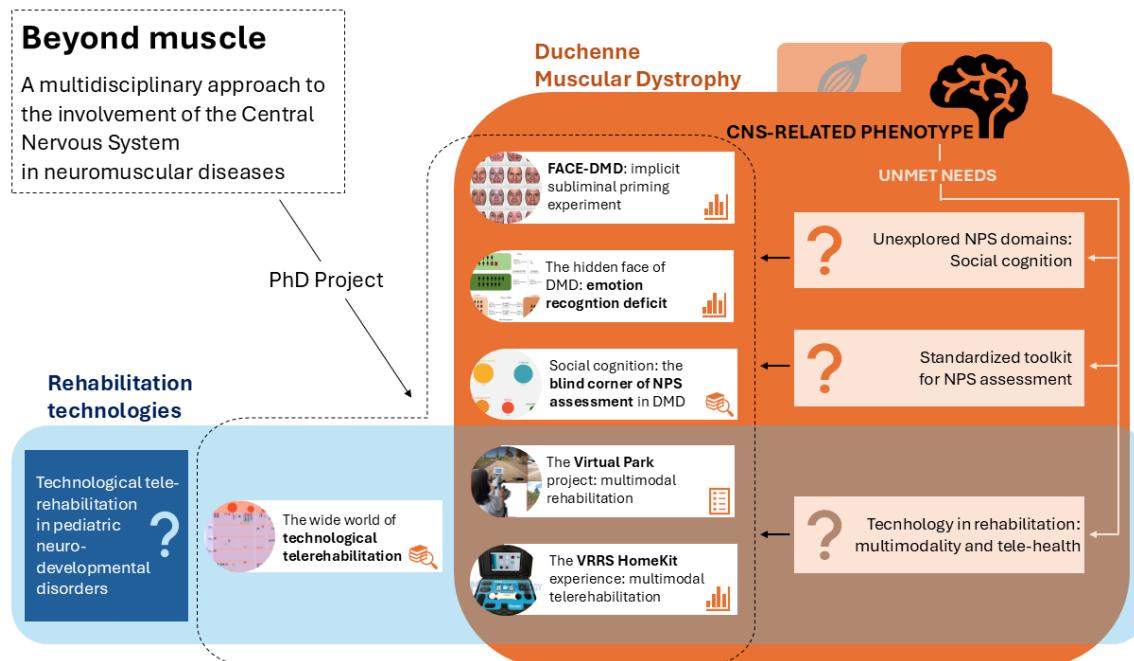
Chapter 2 focused on the clinical assessment of social cognition, one of the less explored domains of the neuropsychological phenotype of DMD patients, even if potentially linked to the expression profile of the isoforms of dystrophin. The chapter includes a scoping review that provides an overview of the standardized tools previously adopted and described in the literature for the neuropsychological assessment of DMD patients, the application of an innovative clinical protocol including the assessment of social cognition, and a specific experimental setting based on implicit cognition and subliminal priming developed to further explore the social cognition deficit and its possible neural substrate.

Chapter 3 is dedicated to the challenges of implementing technologies in the rehabilitation of pediatric neuropsychiatric disorders, in general, and neuromuscular disorders, in particular. It includes a comprehensive overview of the multifaceted landscape of technologies applied to the rehabilitation of pediatric neurological and neurodevelopmental diseases, in particular their implementation to telerehabilitation. Furthermore, starting from this background, the chapter address the issue of the innovative multi-modal technological interventions with the report of a case series and a clinical protocol, both based on technological devices applied to the rehabilitation of DMD patients.

This doctoral project contributed to the study of the wide spectrum of neuropsychological manifestations of DMD. Our results provide preliminary evidence of a possible involvement of

social cognition skills, in particular in emotion recognition. Defining the neuropsychological profile of DMD patients in detail is critical for improving their rehabilitative care and providing tailored support for their academic and daily life goals. Furthermore, some of the studies included in this project explored another area with great potential for enhancing care in DMD: the integration of technological devices and telerehabilitation. The adoption of advanced technologies and multidomain interventions have the potential to revolutionize the neurodevelopmental rehabilitation by creating immersive, adaptive, and engaging therapeutic environments. By testing the potential of these technologies, this project represented a pilot-experience of more effective interventions that are tailored to the unique challenges faced by DMD patients, enhancing their overall quality of life and long-term outcomes.

## Graphical abstract



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## 1. Introduction

Neuromuscular diseases (NMDs) encompass a broad spectrum of pathologies that primarily affect the motor unit (motor neurons, motor endplate, skeletal muscle) and manifest at various ages. Most of the forms with a pediatric onset are caused by a genetically based process. Several genes and pathways are involved, but their pathological modifications generally lead to altered muscular function (various muscular atrophy and weakness patterns, up to the complete loss of functionality) and subsequent sequelae (joint contractures, skeletal deformities, osteoporosis, and respiratory failure). Cardiac involvement is a part of the clinical picture in many of these diseases. These chronic manifestations are associated with lifelong disability and reduced life expectancy<sup>1-5</sup>.

Even if all NMDs share a primary affection for the motor unit, they differ in the timing of the onset, comorbidities, pattern of manifestations, and therapeutic options. Molecular diagnostic techniques brought further phenotypic variability, allowing the identification of altered genes in previously idiopathic conditions and broadening the clinical spectrum of NMDs. These findings and the knowledge about the protein expression also raised attention on the effects of the causative genetic mutations of NMDs on other structures than the motor unit. The possible involvement of the Central Nervous System (CNS) is a challenging issue for the clinical practice in child neuropsychiatry. This kind of involvement is traditionally considered part of the clinical picture of Mitochondrial Diseases<sup>6</sup> Congenital Muscular Dystrophies (CMDs) (e.g., dystroglycanopathies, LAMA2 CMD)<sup>7</sup>, Myotonic Dystrophy Type 1<sup>8</sup>, and Duchenne Muscular Dystrophy (DMD)<sup>9</sup>. In these diseases, it is known that the proteins disrupted by pathogenic mutations are also expressed in the CNS, leading to malformations or neuropsychological manifestations. Still, some new issues arose recently.

Firstly, the effect of these altered genes on CNS needs to be characterized in detail, identifying the involved structures/impaired functions and the course of the manifestations (stable v.s. progressive). Moreover, in the pediatric-onset forms, it is fundamental to assess the possible effect on neurodevelopment. For example, neuropsychological impairment and increased risk of neurodevelopmental disease are consistently reported in Duchenne Muscular Dystrophy (DMD), the most frequent pediatric NMD characterized by mutations in the dystrophin gene. In DMD, the differential isoform expression of this protein (both along neurodevelopment and across the different structures) provides clues about the nature of CNS involvement<sup>10</sup>. A few studies based on genetics, neuropsychological assessments, and neuroimaging techniques are available, but some issues are still open<sup>11,12</sup>.

Secondly, the availability of new therapies<sup>13</sup> that effectively prevent premature death and disability unveiled new disease phenotypes. It also opened the possibility of assessing aspects that were previously overshadowed by the muscular impairment. The example of Spinal Muscular Atrophy is clarifying: the availability of specific therapy (i.e., Nusinersen) prevented the early mortality of the most severe forms and made it possible to observe the development of these children. The question of how the altered ubiquitous SMN1 protein can interfere with neurodevelopment remains unanswered. Currently, with the advent of new specific therapies (e.g. gene therapy) for DMD, it is plausible that a similar scenario would emerge also for this disease, reducing the motor disability and thus “clearing space” to the more subtle manifestations of the disease such as the neuropsychological and neurodevelopmental symptoms.

Thus, based on the current literature, some crucial aspects of the nature of CNS involvement still need to be cleared, even in the most common pediatric NMDs whose neuropsychological-neurodevelopmental profile is known. The perspective of the ever-wider availability of new effective therapies for these diseases raises a two-fold issue. Firstly, as previously exposed, the increased survival and the reduced disability give clinicians the space to assess and study some unknown aspects of NMD. On the other hand, patients, families, and associations now face a longer life expectancy with a lower burden of motor disability than before. The importance of completing an appropriate school curriculum is now stressed to get work and personal independence. Thus, it is crucial to give precise information about the neuropsychological outcome.

This PhD project focused on the neuropsychological profile of pediatric patients with Duchenne Muscular Dystrophy and the implementation of technological devices in their rehabilitation. Duchenne muscular dystrophy (DMD) is an X-linked progressive muscular disorder occurring in a frequency of about 1:3500–9300 newborn boys (Orpha: 98896; Orpha: 98895). Even if rare, DMD is the most common neuromuscular disorder with a pediatric onset. Mutations in the DMD gene on the X chromosome, encoding for dystrophin, cause the disease. Its phenotype is characterized by progressive muscle degeneration with loss of muscular strength, leading to severe mobility limitations up to the loss of walking. Progressive motor impairment is common in all patients. However, clinical trajectories may differ in the timing of progression of motor impairment. Furthermore, these patients' neuropsychological, psychiatric, and neurodevelopmental characteristics add further phenotypic variability (see Section 1.1). The choice of this population was driven by the known relationship between dystrophin deficiency and neurodevelopmental disorders and neuropsychological impairments, which are often

overshadowed by the more apparent motor disabilities. With the advent of novel treatments, such as gene therapy, which may significantly reduce motor impairments, there is an even more urgent need to address the cognitive and neuropsychological manifestations of the disease. The project was conducted within the Pediatric Neuroscience Center of the IRCCS Mondino Foundation, ideal for such research due to the presence of the specialized *Center for neuromuscular diseases in childhood and adolescence*. The center's holistic approach — rooted in the academic tradition of Child and Adolescent Neuropsychiatry—integrates neurobiological perspective with the concept of somatic-psychic unity, ensuring comprehensive care for neurodevelopmental disorders.

The primary goal of the project was to address some of the unmet needs in DMD (see Section 1.2) by further describing the CNS-related phenotype, particularly exploring unexplored domains like social cognition, contributing to the development of a standardized toolkit for neuropsychological assessment, and investigating the potential of integrating technology into rehabilitation. The feasibility of this project is mainly based on the access to the large clinical database of the Foundation, an active cohort of DMD patients referring to the center, the intellectual environment of an Institute devoted to translational research, and its participation in national and international research networks focused on neuromuscular disorders and rehabilitation.

## 1.1.CNS-related phenotype of DMD

### 1.1.1. *Profile of expression of dystrophin isoforms*

In DMD, the CNS involvement in this pathology is related to the molecular features of the DMD gene. It includes at least seven promoters and codifies for at least eight isoforms (named Dp427p,c,m, Dp260, Dp140, Dp116, Dp71 e Dp40 based on their relative size), with differential tissue expression. The pathogenic role of the isoforms is better understood considering three factors: the ways they are affected by the mutations in the DMD gene, their tissue distribution, and the neuro-developmental regulation of their expression.

The main tissues where dystrophin is expressed are muscle (Dp427m), the CNS (Dp427p,c, Dp140, Dp71, Dp40), retina (Dp260), the Schwann cells of the peripheral nerve (Dp116), and Kidney (Dp140).

Name	Acronym	Protein length	Amino acids	mRNA	Promoter located in	Expression	site
<b>Dp427c</b>	brain or C-dystrophin	427 kDa	3677	14,069 bp	5' Dp427c	brain	cortical dystrophin
<b>Dp427m</b>	M-dystrophin	427 kDa	3685	13,993 bp	5' Dp427m	muscle	muscle dystrophin
<b>Dp427p</b>	P-dystrophin	427 kDa	3681	14 kb	5' Dp427p	Purkinje cells	Purkinje dystrophin
<b>Dp260</b>	R-dystrophin	260 kDa			intron 29	retina	retinal dystrophin
<b>Dp260</b>	R-1		2344	9773 bp	intron 29	retina	Dp260-1
<b>Dp260</b>	R-2		2341	9916 bp	intron 29	retina	Dp260-2
<b>Dp140</b>		140 kDa	1225	7410 bp	intron 44	central nervous system and kidney	Dp140
<b>Dp140</b>			1243	7378 bp	intron 44	kidney	Dp140b
<b>Dp140</b>			1,23	7339 bp	intron 44	cerebellum and kidney	Dp140ab
<b>Dp140</b>			1115	7050 bp	intron 44	cerebellum	Dp140c
<b>Dp140</b>			1133	7048 bp	intron 44	cerebellum and kidney	Dp140bc
<b>Dp116</b>	S-dystrophin	116 kDa	956	5623 bp	intron 55	Schwann cells	apo-dystrophin 2
<b>Dp71</b>	liver or G-dystrophin	71 kDa (70.4)	617	4623 bp	intron 62	ubiquitous	apo-dystrophin 1
<b>Dp71</b>		72.2 kDa	635	4591 bp	intron 62	ubiquitous	Dp71b
<b>Dp71</b>		68.9 kDa	604	4584 bp	intron 62	ubiquitous	Dp71a
<b>Dp71</b>		70.8 kDa	622	4552 bp	intron 62	ubiquitous	Dp71ab
<b>Dp40</b>		40 kDa	340	2.2 kb	intron 62	ubiquitous	apo-dystrophin 3

**Figure 1- Organization of dystrophin locus and localization of the isoforms.**

(adapted from: Hendriksen JGM, Thangaraj M, Kan HE, Muntoni F; ENMC 249th workshop study group. 249th ENMC International Workshop: The role of brain dystrophin in muscular dystrophy: Implications for clinical care and translational research, Hoofddorp, The Netherlands, November 29th-December 1st 2019. Neuromuscul Disord. 2020 Sep;30(9):782-794. doi: 10.1016/j.nmd.2020.08.357. Epub 2020 Aug 15. PMID: 32912717)

The causative mutations of DMD – mainly deletions (65%), duplications (10%), and small mutations (25%)<sup>14</sup> variously affect the expression of the dystrophin isoforms based on the level they occur. Specifically, proximal mutations (within exon 1-43) only alter the correct transcription of Dp427p,c,m, and Dp260, while distal mutations may also influence the expression of the shorter isoforms (Dp140 or Dp71/Dp40). Some “mutational hotspots” have been described for deletions (exon 45-55) and duplications (exon 2-22). This aspect, combined with the incidence of the main types of mutations, leads to split patients into two subgroups of similar size, considering the isoforms expressed in CNS: a group in which only the expression of Dp427 is compromised (Dp140+), and another in which the mutation affect the expression of both Dp427 and Dp140 (Dp140-) – mutations interfering also with Dp 71/Dp40 are rare. The clinical impact of such a distinction on the neuropsychological outcome is discussed below (see section 1.1.2).

The detailed characterization of the localization and function of dystrophin has been provided by research in both animal models and humans. Conversely, only a few reports studying CNS isoforms in humans are available, and most of our knowledge about it derives from animal models and cell culture studies<sup>15</sup>. Recently, Doorenweerd and colleagues published a study based on transcriptomic data from adult and developing human brain datasets (Allen Human Brain and BrainSpan atlases), which analyzed the expression patterns of dystrophin isoforms across the human brain and along the neurodevelopment<sup>10</sup>. The results of this study suggested an intriguing change of perspective about what was traditionally considered to be the CNS localization of dystrophin (i.e. cortex, hippocampus, amygdala, and cerebellum). Precisely, the average expression of the protein was calculated for 22 anatomical brain regions and compared relative to the same value referred to the whole brain. Such an analysis provided a map of dystrophin expression in the human brain, characterized by low expression in the pons and cerebellum, and relatively high expression in the amygdala and hippocampus. The cerebral cortex appeared to have an average degree of protein expression, with the temporal and frontal cortex presenting higher values than the parietal and occipital cortex. Such a finding may lead to rethinking the research approach to the neuropsychological phenotype and applying an interpretative model that moves the focus from the cerebellar pathways, as suggested in previous studies<sup>16</sup> to the role of these structures.

The same study also provided a neurodevelopmental perspective. A subset of the atlas containing information about brains from donors of different ages (range: eight weeks post-conception to 40 years) was studied to determine how the expression of the isoforms varies across the life stages. Interestingly, a clear differential pattern emerged: short isoforms show high (Dp140) or intermediate (Dp71) representation in the early stages of life, while the expression of the full-length protein is always relatively low in CNS. Furthermore, the function of Dp140/Dp71 across the neurodevelopment was studied, analyzing their co-expression with other genes contained in the database. The results suggested that Dp427 is involved in synaptic transmission, coherently with preclinical studies<sup>79</sup> which demonstrated the function of this isoform in anchoring GABA A receptors to the postsynaptic membrane. Moreover, a role in the early development of neural tissue (axon guidance and neuron differentiation), and cerebral vascularization was hypothesized for Dp140/Dp71.

Finally, it is worth mentioning that a few cases of DMD distal mutations related to cognitive disability without a dystrophic phenotype were reported<sup>17,18</sup>.

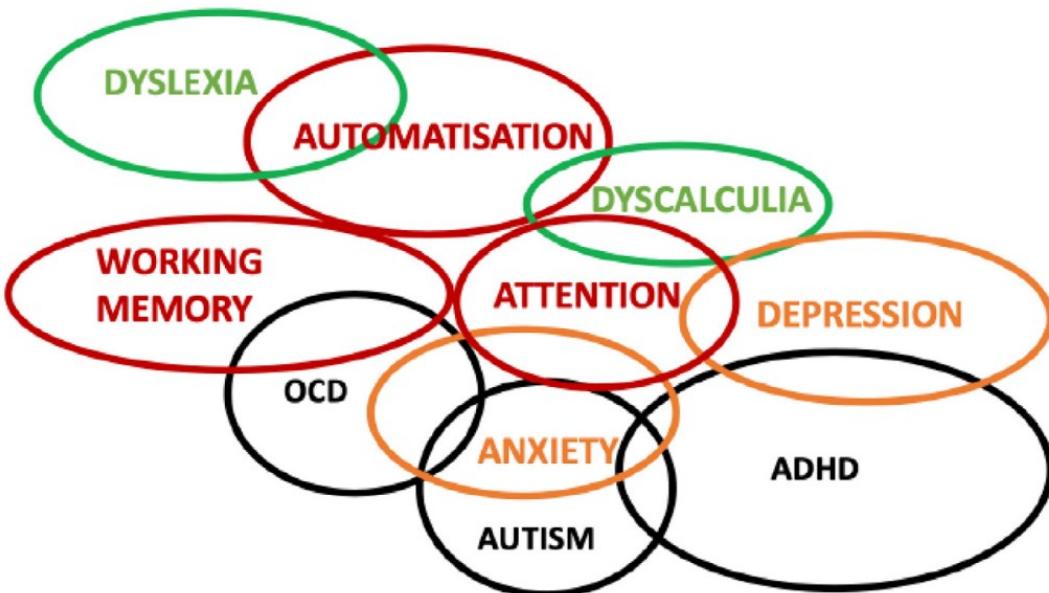
### 1.1.2. Neuropsychological, neurodevelopmental, and neuropsychiatric profile

The neuropsychological profile of DMD and the neurodevelopmental comorbidities have been detailed over the last decades. The presence of cognitive impairment<sup>19–23</sup>, with a lower mean IQ (about -1 SD shift) compared to the general population, and language difficulties have been consistently reported.

Further evidence has provided a precise characterization of the neuropsychological difficulties: deficits in executive functions were reported, such as multitasking, problem-solving, inhibition, and working memory<sup>23</sup>. Some studies examined the hypothesis of the involvement of a cerebellar network, based on the neurobiological findings from the animal models and some clinical evidence (i.e. the description of implicit learning deficit).

These problems can be detected early in life and they tend to be stable over time<sup>23,24</sup>.

Furthermore, the incidence of other neurodevelopmental disorders is higher in DMD patients than in the general population. Data from a recent meta-analysis<sup>9</sup> confirmed the previous data about the diagnostic features and the prevalence of such comorbidities: ASD 3– 15%, ADHD 11–32%. A higher risk of presenting other neuropsychiatric disorders (i.e. obsessive-compulsive disorder, anxiety, and epilepsy) was reported too. A comprehensive term (“big ten of Duchenne”) was proposed by Hendriksen to encompass the critical issues in this field reference (see Figure 2).



**Figure 2 - The “big ten of Duchenne”:** the ten areas of interest of the CNS involvement in DMD and their pattern of comorbidity are schematized in the figure.

(source: Hendriksen JGM, Thangaraj M, Kan HE, Muntoni F; ENMC 249th workshop study group. 249th ENMC International Workshop: The role of brain dystrophin in muscular dystrophy: Implications for clinical care and translational research, Hoofddorp, The Netherlands, November 29th-December 1st 2019. *Neuromuscul Disord*. 2020 Sep;30(9):782-794. doi: 10.1016/j.nmd.2020.08.357. Epub 2020 Aug 15. PMID: 32912717)

As outlined in the previous section, the relationship between the expression of dystrophin isoforms in CNS and the neuropsychiatric outcome has been investigated. Namely, this field of research explored the hypothesis of a detrimental effect of the mutations affecting both the full-length (Dp427) and the shorter isoforms of dystrophin (mainly Dp140), given the higher expression of the latter in CNS. A greater risk of manifesting neuropsychological and neurodevelopmental disorders was then described for DMD patients with distal mutations<sup>25-29</sup>.

It is worth highlighting that the last guidelines for the diagnosis and management of DMD include a “Psychosocial care” section<sup>30</sup>, which emphasizes the impact of CNS involvement in patients’ lives. Therefore, the guidelines suggest addressing these issues with the inclusion into the standard of care of regular diagnostic and follow-up assessments of neuropsychological functions, mental health, and quality of life. The need for an early and robust diagnosis of brain-related comorbidities is raised by the associations representing patients and families, aiming to provide proper care and support even to the more specific and subtle difficulties.

Nonetheless, the variety of diagnostic tools and the absence of a specific test battery make it challenging to implement the suggested psychosocial assessment into clinical practice.

### 1.1.3. Neuroimaging

The anatomical and functional substrate of CNS involvement in DMD has been investigated through the years adopting various neuroimaging techniques.

The first report<sup>31</sup> included a cohort of 30 DMD patients and it was based on CT scans. A significant percentage of the subjects showed signs of cerebral atrophy, cortical atrophy, and ventricular dilation. Consistently, similar anomalies were described in a smaller group of patients assessed by MRI<sup>32</sup> and later confirmed by quantitative MRI study<sup>33</sup> which provided evidence of lower total brain and gray matter volume in a larger group of DMD patients compared to age-matched healthy controls. In the same study, the two groups were compared based on DTI parameters to explore the white matter involvement: DMD patients presented lower fractional anisotropy and higher mean and radial diffusivity than healthy controls.

Interestingly, the subset of patients with mutations affecting the expression of Dp140 contributed most to the grey matter volume differences, supporting the hypothesis of the pathogenetic role of the short isoforms in CNS. These results were further confirmed in a similar study on a large cohort of Indian patients<sup>34</sup>.

Since cerebral expression of dystrophin seems to have a role in cerebral vasculature (see “Neurobiology”), the same cohort was later studied by applying arterial spin labeling (ASL) cerebral blood flow images<sup>35</sup>. Results showed alterations in DMD: patients had a lower cerebral blood flow than controls, independently of grey matter volume. Once again, the subset analysis based on the predicted expression of the isoforms, showed that patients likely missing both full-length and shorter dystrophin presented lower cerebral perfusion.

A few studies based on PET scans also showed reduced glucose metabolism involving various anatomical regions (sensorimotor area, temporal neocortex, medial temporal structures, and cerebellum)<sup>36,37</sup>.

Recently, advanced MRI techniques were adopted to explore functional and structural connectivity in DMD patients. The results from studies based on DWI tractography<sup>12</sup> and resting-state functional MRI<sup>38,39</sup> provided evidence of altered connectivity involving cortical-subcortical-cerebellar tracts and the Default Mode Network.

These studies represent examples of a multidisciplinary approach to the research about CNS involvement in DMD because they included advanced imaging techniques, genetic information, and data from neuropsychological assessments.

## 1.2.Unmet needs in DMD

### 1.2.1. From “muscular” to “neuromuscular”: the shift of paradigm from DMD to DND

During the 249th ENMC International Workshop, the parents’ and patients’ representatives suggested changing the name pathology from DMD to Duchenne Neuromuscular Dystrophy<sup>9</sup>. Such a proposal aims to stress the impact of the pathogenetic process not only on the muscular tissue but also on the CNS.

Since the first radiological and neuropsychological features were described, research has provided an ever-growing insight into such an involvement. The disruption of dystrophin also involves the development and the structure of the CNS, and it causes alterations that can be detected by clinical assessments and neuroimaging.

Thus, CNS involvement must be considered as a part of a complex phenotype that goes beyond the sole progressive muscular wasting. Interestingly, these two aspects of the DMD seem to follow different patterns. The neuropsychological difficulties are reported to have variable severity and to be stable over time, while motor impairment is progressive, and invariably leads to a severe disability.

Moreover, the application of the standard of care, and the perspective of new effective therapies raises a two-fold issue. Firstly, the increased survival and the reduced disability give clinicians the space to assess and study some unknown aspects of DMD. On the other hand, patients, families, and associations now face a longer life expectancy with possibly a lower burden of motor disability. The importance of completing an appropriate school curriculum is now stressed to get work and personal independence<sup>40</sup>. Thus, it is crucial to give precise information about the neuropsychological outcome.

Therefore, the characterization of the CNS involvement in DMD is currently the aim of several lines of research, such as the BIND (Brain Involvement iN Dystrophinopathies) project which started in January 2020 and was awarded a European Union “Horizon 2020” grant. Some preliminary results of this vast project were recently presented: interestingly, the study was focused on the neurobiological and neurodevelopmental features of the dystrophin expression, and the emotional side of the CNS phenotype of the disease, exploring anxiety in DMD patients.

### 1.2.2. Towards a standardized toolkit for neuropsychological assessment

As mentioned before, the revised Standards of Care recommend that patients should be appropriately referred for neuropsychiatric and neuropsychological treatment and care. Consistent screening of the frequently comorbid neuropsychiatric and neurodevelopmental disorders in DMD patients is therefore important<sup>9</sup>. The Standards of Care mention the importance of early and adequate assessment for neuropsychological functioning in dystrophinopathies, but they do not provide a detailed description of which tools and procedures should be adopted for this purpose.

Even looking at the recent literature, a heterogeneous scenario emerges. A large number of different psychological instruments are currently being used in DMD. 32 studies on intellectual functioning in DMD patients were reviewed and the authors found over 8 different instruments being used<sup>21</sup>. Additionally, another larger review<sup>41</sup> analyzed 51 DMD studies and identified a total of 61 adopted neuropsychological tools. These studies reflected the lack of a standardized approach to this clinical issue. Moreover, it raises problems of comparability and epidemiology: the use of so various diagnostic instruments may lead to different prevalence rates of comorbidity. Recently, a European multicenter study conducted within the previously mentioned BIND project surveyed seven of the specialized centers included in the network about the neuropsychological batteries in use in each clinic. In particular, this study focused on three domains: intelligence/neurocognitive functioning, behavioral/psychiatric functioning, and learning disabilities over pediatric age and adulthood.

The previously described heterogeneity was confirmed, even in the assessments used in clinical practice in the surveyed centers (e.g. 79 tests emerged to be used for psychological and cognitive assessment of school-aged patients). The most frequently used instruments were the Wechsler Intelligence Scales, Tower of London, Conners behavior rating, and Child Behavior Checklist (CBCL). The authors also highlighted some peculiarities of such a multifaceted scenario:

- cognitive skills were often tested by general scales (e.g. Wechsler scale)
- language, which is known to be frequently impaired in DMD, was only routinely performed in a minority of the surveyed centers
- common tests (e.g. the CBCL) were usually adopted to test other aspects of neurobehavioral and neuropsychological functioning, even if they were not entirely suitable for DMD boys as some of the items are less applicable to them due to progressive muscle wasting<sup>41</sup>.

The study also provided an age-group analysis that highlighted an interesting clinical-epidemiological issue: neurodevelopmental diagnoses (e.g. ADHD and ASD) are made more frequently in younger children with DMD, while school-aged boys are more often diagnosed with cognitive deficits or learning problems. Conversely, psychiatric diagnoses like anxiety and depression are more often given to young adults. The question of whether these findings reflect the different perspectives of pediatric and adult neuropsychiatric specialists or a change in psychological functioning during the transition to adulthood remains open.

Despite the limitation of a relatively small number of centers participating in the survey, the data from this study were representative of the expertise of specialized centers for neuromuscular diseases. Thus, the described heterogeneity of tools may be a proxy of the complexity of CNS-related comorbidities in dystrophinopathies<sup>9</sup>, but on the other hand, it raises the need to identify a standardized and shared toolkit for neuropsychological assessment.

The impact of brain-related comorbidities on the quality of life for both individuals with DMD and their carers is becoming increasingly evident. Combined also with the fact that life expectancy is prolonged due to better anticipatory, the development of a Standard Operating Procedure (SOP) for the assessment of psychological functioning in DMD is imperative. Work is in progress to develop a toolkit, stratified into a core set for clinical settings and another with tests more suitable for research settings to better understand the complexity of CNS involvement and the correlation among different cognitive and behavioral variables.

#### *1.2.3. Expanding the neuropsychological profile: social cognition*

As exposed in the previous chapter, the profile of expression of dystrophin isoforms in CNS is characterized by a major involvement of subcortical structures (e.g. amygdala, hippocampus) related to many neuropsychological functions. Based on this finding, it is plausible that the investigations on the neuropsychological profile of DMD patients should be broadened to include such functions. In this area, the social cognition domain is particularly intriguing.

Children with MDs may show issues in “reading others,” (i.e. understanding others’ points of view, non-literal language) and understanding nonverbal communication cues, such as facial expressions<sup>42,43</sup>. There are only sporadic reports about children with DMD showing a deficit in SC skills, in particular, facial emotion recognition and ToM<sup>44,45</sup>. Another study comprehensively assessed SC in a broader diagnostic sample (i.e. pediatric patients with muscular dystrophies), adjusting the results for the general intelligence level, behavioral and emotional symptoms,

diagnosis-related variables, and physical functionality<sup>46</sup>. The preliminary findings demonstrate that SC in children with DMD is impaired compared with healthy-matched children. Interestingly, the analysis of the study by Garcia et al. provided insight into some of the possible influencing factors: the level of general intelligence seemed to explain the differences found in SC performance between dystrophic patients and healthy controls for aspects related to ToM, but not for emotion recognition. Furthermore, these differences were also independent of behavioral and emotional symptomatology. The SC performance of pediatric patients with MDs was not influenced by any clinical variables related to diagnosis or physical functionality. However, this body of evidence is thus based on preliminary results, and it is still insufficient, suggesting that there is space for further investigation in this field of scientific and clinical knowledge.

The neuropsychological domain of social cognition (SC) is defined as the cognitive ability to interpret social situations appropriately and act accordingly<sup>47,48</sup>. Facial recognition of emotions and theory of mind (ToM) are the two most analyzed subcomponents. ToM is described as the metacognitive and socioemotional ability to attribute and understand others and one's own beliefs, emotions, desires, and intentions. This complex construct is typically split into two components: affective and cognitive<sup>49</sup>:

- Affective ToM: includes the ability to emphatically understand the other person's emotional state and react consequently
- Cognitive ToM: encompasses the understanding of other people's beliefs, knowledge, and intentions.

Thus, there are many factors influencing the development and the features of SC skills, and the question about the physiopathological process underlying a possible deficit in this domain is still to be cleared. SC is related to other neuropsychological domains (e.g., general intelligence, language, executive functions) and neurodevelopmental factors, and it is also a necessary skill for good social-emotional functioning. The progressive motor disability can significantly impair the social interactions of children with DMD, reducing engagement with peers and negatively affecting their emotional well-being and self-esteem. Moreover, the delay (or the lack) in acquiring age-appropriate motor abilities has a direct impact on the proper development of SC skills<sup>50,51</sup>. Even psychological symptomatology (e.g. internalizing symptoms such as depression and anxiety) plays a role in the SC functions, leading to a poorer ability to understand others' emotions<sup>52</sup>. Lastly, as previously mentioned, the involvement of subcortical structures in the

lack of dystrophin within the CNS can be the neuroanatomical substrate of this impairment. The impact of these factors is summarized below:

- *Motor development:* a growing body of research focuses on the fundamental implications that the early reaching of motor milestones has on other areas of child development<sup>50,51</sup>. From this perspective, having its roots in Piaget's research, motor development, and sensory functions are the fundamental tools the child has to learn about the world<sup>53</sup>. This is an interactive process that starts the consequent development of perceptual, cognitive, and social functioning skills. The stimuli derived from these abilities in turn feed back into the motor domain<sup>54,55</sup>. Evidence lacked about the direct impact of motor development on the acquisition of SC in DMD patients, but other child populations with motor impairments (e.g. developmental coordination disorder) were investigated in studies providing results that confirmed this theoretical framework<sup>50,54,56</sup>. An intriguing model linked to this process is the concept of "embodied cognition": the sensory-motor representation of one's own body is, in this hypothesis, necessary for proper emotional processing and recognition<sup>57-59</sup>. If supported by scientific evidence, this hypothesis may shed some light on the poorer SC performance in children with neuromuscular disorders due to their limitations in movement functionality.
- *Behavioral and emotional symptomatology:* pediatric patients with DMD often show more internalizing and externalizing problems compared with healthy children of the same age; difficulties related to attention and social problems are also reported. Living with a progressive disease from a young age has not only an impact on physical performance and well-being, but also directly impacts their social behavior and, consequently, the mental health of these children<sup>60</sup>. Previous research with children with motor impairment has pointed out that internalizing symptoms can interfere with SC abilities, specifically a poorer understanding of others' emotions<sup>52</sup>. In the previously mentioned study about a larger cohort including muscular dystrophies<sup>46</sup>, the influence of the main emotional and behavioral symptoms on SC differences between patients and healthy controls was investigated. Interestingly, such differences were confirmed even after adjusting for this symptomatology.
- *General cognition and specific neuropsychological skills:* previous research raised the hypothesis that social dysfunction in some neuromuscular patients may be due to deficits in general cognitive performance rather than specific SC deficits<sup>61</sup>. More specifically, it has been speculated that there is a strong correlation between nonverbal fluid intelligence and SC, for both emotion recognition<sup>62</sup> and ToM-related skills<sup>63,64</sup>. The

already cited work by Garcia et al. analyzed the SC impairment in a cohort of patients with muscular dystrophies eliminating the possible effect of the nonverbal fluid intelligence level. Consistently with the literature, some ToM measures (e.g. TM NEPSY-II, TM Verbal task, and RMET-C) kept being statistically different even after the adjustment. It has been hypothesized that general intelligence could be influencing the response to some items, such as the verbal ones related to metaphors or idioms, in which a greater involvement of cognitive abilities and verbal skills is required. Conversely, the mentioned study proved that the level of general intelligence was not a determining covariate for the differences found in the rest of the SC components (i.e. facial emotion recognition and ToM tasks). Moreover, such tasks are more representative of children's everyday lives because they include compensatory contextual cues and involve social knowledge<sup>65</sup>. Therefore, despite the described correlation between nonverbal fluid intelligence and SC reported in the literature, it appears that cognitive ability may not be a determining factor in the development of the specific SC skills of these patients. This raises the possibility that SC development in children with DMDs might be different and mediated by other unidentified variables. Interestingly, a recent study linked the two previously addressed factors, showing that children with intellectual disability frequently have impairments in imitation skills, with an impact on the sensory-motor function, leading to poor performance in nonverbal SC tasks<sup>66</sup>. These findings may provide a key for interpreting the SC involvement in other neurodevelopmental conditions with motor dysfunction. While still not explaining the neurobiological basis of SC difficulties in pediatric patients with DMD, they offer new insights into the possibly impaired social skills and difficulties in the emotional adjustment of these patients. Finally, other specific neuropsychological deficits (e.g., language and executive functions) that are typically associated with DMD are strongly related to SC abilities, especially with ToM<sup>67</sup>.

- *Neuroanatomy:* the introduction of the study “FACE-DMD “ (see below section 2.3) provides an extended discussion about the possible role of social cognition of the neural network involving the subcortical structures that are mostly affected by the lack of dystrophin in CNS

If SC impairment is confirmed to be a part of the CNS-related phenotype of DMD, including it as a target for the specific re-habilitative treatment of the disease should be essential. Environmental stimulation is indispensable for the development of SC<sup>68</sup>. In this background, routine screening protocols in the early stages of development of these patients must include

an assessment of SC skills followed, whenever necessary, by early neuropsychosocial interventions<sup>69–71</sup>. As suggested by the standard of care, it is recommended that such a clinical approach should be adopted starting from the neuromuscular diagnosis by the specialized centers. Currently, there is no standardized approach to these impairments. Psychosocial approaches (e.g. social support networks, facilitation of personal pragmatic tools for interaction) proved to reduce the internalizing symptomatology that these pediatric patients frequently experience<sup>60,72,73</sup>. In addition, specific neuropsychological training for children in SC is effective<sup>74</sup>, in promoting more functional and social adaptation-related aspects<sup>75,76</sup> and, ultimately, has an impact on improving quality of life<sup>74–78</sup>.

#### 1.2.4. Use of technology for DMD: assessment and rehabilitation

Technological devices play a crucial role in the clinical assessment and rehabilitation of Duchenne Muscular Dystrophy (DMD), improving diagnosis, monitoring, and treatment of the condition. An overview of the various applications of technology in this field is exposed below:

- **Clinical Assessment:** in assessing DMD, technological advancements have made the process more accurate, objective, and continuous.
  - Wearable Sensors<sup>79,80</sup>:
    - Accelerometers and gyroscopes are used to track movement and muscle function over time. These devices monitor daily activity patterns, gait, balance, and the progression of muscle weakness, allowing clinicians to detect subtle changes before they become clinically significant.
    - Smart insoles with pressure sensors measure foot pressure distribution and gait anomalies, key markers in tracking the progression of DMD.
  - Digital Biomarkers<sup>81</sup>: using data from wearables and smartphones, digital biomarkers are being developed to assess the progression of DMD more efficiently. These include metrics like walking speed, steps per day, and time spent in certain postures, providing real-time, passive monitoring.
  - 3D Motion Capture: Optical motion capture systems (using cameras and markers) assess complex movements like walking and running, measuring joint angles, gait dynamics, and range of motion. This technology can reveal biomechanical compensations as muscles weaken.

- Genetic Testing and AI: Next-generation sequencing and *machine learning* algorithms can help in genetic assessment. AI tools are also used to correlate clinical symptoms with genetic variants to tailor therapy and predict progression.
- **Rehabilitation**: technological devices aid rehabilitation by improving muscle function, providing compensatory support, and optimizing therapy.
  - Robotics and Exoskeletons<sup>82,83</sup>:
    - *Robotic-assisted devices* and *wearable exoskeletons* help maintain mobility and improve muscle strength through repetitive, controlled movements. They provide assistance in walking and standing, delaying the need for full-time wheelchair use.
    - Devices such as *soft exosuits* offer powered assistance for specific joints like the hip or knee, providing gait support and reducing muscle strain during walking or standing exercises.
  - Virtual Reality (VR) and Augmented Reality (AR)<sup>84</sup>:
    - VR-based rehabilitation offers an engaging way to encourage movement through interactive games and simulations. These systems can be tailored to specific motor tasks, promoting neuroplasticity and compensatory strategies.
    - AR can be used to enhance physical therapy sessions, overlaying visual cues to guide patients through movements and exercises correctly.
  - Telemedicine and Remote Monitoring<sup>85</sup>:
    - Telemedicine platforms enable continuous monitoring and remote physical therapy sessions. With advancements in the Internet of Medical Things (IoMT), clinicians can remotely monitor patients' activity levels, mobility, and adherence to therapy, adjusting rehabilitation protocols as needed.
    - Home-Based Devices: Portable muscle stimulators, home spirometry kits, and wearable rehabilitative devices enable patients to conduct physical therapy or monitor their condition at home, reducing hospital visits and enabling long-term care in familiar environments.
- **Research and Clinical Trials**: technological devices also contribute to clinical trials for new DMD treatments by providing objective data.

- Wearable devices<sup>86</sup> in clinical trials help measure outcomes like muscle function, fatigue, and mobility in real-world settings, providing data points that reflect the patient's daily life, rather than relying solely on episodic clinic visits.
- Digital Health Platforms: advanced platforms integrate wearable data with patient-reported outcomes and imaging results, offering a comprehensive picture of disease progression. This data is crucial for understanding treatment efficacy and tailoring individual rehabilitation protocols.

### 1.3. Objectives of the project

In this framework, this project pointed to a precise characterization of the CNS involvement in DMD, to better insight into the pathophysiology of the disease and expand the horizon of diagnostic assessments and rehabilitative courses. Therefore, the project was declined around the following work packages:

- Work package 1 (WP1) – characterizing the phenotypes: the aim of WP1 was the assessment and the definition of all the aspects that define the profiles of CNS involvement in DMD. Specifically, this WP addressed the identification of possible disease-specific profiles (including neuropsychology and neuroimaging) and the subsequent arrangement of specific protocols for diagnosis and follow-up assessments.
- Work package 2 (WP2) – expanding rehabilitation: WP2 addressed rehabilitation. Specific motor rehabilitation plans are usually activated for patients with DMD. After the study of the specific neuropsychological profile of DMD, we aimed to explore the feasibility and potential benefit of an integrated motor-neuropsychological rehabilitative approach.
- Work package 3 (WP3) – implementing telehealth and new technologies: with WP3, this project also addressed the theme of telerehabilitation, which could substantially impact the healthcare of these patients. The motor impairment often implies difficulties in getting to clinical centers and undergoing procedures and assessments. Once the rehabilitation protocols were defined, we aimed to implement them in clinical practice exploiting the potential of innovative telehealth technology.

The aims of each work package were pursued with different strategies, summarized as follows:

- Work package 1 (WP1) – characterizing the phenotypes: we planned a systematic review, and two clinical studies focused on unexplored neuropsychological domains in DMD.
- Work package 2 (WP2) – expanding rehabilitation: the neurodevelopmental and neuropsychological involvement of DMD was the base to design and validate a multimodal approach to rehabilitation. In particular, we integrated the “Neuromuscular” perspective (see section 1.2.1) into the currently adopted rehabilitation plans mainly focused on motor functions and addressing neuropsychological impairment due to CNS involvement.
- Work package 3 (WP3) – implementing telehealth and new technologies: the possibility of implementing the intervention designed in WP2 by adopting innovative technologies and telehealth devices was explored. Starting from the existing resources of our center (e.g., telehealth software, devices for telerehabilitation) and our participation in the national network of pediatric research institutes for pediatric rehabilitation, we developed two different pilot experiences with technological and telehealth devices.

We expected this project to contribute to connecting the pieces of the complex puzzle of CNS involvement in DMD, integrating and further exploring the acquired knowledge in this field. We aimed at obtaining outcomes that could be a helpful addition to what is known about DMD from a multifold perspective better to address the needs of patients and their families and improve their quality of life. Specifically, we believed that the results of our project could provide:

- improved clinical knowledge, obtaining new information about:
  - o the neuropsychological profiles. The combined use of routine tests (e.g. cognitive scales) and specific tools allows for defining the phenotype. In particular, we expect to assess the functions related to the neurological networks that are supposed to be affected by each pathology (based on neuropathological or neuroimaging evidence);
  - o the neurobiological substrate of CNS involvement. The use of innovative neuroimaging techniques makes it possible to explore the functional and neuroanatomical impact of pathological NMDs genetic alterations within CNS structures;
- New neuropsychological protocols: disease-specific batteries, tailored to the phenotype, should be included in the standard diagnostic and follow-up assessments, broadening the perspective of the standard of care in NMDs.

- New rehabilitation strategies: we believe that such a broad view should be extended to the rehabilitation approach, which should address this pathology's specific neuropsychological frailties with the help of innovative telehealth technologies. Specific programs of cognitive telerehabilitation can be designed starting from what would emerge from studying these patients' clinical profiles.

#### 1.4.Ethics statements

Written informed consent was provided by the participant's legal guardian/next of kin to be included in the two prospective clinical studies.

All studies and experimental protocols were approved by the local EC (Comitato Etico Policlinico "San Matteo" Referente Area Pavia), the EC of the other participating centers (for multicentric studies), or the national EC, where required. Informed consent forms compliant with the Declaration of Helsinki to participate in the research were predisposed for participants, their parents, or their legal representatives.

## 2. The clinical assessment: unexplored domains in the neuropsychological phenotype of DMD

This Chapter is dedicated to the clinical assessment of unexplored domains of the neuropsychological phenotype of DMD patients, potentially linked to the expression profile of the isoforms of dystrophin.

The scoping review presented in Section 2.1 is an overview of the standardized tools previously adopted and described in the literature for the neuropsychological assessment of DMD patients, with a focus on the unexplored but possibly impaired domains. The results of the review have been presented in the article *Social Cognition: the blind corner of neuropsychological assessment in Duchenne (Neuro)Muscular Dystrophy - A Scoping Review* (S. Parravicini, M.I. Dainesi, C.A. Quaranta, A. Berardinelli) - Neuroscience and Biobehavioral Reviews, August 2024 – under review.

My contributions to this study have been conceptualization, methodology, investigation, formal analysis, visualization, and manuscript writing.

The application of an innovative clinical protocol including the assessment of unexplored neuropsychological domains is described in Section 2.2. The study was focused on the preliminary individuation of a social cognition deficit in our cohort of patients. The results of the study have been presented in the article *The Hidden Face of Duchenne (Neuro)Muscular Dystrophy. Preliminary evidence of social cognition impairment as a feature of the neuropsychological phenotype of DMD* (S. Parravicini, C.A. Quaranta, M.I. Dainesi, A. Berardinelli) – Frontiers in Psychology, September 2024 – under review

My contributions to this study have been conceptualization, methodology, investigation, formal analysis, visualization, and manuscript writing.

The study presented in Section 2.3 is the development of the previous step of the project reported in Section 2.2. A specific experimental setting based on implicit cognition and subliminal priming was developed to further explore the social cognition deficit and its possible neural substrate. The study was declined in the multicentric clinical study *FACE-DMD: facial aspect and implicit cognition of Emotions in Duchenne Muscular Dystrophy* (approved by the local EC of the IRCCS Mondino Foundation on November 2023); the preliminary results of the

application of the protocol on a small cohort of DMD patients have been reported in a graduation thesis (Dr S. Khalil; Supervisors: Prof. R. Borgatti, Dr. A. Berardinelli, co-supervisor: Dr. S. Parravicini - Degree in Medicine and Surgery, University of Pavia). The study is currently ongoing and the recruitment of subjects in the centers included in the partnership (IRCCS “Stella Maris” Foundation, IRCCS “E. Medea”, University of Udine, and the University Hospital “Città della Salute e della Scienza”) is still active.

My contributions to this project have been conceptualization, methodology, investigation, formal analysis, visualization, and graduation thesis co-supervision.

## 2.1. Social Cognition: the blind corner of neuropsychological assessment in Duchenne (Neuro)Muscular Dystrophy - A Scoping Review

11,8719,23,88–90

As exposed in the general introduction, DMD is an X-linked progressive muscular disorder caused by mutations in the DMD gene on the X chromosome, encoding for the dystrophin protein. Recent pathological studies have clarified the expression pattern of dystrophin in the human central nervous system (CNS). In particular, the highest levels of dystrophin expression were found in sub-cortical structures such as the amygdala and hippocampus<sup>10</sup>. The same study also reported a relatively low level of dystrophin expression in the human cerebellum, suggesting a reconsideration of the role of this region in the neuropsychological phenotype of DMD, derived from protein expression studies in the animal model and supported by some neuropsychological studies.

The amygdala's involvement in various neurological pathologies<sup>91,92</sup> leads to a deficit in the emotional interpretation of facial expressions. This element is not reported in the classic clinical picture of Duchenne Muscular Dystrophy (DMD), and evidence of a deficit in this function was reported in a single paper<sup>75</sup>.

In DMD, social interaction difficulties are generally interpreted as secondary to the progression of the disease or attributed to concomitant disorders (e.g., intellectual disability).

However, interpreting the emotional value of facial expressions is also crucial in social functioning and interpersonal relationships<sup>93</sup>.

The current indications for diagnosis and management of DMD recommend the assessment of the neuropsychological profile at diagnosis. In particular, the adoption of standardized tests to assess children's cognitive development, academic skills, social functioning, and emotional and behavioral regulation is suggested. Furthermore, the clinical follow-up should include re-evaluations to monitor the children's neurodevelopmental trajectories and their intervention response<sup>94</sup>.

The literature highlighted how heterogeneously this recommendation was declined in clinical practice and research. Previous reviews reported the adoption of a wide variety of tools for assessing cognitive and neuropsychological profiles in DMD<sup>21,41</sup>. The multicentre study Brain INvolvement in Dystrophinopathies<sup>95</sup> (including seven European neuromuscular centers from the UK, Denmark, France, Italy, Netherlands, and Spain) recently surveyed five of the seven participating centers to describe which tests are used in clinical practice for the assessment of the neuropsychological profile of patients with DMD<sup>96</sup>.

This study was representative of specialized centers' expertise for neuromuscular disorders and extremely useful in understanding the topic's complexity. The heterogeneity of tools used highlighted the complexity of the CNS-related phenotype of dystrophinopathies and the need for a shared and standardized tool kit to support comparative work.

A test battery, with a core set of clinical setting tools and other tests more suitable for research purposes, was outlined in the same paper. Such a tool kit would include: general cognitive scales (i.e., Wechsler Intelligence Scales) and tests assessing the domains that were described as predominantly affected in dystrophinopathies (i.e., memory, attention, executive functioning, language, academics, ASD, ADHD, OCD, anxiety and depression)<sup>9,97,98</sup>.

In this background, assessment of social cognition skills is not widespread in clinical practice or research contexts. Among the tools classified in the BIND project survey, only a minority had the specific purpose of investigating these skills. In particular, a few tests designed to evaluate general neuropsychological functions (i.e., NEPSY-II) or specific pathologies (e.g., ADOS-2, ADI-R) include items dedicated to assessing social cognition.

Recently, one cross-sectional study tested a sample of DMD/BMD children and adolescents with a neuropsychological battery, including the Social Perception Domain of the NEPSY-II, the Reading the Mind in the Eyes Test, and the Strange Stories test<sup>45</sup>. To the best of our knowledge, this is the first study, together with the paper by Hinton et al.<sup>75</sup> mentioned above, to demonstrate an impairment in social cognition skills in dystrophinopathies.

The impact of CNS-related comorbidities on the quality of life for both individuals with DMD and their carers, and the need for a standardized neuropsychological assessment in DMD are addressed in Chapter 1.

Thus, the present scoping review aimed to:

- Determine the coverage of the body of literature about this topic.
- Defining the features of the neuropsychological protocols adopted to study the CNS involvement in DMD and identifying unexplored areas that could be relevant based on the recent findings about dystrophin expression in human CNS.

## Methods

### *Literature search*

The author and his collaborators undertook a systematic search from three electronic databases, Medline/PubMed, EMBASE, and Web of Science, on November 2023, according to

the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement<sup>99</sup>. Different combinations of keywords were selected from the previously published papers about the topic of our interest. In particular, such keywords referred to four main clusters: “Duchenne muscular dystrophy”, “children”, “cognitive”, and “neuropsychological”. Other synonyms or related terms were included (see Appendix for the complete search strings). In addition, the references of the included studies were also considered to identify additional eligible studies and to ensure a comprehensive data collection. The author and his collaborators aimed to review peer-reviewed studies published in an international context. Thus, only studies from academic journals, reported in English and available for full text, were included. Considering the new findings about the features of the involvement of CNS in DMD and the relatively recent interest in studying the complexity of the neuropsychological phenotype of the disease, articles published starting from 2000 were considered.

#### *Study selection process*

After automatically removing duplicates, the author and his collaborators independently screened the titles and abstracts of 3566 articles. The resulting 109 articles were then screened by full-text according to eligibility criteria (see below), previously reassigning to each reviewer a different set of papers to be reviewed compared to the title/abstract-selection stage. In case of discrepancies, articles were discussed between the two reviewers to determine their inclusion or exclusion. References of the included studies were eventually reviewed to identify additional eligible studies. The process led to selecting 65 papers that met the inclusion criteria (publication date range: 2000-2023, from 19 countries)<sup>12,20,22-27,29,37,42,44,67,70,87,100-149</sup>. The overall process for selecting studies is shown in Figure 3.

The selection process was performed using the software Rayyan®, which includes specific tools for collaborative systematic reviews<sup>150</sup>.

The researchers adopted a PICO framework to set the inclusion and exclusion criteria:

- Population: studies were included when considering samples of children aged 0-18 diagnosed with Duchenne Muscular Dystrophy.
- Interventions: the search was focused on studies including a standardized neuropsychological assessment. The articles were selected if they included details about the adopted battery of neuropsychological tests/scales.
- Comparison: studies including only DMD samples, healthy controls, or other non-distrophin-related neuropsychiatric diseases were included.

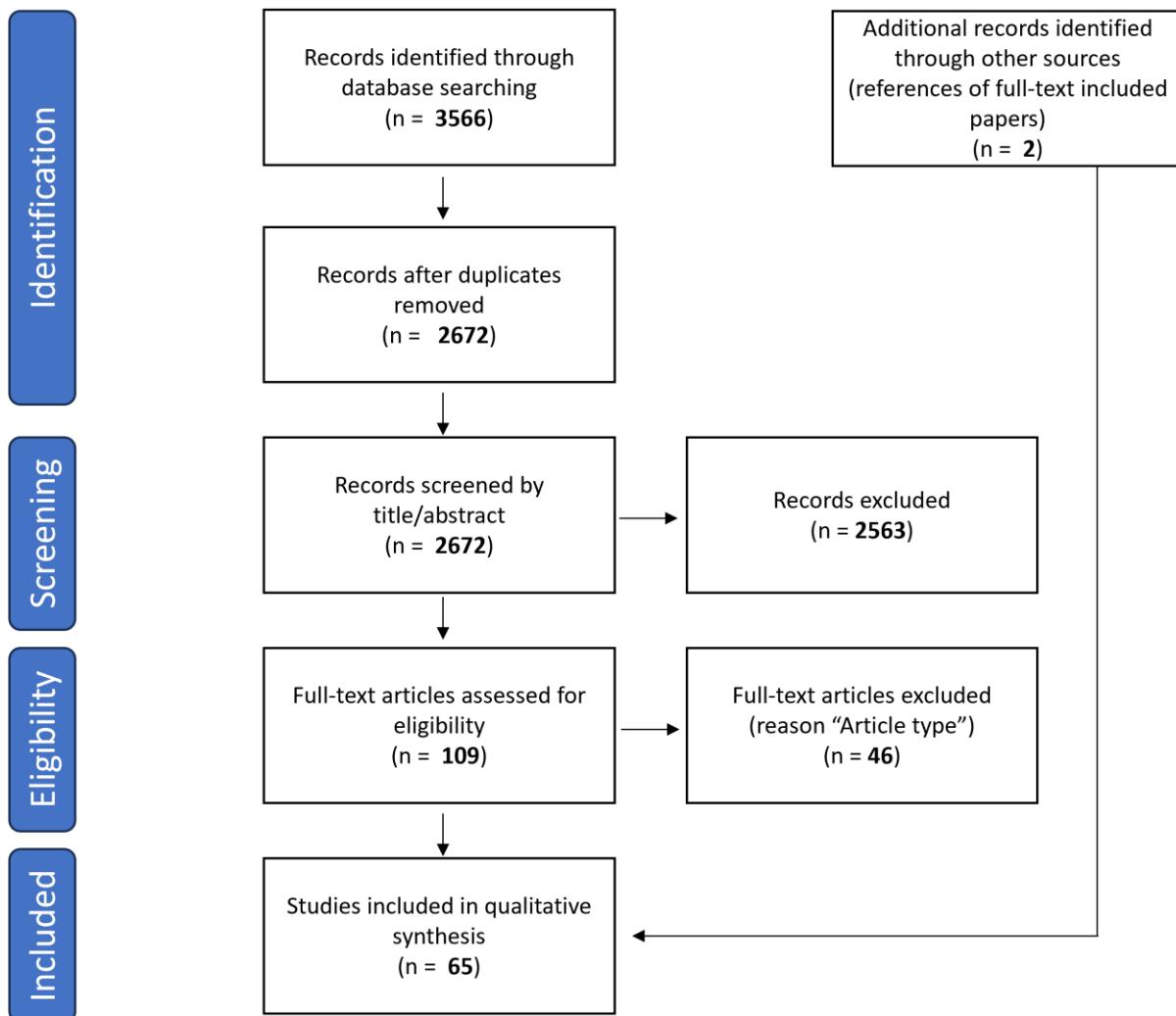
- Outcomes: studies were included if they aimed to define the features of the neuropsychological phenotype of DMD or if they described protocols adopted in routine clinical practice.

The following exclusion criteria were considered: (1) case reports, reviews, book chapters, and conference abstracts; (2) samples including patients aged >18 years or diagnosed with other neuropsychiatric disorders; (3) studies not describing batteries standardized tests/scales for neuropsychological assessment, (4) studies about other topics (e.g., studies on animals, other disciplines)

#### *Data extraction*

For each paper included, the author and his collaborators recorded the following information in a dedicated database: author, title, year of publication, age range and diagnosis of the sample, study design, sample size, and details about the battery of neuropsychological tests adopted in the study (i.e., name, main aim, age range, and structure of each test or scale). Each test was then classified based on its primary aim. We applied seven labels (“attention and executive functioning”, “language”, “memory and learning”, “sensorimotor”, “social perception”, “visuospatial processing”, and “other”) derived from the structure of the NEPSY-II scale into neuropsychological sub-domains; this framework is based on the work of Marit Korkman and inspired by the model of the neuropsychological functions of Alexander Luria<sup>151</sup>. The label “other” was applied if classifying the main aim of a test into one of the previous groups was impossible. If a test or a scale was structured to assess more than a neuropsychological function, more than a label could be assigned.

When a test included sub-items assessing specific neuropsychological fields (e.g., Wechsler scales), we assigned multiple labels to reflect this multifaceted structure. If a scale was used partially, it was classified separately.



**Figure 3- PRISMA Flow Diagram:** the flow diagram represents the stages of the search strategy and the selection process of the articles included in the review, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

## Results

The application of 96 different tools emerged from the data extraction process. The most recurring tests were Wechsler scales (i.e., WPSSI, WISC, and WAIS), entirely administered for a comprehensive cognitive assessment or partially used to focus on specific neuropsychological functions. The use of the other tools was more scattered, and most of them recurred only once.

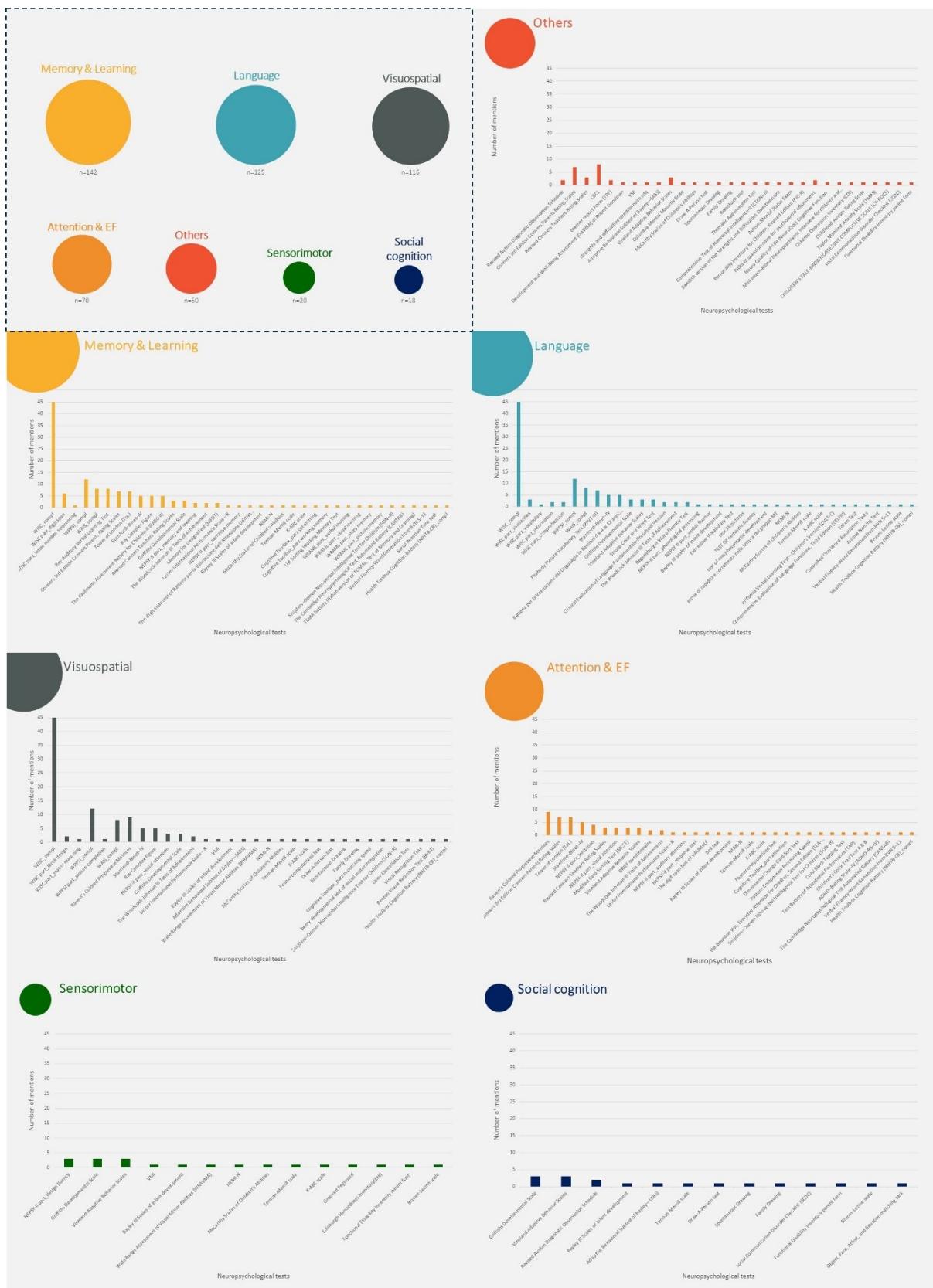
After the labeling process, the group including the references to tools for assessing memory skills and learning abilities was the largest (n=142), followed by the “language” (n=125), “visuospatial” (n=116), and “attention and executive functioning” (n=70) groups; only a minority of the identified papers used tools aimed at assessing sensorimotor functions (n=20).

Thirty tests and scales were included in the “Other” group (n=50). Even if a specific classification within the framework we adopted was not applicable, some recurrences emerged

when analyzing the primary purpose of the assessments included in this set. In particular, this group included functional scales, diagnostic tools for autism spectrum disorders, projective psychological tests, and scales assessing psychiatric symptoms (e.g., anxious, depressive, or obsessive-compulsive symptoms).

Finally, the least assessed function was social cognition (n=18). Almost all tests and scales in this group were not specifically designed to assess social cognition. Still, they included an assessment of social competence (e.g., the social skills section of the Vineland Adaptive Behaviour Scale). The only paper adopting a tool focused on social cognition skills, namely visual affect recognition, was the previously mentioned paper by Hinton et al.<sup>22</sup>.

In the Appendix a complete list of the identified tests and scales, their assigned labels, and their frequency across the reviewed papers is provided; the infographic in Figure 4 is an overview of each test's frequency of use, split into the different neuropsychological domains.



**Figure 4 - Overview of neuropsychological test usage in DMD:** the infographic represents the recurrence of the neuropsychological test across the articles included in the review. The tests were classified based on the neuropsychological domains framework based on the work of M. Korkman and A. Luria. More than a label could be assigned to each test/subtest. The circle's size is proportional to the number of papers mentioning neuropsychological tests referring to each area. (EF=Executive Functioning)

## Discussion

CNS involvement in DMD has recently been increasingly explored and characterized. The current guidelines for clinical management, the development of new therapeutic options, and the demands of the patients' associations raised the need to redefine the disease's features. In a word, a shift from a "muscular" to a "neuromuscular" disease was urged.

Current clinical management guidelines, new therapeutic options, and patient association demands require a redefinition of DMD features. This shift arises from observations of dystrophin isoform expression in the human CNS, suggesting the involvement of less explored neuropsychological domains in the CNS-related phenotype of DMD. Our scoping review reveals significant heterogeneity in neuropsychological assessment tools for DMD patients, with the Wechsler scales being the most frequently used. These scales primarily evaluate cognitive skills such as language, visual-spatial abilities, memory, attention, and executive functioning<sup>2</sup>. These domains align with the neuropsychological functions traditionally recognized as impaired in DMD. However, tools specifically designed to assess social cognition are rarely utilized in clinical or research settings.

Emerging data from the Human Brain Atlas have highlighted high levels of dystrophin expression in the amygdala and hippocampus, brain regions integral to social functions and networks like the extra-geniculostriatal network<sup>13</sup>. The complexity of neural networks, which often overlap and share CNS structures, complicates pinpointing the exact cause of impairments in functions such as executive and social functioning. Functional neuroimaging has identified brain regions implicated in these functions, including the prefrontal and parietal cortex, basal ganglia, thalamus, cerebellum, and the Default Mode Network. These deficits are prevalent across various neuropsychiatric conditions, further complicating precise identification.

While clinical features and animal models have suggested cerebellar dysfunction, subsequent analyses of dystrophin expression in the human brain have led to reconsiderations. Despite this, involvement at different network levels remains plausible. More specific assessments are required to elucidate the pathological basis of social cognition deficits and determine their inclusion in DMD's CNS-related phenotype. Advanced neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) and tractography, have been utilized to describe altered connectivity within the Default Mode Network and cortico-subcortical-cerebellar tracts<sup>59,99</sup>. These techniques could further explore structural and functional involvements at finer levels.

The evolving understanding of DMD necessitates a more comprehensive approach to neuropsychological assessment, including tools for social cognition.

## Conclusions

Our scoping review of the papers assessing the CNS-related profile of DMD patients covered more than twenty years and revealed a scattered scenario regarding adopted neuropsychological batteries. This finding reflects the heterogeneous landscape of both research and clinical settings, with significant differences in the type of adopted tools and the explored domains<sup>96</sup>. Moreover, an imbalance in the assessment of the various domains emerged. Some areas (e.g., language or attention) that became part of the “classical” description of the DMD CNS-related phenotype were more intensively investigated. Nevertheless, brain networks and neuropsychological functions (e.g., social cognition skills) that might be part of the clinical picture based on the physiopathological basis of the disease might have been overlooked.

This feature is still not widely explored in research settings or clinical practice. Thus, our work raises the need for a comprehensive assessment of social cognition for age monitoring during the whole follow-up with standardized and specific neuropsychological batteries (e.g., the NEPSY-II scale).

Better neuropsychological characterization could reveal a related misrecognized clinical (neuro) DMD phenotype, guiding clinical and rehabilitation practice.

## 2.2.A multidisciplinary approach: the neuroimaging protocol

Starting from the study reported in section 2.1, we expanded the scope of our research to a multidisciplinary approach to exploring the social cognition domain. Our goal was to contribute to a line of research that recently adopted a multidisciplinary approach to explore CNS involvement in DMD. As mentioned in the introduction (see section 1.1.3), a few studies recently used advanced MRI techniques and batteries of neuropsychological tests to study the relationship between neuroimaging and clinical data.

Firstly, we performed a preliminary literature search, looking for studies exploring the same area with multidisciplinary assessments. Thus, we used the search engine PubMed to explore the literature: we applied multiple searches sequentially combining the MeSH term “Duchenne Muscular Dystrophy” with the MeSH terms “Neuropsychological test”, “diffusion tractography”, “fMRI”, and “arterial spin labeling”. Subsequently, the findings of this preliminary search were discussed together with skilled neuropsychologists and neuroradiologists from our center. This step allowed us to define the composition of the protocol in a way that made it feasible in our routine clinical setting.

Our literature survey yielded about 57 papers, that we preliminary screened based on the titles and the abstracts to select the most relevant to our purpose. Then we explored the methods of such studies, focusing on the features of the neuropsychologic and neuroimaging assessments. We particularly valued papers presenting a multidisciplinary approach and the association between these two areas.

We found that only a few studies adopted advanced neuroimaging techniques to assess structural and functional connectivity, none of them presenting data from multiple methods of acquisition.

Based on these explorative findings, we discussed the protocol design with skilled neuropsychologists and neuroradiologists from our center. As a project joining multiple neuroimaging techniques was lacking, we focused on a wide protocol to acquire multiple advanced MRI data with a reasonable acquisition time. We decided to value the acquisition time to address as far as possible the issue of the pediatric patients' cooperation to the proposed assessments.

An overview of the features of the main studies we selected is provided in Table 1.

REFERENCE	POPULATION	MRI PROTOCOL	NPS PROTOCOL
Doorenweerd, et al, 2021 <sup>38</sup>	133 DMD + 24 matched HC	<ul style="list-style-type: none"> <li>• T1-weighted</li> <li>• Resting-state fMRI</li> </ul> <p>[3T scan]</p>	<ul style="list-style-type: none"> <li>• Peabody Picture Vocabulary test</li> <li>• Reading composite score: based on the monosyllabic word reading test and the 1-minute reading test derived from CB&amp;WL or the Test of Word Reading Efficiency</li> <li>• Information processing composite score: based on 2 subtests from the Kaufman Assessment Battery (number recall for auditory working memory and block counting for conceptual thinking) and 1 subtest from the Wechsler Intelligence Scale for Children (symbol search)</li> <li>• Emotional and behavioral problems composite score: four problem-based subscales from the Dutch or English version of the Strengths and Difficulties Questionnaire for parents</li> </ul>
Biagi, et al., 2021 <sup>12</sup>	5 DMD subjects + 5 BMD subjects + 5 age-matched HC	<ul style="list-style-type: none"> <li>• T1-weighted</li> <li>• DWI tractography</li> </ul> <p>[1.5 T scan]</p>	<ul style="list-style-type: none"> <li>• Wechsler cognitive scales</li> <li>• Executive functioning: NEPSY (Inhibition test) and Tower Of London</li> </ul>
Preetish- Kumar, et al., 2021 <sup>34</sup>	60 DMD + 40 HC	<ul style="list-style-type: none"> <li>• T1-weighted</li> <li>• DWI</li> </ul>	<ul style="list-style-type: none"> <li>• Wechsler Intelligence Scale for children (WISC-IV)</li> <li>• Memory (Rey auditory verbal learning test, Memory for design test)</li> </ul>
Lv, et al., 2011 <sup>39</sup>	10 DMD + 15 HC	<ul style="list-style-type: none"> <li>• T1-weighted</li> <li>• resting state functional MRI</li> </ul>	NA
Doorenweerd, et al., 2014 <sup>33</sup>	29 DMD	<ul style="list-style-type: none"> <li>• T1-weighted</li> <li>• DWI</li> </ul> <p>[3T scan]</p>	<ul style="list-style-type: none"> <li>• Peabody Picture Vocabulary test</li> <li>• Reading composite score: based on the monosyllabic word reading test and the 1-minute reading test derived from CB&amp;WL or the Test of Word Reading Efficiency</li> <li>• Information processing composite score: based on 2 subtests from the Kaufman Assessment Battery (number recall for auditory working memory and block counting for conceptual thinking) and 1 subtest from the Wechsler Intelligence Scale for Children (symbol search)</li> <li>• Emotional and behavioral problems composite score: four problem-based subscales from the Dutch or English version of the Strengths and Difficulties Questionnaire for parents</li> </ul>
Doorenweerd N, et al. 2016	26 DMD + 19 age- matched HC	<ul style="list-style-type: none"> <li>• T1-weighted</li> <li>• pseudo- continuous ASL</li> </ul> <p>[3 T scan]</p>	NA

**Table 1 - Overview of the selected papers from the literature preliminary review:** the features of the studies are summarized in the table (included population, adopted neuroimaging techniques, specifics of the neuropsychological batteries).

Thus, we assembled a 3T MRI protocol to acquire multiple advanced data at the same time, to expand the results obtained from the administration of the neuropsychological battery described in the previous study, which encompassed “traditional” assessments (i.e. general cognitive profile, executive functions, memory, attention) but also less explored functions like the theory of mind and emotion recognition.

The details of the protocol are summarized as follows:

- *Objective*: applying advanced MRI techniques to explore the presence of significant differences in structural and functional connectivity between DMD patients (clinical group) and healthy subjects (control group).
- *Endpoint*: we planned to acquire quantitative structural and functional connectivity parameters by a 3 Tesla Magnetic Resonance (MRI) study including the following sequences: volumetric T1 sequence, DTI study, rs-fMRI, ASL perfusion study. We aim to focus the analysis on the functional traits and networks including the limbic system.
- *Procedure*: the project includes a single MRI acquisition session (total duration: about 45 minutes) at the department of Pediatric Neuroradiology of the IRCCS “Mondino” Foundation. For the DMD patients, we foresaw an assessment in an inpatient or outpatient setting, based on the individual clinical follow-up program; for healthy subjects, outpatient sessions were programmed.

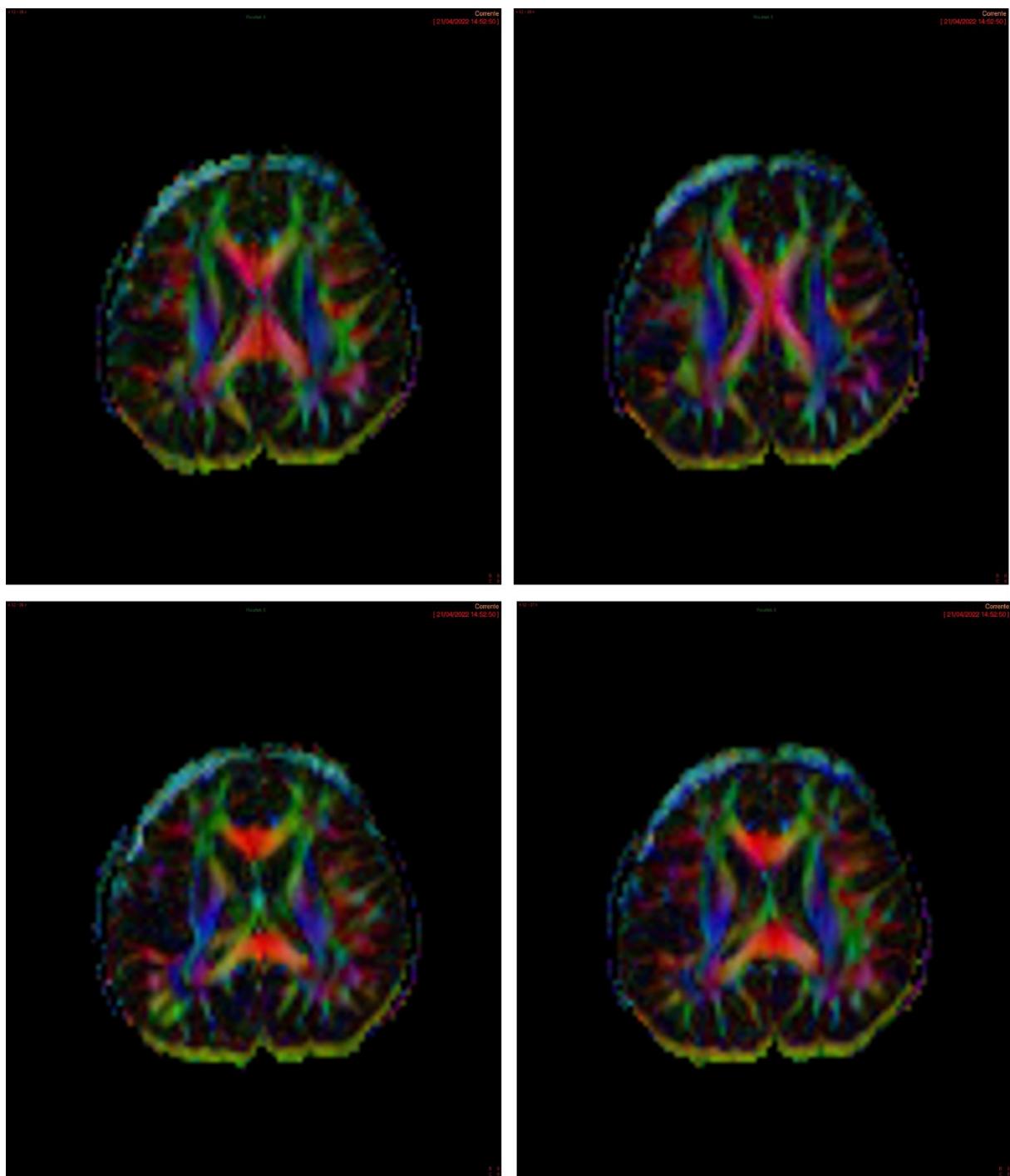
Currently, 12 patients have been accepted to undergo the neuroimaging protocol. Unfortunately, the compliance to this part of the study was lower than expected. More than half of the patients screened for eligibility from the clinical population of our center declined the proposal. The main reasons were: (1) the anxiety and fear expressed by the children about the procedure (2) the resistance to undergo a non-necessary medical procedure, even if relatively noninvasive. Furthermore, the recruitment of healthy controls resulted in being even harder: at the moment, only 3 subjects with no neuropsychiatric disorders accepted to participate in the study.

Moreover, the patients of the neuroimaging cohort were spread through a quite wide age span (from 10 to 19 years old at the time of MRI acquisition). When this type of neuroimaging study, especially the DTI tractography, is performed involving the neurodevelopment age, groups of patients with adequate numerosity and concentrated in a relatively narrow age span are required. Besides, having the possibility of comparing the results with a cohort of healthy

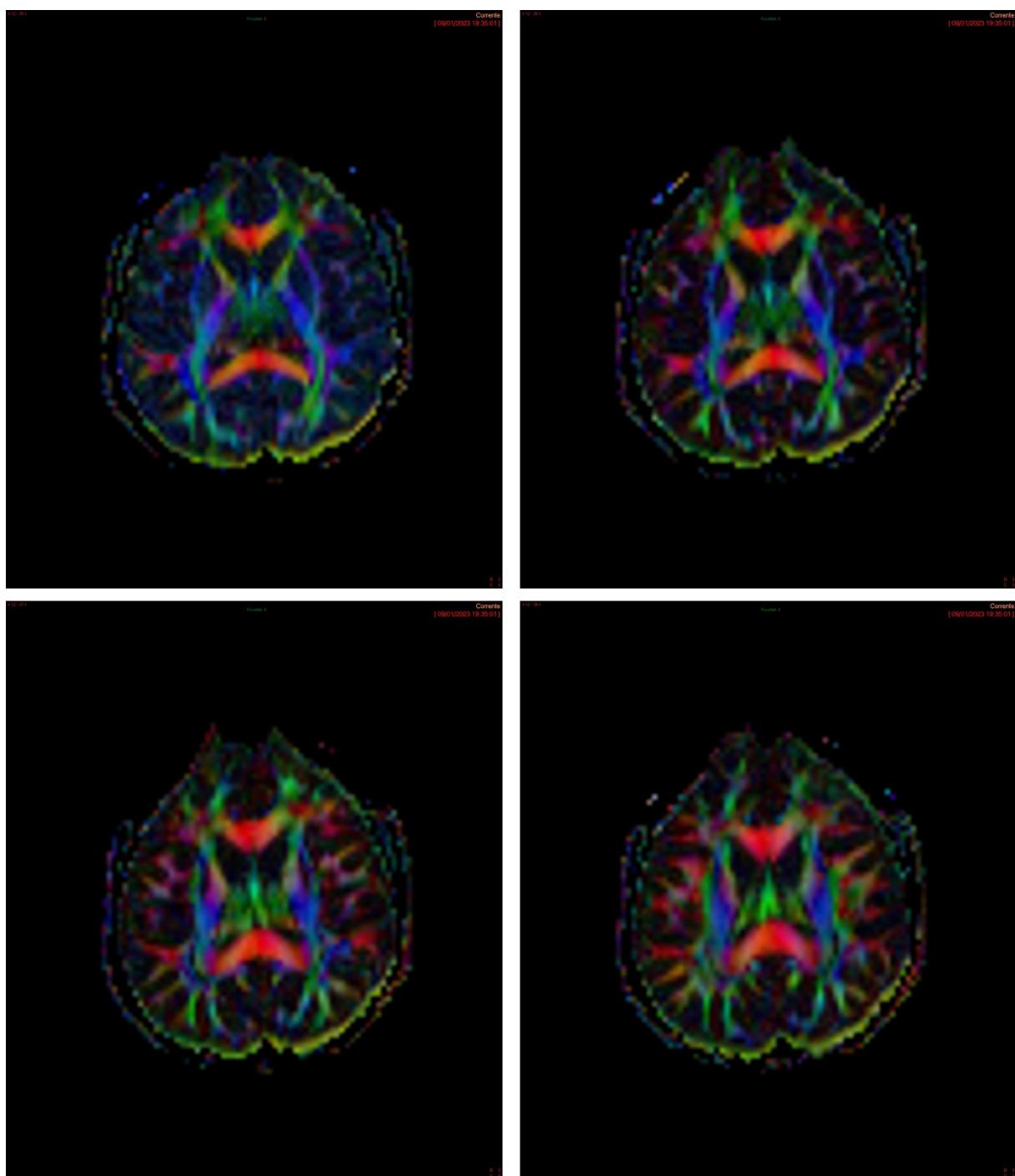
controls, comparable in terms of age span, sex, and numerosity, is fundamental. Thus, at the moment, no reliable analysis could be performed on the data we acquired.

Some examples of the DTI tractography scans of two significative patients are provided in Figures 5 and 6:

- *Patient 1:* 11 years old at the time of MRI acquisition, exon 48-52 deletion with theoretical depletion of the CNS-related dystrophin isoforms, normal intellective level, and performance under the normality range in social cognition skills.
- *Patient 2:* 15 years old at the time of MRI acquisition, exon 3-29 deletion, no theoretical depletion of the CNS-related dystrophin isoforms, normal intellective level, and performance within the normality range in social cognition skills.



**Figure 5 – Examples of DTI tractography images in DMD patients (patient 1):** DTI tractography scan of patient 1. Significant coronal cuts showing the geniculostriatal and extrageniculostriatal tracts are provided in the figure.



**Figure 6 - Examples of DTI tractography images in DMD patients (patient 2):** DTI tractography scan of patient 1. Significant coronal cuts showing the geniculostriatal and extrageniculostriatal tracts are provided in the figure.

## 2.3.The hidden face of Duchenne (Neuro)Muscular Dystrophy. Preliminary evidence of social cognition impairment as a feature of the neuropsychological phenotype of DMD

1091,92759421,4195969,97,98

Recently, one cross-sectional study tested a sample of DMD/BMD children and adolescents with a neuropsychological battery, including the Social Perception Domain of the NEPSY-II, the Reading the Mind in the Eyes Test, and the Strange Stories test<sup>45</sup>. To the best of our knowledge, this is the first study, together with the paper by Hinton et al.<sup>75</sup> mentioned above, to demonstrate an impairment in social cognition skills in dystrophinopathies.

The results of the systematic review reported in section 2.1 proved that social cognition skills have been almost entirely excluded from the traditional neuropsychological assessment of children and kids with DMD, both in clinical and research settings. Starting from these findings, we designed a clinical study to test social cognition skills in a cohort of DMD patients using a standardized neuropsychological battery. Among the available tools, NEPSY-II has been diffusely adopted to assess social cognition abilities such as the theory of mind (i.e., the ability to share another person's perspective or mentally represent someone else's intention) and affect recognition (i.e., the ability to perceive and interpret social cues to interpret the emotional meaning of others' behavior) in other pediatric neuropsychiatric diseases. Thus, we adopted this battery for our study,

As mentioned, only two previous studies demonstrated a possible impairment in social cognition skills in dystrophinopathies<sup>46,75</sup>.

Thus, this study aims to provide further data about the possible impairment of social cognition skills in DMD children, identifying unexplored areas that could be relevant based on the recent findings about dystrophin expression in human CNS.

### Methods

We designed a cross-sectional study that included DMD patients identified from the clinical database and the incident cases of the child neuropsychiatry department of a specialized center in northern Italy (IRCCS Mondino Foundation, Pavia, Italy). The subjects were selected based on the following inclusion criteria: male sex, availability of DMD genetic diagnosis data, age between 7 and 16 and 11 (end values included), and good knowledge of the Italian language. Subjects diagnosed with other concomitant genetic diseases were excluded from the study.

The inclusion in the study of all subjects is free and subject to the acquisition of the informed consent of the parents / legal representatives and - whenever possible - the subject's consent. The study protocol was approved by the local Ethics Committee and was conducted according to the Declaration of Helsinki.

#### *Study protocol*

Based on the available literature and the results of our review, we assembled a neuropsychological battery encompassing standard assessments (i.e., general cognitive profile, executive functions, memory, attention) but also less explored social cognition skills such as theory of mind and emotional recognition. In particular, we adopted two standardized tests designed for a general cognitive and neuropsychological assessment:

- Wechsler cognitive scales: we adopted the Wechsler Intelligence Scale for Children IV<sup>152</sup>. All the items were administered to calculate the general IQ and the other indexes (Verbal Comprehension Index (VCI), Perceptual Reasoning Index (PRI), Working Memory Index (WMI), and Processing Speed Index (PSI))
- NEPSY-II<sup>151</sup>: selected tasks from the Attention and Executive Functioning (Visual Attention, Design Fluency, Auditory Attention and Response Set, Inhibition and Animal Sorting), Memory and Learning (Memory List), and Social Cognition (Theory of Mind and Affect Recognition) domains were adopted.

#### *Statistical planning and data analysis*

At the time of the project conceptualization, no other studies had investigated social cognition in DMD samples. Therefore, the sample size and effect size were hypothesized based on preliminary data derived from the results of NEPSY-II adoption in a small group of DMD patients (n=11) during routine clinical assessment. In particular, the mean performance in the Social Cognition tasks (Theory of Mind and Affect Recognition) and the median standard score value were 5.00 (SD 3.95) and 4.60 (SD 2.95). We applied the results from both tasks to calculate the effect size, and we adopted the larger resulting sample size (i.e., the one derived from the preliminary results of the Theory of Mind task). The software we used was G \* Power Version 3.1.9.6103,104, and an “a priori” analysis of the required sample size was performed (alpha error 0.05, power 0.95, one tail; considering an effect size d = 1.27).

A minimum necessary sample size of 9 patients was obtained. As the procedures were not invasive and the assessments were planned to be included in the routine check-up program, we

expected a minimum dropout. Thus, it was assumed to adopt an oversampling of 20% (minimum initial sample: 11 patients).

The scores obtained from the administration of standardized tests were analyzed by an independent samples t-test (or non-parametric analog) to verify the presence of significant differences between the performance of DMD patients and the normative data.

## Results

### *Descriptives*

All patients were males without cardiac-respiratory involvement. The overall general features of the sample are summarized below in Table 2; the descriptive analysis of the sample split into two subgroups based on the theoretical depletion of the Dp140 isoform of dystrophin is also provided.

	DMD	Dp140+	Dp140-
<i>Number of patients</i>	22 (100%)	9 (41%)	13 (59%)
<i>Age [y]*</i>	12.27 (3.07; 7 – 17)	13.33 (3.32; 7-17)	11.54 (2.79;7-16)
<i>Age at diagnosis [y]*</i>	3.64 (1.71;1-7)	3.22 (1.30; 1-5)	4.92 (1.94;1-7)
<i>Assuming corticosteroids**</i>	95.5% (21/1)	89% (1/8)	100% (0/13)
<i>Duration of the therapy with corticosteroids [m]*</i>	95.48 (43.36;17-184)	111.13 (52.55;41-184)	85.85 (35.47;17-143)
<i>Ambulatory**</i>	73% (16/4)	56% (5/4)	85% (11/2)

**Table 2 - Clinical overview of the sample:** data about age, school attendance, age at symptoms onset/diagnosis, therapy, ambulation, and cardiac/respiratory function are summarized in the table.

(Tot = all included DMD patients; Dp140+ = subgroup of DMD patients with mutation theoretically not affecting the expression of Dp140; Dp140- = subgroup of DMD patients with mutation theoretically affecting the expression of Dp140)

\*mean (SD; range)

\*\* % of patients presenting the condition mentioned in the first column (n°pts presenting the condition/n° patients not presenting the condition)

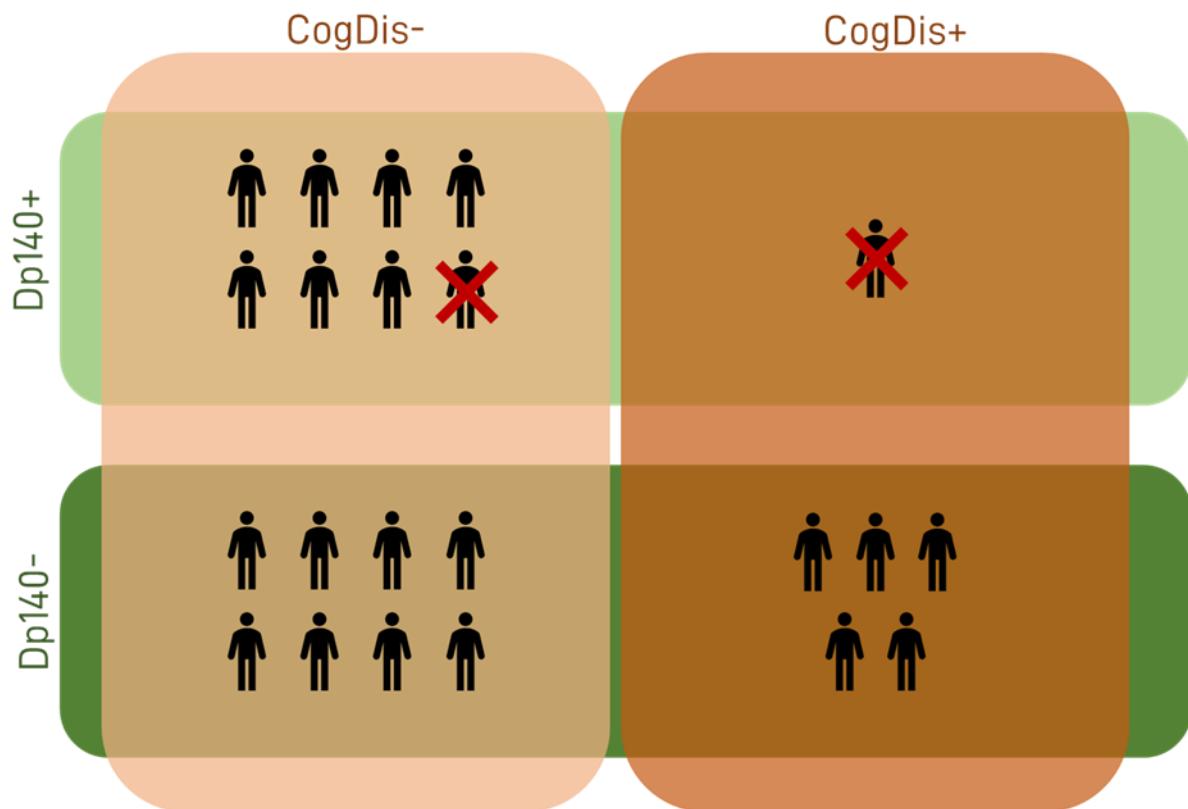
The mean overall IQ of the included patients was 83.73, which aligns with the one-standard-deviation shift described in the literature for DMD patients compared to the general population (see Table 3).

Wechsler indexes	Tot	Dp140+	Dp140-	Student's t-test (p-value)
<i>Full Intellectual Quotient*</i>	83.73 (22.57; 40-127)	92.11 (17.04;67-127)	77.92 (24.68;40-115)	1.491 (0.152)
<i>Verbal Comprehension Index*</i>	93.50 (22.00; 54-126)	94.56 (19.91; 54-121)	92.77 (24.10;56-126)	0.183 (0.857)
<i>Visual-Spatial Index*</i>	95.77 (23.92;48-139)	105.11 (16.68;85-139)	89.31 (26.55;48-128)	1.577 (0.131)
<i>Working Memory Index*</i>	73.32 (20.11;46-123)	83.44 (23.46;55-123)	66.31 (14.50;46-85)	2.124 (0.046)
<i>Processing Speed Index*</i>	82.36 (15.06;53-106)	90.22 (8.96;74-103)	76.92 (16.28;53-106)	2.218 (0.038)

**Table 3- Cognitive assessment:** an overview of the scores obtained by administering Wechsler's scales for general cognitive assessment.

(Tot = all included DMD patients; Dp140+ = subgroup of DMD patients with mutation theoretically not affecting the expression of Dp140; Dp140- = subgroup of DMD patients with mutation theoretically affecting the expression of Dp140)

\*mean values (SD; range)



**Figure 7 - Sample descriptives:** the features of our sample in terms of cognitive profile and expression of the Dp140 isoform of dystrophin are summarized in the figure.  
 Legend: Dp140+ = subgroup of DMD patients with mutation theoretically not affecting the expression of Dp140;  
 Dp140- = subgroup of DMD patients with mutation theoretically affecting the expression of Dp140; CogDis+ =  
 subgroup of DMD patients with IQ<71; CogDis- = subgroup of DMD patients with IQ>70

#### *Social cognition:*

We administered the sub-items “Theory of mind” and “Affect recognition” from the “Social Cognition” domain of NEPSY-II to our sample; two boys were not able to complete the assessment because of their severe cognitive deficit. Thus, the total number of subjects included in this analysis was 20.

Our sample size was limited, and a control group was not included. Thus, we analyzed the median standard scores and compared them to the reference score reported in the normative data of the test (i.e., 10). The overall scores obtained by our subjects were significantly lower than the reference in both sub-items; such difference was also confirmed when the sample was split based on the presence of Dp140 depletion (Dp140+ vs. Dp140-) or cognitive deficit (CogDis+ vs CogDis-; threshold: total QI<71) and the same analysis was singularly repeated on each subgroup (see Table 4)

NEPSY-II “Social cognition” subtests	tot	One sample -test**	Dp 140+	One sample t-test**	Dp 140-	One sample t-test**	Cog Dis+	One sample t-test**	Cog Dis-	One sample t-test**
Number of subjects	20	***	7	***	13	***	5	***	15	***
<i>Theory of mind*</i>	5;5.55 (3.99;1-12)	13.5 (0.001)	5;5.29 (4.50;1-12)	2.500 (0.030)	5;5.69 (3.88;1-12)	5.000 (0.004)	2;2.40 (1.67;1-5)	0.000 (0.029)	6;6.60 (4.01;1-12)	13.500 (0.008)
<i>Affect recognition*</i>	5;4.95 (3.35;1-10)	0.000 (<.001)	6;7.00 (2.58;4-10)	0.000 (0.029)	2;3.85 (3.26;1-10)	0.000 (0.001)	1;1.20 (0.45;1-2)	0.000 (0.024)	6;6.20 (2.91;1-10)	0.000 (0.001)

**Table 4 - Social Cognition - part 1:** an overview of the standard scores obtained by administering the specific battery of the NEPSY-II scale. The standard scores obtained by DMD were tested for differences from the reference scores (test value: 10) by a non-parametric test due to a deviation from the assumption of normality (Shapiro-Wilk test). (Tot = all included DMD patients; Dp140+ = subgroup of DMD patients with mutation theoretically not affecting the expression of Dp140; Dp140- = subgroup of DMD patients with mutation theoretically affecting the expression of Dp140; CogDis + = subgroup of DMD patients with general QI<71 at the Wechsler scale; CogDis - = subgroup of DMD patients with general QI>70 at the Wechsler scale)

Note. For all tests, the alternative hypothesis specifies that the median is less than 10.

\*median; mean standard scores (SD; range)

\*\*Wilcoxon signed-rank test (p-value)

Then, the differences between each couple of subgroups were tested to explore the role of the cognitive deficit and the expression of the Dp140 dystrophin isoform. The only significant difference emerged in the “affect recognition” sub-item, with the subset of patients with cognitive deficit scoring worse than those without it. Nevertheless, it is noteworthy that a similar difference in the “affect recognition” scores also emerged between the Dp140+ (better) and the Dp140- (worse) subgroups, with a p-value close to significance (see Table 5 and Figure 8).

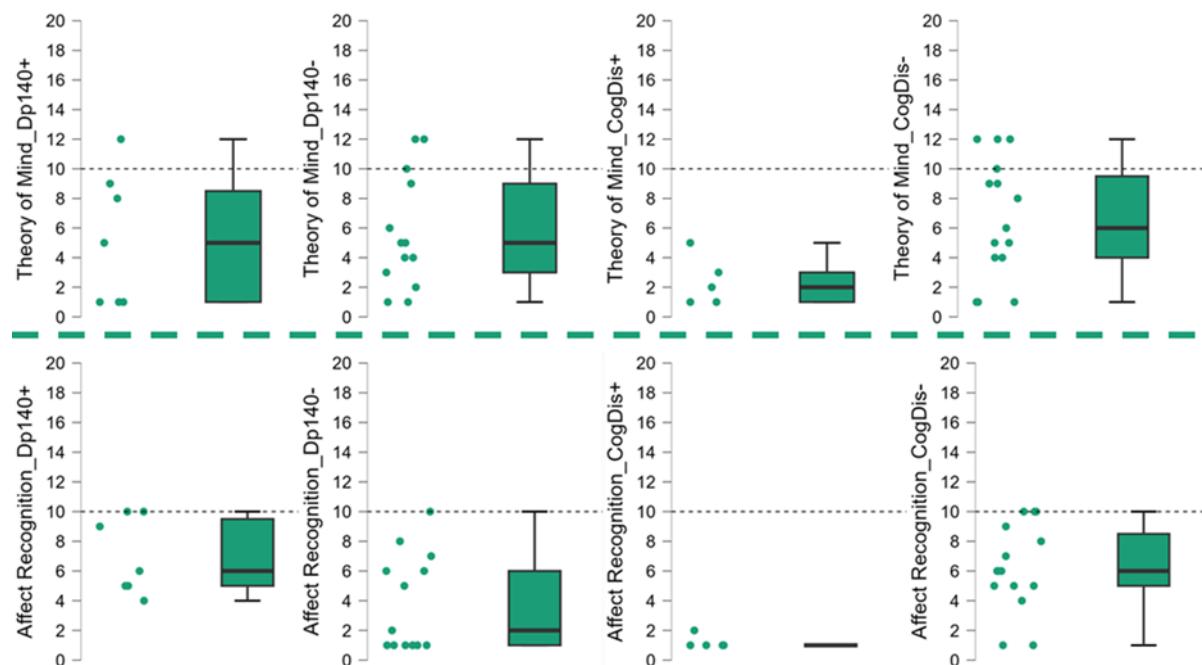
NEPSY-II "Social cognition" subtests	<b>tot</b>	<b>Dp140+</b>	<b>Dp140-</b>	<b>independent samples t-test**</b>	<b>Cog Dis+</b>	<b>Cog Dis-</b>	<b>independent samples t-test**</b>
Number of subjects	20	7	13	***	5	15	***
<i>Theory of mind*</i>	5;5.55 (3.99; 1-12)	5;5.29 (4.50; 1-12)	5;5.69 (3.88; 1-12)	40.500 (0.718)	2;2.40 (1.67;1-5)	6;6.60 (4.01; 1-12)	60.000 (0.052)
<i>Affect recognition*</i>	5;4.95 (3.35; 1-10)	6;7.00 (2.58; 4-10)	2;3.85 (3.26; 1-10)	68.000 (0.076)	1;1.20 (0.45;1-2)	6;6.20 (2.91; 1-10)	69.000 (0.006)

**Table 5 - Social Cognition - part 2:** an overview of the differences in standard scores obtained by administering the specific battery of the NEPSY-II scale between subgroups based on the presence/absence of Dp140 depletion and cognitive deficit. The standard scores were tested for differences by a non-parametric test due to deviation from the assumption of normality (Shapiro-Wilk test).

(Tot = all included DMD patients; Dp140+ = subgroup of DMD patients with mutation theoretically not affecting the expression of Dp140; Dp140- = subgroup of DMD patients with mutation theoretically affecting the expression of Dp140; Cognitive Deficit + = subgroup of DMD patients with general QI<71 at the Wechsler scale; Cognitive Deficit - = subgroup of DMD patients with general QI>70 at the Wechsler scale )

\*median standard scores; mean standard scores (SD; range)

\*\* Mann-Whitney U test (p-value)



**Figure 8 - Social Cognition - part 1:** the graphs refer to the data shown in Table 4 and represent the performance of our sample in the "social cognition" items of the NEPSY-II scale, showing the data dispersion and the differences between the standard scores from the reference value (=10).

Legend: Dp140+ = subgroup of DMD patients with mutation theoretically not affecting the expression of Dp140; Dp140- = subgroup of DMD patients with mutation theoretically affecting the expression of Dp140; CogDis + = subgroup of DMD patients with general QI<71 at the Wechsler scale; CogDis - = subgroup of DMD patients with general QI>70 at the Wechsler scale

Lastly, the same analysis was applied to the subset of subjects with total QI>70 (CogDis-) to assess the role of the expression of the Dp140 dystrophin isoform without the possible confounding effect of cognitive disability. No significant differences emerged, but interestingly, the divergence between the Dp140+ and the Dp140- subgroups in the “affect recognition” scores aligned with the results of the previous analysis, even excluding the subjects with a cognitive deficit, with a worse performance in the Dp140- subgroup (see Table 6 and Figure 9 and 10).

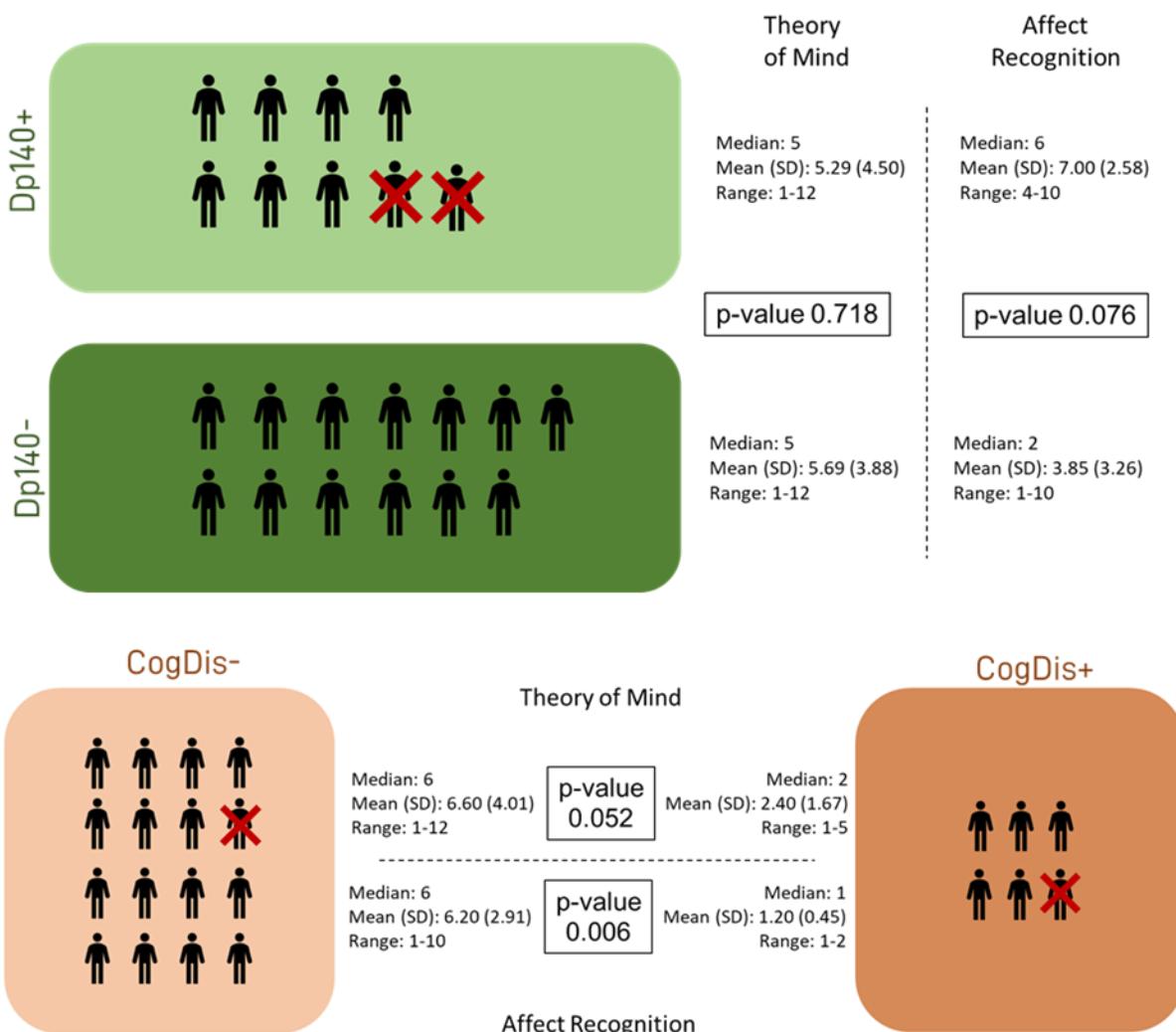
NEPSY-II “Social cognition” subtests	<b>tot</b>	<b>Dp140+</b>	<b>Dp140-</b>	<b>independent samples t-test**</b>
Number of subjects	15	7	8	***
<i>Theory of mind*</i>	6;6.60 (4.014;1-12)	5;5.286 (4.499; 1-12)	7.50; 7.75 (3.412;4-12)	-1.205(0.250)
<i>Affect recognition*</i>	6;6.20 (2.908;1-10)	6;7.00 (2.582;4-10)	6;5.50 (3.162;1-10)	0.996 (0.337)

**Table 6 - Social Cognition - part 3:** an overview of the differences in standard scores obtained by the administration of the specific battery of the NEPSY-II scale between subgroups based on the presence/absence of Dp140 depletion (only subjects without cognitive deficit, i.e., QI>70).

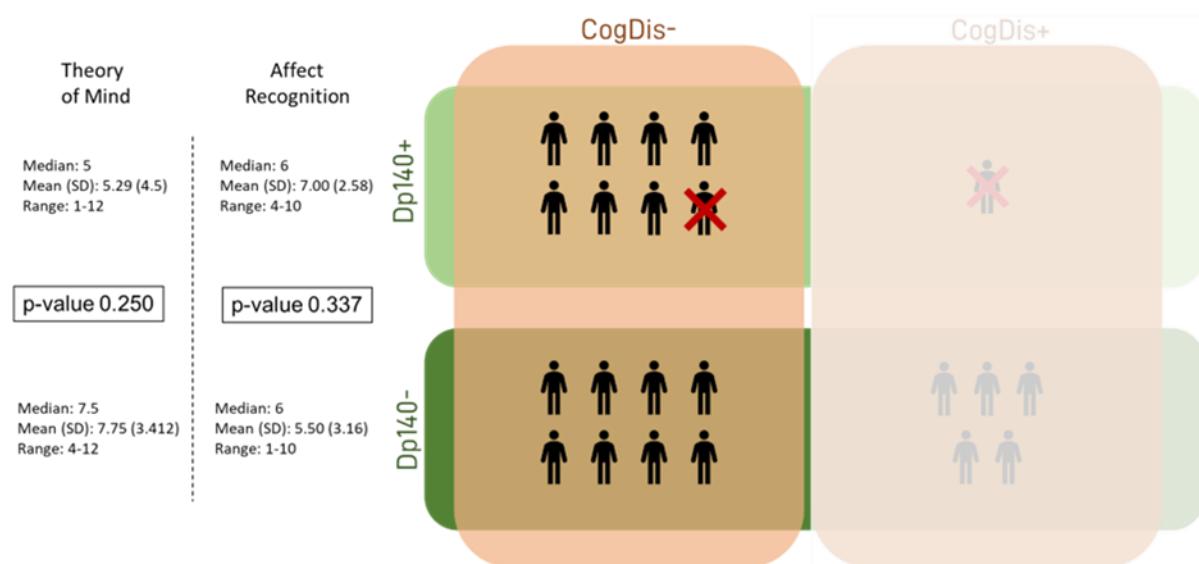
(Tot = all included DMD patients; Dp140+ = subgroup of DMD patients with mutation theoretically not affecting the expression of Dp140; Dp140- = subgroup of DMD patients with mutation theoretically affecting the expression of Dp140)

\*median standard scores; mean standard scores (SD; range)

\*\* Student's t-test (p-value)



**Figure 9 - Social Cognition - part 2:** the figure refers to the data in Table 5. It represents the differences in standard scores obtained in our sample by administering the specific battery of the NEPSY-II scale. The differences were tested between subgroups based on the presence/absence of Dp140 depletion and cognitive deficit.  
 Legend: Dp140+ = subgroup of DMD patients with mutation theoretically not affecting the expression of Dp140; Dp140- = subgroup of DMD patients with mutation theoretically affecting the expression of Dp140; CogDis + = subgroup of DMD patients with general QI<71 at the Wechsler scale; CogDis - = subgroup of DMD patients with general QI>70 at the Wechsler scale



**Figure 10 - Social Cognition - part 3:** this figure refers to the data in Table 5. It represents the differences in standard scores obtained in our sample by administering the specific battery of the NEPSY-II scale. The differences were tested between subgroups based on the presence/absence of Dp140 depletion and cognitive deficit.  
 Legend: Dp140+ = subgroup of DMD patients with mutation theoretically not affecting the expression of Dp140; Dp140- = subgroup of DMD patients with mutation theoretically affecting the expression of Dp140; CogDis + = subgroup of DMD patients with general QI<71 at the Wechsler scale; CogDis - = subgroup of DMD patients with general QI>70 at the Wechsler scale

## Discussion

Neuropsychological batteries in the evaluation of DMD patients are very heterogeneous, and the most commonly adopted tools are Wechsler scales and tests assessing the functions traditionally described as impaired in DMD. Thus, the neuropsychological domains mainly assessed were language, visual-spatial skills, memory, attention, and executive functioning.

Social cognition skills are rarely included in the neuropsychological assessment of DMD patients in clinical practice or research settings.

With this background, we designed a clinical protocol that was, to our knowledge, one of the first studies investigating this domain in DMD patients. Interestingly, our social cognition assessment yielded intriguing results, in line with those recently published in a similar experimental setting<sup>45</sup>. Compared to the standard data, we consistently observed poor performances in our sample in the tasks assessing the theory of mind and recognizing the affective meaning of facial expressions.

In particular, among the social cognition skills we investigated, the impairment of affect recognition appeared more evident in our sample when comparing Dp140+ and Dp140- groups.

This finding is preliminary and should be taken with caution, given the low numerosity and the absence of significance when analyzing without the influence of cognitive disability. Nevertheless, it provides additional evidence of a possible specific impairment in this area as another feature of the CNS phenotype of DMD, consistent with a few other previous reports<sup>45,75</sup>.

Previous studies have not largely explored this area, and there is no clinical evidence of such an impairment (except for the increased risk of comorbidity with ASD in DMD patients). However, the recent data from the Human Brain Atlas pointed out the high levels of dystrophin expression in the amygdala and hippocampus. These structures are involved in the brain networks underlying the social functions (e.g., the extra-geniculostriatal network)<sup>153</sup>.

A common consideration about these hypotheses should be made. Various neural networks underlie all the mentioned functions, sometimes overlapping and involving the same CNS structures. Based mainly on functional neuroimaging, it has been possible to identify the brain regions involved in executive (involving the prefrontal and parietal cortex, basal ganglia, thalamus, and cerebellum) and social functioning (amygdala, portions of occipital-temporal-frontal cortex, the Default Mode Network). Furthermore, these deficits are common to many neuropsychiatric diseases. Consequently, identifying the exact cause of an impairment widely involving these functions may not be obvious. For example, the hypothesis of cerebellar dysfunction was supported by clinical features (e.g., deficit in cognitive functions or memory) and evidence from animal models. Still, it was reconsidered based on analyzing dystrophin expression in the human brain. Nevertheless, the involvement of the same networks at another level remains plausible.

Future development of these preliminary findings might involve designing an experimental setting to specifically explore the behavioral correlates of the involvement of those CNS networks, including the subcortical structures characterized by a higher dystrophin expression.

## 2.4.FACE-DMD: Facial Aspect and implicit Cognition of Emotions in Duchenne Muscular Dystrophy

Recent anatomopathological studies have clarified the expression pattern of dystrophin in the human central nervous system (CNS). In particular, the highest levels of dystrophin expression have been found in sub-cortical structures such as, for example, amygdala, hippocampus, etc.<sup>10</sup>.

The involvement of the amygdala in several neurological diseases<sup>154,155</sup> involves a deficit in the emotional interpretation of facial expressions. This element is not reported in the classic clinical picture of Duchenne Muscular Dystrophy (DMD). However, the preliminary results of a study conducted in our center (CNSDMD Study) with the NEPSY battery (item “Emotion Recognition”) on DMD patients suggest a deficit in this function, reported in a few articles<sup>45,75</sup>.

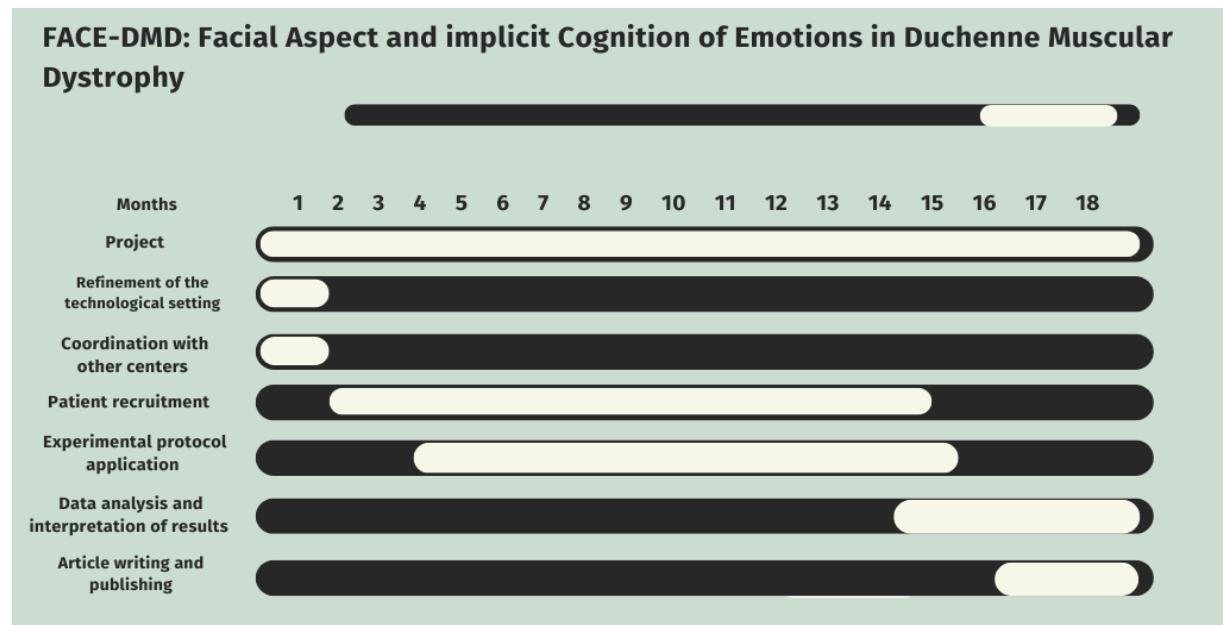
In DMD, difficulties in social interaction are generally interpreted as secondary to the progression of the disease or attributed to concomitant disorders (e.g. intellectual disability).

However, the interpretation of the emotional valence of facial expressions is also crucial in social functioning and interpersonal relationships<sup>93</sup>. This function is based on two processes (conscious-explicit and unconscious-implicit recognition) that underlie different neural substrates, interconnected but distinct<sup>153</sup>: the geniculostriatal system and the extra-geniculostriatal system. Studies on patients with striatal lesions have allowed us to identify the specific activation of subcortical structures during tasks of unconscious perception of visual stimuli of emotional significance<sup>156</sup>.

The impairment of the extra-geniculostriatal system could therefore also play a role in the difficulties of social interaction of DMD patients. These structures are specifically responsible for implicit emotional processing functions. It is possible to study this phenomenon through backward masking techniques, testing in particular the influence of subliminal emotional priming<sup>157</sup>. Several studies have shown that it is possible to measure the behavioral effects of priming following the presentation of subliminal visual stimuli, therefore not perceived at a conscious level<sup>158,159</sup>. In this context, the adoption of an emotional priming paradigm in patients affected by DMD would allow us to specifically document the possible neuropsychological manifestation of an impairment of the subcortical structures (in particular the amygdala and thalamus).

## Methods

The study is a multi-center experimental case-control study involving the IRCCS Mondino Foundation (Pavia), the IRCCS “Eugenio Medea” (Bosisio Parini), “Città della Salute e della Scienza” - University of Turin (Turin), University of Udine and IRCCS Fondazione Stella Maris (Pisa). The study has a planned duration of about 18 months from the approval of the Ethics Committee of the coordinating center (January 2024). Figure 11 shows the planned timeline of the study.



**Figure 11 – Project FACE DMD:** the Gantt chart represented in the figure is the planned timeline of the project

### *Study population*

The study included three groups: the DMD group (cases), the healthy control group, and the control group with different neuromuscular diseases. The criteria used to select the participants are outlined below.

### Criteria for the DMD group

Patients were identified from the clinical database of the participating centers, from individuals diagnosed with DMD, and from incident cases referred to the different centers. They were selected according to the following criteria:

- Inclusion: male sex; availability of the genetic diagnosis of DMD; age between 6 and 25 years and 11 months (inclusive); good knowledge of the Italian language.
- Exclusion: other concomitant genetic diseases, psychiatric comorbidities such as ADHD, patients diagnosed with autism spectrum disorder and other disorders that may affect emotional processing, presence of visual impairments that do not allow the procedure to be performed.

#### Healthy control group criteria

Healthy controls were recruited from a variety of sources (e.g. acquaintances of patients, local schools, or clubs) and matched for gender and age. They were pre-screened for possible concomitant neuropathologies by an experienced clinician by collecting anamnestic information and performing an objective examination. They were selected according to the following criteria:

- Inclusion: male sex, age between 6 and 25 years and 11 months (inclusive), good knowledge of the Italian language.
- Exclusion: presence of neuropsychiatric disorders, presence of concomitant genetic disorders, presence of visual disorders that did not allow the procedure to be performed.

#### Criteria for the control group with different neuromuscular disorders

Patients were selected from the clinical database of the participating centers, among those diagnosed with neuromuscular disease, and among the incident cases referred to the different centers. They were selected according to the following criteria:

- Inclusion: male sex, age between 6 and 25 years and 11 months (inclusive), availability of a clinical and/or genetic diagnosis of neuromuscular disease, good knowledge of the Italian language.
- Exclusion: diagnosis of dystrophinopathy (DMD, BMD or intermediate forms), presence of other concomitant genetic diseases, psychiatric comorbidities such as ADHD, patients diagnosed with autism spectrum disorder and other disorders that may affect emotional processing, presence of visual impairments that do not allow the procedure to be performed.

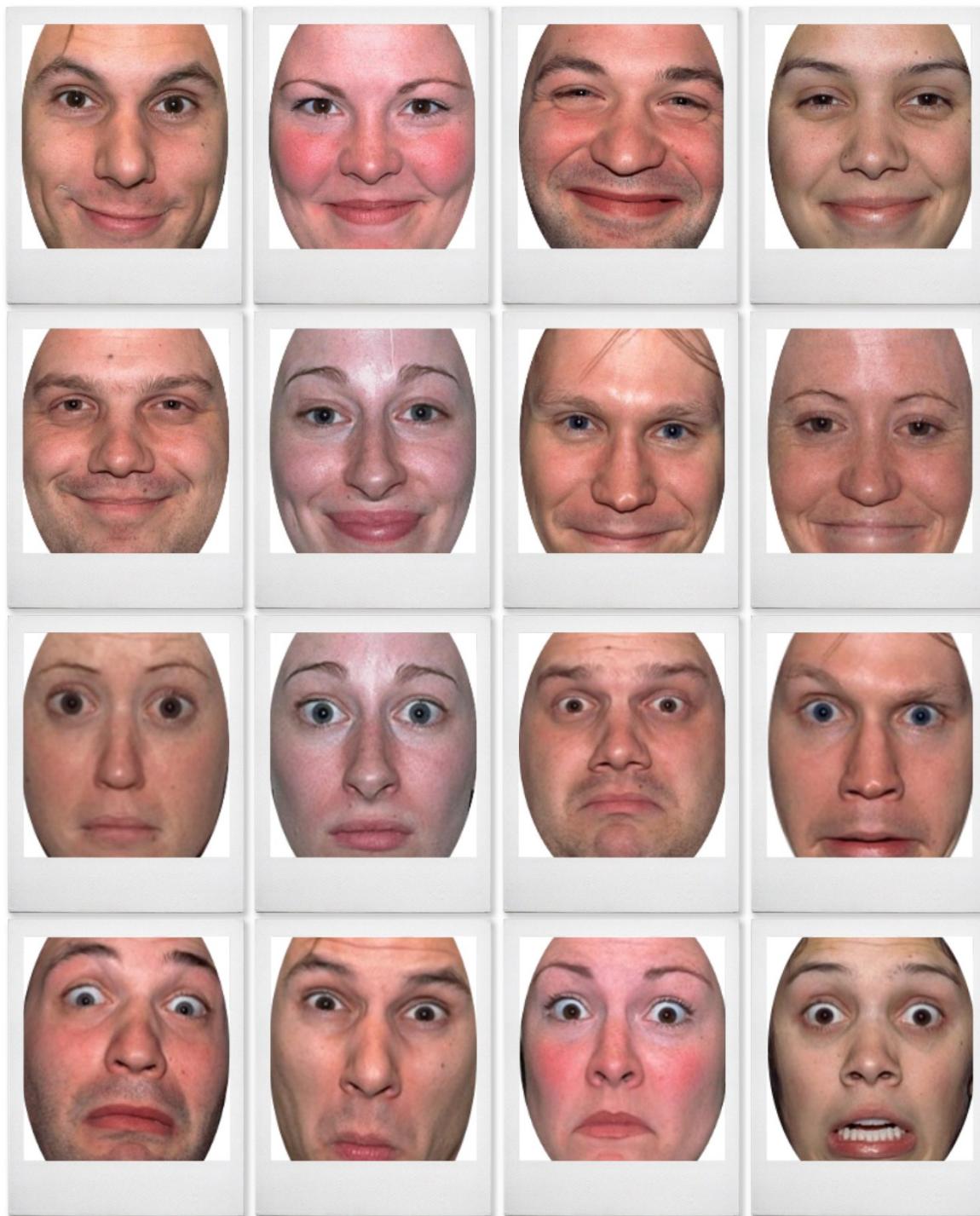
The inclusion of all subjects in the study was free of charge and subject to the informed consent of the parents/legal representatives and, where possible, the consent of the subject.

### Sample size

Sample size estimation analyses were performed using Gpower software (option: Cohen's f calculated as in SPSS). In a mixed design Anova 2x2x3 test with task (emotion vs. gender recognition) and priming condition (congruent vs. incongruent) as within-subjects factors and group (DMD vs. clinical controls vs. healthy controls) as between-subjects factors, a moderate to large estimated effect size (Cohen's f) of 0.35 (obtained by further assuming an equal standard deviation in all groups of 20), a power of 80% and an alpha value of 0.05, the total number of subjects to be recruited is 84. Taking into account the possible exclusion of a small percentage of subjects (<10%) who may not complete the tests, it was considered necessary to recruit 30 subjects per group (90 in total).

### *Tools*

The task was programmed using E-prime 3.0 software and the stimuli were displayed on a 15-inch screen at a distance of approximately 50 cm from the participants. The 16 expressions (Figure 12) used were prototypes of fear and happiness derived from 8 model faces (four male and four female) taken from the NimStim Face Stimulus Set<sup>160,161</sup>. A PC with technical characteristics (graphics card, communication protocols with the monitor, process) suitable for the experiment was used, in particular an HD monitor with a high refresh rate. Study data were collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at Fondazione Mondino. REDCap is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.



**Figure 12 – The “emotion recognition” stimuli:** the figure shows the 16 facial expressions used in the experiment. The pictures were prototypes of fear and happiness derived from 8 model faces (four male and four female) taken from the NimStim Face Stimulus Set

### Procedure

The study involved presenting visual stimuli to the subject via a monitor with appropriate resolution and refresh rate characteristics connected to a dedicated laptop with appropriate GPU and peripheral connection characteristics. A total of 4 tests were performed:

- a test of implicit emotion processing (facial expressions): 4 blocks (1 practice + 3 tests) of 32 items, except for the practice block which contains 16 trials; in each item, the stimuli were presented sequentially. Subjects were asked to indicate the target emotion, which was preceded by a face expressing the same emotion (congruent priming) or a different emotion (incongruent priming);
- an explicit emotion processing test: superimposable on the previous one, with the prime presented for a sufficient time to ensure conscious perception;
- a control test: superimposable on the previous one, but with a priming stimulus of a non-emotional valence. Specifically, subjects were asked to identify the gender of faces. The structure and stimuli are identical to those of the emotion processing test. Subjects were asked to indicate the gender of the target, preceded by a face of the same gender (congruent priming) or of the opposite gender (incongruent priming). The control test was also administered in the two versions of implicit vs. explicit recognition.

The experimental procedure was carried out at the sites of the participating clinical centers in a session of approximately 45-60 minutes, either in specially scheduled outpatient sessions or as part of the routine clinical check-ups scheduled in outpatient or inpatient settings.

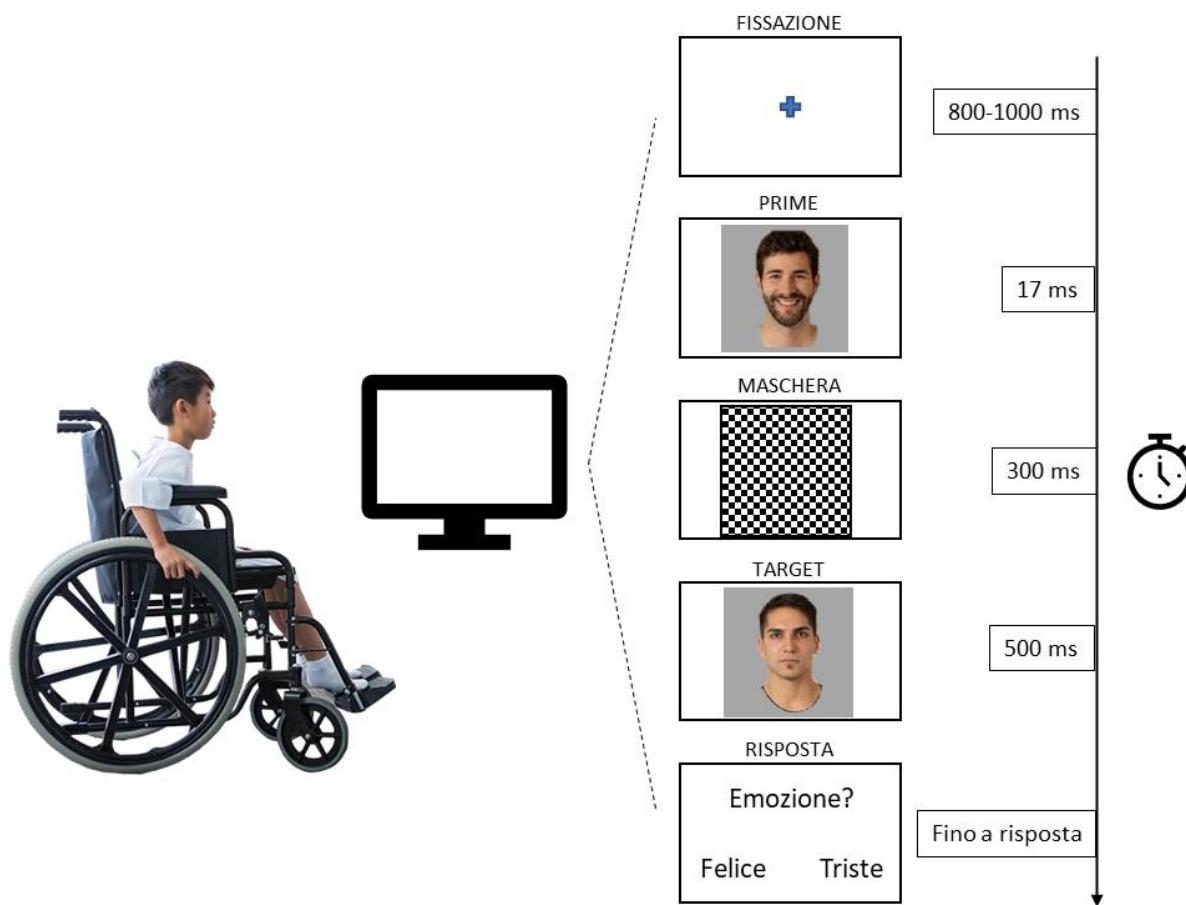
The prime in the subliminal version was 17 ms, while the prime in the supraliminal version was 30 ms.

All tasks were preceded by prompts that clearly explained to the subject what they were about to be shown, and they were given instructions on how to answer.

The tasks were structured as follows: facial expressions were presented in the order shown in Figure 13. First, a cross was shown to fix the participant's attention to the center of the screen for 1000-1500 ms. This was followed by the prime expression (of happiness or fear). By setting the refresh rate to 60 Hz (min 59; max 61), the actual duration of the prime varies between 16 and 17 ms (or between 33 and 34 ms). This was followed by a mask that remains on the screen for 300 ms, then the target for 500 ms. After the target, there was a blank until the response (unless the subject responded during the presentation of the target). Subjects were asked to

respond to the target as quickly as possible by pressing a key between 'z' and 'm'. The z/m response keys were counterbalanced across subjects so that the association between response and key was set one way for 50% of the sample (e.g. joy 'z' and fear 'm') and the opposite way for the other 50% of the sample (e.g. joy 'm' and fear 'z'). The faces were paired so that the identity of the prime was always different from that of the target. In emotional priming, the gender of the face remained unchanged between prime and target (all female/all male), just as in gender priming, the emotion of the face remained unchanged between prime and target (all happy/all fearful). In both tasks, the prime was 100% morphing (i.e. 100% emotional expressiveness), while the target was 70% morphing (i.e. the faces have 30% neutral facial features and 70% emotional features, which should make recognition slightly more difficult).

Data on neuropsychological functioning were obtained by administering the NEPSY-II (social cognition section)<sup>151</sup> and Wechsler tests<sup>152</sup> (WAIS, WISC, or WPPSI, depending on the age of the subject). Data were extracted retrospectively if the tests were administered within the 6 months before recruitment as part of normal clinical practice controls. Otherwise, the neuropsychological test battery was administered at the sites of the participating clinical centers, in a session of approximately 120 minutes, during specially scheduled outpatient sessions, or as part of routine clinical controls in the outpatient or inpatient setting. For the VABS-II socialization section, data were also collected retrospectively if the tests were administered within 6 months before recruitment, otherwise, sessions of approximately 30 minutes are scheduled for collection.



**Figure 13 - The subliminal priming protocol:** the figure represents a schematic representation of the structure of the experimental process of subliminal priming.

#### Data Analysis

It was expected that DMD subjects would perform worse than the other two groups (where no relevant difference is expected). Considering the 96 items on which each subject was tested for implicit emotion processing (32 for 3 blocks, excluding the adaptation battery from the analyses), the percentage of correct responses on the 96 measures was taken as the outcome value (range from 0 to 100) for each priming condition. Assuming that the subjects with DMD would respond slightly more than randomly (approximately 60 percent correct), the expected number of correct responses was 58 out of 90, whereas it was higher for the other two groups (77 out of 96 responses, i.e. approximately 80 percent). In addition, the average time to give a correct answer for each condition was taken into account. Responses under 150 ms were excluded as anticipations and over 5 sec as non, so the percentage was calculated on the total number of responses considered valid.

The variables collected were described in terms of mean and standard deviation (or median and interquartile range, if appropriate) if continuous, and in terms of counts and percentages if categorical. The distribution of continuous variables was examined using Shapiro-Wilk tests or graphs.

The percentage of correct responses (accuracy) on tests assessing explicit emotion recognition was calculated for each emotional/gender priming condition (i.e. congruent vs. incongruent with target) to produce a score ranging from 0 to 100 and analyzed as a continuous variable. The same type of calculation was performed for the mean of the correct response times (TR) for each condition.

Accuracy (%) and response times were analyzed separately with a 2x2x3 mixed-design ANOVA, with congruence (2 levels, congruent vs. incongruent) as the repeated within-subjects variable and group (3 levels, DMD, controls, patients with other neuromuscular disease) as the group factor. If, due to the nature of the variable, it did not meet the normality conditions, the use of an appropriate non-parametric test is considered instead of the mixed design ANOVA with 2 priming conditions (i.e. congruent vs. incongruent) for three groups.

Post-hoc comparisons were made using pairwise tests with Tukey HSD correction.

The data from the explicit vs. implicit recognition tests were analyzed with separate mixed-design ANOVAs.

## Results

Twenty-one patients were included in the study: 11 healthy controls (HC), 5 controls with other non-dystrophinopathic neuromuscular diseases (NMD-C), and 5 DMD patients.

The healthy controls were recruited through voluntary participation in the project by friends, acquaintances, and children of the medical staff of the IRCCS Fondazione Mondino. The subjects were heterogeneous in age, with a minimum of 8 years and a maximum of 25 years. None of them suffered from any disease and none were on any long-term medication. The data should therefore be considered 'free' of any influence. All subjects were educated. All of them were ambulant.

For the recruitment of participants with other neuromuscular diseases, an attempt was made to create as homogeneous a group as possible so that the data obtained could be related to the phenotype of the individual disease and to avoid confounding bias. Therefore, two patients with

SMA 2 (spinal muscular atrophy type 2), two patients with SMA 3 (spinal muscular atrophy type 3), and one patient with myasthenia congenita due to CHRNE gene mutation on chr 17 were recruited into this group (see Table 7).

Spinal muscular atrophy is a hereditary disease that usually begins in childhood mainly affecting spinal motor neurons. Genetically, 95% of cases have an allelic alteration in chromosome 5q11.2-13.3, corresponding to the SMN1 (survival of motor neuron 1) gene, which encodes a ubiquitous protein involved in the process of apoptosis, but also with functions in the regulation of DNA transcription. The different clinical forms of SMA are characterized by the type of mutation in the SMN1 gene and by allelic differences in a second gene, called SMN2, which acts as a modifier gene. SMA 2 usually begins in the first two years of life and has a slower progression than type 1, but is still fatal. SMA 3 has an even later onset, but still in childhood. The clinical picture is characterized by motor deficit and atrophy, particularly of the cingulate muscles<sup>10</sup>. The prognosis varies depending on which muscle district is affected. Recent studies highlighted that the general cognitive functioning of SMA patients is mainly within the range of normality, with a profile characterized by specific deficits in some areas (e.g., attention and executive functions)<sup>162</sup>. Nonetheless, those findings defined a picture where patients with SMA2 and 3 are likely to be less cognitively affected than those with SMA1 and have relational-communicative skills comparable with their non-affected peers. On the other hand, the studies<sup>163-165</sup> specifically investigating the cognitive profile of SMA1 patients (excluded from our control group) reported significant deficits even in domains not directly affected by the motor impairment (i.e. attention, language, executive functions). Interestingly, a recent international survey<sup>166</sup> showed a high percentage of treated early-onset SMA patients with neurodevelopmental comorbidities. Besides, a study based on newborn SMA screening in Germany<sup>167</sup> described a significant subset of early-treated children identified through the screening showing cognitive development impairment. This finding is still controversial, as a similar study on a Belgian cohort of patients identified by screening yielded opposite results<sup>168</sup>.

Several drugs are approved for the treatment of SMA. However, the use of these drugs depends on factors such as age, SMN2 copy number, type of SMA, and use of previous treatments. Of the four SMA patients recruited into the study, three are taking Nusinersen (brand name Spinraza) and one is taking Risdiplam.

The congenital myasthenic syndromes (CMS) include various disorders linked by abnormal signal transmission at the motor endplate because of defects in single or multiple proteins. The

diagnosis of a CMS can be suspected based on some clinical signs: the onset at birth to early childhood, weakness and fatigability affecting especially the ocular and other cranial muscles, a positive family history, and a decremental EMG response or an abnormal single-fiber EMG. The convergence of clinical and EMG studies to a candidate gene is a great support to the genetic diagnostic workflow of a specific CMS: BGS studies or commercial testing for CMS mutations in previously identified CMS genes are best used when targeted on specific clinical clues. Current therapies for the CMS include cholinergic agonists, namely pyridostigmine and 3,4-diaminopyridine (3,4-DAP), long-lived open-channel blockers of the AChR ion channel, and adrenergic agonists.

FEATURES OF NMD PATIENTS CONTROL GROUP				
	Age	Disease	Age of Diagnosis	Ambulation
Subject 1	14y 1m	SMA 3	5y	Yes
Subject 2	19y 11m	SMA 2	3y	No
Subject 3	7y 7m	SMA 2	1y	No
Subject 4	20y	SMA 3	5y	Yes
Subject 5	10y 11m	Congenital myasthenia	9y	Yes

**Table 7 – NMD controls cohort:** the main general features of the subjects recruited as controls with neuromuscular disorders are summarized in the table.

All the DMD patients enrolled in the project were on daily deflazacort treatment. Of these, four were ambulatory at the time of the various tests and one was non-ambulatory, confined to a wheelchair. Of these, four had a deletion of the dystrophin gene as a mutation and one had a duplication. All the deletions affected three isoforms: Dp427, Dp260, and Dp140 (Dp140-). The duplication affected only Dp427 isoform (Dp140+). See Table 8 for more details.

DMD PATIENTS FEATURES							
	Age	Type of mut.	Isoforms affected	Age of Diagnosis	Amb.	Therapy	Age at the start of therapy
P1	18y 8m	Dup.	Dp427	3	No	Daily deflazacort	7
P2	12y 7m	Del.	Dp427Dp260 Dp140	5	Yes	Daily deflazacort	6
P3	8y 8m	Del.	Dp427Dp260 Dp140	3	Yes	Daily deflazacort	5
P4	13y 9m	Del.	Dp427Dp260 Dp140	8	Yes	Daily deflazacort	8
P5	11y 6m	Del.	Dp427Dp260 Dp140	5	No	Daily deflazacort	6

**Table 8 - DMD patients cohort:** the major clinical features of the subjects included into the DMD cohort are summarized in the table.

(Legend: P= patient; Del.= deletion; Dup.= duplication; Amb.= ambulation; DMD=Duchenne Muscular Dystrophy)

In all three groups, an attempt was made to maintain a certain age heterogeneity, while respecting the inclusion/exclusion criteria, but the controls were slightly older in terms of age range, even if still comparable (Mean; Std. Deviation [range]: HC group 16.182; 6.585 [8-25], DMD group 12.400; 3.647 [8-18], NMD-C group 14.000; 5.612 (7-20) )

By the project protocol, the Wechsler IQ scales were administered to all participants to exclude subjects with an IQ below 70, i.e. with intellectual disability, so as not to invalidate the study results. The WAIS-IV and WISC-IV scales were administered based on age, whereas the WPPSI scale did not need to be administered as no preschool children were recruited. The raw scores of each subtest were transformed into weighted scores according to the age of the subject to compare the different results and to obtain the IQ score. In addition, the social cognition section of the NEPSY-II was administered to all participants, although some were over 16 years of age, again as described in the Materials and Methods section. This subscale consists of a Theory of Mind section and an Emotion Recognition section. Finally, the families of the participants were

administered the Socialisation subscale of the VABS-II by direct interview. This subscale consists of a section on interpersonal relationships, a section on play and leisure, and a section on social rules, from which the Deviation IQ Score can be derived. The scores obtained were converted into age-weighted scores. The results are presented in terms of mean and standard deviation in Table 9.

<b>Neuropsychological assessment (main scores)</b>				
<b>Test</b>	<b>Score</b>	<b>HC</b>	<b>DMD</b>	<b>NMD-C</b>
Wechsler Index: Full Intellectual Quotient score	<b>Mean; Std. Deviation (range)</b>	<b>112.091;</b> 15.030 (80-136)	<b>79.800;</b> 8.379 (70-88)	<b>105.800;</b> 12.716 (91-122)
NEPSY subtest: Total Theory of Mind (standard score)	<b>Mean; Std. Deviation (range)</b>	<b>10.182;</b> 3.188 (3-14)	<b>4.600;</b> 2.608 (1-7)	<b>8.000;</b> 3.391 (3-12)
NEPSY subtest: PS- Emotions Recognition (standard score)	<b>Mean; Std. Deviation (range)</b>	<b>8.909;</b> 2.300 (5-12)	<b>2.800;</b> 1.643 (1-4)	<b>7.400;</b> 4.159 (1-12)
VABS-II Socialisation subscale (Deviation IQ score)	<b>Mean; Std. Deviation (range)</b>	<b>114.455;</b> 12.283 (95-132)	<b>82.200;</b> 31.284 (31-110)	<b>101.800;</b> 18.458 (70-117)

**Table 9 – Neuropsychological assessment:** the table summarizes the main results obtained from the administration of Wechsler cognitive scales, NEPSY-II subtest, and VABS-II

Finally, all participants completed all the priming tests. These were administered in 50% of the cases from the emotional processing test (implicit before and explicit after) and in the other 50% of the cases from the non-emotional processing test (implicit before and explicit after). This was done to avoid the results being affected by better performance on the first test administered and poorer performance on the second test, due to tiredness or listlessness. In this way, the data obtained are cleansed of this possible bias. The administration environment of the priming tests was kept as similar as possible for the different participants, with the same lighting and acoustic characteristics.

At the end of each priming test, the E-prime 3 software automatically generates a results file with various parameters, including those of interest to us: target.ACC, which indicates the

accuracy of the response (1= correct; 0= incorrect); target.RT, which indicates the response time (in ms); Group, which indicates the group to which the subject belongs (HC, NMD-C, or DMD); Congruency, which indicates whether the prime is congruent or incongruent with the target; and finally ExperimentName, which indicates the type of test administered (task\_E\_17 and task\_E\_30 for the emotional processing test, implicit and explicit respectively; task\_S\_17 and task\_S\_30 for the non-emotional processing test, implicit and explicit respectively). An example of the software output of a DMD patient is shown in Figure 14. The outputs of the different tasks are merged for each subject or group using the E-Merge 3 option of the E-prime 3 software. Once the final output is obtained, it is filtered by excluding responses with the target.RT below 150 ms and above 5000 ms, according to the study protocol, and by eliminating responses corresponding to the first trial phase of each task. This filtering is possible thanks to an option, 'Filter', provided by the software itself. Another option, 'Analyze', provides results for each subject in terms of accuracy percentage (i.e. percentage of correct responses to the target) and average response time, broken down for each task by congruence. Figure 15 shows examples of the above.

	ExperimentName	Group	Subject	congruency	target.ACC	target.RT
1	task_E_17	DMD	25	incongruent	1	3540
2	task_E_17	DMD	25	incongruent	1	1687
3	task_E_17	DMD	25	congruent	0	1463
4	task_E_17	DMD	25	congruent	1	1069
5	task_E_17	DMD	25	incongruent	1	987
6	task_E_17	DMD	25	incongruent	1	759
7	task_E_17	DMD	25	incongruent	1	652
8	task_E_17	DMD	25	congruent	0	602
9	task_E_17	DMD	25	congruent	0	663
10	task_E_17	DMD	25	congruent	1	873
11	task_E_17	DMD	25	congruent	1	811
12	task_E_17	DMD	25	incongruent	1	968
13	task_E_17	DMD	25	congruent	1	1471
14	task_E_17	DMD	25	incongruent	1	685
15	task_E_17	DMD	25	congruent	1	768
16	task_E_17	DMD	25	congruent	0	1837
17	task_E_17	DMD	25	incongruent	0	1628
18	task_E_17	DMD	25	congruent	1	648
19	task_E_17	DMD	25	incongruent	0	663
20	task_E_17	DMD	25	incongruent	1	541
21	task_E_17	DMD	25	congruent	1	846

**Figure 14 – Software output:** the figure is an example of the E-Prime software output of a DMD patient.

The screenshot shows the 'Analyze' dialog box from the E-Prime software. The 'Variables' list includes: target.AC, target.CRESP, target.DEVICE, target.DurationError, target.OnsetDelay, target.OnsetTime, target.OnsetToOnsetTime, target.RESP, target.RT, target.RTTIME, target\_type, Trial, and type. 'target.RT' is selected and highlighted in blue. The 'Columns' section lists Group, ExperimentName, and congruency. The 'Rows' section lists Subject. The 'Data' section lists target.AC:Mean and target.RT:Mean. The 'Comments' and 'Filters' sections are empty. At the bottom are buttons for Save Analysis..., Load Analysis..., Filter..., Run... (highlighted in blue), New, and Close.

target.AC:Mean, target.RT:Mean by Subject and Group, ExperimentName, congruency							
	DMD	DMD	DMD	DMD	DMD	DMD	DMD
	task_E_17	task_E_17	task_E_30	task_E_30	task_S_17	task_S_17	task_S_30
Subject	Stats	congruent	incongruent	congruent	incongruent	congruent	incongruent
25	Mean target.AC	0,71	0,63	0,53	0,56	0,85	0,85
25	Mean target.RT	755,90	863,17	508,21	594,83	762,69	706,90
							621,04
							701,69

**Figure 15 – E-Prime analysis:** the figure is an example of the function “Analyze” of the E-Prime software and the relative output.

All the results obtained in the different sessions are presented in terms of percentage accuracy (see Figure 16) and response times (see Figure 17). The mean, standard deviation, minimum, and maximum are given for each.



		EMO_Percentage accuracy congruent implicit priming				EMO_Percentage accuracy incongruent implicit priming				EMO_Percentage accuracy congruent explicit priming				EMO_Percentage accuracy incongruent explicit priming			
Group		HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C	
Valid	11	5	5	5	11	5	5	11	5	5	11	5	5	5	5	5	
Missing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Mean	0.922	0.770	0.916	0.936	0.750	0.904	0.912	0.668	0.924	0.923	0.682	0.914	0.682	0.916	0.682	0.914	
SD	0.075	0.167	0.121	0.085	0.130	0.074	0.084	0.177	0.114	0.087	0.148	0.086	0.148	0.086	0.148	0.086	
Minimum	0.710	0.510	0.710	0.600	0.790	0.700	0.490	0.720	0.700	0.550	0.550	0.770	0.550	0.770	0.550	0.770	
Maximum	0.980	1.000	1.000	0.900	0.960	1.000	0.880	0.980	1.000	0.980	1.000	0.980	1.000	0.980	1.000	0.980	
		GEN_Percentage accuracy congruent implicit priming				GEN_Percentage accuracy incongruent implicit priming				GEN_Percentage accuracy congruent explicit priming				GEN_Percentage accuracy incongruent explicit priming			
Group		HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C	
HC	11	5	5	5	11	5	5	11	5	5	11	5	5	5	5	5	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0.934	0.868	0.946	0.928	0.886	0.948	0.918	0.790	0.940	0.924	0.800	0.916	0.800	0.916	0.800	0.916	0.800	
0.076	0.112	0.084	0.052	0.063	0.055	0.085	0.267	0.066	0.066	0.271	0.102	0.271	0.102	0.271	0.102	0.271	
0.790	0.690	0.810	0.850	0.810	0.890	0.740	0.320	0.850	0.810	0.330	0.770	0.330	0.770	0.330	0.770	0.330	
1.000	0.980	1.000	1.000	0.960	1.000	1.000	0.980	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	

**Figure 16 – Subliminal priming task results (percentage of accuracy):** the figure is an overview of the results of the three subgroups of subjects in the emotional and non-emotional tasks in terms of percentage of accuracy. All the combinations of implicit/explicit and congruent/incongruent priming are represented.

Legend: EMO=stimuli of emotional significance ; GEN=stimuli of non-emotional significance (gender recognition)



		EMO_RT congruent implicit priming			EMO_RT incongruent implicit priming			EMO_RT congruent explicit priming			EMO_RT incongruent explicit priming		
Group		HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C
Valid	11	5	5	5	11	5	5	11	5	5	11	5	5
Missing	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean	872.374	1379.952	1167.042	895.554	1466.020	1208.170	798.137	1192.064	1185.206	823.472	1277.452	1167.754	
SD	272.080	775.277	706.121	257.023	821.872	645.286	214.680	627.396	594.414	228.268	670.394	576.173	
Minimum	610.920	711.690	751.210	626.540	703.520	800.080	574.190	508.210	752.130	579.690	594.830	716.170	
Maximum	1462.790	2385.450	2423.000	1328.150	2503.720	2353.910	1177.420	2047.470	2230.650	1192.810	2238.870	2166.410	
		GEN_RT congruent implicit priming			GEN_RT incongruent implicit priming			GEN_RT congruent explicit priming			GEN_RT incongruent explicit priming		
Group		HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C	HC	DMD	NMD-C
11	5	5	5	5	11	5	5	11	5	5	11	5	5
0	0	0	0	0	0	0	0	0	0	0	0	0	0
733.732	1102.516	926.314	727.045	1101.564	916.942	726.415	1108.492	968.266	708.745	1089.030	1037.312		
340.978	334.268	530.956	320.916	359.299	551.804	384.911	415.988	609.601	377.739	401.594	700.065		
447.080	762.690	600.830	456.480	706.900	566.940	486.420	521.040	517.630	467.920	669.310	515.710		
1626.940	1511.200	1867.310	1581.810	1476.550	1888.040	1805.460	1530.770	2037.460	1783.790	1612.970	2264.960		

**Figure 17 - Subliminal priming task results (response time):** the figure is an overview of the results of the three subgroups of subjects in the emotional and non-emotional tasks in terms of response time. All the combinations of implicit/explicit and congruent/incongruent priming are represented.

Legend: EMO=stimuli of emotional significance ; GEN=stimuli of non-emotional significance (gender recognition)

### Statistical Analysis

A one-way ANOVA test was used to compare the average IQ scores between the three groups. As the one-way ANOVA test gave a significant result ( $F=10.279$ ;  $p=0.001$ ;  $\eta^2=0.533$ ), a post hoc comparison test was carried out to show which of the three groups had a significant difference. These analyses showed that, although there were no subjects with cognitive impairment, DMD patients had a significantly lower mean IQ than controls, both HC and NMD-C, in agreement with the literature<sup>101</sup> (see Table 10).

<b>Wechsler scales – total IQ scores</b>					
<b>Post Hoc Comparisons - Group</b>					
<b>Groups</b>		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
<b>HC</b>	<b>DMD</b>	<b>32.291</b>	<b>7.176</b>	<b>4.500</b>	<b>&lt;.001</b>
HC	NMD-C	6.291	7.176	0.877	0.661
<b>DMD</b>	<b>NMD-C</b>	<b>-26.000</b>	<b>8.415</b>	<b>-3.090</b>	<b>0.017</b>

**Table 10 – IQ results (post-hoc comparison):** Results of post hoc comparisons of one-way ANOVA on IQ mean.  
Note. P-value adjusted for comparing a family of 3.

A one-way ANOVA test and a post-hoc comparison were also used to compare NEPSY scores. The results confirm the poorer performance in the social cognition tasks of the NEPSY in DMDs compared to both HCs and NMD-Cs. In particular, in the Theory of Mind (ToM) section, there is a significant difference only between HC and DMD subjects, but DMDs score lower than both control groups. The lack of significance between DMD and NMD-C may be related to the small sample size of the two samples. In the Emotion Recognition (ER) section, however, there is a significant difference between the DMD group and the other two groups, with the former performing worse than the latter. The results of the post hoc comparisons are shown in Tables 11 and 12.

<b>NEPSY - Theory of Mind section</b>					
<b>Post Hoc Comparisons - Group</b>					
<b>Groups</b>		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
<b>HC</b>	<b>DMD</b>	<b>5.582</b>	<b>1.681</b>	<b>3.321</b>	<b>0.010</b>
HC	NMD-C	2.182	1.681	1.298	0.414
DMD	NMD-C	-3.400	1.971	-1.725	0.223

**Table 11 – NEPSY-ToM results (post-hoc comparison):** Results of post hoc comparisons of one-way ANOVA on NEPSY score in the ToM section ( $F=5.551$ ;  $p=0.013$ ;  $\eta^2 p=0.381$ ). Note. P-value adjusted for comparing a family of 3.

<b>NEPSY - Emotion Recognition section</b>					
<b>Post Hoc Comparisons - Group</b>					
<b>Groups</b>		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
<b>HC</b>	<b>DMD</b>	<b>6.109</b>	<b>1.466</b>	<b>4.168</b>	<b>0.002</b>
HC	NMD-C	1.509	1.466	1.030	0.568
<b>DMD</b>	<b>NMD-C</b>	<b>-3.400</b>	<b>1.719</b>	<b>-2.677</b>	<b>0.039</b>

**Table 12 - NEPSY-ToM results (post-hoc comparison):** Results of post hoc comparisons of one-way ANOVA on NEPSY score in ER section ( $F=8.729$ ;  $p=0.002$ ;  $\eta^2 p=0.492$ ). Note. P-value adjusted for comparing a family of 3.

On the other hand, the comparison of the VABS deviation IQs, again using the same statistical test, confirms, from the point of view of adaptive functioning, the deficit in socialization skills reported by the families in comparison with the HCs (statistically significant difference) and the NMD-Cs (not statistically significant difference, but with a significantly lower score in the DMD group than in the NMD-C group), although the means are all within a range considered to be normal (see Table 13).

<b>VABS – II Socialization section (deviation IQ)</b>					
<b>Post Hoc Comparisons - Group</b>					
<b>Groups</b>		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
<b>HC</b>	<b>DMD</b>	<b>32.255</b>	<b>10.473</b>	<b>3.080</b>	<b>0.017</b>
HC	NMD-C	12.655	10.473	1.208	0.464
<b>DMD</b>	<b>NMD-C</b>	-19.600	12.280	-1.596	0.273

**Table 13 – VABS-II results (post-hoc comparison): results (post-hoc comparison):** results of post hoc comparisons of one-way ANOVA on VABS score ( $F=4.776$ ;  $p=0.022$ ;  $\eta^2 p=0.347$ ) Note. P-value adjusted for comparing a family of 3.

Instead, a 2x2x3 mixed design ANOVA was used to analyze the results of the experimental emotional priming test compared to the non-emotional priming test. Congruence (2 levels, congruent vs. incongruent) was the repeated within-subjects variable, and group (3 levels, DMD, HC, NMD-C) was the group factor. This was done separately for both accuracy percentage and response time. The ANOVA tests performed on accuracy percentage compared the congruent stimulus with the incongruent stimulus in the different settings, i.e. emotional priming (implicit and explicit) and non-emotional priming (implicit and explicit). To make this comparison, repeated measures ANOVA tests were used to determine whether or not there was a statistically significant difference between the means of three or more groups, with the same subjects appearing in each group.

The results of the ANOVA tests showed no significant differences between congruent and incongruent priming, nor between implicit and explicit priming. However, in the emotional priming task, there was a significant difference between the scores of the DMD group and the other two control groups in both the explicit and implicit settings. The difference was not present in the non-emotional priming task. The results are summarized in the tables below.

	F	p	$\eta^2 p$
Implicit emotional priming (% accuracy): congruent vs incongruent	5.047	<b>0.018</b>	0.359
Explicit emotional priming (% accuracy): congruent vs incongruent	9.565	<b>0.001</b>	0.515
Implicit non-emotional priming (% accuracy): congruent vs incongruent	1.585	0.232	0.150
Explicit non-emotional priming (% accuracy): congruent vs incongruent	1.562	0.237	0.148
Congruent emotional priming (% accuracy): explicit vs implicit	6.069	<b>0.010</b>	0.403
Incongruent emotional priming (% accuracy): explicit vs implicit	8.947	<b>0.002</b>	0.499
Congruent non-emotional priming (% accuracy): explicit vs implicit	1.654	0.219	0.155
Incongruent non-emotional priming (% accuracy): explicit vs implicit	1.579	0.234	0.149

Table 14: between subjects effects(Groups=HC, DMD, NMD-C) of repeated measures ANOVA

<b>ANOVA-Implicit emotional priming (% accuracy): congruent vs incongruent</b>					
<b>Post Hoc Comparisons - Group</b>					
<b>Groups</b>		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>holm</sub></b>
HC	DMD	0.169	0.054	3.109	<b>0.018</b>
HC	NMD-C	0.019	0.054	0.351	0.730
DMD	NMD-C	-0.150	0.064	-2.352	<b>0.060</b>

Table 15 - Implicit emotional priming (congruent vs incongruent): post hoc comparisons of the repeated measures ANOVA in the implicit emotional priming setting, comparing congruent vs incongruent stimulus. Note. P-value adjusted for comparing a family of 3; results are averaged over the levels of: RM Factor 1.

<b>ANOVA - Explicit emotional priming (% accuracy): congruent vs incongruent</b>					
<b>Post Hoc Comparisons - Group</b>					

Groups		Mean Difference	SE	t	p <sub>holm</sub>
HC	DMD	0.242	0.058	4.145	0.002
HC	NMD-C	-0.002	0.058	-0.030	0.977
DMD	NMD-C	-0.244	0.069	-3.560	0.004

**Table 16 - Explicit emotional priming (congruent vs incongruent):** Post hoc comparisons of the repeated measures ANOVA in the explicit emotional priming setting, comparing congruent vs incongruent stimulus. Note. P-value adjusted for comparing a family of 3; results are averaged over the levels of: RM Factor 1.

<b>ANOVA - Congruent emotional priming (% accuracy): explicit vs implicit</b>					
<b>Post Hoc Comparisons - Group</b>					
Groups		Mean Difference	SE	t	p <sub>holm</sub>
HC	DMD	0.198	0.060	3.293	0.012
HC	NMD-C	-0.003	0.060	-0.053	0.958
DMD	NMD-C	-0.201	0.070	-2.853	0.021

**Table 17 - Congruent emotional priming (explicit vs implicit):** Post hoc comparisons of the repeated measures ANOVA in the congruent emotional priming setting, comparing explicit vs implicit stimulus. Note. P-value adjusted for comparing a family of 3; results are averaged over the levels of: RM Factor 1.

<b>Incongruent emotional priming (% accuracy): explicit vs implicit</b>					
<b>Post Hoc Comparisons - Group</b>					
Groups		Mean Difference	SE	t	p <sub>holm</sub>
HC	DMD	0.214	0.052	4.125	0.002

HC	NMD-C	0.021	0.052	0.397	0.696
DMD	<b>NMD-C</b>	<b>-0.193</b>	<b>0.061</b>	<b>-3.179</b>	<b>0.010</b>

**Table 18 - incongruent emotional priming (explicit vs implicit):** Post hoc comparisons of the repeated measures ANOVA in the incongruent emotional priming setting, comparing explicit vs implicit stimulus. Note. P-value adjusted for comparing a family of 3; results are averaged over the levels of: RM Factor 1.

When the significant difference between the DMD group and the other two groups in terms of the percentage of accuracy on the emotional tasks is examined with a one-way ANOVA, it is confirmed that DMD patients perform significantly worse than the other two groups in both settings (implicit and explicit) in both congruent and incongruent priming. The results are shown in the tables below.

<b>One-way ANOVA - EMO-% accuracy in implicit congruent priming setting</b>					
<b>Post Hoc Comparisons - Group</b>					
<b>Groups</b>		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
HC	DMD	<b>0.152</b>	<b>0.061</b>	<b>2.507</b>	<b>0.055</b>
HC	NMD-C	0.006	0.061	0.096	0.995
DMD	NMD-C	-0.146	0.071	-2.056	0.128

**Table 19 – emotional implicit congruent priming (group comparison):** post hoc comparisons of the one-way ANOVA in the implicit congruent emotional priming setting, comparing groups' results ( $F= 3.405$ ;  $p=0.056$ ;  $\eta^2 p=0.274$ ). Note. P-value adjusted for comparing a family of 3.

<b>One-way ANOVA - EMO-% accuracy in implicit incongruent priming setting</b>					
<b>Post Hoc Comparisons - Group</b>					
<b>Groups</b>		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
HC	DMD	<b>0.152</b>	<b>0.061</b>	<b>2.507</b>	<b>0.055</b>

<b>HC</b>	<b>DMD</b>	<b>0.186</b>	<b>0.051</b>	<b>3.654</b>	<b>0.005</b>
HC	NMD-C	0.032	0.051	0.635	0.803
<b>DMD</b>	<b>NMD-C</b>	<b>-0.154</b>	<b>0.060</b>	<b>-2.575</b>	<b>0.048</b>

**Table 20 - emotional implicit incongruent priming (group comparison):** Post hoc comparisons of the one-way ANOVA in the implicit incongruent emotional priming setting, comparing groups' results ( $F=6.818$ ;  $p=0.006$ ;  $\eta^2 p=0.431$ ). Note. P-value adjusted for comparing a family of 3.

<b>One-way ANOVA - EMO-% accuracy in explicit congruent priming setting</b>					
<b>Post Hoc Comparisons - Group</b>					
<b>Groups</b>		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
<b>HC</b>	<b>DMD</b>	<b>0.244</b>	<b>0.063</b>	<b>3.850</b>	<b>0.003</b>
HC	NMD-C	-0.012	0.063	-0.192	0.980
<b>DMD</b>	<b>NMD-C</b>	<b>-0.256</b>	<b>0.074</b>	<b>-3.447</b>	<b>0.008</b>

**Table 21 - emotional explicit congruent priming (group comparison):** Post hoc comparisons of the one-way ANOVA in the explicit congruent emotional priming setting, comparing groups' results ( $F=8.490$ ;  $p=0.003$ ;  $\eta^2 p = 0.485$ ). Note. P-value adjusted for comparing a family of 3.

<b>One-way ANOVA - EMO-% accuracy in explicit incongruent priming setting</b>					
<b>Post Hoc Comparisons - Group</b>					
<b>Groups</b>		<b>Mean Difference</b>	<b>SE</b>	<b>t</b>	<b>p<sub>tukey</sub></b>
<b>HC</b>	<b>DMD</b>	<b>0.241</b>	<b>0.056</b>	<b>4.312</b>	<b>0.001</b>
HC	NMD-C	0.009	0.056	0.156	0.987
<b>DMD</b>	<b>NMD-C</b>	<b>-0.232</b>	<b>0.065</b>	<b>-3.544</b>	<b>0.006</b>

**Table 22 - emotional explicit incongruent priming (group comparison):** Post hoc comparisons of the one-way ANOVA in the explicit incongruent emotional priming setting, comparing groups' results ( $F=10.084$ ;  $p=0.001$ ;  $\eta^2 p=0.528$ ). Note. P-value adjusted for comparing a family of 3.

Regarding the analysis of response times, the descriptive analysis (Figure 17 above) shows a slight difference between the times of incongruent (longer) and congruent (shorter) priming only for the emotional stimulus. However, a repeated measures ANOVA comparing congruent vs. incongruent in all settings (implicit and explicit emotional priming, implicit and explicit non-emotional priming) and implicit vs. explicit in all settings (congruent and incongruent emotional priming, congruent and incongruent non-emotional priming) shows no significance concerning the effect of the 3 groups on the variation of the parameter studied each time. Thus, the difference found in the descriptive analysis is so small that the results are not significant. The descriptive analysis also shows slightly longer response times in the DMD group than the other two groups, but when the differences between the three groups are analyzed with a one-way ANOVA in the different settings, nothing significant emerges. Again, this may be due to the sample size being too small to detect significant differences.

## Discussion

### *General considerations*

This project is, to the best of our knowledge, the first to study the neuropsychological phenotype of DMD using a subliminal priming protocol. As previously mentioned, the study has been carried out as a monocentric study, and the recruitment is still active in the other centers of the network. Thus, the number of participants is smaller than the sample size required to draw meaningful conclusions. However, even these preliminary data show a clear difference between the group of DMD patients and the controls in both groups, HC and NMD-C, both at the clinical level, in the cognitive and social perception assessment tests, and at the experimental level, in the priming setting. The main difficulties in recruiting the different participants were encountered in the DMD group and the NMD-C group. In particular, for the DMD group, some of the patients recruited were scheduled to be admitted to the IRCCS Fondazione Mondino and some were patients who lived close to the center since the database of patients attending the center did not allow the selection of patients who lived far away due to their motor difficulties and the consequent difficulty in moving and reaching the center. However, this has eliminated the selection bias caused by the targeted selection of patients for the study. The DMD patients enrolled in the project so far are all patients on daily deflazacort treatment, without major

cardio-respiratory complications. Of these, 4 were ambulatory at the time of the various tests and 1 was non-ambulatory, confined to a wheelchair.

None of the DMD patients had cognitive impairment but they overall had a significantly lower mean IQ than the controls (both HC and NMD-C), as expected based on the literature. For three of the five patients, the cognitive test data were collected within the same session in which all the tests were administered, whereas for two patients they were collected retrospectively as they had been performed recently. As far as the NEPSY-II social perception subscale scores are concerned, all data were collected at the same time as the rest of the data. Similarly, within the same session or admission, the parents or relatives of the DMD patients underwent the interview to assess the socialization part of the VABS-II scale. All the data collected in the different data sets were converted into weighted scores according to the different scoring tables calibrated in Italian and entered into the database created on REDcap so that they could be compared. In the case of DMD patients, the computer priming tests were carried out in the same room, thus respecting the equality of the administration environment.

About the NMD controls, as mentioned above, four patients with SMA and one patient with congenital myasthenic syndrome were recruited. Two patients were unable to reach the IRCCS Fondazione Mondino so we decided to travel to the site of the Italian Association for Muscular Dystrophy (UILDM) in Bergamo (BG), close to their residence and well known to the patients and families. For this reason, it was not possible to use the same administration environment as for the priming tests, but we were able to reconstruct the acoustic and light characteristics. The SMA 2 patients, both of whom were non-ambulatory, were the most challenging. In particular, one of the two patients still had some motor capacity in the upper limbs, so he was able to perform the computer tests and the manual subtests of the Wechsler scales (in particular the 'Block Design', 'Symbol search' and 'Coding' subtests) on his own, while the other patient, due to the severe motor impairment in upper limbs, required a third person to act on pressing the keys necessary for the performance of the priming tests on his behalf. To homogenize the test administration as much as possible, the participant told the experimenter the letter to press, rather than the emotion or gender to select. Regarding the administration of manual subtests, we carried out a thorough search in the main search engines, such as PubMed, Nature, and Elsevier, to find literature suggesting how to compensate for the availability of these subtests in these patients, mainly finding that the Verbal Comprehension Index (VCI) and Working Memory Index (WMI) were considered as indicators of total IQ. In the context of our study, we preferred to replace these subtests with others, following the instructions in the administration manual. In particular, the 'Block Design' subtest was replaced by the 'Picture completion' subtest, the

'Coding' subtest by the 'Cancellation' subtest, while the 'Symbol search' subtest was kept. For all NMD-C, the different tests were administered in the same session.

Finally, for the recruitment of the HCs, we turned mainly to the relatives of the patients of the other two groups, to the students of Medicine and Surgery, Dentistry and Engineering at the University of Pavia, and the children of the medical staff of the Foundation. There were no major problems in carrying out the tests and collecting the data. All patients accepted and enjoyed the tests.

### *The social cognition domain*

Our results supported the hypothesis of a deficit in the ability to recognize emotions in DMD patients compared with controls in both groups. This was particularly evident in the social cognition tasks of NEPSY: in the Theory of Mind section there is a significant difference with the HC group, but not with the NMD-C group, probably due to the small number of samples in both groups; in the Emotion Recognition section, however, there is a significant difference with both groups. Thus, among the social cognitive tasks, we confirm a deficit that is most detectable in emotion recognition, confirming the preliminary results already obtained in the previous clinical study (see section 2.2). Indeed, although our sample is small, it is "clean" in the sense that none of the DMD patients has an intellectual disability. The data obtained from the VABS-II questionnaire also support our hypothesis, as they report a statistically significant deficit in the socialization skills of DMD patients compared with the other two groups. This finding is particularly interesting as it is less described in the literature, but it is congruent with the NEPSY results. Finally, regarding the results obtained in the emotional and non-emotional priming tests, a statistically significant difference was found in the emotional tasks of the DMD patients compared with those of the other two groups (with the former performing worse), which further supports the hypothesis of a deficit in the ability in recognizing the emotional value of facial cues. This difference is present in both settings, implicit and explicit, with congruent and incongruent emotional priming. In contrast, there is no significant difference in non-emotional priming tasks. . As other cortical structures involved in higher level processing (e.g. posterior superior temporal sulcus) are preferentially activated in the interpretation of facial expressions, our finding must not be interpreted as specifically supporting the hypothesis of an extrageniculostriatal involvement. Any selectivity of the effects for the subliminal task would instead have pointed to this scenario. However, we did not obtain statistically significant results to support this hypothesis, probably because of the small number of participants recruited.

Based on the data analyzed, there does not seem to be a major difference in performance between the implicit and explicit settings, nor between congruent and incongruent priming. However, more data are necessary to further test this hypothesis and possibly investigate the CNS structures that might be involved and cause this deficit. Also, in a very recent study<sup>169</sup> the ability to recognize emotions is correlated with the age of the subject, stating that there is a continuous development of the neural mechanisms underlying the processing of faces and emotions during adolescence and young adulthood. It is not possible to test this hypothesis in this study, as the number of participants is very small, but it will be an important point for reflection and analysis when we expand the sample.

## Conclusions

From the data obtained and direct observations, DMD patients have a significant deficit in emotion recognition and socialization skills that does not correlate with the motor deficit that characterizes the disease, nor with an impairment of their visual system. These findings are compliant with the hypothesis of possible involvement of the extra-geniculostriate system, particularly the amygdala and thalamus, secondary to the dystrophin deficiency in the CNS. However, to obtain statistically significant results, it is necessary to analyze a larger sample. Moreover, as the priming task is also based on the ability of maintaining attention on visual stimuli, it is plausible that the higher frequency of neurodevelopmental disorders, including attention impairment, may have played a role in the worse performance of DMD patients compared to HC.

However, based on these preliminary data, it is already possible to start addressing the issue of how to help these patients in this aspect, or how to implement the guidelines for managing the disease, also focusing on rehabilitation approaches in this sense and extending the multidisciplinary approach. In particular, tools already used in the context of other neuropsychiatric frameworks, such as ASD, could be used to work on this aspect. For example, by using; social stories, i.e. short stories of invented characters, written by the child together with the therapist or the child's parents, useful to explain a specific emotional situation clearly and simply, to develop social skills and interpersonal relationships; the CAT-kit (Cognitive Affective Training kit), a tool developed by Attwood, Callesen, and Nielsen in 2008 for cognitive affective training and to improve understanding of the cognitive and emotional aspects present in communication<sup>105</sup>; simple games in which the child is first asked to identify an emotion and then to imitate the facial expressions associated with it. This would help to improve the quality of life of these patients and be of strong support to their families and caregivers.



### 3. Implementing new technologies: issues and perspectives

This chapter is dedicated to the declination of the findings of the clinical assessment into the context of rehabilitation. This stage of the project was focused on the challenges of implementing technologies in the rehabilitation of pediatric neuropsychiatric disorders, in general, and neuromuscular disorders, in particular. In this stage of the project, we first aimed to obtain a reliable overview of the application of technology to the rehabilitation of pediatric neurological and neurodevelopmental diseases, in particular its implementation in the context of telerehabilitation. Subsequently, also based on this insight and the preliminary evidence derived from our clinical studies, we designed a clinical protocol to test an innovative multimodal technological intervention for the rehabilitation of DMD patients.

The systematic review presented in Section 3.1 is a comprehensive overview of the multifaceted landscape of technological telerehabilitation applied to pediatric neurological and neurodevelopmental disorders. The analysis was focused on describing the treated pathologies, the type of adopted technologies, the grade of evidence of the described results, and the impact on the role of caregivers. The results of the review have been presented in the article *The wide world of technological telerehabilitation for pediatric neurologic and neurodevelopmental disorders – a systematic review* (Del Lucchese B., Parravicini S, [...] Cioni G., Sgandurra G. and the Italian Neuroscience and Neurorehabilitation Network) *Frontiers in Public Health* (12:1295273. doi: 10.3389/fpubh.2024.1295273), April 2024

My contributions to this study have been conceptualization, methodology, investigation, formal analysis, visualization, and manuscript writing.

The protocol presented in Section 3.2 is an example of the potential role of technology in the rehabilitation setting of DMD. We designed a clinical protocol for a multimodal rehabilitative intervention involving Virtual Reality software for cognitive training integrated into an assisted cycloergometer (TheraTrainer). The clinical protocol is part of the project *Valutazione dell'impatto di un intervento riabilitativo basato su pedalata con Virtual Park sul rallentamento del declino funzionale motorio del paziente neuromuscolare in età evolutiva* [eng: *Evaluation of the impact of a rehabilitation intervention based on pedaling with Virtual Park on slowing down the motor functional decline of neuromuscular patients in developmental age*] submitted to the National EC on July 2024 as a within the consortium of the *Fit4Medical Robotics* project.

My contributions to this study have been conceptualization, methodology, and protocol writing.

The case series presented in Section 3.3 is the report of a pilot experience of the implementation of a technological device for multimodal telerehabilitation in the therapeutic setting of DMD. We designed an intervention protocol for personalized multimodal (motor and cognitive) training based on a commercial device for telerehabilitation (VRRS Home-Kit, Khymeia).

This experience is part of the projects *Valutazione e Teleriabilitazione di abilità cognitive e motorie nelle lesioni cerebrali e/o nelle disabilità funzionali neuropsichiche in età evolutive* [eng: *Assessment and Telerehabilitation of cognitive and motor skills in brain lesions and/or functional neuropsychic disabilities in developmental age*] and *Sviluppo e implementazione di percorsi diagnostico-riabilitativi per le patologie pediatriche basati su tecnologie omiche, telemedicina e teleriabilitazione* [eng: *Development and implementation of diagnostic-rehabilitation pathways for pediatric pathologies based on omics, telemedicine and telerehabilitation*] conducted as partner of the Italian Neuroscience and Neurorehabilitation.

My contributions to this study have been investigation, coordination, and supervision.

### 3.1.The wide world of technological telerehabilitation for pediatric neurologic and neurodevelopmental disorders – a systematic review

This systematic review is a comprehensive overview of the multifaceted landscape of technological telerehabilitation applied to pediatric neurological and neurodevelopmental disorders.

#### *Telerehabilitation: main features and conveniences*

The recent development and availability of Internet and Communication Technologies (ICTs) have fostered the possibility of applying technology-based solutions to provide health services both during hospitalization and after discharge from the hospital<sup>170</sup>, as well as for children with neurodevelopmental disabilities or neurological conditions. The World Health Organization (WHO) defines telehealth as the “delivery of health care services, where patients and providers are separated by distance. Telehealth uses information communication technology for the exchange of information for the diagnosis and treatment of diseases and injuries, research and evaluation, and for the continuing education of health professionals”<sup>171</sup>. Over the past three years, an increasing interest in developing and applying user-friendly technological systems has become even more highlighted. The unexpected COVID-19 pandemic has driven the introduction of security measures and restrictions to preserve public health, substantially impacting clinical activities and rehabilitation services for neurodevelopmental disabilities<sup>172</sup>. Such abrupt interruption or the reduction of access to non-emergency face-to-face diagnostic and rehabilitative procedures have had adverse short- and long-term consequences for patients with neuropsychological and motor disorders and their caregivers<sup>173</sup> (4), thus pushing forward the uptake of telehealth, as the only way to continue the clinical practice, with promising results<sup>174–177</sup>. Among different applications of the technologies in clinical practice (assessment, consultation, monitoring), ICTs have become a valuable option for rehabilitation, enabling timely and tailored therapeutic interventions<sup>178</sup>.

Telerehabilitation programs foster access to rehabilitative services and permit the delivery of a wide range of neuropsychological, motor, speech, and communication interventions, even for patients unable to frequently attend a clinical institution (distance from the hospital, parental work employment, etc.), by overcoming geographic barriers. In this scenario, new technologies guarantee significant time- and cost-saving, shortening hospitalization and delivering the rehabilitative process at home, in a more ecological context (American Telemedicine Association, 2017) therefore enforcing the generalization of the achieved competencies.

Another great advantage provided by using innovative technologies in clinical practice to foster therapies tailored to patients' needs concerns both the possibility of collecting comprehensive and accurate quantitative data, thus supporting better intervention monitoring, and of offering multi-domain activities, also integrating peripheral devices (i.e. sensors). Using innovative technologies in clinical practice also gives the possibility to propose neuropsychological and motor activities in a playful and motivating context, thus enhancing participation and enjoyment, especially for the pediatric population, while maintaining high levels of efficiency<sup>179,180</sup>. Such telerehabilitation pathways allow for increased dosage and intensity of the intervention<sup>181</sup> and ensure caregivers' involvement in the rehabilitation process. The parental role in rehabilitation interventions is described as the set of tasks or responsibilities attributed to caregivers during the intervention<sup>182</sup>, placed on a continuum from a passive to an active involvement<sup>183</sup>, in passive roles, parents comply with interventions driven by the expert professional, ensuring children's attendance at rehabilitative sessions and supporting their enthusiasm and motivation to participate; conversely, in more active roles, parents are involved as "leaders", bringing a personal contribution to the intervention sessions and also collaborating in the decision-making steps. Both intensity and parental involvement are described as features supportive of the rehabilitation effectiveness in children with neurodevelopmental disorders, according to the main scientific literature and guidelines<sup>184–186</sup>.

Telerehabilitation yielded promising results in enhancing cognitive, motor, speech, and communication abilities, but such intervention protocols still need to be routinely included in routine clinical practice. Several barriers exist to the adoption of ICT technologies in pediatric intervention programs, both from the perspective of healthcare providers and families, such as limited access to the technology, cost implications, technological competency, privacy and data security concerns, lack of face-to-face interactions, just to name some of them<sup>186</sup>. Addressing these barriers is therefore crucial for facilitating the successful implementation and acceptance of telerehabilitation in pediatric care, thus improving, as described above, the access to clinical services and outcomes for children with neurodevelopmental disabilities.

### *ICTs technologies classification*

The progress of digital technologies (namely, associated with the use of computers, smartphones, the internet, and other digital devices and platforms) enabled the delivery of rehabilitation services via ICTs<sup>187</sup>, by offering a vast world of possibilities, from interventions

targeting separately motor, neuropsychological, speech and communication functions, to integrated rehabilitation pathways.

Despite the benefits offered by digital technologies and the increase in their use, strongly driven by the pandemic emergency, a standardized taxonomy able to classify the different existing digital technologies for telerehabilitation is still lacking.

In general, technologies can be classified based on their attributes and functionalities, depending on the context and the intended use. Likewise, this applies to digital health technologies; for instance, Camden and Silva<sup>188</sup> drafted a general classification of pediatric telehealth strategies able to offer personalized and home-based intervention based on the devices' complexity from low-tech (e.g., phone calls and video/photo sharing), to high-tech solutions (e.g., specialized programs/serious games, virtual reality and sensors). A different example of digital technology classification for motor rehabilitation in children has been proposed by The European Academy of Childhood Disability (EACD)<sup>189</sup>. In this case, the classification involved three categories: 1) robotic devices and treadmills with body weight support systems; 2) virtual reality/gaming systems; 3) telehealth and phone/tablet apps. However, this classification does not consider many other evidence-based technologies that, to date, are utilized for rehabilitation interventions, mainly for cognitive functions.

Considering the wide world of technological devices for the intervention in children with neurodevelopmental disabilities, this systematic review aims to investigate the main features (e.g., type of adopted technology, functional domains identified as outcomes, caregiver involvement, dosage) supporting the effectiveness of telerehabilitation protocols, to guide clinical practice, path further future studies, and support the use of innovative solutions for inclusive development.

## Methods

### Search Strategy

The author and his collaborators undertook a systematic search from four electronic databases Medline/PubMed, EMBASE, and Web of Science in February 2023, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement<sup>99</sup>. Different combinations of keywords selected from analyzing recent scientific literature were used, particularly referring to four main clusters: “neurodevelopmental disabilities”, “children”, “telerehabilitation” and “home-based intervention”. Terms related to such constructs and definitions were also included (see Appendix 1 for the complete search string). In addition, the references of the included studies were also considered to identify additional eligible studies

and to ensure a comprehensive data collection. To exclude non-peer-reviewed studies, the author and his collaborators included studies published in academic journals, reported in English, and available for full text. Considering that the development and the implementation of technological devices in telerehabilitation are relatively recent, articles published from 2000 were considered. The methodological quality of the included studies was assessed according to the National Health and Medical Research Council Evidence Hierarchy (NHMRC, 2009). This systematic review was registered on PROSPERO (CRD42020210663).

### *Inclusion and exclusion criteria*

#### Population

Studies were included when considering samples of children aged 0-18 years with motor, neuropsychological, cognitive, and speech-communication impairments due to neuropsychiatric conditions such as neurodevelopmental disorders (including Specific Learning Disorders, Developmental Coordination Disorder, Language Disorder, Autism Spectrum Disorder, Attention Deficit Hyperactivity Disorder and Developmental Delay/Intellectual Disabilities (according to ICD 10 or DSM 5-IV TR) genetic syndromes, prematurity, congenital or acquired brain lesions, and neuromuscular diseases.

#### Interventions

The selected studies focused on telerehabilitation programs to improve motor, neuropsychological, cognitive, and speech-communication functions. Interventions had to be delivered entirely or partially (with almost a 50% percentage) in an ecological context such as home or school and through ICTs. According to the technologies classification reported in the following section, rehabilitation programs including virtual reality, active video gaming devices (i.e., Xbox, Kinect, Playstation), telemedicine and telemonitoring tools, computer-based programs and web-based platforms (i.e., CogMed RIDInet) were considered. Interventions should be monitored by health professional staff (such as psychologists, neuropsychiatrists, speech therapists, motor therapists, physiotherapists, and occupational therapists). Any frequency, intensity, and duration of the training was included. Moreover, the studies needed to have a pre-post treatment design or the presence of a control group (both active or waitlist).

### **Classification of ICTs**

Starting from the EACD classification, in this study we have defined a novel taxonomy for digital technologies to consider all the domains handled by the clinicians. Our proposal includes i) Virtual reality and active video gaming devices (i.e., Xbox, Kinect, Playstation); ii) Telemedicine and Telemonitoring devices; iii) Computer-based program and web-based platform (i.e., CogMed RIDInet); iv) other. Specifically, ‘other’ refers to purely robotic/treadmill systems that are difficult to transport and not entirely suitable for home-based treatment. This categorization manages to encompass all devices targeting purely motor, neuropsychological, or speech treatments but also integrated ones, thus combining motor and cognitive or cognitive and speech functions.

### **Outcomes**

Studies were included when quantitative measures of the efficacy of telerehabilitation interventions (i.e., standardized tests and scales administered to the child, clinicians/caregivers/self-report questionnaires, instrumental measurements) were adopted to assess neuropsychological, motor, cognitive, and speech-communication outcomes. Quality of life and daily life functioning were also considered as admissible outcomes.

The following exclusion criteria were considered: (1) case reports, book chapters, conference abstracts, protocol studies, reviews; (2) diagnostic or prognostic studies (3) participants aged > 18; (4) samples with other medical, psychiatric or neurological conditions (5) interventions not based on ICTs; (6) totally “clinic-based” interventions; (7) interventions not primarily targeting neuropsychological, motor, speech and communication skills; (8) quantitative outcome measures on the efficacy of the training not applied.

Feasibility studies were excluded unless they had pre- and post-treatment clinical measures as secondary outcomes.

### ***Study selection process***

After automatically removing duplicates, pairs of independent reviewers screened the titles and abstracts of 1427 articles. The resulting 170 articles were then further full-text screened according to eligibility criteria, previously reassigning the set of papers to be reviewed by each

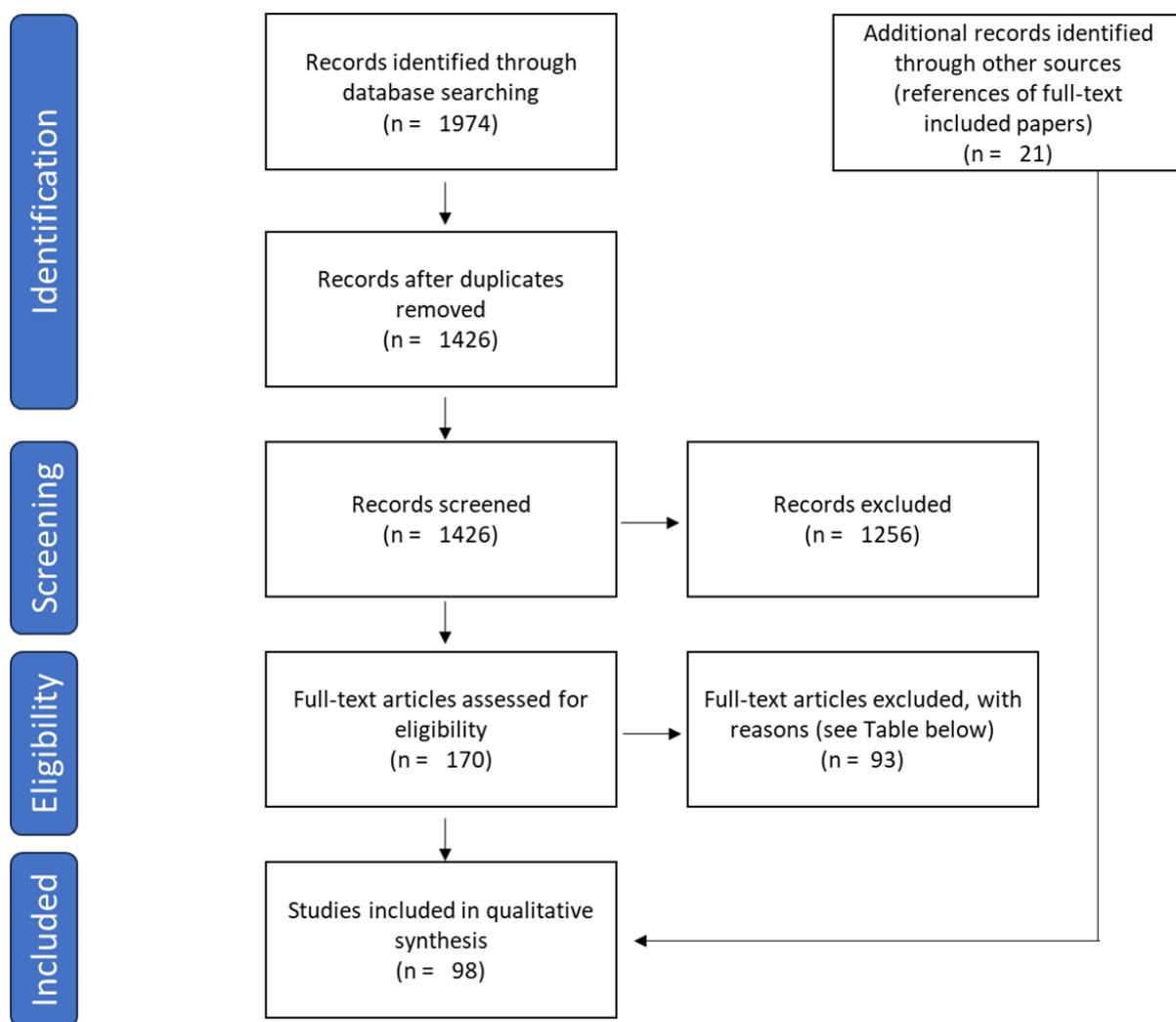
pair of reviewers (compared to the title/abstract selection stage). In case of discrepancies, articles were discussed between the two reviewers to determine their inclusion or exclusion. If consensus could not be reached, a third reviewer was therefore consulted. References of the included studies were eventually reviewed to identify additional eligible studies. The process led to the selection of 98 papers that met the inclusion criteria. The overall process for selecting studies is shown in Figure 18 and Table 23.

#### *Data extraction*

For each paper included, the reviewers recorded in a dedicated database the following information: first author, title, year of publication, quality of the study (according to NHMRC Evidence Hierarchy), age range and diagnosis of the sample, study design, sample size, type of technologies used for intervention (see Introduction for the adopted classification), target functions of the rehabilitation program (motor, neuropsychological, speech/communication skills), direct target recipients of the interventions, intensity, frequency and duration of each treatment and outcome measures.

In particular, the framework proposed in a previously published review<sup>183</sup> has been adopted to classify the parental role in the rehabilitation process. Such classification includes eight different categories (Bringer, Supporter, Informer, Observer, Learner, Implementer, Adaptor, Collaborative Decision Maker), defining, in this order, a spectrum from passive to active responsibility.

Furthermore, considering the high heterogeneity of the studies, primary outcome measures were extracted and classified by two independent reviewers according to the International Classification of Functioning, Disability and Health - Children&Youth Version (ICF-CY) domains, and core-set outcome measures that could be assigned to more than one ICF domain or core sets were classified considering the most prevalent one.



**Figure 18 - PRISMA Flow Diagram:** the flow diagram represents the stages of the search strategy and the selection process of the articles included in the review, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

<b>REASONS FOR EXCLUSION OF FULL-TEXT ASSESSED ARTICLES</b>	
Exclusion criteria	n° of excluded papers
Reviews, case reports, book chapters, conference abstracts, and protocol studies. [tag: article type]	29
Studies not including intervention based on technological devices (e.g. rehabilitation softwares, commercial videogames, sensors). [tag: technology]	26
Studies not applying quantitative outcome measures (assessed functions: motor function, neuropsychological functions, language, quality of life/daily life functioning). Feasibility studies not included [tag: outcome]	10
Studies not including totally or partially “home/school-based” interventions. [tag: intervention]	14
Studies including >18-year-old subjects or patients with non-neuropsychiatric disorders. [tag: population]	11
Studies about interventions not primarily targeting motor functions, neuropsychological functions or language. [tag: intervention target]	2
Studies on animals or about other disciplines. [tag: topic]	1
<b>TOT</b>	<b>93</b>

**Table 23 - Reasons for full-text exclusion:** the table provides an overview of the articles excluded per full-text examination, with details about the reasons for exclusion.

## Results

The overall study selection process yielded 98 papers published between 2001 and 2023 (see Appendix for the detailed list of the included papers).

The selected papers differed widely in all the considered parameters (i.e., study design, population, adopted technology, and outcome measures); thus, we analyzed the evidence grade, classifying them based on the NHMRC Levels (2009). None of the reviewed papers were

included in Level I.

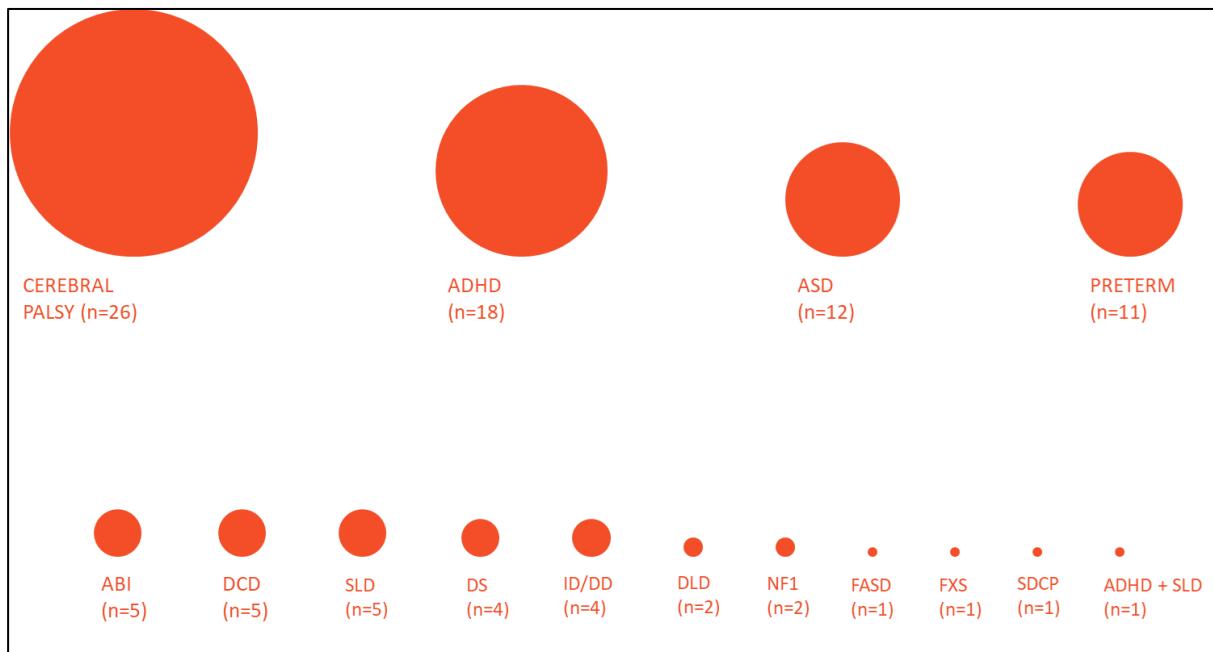
More than half of the studies (52/98) were designed as randomized controlled trials (RCTs). Therefore, they were classified as Level II, while Level IV emerged as the second largest group (25/98), including case series with either post-test or pre-/post-test outcomes. The remaining papers were assigned to the sub-classification of Level III, depending on whether they described pseudorandomized-controlled trials (Level III-1; 6/98) or comparative studies with or without concurrent controls (respectively Level III-2; 14/98 and Level III-3; 1/98). Furthermore, we verified the presence and the features of the control groups. While a subset of the included studies (29/98 - 30%) was designed without control groups, in most papers (69/98 - 70%), the subjects were compared to a group of healthy controls (5/69) or subjects undergoing treatment as usual (i.e., rehabilitative sessions not including telerehabilitation - 17/69), no treatment/waitlist (21/69), placebo treatments (11/69), or same/different telerehabilitation treatment with different features (e.g. frequency and duration of the rehabilitative sessions - 11/69); a small minority (4/69) of the studies were designed with more than a control group: two papers included a no-treatment/waitlist and a placebo group, while the other two included a placebo and a same/different telerehabilitation treatment group.

The applied population criteria also yielded a heterogeneous representation of the neuropsychiatric conditions treated via technological tools for telerehabilitation (see Figure 19). Based on the epidemiology of this nosographic group, the most numerous papers (47/98 - 48%) included papers describing interventions for patients with neurodevelopmental disorders. "Neurodevelopmental disorders" is an umbrella term, including various diseases with different clinical features; thus, a more specific analysis was performed: the two most represented pathologies were Attention Deficit and Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) (respectively, 18/98 - 18%, and 12/98 - 12%), followed by the Developmental Coordination Disorder (DCD) (5/98 - 5%), the Specific Learning Disabilities (SLD) (5/98 - 5%) and the Developmental Delay/Intellectual Disability (DD>ID) (4/98 - 4%); a few papers about Developmental Language Disorders (DLD) (2/98 - 2%) and a sample of patient presenting a combination of SLD and ADHD (1/98 - 1%) were included too.

Besides neurodevelopmental disorders, two other significant subgroups emerged, including papers about technological telerehabilitation protocols in patients with cerebral palsy (26/98 - 27%) and preterm newborns (11/98 - 11%).

The group of paper not classified in the previous categories consisted of a collection of other conditions, such as acquired brain lesions (5/98 - 5%), Down Syndrome (4/98 - 4%), Type 1

Neurofibromatosis (2/98 - 2%), Fragile-X Syndrome, fetal alcohol spectrum disorders, and speech disorders associated to cleft palate (1/98 - 1% each).



**Figure 19 - The landscape of neurological and neurodevelopmental disorders:** the figure represents the distribution of the reviewed papers according to the nosographic classification of their populations. The diameter of the bubbles is proportional to the numerosity of the groups.

ADHD=Attention Deficit and Hyperactivity Disorder; ASD=Autistic Spectrum Disorder; ABI=Acquired Brain Injury; DCD=Developmental Coordination Disorder; SLD=Specific Learning Disabilities; DS=Down Syndrome; ID/DD=Intellectual Disability/Developmental Delay; NF1: Type 1 Neurofibromatosis; FASD: Fetal Alcohol Spectrum Disorder; FXS: Fragile-X Syndrome; SDCP: speech disorder associated to cleft palate

Such heterogeneity also emerged when the populations of the reviewed papers were analyzed in terms of age range (from 3 months to 18 years) and sample size (from 3 to 180 patients).

Papers about totally clinic-based rehabilitative care were excluded from the review. Thus, the settings were analyzed based on the type of adopted ecological environment (home or school) and the direct recipient of the intervention (patient or caregiver or patient+caregiver/teacher). Almost all studies directly targeted patients (87/98 - 89%) in a home-based setting (88/98 - 90%).

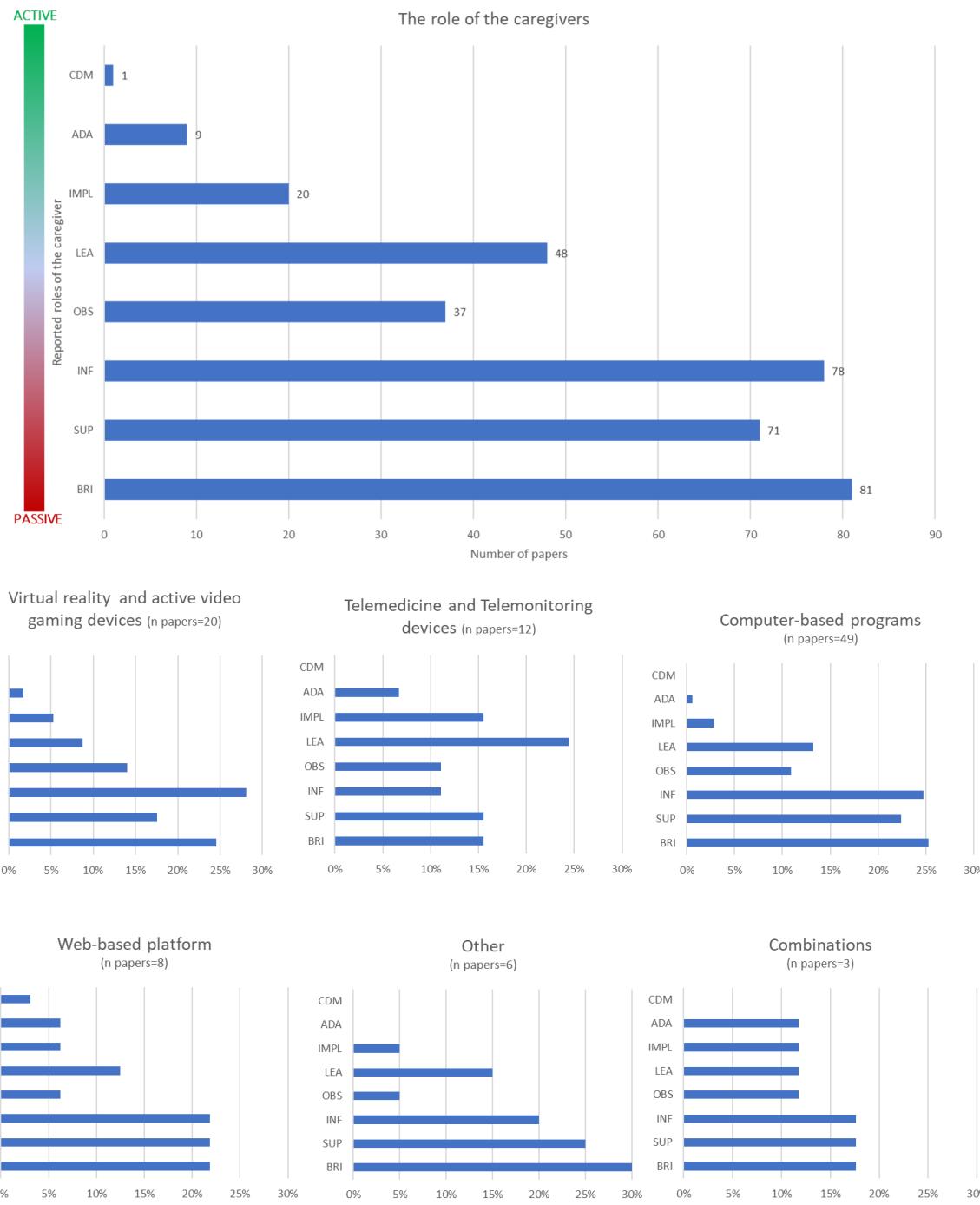
However, the vast majority of the included papers (89/98 - 91%) explicitly mentioned the role of the caregivers in the tele-rehabilitative sessions. We adopted the framework proposed in a previously published review (14) to classify the type of roles that parents assumed in the

intervention, as described in the method section. More than one label could be assigned to a single paper to describe the features of the caregiver involvement completely.

In most papers, the caregivers were described as the subjects having the responsibility to ensure the child's attendance to the rehabilitative sessions, encourage/motivate them to complete the intervention, and share information (e.g., child's behavior, family needs) with the therapists or the researchers (in detail: "Bringer" 81/98; "Supporter" 71/98; "Informer" 78/98). As this review was focused on telerehabilitation, many interventions included pre-training sessions to show and teach caregivers how to use the technological devices or conduct the rehabilitative session at home; besides, such an approach was the milestone of the interventions targeting directly caregivers<sup>190-193</sup>. Thus, a significant subset of papers was classified into the "Observer" and "Learner" categories (in detail: "Observer" 37/98; "Learner" 48/98). The "Implementer" label was applied (in detail: "Implementer" 20/98) when caregivers were reported to play an active role in the telerehabilitation activities but not for every home-based task, even if it was described as part of the intervention (e.g., we did not use this label when caregivers were merely asked to install software and supervise its use). A smaller subset of papers outlined a therapeutic relationship where professionals and caregivers share ideas to adapt the rehabilitative program ("Adaptor" 9/98)<sup>177,191,193-199</sup> or have an active dialogue to set the focus of the intervention ("Collaborative Decision Maker" 1/98)<sup>199</sup>.

Furthermore, we cross-applied the classification of the caregivers' role and the taxonomy of technologies to explore the influence of the different settings on the features of the therapeutic relationship: the occurrence of the "caregivers' role" labels across the papers describing "Virtual reality and active video gaming devices", "Computer-based programs", "Web-based programs", and "other devices (e.g., purely robotic/treadmill systems, sensorized tools)" reflected the general distribution. Otherwise, the interventions based on "Telemedicine and Telemonitoring devices" or combinations of the previously mentioned technologies seemed to assign active roles to the caregivers more frequently.

An overview of the analysis of the role of the caregiver is provided in Figure 20.



**Figure 20 - The role of the caregivers and the impact of technologies:** the classification of the caregivers' role is summarized in the bar graphs above. The upper one represents the distribution of the labels applied to the involvement of the caregivers described in the reviewed papers (more than one label could be applied to a single article). The labels are reported on the axis according to the spectrum from “passive” to “active”, which is represented alongside the bar graph. The lower ones represent the results of the cross-application of the classification of the caregivers' role and the technologies taxonomy (results are expressed in percentages of the numerosity of each technology subgroup, which is reported in the titles of the bar graphs).

BRI= Bringer; SUP=Supported; INF=Informer; OBS=Observer; LEA=Learner; IMPL=Implementer; ADA=Adapter; CDM=Collaborative Decision Maker

We also characterized the rehabilitative setting based on the role of the therapist: in 65/98 studies, the program did not require the direct intervention of the therapist to administer or monitor the intervention; more precisely, a subset of these papers (41/65) described adaptive device automatically modulating the level of difficulty of the exercise based on child's performance, while the remaining (24/65) reported pre-determined interventions with no monitoring or adaptations needed. Otherwise, 33/98 studies described the involvement of a professional who monitored and adjusted the intervention in a synchronous (9/33) or asynchronous (24/33) setting.

The selection criteria excluded the totally "clinic-based" rehabilitative programs. Still, a subgroup of papers (7/98 - 7%)<sup>196,197,200–204</sup> describing hybrid interventions (i.e., partially administered via telerehabilitation and during "in clinic" sessions) was included in the review. The remaining papers (91/98 - 93%) were identified as entirely administered via telerehabilitation; a sub-classification was applied to the latter group to differentiate the home-based (82/98) from the school-based programs (9/98)<sup>205–213</sup>.

The workload of the rehabilitative interventions was once again largely variable, both within and between papers, in terms of frequency and duration of the sessions and the total duration of the intervention. Thus, we calculated a "treatment intensity index" by dividing the minimum total rehabilitative workload described in the papers (in minutes) by the total time span of the intervention (in weeks); eight articles<sup>177,193,214–219</sup> did not contain sufficiently detailed information to calculate the index. Such a parameter provided a comparable measure to classify the interventions' dosage; the classification results are summarized below in Figure 21.

ICTs were analyzed using a previously published classification system to define the heterogeneous landscape of the adopted devices. The most common tools (58/98 - 50%) were "computer-based programs and web-based platforms" (e.g., Cogmed, BrainGame Brian), followed (20/98 - 21%) by "virtual reality and active video-gaming" including commercially available video-gaming consoles (e.g., Nintendo Wii, Sony Playstation, Microsoft Xbox) and research devices based on virtual reality. A third subset of papers (12/98 - 12%) analyzed rehabilitative interventions administered via "telemedicine or telemonitoring devices" (e.g., telehealth platforms, video-call platforms). A minority of studies adopted "other devices" such as research prototypes or sensorized and tele-monitored machines, and a combination of the previous categories (respectively: 6/98 - 6%; 3/98 - 3%). The outlook of the adopted ICTs and their categorization is provided in Table 24).

CATEGORY	REHABILITATION TOOLS
Virtual reality and active video gaming	Nintendo Wii Fit, Microsoft Xbox Kinect, VR videogame using a sensing glove, Sony PlayStation, Move and Eye motion input devices
Telemedicine or telemonitoring devices	App - phonetic training program, Zoom, video calls, video recording, RUBI-Parent Training via Telehealth, Parent Coaching Telehealth intervention
Computer-based or web-based programs	Cogmed Working Memory Training, XtraMath, Scientific Brain Training, Luminosity cognitive training, EVO platform, Braingame Brian training, Tobii X2-60, Gaming Open Library for Intervention in Autism at Home(GOLIAH), TALi Health, Mind Reading Software, The Number Race, Focus Pocus, NeuroScouting, Reading Trainer®, The Emotion Trainer, Computer-Assisted Arm Rehabilitation (CAAR), ABRACADABRA program, "Move it to improve it" (Mitii), MoveHero, RuntheRAN, Web App "I bambini contano"
Other devices	Home-based virtual cycling training (hVCT), home-based intelligent stretching robot, MOTOMed gracile, Self-Initiated Prone Progression Crawler (SIPPC) robotic system, Google glasses+Android app, CareToy platform.
Combination of the previous categories	Microsoft Xbox360+Kinect; Sony PlayStation3 + Move and Eye input devices; Google glasses+Android app, Focus Pocus+ EEG hardware, Computer videogames + EEG (neurofeedback), Pre-recorded video clip+Kinect 3D camera,+video-connection

**Table 24 - The taxonomy of telerehabilitation technologies:** the technological tools adopted in the reviewed studies are reported in the table and classified according to the framework we applied for the qualitative description.

The rehabilitative interventions were analyzed based on the skills (neuropsychological, motor, or speech and communication abilities) they were designed to address, and the type of outcome measures adopted to assess their effectiveness.

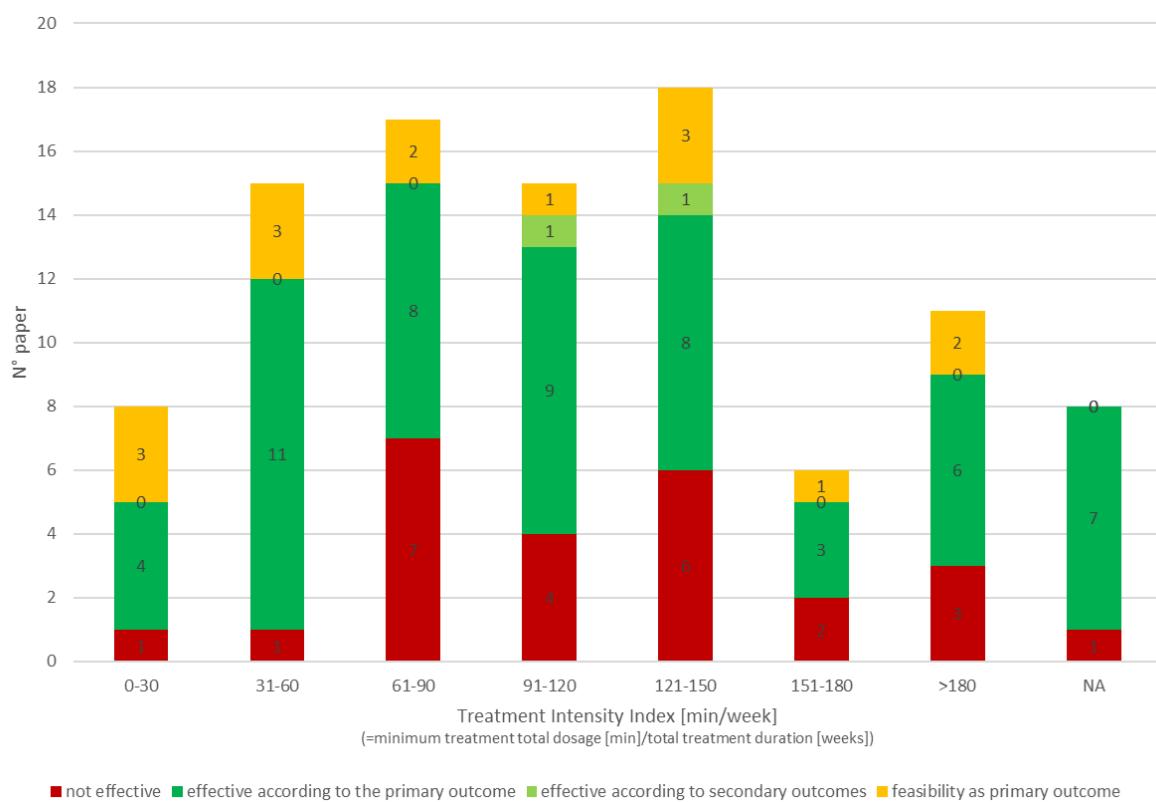
Most of the described protocols were designed to train functions of a single domain, in particular neuropsychological (e.g., cognitive skills, executive functions, academic skills) or motor (e.g., gross motor functions, balance, coordination) functions (respectively 53/98 - 54%; 34/98 - 35%). Only a small minority (3/98 - 3%) of the reviewed paper described rehabilitative tools aiming to train speech and communication skills specifically. Moreover, we identified a

subset of papers reporting multimodal telerehabilitation tools that simultaneously targeted neuropsychological and motor (5/98 - 5%) or speech and communication (3/98 - 3%) skills.

Each primary outcome measure of the paper selected (274 variables in total) was classified based on the assessed function, into the four broad components of the *International Classification of Functioning, Disability and Health for Children and Youth* (ICF-CY). Most of the tools adopted to assess the outcome of neuropsychological and motor rehabilitative tools fell into the “Body Functions” category, mainly because the trained skills could be classified as “global/specific mental functions” (128/169) or “movement functions” (35/169), thus this domain resulted in being the largest (164/274 - 59,9%). The “Activities and Participation” domain is less represented as 91/274 (33,2%) outcome measures could be classified, including mostly “mobility” (30/91) and “learning and applying knowledge” (44/91) chapters. No papers primarily assessing skills specifically attributable to the “Body structure” and “Environmental Factors” were identified. However, a subgroup of papers adopted a composite battery of primary outcome measures, assessing beyond parameters classifiable into the “Body Functions” or “Activities and Participation” variables into the “Body structures” domain (5/274 - 1,8%) categories. The remaining reviewed articles (14/274 - 5,1%) reported “feasibility” as the main outcome measure, therefore they were not included in this analysis.

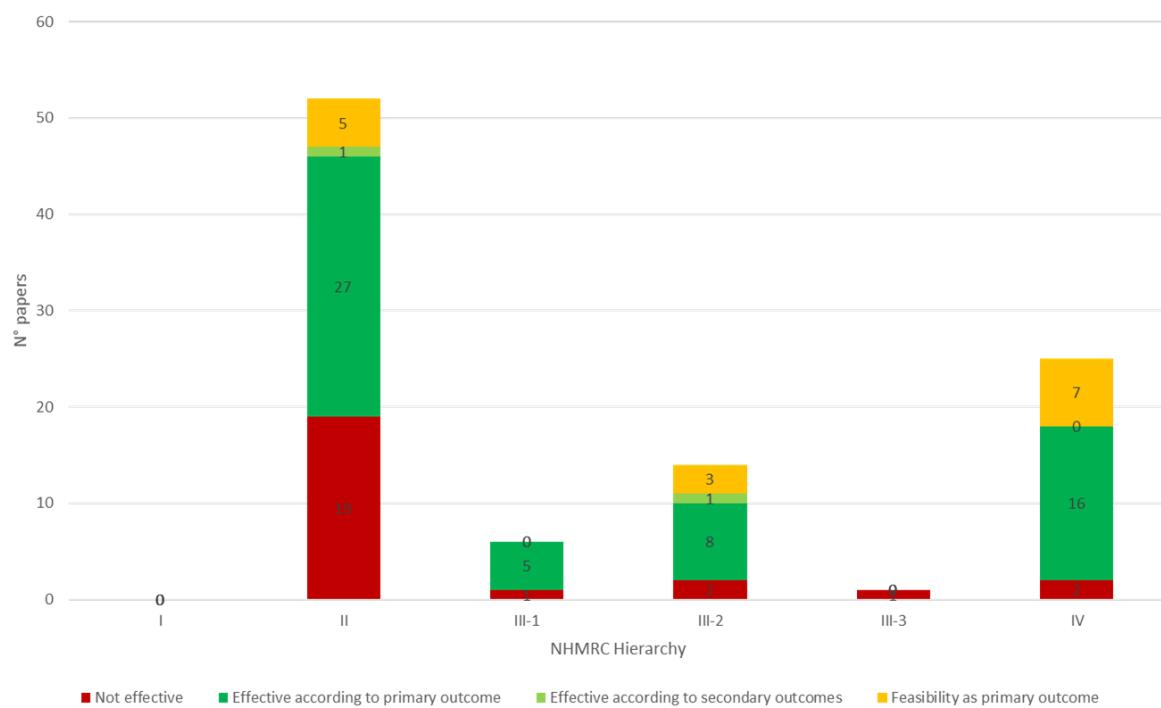
We eventually classified the included papers based on their results (i.e., non-efficacy, efficacy based on the primary outcome/other outcomes, feasibility). Overall, 59% of the reviewed papers documented the effectiveness of the intervention based on the primary outcome (57%) or secondary outcomes (2%); the subgroup including the studies having feasibility as the primary outcome was not included in the efficacy categorization.

The results of this analysis, subclassified per grade of evidence and “Intensity index”, are summarized in Figures 21 and 22.



**Figure 21 - Rehabilitative workload of technological telerehabilitation interventions:** the workload of the rehabilitative interventions is represented in the bar graph based on the “treatment intensity index” we applied by dividing the minimum total rehabilitative workload described in the papers (in minutes) by the total time span of the intervention (in weeks). Each bar represents a 30-minute step. Bars are segmented in different colors according to the classification of effectiveness.

NA=articles not containing sufficiently detailed information to calculate the index.

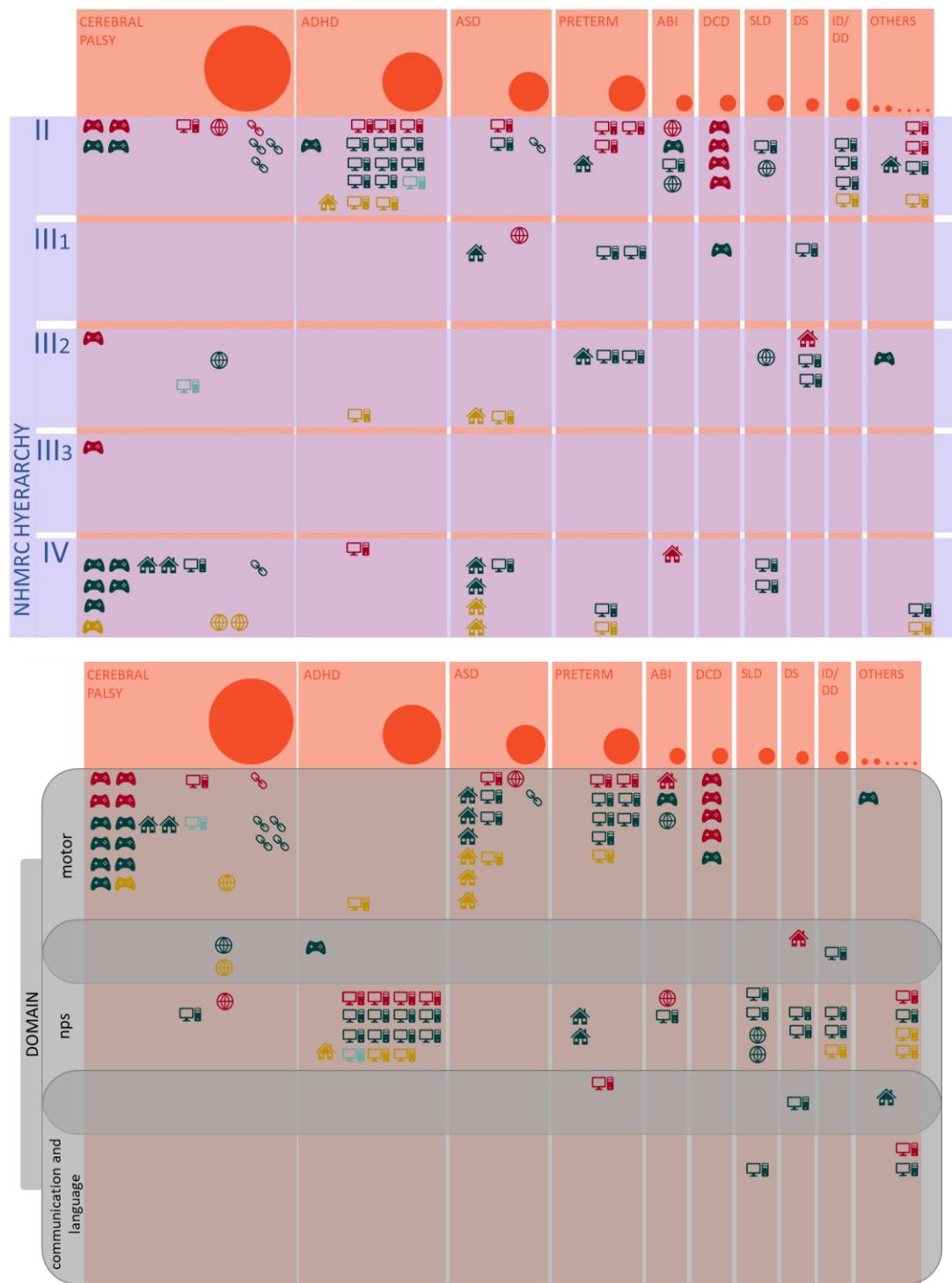


**Figure 22 - Evidence grade and effectiveness of technological telerehabilitation interventions:** the bar graph summarizes the qualitative description of the evidence grade and the effectiveness of the reviewed papers. The study design was classified according to the NHMRC Hierarchy and effectiveness was labeled according to the outcomes.

Bars are segmented in different colors according to the classification of effectiveness.

NHMRC=National Health and Medical Research Council

A global overview of the main analyzed variables of the reviewed papers is represented as an infographic in Figure 23.



**Figure 23 - The landscape of technologic telerehabilitation for pediatric neurologic and neurodevelopmental disorders:** the infographic summarizes the main analyzed variables of the reviewed papers. The bubbles' diameter and the orange columns' width are proportional to the number of identified papers per diagnostic group. The icons represent the classification of the adopted technological devices (see below); every icon corresponds to a single paper. The colors correspond to the classification of the efficacy of the interventions described in each paper (i.e., red=not effective; dark green=effective based on primary outcome; light green=effective based on secondary outcomes; gold=feasibility as primary outcome).

**Legend:**  
 🎮 = Virtual reality and active video gaming devices 🏠 = Telemedicine and Telemonitoring devices 📱 = Computer-based program  
 🌐 = Web-based platform 💾 = other devices

ADHD=Attention Deficit and Hyperactivity Disorder; ASD=Autistic Spectrum Disorder; ABI=Acquired Brain Injury; DCD=Developmental Coordination Disorder; SLD=Specific Learning Disabilities; DS=Down Syndrome; ID/DD=Intellectual Disability/Developmental Delay; NPS=Neuropsychological domain

## Discussion

For the scoping purpose of this review, we adopted a wide-scope search strategy to encompass as extensively as possible the multifaceted field of technological telerehabilitation for pediatric neurologic and neurodevelopmental disorders. Consequently, the paper selection process yielded many papers composing a heterogeneous landscape, mainly in terms of population and study design. The two most numerous sub-groups of articles included samples of patients affected by cerebral palsy and neurodevelopmental diseases, as expected based on the epidemiology of pediatric neuropsychiatric disorders. Besides, two other recurring conditions were Acquired Brain Injuries and Preterms. At the same time, the remaining few included a group of other pathologies studied in a single or a couple of papers. Notably, the distribution of the studies about neurodevelopmental disorders is unbalanced in favor of ADHD and ASD, while other disorders with high prevalence (e.g., SLD) were less represented. Furthermore, our search did not intercept other common neuropsychiatric conditions (e.g., epilepsy, neuromuscular diseases) in the reviewed paper. This finding may be due to the features of the search string. However, it suggests that there are areas where the application of technological telerehabilitation is still to be explored.

Despite the majority of the protocols being structured as RCTs, sample sizes and the study design differed widely. The diversity in the pathogenesis of the diseases and the variability in the study design and the adopted outcome measures made it unfeasible to do a meta-analysis to compare the results of the included studies. Nonetheless, our qualitative description yielded a prevalence of papers reporting efficacy according to the selected primary/secondary outcome measures in every NHMRC Hierarchy Class. This distribution might be influenced by publication biases. Still, it also provides preliminary support for the effectiveness of this kind of rehabilitative approach, even if it needs to be confirmed by specific meta-analyses focused on single domains of intervention or technological devices.

Our review aimed to provide a comprehensive description of the features of the telerehabilitation setting in this field, and we decided to focus on (1) the role of caregivers and professionals, (2) the types of adopted technologies, (3) the intensity of the interventions and (4) the functional domains identified as therapeutic target.

We characterized the role of the caregiver by applying to the reviewed papers a previously published classification that described a spectrum from “passive” to “active” roles<sup>183</sup>. Even if the direct target of the intervention was the patient himself, almost all studies explicitly mentioned the involvement of caregivers in the intervention, suggesting that the tele-

rehabilitative approach for pediatric diseases intrinsically supports a therapeutic relationship between families and professionals. However, our qualitative classification showed a “pyramidal” distribution, with “passive” labels (e.g., Implementer, Supporter, Informer) being more frequently applied than the “active” ones (e.g., Adaptor, Collaborative Decision Maker). The cross-application of this classification and the technologic taxonomy gave us a more detailed insight into this finding, even if the unbalanced numerosity of the “technologies” subgroups made a statistical comparison unfeasible. The occurrence of the “caregivers’ role” labels across the papers describing “Virtual reality and active video gaming devices”, “Computer-based programs”, “Web-based programs”, and “other devices” reflected the general distribution. In contrast, the interventions based on “Telemedicine and Telemonitoring devices” or combinations of the previously mentioned technologies seemed to assign active roles to the caregivers more frequently. We also classified the other side of the therapeutic relationship, by analyzing the professionals’ role in designing, administering, and modulating the interventions. Notably, most studies described programs that do not require the direct intervention of the therapist to administer or monitor the intervention.

Many factors may have influenced this finding. Firstly, computer/web-based programs and devices for virtual reality and active video gaming emerged to imply more “passive” roles, as caregivers in these interventions are mainly required to supervise and support the use of the tool by the child. As these technologies were the most frequently mentioned in the reviewed papers, the features of their setting may have twisted the general description. Secondly, a significant subset of articles described technological tools having the possibility of modulating the level of difficulty of the exercise based on the child’s performance with no professional interventions needed. The intrinsic adaptivity of the technological devices was emphasized because of their potential to provide a dynamic intervention, reducing the workload of professionals and fostering the effectiveness of the rehabilitative intervention<sup>220</sup>. However, the usability of technologies can still be a barrier to the acceptance of the telerehabilitation approach by the families<sup>221</sup>, and, as mentioned above, “active” caregivers’ roles imply collaborative interaction with the therapist.

Regarding the analysis of the adopted technologies for telerehabilitation, to date, a standardized taxonomy able to classify is still lacking. We integrated previously published classifications to define a novel taxonomy for digital technologies that could consider all the domains handled by clinicians. The categories we proposed encompass all devices targeting purely motor, neuropsychological, or speech treatments but also integrated ones, thus, combining motor and cognitive or cognitive and speech functions.

The most commonly adopted ICTs were computer-based/web-based programs and virtual reality and active video-gaming devices, while a smaller subset of papers described telemedicine/telemonitoring devices or tools combining different technologies. Some issues may be raised from this situation. As mentioned above, the computer-based/web-based and virtual reality/active video-gaming types of technologies appeared to be related to a more “passive” role of the caregiver. Besides, more advanced integrated technologies (e.g. equipped with wearable sensors or remotely monitorable) are not yet very diffused across the clinical studies.

The data about the rehabilitative interventions workload - in terms of frequency and duration of the sessions, and total duration of the intervention - were once again largely variable, both within and between papers. The “treatment intensity index” we applied provided an approximate but comparable measure to classify the dosage of such diverse interventions. Interestingly, the majority of the interventions (70/98) included a weekly workload of 60 minutes or more. This finding might be due to the research setting, prioritizing shorter and more intense interventions. However, it also suggests the potentiality of the home-based setting in integrating the in-clinic session increasing the dosage of the intervention.

The description of the main features of the technological telerehabilitative setting was completed by the analysis of the interventions based on the skills they were designed to address, and the type of outcome measures adopted to assess their effectiveness.

Overall, a prevalence of a single-domain intervention emerged, in particular, focused on neuropsychological or motor functions. Interestingly, we also identified a subset of papers reporting multimodal telerehabilitation tools that simultaneously targeted neuropsychological and motor or speech and communication skills.

We aimed to further characterize the objectives of the interventions classifying the main outcome measures, based on the assessed function, into the four broad components of the ICF-CY. As outlined in the Results section, most of the primary outcome measures of the reviewed telerehabilitation programs could be classified in the “Body function”, according to reviews on ICF domains mainly targeted by interventions<sup>222</sup>, even though family and child goals tend to be focused on activities and participation. It is therefore of utmost importance to conceptualize technological treatment pathways that conceive both the improvement of function and quality of life integrated as primary goals and targets of the intervention.

To the best of our knowledge, this is the first systematically conducted review providing a wide-scope overview of the heterogeneous landscape of technological telerehabilitation for pediatric neurologic and neurodevelopmental disorders. Our results provide a detailed qualitative description that can be a base for planning future policies and research, considering the promising results in terms of the effectiveness of telerehabilitation protocols. In particular, the following issues should be addressed based on the features emerged from this review: (1) the description of a relatively “passive” caregiver role across the studies advocate for a further exploitation of the potentials of the technological telerehabilitation approach as a setting where caregivers and professionals can cooperate in an actual active family-centered care; (2) the creation of a standardized classification shared by the different professional figures involved in this field (e.g. by a consensus panel) is needed to improve clinical practice, scientific research, and comparative work; (3) given the vast heterogeneity of the interventions, the efficacy of this approach needs to be confirmed by specific meta-analysis focused on comparable domains of interventions or technological devices; (4) the potential of adopting advanced technologies and multidomain interventions should be further explored, to address the clinical needs of the most common pediatric neurological and neurodevelopmental diseases often including complex and multifaceted impairments.

### 3.2.The Virtual Park project

#### *Scientific background*

Until about twenty years ago it was believed that physical exercise in muscular dystrophies could be harmful to the structure of the muscle fiber cell. It is now certain and validated that an active lifestyle and the practice of controlled and regular physical activity are to be considered therapeutic in neuromuscular pathologies to optimize muscular and cardio-respiratory function and prevent atrophy from "non-use" with a positive impact also in terms of quality of life<sup>223-227</sup>. There are still many doubts about the optimal type, intensity, and frequency of training, in the absence of defined and specific guidelines<sup>228</sup>. Pedaling exercise (cycling), with the lower or upper limbs, is an aerobic activity performed in a seated position and easily accessible for the patient affected by neuromuscular pathology in different phases of the disease. In literature, cycling has been proposed to subjects affected by Duchenne muscular dystrophy in some clinical studies both in intensive (5 days/week) and extensive (3 days/week) modalities for periods varying between two and six months, demonstrating the feasibility and safety of this approach for both ambulant and non-ambulant subjects, with positive effects in preserving motor function and slowing down its deterioration<sup>229-231</sup>. Currently, the indication to intervene in support of neuropsychological deficits is reported, for example, in the guidelines for the treatment of Duchenne muscular dystrophy<sup>30</sup>. However, there is a lack of descriptions in the literature of specific interventions in this sense in pediatric patients with neuromuscular pathologies. To the best of our knowledge, only one study has been published so far, on a small sample of patients with Duchenne muscular dystrophy, focused on neuropsychological training based on digital tools<sup>232</sup>.

The VirtualPark system has already been validated in previous research projects by CNR-STIIMA. Specifically, several versions of the system designed for different patient populations have been validated. Common to all previous versions is the cycle ergometer used: model COSMED E100-E5, medically certified. The VR application and the devices for interaction and the detection of the associated physiological response differ depending on the context of use.

The first version of the system (Goji Aging Interest Project, later included in the Young Researchers Project GR-2013-02356043) created for elderly people with mild cognitive decline offered a semi-immersive experience, thanks to the use of a large screen projected onto the wall. The virtual environment consisted of two scenes: a park in which navigation occurred via the actual pedaling speed and a scenario in which the user had to cross the road, slowing down when appropriate. The physiological response was detected via a sensorized t-shirt worn by the

patient. A third scene, set in a supermarket, was dedicated to cognitive training. The system was the subject of a pilot study ( $n=10$ , Goji) and a randomized clinical study ( $n=80$ , GR) at the Santa Lucia Foundation in Rome, proving to be acceptable and pleasant for the elderly people involved. In the Goji project, following 6 weeks of physical and cognitive training, older adults in the experimental group reported slight improvements in neuropsychological tests and a significant improvement in the concentration of biological markers indicating oxidative stress and, consequently, cognitive decline<sup>233</sup>. In the second study, it emerged that the groups ( $n = 40$ ) that performed physical activity, either alone or in combination with cognitive training, after 12 weeks, had improvements in neuropsychological tests, which were maintained at follow-up<sup>234</sup>.

A second version of the prototype was designed for endurance training of elderly people with chronic respiratory diseases in collaboration with the IRCCS INRCA of Casatenovo (Lecco). Similarly to the first version, the virtual environment was projected onto a wall screen placed in front of the COSMED cycle ergometer. The system also integrated a medically certified wrist pulse oximeter to measure oxygen saturation during exercise. A group of 14 elderly patients with chronic obstructive pulmonary disease used the system as part of a 3-week in-clinic rehabilitation program. Patients experienced improvements in terms of endurance in line with traditional rehabilitation protocols. The subjective experience was positive, and involvement remained high throughout the treatment<sup>235</sup>.

Two immersive versions of this system were also created in which the virtual environment that the patient explores while pedaling was displayed through a virtual reality viewer. The first involved the use of the cycle ergometer for a dual-task task. In a Cave Automatic Virtual Environment (CAVE), installed at the Istituto Auxologico Italiano, the elderly user had the task of pedaling in a virtual park and identifying a series of target objects/animals that appeared along the path among different distractors. The system was validated from the usability point of view by enrolling frail elderly people, proving to be acceptable and user-friendly<sup>236</sup>. The second system, instead, implements a training protocol for post-COVID patients. A preliminary study carried out at the MSWiA Specialized Hospital in Głuchołazy involved 22 adult post-COVID patients. Motivation, positive emotions, and the state of flow (which represents how much the user is involved in the activity) increased over time (3 weeks), demonstrating how the use of virtual reality is perceived as motivating and engaging and has a positive impact on the rehabilitation experience<sup>237</sup>.

Finally, a version for home use was created and tested (ARTEDIA project). In this case, the virtual environment is displayed on a tablet anchored to the cycle ergometer, thus ensuring a

compact and easy-to-transport setup. In addition to navigation in the park, the virtual environment also offers a cognitive task, based on the Go-no Go task. The user must, via a button positioned on the handlebars of the cycle ergometer, recognize certain stimuli and identify them by pressing the button. To enable home use, the application was integrated into a commercial tele-rehabilitation platform (Khymeia VRSS)<sup>238</sup>. The feasibility study, in this case, involved post-stroke patients<sup>225</sup>, children and adolescents with neurological disorders<sup>229</sup>, and post-COVID patients<sup>238</sup>. The entire system was judged positively by all categories of patients, who had good adherence to the intervention protocol and appreciated the possibility of carrying out rehabilitation activities at home. In some cases, the perceived effort for using the system was high due to problems caused by the lack of a sufficiently stable internet connection and some updates to the telerehabilitation platform that limited, at least temporarily, access to the rehabilitation programs.

#### *Innovation and potential impact*

The new device, the subject of this study, was designed starting from the previous versions to be used by pediatric users with neuromuscular pathologies. The adaptations concern both the hardware setup and the software.

The THERA-Trainer cycle ergometer, used in the new version of the system, differs from the one used in previous studies because:

- the patient is seated on a chair or wheelchair
- the cycle ergometer is adaptable to different heights through adjustments to the seat, pedals and
- handles, ensuring use also by children and adolescents;
- the device can be used with both the lower limbs and the upper limbs, which makes it suitable also for patients with poor mobility of the lower limbs;
- the cycle ergometer is motorized and allows passive, assisted, and active movement;
- the virtual reality environment includes two scenarios, one naturalistic and one urban;
- the cognitive tasks coupled with the physical task of pedaling were specifically designed with the needs of neuromuscular and developmental patients in mind. There are four tasks and they focus on: attention, inhibition, navigation, and working memory.

The risk analysis was drawn up based on the ALARP (As Low As Reasonably Practicable) principle. The risk analysis reported in this document concerns in particular the risks associated with the association of the cycle/armoergometer with the virtual reality application. A specific

risk analysis associated with the use of the cycle/arm ergometer was not carried out as it is a medical device with CE medical certification that is used per its intended use. Furthermore, it is specified that the operation of the cycle ergometer is not controlled by external software and cannot be modified by it in any way.

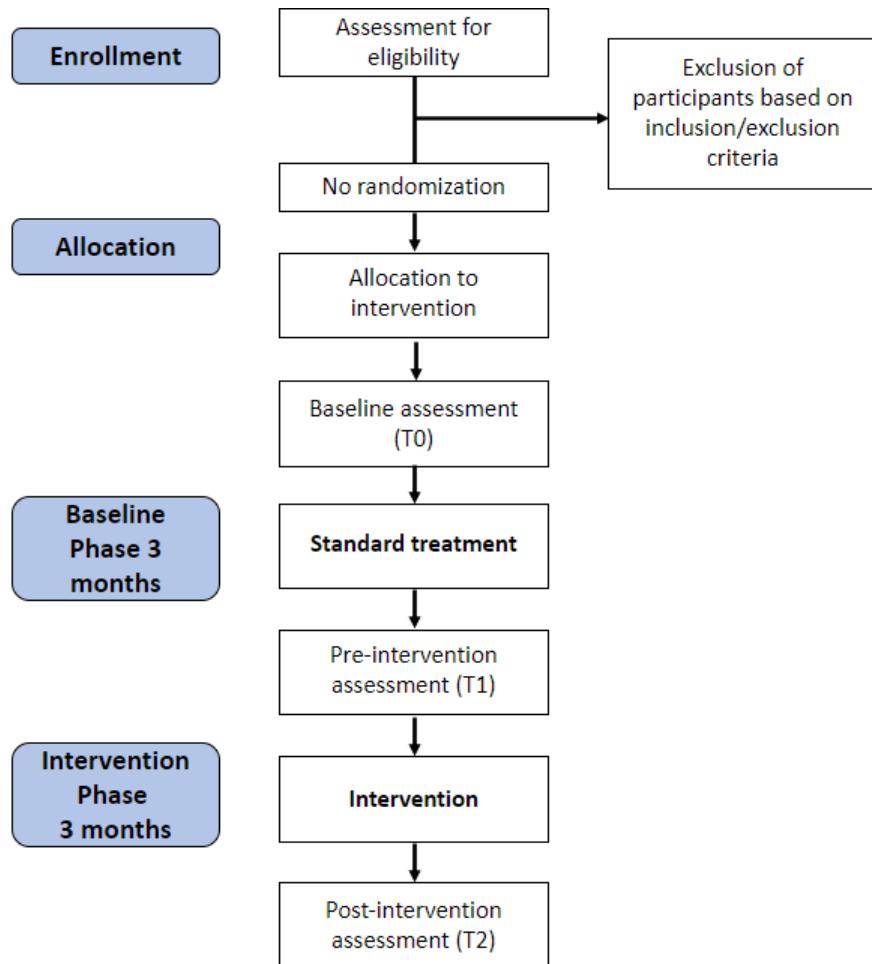
### **Objectives**

This clinical study aims to evaluate the performance and safety of VirtualPark, a technological device that allows a multimodal rehabilitation intervention in a group of developmental patients affected by neuromuscular diseases. The device under study, in particular, allows it to intervene in parallel on the motor domain (via cycle-/armoergometer) and on the neuropsychological domain (via the integrated virtual reality serious game platform). The possibility of integrating a multimodal technology has considerable potential in the pediatric age, where the dynamics of neuropsychomotor development imply the fundamental role of the global approach to rehabilitation intervention. Furthermore, the integration of motor and cognitive rehabilitation in a playful context is a cornerstone of the care of pediatric patients with neurological and neurodevelopmental disorders. This model also applies to neuromuscular diseases (e.g. Duchenne muscular dystrophy), of which recent scientific literature describes in ever greater detail the specific neuropsychological profiles, in addition to the known deficits in motor function<sup>11,166,239,240</sup>.

The objectives of this study are: to evaluate the impact of a multimodal technological device on the slowing of the functional motor decline of neuromuscular patients compared to standard care and to evaluate its effects on engagement and some neuropsychological functions.

### **Methods**

The study is configured as a multicenter clinical study in the pilot phase. Figure 24 shows the flow chart that summarizes the phases of the study, which are described in detail below.



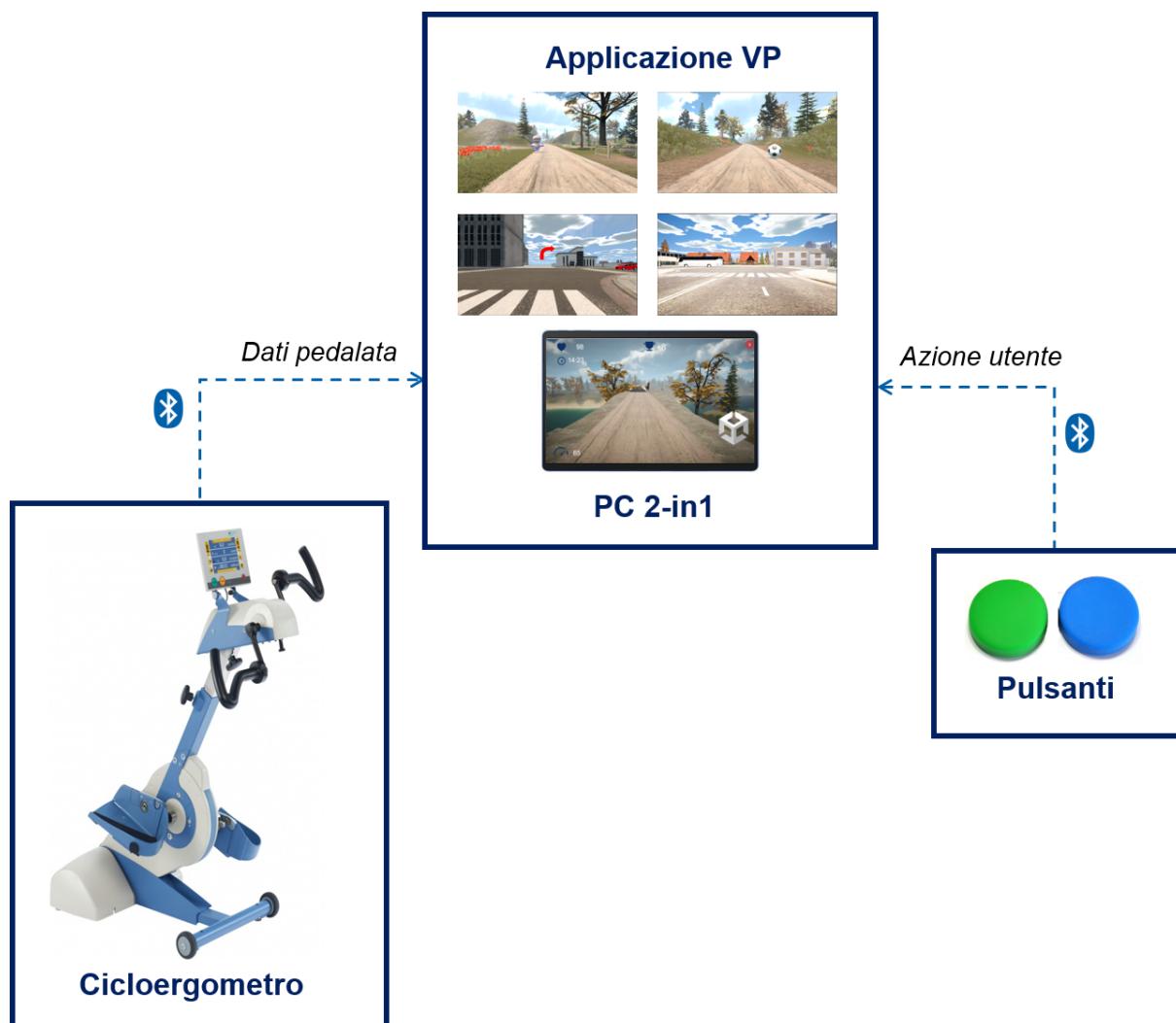
**Figure 24 - Structure of the study:** the flowchart summarizes the structure and the main steps of the study

#### *Technical overview of the device*

The protocol is based on a device called VirtualPark.

The VirtualPark device allows multimodal dual-task training (physical + cognitive) on a cycle/arm ergometer in a virtual reality (VR) environment.

The device consists of a cycle/arm ergometer for performing physical training and a virtual reality application. The device also integrates two buttons that constitute the user interface. The integration of these elements allows the creation of a system for dual-task training, which combines physical and cognitive training at the same time (Figure 25).



**Figure 25 - the Virtual Park device:** the main tools included in the integrated Virtual Park device are represented in the figure

The cycle ergometer is a commercial device produced by the THERA-Trainer company. In particular, the THERA-Trainer Tigo model with a 7" color screen is a device for the lower limbs combined with a device module for the upper limbs and therefore allows training in both modes. THERA-Trainer tigo is a Class IIa medical device. The device is motorized and allows passive, assisted, and active training. The training mode and exercise parameters (duration, assistance level, etc.) can be set for each session directly from the THERA-Trainer panel.

The RV environment is a Windows application developed in Unity by CNR-STIIMA. The application is installed on a 2-in-1 Microsoft Surface Pro PC, anchored to the cycle ergometer via a 3D-printed ad-hoc support. The application includes four cognitive tasks, each focused on a specific domain (attention, inhibition, working memory, and navigational skills), set in two

scenarios. The scenarios represent a park and a city. The user navigates within the scenarios using the real speed detected by the ergometer and sent via Bluetooth protocol.

The user interface consists of Puck.js buttons through which the user interacts in carrying out the cognitive task. In particular, pressing the button corresponds to an action in the RV environment that is different depending on the exercise being performed. For example, the user presses the button to signal the identification of a target. The buttons are positioned on the cranks to allow their use during training of both the upper and lower limbs.

The VirtualPark device is designed to perform multimodal dual-task training in an indoor environment. It is characterized by a compact setup that makes it easily transportable and usable by operators and end users. The device is designed for use by patients aged between 6 and 17 with neuromuscular pathologies. The pedaling exercise can be performed with both the upper and lower limbs. Cognitive training includes four exercises, each focused on a specific domain: attention, inhibition, working memory, and navigational skills (Figures 26 and 27). The details about the cognitive tasks are provided below:

- *Visual attention, Inhibition, and cognitive flexibility:* the first task focuses on the domains of visual attention, inhibition, and cognitive flexibility and is set in the park scenario. It is a Go – No Go task in which the patient must identify – by pressing the button – target stimuli (e.g. monsters) that appear along the path, discriminating them from non-target stimuli (e.g. animals). There are four modes, each with 3 levels of difficulty with increasing target appearance frequency (level 1: 15 s, Level 2: 10 s, Level 3: 5 s):
  - In mode 1, monsters and animals do not have a colored aura; when the child hits an incorrect target, he receives an error message. The instruction that the child receives is: “Now hit all the monsters you see”.
  - In mode 2, monsters and animals have a blue-colored aura or a red-colored aura; when the child hits an incorrect target, he receives an error message. The instruction the child receives is: “Now hit all the characters you see with a red aura”.
  - In mode 3, monsters and animals have a blue-colored aura or a red-colored aura; when the child hits an incorrect target, he receives an error message. The instruction the child receives is: “Now hit all the characters you see with a blue aura”.
  - In mode 4, monsters and animals have a blue-colored aura or a red-colored aura. The correctness criterion will be set randomly by the system and variable

during the test; when the child hits an incorrect target, he receives an error message. The instruction the child receives is: "Now the rule for hitting the characters can be any of the previous three, try to understand which one it is. The rule can change as you go along the path."



**Figure 26 - Task 1 – Attention, inhibition and cognitive flexibility, memory**

- *Working memory:* the second task trains working memory and is inserted within the park scenario. The goal is to collect a series of target objects that appear along the path, together with other distractor objects. The list of target objects (e.g. oranges, newspaper, ball) is shown at the beginning of the exercise. The patient must memorize the objects to be collected and identify them within the park by pressing the button. The difficulty levels require a greater number of objects to be identified and the insertion of objects similar to those on the list (e.g. oranges vs. apples). The task has two modes:
  - Mode 1: the list of objects to be collected is presented in graphic form ("list" of images of the objects to be collected)
  - Mode 2: the list of objects to be collected is presented in textual form ("list" of names of the objects to be collected).
- *Inhibition:* the third task is set in the city scenario that is characterized by a series of intersections and straight lines. At the intersections, the patient must decide which type of turn to make (right/left) by pressing the right or left button respectively. In the first level, the correct direction is the one indicated by the arrow. In the second level, the correct direction is the one opposite to the one indicated by the arrow. In the third level a second factor (color) is added: if the color is yellow the correct direction is the one indicated by the arrow, if the color is blue the correct direction is the opposite one.

- **Navigation:** the fourth task is inserted in the city scenario and trains navigation skills. In the first part of the task the patient follows a predefined path (the turns are made automatically); the child/young person receives the instruction: “Pay attention to the path and to what you see around you while you pedal because then you will have to do the road again by yourself”. In the second part of the task, the patient must repeat the path by selecting with the buttons the direction to take at each intersection. The difficulty is defined by the number of turns and the number of reference points inserted in the path (e.g. buses near the turn). The task includes a predefined number of paths from which the operator can choose. Each path has a different objective, for example: “get to school”, or “get to the playground”, and is made up of a certain number of steps (each consisting of a limited number of turns/reference points). For example, the first step of the “get to school” path starts from the child’s home to the first traffic light. In each session, the user retraces the previous steps and adds a step until the final path is completed.



**Figure 27 - the Virtual Park Software:** the figure shows an example of the patient's experience with the training software, with some of the possible scenarios and tasks included in the rehabilitative intervention

The device is produced and developed by the Institute of Intelligent Industrial Systems and Technologies for Advanced Manufacturing of the National Research Council (CNR-STIIMA)

Each device is equipped with a label showing the main data of the device and a progressive number that uniquely identifies each device.

Adequately trained healthcare personnel will be present during the sessions of use with patients. Before the start of the trial, the CNR-STIIMA technical staff will conduct a training day to instruct the clinical staff of the two centers on the use of the VirtualPark device. The user

manuals for the individual devices and the integrated system will be provided and left available. No additional skills or experience are required in addition to what is already required for carrying out the rehabilitation activity.

Additional information about the device is provided in the User Manual included in section 7.1 (“Supplementary Materials”) of the “Appendix”.

#### *Clinical protocol*

The expected duration of the trial is 24 months. The study has a nature:

- exploratory: the investigation aims to test for the first time the applicability and effects of a multimodal rehabilitation protocol in a population of pediatric patients with neuromuscular diseases
- non-“first-in-human”: for the study a medical device (cycle ergometer) integrated with a non-immersive virtual reality system (Virtual Park) previously tested on human subjects is applied
- multicentric: involves the IRCCS “Fondazione Mondino” (Pavia), “E.Medea” (Bosisio Parini), and Fondazione “Stella Maris” (Pisa)
- prospective: involves the administration of a rehabilitation intervention and the evaluation of cases over time
- pilot phase: the investigation applies a protocol on a small sample of patients

The evaluations will be carried out at three time points:

- T0 at the beginning of the study;
- T1 at the end of the baseline period and before the intervention
- T2 at the end of the intervention

A main endpoint was established, and declined as follows:

Endpoint 1: slowing of the decline curve of motor function parameters  
*Measured variables:* pre-/post-intervention changes in the Motor Function Measure-32 (MFM-32) scale. The Motor Function Measure-32 is a motor skills assessment scale validated for patients with neuromuscular disease from 6 years of age. The instrument consists of 32 items (scored from 0 to 3) divided into 3 dimensions: D1- standing and transfers (13 items), D2- axial and proximal motor skills (12 items), and D3- distal motor skills (7 items). The results are expressed as a percentage in relation to the maximum score. The result of each dimension

corresponds to the sum of the scores obtained by the subject in the items of this dimension divided by the maximum score of the same dimension and multiplied by 100. For the MFM-32 the total score corresponds to the sum of all the scores attributed to the subject (all dimensions together) divided by 96 and multiplied by 100<sup>241</sup>.

Secondary endpoints were then established:

Endpoint 2: improvement of motor performance, differentiated for ambulant or non-ambulant subjects:

- Ambulatory subjects: NSSA scale, timed functional tests, and 6MWT.  
The North Star Ambulatory Assessment (NSSA) scale was developed specifically for ambulant children with dystrophinopathy starting from 5 years of age to obtain information on their motor and functional abilities. The instrument consists of 17 items scored from 0 to 2. The final score is given by the sum of the scores obtained in all the items. In addition, 2 timed tests are recorded: the time to get up from the ground and the time required to walk/run 10 meters<sup>242</sup>. Timed functional tests (TFT) are administered in a standardized manner in subjects with Duchenne muscular dystrophy to measure the time taken to perform the following 4 activities: moving from a supine position to a standing position, walking/running for 10 meters, climbing and descending a standardized 4-step staircase. In addition to the time taken to perform the task, the scoresheet also records a qualitative grading from 1 to 6<sup>243</sup>. The 6-Minute Walking Test (6MWT) was born in the cardio-respiratory field but is now used transversally in various fields of motor rehabilitation where it is necessary to quantify the patient's walking resistance. The test measures the distance that a patient can travel, walking on a flat surface along a standardized path, in 6 minutes<sup>244</sup>. In addition to the total distance traveled, the distances traveled for each minute are also collected and any falls/pauses are recorded.
- Non-ambulant subjects: PUL 2.0 scale. The Performance Upper Limb Module 2.0 scale (PUL version 2.0) was developed to evaluate the motor performance of the upper limbs of subjects with dystrophinopathies from 5 years of age. This latest version of the instrument consists of an entry item useful for defining the functional level of the patient and scored separately (from 0 to 6) and 22 other items (scored from 0 to 2). The final score consists of 2 values: the entry-level score and the total score given by the sum of the scores obtained in the individual items<sup>245</sup>.

Endpoint 3: Improvement in endurance after 6 months of intervention.

*Measured variables:* Change in performance on the Assisted 6-minute cycling test (A6MCT). The A6MCT is a submaximal endurance test performed by pedaling with a cycle ergometer in passive mode for both upper and lower limbs created specifically for subjects with Duchenne muscular dystrophy, both ambulatory and non-ambulatory. The final score records the number of complete revolutions performed in 6 minutes; the number of revolutions per minute and any breaks requested by the subject are also recorded<sup>246</sup>.

Endpoint 4: improvement of the neuropsychological functions trained (inhibition, working memory, visuospatial orientation).

*Measured variables:* pre-/post-intervention changes in the specific items of the NEPSY-II scales<sup>151</sup>. The NEPSY-II scale is a battery of tests that allows a neuropsychological evaluation of subjects in pediatric age, about specific cognitive domains. It is composed of 33 tests that refer to 6 different cognitive domains (Attention and Executive Functions, Language, Memory and Learning, Sensorimotor Functions, Social Perception, and Visuospatial Processing). For the purposes of the study, the items “list memory” and “drawing memory” (Memory and Learning domain), the item “Inhibition” (Attention and Executive Functions domain), and the item “find the way” (Visuospatial Processing domain) will be used.

Endpoint 5: improvement of effort tolerance.

*Measured variables:* heart rate (HR) and heart rate variability (HRV) collected with a wearable sensor (band) Movesense Medical (class IIa MDR 2017/745) or similar medically certified device.

Endpoint 6: measurement of user engagement concerning the adoption of the proposed technology. This investigation aims to obtain feedback from participants on the overall experience, as well as to understand whether the proposed technology has the characteristics for implementation in real clinical settings.

*Measured variables:* the User Engagement Scale-Short Form (UES-SF) questionnaire will be used to measure engagement. This is a self-report questionnaire, composed of 12 items rated on a 5-point Likert scale (1 = strongly disagree; 5 = strongly agree). In particular, the UES-SF questionnaire investigates the following four factors: perceived usability, aesthetic appeal, focused attention, and reward. This questionnaire will be administered to the study participants in the Italian version, for which the scale has been translated and validated<sup>247</sup>. The evaluation will be carried out exclusively during the intervention period (between T1 and T2) every week

(every 3 sessions); the compilation will take place at the end of the rehabilitation session with the multimodal technological device.

To carry out careful monitoring during training sessions, we will use the BORG Rating of Perceived Exertion (RPE) scale, widely used in literature to indirectly modulate training intensity<sup>248</sup>. The BORG RPE scale represents a useful tool for evaluating the perception of effort during training. The scale is composed of 15 grades ranging from number 6 to number 20, which correspond to verbal expressions that can help subjects to identify more precisely the number related to their sensation of fatigue; for example, the lower limit of the scale, i.e. number 6, corresponds to the word "no fatigue" while the upper limit of the scale, i.e. number 20, corresponds to "maximum fatigue". These grades are correlated with the heart rate values during physical effort. The lowest value of the scale corresponds approximately to the heart rate at rest (about 60), while the highest value corresponds to a maximum heart rate, approximately 200 bpm. RPE is often used to assess exercise intensity by combining overall perceptions and responses with the degree of fatigue.

#### *Study population*

For the clinical study, cases will be recruited among patients affected by neuromuscular diseases (dystrophinopathies, muscular dystrophies, congenital myopathies) referred to the participating centers, identified as follows:

- Criteria for the group of cases: Patients identified from the clinical database of the involved facilities, among subjects diagnosed with neuromuscular disease (dystrophinopathies, congenital muscular dystrophies, congenital myopathies), and among incident cases referred to the different centers. They will be selected based on the following criteria:
  - inclusion: availability of certain data of clinical or molecular genetic diagnosis of neuromuscular disease; age between 6 years and 17 years and 11 months (extremes included); height compatible with the use of Thera-Trainer (120 cm); Performance Upper Limb Module 2.0 evaluation: Entry level  $\geq 2$
  - exclusion: other concomitant genetic diseases, presence of visual impairments that do not allow access to the experimental virtual reality protocol, concomitant impairment of cardio-respiratory function such as to contraindicate training with a cycle ergometer, severe osteoporosis.

The analyses on motor and neuropsychological function will be carried out for subgroups of pathology, to limit confounding factors related to the different pathogenic processes. All procedures will be standardized to ensure repeatability.

Enrollment in the clinical study is free and subject to the acquisition of informed consent from participants. Since the participants are minors, inclusion in the study is conditional on obtaining informed consent from the parents or legal representative(s) of the participant and – where possible – the assent of the subject (see Annex D(a) and Annex D(b)). Recruitment will be carried out at the IRCCS “E.Medea” (Bosisio Parini), “Fondazione Mondino” (Pavia), and “Fondazione Stella Maris” (Pisa).

Eligible subjects will be identified following the inclusion and exclusion criteria mentioned above.

Participation in the research voluntarily will be possible only after the parents of the minors have had an exhaustive interview with the study investigators and have carefully read the information document relating to informed consent, with detailed information about the procedures envisaged and the medical devices used. The study investigators will ensure that the consent form is properly signed and dated in all parts and that there are no doubts regarding the procedures, either by the parents or by the children or young adults, before any procedure foreseen by the study protocol is carried out. Each subject will, however, be able to request any information and clarification and the healthcare personnel will be at their disposal at any stage of the trial.

At any time the parent will be able to withdraw from the study, without any penalty. Current and future care at the reference clinical center will not be compromised, therefore the same care and assistance will be guaranteed. Any changes to the clinical protocol will be promptly communicated to the participants.

### *Procedures*

The subjects included in the study will undergo an initial assessment (T0) of motor and neuropsychological functions. The subjects will then enter the intervention phase, with these characteristics:

- Phase 1 baseline: patients will follow the standard treatment (outpatient treatment if planned or no treatment) for 3 months;

- Phase 2 intervention with Virtual Park: for the following 3 months, patients will follow the rehabilitation program based on Virtual Park in addition to standard care (outpatient treatment if planned or no treatment);

The Virtual Park device allows the administration of motor tasks (aerobic training via cycle ergometer) and neuropsychological tasks (training on inhibition, working memory, and navigational skills). The Virtual Park intervention includes 3 sessions per week. Each session consists of: a warm-up phase of approximately 5-10 minutes (also passive), a central phase of at least 20 minutes (active or active-assisted), and a final phase of 5-10 minutes (also passive); during the central phase, it is possible to take breaks as needed. The cognitive tasks are performed during the central phase; the choice and change of tasks are at the operator's discretion.

The assessments will be carried out after the baseline phase (T1) and at the end of the intervention period with Virtual Park (T2).

#### *Plan of data analysis*

The collected data will be analyzed using specialized statistical software. Descriptive statistics of the collected variables will be calculated. The statistical analysis involves the comparison of the two conditions (treatment/no treatment) within the same group in which each participant acts as his/her own control. The comparison between the post-baseline (T0-T1) and post-treatment (T1-T2) difference is performed using ANCOVA repeated measures between factors. The sample size calculation was performed starting from the results in terms of MFM reported by a similar study<sup>229</sup>, which reports an effect size of 0.69, and considering a power of 90% and alpha equal to 0.05. The estimated sample size necessary for the purposes of the study is equal to 20. Considering a drop-out rate equal to 10%, 22 patients will be recruited.

#### *Data storage and management*

The data, in particular personal and health data and only to the extent that they are indispensable in relation to the objective of the trial, will be processed in compliance with EU Regulation 2016/679 (GDPR) and Legislative Decree 10 August 2018, no. 101.

At the time of data collection, whether obtained from standardized clinical assessments or obtained from the device report, the Data Controllers will pseudonymize the data collected, identify the subjects recruited with codes, or adopt other solutions compliant with point 5.4 of the Provision of the Privacy Guarantor no. 146/2019. The use of encryption techniques allows

the data subject's information to be stored and processed in a form that prevents identification by any person outside the Center. Only the Project Manager of each center and authorized personnel will be able to connect each code to the name of the participating subject.

The subjects, if they wish, will be able to learn what information will be archived and in what way. Access to the data will be permitted only to personnel authorized by the center involved in the trial. Upon request of the individual subject, at the end of the research, the results of the study in general and also in particular those specific to him/her may be communicated to the individual subject.

The data will be processed according to the following methods:

- The personal data will be known only to the individual clinics, responsible for recruiting the patients and carrying out the therapy sessions. The data previously in possession of the clinics (e.g. medical records) remain the exclusive property of the individual clinics.
- Each clinic will be responsible for the process of pseudonymization of the data collected during the trial and for ensuring the security of the pseudonym mapping table.
- Each clinic will appoint personnel in charge who have the authorization to access the pseudonymization table.
- The methods of storage and processing of personal data will be the responsibility of the individual centers.
- The instrumental data collected through the device during the trial will be identified only by the pseudonym. No personal data will be saved within the device.
- Pseudonymized data will be shared on a cloud platform between centers. Access to the data will be granted to authorized personnel with appropriate read and read/write rights.  
The cloud platform identified will be Microsoft 365, compliant with GDPR.

Only anonymized data will be made public.

### 3.3.The VRRS home-kit experience: a pilot experience of a multimodal tele-rehabilitation protocol

*Telerehabilitation* refers to a broad spectrum of rehabilitation and enabling services, which includes assessment, monitoring, intervention, supervision, educational aspects, consultancy, and counseling<sup>249</sup>. It is an innovative model of care that allows for the integration of traditional home care and rehabilitation activities with those provided through technological solutions. Telerehabilitation integrates the traditional patient-rehabilitator face-to-face approach and allows the telepresence of the therapist in the home context of patients even in situations where it is difficult for them to reach the treatment centers.

The technology used for telerehabilitation interventions is based on video-conferencing tools (Skype, Zoom, Google Meet, etc.), tools for monitoring and evaluating clients, and/or virtual environments and/or software created for the provision of exercises or virtual reality (Lange, et al., 2009). Treatment methods can vary depending on the needs: the clinician can conduct the session in real-time through audio and video connection (synchronous mode) or send material and video to the patient, who can use it at a later time (asynchronous mode).

To implement a telerehabilitation intervention, it is essential to have a good level of skill in using information technology and to have a computer, a webcam, and a stable Wi-Fi network; to take advantage of more advanced technologies, it will be necessary to use additional devices such as wearable sensors, virtual reality helmets, etc. If specific platforms or software are used, these must allow not only the provision of exercises but also the monitoring of results during the various sessions, through data recording and analysis mechanisms<sup>250</sup>.

To date, the provision of telerehabilitation services is possible not only through the use of Internet connection networks and the most varied means of telecommunication (smartphones, tablets, and PCs) but also through the use of virtual reality devices of sensors, accelerometers, gyroscopes, etc., used for the detection of physiological and/or environmental data. Thanks to the possibility of using such innovative technologies, the study variables can be better defined and controlled, the difficulties graduated and the results recorded, with a positive effect on the quality of the intervention, which thus becomes more precise and customizable. To date, the use of virtual reality devices in telemedicine, in addition to offering the possibility of evaluating and monitoring patients remotely, and maintaining a direct relationship between clinician and patient, can also provide a fun environment for patients, arousing more active participation in the required exercises, thus promoting the recovery process<sup>251</sup>.

Although applications have been observed primarily in the field of physiotherapy, there is a growing body of evidence that supports the effectiveness of telerehabilitation also for neurological, speech therapy, cardiac, occupational, and psychological treatments. The number of patients treated with this modality has increased exponentially over the years, with a rapid expansion not only of the fields of application but also of the clinical conditions and ages of the patients.

### *Telemedicine and telerehabilitation in DMD*

Telemedicine services for outpatients with neuromuscular or musculoskeletal disorders were underused before the COVID-19 pandemic<sup>252</sup>. The health emergency has led to a greater emphasis on telemedicine and telerehabilitation in all healthcare worldwide. The use of telemedicine and telerehabilitation tools has allowed patients to continue to access, albeit remotely, the clinical, rehabilitative, and rehabilitation services essential for the management of their pathology.

The use of telemedicine in neuromuscular pathologies during a health emergency has been shown to lose its potential if used for diagnostic purposes due to two factors: the difficulty in objectively assessing mild neurological signs (such as muscle weakness) and the adequate administration of standardized assessment tools<sup>253</sup>.

On the contrary, telemedicine has appeared adequate for the treatment of the pathologies in question. During the telerehabilitation sessions, it was important to adapt the activities carried out in person to a condition of remote interaction as much as possible, maintaining good continuity in pursuing specific therapeutic objectives. During remote rehabilitation, therapists were able to conduct activity sessions aimed at muscle activation and maintaining a good joint range, proposing active or passive stretching exercises (performed with the help of the reference figure) to continue to counteract the onset of dysmorphisms or tendon retractions.

The applicability and effectiveness of telerehabilitation in DMD are little studied: clinical studies dealing with this topic appear to be limited and attributable only to the period of the health emergency caused by COVID-19. Among these, we find the study conducted by Kenis-Coskun and collaborators in 2021<sup>254</sup>, which compared the effectiveness of using live telerehabilitation compared to a home video exercise program in 19 ambulant subjects with DMD aged between 6 and 15 years. In both programs, the same stretching and muscle activation exercises (upper limbs, lower limbs, and trunk) were used three times a week with a duration of 30 minutes per

session and for a total period of 8 weeks. Before and after treatment, the following were assessed: muscle strength with the use of a portable dynamometer, motor function through the NSAA, and 6MWT. The results of this study show no significant changes in the global motor function of the subjects participating in both groups before and after the start of treatment; the primary outcome measures are comparable between the two groups. The telerehabilitation approach in this study seems to have found greater compliance compared to the video streaming exercise training. The reason for the higher participation rate of the telerehabilitation group could be traced back to the presence of a therapist via videoconference during the session who was able to explain the movements, support the patients, and give them instructions to correct the movements.

In another study<sup>255</sup>, conducted during the COVID19 pandemics, the possibility of implementing respiratory exercises through the use of an online video program in home rehabilitation routines in patients with DMD was evaluated. In this study, the outcomes of respiratory exercises on respiratory function were not investigated, but the degree of participation of the subjects and the feasibility of carrying out the intervention program. The 37 participants, all ambulant and with an average age of 11 years, stated that they found the exercises easy to perform and that they were able to include the training in their home rehabilitation routine. The study shows that this intervention method is well accepted by patients with DMD. The results indicate that respiratory telerehabilitation can be implemented in the home rehabilitation of patients with DMD.

A systematic review<sup>84</sup> was conducted in 2022 to investigate the effectiveness of using virtual reality systems in the rehabilitation of the upper limbs of individuals with DMD. Seven clinical trials were included in the review, all of which used non-immersive virtual reality using devices such as smartphones, Kinect systems, game programs, and arm support systems. No adverse effects of using virtual reality were described in the studies included in this review. Collectively, improved functionality, quality of life, and motivation of subjects participating in intervention programs were reported. Some studies showed better responses in virtual environments compared to the real world or when subjects used touch interfaces. Although people with DMD may benefit from the use of virtual reality technologies, the use of these technologies should be carefully selected and consider the unique abilities and needs of participants.

### *Unmet needs in DMD rehabilitation*

This pilot experience focused on a multimodal intervention aimed at rehabilitating some of the neuropsychological functions involved in the central phenotype of DMD (see Chapter 1) and the stability of the trunk muscles.

DMD is characterized by the loss of muscle strength that initially affects the proximal muscles of the pelvic girdle and lower limbs and then progresses to the trunk and the muscles responsible for maintaining an upright posture<sup>256</sup>.

Trunk stability is a primary component for the correct functionality of movement. Each motor task requires postural control, therefore an orientation and stability component, which change based on the environment and the task itself. For these processes to occur, a complex interaction between the musculoskeletal and nervous systems occurs. The musculoskeletal components include joint ROM, spinal flexibility, muscle properties, and biomechanical relationships between body segments. The essential nervous components for postural control are instead: motor processing (which includes the organization of the body muscles in neuromuscular synergies), sensory and perceptual processing (which includes visual, vestibular organization and integration), and the somatosensory system.

Good stability is essential in maintaining a sitting posture and in particular in maintaining a stable upright posture. Appropriate activation of the trunk and abdominal muscles, defined as “core stability” is important for efficient postural control, including postural compensations for breathing-induced body movements<sup>257</sup>.

Stability of the trunk and pelvic muscles is also essential during walking. Trunk stability appears to be relatively stable in the walking phase; this begins to decrease during the non-walking phase<sup>256,258</sup>. This aspect was confirmed in the study conducted by Sá et al. in which trunk control was studied in ambulant and non-ambulant patients with DMD. The results of this research show that ambulant patients have a higher level of trunk control than non-ambulant patients; however, the study showed that more than half of the patients with functional levels on the Vignos scale equal to 7 had total trunk control.

Good strength in the trunk muscles is therefore essential, in addition to motor functions, also for correct postural alignment. Muscle weakness associated with the strength imbalance that occurs in DMD leads to postural asymmetries that can progress to spinal deformities such as scoliosis, especially after the loss of ambulation. These spinal dysmorphisms in turn have a detrimental effect on functional independence, postural control, and balance.

The trunk muscles also provide support during upper limb movements. Recent studies have shown a possible correlation between trunk stability, upper limb functionality, and the course of the disease. According to these, children with DMD with better upper limb functional performance are those who have better trunk control and this suggests the possible association between trunk stability and upper limb function. Similarly, reduced upper limb strength and a more advanced stage of the disease are related to impaired trunk control<sup>256,259</sup>. Patients at the same stage of the disease may still have different levels of trunk control, however, it remains established that a more advanced stage of the disease is associated with less trunk control. Due to muscle weakness, upper limb function in daily activities becomes increasingly difficult and when the trunk muscles are increasingly compromised, patients perform compensatory movements that require lateral flexion and flexion-extension of the trunk<sup>256,258</sup>. Due to the establishment of compensatory movements, the demands on the trunk muscles increase and as the weakness of the muscles themselves progresses, the movements become increasingly difficult until the subjects lose the ability to perform the various tasks.

The muscles of the trunk support an important basic function, namely breathing. As the disease progresses, respiratory function is also affected by the progressive weakness of the diaphragm and intercostal muscles. The deterioration of the respiratory muscle function affects the ability to cough and therefore the ability to expel secretions, a factor that can lead to infections such as pneumonia and respiratory failure<sup>260</sup>.

Studies on trunk assessment are limited and the few present focus on its correlation with upper limb functionality and walking<sup>256,258,259,261–264</sup> or on the assessment of scoliosis and its treatment with methods such as surgery or the use of orthoses.

Also in clinical practice, routine assessments of patients with DMD focus mainly on limb functionality and global motor functionality as well as rehabilitation intervention.

In the scientific literature, there is a single clinical trial conducted on subjects with DMD aimed at studying the effect of trunk training on trunk function, upper limb function, and lung function<sup>265</sup>. This trial included 26 children with DMD aged between 5 and 16 years and all under pharmacological treatment. The subjects were randomly assigned into two groups. The study group ( $N = 13$ ) performed a trunk-oriented exercise program and the conventional exercise program, while the control group ( $N = 13$ ) was subjected only to the conventional exercise program. The latter included stretching and active or assisted strength exercises (upper and lower limbs, abdominal muscles, and back muscles). The trunk-specific program included stretching and stabilization exercises (active or active-assisted), arm exercises in a static trunk

position, and trunk-oriented exercises (active or active-assisted). The training sessions were performed every day for eight weeks, twice a day, with an average duration of 45 minutes per session. To ensure that the number of training sessions in the two groups was equal, the study group's daily program included one trunk-oriented exercise session and one conventional exercise session. Trunk control was assessed using the MSCT trunk control measurement scale, arm function was assessed with the PUL test, and respiratory function was assessed using the pulmonary function test. Changes in trunk control scores, arm function (total and distal level scores), and respiratory function scores (forced vital capacity forced expiratory volume in one second, and peak expiratory flow volume percentages) were compared, with significant differences between the two groups after the eight-week intensive training. The control group showed stability in MSCT and PUL scores and a worsening of forced vital capacity and expiratory volume, while the study group improved in all outcome measures. In this study, trunk-oriented exercises in DMD were found to have positive effects on trunk function, upper limb function, and respiratory function. The findings from this study highlight the importance of adding trunk-oriented exercises to conventional exercise programs to maintain and improve global muscle strength and respiratory function in DMD.

In the clinical studies indicated above, the motor function of the trunk was evaluated through the use of specific tests: Trunk Control Measurement Scale (TCMS), and Trunk Control Segmental Assessment (SATCo).

## Methods

### *Inclusion criteria*

The protocol was designed for patients diagnosed with Duchenne Muscular Dystrophy aged between 7 and 12 years, able to walk independently and with functional levels of the Vignos scale between 1 and 6.

### *Tools*

The VRSS HomeKit device, supplied by the “C. Mondino” institute, was used to create and apply the rehabilitation intervention.

HomeKit is a virtual reality device used for cognitive, motor, speech, and respiratory tele-training<sup>266</sup>.

VRSS HomeKits are complete with sensors to allow the rehabilitation program to be carried out. In our study, the VRSS K-sensor 1 applied to the trunk was used to record the movements made by the subject during the training sessions.

Tele-training with VRSS Home Kit can be carried out at home in two ways:

Online: the therapist connects via integrated videoconference and takes remote control of the device at the patient's home, interacting with it in real-time.

Offline, the patient performs the personalized exercise schedule, guided by the Smart Virtual Assistant, which accompanies him in the interactive mode in real-time throughout the training session.

Every activity performed by the patient with the VRSS HomeKit home device and the related data are automatically recorded by the system and can be consulted by the therapist at any time to monitor the patient's activity over time.



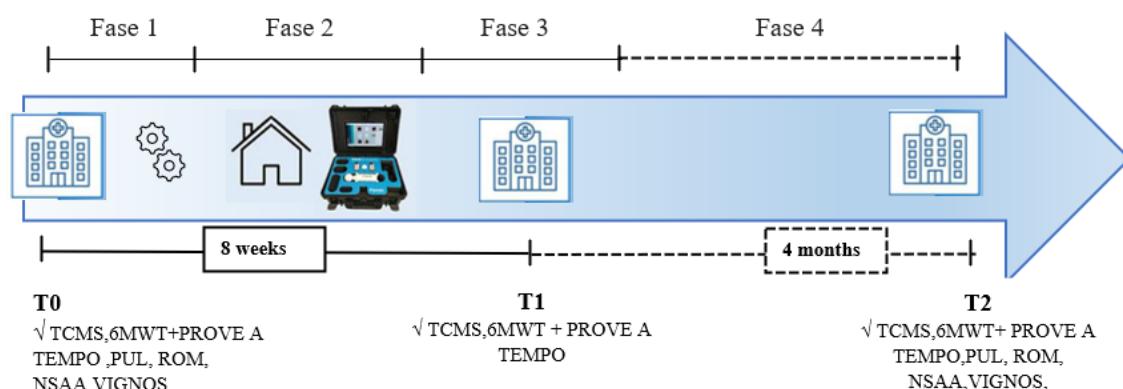
Figure 28 - Khymelia home-kit

### *Intervention*

The trunk-oriented intervention protocol was created to evaluate any effects of physical exercises on the functionality of the trunk and the maintenance of the subjects' motor framework.

Remote training was used to facilitate the meeting with patients from different regions and provinces, thus limiting the difficulties in regularly reaching the reference centers. Furthermore, given the positive feedback on the use of virtual reality technological devices in the treatment of various pathologies, including DMD, the proposal of this teletraining aims to evaluate its feasibility and effectiveness, to possibly include it in the routine rehabilitation practice of subjects affected by DMD. To evaluate these latter aspects, a satisfaction questionnaire was developed to be administered to patients and caregivers after the treatment (see Appendix).

The intervention protocol consists of 6 active trunk mobilization exercises (see Appendix) selected from the specific trunk motor module of the VRRS Home Kit system. The proposed program was designed to be carried out for a total period of 8 weeks, 3 days a week, and with a duration of approximately 20 minutes per session.



**Figure 29 – VRRS HomeKit protocol:** the steps of the intervention protocol are summarized in the figure

The phases of the study are presented below:

- **Phase 1:** Before setting up and starting the motor training, the global motor assessment of the patient is performed to verify that the demographic and residual motor function indices fall within the inclusion criteria of the study. This assessment includes:
  - Measurements of passive joint range of motion
  - Postural assessment
  - North Star Ambulatory Assessment (NSAA)

- Timed function test
- 6 Minute Walk Test (6MWT)
- Performance of Upper Limb (PUL)
- Vignos Scale
- Trunk Control Measurement Scale (TCMS)

After the assessment, the tele-training device is programmed considering the patient's motor performance. Before starting the training, patients, and caregivers are trained on the use and functionality of the system.

- **Phase 2:** Once the device is delivered to the patient's home, the rehabilitation program begins. In this tele-training study, the off-line mode was chosen: the patient performs the personalized exercise schedule, guided by the system's Smart Virtual Assistant, which accompanies him in real-time interactive mode throughout the training session. Each activity performed by the patient with the VRSS HomeKit home device and the related data are automatically recorded by the system and consulted remotely by the therapist at any time. Based on the data obtained, the therapist can then decide whether to keep the exercise program unchanged or possibly modify it, adapting it to the subject's needs and functionality. Throughout the rehabilitation treatment period, the therapists maintain contact with the families regarding the progress of the training and remain available for any clarifications and difficulties in using the device. At the end of the intervention, the family is contacted again by the therapists to schedule the date for the return of the device and the motor reassessment in an outpatient setting.
- **Phase 3:** At the end of the 8 weeks of treatment, the motor assessment is performed and the following assessment scales are administered:

- TCMS
- NSAA
- Timed function test

The results obtained from the assessments at T0 and T1 are compared to evaluate any effects of training on the trunk and motor function of the subject.

- **Phase 4:** the global reassessment is performed during routine follow-ups to verify whether or not the effects of the intervention are also in the long term (T2). At T2, the administration of the complete battery of evaluation scales used at T0 is planned.

The intervention protocol consisted of 6 active trunk mobilization exercises (see Appendix). During training, the movements of flexion-extension, lateral inclination, and rotation of the trunk are stimulated. The exercises aimed to mobilize the dorsal lumbar section of the spine, helped improve posture, and slowed down the progression of the loss of strength of the trunk muscles.

The exercise program was set up in agreement with the patients to be carried out 3 days a week. On the day set for the execution of the exercises, the patient connects to the software via an Internet connection to carry out the training session lasting a total of 20 minutes.

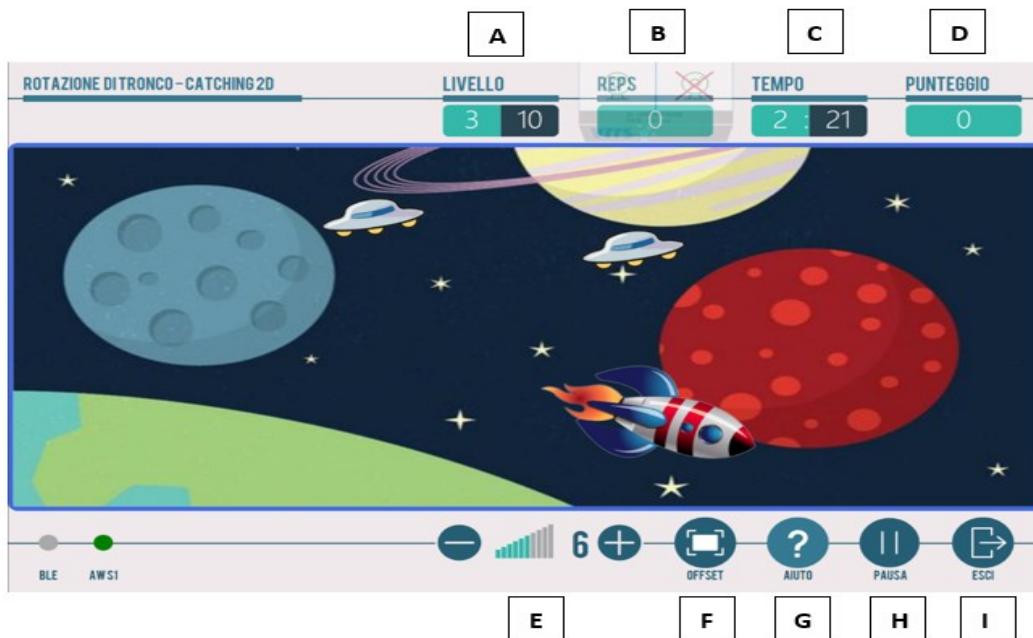
After having correctly placed the device on a support at the appropriate height and worn the sensor on the appropriate band positioned 10 cm below the axillary cavity (fig. 2), the patient, using the touch interface of the display, starts the motor exercise program.

The patient performs the personalized exercise program, guided by the Smart Virtual Assistant, which accompanies him in interactive real-time mode throughout the training session.

During the activity, the sensor positioned on the trunk records the subject's movements to transform them into actions within the game. In figure 30, an exercise is shown as an example: by rotating the trunk to the left and right and tilting it forward and backward, the subject, represented by a spaceship, must hit the objects that appear on the screen, before they land on the ground.

While performing the exercise, the subject can observe: the difficulty level and possibly the progression of the task (A), the number of repetitions (B), the duration of the exercise (C), and the scores that are calculated based on the errors made (D).

Furthermore, the subject can change the sensitivity level (E), reset the calibration (F), select the help button to read the exercise instructions (G), pause the exercise (H), and exit the game (I). Once all the exercises have been completed, the daily program is finished.



**Figure 30 – the VRSS HomeKit exercises:** the figure is an example of the user interface shown during an exercise session

### Outcome measures

The following outcome measures were adopted:

- North Star Ambulatory Assessment (NSAA): is a rating scale used to evaluate motor and functional abilities in ambulant children with DMD. It is composed of 17 items, each of which can be rated on a 3-point scale: 2 – the subject reaches the goal without any assistance, 1 – reaches the goal independently of the physical assistance of another person but using compensations, 0 – Fails to reach the goal independently. The total score is obtained by adding the scores of all the individual items. The score can vary from a minimum of 0 to 34 which represents the maximum functional level.
- Timed function tests: they include the time taken to: rise from supine to standing, run/walk 10 m, climb and descend 4 standard-sized steps. Timed tests provide a measure of functional capacity in patients.
- 6 Minute Walk Test (6MWT): the test measures in meters the distance a subject can walk in 6 minutes on a flat surface. The 6 MWT was originally developed as a test to evaluate endurance and exercise tolerance in patients with heart failure or respiratory diseases. It was later adapted and standardized for ambulant subjects with DMD and represents a reliable tool for assessing the degree of functional limitation of the subject.

- Performance of Upper Limb (PUL): is a test specifically developed for the evaluation of upper limb performance in DMD, both in ambulant and non-ambulant patients. The test consists of 22 items and evaluates upper limb abilities at both proximal and distal levels. The total score ranges from 0 to a maximum score of 74, which represents the highest level of upper limb motor function.
- Trunk Control Measurement Scale (TCMS): is an assessment tool, developed to evaluate the performance of the trunk in a sitting position in subjects with DMD. The TCMS is composed of 15 items that evaluate two parameters related to trunk control, namely static and dynamic balance of the sitting position. This scale also evaluates the compensatory movements of the trunk during limb movements. Total scores range from 0 to 58; higher scores indicate good levels of trunk control.
- Scala Vignos: it is an ordinal scale of severity consisting of 10 levels used to define the level of functionality of the lower limbs. Level 1 represents the maximum degree of functionality: the subject can walk and climb stairs independently. Level 10 represents the maximum level of impairment: the patient is bedridden. Ambulatory patients obtain scores between 1 and 6 while non-ambulatory patients receive scores between 7 and 10.

### Case series

This study was a pilot experience, carried out to test this technology in patients with neuromuscular diseases, as part of a larger project that included other diagnostic categories. In this chapter, the characteristics of the initial assessment and the application of the protocol in two patients with DMD will be presented.

After carrying out the global motor assessment of the two subjects and verifying that the indices regarding the chronological age and the residual motor function fell within the inclusion criteria of the project, the tele-training device was programmed.

A pre-training session was performed for both patients together with the therapists to evaluate the suitability of the set program or otherwise to make changes. Furthermore, instructions were provided by the therapists for the correct execution of the exercises.

During this meeting, patients and caregivers were trained on the use and functionality of the system; they were provided with a paper manual with instructions for using the tool at home and the patients were also given an 8-week calendar on which the days established for the training were marked (Figure 31).

The training was set for 8 weeks; together with the patients, the 3 days of the week for carrying out the program were chosen. Each motor training session lasted 20 minutes.

The motor training was personalized for each patient.



**Figure 31 – Training calendar:** an example of the training calendar provided to the patients is shown in the figure. The scheme includes the programmed days for training and a “note” space for reporting the reasons for missed sessions..

### Case 1

Boy with Duchenne Muscular Dystrophy, born in 2011. The patient is undergoing daily therapy with deflazacort, givinostat, and perindopril.

The boy walks independently with a slight waddling gait and at times with support on the forefoot (right>left). The patient can go up and down the stairs with an alternating step without using support and compensations. He gets up from the ground quickly by placing a hand on the knee but without using bottom-up or trunk rotations. Given the patient's abilities, the functional level on the Vignos Scale assigned to him is equal to 1.

At postural assessment, in the upright position, a slight collapse of the plantar arch is observed bilaterally. The spine and pelvis appear in line on the frontal plane. Sometimes the maintenance of the load on the left is observable.

At ROM assessment, the measurement of the ankle in passive dorsal flexion of the feet is 92° on the right and 93° on the left.

Respiratory activity appears adequate with a functional cough.

During the interview, the boy reported good general health conditions and stability of the functional motor picture. S. reported performing daily aerobic activity; no myalgia or cramps were reported, nor episodes of falls.

During hospitalization, the following evaluation scales were administered:

- TIMED FUNCTION TESTS:
  - Walking 10 m: 6,38 s
  - 4 steps (up): 1,44 s
  - 4 steps (down): 1,66 s
  - Sitting to standing (chair): 0,47 s
  - Sitting to standing: 2,81 s
  - Lying to standing: 3,9 s
- PUL: 74/74
- 6 MWT: 603 m
- NSAA: 33/34
- TCMS: total score 54/58
  - Static balance: 20/20
  - Dynamic balance: 24/28
  - Dynamic reaching: 10/10

PERSONALIZED REHABILITATION PROGRAM: the exercises were programmed in the progression of difficulty, setting level 2 as the base and level 8 as the maximum achievable difficulty level. The progression of difficulty was set after completing 1 repetition of exercises. The sensitivity was adapted to the subject's abilities and the level and complexity of the exercise to avoid excessive fatigue and the risk of falls. The exercises were performed in an upright position.

In the same training session, cognitive exercises lasting a total of 10 minutes were also performed. The activities were chosen together with the patient and adapted to his abilities.

## Case 2

Boy with Duchenne Muscular Dystrophy, born in 2010, undergoing daily therapy with deflazacort and perindopril.

The boy walks independently with a slightly waddling gait and at times with flat feet. He gets up from the ground with a complete Gowers maneuver. The boy can climb the stairs with a paired step, requiring the support of the handrail and the support of the wall. The patient can go down the stairs with an alternate step, using the handrail as support. For these motor skills, the patient was assigned a functional level of 2 on the Vignos Scale.

Concerning the postural assessment, in the bipedal orthostatic position, slight valgus of the hindfoot and collapse of the plantar arch are evident bilaterally. The load slightly prevails on the left AI. The spine and pelvis appear in line on the frontal plane. There is a slight accentuation of the lumbar lordosis.

The assessment in the supine position shows an increase in muscle-tendon tension in the m. tensor fascia lata for left>right and in the triceps surae; the measurement of the tibiotarsal joint in passive dorsiflexion of the feet is 94° on the right and 95° on the left. Respiratory activity appears adequate with a functional cough.

During the interview, the boy reports good health conditions; no myalgia or cramps are reported, nor episodes of falls. The boy continues with neuromotor therapy once a week and attends a swimming course once a week, in which he willingly participates.

During hospitalization, the following evaluation scales were administered:

- TIMED FUNCTION TESTS:
  - Walking 10 m: 6,72 s
  - 4 steps (up): 4,56 s
  - 4 steps (down): 2,63 s
  - Sitting to standing (chair): 1,09 s
  - Sitting to standing: 3,31 s
  - Lying to standing: 4,19 s
- PUL: 74/74
- 6 MWT: 429 m
- NSAA: 28/34
- TCMS: total score 52/58
  - Static balance: 19/20
  - Dynamic balance: 23/28
  - Dynamic Reaching: 10/10

**PERSONALIZED REHABILITATION PROGRAM:** the exercises were programmed with progressive difficulty levels, setting level 2 as the base and level 6 as the maximum level. The difficulty progression was set after completing 2 repetitions. The sensitivity was adapted to the subject's abilities and the difficulty level of the exercise to ensure safety and avoid excessive fatigue. The exercises were performed in an upright position.

In the same training session, cognitive exercises lasting a total of 10 minutes were also performed. The activities were chosen together with the patient and adapted to his abilities.

Once the device was delivered to the patients' homes, they began the telerehabilitation program.

On the corresponding days, the children performed the personalized exercise schedule; during the sessions, the subjects were guided by the system's Smart Virtual Assistant, which accompanied them in real-time interactive mode throughout the training session. Each activity performed with the VRSS HomeKit home device and the related data were automatically recorded by the system and consulted remotely by the therapist during the intervention program.

The therapists made telephone contact with the families to find out how the training was progressing 3 and 6 weeks after the start of the training. During the first telephone interview, it emerged that, while patient 2 continued his path with emphasis and performed the exercises on the corresponding days, patient 1 showed little interest in the program, reporting that he was too tired to be able to perform the exercises every day upon returning from the summer camp.

At the second telephone interview, which took place 6 weeks after the start of the training, both families reported a picture that overlapped with the one described above: patient 2 showed good tolerance to the treatment while Patient 1 performed the program discontinuously and needed constant prompting from the caregivers to perform the exercises on the corresponding days.

The therapists remained available for any clarifications and/or difficulties in using the device throughout the training period.

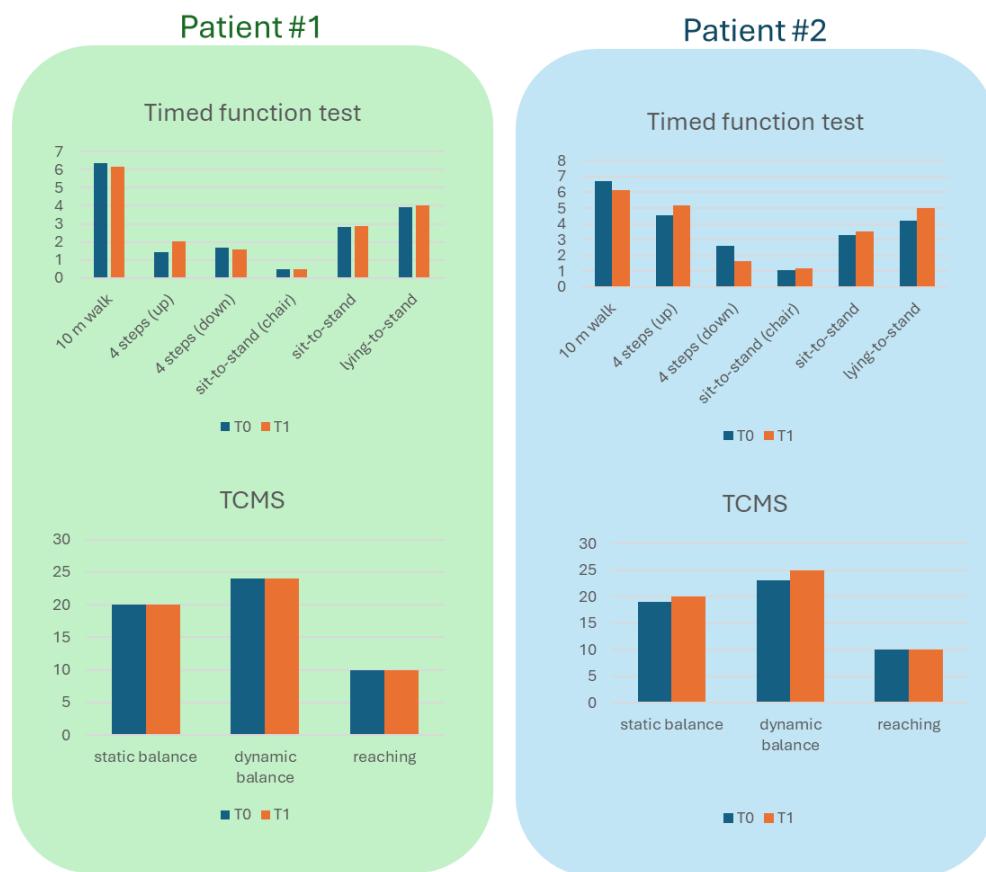
At the end of the intervention, the family was contacted again by the therapists to schedule the date for the return of the device and the motor reassessment in an outpatient setting; during this meeting, a final in-person training session was performed and satisfaction questionnaires relating to the teletraining carried out were administered to both patients and caregivers.

## Results

The study was conducted on two patients with DMD. At the end of the intervention program, the following evaluation scales were administered: NSAA, timed trials, and TCMS. The results of the two patients for each outcome measure used (pre- and post-treatment) are reported below in Table 25 and Figure 32

		Patient 1		Patient 2	
		T0	T1	T0	T1
Timed function tests [s]	Walking 10 m	6.38	6.15	6.72	6.16
	4 steps (up)	1.44	2.01	4.56	5.20
	4 steps (down)	1.66	1.60	2.63	1.63
	Sitting to standing (chair)	0.47	0.50	1.09	1.20
	Sitting to standing	2.81	2.90	3.31	3.50
	Lying to standing	3.9	4.0	4.19	5.0
NSAA		33/34	33/34	28/34	28/34
TCMS	Static balance	20/20	20/20	19/20	20/20
	Dynamic balance	24/28	24/28	23/28	25/28
	Dynamic Reaching	10/10	10/10	10/10	10/10

**Table 25 – Motor function (results):** the scores of the outcome measures to assess motor function before and after the intervention are summarized in the table



**Figure 32 - Motor function (results):** the variation of the scores assessing the evolution of motor function before and after the intervention are represented in the graphs

Compared to the timed tests, for patient 1 there was a slight reduction in the times for walking 10 m (-0.23 s) and for descending 4 steps (-0.06 s); while there was an increase in the times for climbing 4 steps (+ 0.57 s) and for moving from a sitting to an upright position (+0.09 s). The times for getting up from a chair and for moving from a supine to an upright position remained unchanged.

About patient 2, there was a reduction in the times for walking 10 m (-0.56 s) and for descending 4 steps (-1 s). There was an increase in the times for the remaining items: climbing 4 steps (+1.3 s), getting up from a chair (+0.11 s), getting up from a sitting position from the floor (+0.19 s), and moving from a supine to an upright position (+1.21).

For both, no variations in the execution mode were recorded in the tests described. In some tests, the minimal variations in the execution time are not considered significant, probably related to latency at the beginning of the test and/or to transient lower collaboration. The most important increases or reductions in the times found will be monitored in the next follow-up.

At the reassessment at T1, the global motor function of both patients remained stable, as demonstrated by the results of the NSAA standardized tests. No changes in the way the tests were performed were recorded for either patient.

During the evaluation of the trunk function and the last training session performed in the presence of patient 1, the impulsive and hasty attitude of the boy already observed during the first evaluation emerged. The boy performed the tests with reduced attention to the task and sometimes anticipated its execution before completing the explanation; this behavior could have affected the outcome of the motor performance related to the trunk. However, the scores on the TCMS scale appear unchanged compared to the evaluation carried out at T0. The motor picture related to the trunk remained stable. The patient completed all the tests of the measurement scale, using the same compensation methods that emerged at the first evaluation.

As regards patient 2, at the administration of the TCMS scale at T1, the trunk function seems to have improved both in static and dynamic balance. An increase of 3 points was observed compared to the first evaluation. The patient was able to perform all the tests, including those related to trunk flexion and extension in the absence of support that scared him at the first evaluation. The boy showed greater confidence and fluidity in his movements during the various tests. The trunk flexion movements that the boy uses as compensation in some tests in which he is asked to lift his pelvis from both sides while sitting without touching the support surface (a

few centimeters away from the right femoral head) with his right elbow and to return to the starting position are still visible.

Motor training, by stimulating the movements of flexion, extension, lateral inclination, and rotation of the trunk, helped patient 2 to increase his confidence in movement, an aspect that also had a positive impact on the fluidity and speed of movement.

The improvements in these movement components were reported in the interview by both the boy and his mother and were also clinically confirmed by the therapists during the last in-person training session: the patient appeared much more confident and faster in performing all the exercises.

Of the total number of training sessions performed, patient 1 performed 17 of the 24 sessions planned, while patient 2 performed 22/24.

At the end of the intervention program, satisfaction questionnaires regarding the training performed were administered to caregivers and patients.

For both parents and patients:

- The overall duration of each exercise session was adequate
- The type of exercises appeared suitable for the subjects' abilities
- The training sessions did not appear to have caused excessive fatigue in the subjects.  
Both boys never needed to take breaks during the sessions.
- No difficulty emerged in using the tele-training system.
- The training did not interfere with the management and organization of the routine and any commitments.
- The help of the parent was necessary to be able to carry out the exercises, in particular in activating them and in recalibrating the sensor at the beginning of each exercise.

For the parents, the tele-training program appeared useful, and they expressed themselves in favor of continuing the tele-training intervention.

The patients were not very satisfied with the motor tele-training: for both of them, the task appeared to be not very motivating after only the first 3 weeks. The motor exercises were considered boring and for this reason, patient 1 did not consistently carry out the training. Both subjects preferred the cognitive exercises to the motor ones.

The idea of using a digital device to be able to do therapy instead of the routine one was appreciated by the subjects. The dissatisfaction that emerged can be traced back to the

reduced variability and typology of the exercises: both would have preferred to carry out the proposed activities using different and more motivating scenarios.

## Conclusions

The development of a multidisciplinary approach in DMD that includes pharmacology, physiotherapy, orthopedic surgery, cardiology prevention, and respiratory assistance, has allowed in the last decade to limit the effects of the disease and improve life expectancy. Among these interventions, neuromotor rehabilitation and pharmacology are two important pillars in the treatment of this pathology.

Intervention in DMD is aimed at preventive, curative (where possible), and compensatory purposes; the objectives are aimed at preserving the maximum degree of autonomy and participation capacity of the patient for as long as possible, as well as counteracting the worsening of the signs.

Studies in the scientific literature regarding neuromotor rehabilitation in DMD focus mainly on the functionality of the upper and lower limbs to the detriment of the functionality of the trunk.

The trunk muscles play important roles, which is why many studies concern the evaluation and treatment of this district in some neurological pathologies, such as infantile cerebral palsy. Due to the important basic functions related to the trunk, specific interventions on this body district have therefore been included in the treatment programs of these pathologies.

Studies regarding the evaluation and treatment of the trunk in DMD appear to be limited: in only one study<sup>265</sup> the importance of trunk-oriented physical exercises in DMD and their positive effects on its functionality, that of the upper limbs and on respiratory function were investigated.

Based on these considerations, a telerehabilitation intervention protocol aimed at the trunk was created to evaluate the effects of physical training on the trunk and global motor function.

Remote tele-training was used to limit the difficulties in regularly reaching the reference centers. Furthermore, given the positive results in the use of virtual reality technological devices in the treatment of various pathologies, including DMD, the proposal of this tele-training had as a secondary objective that of evaluating its feasibility and effectiveness, to possibly include it in the routine rehabilitation practice of subjects affected by DMD.

The two patients were evaluated before and after the 8-week training using the following standardized scales as outcome measures: the NSAA scale and timed tests, to measure global motor skills and the TCMS scale, to evaluate trunk control.

Considering the progressive nature of the pathology and the level of motor functioning of the subjects included in the study, at the end of the treatment there was a substantial maintenance of global motor functions in both subjects and a single patient as well as an improvement in trunk functionality. It is crucial to mention that the 8-week period of rehabilitation is a relatively short time span to appreciate possible functional modifications in a DMD patients. Based on the disease's natural history<sup>267-269</sup>, a significant variation in the motor function is detectable in a 2-month period only in the late fast declining phase of the disease. Thus, a pilot experience like ours can reliably provide preliminary information only about the compliance/feasibility of technological rehabilitation.

To evaluate the possible effects of motor training even in the long term, re-evaluations will be necessary after some time from the intervention.

The preliminary data of the two patients are encouraging, but a more extensive application of similar protocols is necessary to obtain conclusive results on the real effectiveness of technological devices such as the one tested in this study.

Thanks to the questionnaires administered at the end of the treatment, it was possible to report the comments of the patients and their caregivers regarding the treatment carried out and highlight some of its limitations.

First of all, an internet connection is essential to carry out the training, so that the data are recorded by the device and can be consulted remotely by the therapists.

The presence of a family member of the patient, trained in its use, was necessary to facilitate the virtual session, especially to activate the exercise and recalibrate the sensor used at the start of each activity.

The limited typology of motor exercises and the little variable scenarios made the task not very motivating after a limited period from the start of the training. On the contrary, the inclusion of some cognitive exercises allowed to differentiate and encourage the training and was well received by both patients.

Given the involvement of the CNS in DMD and the presence of mild-medium cognitive impairment in some subjects, the multimodal potential (motor and cognitive treatment) of these devices should be emphasized in future research.

Despite the limitations reported by the subjects participating in the study, home tele-training allowed the family to better organize their time to carry out the training, in this way, it was easy to manage their commitments and avoid going to the reference center to carry out the therapy.

Furthermore, this intervention demonstrated how the system used is highly flexible thanks to the possibility of customizing the exercises based on the patient's abilities.

Some of the limitations that emerged from the use of the VRSS Home Kit suggest the need to make technical changes to improve the teletraining experience with this device. For example, optimizing the internet connection time needed to save data on the device remotely and facilitating the use of the device independently by the subject without having to constantly depend on external help to activate the exercises and recalibrate the sensors used. Furthermore, to make the motor task more motivating and variable, it could be useful to increase the type of exercises using more motivating and captivating scenarios.

Overall, the tele-training intervention was tolerated by the patients and completed by both subjects in terms of exercises performed and availability. The promising results, combined with these aspects, could demonstrate, in future research, the feasibility of this tele-training intervention.

This study provides a pilot experience, therefore only after studies conducted on a larger number of patients will it be possible to judge its effectiveness. This study lays an initial basis for the applicability of a multimodal rehabilitation intervention, which includes a specific target on the trunk and on cognitive aspects in DMD, which to date are an area of intervention still little explored in the scientific literature.

## 4. General Discussion

This doctoral thesis aimed at assessing the clinical features of unexplored neuropsychological domains possibly involved in the CNS-related phenotype of DMD (first objective, Chapter 2), and exploring the possibility of implementing technological devices in the rehabilitative setting in this disease (second objective, Chapter 3).

The multidisciplinary approach of this work reflects the clinical care of the Child and Adolescence Neuropsychiatry Unit where this PhD project was conceptualized and conducted. Such an approach is based on the integration of different healthcare professionals combining their skills and perspectives to define and sustain the overall development of each patient. This is also a translational research project, as it is based on the day-by-day exchange of knowledge, competence, and approaches between basic (e.g., neuroscientists and engineers) and clinical researchers (e.g. neuropsychiatrists, neuropsychologists, language and neurodevelopmental therapists) to deliver appropriated and personalized care to the patient.

In this background, the first part of the project (Chapter 2) was focused on studying unexplored neuropsychological domains possibly involved in the CNS-related phenotype of DMD. In particular, the chapter focused on the currently adopted batteries of standardized neuropsychological tools (section 2.1) and the evaluation of social cognition skills in a clinical and experimental setting that shed light on its neural correlates (sections 2.2 and 2.3).

The second part (Chapter 3), was focused on treatment, exploring the possibility of implementing innovative technological devices for the rehabilitation of motor and neuropsychological skills. In particular, the chapter included the report of two pilot experiences with multimodal technological devices for telehealth (Khymeia) and multimodal (VirtualPark) rehabilitation.

In this chapter, the main outcomes from the original studies (Chapters 2 and 3) are discussed to summarize them within the general framework of this PhD project.

### 4.1. Summary of results

#### 4.1.1. *Insights from the studies on neuropsychological profile and social cognition impairment*

The study of the phenotype related to the involvement of the CNS in DMD is the fundamental pillar on which the paradigm shift that can be summarized in the formula "from Duchenne Muscular to Neuromuscular Dystrophy" is based. This change of perspective is strongly

supported by patient associations and families and corresponds to increased attention to these aspects also by the scientific community. The description of a neuropsychological correlate of DMD is not recent and the higher incidence of cognitive frailty, neurodevelopmental disorders (e.g. intellectual disability, ADHD, autism spectrum disorders), and emotional difficulties in this population has long been known, particularly in the subpopulation with a genotype compatible with the reduced expression of dystrophin isoforms expressed in the CNS. The recent discoveries regarding the expression profile of dystrophin isoforms in human tissues have allowed us to hypothesize the involvement of neuropsychological domains not traditionally included in clinical assessment. The greater “weight” of subcortical structures such as the amygdala and hippocampus in the dystrophin expression profile pushes us to investigate, in addition to the already described disorders affecting language, memory, and executive functions, also the functions potentially connected to an impairment of these structures. In this project, we have therefore focused on exploring this possible gap in the increasingly in-depth description of the neuropsychological profile of patients living with DMD. In Chapter 2, the studies dedicated to the clinical evaluation of some possible deficits in neuropsychological domains that have been unexplored so far have been described; the main results are summarized below.

Our systematic review confirmed these premises and revealed a significant heterogeneity in neuropsychological assessment tools for DMD patients, with the Wechsler scales being the most frequently used. These scales primarily evaluate cognitive skills such as language, visual-spatial abilities, memory, attention, and executive functioning. These domains align with the neuropsychological functions traditionally recognized as impaired in DMD. However, tools specifically designed to assess social cognition are rarely utilized in clinical or research settings and there were only a few reports of observed deficits in this domain.

With this background, we designed a clinical protocol that was, to our knowledge, one of the first studies investigating this domain in DMD patients. Interestingly, our social cognition assessment yielded intriguing results, in line with those recently published in a similar experimental setting<sup>45</sup>. Compared to the standard data, we consistently observed poor performances in our sample in the tasks assessing the theory of mind and recognizing the affective meaning of facial expressions. In particular, among the social cognition skills we investigated, the impairment of affect recognition appeared more evident in our sample when comparing Dp140+ and Dp140- groups. This finding is preliminary and should be taken with caution, given the low numerosity and the absence of significance when analyzing without the influence of cognitive disability. Nevertheless, it provided additional evidence of a possible

specific impairment in this area as another feature of the CNS phenotype of DMD, consistent with a few other previous reports<sup>45,75</sup>.

Previous studies have not largely explored this area, and there is no clinical evidence of such an impairment (except for the increased risk of comorbidity with ASD in DMD patients). However, as mentioned above, the recent data from the Human Brain Atlas pointed out the high levels of dystrophin expression in structures (e.g. the amygdala and hippocampus) involved in the brain networks underlying the social functions (e.g., the extra-geniculostriatal network).

Starting from these findings, we developed a protocol that was, to the best of our knowledge, the first to study the neuropsychological phenotype of DMD using a subliminal priming protocol. The preliminary results of the study (the project was designed as a multicentric clinical trial and the recruitment is still active) showed a difference between the group of DMD patients and the controls in both groups, HC and NMD-C, both at the clinical level, in the cognitive and social perception assessment tests, and at the experimental level, in the priming setting.

In particular, the results of this second study supported the previous hypothesis of a deficit in the ability to recognize emotions in DMD patients compared with controls in both groups. This was particularly evident in the social cognition tasks of NEPSY: in the Emotion Recognition section, however, there is a significant difference with both groups. The data obtained from the VABS-II questionnaire also pointed in the same direction of this hypothesis, as they report a statistically significant deficit in the socialization skills of DMD patients compared with the other two groups. The results obtained in the emotional and non-emotional priming tests, a significant difference was found in the accuracy of the emotional tasks of the DMD patients compared with those of the other two groups (with the former performing worse), which further supports the hypothesis of a deficit in the ability in recognizing the emotional value of facial cues. This difference was present in both settings, implicit and explicit, with congruent and incongruent emotional priming. In contrast, there was no significant difference in non-emotional priming tasks.

Regarding the role of the structures underlying implicit emotional recognition, a major difference in performance between the implicit and explicit settings did not emerge from our preliminary data. However, more data are necessary to further test this hypothesis and possibly investigate the CNS structures that might be involved and cause this deficit.

A common consideration about these hypotheses should be made. Various neural networks underlie all the mentioned functions, sometimes overlapping and involving the same CNS structures. Furthermore, these deficits are common to many neuropsychiatric diseases.

Consequently, identifying the exact cause of an impairment widely involving these functions may not be obvious.

More specific assessments are required to elucidate the pathological basis of social cognition deficits and determine their inclusion in DMD's CNS-related phenotype. Advanced neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) and tractography, have been utilized to describe altered connectivity within the Default Mode Network and cortico-subcortical-cerebellar tracts. These techniques could further explore structural and functional involvements at finer levels.

#### *4.1.2. Contribution to the development of a standardized toolkit for clinical neuropsychological assessment*

The current indications for the diagnosis and management of DMD include a section focused on the psychosocial care of patients, urging the inclusion of a neuropsychological assessment in the standards of care for this pathology. The integration of psychosocial care into the neuromuscular clinic is promoted as a fundamental requisite to address and understand the issues related to the neurodevelopmental comorbidities of DMD.

According to these indications, the clinical timeline should include a baseline assessment at diagnosis (and, of course, whenever concerns about developmental progress arise) and follow-up re-evaluation every 2-3 years. The use of standardized tests, administered by trained clinical specialists (e.g. neuropsychologists or neuropsychiatrists) to monitor the evolution of the neuropsychological profile of these patients is supported by the guidelines.

Recently, a multi-center study including five of the seven participating clinics of the Brain Involvement In Dystrophinopathy (BIND) project was conducted to map the neuropsychological batteries adopted in clinical routine in specialized centers. The results of the survey showed a scattered scenario, with a wide diversity of tests being used for different age groups and different domains. Consensus in using the Wechsler scales to assess cognitive skills was reported, but conversely, all other domains (e.g. memory, attention, behavioral problems, learning skills) emerged to be tested with a variety of different tools. This heterogeneity, according to the authors, urges the importance of developing a Standard Operating Procedure (SOP) to improve both clinical practice and scientific research in different countries and improve comparative work. Section 2.1 includes the results of our systematic review of the papers assessing the CNS-related profile of DMD patients. The search covered more than twenty years and confirmed the scattered scenario outlined by the previously mentioned survey regarding the adopted neuropsychological batteries. Moreover, an imbalance in the assessment of the various domains emerged. Some areas (e.g., language or attention) that became part of the “classical” description of the DMD CNS-related phenotype were more intensively investigated. Nevertheless, brain networks and neuropsychological functions, like social cognition skills, that are possibly part of the clinical picture based on the physiopathology of the disease might have been overlooked. This feature is still not widely explored in research settings or clinical practice, but the evolving understanding of DMD necessitates a more comprehensive approach to neuropsychological assessment. Thus, our work raises the need for a comprehensive assessment of social cognition for age monitoring during the whole follow-up with standardized and specific neuropsychological batteries (e.g., the NEPSY-II scale).

Better neuropsychological characterization could reveal a related misrecognized clinical (neuro) DMD phenotype, guiding clinical and rehabilitation practice.

#### 4.1.3. Toward the use of new technologies in integrated rehabilitation

One of the aims of the project was to explore the application of technologies to the rehabilitative setting of neuromuscular diseases, with a particular focus on DMD. We explored in particular two of the most promising features of the technological devices applied to this field: multimodality and telehealth.

Section 3.1 includes the wide-scope systematic review we performed to encompass as extensively as possible the multifaceted field of technological telerehabilitation for pediatric neurologic and neurodevelopmental disorders. This search revealed a heterogeneous landscape, with a larger application of this kind of technology in cerebral palsy and neurodevelopmental diseases, as expected based on the epidemiology of pediatric neuropsychiatric disorders. Notably, the distribution of the studies about neurodevelopmental disorders was unbalanced in favor of ADHD and ASD, while other disorders with high prevalence (e.g., SLD) were less represented. Furthermore, our search did not intercept other common neuropsychiatric conditions (e.g., epilepsy, neuromuscular diseases) in the reviewed paper. This finding may be due to the features of the search string, but it also suggests that there are areas where the application of technological telerehabilitation is still to be explored. Our review aimed to provide a comprehensive description of the features of the telerehabilitation setting in the field of pediatric neurodevelopmental disorders, thus we focused on the role of caregivers and professionals, the types of adopted technologies, and the functional domains identified as therapeutic targets.

Even if the direct target of the intervention was the patient himself, almost all studies explicitly mentioned the involvement of caregivers in the intervention, suggesting that the tele-rehabilitative approach for pediatric diseases intrinsically supports a therapeutic relationship between families and professionals. However, our qualitative classification showed a “pyramidal” distribution, with “passive” roles (e.g., Implementer, Supporter, Informer) being more frequently applied than the “active” ones (e.g., Adaptor, Collaborative Decision Maker). The cross-application of this classification and the technological taxonomy gave us a more detailed insight into this finding. The interventions based on “Telemedicine and Telemonitoring devices” or combinations of the previously mentioned technologies seemed to assign active roles to the caregivers more frequently. We also classified the other side of the therapeutic relationship, by analyzing the professionals’ role in designing, administering, and modulating the interventions: most studies described programs that do not require the direct intervention of the therapist to administer or monitor the intervention and the intrinsic adaptivity of the

technological devices was emphasized because of their potential in providing a dynamic intervention, reducing the workload of professionals and fostering the effectiveness of the rehabilitative intervention. However, the usability of technologies can still be a barrier to the acceptance of the telerehabilitation approach by the families and, as mentioned above, “active” caregivers' roles imply collaborative interaction with the therapist.

Regarding the analysis of the adopted technologies for telerehabilitation, to date, a standardized taxonomy able to classify is still lacking. We integrated previously published classifications to define a novel taxonomy for digital technologies. The most commonly adopted ICTs were computer-based/web-based programs and virtual reality and active video-gaming devices, while a smaller subset of papers described telemedicine/telemonitoring devices or tools combining different technologies. Some issues may be raised from this situation: the computer-based/web-based and virtual reality/active video-gaming types of technologies appeared to be related to a more “passive” role of the caregiver. Besides, more advanced integrated technologies (e.g. equipped with wearable sensors or remotely monitorable) are not yet very diffused across the clinical studies.

We completed the description of the technological tele-rehabilitative interventions with the analysis of the skills they were designed to address. Overall, a prevalence of a single-domain intervention emerged, mainly focused on neuropsychological or motor functions. Interestingly, we also identified a subset of papers reporting multimodal telerehabilitation tools.

Focusing on neuromuscular diseases, telemedicine services were underused before the COVID-19 pandemic; moreover, they resulted in being still unhelpful as diagnostic tools because of the difficulty in objectively assessing subtle neurological signs (such as muscle weakness) and the adequate administration of standardized assessment tools.

Conversely, telehealth appeared to fit with the rehabilitative setting. The applicability and effectiveness of telerehabilitation in DMD were not extensively studied, but the COVID-19 pandemic boosted the emergence of some experiences in this field. Some studies tested the application of various telerehabilitation technologies (e.g. home-video-based motor training, online video-based respiratory training, and virtual reality systems for the rehabilitation of the upper limbs).

The second part of this project was focused on the implementation of two devices, exploiting two of the most promising features of the technologies applied to rehabilitation: the multimodality and the telehealth setting.

Firstly, the VirtualPark protocol was developed to study a technological device that allows a multimodal rehabilitation intervention in a group of developmental patients affected by neuromuscular diseases. The device under study allows it to intervene in parallel on the motor domain (via cycle-/armoergometer) and on the neuropsychological domain (via the integrated virtual reality serious game platform). The possibility of integrating a multimodal technology has considerable potential in the pediatric age, where the holistic approach to the neuropsychomotor development process is fundamental for rehabilitative intervention. Furthermore, the integration of motor and cognitive rehabilitation in a playful context is a cornerstone of the care of pediatric patients with neurological and neurodevelopmental disorders. As previously exposed, this model also applies DMD and its specific neuropsychological correlates.

Secondly, a telerehabilitation intervention protocol aimed at both the motor (trunk muscular function) and cognitive (executive functions) training was created by exploiting a telehealth technological device (VRSS Khymeia home kit). Two patients were evaluated before and after the 8-week training and, considering the progressive nature of the pathology and the level of motor functioning of the included subjects, at the end of the treatment, there was substantial maintenance of global motor functions in both subjects and a single patient also experienced an improvement in trunk functionality. The time span was too short to detect an effect on cognitive function, but the inclusion of multimodal training was generally well-tolerated and described as engaging by patients. Still, some limitations emerged from the questionnaires administered at the end of the treatment limitations: the constant need for an internet connection, the necessity of the supervision of a trained caregiver, and the limited variety of exercises and scenarios had been reported as frequent obstacles to the compliance to the treatment.

Despite these limitations, home tele-training allowed the family to better organize their time to carry out the training, in this way, it was easy to manage their commitments and avoid going to the reference center to carry out the therapy. Furthermore, this intervention demonstrated how this kind of system is flexible and open to the customization of the exercises based on the patient's abilities. Our experience with the VRSS Khymeia Home Kit also highlighted some of the issues frequently connected to the use of commercial devices on a pediatric population: the need for structural adaptation to the proportion of children's bodies (in particular when affected by motor impairments), the importance of an easy user experience to promote as far as possible the unsupervised use of the device, and the development of the scenarios to maintain engagement and promote compliance to the rehabilitative programs.

Overall, the tele-training intervention was tolerated by the patients and completed by both subjects in terms of exercises performed and availability. The promising results, combined with these aspects, could demonstrate, in future research, the feasibility of this tele-training intervention.

These studies provide a pilot experience of the applicability of a multimodal rehabilitation intervention, potentially including specific motor and neuropsychological targets less explored in scientific literature.

Designing a rehabilitative intervention specifically targeting social cognition skills in DMD to be integrated in such a multimodal approach might be an intriguing future development of this project. Virtual reality is one of the most promising tools in this field. Despite its use in DMD was described in literature exclusively as a motor rehabilitation tool<sup>84</sup>, it was already successfully applied for rehabilitating social cognition skills in pediatric neurological disorders, (e.g. Williams Syndrome and cerebellar malformations<sup>270,271</sup>, by combining the ecological validity of “real-world-like” interactions with the precision of a controlled laboratory environment. Unlike traditional therapeutic methods, VR allows researchers and clinicians to create immersive, interactive scenarios that closely mimic real-life social contexts, such as navigating peer interactions, interpreting emotional expressions, or practicing conversational turn-taking. This ecological soundness ensures that skills learned in VR are more likely to generalize to real-world settings. At the same time, VR environments enable strict control over variables of interest—such as the complexity of the social task, timing of interactions, or the type of feedback provided—ensuring that the intervention is both targeted and measurable. Furthermore, VR's capacity for reproducibility and customization enhances its utility, allowing clinicians to tailor interventions to the specific needs and abilities of each child while simultaneously standardizing research protocols.

## 4.2.Final remarks

This doctoral project contributes to the study of the wide spectrum of neuropsychological manifestations of DMD. These cognitive and behavioral challenges can vary widely, ranging from deficits in attention, memory, and language to difficulties in executive functions, learning disabilities, and emotional regulation. Our results provide preliminary evidence of a possible involvement of social cognition skills, in particular in emotion recognition. Defining the neuropsychological profile of DMD patients in detail is critical for improving their rehabilitative care and providing tailored support for their academic and daily life goals.

Accurately identifying and addressing neuropsychological issues in DMD is crucial. These features of the disease can significantly impact the patient's quality of life, affecting their school performance, social relationships, and emotional well-being. Personalized intervention plans, informed by detailed neuropsychological assessments, allow healthcare professionals and caregivers to develop strategies that target these specific challenges.

Despite the importance of neuropsychological assessment in DMD, current clinical and research practices are highly heterogeneous. This inconsistency presents a significant barrier to delivering standardized and effective care. The specialized clinical centers and the research studies employ different tools and protocols, making it difficult to compare results across studies or consistently gather data about patients' neuropsychological development over time. This diversity underscores the urgent need to develop a shared, standardized toolkit of neuropsychological scales specifically designed for patients with DMD. Such a toolkit would allow for reliable, longitudinal assessment of cognitive and behavioral outcomes, ensuring that interventions can be adjusted over time to match the patient's evolving needs. A shared approach would also facilitate collaboration between research teams and healthcare providers, fostering a more cohesive understanding of the neurodevelopmental aspects of DMD.

Some of the studies included in this project explored another area with great potential for enhancing care in DMD: the integration of technological devices and telerehabilitation. In this setting, caregivers, clinicians, and rehabilitation specialists can cooperate, creating an active family-centered care environment. Telerehabilitation offers the opportunity for continuous monitoring and intervention, providing patients with access to rehabilitative services regardless of geographical barriers. Furthermore, it allows family members to take a more active role in the care process, ensuring that neuropsychological interventions are reinforced in daily routines and that progress is tracked in real time. This approach aligns well with the personalized care

model that DMD patients require, as it fosters a more holistic and integrated approach to management.

Moreover, the adoption of advanced technologies and multidomain interventions in DMD care should be further explored. Technologies like virtual reality (VR), robotics, and artificial intelligence (AI) have the potential to revolutionize neuropsychological rehabilitation by creating immersive, adaptive, and engaging therapeutic environments. In particular, VR is a particularly promising tool for the specific rehabilitation of social cognition skills, as it was positively adopted with this purpose in other pediatric neurological diseases, exploiting its possibility of implementing ecological but still controlled settings. These innovations, combined with multidimensional interventions addressing motor, cognitive, and emotional domains, could lead to more comprehensive treatment strategies for DMD patients, particularly those with complex, multifaceted impairments. By exploiting the full potential of these technologies, clinicians and researchers can develop more effective interventions that are tailored to the unique challenges faced by DMD patients, enhancing their overall quality of life and long-term outcomes.

## 5. Appendix

### 5.1. Supplementary materials

#### 5.1.1. Social Cognition: the blind corner of neuropsychological assessment in Duchenne (Neuro)Muscular Dystrophy - A Scoping Review

### SUPPLEMENTARY 1: databases, search strings, and PICO framework

#### DATABASES:

Medline (PubMed), EMBASE, Web of Science (date: 01/01/2000 → 30/11/2023)

#### SEARCH STRINGS:

PubMed String: ((*duchenne muscular dystrophy*[MeSH Terms]) AND (Child\* OR children OR preschooler OR scholar OR childhood OR pediatric OR paediatric OR student OR kindergartner OR toddler OR infant)) AND (cognitive[Title/Abstract] OR neuropsychological[Title/Abstract] OR "executive functions"[Title/Abstract] OR memory[Title/Abstract] OR attention[Title/Abstract] OR language[Title/Abstract] OR "learning abilities"[Title/Abstract] OR reading[Title/Abstract] OR comprehension[Title/Abstract] OR math\*[Title/Abstract] OR arithmetic[Title/Abstract] OR writing[Title/Abstract] OR decoding[Title/Abstract] OR "working memory"[Title/Abstract] OR inhibition[Title/Abstract] OR updating[Title/Abstract] OR shifting[Title/Abstract] OR monitoring[Title/Abstract] OR reasoning[Title/Abstract] OR "cognitive flexibility"[Title/Abstract] OR planning[Title/Abstract] OR prax\*[Title/Abstract] OR "social cognition"[Title/Abstract] OR "mental retardation"[Title/Abstract] AND assess\*[Title/Abstract] OR evaluat\*[Title/Abstract] OR "test"[Title/Abstract] OR "scale"[Title/Abstract])

EMBASE string: '*duchenne muscular dystrophy*' AND (child\* OR children OR preschooler OR scholar OR childhood OR pediatric OR paediatric OR student OR kindergartner OR toddler OR infant) AND (cognitive:ab,ti OR neuropsychological:ab,ti OR 'executive functions':ab,ti OR memory:ab,ti OR attention:ab,ti OR language:ab,ti OR 'learning abilities':ab,ti OR reading:ab,ti OR comprehension:ab,ti OR math\*:ab,ti OR arithmetic:ab,ti OR writing:ab,ti OR decoding:ab,ti OR 'working memory':ab,ti OR inhibition:ab,ti OR updating:ab,ti OR shifting:ab,ti OR monitoring:ab,ti OR reasoning:ab,ti OR 'cognitive flexibility':ab,ti OR planning:ab,ti OR prax\*:ab,ti OR 'social cognition':ab,ti OR 'mental retardation':ab,ti)

Web of Science string: *Duchenne Muscular Dystrophy* (Topic) AND (Child\* OR children OR preschooler OR scholar OR childhood OR pediatric OR paediatric OR student OR kindergartner OR toddler OR infant) (Topic) AND (cognitive OR neuropsychological OR "executive functions" OR memory OR attention OR language OR "learning abilities" OR reading OR comprehension OR math\* OR arithmetic OR writing OR decoding OR "working memory" OR inhibition OR updating OR shifting OR monitoring OR reasoning OR "cognitive flexibility" OR planning OR prax\* OR "social cognition" OR "mental retardation") (Topic)

#### PICO FRAMEWORK:

POPULATION	INTERVENTIONS	COMPARISON	OUTCOMES
Studies were included when considering samples	The search was focused on studies including a	Studies including only DMD samples, healthy controls, or	Studies were included if they aimed to define the features of the

of children aged 0-18 diagnosed with Duchenne Muscular Dystrophy.	standardized neuropsychological assessment. The articles were selected if they included details about the adopted battery of neuropsychological tests/scales.	other non-distrophin-related neuropsychiatric diseases were included	neuropsychological phenotype of DMD or if they described protocols adopted in routine clinical practice
The following exclusion criteria were considered: (1) case reports, reviews, book chapters, and conference abstracts; (2) samples including patients aged >18 years or diagnosed with other neuropsychiatric disorders; (3) studies not describing batteries standardized tests/scales for neuropsychological assessment, (4) studies about other topics (e.g., studies on animals, other disciplines)			

## SUPPLEMENTARY 2: classification of neuropsychological tests and scales

The classification of the neuropsychological tests and scales adopted in the papers included in the review is summarized in the following tables (each table refers to a domain, according to the Korkman's framework).

ATTENTION AND EXECUTIVE FUNCTIONING
Raven's Colored Progressive Matrices
Conners 3rd Edition Conners Parents Rating Scales
Tower of London (ToL)
Stanford–Binet–IV
NEPSY-II (partial): Inhibition
NEPSY-II (partial): visual attention
NEPSY-II (partial): auditory attention
NEPSY-II (partial): response test
Revised Conners Teachers Rating Scales
Modified Card Sorting Test (MCST)
Vineland Adaptive Behavior Scales
BRIEF questionnaire
The Woodcock-Johnson III Tests of Achievement
Leiter International Performance Scale – R
The digit span test of VAUMeLF

Bell test
Bayley III Scales of infant development
NEMI-N
Terman-Merrill scale
K-ABC scale
Posner computerized test
Cognitive Toolbox_part attention
Dimensional Change Card Sort Test
Pattern Comparison Processing Speed
the Bourdon Vos, Everyday Attention for Children, Second Edition (TEA-Ch)
Snijders-Oomen Non-verbal Intelligence Test for Children (SON-R)
Corsi Block Tapping Test
Test Battery of Attentional Performance (TAP).
Children's Color Trail Test A & B
ADHD-Rating Scale-IV (ADHD-RS-IV)
The Cambridge Neuropsychological Test Automated Battery (CANTAB)
Verbal Fluency-Word Generation from BVN 5-11
Health Toolbox Cognition Battery (NIHTB-CB) (complete):

<b>LANGUAGE</b>
WISC (complete)
WISC (partial): similarities
WISC (partial): vocabulary
WISC (partial): information
WISC (partial): comprehension
WPPSI (complete)
WAIS (complete)
Peabody Picture Vocabulary Test (PPVT-III)
Stanford-Binet-IV

Batteria per la Valutazione del Linguaggio in Bambini dai 4 ai 12 anni; BVL 4_12
Griffiths Developmental Scale
Vineland Adaptive Behavior Scales
Stroop Color and Word Test
Clinical Evaluation of Language Fundamentals–Preschool Version
The Woodcock-Johnson III Tests of Achievement
Regensburger Word Fluency Test
NEPSY-II (partial):phonological processing
NEPSY-II (partial):verbal fluency
Bayley III Scales of infant development
Expressive Vocabulary Test
test 36 pictures
TEST OF semantic fluency
test of morphosyntactic development
prove di rapidità e correttezza nella lettura del gruppo MT
NEMI-N
McCarthy Scales of Children's Abilities
Terman-Merrill scale
K-ABC scale
California Verbal Learning Test – Children's Version (CVLT-C)
Comprehensive Evaluation of Language Functions, Third Edition (CELFIII)
Token Test
Controlled Oral Word Association Tests
Animal Naming Test
Verbal Fluency-Word Generation from BVN 5–11
Brunet-Lezine scale
Health Toolbox Cognition Battery (NIHTB-CB) (complete)

## MEMORY AND LEARNING

WISC (complete):
WISC (partial):digit span
WISC (partial):letter number sequencing
WPPSI (complete):
WAIS (complete):
Rey Auditory Verbal Learning Test
Conners 3rd Edition Conners Parents Rating Scales
Tower of London (ToL)
Stanford-Binet-IV
Rey Complex Figure
The Kaufmann Assessment Battery for Children-II (KABC-II)
Revised Conners Teachers Rating Scales
Griffiths Developmental Scale
NEPSY-II (partial):memory and learning
NEPSY-II (partial):narrative memory
The Woodcock-Johnson III Tests of Achievement
Memory for DesignsTest (MFDT)
Leiter International Performance Scale – R
The digit span test of Batteria per la Valutazione dell'Attenzione Uditiva e della Memoria di Lavoro Fonologica nell'Età Evolutiva
Bayley III Scales of infant development
NEMI-N
McCarthy Scales of Children's Abilities
Terman-Merrill scale
K-ABC scale
Cognitive Toolbox_parz set-shifting
Cognitive Toolbox_parz working memory
List Sorting Working Memory Test
WRAML (partial): verbal learning
WRAML (partial): visual learning

WRAML (partial): story memory
WRAML (partial): picture memory
Snijders-Oomen Non-verbal Intelligence Test for Children (SON-R)
The Cambridge Neuropsychological Test Automated Battery (CANTAB)
TEMA battery (Italian version of TOMAL, Test of Memory and Learning)
Verbal Fluency-Word Generation from BVN 5-11
Serial Reaction Time task
Health Toolbox Cognition Battery (NIHTB-CB) (complete):

<b>SENSORIMOTOR</b>
NEPSY-II (partial):design fluency
Griffiths Developmental Scale
Vineland Adaptive Behavior Scales
VMI
Bayley III Scales of infant development
Wide Range Assessment of Visual Motor Abilities (WRAVMA)
NEMI-N
McCarthy Scales of Children's Abilities
Terman-Merrill scale
K-ABC scale
Grooved Pegboard
Edinburgh Handedness Inventory(EHI)
Functional Disability Inventory parent form
Brunet-Lezine scale

<b>SOCIAL COGNITION</b>
Griffiths Developmental Scale
Vineland Adaptive Behavior Scales

Revised Autism Diagnostic Observation Schedule
Bayley III Scales of infant development
Adaptive Behavioral Subtest of Bayley—(ABS)
Terman-Merrill scale
Draw-A-Person test
Spontaneous Drawing
Family Drawing
social Communication Disorder Checklist (SCDC)
Functional Disability Inventory parent form
Brunet-Lezine scale
Object, Face, Affect, and Situation matching task

<b>VISUOSPATIAL</b>
WISC (complete):
WISC (partial): Block design
WISC (partial): matrix reasoning
WPPSI (complete):
WPPSI (partial):picture completion
WAIS (complete):
Raven's Colored Progressive Matrices
Stanford-Binet-IV
Rey Complex Figure
NEPSY-II (partial): visual attention
Griffiths Developmental Scale
The Woodcock-Johnson III Tests of Achievement
Leiter International Performance Scale – R
VMI
Bayley III Scales of infant development
Adaptive Behavioral Subtest of Bayley—(ABS)

Wide Range Assessment of Visual Motor Abilities (WRAVMA)
NEMI-N
McCarthy Scales of Children's Abilities
Terman-Merrill scale
K-ABC scale
Posner computerized test
Draw-A-Person test
Spontaneous Drawing
Family Drawing
Cognitive Toolbox_parz processing speed
beery developmental test of visual motor integration
Snijders–Oomen Non-verbal Intelligence Test for Children (SON-R)
Color Cancellation Test
Visual Recognition Test
Benton Visual Retention Test (BVRT)
Health Toolbox Cognition Battery (NIHTB-CB) (complete):

<b>OTHER</b>
Revised Autism Diagnostic Observation Schedule
Conners 3rd Edition Conners Parents Rating Scales
Revised Conners Teachers Rating Scales
CBCL
teacher report Form (TRF)
Development and Well-Being Assessment (DAWBA) di Robert Goodman
YSR
strengths and difficulties questionnaire sdq
Adaptive Behavioral Subtest of Bayley—(ABS)
Vineland Adaptive Behavior Scales
Columbia Mental Maturity Scale

McCarthy Scales of Children's Abilities
Draw-A-Person test
Spontaneous Drawing
Family Drawing
Rorschach test
Thematic Apperception test
Comprehensive Test of Nonverbal Intelligence-II (CTONI-II)
Swedish version of the Strengths and Difficulties Questionnaire
Autism Mental Status Exam
Personality Inventory for Children, Revised Edition (PIC-R)
PARS-III questionnaire for psychosocial adjustment.
Neuro Quality-of-Life (NeuroQoL) Cognitive Function.
Mini International Neuropsychiatric Interview for children and adolescents (MINI KID)
Children Depression Inventory (CDI)
Childhood Autism Rating Scale
Taylor Manifest Anxiety Scale (TMAS)
CHILDREN'S YALE-BROWN OBSESSIVE COMPULSIVE SCALE (CY-BOCS)
social Communication Disorder Checklist (SCDC)
Functional Disability Inventory parent form

### 5.1.2. The wide world of technological telerehabilitation for pediatric neurologic and neurodevelopmental disorders – a systematic review

The details (authors, title, publication year) of the articles selected after the screening process and included in the qualitative analysis are reported in the table below:

Author	Title	Publication Year
Aarnoudse-Moens, et al.	Executive Function Computerized Training in Very Preterm-Born Children: A Pilot Study	2018
Alsaif, et al.	Effects of interactive games on motor performance in children with spastic cerebral palsy	2015
Anderson, et al.	Long-Term Academic Functioning following Cogmed Working Memory Training for Children Born Extremely Preterm: A Randomized Controlled Trial	2018
Bailey, et al.	A trial of online ABRACADABRA literacy instruction with supplementary parent-led shared book reading for children with autism	2022
Baque, et al.	Randomized controlled trial of web-based multimodal therapy for children with acquired brain injury to improve gross motor capacity and performance.	2017
Bearss, et al.	Feasibility of Parent Training via Telehealth for Children with Autism Spectrum Disorder and Disruptive Behavior: A Demonstration Pilot	2018
Benzing, et al.	The effect of exergaming on executive functions in children with ADHD: a randomized clinical trial	2019
Bikic, et al.	A double-blind randomized pilot trial comparing computerized cognitive exercises to Tetris in adolescents with attention-deficit/hyperactivity disorder	2017
Bilde, et al.	Individualized, home based intercative training of cerebral palsy children delivered through the internet	2011
Chacko, et al.	A randomized clinical trial of Cogmed Working Memory Training in school-age children with ADHD: A replication in a diverse sample using a control condition	2014
Chen, et al.	Efficacy of home-based virtual cycling training on bone mineral density in ambulatory children with cerebral palsy	2012
Chen, et al.	Efficacy of an integrated intervention with vocabulary and phonetic training for Mandarin-speaking children with developmental language disorders	2022
Chen, et al.	Muscle strength enhancement following home-based virtual cycling training in ambulatory children with cerebral palsy	2012
Chen, et al.	Home based tele assisted robotic rehabilitation of joint impairments in children with cerebral palsy	2014
Chiu, et al.	Upper limb training using Wii Sports Resort for children with hemiplegic cerebral palsy: a randomized, single-blind trial	2014
Chiu, et al.	Balance and mobility training at home using Wii Fit in children with Cerebral Palsy: a feasibility study	2018
Cohen, et al.	Effects of computer-based intervention through acoustically modified speech (Fast ForWord-FFW) in severe mixed receptive-expressive language impairment: outcomes from a randomized controlled trial	2005
Corti, et al.	Home based cognitive training in pediatric patients with acquired brain injury: preliminary results on efficacy of a randomized clinical trial	2020
Cristinziano, et al.	Telerehabilitation during COVID-19 lockdown and gross motor function in cerebral palsy: an observational study.	2022
Da Silva, et al.	Serious Game Platform as a Possibility for Home-Based	2021

	Telerehabilitation for Individuals With Cerebral Palsy During COVID-19 Quarantine - A Cross-Sectional Pilot Study.	
Damiano, et al.	Task-Specific and Functional Effects of Speed-Focused Elliptical or Motor-Assisted Cycle Training in Children With Bilateral Cerebral Palsy: Randomized Clinical Trial.	2017
Davis, et al.	Proof-of-concept study of an at-home, engaging, digital intervention for pediatric ADHD.	2018
De Vries, et al.	Working memory and cognitive flexibility-training for children with an autism spectrum disorder: a randomized controlled trial	2015
Di Lieto, et al.	Adaptive Working Memory Training Can Improve Executive Functioning and Visuo-Spatial Skills in Children With Pre-term Spastic Diplegia	2021
Dovis, et al.	Improving Executive functioning in children with ADHD: Training multiple Executive Functions within the context of a computer Game. A randomized double blind placebo controlled trial	2015
Egeland, et al.	Few Effects of Far Transfer of Working Memory Training in ADHD: A Randomized Controlled Trial	2013
Ferguson, et al.	The efficacy of two task-orientated interventions for children with Developmental Coordination Disorder: Neuromotor Task Training and Nintendo Wii Fit Training	2013
Garnett, et al.	Parent perceptions of a group telepractice communication intervention for autism	2022
Golomb, et al.	In home virtual reality videogame telerehabilitation in adolescents with hemiplegic cerebral palsy	2010
Goodwin, et al.	INTERSTAARS: attention training for infants with elevated likelihood of developing ADHD:a proof of concept randomised controlled trial	2021
Graucher, et al.	From Clinic Room to Zoom: Delivery of an Evidence-Based, Parentmediated Intervention in the Community Before and During the Pandemic	2022
Gray, et al.	Effects of a computerized working memory training program on working memory,attention, and academics in adolescents with severe LD and comorbid ADHD: a randomized controlled trial	2012
Grunewaldt, et al.	Working Memory Training Improves Cognitive Function in VLBW Preschoolers	2013
Grunewaldt, et al.	Computerized working memory training has positive long-term effect in very low birthweight preschool children	2015
Hammond, et al.	An investigation of the impact of regular use of the Wii Fit to improve motor and psychosocial outcomes in children with movement difficulties: a pilot study	2012
Hardy, et al.	Computerized Working Memory Training for Children With Neurofibromatosis Type 1 (NF1): A Pilot Study	2021
Hessl, et al.	Cognitive training for children and adolescents with fragile X syndrome: a randomized controlled trial of Cogmed	2019
Howie, et al.	Understanding why an active video game intervention did not improve motor skill and physical activity in children with developmental coordination disorder: a quantity or quality issue?	2017
Howie, et al.	An active video game intervention does not improve physical activity and sedentary time of children at-risk for developmental coordination disorder: a crossover randomized trial	2015
Jaekel , et al.	Preterm children's long-term academic performance after adaptive computerized training: an efficacy and process analysis of a randomized controlled trial	2021
Jirikowic, et al.	Virtual Sensorimotor Training for Balance: Pilot Study Results for Children With Fetal Alcohol Spectrum Disorders	2016
Johnstone, et al.	A pilot study of combined working memory and inhibition training for children with AD/HD	2009
Jouen, et al.	GOLiah(gaming open library for intervention in autism at home); a 6 month single blind matched controlled exploratory study	2017

Kassee, et al.	Home based nintendo wii training to improve upper limb function in children ages 7 to 12 with spastic hemiplegic cerebral palsy	2017
Kirk, et al.	Computerised attention training for children with intellectual and developmental disabilities: a randomised controlled trial	2016
Kirk, et al.	Impact of attention training on academic Achievement executive functionig and behavior: a randomized controlled trial	2017
Klingberg, et al.	Computerized Training of Working Memory in Children With ADHD—A Randomized, Controlled Trial	2005
Kollins, et al.	A novel digital intervention for actively reducing severity of paediatric ADHD (STARS-ADHD): a randomised controlled trial	2020
Kolobe, et al.	Robot Reinforcement and Error-Based Movement Learning in Infants With and Without Cerebral Palsy	2019
Lacava, et al.	Using assistive technology to teach emotion recognition to students with Asperger Syndrome	2007
Lanfranchi, et al.	Parent-based training of basic number skills in children with Down syndrome using an adaptive computer game	2021
Lee, et al.	Effects of working memory training on children born preterm	2016
Levac, et al.	Active Video Gaming for Children with Cerebral Palsy: Does a Clinic-Based Virtual Reality Component Offer an Additive Benefit? A Pilot Study	2018
Løhaugen, et al.	Computerized Working Memory Training Improves Function in Adolescents Born at Extremely Low Birth Weight	2010
Lorentzen, et al.	Twenty weeks of home-based interactive trainig of children with cerebral palsy improves functional abilites	2015
Luna-Oliva, et al.	Kinect Xbox 360 as a therapeutic modality for children with cerebral palsy in a school environment: a preliminary study	2013
Luo, et al.	A randomized controlled study of remote computerized cognitive, neurofeedback, and combined training in the treatment of children with attention-deficit/hyperactivity disorder	2022
MacIntosh, et al.	The design and evaluation of electromiography and inertial biofeedback in hand motor therapy gaming	2020
Magnan, et al.	Audio-visual training in children with reading disabilities	2006
Meguid, et al.	Influence of Covid 19 pandemic lockdown on a sample of egyptian children with down syndrome	2022
Meyer, et al.	Computer-based inhibitory control training in children with Attention-Deficit/Hyperactivity Disorder (ADHD): Evidence for behavioral and neural impact	2020
Molinaro, et al.	Action Observation Treatment in a telerehabilitation setting	2020
Nuara, et al.	Efficacy of a home-based platform for child-to-child interaction on hand motor function in unilateral cerebral palsy	2019
Pascoe, et al.	Child motivation and family environment influence outcomes of working memory training in extremely preterm children	2019
Pecini, et al.	Telerehabilitation in developmental dyslexia: methods of implementation and expected results.	2018
Pecini, et al.	Training RAN or redaing? A telerehabilitation study on developmental dyslexia	2019
Penev, et al.	A Mobile Game Platform for Improving Social Communication in Children with Autism: A Feasibility Study	2021
Piovesana, et al.	Randomized controlled trial of a web-based multi-modal therapy program for executive functioning in children and adolescents with unilateral cerebral palsy.	2017
Piovesana, et al.	A randomised controlled trial of a web-based multi-modal therapy program to improve executive functioning in children and adolescents with acquired brain injury	2017
Preston, et al.	A pilot single-blind multicentre randomized controlled trial to evaluate the potential benefits of computer-assisted arm rehabilitation gaming technology on the arm function of children with spastic cerebral palsy	2016
Preston, et al.	Feasibility of school-based computer-assisted robotic gaming	2014

	technology for upper limb rehabilitation of children with cerebral palsy	
Prins, et al.	“Braingame Brian”: Toward an Executive Function Training Program with Game Elements for Children with ADHD and Cognitive Control Problems	2013
Pulina, et al.	Improving spatial simultaneus working memory in DOWN Syndrome:effect of a training program led by parents instead of an expert	2015
Ramstrand, et al.	Can balance in children with cerebral palsy improve through use of an activity promoting computer game?	2012
Re, et al.	Response to a Specific and Digitally Supported Training at Home for Students With Mathematical Difficulties	2020
Ronimus, et al.	Supporting struggling readers with digital game-based learning	2019
Sabel, et al.	Active video gaming improves body coordination in survivors of childhood brain tumours	2016
Sandlund, et al.	Training of goal directed arm movements with motion interactive video games in children with cerebral palsy - a kinematic evalutation	2014
Saniee, et al.	Developing set-shifting improvement tasks (SSIT) for children with high-functioning autism	2019
Sella, et al.	Training basic numerical skills in children with Down syndrome using the computerized game "the number race"	2021
Serrano-Gonzalez, et al.	Action Observation Training to Improve Activities of Daily Living and Manipulation Skills in Children with Acquired Brain Injury Secondary to an Oncologic Process: A Prospective Case Series Clinical Study	2022
Sgandurra, et al.	A pilot study on early home-based intervention through an intelligent baby gym (CareToy) in preterm infants	2016
Sgandurra, et al.	A randomized clinical trial in preterm infants on the effects of a home-based early intervention with the CareToy System	2017
Silver, et al.	Evaluation of a new computer intervention to teach people with autism or Asperger syndrome to recognize and predict emotions in others	2001
Simone, et al.	Computer-assisted rehabilitation of attention in pediatric multiple sclerosis and ADHD patients: a pilot trial	2018
Soderqvist, et al.	Computerized training of non-verbal reasoning and working memory in children with intellectual disability	2012
Steiner, et al.	Computer-Based Attention Training in the Schools for Children With Attention Deficit/Hyperactivity Disorder: A Preliminary Trial	2011
Straker, et al.	A crossover randomised and controlled trial of the impact of active video games on motor coordination and perceptions of physical ability in children at risk of Developmental Coordination Disorder	2015
Swenney, et al.	Randomized controlled trial comparing Parent Led Therapist Supervised Articulation Therapy (PLAT) with routine intervention for children with speech disorders associated with cleft palate.	2020
Tse, et al.	Teletherapy delivery of caregiver behavior training for children with attention-deficit hyperactivity disorder.	2015
Ura, et al.	Parent-Coaching Telehealth Intervention for Youth with Autism Spectrum Disorder: A Pilot Program	2021
Van der Molen, et al.	Effectiveness of a computerised working memory training in adolescents with mild to borderline intellectual disabilities	2010
van Dongen-Boomsma, et al.	Working memory training in young children with ADHD: a randomized placebo-controlled trial	2014
van Houdt, et al.	Executive function training in very preterm children: a randomized controlled trial	2020
Voss, et al.	Effect of Wearable Digital Intervention for Improving Socialization in Children With Autism Spectrum Disorder: A Randomized Clinical Trial	2019
Wang, et al.	Commercial exergaming in home-based pediatric constraint-induced therapy: a randomized trial	2021
Yoncheva, et al.	Computerized cognitive training for children with neurofibromatosis type 1: a pilot resting-state fMRI study	2017
Zhang, et al.	Comparing the transfer effects of three nonpharmacological interventions in children with AD/HD: a single-case experimental design	2020

### 5.1.3. The Virtual Park protocol

## SUPPLEMENTARY 1 . Virtual Park: User Manual

This document contains the description of the VirtualPark device in terms of hardware and software components, and the instructions for correct use and maintenance. We invite you to read this document completely before any use of the device. It is recommended to always keep the manual together with the device and to reread it periodically.

### *Device Presentation*

The VirtualPark device enables dual-task training (physical + cognitive) on a cycle ergometer in a virtual reality (VR) environment.

The device consists of a cycle ergometer and a VR environment for dual-task training of individuals with disabilities. The device combines physical exercise, for the upper and lower limbs, and cognitive stimulation, through a virtual reality application. The cycle ergometer is a commercial device, certified as a medical device, which allows passive, active or assisted pedaling exercises, even against resistance. The VR application is developed by CNR-STIIMA, the manufacturer of the VirtualPark device. It offers cognitive tasks contextualized to the pedaling activity in virtual reality scenarios that simulate real-life situations. The system also integrates two buttons to allow the user to interact during the execution of the cognitive task.

Using VirtualPark allows you to benefit from the effect of an integrated multimodal technology, with considerable potential in developmental age. A rehabilitation treatment based on VirtualPark allows you to follow a training program aimed at slowing down the functional motor decline based on standard guidelines, also thanks to the use of a medically certified device. Further expected benefits derive from the enhancement of physical exercise with the possibility of performing cognitive stimulation exercises in a playful mode, thanks to the presence of the virtual reality software VirtualPark. These benefits, which constitute secondary endpoints of the clinical investigation, include neuropsychological functions (memory, inhibition, cognitive flexibility, navigational skills), endurance and effort tolerance, and engagement. Further details are included in the Clinical Investigation Plan.

### *Intended use*

The VirtualPark device is designed for dual-task training in an indoor environment. It is characterized by a compact setup that makes it easily transportable and usable by operators and end users. The device is designed for use by patients aged between 6 and 18 years with neuromuscular pathologies. The pedaling exercise can be performed with both the upper and lower limbs. Cognitive training consists of a series of exercises, each focused on a specific executive function.

### *Safety*

The device is designed for indoor use. To ensure the safety of the patient during its use, it is necessary to follow the instructions in the user manual of the cycle ergometer (User\_manual\_thera-trainer\_tigo\_veho\_italian\_0). The main indications are given below:

The THERA-Trainer should only be used if it is undamaged and functioning properly.

Follow the user manual.

Position the THERA-Trainer so that the power plug is easily accessible and can be quickly disconnected from the power outlet in an emergency.

Before starting each training session, check that the safety devices are working perfectly.

THERA-Trainer and the patient's chair/wheelchair must be placed on a flat and non-slip surface; make sure that the chair/wheelchair does not tip backward.

After each training session, disconnect the power plug.

The PC, a 2-in-1 with touch screen, is a consumer electronic product; for information on safety and health, please refer to the manufacturer's instructions (<https://support.microsoft.com/it-it/topic/istruzioni-e-avvisi-di-sicurezza-per-il-prodotto-726eab87-f471-4ad8-48e5-9c25f68927ba>). The buttons used to interact with the virtual environment are the only input devices required during use.

The VirtualPark device is not used in specific treatments and therefore does not cause any risk of interference with them.

Installation of the device at the clinical investigation site requires the assembly of accessories by specialized technical personnel from CNR-STIIMA (manufacturer). This operation must not be repeated during the use of the device, excluding risks due to errors that may be made when reassembling parts.

Before each use, check that the device has not been tampered with or has obvious physical damage. The user is advised to verify that, at the time of use, the device ensures the performance expected by the manufacturer, and if necessary to notify the manufacturer if the device does not provide a valid result.

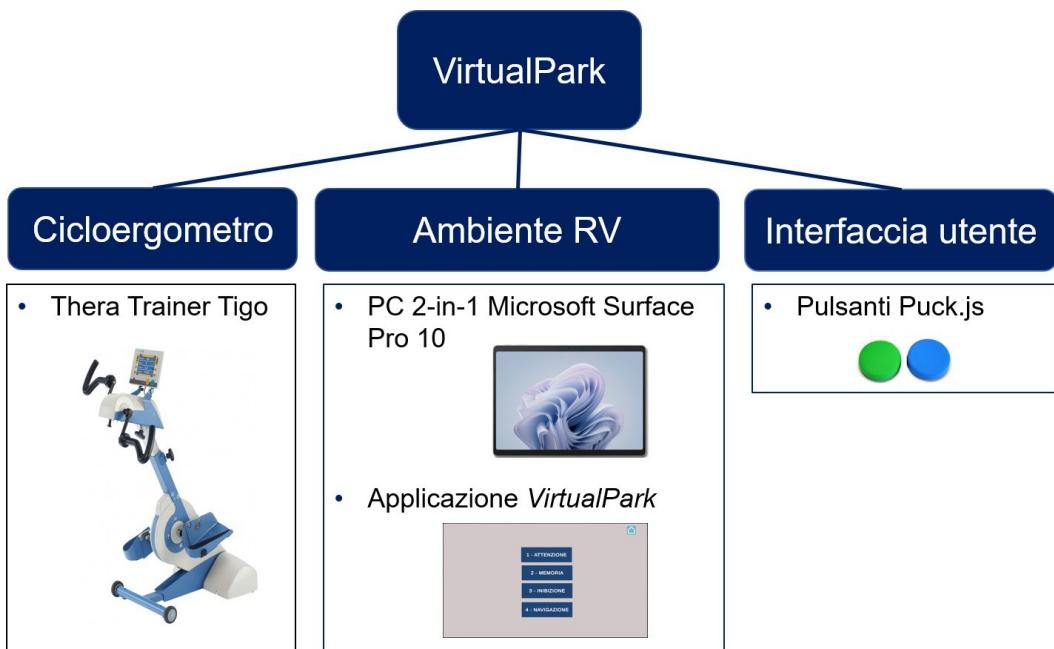
To ensure maximum patient safety during the use of VirtualPark, a risk analysis was carried out dedicated primarily to the risks associated with the combination of the cycle ergometer and the virtual reality environment, and risk mitigation procedures were implemented. Furthermore, the list of General Safety and Performance Requirements applicable to the device with the demonstration of compliance was drawn up. Please refer to the Investigator's Dossier for further details.

There are no contraindications relating to the use of the VirtualPark device in addition to what is indicated in the Thera-Trainer device User Manual and not foreseen by the clinical investigation plan provided that the device is used according to its intended use.

## VIRTUAL PARK (SYSTEM)

### *Functional elements*

The VirtualPark device consists of: a cycle ergometer, VR environment, and user interface. Figure S1 shows a diagram that includes the main elements that make up the VirtualPark system.



**Figure S 1 – Virtual Park components:** the diagram summarize the various components of the Virtual Park device

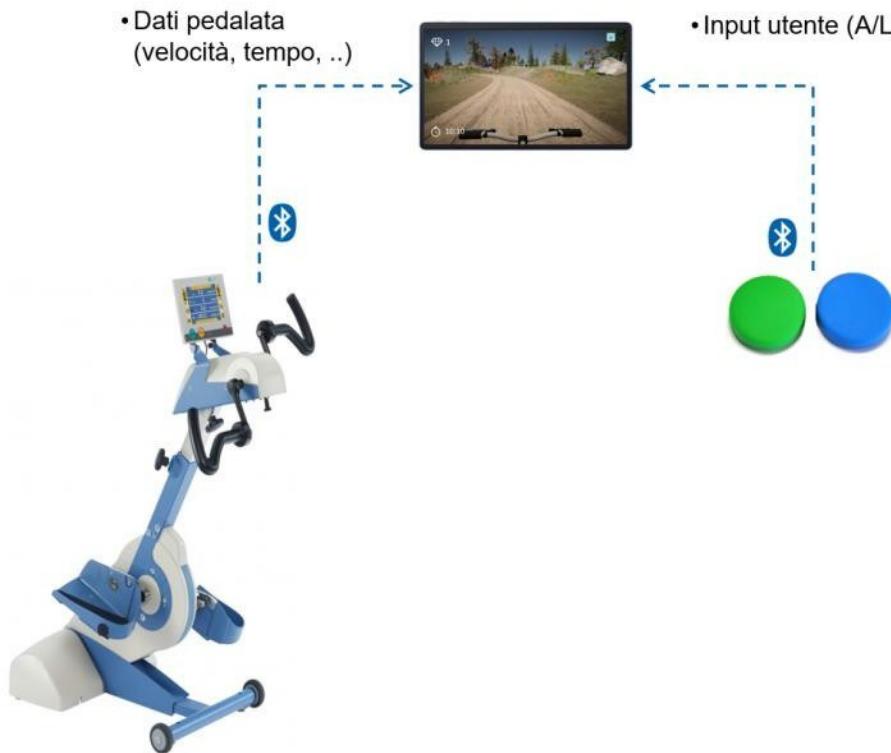
The **cycle ergometer** is a commercial device produced by the THERA-Trainer company. In particular, the THERA-Trainer tigo model is a lower limb device combined with an upper limb device module and therefore allows training in both modes. THERA-Trainer tigo is a Class IIa medical device. This document refers to the user manual provided by the manufacturer and is attached to the documentation (User\_manual\_thera-trainer\_tigo\_veho\_italian\_0).

The **RV application** is a Windows application developed by CNR-STIIMA. The application is installed on a 2-in-1 Microsoft Surface Pro 10 PC, which can be anchored to the cycle ergometer via an ad-hoc 3D-printed support, supplied as an accessory of the system.

The **user interface** consists of Puck.js buttons (<https://www.espruino.com/Puck.js>) through which the user interacts in carrying out the cognitive task. In particular, pressing the button corresponds to an action in the VR environment that is different depending on the exercise being performed. For example, the user presses the button to signal the identification of a target. The buttons can be held in the patient's hand or positioned on the cranks in special housings provided as an accessory for the system.

#### Connections

The hardware components (cycle ergometer and buttons) communicate with the software (RV environment) that manages the data exchange and integrates the different signals in the application. The diagram in Figure S2 shows these connections. The communication, for all devices, is based on Bluetooth communication protocols.



**Figure S 2 – Virtual Park connections:** the connectivity scheme of the Virtual Park device is represented in the figure

**RV Application – Cycle ergometer** The PC receives training data from the THERA-Trainer device via a specially developed Bluetooth communication protocol. The device is paired by qualified technical personnel when the VirtualPark system is installed. The connection/disconnection is managed at each use by the RV application. No additional action is required by the user. No data or commands can be sent to the THERA-Trainer device, which can only be controlled from the dedicated integrated control panel, as indicated in the user manual.

**RV Application – Puck.js Buttons** The Espruino Puck.js buttons are programmed to send a key press signal that emulates the pressing of the A and L keys on the keyboard, which are read by the software via the standard input device management libraries. Pairing is performed during installation. In the event of a failed connection, the user is invited to consult the relevant section of this manual.

## VIRTUAL PARK (APPLICATION)

### Main features

The virtual reality application, VirtualPark, is developed using the Unity game engine (<https://unity.com/>) version 2021.3.11f and compiled for the Windows operating system. The main features of the application are described below.

The VirtualPark application consists of two 3D scenarios: a park and a city. The “park” scenario is characterized by naturalistic elements (trees, streams, flowers, bushes); within this area, navigation takes place along a circular path. The real pedaling speed measured by the cycle ergometer (in revolutions per minute) is converted into a speed value (in meters per second)

used to move the virtual bicycle along a predefined trajectory. The “city” scenario presents the characteristic elements of an urban context (various buildings, parks, parking lots, cars, etc.); the path along which the user moves is made up of a series of roads. Navigation occurs, similarly to the first scenario, through the real pedaling speed and along a predefined trajectory. In addition to the graphic elements, the two scenarios also feature sound effects: background noises that contribute to the realism of the scene and sound feedback that is played in correspondence with specific events (e.g. to signal a correct or incorrect answer).

The two scenarios are shown in Figure S3.



**Figure S 3 – Virtual Park scenarios:** the figure shows some of the scenarios included in the Virtual Park VR environment (“City” and “Park”)

Four cognitive tasks are associated with the two scenarios, each focused on a specific cognitive domain of interest.

**Communication with external devices** (THERA-Trainer, Puck.js) is managed at the C# program level. The management of the connection/disconnection of the device occurs automatically when the application is started and when the data stream starts at the beginning of the training session. The encoding of the data sent by the cycle ergometer and read by the program is managed through classes implemented ad-hoc starting from the APIs provided by the THERA-Trainer manufacturer. The data are recorded starting from the moment the exercise is started: some of them are displayed in the user interface (e.g. progress speed, remaining time), and others are saved for the generation of the report file. External devices are connected automatically when the application is started. The connection of the Puck.js buttons occurs

automatically after the first installation; once the device has been correctly paired with the PC (pairing) it will be recognized by the VirtualPark application that will interpret it as a standard input device, in particular as a Bluetooth keyboard characterized by a single key (corresponding to the letter A or L). The interaction can therefore take place, if deemed appropriate, also with any standard keyboard. For a better use of the system, we recommend using the buttons provided.

The **graphical interface** (Graphical User Interface) allows the therapist to create the patient profile, specify the ID, load the profile of an existing user, and select tasks and relative configuration. During the exercise, the patient displays, superimposed on the 3D scenario, the data of the training session such as remaining time, speed, and score.

The patient profile, once created, is saved in the Data folder of the Streaming Assets folder within the VirtualPark\_Data folder located in the directory where the application is installed. For each patient, a folder is created whose name is the ID, and within which the configuration and report files specific to each session will be saved.

### Cognitive tasks

Visual attention, Inhibition, and cognitive flexibility: the first task focuses on the domains of visual attention, inhibition, and cognitive flexibility and is set in the park scenario. It is a Go – No Go task in which the patient must identify – by pressing the button – target stimuli (e.g. monsters) that appear along the path, discriminating them from non-target stimuli (e.g. animals). There are four modes, each with 3 levels of difficulty with increasing target appearance frequency (level 1: 15 s, Level 2: 10 s, Level 3: 5 s):

- In mode 1, monsters and animals do not have a colored aura; when the child hits an incorrect target, he receives an error message. The instruction that the child receives is: "Now hit all the monsters you see".
- In mode 2, monsters and animals have a blue-colored aura or a red-colored aura; when the child hits an incorrect target, he receives an error message. The instruction the child receives is: "Now hit all the characters you see with a red aura".
- In mode 3, monsters and animals have a blue-colored aura or a red-colored aura; when the child hits an incorrect target, he receives an error message. The instruction the child receives is: "Now hit all the characters you see with a blue aura".
- In mode 4, monsters and animals have a blue-colored aura or a red-colored aura. The correctness criterion will be set randomly by the system and variable during the test; when the child hits an incorrect target, he receives an error message. The instruction the child receives is: "Now the rule for hitting the characters can be any of the three previous ones, try to understand which one it is. The rule can change as you progress along the path."
- Working memory: the second task trains working memory and is inserted within the park scenario. The goal is to collect a series of target objects that appear along the path, together with other distractor objects. The list of target objects (e.g. oranges, newspaper, ball) is shown at the beginning of the exercise. The patient must memorize the objects to collect and identify them within the park by pressing the button. The difficulty levels require a greater number of objects to identify and the insertion of objects similar to those on the list (e.g. oranges vs. apples). The task has two modes:

- Mode 1: the list of objects to collect is presented in graphic form (“list” of images of the objects to collect)
- Mode 2: the list of objects to collect is presented in textual form (“list” of names of the objects to collect).

Inhibition: the third task is set in the city scenario which is characterized by a series of intersections and straights. At the intersections, the patient must decide which type of turn to make (right/left) by pressing the right or left button respectively. In the first level, the correct direction is the one indicated by the arrow. In the second level, the correct direction is the one opposite to the one indicated by the arrow. In the third level a second factor is added (color): if the color is yellow the correct direction is the one indicated by the arrow, if the color is blue the correct direction is the opposite one. Navigation: The fourth task is set in the city scenario and trains navigation skills. In the first part of the task the patient follows a predefined path (the turns are made automatically); the child/young person receives the instruction: “Pay attention to the path and to what you see around you while you pedal because then you will have to do the road again by yourself”. In the second part of the task, the patient must repeat the path by selecting with the buttons the direction to take at each intersection. The difficulty is defined by the number of turns and the number of landmarks inserted in the path (e.g. bus near the turn). The task includes a predefined number of paths from which the operator can choose. Each path has a different goal, e.g. “get to school”, or “get to the playground”, and consists of a certain number of steps (each consisting of a limited number of turns/landmarks). For example, the first step of the path “get to school” starts from the child’s house and ends at the first traffic light. In each session, the user retraces the previous steps and adds a step until the final path is completed.

## TRAINING SESSION PREPARATION

The VirtualPark system is assembled by specialized technical personnel before first use. The THERA-Trainer device is installed according to the instructions in the user manual attached to the documentation (User\_manual\_thera-trainer\_tigo\_veho\_italian\_0).

The CNR-STIIMA technical personnel will assemble the accessories, PC supports, and buttons before the device is installed at the final use site. This operation will not need to be repeated unless extraordinary maintenance is required, for which it is recommended to contact the CNR-STIIMA personnel.

During the installation of the device, the CNR-STIIMA personnel will also set up and verify the connections between the THERA-Trainer and Puck.js devices and the VR application.

Once the connection has been set up during installation, during use of the system it is sufficient to select the THERA-Soft/Group therapy program from the THERA-Trainer panel and wait for the automatic connection.

The buttons are also connected automatically after the first installation.

It is not necessary to repeat these operations after the first installation. In case of problems, consult the relevant section (§ 6).

Before starting the training session, turn on the PC and make sure that the PC battery charge level is sufficient; if necessary, place the PC and the buttons in the appropriate supports.

Position the patient on the chair/wheelchair, making sure that he/she does not tip over, at an adequate distance from the THERA-Trainer. Depending on the type of training, place the legs in the pedals and secure them, or place the arms in the armrests, or grip the handles. Consult the THERA-Trainer user manual for further details.

Once these operations are completed, you can start the training session by following the instructions below.

## EXECUTION OF THE TRAINING SESSION

The training session is started in parallel via the THERA-Trainer panel (start of pedaling exercise) and the VirtualPark application from the PC (start of dual-task exercise).

THERA-Trainer allows you to perform active or passive training of the lower or upper limbs. For details on the settings of the physical training session, please refer to the THERA-Trainer user manual.

Start the VirtualPark application from the PC desktop by double-clicking on the application icon. Using the graphical interface, create the patient profile by selecting the USER option. You can select a user from the list of existing users or create a new profile. Each patient is identified by an alphanumeric code (ID) set by the operator. The date of the last session and the total number of sessions are also displayed (see Figure S4).



**Figure S 4 – Virtual Park Interface (Therapist):** the figure shows an example of the therapist interface of the Virtual Park software (user profile).

Turn on the THERA-Trainer device by pressing the START button (if the device is on standby).

Select the Thera-soft/Group Therapy program. The system will start searching for the connection to the PC.

Wait for the connection to take place. If the connection has been established correctly, you will see the icon at the bottom right of the THERA-Trainer panel and you will hear connection feedback from the PC.



**Figure S 5 – Thera Trainer panel:** the figures are examples of the screen interface of Thera Trainer cycloergometer (PC connections and exercise parameters)

In addition, the Home screen of the Virtual-Park application will display a message indicating that the connection was successful. Otherwise, an error message will be displayed. The same error message is also displayed on the THERA-Trainer panel.

From the settings of the Thera-soft/Group Therapy program, configure the exercise session for the patient. The Thera-soft/Group Therapy program corresponds – in terms of exercise settings – to the Neuro program but also allows connection to external software. The Neuro program is a training program for users with neurological diseases and has the following characteristics:

- Training type: set resistance.
- The improvement in the set resistance within the total laps remains constant.
- A higher pedaling frequency corresponds to greater power

The exercise parameters are:

- Duration: from 1 to 180 minutes
- Motor force: from 2 to 22 Nm
- Spasm recognition: fine – medium – strong

For further details on the training options, consult the THERA-Trainer user manual attached to the documentation (User\_manual\_thera-trainer\_tigo\_veho\_italian\_0).

From the VirtualPark application interface, choose the task from those proposed and then configure the exercise.



**Figure S 6 – Virtual Park interface (Therapist):** the figure shows an example of the therapist interface of the Virtual Park software for task selection (left) and configuration (right)

Start the dual-task exercise from the Virtual-Park application by selecting the SAVE button.

Start the exercise from the THERA-Trainer panel by pressing the START button. Select the lower or upper limbs option from the panel.

During the exercise, the user advances in the virtual scenario at the same pedaling speed detected by the THERA-Trainer. The user interface displays training data (speed, remaining time), information, and feedback related to the cognitive task (score, correct/incorrect answer feedback), as shown in Figure S7.



**Figure S 7 – Virtual Park interface (patient):** the figure shows an example of the user interface with the virtual Park VR environment (Park).

The patient interacts with the application, e.g. to signal the identification of a target, via the buttons located on the device knobs.

During the session, it is possible to interrupt or pause the training by pressing the STOP button on the THERA-Trainer panel. The Virtual-Park application graphic interface will show the patient the interruption of the exercise and the visualization of the scenario and the cognitive task will be interrupted.

If the training is paused, it is possible to resume the training or interrupt it by pressing the STOP button again on the THERA-Trainer interface.

In this second case, the End of exercise screen will allow you to return to the Home screen or close the application. In the absence of direct actions by the user, the exercise ends once the predefined duration has been reached.

At the end of each exercise, a summary of the performance is shown. Choose whether to continue the training session with a new task (restart from point 6) or return to the “Home” screen, to select a new user (point 1) or to close the application.

You can close the application, turn off the PC without risk and, if necessary, remove it from the support (e.g. to charge the battery) and disconnect the power cable of the cycle ergometer to turn off the device. In the case of training with lower limbs, proceed by releasing the protections on the pedals and having the user get off the cycle ergometer.

## ERROR MESSAGES

Error messages related to the THERA-Trainer device are displayed in the associated control panel and managed by the THERA-Trainer; for these, please refer to the user manual (User\_manual\_thera-trainer\_tigo\_veho\_italian\_0).

Other system errors are associated with connection errors between the application and external devices (Thera-Trainer cycle ergometer or Puck.js buttons). For these errors, you must restart the application and check what is causing the error.

Connection error Thera-Trainer cycle ergometer - VirtualPark application. If the connection failure symbol or a “Connection failed” message appears on the THERA-Trainer panel, the application has failed to establish a connection with the cycle ergometer. Try the following:

Close the application, and change the program from the THERA-Trainer panel using the +/- buttons. Restart the application, and try connecting again by selecting the Thera-soft or Group Therapy program from the THERA-Trainer panel. If the data displayed by the RV application is not consistent with what is shown on the THERA-Trainer control panel, although it appears to be connected, please contact the manufacturer.

Verify that Bluetooth is active on the PC using the appropriate Windows Bluetooth and other devices panel.

Verify that the PC is correctly paired with the cycle ergometer following the procedure performed during installation, summarized below.

Call up the submenu by pressing the STOP button and the – button to decrease the power (on the left of the panel under the weight symbol).

Select *System > Wireless connection > THERA-soft*

In the panel that appears (see Figure S8) verify that the Current server: item corresponds to a device (the name of the device may change from PC to PC).



**Figure S 8 – PC/Cycloergometer pairing:** the figure shows an example of the screen message that appears during the PC-Cycloergometer pairing process

If it does not appear, select “Search for devices” and wait until the device name appears.

Confirm; at this point, the PC will appear under the *Current server*:

If it does not appear, contact the manufacturer.

**Puck.js button connection error – VirtualPark application.** If you encounter problems with one or both buttons (i.e. user input is not detected during the execution of the task) the connection with the PC may have been lost. To restore the correct operation, perform the following operations.

Verify that Bluetooth is active on the PC using the appropriate Windows Bluetooth and other devices panel.

Verify that the two buttons are paired and connected to the PC using the appropriate Windows Bluetooth and other devices panel; the buttons must appear in the list of connected devices. The buttons are identified as “Puck.js XXXX” where XXXX is 4 digits specific to each button. If not present, pair the buttons.

If you need to pair the buttons from the Bluetooth and other devices panel, select Add device – Bluetooth and wait for the Puck.js button identified as “Puck.js XXXX” to appear in the list of available devices. Connect the device and try typing the letter A or L in any text field by pressing the button.

If the buttons do not appear in the list of available devices, try removing the battery from the button and reinserting it. Then repeat the previous step.

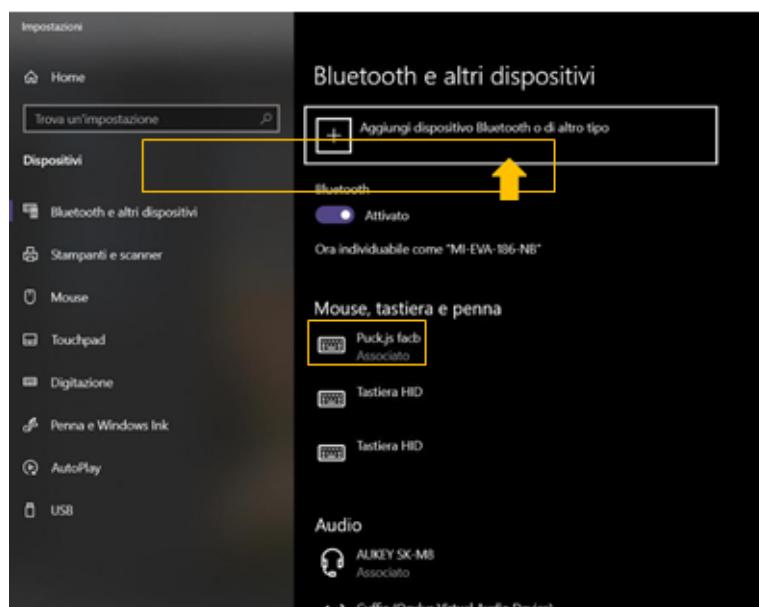
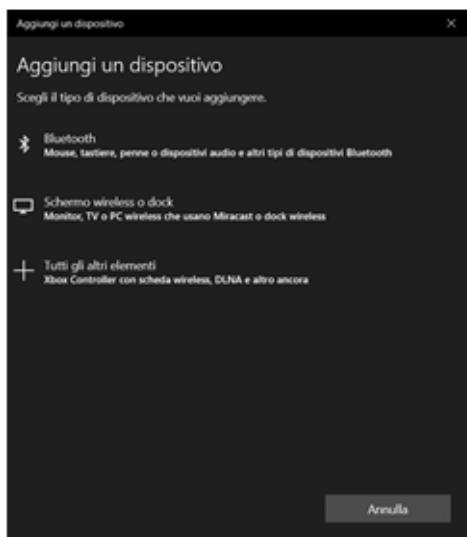


Figura 14 Pairing e verifica connessione pulsanti Bluetooth



**Figure S 9 – Bluetooth button search and connection:** the figures shows an example of the screen message that appears during the Bluetooth button search and connection process

## MAINTENANCE AND CLEANING

Keep the VirtualPark device clean and regularly maintained. Before each use, it is recommended to visually inspect for damaged components and to check that all parts of the device (cycle ergometer, PC, and buttons) are properly positioned and fixed.

About cleaning the THERA-Trainer cycle ergometer, it is recommended to clean/disinfect the device and the grip cuffs regularly, following the anti-infection measures in force in the factory and following the detailed instructions in the user manual.

(User\_manual\_thera-trainer\_tigo\_veho\_italian\_0). In particular, refer to paragraph 16 CLEANING AND DISINFECTION. The THERA-Trainer device is not subject to maintenance (paragraph 17 MAINTENANCE).

To avoid weakening or damaging the power cables (THERA-Trainer and PC) avoid twisting or crushing the cable, do not wrap the cable too tightly, and inspect the cable regularly.

Periodically check the battery charge level of the Puck.js buttons and replace them if necessary.

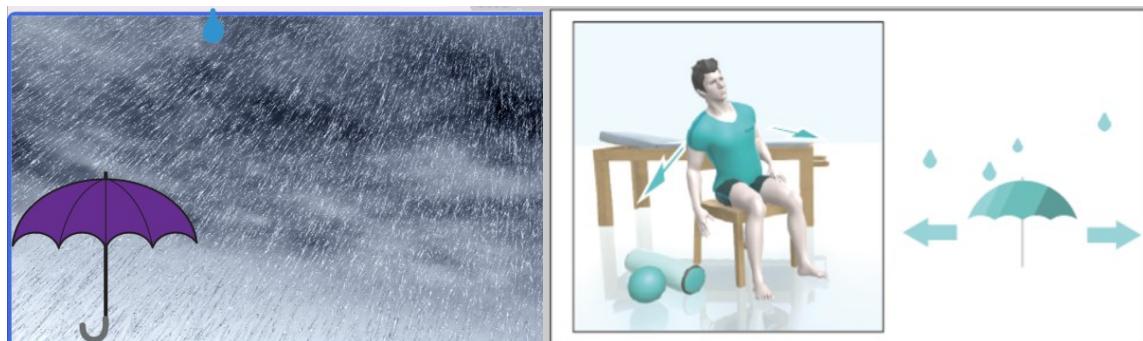
#### 5.1.4. The Khymenia home-kit experience: a pilot experience of a multimodal tele-rehabilitation protocol

### SUPPLEMENTARY 1 – Intervention protocol

#### MOTOR TRAINING

##### Exercise #1

Name: Inclinazione di tronco Catching



SCOOPO: Aim: Mobilization of the dorsal lumbar tract of the spine. Helps improve posture and flexibility of the trunk when sitting and standing.

Instructions: In a standing or sitting position, with the lower limbs slightly apart, lean the trunk to the right and left to pick up objects. In this exercise, the subject is represented by an umbrella. The task is to collect all the droplets before they hit the ground.

Key Points: Make sure that the movement is controlled both on the way out and on the way back. Keep the head aligned with the trunk and look forward. Make sure that the shoulders are relaxed and low. If the exercise is performed in a sitting position, make sure not to rest the trunk on the backrest.

##### Exercise #2

Name: Rotazione di tronco Catching



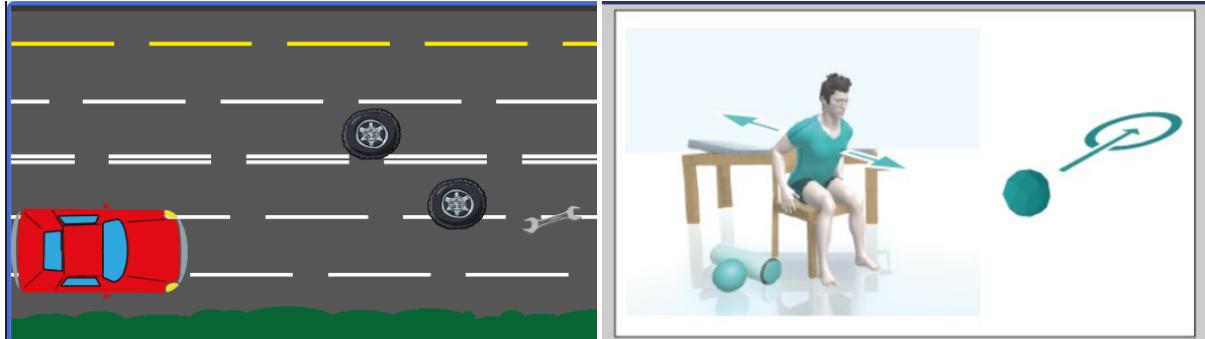
Aim: Mobilization of the dorsal lumbar region of the spine. Helps improve posture and flexibility of the trunk while sitting and standing

Instructions: Standing or sitting, with the lower limbs slightly apart, rotate the trunk to the right and left to pick up objects. In this exercise, too, the subject is represented by an umbrella. The purpose is to collect all the droplets.

**Key Points:** Make sure the movement is controlled both on the way out and on the way back and that the head remains still during the movement. Make sure the shoulders are relaxed and low.

#### Exercise #3

**Name:** Flesso-estensione di tronco Catching



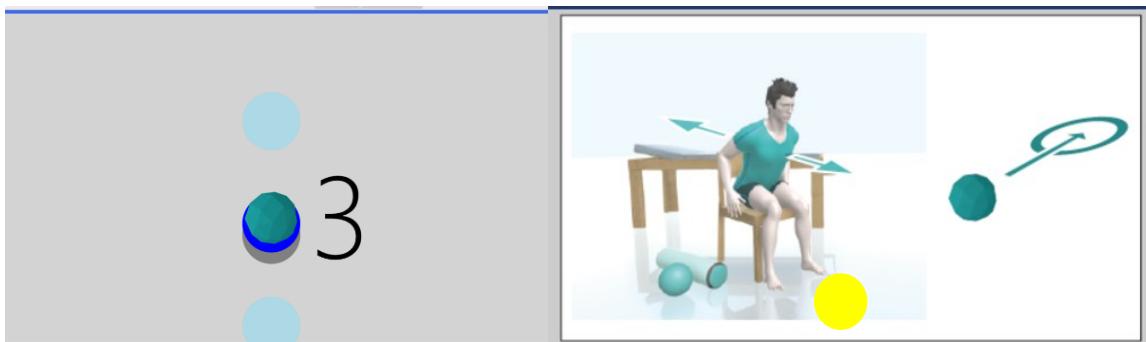
**Purpose:** Mobilization of the dorsal lumbar spine. Helps improve posture and trunk flexibility when sitting and standing

**Instructions:** In a standing or sitting position, with the lower limbs slightly apart, lean the trunk forward and backward to pick up objects. The subject must pick up all the elements (keys and wheels) that appear on the screen.

**Key Points:** Emphasize that the movement is slow and controlled. Maintain a forward gaze. If the exercise is performed in a standing position, avoid leaning backward by raising the tips of the toes. If the task is performed in a seated position, make sure to maintain a correct position and do not rest the trunk on the backrest during the exercise.

#### Exercise #4

**Name:** Flesso-estensione di tronco Reaching



**Aim:** Mobilization of the dorsal lumbar section of the spine. Helps improve posture and activate the flexor and extensor muscles of the trunk.

**Instructions:** In a sitting or standing position, with the lower limbs slightly apart, lean the trunk forward and backward to reach the blue dot, once reached hold the position for 3 seconds and then return to the starting position (yellow dot).

**Key Points:** Emphasize that the movement is slow and controlled. Keep your gaze forward. If performed in a standing position, avoid leaning back, or lifting the tips of your feet.

### Exercise #5

*Name:* Inclinazione di tronco Reaching 2D



*Aim:* Mobilization of the dorsal lumbar section of the spine. Helps improve posture, and flexibility of the trunk while sitting and standing.

*Instructions:* In a standing or sitting position, with the lower limbs slightly apart, lean the trunk forward, backward, and sideways to reach the blue dot. Once reached, hold the position for 3 seconds and then return to the starting position.

*Key Points:* Emphasize that the movement is slow and controlled. Keep your gaze forward. Avoid leaning backward, lifting your toes, or leaning on the back support of the chair.

### Exercise #6

*Name:* Rotazione tronco Catching 2D



*Aim:* Mobilization of the dorsal lumbar tract of the spine. Helps improve posture and flexibility of the trunk while sitting and standing

*Instructions:* In a standing or sitting position, with the lower limbs slightly apart, combine rotation and flexion-extension movements of the trunk to pick up objects. In this exercise the subject is represented by a missile; the task is to hit all the UFOs before they land.

*Key Points:* Emphasize that the movement is slow and controlled. Keep your gaze forward. Avoid leaning back, lifting your toes, or leaning on external supports.

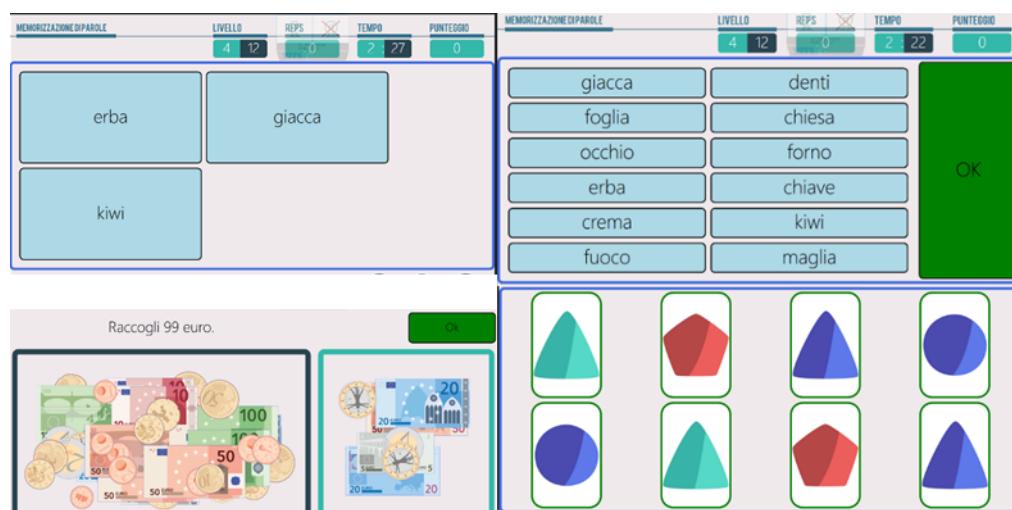
## COGNITIVE TRAINING

In parallel with the motor training, cognitive training was included in the rehabilitation program of both patients, which included the following activities:

- Activities in which short-term memory of both numbers and words is stimulated, such as memorizing a certain number of words that the subject must find in a predetermined time within a list (fig. 5) or collecting a certain amount of money while keeping it in mind (fig. 6).
- Visual memory games, such as memory (fig. 7) or memorizing beats to be copied.

These exercises were also set with progressive levels of difficulty. To carry out the activities, the subject used the touch interface of the device.

In Figure S10 some examples of cognitive activities included in the program are shown.



**Figure S 10 - VRRS HomeKit cognitive training:** the figure includes some examples of screenshots taken from the cognitive exercise included in the training protocol (e.g. “memory of words”, “pick up money to 100”, “Memory card game”)

## SUPPLEMENTARY 2 – Satisfaction questionnaires

All questions are based on the Likert scale model.

### PARENTS FORM:

*Quanto è soddisfatto del training di teleriabilitazione utilizzato?*

1: Per niente soddisfatto

5: Molto soddisfatto

Quali modifiche apporterebbe?.....

*Cosa ne pensa della durata degli esercizi ?*

1: Per niente adeguato

5: Molto adeguato

*Quanto si affaticava suo figlio dopo l'esecuzione degli esercizi?*

1: Molto affaticato

5: Per niente affaticato

*Suo figlio necessitava di fare delle pause durante l'esecuzione degli esercizi?*

1: Sempre

5: Mai

Se sì, dopo quanto tempo?.....

*Rispetto le capacità di suo figlio, gli esercizi erano?*

1: Molto difficili

5: Molto facili

*Come'è stato organizzare la settimana e gli impegni per poter svolgere gli esercizi?*

1: Molto difficile

5: Molto facile

*È stato richiesto un vostro supporto affinchè vostro figlio potesse utilizzare il dispositivo?*

1: Sempre

5: Mai

Se si, per quale motivo?.....

*Avete avuto difficoltà nell'utilizzo del dispositivo?*

1: Sempre

5: Mai

*Secondo voi potrebbe essere utile proseguire il training a domicilio?*

1: Poco utile

5: Molto utile

Perché ?.....

## PATIENTS FORM:

*Quanto sei soddisfatto del training di teleriabilitazione utilizzato?*

1: Per niente soddisfatto

5: Molto soddisfatto

Cosa miglioreresti?.....

*Cosa ne pensi della durata degli esercizi ?*

1: Per niente adeguati

5: Molto adeguati

*Quanto ti affaticavi durante gli esercizi?*

1: Molto affaticato

5: Per niente affaticato

*Dovevi fare delle pause durante l'esecuzione degli esercizi?*

1: Sempre

5: Mai

Se sì, dopo quanto tempo?.....

*Secondo te, come erano gli esercizi?*

1: Molto difficili

5: Molto facili

*Com'è stato organizzare la settimana e gli impegni per poter svolgere gli esercizi?*

1: Molto difficile

5: Molto facile

*Hai avuto bisogno di un aiuto per poter svolgere gli esercizi?*

1: Sempre

5: Mai

Se sì, per quale motivo?.....

*Hai avuto difficoltà nell'utilizzo del dispositivo?*

1: Sempre

5: Mai

*Secondo te potrebbe essere utile proseguire il training a domicilio?*

1: Poco utile

5: Molto utile

Perché?.....

## 5.2. Dissemination

A list of all the dissemination material originating from the project (publications, oral communications, lectures, posters, graduation thesis) is provided below:

Title	Type	Venue / Journal
<b>S. Parravicini Neuropsychology of Duchenne Muscular Dystrophy</b> (within the workshop: <i>From muscular to neuromuscular dystrophies: the CNS involvement in dystrophinopathies</i> )	Lecture	22 <sup>nd</sup> AIM National Congress, Matera, October 2022 (organizer: Italian Association of Myology)
<b>S. Parravicini, C.A.Quaranta, M.I. Dainesi, S.Khalil, A.Berardinelli</b> <i>The hidden face of Duchenne (Neuro)Muscular Dystrophy: social cognition impairment as a feature of the neuropsychological phenotype of DMD</i>	Oral Communication	24 <sup>th</sup> AIM National Congress, Rome, June 2024 (organizer: Italian Association of Myology)
Del Lucchese B, <b>Parravicini S</b> , [...]Cioni G, Sgandurra G and the Italian Neuroscience and Neurorehabilitation Network <i>The wide world of technological telerehabilitation for pediatric neurologic and neurodevelopmental disorders – a systematic review</i>	Article – Systematic review	Frontiers in Public Health (12:1295273. doi: 10.3389/fpubh.2024.1295273), April 2024
<b>S. Parravicini, M.I. Dainesi, C.A. Quaranta, A. Berardinelli</b> <i>Social Cognition: the blind corner of neuropsychological assessment in Duchenne (Neuro)Muscular Dystrophy - A Scoping Review</i>	Article – Systematic review	Neuroscience and Biobehavioral Reviews (Print ISSN: 0149-7634 Online ISSN: 1873-7528 – IF 7.5), August 2024 – under review
<b>S. Parravicini, C.A. Quaranta, M.I. Dainesi, A. Berardinelli</b> <i>The hidden face of Duchenne (Neuro)Muscular Dystrophy. Preliminary evidence of social cognition impairment as a feature of the neuropsychological phenotype of DMD</i>	Article – Research paper	Frontiers in Psychology (ISSN=1664-1078;DOI=10.3389/fpsyg.2024.1504174), January 2025
<b>S. Parravicini, A. Gardani, V. Vacchini, A. D'Errico, A. Berardinelli</b> <i>Testing multimodal telerehabilitation in two patients with DMD</i>	Poster	4th eNMD Congress – eHealth and innovation to overcome barriers in neuromuscular diseases “Digital biomarkers and digital therapies: new solutions and new avenues in NMD”, Munich, November 2023
B. Del Lucchese, <b>S. Parravicini, S. Filogna, G. Mangani, E. Beani, M.C. Di Lieto, A. Bardoni, M. Bertamino, M. Papini,</b>	Poster	36th European Academy of Childhood Disability (EACD) Meeting, Bruges, May

C.Tacchino, F.Fedeli, G.Cioni, G.Sgandurra, on behalf of the Italian Neuroscience and Neurorehabilitation Network <i>Technological telerehabilitation for children with neurologic and neurodevelopmental disorder: new opportunities and open challenges</i>		2024
C.A. Quaranta, [...], <b>S. Parravicini</b> , A.L. Berardinelli <i>Verso la Distrofia Neuromuscolare di Duchenne: possibile coinvolgimento delle funzioni di "social cognition" nel fenotipo neuropsicologico della DMD</i>	Poster	2° RESIDENTS' DAYS "Dalle evidenze scientifiche alla formazione specialistica", Bari, December 2023 (organizer: Italian Society of Child and Adolescence Neuropsychiatry)
S. Khalil (supervisor: R. Borgatti, co-supervisor: A. Berardinelli, <b>S. Parravicini</b> ) <i>FACE-DMD: facial aspect and implicit cognition of Emotions in Duchenne Muscular Dystrophy</i>	Graduation Thesis	Faculty of Medicine and Surgery, University of Pavia (Italy), June 2024

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