To my daughter Diletta

GENERAL INTRODUCTION

The PhD thesis, entitled “Cerebro-cerebellar networks in DMD children: neuropsychological, genetic and neuroimaging aspects”, is organized in a “General section” and in the “Experimental study”.

In the general section we provide a review of the literature concerning the main topics of the research and the state of art from which the experimental study starts.

The experimental study is subvidided into three parts:

1) *The neuropsychological study*: after the description of the preliminary neuropsychological results concerning a sample of 40 DMD boys without intellectual disability evaluated in a multicenter setting during school age, this part is further subdivided into three section.

   Section A: this section concerns the results of the follow up neuropsychological reassessment of the DMD children previously evaluated;

   Section B: the section shows the neuropsychological findings regarding an additional cohort of 17 DMD boys;

   Section C: this section provides the results deriving from the administration of a questionnaire for 47 parents deepening the neuropsychological functioning of DMD children in home environment.

2) *The genetic study*: in this ancillary study we analyze the role of genetic modifiers as possible influential factors for neuropsychological profile in a small group of the DMD boys already involved in the neuropsychological study.

3) *The neuroimaging study*: this is a pilot study in which we aimed to identify possible functional neuroimaging biomarkers of the neuropsychological impairments in DMD.

In the final part of the experimental study, we provide an overall discussion of the obtained results, we present advantages and limitations of the study and we suggest the future perspectives of the research.

The overall organization of the PhD thesis is shown in the “Index”.

Table of the main abbreviations

DMD	Duchenne muscular dystrophy
BMD	Becker muscular dystrophy
ADHD	Attention Deficit Hyperactivity Disorder
IQ	Intelligence Quotient
FIQ	Full Intelligence Quotient
VCI	Verbal Comprehension Index
PRI	Perceptual Reasoning Index
WMI	Working Memory Index
PSI	Processing Speed Index
TOL	Tower of London
MCST	Modified Card Sorting Test
BRIEF-2	Behavior Rating Inventory of Executive Functions
SD	Standard Deviation
GWAS	Genome Wide Association Study
HWE	Handy-Weinberg Equilibrium
MRI	Magnetic Resonance Imaging
DWI	Diffusion Weighted imaging
HARDI	High Angular Resolution Diffusion Imaging
ROI	Region Of Interest
FA	Fractional Anisotropy
WM	White Matter
CPCT	Corticopontocerebellar Tract
CTT	Cerebellar-thalamic Tract
SLF	Superior Longitudinal Fasciculus
FARCPCT	Fractional Anisotropy of the Right CPCT
FALCPCT	Fractional Anisotropy of the Left CPCT

FARCTT	Fractional Anisotropy of the Right CTT
FALCTT	Fractional Anisotropy of the Left CTT
FARSLF	Fractional Anisotropy of the Right SLF
FALSLF	Fractional Anisotropy of the Left SLF

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GENERAL SECTION

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GENERAL SECTION

1. Introduction

Duchenne muscular dystrophy (DMD) is a hereditary, X-linked muscular disorder affecting about 1 in 3500 boys (Emery, 1991). The disease is due to mutations in *DMD* gene resulting in lack of the gene product dystrophin, which is part of a protein complex located in the muscle cell membrane (Hoffman et al., 1987).

This multisistemic disease causes not only an involvement of muscular, cardiorespiratory, endocrine, gastrointestinal, and nutritional aspects but also cognitive difficulties and neuropsychological alterations. Therefore, a coordinated and multidisciplinary approach to care is essential for optimum management of the clinical manifestations and complications of DMD (Birnkrant et al., 2018).

Because of the major impact of motor and cardiorespiratory aspects on patients' duration and quality of life, more sensitive diagnostic techniques and earlier therapeutic interventions have been widely studied in the last years, and important outcome measures have been established in these issues, thanks to several carried out clinical trials.

Psychological, neurocognitive and learning aspects have been less deepened in DMD; however, misdiagnosed neuropsychological deficits can have a negative impact on the global functioning of these children.

In this “general section” we provide a review of the literature regarding the neuropsycohological, genetic and neuroimaging aspects, which are the topics of the PhD research.

2. Duchenne muscular dystrophy (DMD) and neuropsychological involvement

Several studies have reported cognitive difficulties and neuropsychological alterations in DMD patients. In fact, one in three DMD boys exhibits a cognitive impairment (Ogasawara 1989; Billard et al., 1992), with the mean Intelligence Quotient (IQ) approximately 1.0-1.5 standard deviations (SDs) below the mean (Cohen et al., 1968), and a high degree of impairment in verbal versus nonverbal performances both in older and younger patients (Cotton et al., 2001). Furthermore, DMD children often show a language involvement (Marini et al., 2007) and suffer from deficits in executive functions, such as attentional control, inhibition and working memory (Mento et al., 2011), with a possible negative impact on academic skills (Billard et al., 1992; Billard et al., 1998; Hendriksen et Vles, 2006; Lorusso et al., 2013; Astrea et al., 2015). DMD boys can also have significant psychiatric and neurodevelopmental comorbidities with attention deficit hyperactivity disorder (ADHD) being the most common (Pane et al., 2012).

Longitudinal studies on neurocognitive aspects in DMD children have shown that IQ tend to remain stable with increasing age (Cotton et al., 2001; Cotton et al., 2005), and cognitive and language skills do not vary with age also in very young DMD boys (Connolly et al., 2014). A correlation between early neurodevelopmental assessments and the cognitive scales has been found in a cohort of preschool DMD young boys followed until school age, with a clear concordance between subscales exploring similar domains on the two scales (Chieffo et al., 2015).

However, to our knowledge, no longitudinal studies have assessed higher and more complex cognitive functions, as executive functions, at different times in the same sample of patients.

A possible role of cerebellum and of a complex cerebro-cerebellar network, also involving cortical and subcortical structures (as basal ganglia), has been hypothesised for cognitive deficits in DMD (Cyrulnik & Hinton, 2008). The cerebellum has been traditionally associated with motor control and coordination, but several studies have extended its contribution to cognitive functions. In particular, the cerebellar-cerebro networks would be involved in language, abstract reasoning, emotions and the ability to process logical sequences (Schmahmann & Sherman, 1998; Riva et al., 2000). Therefore, a cerebellar dysfunction can give rise to different degree of impairments in language, visuospatial tasks, mental flexibility, speed and information processing and attention modulation, as well as affects the patient's ability to control many tasks automatically (Koziol et al., 2014).

The hypothesis of a cerebro-cerebellar network involvement in DMD is based on the localisation of dystrophin isoforms in normal brain. In fact, two alternative full-length isoforms (Dp427B and Dp427P) are expressed mainly in cortical neurons, both also in cerebellum, and two carboxy-terminal dystrophin proteins, Dp71 and Dp140, are both espressed in the brain, particularly in the Purkinje cells of the cerebellar cortex (Huard & Tremblay, 1992; Tinsley et al., 1993; Lidov et al., 1995; Anderson et al., 2002). Dp140 is expressed mainly in fetal tissue and in low quantity in adult brain and probably plays a role in the regulation of neuroglial specific gene expression. Dp71 expression gradually increases from the embryo until adult stage, becoming the major product of dystrophin in the brain, particularly in the hippocampus and in some layers of the cerebral cortex. The Dp71 function remains unknown but a role in the formation and/or stabilisation of the dystrophin- associated complex and in glutaminergic synaptic maturation and function is supported by studies (Lidov et al., 1990; Daoud et al., 2008). Recently, results from a study conducted on humans by using RNA-sequencing-derived exon-level expression data of dystrophin isoforms, and spatial gene expression data emphasized the expression patterns of dystrophin isoforms in the amygdala

and the hippocampus and showed the absence of the Purkinje isoform Dp427 in cerebellum (Doreenweerd et al., 2017a).

Commonly, it is known that mutations located upstream from exon 44 of *DMD* gene affect only Dp427 and Dp260 isoforms and preserve Dp140, while mutations located in (or extending to) the genomic region corresponding to exons 45-55 of the DMD gene are considered to affect Dp140, but not Dp71. Finally, mutations located on the 3' side of exon 63 are considered to affect all dystrophin isoforms, including Dp71 (Muntoni et al., 2003; Daoud et al., 2009; D'Angelo et al., 2011).

Several reports have described mutations in the second part of the dystrophin gene, affecting Dp140 and/or Dp71 coding region, as a factor that contributes to the presence and severity of intellectual disability and neuropsychological alterations (Moizard et al., 1998; Felisari et al., 2000; Daoud et al., 2008; Daoud et al., 2009; Taylor et al., 2010; D'Angelo et al., 2011; Snow et al., 2013; Ricotti et al., 2016).

However, the involvement of Dp140 is certain in case of mutations located in the promoter or in/dowstream the translation start codon (exon 51), while the effect on Dp140 expression is hard to predict for mutations placed between the Dp140 promoter (intron 44) and exon 51 (Muntoni et al., 2003). Therefore, an alternative subdivision of mutations along the *DMD* gene, according to the real involvement of Dp140 and that takes into account this "grey area", has been suggested and may be applied also for studies of genotype-phenotype correlation.

3. DMD and genetic aspects

The genetic bases of dystrophinopathies are mutations in the *DMD* gene on chromosome Xp21, mainly delections and, more rarely, duplications and point mutations. Mutations that maintain the reading frame generally result in abnormal but partly functional dystrophin and are associated with BMD; in DMD patient, on the contrary, mutations in *DMD* gene usually disrupt the open reading frame of the transcript, with the consequence of an absence of the dystrophin protein (Muntoni et al., 2003).

The multisistemic involvement observed in DMD take into account the alternative splicing events which commonly occur in a tissue-specific way and that generated many dystrophin isoforms. These include the above-mentioned brain dystrophin isoforms Dp140 and Dp71, which are detected also in other tissues, as retina, kidney, liver, lung and cardiac muscle (Muntoni et al., 2013).

Despite the homogeneous molecular mechanism, a relevant phenotype variability is commonly observed among DMD boys, for example in terms of age at loss of indipendent ambulation, age of onset and rate of progression of respiratory insufficiency and dilated cardiomyopathy. This variability in phenotype severity can be explained by environmental factors, such as implementation of standards of care, but also by type of mutations or molecular mechanisms. It is known that DMD boys with deletions generally show worse motor outcome than those with duplications and small mutations (Pane et al., 2014; Bello et al., 2016a); moreover, exceptions to the reading frame rule may produce trace protein amounts.

However, also DMD patients completely lacking dystrophin may have a wide-range of clinical manifestations, suggesting that other genetic variants may produce a modifier effects on the phenotype of DMD, interacting with the disease-causing mutation (Bello et al., 2019).

These genetic modifiers have been identified both on animal models and on human samples, by hypotesis-driven strategies or by genome wide association studies (GWAS), the formers concentrated on selective variants in genes with a known role in the pathogenesis of the disease, while the latter potentially capable to identify new modifiers gene (Bello et al., 2019). Some common polymorphisms have been reported as possible modifiers for DMD in both humans and animal models in several genes, for example *LTBP4*, *SPP1* (Barp et al., 2015; Bello et al., 2015), *ACTN3* (Zatz et al., 2014), *TRAF6* (Hindi et al., 2014), *CD40* (Bello et al., 2016b) *Anxa6* (Quattrocelli et al., 2017), *TCTEX1D* (Spitali et al., 2020).

The more widely studied modifiers were polymorphisms in the *SPP1* and in the *LTBP4* gene.

In particular, the G allele of the SNP rs28357094, situated in the promoter of the *SPP1* gene and encoding osteopontin, was associated with greater weakness and younger age at loss of ambulation in DMD (Pegoraro et al., 2011; Bello et al., 2015), a poor response to gluococorticoid corticosteroid treatment (Bello et al., 2015), but a protective effect on dilated cardiomyoparthy onset (Barp et al., 2015). The association of the G allele with rapid disease progression was not confirmed by another recent study performed on European and American cohorts (Van den Bergen et al., 2015).

Regarding the *LTBP4* gene, individuals homozygous for the IAAM *LTBP4* haplotype remained ambulatory significantly longer than those heterozygous or homozygous for the VTTT haplotype, with an additive effect on steroid therapy (Flanigan et al., 2013); moreover, a protective effect on dilated cardiomyopathy onset of the recessive T allele at LTBP4 rs10880 was found, in particular for steroid-treated patients (Barp et al., 2015).

A positive effect on age at loss of ambulation was recently found for the minor allele at rs1883832, in the 5'-untranslated region of *CD40* gene, by a genome-wide association study in a sub-cohort of European American ancestry from the Cooperative International Research Group Duchenne Natural Study (Bello et al., 2016b).

The protein products of the main modifiers are involved in muscle pathology as tissue repair, remodeling and regeneration, and in fibrosis or regulation of the infiammatory response (Bello et al., 2019). However, some of these genes and their protein products can also be widely distributed in many tissues and cells, including the brain, where they can have neuroprotective functions and may be involved in the pathogenesis of neurodegenerative disorders.

For example, the product of *SPP1* gene, osteopontin, is a constituent of the extracellular matrix of the central nervous system, with a possible role in pathogenesis of neurodegenerative diseases or in neuroprotection by regulating the activation and function of microglia (Yu et al., 2017).

In the rat brain, the mRNA of *LTBP4* has been demonstrated to have a widespread distribution in the cerebral cortex and it has been expressed in the hippocampus, the forebrain and the thalamus (Dobolyi & Palkovits, 2008).

Finally, the product of *CD40* gene, a member of the TNF receptor superfamily, is involved in neuroinflammation and neurodegeneration (Togo et al., 2000; Kempuraj et al., 2016) and it has been recently identified in a mouse model as a major regulator of dendrite growth and elaboration in the developing brain (Carriba et al., 2017).

4. DMD and neuroimaging

Magnetic Resonance Imaging (MRI) has shown a reduced brain volume in subjects with DMD, attributed to mild atrophy (Al-Qudah et al., 1990), or, more recently, to an altered brain maturation (Doorenweerd et al., 2014). Total brain and gray matter volumes would be more severely affected in patients with an impairment of Dp140, suggesting a role for this dystophin isoform in cerebral development (Doorenweerd et al., 2014).

The structural abnormalities may be associated to an altered cerebral metabolism: for example, glucose hypometabolism, measured by positron emission topography (PET) analysis, was found in DMD brain areas that are typically enriched in dystrophin, as the medial temporal structures, the cerebellum, the sensori motor and lateral temporal cortex on the right side (Bresolin et al., 1994; Lee et al. 2002). Moreover, a H^1 magnetic resonance spectroscopy (MRS) study by Rae et al. showed a significant increase in the ratio of choline-containing compounds to N-acetylaspartate (Cho/NAA) and Cho/creatine (Cr) in the left cerebellum in DMD boys compared to age-matched normal boys. Within the DMD group, the abnormal Cho/NAA group was significantly older than the normal Cho/NAA group, which reflects the progressive nature of the disease (Rae et al., 1998). In contrast, a more recent study showed a decrease in absolute choline levels in both cerebellar white matter and temporo-parietal cortex in DMD; moreover, small, but significant, metabolic abnormalities for glutamate and total N-acetyl compounds in the temporo-parietal region were found applying quantitative MRS (Kreis et al., 2011).

Among functional neuroimaging, a resting-state functional magnetic resonance imaging (RS-fMRI) study showed a reduction of local synchronisation of spontaneous activity in the motor cortex in patients with DMD (Lv et al., 2011). Moreover, the application of pseudo-continuous arterial spin labeling (ASL) cerebral flow images has demonstrated a reduction of

cerebral perfusion in DMD, regardless of the reduced grey matter volume (Doorenweerd et al., 2017b).

Because of the possibility of analysing brain connectivity and dynamic cerebral organisations, Diffusion Weighted Imaging (DWI) with tractography has widespread potential implications in the fields of cognitive neuroscience and neurobiology.

Recently, tractography has been widely applied in developmental age, both in neurodevelopmental disorders, as ADHD (Sudre, 2017), and autism spectrum disorders (Conti et al., 2016 and 2017; Qin et al., 2018; Payabvash et al., 2019), and in neurological disorders, as leukodystrophies, cerebral palsy or cerebellar diseases (Poretti et al., 2007; Escolar et al., 2009; Poretti et al., 2014; Pannek et al., 2014; Fiori et al., 2016a; Kimiskidis et al., 2017).

Regarding neuromuscular diseases, tractography has been applied in disorders with a known brain involvement. In particular, alterations of white matter microstructure in projection, association and commissural fibres have been described in myotonic dystrophy type 1 (Okkersen et al., 2017; van Dorst et al., 2019; Labayru et al., 2019) and alterations of diffusion coefficient values in white matter have been revealed for merosin-deficient congenital muscular dystrophy (Sijens et al., 2007; Ip et al., 2012).

Tractography has been also tried in DMD samples with evidence of microstructural differences in scalar measures (Fractional Anisotropy, FA, and Mean Diffusivity, MD) between DMD subjects and controls in the occipital areas (Doorenweerd et al., 2014) and in the splenium of corpus callosum, with a correlation with intellectual quotients (Fu et al., 2016). Alterations in diffusion of the prefrontal cortex and hippocampus emerged also in *mdx* mice (Xu et al., 2015).

EXPERIMENTAL STUDY

1. Neuropsychological study

1.1 Introduction and preliminary results

Cognitive difficulties and neuropsychological alterations have been reported in DMD children (Moizard et al., 1998; Felisari et al., 2000; Snow et al., 2013) and may be due to the lack of specific dystrophin isoforms in the brain, taking account on the possible involvement of cerebellum and of a complex cerebro-cerebellar network (Cyrulnik & Hinton, 2008).

The PhD project comes from the neuropsychological data collected before the beginning of the PhD by a group of Centres from the DMD Italian Network (IRCCS Stella Maris Foundation, Pisa; Policlinico Gemelli, Catholic University of Sacred Hearth, Roma; Bambino Gesù Children's Hospital, IRCCS, Roma; Neuropsychiatric Division, C. Besta IRCCS, Milan).

The data previously collected have been developed and discussed in a multicenter setting and produced two scientific reports, published during the second year of the PhD (Battini et al., 2018; Vicari et al., 2018).

Both the studies analysed samples of DMD boys without intellectual and behavior disability during school age in order to assess profiles of neuropsychological functions and to deepen the issue of a possible role of cerebellar-thalamo-cortical network. To examine this selected DMD sample has allowed a targeted neurocognitive detection free from the potential bias of a more global cognitive deficit.

In the first study, 40 DMD boys without intellectual disability (range of age: 6 years to 11 years and 6 months) were evaluated by Wechsler Intelligence scale and a battery of tests including tasks assessing working memory and executive functions.

The results showed deficits in specific aspects of executive functions such as working memory, inhibition, multitasking and problem solving. In fact, regarding intellectual functioning, the worst performances were observed in Working Memory Index (WMI) and in the related subtests. The impairment in “rules and moves” of the Tower on London (TOL) test underlighted an involvement of working memory and planning abilities and the impairment in the Switching task of the Inhibition test (NEPSY-II) demonstrated difficulties in inhibition and response switch. Finally, the impairments in “maintaining the set” at the Modified card Sorting Test (MCST) and in “violation of rules” al TOL were related to abstract reasoning and planning and may resemble working memory skills (Battini et al., 2018).

The second study aimed at comparing implicit learning sequence in DMD boys without intellectual disability and age-matched typically developing children using a modified version of the Serial Reaction Time task (SRTT). Thirty-two DMD children and 37 controls of comparable chronological age were studied. The reaction times (RTs) on the last ordered block (O4) and on the random block (R5), usually taken as a measure of implicit learning effect, were different between controls and DMD patients. In fact, an important change of the RT from block O4 (ordered) to block R5 (random) was found in the control group, but not in DMD children. These results suggested that the DMD boys show a reduced rate of implicit learning even if in the absence of global intellectual disability (Vicari et al., 2018).

These overall results supported the hypothesis of a specific involvement of cerebellum as part of a more complex network resulting from the interplay of cerebellar, cortical and subcortical neural system interconnected by reciprocal projections (Battini et al., 2018).

In both studies, the DMD sample was subdivided on the basis of the site of mutation in those with proximal and distal mutation, but no correlations with genotype-neuropsychological phenotype were found (Battini et al., 2018; Vicari et al., 2018).

1.2 Aims

Starting from these results previously obtained on the cohort of 40 DMD boys (Battini et al., 2018), we aimed to:

A) assess longitudinally the DMD boys without intellectual disability previously evaluated in order to recognise the trend of deficits in executive functions, better defining the neuropsychological natural history in these patients;

B) extend neuropsychological evaluation of executive functions and working memory in a another group of DMD Italian children without intellectual disability during school age to confirm the involvement of the cerebellar-thalamo-cortical network in a larger cohort of DMD patients than those previously evaluated;

C) expand the neuropsychological protocol adding the Behavior Rating Inventory of Executive Functions (BRIEF-2) Parent form in order to assess executive functioning in home environment for DMD boys without intellectual disability.

As already tried in our previous study (Battini et al., 2018), where no correlations with genotype-neuropsychological phenotype were found, we were also interested in analysing a possible correlation between the neuropsychological findings and the site of mutations in *DMD* gene. In this research, we analysed a wider sample and we subdivided the cohort using a different and more analytical classification compared to the previous study.

1.3 <u>Section A:</u> Follow up study

A.1 Subjects and Methods

A.1.1 Subjects

This study was a multicenter research involving the same specialised Centres, members of DMD Italian Network, engaged in the above-mentioned studies (Battini et al., 2018; Vicari et al., 2018): a) IRCCS Stella Maris Foundation, University of Pisa; b) Policlinico Gemelli,

Catholic University of Sacred Hearth, Roma; c) Bambino Gesù Children's Hospital, IRCCS, Roma; d) Neuropsychiatric Division, C. Besta Institute, Milan.

The sample of DMD children previously evaluated has been longitudinally re-assessed after 3 years from the first evaluation using the same neuropsychological protocol; the new cohort included 33 subjects because 7 patients dropped-out. Some parents' patients refused the new assessment (N. 4) or addressed to another Hospital (N. 3).

The inclusion criteria were: i) DMD boys with proven mutation in the dystrophin gene; ii) primary school age (6-12 yrs); iii) no cognitive impairment (IQ<70) or any associated neuropsychiatric disorders (drug-resistant epilepsy, autism spectrum, attention deficit and hyperactivity) or any additional neurosensory deficits; iv) steroid treatment and/or other experimental drug stable for at least six months (Battini et al. 2018).

According to their full IQ (FIQ), the DMD children have been subdivided into two groups (FIQ<85 and FIQ≥85). In order to explore a possible genotype-neuropsychological phenotype correlation, four groups should be considered, according to the predicted effect on dystrophin expression (Group 1: mutations located upstream from or in exon 44; Group 2: mutations located in or extending to the region corresponding to exons 45-50; Group 3: mutations located in or extending to the region corresponding to exons 51-62; Group 4: mutations located in or downstream exon 63). However, as it is known that the mutations extended beyond exon 63 affect Dp71 and are commonly associated to severe cognitive impairment, patients with this type of mutation have not been included in the study.

All parents' patients included in the study signed a written informed consent.

A.1.2 Neuropsychological protocol

Each patient received a wide and comprehensive battery of neuropsychological tests assessing intellectual functions and executive functions and language involving the cerebello-thalamo-cortical network, as already reported in our previous work (Battini et al 2018).

Intellectual functioning.

The Wechsler Intelligence Scale for Children (WISC-IV) was used not only to obtain the FIQ but also to measure skills and cognitive abilities related to a possible cerebellar involvement. The scale is based on a factorial model of intelligence and it is divided into 10 subtests (Orsini et al., 2012). In addition to subtest scores a composite score relating to specific cognitive areas may also be reported: the Verbal Comprehension Index (VCI) measures the child's use and understanding of language using subtests that assess abstract reasoning, vocabulary development, and common sense reasoning; the Perceptual Reasoning Index (PRI) assesses nonverbal reasoning and problem solving; the WMI measures child's ability to recall, manipulate, and sequence auditory information; the Processing Speed Index (PSI) assesses the speed and accuracy of visual motor integration.

Executive functions and Language.

Some tests or specific items from general neuropsychological developmental assessment scales have been selected in order to obtain information about specific aspects of cognitive functions that were relevant to a possible cerebellar role. The Inhibition subtest (Denomination, Inhibition and Switching subtest) and the Design Fluency have been chosen from the NEPSY-II (Brooks et al., 2010; Urgesi et al., 2011) in order to explore processing speed, inhibitory control, cognitive flexibility, and initiation and productivity, respectively.

We administered TOL (Sannio Fancello et al., 2006) to evaluate planning and problem solving and the MCST (Sannio Fancello et al., 2003), a simplified version of the Wisconsin Card Sorting Test for children, to assess categorising ability.

We used Verbal Fluency-Word Generation from BVN 5-11 (Bisiacchi et al., 2005) phonological and semantic Criteria because of cerebellar involvement in these specific linguistic functions.

All the subjects were assessed individually and each assessment took approximately 4 hours.

A.1.3 Data and statistical analysis

Continuous variables were presented as the mean and standard deviation. All the tasks have been standardised on Italian population of children aged between 6 and 12 years; row scores are thus transformed in z or scaled scores and T scores according to the reference data (Orsini et al., 2008; Brooks et al., 2009; Urgesi et al., 2011; Sannio Fancello et al., 2006; Sannio Fancello et al., 2003; Bisiacchi et al., 2005).

The mean FIQ, VCI, PRI, WMI and PSI were considered in the normal range according to the Diagnostic Mental Index if the mean value was 100 and the SD was 15. The specific Wechsler subtest was measured as mean 10 and SD 3. The same criteria were measured for the NEPSY's Substest (mean = 10; SD = 3, cut-off = 7). In contrast, TOL results were expressed with T Score (mean = 50; SD = 10), for MCST test results were used z normalized (mean = 0; SD = 1). The individual z scores were then averaged to allow a comparison between different tests and different age and group of patients and z score values < -1.6 were considered pathological.

In consideration of the sample size, we decided to use non parametric tests.

Wilcoxon test was used to define if the cognitive profile was stable over time in our sample who performed a second neuropsychological assessment after 3 years from the previous. Mann–Whitney U-test was used to compare the distribution of variables among the two groups in which the sample was subdivided according to the FIQ and Kruskal Wallis test was used to compare the distribution of variables among the three groups according to the site of mutation of *DMD* gene. Moreover, Pearson correlation test was performed to evaluate possible correlation between scores difference from baseline and other variables such as age and score at baseline. Version 23 of the SPSS software (SPSS, Inc.) was used for all statistical analyses, setting the significance at $p < 0.05$.

A.2 Results

Thirty-three DMD subjects (mean age at follow up: 10 years and 7 months) performed a new assessment three years apart from the baseline previously reported.

A.2.1 Intellectual functioning

WISC-IV profile in the cohort study at baseline and follow-up assessment is shown in Table 1.

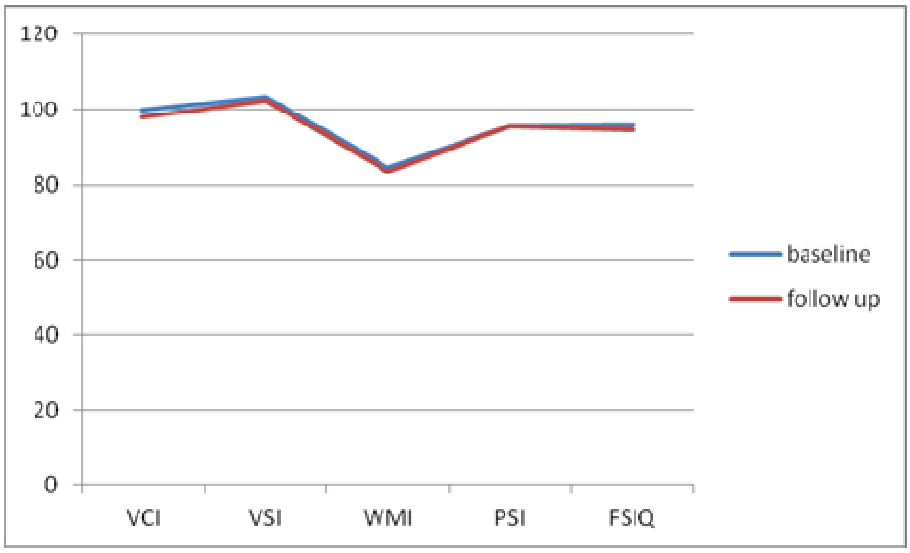
Table 1. WISC-IV assessment in DMD cohort

	Baseline *Mean (SD)*	**Follow-up** *Mean (SD)*	**P value**
WISC-IV Index			
Verbal Comprehension Index	99.94 (15.14)	98 (13.37)	0.082
Perceptual Reasoning Index	103.39 (13.40)	102.36 (13.93)	0.204
Working Memory Index	84.36 (17.28)	83.61 (12.21)	0.928
Processing Speed Index	95.33 (11.71)	95.45 (13.69)	0.645
Full Intellectual Quotient	95.67 (13.67)	94.45 (10.44)	0.065
WISC-IV Subtest			
Similarities	9.82 (3.05)	9.76 (2.69)	0.727
Vocabulary	9.94 (3.36)	9.88 (2.68)	0.063
Comprehension	10.21 (2.84)	9.12 (2.79)	0.332
Block Design	10.45 (3.35)	10.12 (2.84)	0.646
Picture Concepts	11.09 (3.17)	11.15 (2.36)	0.791
Matrix Reasoning	10.15 (2.93)	10.30 (2.91)	0.992
Digit Span	7.21 (3.07)	7.61 (2.13)	0.068
Letter-Number sequencing	7.61 (3.68)	6.91 (2.91)	0.857
Coding	9.00 (2.85)	8.15 (2.51)	0.942
Symbol Research	9.36 (2.42)	10.24 (2.77)	0.395

No statistically significant differences emerged between baseline and follow-up assessment. These results confirmed therefore the stability of the cognitive impairment over time and the worst performance in the WMI at WISC-IV (mean score at baseline=84.36; SD= 17.28; mean score at follow up=83.61; SD= 12.21) and in the related subtests Digit Span (mean score at baseline=7.21; SD= 3.07; mean score at follow up=7.61; SD= 2.13) and Letter-Number

Sequencing (mean score at baseline=7.61; SD= 3.68; mean score at follow up=6.91; SD= 2.91). (Table 1 and Figure 1).

Figure 1. WISC-IV profile in DMD cohort at baseline and after 3 years follow-up



VCI: verbal comprehension index; VSI: visual spatial index; WMI: working memory index; PSI: processing speed index; FSIQ: Full scale Intelligence Quotient.

A.2.2 Executive functions and language

The mean scores of the tasks assessing executive functions and language in DMD cohort at baseline and follow-up assessment are reported in Table 2.

Table 2. Executive functions and language assessment in DMD cohort

	Baseline *Mean (SD)*	**Follow-up** *Mean (SD)*	**P value**
Inhibition Test (NEPSY-II) (standard score)			
Denomination Total	7.76 (2.48)	7.64 (2.97)	0.413
Inhibition Total	9.09 (3.29)	8.09 (3.32)	0.061
Switching Total	**5.81 (4.35)**	**7.34 (2.86)**	0.306
Graphic Fluency Test NEPSY II (standard score)			
Graphic Fluency	9.52 (4.00)	8.67 (3.55)	0.173
TOL Test (T score)			
Total corrected	**38.22 (18.70)**	**49.84 (14.43)**	0.617
Number of moves	72.84 (16.85)	79.59 (16.87)	0.602
Violation of rules	**79.10 (21.89)**	**74.84 (21.41)**	0.918
Time decision	**42.47 (6.22)**	**51.28 (10.16)**	0.062

Time execution	**42.66 (10.50)**	**50.78 (16.12)**	0.182
Time total	**41.94 (9.53)**	**51.03 (16.04)**	0.067
MCST Test (z score)			
Categories	-0.17 (1.08)	-0.76 (1.07)	0.407
Correct answers	-0.23 (1.09)	-0.64 (1.32)	0.541
Categorial efficiency	-0.77 (1.02)	-1.05 (0.98)	0.061
Total errors	0.16 (0.92)	0.64 (1.04)	0.737
Perseveration errors	-0.18 (0.60)	0.65 (1.48)	0.268
Non perseveration errors	0.48 (0.99)	0.59 (1.20)	0.728
Failure	2.96 (5.20)	2.65 (3.80)	0.289
Verbal Fluency Test BVN (z score)			
Semantic	-0.78 (1.00)	-0.54 (0.89)	0.257
Phonological	-0.46 (0.77)	.0.93 (0.79)	0.688

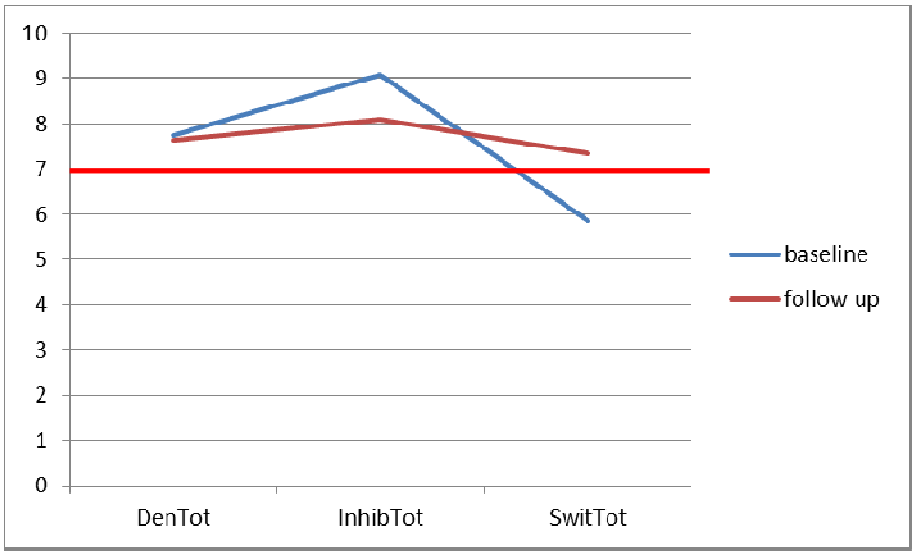
The slight maturation in performances is written in bold

No statistically significant differences emerged between baseline and follow-up assessment. However, if we considered the mean scores, we observed a slight increase in Switching total mean score of the Inhibition test (NEPSY-II) over time (mean score at baseline below average=5.81; SD= 4.35; mean score at follow up in the borderline range=7.34; SD= 2.86).

At TOL test, "total correct answers" mean score increased (mean score at baseline=38.22; SD= 18.70; mean score at follow up=49.84; SD= 14.43), as well as "time decision" (mean score at baseline=42.47; SD= 6.22; mean score at follow up=51.28; SD= 10.16), "time execution" (mean score at baseline=42.66; SD= 10.50; mean score at follow up=50.78; SD= 16.12), and "time total" (mean score at baseline=41.94; SD= 9.53; mean score at follow up=51.03; SD= 16.02); "violation of rules" mean score decreased (mean score at baseline=79.10; SD= 21.89; mean score at follow up=74.84; SD= 21.41) (Table 2 and Figure 2.A/B).

The improvement of scores in "time decision", "time execution" and "time total" subtests must be considered related to a reduction in impulsivity for DMD subjects.

Figure 2.A/B. A: Inhibition Test (NEPSY-II) profile in DMD cohort at baseline and after 3 years follow-up. B: TOL Test profile in DMD cohort at baseline and after 3 years follow-up



A. Den Tot: Denomination total; Inhib tot: inhibition total; Swit tot: Switching total (NEPSY II).

B. t_totcorr: total corrected; t_moves: number of moves; t_rules: violation of rule; t_t.dec: time decision; t_t.esec: time execution; t_t.tot: time total (TOL).

The youngest patients tended to improve their performances in specific tasks at follow up reassessment: a statistically significant correlation between age at baseline and the point spreads considering baseline and follow up scores emerged in the Switching total of the

Inhibition test (NEPSY-II) (p=0.001; r=0.61) and in the “total correct answers” of TOL test (p=0.015; r=0.42).

Patients with an impairment of scores at baseline tended to improve their performances at follow up reassessment. An inversely proportional correlation between scores at baseline and the point spreads considering baseline and follow up scores emerged in all the neuropsychological tests (p <0.001). This trend was particularly evident for switching subtest from Inhibition Test (NEPSY-II) and for some subtests of TOL test. In fact 17 subjects in 20 (85%) with an impairment of scores at baseline increased their score in Switching total from Inhibition Test (NEPSY-II) at follow up reassessment. At TOL test, all the patients with an impairment of score at baseline improved their performance in “total correct answers”, “time decision”, “time execution”, and “time total”.

The improvement of scores in these latter subtests must be considered related to a reduction in impulsivity for DMD subjects.

A.2.3 Comparison between groups according to site of mutation and FIQ

At the follow up assessment in the sample of 33 DMD boys 25 subjects (76%) have a FIQ≥85 and 8 (24%) have a FIQ<85.

According to site of mutation subdivision, Group 1 included 14 subjects (42.4%), Group 2, 12 patients (36.4%), and Group 3, 7 (21.2%).

In order to compare the subgroups by site of mutation and FIQ, we considered all the 66 evaluations in the analysis as there are no differences between the baseline and the follow-up scores and to increase the statistical power.

The mean scores and SDs of WISC-IV and executive functions and language subtests in different subgroups according to the site of mutation and FIQ are reported in Table 3.

No significant differences emerged among the two subgroups subdivided on FIQ regarding executive functions and language subtests.

Instead, significant differences emerged among the three groups subdivided on site of mutation in WMI (p=0.021), Digit Span (p=0.042), and Coding (p=0.035) of WISC-IV where lower scores were observed in Group 3 and for Switching Total (p=0.018) of the Inhibition test (NEPSY-II) where higher scores were observed in Group 1. No other significant differences emerged among groups subdivided on site of mutation.

Neither FIQ at baseline or site of mutation had a correlation with the point spreads between baseline and follow up scores in all the performed subtests.

Table 3. WISC-IV and Executive functions and language assessments in DMD cohort subdivided into groups according to site of mutation and FIQ

	Site of mutation			**Intellectual quotient**	
	Group 1 *28 assessments Mean (SD)*	***Group 2*** *24 assessments Mean (SD)*	***Group 3*** *14 assessments Mean (SD)*	**FIQ <85** *16 assessments Mean (SD)*	**FIQ≥85** *50 assessments Mean (SD)*
WISC-IV Index					
Verbal Comprehension Index	102.93 (16.023)	96.33 (14.25)	95.57 (7.62)	85.64 (8.33)	101.64 (13.66)
Perceptual Reasoning Index	106.21 (12.79)	99.87 (13.12)	101.36(15.27)	91.82 (6.95)	105.09 (13.52)
Working Memory Index	83.29 (16.32)*	89.46 (12.05)*	76.00 (12.94)*	67.27 (12.18)	87.33 (13.03)
Processing Speed Index	98.14 (12.64)	94.54 (12.45)	91.36 (12.57)	87.36 (12.05)	97.00 (12.04)
Full Intellectual Quotient	98.46 (12.66	94.13 (10.25)	89.86 (10.04)	78.82 (4.53)	98.31 (10.37)
WISC-IV Subtest					
Similarities	10.25 (2.76)	9.71 (3.00)	9.00 (2.80)	7.55 (2.50)	10.24 (2.72)
Vocabulary	10.64 (3.21)	9.12 (3.22)	9.79 (1.85)	7.27 (2.41)	10.44 (2.86)
Comprehension	10.57 (3.18)	9.00 (2.64)	9.00 (2.04)	8.00 (1.73)	10.00 (2.92)
Block Design	10.32 (3.13)	10.29 (3.35)	10.21 (2.69)	8.64 (2.46)	10.62 (3.11)
Picture Concepts	11.71 (2.27)	10.17 (3.14)	11.57 (2.74)	9.09 (2.11)	11.53 (2.72)
Matrix Reasoning	10.96 (2.63)	10.08 (2.75)	9.00 (3.37)	8.64 (2.46)	10.55 (2.83)
Digit Span	6.86 (2.37)*	8.83 (2.29)*	6.07 (2.70)*	5.45 (3.01)	7.80 (2.39)
Letter-Number sequencing	7.61 (3.69)	7.63 (3.09)	5.93 (2.67)	3.64 (2.69)	7.98 (2.94)
Coding	9.39 (2.35)*	8.54 (3.05)*	7.00 (2.11)*	6.91 (2.98)	8.91 (2.54)

Symbol Research	9.25 (2.86)	9.42 (2.54)	10.07 (2.99)	8.82 (2.09)	10.00 (2.69)
Inhibition Test (NEPSY-II) (standard score)					
Denomination Total	7.56 (2.34)	6.45 (2.11)	6.11 (1.34)	6.34 (2.46)	8.11 (3.04)
Inhibition Total	9.25 (2.86)	7.92 (2.96)	8.43 (2.62)	7.18 (2.89)	8.87 (2.81)
Switching Total	8.00 (3.87)*	5.48 (3.69)*	5.50 (2.38)*	6.45 (3.27)	6.59 (2.81)
Graphic Fluency Test NEPSY II (standard score)					
Graphic Fluency	9.11 (2.53)	8.83 (3.59)	8.57 (2.38)	7.64 (2.16)	9.15 (2.98)
TOL Test (T score)					
Total corrected	48.19 (17.97)	38.17 (17.28)	44.71 (15.84)	36.60 (22.68)	45.04 (16.43)
Violation of rules	70.00 (21.67)	84.58 (10.21)	76.21 (23.33)	80.20 (23.83)	76.20 (21.60)
Time decision	47.44 (11.90)	46.67 (8.31)	46.71 (5.72)	45.20 (7.63)	47.33 (9.76)
Time execution	45.96 (11.34)	49.75 (15.28)	46.21 (16.46)	45.10 (11.39)	47.84 (14.41)
Time total	45.15 (12.50)	48.08 (14.40)	47.14 (15.91)	44.90 (12.32)	46.98 (14.17)
MCST Test (z score)					
Categories	-0.33 (1.61)	-0.31 (1.27)	-0.59 (1.07)	-0.74 (0.76)	-0.32 (1.18)
Correct answers	-0.50 (1.16)	-0.37 (1.13)	-0.32 (1.49)	-0.75 (0.89)	-0.31 (1.26)
Categorial efficiency	-0.51 (1.23)	-0.84 (1.41)	-1.12 (0.95)	-1.55 (0.37)	-0.63 (1.30)
Total errors	0.35 (1.11)	0.12 (0.99)	0.53 (1.11)	0.42 (0.73)	0.29 (1.09)
Perseveration errors	0.35 (1.37)	0.08 (0.93)	0.04 (0.99)	0.13 (0.93)	0.19 (1.17)
Non perseveration errors	0.38 (0.97)	0.28 (4.22)	0.78 (1.05)	0.31 (0.85)	0.46 (1.18)
Failure	2.54 (4.91)	2.88 (4.22)	2.16 (3.54)	3.63 (3.76)	2.38 (4.41)
Verbal Fluency Test BVN (z score)					
Semantic	-0.50 (0.94)	-0.70 (1.03)	-0.84 (1.02)	-1.23 (0.91)	-0.55 (0.97)
Phonological	-0.47 (0.82)	-0.92 (0.77)	-0.88 (0.78)	-0.43 (1.13)	-0.76 (0.76)

* = statistically significant differences

The results about the follow up data on the 33 DMD subjects are described in an ongoing paper (NMD 2020, submitted).

A.3 Commentary

The follow up neuropsychological data show difficulties in the manipulation of stored information, in the ability to inhibit automatic responses and switch the tasks, in abstract reasoning and planning capacity for DMD subjects without intellectual disability, after a re-assessment 3 years apart from the first evaluation. In fact, despite a slight maturation trend over time in some tasks assessing executive functions, particularly evident for subjects who show a score impairment at baseline and for the youngest boys, specific difficulties persist, confirming a characteristic neuropsychological functioning in DMD boys without intellectual disability.

These findings are consistent with our previous results (Battini et al., 2018) and support the hypothesis of a fronto-striatal-cerebellar network involvement in DMD.

With respect to our previous study (Battini et al., 2018), this research deepen the understanding of the role of dystrophin in the brain. A more detailed description of subgroups of subjects according to the real expression of Dp140, let us to reveal possible correlations between genotype and neuropsychological phenotype, and a more general neuropsychological impairment emerged in DMD boys without Dp140 expression (NMD 2020, submitted).

1.4 Section B: Total cohort study

B.1 Subjects and Methods

B.1.1 Subjects

Seventeen new DMD patients (mean age: 8 years and 7 months) have been recruited according to the inclusion criteria already used in the follow up study. Therefore, a total cohort of 57 subjects has been analyzed.

As already described for the follow up study, the DMD children have been subdivided into two groups according to their FIQ (FIQ<85 and FIQ≥85) and into three groups according to site of mutation (Group 1: mutations located upstream from or in exon 44; Group 2: mutations located in or extending to the region corresponding to exons 45-50; Group 3: mutations located in or downstream exon 51) (see section A).

All parents' patients included in the study signed a written informed consent.

B.1.2 Neuropsychological protocol

The new enrolled DMD children performed the same neuropsychological assessment used for the follow up study, assessing intellectual functions (WISC-IV) and executive and language functions: the Inhibition subtest and the Design Fluency have been chosen from the NEPSY-II (Brooks et al., 2010; Urgesi et al., 2011); TOL (Sannio Fancello et al., 2006); MCST (Sannio Fancello et al., 2003), Verbal Fluency-Word Generation from BVN 5-11 (Bisiacchi et al., 2005) phonological and semantic criteria (see Section A).

All the subjects were assessed individually and each assessment took approximately 4 hours.

B.1.3 Data and statistical analysis

For the analysis of neuropsychological data see Section A.

In consideration of the sample size, we decided to use non parametric tests.

Mann–Whitney U-test was used to compare the distribution of variables among the two groups in which the sample was subdivided according to the IQ and Kruskal Wallis test was used to compare the distribution of variables among the three groups according to the site of mutation of *DMD* gene. Version 23 of the SPSS software (SPSS, Inc.) was used for all statistical analyses, setting the significance at $p < 0.05$.

B.2 Results

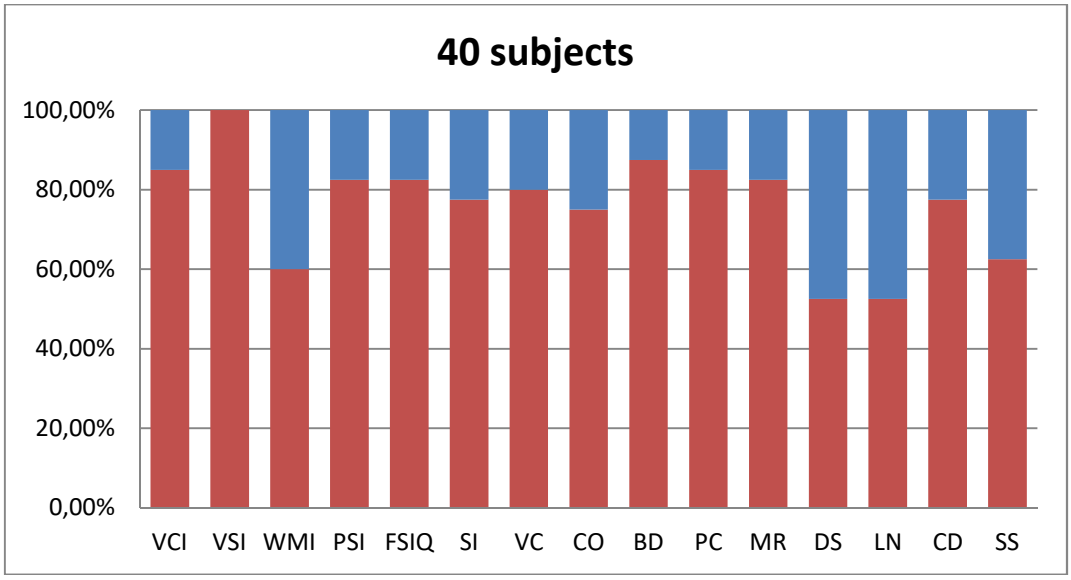
B.2.1 Intellectual functioning

We found similar trends in the cohort of 57 DMD patients compared to the previous sample of 40 DMD boys.

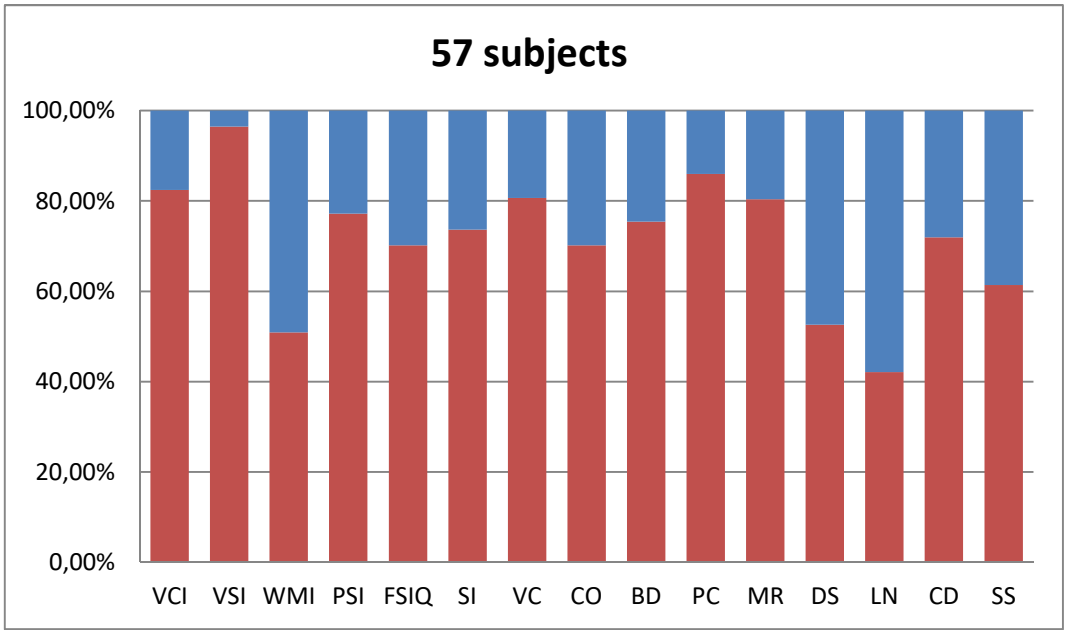
In the total cohort, we confirmed the worst performance in WMI (mean score=85.58; SD=16.81) and in the related subtests (Digit Span - mean score=7.66; SD=2.81 – and Letter-Number Sequencing – mean score= 7.46; SD=3.6).

Intellectual functioning profile in the two groups (40 DMD subjects/57 DMD subjects) was reported in Figure 3.A/B.

Figure 3.A/B WISC-IV profile in DMD cohort (40 subjects/57 subjects)



A. Previous cohort of 40 DMD children (modified from Battini et al., 2018)



B. Total cohort of 57 DMD children

Red bars represent the percentage of normal values standardized as 100%, blue bars represent pathological values.
VCI: verbal comprehension index; VSI: visual spatial index;WMI: working memory index; PSI: processing speed index; FSIQ: Full scale Intelligence Quotient; SI: similarities; VC: vocabulary; CO: comprehension; BD: block design; PC: picture concepts; MR: matrix reasoning; DS: digit span; LN: letter-number sequencing; CD: coding digit; SS: symbol search.

B.2.2 Executive functions and language

We found similar trends in the cohort of 57 DMD patients compared to the previous sample of 40 DMD boys.

Relating to Executive Functions, the overall sample of 57 DMD subjects confirmed the worst performances in the Switching task of the Inhibition test (NEPSY II) compared to the Denomination and the Inhibition tasks (mean score Switching Total=6.13; SD=3.8; mean score Denomination Total=7.77; SD=2.58; mean score Inhibition Total=8.5; SD=3.13).

In TOL worst performances were confirmed especially in “number of moves” (mean scores=76.80; SD=16.66) and “violation of rules” (mean score=78.64; SD=21.48).

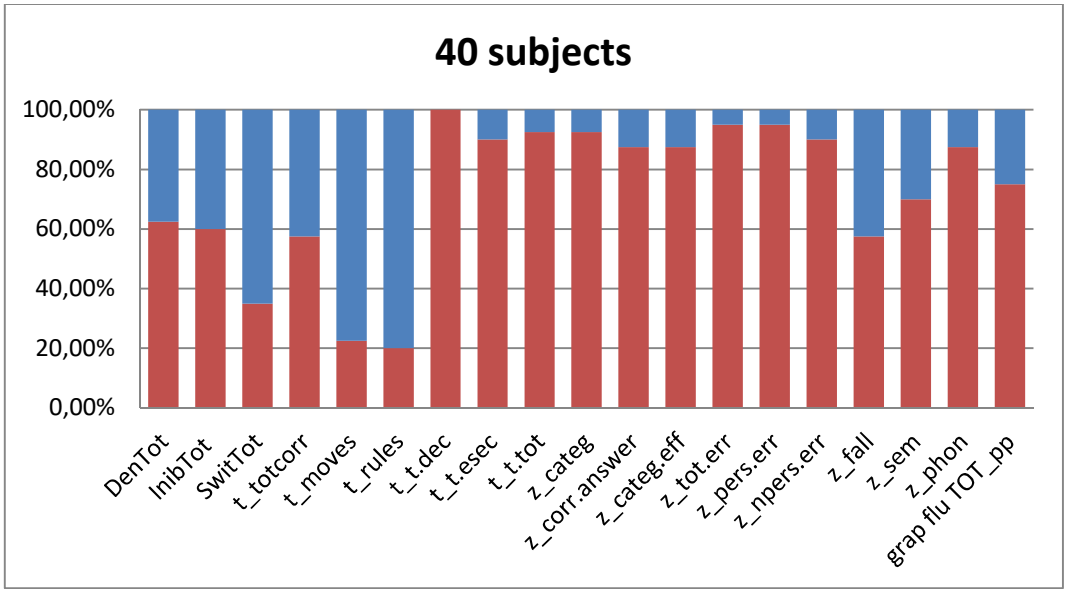
In the MCST, the worst performance was again in “failure” (mean score=2.59; SD=4.31).

We confirmed good performances in Graphic Fluency Test (NEPSY II) (mean score=8.98; SD=3.14).

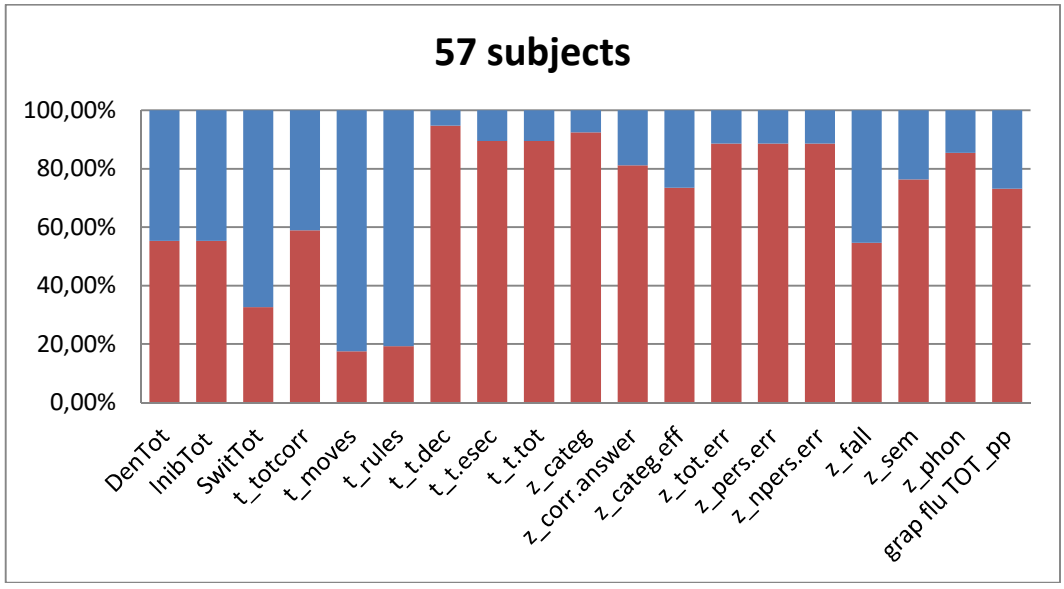
Concerning language abilities, the scores of global group of 57 DMD subjects ranged between -2.7 and +1.87 z score in Semantic fluency test and between -2.33 and +3.84 z score in Phonetic fluency test, with an impairment in 24.6% and 14.5% of patients, respectively.

Executive functions and language profile in the two groups (40 DMD subjects/57 DMD subjects) was reported in Figure 4.A/B.

Figure 4.A/B Executive functions and language profile in DMD cohort (40 subjects/57 subjects)



A. Previous cohort of 40 DMD children (modified from Battini et al., 2018)



B. Total cohort of 57 DMD children

Red bars represent the percentage of normal values standardized as 100%, blue bars represent the percentage of pathological values.
Den Tot: Denomination; Inhib tot: inhibition total; Swit tot: switching total (NEPSY II). t_totcorr: total corrected; t-moves: number of moves; t_rules: violation of rule; t_t.dec: time decision; t_t.esec: time esecution; t_t.tot: time total (TOL). z_categ: categories; z_corr.answer: correct answers; z_categ.eff: categorial efficiency; z_tot.err: total error; z_persev.err: perseveration errors; z_npers.err: non perseveration errors; z_fail: failure (MCST). Grap flu TOT_pp: graphic fluency (Nepsy-II). Phonological (phon) and semantic (sem) fluency has been also described.

B.2.3 Comparison between groups according to site of mutation and FIQ

As the analysis did not reveal any differences between the follow-up and the baseline, all the evaluations were taken into account for the statystical analyses. Therefore, 90 assessments were included in the statistical analysis: the 33 evaluations at baseline, the 33 evaluations at follow-up, the 7 evaluations at baseline of patients lost at follow-up and the new 17 evaluations.

With regard to site of mutation, 40 evaluations (44.4%) derived from patients of Group 1, 30 (33.4%) from patients of Group 2, and 20 (22.2%) from patients of Group 3.

In 22 evaluations (24%) FIQ was <85, and in 68 evaluations (76%) FIQ was $\geq$85.

Regarding intellectual functioning, when we divided the subgroups according to site of mutation, the best performances were observed in Group 1 and the worst performances in Group 3 in most cases. Statistically significant differences were found in WMI (p=0.044) and its related subtest Digit Span (p=0.018), where the worst performances were observed in Group 3.

Concerning executive functions and language assessments, the best performances were observed in Group 1 and the worst performances in Group 3 in most cases. Statistically significant differences were found in Switching total of the Inhibition subtest (NEPSY-II) (p=0.037) and in Violation of rules at TOL test (p=0.047), where the best performances were observed in Group 1.

When we subdivided the whole cohort in the two subgroups according to FIQ, the best performances were observed for FIQ$\geq$85 evaluations and the worst performances for FIQ<85 in most cases. Statistically significant differences were found in “Correct answers” of MCST Test (p= 0.023) and “Graphic Fluency Total” of NEPSY II (p=0.049), where the best performances were observed for FIQ$\geq$85 evaluations.

Table 4 shows details of the mean scores and SDs of WISC-IV, executive functions and language subtests in different subgroups according to site of mutation and FIQ.

Table 4. WISC-IV and Executive functions and language assessments in DMD cohort subdivided into groups according to site of mutation and FIQ

	Site of mutation			Intellectual quotient	
	Group 1 40 assessments *Mean (SD)*	***Group 2*** 30 assessments *Mean (SD)*	***Group 3*** 20 assessments *Mean (SD)*	**FIQ <85** 22 assessments *Mean (SD)*	**FIQ≥85** 68 assessments *Mean (SD)*
WISC-IV Index					
Verbal Comprehension Index	99.95 (16.40)	97.57 (14.66)	94.70 (9.61)	84.64 (7.29)	102.31(13.68)
Perceptual Reasoning Index	103.55 (15.43)	99.70 (14.02)	99.15 (16.48)	89.82 (9.02)	105 (13.54)
Working Memory Index	82.68 (15.43)*	89.97 (13.74)*	81.55 (15.79)*	72.05 (12.12)	89 (13.83)
Processing Speed Index	95.73 (13.71)	92.97 (12.63)	91.55 (10.70)	87.95 (11.89)	95.79 (12.47)
Full Intellectual Quotient	95.38 (14.71)	93.73 (11.77)	90.25(10.60)	79.05 (3.91)	98.43 (11.18)
WISC-IV Subtest					
Similarities	10.03 (2.82)	9.90 (3.20)	9.30 (2.83)	7.73 (2.12)	10.50 (2.85)
Vocabulary	10.28 (3.00)	9.33 (3.17)	9.65 (1.63)	7.68 (1.94)	10.51 (2.72)
Comprehension	9.68 (3.63)	9.20 (2.62)	8.40 (2.93)	6.91 (2.72)	9.99 (2.95)
Block Design	9.90 (2.97)	10.37 (3.39)	9.50 (3.19)	7.86 (2.40)	10.65 (3.07)
Picture Concepts	11.38 (2.19)	10.37 (3.17)	11.10 (2.97)	9.05 (2.28)	11.60 (2.58)
Matrix Reasoning	10.48 (2.59)	9.97 (2.95)	9.15 (3.35)	8.50 (2.43)	10.50 (2.89)
Digit Span	7.13 (2.28)*	8.73 (2.33)*	7.05 (3.02)*	6.50 (2.79)	8.01 (2.40)
Letter-Number sequencing	7.10 (3.61)	7.93 (3.14)	6.80 (3.07)	4.29 (2.35)	8.24 (3.04)
Coding	9.15 (2.54)	8.43 (3.05)	7.70 (2.54)	8.23 (3.25)	8.71 (2.58)
Symbol Research	9.45 (3.02)	8.87 (2.50)	9.45 (2.96)	7.77 (2.72)	9.74 (2.71)
Inhibition Test (NEPSY-II) (standard score)					
Denomination Total	8.91 (2.56)	7.66 (2.57)	8.76 (2.49)	7.57 (2.87)	8.59 (2.82)
Inhibition Total	8.93 (3.00)	7.55 (2.86)	8.35 (2.35)	7.32 (2.12)	8.38 (3.07)
Switching Total	7.76 (3.65)*	5.50 (3.43)*	5.95 (2.57)*	6.84 (3.06)	6.52 (3.62)
Graphic Fluency Test NEPSY II (standard score)					
Graphic Fluency	9.18 (2.97)	8.77 (3.30)	8.63 (2.11)	7.95 (2.54)*	9.24 (2.97)*
TOL Test (T score)					
Total corrected	47.50 (16.78)	40.63 (16.80)	44.70(13.62)	40.33 (18.02)	45.84 (15.54)
Number of moves	75.05 (19.42)	80.57 (14.95)	78.95(13.15)	81.43 (12.72)	76.69 (17.69)

Violation of rules	71.08 (20.88)*	83.47 (20.04)*	79.17(22.39)*	75.53 (21.57)	77.74 (21.50)
Time decision	47.87 (13.24)	46.50 (7.82)	47.45 (8.75)	47.71 (9.54)	47.19 (10.99)
Time execution	46.49 (11.48)	48.50 (13.99)	45.50(13.79)	47.43 (11.71)	46.79 (13.20)
Time total	46.36 (12.88)	47.23 (13.09)	46.75(14.05)	47.95 (11.76)	46.37 (13.51)
MCST Test (z score)					
Categories	-0.50 (1.01)	-0.27 (1.30)	-0.32 (1.14)	-0.59 (1.12)	-0.32 (1.14)
Correct answers	-0.59 (1.11)	-0.35 (1.25)	-0.48 (1.41)	-0.91 (1.09)*	-0.24 (1.24)*
Categorial efficiency	-0.84 (1.13)	-0.97 (1.33)	-1.04 (1.06)	-1.38 (0.68)	-0.79 (1.25)
Total errors	0.47 (1.06)	0.27 (1.09)	0.29 (1.02)	0.73 (0.95)	0.26 (1.06)
Perseveration errors	0.55 (1.44)	0.35 (1.31)	-0.49 (0.91)	0.78 (1.43)	0.22 (1.25)
Non perseveration errors	0.37 (0.89)	0.25 (1.31)	0.50 (1.09)	0.30 (0.90)	0.37 (1.13)
Failure	2.56 (4.24)	3.14 (4.45)	1.75 (3.29)	2.58 (3.38)	2.55 (4.30)
Verbal Fluency Test BVN (z score)					
Semantic	-0.56 (1.06)	-0.69 (1.03)	-0.69 (0.97)	-0.99 (1.02)	-0.54 (1.00)
Phonological	-0.49 (1.12)	-0.91 (0.73)	-0.95 (0.95)	-0.42 (1.55)	-0.82 (0.76)

* = statistically significant differences

B.3 Commentary

The results confirm a characteristic neuropsychological profile in a wider cohort of DMD patients without intellectual disability than previously studied (Battini et al., 2018). In fact, we found similar trends in the cohort of 57 DMD patients compared to the previous sample of 40 DMD boys, both for intellectual functioning and for executive functions and language assessment.

The comparison between groups according to site mutations confirm possible genotype-neuropsychological phenotype correlations also in this wider cohort, with a major neuropsychological impairment in DMD boys without Dp140 expression.

Moreover, the enlargement of the sample let us to reveal, as expected, a possible effect of the intellectual functioning on the performances at neuropsychological tasks; in fact, DMD boys with a better FIQ tend to obtain better performances also in other neuropsychological tests.

1.5 Section C: BRIEF-2 study

C.1 Subjects and Methods

C.1.1 Subjects

All the 17 new enrolled children' parents and 30 children's parents of the follow up study were involved in this study. A total of 47 children' parents were included. As already described for the previous studies, the DMD children involved were subdivided into two groups according to their FIQ (FIQ<85 and FIQ≥85) and into three groups according to site of mutation (Group 1: mutations located upstream from or in exon 44; Group 2: mutations located in or extending to the region corresponding to exons 45-50; Group 3: mutations located in or downstream exon 51) (see Section A).

With regard to FIQ, 15 boys showed a FIQ<85 and 32 a FIQ≥85.Concerning site of mutation, 23 DMD children belonged to Group 1, 13 to Group 2 and 11 to Group 3.

C.1.2 BRIEF-2 questionnaire

The Behavior Rating Inventory of Executive Functions, Second Edition (BRIEF-2) - Parent Form (Marano et al., 2016) was administered in order to assess everyday behavior and executive functioning in home environment. It is composed by 63 items and includes 9 subscales (Inhibit, Self-Monitor, Shift, Emotional Control, Initiate, Working Memory, Plan/Organise, Task Monitor, and Organisation of Materials). The subscales form three broad indexes (Behavioural, Emotion and Cognitive Regulation Index) and a Global Executive Composite Index. Higher BRIEF scores represent poorer executive functions.

C.1.3 Data and statistical analysis

T scores were used to interpret the child's level of executive functioning as reported by parents (mean = 50, SD = 10; pathological values >65).

In consideration of the sample size, we decided to use non parametric tests.

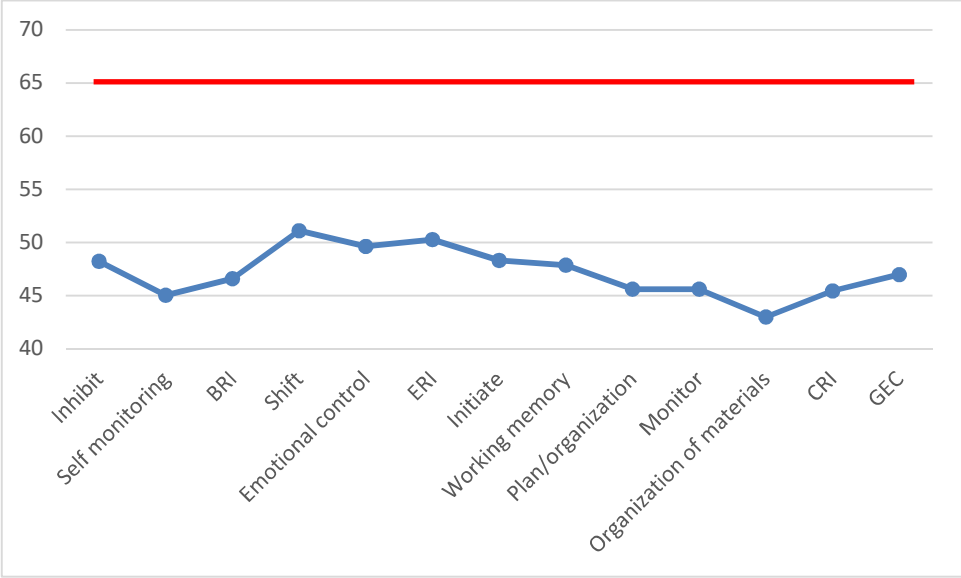
Mann–Whitney U-test was used to compare the distribution of variables among the two groups in which the sample was subdivided according to the FIQ and Kruskal Wallis test was used to compare the distribution of variables among the three groups according to the site of mutation of *DMD* gene. Version 23 of the SPSS software (SPSS, Inc.) was used for all statistical analyses, setting the significance at $p < 0.05$.

C.2 Results

The mean scores were in the normal range in all the subtests, but the worst mean performance was observed in "Shift" (mean score=51.11; SD=12.97) and the best one in "Organization of Materials" (mean score=43; SD=7.18) (Figure 5).

The highest percentage of T-score over 65 was in "Shift" (24.32%), followed by "ERI" (13.51%), and "Emotional control", "Initiate", and "Working Memory" (10.8 % for all the three indexes).

Figure 5. BRIEF-2 mean scores



When we subdivided the sample according to site of mutation, we found statistically significant difference in "Working Memory" (p=0.033), where the highest T score was observed in Group 3.

No statistically significant differences were found between groups subdivided on FIQ. The mean scores obtained at BRIEF-2 in groups subdivided according to site of mutation and FIQ are shown in Table 5.

Table 5. BRIEF-2 mean scores in subgroups according to site of mutation and FIQ

	Site of mutation			**Intellectual quotient**	
	Group 1 23 subjects *Mean (SD)*	***Group 2*** 13 subjects *Mean (SD)*	***Group 3*** 11 subjects *Mean (SD)*	**FIQ <85** 15 subjects *Mean (SD)*	**FIQ≥85** 32 subjects *Mean (SD)*
Inhibit	47.78 (10.56)	46.08 (4.15)	51.73 (8.19)	46.20 (5.67)	49.19 (9.82)
Self monitoring	42.83 (9.94)	46.15 (5.54)	48.36 (9.92)	46.47 (7.36)	44.37 (9.79)
BRI	45.26 (9.64)	45.85 (4.08)	50.27 (8.74)	46 (5.25)	46.88 (9.52)
Shift	51.04 (13.31)	46 (9.59)	57.27(14.07)	50.87 (11.40)	51.22 (13.82)
Emotional control	49.83 (10.40)	48.08 (8.50)	51.09 (9.81)	49.60 (8.71)	49.66 (10.16)
ERI	50.39 (11.91)	46.69 (9.15)	54.27(11.88)	50.33 (10.38)	50.25 (11.88)
Initiate	48.13 (9.92)	46.46 (9.13)	50.91(10.55)	46.87 (8.42)	49 (10.41)
Working memory	47.57 (11.36)*	43 (6.23)*	54.27(10.73)*	47.80 (8.18)	47.91 (11.75)
Plan/organization	44.35 (10.85)	44.77 (8.28)	49.27(11.98)	47 (10.37)	44.97 (10.62)
Organization of materials	43.35 (8.55)	41.15 (2.94)	44.45 (7.70)	43.27 (4.88)	42.88 (8.11)
Monitor	45.87 (10.99)	43.08 (5.45)	48.08 (7.20)	44.53 (7.43)	46.13 (9.64)
CRI	45.04 (10.81)	42.85 (6.31)	49.36 (9.22)	45.40 (7.82)	45.47 (10.32)
GEC	46.65 (11.45)	44 (6.82)	51.18 (9.95)	46.27 (7.59)	47.31 (11.25)

C.3 Commentary

Despite the assessment of DMD children without intellectual disability may show possible neuropsychological weaknesses, the results obtained by the BRIEF-2 questionnaire

demonstrate that the parents focus little attention on cognitive and neuropsychological abilities of their children.

As a good cognitive level may mask possible underlying executive dysfunctions, DMD boys without intellectual disability are a particularly vulnerable population. In fact, not detected and recognized neuropsychological impairments may have anyway a negative impact on the global functioning of these children.

2. Genetic study

2.1 Introduction

As known, the site of mutation in *DMD* gene may have an impact on the cognitive and neuropsychological functioning in DMD boys. Also our neuropsychological results confirm that a more evident neuropsychological impairment is observed in DMD children without Dp140 expression.

The role of common polymorphisms in genes remote from the *DMD* gene that may act as genetic modifiers and modulate the disease severity (Zatz et al., 2014; Hindi et al., 2014; Barp et al., 2015; Bello et al., 2015; Bello et al., 2016b; Quattrocelli et al., 2017; Spitali et al., 2020) has been widely studied to explain the variability in clinical phenotype severity that is commonly observed in DMD, in term of age at loss of independent ambulation, age of onset and rate of progression of respiratory insufficiency and dilated cardiomyopathy.

To our knowledge, the role of these genetic modifiers as possible influential factor for neuropsychological profile has never been deepened in DMD population.

However, in addition to being commonly involved in response to tissue damage, in tissue repair and regeneration, and in regulation of the inflammatory response (Bello et al., 2019), some of these genes and their protein products can also be expressed in the brain, with a possible role in pathogenesis of neurodegenerative diseases, in neuroinflammation or in neuroprotection (Yu et al., 2017; Dobolyi & Palkovits, 2008; Togo et al., 2000; Kempuraj et al., 2016; Carriba et al., 2017).

These data suggest the possibility to identify modifiers which could influence also the neuropsychological profile in DMD.

2.2 Aims

In addiction to role of the site of mutation in *DMD* gene, in this ancillary research to the neuropsychological study, we wanted to analyse polymorphisms in genes which have been already reported as modifiers for DMD in a subgroup of DMD children already involved in the neuropsychological study in order to identify possible biological biomarkers of their deficits.

2.3 Subjects and Methods

2.3.1 Subjects

Blood samples from 20 of the 57 children involved in the neuropsychological study have been collected and were analysed at the Laboratory of Neuromuscular Centre in Padova.

The mean age of the sample at baseline evaluation was 8.08 years and the mean FIQ was 94.9.

2.3.2 Genetic testing

We applied a candidate gene association strategy to identify possible genetic modifiers and we concentrated on selected variants in genes which have been already reported as modifiers for DMD. Genotypes at the SNPs rs28357094 (T/G nucleotide substitution at position -66 in the promoter region of the *SPP1* gene), rs2303729 (*LTBP4* V194I), rs1131620 (*LTBP4* T787A), rs10880 (*LTBP4* T1140M), and rs1883832 (T/C nucleotide substitution adjacent to where translation starts in the 5' UTR of *CD40* gene) were determined by Applied BioSytems TaqMan SNP genotyping assays and end-point allelic discrimination on an ABI-7000 SDS instrument.

For *SPP1* rs28357094, a dominant model for the minor allele G was adopted (Pegoraro et al., 2011). *LTBP4* haplotype was reconstructed based on rs2303729, rs1131620, and rs10880 genotypes. Genotype at the fourth SNP rs1051303 (T820A) was imputed from rs1131620 genotype, assuming no recombination events due to very strong linkage disequilibrium (LD).

Patients were assigned to genotype groups according to recessive inheritance models for rs10880 and were subdivided into VTTT homozygotes, IAAM homozygotes, and "other" for other haplotype configurations (e.g. VTTT/IAAM heterozygotes, other rare haplotypes) (Flanigan et al., 2013; Barp et al., 2015).

Regarding *CD40* rs1883832, an additive model for the minor allele T was applied (Bello et al., 2016b).

Dystrophin gene mutations were classified based on the predicted effect on Dp140 expression: "Dp140-" for those in which the Dp140 promoter or translation start ATG was lost, or there were frameshift/nonsense mutations within the Dp140 ORF; "Dp140+" for those with an intact promoter and ORF for Dp140; and "dubious" for mutations situated in between the Dp140 promoter (intron 44) and translation start codon (exon 51), for which the actual effect on Dp140 expression is hard to predict.

2.3.3 Data and statistical analysis

Distributions of test scores were summarised as mean ± SD. Score differences by genotype group were tested by ANOVA, with scores as dependent variables and concurrent effects of genotypes as independent variables. In the ANOVA models, the effect of DMD mutations on Dp140 expression was modelled quantitatively as follow: Dp140- = 0; Dp140 "dubious" = 0.5; and Dp140+ = 1. Statistical tests were performed with R v. 3.5.3. Statistical significance was set at $p < 0.05$.

2.4 Results

2.4.1 *SPP1* rs28357094 genotyping

Regarding *SPP1* rs28357094 genotyping, in our cohort there were 15 homozygotes for the T allele (75%), 5 T/G heterozygotes (25%) and no homozygotes for the G allele. This distribution was close to expected minor allele frequency (MAF) in a European population

(1000 Genomes Project Consortium et al., 2012) and consistent with Hardy-Weinberg equilibrium (HWE).

The mean scores and SDs of WISC-IV and Executive functions and language subtests at baseline evaluation in DMD cohort subdivided on *SPP1* rs28357094 genotyping are reported in Table 6.

Table 6. DMD cohort subdivided on *SPP1* rs28357094 genotyping

	SPP1 rs28357094 genotyping	
	TG 15 subjects *Mean (SD)*	*TT* 5 subjects *Mean (SD)*
WISC-IV Index		
Verbal Comprehension Index	97.2 (11.19)	99.6 (17.14)
Perceptual Reasoning Index	101.4 (18.15)	105.33 (15.85)
Working Memory Index	91.6 (15.21)	85.4 (16.50)
Processing Speed Index	88.8 (12.01)	90 (10.74)
Full Intellectual Quotient	94 (9.75)	95.2 (17.71)
WISC-IV Subtest		
Similarities	9 (1.87)	9.93 (3.73)
Vocabulary	9.4 (1.95)	10 (3.30)
Comprehension	10.2 (3.20)	9.87 (2.90)
Block Design	9 (4.19)	10.13 (3.11)
Picture Concepts	13.2 (1.48)	11.6 (2.75)
Matrix Reasoning	8.6 (4.04)	10.87 (3.23)
Digit Span	7.2 (3.35)	7.13 (2.95)
Letter-Number sequencing	10 (2)	8 (3.30)
Coding	8.4 (2.88)	8.33 (2.82)
Symbol Research	7.8 (1.92)	8.33 (2.66)
Inhibition Test (NEPSY-II) (standard score)		
Denomination Total	9 (1.87)	7.73 (2.49)
Inhibition Total	8.4 (1.95)	9.6 (3.50)
Switching Total	3.8 (3.83)	7.33 (4.08)
Graphic Fluency Test NEPSY II (standard score)		
Graphic Fluency	7.6 (3.91)	8.87 (2.95)
TOL Test (T score)		
Total corrected	48.6 (14.19)	43.4 (15.06)
Number of moves	61.2 (17.96)*	79.73 (16.38)*
Violation of rules	76.6 (23.02)	77.47 (25.53)
Time decision	41.2 (3.96)	46.27 (6)
Time esecution	43 (5.43)	49.67 (12.38)
Time total	40.4 (2.41)*	49 (11.06)*
MCST Test (z score)		
Categories	-0.38 (1.27)	-0.21 (1.26)
Correct answers	-0.05 (1.66)	-0.44 (1.14)
Categorial efficiency	-1.06 (1.04)	-0.70 (0.78)

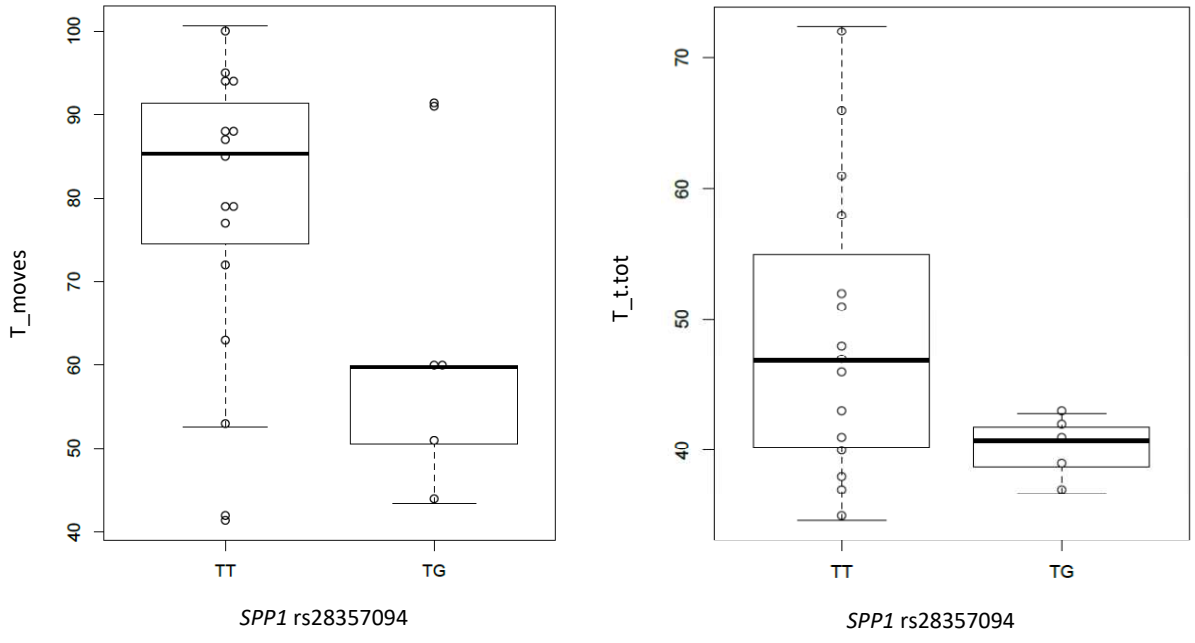
Total errors	-0.05 (1.32)	0.50 (0.95)
Perseveration errors	0.51 (2.42)	0.20 (1.05)
Non perseveration errors	-0.04 (0.71)	0.63 (1.90)
Failure	-0.18 (0.40)	0.58 (2.27)
Verbal Fluency Test BVN (z score)		
Semantic	-0.72 (1.14)	-1.07 (1.25)
Phonological	-0.67 (0.60)	-0.85 (0.95)

* = statistically significant differences

At TOL test, T/G heterozygotes patients reported a reduced "number of moves" than T/T homozygotes with a statistically significant difference (p=0.041; η^2=0.21 - "large"). A statistically significant difference emerged also in "time total" where T/G heterozygotes took less time to complete the task (p=0.039; η^2=0.08 – "medium/large").

The significant differences in groups subdivided on *SPP1* rs28357094 genotyping are shown in Figure 6. A/B.

Figure 6.A/B. T scores in "number of moves" and "time total" at TOL test in groups subdivided on *SPP1* rs28357094 genotyping



2.4.2 *LTBP4* genotyping

With regard to *LTBP4* genotyping, 14 patients (70%) were homozygotes for the VTTT/VTTT haplotype, and 2 (10%) for the IAAM/IAAM haplotype. Other haplotype configurations (e.g. VTTT/IAAM heterozygotes, other rare haplotypes) were found in 4 patients (20%).

These findings were close to the expected distribution for a European population (1000 Genomes Project Consortium et al., 2012) and consistent with HWE.

The mean scores and SDs of WISC-IV and Executive functions and language subtests at baseline evaluation in DMD cohort subdivided on LTBP4 genotyping were reported in Table 7.

Table 7. DMD cohort subdivided on *LTBP4* genotyping.

	LTBP4 genotyping		
	VTTT/VTTT 14 subjects *Mean (SD)*	*Other* 4 subjects *Mean (SD)*	*IAAM/IAAM* 2 subjects *Mean (SD)*
WISC-IV Index			
Verbal Comprehension Index	100.86 (17.34)	98.5 (11.36)	87 (1.42)
Perceptual Reasoning Index	104.64 (16.98)	105.75 (18.30)	99.5 (9.19)
Working Memory Index	86.07 (17.48)	88.75 (15.95)	89.5 (10.61)
Processing Speed Index	91.21 (11.13)	83 (10.86)	92.5 (2.12)
Full Intellectual Quotient	96 (18.07)	93.75 (12.26)	89.5 (0.71)
WISC-IV Subtest			
Similarities	10.21 (3.75)	9 (2.16)	7.5 (0.71)
Vocabulary	10.21 (3.26)	10.25 (0.5)	6.5 (2.12)
Comprehension	10 (2.96)	10 (3.65)	9.5 (2.12)
Block Design	9.5 (3.61)	10.5 (3.32)	11 (1.41)
Picture Concepts	12.07 (2.53)	13.25 (1.71)	9 (2.83)
Matrix Reasoning	10.71 (3.40)	9 (4.55)	10 (2.83)
Digit Span	6.86 (3.03)	7.25 (2.63)	9 (4.24)
Letter-Number sequencing	8.5 (3.44)	9 (2.94)	7.5 (0.71)
Coding	8.57 (2.98)	7.25 (2.22)	9 (2.83)
Symbol Research	8.5 (2.56)	7 (1.83)	8.5 (3.54)
Inhibition Test (NEPSY-II) (standard score)			
Denomination Total	7.79 (2.58)	9 (2.16)	8 (1.41)
Inhibition Total	8.79 (3.14)	9.25 (2.87)	13 (2.83)
Switching Total	5.93 (3.40)	7.75 (5.32)	7.5 (9.19)
Graphic Fluency Test NEPSY II (standard score)			
Graphic Fluency	8.29 (2.84)	7.75 (2.87)	12 (5.66)
TOL Test (T score)			
Total corrected	41.36 (14.47)	51.75 (14.01)	54 (15.56)
Number of moves	81.36 (14.56)*	60.5 (18.84)*	60.5 (26.17)*
Violation of rules	77.5 (24.26)	78.75 (25.99)	72.5 (38.89)
Time decision	45.29 (6.29)	42.25 (4.19)	48.5 (6.36)

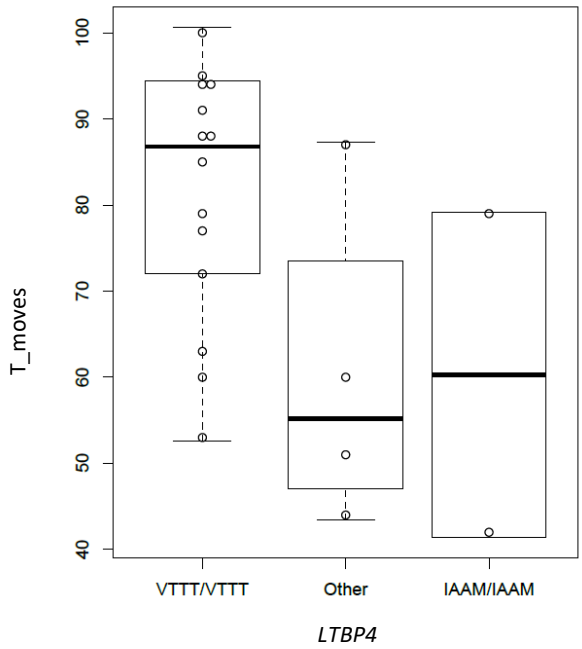
Time esecution	48.43 (13.29)	46 (5.94)	49 (2.83)
Time total	47.64 (12.01)	43 (3.74)	49 (4.24)
MCST Test (z score)			
Categories	-0.51 (1.11)	-0.16 (1.36)	1.40 (0.94)
Correct answers	-0.57 (1.12)	-0.03 (1.80)	0.64 (0.74)
Categorial efficiency	-0.98 (0.75)	-0.67 (0.86)	0.24 (0.28)
Total errors	0.44 (1.09)	0.54 (1.00)	-0.52 (0.79)
Perseveration errors	0.96 (1.18)	1.19 (2.36)	-0.26 (0.34)
Non perseveration errors	0.71 (1.08)	-0.025 (0.73)	-0.37 (0.66)
Failure	0.43 (2.16)	-0.23 (0.45)	1.38 (3.19)
Verbal Fluency Test BVN (z score)			
Semantic	-1.02 (1.21)	-0.65 (1.19)	-1.35 (1.87)
Phonological	-0.89 (0.90)	-0.58 (0.68)	-0.69 (1.39)

* = statistically significant differences

At TOL test, VTTT/VTTT homozygotes patients reported worse performances in "total correct–answers" than IAAM/IAAM homozygotes and other genotypes. In particular, an almost significant difference emerged for "number of moves" where higher scores (corresponding to a worse performance) were observed in VTTT/VTTT homozygotes ($p=0.053$; $\eta^2=0.52$ – "large").

The significant differences in groups subdivided on LTBP4 genotyping are shown in Figure 7.

Figure 7. T scores in "number of moves" at TOL test in groups subdivided on *LTBP4* genotyping



2.4.3 *CD40* rs1883832 genotyping

Concerning *CD40* rs1883832 genotyping, 11 patients (55%) were homozygotes for the C allele, 8 were C/T heterozygotes (40%) and only 1 was homozygotes for the T allele (5%).

These findings were close to the expected distribution for a European population (1000 Genomes Project Consortium et al., 2012) and consistent with HWE.

The mean scores and SDs of WISC-IV and Executive functions and language subtests at baseline evaluation in DMD cohort subdivided on CD40 genotyping were reported in Table 8.

Table 8. DMD cohort subdivided on *CD40* rs1883832 genotyping

	CD40 rs1883832 genotyping		
	CC 11 subjects *Mean (SD)*	*CT* 8 subjects *Mean (SD)*	*TT* 1 subject
WISC-IV Index			
Verbal Comprehension Index	92.73 (12.40)	109.25 (15.38)	86
Perceptual Reasoning Index	97.91 (13.28)	114 (16.41)	98
Working Memory Index	82 (14.84)	95.13 (15.71)	76
Processing Speed Index	88 (8.38)	93 (13.71)	82
Full Intellectual Quotient	88.18 (11.33)	105.75 (16.42)	82
WISC-IV Subtest			
Similarities	8.55 (2.58)	11.63 (3.66)	7
Vocabulary	8.82 (3.16)	11.25 (2.43)	10
Comprehension	9 (2.37)	11.75 (2.71)	6
Block Design	8.45 (2.62)	12 (3.38)	8
Picture Concepts	10.73 (2.53)	13.88 (1.36)	11
Matrix Reasoning	10 (3.46)	10.75 (3.92)	10
Digit Span	6.36 (3.07)	8.38 (2.72)	6
Letter-Number sequencing	7.64 (3.14)	10 (2.73)	6
Coding	8.55 (3.36)	8.13 (2.10)	8
Symbol Research	7.45 (1.75)	9.5 (2.93)	6
Inhibition Test (NEPSY-II) (standard score)			
Denomination Total	7.55 (2.38)	8.63 (2.50)	9
Inhibition Total	8.64 (3)	9.75 (3.41)	13
Switching Total	5 (3.40)*	7.5 (4.28)*	14*
Graphic Fluency Test NEPSY II (standard score)			
Graphic Fluency	9.09 (3.709	8.38 (2)	4
TOL Test (T score)			
Total corrected	38.64 (15.39)	54.13 (8.64)	36
Violation of rules	81.36 (13.95)	65 (20.70)	87
Number of moves	80.91 (11.84)	69.38 (26.37)	100
Time decision	45.91 (6.06)	44.13 (6.24)	42
Time esecution	50.09 (14.56)	44.88 (5.06)	50
Time total	49.64 (12.54)	42.88 (5.69)	48
MCST Test (z score)			
Categories	-0.48 (1.27)	0.10 (1.24)	-0.6
Correct answers	-0.79 (1.09)	0.25 (1.35)	-0.11)

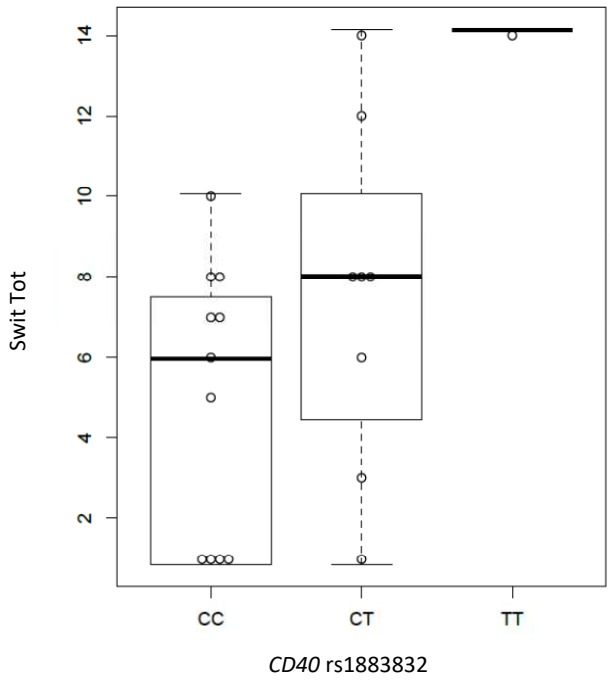
Categorial efficiency	-1.11 (0.64)	-0.41 (0.92)	-0.58
Total errors	0.53 (1.21)	0.17 (0.87)	0.13
Perseveration errors	0.24 (1.25)	0.37 (1.83)	0.03
Non perseveration errors	0.80 (1.89)	0.04 (0.69)	0.14
Failure	0.36 (2.14)	0.49 (2.02)	0
Verbal Fluency Test BVN (z score)			
Semantic	-1.28 (1.01)	-0.48 (1.31)	-1.67
Phonological	-0.71 (0.97)	-1.03 (0.73)	0

* = statistically significant differences

A significant difference emerged among groups subdivided on *CD40* rs1883832 genotyping for Switching Total of the Inhibition test (NEPSY-II) where lower scores were observed in C/C homozygotes patients (p=0.031; η^2=NaN).

The significant differences in groups subdivided on *CD40* rs1883832 genotyping are shown in Figure 8.

Figure 8. T scores in "Switching total" of the Inhibition test (NEPSY-II) in groups subdivided on *CD40* rs1883832 genotyping



2.5 Commentary

These results suggest that some polymorphisms in genes known as genetic modifiers of phenotype variability for motor and cardiac aspects in DMD boys may play a role also in the modulation of their neuropsychological phenotype. Specifically, these genetic variants seem to have an effect on executive functioning as switching, planning and problem solving abilities.

Therefore, our genetic data, even if with the limit of the small sample, suggest that genetic mechanisms other than site of mutation in *DMD* gene may have a role as potential biological biomarkers of the neuropsychological profile for our cohort of DMD patients without cognitive disability.

3. Neuroimaging study

3.1 Introduction

DWI uses water molecules diffusion patterns to provide non-invasively image contrast and reveal in vivo details of white matter (WM) microstructure. In particular, tractography can be applied to map WM pathways and trajectories in the brain (Jones & al., 2013; Tournier, 2019). At the moment, WM tracts which include crossing fibers, as the afferent and efferent cerebellar tracts, may be accurately reconstructed (Tournier et al., 2008; Kamali et al., 2010; Tournier et al., 2013; Palesi et al., 2015), thanks to developments of new techniques of data acquisition, such as high-angular-resolution diffusion imaging (HARDI), and progress in postprocessing software (Tournier et al., 2008; Tournier et al., 2013).

Quantitative analysis of WM organization can also be performed measuring scalar measures, such as Fractional Anisotropy (FA), in specific anatomic regions of interest (ROIs) or within/along reconstructed WM tracts (Jones et al., 2013).

Tractography has been widely applied for neurodevelopmental and neurological disorders and it has also been tried in neuromuscular disorders, including DMD, with a small scientific contribution (Doorenweerd et al., 2014; Fu et al., 2016).

In the last years, IRCCS Stella Maris MRI Laboratory has acquired a large experience in the use of conventional and advanced MRI techniques in children with neurological disorders. The connectomic whole brain approach and reconstruction of specific tracts by HARDI approach have been already developed in the Laboratory and applied in different pathological conditions, in particular in children with primary cerebellar disease, language disorders as childhood apraxia of speech (CAS) (Fiori et al., 2016a; Fiori et al., 2016b) and autism (Conti et al., 2016; Conti et al., 2017).

The overall above-described neuropsychological results of this research (see 1. *Neuropsychological study*) strengthen the involvement of cerebello-cerebro networks in the

neuropsychological profile of DMD children and support the application of DWI tractography in this population.

3.2 Aims

Since we succeded in better defining the neuropsychological functioning in DMD without intellectual disability, and we highlighted potential biological genetic modifiers (see previous studies), in this pilot study our ambition was to reveal also possible functional neuroimaging biomarkers of the neuropsychological profile of DMD.

In this perspective, in this study we aimed to explore structural connectivity in DMD children focusing on cortico-subcortical tracts that connect the frontal cortex, the basal ganglia and the cerebellum via the thalamus and on tracts that have been described to be possibly involved in executive functions. Mean FA along each tract was calculated. FA is a normalised scalar measure that describes the degree of diffusion anisotropy and varies between 0 (equal diffusion in all directions) and 1 (highly directional diffusion). It was used as the measure of altered white matter connectivity, and compared among DMD, BMD children and in sex- and age-matched controls. As FA reflects fibre density, axonal diameter and myelination, we hypothesized that DMD boys had lower FA in the analyzed tracts than the other groups of subjects.

Moreover, we explored possible correlations between altered FA values and clinical measures of neuropsychological function in each DMD and BMD group, in order to identify possible functional neuroimaging biomarkers of the neuropsychological profile. We hypothesized that reduced FA corresponded to higher functional impairment.

3.3 Subjects and Methods

3.3.1 Subjects

Fifteen subjects participated in the study. The rarity of the DMD disease, the difficulty for the patients' families to move from long distances, the participation of many DMD patients in experimental trials which already involve several clinical monitoring, or the refusal of patients to participate in a study involving a brain MRI protocol which is not part of the routine follow up, compromised the recruitment of DMD children. Therefore, only 5 DMD boys (mean age: 10.1 years old; range: 7-13 years) were enrolled in the study. Three of them were recruited from IRCCS Stella Maris Foundation, Pisa, and have already participated to the above-mentioned neuropsychological study, and 2 were enrolled from IRCCS Institute of Neurological Sciences, Bellaria Hospital, Bologna. Because of the involvement of dystrophin in all dystrophinopathies, additional 5 BMD boys (mean age: 13.1 years old; range: 11-15 years) were also recruited in the study from IRCCS Stella Maris Foundation. BMD is due to mutations in *DMD* gene which result in partly functional dystrophin. The variable expression of the protein reflects the widely variable clinical phenotype observed in BMD children, in terms of motor and cardiorespiratory outcome (Flanigan, 2014). In literature, the neuropsychological functioning of BMD boys has been less widely explored than DMD (Young et al., 2008; Banihani et al., 2016). We may speculate that the BMD neuropsychological phenotype could be similar but variable compared to DMD.

The inclusion criteria for the study were the following: i) DMD and BMD boys with proven mutation in the dystrophin gene; ii) the availability of a brief neuropsychological evaluation assessing cognitive and neuropsychological functioning; iii) no cognitive impairment (IQ<70) or any associated neuropsychiatric disorders (drug-resistant epilepsy, autism spectrum, attention deficit and hyperactivity) neither any additional neurosensory deficits; iv) steroid treatment and/or other experimental drug stable for at least six months.

Moreover, 5 age-matched typically developing (TD) boys (mean age: 9.5 years old; range: 7-12 years) were enrolled.

All the children were recruited only if they have not MRI contraindications and if they were be able to lie supine for at least 30/40 minutes. The MRI were in fact performed without narcosis.

The study was approved by Tuscany Pediatric Ethics Committee on September 26th 2017 and a specific informed consent form has been signed by all parents and subjects included in the study.

3.3.2 MRI acquisition

MRI data were acquired by using a 1.5T MRI scanner (1.5T GE HDx). The acquisition protocol consisted of: (1) Isotropic high-resolution T1-weighted sequence (3D BRAVO) with slice thickness = 1 mm, Field of view (FOV) = 256 mm X 256 mm, matrix = 256 X 256; Time of repetition (TR)/ Time of echo (TE) = 450/5.18 ms, flip angle (fa) = 13°; (2) Isotropic diffusion weighted sequence using a 2D single-shot EPI; including 30 non-collinear encoding directions with b value of 1000 s/mm^2 and one additional volume without diffusion gradients (b0), slice thickness = 3 mm; FOV = 240 mm X 240 mm; matrix = 80 x 80; TR/TE = 13000/115.8 ms.

3.3.3 MRI analysis

Brain tissue segmentation was performed using FreeSurfer (Fischl, 2012) based on 3D T1-weighted images. WM, gray matter, cerebrospinal fluid (CSF) and subcortical gray matter structures were obtained for each subject (5 type tissue segmentation).

DWI data were preprocessed to correct image artifacts caused by involuntary head motion, cardiac pulsation, and intensity inhomogeneities by using FSL tools. After preprocessing, a color-encoded track-density was generated to find ROI used to tract reconstruction. Fiber

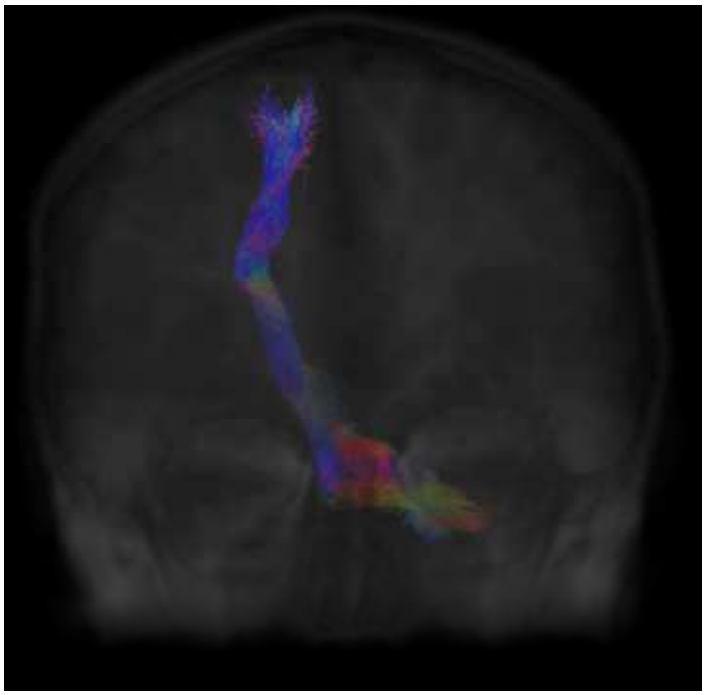
tractography (MRtrix package) was performed using constrained spherical deconvolution (CSD) with a maximum number of streamlines of ten thousand. The 5 type tissue segmentation was used to correct tractography and to discard streamlines that are anatomically unfeasible. Corticopontocerebellar tract (CPCT), cerebellar-thalamic tract (CTT) and Superior Longitudinal Fasciculus (SLF) were selected in each hemisphere of all subjects. Mean FA value along the tracts were extracted.

Tracts were checked by 2 experienced raters on all subjects to verify trajectory and anatomic landmarks described in atlases of human WM and to check false-positive streamlines within the pathways.

Corticopontocerebellar tract

The CPCT of left hemisphere was selected setting a seeding ROI in the right middle cerebellar peduncle and an inclusion ROI in the left posterior limb of the internal capsule (Fiori et al., 2016). An add inclusion ROI was positioned in a frontal WM area of left hemisphere. The CPCT of right hemisphere was obtained using the homologous ROIs in the contralateral hemispheres. Figure 9 shows the right CPCT obtained in one representative subject.

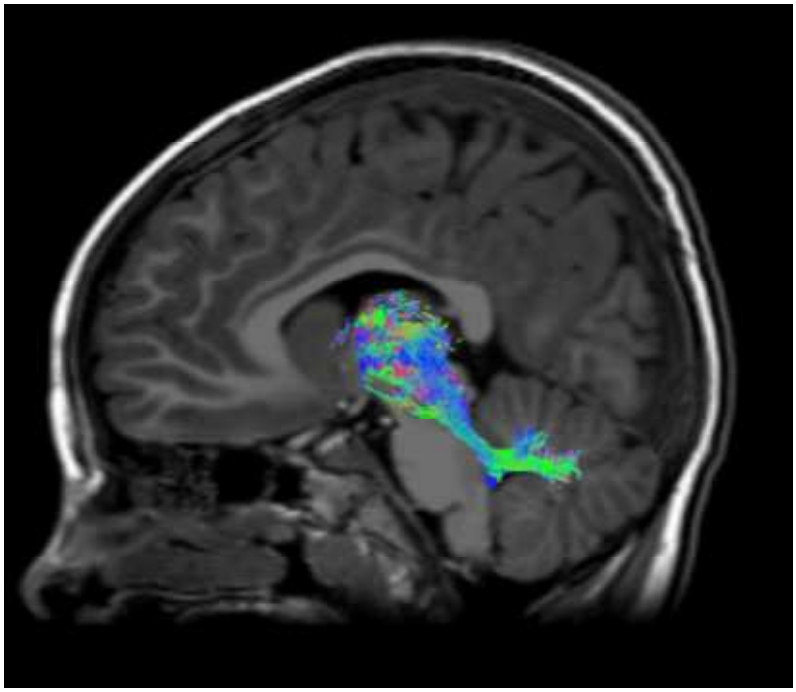
Figure 9. Example of Right Corticopontocerebellar tract, obtained in one representative subject



Cerebellar-thalamic tract

To define the left CTT, a seed in the right superior cerebellar peduncle and an inclusion ROI on the left thalamic WM were chosen (Fiori et al., 2016). For the right CCT, homologoue ROIs were selected in the contralateral hemispheres. Figure 10 shows an example of CTT in a representative subject.

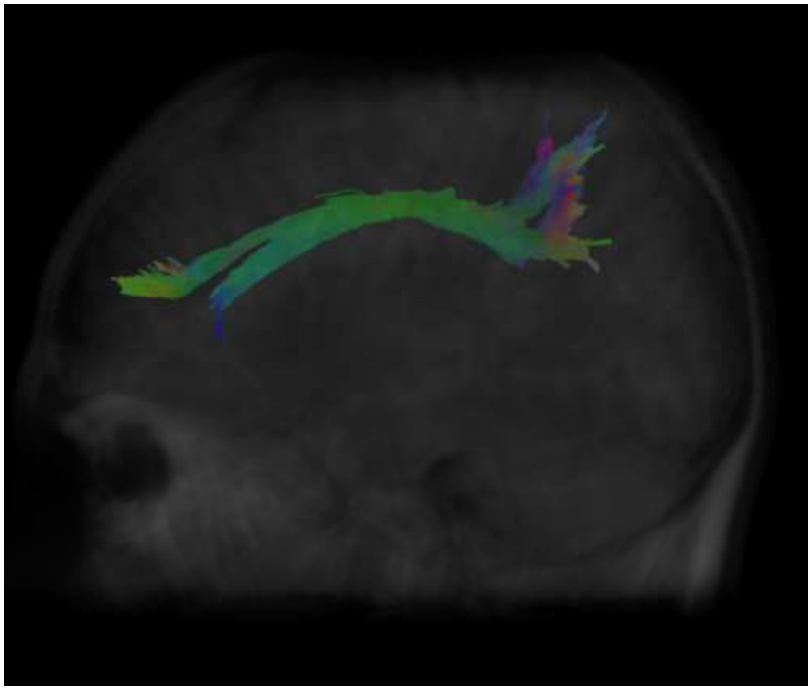
Figure 10. Example of Right Cerebellar-thalamic tract tract, obtained in one representative subject



Superior Longitudinal Fasciculus

As in Kamali et al., 2015, to identify SLF, for each hemisphere, the first ROI was placed over the green association bundles just superolateral to the cingulum on the color-encoded track-density at the most posterior part of the corpus callosum. The second ROI was placed over the fibers generated on the superolateral aspect of the cingulum at the coronal plane passing though the mid thalamus. An example of SLF is shown in Figure 11.

Figure 11. Example of Right Superior Longitudinal Fasciculus, obtained in one representative subject



3.3.4 Neuropsychological assessment

The brief neuropsychological assessment protocol included cognitive and executive functioning assessment. Concerning cognitive evaluation, we performed WISC-IV and FIQ and WMI were available for all the subjects. With regard to executive functions, Inhibition test of NEPSY-II and TOL test were administered.

3.3.5 Statistical analysis

For all subjects, the number of streamlines and FA were calculated for each reconstructed tract (CTT, CPCT and SLF). Within each subject, a paired sample t test was used to compare mean FA and number of streamlines between the right and left side of each tract. For each subject, a complete set of neuropsychological clinical measures was included in the analyses. For the analysis of neuropsychological data see Section A of *1. Neuropsychological study*. A general linear model was used to determine the difference among groups for FA and clinical measures, and post hoc pair-wise comparisons were performed. Effect size was calculated for significant differences. For tracts that showed altered connectivity among groups, the relationship with clinical measures was explored. Statistical analyses were performed by using SPSS, Version 2.0 (IBM, Armonk, New York). Results were considered significant at $p < 0.05$.

3.4 Results

3.4.1 Fiber tracts reconstruction and differences in FA within and between groups

All fiber tracts were successfully extracted on each hemisphere of each subjects.

The number of streamlines was >10 in each of the examined tracts. The mean number of streamlines generated for all the examined tracts in both hemispheres in the three groups of subjects (DMD, BMD and TD) is described in Table 9.

Table 9. Mean number of streamlines in the examined tracts in DMD, BMD and TD

	nCTT *Mean (SD)*		nCPC *Mean (SD)*		nSLF *Mean (SD)*	
	Left	**Right**	**Left**	**Right**	**Left**	**Right**
DMD	10000 (0)	8527 (2936.65)	1076.20 (1251.45)	155.60 (212.51)	10000 (0)	10000 (0)
BMD	6479.60 (4011.54)	5161.80 (4523.57)	798.40 (322.81)	772 (768)	10000 (0)	10000 (0)
TD	8389.80 (2265.85)	9906 (210.19)	1283 (815.39)	416.60 (3376.81)	10000 (0)	10000 (0)

nCTT: number of streamlines in cerebellar-thalamic tracts; nCPC: number of streamlines in corticopontocerebellar tracts; nSLF: number of streamlines in superior longitudinal fasciculus. DMD: Duchenne muscular dystrophy children; BMD: Becker muscular dystrophy children; TD: typical developing children. The SD equal to 0 means that all tracts of all suabjects were selected finding the maximum number of allowed streamlines (10.000).

No statistically significant differences for number of streamlines of all the examined tracts were found between the left and the right side within each group of children. Moreover, no statistically significant differences were found for number of streamlines of all the tracts between the three groups of subjects.

When we compared the mean FA of the left and right side for all the examined tracts in each group of subjects, we found a statistically significant difference between the mean FA of the left CPCT (FALCPCT) and the mean FA of the right CPCT (FARCPCT) in the DMD group ($p=0.007$), with FARCPCT lower than FALCPCT. No other statistically significant difference emerged over groups.

Mean FA resulted lower in DMD compared to both BMD and TD groups for all the examined tracts, with the exception of the right SLF (FARSLF), which showed no statistically different values in FA between DMD and TD.

A statistically significant difference emerged between DMD and BMD in the mean FA value of the right CTT (FARCTT) (p=0.002) and in the mean FA value of the right CPCT (FARCPCT) (p=0.037). The effect size was varied between "very large" and "huge" for FARCPCT (d=1.65), while was varied between "small" and "medium" for FARCTT (d=0.29).

Moreover, a statistically significant difference emerged between DMD and TD in the mean FARCTT (p=0.007) and in the FARCPCT (p=0.008). The effect size was varied between "small" and "medium" both for FARCPCT (d=0.22) and for FARCTT (d=0.24).

No statistically significant differences emerged among groups for FA values of bilateral SLF.

The results are shown in Table 10.

Table 10. Mean FA in all the examined tracts in DMD, BMD and TD

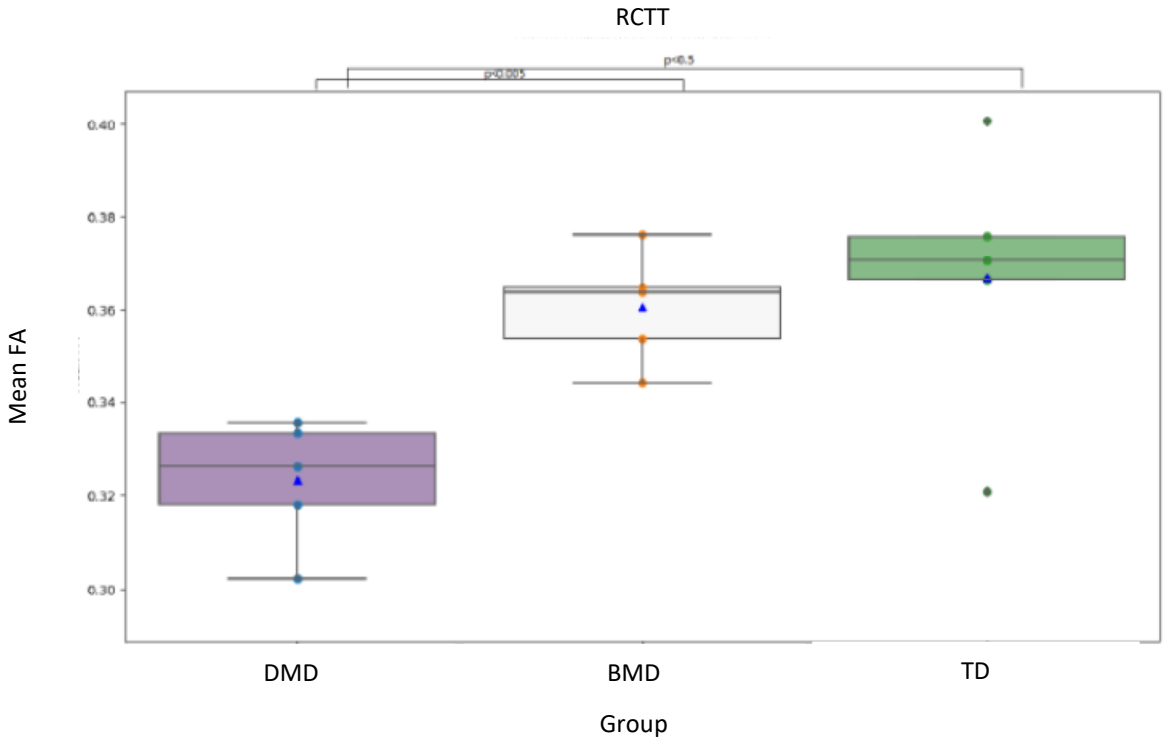
	DMD *Mean (SD)*	**BMD** *Mean (SD)*	**TD** *Mean (SD)*
FALCTT	0.34 (0.03)	0.36 (0.03)	0.36 (0.02)
FARCTT	0.32 (0.01)*	0.36 (0.01)*	0.37 (0.02)*
FALCPCT	0.48 (0.01) }	0.49 (0.20)	0.49 (0.03)
FARCPCT	0.46 (0.02)* }	0.49 (0.03)*	0.49 (0.01)*
FALSLF	0.37 (0.04)	0.41 (0.66)	0.39 (0.47)
FARSLF	0.36 (0.13)	0.38 (0.35)	0.36 (0.03)

FALCTT: FA of the left cerebellar-tahalmic tract; FARCTT: FA of the right cerebellar-thalamic tract; FALCPCT: FA of the left corticopontocerebellar tract; FARCPCT: FA of the right corticopontocerebellar tract; FALSLF: FA of the left superior longitudinal fasciculus; FARSLF: FA of the right superiori longitudinal fasciculus. DMD: Duchenne muscular dystrophy children; BMD: Becker muscular dystrophy children; TD: typical developing children.

* = statistically significant differences; } = statistically significant difference within DMD group.

Interestingly, with regard to FARCTT, a possibly “gradient effect” was observed, with mean FA of BMD children resulting in the middle between mean FA of DMD and mean FA of controls (Figure 12).

Figure 12. “Gradient effect” for mean FA in right CTT



Boxplot represent data of each single subject (cyan, orange and green points) of the three groups respectively (Duchenne muscular dystrophy children -DMD; Becker muscular dystrophy children -BMD; typical developing children -TD). The box represents the interval between the 25° and 75° percentile of data, the line corresponds to the median, the trinagles to the mean value. RCTT: right cerebellar-thalamic tract.

3.4.2 Correlations between FA fiber tracts and neuropsychological profile

On the whole, the neuropsychological profile of the small group of BMD children resulted comparable to that described in the neuropsychological study for DMD. In fact, regarding intellectual functioning, a major impairment in WMI compared to the general intellectual functioning was observed (mean score=88; SD=3.67). Worse performances were confirmed in the Switching task of the Inhibition test (NEPSY-II) (mean score=6; SD=1.87) compared to

the other tasks; in TOL test, the BMD boys reported impairments in "total correct answers" (mean score=36.60; SD=13.35).

No significant difference emerged between the BMD and DMD groups enrolled in the study in neuropsychological measures, except for the Denomination task of the Inhibition test (NEPSY-II), where BMD obtained worse performances (p=0.014).

Table 11 shows mean scores and SDs of cognitive and executive functioning tests in BDM and DMD groups.

Table 11. Intellectual and executive functioning in DMD and BMD cohorts

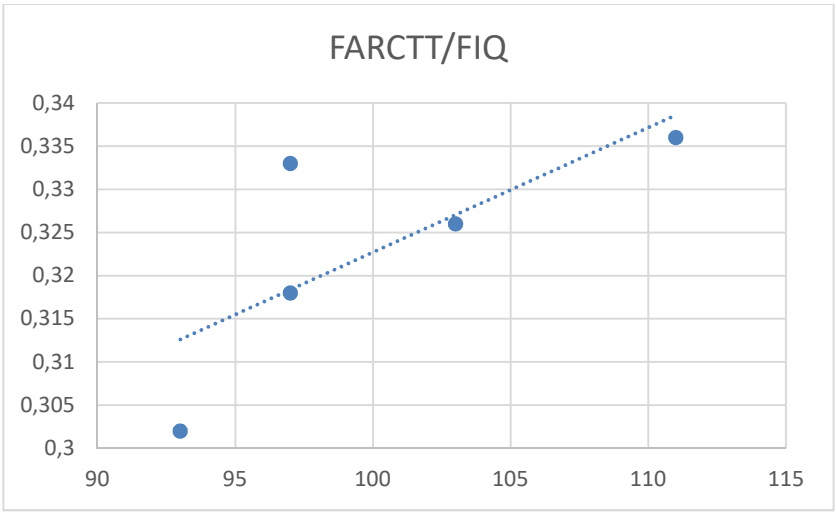
	DMD 5 subjects *Mean (SD)*	**BMD** 5 subjects *Mean (SD)*
WISC-IV Index		
Working Memory Index	86.20 (11.54)	88 (3.67)
Full Intellectual Quotient	100.20 (7.01)	95.20 (14.18)
Inhibition test (NEPSY-II) (standard score)		
Denomination Total	7.20 (1.64)*	6.40 (0.55)*
Inhibition Total	7.60 (2.07)	7.40 (1.34)
Switching Total	5.60 (2.70)	6 (1.87)
TOL test (T score)		
Total corrected	45.40 (11.01)	36.60 (13.35)

*= statistically significant differences

Concerning DMD, significant correlations emerged between FARCTT and FIQ (p=0.044; ρ_s= 0.821), Denomination Total (p=0.044; ρ_s= 0.821) and Inhibition Total (p=0.019; ρ_s= 0.900) of the Inhibition test (NEPSY-II) (Figure 13.A/B/C). No other significant correlation emerged between FARCTT and neuropsychological tests.

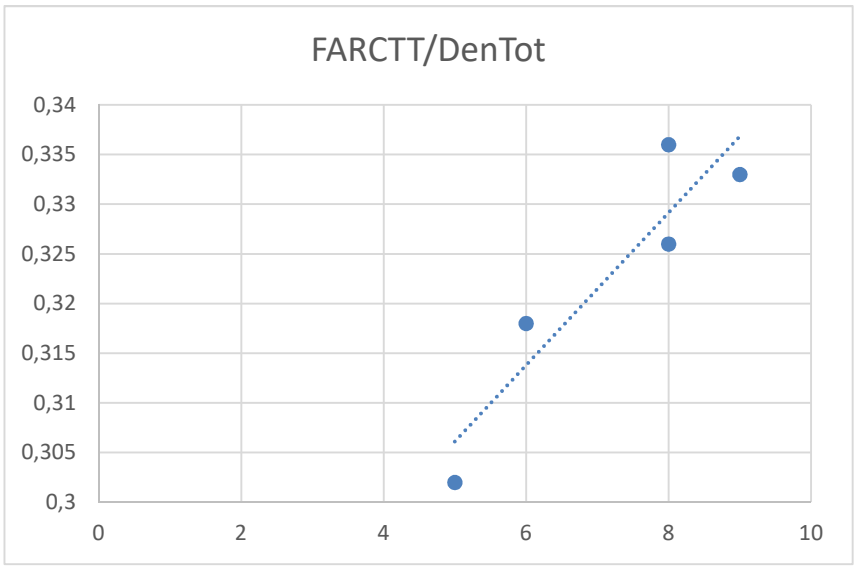
Figure 13.A/B/C. Significant correlations between FARCTT and neuropsychological tests in DMD

A.



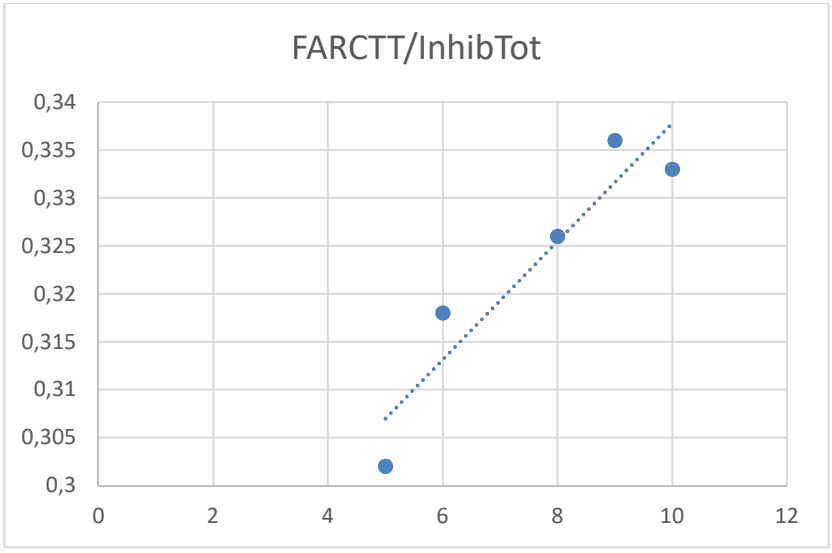
FARCTT: FA of the right cerebellar-thalamic tract; FIQ: Full Intellectual Quotient (WISC-IV).

B.



FARCTT: FA of the right cerebellar-thalamic tract; DenTot: Denomination Total (Inhibition test- NEPSY II).

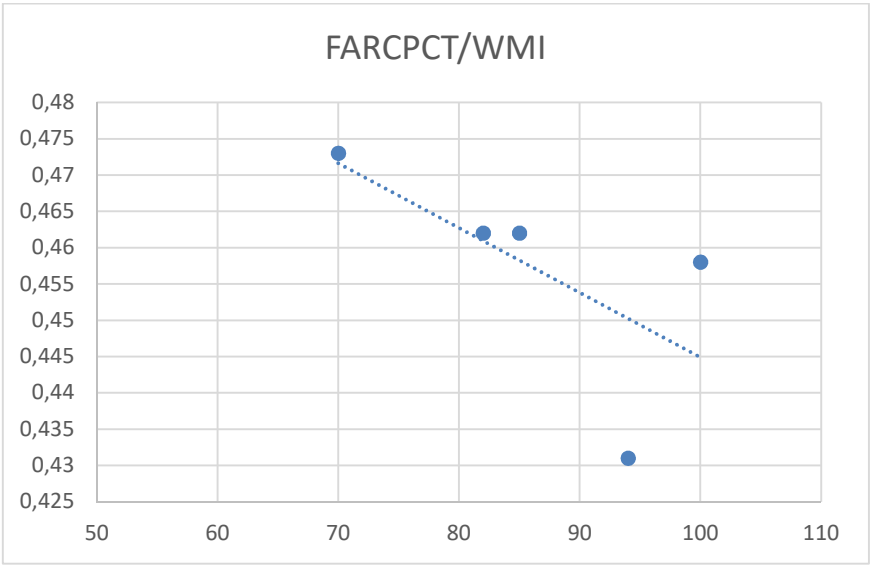
C.



FARCTT: FA of the right cerebellar-thalamic tract; InhibTot: Inhibition Total (Inhibition test- NEPSY II).

Moreover, as surprising result, we found a negative correlations between FARCPCT and WMI ($p=0.027$; $\rho_s= -0.872$). (Figure 14). No other significant correlation emerged between FARCPCT and neuropsychological tests.

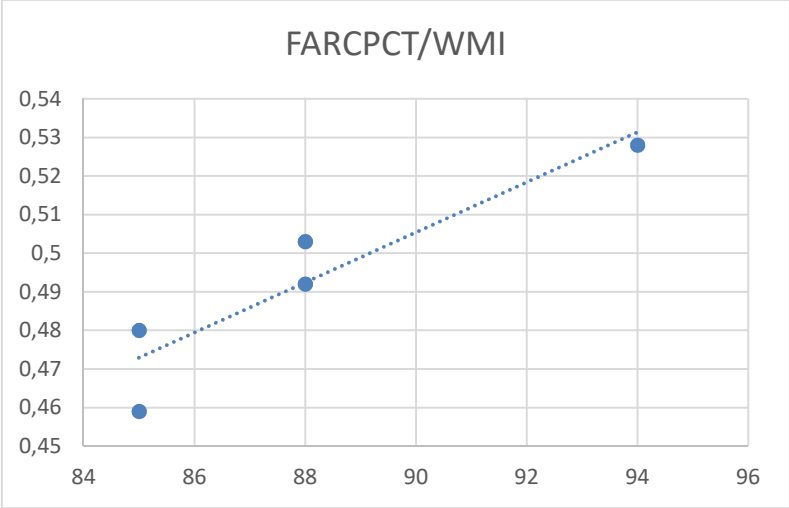
Figure 14. Significant correlations between FARCPCT and neuropsychological tests in DMD



FARCPCT: FA of the right corticopontocerebellar tract; WMI: Working Memory Index (WISC-IV).

Regarding BMD, a significant correlation emerged between FARCPCT and WMI (p=0.007; ρ_s= 0.949) (Figure 13. A/B) (Figure 15). No other significant correlation emerged between FARCPCT nor FARCTT and neuropsychological tests.

Figure 15. Significant correlations between FARCPCT and neuropsychological tests in BMD



FARCPCT: FA of the right corticopontocerebellar tract; WMI: Working Memory Index (WISC-IV).

The results about the neuroimaging study are described in an ongoing paper (Frontiers in Neuroscience, 2020).

3.5 Commentary

The results suggest possible alterations in WM microstructural integrity in DMD children, in particular for cerebellar connectivity, probably due to the lack of cerebral dystrophin protein already during neural development. As we found possible correlations between FA and neuropsychological measures, we suppose that an altered connectivity in specific tracts involved in cerebro-cerebellar loop may render less efficient some neuropsychological functions in DMD. The role of dystrophin is supported also by the findings obtained in BMD children who showed an intermediate mean FA values in some of the analyzed tracts and less

widespread correlations with neuropsychological results compared to DMD boys. This reflect the well known variable clinical and motor phenotype observed in BMD due to the partial but variable expression of dystrophin protein in these subjects

The surprising results obtained for working memory abilities in DMD need further investigations but could also be explained by mechanisms of maladaptive plasticity.

However, the overall results support the hypothesis of the involvement of cerebellar-thalamo-cortical networks for the neuropsychological profile of DMD and help to identify possible functional neuroimaging biomarkers for the neurocognitive profile of DMD without intellectual disability (Frontiers in Neuroscience 2020, ongoing).

4. Discussion

In this section, we provide a discussion of the overall results derived from the PhD research, recalling and developing the brief comments described in the “commentary” at the end of each studies.

Starting from the neuropsychological aspects, possible cognitive and neuropsychological impairments have been described in DMD children, may be due to the involvement of cerebellum and of a complex cerebro-cerebellar network (Cyrulnik & Hinton, 2008), based on the localisation of dystrophin isoforms in normal brain (Huard & Tremblay 1992; Tinsley et al., 1993; Lidov et al., 1995; Anderson et al., 2002).

The PhD project started from the neuropsychological data collected in a multicenter setting started before the beginning of the PhD in a cohort of DMD boys without intellectual disability during school age, showing an impairment of multitasking, problem solving, inhibition and working memory and an implicit learning deficit in these children.

The PhD research aimed then to deepen this issue assessing longitudinally the DMD boys previously evaluated and extending neuropsychological evaluation in another group of DMD children without intellectual disability in order to define the neuropsychological natural history and to confirm the involvement of the cerebellar-thalamo-cortical network in a larger cohort of DMD patients than those previously evaluated.

The results obtained by our neuropsychological study confirm a characteristic neuropsychological profile in a wider cohort of DMD patients without intellectual disability than previously studied (Battini et al., 2018), and the stability of such profile after a re-assessment 3 years apart from the first evaluation.

Regarding the intellectual functioning, the results confirm the worst performance in the WMI and in the related subtests Digit Span and Letter-Number Sequencing. This clear difficulty in

the manipulation of stored information emerged both for the whole DMD sample and for the follow-up reassessment and has been already decribed in literature (Hinton et al., 2000; Hinton et al., 2001; Hinton et al., 2004). The overall stability of the cognitive impairment over time is also in accord with literature (Cotton et al., 2001; Cotton et al., 2005; Connolly et al., 2014; Chieffo et al., 2015).

Concerning executive functions, a typical profile emerges for DMD children without intellectual disability, both in the whole sample and regarding the follow up re-assessment. In particular, difficulties are confirmed in the "Switching subtest" of Nepsy-II, that tests the ability to inhibit automatic responses and switch the tasks. The impairments in "maintaining the set" at the MCST test and in"violations of rules" with short time of decision and execution at TOL test confirm poor abstract reasoning and planning capacity, as well as impulsiveness for these patients.

These findings are consistent with our previous results and contribute to support the hypothesis of a frontostriatal-cerebellar network involvement in DMD (Battini et al., 2018). In fact, it is known that dystrophin isoforms are usually expressed not only in cerebellum but also in other cortical and subcortical areas (Lidov et al. 1990; Huard & Tremblay 1992; Tinsley et al., 1993, Lidov et al., 1995; Anderson et al., 2002; Daoud et al., 2008). Moreover, tasks requiring executive functions explored by our neuropsychological protocol are believed to activate cortico-subcortical circuits which connect the prefrontal cortex, the basal ganglia and the cerebellum via the thalamus (Heyder et al., 2004).

Even if no statistically significant differences emerged between baseline and follow up mean scores in tasks assessing executive functions, a slight maturation trend has been observed.

This is particularly evident considering that patients with a score impairment at baseline tend to improve their performances at follow up reassessment in all the neuropsychological tests.

The consolidation of neuropsychological skills that emerged in our cohort of DMD boys does not surprise. In fact, other studies have shown a development and maturation of executive

functioning, using neuropsychological or neuroimaging approaches, both in normotypical children and adolescents (Igazság et al., 2019; Baron Nelson et al., 2019; Wierenga et al., 2019; Engelhardt et al., 2019; Richardson et al., 2018), and in clinical populations, as, for example, children born preterm (Stålnacke et al., 2019), or children with autism spectrum disorders (Kouklari et al., 2018) and ADHD (Skogli et al., 2017). Our results suggest that a similar neuropsychological maturation can be observed also in DMD children and, to our knowledge, our description is the first in this specific population.

In particular, in our sample, the increase in Switching total mean score of the Inhibition test (NEPSY-II) over time expresses an improvement of cognitive flexibility, particularly evident for the youngest boys, aged 6-8 years-old at baseline. This result is in accordance with literature in which emerged that not until 7 to 9 years of age switch flexibility begin operating (Davidson et al., 2006; Gupta et al., 2009; Diamond, 2013). Furthermore, the variations of mean scores in “total correct answers”, “time decision”, “time execution”, and “time total” observed at TOL test suggest a reduction in impulsivity and a consequent increased capacity of planning and problem solving over time in this cohort of patients. With increasing age, a better emotional and behavioral control, inherent in growth, could have helped these children to develop greater attention capabilities.

As already described for motor aspects in reports showing that some activities can be delayed and achieved by DMD boys at a later age than typically development children while others are rarely achieved (Connolly et al., 2013; Pane et al., 2013; De Sanctis et al., 2015; Coratti et al., 2019), a progressive maturation in neuropsychological aspects may be detected. However, specific difficulties in some tasks may persist, defining a specific neuropsychological functioning and outlining the neuropsychological natural history of DMD children without intellectual disability.

With a view to better define the neuropsychological functioning of our sample, we were interested in providing a window into the everyday behavior of these DMD children and assessing executive functioning in the home environment, by the BRIEF-2 questionnaire.

In our sample, the main difficulties were observed in the ability to adjust to changes in routine or task demands ("Shift"), in modulate emotions ("Emotional control", "Emotion Regulation Index"), initiate problem solving or activities ("Initiate") and hold information in mind for the purpose of completing a task ("Working memory"). These results are partly according to literature, where clinical significant executive difficulties recently emerged on the "Shift", "Emotional Control", and "Behavior Regulation" indices of the BRIEF in a cohort od dystrophinopathic patients (Fee et al., 2019). However, the mean scores resulted in the normal range for our DMD subjects in all the subtests. These results demonstrate that the DMD boys'parents focus their attention mainly on motor functions rather than on cognitive and neuropsychological abilities of their children. However, as demonstrated by our results, the assessment of DMD children through specific neuropsychological tests may highlight possible executive dysfunctions, even for boys without a cognitive impairment.

The deepening of the role of dystrophin in the brain by the identification of the neuropsychological involvement for DMD boys could have not only a value of a mere description, taking into account the negative impact that executive dysfunctions may have on the global functioning of the children, both at an academic and an adaptive level. In this perspective, DMD boys without intellectual disability are a particularly vulnerable population because a good cognitive level may mask possible underlying neuropsychological weaknesses that, if not detected and correctly recognized, may have effects on the quality of life of these children.

This is even more true if we consider that the development of emotion regulation is strongly supported by several core executive functions and cognitive and emotion regulation appear to work in concert (Rueda and Paz- Alonso, 2013). Difficulties with emotion regulation can be

related to mood disruption and behavioural problems (Cole et al., 2004) and DMD patients are particularly at risk of developing mood problems because of the motor disabilities due to the underlying disease.

We believe that this issue will become increasingly relevant in the coming years, with the increase in life expectancy of DMD patients. The implementation of care recommendations (including corticosteroids, cardiac medications and assisted ventilation) has led in fact to a growing adult DMD population (Koeks et al., 2017), with several patients who have to face employments and social life.

In this research, we were also interested in defining a possible correlation between genotype and neuropsychological phenotype. In order to better define the effect of the real involvement of Dp140 on the neurocognitive profile, we divided our cohort in three subgroups according to the expression of this protein.

Regarding the follow up data, this more detailed description revealed possible correlations between the Dp140 expression and the neuropsychological phenotype: in fact, DMD boys without Dp140 expression show a specific impairment in cognitive performances regarding working memory, and in Coding, a subtest of WISC-IV assessing processing speed; furthermore, children with a certain Dp140 expression demonstrate better results in tasks requiring cognitive flexibility.

Possible genotype-neuropsychological phenotype correlations were confirmed also when we considered the total cohort of DMD boys without intellectual disability. In particular, the DMD boys who do not express Dp140 show greater difficulties in the manipulation of stored information than children with a certain Dp140 dystrophin expression, while this last subgroup exhibits better performances in switching the tasks, and are more accurate in tasks requiring planning and problem solving. As expected, in addition to the role of cerebral dystrophin isoforms, also the intellectual functioning shows an effect in influencing the

neuropsychological profile in the total cohort. In fact, children with a better FIQ tend to obtain better performances also in other neuropsychological tasks.

The results obtained by patients of the “grey area”, where the effect on Dp140 expression is hard to predict, were globally undetermined. In fact, the mean scores resulted as intermediate compared to the mean scores of patients with a certain presence or absence of Dp140 expression only in about a one-third of the examined items. Therefore, we can speculate that the identification of this “grey area” could better define the DMD subjects with mutations placed in the far side of *DMD* gene, expressing or not expressing the Dp140 dystrophin, but the results were undetermined because the presence or the absence of Dp140 expression is dubious in this area.

These overall results are in agreement with literature, where rearrangements in the dystrophin gene involving cerebral dystrophin isoforms tend to be more commonly associated with not only cognitive but also with a more general neuropsychological impairment (Moizard et al., 1998; Felisari et al., 2000; Daoud et al., 2008; Daoud et al., 2009; Taylor et al., 2010; D’Angelo et al., 2011; Snow et al., 2013; Ricotti et al., 2016). Moreover, DMD subjects with mutations predicted to affect Dp140 expression showed an impairment in processing speed ability that may be related to attention problems (Pane et al., 2012).

Genetic mechanisms other than site of mutation, in particular the modifier effect of specific genetic loci (e.g. in *LTBP4*, *SPP1*, *CD40* genes) have been widely described to explain variability in DMD clinical phenotype in terms of age at loss of independent ambulation, response to glucocorticoid treatment, and age of onset of dilated cardiomyopathy (Barp et al., 2015; Bello et al., 2015). However, to our knowledge, the possible role of genetic modifiers for the neuropsychological variability in DMD populations has never been explored. Only very recently, hippocampal synaptic and membrane function has been deepened in DBA/2J-*mdx* mice model, and an after-hyperpolarization (mAHP) in CA1 pyramidal neurons, that may result in reduced excitability of the neural network, has been described (Bianchi et al., 2020).

In the DBA/2J-*mdx* mice a polymorphism in *LTBP4* was identified as a genetic modifier, responsible for a more severe dystrophic penothype than BL10-*mdx* mice including impaired muscle function and regeneration, decreased muscle weight, and elevated levels of fibrotic tissue in skeletal muscles (van Putten et al., 2019).

Because of the possible distribution of genetic modifiers and their protein products in the brain and of their involvement in neuroprotection and in the pathogenesis of neurodegenerative disorders (Yu et al., 2017; Dobolyi and Palkovits 2008; Togo et al., 2000; Kempuraj et al., 2016; Carriba et al. 2017), our genetic study was aimed at analysing polymorphisms as potential modifiers for DMD, in order to explore possible biological biomarkers of the neurocognitive profile of our cohort of patients without cognitive disability at school age.

The results suggest that some genetic variants may play a role in the modulation of the neuropsychological phenotype; in particular, no effects seem to be observed on the cognitive functioning, while a greater effect may be found on executive functions, as switching and planning abilities. Statistically significant differences were indeed found considering *CD40* genotypes in“Switching total” of the Inhibition test of NEPSY-II and, regarding *SPP1* and *LTBP4* genotypes, in “number of moves” and “time total” of TOL.

In view of these promising results, our ambition was also to identify, as pilot research, possible functional neuroimaging biomarkers of the neuropsychological impairments in DMD, which could help the clinicians to recognize earlier problems in the area of cognition with a major impact on social and school integration, and to adopt appropriate rehabilitative interventions from the beginning.

Because of the possibility of analyzing WM microstructure and brain connectivity, DWI with tractography has been widely applied in neurodevelopmental disorders and in neurological diseases, including neuromuscular disorders, and has been already tried also in DMD, but

with a small scientific contribution (Sudre, 2017; Qin et al., 2018; Payabvash et al., 2019; Poretti et al., 2007; Escolar et al., 2009; Poretti et al., 2014; Kimiskidis et al., 2017; van Dorst et al., 2019; Labayru et al., 2019; Ip et al., 2012; Doorenweerd et al., 2014; Fu et al., 2016).

In our functional neuroimaging study, we explored WM microstructure in tracts that are known or supposed to be involved in executive functions networks by investigating FA, as the measure of disrupted connectivity, in a group of DMD children without intellectual disability compared to BMD boys and controls.

In particular, we explored two cerebellar tracts, separately on the right and left sides: the CTT, which is the main efferent pathway from cerebellum, and the CPCT, which is the major input of the cerebellum from the cerebral cortex, focusing specifically on the fibers originating from the frontal area. Moreover, the SLF, involved in fronto-striatal connections and in executive functions, as already described in literature (Catani et al., 2012), has been included, separately on the right and left sides.

The overall mean FA resulted lower in DMD children than in the other groups of subjects for the examined tracts, suggesting a possible alteration in WM microstructural integrity. More specifically, the cerebellar connectivity seemed to be more compromised compared to SLF in the DMD group.

Even if with few and explorative contributions, possible altered WM connectivity has been already described in DMD boys (Doorenweerd et al., 2014; Fu et al., 2016). We suppose that the lack of cerebral dystrophin protein in DMD children, already during neural development, may be responsible for reduced fiber coherence, and altered myelination and axonal density in the explored tracts. This speculation may be supported also by the results obtained in BMD children, who showed an intermediate mean FA in some of the examined tracts, with a sort of "gradient effect" between DMD and controls, at least in tracts that might be responsible for specific cerebral symptoms (i.e. neuropsycological dysfunction), which have been previously described (see *1. Neuropsychological study*). As we known, BMD boys have partial but

variable expression of dystrophin protein that reflect the widely variable phenotype and their clinical spectrum (Flanigan, 2014). A greater effect regarding differences between DMD and BMD groups seem to be observed in CPCT than in CTT, but the results must be confirmed in wider samples.

In order to explore possible functional correlates of altered connectivity for specific tracts, we thus studied the relationship between mean FA values in the cerebellar tracts that showed reduced FA and neuropsychological measures. Indeed, we demonstrated a less widespread involvement in BMD compared to DMD. In detail, regarding DMD children, we observed significantly lower FARCTT in boys with lower FIQ and a major impairment in inhibition abilities. With respect to BMD, we demonstrated significantly lower FARCPCT in boys with lower WMI.

Surprisingly, FARCPCT seem to show an opposite effect on the working memory abilities for DMD boys, since significantly lower FA correlate to higher WMI performance. This result may be due to the small sample size and must be verified in a next wider analysis, but could also underline mechanisms of maladaptive neural plasticity for DMD which may produce abilities globally slightly reduced in DMD compared to BMD children.

Anyhow, these findings support the hypothesis of the involvement of cerebellar-thalamo-cortical loops for the neuropsychological profile of DMD, as the CTT and the CPCT are involved in the network and the related brain structures are known to be implied in executive functions.

It has long been known that the frontal cortex, in particular the prefrontal area, plays a central role in global aspect of general intelligence (Barbey et al., 2013) and executive functions (Diamond, 2013) across the lifespan; thanks to connections between the prefrontal cortex and other brain regions, the neural substrates of executive functions include also the parietal cortex, the anterior cingulate cortex, and subcortical regions as the striatum and the cerebellum (Fiske & Holmboe, 2019). Moreover, the superior cerebellar peduncle, involved in

CTT, and the posterior limb of internal capsule, involved in CPCT, are thought to be key components of the circuit (Schmahmann & Pandya, 1995). Recent data have also emphasized a role of the thalamus, in particular the mediodorsal nucleus, in cognition and executive functions because of its significant interconnectivity with the prefrontal cortex (Ouhaz et al., 2018; Wolff & Vann, 2019).

More in detail, a bilateral contribution of cerebellum in DMD seem to be suggested by our findings. In fact, both the CTT which originates from the right cerebellum and the CPCT fibers which, originating from the right frontal cortex, projects to the pontine nuclei and cross the midline thus terminating in the contralateral half of the cerebellum (Nolte, 2002), resulted more damaged in DMD than in BMD and controls. In literature, a bilateral contribution of cerebellum in executive functions is reported. For example, a cross cerebral-cerebellar circuitry with left prefrontal cortex predominantly involved and strong right cerebellum activation has been shown for verbal working memory (Emch et al., 2019). However, an fMRI study has demonstrated a bilateral cerebellar activation for working memory paradigms, while other executive function tasks showed converging activation in lobules VI, Crus I and left VIIB of cerebellum (Stoodley et al., 2012). Moreover, left and right cerebellum involvement in switching attention has been demonstrated (Berninger et al., 2017).

To our knowledge, this is the first study to specifically explore the role of the cerebellar-thalamo-cortical network in DMD boys using DWI with HARDI approach. Our results suggest that altered WM connectivity and reduced fibre organization in cerebellar tracts, probably due to the lack of dystrophin in the brain, may render less efficient some neuropsychological functions in children affected by dystrophinopathies. The findings lead us to identify possible functional neuroimaging biomarkers for the neuropsychological profile of DMD without intellectual disability.

5. Conclusion and future perspectives

Our overall results confirm that some aspects of neuropsychological function, concerning in particular executive functioning, can be impaired in DMD boys, even without intellectual disability, and help to better outline the cognitive natural history in these patients. In fact, in addition to the known stability of cognitive function, our longitudinal findings suggest also the overall stability of the neuropsychological profile over time.

Since our results deriving from a specific questionnaire for DMD boys' parents show that neuropsychological deficits may be not correctly recognized in the home environment, we believe that the detection of possible neuropsychological impairments in DMD boys without intellectual disability may be an important challenge. Actually, a misdiagnosed neuropsychological deficit can have a negative impact on the global functioning of these children.

Moreover, we confirm that the site of mutation may have an impact on the neuropsychological functioning, with difficulties in cognition, expecially in working memory abilities, observed in case of rearrangements in the dystrophin gene involving cerebral dystrophin isoforms, and better performances in executive functions for DMD children who express the Dp140 dystrophin.

The ancillary genetic study suggests that genetic modifiers may also play a role in the modulation of the neuropsychological phenotype in DMD.

The hypothesis of the role of the cerebellar-thalamo-cortical network in neuropsychological aspects of DMD is supported by our neuroimaging findings. In our pilot study, in fact, we seem to identify possible functional neuroimaging biomarkers for the neuropsychological profile of DMD without intellectual disability, thanks, in particular, to the involvement of cerebellar connectivity.

Regarding the neuropsychological study, the follow up data is limited to 33 DMD subjects out of the 40 already tested in our previous study (Battini et al., 2018). We will be able to extend the analysis to the whole cohort of 57 DMD children only when three years will be passed after the first evaluation of the new enrolled patients. We are particularly concerned about this issue because we would like to outline the trend of the neuropsychological deficits over time in the whole recruited sample.

The limitation of the genetic and neuroimaging sections is the reduced sample which could have conditioned the statistical analysis.

Regarding the genetic study, blood samples for the genetic testing were available only for 20 DMD patients. When we genotyped the sample according to *SPP1, LTBP4* and *CD40* genes, we obtained even smaller subgroups; actually, with regard to *CD40* gene rs1883832 genotyping, only 1 patient was homozygote for the T allele. In any case, the overall effect size seemed to be medium/large.

A larger cohort of DMD patients without intellectual disability could not only increase the results obtained considering candidate genes, but also lead us to apply GWAS to potentially identify novel and unsuspected modifier genes.

In the neuroimaging study, the enrollment of DMD children has been limited not only by the rarity of the disease but also by the difficulty for the patients' families to move from long distances, the participation of many DMD patients in experimental trials which already involve several clinical monitoring, or the refusal of some patients to undergo a brain MRI protocol which is not part of the routine assessment. The small sample size limits the possibility to correlate the neuroimaging data with the neuropsychological findings and the interpretation of results, in particular if we consider some outliers in FA measures which emerged from the data analysis. Also the unexpected negative correlation between FARCPCT and WMI in DMD children needs to be further deepened. The enlargement of the sample could help us to definitively establish the role of cerebellar connectivity in

neuropsychological profile for dystrophinopathic patients. Furthermore, DMD and BMD children enrolled in the study underwent only a brief neuropsychological assessment, but a more detailed evaluation could help not only to better describe the role of WM abnormalities but also, for BMD children, to define their neuropsychological profile. In literature, the neuropsychological functioning of BMD has been less widely deepened compared to DMD children until now (Young et al., 2008; Banihani et al., 2016).

However, on the other hand, the small sample size has been overall due to the difficult recruitment of DMD children who fullfil the inclusion criteria, despite the involvement in this project of several Italian Centers specialized in neuromuscular disorders. This issue suggests that the real amount of DMD boys with cognitive impairment and associated neuropsychiatric disorders may be larger than that reported in literature (Billard et al., 1992; Ricotti et al., 2016), and support the necessity of an update on the neurodevelopmental, emotional, and behavioural problems in the DMD population.

In conclusion, the direct consequences of a recognized “weak brain”, in addition to muscle weakness well known in the natural history of the disease, are manifold and all relate to missing educational opportunities and significant negative impact on quality of life.

As future perspectives, by means of biomarkers, our main challenge is to early recognize possible neuropsychological impairments and to plan specific treatments, also based on new technological devices as home-based treatment and tele-rehabilitation.

References

Al-Qudah AA, Kobayashi J, Chuang S, Dennis M, Ray P. Etiology of intellectual impairment in Duchenne muscular dystrophy. Pediatr Neurol. 1990; 6: 57–59.

Anderson JL, Head SI, Rae C, Morley JW. Brain function in Duchenne muscular dystrophy. Brain 2002;125(Pt 1):4-13.

Astrea G, Pecini C, Gasperini F, Brisca G, Scutifero M, Bruno C, Santorelli FM, Cioni G, Politano L, Chilosi AM, Battini R. Reading impairment in Duchenne muscular dystrophy: A pilot study to investigate similarities and differences with developmental dyslexia.Res Dev Disabil. 2015 Oct-Nov;45-46:168-77.

Banihani R, Baskin B, Halliday W, Kobayashi J, Kawamura A, McAdam L, Ray PN, Yoon G. A Novel Mutation in DMD (c.10797+5G>A) Causes Becker Muscular Dystrophy Associated with Intellectual Disability. J Dev Behav Pediatr. 2016 Apr;37(3):239-44. doi: 10.1097/DBP.0000000000000262.

Baron Nelson M, O'Neil SH, Wisnowski JL, Hart D, Sawardekar S, Rauh V, Perera F, Andrews HF, Hoepner LA, Garcia W, Algermissen M, Bansal R, Peterson BS. Maturation of Brain Microstructure and Metabolism Associates with Increased Capacity for Self-Regulation during the Transition from Childhood to Adolescence. J Neurosci. 2019 Oct 16;39(42):8362-8375. doi: 10.1523/JNEUROSCI.2422-18.2019. Epub 2019 Aug 23.

Barbey AK, Colom R, Grafman J. Dorsolateral prefrontal contributions to human intelligence. Neuropsychologia. 2013 Jun;51(7):1361-9. doi: 10.1016/j.neuropsychologia.2012.05.017. Epub 2012 May 23.

Barp A, Bello L, Politano L, Melacini P, Calore C, Polo A, Vianello S, Sorarù G, Semplicini C, Pantic B, Taglia A, Picillo E, Magri F, Gorni K, Messina S, Vita GL, Vita G, Comi GP, Ermani M, Calvo V, Angelini C, Hoffman EP, Pegoraro E. Genetic Modifiers of Duchenne Muscular Dystrophy and Dilated Cardiomyopathy. PLoS One. 2015 Oct 29;10(10):e0141240.

Battini R, Chieffo D, Bulgheroni S, Piccini G, Pecini C, Lucibello S, Lenzi S, Moriconi F, Pane M, Astrea G, Baranello G, Alfieri P, Vicari S, Riva D, Cioni G, Mercuri E. Cognitive profile in Duchenne muscular dystrophy boys without intellectual disability: The role of executive functions.Neuromuscul Disord. 2018 Feb;28(2):122-128. doi: 10.1016/j.nmd.2017.11.018. Epub 2017 Dec 6.

Bello L, Kesari A, Gordish-Dressman H, Cnaan A, Morgenroth LP, Punetha J, Duong T, Henricson EK, Pegoraro E, McDonald CM, Hoffman EP; Cooperative International Neuromuscular Research Group Investigators. Genetic modifiers of ambulation in the Cooperative International Neuromuscular Research Group Duchenne Natural History Study. Ann Neurol. 2015 Apr;77(4):684-96.

Bello L, Morgenroth LP, Gordish-Dressman H, Hoffman EP, McDonald CM, Cirak S, CINRG investigators. DMD genotypes and loss of ambulation in the CINRG Duchenne Natural History Study. Neurology 2016a; 87(4): 401-9.

Bello L, Flanigan KM, Weiss RB,United Dystrophinopathy Project, Spitali P, Aartsma-Rus A, Muntoni F, Zaharieva I, Ferlini A, Mercuri E, Tuffery-Giraud S, Claustres M, Straub V, Lochmüller H, Barp A, Vianello S, PegoraroE, Punetha J, Gordish-Dressman H, Giri M, McDonald CM, Hoffman EP, Cooperative International Neuromuscular Research Group. Association Study of Exon Variants in the NF-κB and TGFβ Pathways Identifies CD40 as a Modifier of Duchenne Muscular Dystrophy.Am J Hum Genet. 2016b Nov 3 99 (5), 1163-1171.

Bello L, Pegoraro E. The "Usual Suspects": Genes for Inflammation, Fibrosis, Regeneration, and Muscle Strength Modify Duchenne Muscular Dystrophy. J Clin Med. 2019;8(5):649. Published 2019 May 10. doi:10.3390/jcm8050649

Berninger VW, Richards TL, Abbott RD. Brain and Behavioral Assessment of Executive Functions for Self-Regulating Levels of Language in Reading Brain. J Nat Sci. 2017;3(11):e464.

Bianchi R, Eilers W, Pellati F, Corsi L, Foster H, Foster K, Tamagnini F. Hippocampal Synaptic and Membrane Function in the DBA/2J-mdx Mouse Model of Duchenne Muscular Dystrophy. Mol Cell Neurosci. 2020 Apr;104:103482. doi: 10.1016/j.mcn.2020.103482. Epub 2020 Mar 19.

Billard C, Gillet P, Signoret JL, Uicaut E, Bertrand P, Fardeau M, Barthez-Carpentier MA, Santini JJ. Cognitive functions in Duchenne muscular dystrophy: a reappraisal and comparison with spinal muscular atrophy. Neuromuscul. Disord. 1992; 2(5-6): 371–378.

Billard C, Gillet P, Barthez M, Hommet C, Bertrand P. Reading ability and processing in Duchenne muscular dystrophy and spinal muscular atrophy. Dev Med Child Neurol. 1998 Jan;40(1):12-20.

Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, Case LE, Clemens PR, Hadjiyannakis S, Pandya S, Street N, Tomezsko J, Wagner KR, Ward LM, Weber DR, DMD Care Considerations Working Group. Diagnosis and Management of Duchenne Muscular Dystrophy, Part 1: Diagnosis, and Neuromuscular, Rehabilitation, Endocrine, and Gastrointestinal and Nutritional Management. Lancet Neurol 2018 Mar, 17 (3), 251-267.

Bisiacchi PS, Cendron M, Gugliotta M, Tressoldi PE, Vio C. BVN 5-11 - Batteria di valutazione neuropsicologica per l'età evolutiva. Erickson. 2005.

Bresolin N, Castelli E, Comi GP, Felisari G, Bardoni A, Perani D, Grassi F, Turconi A, Mazzucchelli F, Gallotti D, Moggio M, Prelle A, Ausenza C, Fazio G, Scarlato G. Cognitive impairment in Duchenne muscolar dystrophy. Neuromuscul Disord 1994; 4:359-369.

Brooks BL, Sherman EMS, Strauss E. NEPSY-II: A Developmental Neuropsychological Assessment, Second Edition. Child Neuropsychol 2010;16(1):80-101.

Carriba P, Davies AM. CD40 is a major regulator of dendrite growth from developing excitatory and inhibitory neurons. Elife. 2017;6:e30442. Published 2017 Nov 7. doi:10.7554/eLife.30442

Catani M, Dell'acqua F, Bizzi A, Forkel SJ, Williams SC, Simmons A, Murphy DG, Thiebaut de Schotten M. Beyond Cortical Localization in Clinico-Anatomical Correlation. Cortex. Nov-Dec 2012;48(10):1262-87. doi: 10.1016/j.cortex.2012.07.001. Epub 2012 Aug 23.

Chieffo D, Brogna C, Berardinelli A, D'Angelo G, Mallardi M, D'Amico A, Alfieri P, Mercuri E, Pane M. Early Neurodevelopmental Findings Predict School Age Cognitive Abilities in Duchenne Muscular Dystrophy: A Longitudinal Study. PLoS One. 2015 Aug 14;10(8):e0133214. eCollection 2015.

Cohen HJ, Molnar GE, Taft LT. The genetic relationship of progressive muscular dystrophy (Duchenne type) and mental retardation. Dev. Med. Child Neurol. 1968; 10, 754–765.

Cole PM, Martin SE, Dennis TA. Emotion regulation as a scientific construct: methodological challenges and directions for child development research. Child

Dev. 2004 Mar-Apr;75(2):317-33. doi: 10.1111/j.1467-8624.2004.00673.x.

Connolly AM, Florence JM, Cradock MM, Malkus EC, Schierbecker JR, Siener CA, Wulf CO, Anand P, Golumbek PT, Zaidman CM, Miller JP, Lowes LP, Alfano LN, Viollet-Callendret L, Flanigan KM, Mendell JR, McDonald CM, Goude E, Johnson L, Nicorici A, Karachunski PI, Day JW, Dalton JC, Farber JM, Buser KK, Darras BT, Kang PB, Riley SO, Shriber E, Parad R, Bushby K, Eagle M, MDA DMD Clinical Research Network. Motor and Cognitive Assessment of Infants and Young Boys With Duchenne Muscular Dystrophy: Results From the Muscular Dystrophy Association DMD Clinical Research Network Neuromuscul Disord. 2013 Jul;23(7):529-39. doi: 10.1016/j.nmd.2013.04.005. Epub 2013 May 28.

Connolly AM, Florence JM, Cradock MM, Eagle M, Flanigan KM, McDonald CM, Karachunski PI, Darras BT, Bushby K, Malkus EC, Golumbek PT, Zaidman CM, Miller JP, Mendell JR; MDA DMD Clinical Research Network. One year outcome of boys with Duchenne muscular dystrophy using the Bayley-III scales of infant and toddler development. Pediatr Neurol. 2014 Jun;50(6):557-63.

Conti E, Calderoni S, Gaglianese A, Pannek K, Mazzotti S, Rose S, Scelfo D, Tosetti M, Muratori F, Cioni G, Guzzetta A.Lateralization of Brain Networks and Clinical Severity in Toddlers With Autism Spectrum Disorder: A HARDI Diffusion MRI Study. Autism Res. 2016 Mar;9(3):382-92. doi: 10.1002/aur.1533. Epub 2015 Aug 17.

Conti E, Mitra J, Calderoni S, Pannek K, Shen KK, Pagnozzi A, Rose S, Mazzotti D, Scelfo D, Tosetti M, Muratori F, Cioni G, Guzzetta A. Network Over-Connectivity Differentiates Autism Spectrum Disorder From Other Developmental Disorders in Toddlers: A Diffusion MRI Study. Hum Brain Mapp. 2017 May;38(5):2333-2344. doi: 10.1002/hbm.23520. Epub 2017 Jan 17.

Coratti G, Brogna C, Norcia G, Ricotti V, Abbott L, D'Amico A, Berardinelli A, Vita GL, Lucibello S, Messina S, Sansone V, Albamonte E, Colia G, Salmin F, Gardani A, Manzur A, Main M, Baranello G, Arnoldi MT, Parsons J, Carry T, Connolly AM, Bertini E, Muntoni F, Pane M, Mercuri E. Longitudinal natural history in young boys with Duchenne muscular dystrophy. Neuromuscul Disord. 2019 Nov;29(11):857-862. doi: 10.1016/j.nmd.2019.09.010. Epub 2019 Sep 26.

Cotton S, Voudouris NJ, & Greenwood KM. Intelligence and Duchenne muscular dystrophy: full-scale, verbal, and performance intelligence quotients. Developmental Medicine & Child Neurology 2001; 43, 497-501.

Cotton SM, Voudouris NJ, Greenwood KM. Association between intellectual functioning and age in children and young adults with Duchenne muscular dystrophy: further results from a meta-analysis. Dev Med Child Neurol. 2005 Apr;47(4):257-65.

Cyrulnik SE, Hinton VJ. Duchenne muscular dystrophy: a cerebellar disorder? Neurosci Biobehav Rev. 2008;32(3):486-96. Review.

D'Angelo MG, Lorusso ML, Civati F, Comi GP, Magri F, Del Bo R, Guglieri M, Molteni M, Turconi AC, Bresolin N. Neurocognitive profiles in Duchenne muscular dystrophy and gene mutation site. Pediatr Neurol. 2011 Nov;45(5):292-9.

Daoud F, Candelario-Martínez A, Billard JM, Avital A, Khelfaoui M, Rozenvald Y, et al. Role of mental retardation-associated dystrophin-gene product Dp71 in excitatory synapse organization, synaptic plasticity and behavioral functions. PLoS One 2008; 4, e6574.

Daoud F, Angeard N, Demerre B, Martie I, Benyaou R, Leturcq F, et al. Analysis of Dp71 contribution in the severity of mental retardation through comparison of Duchenne and Becker patients differing by mutation consequences on Dp71

expression. Human Molecular Genetics 2009;18, 3779–94.

Davidson MC, Amso D, Anderson LC, Diamond A. Development of Cognitive Control and Executive Functions From 4 to 13 Years: Evidence From Manipulations of Memory, Inhibition, and Task Switching Neuropsychologia. 2006;44(11):2037-78. doi: 10.1016/j.neuropsychologia.2006.02.006. Epub 2006 Mar 31.

De Sanctis R, Pane M, Sivo S, Ricotti V, Baranello G, Frosini S, Mazzone E, Bianco F, Fanelli L, Main M, Corlatti A, D'Amico A, Colia G, Scalise R, Palermo C, Alfonsi C, Tritto G, Romeo DM, Graziano A, Battini R, Morandi L, Bertini E, Muntoni F, Mercuri E. Suitability of North Star Ambulatory Assessment in Young Boys With Duchenne Muscular Dystrophy Neuromuscul Disord. 2015 Jan;25(1):14-8. doi: 10.1016/j.nmd.2014.09.015. Epub 2014 Oct 6.

Diamond A. Executive functions.
Annu Rev Psychol. 2013;64:135-68. doi: 10.1146/annurev-psych-113011-143750. Epub 2012 Sep 27.

Dobolyi A, Palkovits M. Expression of latent transforming growth factor beta binding proteins in the rat brain. J Comp Neurol. 2008;507(3):1393–1408. doi:10.1002/cne.21621

Doorenweerd N, Straathof CS, Dumas EM, Spitali P, Ginjaar IB, Wokke BH, Schrans DG, van den Bergen JC, van Zwet EW, Webb A, van Buchem MA, Verschuuren JJ, Hendriksen JG, Niks EH, Kan HE. Reduced cerebral gray matter and altered white matter in boys with Duchenne muscular dystrophy. Ann Neurol. 2014 Sep;76(3):403-11.

Doorenweerd N, Ahmed Mahfouz,Maaike van Putten,Rajaram Kaliyaperumal,Peter A C T' Hoen,Jos G M Hendriksen,Annemieke M Aartsma-Rus,Jan J G M Verschuuren,Erik H Niks,Marcel J T Reinders,Hermien E Kan,Boudewijn P F Lelieveldt. Timing and Localization of Human Dystrophin Isoform Expression Provide Insights Into the Cognitive Phenotype of Duchenne Muscular Dystrophy Sci Rep. 2017a Oct 3; 7 (1), 12575.

Doorenweerd N, Dumas EM, Ghariq E, Schmid S, Straathof CS, Roest AA, Wokke BH, van Zwet EW, Webb AG, Hendriksen JG, van Buchem MA, Verschuuren JJ, Asllani I, Niks EH, van Osch MJ, Kan HE. Decreased cerebral perfusion in Duchenne muscular dystrophy patients. Neuromuscul Disord. 2017b Jan;27(1):29-37. doi: 10.1016/j.nmd.2016.10.005. Epub 2016 Oct 17.

Emch M, von Bastian CC, Koch K. Neural Correlates of Verbal Working Memory: An fMRI Meta-Analysis. Front Hum Neurosci. 2019;13:180. Published 2019 Jun 12. doi:10.3389/fnhum.2019.00180

Emery AE. Population frequencies of inherited neuromuscular diseases a world survey. Neuromus. Dis. 1991; 1, 19–29.

Engelhardt LE, Harden KP, Tucker-Drob EM, Church JA. The neural architecture of executive functions is established by middle childhood. Neuroimage. 2019 Jan 15;185:479-489. doi: 10.1016/j.neuroimage.2018.10.024. Epub 2018 Oct 9.

Escolar ML, Poe MD, Smith JK, Gilmore JH, Kurtzberg J, Lin W, Styner M. Diffusion tensor imaging detects abnormalities in the corticospinal tracts of neonates with infantile Krabbe disease. AJNR Am J Neuroradiol. 2009 May;30(5):1017-21.

Fee RJ, Montes J, Hinton VJ. Executive Functioning in the Dystrophinopathies and the Relation to Underlying Mutation Position. J Int Neuropsychol Soc. 2019 Feb;25(2):146-155. doi: 10.1017/S1355617718000942. Epub 2018 Dec 4.

Felisari G, Martinelli Boneschi F, Bardoni A, et al. Loss of Dp140 dystrophin and intellectual impairment in Duchenne dystrophy. Neurology. 2000 Aug 22;55(4):559-64.

Fiori S, Poretti A, Pannek K, Del Punta R, Pasquariello R, Tosetti M, Guzzetta A, Rose S, Cioni G, Battini R. Diffusion Tractography Biomarkers of Pediatric Cerebellar Hypoplasia/Atrophy: Preliminary Results Using Constrained Spherical Deconvolution. AJNR Am J Neuroradiol. 2016a May;37(5):917-23.

Fiori S, Guzzetta A, Mitra J, Pannek K, Pasquariello R, Cipriani P, Tosetti M, Cioni G, Rose SE, Chilosi A. Neuroanatomical correlates of childhood apraxia of speech: A connectomic approach. Neuroimage Clin. 2016b Nov 4;12:894-901. eCollection 2016.

Fischl B. FreeSurfer. Neuroimage. 2012;62(2):774-781. doi: 10.1016/jneuroimage.2012.01.021

Fiske A, Holmboe K. Neural substrates of early executive function development. Dev Rev. 2019;52:42-62. doi:10.1016/j.dr.2019.100866

Flanigan KM, Ceco E, Lamar K-M, Kaminoh Y, Dunn DM, Mendell JR, King WM, Pestronk A, Florence JM, Mathews KD, Finkel RS, Swoboda KJ, Gappmaier E, Howard MT, Day JW, McDonald C, McNally EM, Weiss RB, United Dystrophinopathy Project LTBP4 Genotype Predicts Age of Ambulatory Loss in Duchenne Muscular Dystrophy. Ann Neurol. 2013Apr, 73 (4), 481-8.

Fu Y, Dong Y, Zhang C, Sun Y, Zhang S, Mu X, Wang H, Xu W, Wu S. Diffusion tensor imaging study in Duchenne muscular dystrophy. Ann Transl Med. 2016 Mar;4(6):109.

1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. An integrated map of genetic variation from 1.092 human genomes. Nature. 2012 Nov 1;491(7422):56-65. doi: 10.1038/nature11632.

Gupta R, Kar BR, Srinivasan N. Development of task switching and post-error-slowing in children. Behav Brain Funct. 2009 Sep 15;5:38. doi: 10.1186/1744-9081-5-38.

Hendriksen JG, Vles JS. Are males with Duchenne muscular dystrophy at risk for reading disabilities? Pediatr Neurol. 2006 Apr;34(4):296-300.

Heyder K, Suchan B, Daum I. Cortico-subcortical contributions to executive control. Acta Psychol (Amst). 2004 Feb-Mar;115(2-3):271-89.

Hindi SM, Sato S, Choi Y, Kumar A. Distinct roles of TRAF6 at early and late stages of muscle pathology in the mdx model of Duchenne muscular dystrophy. Hum Mol Genet. 2014 Mar 15;23(6):1492-505.

Hinton VJ, De Vivo DC, Nereo NE, Goldstein E, Stern Y. Poor verbal working memory across intellectual level in boys with Duchenne dystrophy. Neurology. 2000 Jun 13;54(11):2127-32.

Hinton VJ, De Vivo DC, Nereo NE, Goldstein E, Stern Y. Selective deficits in verbal working memory associated with a known genetic etiology: the neuropsychological profile of Duchenne muscular dystrophy. J Int Neuropsychol Soc. 2001 Jan;7(1):45-54.

Hinton VJ, De Vivo DC, Fee R, Goldstein E, Stern Y. Investigation of Poor Academic Achievement in Children with Duchenne Muscular Dystrophy. Learn

Disabil Res Pract. 2004 Aug;19(3):146-154.

Hoffman EP, Brown Jr RH, Kunkel LM, 1987. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 1987; 51, 919–928.

Huard J, Tremblay JP. Localization of dystrophin in the Purkinje cells of normal mice. Neuroscience Letters 1992; 137, 105-108.

Igazság B, Demetrovics Z, Cserjési R. The developmental trajectory of executive functions and their stress sensitivity in adolescence. Psychiatr Hung. 2019;34(3):300-310.

Ip JJK, Hui PKT, Chau MT, Lam WWM. Merosin-deficient Congenital Muscular Dystrophy (MDCMD): A Case Report With MRI, MRS and DTI Findings. J Radiol Case Rep. 2012;6(8):1–7. doi:10.3941/jrcr.v6i8.997

Jones DK, Knösche TR, Turner R.White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI. Neuroimage. 2013 Jun;73:239-54. doi: 10.1016/j.neuroimage.2012.06.081. Epub 2012 Jul 23. PMID: 22846632

Kamali A, Kramer LA, Frye RE, Butler IJ, Hasan KM.. Diffusion tensor tractography of the human brain cortico-ponto-cerebellar pathways: a quantitative preliminary study. J Magn Reson Imaging 2010;32:809–17 doi:10.1002/jmri.22330 pmid:20882611

Kamali A, Yousem DM, Lin DD, Sair HI, Jasti SP, Keser Z, Riascos RF, Hasan KM. Mapping the trajectory of the stria terminalis of the human limbic system using high spatial resolution diffusion tensor tractography. Neurosci Lett. 2015 Nov 3;608:45-50. doi: 10.1016/j.neulet.2015.09.035. Epub 2015 Oct 3.

Kempuraj D, Thangavel R, Natteru PA, Selvakumar JP, Saeed D, Zahoor H, Zaheer S, Iyer SS, Zaheer A. Neuroinflammation Induces Neurodegeneration J Neurol Neurosurg Spine. 2016, 1 (1).

Kimiskidis VK, Papaliagkas V, Papagiannopoulos S, Zafeiriou D, Kazis D, Tsatsali-Foroglou E, Kouvatsou Z, Kapina V, Koutsonikolas D, Anogianakis G, Geroukis T, Bostantjopoulou S. Investigation of the motor system in two siblings with Canavan's disease: a combined transcranial magnetic stimulation (TMS) - diffusion tensor imaging (DTI) study. Metab Brain Dis. 2017 Apr;32(2):307-310.

Koeks Z, Bladen CL, Salgado D, van Zwet E, Pogoryelova O, McMacken G, Monges S, Foncuberta ME, Kekou K, Kosma K, Dawkins H, Lamont L, Bellgard MI, Roy AJ, Chamova T, Guergueltcheva V, Chan S, Korngut L, Campbell C, Dai Y, Wang J, Barišić N, Brabec P, Lähdetie J, Walter MC, Schreiber-Katz O, Karcagi V, Garami M, Herczegfalvi A, Viswanathan V, Bayat F, Buccella F, Ferlini A, Kimura E, van den Bergen JC, Rodrigues M, Roxburgh R, Lusakowska A, Kostera-Pruszczyk A, Santos R, Neagu E, Artemieva S, Rasic VM, Vojinovic D, Posada M, Bloetzer C, Klein A, Díaz-Manera J, Gallardo E, Karaduman AA, Oznur T, Topaloğlu H, El Sherif R, Stringer A, Shatillo AV, Martin AS, Peay HL, Kirschner J, Flanigan KM, Straub V, Bushby K, Béroud C, Verschuuren JJ, Lochmüller H. Clinical Outcomes in Duchenne Muscular Dystrophy: A Study of 5345 Patients from the TREAT-NMD DMD Global Database.J Neuromuscul Dis. 2017;4(4):293-306. doi: 10.3233/JND-170280.

Kouklari EC, Tsermentseli S, Monks CP. Hot and cool executive function in children and adolescents with autism spectrum disorder: Cross-sectional developmental trajectories. Child Neuropsychol. 2018 Nov;24(8):1088-1114. doi: 10.1080/09297049.2017.1391190. Epub 2017 Oct 20.

Koziol LK, Budding D, Andreasen N, D'Arrigo S, Bulgheroni S, Imamizu H, Ito M, Manto M, Marvel C, Parker K, Pezzulo G, Ramnani N, Riva D, Schmahmann J,

Vandervert L, Yamazaki T. Consensus Paper: The Cerebellum's Role in Movement and Cognition. Cerebellum 2014 Feb; 13 (1), 151-77.

Kreis R, Wingeier K, Vermathen P, Giger E, Joncourt F, Zwygart K, Kaufmann F, Boesch C, Steinlin M. Brain metabolite composition in relation to cognitive function and dystrophin mutations in boys with Duchenne muscular dystrophy. NMR Biomed. 2011 Apr;24(3):253-62.

Labayru G, Diez I, Sepulcre J, Fernández E, Zulaica M, Cortés JM, López de Munain A, Sistiaga A. Regional Brain Atrophy in Gray and White Matter Is Associated With Cognitive Impairment in Myotonic Dystrophy Type 1. Neuroimage Clin. 2019;24:102078. doi:10.1016/j.nicl.2019.102078

Lee JS, Pfund Z, Juhász C, Behen ME, Muzik O, Chugani DC, Nigro MA, Chugani HT. Altered regional brain glucose metabolism in Duchenne muscular dystrophy: a pet study. Muscle Nerve. 2002 Oct;26(4):506-12.

Lidov HGW, Byers TJ, Watkins SC, Kunkel LM. Localization of dystrophin to postsynaptic regions of central nervous system cortical neurons. Nature. 1990 Dec 20-27;348(6303):725-8.

Lidov HG, Selig S, Kunkel LM. Dp140: a novel 140 kDa CNS transcript from the dystrophin locus. Hum Mol Genet. 1995; 4(3):329-35.

Lorusso ML, Civati F, Molteni M, Turconi AC, Bresolin N, D'Angelo MG. Specific profiles of neurocognitive and reading functions in a sample of 42 Italian boys with Duchenne Muscular Dystrophy. Child Neuropsychol. 2013;19(4):350-69.

Lv SY, Zou QH, Cui JL, Zhao N, Hu J, Long XY, Sun YC, He J, Zhu CZ, He Y, Zang Y.F.Decreased gray matter concentration and local synchronization of spontaneous activity in the motor cortex in Duchenne muscular dystrophy. Am. J. Neuroradiol 2011;.32, 2196–2200.

Marano A et al. Behavior Rating Inventory of Executive Functions, Second Edition.Hogrefe, 2016.

Marini A, Lorusso ML, D'Angelo MG, Civati F, Turconi AC, Fabbro F, Bresolin N. Evaluation of narrative abilities in patients suffering from Duchenne Muscular Dystrophy. Brain Lang. 2007 Jul;102(1):1-12. Epub 2007 Apr 10.

Mento G, Tarantino V, Bisiacchi PS. The neuropsychological profile of infantile Duchenne muscular dystrophy. Clin Neuropsychol. 2011 Nov;25(8):1359-77.

Moizard MP, Billard C, Toutain A, Berret F, Marmin N, Moraine C. Are Dp71 and Dp140 brain dystrophin isoforms related to cognitive impairment in Duchenne muscular dystrophy? Am J Med Genet 1998; 80: 32–41.

Muntoni F, Melis MA, Ganau A, Dubowitz V. Transcription of the dystrophin gene in normal tissues and in skeletal muscle of a family with Xlinked dilated cardiomyopathy. Am J Hum Genet 1995; 56: 151–57.

Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. Lancet Neurol. 2003 Dec;2(12):731-40.

Nolte J. The human brain. An introduction to its functional anatomy, 5th edition. St. Louis, MO: Mosby; 2002. p 650.

Ogasawara A. Downward shift in IQ in persons with Duchenne muscular dystrophy compared to those with spinal muscular atrophy. Am. J. Ment. Retard. 1989; 93, 544–547.

Okkersen K, Monckton DG, Le N, Tuladhar AM, Raaphorst J, van Engelen BGM. Brain Imaging in Myotonic Dystrophy Type 1: A Systematic Review. Neurology. 2017;89(9):960–969.

Ouhaz Z, Fleming H, Mitchell AS. Cognitive Functions and Neurodevelopmental Disorders Involving the Prefrontal Cortex and Mediodorsal Thalamus. Front Neurosci. 2018;12:33. Published 2018 Feb 6. doi:10.3389/fnins.2018.00033

Orsini, A., Pezzuti, L., Picone, L. WISC-IV. Contributo alla taratura italiana. Firenze: Giunti O.S. Organizzazioni Speciali. 2012.

Palesi F, Tournier JD, Calamante F, Muhlert N, Castellazzi G, Chard D, D'Angelo E, Wheeler-Kingshott CA. Contralateral cerebello-thalamo-cortical pathways with prominent involvement of associative areas in humans in-vivo. Brain Struct Funct 2015;220:3369–84 doi:10.1007/s00429-014-0861-2 pmid:25134682

Pane M, Lombardo ME, Alfieri P, D'Amico A, Bianco F, Vasco G, Piccini G, Mallardi M, Romeo DM, Ricotti V, Ferlini A, Gualandi F, Vicari S, Bertini E, Berardinelli A, Mercuri E. Attention deficit hyperactivity disorder and cognitive function in Duchenne muscular dystrophy: phenotype-genotype correlation. J Pediatr. 2012 Oct;161(4):705-9.e1.

Pane M, Scalise R, Berardinelli A, D'Angelo G, Ricotti V, Alfieri P, Moroni I, Hartley L, Pera MC, Baranello G, Catteruccia M, Casalino T, Romeo DM, Graziano A, Gandioli C, Bianco F, Mazzone ES, Lombardo ME, Scoto M, Sivo S, Palermo C, Gualandi F, Sormani MP, Ferlini A, Bertini E, Muntoni F, Mercuri E. Early neurodevelopmental assessment in Duchenne muscular dystrophy. Neuromuscul Disord. 2013 Jun;23(6):451-5. doi: 10.1016/j.nmd.2013.02.012. Epub 2013 Mar 25.

Pane M, Mazzone ES, Sormani MP, et al. 6 minute walk test in Duchenne MD patients with different mutations: 12 month changes. PLoS One 2014; 9(1): e83400.

Pannek K, Boyd RN, Fiori S, Guzzetta A, Rose SE. Assessment of the structural brain network reveals altered connectivity in children with unilateral cerebral palsy due to periventricular white matter lesions. Neuroimage Clin. 2014 Jun 4;5:84-92.

Pegoraro E, Hoffman EP, Piva L, Gavassini BF, Cagnin S, Ermani M, Bello L,Soraru G, Pacchioni B, Bonifati MD, Lanfranchi G, Angelini C, Kesari A, Lee I, Gordish-Dressman H, Devaney JM, McDonald CM, Cooperative International Neuromuscular Research Group. SPP1 Genotype Is a Determinant of Disease Severity in Duchenne Muscular Dystrophy. Neurology. 2011 Jan 18, 76 (3), 219-26.

Poretti A, Boltshauser E, Loenneker T, Valente EM, Brancati F, Il'yasov K, Huisman TA.
Diffusion tensor imaging in Joubert syndrome.AJNR Am J Neuroradiol. 2007 Nov-Dec;28(10):1929-33.

Poretti A, Meoded A, Bunge M, Fatemi A, Barrette P, Huisman TA, Salman MS. Novel diffusion tensor imaging findings in Krabbe disease. Eur J Paediatr Neurol. 2014 Mar;18(2):150-6.

Qin B, Wang L, Zhang Y, Cai J, Chen J, Li T. Enhanced Topological Network Efficiency in Preschool Autism Spectrum Disorder: A Diffusion Tensor Imaging Study. 2018 Jun 27 eCollection 2018. Front Psychiatry , 9, 278.

Quattrocelli M, Capote J, Ohiri JC, Warner JL, Vo AH , Earley JU , Hadhazy M, Demonbreun AR ,Spencer MJ, McNally EM. Genetic Modifiers of Muscular Dystrophy Act on Sarcolemmal Resealing and Recovery From Injury. PLoS Genet. 2017 Oct 24 eCollection Oct 2017, 13 (10), e1007070.

Rae C, Scott RB, Thompson CH, Dixon RM, Dumughn I, Kemp GJ, Male A, Pike M, Styles P, Radda GK. Brain biochemistry in Duchenne muscular dystrophy: a H1 magnetic resonance and neuropsychological study. J Neurol Sci. 1998 Oct 8;160(2):148-57.

Richardson C, Anderson M, Reid CL, Fox AM. Development of inhibition and switching: A longitudinal study of the maturation of interference suppression and reversal processes during childhood. Dev Cogn Neurosci. 2018 Nov;34:92-100. doi: 10.1016/j.dcn.2018.03.002. Epub 2018 Mar 6.

Ricotti V, Mandy WP, Scoto M, Pane M, Deconinck N, Messina S, Mercuri E, Skuse DH, Muntoni F. Neurodevelopmental, emotional, and behavioural problems in Duchenne muscular dystrophy in relation to underlying dystrophin gene mutations. Dev Med Child Neurol. 2016 Jan;58(1):77-84.

Riva D, Giorgi C. The contribution of the cerebellum to mental and social functions in developmental age. Fiziol Cheloveka. 2000 Jan-Feb;26(1):27-31.
Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. Brain 1998;121:561– 79.

Rueda MR, Paz-Alonso PM. Executive functiona and emotional development. Encyclopedia of early childhood development. 2013 Jan; Executive functions.

Sannio Fancello G., Cianchetti C. MCST: Modified card sorting test : adattamento italiano per l'età evolutiva del Wisconsin card sorting test : manuale. Giunti O.S., Organizzazioni Speciali. 2003.

Sannio Fancello G., Vio C., Cianchetti C. TOL Torre di Londra: test di valutazione delle funzioni esecutive (pianificazione e problem solving). Erickson. 2006.

Schmahmann JD, Pandya DN. Prefrontal cortex projections to the basilar pons in rhesus monkey: implications for the cerebellar contribution to higher function. Neurosci Lett. 1995;199(3):175-178. doi:10.1016/0304-3940(95)12056-a

Sijens PE, Fock JM, Meiners LC, Potze JH, Irwan R, Oudkerk M. MR Spectroscopy and Diffusion Tensor Imaging of the Brain in Congenital Muscular Dystrophy With Merosin Deficiency: Metabolite Level Decreases, Fractional Anisotropy Decreases, and Apparent Diffusion Coefficient Increases in the White Matter. Brain Dev. 2007;29(5):317–321. doi:10.1016/j.braindev.2006.10.004

Skogli EW, Andersen PN, Hovik KT, Øie M. Development of Hot and Cold Executive Function in Boys and Girls With ADHD. J Atten Disord. 2017 Feb;21(4):305-315. doi: 10.1177/1087054714524984. Epub 2016 Jul 28.

Snow WM, Anderson JE, Jakobson LS. Neuropsychological and neurobehavioral functioning in Duchenne muscular dystrophy: a review. Neurosci Biobehav Rev. 2013 Jun;37(5):743-52. Review.

Spitali P, Zaharieva I, Bohringer S, Hiller M, Chaouch A, Roos A, Scotton C, Claustres M, BelloL, McDonald CM , Hoffman EP, CINRG Investigators, Koeks Z, Suchiman HE, Cirak S, Scoto M, Reza M,AC 't Hoen P, Niks HE, Tuffery-Giraud S, Lochmüller H, Ferlini A, Muntoni F, Aartsma-RusA. TCTEX1D1 Is a Genetic Modifier of Disease Progression in Duchenne Muscular Dystrophy. Eur J Hum Genet 2020 Jan 2[Online ahead of print]

Stålnacke J, Lundequist A, Böhm B, Forssberg H, Smedler AC. A longitudinal model of executive function development from birth through adolescence in children born very or extremely preterm. Child Neuropsychol. 2019 Apr;25(3):318-335. doi: 10.1080/09297049.2018.1477928. Epub 2018 May 30.

Stoodley CJ, Valera EM, Schmahmann JD. Functional topography of the cerebellum

for motor and cognitive tasks: an fMRI study. Neuroimage. 2012;59(2):1560-1570. doi:10.1016/j.neuroimage.2011.08.065

Sudre G, Szekely E, Sharp W, Kasparek S, Shaw P. Multimodal Mapping of the Brain's Functional Connectivity and the Adult Outcome of Attention Deficit Hyperactivity Disorder. Proc Natl Acad Sci U S A. 2017 Oct 31 114 (44), 11787-11792.

Taylor PJ, Betts G A, Maroulis S, Gilissen C, Pedersen RL, Mowat DR, Johnston HM, Buckley MF. Dystrophin gene mutation location and the risk of cognitive impairment in Duchenne muscular dystrophy. PLoS One 2010; 5, e8803.

Tinsley JM, Blake DJ, Pearce M, Knight AE, Kendrick-Jones J, Davies KE. Dystrophin and related proteins. Curr Opin Genet Dev. 1993;3(3):484-90. Review.

Togo T, Akiyama H,Kondo H, Ikeda K, Kato M, Iseki E, Kosaka K. Expression of CD40 in the Brain of Alzheimer's Disease and Other Neurological Diseases. Brain Res. Dec 1 2000. 885 (1), 117-21

Tournier JD, Yeh CH, Calamante F, Cho KH, Connelly A, Lin CP. Resolving crossing fibers using constrained spherical deconvolution: validation using diffusion-weighted imaging phantom data. Neuroimage 2008;42:617–25 doi:10.1016/j.neuroimage.2008.05.002 pmid:18583153

Tournier JD, Calamante F, Connelly A. Determination of the appropriate b value and number of gradient directions for high-angular-resolution diffusion-weighted imaging. NMR Biomed 2013;26:1775–86 doi:10.1002/nbm.3017 pmid:24038308

Tournier JD. Diffusion MRI in the brain - Theory and concepts. Prog Nucl Magn Reson Spectrosc. 2019;112-113:1-16. doi:10.1016/j.pnmrs.2019.03.001

Urgesi C, Campanella F, Fabbro F. NEPSY-II. Second edition. Contributo alla taratura italiana. Firenze: Giunti O.S. Organizzazioni Speciali. 2011

van den Bergen JC, Hiller M, Böhringer S ,Vijfhuizen L, Ginjaar HB ,Chaouch A, Bushby K, Straub V , Scoto M, Cirak S, Humbertclaude V, Claustres M, Scotton C, Passarelli C, Lochmüller H, Muntoni M ,Tuffery-Giraud S, Ferlini A, Aartsma-Rus AM, Verschuuren JGJM, Ac 't Hoen P,Spitali P. Validation of Genetic Modifiers for Duchenne Muscular Dystrophy: A Multicentre Study Assessing SPP1 and LTBP4 Variants J Neurol Neurosurg Psychiatry. 2015 Oct, 86 (10), 1060-5.

van Dorst M, Okkersen K, Kessels RPC, Meijer FJA, Monckton DG, van Engelen BGM, Tuladhar AM, Raaphorst J, OPTIMISTIC consortium. Structural White Matter Networks in Myotonic Dystrophy Type 1. 2019;21:101615. doi:10.1016/j.nicl.2018.101615

van Putten M, Putker K, Overzier M, Adamzek WA, Pasteuning-Vuhman S, Plomp JJ, Aartsma-Rus A. Natural Disease History of the D2 -mdx Mouse Model for Duchenne Muscular Dystrophy. FASEB J. 2019 Jul;33(7):8110-8124. doi: 10.1096/fj.201802488R. Epub 2019 Apr 1.

Vicari S, Piccini G, Mercuri E, Battini R, Chieffo D, Bulgheroni S, Pecini C, Lucibello S, Lenzi S, Moriconi F, Pane M, D'Amico A, Astrea G, Baranello G, Riva D, Cioni G, Alfieri P. Implicit learning deficit in children with Duchenne muscular dystrophy: Evidence for a cerebellar cognitive impairment?PLoS One. 2018 Jan 6;13(1):e0191164. doi: 10.1371/journal.pone.0191164. eCollection 2018.

Wierenga LM, Bos MGN, van Rossenberg F, Crone EA. Sex Effects on Development of Brain Structure and Executive Functions: Greater Variance than Mean Effects. J Cogn Neurosci. 2019 May;31(5):730-753. doi: 10.1162/jocn_a_01375. Epub 2019 Feb 6.

Wolff M, Vann SD. The Cognitive Thalamus as a Gateway to Mental Representations. J Neurosci. 2019;39(1):3-14. doi:10.1523/JNEUROSCI.0479-18.2018

Xu S, Shi D, Pratt SJP, Zhu W, Marshall A, Lovering RM. Abnormalities in brain structure and biochemistry associated with mdx mice measured by in vivo MRI and high resolution localized (1)H MRS. Neuromuscul Disord. 2015 Oct;25(10):764-72.

Young HK, Barton BA, Waisbren S, Portales Dale L, Ryan MM, Webster RI, North KN. Cognitive and psychological profile of males with Becker muscular dystrophy. J Child Neurol. 2008 Feb;23(2):155-62. Epub 2007 Dec 3.

Yu H, Liu X, Zhong Y. The Effect of Osteopontin on Microglia. Biomed Res Int. 2017;2017:1879437. doi:10.1155/2017/1879437

Zatz M, Pavanello RC, Lazar M, Yamamoto GL, Lourenço NC, Cerqueira A, Nogueira L, Vainzof M. Milder course in Duchenne patients with nonsense mutations and no muscle dystrophin. Neuromuscul Disord. 2014 Nov;24(11):986-9.

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