9-2018

Underlying Contribution of Executive Functioning to Cognition and Academic Achievement in Individuals with Dystrophinopathy

Robert Fee

The Graduate Center, City University of New York

How does access to this work benefit you? Let us know!

Follow this and additional works at: https://academicworks.cuny.edu/gc_etds

Part of the Clinical Psychology Commons, Cognitive Neuroscience Commons, Developmental Neuroscience Commons, Genetics Commons, and the Psychiatry and Psychology Commons

Recommended Citation

Fee, Robert, "Underlying Contribution of Executive Functioning to Cognition and Academic Achievement in Individuals with Dystrophinopathy" (2018). CUNY Academic Works.
https://academicworks.cuny.edu/gc_etds/2779

This Dissertation is brought to you by CUNY Academic Works. It has been accepted for inclusion in All Dissertations, Theses, and Capstone Projects by an authorized administrator of CUNY Academic Works. For more information, please contact deposit@gc.cuny.edu.
UNDERLYING CONTRIBUTION OF EXECUTIVE FUNCTIONING TO COGNITION AND ACADEMIC ACHIEVEMENT IN INDIVIDUALS WITH DYSTROPHINOPATHY

by

ROBERT J. FEE

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2018
UNDERLYING CONTRIBUTION OF EXECUTIVE FUNCTIONING TO COGNITION AND ACADEMIC ACHIEVEMENT IN INDIVIDUALS WITH DYSTROPHINOPATHY

by

ROBERT J. FEE

This manuscript has been read and accepted for the Graduate Faculty in Clinical Psychology to satisfy the dissertation requirement for the degree of Doctor of Philosophy.

Jennifer L. Stewart, Ph.D.

Date

Chair of Examining Committee

Richard Bodnar, Ph.D.

Date

Executive Officer

Veronica J. Hinton, Ph.D.

Valentina Nikulina, Ph.D.

Justin Storbeck, Ph.D.

Paul Mattis, Ph.D., ABPP

Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK
ABSTRACT

Underlying contribution of executive functioning to cognition and academic achievement in individuals with dystrophinopathy

by

Robert J. Fee

Advisor: Jennifer L. Stewart, Ph.D. and Veronica J. Hinton, Ph.D.

Committee members: Paul Mattis, Ph.D., Valentina Nikulina, Ph.D., Justin L. Storbeck, Ph.D.

Dystrophinopathy is a genetic disorder that results in the lack of or abnormal expression of the protein dystrophin. It is a disorder that alters cell structure and function, impacts the developing brain and brain function, presents with multi-domain cognitive deficits, and influences both mood and behavior. Cognitive impairments appear to be more localized to specific areas of functioning rather than a global deficit; however, deficits have been identified across multiple cognitive domains including language and aspects of executive functioning. A careful examination of the cognitive phenotype and its association to mutations affecting CNS isoforms is necessary to clarify the neuropsychological profile. Executive functions that may contribute to overall IQ performance have not been fully examined for their contribution to cognitive efficiency as well as to real world functioning. A three-step investigation was used to examine potential areas of executive weakness, the association with mutation position, and their impact on everyday functioning for children with dystrophinopathy. First, an investigation of executive skills including behavior was conducted in children with dystrophinopathy. Additionally, the association between these executive skills and the dystrophin gene mutation
position was studied. Second, the implications of these executive deficits to real world functioning were studied by examining academic performance in boys with dystrophinopathy as well as the association with mutation position. Finally, given the consistent finding of reduced digit span, specific executive weaknesses were examined for their contribution to digit span performance to further specify compromised cognitive constructs. Clearly defining cognitive functioning among individuals affected by the dystrophinopathies and the association with molecular abnormalities will help to further understand how the absence of dystrophin in the central nervous system (CNS) impacts brain development and function, influencing cognition and everyday functioning for individuals with the disorder.
ACKNOWLEDGMENTS

This dissertation project is dedicated to:

My mother,

who gave me unconditional love and support over years of hard work, she is the epitome of

compassion, sacrifice, and strength;

My cousins, especially Richard,

who gave me the opportunity to grow and persevere, and provided a second home with love

and support;

My mentor, Veronica J. Hinton,

who guided my professional identity, but equally took on supportive roles of friend/family

when needed;

My teachers,

who built foundations, promoted skills, and showed me the importance of individual strengths

And last but definitely not least, my wife Despina,

who changed my life for the better providing the perfect mix of fun, love, and life; we created a

family starting with my incredibly strong and beautiful son, Neo.
TABLE OF CONTENTS

I. INTRODUCTION........................................................................................................1-52

Clinical presentation of the Dystrophinopathies.........................................................3

Physical phenotype in the Dystrophinopathies.........................................................3

CNS involvement in Dystrophinopathies.................................................................5

Dystrophin gene mutation position and cognition....................................................10

Cognitive phenotype in the Dystrophinopathies......................................................12

Global Conceptual Processing.................................................................................13

Attention and Processing Speed..............................................................................17

Executive functioning..............................................................................................21

Memory....................................................................................................................24

Language processing.................................................................................................26

Visuospatial processing............................................................................................30

Academic Achievement............................................................................................31

Motor skills................................................................................................................33

Neuropsychiatric presentation in the dystrophinopathies........................................35

Mood..........................................................................................................................35

Behavior....................................................................................................................37

Social..........................................................................................................................37

Attention and Executive behavior problems.........................................................39

Quality of Life............................................................................................................42

Hypotheses and Aims...............................................................................................50-52
II. PART ONE (AIM 1 AND 2): Executive functioning in the dystrophinopathies and the relation to underlying mutation position

Introduction ................................................................. 54
Methods ............................................................ 56
Results ............................................................... 61

III. PART TWO (AIM 3): Executive functions and academic achievement in the dystrophinopathies

Introduction ................................................................. 74
Methods ............................................................ 78
Results ............................................................... 84

IV. PART THREE (AIM 4): Weaknesses in executive functioning contribute to digit span performance

Introduction ................................................................. 102
Methods ............................................................ 104
Results ............................................................... 110

V. DISCUSSION ............................................................................. 121

VI. TABLES/FIGURES ........................................................................

PART ONE ............................................................................ 67-71
PART TWO ............................................................................ 93-100
PART THREE ........................................................................ 116-120

VII. APPENDICES ........................................................................

A. INSTITUTIONAL REVIEW BOARD APPROVALS

VIII. BIBLIOGRAPHY ......................................................................

INTRODUCTION ........................................................................ 145
PART ONE ..............................................................................165
PART TWO ..............................................................................169
PART THREE .........................................................................177
DISCUSSION .........................................................................184
I. INTRODUCTION

The dystrophinopathies constitute a spectrum of muscle diseases characterized by the abnormal expression of the protein dystrophin in muscle, as well as other tissue types including the brain. The diseases are associated with a genetic mutation at Xp21 on the short arm of the X chromosome; thus the majority of those affected are males (Hoffman, 1993). Phenotypic expression of the disease can be variable and is likely dependent upon the type of mutation and the effects on functional dystrophin production. In muscle, absence of dystrophin from the muscle cell sarcolemma leads to muscle cell breakdown with use, resulting in progressive muscular weakness for the individuals affected.

Neurodevelopmental difficulties are quite common in the disorder. Absence of, or incomplete isoforms of, dystrophin has been hypothesized to impact brain functioning (Anderson, Head, & Morley, 2012; Mehler, 2000). Cognitive impairments appear to be more localized to specific areas of functioning rather than a global deficit (Cotton, Voudouris, & Greenwood, 2005). Deficits have been identified across multiple cognitive domains including language processing, memory, and in some aspects of attention and executive functioning; however, there has not been adequate evidence examining the cognitive mechanisms underlying observed weaknesses, particularly in areas of basic cognitive functioning. There has been consistent evidence of a verbal weakness across affected individuals (Billard et al., 1992; Cotton, Voudouris, & Greenwood, 2001) as well as a replicated deficit on digit span tasks (D'Angelo & Bresolin, 2006; Hendriksen & Vles, 2006; Hinton, De Vivo, Nereo, Goldstein, & Stern, 2001; Hinton, Fee, Goldstein, & De Vivo, 2007). Further, unlike motor skills, cognitive
deficits have not been shown to progressively worsen with age (Cotton et al., 2005; Hinton, Kim, Fee, Goldstein, & DeVivo, 2017).

Additionally, rates of behavioral problems are increased in individuals affected by the dystrophinopathies. Social skill deficits, depression and attention deficits (Darke, Bushby, Le Couteur, & McConachie, 2006; Hendriksen & Vles, 2008; Hinton, Nereo, Fee, & Cyrulnik, 2006a; Poysky, 2007) have been found at increased rates across samples of boys with dystrophinopathy. Although some of the behaviors observed may be reactive responses to living with the disorder, in general, the cognitive and behavioral deficits observed are hypothesized to be linked to the missing dystrophin isoforms in the brain (Anderson, Head, Rae, & Morley, 2002; Mehler, 2000).

In order to better understand the brain-behavior relationships in the disorder, careful examination of the cognitive phenotype is necessary to make definitive associations. Clearly defining cognitive functioning among individuals affected by the dystrophinopathies may help to further understand the possible roles of dystrophin in the central nervous system (CNS). The differences in cognition and behavior likely reflect how brain areas function differently within this population and thus impact the development of specific cognitive skills.

The underlying hypothesized mechanisms of the disorder, performance across domains of cognitive processing, and behavioral presentation will be thoroughly reviewed. The goal will be to further delineate a neuropsychological profile by specifying underlying contributions to overall cognition and academic functioning that will aid future research in exploring possible associated dystrophin CNS origins and functions as well as contribute to identifying possible targets for cognitive remediation.
Clinical presentation of Dystrophinopathy

Physical Phenotype in the dystrophinopathies

The dystrophinopathies were first clinically described by Edward Meryon in 1851 and further documented by Duchenne de Boulogne in 1868 as a progressive disease of muscle atrophy with impairments in mental capacities (Duchenne, 1968; Waclawik, 2014). Phenotypic presentation of dystrophinopathy has a degree of variability, but clinical course is generally consistent across individuals. The complete absence of dystrophin results in the most severe form of the disease known as Duchene Muscular Dystrophy (DMD). On the other end of the spectrum, the milder form of the disease called Becker Muscular Dystrophy (BMD) is associated with partial but functional dystrophin production (Muntoni, Torelli, & Ferlini, 2003). DMD is the most common variant occurring in approximately 1 in 3500 live male births, whereas BMD affects approximately 1 in 18,000 (Wicklund, 2013). The disorder is not specific to a particular race, but there is some inconsistency in the prevalence rates due to variability in methods of diagnostic confirmation (Mah et al., 2014).

Dystrophinopathy is suspected when there are signs of proximal muscle weakness indicative of a myopathy (Sarrazin, von der Hagen, Schara, von Au, & Kaindl, 2014), calf hypertrophy, and specimen analysis showing highly elevated creatine kinase levels (Dalton, Goldman, & Sampson, 2015). The Gower maneuver, a rising movement from a seated position, is used as a diagnostic sign to indicate weakness of the hip and knee extensor, a defining clinical characteristic of dystrophinopathy (Wicklund, 2013). To confirm diagnosis, molecular assessment is generally the first line of confirmation using the current advanced methods of gene sequencing; however, although typically a last resort because of the invasiveness of the procedure, muscle biopsy may be utilized in some cases for a definitive
diagnosis to examine the level of dystrophin through immunohistochemistry or western blot analysis (Dalton et al., 2015). Genetic testing or muscle biopsy and the clinical presentation of impaired functional ambulation is often used to distinguish the particular phenotype of dystrophinopathy. The rapid progression of muscle weakness through the school-age years with advancing motor difficulties affecting the muscles of the hips, pelvic area, thighs and shoulders resulting in eventual gait cessation is indicative of DMD whereas prolonged ambulation is a distinguishing feature of the less severe BMD (Bushby et al., 2010a; Bushby & Gardner-Medwin, 1993). Generally for DMD, individual mobility becomes confined to the use of a wheelchair by the start of the adolescent years. However, with the current standard of care for the treatment of the dystrophinopathies with glucocorticosteroids, clinical evidence has shown an increase in muscle strength and function that prolongs ambulation, delays the progression of complications such as cardiac and respiratory problems, and aids in the prevention of scoliosis (Goemans & Buyse, 2014). For most individuals with DMD, wheelchair dependence leads to increased severity of contractures and the development of kyphoscoliosis, which ultimately necessitates spinal fusion for respiratory functioning (Alexander et al., 2012; Wicklund, 2013). With advancing age into adulthood and further muscle weakness, a multidisciplinary medical team becomes a necessity to manage pulmonary and cardiac functioning. Weakness in respiratory muscles leads to an inefficiency in respiratory functioning, leaving patients at an increased risk for respiratory infections, breathing difficulties during sleep and eventually respiratory failure (Khirani et al., 2014). Additionally, myocardial complications due to the damaged cellular pathways (Shirokova & Niggli, 2013) lead to atrial and ventricular arrhythmias which are associated with cardiac fibrosis and cardiomyopathy (Chiang et al., 2016). Mechanical ventilation and cardioprotective agents are
common interventions utilized in the later stages of the disease as well as other treatments from a team of providers including cardiologists, pulmonologists, orthopedists, nutritionists, and psychologists (Goemans & Buyse, 2014; Wicklund, 2013). Life expectancy has increased to the third decade of life for DMD due to improvements in healthcare and corticosteroids (Bushby et al., 2010b). BMD is associated with a more variable disease course, much slower progression of muscle weakness and an extended life span, with a mean age of death in the mid-40s (Bushby & Gardner-Medwin, 1993; Sahenk & Rodino-Klapac, 2014). Techniques of genetic editing have been experimentally employed to attempt to restore levels of the missing protein in the dystrophinopathies utilizing DMD-patient-derived pluripotent stem cells. Genome wide mutation analysis using TALEN or CRISPR technology has been found to be effective when targeted to a specific unique sequence such that researchers have been able to make specific changes to the DNA sequence and replace full length dystrophin (Li et al., 2015). Advances in genetics utilizing other therapeutic procedures such as multiexon skipping therapy also show promise in the production of functional protein (Kole & Krieg, 2015; Nakamura et al., 2016).

**CNS involvement in the Dystrophinopathies**

Dystrophin is a very large membrane-bound cytoskeletal protein. Dystrophin comprises an integral part of a large dystrophin-glycoprotein complex (DPC). As well, there are several isoforms (protein variants) of dystrophin of different lengths and configurations. Different dystrophin isoforms are expressed in different cell types. Full-length dystrophin (Dp427) has been found in both muscle and brain. Additional dystrophin isoforms have been expressed in the retina (Dp260), kidney and CNS (Dp140), peripheral nervous system (Dp116), the brain (Dp40), and in a number of other tissues excluding muscle (Dp71) (Anderson et al.,
2012; Austin, Morris, Howard, Klamut, & Ray, 2000; Lidov & Kunkel, 1997; Tozawa et al., 2012).

In muscle, dystrophin is a protein that connects the cellular matrix to the subsarcolemmal actin cytoskeleton. The main role of dystrophin in the muscle cell sarcolemma has been shown to be stabilization and protection from enduring damage to the cell membrane with applied force. Functional studies have demonstrated that muscles deprived of dystrophin are more susceptible to damage from acute eccentric contractions. More recent evidence has found that dystrophin may also have a role in regulating various signaling pathways necessary for muscle function (Allen, Whitehead, & Froehner, 2016). Similar to its role in muscle tissue, dystrophin is part of the DPC in the brain where it has been localized to both neurons and glia and research has suggested that dystrophin plays a role in both the structure and functioning of synapses (Hendriksen et al., 2015; Perronnet & Vaillend, 2010).

Dystrophinopathy can result from a variety of mutations in the dystrophin gene resulting in the loss of, or decreased function of, the full-length dystrophin isoform normally found in muscle. The presence of disease variability is likely due to genetic modifiers, but still very much under scientific investigation (Anderson et al., 2012; Flanigan et al., 2009). Dystrophinopathy results from a variety of possible mutations in the dystrophin gene, and location of those mutations may selectively affect the production of different smaller dystrophin isoforms localized to specific cell types. Absence of those isoforms from brain tissue likely impacts CNS functioning.

Within animal models, research has found that dystrophin may have specific roles in the CNS including the anchoring of molecules for neuronal function (Lidov, 1996), aiding in the adaptation of the cytoskeleton, organization of receptor clusters on the membrane namely
GABA\textsubscript{A} and acetylcholine afferents (Cohen, Quarta, Fulgenzi, & Minciacchi, 2015; Lidov, Byers, Watkins, & Kunkel, 1990; Yoshihara, Onodera, Iinuma, & itoyama, 2003; Zaccaria et al., 2001), and stabilizing the postsynaptic neuron following maturation and synaptic plasticity (Brunig, Suter, Knuesel, Luscher, & Fritschy, 2002). Additionally, the dystrophin-glycoprotein complex has been implicated as a signaling complex that aids in cellular communication in the neuron (Anderson et al., 2012; Anderson et al., 2002; Muntoni et al., 2003). A lack of or insufficient amount of dystrophin may thus impact the nerve cell on multiple levels including the structure and functional organization of the membrane, ion channels and the synapse as well as signal integration and communication (Albrecht & Froehner, 2002; Anderson et al., 2012; Mehler, 2000; Vaillend, Billard, & Laroche, 2004).

This lack of dystrophin in the brain likely causes a cascade of functional abnormalities that impacts synaptic formation and the fine-tuning of synapses as the individual develops. These activity-dependent mechanisms important for learning, especially during critical periods of development, are likely altered by the disordered connections and structural anomalies present in the brain lacking dystrophin. Evidence from several animal studies suggests that the expression of dystrophin is developmentally regulated and different dystrophin isoforms are predominantly expressed in different brain regions and are likely to have a role in early fetal development (Sogos, Curto, Reali, & Gremo, 2002). Dystrophin has been linked to the development of neurons in the fetal cerebral cortex and cerebellar Purkinje neurons (Bardoni et al., 2000). The cellular and tissue expression of dystrophin isoforms is quite variable, depending largely on the disease mutations (Nichols, Takeda, & Yokota, 2015). Based on immunohistochemical studies of the brain in animal models of dystrophinopathy, dystrophin has been shown to be localized in post-synaptic densities in cerebral cortex, hippocampus,
cerebellum, and brainstem (Nichols et al., 2015). Within these structures, the evidence suggests that dystrophin localizes to specific cell areas. There is evidence of increased levels of dystrophin in post-synaptic structures (Gorecki, Lukasiuk, Szklarczyk, & Kaczmarek, 1998; Kim, Wu, & Black, 1995) specifically an abundance in Purkinje cells. (Kim, Wu, Xu, & Black, 1992; Lidov, Byers, & Kunkel, 1993; Lidov et al., 1990; Perronnet & Vaillend, 2010). Even more specific, dystrophin has been associated with GABAa channels within Purkinje cells in the mouse dystrophin knockout models (mdx) that has been shown to interfere with cellular communication (Anderson, Head, & Morley, 2003; Anderson et al., 2002; Knuesel et al., 1999). Structural cellular abnormalities lead to dysfunction including metabolic abnormalities (Lee et al., 2002) as well as irregularities in signaling impacting synaptic plasticity (Anderson et al., 2003; Anderson, Head, & Morley, 2004). The abnormal dystrophin expression alters nerve cell structure and function and ultimately influences brain circuitry within identified regions including cortical areas, hippocampus, and cerebellum.

Glucose hypometabolism indicating lowered synaptic activity has been found in positron emission tomography (PET) studies localized to medial temporal structures, bilateral cerebellum, and sensorimotor/lateral temporal cortex (Bresolin et al., 1994; Jueptner & Weiller, 1995; Lee et al., 2002). 1H magnetic resonance spectroscopy (MRS) in DMD patients, mdx mice and autopsy studies have shown elevated concentrations of choline compounds implying possible gliosis or developmental abnormalities (Kreis et al., 2011; Lee et al., 2002; Rae et al., 1998). Increased choline compounds were also found in cerebellar white matter in DMD patients (Kreis et al., 2011) on MRS. Abnormalities in electroencephalographic (EEG) activity across multiple studies have also been shown in DMD patients, suggesting possible ineffective neuronal function and communication (Anderson et al., 2002; Etemadifar &
This pattern of brain dysfunction likely defines the pattern of cognitive and behavioral impairments that can be observed in children with dystrophinopathy (Cohen et al., 2015; Perronnet & Vaillend, 2010). A few studies using magnetic resonance imaging (MRI) have found no obvious focal changes in individuals with dystrophinopathy (Bresolin et al., 1994), while others have found dilated ventricles and some cortical atrophy (al-Qudah, Kobayashi, Chuang, Dennis, & Ray, 1990; Septien et al., 1991). Recent imaging work has found reduced brain volume that is hypothesized to be the result of developmental abnormalities in the brain rather than atrophy. Global reductions in grey matter and microstructural changes in white matter were also found indicating some level of structural disorganization that the authors hypothesize indicate a level of whole brain dysfunction (Doorenweerd et al., 2014). A more recent study found microstructural abnormalities in the splenium of the corpus callosum that was positively correlated with verbal intelligence, suggesting a link to the cognitive deficits found in the disorder (Fu et al., 2016).

As previously noted, research has also been able to identify specific dystrophin isoforms related to CNS function. Loss of Dp427 has been associated with functional impairments in synaptic inhibition (Perronnet & Vaillend, 2010) as well as reductions in GABA_A receptors in hippocampus, cerebellum, and amygdala, likely impacting functioning in these areas including long-term plasticity (Anderson et al., 2004; Vaillend et al., 2004). Dp71, the most plentiful dystrophin product in the CNS, expressed in the hippocampus (Daoud, Candelario-Martinez, et al., 2009) and Dp140 which is mainly expressed in the fetal brain (Bardoni et al., 2000; Waite, Brown, & Blake, 2012), both isoforms have also been associated with greater cognitive impairments (Anderson et al., 2002; Bardoni et al., 2000; Taylor et al., 2010; Wingeier et al., 2011)
Thus, individuals with dystrophinopathy present clinically with genetically based cognitive and behavioral abnormalities. Dystrophin deficiencies influence how the brain develops its structures and functions and likely increases vulnerabilities to environmental insult. As a result of dystrophin abnormalities in the CNS, there may be a wide array of dysfunction in both neuronal structure and neuronal communication impacting brain development and cognitive and behavioral processes.

*Dystrophin gene mutation position and cognition*

There is growing empirical evidence of an association between mutation location and cognitive functioning. Mutations affecting shorter isoforms localized in distal portions of the gene have been shown to be more frequently linked to reduced intellectual functioning (Bushby et al., 1995; Bushby & Gardner-Medwin, 1993; Felisari et al., 2000; Moizard et al., 1998; Rapaport et al., 1991; Ricotti et al., 2016; Taylor et al., 2010), but the findings for an association to specific mutation site (Bardoni et al., 2000; Daoud, Angeard, et al., 2009; Moizard et al., 2000; Rasic et al., 2014; Wingeier et al., 2011) have been variable and there is a lack of evidence for specific types of cognitive deficits. Research has identified dystrophin isoforms as having a role in CNS function. Loss of Dp427 has been associated with functional impairments in synaptic inhibition (Perronnet & Vaillend, 2010) as well as reductions in GABA_A receptors in hippocampus, cerebellum, and amygdala, likely impacting functioning in these areas including long-term plasticity (Anderson, Head, & Morley, 2004; Vaillend, Billard, & Laroche, 2004). Dp71, the most plentiful dystrophin product in the CNS, expressed in the hippocampus (Daoud, Candelario-Martinez, et al., 2009) and Dp140 which is mainly expressed in the fetal brain (Bardoni et al., 2000; Waite, Brown, & Blake, 2012), both isoforms have also been associated with greater cognitive impairments (Anderson, Head, Rae, & Morley, 2002;
Bardoni et al., 2000; Taylor et al., 2010; Wingeier et al., 2011). Mutations that are more distal have been linked to the expression of these CNS dystrophin isoforms including Dp71 and Dp140. The frequency of intellectual impairment appears to be related to dysfunction within these isoforms (Daoud, Angeard, et al., 2009; Lenk, Hanke, Thiele, & Speer, 1993; Moizard et al., 2000; Taylor et al., 2010; Tuffery et al., 1995). A number of studies have examined abnormalities in Dp71, typically distal of exon 63, and severe intellectual impairments have been repeatedly reported to be related to this distal position (Daoud, Angeard, et al., 2009; Moizard et al., 2000; Rasic et al., 2014). Similarly, Dp140, localized to exon 44-45, has also been extensively examined and a strong association has been linked to cognitive impairments, but the extent of the impairments in performance has been more variable, but still deficient (Bardoni et al., 2000; Daoud, Candelario-Martinez, et al., 2009; Moizard et al., 1998; Wingeier et al., 2011). Ricotti et al (2016) recently examined the prevalence of cognitive and behavioral deficits within specific gene regions and found higher rates of intellectual disability and behavioral difficulties in individuals with mutations downstream. The group showed that 64% of participants with mutations affecting Dp71 had intellectual impairment compared to 25% with mutations influencing Dp140, and 15% with mutation affecting Dp427, the long dystrophin isoform. They were also able to show a strong association with impaired working memory and mutations disrupting shorter isoforms downstream (Ricotti et al., 2016). Most other studies have focused only on overall cognitive functioning in relation to mutation site without specifically examining individual cognitive domains. The prevalence of ADHD has been associated with mutations impacting Dp140 and Dp71 expression indicating that a risk of increased behavioral difficulties may also be related to downstream mutations (Pane et al., 2012). Thus, the frequency and severity of cognitive dysfunction as well as behavioral
problems appears to be related to mutations affecting Dp71 and Dp140, both downstream from exon 43. An examination of the relationship between mutation location and specific areas of cognition will only further help to clarify the strength of this relationship and may add to potential targets for therapeutic interventions.

**Cognitive phenotype in the dystrophinopathies**

The neuropsychological presentation observed in individuals with dystrophinopathy is likely related to the decreased level of dystrophin and dystrophin isoforms regulating neurological functioning, but a clear genotype-phenotype correlation for cognitive deficits has not been established; the assumption that specific mutations interfere with dystrophin production resulting in cognitive impairments based on an associated mutation position is still under investigation (Taylor et al., 2010; Vojinovic et al., 2015). The dystrophin gene encodes several different dystrophin isoforms specific to cell types hypothesized to be related to the presence or severity of cognitive difficulties (Waite et al., 2012). Additionally, the interaction of individual, developmental and social factors including family dynamics, education and socioeconomic status also need to be considered as plausible contributors to performance.

The identification of cognitive dysfunction in humans with dystrophinopathies can be traced back to early clinical descriptions of the disorder (Duchenne, 1868) and over time research has found more evidence of specific deficits within cognitive domains (Cotton et al., 2001; Snow, Anderson, & Jakobson, 2013). There is an increased prevalence of intellectual disability, with some studies demonstrating that 20% to 35% of individuals in the dystrophinopathy population exhibit global cognitive impairments (Bresolin et al., 1994; Cotton et al., 2001; D'Angelo & Bresolin, 2006; Snow et al., 2013). Notable impairments in individuals with DMD are consistent across studies and many researchers have reported a
distinguishable split between verbal and nonverbal processing with a higher degree of impairment on verbal measures. Meta-analyses also supported this discrepancy (Cotton, Crowe, & Voudouris, 1998; Cotton et al., 2001). Performance in academic areas is also reported to be at risk (Billard, Gillet, Barthez, Hommet, & Bertrand, 1998; Dorman, Hurley, & D'Avignon, 1988; Hendriksen & Vles, 2006; Hinton, DeVivo, Fee, Goldstein, & Stern, 2004), as is an increase in behavioral problems (Hendriksen & Vles, 2008; Hinton, Nereo, et al., 2006a; Poysky, 2007). Of note, these cognitive, and many of the behavioral, impairments appear to develop independently from the advancing motor declines and thus the cognitive phenotype in dystrophinopathy may be distinct from what is observed in other degenerative diseases (Billard et al., 1992). Most of the published studies in the dystrophinopathies utilize diverse measurements and methods to assess cognitive constructs and a number of studies employed measures with motor components that may have influenced performance scores. Additionally, the documented results are generally based on cross-sectional study designs and many studies draw conclusions using small samples with no comparison groups. The limitations of each study must be further evaluated before generalizations about the group can be made.

Global Conceptual Processing

Across studies of patients with dystrophinopathy, global intellectual abilities are reduced one standard deviation from the mean of the general population. Yet most children with dystrophinopathy have been shown to have an IQ within the normal range. Meta-analytic data from 1231 participants (age ranging from 2 to 27 years) with DMD in 32 published studies found that overall IQ estimates are normally distributed in the population but are shifted downward, with 35 percent of the sample falling in the intellectually disabled range.
The data also showed an overall significant group difference between verbal and nonverbal composites with affected individuals scoring lower on verbal measures (Cotton et al., 2001). This finding has been replicated across several subsequent studies (Cyrulnik, Fee, Batchelder, et al., 2007; Wicksell, Kihlgren, Melin, & Eeg-Olofsson, 2004; Young et al., 2008). The patients with dystrophinopathy have thus been shown to have more impairment in verbal areas than in nonverbal areas (Cotton et al., 2001; Hinton, De Vivo, Nereo, Goldstein, & Stern, 2000; Wicksell et al., 2004; Young et al., 2008), but there is much variability in the findings as to the degree of this discrepancy. This presentation has been generally based on composite scores of intellectual estimates that do not parse out individual weaknesses. Additionally, these research samples may be based on samples of convenience or individuals with clinical issues seeking treatment and thus may not be representative of the realistic level of functioning in the group. These findings are informative as to the degree of verbal weakness in DMD, given that performance composites are composed of motor and speed measures, and this population is at a disadvantage because of their progressing motor weaknesses. Despite an abundance of evidence supporting a split in conceptual processing, there are still conflicting findings reporting that some individuals show no difference between type of ability or that specific verbal abilities are comparatively better than nonverbal abilities (Marini et al., 2007; Wingeier et al., 2011). This discrepancy across the research may speak to age related changes or to the impact of changes in motor skills. Additionally, the variability in overall functioning may speak to an underlying specific deficit impacting overall cognitive function. Despite some conflicting evidence, there appears to be a significant trend across the literature of weaker conceptual abilities with differentiated verbal and nonverbal processing.
Global processing over time

To further characterize general intellectual functioning in the dystrophinopathies, it is important to address developmental changes in cognitive capacity given the progressive nature of the disorder. Unlike that observed in muscle, there appears to be no evidence for a progression of cognitive symptoms. However, most of the research is cross sectional comparing different age groups including very young children to adolescents and the data is even more restricted for older age individuals. To date, the longitudinal evidence is limited to support the stability or fluctuation of cognitive abilities over time in this population. Yet, age group comparative data has shown a pattern of stabilization of conceptual skills with age followed by possible improvement (Bresolin et al., 1994; Sollee, Latham, Kindlon, & Bresnan, 1985). Moreover, the verbal processing weaknesses seen early in childhood appear to lessen with time and eventually approximate age appropriate levels. Results from a large meta-analysis of cross sectional studies that included 1217 individuals with DMD provided evidence for possible improvements in intellectual functioning with increasing age of the participant. Analyzing five age groups (age 9 and younger, 9 to 11 years of age, 11 to 14 years of age, 14-19 years of age and 20 years and older), the compilation of data revealed that mean verbal processing scores increased with age. Also, the analysis showed a greater mean discrepancy of verbal and nonverbal processing in the younger age groups, whereas those aged 14 and older had smaller discrepancies. Contrary to previous findings of a decrease in performance abilities likely due to progressing motor decline, this combined data showed better performance in older affected individuals (Cotton et al., 2005). Additional cross sectional evidence from Hinton and colleagues assessing a group of 150 boys with DMD found overall improvements in both cognitive and behavioral performance across time. The research group also demonstrated these
improvements in a subsample of 20 boys with DMD who were followed longitudinally across three time points over a period of five years (Hinton et al., 2017). Thus based on the evidence, general cognitive skills appear to improve with age and the weaker verbal areas may mature and catch up to age appropriate levels. These findings do not support the previous theories that verbal improvements were more a function of declines in performance abilities due to motor constraints. In summary, within the dystrophinopathies, overall conceptual abilities have been found to be weaker than the general population paired with a discrepancy between verbal and nonverbal processing such that verbal skills are less developed than performance skills. However, this pattern of verbal weakness has been shown to be prevalent in younger affected children and with increasing age these deficits appear to lessen.

A major limitation in the current research includes limited longitudinal evidence to track the progression of cognitive skills; most current conclusions are based on age comparison studies. Additionally, it is important to note that most published studies focus on combined samples of both phenotypes of the dystrophinopathies (DMD and BMD). However, the frequency and extent of cognitive impairments in boys with dystrophinopathy is quite variable, so there also may be differences in processing within subtypes of the disorder. To date only a few studies have examined intellectual functioning in the population with BMD alone (Fee RJ & Hinton, 2016; Young et al., 2008). There are thus still many gaps in the current understanding of general intellectual functioning in the group with dystrophinopathy, including a lack of evidence for the trajectory of cognition as the disorder progresses as well as a characterization of the genotypic profile with the cognitive profile. Greater comprehensive knowledge of overall conceptual processing may aid in the understanding of the role of dystrophin in brain functioning; clarification of the functional role of the protein in the brain is
needed to provide a better understanding of possible disruption in neuroanatomical circuitry given the pattern of a weak capacity to process verbal or linguistic information.

**Attention and Processing Speed**

Attentional capacity is the most basic cognitive ability and is inherent in most cognitive processing. Attention can be referred as an allocation of resources that increase or in some cases decrease with situational and stimulus demands. Attention can be viewed in close relation with the speed of processing information. There are also different types of attention, including the ability to focus: (1) on one particular task (selective attention); (2) for a prolonged period of time (sustained attention); and (3) on many tasks at the same time (Petersen & Posner, 2012). Methods of assessing attention in clinical populations typically fall within routes of auditory or visual cueing. Attention is also often measured within the context of other cognitive domains.

In reviewing performance on verbal measures of attention by children with dystrophinopathies, basic attention is often simply assessed by short-term recall of a verbally presented string of digits. Poor performance on digit span is a constant finding across multiple studies, regardless of methodological approach. Boys with dystrophinopathy performed more poorly on digit span when compared to normative data (Dorman et al., 1988; Wingeier et al., 2011), controls matched for age and IQ (Anderson, Routh, & Ionasescu, 1988; Cotton et al., 1998; Wicksell et al., 2004), unaffected siblings (Hinton et al., 2001; Hinton, DeVivo, et al., 2004; Leaffer, Fee, & Hinton, 2016), or disabled peers (i.e., spinal muscular atrophy or cerebral palsy) (Billard et al., 1998; Billard et al., 1992; D'Angelo et al., 2011; Hinton et al., 2007; Ogasawara, 1989; Whelan, 1987). Moreover, performance on digit span remained the overall lowest score across intellectual level in DMD boys, such that it was relatively poorer
than other measures even among those with high intellectual levels (Hinton, De Vivo, et al., 2000). Thus, poor digit span is the most consistent finding across studies in the dystrophinopathies; however, the underlying cognitive construct contributing to this performance is still under debate.

Hinton and colleagues have argued that the poor digit span performance reflects a core deficit in verbal span, or decreased phonological storage, and that this deficit may have wide ranging detrimental effects on language and academic skill development among boys with dystrophinopathy, accounting for the more widespread language deficits seen in younger boys (Cyrulnik, Fee, Batchelder, et al., 2007; Cyrulnik et al., 2008; Hinton, DeVivo, et al., 2004). This hypothesis focused primarily on the phonological storage of the information, rather than on more generalized attention mechanisms. Hinton postulated the verbal span hypothesis based on evidence that boys with dystrophinopathy had average performance across measures of embedded attention such as list learning and visual learning tasks, findings suggesting span of attention was not compromised (Hinton, De Vivo, et al., 2000; Hinton et al., 2007), yet they did poorly on tasks of verbal contextual memory (Donders & Taneja, 2009; Hinton, De Vivo, et al., 2000; Hinton et al., 2001). Moreover, among school-aged boys with dystrophinopathy, performance on many language measures was found to be equivalent to that of controls, yet the boys with dystrophinopathy consistently did more poorly on measures requiring holding of verbal information, such as following multi-step commands (Hinton et al., 2001; Hinton et al., 2007), and sentence repetition, while the younger boys with dystrophinopathy had more widespread language deficits (Cyrulnik, Fee, Kiefel, Batchelder, & Hinton, 2006).

Others have suggested that the poor performance on digit span primarily reflects more of an executive deficit (Anderson et al., 1988; Cotton, 1998; Donders & Taneja, 2009; Mento,
In a recent publication, the Hinton lab examined performance on digit span in depth and again found that both digit span forward and backward are compromised in dystrophinopathy, suggesting that both verbal span and working memory may contribute independently to academic reading performance (Leaffer et al., 2016). Thus, although verbally presented information that requires attention has been shown to be an area of weakness in the dystrophinopathies, these deficits are not generalized to all measures within this route of administration; therefore, the underlying cause of these inefficiencies in processing needs to be further examined to rule out potential deficits in basic attention.

In terms of visual attention, only a few published studies have experimentally manipulated the construct in the patients with dystrophinopathy. Automatic, exogenous stimuli, and voluntary, endogenous stimuli, attention are commonly assessed to measure the orienting of attention or ability to focus attention. A deficit in voluntary orienting of visual attention was found in two studies of DMD using Posner’s computerized measure of attention when compared to typically developing peers. More specifically, DeMoura et al. (2009) compared performance on the Posner test with a sample of 25 children with DMD and a comparison group of mean similar age. Decreased reaction time and more inversion errors was noted in the group with DMD and the group found a much larger impairment in the younger children with DMD. The authors attributed the pattern of performance to a delay in the maturation of voluntary attentional systems. Assessing the same paradigm, Piccini et al. (2015) expanded on this work and assessed 20 children with DMD and compared performance to two control groups divided by age. When compared to the age matched sample, the group with DMD had significantly slower mean weighted reaction times when responding to a visual stimulus, but were not significantly different from the young (ages 6-9) control group. Both authors
suggested a delay in the development of both automatic and voluntary orienting attention systems as a potential contributor to the known cognitive difficulties in the population (De Moura, Valle, Resende, Reed, & Pinto, 2010; Piccini et al., 2014; Sollee et al., 1985). However, three major limitations should be considered before broad generalization can be made from these studies including the small sample size in both studies, utilizing reaction time as a performance measure that may have been influenced by participants’ motor deficits, and failure to control for overall IQ. Another study has supported the finding that young children with DMD have weaknesses in visual attention using a visual search task, yet among the group there was evidence of generalized deficits rather than selective findings across the battery of tasks given (Cyrulnik, Fee, Batchelder, et al., 2007). However, in another study of 14 children with DMD compared to 13 children with juvenile rheumatoid arthritis, visual attention (measured by selecting a visual target among distractors) and auditory attention (measured by identifying a target word among a list of rapid verbally presented words) were both found to be within normal limits, but given the small sample size and lack of validated dependent variables, any conclusions based on this finding is limited (Mento, Tarantino, & Bisiacchi, 2011a). The authors noted no difficulties in the selective attention of information in the group with DMD, but reported increased inefficiencies in more complex tasks of attention that required manipulation of the information (Mento et al., 2011a). However, this reference to deficits in complex attention and executive dysfunction was based on timed measures requiring motor execution, which may be impacted by the physical aspects of the disorder.

Processing speed measures cognitive efficiency and is related to the ability to process information quickly and automatically. Speed of processing is difficult to assess in this population due to the motor confounds in many of the measures of processing speed.
Variability in performance of complex attention was reported in individual ability to rapidly attend to and substitute symbols for numbers (Cotton et al., 1998; Whelan, 1987). Visual attention and processing speed determined by visual-motor sequencing tasks (Trail Making Test) (Wicksell et al., 2004) as well as a measure of rapid generation of visual patterns (DKEFS design fluency) (Donders & Taneja, 2009) was found to be poor in children with DMD. However, performances on these measures are likely impacted by declining motor abilities and may therefore not be an appropriate measure of attention and speed of processing within this population.

Thus, there appears to be conflicting evidence implying deficits in aspects of attention in the dystrophinopathies. Most of the research has not focused systematically on the construct of attention and its multiple constructs including sustained attention, and more complex attention such as divided attention. Although there is evidence of poor performance on tests of immediate verbal recall, a common measure of basic attention, and a suggestion of limited visual attention, research still needs to further examine the constructs of selective and sustained attention as well as more complex attention to definitively rule out an underlying attentional deficit within this population. There is thus still a need to explore specific cognitive attentional systems that may help to further characterize the cognitive phenotype in this disorder.

*Executive Functioning*

Executive functioning skills including cognitive flexibility, working memory, planning and goal setting are associated with higher order cognitive control processes that regulate the efficiency of performance in a number of cognitive domains. These skills, including attentional control, continue to mature well into early adulthood and are associated with the development of frontal brain regions (Spencer-Smith & Anderson, 2009). Executive function skills along
with attention are a network of interacting cognitive systems working together for cognitive efficiency.

Children with DMD have been found to have deficits in many executive areas, but there are discrepancies in results and confounding variables interfering with accurate measurement of this construct. Word retrieval (measuring efficiency in thought processes, flexibility and planning as well as response inhibition) has been shown to be impaired (Cotton et al., 1998; Donders & Taneja, 2009; Wicksell et al., 2004). However, in studies wherein overall IQ was employed as a covariate, there were no significant differences on performance on the executive measures between the groups (Donders & Taneja, 2009; Hinton et al., 2007). Deficits have also been documented in set shifting and the efficiency of strategic planning and problem solving (Mento et al., 2011a; Wicksell et al., 2004), with weaker performance across multiple measures evident within the DMD group, but individual differences in performance were not significant across many of the measures, suggesting overall IQ may have impacted the results. Additionally, motor deficits due to illness likely impacted the timed measures. In contrast, Hinton and colleagues found no deficit in abstract conceptual skills including mental flexibility as evidenced by average performance on the Children’s Category Test (Hinton et al., 2001). The evidence for deficits in executive areas is thus not clear and needs to be further evaluated especially since these skills are necessary for success in a number of other cognitive areas.

Working memory, a higher order cognitive process, has been shown to be impaired within this population. Most studies rely only on digit span performance as evidence of a deficit in this cognitive skill. Specifically, the mental manipulation of numbers backwards has been found to be impaired across studies (Billard et al., 1992; Hinton, De Vivo, et al., 2000; Hinton et al., 2001; Whelan, 1987). However, as recently shown by Hinton and colleagues,
forward span is also poor (Leaffer et al., 2016) within boys with DMD, suggesting that basic attention may also be compromised, contributing to the overall performance impairment. As previously noted, it has been proposed by a series of publications by Hinton and colleagues that the core cognitive deficit in the dystrophinopathy group is a limited verbal span. This hypothesis is based on the pattern of poor performance on digit span, weak story memory, and impaired sentence repetition suggesting a reduced capacity in linguistic load rather than a primary deficiency in executive functioning (Hinton et al., 2007). However, other studies have challenged that hypothesis and have found no deficits in backward digit span or immediate verbal recall, but did find difficulties on strategic planning, as measured by the Tower Test and the Rey Complex Figure Test (Mento et al., 2011a; Wicksell et al., 2004), rapid word retrieval measured by verbal fluency (Donders & Taneja, 2009; Mento et al., 2011a) and shifting measured by Trails B (Wicksell et al., 2004) suggesting a deficit in executive control. However, a number of these measures rely heavily on motor and time demands that likely impeded performance and thus may not be appropriate measurements of planning and organizational abilities in this population. Sample sizes were small in these studies and included participants of various ages with varying motor deficits. These studies also failed to adequately control for IQ in the analysis which may have impacted the results. Additionally, a number of these studies analyzed batched cognitive measures together, especially the executive functioning construct wherein the defined spectrum of what defines these skills is still ambiguous. Thus, the idea of deficiencies of executive skills in both verbal and nonverbal domains within this population remains to be thoroughly tested. Aspects of attention and executive functioning are embedded in a number of cognitive abilities and reduced capacity in
this area may impact individual capacity to develop other cognitive skills and interact with the environment for academic and behavioral/social learning.

Memory

Learning and memory performance in the dystrophinopathies has been shown to be variable with somewhat better processing of nonverbal than verbal information. As in other cognitive domains, there has been variability reported across studies, but aspects of memory are generally noted to be impacted in some way (Snow et al., 2013). Impaired immediate short-term verbal recall is the most common finding across the literature, but as previously stated this was typically measured by digit span (Cotton et al., 1998; Mento et al., 2011a; Ogasawara, 1989; Whelan, 1987; Wicksell et al., 2004) and the underlying cognitive construct impaired does not appear to be a true memory deficit but rather a limitation in verbal span, given the lack of impairment on other memory measures (Cyrulnik & Hinton, 2008; Hinton et al., 2001; Hinton et al., 2007). Immediate recall of rote verbal information was found to be within normal limits with intact learning with repetition (Hinton, De Vivo, et al., 2000; Hinton et al., 2001; Hinton et al., 2007; Wicksell et al., 2004). Long-term rote verbal recall and recognition was found to be at expected levels as well (Hinton, De Vivo, et al., 2000; Hinton et al., 2001; Hinton et al., 2007; Mento et al., 2011a; Wicksell et al., 2004). The learning and retention of contextual information (stories) were found to be impaired both on immediate recall (Cyrulnik et al., 2008; Hinton, De Vivo, et al., 2000; Hinton et al., 2001; Wicksell et al., 2004) and delayed recall (Donders & Taneja, 2009; Wicksell et al., 2004). But, the information that was encoded from the story was recalled over time (Donders & Taneja, 2009). Immediate repetition of sentences was also found to be an area of weakness especially as the length of the sentence increased (Hinton et al., 2007). The pattern of deficits across these verbal memory measures
have been hypothesized to be the product of a verbal working memory deficit likely due to limited phonological store as evidenced by poor group performance on measures of increased linguistic information (Hinton et al., 2001; Hinton et al., 2007) rather than a true memory deficit. A verbal memory deficit was also reported in DMD cases with mutations in distal portions of the gene, but these impairments were better explained by overall IQ variations (D'Angelo et al., 2011). Thus although aspects of verbal memory have been shown to be weak in several studies, the pattern of deficits with intact rote verbal memory do not indicate a clear memory deficit. To reiterate, replicated deficits in memory in the children with DMD appear to be restricted to verbal short term memory and contextual memory of large amounts of verbal information; this pattern of impairment for recall of linguistic information seems to suggest a limitation in verbal store rather than a memory impairment. This deficit may also reflect an inefficiency in the processing of verbal information.

Learning and memory for nonlinguistic information is reported to be variable across studies, but reported results are negatively biased by motor and potential socialization confounds. No differences were found between children with DMD and unaffected comparison groups on visual learning and picture memory (Hinton, De Vivo, et al., 2000; Hinton et al., 2001) or visuo-spatial memory when compared to a juvenile rheumatoid arthritis sample controlling for motor confounds (Mento et al., 2011a). Although when children with DMD were asked to recall a complex two-dimensional image, recall and retention were impaired (Wicksell et al., 2004): (1) this measure requires graphomotor construction, an ability that may be limited given the children’s motor weaknesses; and (2) no recognition component was given to control for motor deficits and to decipher if the information was actually encoded. Impairments have also been noted in the retention of nonverbal information with faces
(Donders & Taneja, 2009; Wicksell et al., 2004); however this may be a factor of the known deficits in socialization skills in the population (Hinton, Fee, De Vivo, & Goldstein, 2006; Hinton, Nereo, et al., 2006a) rather than a true visual memory deficit. Nonverbal memory appears to be intact; although the data for a nonverbal memory deficit is inconsistent and confounded. As with other areas, memory still needs to be further explored with larger samples and considering the impact of overall IQ, further examining differences among age groups, and utilizing measures that adequately assess the various components of memory. The results across studies suggest that the findings are not clear; the observed impairments appear not to be due to a true deficit in memory, but may signify weaknesses in broader attentional mechanisms needed to adequately perform the memory tasks.

**Language Processing**

Throughout the literature, linguistic processing is an area of established relative weakness when compared to nonverbal processing, with inefficiency noted in both expressive and receptive aspects of language. Overall, verbal processing is reported to be weaker than nonverbal processing in most comprehensive reviews of cognition in children with dystrophinopathy (Cotton et al., 2001; Snow et al., 2013). Documented delays in the attainment of language milestones (Karagan & Zellweger, 1978) have also been indicated as a predictor of later impairments in cognitive functioning (Cyrulnik, Fee, De Vivo, Goldstein, & Hinton, 2007). Generalized language deficits have been noted in studies of toddlers (Connolly et al., 2013) and pre-school age children with DMD (Chieffo et al., 2015; Cyrulnik et al., 2008; Sollee et al., 1985). Furthermore, reading and writing disabilities have been reported at elevated rates (Billard et al., 1998; Dorman et al., 1988; Hendriksen & Vles, 2006; Karagan, Richman, & Sorensen, 1980; Leibowitz & Dubowitz, 1981) in the population with DMD when
compared to the normal population. Dorman and colleagues (1988) found that half of the older children with DMD aged 10 to 19 had deficits in reading and phonological processing (Dorman et al., 1988). Another study with 25 males with DMD found that 40% of the participants had moderate to severe reading problems (Hendriksen & Vles, 2006). In assessing a group of 24 children with dystrophinopathy, there was an increased frequency for reading (21%), spelling (32%) and arithmetic (26%) learning disabilities that were significantly higher than that found in the general population (Young et al., 2008). Additionally, it has been shown that children with dystrophinopathy are at risk for poor academic achievement across academic areas with a weakness for verbally presented material (Hinton, DeVivo, et al., 2004). Despite the small samples of children with DMD used in these studies, these studies have shown that there is an increased risk for the development of a specific learning disability within the population, with higher risks for reading disabilities.

Crystallized aspects of language have generally been found to be within normal limits for most children with DMD (Billard et al., 1992; Bushby et al., 1995; Dorman et al., 1988; Hinton, De Vivo, et al., 2000). Acquisition and use of grammar, language production, and understanding verbal concepts have been documented to develop appropriately in the children with DMD (Hinton et al., 2007). However, expressive language delays have been noted in young children with DMD and typically these children are referred for early speech interventions (Cyrulnik, Fee, Batchelder, et al., 2007; Cyrulnik, Fee, De Vivo, et al., 2007; Kaplan, Osborne, & Elias, 1986). Word retrieval issues have been described as well with deficiencies in confrontation naming (Dorman et al., 1988), reduced rapid naming (Astrea et al., 2015), and restrictive fluency with semantic cueing (Donders & Taneja, 2009). However, a number of other studies have documented relatively intact naming (Billard et al., 1998; Hinton
et al., 2001; Marini et al., 2007) and semantic verbal fluency (Billard et al., 1998; Hinton et al., 2001; Marini et al., 2007). Expressive language difficulties thus may be related to the early delays in language attainment; as language skills and vocabulary improve over time, so does individual ability to express ideas and concepts.

Circumscribed deficits in receptive language are the most prominent finding in the literature with evidence of elevated difficulties when linguistic demand increases (Billard et al., 1998; D’Angelo et al., 2007; Hinton, De Vivo, et al., 2000; Hinton et al., 2001; Hinton, DeVivo, et al., 2004; Lorusso et al., 2013), but as previously described the issues may indicate limited linguistic capacity rather than a language disorder. Evidence of impairments in sentence repetition (Hinton et al., 2007) as well as syntactic comprehension have been well documented (Hinton, De Vivo, et al., 2000; Hinton et al., 2001; Lorusso et al., 2013); this specific processing difficulty may be partially related to the well-established deficits found in verbal working memory (Snow et al., 2013). Comprehension deficits due to increasing auditory working memory load were most evident in individual performance on the Token Test, wherein difficulties were present following lengthy and more complex commands (Hinton et al., 2001). Deficiencies in phonological processing and morphosyntactic processing (Billard et al., 1998; Dorman et al., 1988; Fabbro et al., 2007; Hinton et al., 2001) are also consistently cited in children with DMD and have been noted to impact reading (Astrea et al., 2015). Children with DMD were reported to perform significantly worse on measures of phonological awareness and phonological memory, data suggesting a phonological processing deficit (Hinton, 2005; Hinton, Fee, & Stern, 2000).

Reading skills in children with DMD are below expectation. Hinton and colleagues demonstrated that reading performance was significantly lower than a nonverbal IQ measure in
the group with dystrophinopathy, but not among their unaffected sibling controls (Hinton 2004). Speech narratives of boys with DMD had limited content and deficits were described at the sentence level, as well as within the context of more complex linguistic composition (D’Angelo et al., 2007; Marini et al., 2007). Reading difficulties are reported to be much more common in samples of children with DMD (Billard et al., 1998; Hendriksen & Vles, 2006). In a recent study, 42 percent of boys with DMD had disabilities in both reading and spelling (Astrea et al., 2015). Reading speed and accuracy was deficient in a group of children with DMD as well as deficits in text decoding and spelling, findings further illustrating deficits in phonological retrieval. This pattern of deficits was compared with the deficits observed in those diagnosed with developmental dyslexia and revealed significant similarities, but decreased severity in the children with DMD (Astrea et al., 2015); this evidence supports a compromised language profile in individuals with DMD such that the pattern of linguistic deficits mirror those found in developmental dyslexia.

In summary, linguistic weaknesses have been well defined across the neuropsychological literature examining dystrophinopathy. Additionally, there is some evidence of age related improvements in regards to linguistic deficits, but the research is inconsistent and based on cross-sectional studies (Cotton et al., 2005). Early language delays are prominent, and both receptive and expressive aspects of language appear susceptible to deficiencies that likely increase with linguistic complexity. Limitations in individual capacity to process linguistic information likely interact with other cognitive domains and adversely impact complex language based everyday cognitive functioning. The interaction of impairments in phonological processing and reading deficits in DMD are similar to those found
in developmental dysphonetic dyslexia (Astrea et al., 2015; Hinton et al., 2007; Mento et al., 2011a).

**Visuospatial Processing**

The majority of the evidence in the literature reports that visuospatial processing appears to be the least affected cognitive domain in children with DMD. It has been consistently revealed across the dystrophinopathy research that visuospatial index scores are higher than verbal IQ scores with some degree of variability (Cotton et al., 2001; Snow et al., 2013). When compared to unaffected controls, children with DMD typically performed similarly to their peers on measures of visuospatial skills (Cotton et al., 1998; Dorman et al., 1988; Hendriksen & Vles, 2006; Hinton et al., 2001). However in line with other domains of functioning, visuospatial skills have been found to be weak in the younger populations with DMD (Cyrulnik et al., 2008). Yet, spatial processing has been found to be relatively intact in children with DMD as they get older (Hinton, De Vivo, et al., 2000; Hinton et al., 2001; Mento et al., 2011a). Deficits have been reported in nonverbal tasks of attention (De Moura et al., 2010; Piccini et al., 2014) and also nonverbal executive functioning (Donders & Taneja, 2009; Wicksell et al., 2004); however: (1) visuomotor abilities were needed in those described executive measures, (2) studies had small sample sizes; and (3) this research did not control for IQ, introducing potential confounds that question the results. Thus, visuospatial processing is noted across the majority of studies to remain relatively intact in individuals with dystrophinopathy. Visuospatial performance impairments may be due to deficits in other cognitive domains of functioning and not reflective of visuospatial processing in isolation.
**Academic Achievement**

Performance on academic measures in the dystrophinopathies generally is proportionate to general conceptual processing. Across most of the research assessing academics, children with DMD generally fall below expected age and grade levels (Billard et al., 1992; Hendriksen & Vles, 2006; Hinton et al., 2001; Hinton, DeVivo, et al., 2004; Leibowitz & Dubowitz, 1981; Worden & Vignos, 1962). There are likely environmental contributors that need to be considered for the decreased performance such as educational placement, school setting, neighborhood and the psychosocial stressors of having a physical disability, but these factors do not fully explain the observed cognitive profile. Based on the established weakness in linguistic processing, it would be expected that a significant discrepancy exists in skill development, with somewhat weaker performance in verbal areas. Surprisingly, computational math skills were found to be weak in the children with DMD when compared to unaffected siblings (Hinton et al., 2001; Hinton, DeVivo, et al., 2004). After controlling for general intellectual functioning, performance on mathematics still remained poor. Like other academic subjects, math requires subskills such as an understanding of facts and an ability to recognize and relate quantities, requiring some degree of verbal and auditory learning. The difficulties in mathematics in the population with DMD needs to be further explored to determine the types of errors whether there is a deficit in mastering math facts, deficits in understanding the language and symbol system of math, a calculation weakness or a contributing visual spatial deficit. It will be important to decipher if the types of errors made by children with dystrophinopathy further reflect an underlying verbal weakness rather than a true math disorder in the population. Overall, when academic composite scores were compared to nonverbal IQ scores, the academic scores of children with DMD were depressed compared to their
unaffected siblings. After examining relative contributions to academic achievement performance, Hinton and colleagues (2004) found that individual intellectual functioning and performance on the digit span subtest were the most influential (Hinton, DeVivo, et al., 2004). This finding is consistent with other studies demonstrating that academic scores were predicted by digit span and arithmetic scores (Billard et al., 1998) and that digit span or verbal working memory performance is critical to the acquisition of mathematical and language skills (Helland & Asbjørnsen, 2004).

A review of reading performance in the dystrophinopathies was previously addressed with an increased rate of reading disabilities and delays in developing reading skills. Difficulties in comprehension with increasing syntactic complexity, reading speed and accuracy, and phonological processing deficits have been identified in several studies (Astrea et al., 2015; Billard et al., 1998; Dorman et al., 1988; Fabbro et al., 2007; Hendriksen & Vles, 2006; Hinton, 2005; Hinton, DeVivo, et al., 2004). In academic reading measures, scores on the decoding of nonwords (Billard et al., 1998; Dorman et al., 1988; Hinton, DeVivo, et al., 2004), single word reading and passage comprehension (Hinton, DeVivo, et al., 2004) were reduced when compared to control groups. The performance across language based measures provided further evidence for a linguistic disorder with underlying phonological processing deficits that appear to be similar to a developmental dyslexia (Astrea et al., 2015; Billard et al., 1998; Hinton, DeVivo, et al., 2004). Spelling was also found to be poor in the children with DMD (Dorman et al., 1988; Hinton, DeVivo, et al., 2004). In 26 boys with DMD, Hinton and colleagues demonstrated that both IQ and verbal span contributed significantly to academic performance, but performance on an executive task did not, nor did parental ratings of the children’s behavior. In a later, larger study by Hinton and colleagues, the contribution of both
forward and backward digit span was shown to be associated with reading performance (Leaffer et al., 2016). Overall, across studies there appears to be a generalized weakness in academic measures in children with DMD that is likely due to the contribution of specific cognitive weaknesses impacting the multiple skills needed for success in academic subjects. Hinton has hypothesized that the core deficit in verbal span may contribute to performance in academic areas. She proposed that because a great deal of material across subject areas is verbally presented, individuals may not grasp necessary concepts for academic success (Hinton, DeVivo, et al., 2004). Yet the role of specific attentional measures on academic performance has not been investigated, and appears to reflect a major gap in the research literature, as poor attentional control has been shown to be associated with poorer academic achievement in general (Biederman et al., 2004; Stevens & Bavelier, 2012).

*Motor skills*

Motor skills are expected to decline with age in the dystrophinopathies given the bilateral progressive muscle weakness in a proximal to distal direction. Reduced muscle functioning occurs early in development by the toddler and preschool ages and strength declines rapidly by the second decade of life (Bushby et al., 2010a; Connolly et al., 2014; Connolly et al., 2013). Lower extremities are usually the first to be affected, with greater gait changes and utilization of compensatory movements as the disease progresses (Martini et al., 2015). Connolly and colleagues (2014) assessed motor functioning in infants and toddlers with dystrophinopathy and discovered that gross motor skills were poor and matured less with age relative to peers (Connolly et al., 2013); additionally, the researchers found after a one year follow-up that these motor skills declined even at this very young age (Connolly et al., 2014). In another study, 30 percent of the children with DMD were delayed in ambulation, with an
inability to walk by 18 months of age, and 8 percent did not reach ambulation until 24 months and later (Sarrazin et al., 2014). Motor constraints may impact individual ability to learn, develop certain perceptual skills and may even impact behavior and socialization. One study found similar perceptual learning in children with DMD when compared to controls as measured by mirror-tracing and rotary pursuit tasks (Nakafuji & Tsuji, 2001). Fine motor speed and dexterity were found to be weak in younger children with DMD; walking attainment delays were found to be associated with impaired reasoning, but not receptive vocabulary (Cyrulnik, Fee, Batchelder, et al., 2007). Poor motor skills have been associated with cognitive impairment in children with specific learning disorders (Bishop, 2002; Hill, 2001) and thus this evidence in boys with DMD may further speak to the interplay of motor and cognitive skills and the impact of the absence of dystrophin in specific brain regions. One interesting study tested the planning and execution of movement in children with DMD and discovered that the children with DMD did worse on the execution of meaningful movements when compared to individuals with juvenile rheumatoid arthritis. The study found impairment in the planning of motor sequences with poorer performance when asked to imitate a sequence than when given a verbal command. The authors suggest a type of apraxia due to a failure in retrieval of the sequence of movements in which performance may actually be aided by linguistic and semantic cueing (Mento et al., 2011a). However, performance may be more related to progressive motor weakness than a motor planning deficit given that motor weakness has been shown to be present even in very young children and motor skills have been shown to worsen over time (Connolly et al., 2014). Sensorimotor functioning has yet to be thoroughly explored in this population to assess for changes as the disorder progresses. There is limited research examining the interaction between motor and cognitive processing. Further evaluation is thus
needed to explore the synergistic effects of deficits in motor and cognitive development in the
dystrophinopathies.

*Neuropsychiatric presentation in the dystrophinopathies*

As with the cognitive phenotype, there is a spectrum of both internalizing and
externalizing symptoms in children with dystrophinopathy with some reaching clinically
significant levels. Pathological presentation is quite variable and, as with any population,
influenced by a number of individual factors including biological, psychological, and social.
According to pediatric epidemiologic studies, 15 to 30 percent of children with chronic
medical conditions have been shown to be at an increased risk for developing mood and
behavioral pathology (Glazebrook, Hollis, Heussler, Goodman, & Coates, 2003; Hysing,
Elgen, Gillberg, & Lundervold, 2009; Lavigne & Faier-Routman, 1992). Similar outcomes of
psychological adjustment are shared by many chronic illnesses despite different characteristics,
trajectories, and treatment (Hysing, Elgen, Gillberg, Lie, & Lundervold, 2007; Wallander &
Varni, 1998). Within the children with dystrophinopathy, the most relevant risk factors include
age, the stage of disease progression, cognitive functioning, and environmental influences
including family, socioeconomic status, and education. Individuals with neuromuscular
disorders are thus highly susceptible to significant changes in mood, behavior and changes
within family functioning. Nonetheless, the data suggest that there may well be behavioral
symptoms that are related to the underlying etiology, in addition to those that are reactive
responses to living with the disorder.

*Mood*

Studies assessing significant changes in mood in children with dystrophinopathy are
few in number. Past research found increased prevalence of emotional difficulties as well as
social isolation, but there was a great deal of variability within the DMD samples studied (Firth & Wilkinson, 1983; Harper, 1983; Leibowitz & Dubowitz, 1981; Thompson, Zeman, Fanurik, & Sirotkin-Roses, 1992). For example, one study documented higher rates of depressive disorders in older boys with DMD when compared to an unaffected control group. Using clinical scales filled out by parents, teachers, and an interview with a psychiatrist, 52 percent (out of 12 children) of the older boys with DMD met criteria for a depressive disorder. A common finding amongst the parent and teacher reports was that poor peer relationships was a significant contributor to behavioral disturbance (Fitzpatrick, Barry, & Garvey, 1986). However, many of these earlier studies based findings on small samples and subjective information reported by caregivers. As well, the studies are somewhat dated, as there were fewer support systems in place for the families and children with the disorder in the 1980s than there are currently.

More recent studies have found better emotional outcomes in the population. For example, in a large study of 287 boys with dystrophinopathy, behavioral adjustment using a standardized parent completed measure, Personal Adjustment and Role Skills Scale (PARS-III), Hendriksen et al. (2009) found that when compared to other chronic conditions, most children with DMD were actually well adjusted with decreased depressive and anxious symptoms, but around 17% of boys had more problems with peer social relationships. Boys between the ages of 8 and 10 had more adjustment problems and the significant interpersonal difficulties increased with age (Hendriksen et al., 2009; Poysky, 2007). In another study, 17% of a sample of 181 boys with DMD were rated by their parents as having increased anxiety and depressive symptoms and the elevated rates were much higher in the older children, but the group was not statistically different from the sibling comparison group (Hinton, Nereo, et al.,
No depressive symptomology for the group has been found in other studies, although some individuals do report it (Nereo & Hinton, 2003). Increased rates of obsessive-compulsive disorder and other anxiety-related disorders have also been documented indicating possible susceptibility for anxiety disorders (Filippo, Parisi, & Roccella, 2012; Hendriksen & Vles, 2008; Poysky, 2007), but these data were based on parental report of psychiatric diagnosis in a questionnaire. Although the data are limited and often based on subjective information, there is some evidence for an elevated risk for internalizing problems as children age and experience more physical disability. Mood symptoms may be a reactive response to the progressing declines associated with the disorder, but for the most part children with DMD appear to be overall well adjusted. A commonality discovered among the studies, however, is that psychosocial factors appear to be a large contributor to maladjustment in this population. More evidence assessing the psychological adjustment of the older boys with the disorder beyond parental reports would provide knowledge regarding mood changes that may contribute to cognitive as well as overall functioning.

**Behavior**

**Social**

The evidence for behavioral problems in the dystrophinopathies is much more consistent and abundant in the literature. Higher rates of deficits in social functioning have been seen across studies (Donders & Taneja, 2009; Fitzpatrick et al., 1986; Hendriksen et al., 2009; Hinton & Fee, 2000; Hinton, Nereo, et al., 2006a). Across age groups and type of dystrophinopathy, social problems have been reported. Both children with DMD and BMD showed significant impairments in social interactions and communication; socialization skills have been described as a weakness in this population (Darke et al., 2006). Similarly, Hinton et
al. (2006) found in a large sample of 181 boys with DMD that one third of the sample had significant social difficulties when compared to unaffected siblings, a cerebral palsy comparison sample, and the normative population. Socialization difficulties were also found to be much more prevalent in the younger sample with DMD whereas symptoms of depression and anxiety were higher in the older children (Hinton, Nereo, et al., 2006a; Poysky, 2007). However, an earlier study with an adolescent sample with DMD found that the psychosocial problems persisted even at older ages and the adjustment problems were related to stress levels reported by family members (Reid & Renwick, 2001). Children with BMD have also been shown to present with more behavioral problems than other neuromuscular disorders (Darke et al., 2006). When other chronic conditions were compared to children with DMD, social difficulties were still found to be much more common in the children with DMD and the rate of social problems increased with age (Birnkrant, Bennett, Noritz, & Birnkrant, 2011; Hendriksen et al., 2009).

Related to the socialization deficits, higher rates of autism spectrum disorders have been documented in children with DMD when compared to the general population. Rates ranging from 3% to 19% of children with DMD have been shown in both clinic and research samples to reach clinical criteria for a spectrum disorder (Banihani et al., 2015; Hendriksen & Vles, 2008; Hinton, Batchelder, Cyrulnik, Fee, & Kiefel, 2006; Hinton et al., 2009; Wu, Kuban, Allred, Shapiro, & Darras, 2005). Several case studies have also documented symptomology in children with DMD that meet a diagnosis for spectrum disorder, including impairments in language, social interactions and even stereotyped behaviors (Komoto, Horikawa, Nakao, & Shibata, 1986; Komoto, Usui, & Hirata, 1984; Zwaigenbaum & Tarnopolsky, 2003). In a small study, 38% of boys with BMD also met criteria for a spectrum
disorder diagnosis (Darke et al., 2006). A number of spectrum type symptoms were analyzed and it was found that children with dystrophinopathy had significant difficulties with reciprocal conversation and social interactions whereas stereotypical behaviors were less prevalent (Hinton et al., 2009). It is evident across studies that many children may not meet full criteria for a spectrum disorder, but many do fall on a continuum of spectrum disorder symptoms with poor socialization, withdrawal, language delays, and even mild repetitive or restrictive behaviors. These deficits in socialization skills are an area that needs further study to determine the possible relationship with the verbal cognitive weaknesses and to further investigate the possible connection to dysfunction in neural networks.

*Attention and Executive Behavior Problems*

Attentional behavior problems are typically defined by a persistent pattern of inattention and/or hyperactivity in multiple settings usually described by parents, caregivers or teachers. A diagnosis of an attentional behavioral disorder is usually based on parental and teacher report on clinical behavior inventories that assess symptomology. The most common behavioral pathology reported in the dystrophinopathies is attention deficit hyperactivity disorder, with a reported rate of 12% to 50% (Banihani et al., 2015; Hendriksen & Vles, 2008; Pane et al., 2012; Steele et al., 2008). In two studies, one of 59 (Banihani et al., 2015) and one of 103 (Pane et al., 2012) boys with dystrophinopathy, an ADHD diagnosis was based on assessment with the Diagnostic and Statistical Manual IV (DSM-IV) criteria paired with elevated ratings on the Conners rating scales completed by both parents and teachers (Banihani et al., 2015; Pane et al., 2012). In 38 of the 103 patients with dystrophinopathy (37%), ADHD was diagnosed, with 16 having more inattention than hyperactivity problems and 19 meeting
criteria for a combined presentation (Pane et al., 2012). Similarly, 32% of the 59 boys with DMD met criteria for ADHD in the other study (Banihani et al., 2015).

The Schedule for Affective Disorders and Schizophrenia for School Age Children (KSADS), a clinical interview, along with parental report using the Conners and Child Behavior Checklist was used in a small sample of 10 children and adolescents with dystrophinopathy who were randomly recruited from a center for children with special needs; the study found that half of the sample met criteria for ADHD, but results may have been influenced by ascertainment bias given that the sample was selected from a clinic population seeking various interventions (Steele et al., 2008). Another large study of 351 boys with DMD based the ADHD diagnosis on parental report of a formal diagnosis made by a medical professional (Hendriksen & Vles, 2008). Again an increased prevalence of ADHD was found in 12% of the sample as reported by parents. Limitations to this study, however, included the method of report of diagnosis of ADHD based on parental report, which may have been impacted by subjective opinion of behavioral problems, rather than a formal diagnosis. A sample of 181 children with dystrophinopathy found that 24% were rated high on the Child Behavior Checklist attention scale suggesting clinically significant levels of problems, and the rates were higher than those endorsed by unaffected sibling controls. However, when this group was compared to a sample with cerebral palsy, the findings were not significantly different (Hinton, DeVivo, et al., 2004).

Some of this evidence has attributed symptoms of ADHD to the corticosteroid treatment in patients with DMD (Merlini et al., 2003; Mesa, Dubrovsky, Corderi, Marco, & Flores, 1991), but others have found no significant relationship between the pharmacological treatment and behavior changes (Banihani et al., 2015; Fee & Hinton, 2002; Hendriksen &
Vles, 2008; Pane et al., 2012). Although based on various methods of clinical assessment across studies, there appears to be an elevated risk for an attentional disorder within the children with dystrophinopathy.

Also, parent responses on the Behavior Rating Inventory of Executive Functioning (BRIEF), a measure of executive skills in everyday life, indicated in one study that the children with DMD had higher ratings of executive difficulties when compared to unaffected siblings, but these differences were only significant on the Shift Scale (measuring flexibility and resistance to change), and none of the children received ratings in the clinical range (Donders & Taneja, 2009). Similar findings on the BRIEF were found in 25 children with dystrophinopathy when compared to matched sibling controls with elevated ratings on the shift and planning/organization scales (Kiefel, Batchelder, Fee, & Hinton, 2006). Hinton and colleagues also found that parental report of executive dysfunction on the BRIEF did not statistically distinguish performance on standardized clinical measures of working memory and/or executive functioning in boys with dystrophinopathy. However, boys rated with clinically elevated BRIEF scores did exhibit poorer performance across the executive cognitive measures (Fee, Leaffer, Vega Villar, & Hinton, 2016).

Other behavioral scales have had difficulty measuring behavioral symptoms given physical limitations, cognitive difficulties, and everyday environmental stressors experienced by children with DMD (Poisky, 2007); thus, the diagnostic numbers may be an inaccurate representation of the sample. As noted earlier, the pattern of cognitive deficits found in boys with DMD suggests underlying attention and/or dysexecutive difficulties that may significantly interfere with optimal functioning (S. Cotton et al., 1998; De Moura et al., 2010; Donders & Taneja, 2009; Wicksell et al., 2004). The combination of cognitive performance and behavioral
ratings of inattention and executive difficulties may further be indicative of an overarching deficit in these areas, resulting in greater impairments in overall functioning.

**Quality of Life**

Despite the increased rates of mood and behavioral difficulties in children with dystrophinopathy, it should be noted that the majority of affected children are well adapted and do have a positive outlook. Nearly 80 percent of the sample with DMD were reported by their parents not to have behavioral problems or social skills deficits (Hinton et al., 2009; Hinton, Nereo, et al., 2006a; Nereo & Hinton, 2003). As the disorder progresses and individuals become wheelchair dependent, quality of life has been shown to decrease (Baiardini et al., 2011; Wei, Speechley, Zou, & Campbell, 2016); however, other studies have shown that the majority of the children with DMD even as they age to levels of severe physical disability generally reported positive affect and good ratings of life satisfaction (Bach, Campagnolo, & Hoeman, 1991). Additionally, a good quality of life has been reported amongst adult patients with DMD (Elsenbruch, Schmid, Lutz, Geers, & Schara, 2013; Rahbek et al., 2005). Thus, although the disorder is devastating with progressing physical limitations as well as associated cognitive deficits, the majority of individuals with DMD are quite resilient. It was found that familial and social factors contributed the most to positive adaptation in boys with DMD whereas disease progression and estimated IQ did not. The more socialized the individual and the less stress within the family made the greatest impact on positive outcome (Fee & Hinton, 2011). To conclude, children with dystrophinopathy are at risk for mood and behavioral issues as in a number of chronic conditions, but most individuals have been reported to be well adjusted and family and friendships have been shown to have the largest impact on improved quality of life outcomes.
**Summary and Discussion**

Dystrophinopathy is linked to relatively average general intellectual functioning paired with poor reading abilities and weakness in the capacity to process verbal/linguistic information (deficits in verbal/digit span, verbal working memory, and following lengthy verbal instructions) (Billard et al., 1998; Dorman et al., 1988; Fabbro et al., 2007; Hinton, 2005; Hinton, DeVivo, et al., 2004; Hinton et al., 2007). This inability to attend to larger amounts of information is illustrative of how attention difficulties could contribute to functional communication difficulties. A basic level of attention is required for successful performance on most cognitive tasks. Although other aspects of cognitive processing have been shown to be relatively intact, the DMD literature is far from consistent and could be potentially explained in part by variability in attentional control. Because attentional processing contributes to most if not all cognitive domains, it is still unclear whether compromised attention underlies evident deficits due to potential confounds: (1) small sample sizes; (2) lack of comparison samples; (3) inclusion of participants of various ages with varying motor deficits; (4) failure to control for IQ; (5) batched cognitive measures to assess executive functioning; and (6) failure to control for motor deficits. Moreover, interpretation of cognitive findings may have neglected to account adequately for the role of attentional mechanisms. For instance, deficits in auditory comprehension may in fact reflect an inability to attend to larger amounts of information. Also, true deficits observed may be an interaction of combined attentional, executive and language difficulties resulting in more significant dysfunction in complex language-based cognitive functioning than would be predicted by a decreased verbal span in isolation. These interacting weaknesses likely interfere with individual capacity to complete functional activities including the capacity to follow multistep
commands, plan and organize ideas and succeed in academic activities. They may combine to be the underlying basis for the reading difficulties observed in this population (Astrea et al., 2015). Working memory has been shown to predict reading attainment and impairments in working memory have been associated with learning difficulties in literacy (Gathercole, Alloway, Willis, & Adams, 2006; Gathercole & Pickering, 2000). Increased load in academic settings is hypothesized to impose large demands on working memory and a limited capacity in working memory thus results in poor academic performance (Gathercole et al., 2004). What is evident from most studies of cognition in individuals with dystrophinopathy is that nonverbal processing and visuospatial skills appear to be relatively intact across measures and may be a relative strength in the population, but discrepancies still remain. These include evidence of a possible visual attention deficit as was demonstrated by reduced reaction time and errors in attending to voluntary, endogenous visual stimuli (De Moura, Valle, Resende, Reed, & Pinto, 2010; Piccini et al., 2014). However, the interpretation of these deficient visual attention results are limited given potential confounds noted previously. Thus, these studies do not sufficiently evaluate functioning in attentional systems, but do provide evidence for a potential weakness in attention that needs to be further explored in the dystrophinopathies.

Attention has thus been shown to be weak in both verbal and nonverbal areas suggesting that an attentional impairment may exist in individuals with dystrophinopathy. Learning and memory also has been found to be variable with better performance on nonverbal measures. Studies have demonstrated adequate individual ability in the encoding and retrieval of rote verbal information, but memory for contextual information has been shown to be weak which may reflect either a proposed limitation in phonological load, or a limitation in attending
to more complex stimuli, or a combination of the two. More carefully designed studies are needed to tease out the cognitive contributions to task performance.

With respect to mood and behavior, children with dystrophinopathy have an increased risk for depressive symptoms and anxiety especially as the disease progresses, which is not unexpected given the increasing frequency and severity of health stressors. Psychological difficulties may contribute to functional disability. Emotional distress and anxiety can influence an individual’s ability to attend to information, but are not likely the sole contributor to the pattern of deficits in the population. Children with dystrophinopathy are also at increased risk for attentional and executive behavioral problems with an increased prevalence of ADHD when compared to the general population. It is important to note that the aforementioned cognitive and behavioral issues are likely not separate entities but rather aspects of a unique neuropsychological profile reflecting individual strengths and weaknesses interacting with the effects of the illness.

The bases for these neuropsychological findings are however incomplete as fundamental cognitive processes have yet to be adequately explored. The effect of the disorder on every cognitive domain is still not clear because basic cognitive functions such as attention have not been fully examined. Multiple aspects of attention are inherent in most of the cognitive tasks presented. The ability to selectively receive and process incoming information is a necessity for successful performance on any cognitive task. The evidence for poor performance on a basic passive span task (digit span) across studies suggests the possibility of an inability to allocate resources to stimuli especially when the volume of information presented increases. There is also evidence that voluntary attention systems are compromised on visual attention tasks in this population, but too many limitations due to methodological
confounds prevent generalizability. Thus, selective attention, the voluntary ability to focus cognitive resources, may well be impaired in this population impacting performance in other cognitive areas.

The experimentation of selective attention in children with dystrophinopathies is quite limited. For example, the effect of task-irrelevant stimuli on performance has not been carefully investigated. Similarly, complex aspects of attention have also not been explored, including the ability to maintain attention over time. Sustained attention is inherent in a number of the utilized cognitive measures in the research especially with long testing periods, but this also has not been examined as an independent construct. Orienting to sensory stimuli, achieving and maintaining alertness, as well as selecting among conflicting stimuli are all attentional mechanisms that have not been adequately explored within the population.

Attention is also closely associated with executive functioning and working memory. The construct of attentional control has been defined in multiple models as an aspect of executive functioning and also working memory capacity (e.g., McCabe, Roediger, McDaniel, Balota & Hambrick, 2010). Selective attention and working memory have been viewed as distinct constructs, but studies have also found significant overlap between these cognitive abilities. Attentional control is particularly important for the active maintenance of information when faced with distraction. Working memory capacity is related to the ability to control attention especially in tasks that have elements of potential interference or distraction. As the amount of information presented in a task increases, the potential for interference also increases. The consistent finding across studies in children with dystrophinopathy is that as the amount of information increases in a task, performance suffers. This is primarily evident on span tasks where performance on both digit span forward and backward was found to be weak.
This can also be seen with the circumscribed deficits within aspects of language. The language deficits found do not fit the pattern of a specific language disorder given that performance on many aspects of language (including vocabulary, syntactical understanding, semantic knowledge, and fluency) was at expected levels; but the evidence points to a specific processing deficit likely related to underlying difficulties within attention and working memory. The hypothesized model of a limited verbal span core makes logical sense given the pattern of deficits observed in the population. However, attentional control is particularly important for the active maintenance of task goals. Selective attention to stimuli while ignoring irrelevant information is the initial stage that supports working memory performance. The individual must first focus on presented stimuli before that information is encoded, maintained, and finally retrieved. The verbal span hypothesis fails to account for the possibility that attentional processes namely selective attention may be compromised leading to the observed deficits rather than a limitation in capacity. Attention and working memory are thus likely overlapping constructs such that selective attention influences working memory performance. These hypothesized deficits in attention and/or working memory impact cognitive efficiency across many aspects of life in individuals with dystrophinopathy including areas of cognition, academics and social functioning.

Executive functioning is characterized as a set of distinct functions associated with the voluntary control of behavior. Some studies have alluded to an executive dysfunction in the dystrophinopathies. Researchers have suggested that the poor performance on digit span across the literature primarily reflects more of an executive deficit (Anderson et al., 1988; Cotton et al., 1998; Donders & Taneja, 2009; Mento et al., 2011a; Wicksell et al., 2004) than a limitation in linguistic load. Deficits in reading have also been discussed within the context of potential
working memory impairment (Gathercole, Alloway, Willis, & Adams, 2006). However both constructs – executive deficits and limitations in linguistic load- are likely part of the same system impacting performance. Working memory is a multidimensional cognitive construct associated with many cognitive processes including aspects of long-term memory and language. Working memory involves an active manipulation of information that is maintained by focused attention. As evidenced in the recent publication by Hinton and colleagues (Leaffer et al., 2016), where hypotheses about digit span performance were tested using Baddeley’s model of working memory (Baddeley, 2003), verbal span as well as executive processes contributed independently to performance illustrating an interaction of distinct cognitive constructs. Taken together, the evidence conveys the need for a more in-depth examination in a manner that better evaluates these cognitive areas. Narrowing down specificities within the neuropsychological profile will lead to a better understanding of the disorder and will also contribute to identifying the possible role of dystrophin in the brain and how it impacts functioning.

Within the brain, posterior and anterior networks have been hypothesized in models of attention and executive functioning. Posterior networks have been associated with orienting and shifting attention whereas anterior regions are described as a detection system for sensory information as well as a necessary component for memory and higher order cognitive abilities. The frontal cortex and anterior cingulate region have been described as a voluntary control system responsible for controlling attention. Both the anterior and posterior systems work together for optimum cognitive performance (Posner & Petersen, 1990). Attentional control and executive functioning have both been associated with frontal brain regions. Frontocerebellar circuits (Breitenstein et al., 2005; Cyrulnik & Hinton, 2008; O'Reilly &
Norman, 2002) have been hypothesized to be brain regions where dystrophin may play an active role. The lack of dystrophin in the brain likely causes a cascade of functional abnormalities that impacts synaptic formation and the fine-tuning of synapses as the individual develops. Additionally, the pattern of neuropsychological evidence provides even further evidence to hone in on the functional role of dystrophin in specific brain networks. Functional brain mapping has validated a cerebro-cerebellum network that is activated in verbal working memory tasks (Marvel & Desmond, 2010a, 2010b) where two distinct pathways are activated including the dorsal dentate of the cerebellum, a motor tract, projecting to frontal motor regions, and a cognitive tract with the ventral dentate projecting to frontal cognitive areas (Chen, 2005; Kirschen, Chen, Schraedley-Desmond, & Desmond, 2005; Marvel & Desmond, 2010a). Based on the pattern of cognitive deficits, it has been hypothesized that the neuropsychological profile in the dystrophinopathies is a product of the disruptions in cerebro-cerebellar pathways (Cyrulnik & Hinton, 2008). Individuals with other neurological insults to the cerebellum have been shown to have similar patterns of deficits (Levisohn, Cronin-Golomb, & Schmahmann, 2000; Wingeier et al., 2011). Given this evidence and the findings that dystrophin plays a functional role in specific brain regions, it may be justified to assume that both frontal cortical areas and the cerebellum are involved in this disorder. Evidence does suggest a disruption in cognitive processes likely resulting from the underlying functional and biochemical abnormalities provoked by the lack of dystrophin, and future fMRI studies could examine this. The correlation between cognitive and behavioral data and specific brain areas supports the idea that the disorder impacts a network of interacting brain regions rather than just one isolated region.
In conclusion, the dystrophinopathies are a devastating group of diseases that impact physical, cognitive, and behavioral functioning. Much has been researched about the physical and cognitive changes due to dystrophin abnormalities, but there is still much to be learned about the contributions to the diversity of outcome in functioning in this population. Many neuropsychological measures are multifactorial requiring aspects of many different cognitive abilities. A careful evaluation of the constituent requirements of each cognitive task is important for accurate interpretation of performance. While a model for limited verbal span is quite attractive to describe the cognitive presentation, it cannot fully describe the underlying neuropsychological profile until limitations in attentional capacity have been addressed. Because of the combined evidence of compromised attention and possible executive dysfunction on performance measures along with report of attentional and executive behavioral problems across settings, the findings warrant a more detailed investigation of attentional and executive domains within individuals with dystrophinopathy.

It is thus hypothesized that the span deficits observed in the dystrophinopathies are due to the overlapping system of constructs of attention, working memory, and aspects of executive functioning. Attention is both associated and embedded within working memory and executive functioning tasks. It is also hypothesized that these constructs contribute to the underlying cognitive inefficiency in the dystrophinopathies impacting overall cognition and academic functioning. Boys with DMD are thus at an increased risk for learning difficulties because of these underlying weaknesses. The aims of the current study are to further understand the genotype-phenotype relationship in the dystrophinopathies, thereby identifying possible mechanisms behind cognitive deficits and contributing to the localization of cognitive networks in the brain. The current study will address these aims: (1) The pattern of deficits
observed among children with dystrophinopathy highlights specific areas of weakness that may impact the efficiency of cognitive processing and behavior, suggestive of deficiencies mediated by frontal brain systems. To further test this, we will administer a battery of tests known to recruit frontal lobe systems (including measures of executive attention, set shifting, working memory, processing speed and measures of executive deficits in everyday life) and hypothesized that if generalized frontal brain systems are involved, performance on all measures of executive functioning will be lower than expected. We will determine if individuals with dystrophinopathy have generalized executive difficulties in both cognitive processing and behavior that contribute to overall function, and (2) will investigate if there is an association between this cognitive profile and mutation positions affecting CNS isoforms. (3) We will then analyze these executive constructs for independent contributions to real world functioning measured by academic achievement. Additionally, we will examine the relationship of academic weaknesses with the molecular abnormalities (mutation positions affecting CNS isoforms). (4) Finally, we will examine the contribution of these executive constructs to total digit span performance as well as the subtests of digit forward and digit backward given the consistent finding across the literature of compromised performance on digit span, while controlling for motor, demographic and IQ confounds.

Power Analysis

To ensure that the sample size was adequate for the four aims to detect clinical significance, a power analysis calculation was conducted for the different analyses. The multiple regression analyses in aims 3 and 4 will require the largest number of participants. For an alpha of .05 (adjusted to .01) and an effect size value of .25 (Cohen, Cohen, West, & Aiken,
2013; Suresh & Chandrashekara, 2012) indicating a moderate difference, a multiple regression analysis requires a minimum sample size of 50 participants to achieve power at least .80 for the detection of a significant model (Lenth, 2009). From a clinic sample of 75 cases, the study aimed for 70% enrollment to meet the required sample size.
II. PART ONE (AIM 1 AND 2):

Executive functioning in the dystrophinopathies and the relation to underlying mutation position

ABSTRACT

Aim: To investigate executive skills in children with dystrophinopathy and to examine the association between executive functions and dystrophin gene mutation position.

Methods: Fifty boys with dystrophinopathy (mean age 11 y 0 m) completed measures of IQ, working memory and executive functioning (including Digit Span and measures from the NIH Toolbox). Parents completed the Behavior Rating Inventory of Executive Function (BRIEF). Mutation positions were categorized into three groups (upstream exon 30, 31-62, and downstream exon 63). Paired-samples t-test compared performance on executive measures to IQ and a one-way (3-group) ANOVA compared cognitive performance with mutation location.

Results: Mean performance on all executive measures was significantly lower than IQ. Parents were also more likely to rate their child with dystrophinopathy as having clinically significant executive difficulties on the Shift, Emotional Control, and Behavior Regulation indices of the BRIEF. Those with a downstream mutation position had significantly poorer performance on IQ, total digit span, and digits forward, but not on other measures of executive function including behavior.

Interpretation: Individuals with dystrophinopathy have executive skill deficits, but they are not generally associated with distal mutations. These results suggest boys with dystrophinopathy have weaknesses in overlapping brain frontal systems that affect overall cognitive efficiency.
INTRODUCTION

Dystrophinopathies are muscle diseases caused by the absence or abnormal expression of the protein dystrophin. In the brain, dystrophin is localized to both neurons and glia and it appears to play a role in both the development and function of brain structures (Lidov, 1996). Dystrophin is normally localized in the cerebral cortex, hippocampus, and cerebellum where it may serve to anchor molecules for neuronal function (Lidov, 1996), organize GABA_A and acetylcholine receptors (Cohen et al., 2015), and stabilize postsynaptic areas. The development of the brain in the absence of dystrophin likely defines the pattern of cognitive and behavioral impairments that are observed in children with dystrophinopathy (Cohen et al., 2015; Lidov, 1996).

Neurocognitive impairments independent of motor declines have been identified within the dystrophinopathy population. There is an increased risk of overall intellectual disability; however, most children with dystrophinopathy have IQ within the normal range (Cotton et al., 2005). Aspects of memory, visuospatial processing, and crystallized language abilities have all been documented to develop appropriately in children with dystrophinopathy (Hinton et al., 2001; Hinton et al., 2007). However, attention and executive functioning as well as linguistic processing are susceptible to impairments, although the findings are somewhat variable across studies. The most consistent finding across the literature is impaired digit span (Hinton et al., 2001; Hinton, DeVivo, et al., 2004; Hinton et al., 2007; Leaffer et al., 2016; Wicksell et al., 2004; Wingeier et al., 2011). Evidence from parent behavior ratings indicate increased rates of attention, executive and social deficits (Banihani et al., 2015; Donders & Taneja, 2009; Hendriksen & Vles, 2008; Hinton, Nereo, Fee, & Cyrulnik, 2006b) among children with dystrophinopathies. Hinton and colleagues have posited a core deficit in verbal working
memory may explain the cognitive profile (Cyrulnik et al., 2008; Hinton, DeVivo, et al., 2004) whereas others have suggested it may reflect more generalized language or executive deficits (Donders & Taneja, 2009; Mento, Tarantino, & Bisiacchi, 2011b; Wicksell et al., 2004).

Full-length dystrophin (Dp427) and smaller isoforms including Dp71 and Dp 140 have been identified as having a role in cognitive function (Daoud, Angeard, et al., 2009; Taylor et al., 2010). Mutations typically distal of exon 63 and affecting the expression of the isoform Dp71 are associated with severe intellectual impairments (Daoud, Angeard, et al., 2009; Ricotti et al., 2016). Similarly, mutations localized to exon 44-45 affecting Dp140 are linked to cognitive impairments, but the extent of the impairments in performance is more variable (Wingeier et al., 2011). Ricotti et al (2015) found higher rates of intellectual disability, behavioral difficulties, and impaired working memory associated with mutations disrupting Dp 71 (Ricotti et al., 2016).

Overall, the pattern of deficits observed among children with dystrophinopathy highlights specific areas of weakness that may impact the efficiency of cognitive processing and behavior, suggestive of deficiencies mediated by frontal brain systems. To further test this, we administered a battery of tests known to recruit frontal lobe systems (including measures of executive attention, set shifting, working memory, processing speed and measures of executive deficits in everyday life) to a diverse cohort of 50 boys with dystrophinopathy. We hypothesized if generalized frontal brain systems are involved, performance on all measures of executive functioning will be lower than expected. The goals of the study were to determine if individuals with dystrophinopathy have generalized executive difficulties in both cognitive processing and behavior that contribute to overall function, and to investigate if there is an association between this cognitive profile and mutation position affecting CNS isoforms.
METHODS

Participants

50 boys with dystrophinopathy participated. Participants were recruited from the Pediatric Neuromuscular Center/MDA clinic at New York Presbyterian Hospital, associated with Columbia University. The clinic serves the greater New York metropolitan area and a wide range of socioeconomic levels. Inclusion criteria were: genetically confirmed diagnosis of dystrophinopathy, between 5 and 17 years of age, English as primary language of participant (not of parents), willingness to participate and in relatively good health other than diagnosis of dystrophinopathy.

Procedure

The study was approved by the institutional review board at Columbia University Medical Center (IRB #AAAA5627) and The Graduate Center of City University of New York and was supported by a grant from the Muscular Dystrophy Association. Members of the clinic team discussed potential participation and the study coordinator followed up and described the protocol in depth with interested families. All parents or guardians gave informed consent and all participants gave assent prior to enrollment.

Evaluations took place in a quiet room in the clinic, breaks were given as needed, and each assessment took approximately 2 hours. All neuropsychological measures were administered to participants in a standardized order. Measures were chosen to assess a broad range of intellectual function and have minimal physical demands. All data were scored and converted to standardized scores. Data were coded without links to identifying information and stored in a secure database. Health Insurance Portability and Accountability Act and IRB regulations to ensure patient confidentiality and security were applied.
Measures

Motor Function

The Brooke and Vignos Scale was used to assess motor functioning (Brooke et al., 1981; Vignos, Spencer, & Archibald, 1963). The scales include measurements of upper and lower extremity functioning. Scores were combined to establish an overall motor functioning score and categorized into minimal impairment (1-5); able to walk, climb stairs, and full use of arms, moderate (6-12); able to walk, but unable to climb stairs and not able to raise hands over head; and severe impairment (13-16); wheelchair bound, limited use of upper extremities. Fine motor abilities were also assessed using a finger-tapping task to ensure participants were able to respond accurately using a computer keyboard. Using a standardized counting device, participants were instructed to tap their index as quickly as possible in 10 seconds. Each motor measure was administered by, or supervised by, a licensed physical therapist.

Intellectual Function

Participants completed two measures that were used as proxies for general intellectual function: The Peabody Picture Vocabulary Test-IV (PPVT-IV) (Dunn & Dunn, 2007), a measure of single word comprehension, and the Comprehensive Test of Nonverbal Intelligence-2 (CTONI-2), a nonverbal measure of analogical reasoning, categorical classification, and sequential reasoning. Both measures are highly correlated with the Full Scale IQ score of the Wechsler Intelligence Scales for Children with ranges from .69 to .88 (Rossen, Shearer, Penfield, & Kranzler, 2005). Raw scores were age standardized and converted to z scores. Scores on the PPVT-4 and CTONI-2 were combined and the mean was used to create a composite IQ score for analysis.
Primary measures were chosen from *The National Institutes of Health Toolbox* (Weintraub et al., 2013). The Toolbox tasks are computer administered, quick and efficient, standardized cognitive instruments that have been validated on a pediatric population (Weintraub et al., 2013). Selected subtests from the NIH Cognitive Toolbox were administered, including those indexing executive attention (Flanker Inhibitory Control Test), set shifting (Dimensional Change Card Sort Test), working memory (List Sorting Working Memory Test), and processing speed (Pattern Comparison Processing Speed Test). The Flanker task measures selective attention/inhibitory control and requires the participant to focus on a stimulus while inhibiting congruent and incongruent stimuli. Accuracy and reaction time are measured. The Dimensional Change Card Sort task measures mental flexibility. Two dimensions are assessed, including both the color and shape of an object, and requires the ability to shift dimensional sets. Scoring is based on both accuracy and reaction time. The List Sorting Working Memory Test measures immediate recall and sequencing of different visually and orally presented stimuli (animals and foods) in size order from smallest to largest, first within a single dimension and then on two dimensions. The score is equal to the number of items recalled and sequenced correctly. The Pattern Comparison Processing Speed Test measures speed of processing. Participants are given 85 seconds to rapidly judge as many item sets as possible to determine whether two pictures presented are the same or not. Participants’ raw score is the number of correct items within 85-seconds.

The *Digit Span subtest* from the Wechsler Intelligence Scale for Children (Wechsler, 2004), was administered as a measure of verbal span and working memory. Participants were asked to repeat numbers in the same order, or in the reverse order, of presentation. Total score
reflects performance on the entire measure, and individual maximum span length of both forward and backward administration was also calculated. All raw scores were age standardized and converted to z scores.

**Behavioral Assessment**

Parent ratings of children’s executive function and self-regulation in everyday life were assessed using the Behavior Rating Inventory of Executive Function (BRIEF) (Gioia, Isquith, Guy, & Kenworthy, 2000). The scale is designed for children 5 to 18 years of age and has been shown to have clinical utility, as well as predictive validity, for diagnosing executive dysfunction. There is an overall Global Executive composite score, as well as two broad scales of Metacognition and Behavioral Regulation, and eight empirically derived clinical scales that measure different aspects of executive functioning (Inhibit, Shift, Emotional Control, Initiate, Working Memory, Plan/Organize, Organization of Materials, and Monitor). The scale was administered in either English or Spanish depending on parent’s or guardian’s stated primary language. Raw scores were age standardized to T scores, and dichotomized based on a T score cut-point of 65 and above (at or above the 94th percentile and indicative of clinical significance, as designated by the BRIEF manual) (Gioia et al., 2000).

**Mutation Position**

Participant medical records were reviewed for the results of genome sequencing and dystrophin gene mutation analysis. Mutation positions were categorized into three groups: upstream of exon 30, exons 31-62 (includes Dp 140) and downstream of exon 63 (includes Dp 71).

**Statistical Methods**
Descriptive demographic analyses were done on standardized scores. Shapiro-Wilk analyses were run to ensure the data were normally distributed for parametric analyses and Levene's test assessed for homogeneity of variances. To examine for potential motor confounds, the Brooke and Vignos Motor categorized groups’ (minimal, moderate and severe) and performance on the Finger Tapping test (dichotomized by performance one standard deviation below the population mean versus above) was compared to performance on the cognitive measures.


To examine whether performance on tests of attention, processing speed, working memory, and executive functioning was significantly different from general intellectual level, paired t-tests were conducted to compare performance on each executive task with individual IQ composite estimates. Alpha was set at .01 to reduce the probability of Type I error.

**Behavioral Assessment:** To determine whether parent reported rates of executive problems were greater than those expected within the general population, the frequency of participants scoring in the “clinically significant” range (T > 65) on each of the scales was determined and compared to the expected frequencies on the measure (based on the normative sample) using chi-square statistics. Alpha was set at .01 to reduce the probability of Type I error.

**Part 2. Mutation position and cognition**

To investigate the association between mutation position and cognition, performance on all neuropsychological measures was compared between the three groups. An analyses of variance (ANOVA) was used to determine differences between the three groups. For a more robust test, the Welch's statistic was used for analysis because of unequal sample sizes in the groups. Chi-
square statistics were used to compare significantly elevated BRIEF scales across the groups. Alpha was again set at .01 to reduce the probability of Type I error.

RESULTS

Demographic information is presented in Table 1. Participants came from diverse backgrounds with a broad range of socioeconomic status and education.

The motor scale identified 38% of the sample as severely impaired, the majority of participants were minimally impaired (56%), but when performance on neuropsychological test measures was compared across the three motor categories (minimal, moderate, and severe), there were no significant differences. Similarly, although 29% of the sample scored below one standard deviation from the population mean on the Finger Tapping test, there was no difference on the cognitive measures when the groups were compared. These results confirm that participants’ performance on the cognitive tests was unlikely confounded by motor limitations.

For subsequent analyses, all assumptions were met for parametric analysis; cognitive measure data were normally distributed with equality of variances.


Neuropsychological performance data are presented in Table 2.

Intellectual functioning

Mean performance on measures of single word comprehension and perceptual reasoning was comparable \((t (50) = -.94, p = .35)\) with scores for each falling in the average range. IQ composite estimates ranged from delayed to high average and mean performance was in the average range \((z = -.34, \text{equivalent to a standard score of 95})\).
Selected tasks of attention/executive function

Comparison of standardized scores on Toolbox tasks and on the Digit Span subtest revealed no significant differences between measures.

In contrast, all executive tasks were significantly lower than estimated IQ, with the biggest differences observed on the Flanker, List Sorting Working Memory Task and Digit Span total subtests (See Table 2). When Digit Span was broken down into maximum span forward and maximum span backwards, maximum forward span did not differ from estimated IQ.

Behavior – Executive

Only 44 parents completed the BRIEF scales. 35 completed it in English and 9 completed it in Spanish, based on the parent or guardian’s primary language. When age, IQ, motor abilities, and all executive measures from the six families whose scales were not completed were compared to those with completed scales, no differences were observed, suggesting the missing data are reflective of the overall sample.

The percentages of participants who scored above clinical cutoff of T > 65 for each scale on the BRIEF are presented in Table 3. Parents were more likely to rate their son with dystrophinopathy as having clinically significant difficulties with executive control on the Shift, Emotional Control and Behavior Regulation indices than expected.

Part 2. Mutation position and cognition

Comparison between the three mutation position groups revealed that those with a mutation downstream of exon 63 (n = 7) had significantly poorer performance on the IQ estimate, total digit span and digits forward, but not on the other executive measures (Figure 1). Post-hoc pairwise testing using the Tukey test revealed that the downstream exon 63 group performed significantly more poorly than the other mutation position groups on these three measures.
(Table 4). There were no significant differences between the mutation position groups on elevated BRIEF Shift, Emotional Control and Behavior Regulation scales.

DISCUSSION

Our study set out to examine a diverse sample of individuals with dystrophinopathy to determine whether cognitive functions subserved by the brain’s frontal circuitry underlie their unique neuropsychological profile and to examine the association between these functions and mutation position. We hypothesized that children with dystrophinopathy would: 1) perform more poorly on a range of measures of executive ability, 2) be rated as having more difficulties in executive skills in their daily life, and 3) have executive skill deficits associated with mutation position.

Results indicated that on direct testing, performance across all aspects of executive function studied was below expected levels for the sample. Lower performance was found across measures assessing attention, executive control, set shifting, processing speed, and working memory, when compared to crystallized abilities reflective of intellectual level. Executive deficits also manifested in the performance of everyday functional activities. Parents from the sample rated their children as having limited flexibility, difficulty transitioning from one activity to the next, and poor emotion regulation. Thus, among children with dystrophinopathy, there is a generalized deficit in executive functioning.

Functional brain mapping studies in healthy individuals have described different patterns of frontal lobe activation that mediate different aspects of executive functioning. Performance of executive tasks recruits networks of brain areas including frontal areas, basal ganglia, parts of the parietal cortex, the anterior cingulate cortex and even the cerebellum.
There is commonality in the activation of frontal regions (dorsolateral, mid-ventrolateral and anterior cingulate cortex) when completing tasks of executive control, processing speed, working memory, flexibility, and problem solving (Duncan & Owen, 2000). Based on our findings of compromised test performance across executive skills and parent ratings in our cohort, we surmise the lack of dystrophin in the brain likely causes a cascade of functional abnormalities affecting a network of interacting brain regions that rely on frontal brain regions. Future functional imaging studies within the group with dystrophinopathy will provide more detailed support of these hypotheses.

Our study also explored the association between genotype and the neuropsychological profile. Consistent with previous findings, overall IQ was significantly lower for those individuals with distal mutations. Surprisingly, and contrary to what we hypothesized, there were no significant differences with mutation segregation and performance on most executive tasks, but a significant difference was observed on digit span. Our results replicate Ricotti et al. (2015) (Ricotti et al., 2016) findings that performance in the Working memory index that includes digit span was significantly lower for those with mutations downstream of exon 63 affecting Dp 71. However, in addition, we found performance on digit span forward (but not backward) was significantly lower for those individuals with distal mutations suggesting a genotypic association with a specific cognitive deficit.

Other research has inferred that forward digit span is not just a measure of basic attention, but an important predictor of verbal memory functioning (Hurlstone, Hitch, & Baddeley, 2014). Further, empirical evidence assessing the general population have found a strong correlation between span and general intelligence (Gignac & Weiss, 2015). Greater span has been demonstrated to strongly influence greater cognitive abilities as well as in the
application of knowledge in everyday functioning such as academics. The current finding that performance on digit span forward was significantly lower for individuals with distal mutations may indicate that reduced span capacity is somehow distinct from other executive tasks within this population. This may support the hypothesis that a deficit in verbal span is fundamentally related to the decreased performance observed on measures of language, academic skill development, and perhaps executive functioning in children with dystrophinopathy (Cyrulnik et al., 2008; Hinton, DeVivo, et al., 2004).

Strengths of our study include the diversity of the sample; our cohort’s varied ethnicity and socioeconomic status is distinct from many samples described in the dystrophinopathy cognitive research. The diversity in our sample also makes our findings more profound for generalizability to the population of those affected by the disorder. Additionally, the measures chosen for the study represent a greater variety of attention/executive skills (including selective attention, set shifting and cognitive flexibility, working memory, and processing speed) than have previously been tested in one study. Our simultaneous collection and analysis of motor data confirmed the cognitive data were not influenced by motor ability.

Although the lack of comparison sample might be considered a weakness of the study, the data were investigated using each participant as his own control, further strengthening the validity of our findings. Finally, sample size was relatively large for the studied population and adequate for the aims of the study to detect clinical significance. For the behavior rating comparison the sample was compared to published norms. It is possible that the general effects of chronic illness, not specific to dystrophinopathy, might impact on parent report of executive skills in everyday functioning, yet it is known that among other groups with chronic illness (such as diabetes), executive functions are not always reported to be elevated (Duke &
Harris, 2014), so we infer our data reflect true executive deficits associated with dystrophinopathy, not generalized consequences of chronic illness.

In conclusion, our findings demonstrate children with dystrophinopathy are at increased risk for having generalized executive deficits. Since well-developed executive skills have been linked to protective health behaviors, reduced risk behaviors, and greater longevity (especially in those with chronic illness), ensuring children receive targeted interventions to help improve their executive control will impact positively on day-to-day functioning and improve overall quality of life.

ACKNOWLEDGMENTS

This work was supported by a grant from the Muscular Dystrophy Association to VJH. We are very grateful to the families who took the time and effort to participate in our project. Special thanks to Sally Dunaway, PT, DPT, Ashwini K. Rao, OTR, EdD, Fiona McMahon, Ruta Patel and Justine Payne for their contribution to the physical therapy assessments.
<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)/% Range</th>
<th>Total (n = 50)</th>
<th>Upstream of exon 30 (n = 17)</th>
<th>Exons 31-62 (n = 26)</th>
<th>Downstream of exon 63 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td>11.00 (3.49)</td>
<td>10.89 (3.54)</td>
<td>11.38 (3.68)</td>
<td>11.30 (2.80)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-17</td>
<td>5-17</td>
<td>5-17</td>
<td>5-17</td>
</tr>
<tr>
<td><strong>Ethnicity %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• White</td>
<td>48%</td>
<td>59%</td>
<td>50%</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>• Hispanic</td>
<td>32%</td>
<td>23%</td>
<td>31%</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>• African American</td>
<td>10%</td>
<td>12%</td>
<td>8%</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>• Asian</td>
<td>8%</td>
<td>6%</td>
<td>7%</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>• Other</td>
<td>2%</td>
<td>0%</td>
<td>4%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><strong>Motor severity %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Minimal</td>
<td>56%</td>
<td>69%</td>
<td>56%</td>
<td>43%</td>
<td></td>
</tr>
<tr>
<td>• Moderate</td>
<td>6%</td>
<td>6%</td>
<td>8%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>• Severe</td>
<td>38%</td>
<td>25%</td>
<td>36%</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td><strong>Finger Tapping Test (Dominant) %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Within normal limits</td>
<td>71%</td>
<td>90%</td>
<td>81%</td>
<td>71%</td>
<td></td>
</tr>
<tr>
<td>• ≤1 SD</td>
<td>29%</td>
<td>10%</td>
<td>19%</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td><strong>Family income (members/MFI) %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Low</td>
<td>45%</td>
<td>12%</td>
<td>50%</td>
<td>71%</td>
<td></td>
</tr>
<tr>
<td>• Middle</td>
<td>26%</td>
<td>53%</td>
<td>19%</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>• High</td>
<td>29%</td>
<td>35%</td>
<td>31%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><strong>Language in Home</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• English only</td>
<td>64%</td>
<td>76%</td>
<td>58%</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>• Bilingual</td>
<td>18%</td>
<td>12%</td>
<td>23%</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>• Spanish only</td>
<td>18%</td>
<td>12%</td>
<td>19%</td>
<td>29%</td>
<td></td>
</tr>
</tbody>
</table>

MFI = Median Family income
<table>
<thead>
<tr>
<th>Measure</th>
<th>Total sample (n = 50) z-score Mean (SD)</th>
<th>Difference from IQ estimate</th>
<th>t statistic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPVT</td>
<td>-.41 (.19)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTONI</td>
<td>-.29 (.84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ Estimate</td>
<td>-.34 (.91)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIH Flanker</td>
<td>-1.14 (.91)</td>
<td>.86</td>
<td>6.04</td>
<td>.00*</td>
</tr>
<tr>
<td>Dimensional Card Sort</td>
<td>-.89 (.73)</td>
<td>.60</td>
<td>4.21</td>
<td>.00*</td>
</tr>
<tr>
<td>List Sort Working Memory</td>
<td>-1.16 (.76)</td>
<td>.88</td>
<td>8.21</td>
<td>.00*</td>
</tr>
<tr>
<td>Pattern Comparison Speed</td>
<td>-.91 (.84)</td>
<td>.63</td>
<td>5.17</td>
<td>.00*</td>
</tr>
<tr>
<td>Total Digit span</td>
<td>-1.11 (1.06)</td>
<td>.75</td>
<td>6.42</td>
<td>.00*</td>
</tr>
<tr>
<td>Digits forward</td>
<td>-.67 (.85)</td>
<td>.30</td>
<td>2.18</td>
<td>.03</td>
</tr>
<tr>
<td>Digits backward</td>
<td>-1.21 (.80)</td>
<td>.84</td>
<td>6.94</td>
<td>.00*</td>
</tr>
</tbody>
</table>

*p < .01

PPVT = Peabody Picture Vocabulary Test-IV; CTONI = Comprehensive Test of Nonverbal Intelligence-2
<table>
<thead>
<tr>
<th>Measure</th>
<th>% T Score &gt; 65</th>
<th>T Score Range</th>
<th>T score Mean (SD)</th>
<th>Compared to standard sample p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRIEF Inhibit</td>
<td>14*</td>
<td>37-78</td>
<td>53.11 (9.69)</td>
<td>.29</td>
</tr>
<tr>
<td>Shift</td>
<td>27*</td>
<td>36-84</td>
<td>58.25 (11.63)</td>
<td>.00*</td>
</tr>
<tr>
<td>Emotional Control</td>
<td>29*</td>
<td>37-85</td>
<td>55.74 (12.45)</td>
<td>.00*</td>
</tr>
<tr>
<td>Initiate</td>
<td>12*</td>
<td>35-73</td>
<td>53.25 (9.27)</td>
<td>.44</td>
</tr>
<tr>
<td>Working Memory</td>
<td>16*</td>
<td>40-74</td>
<td>53.69 (9.54)</td>
<td>.18</td>
</tr>
<tr>
<td>Plan/Organization</td>
<td>23*</td>
<td>37-74</td>
<td>51.36 (10.88)</td>
<td>.04</td>
</tr>
<tr>
<td>Organization Materials</td>
<td>5</td>
<td>34-67</td>
<td>45.15 (7.51)</td>
<td>.67</td>
</tr>
<tr>
<td>Monitor</td>
<td>12*</td>
<td>33-75</td>
<td>50.28 (9.40)</td>
<td>.44</td>
</tr>
<tr>
<td>Behavioral Regulation</td>
<td>27*</td>
<td>36-85</td>
<td>56.67 (11.91)</td>
<td>.00*</td>
</tr>
<tr>
<td>Metacognition</td>
<td>15*</td>
<td>36-69</td>
<td>51.05 (9.01)</td>
<td>.27</td>
</tr>
<tr>
<td>Global Executive Composite</td>
<td>18*</td>
<td>34-72</td>
<td>53.97 (9.40)</td>
<td>.11</td>
</tr>
</tbody>
</table>

*Higher frequency than expected, > 6% of population
* *p < .01

BRIEF = Behavior Rating Inventory of Executive Function
Table 4. Between mutation position group comparisons

<table>
<thead>
<tr>
<th>Measure</th>
<th>Upstream of exon 30 (n = 17)</th>
<th>Exons 31-62 (n = 26)</th>
<th>Downstream of exon 63 (n = 7)</th>
<th>Statistic</th>
<th>( p )</th>
<th>Post hoc*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQ estimate</td>
<td>.18 (.62)</td>
<td>-.46 (.91)</td>
<td>-1.18 (.80)</td>
<td>( F = 7.59 )</td>
<td>.00*</td>
<td>upstream &gt; downstream</td>
</tr>
<tr>
<td>NIH Flanker</td>
<td>-.91 (.91)</td>
<td>-1.33 (.89)</td>
<td>-1.06 (.95)</td>
<td>( F = 1.12 )</td>
<td>.34</td>
<td></td>
</tr>
<tr>
<td>Dimensional Card Sort</td>
<td>-.76 (.73)</td>
<td>-.95 (.73)</td>
<td>-1.00 (.80)</td>
<td>( F = .39 )</td>
<td>.68</td>
<td></td>
</tr>
<tr>
<td>List Sort Working Memory</td>
<td>-1.04 (.62)</td>
<td>-1.14 (.74)</td>
<td>-1.60 (1.12)</td>
<td>( F = 1.23 )</td>
<td>.30</td>
<td></td>
</tr>
<tr>
<td>Pattern Comparison Speed</td>
<td>-.66 (1.05)</td>
<td>-.94 (.58)</td>
<td>-1.54 (.84)</td>
<td>( F = 2.67 )</td>
<td>.08</td>
<td></td>
</tr>
<tr>
<td>Total Digit span</td>
<td>-.61 (.89)</td>
<td>-1.12 (1.01)</td>
<td>-2.20 (.75)</td>
<td>( F = 6.85 )</td>
<td>.00*</td>
<td>upstream &gt; downstream</td>
</tr>
<tr>
<td>Digits forward</td>
<td>-.25 (.63)</td>
<td>-.72 (.89)</td>
<td>-1.43 (.61)</td>
<td>( F = 5.70 )</td>
<td>.00*</td>
<td>upstream &gt; downstream</td>
</tr>
<tr>
<td>Digits backward</td>
<td>-.88 (.83)</td>
<td>-1.26 (.75)</td>
<td>-1.78 (.57)</td>
<td>( F = 3.65 )</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>BRIEF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shift</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WNL</td>
<td>81%</td>
<td>59%</td>
<td>100%</td>
<td>( X^2 = 4.89 )</td>
<td>.09</td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>19%</td>
<td>41%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WNL</td>
<td>81%</td>
<td>59%</td>
<td>83%</td>
<td>( X^2 = 2.74 )</td>
<td>.25</td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>19%</td>
<td>41%</td>
<td>17%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behavior Regulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WNL</td>
<td>80%</td>
<td>65%</td>
<td>83%</td>
<td>( X^2 = 1.35 )</td>
<td>.51</td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>20%</td>
<td>35%</td>
<td>17%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*\( p < .01 \)

+ = post-hoc Tukey HSD

BRIEF = Behavior Rating Inventory of Executive Function
Figure 1. Comparison of cognitive performance of the mutation site groups

DCST = Dimensional Card Sort Test; LSWM = List Sorting Working Memory Test; PCPS = Pattern Comparison Processing Speed Test
ABSTRACT

Objectives: To examine academic performance in dystrophinopathy as a function of dystrophin gene mutation position as well as intellectual function, executive skills, socioeconomic status (SES), behavior and physical ability.

Methods: In a cross-sectional study, boys with dystrophinopathy (ages 5-17; n=50) completed tests of academics (Woodcock-Johnson-III: spelling, reading, calculation and total scores), executive functioning (selective attention/inhibitory control, set shifting, working memory, and processing speed), single word comprehension and nonverbal reasoning. Motor skills were assessed and parents provided demographic information and child behavioral assessments.

Dystrophin gene mutation positions were dichotomized into groups (upstream versus downstream of exon 43, location of central nervous system (CNS) isoforms previously linked to intellectual impairment). Genetic mutation groups were compared on measures of academic achievement, and multiple regression analyses examined unique and joint contributions of executive skills, intelligence quotient (IQ), SES, motor abilities, behavior, and mutation positions to academic outcomes (α = .01).

Results: Academic performance was slightly, yet significantly, lower than IQ and varied as a function of dystrophin gene position, wherein boys possessing the downstream mutation exhibited greater impairment than boys with the upstream mutation. Digit span forward (indexing verbal span) contributed significant variance to total academic achievement, spelling and calculation.
Conclusions: Weak academic performance is associated with dystrophinopathy and is more common in mutations that disrupt CNS isoforms. A specific deficit in verbal span may underlie inefficiencies observed in children with dystrophinopathy and may drive deficits impacting academic abilities, although longitudinal research is warranted to further test this hypothesis.

Keywords: Dystrophin, Cognition, Academics, Executive skills, Verbal working memory, Digit span
INTRODUCTION

The dystrophinopathies are muscle diseases ranging in severity due to the abnormal expression of the protein dystrophin. Various dystrophin isoforms are expressed in different cell types including nerve and glial cells in the brain. In normal brains according to both autopsy and animal studies, dystrophin has been localized to areas of the cortex, hippocampus, and cerebellum (Anderson et al., 2002). The role of dystrophin in the central nervous system (CNS) is still unclear, but abnormal dystrophin likely influences brain circuitry affecting the development of brain structures (Gorecki et al., 1998; Kim et al., 1995; Lidov, 1996; Sogos et al., 2002) and function (Anderson et al., 2003, 2004). Altered or absent dystrophin may cause a cascade of molecular abnormalities affecting cell stabilization, protection from damage, and regulation in signaling pathways (Allen et al., 2016).

Neurodevelopmental difficulties are common in the disorder. The absence of, or incomplete isoforms of, dystrophin have been hypothesized to impact brain functioning (Anderson et al., 2012; Mehler, 2000). Neurocognitive evidence suggests areas of specific impairment rather than generalized deficits. Although there is an increased risk of overall intellectual impairment, most children with dystrophinopathy have average intellectual functioning (Cotton et al., 2001).

There is evidence that cognitive deficits in dystrophinopathy are related to the molecular abnormalities in the disorder. A variety of mutations in the dystrophin gene alters expression of CNS dystrophin isoforms. Full-length dystrophin (Dp427) as well as other dystrophin isoforms including Dp71 and Dp140 are found in the brain and are negatively
associated with cognitive abilities (Bardoni et al., 2000; Daoud, Candelario-Martinez, et al., 2009; Waite, Brown, & Blake, 2012). Mutations affecting Dp71, typically distal of exon 63, and Dp140, localized to exon 44-45, are associated with a greater frequency of intellectual impairment (Daoud, Angeard, et al., 2009; Lenk, Hanke, Thiele, & Speer, 1993; Moizard et al., 2000; Taylor et al., 2010; Tuffery et al., 1995). Ricotti et al (2015) found an increased prevalence of cognitive and behavioral deficits within specific downstream gene regions (Ricotti et al., 2016). Our research group discovered that downstream mutations are linked to lower intelligence quotients (IQ) as well as reduced digit forward span (Fee et al., 2017, in submission), a measure of short-term memory capacity (Oberauer, Süß, Schulze, Wilhelm, & Wittmann, 2000) or basic attention (Hebben & Milberg, 2009; Lezak, Howieson, & Loring). The frequency and severity of cognitive dysfunction thus appears to be related to mutations affecting more distal areas of the gene, located downstream from exon 43.

Performance on academic measures in the dystrophinopathies is generally lower than expected, with most children falling below expected age and grade levels (Billard et al., 1992; Hendriksen & Vles, 2006; Hinton et al., 2001; Hinton, DeVivo, et al., 2004; Leibowitz & Dubowitz, 1981; Worden & Vignos, 1962). Moreover, academic scores of children with dystrophinopathy are depressed relative to nonverbal IQ, and when compared to unaffected siblings across reading, spelling and math (Hinton, DeVivo, Fee, Goldstein, & Stern, 2004). There is substantial evidence for an increased rate of reading disabilities and delays in developing reading skills as well as poor spelling abilities (Astrea et al., 2015; Billard et al., 1998; Dorman et al., 1988; Fabbro et al., 2007; Hendriksen & Vles, 2006; Hinton, DeVivo, et al., 2004). There is less research examining computational math skills, but our studies found performance to be weak when compared to nonverbal IQ and unaffected siblings (Hinton et al.,
Overall, across studies there appears to be a generalized weakness in academic measures in the dystrophinopathies.

Executive functioning deficits may underlie impaired academic achievement evident in children with dystrophinopathies. There is a wide range of cognitive functions ascribed to executive tasks; the ability to focus and shift attention, organize and plan assignments, and problem solve are essential for attaining academic goals. Executive skills have been strongly associated with academic performance in healthy children (Best, Miller, & Naglieri, 2011), wherein specific executive abilities such as inhibition, shifting, and working memory have been linked to performance in specific academic subject areas (St Clair-Thompson & Gathercole, 2006). Working memory and planning (Cutting, Materek, Cole, Levine, & Mahone, 2009; Sesma, Mahone, Levine, Eason, & Cutting, 2009), shifting (van der Sluis, de Jong, & van der Leij, 2007) and cognitive flexibility (Welsh, Nix, Blair, Bierman, & Nelson, 2010) have been shown to predict reading skills. For example, the Dimensional Change Card Sort test predicted reading achievement in children of low-income families (Welsh et al., 2010). Executive skills are also associated with the development of math skills (Clark, Pritchard, & Woodward, 2010; Mazzocco & Kover, 2007), and working memory more specifically has been predictive of math achievement (DeStefano & LeFevre, 2004; Geary, Hoard, Byrd-Craven, Nugent, & Numtee, 2007; Swanson & Sachse-Lee, 2001). Even over time, working memory capacity determined by performance on span tasks has been linked to both mathematics and science skill development (Gathercole, Pickering, Knight, & Stegmann, 2004).

With respect to children with dystrophinopathy, although our prior work has shown that IQ and digit span performance contributed to academic achievement (Hinton, De Vivo, et al., 2001; Hinton, DeVivo, et al., 2004).
2004), the role of other executive skills has not yet been thoroughly explored. In a large cohort of children with dystrophinopathy, contributions of both forward and backward digit span were shown to be associated with reading performance, with forward span having the greatest contribution (Leaffer et al., 2016). Although we have argued that performance on digit span may reflect an underlying “core deficit” in children with dystrophinopathy (Cyrulnik et al., 2008; Hinton, DeVivo, et al., 2004), others have suggested more generalized executive skill deficits (Donders & Taneja, 2009; Mento et al., 2011b; Wicksell et al., 2004). We recently documented generalized deficits across multiple areas of executive functioning in children with dystrophinopathy (Fee et al., 2017, in submission). Surprisingly however, only performance on forward digit span – and no other measure of executive functioning – was associated with mutation position.

In addition to executive functioning abilities, several other factors may contribute to academic performance. First, IQ is strongly related to academic achievement and widely considered a strong predictor of academic performance (Mayes, Calhoun, Bixler, & Zimmerman, 2009; McGrew, Flanagan, Keith, & Vanderwood, 1997). Second, demographic variables such as socioeconomic status (SES) and parental education predict academic performance (Bradley & Corwyn, 2002; Duncan & Murnane, 2011). Third, behavioral indicators (prosocial behavior, motivation, and the extent of individual academic engagement) (Komarraju & Nadler, 2013; Wentzel, 1993), not only impact academic performance, but affect the classroom environment for learning (Wang & Eccles, 2013). Fourth, chronic illness can impact academic performance (Taras & Potts-Datema, 2005) while coping with symptoms and future outcomes (Thies, 1999). Fifth, physical limitations can limit school interactions as well as attendance influencing individual academic functioning (Pinquart & Teubert, 2011).
Among children with dystrophinopathies, any of these factors might play a significant role in academic achievement.

Most studies examining academics in the dystrophinopathies have used homogenous samples that lacked diversity, failing to consider the multiple factors that contribute to academic performance including cognitive ability, educational opportunity, SES, motor deficits, and the psychosocial stressors of having a physical disability. Additionally, the relationship between mutation position and academic skills has not been explored. To clarify specific predictors of individual performance, the contributions of generalized executive deficits as well as other individual factors including IQ, physical limitations, mutation position, behavior and SES were examined with respect to real world functioning, as measured by academic skill level in a diverse, substantial sample of fifty boys with dystrophinopathy. Given prior evidence, we hypothesized that academic skills in the present sample would: (1) be lower than normative data from similar-aged children; (2) be more impaired in boys with downstream genetic mutations than those with upstream mutations; (3) share significant variance and overlap with executive deficits (selective attention/inhibitory control, set-shifting, working memory and processing speed) including digit span. Exploratory analyses examined whether illness severity, SES, IQ, behavioral variables, and demographic variables also impacted academic performance.

METHODS
Sample

Boys with dystrophinopathy (n=50) were recruited and enrolled from the Pediatric Neuromuscular Center and Muscular Dystrophy Association (MDA) clinic located at New York Presbyterian Hospital, the academic hospital associated with Columbia University. The
Clinic population came from the greater New York City (NYC) metropolitan area and a wide range of SES levels. Eligible participants had a genetically confirmed diagnosis of dystrophinopathy, were between the ages of 5 and 17, English as a primary language (not of parents), expressed an interest in research participation, and were deemed in relatively good health other than diagnosis of dystrophinopathy.

Procedures

The study was approved by the institutional review board (IRB) at Columbia University Medical Center (IRB #AAAA5627) and The Graduate Center of City University of New York and was supported by a MDA grant. After an introduction by the treating physician, interested participants were described protocol details by the study coordinator. All parents or guardians gave informed consent and all participants gave assent prior to enrollment.

Evaluations were conducted in a quiet room free of potential distractions, and breaks were provided; assessment duration was approximately 2 hours. Neuropsychological measures were administered to all children in a standardized order, chosen to assess a broad range of intellectual function that emphasized attention/executive skills and minimized potential confounding effects of impaired motor abilities. Data were scored and converted to standardized scores using normative data. Additionally, participant medical records were reviewed for the results of genome sequencing and dystrophin gene mutation analysis, and mutation position was recorded for each participant. Data were coded without links to identifying information, and entered into a secure database; files were then stored in a locked file cabinet. Patient confidentiality was protected and ensured by the Health Insurance Portability and Accountability Act, IRB regulations, and the study team.

Genetic Mutation Groups
Mutations downstream of exon 43 have been associated with intellectual impairments. Participant medical records were reviewed for the results of genome sequencing and dystrophin gene mutation analysis. Mutation positions were categorized into two groups: upstream of exon 43 and downstream of exon 43 (including Dp 140 and Dp 71).

Assessment Measures

Standardized neuropsychological measures with strong normative data were selected for the assessment. Measures were carefully chosen to minimize the amount of motor skills needed and most were able to be answered with a verbal response or by pressing a computer button.

IQ composite: Participants completed two measures that were used as proxies for general IQ: The Peabody Picture Vocabulary Test-IV (PPVT) (Dunn & Dunn, 2007), a measure of single word comprehension, and the Comprehensive Test of Nonverbal Intelligence-2 (CTONI), a nonverbal measure of analogical reasoning, categorical classification, and sequential reasoning. Both measures are highly correlated with the Full Scale IQ score of the Wechsler Intelligence Scales for Children with ranges from .69 to .88 (Rossen et al., 2005). Raw scores were age-standardized and converted to z scores. Scores on the PPVT-IV and CTONI-2 were combined and the mean was used to create a composite IQ score for analysis.

Academic function: The Woodcock-Johnson III Tests of Achievement (Woodcock, McGrew, & Mather, 2001) is a comprehensive and extensively used test battery for assessing academic achievement skills in individuals aged 2-90 years and normed on diverse communities in the U.S population. The measure has strong reliability and validity, possessing correlations ranging from .60 to .70 with other academic and IQ measures. Overall academic
skills as well as subtests of Single Word Reading, Spelling and Calculation were calculated for each child. Age-standardized scores were used in analysis.

*Executive skills:* Selected subtests from The National Institutes of Health (NIH) Toolbox were chosen given the assessment’s wide use among diverse populations and representative normative data for individuals between ages of 3-85 in the U.S. population. Subtests from the Cognitive Toolbox (Hodes, Insel, Landis, & Research, 2013; Weintraub et al., 2013) were administered, including attention (Flanker Inhibitory Control Test), set-shifting (Dimensional Change Card Sort Test), working memory (List Sorting Working Memory Test), and processing speed (Pattern Comparison Processing Speed Test). These measures possessed short administration times and normative data matched the study sample.

First, the Flanker task required the participant to focus on a stimulus in the midst of congruent and incongruent stimuli, recording both reaction time (RT) and accuracy. Second, the Dimensional Change Card Sort task (DCCST) measured set-shifting by assessing two dimensions, both the color and shape of an object; moreover, cognitive flexibility was evaluated by having the individual shift sets by dimensions, recording RT and accuracy. Third, the List Sorting Working Memory Test (LSWMT) measured span and sequencing of visually and orally presented stimuli (animals and foods). The participant organized the items in size order from smallest to largest, first within a single dimension and then on two dimensions. The score was equal to the number of items recalled and sequenced correctly. Fourth, the Pattern Comparison Processing Speed Test (PCPST) measured processing speed. Participants had 85 seconds to rapidly judge as many item sets as possible (whether two pictures presented were the same or different).
**Motor function:** The Brooke and Vignos Scale was used to assess motor functioning (Brooke et al., 1981; Vignos et al., 1963). The scales include measurements of upper and lower extremity functioning. Scores were categorized into minimal impairment (1-5); able to walk, climb stairs, and full use of arms, moderate (6-12); able to walk, but unable to climb stairs and not able to raise hands over head; and severe impairment (13-16); wheelchair bound, limited use of upper extremities. Fine motor abilities were also assessed using a finger-tapping task to ensure participants were able to respond accurately using the computer keyboard for the NIH Toobox measures. Each motor measure was administered by, or supervised by, a licensed physical therapist in the MDA clinic. For regression analysis, Upper and lower extremity motor scores were combined for analysis. Individuals with a gross motor score of 13-16 and finger tapping less than -1.5 standard deviation from the mean were categorized as severely impaired.

**SES:** Family Income was reported by parents in a family history questionnaire by checking off a range of incomes provided for the household. Income was based on reported household income and number of individuals in the home and then compared to the NYC census report for annual median household income; income was categorized into low, middle, high-income levels. Income was recoded for analysis comparing low to middle/high income.

**Behavior:** Parental report behavior measures were examined for potential contributions to cognitive performance. The Behavior Assessment System for Children, second edition (BASC-2) (Reynolds & Kamphaus, 2004), is a multidimensional assessment that evaluates various aspects of behavior and personality from the perspective of the parent. The BASC-2 was used to determine overall behavioral issues both externalizing problems (attention difficulties, conduct problems, hyperactivity) and internalizing problems (anxiety, depression, and somatization) by using the Behavioral Symptoms Index (BSI) score. Parents rated, on a
“never” to “almost always” scale, on how often their child engaged in each behavior. The BASC-2 yielded a T score for the BSI score. Adverse outcome was coded as a score of T > 67 on the scale and dichotomized (elevated versus within normal limits).

Additionally, the Behavior Rating Inventory of Executive Function (BRIEF) (Gioia et al., 2000) was used to assess executive function and self-regulation in everyday life and was examined as a potential marker for deficits in attention/executive skills. The scale is designed for children 5 to 18 years of age and has been well standardized. The measure provided information on different aspects of executive functioning through an overall Global Executive composite score (GEC). T scores were generated for the composite scale and a T score > 65 was categorized as elevated. The BRIEF GEC composite score was dichotomized into elevated or within normal limits and used for analysis.

**Statistical Analysis**

Descriptive demographic analyses were based on standardized scores. Shapiro-Wilk analyses were run to ensure the data were normally distributed for parametric analyses and Levene's test assessed for homogeneity of variances. For regression analysis, variables were entered into a correlation matrix to determine their association with each other. Multivariate outliers were investigated by examination of Mahalanobis Distance and predicted residual values. Multicollinearity was examined by inspecting the correlation coefficients and tolerance/VIF values. Independence of observations was determined by the Durbin-Watson statistic. Independence of errors from regression predictors was confirmed by histogram evaluation and P-P plot of standardized residuals.
**Within-subject analysis:** Paired t-tests were conducted across participants to compare performance on each academic measure (Reading, Spelling, Calculation, Total) with individual IQ composite estimates. Alpha was set at .05.

**Group analysis:** Welch’s test (independent sample t-test for unequal variances and sample sizes) was conducted between mutation groups (downstream of exon 43: n=32; upstream of exon 43: n=18) for the following dependent measures of academic performance: Reading, Spelling, Calculation and Total. Alpha was set at .05.

**Regression analysis:** Four linear regressions were computed for the following outcome variables indexing academic performance: (1) Reading; (2) Spelling; (3) Calculation; and (4) Total. Frequencies and distributions of each variable were determined and each was entered into a correlation matrix to determine whether it was associated with the outcome variable of interest (Table 5). Independent variables were entered as predictors in two steps. Executive function measures including digit span forward and backward were included in the first block of the regression model (Model I). The motor, demographic, SES, IQ, mutation position, and behavior were then added to the overall regression model in step two (Model II). Standardized beta values ($\beta$) were used to indicate the relative influence of each predictor. Alpha was set at .01 to reduce the probability of Type I error.

**RESULTS**

Table 1 illustrates demographic information for the sample, demonstrating that families who agreed to participate came from diverse backgrounds with a broad range of SES and education.
Within-subject analysis: Across participants, Reading, Calculation, and Total academic measures, although well within normal limits, was lower than expected when compared to IQ (Table 2).

Group analysis: Demographic information between groups was not significantly different except for SES, more boys with downstream mutations came from low income families (Table 1). Boys with a downstream mutation exhibited worse performance on Reading, Spelling, Calculation, and Total academic measures than boys with an upstream mutation (Table 3; Figure 1).

Regression analysis: Of note is that 44 of the 50 participants originally enrolled had completed parent questionnaire measures. Of those, 35 completed them in English and 9 completed them in Spanish, based on the parent or guardian’s primary language. To ensure that those participants whose parents did not complete the questionnaires were comparable to those whose parents did complete the scales, multiple exploratory independent sample t-tests were run. Group age, IQ, motor abilities, income, behavioral measures, mutation position and performance on all cognitive measures did not differ between the six families whose scales were not completed and those with completed scales, suggesting the missing data were reflective of the overall sample (Table 4). Regression assumptions were met for subsequent analyses, wherein no standardized residual outliers >2.5 standard deviations were present, there was no evidence of multicollinearity, errors were independent and normally distributed, and relationships between predictor and outcome variables were linear and homoscedastic.

The regression model for the cognitive executive measures (Model I) was significant (F (6, 38) = 5.71, p < .01) with digit span forward as the only significant predictor of Total academic achievement in the model (β = .35, t(44) = 2.55, p < .01). Regression results
including all predictor variables entered (Model 2) are presented in Table 5. For Total academic achievement, the overall model was significant, $F(12, 32) = 4.02, p < .01$, with predictors accounting for 53% of the variance; however, digit span forward was again the only significant predictor in the model, with better performance predicting higher Total scores ($\beta = .45, t(44) = 2.45, p < .01$).

For individual academic areas, the overall models were also significant. Model I was significant for all subject areas, Reading ($F(6, 38) = 4.60, p < .01, R^2_{Adjusted} = .41$), Spelling ($F(6, 38) = 3.12, p < .01, R^2_{Adjusted} = .41$) and Calculation ($F(6, 38) = 6.13, p < .01, R^2_{Adjusted} = .54$); greater digit span forward significantly predicted better Spelling ($\beta = .35, t(44) = 2.48, p < .01$) and better Calculation ($\beta = .38, t(44) = 2.65, p < .01$) performance. Both digit forward ($\beta = .37, t(44) = 3.00, p < .01$) and digit backward ($\beta = .35, t(44) = 2.52, p < .01$) significantly predicted Reading. For Model II, all predictors significantly predicted Reading ($F(12, 32) = 4.32, p < .01, R^2_{Adjusted} = .52$), Spelling ($F(12, 32) = 4.06, p < .01, R^2_{Adjusted} = .45$) and Calculation ($F(12, 32) = 4.47, p < .01, R^2_{Adjusted} = .57$). Higher digit span forward scores significantly predicted better Spelling ($\beta = .37, t(44) = 2.55, p < .01$) and Calculation ($\beta = .42, t(44) = 3.04, p < .01$) performance. Of note is that for Reading, no individual variable contribution was significant, yet the contributions of both digit span forward and digit span backward approached significance ($\beta = .35, t(44)= 2.42, p = .02$, and $\beta = .34, t(44) = 2.21, p = .03$, respectively).

**DISCUSSION**

The goal of this study was to help clarify understanding about academic performance in children with dystrophinopathy. This was done by examining the academic functioning and the
association with mutation position and by determining if known executive weaknesses
(selective attention/inhibitory control, set-shifting, working memory, processing speed)
contribute significantly to academic performance, when studied in combination with motor
limitations, intellectual function, behavior, SES and mutation position.

As expected, and consistent with our previous finding, academic skills are reduced in
children with dystrophinopathy. Although group mean performance on academic tests fell
within normal limits, when compared to measures of crystallized intellectual function,
performance in reading and math was significantly lower (and there was a similar trend for
spelling). Even though the mean difference from IQ across subject areas was less than a
standard deviation and therefore not great enough to meet criteria for a specific learning
disorder, the finding of reduce academic performance nonetheless may represent significant
difficulties for these children in real world functioning. These findings extend and replicate our
prior research (Hinton, De Vivo, et al., 2004) indicating that academics are overall weak across
subject areas for children with dystrophinopathy.

Our findings also revealed, as predicted, that individuals with more downstream
mutations performed more poorly across academic measures. Overall mean performance across
subject areas was significantly lower for those with mutations downstream of exon 43 as
compared to average performance for those upstream. These results are consistent with those
showing IQ is lower in individuals with mutations downstream of exon 43 (Daoud, Angeard, et
al., 2009; Rasic et al., 2014; Taylor et al., 2010; Wingeier et al., 2011), as is digit span
performance (Ricotti et al., 2016) (Fee, 2017, in submission). Thus, mutations that disrupt the
dystrophin isoforms Dp140 and Dp 71 also affect academic performance.
Our work examined the myriad of things that may contribute to real world academic function. Interestingly, our regression model revealed that among boys with dystrophinopathy, academic achievement was best predicted by performance on digits forward even when potential contributing factors including IQ, SES, illness severity, mutation position and behavior were entered in the model. Digits forward was not only predictive of total academic achievement, but also predicted performance in Spelling and Calculations. Moreover, there was a trend toward digits forward also contributing to single word reading. Performance on other tests of executive functioning did not contribute significantly, contrary to our initial hypothesis.

Our current results replicate our previous studies that found that digit span contributed to academic performance in children with dystrophinopathy (Hinton, De Vivo, et al., 2004) and digit span (both forward and backward span) contributed to the variance in reading performance in a large cohort of children with dystrophinopathy (Leaffer et al., 2016). Unlike those studies, the current study extended the investigation by examining the contribution of a variety of executive skills, as measured by individual performance and parent ratings, on academic performance. Although children with dystrophinopathy have generalized weakness across executive functions (Fee et al., in submission), surprisingly, only digit span forward significantly predicted the variance observed on academic tests.

Performance on digits forward likely reflects individual capacity embedded in a number of more complex abilities including executive and academic tasks. The finding that forward digit span, rather than IQ or the other variables, accounted for the largest amount of statistical variance is valuable knowledge for real world functioning. In academics, research has shown that increased load imposes large demands on working memory and thus limited capacity in
working memory results in poor academic performance (Gathercole et al., 2004). Working memory has been demonstrated to be a strong predictor of academic achievement (Alloway & Alloway, 2010). True deficits in the dystrophinopathies are likely an interaction of weakness in multiple areas including specific aspects of executive functioning. Learning is incremental, and difficulty in the acquisition of basic concepts that depend on linguistic capacity results in academic failure as material becomes more complex. In addition, real world functioning in a variety of environments often requires the ability to handle large amounts of novel information, multitasking, and adaptation, all requiring working memory as well as other executive skills.

We have proposed in a series of publications that the core cognitive deficit in the dystrophinopathy group is a limited verbal span. This hypothesis is based on a repeated pattern of poor performance on digit span, weak story memory, and impaired sentence repetition found in our studies suggesting a reduced capacity in linguistic load (Hinton et al., 2001; Hinton et al., 2007). It is also based on the finding that even across intellectual level, performance on tasks of digit span and story memory remains selectively low for children with dystrophinopathy (Hinton, De Vivo, et al., 2000). We expanded our investigation of verbal span and found that both digits forward and backward was poor demonstrating that decreased span as well as working memory was deficient. The combined deficits in verbal span and executive control was found to contribute to poor reading performance (Leaffer et al., 2016).

More recently, we challenged our assumption of a core deficit in verbal span, and found that across executive skills children with dystrophinopathy have a generalized weakness in both cognitive and behavioral executive functioning (Fee, 2017, in submission). Yet, despite finding generalized executive deficits, only limited forward span was associated with mutation position (Fee, 2017, in submission). The current study again challenged the verbal span
hypothesis by predicting that generalized executive deficits would contribute significant variance to academic performance, yet the results unexpectedly found that only forward span predicted academic performance. Taken together, the combined findings of digit span forward being the only executive skill to be associated with mutation position and the only executive skill to contribute significantly to academic performance support the hypothesis of a distinct weakness in the working memory system of individuals with dystrophinopathy. Both executive deficits and limitations in linguistic load are likely part of that system. Together, they reflect an underlying core deficit in verbal span in this group.

Working memory is a multidimensional cognitive construct that requires a storage system and involves an active manipulation of information. It is associated with many cognitive processes including aspects of long-term memory as well as the development of language skills (Brosnan et al., 2002). In dystrophinopathy, span capacity appears to be reduced impacting more complex areas of executive control as well as other areas of functioning (including academics) mediated by a hypothesized working memory system.

The strengths of the current study lie in the design to examine academic outcomes that represents real world functioning. Academics not only measure knowledge based learning but also reflect individual potential for success in future goals. Individual abilities were examined across academic areas in a diverse population with dystrophinopathy to better isolate the source of cognitive weakness. The current study examined a wide range of executive skills in the context of several individual variables including physical functioning and demographics to decipher underlying contribution to performance. We chose a variety of psychometrically strong measures controlling for a number of potential confounds to delineate potential deficits in specific aspects of functioning. The finding of one specific deficit, performance on digits
forward, in the midst of so many potential contributing factors, including IQ, physical abilities, behavior, and demographic factors, predicting academic performance emphasizes the strength of the finding. Additionally, this study is the first to examine molecular associations with real world academic performance. This not only aids in the understanding of the relationship between a disorder with a known molecular cause and selective cognitive impairments, but also a better understanding of the various contributions to academic achievement. Finally, the sample included individuals from diverse backgrounds which may be more representative of the dystrophinopathy population than found in other cognitive studies.

The study also has several limitations. The study utilized a relatively small sample by including only those with fully completed measures. Although the sample size was deemed adequate and produced valid findings, a larger sample for the regression model might provide more robust results and greater confidence in conclusions. Another potential weakness was the lack of a control group. The sample’s performance was compared to normative data; however, each participant was examined as his own control strengthening the internal validity of the study. Finally, our academics measures assessed basic skills of language and arithmetic; more complex academic measures would help to further describe the extent and specifics of academic impairment within the population. Future studies could expand on our results and examine other measures that examine these cognitive constructs further.

This study contributes to further understanding the role of disrupted dystrophin in the brain and its impact on functioning. In addition, the study provides information that could aid in improving real world functioning for children with dystrophinopathy. Weaknesses in working memory disrupts everyday activities, particularly in the academic environment that requires organization and goal-directed behavior. The identification of these specific areas of
weakness provides targets for remediation that can help in the attainment of appropriate developmental gains promoting a better quality of life.

ACKNOWLEDGMENTS

This work was supported by a grant from the Muscular Dystrophy Association to VJH. We are very grateful to the families who took the time and effort to participate in our project. Special thanks to Sally Dunaway, PT, DPT, Ashwini K. Rao, OTR, EdD, Mercedes Vega Villar, Fiona McMahon, Ruta Patel and Justine Payne for their contribution to the physical therapy assessments.
### Table 1. Descriptive Characteristics of the Total Sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 50)</th>
<th>Upstream exon 43 (n = 18)</th>
<th>Downstream exon 43 (n = 32)</th>
<th>Between group comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>11.00 (3.49)</td>
<td>10.29 (3.43)</td>
<td>11.40 (3.52)</td>
<td>$F = 1.18, p = .28$</td>
</tr>
<tr>
<td><strong>Ethnicity %</strong></td>
<td></td>
<td></td>
<td></td>
<td>$X^2 = 2.65, p = .62$</td>
</tr>
<tr>
<td>• White</td>
<td>48%</td>
<td>61%</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>• Hispanic</td>
<td>32%</td>
<td>22%</td>
<td>37%</td>
<td></td>
</tr>
<tr>
<td>• African American</td>
<td>10%</td>
<td>11%</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>• Asian</td>
<td>8%</td>
<td>6%</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>• Other</td>
<td>2%</td>
<td>0%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td><strong>Motor severity %</strong></td>
<td></td>
<td></td>
<td></td>
<td>$X^2 = 1.73, p = .42$</td>
</tr>
<tr>
<td>• Minimal</td>
<td>56%</td>
<td>67%</td>
<td>53%</td>
<td></td>
</tr>
<tr>
<td>• Moderate</td>
<td>6%</td>
<td>6%</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>• Severe</td>
<td>38%</td>
<td>27%</td>
<td>41%</td>
<td></td>
</tr>
<tr>
<td><strong>Finger Tapping Test (Dominant) %</strong></td>
<td></td>
<td></td>
<td></td>
<td>$X^2 = 11.03, p &lt; .01^*$</td>
</tr>
<tr>
<td>• Within normal limits</td>
<td>71%</td>
<td>82%</td>
<td>64%</td>
<td></td>
</tr>
<tr>
<td>• ≤-1 SD</td>
<td>29%</td>
<td>18%</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td><strong>Family income (members/MFI) %</strong></td>
<td></td>
<td></td>
<td></td>
<td>$X^2 = 1.07, p = .59$</td>
</tr>
<tr>
<td>• Low</td>
<td>45%</td>
<td>13%</td>
<td>53%</td>
<td></td>
</tr>
<tr>
<td>• Middle</td>
<td>26%</td>
<td>50%</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>• High</td>
<td>29%</td>
<td>37%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td><strong>Language in Home</strong></td>
<td></td>
<td></td>
<td></td>
<td>$X^2 = 1.07, p = .59$</td>
</tr>
<tr>
<td>• English only</td>
<td>64%</td>
<td>72%</td>
<td>59%</td>
<td></td>
</tr>
<tr>
<td>• Bilingual</td>
<td>18%</td>
<td>17%</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>• Spanish only</td>
<td>18%</td>
<td>11%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>Measure</td>
<td>Total sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>--------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>z-score Mean (SD)</td>
<td>-0.40 (1.17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPVT</td>
<td>-0.29 (.82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTONI</td>
<td>-0.34 (.91)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IQ Estimate</td>
<td>-0.69 (1.35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference from IQ estimate</td>
<td>.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t statistic</td>
<td>2.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>.01*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reading</td>
<td>.68 (1.32)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spelling</td>
<td>-.59 (1.43)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculation</td>
<td>-.89 (1.34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>.00*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .05

PPVT = Peabody Picture Vocabulary Test-IV; CTONI = Comprehensive Test of Nonverbal Intelligence-2
<table>
<thead>
<tr>
<th>Measure</th>
<th>Upstream of exon 43 (n = 18)</th>
<th>Downstream of exon 43 (n = 32)</th>
<th>Statistic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Academics</td>
<td>.02 (.88)</td>
<td>-1.10 (1.42)</td>
<td>$F = 9.16$</td>
<td>.00*</td>
</tr>
<tr>
<td>Reading</td>
<td>.06 (.79)</td>
<td>-1.12 (1.39)</td>
<td>$F = 10.94$</td>
<td>.00*</td>
</tr>
<tr>
<td>Spelling</td>
<td>.14 (.87)</td>
<td>-1.01 (1.54)</td>
<td>$F = 8.47$</td>
<td>.00*</td>
</tr>
<tr>
<td>Calculation</td>
<td>-.32 (1.08)</td>
<td>-1.22 (1.39)</td>
<td>$F = 5.50$</td>
<td>.02*</td>
</tr>
</tbody>
</table>

*p < .05
Figure 1. Boys with a downstream mutation exhibited worse academic performance
Table 4. Characteristics of the Sample for Regression Analysis (n = 44)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SD)/%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>11.04 (3.30)</td>
</tr>
<tr>
<td></td>
<td>5-17</td>
</tr>
<tr>
<td><strong>IQ</strong></td>
<td></td>
</tr>
<tr>
<td>• PPVT</td>
<td>-.28 (1.14)</td>
</tr>
<tr>
<td>• CTONI</td>
<td>-.23 (.78)</td>
</tr>
<tr>
<td><strong>IQ Estimate</strong></td>
<td>-.26 (.91)</td>
</tr>
<tr>
<td><strong>Income (SES) %</strong></td>
<td></td>
</tr>
<tr>
<td>• Low</td>
<td>39%</td>
</tr>
<tr>
<td>• Middle</td>
<td>29%</td>
</tr>
<tr>
<td>• High</td>
<td>32%</td>
</tr>
<tr>
<td><strong>Income (SES) Estimate</strong></td>
<td></td>
</tr>
<tr>
<td>• Low</td>
<td>41% (18)</td>
</tr>
<tr>
<td>• Middle/High</td>
<td>59% (26)</td>
</tr>
<tr>
<td><strong>Motor severity %</strong></td>
<td></td>
</tr>
<tr>
<td>• Minimal</td>
<td>57%</td>
</tr>
<tr>
<td>• Moderate</td>
<td>7%</td>
</tr>
<tr>
<td>• Severe</td>
<td>36%</td>
</tr>
<tr>
<td><strong>Finger Tapping Test(Dominant) %</strong></td>
<td></td>
</tr>
<tr>
<td>• WNL</td>
<td>86%</td>
</tr>
<tr>
<td>• ≤-1 SD</td>
<td>14%</td>
</tr>
<tr>
<td><strong>Motor Estimate</strong></td>
<td></td>
</tr>
<tr>
<td>• Functional</td>
<td>86%</td>
</tr>
<tr>
<td>• Impaired</td>
<td>14%</td>
</tr>
<tr>
<td><strong>Mutation Position</strong></td>
<td></td>
</tr>
<tr>
<td>• Upstream exon 43</td>
<td>39% (17)</td>
</tr>
<tr>
<td>• Downstream exon 43</td>
<td>61% (27)</td>
</tr>
<tr>
<td><strong>Behavior BASC-2</strong></td>
<td></td>
</tr>
<tr>
<td>• Behavior Symptoms Index T score</td>
<td>52.97 (10.71)</td>
</tr>
<tr>
<td><strong>BSI</strong></td>
<td></td>
</tr>
<tr>
<td>• WNL</td>
<td>86%</td>
</tr>
<tr>
<td>• Elevated</td>
<td>14%</td>
</tr>
<tr>
<td><strong>BRIEF</strong></td>
<td></td>
</tr>
<tr>
<td>• General Executive Composite (GEC) T score</td>
<td>53.97 (9.40)</td>
</tr>
<tr>
<td><strong>GEC</strong></td>
<td></td>
</tr>
<tr>
<td>• WNL</td>
<td>82%</td>
</tr>
<tr>
<td>• Elevated</td>
<td>18%</td>
</tr>
</tbody>
</table>

PPVT = Peabody Picture Vocabulary Test-IV; CTONI = Comprehensive Test of Nonverbal Intelligence-2; BASC = Behavior Assessment System for Children-2; BRIEF = Behavior Rating Inventory of Executive Functioning; WNL = Within Normal Limits
Table 5. Matrix of intercorrelations of all variables

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total Academics</td>
<td>1</td>
<td>.96</td>
<td>.94</td>
<td>.91</td>
<td>.49</td>
<td>.245</td>
<td>.52</td>
<td>.40</td>
<td>.54</td>
<td>.34</td>
<td>.52</td>
<td>.96</td>
<td>.81</td>
<td>.08</td>
<td>.40</td>
<td>-.14</td>
<td>.14</td>
<td></td>
</tr>
<tr>
<td>2. Reading</td>
<td>.96</td>
<td>1</td>
<td>.90</td>
<td>.64</td>
<td>.40</td>
<td>.245</td>
<td>.41</td>
<td>.32</td>
<td>.61</td>
<td>.30</td>
<td>.52</td>
<td>.96</td>
<td>.63</td>
<td>.02</td>
<td>.43</td>
<td>-.09</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>3. Spelling</td>
<td>.94</td>
<td>.90</td>
<td>1</td>
<td>.60</td>
<td>.45</td>
<td>.153</td>
<td>.54</td>
<td>.40</td>
<td>.58</td>
<td>.36</td>
<td>.46</td>
<td>.83</td>
<td>.57</td>
<td>.12</td>
<td>.39</td>
<td>-.12</td>
<td>.19</td>
<td></td>
</tr>
<tr>
<td>4. Calculations</td>
<td>.91</td>
<td>.84</td>
<td>.80</td>
<td>1</td>
<td>.47</td>
<td>.213</td>
<td>.50</td>
<td>.40</td>
<td>.70</td>
<td>.40</td>
<td>.00</td>
<td>.70</td>
<td>.48</td>
<td>.00</td>
<td>.32</td>
<td>-.09</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>5. Flanker</td>
<td>.46</td>
<td>.40</td>
<td>.45</td>
<td>.47</td>
<td>1</td>
<td>.42</td>
<td>.50</td>
<td>.36</td>
<td>.49</td>
<td>.31</td>
<td>.41</td>
<td>.86</td>
<td>.39</td>
<td>.33</td>
<td>.106</td>
<td>.340</td>
<td>.23</td>
<td></td>
</tr>
<tr>
<td>6. Dimensional Card</td>
<td>.745</td>
<td>.345</td>
<td>.153</td>
<td>.213</td>
<td>.40</td>
<td>1</td>
<td>.186</td>
<td>.34</td>
<td>.171</td>
<td>-.188</td>
<td>-.011</td>
<td>.248</td>
<td>.47</td>
<td>-.24</td>
<td>.149</td>
<td>-.67</td>
<td>-.1</td>
<td></td>
</tr>
<tr>
<td>7. List Sorting</td>
<td>.52</td>
<td>.51</td>
<td>.54</td>
<td>.70</td>
<td>.40</td>
<td>.171</td>
<td>.46</td>
<td>.210</td>
<td>1</td>
<td>.61</td>
<td>.72</td>
<td>.86</td>
<td>.46</td>
<td>.05</td>
<td>.35</td>
<td>.129</td>
<td>.0</td>
<td></td>
</tr>
<tr>
<td>8. Pattern Comparison</td>
<td>.46</td>
<td>.39</td>
<td>.46</td>
<td>.39</td>
<td>.34</td>
<td>.35</td>
<td>.81</td>
<td>1</td>
<td>.210</td>
<td>.00</td>
<td>.231</td>
<td>.52</td>
<td>.06</td>
<td>.10</td>
<td>.106</td>
<td>.15</td>
<td>.10</td>
<td></td>
</tr>
<tr>
<td>9. Total digit span</td>
<td>.64</td>
<td>.61</td>
<td>.58</td>
<td>.70</td>
<td>.40</td>
<td>.171</td>
<td>.46</td>
<td>.210</td>
<td>1</td>
<td>.61</td>
<td>.72</td>
<td>.86</td>
<td>.46</td>
<td>.05</td>
<td>.35</td>
<td>.129</td>
<td>.0</td>
<td></td>
</tr>
<tr>
<td>10. Digit span forward</td>
<td>.54</td>
<td>.39</td>
<td>.352</td>
<td>.40</td>
<td>.014</td>
<td>-.06</td>
<td>.031</td>
<td>.080</td>
<td>.51</td>
<td>1</td>
<td>.352</td>
<td>.42</td>
<td>.304</td>
<td>-.09</td>
<td>.35</td>
<td>.36</td>
<td>.10</td>
<td></td>
</tr>
<tr>
<td>11. Digit span backward</td>
<td>.52</td>
<td>.52</td>
<td>.48</td>
<td>.60</td>
<td>.268</td>
<td>-.01</td>
<td>.41</td>
<td>.231</td>
<td>.72</td>
<td>.35</td>
<td>1</td>
<td>.53</td>
<td>.231</td>
<td>.03</td>
<td>.31</td>
<td>.140</td>
<td>-.1</td>
<td></td>
</tr>
<tr>
<td>12. Iconography</td>
<td>.59</td>
<td>.66</td>
<td>.63</td>
<td>.76</td>
<td>.40</td>
<td>.240</td>
<td>.60</td>
<td>.52</td>
<td>.60</td>
<td>.42</td>
<td>.53</td>
<td>1</td>
<td>.66</td>
<td>.07</td>
<td>.36</td>
<td>.112</td>
<td>.16</td>
<td></td>
</tr>
<tr>
<td>13. Income</td>
<td>.61</td>
<td>.63</td>
<td>.57</td>
<td>.48</td>
<td>.195</td>
<td>.39</td>
<td>.268</td>
<td>.43</td>
<td>.30</td>
<td>.231</td>
<td>.56</td>
<td>1</td>
<td>-.13</td>
<td>.46</td>
<td>.363</td>
<td>.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. Motor</td>
<td>.092</td>
<td>.024</td>
<td>.121</td>
<td>.064</td>
<td>.237</td>
<td>-.24</td>
<td>.33</td>
<td>.088</td>
<td>-.05</td>
<td>-.08</td>
<td>.034</td>
<td>-.068</td>
<td>-.127</td>
<td>1</td>
<td>-.18</td>
<td>-.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Mutation position</td>
<td>.40</td>
<td>.42</td>
<td>.39</td>
<td>.324</td>
<td>.167</td>
<td>.149</td>
<td>.106</td>
<td>.160</td>
<td>.35</td>
<td>.35</td>
<td>.308</td>
<td>.360</td>
<td>.45</td>
<td>.10</td>
<td>1</td>
<td>.360</td>
<td>.0</td>
<td></td>
</tr>
<tr>
<td>16. BASIC</td>
<td>-.14</td>
<td>-.085</td>
<td>-.123</td>
<td>-.060</td>
<td>-.312</td>
<td>-.07</td>
<td>.046</td>
<td>-.15</td>
<td>.129</td>
<td>.36</td>
<td>.710</td>
<td>.112</td>
<td>.063</td>
<td>-.23</td>
<td>.086</td>
<td>1</td>
<td>-.2</td>
<td></td>
</tr>
<tr>
<td>17. BRIEF</td>
<td>.138</td>
<td>.114</td>
<td>.188</td>
<td>.110</td>
<td>-.013</td>
<td>-.15</td>
<td>.236</td>
<td>.178</td>
<td>-.01</td>
<td>.105</td>
<td>-.064</td>
<td>.158</td>
<td>.138</td>
<td>.18</td>
<td>-.03</td>
<td>.18</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Table 6. Multiple Regression Analyses for Variables Predicting Academic performance (n = 44)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Academic Achievement</th>
<th>Reading</th>
<th>Spelling</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B$ $SE$ $B$ $\beta$</td>
<td>$B$ $SE$ $B$ $\beta$</td>
<td>$B$ $SE$ $B$ $\beta$</td>
<td>$B$ $SE$ $B$ $\beta$</td>
</tr>
<tr>
<td>Flanker Test</td>
<td>.29 .33 .18</td>
<td>.10 .32 .06</td>
<td>.42 .33 .28</td>
<td>.19 .31 .12</td>
</tr>
<tr>
<td>Dimensional Card Sort</td>
<td>.06 .46 .03</td>
<td>.41 .39 .22</td>
<td>-.47 .46 -.27</td>
<td>-.01 .43 -.00</td>
</tr>
<tr>
<td>List Sort Working Memory</td>
<td>.21 .45 .12</td>
<td>.10 .36 .05</td>
<td>.49 .45 .30</td>
<td>-.13 .42 -.07</td>
</tr>
<tr>
<td>Pattern Comparison Processing Speed</td>
<td>-.10 .33 -.06</td>
<td>-.32 .31 -.21</td>
<td>-.01 .32 -.01</td>
<td>.40 .32 .26</td>
</tr>
<tr>
<td>Digit Span Forward</td>
<td>.67 .27 .45*</td>
<td>.55 .26 .35</td>
<td>.69 .27 .37*</td>
<td>.84 .28 .42*</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>.37 .28 .32</td>
<td>.52 .27 .34</td>
<td>.17 .25 .11</td>
<td>.35 .23 .25</td>
</tr>
<tr>
<td>IQ estimate</td>
<td>.32 .45 .24</td>
<td>.59 .39 .48</td>
<td>-.13 .45 -.10</td>
<td>-.21 .46 -.16</td>
</tr>
<tr>
<td>SES estimate</td>
<td>.17 .32 .18</td>
<td>.06 .26 .07</td>
<td>.36 .32 .39</td>
<td>.28 .30 .29</td>
</tr>
<tr>
<td>Motor estimate</td>
<td>.29 .28 .36</td>
<td>-.00 .35 -.01</td>
<td>.44 .28 .37</td>
<td>.33 .30 .32</td>
</tr>
<tr>
<td>Mutation</td>
<td>.24 .25 .17</td>
<td>.20 .21 .15</td>
<td>.36 .25 .29</td>
<td>-.19 .26 -.14</td>
</tr>
<tr>
<td>Behavioral estimate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BASC</td>
<td>-.72 .42 -.24</td>
<td>-.70 .39 -.24</td>
<td>-.49 .43 -.17</td>
<td>-.66 .40 -.22</td>
</tr>
<tr>
<td>BRIEF</td>
<td>-.09 .39 -.16</td>
<td>-.04 .25 -.14</td>
<td>-.11 .32 -.15</td>
<td>-.11 .41 -.17</td>
</tr>
<tr>
<td>Adjusted $R^2$</td>
<td>.53</td>
<td>.52</td>
<td>.45</td>
<td>.57</td>
</tr>
<tr>
<td>$F$</td>
<td>$4.02^*$</td>
<td>$4.32^*$</td>
<td>$4.06^*$</td>
<td>$4.47^*$</td>
</tr>
</tbody>
</table>

*$p < .01$

BASC = Behavior Assessment System for Children-2; BRIEF = Behavior Rating Inventory of Executive Functioning
Figure 2. Higher digit span predicts better academics across subject areas
IV. PART THREE (AIM 4):

*Weaknesses in executive functioning contribute to digit span performance*

ABSTRACT

**Objectives:** To examine digit span performance (digit span forward as a measure of verbal span, and digit span backward as a measure of mental manipulation) in dystrophinopathy as a function of executive skills as well as dystrophin mutation position, IQ, executive skills, SES, behavior and physical ability.

**Methods:** Forty-four boys with dystrophinopathy completed cognitive measures of executive functioning (including Digit Span and NIH Toolbox measures) and IQ. Motor skills were assessed and parents provided demographic information and child behavioral assessments. Dystrophin gene mutation positions were dichotomized into groups (upstream versus downstream of exon 43, location of central nervous system (CNS) isoforms previously linked to intellectual impairment). Multiple regression analyses examined unique and joint contributions of executive skills, intelligence quotient (IQ), SES, motor abilities, behavior, and mutation positions to digit span performance ($\alpha = .01$).

**Results:** Working memory executive measure significantly contributed to total digit span and digits backward; distal mutations also significantly contributed to digits backward. SES only, not the cognitive measures predicted digits forward.

**Conclusions:** The consistent deficient performance in digit span in individuals with dystrophinopathy was predicted by poor working memory; performance in parts of the task appear to be due to two independent constructs. A specific executive deficit in working memory may underlie inefficiencies in cognition observed in children with dystrophinopathy.

**Keywords:** Dystrophinopathy, Cognition, Executive skills, Verbal span, Working memory,
INTRODUCTION

The dystrophinopathies are muscle diseases ranging in severity due to the abnormal expression of the protein dystrophin. Dystrophin is important for maintaining, protecting, and signaling in nerve cells in the brain. In individuals with dystrophinopathies, dystrophin isoforms are missing. The abnormal dystrophin ultimately influences brain circuitry affecting the development of brain structures (Gorecki et al., 1998; Kim et al., 1995; Lidov, 1996; Sogos et al., 2002) and function (Anderson et al., 2003, 2004). Animal models of dystrophinopathy and autopsy studies have found dystrophin in the cerebral cortex, hippocampus, and cerebellum (Nichols et al., 2015). This pattern of brain abnormalities likely defines the pattern of cognitive and behavioral impairments that are observed in children with dystrophinopathy (Cohen et al., 2015; Perronnet & Vaillend, 2010). The cognitive findings suggest specific areas of impairment rather than generalized deficits. Our recent work showed that attention/executive skills are deficient in the children with dystrophinopathy compared to their overall intact intellectual abilities (Fee et al., 2017, unpublished).

Performance on digit span across studies is demonstrated to be relatively poorer than other measures even among those with high intellect (Hinton, De Vivo, et al., 2000). Impaired digit span is the most consistent finding across studies in the dystrophinopathies; however, the underlying cognitive construct contributing to this performance is still under debate. Digit span forward has been evaluated as a measure of nonmeaningful memory or short-term memory capacity (Oberauer et al., 2000) as well as basic attention (Hebben & Milberg, 2009; Lezak et al.). Whereas digit span backward has been consistently categorized as a measure of mental manipulation or working memory capacity (Oberauer et al., 2000). In the dystrophinopathies, the impairments in digit span have been interpreted in different ways. Hinton and colleagues have argued that poor digit span reflects a core deficit in verbal span, or decreased phonological storage, and that this deficit may
have wide ranging detrimental effects on language and academic skill development in boys with
dystrophinopathy (Cyrulnik, Fee, Batchelder, et al., 2007; Cyrulnik et al., 2008; Hinton, DeVivo, et
al., 2004). This hypothesis primarily focuses on limitations in the storage of information. Hinton
postulated the verbal span hypothesis based on evidence that boys with dystrophinopathy performed
in the average range across measures of embedded attention such as list learning and visual learning
tasks, suggesting span of attention was not compromised (Hinton, De Vivo, et al., 2000; Hinton et
al., 2007). However, the children did poorly on tasks of verbal contextual memory (Donders &
Taneja, 2009; Hinton, De Vivo, et al., 2000; Hinton et al., 2001) and on many language measures
requiring increased storage of verbal information, such as following multi-step commands, and
sentence repetition (Hinton et al., 2001; Hinton et al., 2007). Others have suggested that the poor
performance on digit span primarily reflects more of an executive deficit (Anderson et al., 1988;
Cotton, 1998; Donders & Taneja, 2009; Mento, Tarantino, & Bisiacchi, 2011; Wicksell et al.,
2004), but these areas have not been examined for their contribution to task performance. Attention
and executive functioning are areas of weakness in the dystrophinopathies, examining the
contribution of these inefficiencies to poor digit span may help to clarify the specific cognitive
construct deficient in this population.

Although there are a variety of perspectives as to what digit span measures, there is strong
evidence of its correlation with overall intelligence (Gignac & Weiss, 2015). Backward digit span in
particular has a strong correlation with fluid intelligence (Conway & Kovacs, 2013; Gignac, 2014)
and the capacity for digit span forward is needed for successful mental manipulation (Conway &
Kovacs, 2013). A number of executive functions are highly related to intelligence (Ackerman,
Beier, & Boyle, 2005; Conway, Cowan, Bunting, Therriault, & Minkoff, 2002), but involve
independent aspects of ability including processes such as inhibition, planning, and working
memory (Brookshire, Levin, Song, & Zhang, 2004; Miyake & Friedman, 2012) that impact overall
functioning. Digit span performance may be identifying a specific ability within individuals with
dystrophinopathy that is affecting overall cognition.

Our recent study demonstrated that executive skills are weak in children with
dystrophinopathy as well as impaired digit span. For further identification of specific predictors of
performance, we will examine the contribution of these generalized executive deficits to poor digit
span. We predict that the overall generalized executive weaknesses will significantly contribute to
digit span performance whereas illness severity, IQ, behavior, and demographic variables will not.
The executive measures will also be examined for independent contributions to span (forward) as
well as mental manipulation (backwards) given the potential impact on overall cognition. We
predict that specific executive tasks (attention/inhibitory control, set-shifting, working memory, and
processing speed) will be significantly associated with digit span forward and backward
performance such that digits forward will be associated with more basic tasks of attention and digits
backward will likely be associated with attention and working memory given the increased
cognitive demand. Executive control will be needed for the active maintenance of the information
presented in the digit span task particularly as the amount of span increases thus requiring intact
working memory.

METHODS

Sample

Fifty children with dystrophinopathy were recruited and enrolled from the Pediatric Neuromuscular
Center and MDA clinic located at New York Presbyterian Hospital, the academic hospital
associated with Columbia University. The clinic population came from the greater New York
metropolitan area and a wide range of socioeconomic levels. Eligible participants had a genetically
confirmed diagnosis of dystrophinopathy, were between the ages of 5 and 17, English as a primary
language (not of parents), expressed an interest in participating in research, and were deemed in relatively good health other than diagnosis of dystrophinopathy.

**Procedures**

The study was approved by the institutional review board at Columbia University Medical Center (IRB #AAAA5627) and The Graduate Center of City University of New York and was supported by a grant from the Muscular Dystrophy Association. After an introduction to the study by the treating physician, interested participants were described the study details by the study coordinator. All parents or guardians gave informed consent and all participants gave assent prior to enrollment.

All evaluations were conducted in a quiet room free of potential distractions, and breaks were provided; the assessment took around 2 hours. All neuropsychological measures were administered to all children in a standardized order. Measures were chosen to assess a broad range of intellectual function that emphasized attention/executive skills and minimized potential confounding effects of impaired motor abilities. All data were scored and converted to standardized scores using normative data. Additionally, participant medical records were reviewed for the results of genome sequencing and dystrophin gene mutation analysis, and mutation position was recorded for each participant. All data were coded without links to identifying information, entered into a secure database, and files were stored in a locked file cabinet. Patient confidentiality was protected and ensured by the Health Insurance Portability and Accountability Act, IRB regulations, as well as the study team.

**Measures**

Standardized neuropsychological measures with strong normative data were selected for the assessment. Measures were carefully chosen to minimize the amount of motor skills needed and most were able to be answered with a verbal response or by pressing a computer button.
Predictors

Executive Skills: Selected subtests from The National Institutes of Health (NIH) Toolbox was chosen given its wide use among diverse populations and representative normative data for individuals between the ages of 3-85 in the U.S. population. Subtests from the Cognitive Toolbox (Hodes et al., 2013; Weintraub et al., 2013) were administered, including attention (Flanker Inhibitory Control Test), set-shifting (Dimensional Change Card Sort Test), working memory (List Sorting Working Memory Test), and processing speed (Pattern Comparison Processing Speed Test). The measures all had short administration times and the normative data matched the study sample. The Flanker task required the participant to focus on a stimulus in the midst of congruent and incongruent stimuli. Both reaction time and accuracy were calculated. The Dimensional Change Card Sort task (DCCST) measured set-shifting by assessing two dimensions, both the color and shape of an object. Cognitive flexibility was evaluated by having the individual shift sets by dimensions. Both accuracy and reaction time was measured for the task. The List Sorting Working Memory Test (LSWMT) measured span and sequencing of different visually and orally presented stimuli (animals and foods). The participant organized the items in size order from smallest to largest, first within a single dimension and then on two dimensions. The score was equal to the number of items recalled and sequenced correctly. The Pattern Comparison Processing Speed Test (PCPST) measured processing speed. Participants had 85 seconds to rapidly judge as many item sets as possible whether two pictures presented were the same or not.

Potential contributing variable measures

Intellectual Function: Two measures were chosen as proxies for general intellectual function. The Peabody Picture Vocabulary Test-IV (PPVT-IV) (Dunn & Dunn, 2007) is a measure of single word comprehension. Performance on this measure is highly associated with general intellectual level (high correlation with Full Scale IQ score (.71) (Craig & Olson, 1991)), and was used as a verbal IQ
Additionally, the Comprehensive Test of Nonverbal Intelligence-2 (CTONI-2) (Ehrler & McGhee, 2008), a nonverbal measure of intelligence for individuals aged 5 to 89, was administered. The CTONI-2 measures analogical, categorical, and sequential reasoning, using three subtests of geometric designs. The CTONI-2 is correlated with full-scale intelligence measures including the Wechsler full scale IQ (.81) (Rossen, Shearer, Penfield, & Kranzler, 2005). An overall general IQ estimate was calculated by taking the mean of the verbal and nonverbal IQ scores and this estimate was used for analysis.

**Motor Measures:** The Brooke and Vignos Scale was used to assess motor functioning (Brooke et al., 1981; Vignos et al., 1963). The scales include measurements of upper and lower extremity functioning. Scores were categorized into minimal impairment (1-5); able to walk, climb stairs, and full use of arms, moderate (6-12); able to walk, but unable to climb stairs and not able to raise hands over head; and severe impairment (13-16); wheelchair bound, limited use of upper extremities. Fine motor abilities were also assessed using a finger-tapping task to ensure participants were able to respond accurately using the computer keyboard for the NIH Toobox measures. Each motor measure was administered by, or supervised by, a licensed physical therapist in the MDA clinic. For the regression analysis, upper and lower extremity motor scores were combined for analysis. Individuals with a gross motor score of 13-16 and finger tapping less than -1.5 standard deviation from the mean were categorized as severely impaired. The group with relatively functional motor skills were coded as 0 and the impaired group was coded as 1.

**Income:** Family Income was reported by parents in a family history questionnaire by checking off a range of incomes provided for the household. Income was based on reported household income and number of individuals in the home and then compared to the NYC census report for annual median household income; income was categorized into low, middle, high-income
levels. Income was then dichotomized comparing low (coded as 0) to middle/high income (coded as 1).

Behavioral Assessment: Parental report behavior measures were examined for potential contributions to cognitive performance. The Behavior Assessment System for Children, second edition (BASC-2) (Reynolds & Kamphaus, 2004), is a multidimensional assessment that evaluates various aspects of behavior and personality from the perspective of the parent. The BASC-2 was used to determine overall behavioral issues both externalizing problems (attention difficulties, conduct problems, hyperactivity) and internalizing problems (anxiety, depression, somatization) by using the Behavioral Symptoms Index score. Parents rated, on a “never” to “almost always” scale, on how often their child engaged in each behavior. The BASC-2 yielded a T score for the behavioral symptoms composite score. Adverse outcome was coded as a score of T > 67 on the scale and dichotomized (elevated (coded as 1) versus within normal limits (coded as 0)).

Additionally, the Behavior Rating Inventory of Executive Function (BRIEF) (Gioia et al., 2000) was used to assess executive function and self-regulation in everyday life and was examined as a potential marker for deficits in attention/executive skills. The scale is designed for children 5 to 18 years of age and has been well standardized. The measure provided information on different aspects of executive functioning through an overall Global Executive composite score (GEC). T scores were generated for the composite scale and a T score > 65 was categorized as elevated. The BRIEF GEC composite score was dichotomized into elevated (coded as 1) or within normal limits (coded as 0) and used for analysis.

Mutation: Mutations downstream of exon 43 have been associated with intellectual impairments (Daoud, Angeard, et al., 2009; Lenk, Hanke, Thiele, & Speer, 1993; Moizard et al., 2000; Taylor et al., 2010; Tuffery et al., 1995). To examine the potential contribution of mutation
position to the outcome measures, individuals were categorized into two groups: upstream of exon 43 (coded as 1) and downstream of exon 43 (coded as 0) for analysis.

**Outcome Measures**

*Digit span:* The Digit Span subtest from the Wechsler Intelligence Scale for Children (Wechsler, 2004), is part of the working memory scale and measures both basic attention and working memory. Forward digits measured span and the ability to attend to the task. Backward digits measured mental manipulation or working memory. All raw scores were converted to scaled scores.

**Statistical Analysis**

All scores were converted into z scores (mean of 0 and standard deviation of 1) to ensure the analysis utilized the same standardization scale. Descriptive demographic analyses were then completed on standardized scores. Variables were entered into a correlation matrix to determine their association with each other. Multivariate outliers were investigated by examination of Mahalanobis Distance and predicted residual values. Multicollinearity was examined by inspecting the correlation coefficients and tolerance/VIF values. Independence of observations was determined by the Durbin-Watson statistic. Independence of errors from regression predictors was confirmed by histogram evaluation and P-P plot of standardized residuals.

*The contribution of executive constructs to digit span (forward and backward)*

Three linear regressions were computed for the following outcome variables indexing digit span performance: (1) Total digit span; (2) digit forward; and (3) digit backward. Frequencies and distributions of each variable were determined and each was entered into a correlation matrix to determine whether it was associated with the outcome variable of interest. Significant independent variables were entered as predictors in two steps. Significant executive function measures were included in the first block of the regression model (Model I). The significant motor, demographic, SES, IQ, mutation position, and behavior were then added to the overall regression model in step
two (Model II). Standardized beta values (β) were used to indicate the relative influence of each predictor. Alpha was set at .01 to reduce the probability of Type I error.

RESULTS

Demographic information for the analyzed sample are presented in Table 1. The families who agreed to participate in study came from diverse backgrounds with a broad range of socioeconomic status and education.

*The contribution of executive constructs to digit span (forward and backward)*

Of note is that 44 of the 50 participants originally enrolled had completed parent questionnaire measures. Of those, 35 completed them in English and 9 completed them in Spanish, based on the parent or guardian’s primary language. To ensure that those participants whose parents did not compete the questionnaires were comparable to those whose parents did complete the scales, multiple exploratory independent sample t-tests were run. Group age, IQ, motor abilities, income, behavioral measures, mutation position and performance on all cognitive measures did not differ between the six families whose scales were not completed and those with completed scales, suggesting the missing data were reflective of the overall sample (Table 1). Regression assumptions were met for subsequent analyses, wherein no standardized residual outliers >2.5 standard deviations were present, there was no evidence of multicollinearity, errors were independent and normally distributed, and relationships between predictor and outcome variables were linear and homoscedastic.

The regression model for the cognitive executive measures (Flanker, DCCST, LSWMT, PCPST) (Model I) was significant (F (4, 40) = 7.22, p < .01) with LSWMT as the only significant predictor for Total digit span (β = .70, t(44) = 4.00, p < .01). Regression results including all predictor variables entered (Model II) are presented in Table 2. For Total digit span, the overall
Model II was significant, $F(10, 34) = 4.24, p < .01$, with predictors accounting for 48% of the variance; however, LSWMT was again the only significant predictor in the model, with better performance predicting higher Total digit span ($\beta = .63, t(44) = 2.79, p < .01$). Thus, better performance on LSWMT predicted higher total digit span.

For the individual subtests, Model I was not significant ($F(4, 40) = .16, p = .96$) for digit span forward; none of the cognitive executive measures predicted digit span forward. However, Model II with the inclusion of all variables was significant ($F(10, 34) = 3.42, p < .01$), $R^2_{\text{Adjusted}} = .41$) for predicting digit span forward; only low SES ($\beta = .86, t(44) = 3.15, p < .01$) significantly predicted forward span and no other variable was found to be significant. Thus, individuals who came from low-income households had lower digit span forward performance. Finally, for digit span backward, Model I with the cognitive variables was significant ($F(4, 40) = 5.01, p < .01$), $R^2_{\text{Adjusted}} = .31$). It was found that higher LSWMT scores predicted greater digit span backward performance ($\beta = .52, t(44) = 2.73, p < .01$). For Model II, all the predictors also explained a significant amount of variance for digit span backward ($F(10, 34) = 3.33, p < .01$), $R^2_{\text{Adjusted}} = .40$). Again better LSWMT scores ($\beta = .67, t(44) = 2.78, p < .01$) as well as mutation position ($\beta = .41, t(44) = 2.71, p < .01$) significantly predicted digits backward performance. Thus, both better LSWMT performance and having a mutation upstream predicted higher digit span backward performance. Those individuals with a mutation downstream of exon 43 performed more poorly on digit span backward.

**DISCUSSION**

The goals of this study were to help clarify the neuropsychological profile in children with dystrophinopathy by investigating a specific area of known cognitive weakness. Guided by our previous findings that areas of executive functioning are reduced in the population, we set out to
determine if these areas of executive weakness (selective attention/inhibitory control, set-shifting, working memory, and processing speed) are associated with the consistent finding of impaired digit span and further define the construct behind the two components of the task (forward span and backward span).

Since impaired digit span is the most consistent finding across studies, we wanted to determine what skills are needed to performance this task. We utilized a variety of executive measures to facilitate this investigation given that digit span is considered a task of verbal span/attention and working memory; thus we expected other measures utilizing these constructs to strongly correlate with this measure. As hypothesized, the working memory measure did significantly predict both total digit span as well as digit span backward. Other executive measures were not significantly associated with performance on digit span. Surprisingly in our sample, socioeconomic status significantly predicted digits forward, which may indicate the potential impact of SES on cognitive functioning. Although IQ was not found to contribute to digits forward, when the two income group IQ means were compared, there was a significant difference (F(1, 44) = 33.68, p < .01) such that the low income group (M = -.106 (.68)) performed significantly lower than the middle/high income group (M = .16 (.71)). This finding suggests that digits forward may be highly correlated to overall IQ and in fact was in our sample (r (44) = .42, p < .01). Additionally, 12 of the 19 individuals in the low income group came from bilingual or primarily Spanish speaking homes; digit span performance for Spanish speakers has been found to be worse due to syllabic demand (Olazaran, Jacobs, & Stern, 1996) as well as other cognitive and cultural factors (López, Steiner, Hardy, IsHak, & Anderson, 2016).

Our findings built upon our previous evidence that both digits forward and digits backward was reduced in a larger sample with dystrophinopathy when compared to sibling controls (Leaffer et al., 2016). In the current study, the finding that the working memory measure significantly
contributed to total digit span performance gives further evidence that executive functioning plays a significant role in successful performance on the task. Additionally, our results support that digits forward is likely measuring a different construct; notably none of the executive measures were found to predict digits forward, but this may be indicative of a limitation in the study in not utilizing a measure that selectively examined span or capacity. What is evident from the results is that two different cognitive constructs contribute to performance on the task but working memory is required for successful performance.

This study expanded upon our finding of a generalized executive weakness and further identified a specific executive deficit in working memory embedded in consistently impaired digit span performance. We hypothesize that digits forward likely represents a measure of capacity whereas digits backward involves the more complex ability of mental manipulation, both are needed for working memory. Working memory is strongly associated with individual ability to learn even more than IQ (Alloway, 2009). Moreover, it is not influenced by previous educational experience or socioeconomic status, but represents individual capacity to acquire and use knowledge in many daily life activities including academics (Alloway et al., 2005). Working memory is reported to predict academic learning including the subject areas of reading (Cutting et al., 2009; Sesma et al., 2009), comprehension (Nevo & Bar-Kochva, 2015; Pham & Hasson, 2014; Seigneuric, Ehrlich, Oakhill, & Yuill, 2000), and arithmetic (DeStefano & LeFevre, 2004; Geary et al., 2007; Swanson & Sachse-Lee, 2001). Working memory thus is a foundational skill embedded in many cognitive abilities. Reduced working memory appears to underlie the cognitive deficiencies found in the dystrophinopathies and not deficits in attention, but the specific impairment in this multicomponent system needs to be isolated and studied further.

The finding that working memory, an executive skill, contributes significantly to consistently impaired digit span performance may provide further evidence for disruptions in frontal
brain systems. A network of brain regions regulates executive functions. Neural mechanisms of working memory models suggest a complex interaction of several brain systems; however, the prefrontal cortex has been implicated across a number of studies as the control system in working memory that modulates incoming information and guides behavior (Curtis & D'Esposito, 2003; D'esposito & Postle, 2015). The central executive as described by Baddeley and Hitch (Baddeley, 2012; Baddeley, 1986) regulates the temporary storage of information for reasoning processes and signals and coordinates other brain areas (depending on the sensory modality needed for the task) to process the storage of information. Given that a wide variety of skills are needed to perform different executive tasks, it is inaccurate to state that only frontal regions are involved especially for the different working memory tasks. However, when specifically examining span tasks, several studies have indicated dorsolateral prefrontal involvement (Collette & Van der Linden, 2002; D'Esposito, Postle, Ballard, & Lease, 1999; Postle, Berger, & D'Esposito, 1999; Rypma, Prabhakaran, Desmond, Glover, & Gabrieli, 1999; Smith & Jonides, 1999). Reduced working memory as defined by impaired digit span in boys with dystrophinopathy provides additional cognitive evidence that frontal brain regions are likely affected by the disrupted functional dystrophin in the brain. Imaging studies would provide better support for this finding and help to further identify neurological systems affected by dystrophin abnormalities.

There are several elements to this study that strengthen the validity of our findings including the diversity of the sample, which may be more representative of the diversity within the dystrophinopathy population than found in other cognitive studies. Other studies have used samples of convenience without considering the contribution of socioeconomic status, ethnicity, and other demographic variables. Our study considered the contribution of overall IQ, motor deficits, mutation position, and behavioral/emotional functioning to cognitive performance. Although we did not utilize a control group, each participant was their own control, which prevented the influence of
outside variables on performance and improved generalizability. Finally, we chose a variety of psychometrically strong measures controlling for motor weaknesses to delineate potential deficits in aspects of executive functioning unlike other studies of cognition in the dystrophinopathies that examined broad deficits across domains.

The study also has several limitations. The study utilized a relatively small sample by including only those with fully completed measures. Although the sample size was deemed adequate and produced valid findings, a larger sample for the regression would provide more robust results and greater confidence in conclusions. Another potential weakness was the lack of a control group. The sample’s performance was compared to normative data; however, each participant was examined as his own control strengthening the internal validity of the study. Finally, the measures for the study were chosen to examine a variety of attention and executive skills, yet, we failed to include other measures of span such as a spatial span task to validate a span deficit or measures of more sustained attention to rule out deficits in attentional capacity or vigilance. Future studies could expand on our results and examine other measures that examine these cognitive constructs further.

Despite this study’s contribution to further understanding the role of disrupted dystrophin in the brain and its impact on functioning, weaknesses in working memory disrupts everyday activities that requires organization and goal-directed behavior. The identification of this area of weakness provides targets for remediation that can help in the attainment of individual goals and ultimately improve quality of life.

ACKNOWLEDGMENTS

This work was supported by a grant from the Muscular Dystrophy Association to VJH. We are very grateful to the families who took the time and effort to participate in our project.
Table 1. Characteristics of the Sample for Regression Analysis (n = 44)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SD)/% Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>11.04 (3.30)</td>
</tr>
<tr>
<td></td>
<td>5-17</td>
</tr>
<tr>
<td><strong>IQ</strong></td>
<td></td>
</tr>
<tr>
<td>- PPVT</td>
<td>-.28 (1.14)</td>
</tr>
<tr>
<td>- CTONI</td>
<td>-.23 (.78)</td>
</tr>
<tr>
<td><strong>IQ Estimate</strong></td>
<td>-.26 (.91)</td>
</tr>
<tr>
<td><strong>Income (SES) %</strong></td>
<td></td>
</tr>
<tr>
<td>- Low</td>
<td>39%</td>
</tr>
<tr>
<td>- Middle</td>
<td>29%</td>
</tr>
<tr>
<td>- High</td>
<td>32%</td>
</tr>
<tr>
<td><strong>Income (SES) Estimate</strong></td>
<td></td>
</tr>
<tr>
<td>- Low</td>
<td>41% (18)</td>
</tr>
<tr>
<td>- Middle/High</td>
<td>59% (26)</td>
</tr>
<tr>
<td><strong>Motor severity %</strong></td>
<td></td>
</tr>
<tr>
<td>- Minimal</td>
<td>57%</td>
</tr>
<tr>
<td>- Moderate</td>
<td>7%</td>
</tr>
<tr>
<td>- Severe</td>
<td>36%</td>
</tr>
<tr>
<td><strong>Finger Tapping Test(Dominant) %</strong></td>
<td></td>
</tr>
<tr>
<td>- WNL</td>
<td>86%</td>
</tr>
<tr>
<td>- ≤-1 SD</td>
<td>14%</td>
</tr>
<tr>
<td><strong>Motor Estimate</strong></td>
<td></td>
</tr>
<tr>
<td>- Functional</td>
<td>86%</td>
</tr>
<tr>
<td>- Impaired</td>
<td>14%</td>
</tr>
<tr>
<td><strong>Mutation Position</strong></td>
<td></td>
</tr>
<tr>
<td>- Upstream exon 43</td>
<td>39% (17)</td>
</tr>
<tr>
<td>- Downstream exon 43</td>
<td>61% (27)</td>
</tr>
<tr>
<td><strong>Behavior BASC-2</strong></td>
<td></td>
</tr>
<tr>
<td>- Behavior Symptoms Index</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T score</td>
</tr>
<tr>
<td>- WNL</td>
<td>52.97 (10.71)</td>
</tr>
<tr>
<td><strong>BSI</strong></td>
<td></td>
</tr>
<tr>
<td>- WNL</td>
<td></td>
</tr>
<tr>
<td>- Elevated</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td>14%</td>
</tr>
<tr>
<td><strong>BRIEF</strong></td>
<td></td>
</tr>
<tr>
<td>- General Executive Composite (GEC) T score</td>
<td></td>
</tr>
<tr>
<td>- GEC</td>
<td>53.97 (9.40)</td>
</tr>
<tr>
<td>- WNL</td>
<td></td>
</tr>
<tr>
<td>- Elevated</td>
<td>82%</td>
</tr>
<tr>
<td></td>
<td>18%</td>
</tr>
</tbody>
</table>

PPVT = Peabody Picture Vocabulary Test-IV; CTONI = Comprehensive Test of Nonverbal Intelligence-2; BASC = Behavior Assessment System for Children-2; BRIEF = Behavior Rating Inventory of Executive Functioning
<table>
<thead>
<tr>
<th>Variable</th>
<th>Digit span Total</th>
<th></th>
<th></th>
<th>Digit span Total</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE B</td>
<td>β</td>
<td>B</td>
<td>SE B</td>
<td>β</td>
</tr>
<tr>
<td>Flanker Test</td>
<td>.12</td>
<td>.19</td>
<td>.11</td>
<td>.07</td>
<td>.14</td>
<td>.10</td>
</tr>
<tr>
<td>Dimensional Card Sort</td>
<td>-.14</td>
<td>.24</td>
<td>-.11</td>
<td>-.35</td>
<td>.18</td>
<td>-.41</td>
</tr>
<tr>
<td>List Sort Working Memory</td>
<td>.79</td>
<td>.28</td>
<td>.63*</td>
<td>-.33</td>
<td>.21</td>
<td>-.39</td>
</tr>
<tr>
<td>Pattern Comparison Processing Speed</td>
<td>-.36</td>
<td>.22</td>
<td>-.34</td>
<td>.11</td>
<td>.16</td>
<td>.15</td>
</tr>
<tr>
<td>IQ estimate</td>
<td>.19</td>
<td>.24</td>
<td>.23</td>
<td>-.17</td>
<td>.18</td>
<td>-.28</td>
</tr>
<tr>
<td>SES estimate</td>
<td>.12</td>
<td>.51</td>
<td>.06</td>
<td>1.16</td>
<td>.37</td>
<td>.6*</td>
</tr>
<tr>
<td>Motor estimate</td>
<td>-.17</td>
<td>.35</td>
<td>-.08</td>
<td>-.58</td>
<td>.26</td>
<td>-.38</td>
</tr>
<tr>
<td>Mutation</td>
<td>.46</td>
<td>.27</td>
<td>.24</td>
<td>.31</td>
<td>.20</td>
<td>.24</td>
</tr>
<tr>
<td>Behavioral estimate</td>
<td></td>
<td></td>
<td></td>
<td>BASC-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-.41</td>
<td>.29</td>
<td>-.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.03</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BRIEF</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.03</td>
<td>.34</td>
</tr>
<tr>
<td>Adjusted R²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .01

BASC = Behavior Assessment System for Children-2; BRIEF = Behavior Rating Inventory of Executive Functioning
Figure 1. Higher List Sorting Working Memory performance predicted higher total digit span

LSWMT = List Sorting Working Memory Test
Figure 2. Income level predicted digit span forward performance

SES = Socioeconomic status (Income level)
Figure 3. List Sorting Working Memory performance and Mutation position predicted digit span backward

LSWMT = List Sorting Working Memory Test
V. DISCUSSION

Due to the pattern of neuropsychological performance in children with dystrophinopathies, the question of whether there was an underlying core deficit in limited linguistic capacity or a more generalized impairment in executive functions was unclear. To examine this, this dissertation assessed a diverse cohort of boys with dystrophinopathy with a series of measures known to recruit executive brain systems (including measures of selective attention/inhibitory control, set shifting, working memory, processing speed as well as measures of executive deficits in everyday life). Our studies set out to determine the extent of impairment in executive areas and the impact on real world functioning as measured by academics. We also investigated the relationship between these skills and mutation positions affecting CNS isoforms given the probable association of molecular abnormalities affecting functioning within neuronal networks. Thus, our hypotheses were that children with dystrophinopathy would: (1) perform more poorly on a range of measures of executive ability, as well as have difficulties in executive skills in daily life, and (2) these executive skill deficits would be associated with mutations affecting CNS isoforms. Further, given previous evidence of reduced academic skills in children with dystrophinopathy, (3) we expected academic skills in our sample to be weak and be associated with downstream mutation positions. Moreover, with the known contribution of executive skills to academics, we predicted individual executive skills would significantly contribute to academic functioning. (4) Finally, with digit span as the most consistent impairment across the literature, we hypothesized that one or more of these weak executive constructs would underlie total digit span performance as well as contribute to digits forward and digits backward. These studies further clarified specific areas of cognitive weakness within the population with dystrophinopathy as well as examined molecular associations for a better understanding of the possible roles of dystrophin in the central nervous system (CNS).
Specific cognitive impairments and the relation to mutation in CNS isoforms

Study of cognition within a specific domain began broadly given the conflicting hypotheses regarding the cognitive phenotype. Performance was assessed across a variety of purported executive tasks including measures of selective attention/inhibitory control, set shifting, working memory, and processing speed. Children with dystrophinopathy struggled across executive tasks even when compared to their overall general intellectual abilities. Parents were also more likely to rate their child with dystrophinopathy as having clinically significant executive difficulties with greater difficulties in functional areas defined by Shift, Emotional Control, and Behavior Regulation. All of these executive tasks have been associated with activity within frontal brain systems (dorsolateral, mid-ventrolateral and anterior cingulate cortex) (Duncan & Owen, 2000). Although it is simplistic to isolate one region based on measures that require a variety of skills, weakness across all the executive measures suggested a generalized deficit. And based on our findings of compromised test performance across executive skills as well as parent ratings in our cohort, we surmised that the lack of dystrophin in the brain must be contributing to functional abnormalities likely affecting a network of interacting brain regions that rely on frontal brain regions.

Finding a specific area of deficit prompted an investigation into the association of these executive deficits with mutation position. We replicated other studies finding a relationship between reduced IQ and individuals with distal mutations associated with CNS isoforms. However, there were no associations between performances on executive tasks and mutation segregation except for digit span. This result is consistent with those reported by Ricotti et al. (2016) (Ricotti et al., 2016) that showed performance in the Working memory index that includes digit span was significantly lower for those with mutations downstream of exon 63 affecting Dp 71. Those authors did not, however, examine the wide array of executive functions that we did. We also found that
performance on digit span forward, but not backward was significantly lower for those individuals with distal mutations, which has not been previously reported. Thus, our investigation into a wide array of executive skills revealed generalized deficits, but only digit span - specifically digits forward - was associated with dystrophin mutation position, suggesting a genotypic association with a specific cognitive deficit.

*Impact of executive weaknesses on academics and the association to CNS isoform*

Next, after documenting that executive functions are weak in this population, this dissertation examined how those findings translated to children’s everyday functioning by examining their impact on academic skill acquisition. Research informs that executive skills are required for a number of real-world activities in different environments including academics that often require the ability to handle large amounts of novel information, multitasking, and adaptation. Across studies, executive functioning significantly contributes to academic achievement (Best et al., 2011; Gathercole et al., 2004). In the dystrophinopathy population, academics have been found to be lower than expected across subject areas and significantly lower than individual IQ (Hinton, De Vivo, et al., 2004), and the current work replicated and extended those findings to a new group of children with dystrophinopathy. In addition, the current work demonstrated that reduced academics are also associated with more distal mutations suggesting a relationship with disrupted CNS isoforms and is the first study to demonstrate such an association. Given the known relationship between academics and executive skills in the general population, we predicted poor academic performance would be associated with the generalized executive weaknesses in the group with dystrophinopathy. Surprisingly, digit span forward was the only measure that predicted total academic achievement as well as performance in subject areas. Other executive skills and the included relevant variables of IQ, mutation position, illness severity or family income did not
predict academic performance. These results replicated our previous finding that performance on total digit span as well as IQ were the only significant contributors to academic performance (Hinton, De Vivo, et al., 2004). However, that prior work did not examine an array of executive functions as potential contributors to academics, and the current work examined executive skills in much greater depth. The current work also further specified performance on digit span by examining digits forward and backward and found that only forward span was significantly associated with mutation position and only limited forward span predicted academic performance.

Others have shown that digit span predicts academic ability in the general population. Findings have demonstrated that digit span contributes more to reading performance than IQ (Gathercole & Pickering, 2000; Mayes et al., 2009). We have examined the contribution of digit span to academics and reading before as well, but we never before examined the contributions of other executive skills to tease out the specific executive contribution (Hinton, De Vivo, et al., 2004; Leaffer et al., 2016). The current findings highlight the specificity of the digit span contribution within the dystrophinopathy group. The current findings validate the importance of capacity (as measured by the simple task of forward span) for cognitive processing that impact on outcomes such as academic achievement. Even more striking, the current results provide further evidence for a genotypic association with a very specific cognitive deficit, namely the repetition of digits forward.

**Digit span in dystrophinopathy**

Across our investigation of cognition in dystrophinopathy, performance on the digit span measure revealed a significant weakness, an association with mutations affecting CNS isoforms, and predicted academic achievement. In general, digit span forward has been evaluated as a measure of short-term memory capacity (Oberauer et al., 2000) and basic attention (Hebben &
whereas digit span backward has been consistently categorized as a measure of mental manipulation or working memory capacity (Oberauer et al., 2000). Yet, impairments in digit span have been interpreted in different ways. Our prior work examined the contribution of digit span forward and backward in the context of Baddeley’s model of working memory, and found that children with dystrophinopathy struggled primarily with forward span (believed to reflect phonological storage) but there was also a contribution of backward span (believed to implicate the central executive) (Leaffer et al., 2016).

For the current work, we evaluated the contribution of weak performance on the executive measures to digit span performance including digits forward and backward. The consistent deficient performance in digit span was most predicted by poor working memory as measured by the NIH List Sorting Working Memory Test (LSWM), but performance on total digit span appeared to be due to two independent constructs. Digits forward was not predicted by any of the executive tasks; however, low family income was shown to contribute the performance. Both lower IQ and language factors (both found in the low-income group) likely had an indirect contribution to digits forward performance. Whereas digits backward performance was only predicted by LSWM. What is evident from the results is that two distinct cognitive constructs contribute to performance on the digit span task but working memory is a requirement for successful total digit span performance. Additionally, our results supported that digits forward is likely measuring a distinct cognitive construct that is related to both IQ and income, but was not defined by any of the tested executive tasks. We thus hypothesize that digits forward likely represents a measure of capacity whereas digits backward involves the more complex ability of mental manipulation, both are needed for working memory. Working memory is strongly associated with individual ability to learn even more than IQ (Alloway, 2009) and it is not influenced by educational experience or socioeconomic status, but represents individual capacity to acquire and use knowledge in many daily life activities.
including academics (Alloway et al., 2005). Thus, in the dystrophinopathies, we can conclude a specific executive deficit in working memory underlies inefficiencies in cognition and may be associated with mutations affecting CNS isoforms.

A core working memory deficit with limited linguistic load

In our series of publications examining the cognitive profile associated with dystrophinopathy, including our recent findings, we have identified executive weaknesses regulated by a core cognitive deficit in reduced digits forward or limited linguistic load. Poor performance in digit span has been documented across our studies (Hinton et al., 2001; Hinton, DeVivo, et al., 2004; Hinton et al., 2007; Leaffer et al., 2016), as well as weak story memory (Donders & Taneja, 2009; Hinton, De Vivo, et al., 2000; Hinton et al., 2001), and impaired sentence repetition (Hinton et al., 2001; Hinton et al., 2007). Both digits forward and backward were deficient in another study and predicted poor reading performance (Leaffer et al., 2016). Our current findings documented generalized executive weakness in both cognitive and behavioral functioning. Yet, only limited span (as defined by performance on digits forward) predicted performance in real world academics across subject areas. Moreover, only limited forward span was associated with mutations that disrupt CNS isoforms. The evidence is overwhelmingly convincing that there is a core deficit in dystrophinopathy within the working memory system driven by a limitation in capacity.

The evidence points to a core cognitive deficit within the working memory system. There are several models of working memory. Baddeley’s multicomponent working memory model is the most referenced. This model proposes that a central executive allocates and controls executive processes and integrates information from two subsystems, the phonological loop and visuospatial sketchpad (Baddeley, 2012). Weaknesses have now been demonstrated in both the executive system as well as limitations in linguistic storage in children with dystrophinopathy. It would be simplistic
to isolate these deficits to one system, but our data support a weakness within the working memory system that involves a network of interacting abilities affecting real word functioning.

**Compromised Central Executive network**

George Miller in his seminal research theory (1956) posited the potential for a limitation in memory capacity (Miller, 1956) with a span of seven for digits, around six for letters, and approximately five for words, but evidence over time has proven that capacity is quite variable both in number and across individual ability. Span capacity appears to be reduced in individuals with dystrophinopathy affecting performance across a number of abilities and is somehow associated with the dystrophin disruptions altering brain development and function. Span is often described as interchangeable with short-term rote auditory memory processes, but is distinct from long-term memory storage and these likely involves two separate systems of storage (Hale, Hoeppner, & Fiorello, 2002). Span has been demonstrated to even increase with age throughout childhood and not be affected by education or other environmental factors (Orsini et al., 1987; Taras & Potts-Datema, 2005). Our work has shown this developmental trajectory for digit span performance in the dystrophinopathy group such that span increases with age comparable to unaffected siblings, however, performance in the dystrophinopathy group is consistently worse over time compared to siblings (Leaffer E, 2014; Leaffer et al., 2016) (see figure below). Thus, the dystrophinopathy group has the same developmental trajectory of span as the general population, but start out with a reduced verbal span capacity that remains consistently lower than their peers over time.
Span capacity also depends on the sensory route of information presented as well as executive-attention processes that regulate working memory span measures. Storage and rehearsal processes are thus associated with domain-specific aspects of complex cognition (Kane et al., 2004). Since auditory span was examined in dystrophinopathy, we can describe the deficit as reduced linguistic load, but there may be a generalized limitation in capacity that still needs to be further explored in the population. Reduced capacity likely impacts a number of cognitive abilities especially those requiring the initial storage of a string of information and thus may explain the strong association with overall intellectual abilities (Gignac & Weiss, 2015).

As previously described in the Baddeley model, the central executive of the working memory system regulates incoming information via attention and executive control processes. Our evidence suggests a generalized weakness in executive skills in individuals with dystrophinopathy. And specific deficits are even further defined by a deficit within the working memory system that originates with reduced verbal span. The central executive has been localized to frontal regions, but more recent evidence suggests interactions between networks of brain regions (Collette & Van der Linden, 2002). However, the prefrontal cortex (PFC) has been consistently described as a having an
integral role in maintaining information in an active state during working memory tasks. Research has demonstrated a pattern of neural circuitry subserving performance on working memory tasks that involves persistent activity within the PFC (Goldman-Rakic, Cools, & Srivastava, 1996). One theory posits that neurons in the lateral PFC actually store information via this persistent activity before signaling the designated sensory stores. This theory supports a network of consistent communication between the lateral PFC and sensory areas during encoding and maintenance of information, a process that defines the working memory system (Sreenivasan, Curtis, & D’Esposito, 2014). It is hypothesized that the neural mechanisms behind a working memory system are part of a dynamic network of short-term synaptic facilitation and precise tuning of recurrent excitation and inhibition (Barak & Tsodyks, 2014). In dystrophinopathy, it is presumed that there is disruption in brain network dynamics due to disruptions in synaptic activity provoked by dystrophin abnormalities affecting brain circuitry where dystrophin is normally present. This disruption likely underlies the core deficit of reduced verbal span.

*Less about location more about connections*

In dystrophinopathy, mutations affect protein expression, which impacts on protein function, and plays a significant part in the onset and development of disease. Proteins have a major role in neuronal functioning and are necessary for the strengthening of synapses needed for learning and memory (Reva, Antipin, & Sander, 2011). In dystrophinopathy, there are a variety of mutations in the dystrophin gene that alter the structure and function of dystrophin. Dystrophin has an integral role in the dystrophin-glycoprotein complex in stabilizing, protecting and anchoring membrane-bound proteins such as neurotransmitter receptors. Mutations may affect full-length dystrophin or shorter dystrophin variants including CNS isoforms Dp140 and Dp71. Functional studies with dystrophin-deficient mouse models have greatly increased knowledge of the possible roles of
Dp427 and Dp71 function in the brain including roles in the regulation of membrane receptors and channels involved in neuronal and glial functions. However, the role of Dp140 is less clear; it appears to have a role in brain development given its abundance in the fetal brain, but the specific part the isoform plays in CNS cell functions has not been validated (Perronnet & Vaillend, 2010). Alterations in full-length dystrophin or the isoform Dp71 expression may thus change neuronal excitability for neuronal communication, a communication structure that subserves cognitive abilities.

*Full-length dystrophin*

Biochemical abnormalities have been described in both individuals with dystrophinopathy and the dystrophin-deficient *mdx* mouse. The role of dystrophin in the brain is complex and most of what we know comes from animals models. Full-length dystrophin (Dp427) in the brain has been associated with different populations of neurons including cortical and hippocampal pyramidal cells as well as cerebellar Purkinje cells. Evidence suggest that Dp427 plays a significant role in the anchoring and clustering of GABA_\(A\) receptors that are necessary for efficient synaptic signaling (Kueh, Head, & Morley, 2008b; Vaillend & Billard, 2002). The impaired assembly of inhibitory receptor subunits also affects stabilization for communication. The absence of dystrophin is thus linked to the reduced quantity of GABA_\(A\) receptor clusters in the hippocampus, cerebellum, and amygdala (Sekiguchi et al., 2009) as well as in Purkinje cells and hippocampal CA_1_ neurons in the mdx model (Anderson et al., 2012). Human dystrophinopathy research have reported similar GABA_\(A\) abnormalities with instability of the neuronal cytoskeleton at the synapse (Anderson et al., 2012; Vaillend & Billard, 2002) and altered dendritic development (Jagadha & Becker, 1988). Dysfunction in GABA_\(A\) receptors may also cause disruption in secondary processes such as calcium homeostasis (Chavas, Forero, Collin, Llano, & Marty, 2004; Kueh, Head, & Morley, 2008a) and rapid glucose metabolism (Rae et al., 2002) in the brain. Lack of dystrophin thus appears to cause a
chain of abnormalities starting with disrupted GABA$_A$ receptor activation that interferes with the balance of processes needed for synaptic signaling; the consequences may include impairment in long-term plasticity (Anderson et al., 2004) and may well make cells more receptive to necrosis (Anderson et al., 2012). Additionally, excitation via glutamatergic receptors (NMDA receptor) may be abnormally active due to reduced dendritic inhibition (Vaillend & Billard, 2002) impacting the balance between long term potentiation and downregulation or long-term depression. If synapses continually increased in strength as a result of enhanced potentiation, they would eventually reach a maximal point resulting in synaptic function failure. Disrupted cholinergic transmission is also related to these dystrophin abnormalities. Alterations in both inhibitory and excitatory synapse function affects the cholinergic role in stabilizing receptors in central synapses and regulating the efficiency of neuronal networks (Cohen et al., 2015). Thus, the lack of full-length dystrophin disrupts the interplay between glutamate, GABA$_A$, and cholinergic signaling that is needed for synaptic communication.

_Dp71_

The isoform Dp71 is the most abundant dystrophin-gene product in the adult brain (Jung, Filliol, Metz-Boutigue, & Rendon, 1993). Much like full length dystrophin, there appears to be a strong association with Dp71 and neuronal functioning with specific effects on excitatory synaptic organization and function (Perronnet & Vaillend, 2010). Dp71 is found in both neurons and glia specifically localized to perivascular astrocytes and cerebellar Bergman glia cells (Waite et al., 2012). Within perivascular astrocytes, Dp71 are thought to have a role in maintaining the function of the blood brain barrier (Anderson et al., 2012), which indirectly affects neuronal function. In glia cells, Dp71 has been associated with multiple roles including the regulation of water homeostasis, potassium buffering and regulating blood-neural barrier function; all if disrupted would have a significantly negative impact on neuronal communication (Culligan, Glover, Dowling, &
Ohlendieck, 2001). Additionally, Dp71 likely plays an integral part in synaptic organization and function based on its expression at postsynaptic densities. Enhanced glutamatergic transmission in CA1 hippocampal neurons was found in depleted Dp71 mice models (Daoud, Candelario-Martínez, et al., 2009) suggesting a level of dysfunction in excitatory synaptic communication. Dp71 is associated with glutamate receptor clustering including NMDA and AMPA subunits and organization of signaling proteins required for synaptic transmission and plasticity (Perronnet & Vaillend, 2010). Thus alterations in Dp71 have been linked to reduced synapse density and altered morphology of the postsynaptic active zone. Overall, Dp71 has several roles in cellular function in the brain including a significant role in glutamatergic synapse organization and functioning that is likely affecting synaptic transmission.

A Poor Network Connection in Dystrophinopathy

The origin of functional deficits in cognition likely involves disrupted functioning of Dp427, Dp71, and Dp140. Given the crucial role of synaptic plasticity in cognitive processes, the altered synaptic function due to dystrophin deficiencies may explain the documented cognitive deficits. Mutations affecting shorter isoforms have been examined for associations between mutation position and reduced intellectual functioning (Bushby et al., 1995; Bushby & Gardner-Medwin, 1993; Felisari et al., 2000; Moizard et al., 1998; Rapaport et al., 1991; Ricotti et al., 2016; Taylor et al., 2010), but the findings have been variable across studies (Bardoni et al., 2000; Daoud, Angeard, et al., 2009; Moizard et al., 2000; Rasic et al., 2014; Wingeier et al., 2011). Loss of Dp427 has been associated with functional impairments in synaptic inhibition (Perronnet & Vaillend, 2010) as well as reductions in GABA_{A} receptors in hippocampus, cerebellum, and cortex, likely impacting functioning in these areas including long-term plasticity (Anderson et al., 2004; Vaillend et al., 2004). Dp71, the most plentiful dystrophin product in the CNS, expressed in the hippocampus (Daoud, Candelario-Martinez, et al., 2009) and Dp140 which is mainly expressed in
the fetal brain (Bardoni et al., 2000; Waite et al., 2012), both isoforms have also been associated with greater cognitive impairments (Anderson et al., 2002; Bardoni et al., 2000; Taylor et al., 2010; Wingeier et al., 2011). Mutations more distal have been linked to the expression of these CNS dystrophin isoforms including Dp71 and Dp140. The frequency of intellectual impairment appears to be related to dysfunction within these isoforms (Daoud, Angeard, et al., 2009; Lenk et al., 1993; Moizard et al., 2000; Taylor et al., 2010; Tuffery et al., 1995). A number of studies have examined abnormalities in Dp71, typically distal of exon 63, and severe intellectual impairments have been repeatedly reported to be related to this distal position (Daoud, Angeard, et al., 2009; Moizard et al., 2000; Rasic et al., 2014). Reduced Dp71 protein levels have even been shown to be associated with intellectual disability in individuals without a diagnosis of muscular dystrophy (De Brouwer et al., 2014).

Two recent studies (D'Angelo et al., 2011; Ricotti et al., 2016) replicated the results of others and found higher rates of intellectual disability and behavioral difficulties in individuals with mutations downstream. However, even more specific, both studies also showed a strong association with impaired digit span (working memory) and mutations disrupting shorter isoforms downstream (D'Angelo et al., 2011; Ricotti et al., 2016) with higher levels of impairment in those with mutations affecting Dp71 (Ricotti et al., 2016). This dissertation explored the association between genotype and the neuropsychological profile. Our work built upon the evidence that overall impaired cognition is associated with more distal mutations (Daoud, Angeard, et al., 2009; Lenk et al., 1993; Moizard et al., 2000; Taylor et al., 2010; Tuffery et al., 1995). We expanded this investigation by testing whether specific cognitive weaknesses in executive skills are related to mutations downstream of exon 63, affecting Dp71. Overall IQ was significantly lower for those individuals with distal mutations, but contrary to our hypothesis, there were no significant differences with mutation segregation and performance on most executive tasks, but a significant difference was
observed on digit span. Even more specifically, we found that performance on digit span forward was significantly lower for those individuals with distal mutations. This finding suggests there is something distinct about verbal span; verbal span capacity may be necessary to perform more complex tasks that have higher demands on cognitive resources. Span has been strongly correlated with general intelligence (Gignac & Weiss, 2015) and has been demonstrated to strongly influence the application of cognitive abilities in everyday functioning such as academics (Conway & Kovacs, 2013). Even more important, this may give further evidence that verbal span is the core cognitive deficit in individuals with dystrophinopathy and that it is associated with mutations affecting CNS isoforms namely Dp71. This relationship may provide evidence for neuronal disruptions due to lack of dystrophin affecting neuronal communication that promote plasticity and cognitive functioning.

A hypothesized model of cognitive dysfunction in dystrophinopathy

Mutations in the dystrophin gene alters functional dystrophin production, both full-length and dystrophin isoforms (namely Dp71) disrupting CNS processes. Individuals develop with dystrophin deficient brains which likely impacts the development of networks in a variety of brain structures where dystrophin is normally present. At a network level, there is an imbalance in neurotransmitter activity including reduced inhibitory (GABA<sub>A</sub>) (Vaillend & Chaussenot, 2017) and enhanced excitatory (glutamate) synaptic organization and function (Perronnet & Vaillend, 2010) as well as dysregulation in cholinergic stabilization that affects both synaptic efficiency and potentiation (Cohen et al., 2015). The lack of dystrophin is thus associated with altered neuronal function affecting synaptic transmission. Abnormalities in neuronal communication likely affects both efficiency in processing and the amount of information transmitted. Abnormalities in the active excitation of selective neurons and in neural circuits may result in dysfunction in brain systems
where dystrophin has been found including the cerebellum, frontal cortex, and hippocampus. Given the pattern of cognitive deficits across studies including our finding of generalized executive weakness and reduced digit span, a core cognitive deficit can be defined as a limitation in linguistic load mediated by a disrupted working memory system.

Molecular neural models of a working memory system propose increased activation of cells within the prefrontal cortex (Arnsten & Jin, 2014). The working memory system is a product of persistent firing regulated by glutamatergic pyramidal cells (NMDA receptors) maintained by lateral inhibition from GABAergic interneurons (Goldman-Rakic et al., 1996) and depolarization by nicotinic receptors. This microcircuitry in layer III of the dorsolateral PFC is likely affected in dystrophinopathy resulting in dysfunction in the interplay between cholinergic signaling and glutamate and GABA_A receptors. The abnormalities due to altered dystrophin are believed to affect the balance (excitation/inhibition) of processes needed for neuronal communication in the prefrontal cortex, which has been shown to be active in working memory cognitive tasks.

It is simplistic to isolate these deficits to one system, but our data support a weakness within the working memory system that involves a network of interacting abilities affecting functioning. Likewise, mapping these deficits to neuroanatomical correlates likely involves a network of interacting brain regions where dystrophin plays an active role. Frontal systems are associated with executive functioning, working memory and increased regional activation (Goldman-Rakic et al., 1996) as verbal load increases (left inferior frontal gyrus for three elements; left inferior frontal gyrus, dorsal prefrontal cortical areas, and caudate nucleus for additional loads of six elements or more (Rypma, Prabhakaran, Desmond, Glover, & Gabrieli, 1999)). Given that dystrophin is localized primarily to several brain regions namely the cerebellum, hippocampus, and association cortex (including frontal regions) along with the discovered pattern of cognitive deficits, it is hypothesized that the neuropsychological profile in the dystrophinopathies is due (in part) to
disruptions in cerebro-cerebellar pathways (Cyrulnik & Hinton, 2008). Functional brain mapping has validated a cerebro-cerebellum network that is activated in working memory tasks (Marvel & Desmond, 2010a, 2010b) with the activation of a cognitive tract involving the ventral dentate projecting to frontal cognitive areas (Chen, 2005; Kirschen, Chen, Schraedley-Desmond, & Desmond, 2005; Marvel & Desmond, 2010a). Evidence also suggests that the cerebellum directs frontal systems to utilize anticipatory control mechanisms for regulating the amount and use of cognitive resources (Koziol, Budding, & Chidekel, 2012). Our series of studies provide evidence that disruption in executive systems including reduced span and poor verbal working memory may map on to dystrophin deficiencies in a cerebro-cerebellar network.

It is evident from our findings that these selective deficits in the working memory system may have wide-ranging consequences on learning and everyday functioning including academics. If not addressed, over time learning may become more difficult as concepts become more complex, especially since most academic instruction is dependent on verbally based learning that increases in capacity. Our data suggested that in children with dystrophinopathy, this underlying verbal span capacity is reduced. Capacity is a key component to basic learning; learning requires the ability to simultaneously acquire multiple units of information, hold that information in an active state, and then process relevant information (Unsworth, Fukuda, Awh, & Vogel, 2014). Capacity is likewise needed in everyday life to perform daily activities, solve problems and reason about real world solutions. Real-world functioning in different environments including academics often requires the ability to handle large amounts of novel information, multitasking, and adaptation, all requiring working memory as well as other executive skills. These results aid in the identification of the potential origin of cognitive difficulties that can be addressed and improved upon with compensatory strategies in treatment settings and transferred to day-to-day functioning to promote a better quality of life. Although limited in generalizability, recent work examining the possibility of
cognitive training specifically in areas of working memory may help to improve task specific learning such as aspects of academic learning (Klingberg, 2010). Well-developed skills in working memory have been linked to academic performance as well as protective health behaviors and greater longevity especially in those with chronic illness.

**Strengths and weaknesses**

There are several elements to this dissertation that strengthened the validity of the findings including the diversity of the sample, which may be more representative of the diversity within the dystrophinopathy population than found in other cognitive studies. Other studies have used samples of convenience without considering the contribution of socioeconomic status, ethnicity, and other demographic variables. The diversity in our sample made our findings more profound for generalizability to the population of those affected by the disorder. Although we did not utilize a control group, each participant was their own control, which prevented the influence of outside variables on performance and improved generalizability. Sample size was also relatively large for the studied population and adequate for the aims of the study to detect clinical significance.

Additionally, the measures chosen for the study represent a greater variety of attention/executive skills (including selective attention, set shifting and cognitive flexibility, working memory, and processing speed) than have previously been tested in one study. Our simultaneous collection and analysis of motor data confirmed the cognitive data were not influenced by motor ability. And for the behavior rating comparison the sample was compared to published norms. It is possible that the general effects of chronic illness, not specific to dystrophinopathy, might impact on parent report of executive skills in everyday functioning, yet it is known that among other groups with chronic illness, executive functions are not always reported to be elevated, so we infer our data reflect true executive deficits associated with dystrophinopathy, not generalized
consequences of chronic illness. The dissertation thus examined a wide array of executive abilities in the context of several individual variables including physical functioning and demographics to decipher underlying contribution to performance. We chose a variety of psychometrically strong measures controlling for a number of potential confounds to delineate potential deficits in specific aspects of functioning. The finding of one specific deficit, performance on digits forward, in the midst of so many potential contributing factors, including IQ, physical abilities, behavior, and demographic factors, predicting academic performance emphasized the strength of the finding. Additionally, this study was the first to examine molecular associations with specific cognitive skills including executive functioning and academic performance. This not only aids in the understanding of the relationship between a disorder with a known molecular cause and selective cognitive impairments, but also a better understanding of the various contributions to real world functioning.

The dissertation also had several limitations. The study utilized a relatively small sample for regression analysis by including only those with fully completed measures. Although the sample size was deemed adequate and produced valid findings, a larger sample for the regression models would provide more robust results and greater confidence in conclusions. Another potential weakness was the lack of a control group. The sample’s performance was compared to normative data; however as previously stated, each participant was examined as his own control strengthening the internal validity of the study. Academic measures utilized assessed basic skills of language and arithmetic; more complex academic measures would help to further describe the extent and specifics of academic impairment within the population. Finally, although diversity is a strength, our measures may have been impacted by certain individual variables including language for which a percentage of our sample came from Spanish speaking homes. The development of language skills may be different for bilingual individuals and this may have influenced digit span performance;
however, the dissertation results support and replicate findings found in studies with primarily English speaking samples. Future studies could expand on our results and examine other measures that examine these cognitive constructs further.

CONCLUSIONS: The pattern of deficits observed among children with dystrophinopathy is indicative of specific weaknesses that underlie the efficiency of cognitive processing and behavior, likely suggestive of the potential dystrophin deficiencies affecting brain networks. This dissertation set out to clarify the neuropsychological profile and the association to dystrophin gene mutation position. We found that children with dystrophinopathy have generalized executive weaknesses, but only digit span specifically digits forward was associated with mutation position and predicted academic performance. Our findings thus indicate a weakness within the working memory system that involves a network of interacting abilities affecting real word functioning. Mutations in the dystrophin gene affects CNS isoforms that regulate the balance of neuronal signaling. Disruption in the flow of neuronal communication results in a reduction in the amount of information that can be processed. These molecular abnormalities likely underlie the selective cognitive deficits in the population with a core cognitive deficit in verbal span. And the cognitive and behavioral evidence supports that the disorder impacts a network of interacting brain regions rather than just one isolated region. However, the central executive (in frontal systems), part of working memory circuitry, may be more susceptible to network dysfunction in the dystrophinopathies. Limited capacity is embedded within this compromised working memory system that influences performance in more complex cognitive tasks as well as learning in academics. Capacity is thus a necessary component of learning that affects the development of academic skills, performance in everyday tasks, and contributes to the attainment of future goals. Validation by replicated results for the verbal span hypothesis not only clarifies the cognitive phenotype, and a possible association with dystrophin
molecular abnormalities, but also provides potential targets for interventions. The underlying
deficits in working memory can exert a significant impact on day-to-day functioning, but there are
several cognitive techniques that can be learned within the context of remediation or additional
compensatory strategies that can be used in daily life. Techniques that promote and enhance
individual strengths will foster adaptation to the multiple demands in everyday life aiding
individuals to better cope with the stressors associated with chronic illness.

*Post-hoc assessment of results*

Post hoc analyses were conducted to further examine the potential driving group influence
of individuals with a mutation affecting CNS isoform Dp71 \((n = 7)\) to the overall results of the
studies. Examining between group performance without the Dp71 group revealed that only IQ \((F(2, 48) = 6.46, p < .01)\) was statistically different between groups, both total digit span and digit span
forward no longer met significance; however, digit span forward trended toward significance
\((F(2,48) = 3.36, p = .07)\) in the analysis. These results thus emphasize the strength of the association
between digit span forward and mutations affecting Dp71. The original post hoc analysis between
the three groups (upstream exon 30, 31-62 (Dp140), and downstream exon 63 (Dp71)) suggested
similar findings; the only significant difference was between the upstream exon 30 group and
downstream exon 63 group, there were no other significant group differences.

In contrast, when comparing the adjusted groups (without Dp71) on academic performance,
the original findings remained showing a significant difference between upstream and downstream
groups (Dp140 only) across academic measures including total \((F(2, 48) = 7.89, p < .01)\), reading
\((F(2, 48) = 9.08, p < .01)\), spelling \((F(2,48) = 7.54, p < .01)\), and calculation \((F(2,48) = 3.41, p =
.06)\). These results suggest that academic performance is still depressed for individuals with other
CNS isoforms, namely Dp140. For the regression analysis, across the regression models when the
Dp71 group was removed, the findings no longer held that digit span forward significantly predicted
academic performance across measures. However, digit span forward was still the only variable that trended toward significance across the significant regression models (variance ranged from .36 to .65): total academic achievement ($\beta = .28, p = .07$), reading ($\beta = .28, p = .08$), spelling ($\beta = .29, p = .08$), and calculation ($\beta = .28, p = .05$). It appears that individual performance in the Dp71 group, in particular digit span performance, increased the strength of the association between digit span performance and the academic outcomes; poor performance on digit span forward predicted poor academic scores. These series of findings support a strong relationship between performance on digit span forward and individuals with mutations affecting Dp71. In fact, 6 of the 7 individuals with a mutation affecting Dp71 scored more than 1 standard deviation below the mean on digit span forward (digit span forward Mean = -1.43; SD = .61), but IQ was in the low average range (IQ Mean = -1.05, SD = .80). These findings are in line with other studies showing an association with more severe cognitive impairments in individuals with mutations affecting Dp71, but these results may be identifying a specific deficit in span capacity that may be underlying the cognitive weaknesses in individuals with dystrophinopathy.

Additionally, the data were further evaluated for the potential influence of reduced motor skills. Previous analysis showed no significant differences across cognitive measures between both the gross and fine motor categorized groups. When fine motor abilities were assessed as a covariate, the results remained the same with significant between group differences (both when assessing upstream vs. downstream mutation groups as well as the three groups: upstream of exon 30, 31-62, and downstream of exon 63) on IQ ($F(3,47) = 4.25, p < .01$), total digit span ($F(3,47) = 4.07, p < .01$) and digit span forward ($F(3,47) = 4.03, p < .01$) further confirming that motor skills did not confound performance on the cognitive measures. IQ was also further examined for potential contributions to cognitive performance. When paired t-tests were run comparing the verbal IQ and nonverbal IQ measures separately to the cognitive executive measures, the results remained
the same that performance on all executive measures was significantly lower than both IQ measures except for digit span forward, which was not significantly different from both verbal IQ ($t(49) = 1.41, p = .17$) and nonverbal IQ ($t(49) = 2.43, p = .04$). Both IQ measures were then examined separately for their relationship to digit span performance. Both the verbal (crystallized intelligence) (correlations for total at .70, forward at .40 and backward at .56) and nonverbal (fluid intelligence) (correlations for total at .51, forward at .37, and backward at .39) IQ measures had a similar relationship with digit span performance suggesting overall IQ had a significant positive relationship with performance on digit span. This further emphasizes similarities in digit span and IQ performance and the importance of span capacity for overall cognitive processing.

Taken together, these expanded analyses highlight the strength of the relationship between digit span forward and the CNS isoform Dp71. Mutations affecting Dp71 disrupt the balance of inhibition/excitation needed for neuronal signaling. Since Dp71 is the most prominent dystrophin CNS isoform, disruption to these CNS processes would have a greater impact on cognitive functioning than mutations affecting other isoforms. Thus, performance of individuals with mutations affecting Dp71 may reflect disruptions in synaptic transmission underlying the deficit in reduced verbal span, reduced digit span forward, which is driving the impaired performance across cognitive areas including academic performance. In conclusion, reduced verbal span may represent the core cognitive deficit in individuals with dystrophinopathy indicative of impairments in neuronal communication due to the dystrophin deficiencies.
VII. APPENDICES

Approval Notice
Initial Application

08/03/2016

Robert Fee,
The Graduate School & University Center

RE: IRB File #2016-0575
    Cognitive Skills in Dystrophinopathies

Dear Robert Fee,

Your Initial Application was reviewed and approved on 08/03/2016. You may begin this research.

Please note the following information about your approved research protocol:

Protocol Approval Period: 08/03/2016 - 08/02/2017
Protocol Risk Determination: Minimal
Expedited Categor(ies): (5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(4). This listing refers only to research that is not exempt.)

Documents / Materials:

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Version #</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survey/Questionnaire</td>
<td>Measures IRB.pdf</td>
<td>1</td>
<td>04/21/2016</td>
</tr>
<tr>
<td>Survey/Questionnaire</td>
<td>Parent forms IRB.pdf</td>
<td>1</td>
<td>04/21/2016</td>
</tr>
<tr>
<td>Survey/Questionnaire</td>
<td>Battery forms IRB.pdf</td>
<td>1</td>
<td>04/21/2016</td>
</tr>
<tr>
<td>Site Letter of Compliance</td>
<td>NYPresbyterian/Columbia IRB letter of approval</td>
<td>1</td>
<td>04/21/2016</td>
</tr>
<tr>
<td>Survey/Questionnaire</td>
<td>DMD questionnaire IRB.pdf</td>
<td>1</td>
<td>04/21/2016</td>
</tr>
<tr>
<td>Site Letter of Compliance</td>
<td>CITI training</td>
<td>1</td>
<td>05/12/2016</td>
</tr>
</tbody>
</table>
Please remember to:

- Use the **IRB file number** 2016-0575 on all documents or correspondence with the IRB concerning your research protocol.

- Review and comply with CUNY Human Research Protection Program [policies and procedures](http://www.cuny.edu/research/compliance.html).

The IRB has the authority to ask additional questions, request further information, require additional revisions, and monitor the conduct of your research and the consent process.

If you have any questions, please contact:

Janet Echevery
718-997-5415
janet.echevery@qc.cuny.edu
VIII. BIBLIOGRAPHY

INTRODUCTION:


_Neurosci Biobehav Rev, 32_(3), 486-496._


PART TWO


Duchenne and Becker patients differing by mutation consequences on Dp71 expression.

*Human molecular genetics, 18*(20), 3779-3794.


PART THREE


DISCUSSION


Miller, G.A. (1956). The magical number seven, plus or minus two: some limits on our capacity for processing information. Psychological review, 63(2), 81.


