Dystrophin induced cognitive impairment: mechanisms, models and therapeutic strategies

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ABSTRACT

Existence of conserved domains in dystrophin and its associated complexes provide an opportunity to understand the role of dystrophin associated signalling and its association with neuronal metabolism in a variety of model organisms. We critically reviewed the studies till 2013 through established search engines and databases. Thus, we review the role of dystrophin and its isoforms in different animal models at developmental stages in the neuronal metabolism to enhance the therapeutic strategies. Dystrophin interacts with other proteins in such a way that, when affected, it results in co-morbidities including autism and other neuropsychiatric disorders. It is speculated that various signalling molecules may converge to disrupt neuronal metabolism not adequately studied. TGF-β, RhoGAP and CAM mediated signalling molecules are the chief cause of mortalities due to respiratory and cardiac involvement but remain underevaluated targets for cognitive impairment in DMD/BMD. Manipulation of these signalling pathways could be potent intervention in dystrophin induced cognitive impairment while complementary therapeutic approaches may also be helpful in the treatment of cognitive impairment associated with DMD/BMD.

KEYWORDS: Dystrophin, Cognitive impairment, DMD, Neuronal Metabolism, mdx

INTRODUCTION

Becker’s/Duchenne Muscular Dystrophy (BMD/DMD) are among the disorders that are associated with mild to severe mental retardation in one third of the affected individuals predominantly affecting the males due to X linked inheritance. The prevalence rate of 1 per 3500 males strengthens the need of socio psychological rehabilitation of DMD/ BMD patients arising from cognitive impairment. Dystrophin is the key protein involved in the development of BMD/DMD which in addition to muscle is also localized in cerebral cortex, hippocampus in high density.¹⁻³ Tissue specific expression of dystrophin occurs in brain with full length dystrophins DP427 (B) & DP427 (P) in brain and Purkinje cells respectively. C terminal dystrophin isoforms namely DP140, DP71 and, DP40 which are predominantly expressed in brain, also contributes to synaptic plasticity and are reported to cause MCI (abbreviate MCI) in DMD patients.⁴ Dystrophin and components of the dystrophin-associated glycoprotein complex (DGC) are localized at neuromuscular Junctions (NMJ) and play a key role of postsynaptic maturaton.⁵ Dystrophin’s role in the stabilization and anchoring of ion channels and receptors indicates that altered synaptic plasticity and functioning that contribute to cognitive disability in DMD, may be explained by the loss of dystrophin in the brain.

In this review, we discuss the crucial role of dystrophin protein and its isoforms in developing variable degree of neuropsychological impairment. We hereby review the existing dystrophin linked downstream molecular mechanisms to simplify our understanding of the pathways which link dystrophin and its isoforms to the neuronal metabolism that may be responsible for the severity of the mental retardation.

Clinical features of BMD/DMD

BMD/DMD patients have characteristic feature of progressive muscular weakness due to altered dystrophin expression resulting in disrupted sarcolemmal elasticity⁶. Becker’s muscular dystrophy is a milder form of DMD with late age of onset and milder progression. In initial stages, the delay in walking is observed which includes difficulty in climbing stairs accompanied with waddling gait. Pseudohypertrophy occurs in calf muscles with progressive weakness in proximal muscles leading to loss of ambulation. Other manifestations include scoliosis (Curvature of spine), muscle cramps, toe walking, and gastrointestinal (GI) tract problems. Increased levels of creatine kinase (CK) and Gower’s Sign have been long considered as the diagnostic criteria for diagnosing BMD/DMD. Gower’s sign is characterized by inability to get up from squatting position due to proximal muscle weakness.⁶ CK plays a crucial role by forming high energy phosphate phosphocreatine in skeletal muscle. Elevated creatine kinase is an indicator of muscle damage which is usually used as prognostic marker for DMD. Patients with DMD show variable rates of disease progression with its effect on cardiac, cognitive and respiratory functions. Epigenetic factors and sociodemographic variables may also contribute to the severity of disorder.

Though respiratory and cardiac failures are the primary cause of mortality in BMD/DMD patients but other cognitive and behavioural abnormalities including Autism Spectrum Disorders (ASD), Obsessive-Compulsive Disorder (OCD), and Attention Deficit Hyperactivity Disorders (ADHD)⁷⁻⁸ worsen the condition of the subject. Cognitive impairment occurs in one third of patients which necessitates psychosocial management of patients. Language and memory impairment, learning disabilities, speech/swallowing problems occur in the patients resulting in reduced quality of life thus causing complications. The range of cog-
nitive decline is reported in the patients of DMD ascribed to dystrophin deficiency in brain structures resulting in significant decrease in intelligence quotient (IQ).9

Molecular biology of dystrophin associated cognitive impairment
Gene which encodes dystrophin protein (427kd) is the largest known gene in human beings spanning 2.4 million bp in length comprising of 79 exons, with its locus at Xp21 chromosome. Dystrophin consists of four major units which are a) N terminal actin binding domain b) triple helical spectrin repeats c) cystein rich domain for beta dystroglycan binding (BDG) d) C-terminal domain.10 These domains interact with different proteins like alpha dystroglycan, beta dystroglycan, syntrophin, dystrobrevin etc. to form a protein complex called as dystrophin associated protein complex (DAPC). Dystrophin is localized beneath sarcolemma and plays major role in structural maintenance of muscle integrity and is involved in many signalling pathways. Dystrophin has also been reported to play signalling role as analysed by localization of neuronal nitric oxide (nNOS), through an organized interaction with dystrophin associated protein complexes resulting in regulation of blood flow in the skeletal muscle.11 Dystrophin links the cytoskeleton of the cell with sarcolemma in order to provide mechanical support to the muscle dynamics. Abnormal cytoarchitecture in the brains of DMD patients may not withstand the external mechanical forces as well as the forces exerted by the internal dynamic environment.

Dystrophin gene is found to have deletion in 65% and 55% DMD and BMD patients respectively.12 DMD is caused by out-of-frame mutations resulting in deficiency of dystrophin protein since the transcriptional machinery fails to produce mRNA transcript. In-frame deletions in BMD produce a quasi-dystrophin protein resulting in a milder form of DMD. Three independent promoters encode a full length dystrophin protein (Dp427) in a tissue specific manner and truncated isoforms (Dp 71, 116, 140, 260) of dystrophin are encoded by more than four internal promoters again in a tissue specific manner.13 Regulatory promoter sequences for Dp140, Dp71 & Dp40 are located at exon 44 and exon 62 respectively whereas transcription termination occurs at exon 79 for Dp140 and Dp71.14 Absence of Dp140 and Dp71 isoforms which harbour cystein rich and carboxy domain, are considered to be responsible for increased severity of cognitive impairment in DMD. This is believed to alter the hippocampal neurotransmission. Dp71 is a most abundant brain dystrophin which have multifunctional roles. Dp71 is reported to be detected at DAPC, astrocytes (in vitro), glial end-feet blood vessels, hippocampal neurons and retina. Though Dp71 has a primary role of DAPC stabilisation its multifunctional role in angiogenesis, cell division, adhesion, excitatory synapse organization, synaptic plasticity, has been reported. DP40 isoform has been reported to have interaction with presynaptic proteins SNAP25, Syntaxin 1 which are known for exocytosis, docking and vesicle fusion of neuronal synaptic vesicles to the plasma membrane.15

Neuropsychological and neuropsychiatric considerations in DMD/BMD
In addition to the loss of physical strength, dystrophin gene mutation has long been considered as a cause of impaired intellectual functioning in DMD patients. Guillaume B.A. Duchenne de Boulogne in his initial works in 1868 described the presence of intellectual deficits.

An increased prevalence of mental retardation in 20.9% DMD patients as compared with an estimate of 3% in the normal population was described in initial studies.15 This study was replicated by using Wechsler Intelligence Scale for Children (WISC) and Wechsler Adult Intelligence Scale (WAIS) and validated the prevalence of intellectual impairment using global IQ.16 One third of boys affected with DMD manifest intellectual impairment with mean Intelligence Quotient below 1.0–1.5 SD of normal population with verbal intelligence (VIQ) more affected than performance intelligence. BMD, a milder form of DMD, manifests a less frequent cognitive impairment (Approx 10%). In a recent study conducted in Souh India, mean verbal IQ was reported to be 86.59 in 22 male patients without analyzing the presence or absence of distal dystrophin isoforms.17 Cognitive testing has indicated the enhanced severity of mental retardation in the DMD patients when c-terminal dystrophin isoforms are affected. Studies have shown that there is decrease in intelligence quotient of 2 SD from the normal population when the DP71 isoform was found to be affected.18 Mild to moderate degree of mental retardation with intelligence quotient ranging 35–55 was reported in DMD patients bearing DP71 mutation.18-19

Recently, neuropsychological testing has enabled investigators to examine the impairment of specific cognitive deficit which has revealed the presence of deficits in a range of cognitive domains associated with DMD.17,25-27 It has been found that DMD patients significantly underperformed in verbal short-term memory, praxis, executive functioning, narrative, reading, linguistic and phonological abilities suggesting the inclusion of variable range of affected neuropsychology domains.28-32 Studies on the brain specific dystrophin isoforms such as Dp140, Dp71 with respect to cognitive impairment and neuropsychological assessments are compiled in Table 1.

Higher prevalence of psychiatric abnormailities have also been reported in DMD/BMD patients. First report on DMD autism co-morbidity was provided by Komoto et al who reported a five year old boy of DMD who developed autism.29 In another study, cognitive decline was reported in 3.8% of DMD patients.30 Recent study by Hendriksen showed 3.1% presentation of autism spectrum disorder among 351 males with DMD 31 whereas the frequency of ASD in the general population was 0.0016%. Same group has also reported 11.7% ADHD comorbidity in 351 male DMD patients. ADHD has been correlated to the frontal lobe alterations which in case of DMD patients corresponds to attention deficits. Cerebellar link between ADHD and DMD is still to be established. Mutations in the distal region of dystrophin gene which affects brain specific isoforms induces the ADHD features in DMD patients.2 4.8% among 351 males were affected of obsessive compulsive disorder (OCD).31 Role of external, extracellular and intracellular environment may cause epigenetic alterations which may contribute to establish the link between OCD and DMD. A study in a dutch population revealed 5 DMD patients to be severely affected with reading disability among total 25 DMDs.32

Localization of dystrophin in the brain regions responsible for the higher order functioning including cerebellum, hippocampus and cerebral cortex.1,2 indirectly indicates the correlation between dystrophin deficiency and cognitive alterations. Moreover, dystrophin localization at the postsynaptic GABAergic neurons explains its crucial role of maintaining synaptic plasticity.23 Prior investigations have revealed the dystrophin localization at cortical pyramidal neurons but there is lack
Table 1: Overview of studies on dystrophin loci induced cognitive deficits

| S. No | Study Design Based on Dystrophin isoform | Age Range | Region | Diagnostic Criteria Used | Subjects/Type of Controls | Sample size | Inclusion Criteria | Neuropsychological tests | Outcome | Ref. |
|-------|------------------------------------------|------------|--------|--------------------------|---------------------------|-------------|-------------------|--------------------------|---------|------|
| 1     | Mutation affecting all dystrophin product+ DP71 and all dystrophin product –DP71 | Not Mentioned | French Population | Muscle Biopsy, RT-PCR | DMD Normal Control | N = 81 | Position of mutations (deletions, duplications and point mutations) | WPPSI, WPPSI-R, WISC-R, WAIS-R batteries according to Age | Dp71 contributes to severe mental retardation. (shift of 2 SD downwards) | 18 |
| 2     | Point Mutation analysis without Deletion & Duplication Affecting DP71 transcript | 9.5–17.9 | French study | multiplex PCR and Southern blotting | DMD Normal control | N = 12 | Raised serum creatine kinase level, absence of dystrophin on muscle biopsy. Point Mutation | (VIQ) and visuoo-spatial (PIQ) intelligence assessment (WISC-R scale), reading skills assessment (Alouette test) | Severe mental retardation in Dp71 affected subjects. (VIQ <50) | 24 |
| 3     | All Dystrophin isoform predicted specially DP71 & DP140 | NA | Sydney Neuromuscular Centre, Australia | | DMD Normal Control | N = 62 | Deletions, duplications, point mutations | Full Scale Intelligence Quotients (FSIQ), Wechsler Preschool and Primary Scale of Intelligence [WPPSI-R] or Wechsler Intelligence Scale for Children [WISC-III] | Strong association between dystrophin isoform and FSIQ was found. | 25 |
| 4     | Presence or absence of DP140 independent of Age | 3–20 | Bern, Switzerland | Multiplex-PCR, MLPA, Sequencing | DMD Normal control | N = 25 | Deletions, duplications, point mutations | K–ABC, SON–R, WAIS–III, WISC–III | Loss of Dp140 isoform had significantly high cognitive decline. | 22 |
| 5     | Becker type Mutation | 6 years or More | Sydney, Australia and Boston, Massachusetts. | Multiplex PCR, MLPA, Confirmatory Sequencing | BMD | N = 24 Males | Deletion/Mutation | The Wechsler Intelligence Scales, The Wide Range Achievement Test–Revised, The Developmentmental Test of Visual-Motor Integration, The Child Behavior Checklist, and The Conner’s Parent Rating Scale. | Significantly higher reading, spelling, arithmetic difficulties. Less cognitive loss when compared to DMD. | 26 |
| 6     | DMD (DP140 based study plan) | 9.1 years in DMD, 9.6 yrs in control | Italian population | mPCR, MLPA in some. | DMD SMA Osteogenesis imperfecta (OI) | N = 42 DMD,10 SMA & OI | Deletion/ Duplication/ Point Mutation | General Intelligence: WISC-R, Learning disability: Batteria 4–12, Memory: Test di Memoria e Apprendimento battery (an Italian adaptation) | Visuospatial functions and visual memory were impaired in distally mutated dystrophin isoform. | 21 |
| 7     | Analysing Intellectual and Behavioral Functions | 4–16 yrs | South African Cohort | Muscle Biopsy and Mutation analysis | DMD | N = 17 | Genetic Analysis | Griffiths Mental Development Scale, Different test batteries for age group 7–16 | Mild cognitive dysfunction across multiple domains, including visual memory, verbal and nonverbal executive functioning. High rates of general behavior problems | 27 |
| 8     | Specific cognitive deficits | 7–14 years | eastern Sweden | Not mentioned | DMD | N = 20 | | Block Span, Digit Span, Story Recall, Rey Auditory Verbal Learning Test, Spatial Learning Test, Verbal Fluency | Significantly worse on all aspects of memory as well as in learning ability and executive functions | 28 |
of evidence of prefrontal localization of dystrophin. Moreover, increased incidence of dyslexia, dyscalculia and dysgraphia in DMD boys is suggestive of the crucial role of dystrophin and associated protein complexes in cognitive processes which represents the structural and functional abnormalities of cerebellar as an outcome of dystrophin deficiency.

Impairment in the cognitive capacity may be linked to the altered brain metabolism. Magnetic resonance spectroscopy based assessment showed increased brain ratios of inorganic phosphate to adenosine triphosphate, to phosphomonoesters and to phosphocreatine in the DMD boys.24–25 Metabolic basis of altered cognitive capacity in DMD patient was also assessed through positron emission tomography [PET] which demonstrated the correlation between dystrophin localization and hypometabolism as well as region specific architectural abnormality.26 Elevated ratio of choline-containing compounds to N-acetylaspartate (Cho/NA) in the cerebellum was detected in the DMD patients in comparison to the control group suggesting the high levels of choline compounds in the brain regions affected by the loss of dystrophin.27

These studies depict the crucial role of distal dystrophin isoforms DP140, DP71, DP40 in the development of general as well as specific cognitive functions in order to investigate and review the molecular mechanism of dystrophin induced cognitive fitness. Moreover, mechanism of dystrophin deficiency induced metabolic changes in the brain must be comprehensively investigated.

**Molecular mechanisms of dystrophin or dapc signalling in animal models**

Several animal models have provided insights about mechanisms involved in maintaining cognitive function through molecular engagement in nervous system. DMD animal model (mdx), and model organisms like drosophila and c. elegans are used as an important tools to understand this mechanism. Thus, after reviewing the behavioural deficits in the DMD patients, we describe the molecular mechanism of dystrophin induced metabolic changes.

**mdx mice**

mdx mice harbours mutation at a single nucleotide resulting in a disrupted dystrophin leading to less severe DMD/BMD phenotype. Due to dystrophin deficiency mdx mice is widely used in the study of DMD and its associated manifestations include cognitive alterations and altered neuronal circuitry. mdx<sup>Scn-neutralized</sup> series of strains have been generated to obtain dystrophin isoform deficient models with affected DP71, DP116, DP140, DP260, DP427 expression.28 Dystrophin deficiency is associated to the enhanced axodendritic inhibitory synapses and altered architecture of postsynaptic densities (PSDs).29

Clustering and distribution of synaptic proteins such as VGLUT1 and PSD-95 has been observed to be affected in neuronal culture of DP-71 null mice. Vesicular glutamate transporter 1 (VGLUT1) is reported to be expressed in neuron-rich regions of the brain which functions as sodium dependent phosphate transporter and is responsible in glutamate transport, whereas PSD 95 protein is present at postsynaptic densities. Role of Dp71-DAPC interaction is essential in the synaptic transmission and plasticity through clustering glutamate receptors and organizing signalling proteins in association with multi-protein scaffolds. Postsynaptic membrane of GABAergic synapse on Purkinje cells are the site of dystrophin localization, which in case of dystrophin deficiency, leads to reduced functional receptor on GABAergic synapse in dystrophin deficient mdx mice. Reduced functional GABAergic receptors are reported to have enhanced anxiety and defensive freezing behavior in mdx mice and is reported to be ameliorated by using antisense morpholinolo nucleotide.33 Recombinant adenovirus associated restoration of brain dystrophin has also been reported to increase the functionality of GABA<sub>A</sub> receptors in hippocampal, pyramidal and dendritic layers at CA1 region.40 Thus, rescue of dystrophin restores synaptic plasticity through antisense oligonucleotide mediated skipping of abbreviated exons. Stabilized clustering of GABA<sub>A</sub> receptors in the hippocampal region is correlated with increased neurotransmission.41 Episodic memory is attributed to CA1 region of the hippocampus suggesting the crucial role of GABA<sub>A</sub> mediated deterioration of the same.42 At post synaptic GABAergic densities GABA<sub>R</sub> binds to scaffold protein gephyrin43 and collybistin [GTPase exchange factor] 44 leading to activation of cdc42 which is having roles in regulation of actin cytoskeleton organization. Role of dystrophin and Dystrophin-dys troglycan complex becomes evident in maintaining GABA<sub>R</sub>.45

Dystrophin deficiency in mdx mice causes impairments in cognitive and behavioural domains due to altered synaptic plasticity and dystrophin induced abnormalities in synaptic organization. mdx mice can be used as a tool to investigate the dystrophin induced cognitive impairment through various tests of cognitive and behavioural functions. Exploratory activity, reward learning, long-term spatial and fear memories, extinction, object-place associative learning, emotional reactivity, impulsivity, attention, compulsivity, perceptual discrimination, visual discrimination are assessed to evaluate hippocampal, neocortex and cerebellar functions which are sites of dystrophin localization. T-maze experiments have been utilized to assess learning and memory in mdx and other rodents to discriminate the hippocampus and forebrain functions. Table 2 depicts some of the cognitive tests examined in mdx as well as other mice and rodents. Different tools for testing cognitive functioning in the mice and rodent models to mimic disorders including huntington disease, schizophrenia, down syndrome, alzheimer’s disease, parkinson’s disease etc., can be utilized to evaluate the domain wise function in mdx mice.

As described in the human studies dystrophin loss may also alter the metabolic processes in brain leading to the cognitive and behavioural dysfunctioning. Altered biochemical changes including increased inorganic phosphate and pH, have been observed in the mdx brain which may affect the cognitive functioning.46 Moreover, irregularity of glycolytic metabolism is reported to be caused due to altered positive allosteric interactions between phosphofructokinase (PFK) and neuronal nitric oxide synthase (nNOS) in the mdx mice. PFK is the key regulatory enzyme in the metabolic process of glycolysis.47 Further narrowing down our approach to understanding the dystrophin induced metabolic pathways we reviewed the studies carried out in model organisms such as drosophila and c. elegans.

**Drosophila**

Evolutionary conserved DGC (Dys & DGs) components have been studied in Dro sophila which are localized at neuromuscular junction, CNS, PNS, and in ocular system.48 Drosophila dystrophin gene expresses different isoforms like dystrophin like protein (DLP1), DLP2, DLP3 including Dp186, a shorter isoform. DLP2 is expressed in various stages of development
### Table 2: Cognitive and behavioural testing in mice and rodent models

| S. No | Test Name                                                                 | Measurements                                                                 | Brain function                                                                 | Disease targeted   | Strain                  | reference |
|-------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------------|--------------------|-------------------------|-----------|
| 1     | Contextual fear conditioning. Unconditioned fear response. Open-field activity. Water maze | Fear memory, Anxiety, Spatial learning                                           | Hippocampal, neocortex and cerebellum functions                             | DMD/BMD           | mdx                     | 46        |
| 2     | Avoidance Tests Passive Avoidance Test                                    | Rapid one-trial learning                                                        | –                                                                             | DMD/BMD           | Mdx mice                | 47        |
| 3     | Morris Water Maze                                                        | Spatial learning, Visual acuity                                                 | Hippocampus                                                                   | DMD/BMD           | Mdx mice/ C57BL/6 control | 48        |
| 4     | Restraint, Electrical footshock, Elevated plus maze                       | Freezing response, Fear Conditioning                                            | –                                                                             | DMD/BMD           | mdx mice                | 39        |
| 5     | Operant learning task, Delayed spontaneous alternation task in a T-maze   | Learning and memory tasks, spatial working memory                              | Hippocampal and forebrain function                                            | DMD/BMD           | mdx 3 cv                | 40        |
| 6     | The touchscreen testing method, Visual Discrimination and Reversal: The TUNL Task: Working memory and pattern separation. | Reward learning, memory, perceptual discrimination, object-place associative learning, attention, impulsivity, compulsivity, Extinction. | Hippocampal functions, Focus on dentate gyrus and neurogenesis dependent pattern separation | Schizophrenia     | Rats & rodents           | 49        |
| 7     | The Location Discrimination (LD) task:                                    | Visual discrimination                                                           | neurogenesis dependent pattern separation                                     | –                  | –                       | 50        |
| 8     | Transverse-pattern task                                                  | Non-spatial learning                                                            | Hippocampal function                                                          | NMDA induced memory impairment. | CA1-NR1 knockout mice | 51        |
| 9     | Latent Inhibition                                                        | selective attention, Hyperactivity                                              | –                                                                             | ADHD              | Coloboma mouse model    | 52        |
| 10    | Morris Water Maze                                                        | Spatial learning, Visual acuity                                                  | Hippocampal, neocortex                                                       | AD                 | Swiss albino mice       | 53        |
| 11    | Radial Maze                                                              | spatial learning                                                                | –                                                                             | Y chromosome induced complexity     | Inbred mouse strains, NZB and CBA/H, | 54        |
| 12    | Environmental Enrichment                                                  | –                                                                             | Increased sensory motor stimulation                                            | Down’s Syndrome   | Ts65Dn mice             | 55        |

unlike DP186 which is expressed in CNS at the time of embryonic development. Moreover, dystrophin scaffolds and isoforms have role to play in neurotransmitter release at the neuromuscular synapse. Homeostasis of neuromuscular synapse is regulated by postsynaptic dystrophin and its interaction with different proteins. DP186 isoform maintains wild-type presynaptic release levels mainly at postsynaptic motoneuron while DLP2 isoform is reported to be involved in neurotransmitter release at NMJ in retrograde manner through TGF-β signalling. Downstream signalling mechanism of dystrophin isoforms in the maintenance of synaptic plasticity is less understood. CNS specific dystrophin isoform DP186 is involved in retrograde signalling which is essential for formation, maturation, and plasticity of synaptic connections. Yet another mechanism of understanding the role of dystrophin induced synaptic organization is through RhoGAP (Rho GTPase activating protein), encoded by curovless-c (cv-c) gene which is involved in the regulation of neurotransmitter release. RhoGAPs such as RhoGAP68F, p190 RhoGAPs are very specific to the neural development. Expression of RhoGAP68F is confined to the embryonic brain and is associated to the neuronal morphogenesis. Interaction of dystrophin and RhoGAP is necessary at the site of post synaptic junction to maintain homeostasis. RhoGAP negatively regulates RhoGTPases by inactivating its catalytic activity while Rho GEF (Guanine Nucleotide Exchange Factor) like ephexin-1 together with Rho GAP controls the nucleotide state of GTPases between inactivated and activated state. Ephexin-1 plays a crucial and versatile role of synapse remodelling by changing the synaptic structure as well as molecular composition. Presynaptic protein ephexin-1 is required in the process of vesicle release through homeostatic modulation primarily via CDC42 GTPase in convergence with changes in Ca²⁺ flux.
Changes in Ca\(^{2+}\) flux affects multiple enzymes including Ca\(^{2+}\)-calmodulin kinase II (CaMKII) which phosphorylates its substrate including microtubules. Message to the cell via Ca\(^{2+}\) induced phosphorylation of microtubules creates a memory lattice through tubulin proteins. Cellular message may be encoded in the brain through microtubule based memory followed by the processing of information in neurons.

**C. Elegans**

Dys-1 is an ortholog to human dystrophin gene in *Caenorhabditis elegans* which is involved in maintaining muscular integrity and locomotion. Dysfunctional dys-1 gene causes contractile defect, mild fiber degeneration, reduced life span and age dependent muscle cell death. Dys-1 also plays an essential role of maintaining neural organization by interacting with cell adhesion molecules (CAMs) such as SAX7, a homolog of CAM interacts with dys-1 to provide neural integrity and organization. In vertebrates, sax-7 orthologs like L1CAMs, which include L1, neurofascin, neuronal CAM (Nr CAM), and CHL1 are supposed to play a role in nervous system development. NrCAM mutation leads to development of autism in human, whereas L1 deficits are concerned with mental retardations and other X-linked neural diseases. NrCAM plays a crucial role of axon guidance, synapse formation, cell proliferation alteration of which causes psychiatric disorders. Other than autism spectrum disorders it is also involved in loss of visual acuity lack of sociability and cognitive function. Interaction of dystrophin with CAMs provides an insight on the increased comorbidities of psychiatric disorders like autism in DMD patients.

**Neuronal metabolism as a common denominator**

### 6.1 Transforming growth factor-β (TGF-β) in neuronal metabolism

DLP-2 isofrom of drosophila mediates its effect on neurotransmitters release through TGF-β Signaling. TGF-β in *mdx* mice has also shown to be responsible for respiratory processes that chiefly contribute to mortality in DMD. Respiratory function has been shown to be improved in *mdx* mice by inhibition of TGF-β activity. SPP1 gene encodes a protein osteopontin which is a strong regulator of disease severity. Recently, the progression of DMD has been associated with SPP1 genotype. Recent longitudinal studies showed relevance of SPP1 gene polymorphisms as a disease modifier in Duchenne Muscular Dystrophy. Role of TGF-β becomes important as it activates promoter region of SPP1 gene leading to regulation of osteopontin expression corresponding with increased DMD severity through inflammatory changes. TGF-β is a well studied fibrogenic mediator, activation of which leads to destructive metabolic pathways. Myostatin, an evolutionary conserved TGF-β family protein, is a potent inhibitor of muscle growth and, if mutated, leads to hypermuscularity in several organisms suggesting a pivotal role of TGFs in progression of DMD. Transforming Growth Factor-β functions at the time of development and is also supposed to have role in cognition.

Dystrophin deficiency leads to muscle damage followed by a cycle of muscle regeneration and degeneration leading to reduction in regenerative capacity of the muscle stem cells (MuSCs) due to telomere shortening. Telomere shortening may provide a conducive environment comparable to aged cells. Recent study has reported the role of TGF-β in neurogenesis of aged mice and proliferation of neural stem cells through TGF-β/smad 3 signalling. Thus role of TGF-β becomes important and can be correlated through TGF-β/smad 3 signalling. Moreover, TGF-β has also been involved in neuronal metabolism and abnormal levels of which triggers aberrant metabolic pathways. Altered downstream signalling through mTOR is crucial point which distrupts further molecules including Peroxisome proliferator-activated receptor α (PPAR-α). Peroxisome proliferator-activated receptor-gamma coactivator alpha (PGC1α), Hypoxia-inducible factors (HIF-1), PPARs are transcription factors which regulate the cellular metabolism, specifically adipogenesis, glucose metabolism and fatty acid storage. PGC1α are the coactivators of transcription and play essential role in cellular energy metabolism and mitochondrial biogenesis. HIF-1 are the transcription factors which prepare the cells to survive the low oxygen environment by upregulating specific enzymes including glycolytic enzymes.

Moreover, the role of TGF-β2 has been reported in pathophysiology of Alzheimer’s disease. Superphysiological TGF β2 levels are reported to be involved in neuronal cell death. TGF β2 binding with amyloid precursor protein (APP) leads to a death pathway mediated by APP via heterotrophic G protein G\(_{\text{ai}}\), NADPH oxidase, c-Jun N-terminal kinase and caspase 3 and/or related caspases.

TGF-β also plays a key role in reactive oxygen species induced cellular environments. TGF-β is reported to be an anti-proliferative cytokine and is involved in neuronal survival. TGF-β signalling plays an important role in the uptake of glucose by stimulating GLUT transporters in mesangial cells and glucose induced hypertrophy in fibroblast and epithelial cells mediated by Matrix Metalloproteinases (MMP) activation which transforms latent TGF ligand to active TGF Ligands. TGF-β R1 receptor loss or its downregulation prevents increase of cell size and hypertrophy. Anti-TGF strategies for DMD patients can overcome the effect of altered TGF.

**RhoGAP in Neuronal metabolism**

RhoGAP is identified as the main substrate for Src in developing and mature neurons with its role in axon guidance, outgrowth and fasciculation. Src-dependent adhesion signal to actin filaments is the basis for neurotogenes in association with extracellular protein leminin. RhoGAP is also associated in fear memory formation through its association with ROCK protein. RhoGAP/Rock pathway is involved in neuronal development by regulating dendritic and axonal morphology. RhoGAP mediated flux in Ca\(^{2+}\) followed by regulation of CaMKII may be involved in neuronal apoptosis through CaMKII CREB/βc-2 pathway. It is evident from the earlier studies that calcium homeostasis is altered in DMD patients. Thus, Ca\(^{2+}\) homeostasis may also play critical role in downstream signalling of the dystrophin induced impairment by altering the glycolytic pathways since studies support the idea of Ca\(^{2+}\) homeostasis through glycolysis. Ca\(^{2+}\) homeostasis of the cell is also important in maintaining the oxidative stress levels. Figure: 1 illustrate the mechanism of dystrophin loss induced altered signalling pathways and crucial molecules as a target for therapeutics.

**Drug targeting**

Though exon skipping is the most promising approach of intervention for the restoration of dystrophin in muscles, alternative approaches in restoration of brain dystrophin is still being explored. Exon skipping utilizes the function of antisense-oligonucleotides (AOs) which restore the reading frame to produce a quasi-functional dystrophin protein to
create a milder BMD like phenotype. In-vitro and animal model studies of exon skipping have provided a platform for future clinical trials of AOs based exon skipping experiments for muscle dystrophin.

As mentioned earlier in the text, Sekiguchi et al restored the brain dystrophin through intracerebroventricular administration of antisense morpholino oligonucleotides which led to the skipping of mutated exon 23 and rescued the expression of truncated dystrophin at the PSD fraction, amygdala, cortex and hippocampus. Ability of AOs in crossing the blood brain barrier is still being explored. AO based restoration of brain dystrophin can be followed by cognitive and behavioral testing of animal models to validate the efficacy of AOs. Adenovirus mediated restoration of dystrophin has also been tried to restore the altered levels of inhibitory neurotransmitters. Though the strategies to restore the dystrophin are important however altered neuronal metabolism should also be targeted. Metabolic and pathophysiological severity in the progression of DMD correlates with age dependent muscle cell degeneration/regeneration, reduced life span, altered cellular niche. Increasing the activity of telomerase can create a cellular niche with increased regenerative capacity of muscular fibers and muscle stem cells. Manipulation of the cellular niche may play a crucial role to stabilize the dystrophin deficiency induced alterations. Anti-TGF therapy is evolving as a promising target for neuronal metabolism and cognitive impairments. It is speculated that the molecular mechanisms of neuronal metabolism might serve as the common denominator in the complex pathways impacting dystrophin linked functions.

Recent study aimed at metabolic remodeling by treatment with exercise mimetics such as PPAR-delta and AMPK agonists improved muscle function in dystrophin deficient mice. It is evident that genetic and molecular biological approaches have been explored efficiently to diagnose the disorders, but effective approaches such as diet, physical activity and alternative therapeutics including traditional approaches must not be overlooked until innovative therapies are launched, since these treatments are not being investigated rigorously. Among the alternative approaches, polyphenols, which are cost effective and available through the vegetable sources, are being given a special attention due to its effective role in neurodegenerative disorders. The green tea polyphenols epigallocatechin gallate (EGCG), and curcumin, found in the turmeric plant, have been strongly associated with improved

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**Fig. 1:** Dystrophin Loss induced cellular changes: Illustration of proposed dystrophin deficiency induced telomere shortening which creates an environment comparable to aged cell triggering TGF-β/smads signaling to further activate TGF receptors via glucose followed by MMPs. Activation of TGF receptors which is linked to the Rho Protein mediated Ca2+ flux. Altered Ca2+ flux is the basis of disrupted neuronal metabolism. PI3K : Phosphoinositide 3-kinase MMP: Matrix Metalloproteinases CaMK II: Ca2+-calmodulin kinase II PPARγ: Peroxisome proliferator-activated receptor γ, PGC1α: Peroxisome proliferator-activated receptor-gamma coactivator alpha, HIF-1: Hypoxia-inducible factors.
TABLE 3: Traditional and alternative methods of drug targeting approaches

| S.NO | Therapy | Methods | Affected functioning | Effect of Yogic practices | References |
|------|---------|---------|----------------------|--------------------------|------------|
| 1    | YOGA    | Kirtan Kriya or listening to relaxation music | Depression, Cognitive function, telomerase activity | 12 min per day for 8 weeks. Improved the cognitive function by increased telomerase activity | 105 |
|      |         | Regular yoga practice | Chronic Diseases: pulmonological, gastroinestinal, cardiovascular origin | Better overall health status and physical quality of life | 106 |

**ALTERNATIVE THERAPEUTICS**

| S. No | Therapy | Ingredients | Mechanism | Reference |
|-------|---------|-------------|-----------|-----------|
| 2     | HERBAL THERAPY | Ginkgo biloba | Effects on cerebral circulation and neuronal cell metabolism, on the muscarinic cholinergic system, and showed antioxidant activity | 107–108 |
|       |         | Curcumin | Reduces oxidative damage and amyloid pathology in Alzheimer | 109–110 |
|       |         | Huperzia serrata | Anticholinesterase (anti-ChE) alkaloids isolated from plants | 111–112 |
|       |         | Icariin | Chronic cerebral hypoperfusion | 113–114 |
|       |         | Garlic | Affects brain serotonin (5-hydroxytryptamine [5-HT]) levels | 115 |
|       |         | Berries (flavonoids and polyphenols) | Antioxidants | 116 |
|       |         | Ashwagandha | Upregulation of LDL-related protein, muscarinic acetylcholine receptor | 117 |
|       |         | Indian Ginseng | Acetyl cholinesterase inhibitors | 118–119 |
|       |         | Shankpushapi | Free radical scavenging and enzymes acetylcholinesterase, butyrylcholinesterase, glycogen synthase kinase-3 | 120 |
|       |         | Brahmi (Bacopa monniera) | Bacosides AS synaptic activity in memory improvement in inflammation and antioxidant status, reduction in beta amyloid and increases in metal chelation | 121,122 |

**HERBAL THERAPY**

Cognitive functioning, better mood, and its neuroprotective effects. Dietary habits have also been reported to influence the cognitive function. Green tea has also been found to be associated with enhanced cognitive capacity, possibly explaining the low prevalence of cognitive disorders in Japan. Curcumin is also known to alleviate the symptoms of depression through enhancement of neurogenesis in the hippocampus and frontal cortex of the brain. Various traditional and alternative approaches in studying cognitive impairment have been reproduced in Table 3.

Conclusion

Dystrophin deficiency not only triggers alterations in the central cholinergic synaptic functioning and clustering the receptors of the inhibitory neurotransmitters but it may also be speculated to regulate the neuronal metabolism. Molecules such as TGF-β, Rho GAPs, CAMs and telomerase may trigger some crucial downstream signaling pathways altering the metabolic homeostasis of the cellular niche. Transcription factors and coactivators including PPARγ, PGC-1α, HIF-1 may be the potential targets in the dystrophin induced cognitive impairment in the DMD patients.

Authorship Contribution

Akshay Anand: Substantial contributions to conception, design, revising it critically for important intellectual content.

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