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*

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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 maturation.[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 elasticity[6]. 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 uaually 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.[14]

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 intellactual impairment using global IQ.[16] One third of boys affected with DMD manifest intellactual 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.[17,20–22] 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.[20,22–23] 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 abnormalities 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. Cerebeller 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.[7] 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 eastablish 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 [1,3] 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.[33] 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 BMD Normal Control	N = 81	Position of mutations (deletions, duplications and point mutations)	WPPSI, WPPSI-R, WISC-R, WISC III and 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 visuospatial (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 Developmental Test of Visual-Motor Integration, The Child Behavior Checklist, and The Conner's Parent Rating Scale.	Significantly higher reading, spelling, arithmatic 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 (O.I)	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 og 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.[34–35] 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.[36] 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.[37]

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*$^{2cv-5cv}$ series of strains have been generated to obtain dystrophin isoform deficient models with affected DP71, DP116, DP140, DP260, DP427 expression.[38] Dystrophin deficiency is associated to the enhanced axodendritic inhibitory synapses and altered architecture of postsynaptic densities (PSDs).[39]

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[9], 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 morpholino oligonucleotide.[33] Recombinant adenovirus associated restoration of brain dystrophin has also been reported to increase the functionality of $GABA_A$ 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 $GABA_A$ 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 $GABA_A$ mediated deterioration of the same.[42] At post synaptic GABAergic densities $GABA_A$Rs binds to scaffold protein gephyrin[43] 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-dystroglycan complex becomes evident in maintaining $GABA_A$Rs.[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.[56] 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.[57] 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.*

Drosophilla

Evolutionary conserved DGC (Dys & DGs) components have been studied in Drosophila which are localized at neuromuscular junction, CNS, PNS, and in ocular system.[58] Drosophilla 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 Avoidance response	–	DMD/BMD	*Mdx* mice	47
3	Morris Water Maze	Spatial learning Visual acuity	Hippocampus	DMD/BMD	*Mdx* mice/ C57BL/10 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	Hippocampus, neocortex	AD	Swiss albino mice	53
11	Radial Maze	spatial learning	–	Y chromosome induced complexity	Inbred mouse strains, NZB and CBArH,	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.[59] 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.[59–60] 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 reterograde manner through TGF-β signalling.[60] 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.[61] Yet another mechanism of understanding the role of dystrophin induced synaptic organization is through RhoGAP (Rho GTPase activating protein), encoded by crossveinless-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.[62] 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.[63] Presynaptic protein ephexin-1 is required in the process of vesicle release through homeostatic modulation primarily via CDC42 GTPase in convergence with changes in Ca^{2+} flux.

Changes in Ca^{2+} flux affects multiple enzymes including Ca2+-calmodulin kinase II (CaMKII) which phosphorylates its substrate including microtubules.[64] Message to the cell via Ca2+ 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.[65]

C. Elegans

Dys-1 is an ortholog to human dytrophin 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.[66] 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.[67] 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.[68] NrCAM mutation leads to development of autism in human, whereas L1 deficits are concerned with mental retardations and other X-linked neural diseases.[69,70] NrCAM plays a crucial role of axon guidance, synapse formation, cell proliferation alteration of which causes psychiatric disorders.[71] Other than autism spectrum disorders it is also involved in loss of visual acuity[72] lack of sociability and cognitive function.[73] Interaction of dystrophin with CAMs provide 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 isoform of drosophila mediates its effect on neurotransmitters release through TGF-β Signaling. TGF-β in *mdx* mice has also been 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.[74] 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.[75] 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.[76] TGF-β is a well studied fibrogenic mediator[77], activation of which leads to destructive metabolic pathways.[78] Myostatin, an evolutionary conserved TGF-β family protein, is a potent inhibitor of muscle growth and, if mutated, leads to hypermuscularity in several organisms[79–83] 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.[84] Telomere shortening may provide a conducive environment comparable to aged cells.[85–86] 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.[87] Thus role of TGF-β becomes important and can be correlated through TGF-β/smad 3 signaling. 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 γ (PPARγ), Peroxisome proliferator-activated receptor-gamma coactivator alpha (PGC1α), Hypoxia-inducible factors (HIF-1). PPARγ are transcription factors which regulate the cellular metabolism, specifically adipogenesis, glucose metabolism and fatty acid storage.[88] PGC1α are the coactivators of transcription and play essential role in cellular energy metabolism and mitochondrial biogenesis.[89] HIF-1 are the transcription factors which prepare the cells to survive the low oxygen environment by upregulating specific enzymes including glycolytic enzymes.[3]

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 heterotrimeric G protein G_o, NADPH oxidase, c-Jun N-terminal kinase and caspase 3 and/or related caspases.[90]

TGF β also plays a key role in reactive oxygen species induced cellular environments.[91] TGF-β is reported to be an anti-proliferative cytokine and is involved in neuronal survival.[92–93] TGF-β signalling plays an important role in the uptake of glucose by stimulating GLUT transporters in mesangial cells[94] 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.[95] 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 neuritogenes in association with extracellular protein leminin.[96] RhoGAP is also associated in fear memory formation through its association with ROCK protein. RhoGAP/Rock pathway is involved in neural development by regulating dendritic and axonal morphology.[97] RhoGAP mediated flux in Ca^{2+} followed by regulation of CaMKII may be involved in neuronal apoptosis through CaMKII/CREB/Bcl-2 pathway.[98] It is evident from the earlier studies that calcium homeostasis is altered in DMD patients.[99] 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

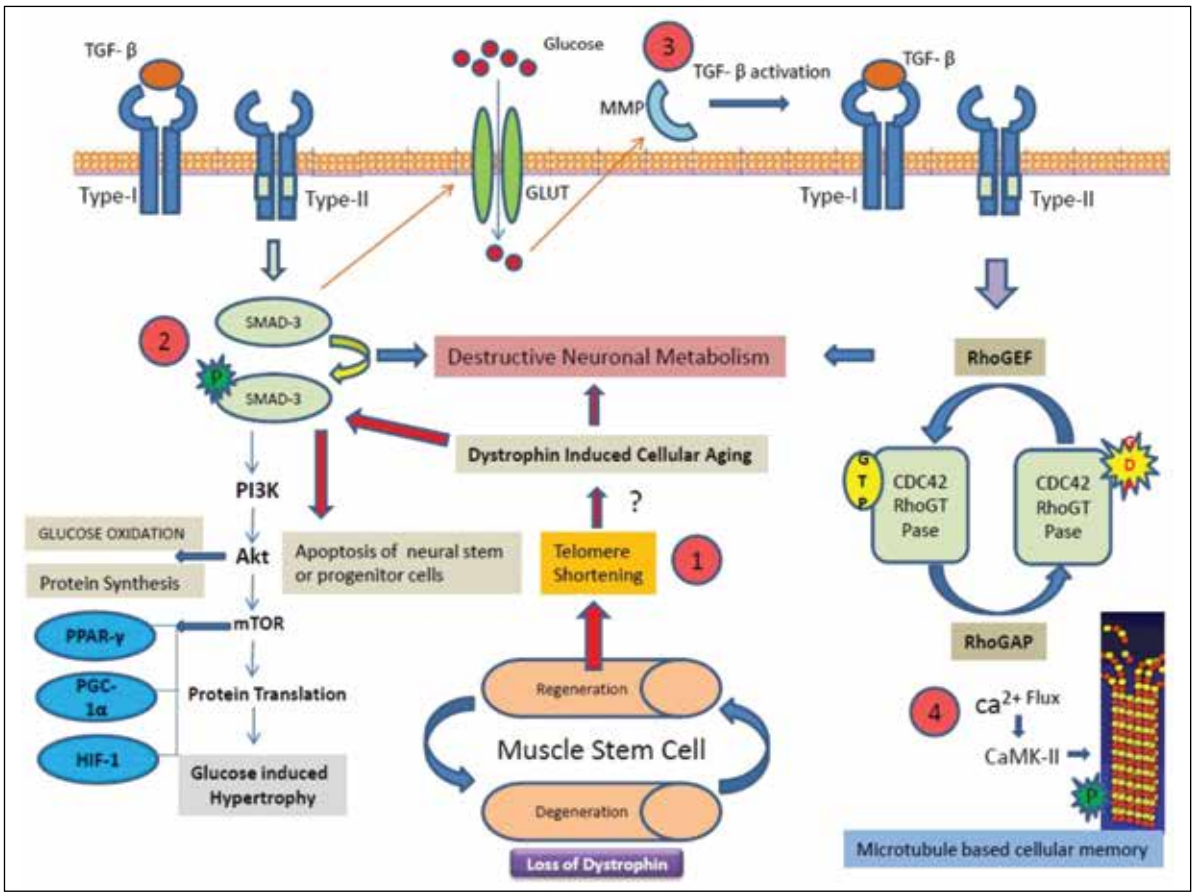

Fig. 1: Dystrophin Loss induced cellular changes: Illustration of proposed dystrophin deficiency induced telomere shortening which creates an environment comparable to aged cell triggering TGF-β/smad 3 signaling to further activate TGF receptors via glucose followed by MMPs. Activation of TGF receptors 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.

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.

Role of nutrition and physical activity is effective for alleviation in cognition deficits in DMD, however, is less investigated. 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.[100]

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.[101] 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

TABLE 3: Traditional and alternative methods of drug targeting approaches

ALTERNATIVE THERAPEUTICS					
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 rheumatological, gastrointestinal, cardiovascular origin	Better overall health status and physical quality of life	106
HERBAL THERAPY					
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, butyrylcholinestrase, 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

cognitive functioning, better mood, and its neuroprotective effects.[102]

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.[103] Curcumin is also known to alleviate the symptoms of depression through enhancement of neurogenesis in the hippocampus and frontal cortex of the brain.[104] Neuroprotection can be achieved by its ability to cross the blood brain barrier.[102] 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 synapse 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 PPAR γ, 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,. Final approval of the version to be published, **Rahul Tyagi:** Substantial contributions to drafting the article, **Manju Mohanty**, **Manoj Goyal**, **K. Ranil D De Silva**, **Nalaka Wijekoon:** Substantial contributions to editing the article.

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Reference

1. Lidov HG, Byers TJ, Watkins SC et al. Localization of dystrophin to postsynaptic regions of central nervous system cortical neurons. Nature. 1990; 20–27: 348(6303): 725-8.
2. Lidov HG, Byers TJ, Kunkel LM. The distribution of dystrophin in the murine central nervous system: an immunocytochemical study. Neuroscience. 1993; 54(1):167–87.
3. Kim JW, Tchernyshyov I, Semenza G L et al. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 2006; 3: 177–85.
4. Haenggi T, Fritschy J M. Role of dystrophin and utrophin for assembly and function of the dystrophin glycoprotein complex in non-muscle tissue. Cell Mol. Life Sci. 2006; 63: 1614–1631.
5. Grady R M, Zhou H, Cunningham JM et al. Maturation and maintenance of the neuromuscular synapse: genetic evidence for roles of the dystrophin--glycoprotein complex. Neuron 2000; 25: 279–293.
6. Petrof BJ, Shrager JB, Stedman HH et al. Dystrophin protects the sarcolemma from

stresses developed during muscle contraction. Proc. Natl. Acad. Sci. USA 1993; 90: 3710–3714.
7. Pane M, Lombardo ME, Alfieri P et al. Attention deficit hyperactivity disorder and cognitive function in Duchenne muscular dystrophy: phenotype-genotype correlation. J. Pediatr. 2012; 161: 705–709.
8. Hendriksen JG, Vles JS. Neuropsychiatric disorders in males with duchenne muscular dystrophy: frequency rate of attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder, and obsessive--compulsive disorder. J Child Neurol. 2008; 23(5): 477–81.
9. Daoud F, Candelario-Martínez A, Billard JM. Role of mental retardation-associated dystrophin-gene product Dp71 in excitatory synapse organization, synaptic plasticity and behavioral functions. PLoS One 2008; 10: 4: e6574.
10. Cirak S, Feng L, Anthony K et al. Restoration of the dystrophin-associated glycoprotein complex after exon skipping therapy in Duchenne muscular dystrophy. Mol. Ther. 2011; 20: 462–467.
11. Percival JM, Anderson KN, Huang P et al. Golgi and sarcolemmal neuronal NOS differentially regulate contraction-induced fatigue and vasoconstriction in exercising mouse skeletal muscle. J. Clin. Invest. 2010; 120: 816–826.
12. Flanigan KM, von Niederhausern A, Dunn DM et al. Rapid direct sequence analysis of the dystrophin gene. Am. J. Hum. Genet. 2003; 72: 931–939.
13. Blake DJ, Weir A, Newey SE et al. Function and genetics of dystrophin and dystrophin-related proteins in muscle. Physiol Rev. 2002; 82: 291–329.
14. Tozawa T, Itoh K, Yaoi T et al. The shortest isoform of dystrophin (Dp40) interacts with a group of presynaptic proteins to form a presumptive novel complex in the mouse brain. Mol Neurobiol 2012; 45: 287–297.
15. Cohen HJ, Molnar GE, Taft LT. The genetic relationship of progressive muscular dystrophy (Duchenne type) and mental retardation. Dev. Med. Child Neurol. 1968; 10: 754–765.
16. Prosser EJ, Murphy EG, Thompson MW. Intelligence and the gene for Duchenne muscular dystrophy. Arch. Dis. Child 1969; 44: 221–230.
17. Perumal AR, Rajeswaran J, Nalini A. Neuropsychological Profile of Duchenne Muscular Dystrophy. Appl Neuropsychol Child. 2013 Nov 26. [Epub ahead of print]
18. Daoud F, Angeard N, Demerre B et al. Analysis of Dp71 contribution in the severity of mental retardation through comparison of Duchenne and Becker patients differing by mutation consequences on Dp71 expression. Hum. Mol. Genet. 2009; 18: 3779–3794.
19. Moizard MP, Toutain A, Fournier D et al. Severe cognitive impairment in DMD: obvious clinical indication for Dp71 isoform point mutation screening. Eur. J. Hum. Genet. 2000; 8: 552–556.
20. Mento G, Tarantino V, Bisiacchi PS. The neuropsychological profile of infantile Duchenne muscular dystrophy. Clin Neuropsychol 2011; 25: 1359–1377.
21. D'Angelo MG, Lorusso ML, Civati F et al. Neurocognitive profiles in Duchenne muscular dystrophy and gene mutation site. Pediatr Neurol 2011; 45: 292–299.
22. Wingeier K, Giger E, Strozzi S et al. Neuropsychological impairments and the impact of dystrophin mutations on general cognitive functioning of patients with Duchenne muscular dystrophy. J. Clin. Neurosci. 2011; 18: 90–95.
23. Anderson SW, Routh DK, Ionasescu VV. Serial position memory of boys with Duchenne muscular dystrophy. Dev. Med. Child. Neurol. 1988; 30, 328–333.
24. Moizard MP, Toutain A, Fournier D et al. Severe cognitive impairment in DMD: obvious clinical indication for Dp71 isoform point mutation screening. Eur. J. Hum. Genet. 2000; 8: 552–556.
25. Taylor PJ, Betts GA, Maroulis S et al. Dystrophin gene mutation location and the risk of cognitive impairment in Duchenne muscular dystrophy. PLoS One 2010; 20; 5: e8803.
26. Young HK, Barton BA, Waisbren S et al. Cognitive and psychological profile of males with Becker muscular dystrophy. J. Child Neurol.2008; 23: 155–162.
27. Donald KA, Mathema H, Thomas KG et al. Intellectual and behavioral functioning in a South African cohort of boys with duchenne muscular dystrophy. J. Child Neurol. 2011; 26: 963–969.
28. Wicksell RK, Kihlgren M, Melin L et al. Specific cognitive deficits are common in children with Duchenne muscular dystrophy. Dev Med Child Neurol. 2004; 46(3): 154–159.
29. Komoto J, Usui S, Otsuki S et al. Infantile autism and Duchenne muscular dystrophy. J. Autism Dev. Disord. 1984; 14: 191–195.
30. Wu JY, Kuban KC, Allred E et al. Association of Duchenne muscular dystrophy with autism spectrum disorder. J. Child Neurol. 2005; 20: 790–795.
31. Hendriksen JG, Vles JS. Neuropsychiatric disorders in males with duchenne muscular dystrophy: frequency rate of attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder, and obsessive-compulsive disorder. J Child Neurol. 2008; 23(5): 477–81.
32. Hendriksen JG, Vles JS. Are males with Duchenne muscular dystrophy at risk for reading disabilities? Pediatr Neurol. 2006; 34(4): 296–300.
33. Sekiguchi M, Zushida K, Yoshida M et al. A deficit of brain dystrophin impairs specific amygdala GABAergic transmission and enhances defensive behaviour in mice. Brain 2009; 132: 124–135.
34. Tracey I, Dunn J F, Radda GK. Brain metabolism is abnormal in the mdx model of Duchenne muscular dystrophy. Brain. 1996; 119: 1039–1044.
35. Tracey I, Scott RB, Thompson CH et al. Brain abnormalities in Duchenne muscular dystrophy: phosphorus-31 magnetic resonance spectroscopy and neuropsychological study. Lancet. 1995; 345: 1260–1264.
36. Lee JS, Pfund Z, Juhász C et al. Altered regional brain glucose metabolism in Duchenne muscular dystrophy: a pet study. Muscle Nerve 2002; 26: 506–512.
37. Rae C, Scott RB, Thompson CH et al. Brain biochemistry in Duchenne muscular dystrophy: a 1H magnetic resonance and neuropsychological study. J. Neurol. Sci. 1998; 60: 148–157.
38. Im WB, Phelps SF, Copen EH et al. Differential expression of dystrophin isoforms in strains of mdx mice with different mutations Hum Mol Genet 1996; 8: 1149–1153.
39. Miranda R, Sébrié C, Degrouard J et al. Reorganization of inhibitory synapses and increased PSD length of perforated excitatory synapses in hippocampal area CA1 of dystrophin-deficient mdx mice. Cereb. Cortex 2009; 19: 876–888.
40. Vaillend C, Perronnet C, Ros C et al. Rescue of a dystrophin-like protein by exon skipping in vivo restores GABAA-receptor clustering in the hippocampus of the mdx mouse. Mol. Ther. 2010; 18: 1683–1688.
41. Dallerac G, Perronnet C, Chagneau C et al. Rescue of a dystrophin-like protein by exon skipping normalizes synaptic plasticity in the hippocampus of the mdx mouse. Neurobiol. Dis. 2011; 43: 635–641.
42. Tritsch NX, Ding JB, Sabatini BL. Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. Nature 2012; 490: 262–266.
43. Fritschy JM, Paysan J, Enna A et al. Switch in the expression of rat GABAA-receptor subtypes during postnatal development: an immunohistochemical study. J Neurosci. 1994; 14: 5302–5324.
44. Poulopoulos A, Aramuni G, Meyer G et al. Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. Neuron 2009; 63: 628–642.
45. Panzanelli P, Gunn BG, Schlatter MC et al. Distinct mechanisms regulate GABAA receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells. J Physiol. 2011; 589: 4959–4980.
46. Perronnet C, Chagneau C, Le Blanc P et al. Upregulation of brain utrophin does not rescue behavioral alterations in dystrophin-deficient mice. Hum. Mol. Genet. 2012; 21: 2263–2276.
47. Coccurello R, Castellano C, Paggi P et al. Genetically dystrophic mdx/mdx mice exhibit decreased response to nicotine in passive avoidance. Neuroreport. 2002; 13: 1219–1222.
48. Sesay AK, Errington ML, Levita L et al. Spatial learning and hippocampal long-term potentiation are not impaired in mdx mice. Neurosci. Lett. 1996; 211: 207–210.
49. Bussey TJ, Padain TL, Skillings EA et al. The touchscreen cognitive testing method for rodents: how to get the best out of your rat. Learn. Mem. 2008; 15: 516–523.
50. Talpos JC, Fletcher AC, Circelli C et al. The pharmacological sensitivity of a touchscreen-based visual discrimination task in the rat using simple and perceptually challenging stimuli. Psychopharmacology (Berl) 2012; 221: 437– 449.

51. Rondi-Reig L, Libbey M, Eichenbaum H et al. CA1-specific N-methyl-D-aspartate receptor knockout mice are deficient in solving a nonspatial transverse patterning task. Proc. Natl. Acad. Sci. U. S. A 2001; 98: 3543–3548.
52. Bruno KJ, Freet CS, Twining RC et al. Abnormal latent inhibition and impulsivity in coloboma mice, a model of ADHD. Neurobiol. Dis. 2007; 25: 206–216.
53. Banik A, Anand A. Loss of learning in mice when exposed to rat odor: a water maze study. Behav. Brain Res. 2011; 216: 466–471.
54. Sluyter F, Marican CC, Roubertoux PL et al. Radial maze learning in two inbred mouse strains and their reciprocal congenics for the non-pseudoautosomal region of the Y chromosome. Brain Res. 1999; 835: 68–73.
55. Begenisic T, Spolidoro M, Braschi C et al. Environmental enrichment decreases GABAergic inhibition and improves cognitive abilities, synaptic plasticity, and visual functions in a mouse model of Down syndrome. Front Cell Neurosci 2011; 23: 29.
56. Tracey I, Dunn JF, Radda GK. Brain metabolism is abnormal in the mdx model of Duchenne muscular dystrophy. Brain. 1996; 119: 1039–1044.
57. Wehling-Henricks M, Oltmann M, Rinaldi C, et al. Loss of positive allosteric interactions between neuronal nitric oxide synthase and phosphofructokinase contributes to defects in glycolysis and increased fatigability in muscular dystrophy. Hum Mol Genet. 2009;18: 3439–3451.
58. Marrone AK, Kucherenko MM, Rishko VM et al. New dystrophin/dystroglycan interactors control neuron behavior in Drosophila eye. BMC Neurosci. 2011; 12: 93.
59. Mariska C, van Der Plas, Gonneke S K et al. Dystrophin Is Required for Appropriate Retrograde Control of Neurotransmitter Release at the Drosophila Neuromuscular Junction Noordermeer. The Journal of Neuroscience 2006; 26: 333–344.
60. Fradkin LG, Baines RA, van der Plas MC et al. The dystrophin Dp186 isoform regulates neurotransmitter release at a central synapse in Drosophila. J. Neurosci. 2008; 28: 5105–5014.
61. Huizhong W T, Mu-ming P. Retrograde signaling at central synapses. PNAS 2001; 98: 11009–11015
62. Pilgram GS, Potikanond S, Baines RA et al. The roles of the dystrophin-associated glycoprotein complex at the synapse. Mol Neurobiol. 2010; 41(1): 1–21.
63. Lei Shi, Amy KYFu, Nancy YI. Multiple roles of the Rho GEF ephexin1 in synapse remodelling. Commun. Integr. Biol. 2010; 3: 622–624.
64. Frank CA, Pielage J, Davis GW. A presynaptic homeostatic signaling system composed of the Eph receptor, ephexin, Cdc42, and CaV2.1 calcium channels. Neuron 2009; 61: 556–569.
65. Craddock TJ, Tuszynski JA, Hameroff S. Cytoskeletal signaling: is memory encoded in microtubule lattices by CaMKII phosphorylation? PLoS Comput. Biol. 2012; 8:e1002421
66. Oh KH, Kim H. Reduced IGF signaling prevents muscle cell death in a Caenorhabditis elegans model of muscular dystrophy. Proc Natl Acad Sci U S A. 2013; 110(47): 19024–9.
67. Zhou S, Chen L. Neural integrity is maintained by dystrophin in C. elegans. J. Cell Biol. 2011; 192: 349-363.
68. Benard C, Hobert O. Looking beyond development: maintaining nervous system architecture. Curr. Top. Dev. Biol. 2009; 87: 175–194.
69. Marui T, Funatogawa I, Koishi S et al. Association of the neuronal cell adhesion molecule (NRCAM) gene variants with autism. Int. J. Neuropsychopharmacol 2009; 12: 1–10.
70. Rosenthal A, Jouet M, Kenwrick S. Aberrant splicing of neural cell adhesion molecule L1 mRNA in a family with X-linked hydrocephalus. Nat Genet. 1992; 2: 107–112.
71. Sakurai T. The role of NrCAM in neural development and disorders--beyond a simple glue in the brain. Mol. Cell Neurosci. 2012; 49: 351–363.
72. Demyanenko GP, Riday TT, Tran TS et al. NrCAM deletion causes topographic mistargeting of thalamocortical axons to the visual cortex and disrupts visual acuity. J. Neurosci. 2011; 31: 1545–1558.
73. Moy SS, Nonneman RJ, Young NB et al. Impaired sociability and cognitive function in Nrcam-null mice. Behav. Brain Res. 2009; 205: 123–131.
74. Pegoraro E, Hoffman EP, Piva L et al. SPP1 genotype is a determinant of disease severity in Duchenne muscular dystrophy. Neurology 2011; 76: 219–226.
75. Bello L, Piva L, Barp A et al. doiImportance of SPP1 genotype as a covariate in clinical trials in Duchenne muscular dystrophy. Neurology 2012; 79: 159–162.
76. Piva L, Gavassini BF, Bello L et al. TGFBR2 but not SPP1 genotype modulates osteopontin expression in Duchenne muscular dystrophy muscle. Pathol. 2012; 228: 251–259.
77. Chen YW, Nagaraju K, Bakay M et al. Early onset of inflammation and later involvement of TGF beta in Duchenne muscular dystrophy. Neurol. 2005; 65: 826–834.
78. Araujo KP, Bonuccelli G, Duarte CN et al. Bortezomib (PS-341) treatment decreases inflammation and partially rescues the expression of the dystrophin-glycoprotein complex in GRMD dogs. PLoS One 2013; 8:e61367.
79. Clop A, Marcq F, Takeda H et al. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nat Genet. 2006; 38: 813–818.
80. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 1997; 387: 83–90.
81. McPherron AC, Lee SJ. Double muscling in cattle due to mutations in the myostatin gene. Proc. Natl. Acad. Sci. USA 1997; 94: 12457–12461.
82. Shelton GD, Engvall E. Gross muscle hypertrophy in whippet dogs is caused by a mutation in the myostatin gene. Neuromuscul. Disord. 2007; 17: 721–722.
83. Schuelke M, Wagner KR, Stolz LE et al. Myostatin mutation associated with gross muscle hypertrophy in a child. N. Engl. J. Med. 2004; 350: 2682–2688.
84. Sacco A, Mourkioti F, Tran R et al. Short telomeres and stem cell exhaustion model Duchenne muscular dystrophy in mdx/mTR mice. Cell 2010; 143: 10559–10571.
85. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat. Rev. Genet. 2005; 6: 611–622.
86. Cheng GL, Zeng H, Leung MK et al. Heroin abuse accelerates biological aging: a novel insight from telomerase and brain imaging interaction. Transl. Psychiatry 2013; 21; 3: e260
87. Pineda JR, Daynac M, Chicheportiche A et al. Vascular-derived TGF-β increases in the stem cell niche and perturbs neurogenesis during aging and following irradiation in the adult mouse. EMBO Mol. Med. 2013; 5:548–562.
88. Blanchard PG, Festuccia WT, Houde VP et al. Major involvement of mTOR in the PPARγ-induced stimulation of adipose tissue lipid uptake and fat accretion. J. Lipid Res. 2012; 53: 1117–1125.
89. Liang H, Ward WF. PGC-1alpha: a key regulator of energy metabolism. Adv. Physiol. Educ. 2006; 30: 145–51.
90. Hashimoto Y, Chiba T, Yamada M et al. Transforming growth factor beta2 is a neuronal death-inducing ligand for amyloid-beta precursor protein. Mol. Cell Biol. 2005; 25: 9304–9317.
91. Barcellos-Hoff MH, Dix TA. Redox-mediated activation of latent transforming growth factor-beta 1. Mol. Endocrinol. 1996; 10: 1077–1083.
92. Hill JJ, Tremblay TL, Cantin C et al. Glycoproteomic analysis of two mouse mammary cell lines during transforming growth factor (TGF)-beta induced epithelial to mesenchymal transition. Proteome Sci. 2009; 7: 2.
93. Konig HG, Kögel D, Rami A et al. TGF-{beta}1 activates two distinct type I receptors in neurons: implications for neuronal NF-{kappa}B signaling. J Cell Biol 2005; 168: 1077–1086.
94. Inoki K, Haneda M, Maeda S et al. TGF-beta 1 stimulates glucose uptake by enhancing GLUT1 expression in mesangial cells. Kidney Int. 1999; 55: 1704–1712.
95. Wu B, Chen Y, Huang J et al. Icariin improves cognitive deficits and activates quiescent neural stem cells in aging rats. J. Ethnopharmacol 2012; 142: 746–753.
96. Brouns MR, Matheson SF, Settleman J. p190 RhoGAP is the principal Src substrate in brain and regulates axon outgrowth, guidance and fasciculation. Nat. Cell Biol. 2001; 3: 361–367.
97. Lamprecht R, Farb CR, LeDoux JE. Fear memory formation involves p190 RhoGAP and ROCK proteins through a GRB2-mediated complex neuron 2002; 36: 727–738.
98. Wei G, Chen YB, Chen DF et al. β-Asarone inhibits neuronal apoptosis via the CaMKII/CREB/Bcl-2 signaling pathway in an in vitro model and AβPP/PS1 mice. J Alzheimers Dis 2013; 33: 863–880.

99. Imbert N, Cognard C, Duport G et al. Abnormal calcium homeostasis in Duchenne muscular dystrophy myotubes contracting in vitro. Cell Calcium. 1995; 18: 177–186.
100. Jahnke VE, Van Der Meulen JH, Johnston HK et al. Metabolic remodeling agents show beneficial effects in the dystrophin-deficient mdx mouse model. Skelet Muscle. 2012; 2(1): 16.
101. Bushby K, Connor E. Clinical outcome measures for trials in Duchenne muscular dystrophy: report from International Working Group meetings. Clin. Investig. (Lond)2011; 1: 1217–1235.
102. Gomez-Pinilla F, Nguyen TT. Natural mood foods: the actions of polyphenols against psychiatric and cognitive disorders. Nutr. Neurosci. 2012; 15: 127–133.
103. Kuriyama S, Shimazu T, Ohmori K et al. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. JAMA 2006; 296: 1255–1265.
104. Kulkarni S, Dhir A, Akula KK. Potentials of curcumin as an antidepressant. ScientificWorldJournal 2009; 9: 1233–1241.
105. Lavretsky H, Epel ES etal. Int A pilot study of yogic meditation for family dementia caregivers with depressive symptoms: effects on mental health, cognition, and telomerase activity. J. Geriatr. Psychiatry 2013; 28: 57–65.
106. Cramer H, Lauche R, Langhorst J et al. Quality of life and mental health in patients with chronic diseases who regularly practice yoga and those who do not: a case-control study. Evid. Based Complement Alternat. Med. 2013: 702914.
107. Ihl R, Tribanek M, Bachinskaya N. Efficacy and tolerability of a once daily formulation of ginkgo biloba extract EGb 761(R) in Alzheimer's disease and vascular dementia: results from a randomised controlled trial. Pharmacopsychiatry 2012; 45: 41–46.
108. Snitz BE, O'Meara ES, Carlson MC et al. Ginkgo biloba for preventing cognitive decline in older adults: a randomized trial. JAMA 2009; 302: 2663–2670.
109. Ishrat T, Hoda MN, Khan MB et al. Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT). Eur. Neuropsychopharmacol 2009; 19: 636–647.
110. Wang HM, Zhao YX, Zhang S et al. PPARgamma agonist curcumin reduces the amyloid-beta-stimulated inflammatory responses in primary astrocytes. J. Alzheimers Dis. 2010; 20: 1189–1199.
111. Wang R, Tang XC. Neuroprotective effects of huperzine A: a natural cholinesterase inhibitor for the treatment of Alzheimer's disease. Neurosignals 2005; 14: 71–82.
112. Ved HS, Koenig ML, Dave JR et al. A potential therapeutic agent for dementia, reduces neuronal cell death caused by glutamate. Neuroreport 1997; 8: 963–968.
113. Wu B, Chen Y, Huang J et al. Icariin improves cognitive deficits and activates quiescent neural stem cells in aging rats. J. Ethnopharmacol 2012; 142: 746–753.
114. Wang X, Li J, Qian L et al. Icariin promotes histone acetylation and attenuates post-stroke cognitive impairment in the central cholinergic circuits of mice. Neuroscience 2013; 236: 281–288.
115. Haider S, Naz N, Khaliq S et al. Repeated administration of fresh garlic increases memory retention in rats. J. Med. Food 2008; 11: 675–679.
116. Chauhan NB, Sandoval J. Amelioration of early cognitive deficits by aged garlic extract in Alzheimer's transgenic mice. Phytother. Res. 2007; 21: 629–640.
117. Ahmed ME, Javed H, Khan MM et al. Attenuation of oxidative damage-associated cognitive decline by Withania somnifera in rat model of streptozotocin-induced cognitive impairment. Protoplasma. 2013; 250, 1067–1078
118. Yadav CS, Kumar V, Suke SG et al. Propoxur-induced acetylcholine esterase inhibition and impairment of cognitive function: attenuation by Withania somnifera. Indian J. Biochem. Biophys. 2010; 47: 117–120.
119. Yeo HB, Yoon HK, Lee HJ et al. (2012). Effects of Korean Red Ginseng on Cognitive and Motor Function: A Double-blind, Randomized, Placebo-controlled Trial. J. Ginseng Res. 2012 36: 190–197.
120. Al-Hazmi MA, Rawi SM, Arafa NM et al. (2013). The potent effects of ginseng root extract and memantine on cognitive dysfunction in male albino rats. Toxicol Ind Health [Epub ahead of print]
121. Saraf MK, Prabhakar S, Anand A. Neuroprotective effect of Bacopa monniera on ischemia induced brain injury. Pharmacol. Biochem. Behav. 2010; 97: 192–197.
122. Stough C, Lloyd J, Clarke J et al. The chronic effects of an extract of Bacopa monniera (Brahmi) on cognitive function in healthy human subjects. Psychopharmacology 2001; 156: 481–484.