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Developments in Genetic Analysis Models on Dysferlin: A Muscle Specific Repair Complex

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ABSTRACT

Many researchers have reported the clinical and genetical studies on Dysferin protein. However protein analysis and structure prediction of Dysferin protein have not been done so far. Moreover, Dysferin in first identified putative muscle specific repair complex. Dysferin act as Ca regulated fusogen that permits rapid resealing of membranes disrupted mechanical stress. So here we report gene analysis by retrieving Homo sapiens gene from NCBI database (according to protein family) and physiochemical characterization, structure production of Dysferlin protein using SPDB Viewer.

Keywords: Dysferlin, Ferlin, Myogenis, Genscan, Gene analysis

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INTRODUCTION

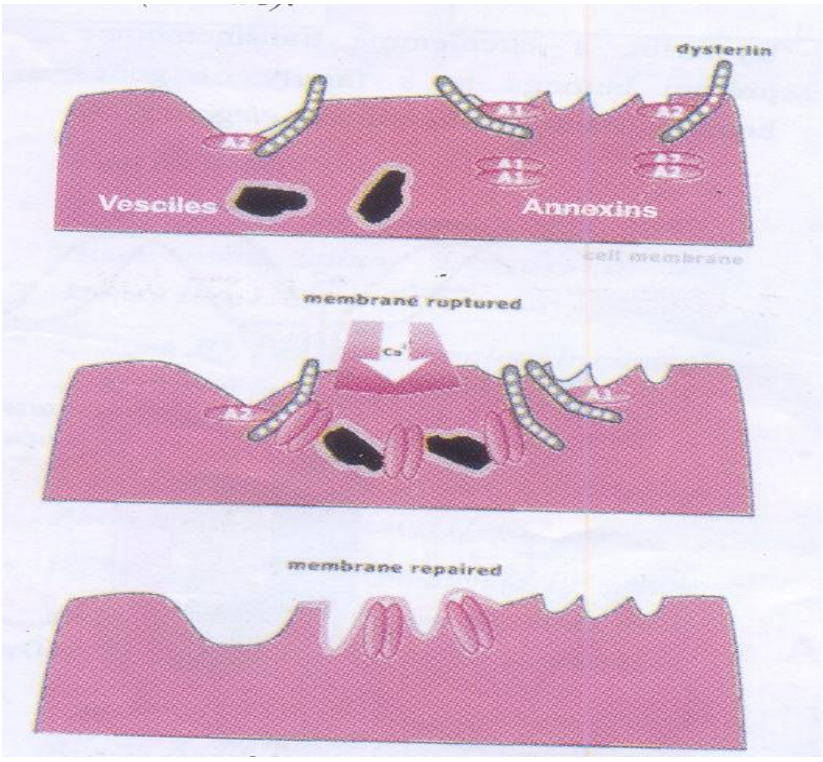
Dysferlin, a sarcolemma transmembrane protein belongs to a family of genes homologues to Caenorhabditis elegans fer-1gene. This gene mediates vesicle fusion in the ruptured plasma membrane of spermatids. Ferlins have been recently identified as a conserved protein family that participates in membrane repair [1-4]. The ferlin family consists of four different genes that encode dysferlin, otoferlin, myoferlin, and Fer1L4. Dysferlin is the first identified putative muscle-specific repair complex. Dysferlin act as Ca+-regulated fusogen that permits rapid resealing of membranes disrupted mechanical stress. Dysferlin is made up of 2,080 amino acids. 6C2 domains of dysferlin are implicated in calcium dependent membrane fusion events [5-11].

Main characteristics of Dysferlin:

- Specialized proteins in muscle cells are the building blocks of the structures constituting the moving machinery of muscle. There are three kinds of muscle viz., skeletal muscle, smooth muscle and cardiac muscle.
- Skeletal muscle is attached to bones and allows the voluntary movement of limbs;
- Smooth muscle is found in internal organs and aids in the involuntary movements that occur in the circulatory, digestive, excretory, reproductive, and respiratory systems;
- Cardiac muscle formal the powerful walls of the heart.
- Muscle proteins indeed are essential for muscle functioning. Numerous muscle proteins are present in the sarcolemma membrane and they play their unique role for normal functioning of the muscle.

Membrane Repair

Membrane repair is an active process of skeletal muscle fibers where dysferlin plays an essential role. During vigorous exercise and when subjected to mechanical stress the plasma membrane of muscle cells gets ruptured, as a result the hydrophobic phospholipids are exposed to hydrophilic environment which is an unfavorable state [4,6,8,11-17]. Normally, the entropic forces draws the membrane ends together to reseal membrane lesions but under certain physiological conditions the membrane tension slows or completely blocks the self sealing process. Alternatively cells are engaged in active traffincking of endomembrane vesicles to damage site and subsequently leads to fusion of vesicles with the plasma membrane by exocytosis. Membrane repair involves both a reduction in membrane tension possibly by local depolymerization of the cortical cytoskeleton and patch formation. [4,5,10,11,18-24]



Cortical granules, yolk granules, endocytic components, lysosomes, and enlargosomes are vesicular membrane compartments that participates in the repair mechanism. These compartments participates depending upon the cell type. Latter, homotypic fusion of membrane vesicles at the rupture site creates a patch which them fuses with the plasma membrane in a Ca^{2+}-dependent process [14,15,18].

Several membrane proteins are required for the active membrane repair process which includes **SNARE** proteins and **SYAPTOTAGMINS.**

- **SNARE PROTEINS –** A family of transmembrane proteins essential in most intracellular membrane fusion processes.
- **SYNAPTOTAGMINS –** Transmembrane proteins containing two highly conserved Ca2+-binding domains that are thought to serve as Ca2+ sensors.

Annexins are a family of cellular proteins found in all kingdoms except bacteria. The main function of annexin is that, it binds calcium-dependently to phospholipids membranes. Annexins is also known as "lipocortin" which suppress phopholipase A2. By this mechanism, glucocorticoids inhibits inflammation. Annexin lack a signal peptide which is necessary for proteins to be transported out of the cell. There are three types of Annexin such as **AnnexinA1, AnnexinA2 and Annexin A5.**

- **AnnexinA1** -It is also known as lipocortin I, plays a role in inflammation processes.
- **AnnexinA2** - It is a pleiotrophic protein, involved in sorting of endsomes and in anticoagulant reactions.
- **AnnexinA5** – It is a cellular protein which is proposed to play a role in anticoagulation and also to inhibit the activity of phospholipaseA1(in-vitro).

Interaction with Caveolin-3

Caveolin-3 is a cardiac and skeletal muscle protein which is a component of Caveolae. Caveolae are small invaginations of the plasma membranes in many vertebrate cell types especially in endothelial cells and adipocytes. Caveolae have several functions in signal transduction and it also play a role in endocytosis, oncogenesis and the uptake of pathogenic bacteria and certain viruses. Dysferlin interacts with caveolin-3 to subserve signaling functions of caveolae [16-22]. Caveolin-3 is localized in the sarcolemma and in plays an important role in the formation of caveolae membranes. This serves as a scaffolding protein to interact with and organize lipid and protein constituents of caveolae membranes [7,9,14,15,22-29]. This serves as a scaffolding protein to interact with and organize lipid and protein constituents of caveolae. Caveolin-3 is important for normal muscle function and its viability. Mutations in CAV3 gene causes an inherited limb girdle type1C(LGMD1C) muscular dystrophy.

Interaction with Myogenin

Myogenin is a basic-helix-loop-helix transcription factor expressed during the development, maintenance and repair of skeletal mauscle. Myogenin belongs to the MyoD family of bHLH transcription factors, which also include MyoD,Myf5, and MRF4. It is also known as myogenic Regulatory Factor (MRF). There is a link between dysferlin and myogenin by which they share a signaling pathway involve in differentiation of skeletal muscle [24,27,29-32]. In adult skeletal muscle, myogenin and dysferlin expressions are coincidental. Myogenin expression are accidental with satellite cell differention and fusion, and dysferlin is expressed in activated satellite cells indication its role in muscle regeneration. Dysferlin also interacts with affixin, an intracytoplasmic protein that accumulates in the disruption site of the membrane and plays an important role in wound healing.

Marker for Multiple Sclerosis

Multiple sclerosis is a chronic inflammatory, demyelinating disease tha affects the central nervous system (CNS). In the normal central nervous system (CNS), dysferlin is only present in endothelial cells of circum ventricular organs. Dysferlin is also expressed in leaky endothelial cells. Iin the inflamed CNS of patients with multiple sclerosis (MS), dysferlin reactivity is induced in endothelial cells. In this case, the

expression of dysferlin is linked with vascular leakage of serum proteins. In MS, dysferlin activity in endothelial cells is not limited to vessels with inflammatory cuffs. And also it is seen in non-inflammatory vessels [6,8,11,15]. Due to inactive lesions or in the normal appearing white matter, dysferlin is not expressed in many blood vessels with perivascular inflammatory infiltrates. In endothelial cells, dysferlin is induced with tumor necrosis factor-alpha (TNF-α). Hence, dysferlin is one of the scientific marker for leaky brain vessels, perivascular inflammatory infiltrates and blood-brain barrier disturbance in multiple sclerosis [22,25,29,32].

DYSF – Defects

DYSF, a novel skeletal muscle gene codes for the protein dysferlin which is located in chromosome 2p13.3-13.1. It is an interesting twist that this gene is associated with two forms of muscular dystrophy,

- Limb-Girdle Muscular Dystrophy type 2B(LGMD2B)
- Miyoshi Myopathy(MM)

Muscular dystrophy is considered as a genetic disease. It is identified by progressive weakness and deterioration of the skeletal muscles, which control muscle movement. There are none major farms of muscular dystrophy. It is proved that various types of muscular dystrophies are resulted form more than 20 mutated genes. Certain types of dystrophies and its symptoms are expressed in the stage of childhood whereas some others appear in adulthood. In specific forms of dystrophies particular muscles are affected with its deserved disorders. In the field of medicine, yet the perfect treatment is not available for curing the disease muscular dystrophy. The ongoing treatments are pointing towards preventing the complications behind its symptoms. Antioxidant and L-glutamic acid tablets are prescribed in order to prevent muscle cell damage. Gene therapy and stem cell therapy trials are still going on in humans.

Limb-Girdle Muscular Dystrophy (Dysferlinopathy)

Limb-girdle muscular dystrophy or Erb's muscular dystrophy is a type of muscular dystrophy that includes Duchenne muscular dystrophy, Becker's muscular dystrophy, and a large number of rarer disorders. Additionally, these disorders are name as "limb-girdle" because of its main symptoms like most severely affected muscles of the hips and shoulders.Generally Limb-girdle is characterized by muscle weakness, myoglobinuria, pain, myotoniam cardiomyopathy, elevated serum CK, and rippling muscles. The muscle weakness is generally symmetric, proximal, and slowly progressive. Normally mental function and pain is not associated with LGMD [15,18,22,26,31,32].

The onset of LGMD can begin in childhood or even at later stage. Usually it is expressed between the age of 10 and 30. Both genders are affected equally. Early progression of limb-girdle muscular dystrophy appears to be faster and severe whereas in the later stages it is vice versa. The distal muscles are affected late in LGMD, if at all. This results in loss of muscle bulk and strength. Basically the patients feel so difficult for their movements and progressed as disabled one. Primarily LGMD weakens the heart and lung muscles, leading to illness. Secondarily it leads to death. LGMD is typically an inherited disorder, though it may be inherited as a dominant, recessive, or X-linked genetic defect. Proteins needed for normal muscle function is affected thus the muscles cannot work properly. Different protein defects leads to various types of Muscular dystrophies.

GENSCAN

It is a program designed to predict complete gene structures, including exons, introns, promoter and poly-adenylation signals, in genomic sequences. It differs from the majority of exisiting gene finding algorithms in that it allows for partial genes as well as complete genes and for the occurrence of multiple genes in a single sequence, on either or both DNA strands. Program versions suitable for vertebrate, nematode (experimental), maize and Arabidopsis sequences are currently available. The vertebrate version also works fairly well for Drosophila sequences. As a rule, internal exons are predicted more accurately than initial or terminal exons, and exons are predicted more accurately than polyadenylation or promoter signals. Predicted promoters, in particular, are not reliable.

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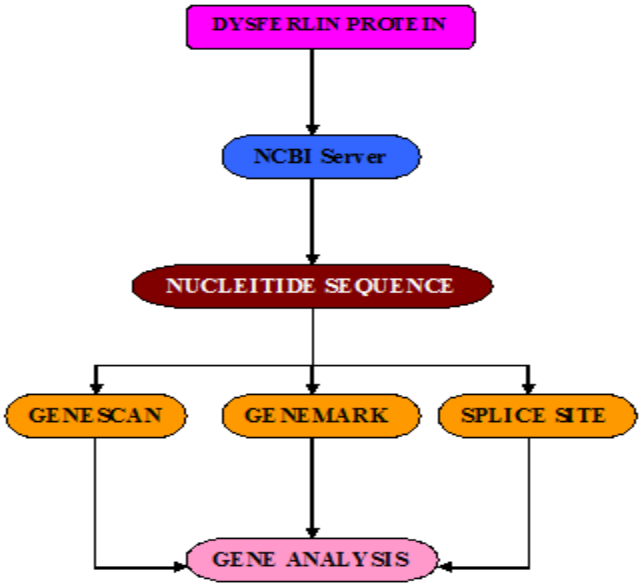
METHODOLOGY FOR GENE ANALYSIS



Prediction coding regions by using GENSCAN

Gene Name	Gn.Ex	Type	S	Begin	End	Length	Fr	CodRg	P	Tscr
DYSF	1.01	Init	+	493	1767	1275	0	1641	0.856	949.35
	1.02	Term	+	2426	3337	912	1	1455	0.940	133.54
	1.03	PlyA	+	3857	3862	6	-	-	-	-

EXPANSION:

Gn.Ex : Gene number, Exon number (for reference)
S : DNA strand (+ = input strand; - = opposite strand)
Begin : Beginning of exon or signal (numbered on input strand)
End : End point of exon or signal (numbered on input strand)
Len : Length of exon or signal (bp)
Fr : Reading frame (a forward strand codon ending at x has frame x mod 3)
CodRg : Coding region score (tenth bit units)
P : Probability of exon
Tscr : Exon score (depends on length, I/Ac, Do/T and CodRg scores)

Genscan predicted two exons having length **1275** and **912** of score **149.39** and **133.4** respectively on a forward reading frame. Since the score having **>100** are considered as a strong splice site.

GENMARK

GeneMark is a system for finding genes in bacterial DNA sequences. The algorithm is based on non-homogeneous 5^{th}-order Markow chains, and it was used to locate the genes in the complete genomes of H. influenzae, M. genitalium, and several other complete genomes.

Prediction coding regions by using GENMARK

Gene Name	SeqLeg	GC-%	Gene	Exon	Strand	Exon Type	Exon	Range	Exon Length	Strat/End
DYSF	3880	52.37%	1	1	+	Initial	1207	1545	339	1 3 - -
				2	+	Internal	1609	1767	159	1 3 - -
				3	+	Internal	2425	3283	859	1 1 - -
				4	+	Internal	3649	3698	50	2 3 - -

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INFERENCE:

GenMark predicted four exons in a forward strand in type initial site, two internal site and one terminal site having the length of **339, 159, 859**, and **50** respectively. Splice Site Prediction tool is a neural network method. Splice sites are the key signal sequences that determine that determine the boundaries of exons. A method for splice site detection should ideally be based on thorough understanding of the complex eukaryotic splicing process. We trained a back propagation feed forward neural network with one layer of hidden units to recognize 5' and 3' splice sites, using a representative data set (Drosophila melanogaster data set). We only consider genes that have constraint consensus splice sites, i.e., 'GT' for the 5' and 'AG' for the 3' splice site. The maximum allowed sequence length is 100000 bases. Neural Network based "consensi" sequences: Extensive analysis of the perception neural network weight matrices have revealed the following "refined" 5' and 3' splice site consensus and non-consensus sequences:

Prediction of by using donar site and acceptor site
By Using SPLICE SITE PREDICTION tool

DONOR SITE PREDICTION

Start	End	Score	Exon Intron
121	135	0.96	tcagtgg**gt**gagtta
328	342	0.55	tctgatg**gt**aaagat
472	486	0.45	tcaagat**gt**aaatgt
886	900	0.76	ctgaaag**gt**gggaac
1095	1109	0.98	acggatg**gt**aaggaa
1267	1281	0.98	ttcaaag**gt**gagaaa
1539	1553	0.40	ccgctgg**gt**acgggg
1761	1775	0.75	caaagag**gt**atagca
1913	1927	0.63	agagcag**gt**gcctca
2184	2198	1.00	tgttctg**gt**aagtgt
2260	2274	0.89	aagtgag**gt**agggag
3171	3185	0.92	ccatgcg**gt**acgcag
3321	3335	0.54	tcaccat**gt**atgcct
3537	3551	0.91	caatgag**gt**aggaag

ACCEPTOR SITE PREDICTION

Start	End	Score	Intron Exon
276	316	0.64	gatcttggtcttaggcttc**ag**gaaggagccatcttctgtga
456	496	0.87	atagtgtgtctttccttca**ag**atgtaaatgtcaaggaatga
709	749	0.58	gcagggatcttccacttcc**ag**ttctggcgctatggagagtg
1894	1934	0.87	cctgtgttatttccactgc**ag**agcaggtgcctcagggcatt
1966	2006	0.49	gcactttctccctcgcacc**ag**acactgcacgtcacacacat
2020	2060	0.53	caccctcctccacgcttac**ag**ccacacacacagtcacacag
2405	2445	0.96	tgggctctccccatctctc**ag**atgcacgggaacaagcagca
2524	2564	0.75	gcgcttccgcctgcctccc**ag**cgagtacgtcatcgtgccct
2674	2714	0.58	catcatcttcgtttcggac**ag**agcaaacagcaacaaggagc
3214	3254	0.95	tttcatctgctgcttcgtt**ag**gctggagggcatgttcagag
3425	3465	0.62	ccagcagctacacccctac**ag**gcttccaggcacctcatcag
3629	3669	0.94	ctcctgcttaccttgctct**ag**gctgtctgcagaagcacctg

The above result shows that the taken genes having considerable amount of both donor and acceptor sites. The best score sites has been showed on the above table. That is for donor site the score of above **0.45-0.98** and acceptor site score of above **0.45-0.95** are taken under the consideration for the boundaries of exon/introns. Dysferlin which is a muscle proteins, a mutation this protein may lead to complication like Limb-girdle and Miyoshi Myopathy, three-dimensional confirmation of the protein is not been predicted using the classical approach like "NMR or Crystallography".

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