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AN INSIGHT THROUGH THE MOST FREQUENTLY MUTATED GENES : THEIR ACTIONS DURING PHYSIOLOGICAL AND PATHOLOGICAL EXPRESSION IN REPORTED CASES OF LIMB GIRDLE MUSCULAR DYSTROPHY (LGMD) GLOBALLY- A LITERATURE REVIEW.

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ABSTRACT Limb girdle muscular dystrophy is a group of genetic disorders characterized by progressive weakness and wasting of muscles, can vary widely in severity and progression depending on the specific genetic mutation involved. The prevalence of LGMD in the world population is estimated to be around 1 in 14,500 to 1 in 123,000 individuals. The global incidence of LGMD is about 1 in 100,000 individuals. This literature review delves into the comprehensive analysis of 15 most frequently mutated genes associated with LGMD, elucidating their chromosomal location, nomenclature, physiological roles under normal conditions and the aberrations observed during pathological expression.

KEYWORDS : LGMD; genes mutated in LGMD; Limb girdle muscular dystrophy genetics

INTRODUCTION:

Limb girdle muscular dystrophy (LGMD) ranks as the fourth most prevalent genetic contributor to muscle weakness. Estimates of prevalence for all forms of LGMD range from one in 14,500 to one in 123,000 [van der Kooi et al 1996, Urtasun et al 1998][41]. The estimated prevalence of primary sarcoglycanopathies is approximately one in 178,000 [Fanin et al 1997][42]. Out of 42 different individual case reports reported individually worldwide by various practioners in Pub-Med, following are the most frequently mutated genes globally. Through this review, we aim to elucidate the detailed pathophysiological mechanisms by examining how these genes (table-1) function under normal physiological conditions and how mutations in these genes can lead to the manifestation of signs and symptoms at specific age groups, as reported in various documenentaions.

Table No: 1: Following Is The Table Of Most Frequently Involved Genes In LGMD Mutations:

Sl. No	Genes	Nomenclature	Chromosome location and exon count
1	**Calpain 3 gene**	Pq4, CANP3, LGMD2, nCL-1,CANPL3, LGMD2A, LGMDD4, and LGMDR1	15q15.1-q21.1; exon count- 26
2	**DYSF gene:**	Dysferlin ,MMD1;FER1L1; LGMD2B; LGMDR2;	2p13.2; Exon count: 58
3	**Sarcoglycans**		
	α-sarcoglycan	50DAG, ADL, DAG2, DMDA2, LGMD2D, LGMDR3, SCARMD1, adhalin,	17q21.33; Exon count: 10
	β-Sarcoglycan	A3b; SGC; LGMD2E; LGMDR4,	4q12; Exon count: 7
	γ-Sarcoglycan	A4; MAM; DMDA; SCG3; 35DAG; DAGA4; DMDA1; LGMD2C; LGMDR5; SCARMD2; gamma-SG	13q12.12 ; Exon count: 10
	δ-Sarcoglycan	50DAG, ADL, DAG2, DMDA2, LGMD2D, LGMDR3, SCARMD1, adhalin,	5q33.2-q33.3; Exon count: 14
4	**GMPPB gene**	GDP-mannose pyrophosphorylase B. LGMDR19; MDDGA14; MDDGB14; MDDGC14;	3p21.31; Exon count: 9
5	**UTRN**	utrophin, DRP; DMDL; DRP1.	6q24.2; Exon count: 76
6	**PSAP**	PROSAPOSIN, GLBA; SAP1; SAP2; PSAPD; PARK24	10q22.1; Exon count: 15
7	**TNPO3**	transportin 3, IPO12; TRNSR; LGMD1F; LGMDD2; MTR10A; TRN-SR; TRN-SR2.	7q32.1; Exon count: 33
8	**FKRP gene**	fukutin related protein .FKTR; MDC1C; LGMD2I; LGMDR9; MDDGA5; MDDGB5; MDDGC5	19q13.32; Exon count: 8
9	**POMT1 gene**	(protein O-mannosyltransferase) RT; LGMD2K; MDDGA1; MDDGB1; MDDGC1; LGMDR11	9q34.13; Exon count: 21
10	**COL6A1, COL6A2, COL6A3 gene**		
	COL6A1	collagen type VI alpha 1 chain .OPLL; BTHLM1; UCHMD1	21q22.3; Exon count: 35.
	COL6A2	collagen type VI alpha 2 chain. UCMD1; BTHLM1; PP3610.	21q22.3; Exon count: 29.

	COL6A3	collagen type VI alpha 3 chain. DYT27; UCMD1; BTHLM1.	2q37.3; Exon count: 44
11	**ANO5 gene**	anoctamin 5. GDD1; LGMD2L; LGMDR12; TMEM16E.	11p14.3; Exon count: 23
12	**DNAJB6**	DnaJ heat shock protein family (Hsp40) member B6. DJ4; MRJ; DnaJ; HSJ2; HHDJ1; HSJ-2; MSJ-1; LGMD1D; LGMD1E; LGMDD1	7q36.3; Exon count: 12 - 2 isoforms present. A and b.
13	**TCAP gene**	titin-cap gene, tele, CMD1N, CMH25,T-cap,LGMD2G,LGMDR7, telethonin	17q12; Exon count: 2
14	**BETA-DYSTROGLYCAN**	Dystroglycan1,A3a;DAG;AGRNR;156DAG;MDDGA9;MDDGC7; MDDGC9;LGMDR16	3p21.31; exon count : 9
15	**DAG COMPLEX: Dystroglycan complex**	-	-

1) Calpain 3 gene - exon 1 [LGMD2A]

Calpain 3 is primarily found in skeletal muscles, specifically in fast and slow twitch fibers, located between the Z disc and M line of the sarcomere. It plays a crucial role in regulating the flexibility and strength of myofibrils. Calpain 3 is a heterodimer, consisting of larger and smaller subunits. The larger subunit acts as an intracellular proteolytic enzyme, breaking down proteins such as titin, filamin C, vinexin, ezrin, and talin, which helps maintain optimal protein levels in the vicinity. In skeletal muscle damage, calpain 3 has a dual role: 1) initiating myofibril disassembly in the initial stages of injury, and 2) aiding in sarcomere remodeling during recovery by degrading unnecessary proteins at the site.

Limb-girdle muscular dystrophy type 2A (LGMD 2A) accounts for 30-40% of all LGMD cases, with a prevalence rate of 6.8-10.2 per million worldwide. The Leiden database and literature report 286 mutations of calpain 3 (161 missense, 74 deletion/insertion, 38 splice site, 13 nonsense). Symptoms of LGMD 2A typically appear between the ages of 6-18 years, although onset can range from 1 to 49 years. Patients with LGMD 2A experience progressive muscle weakness, atrophy of the shoulder and pelvic girdles, and often have elevated creatine phosphokinase (CPK) levels, which may be incidentally discovered or investigated as part of the diagnostic process. Muscle biopsy reveals degeneration and regeneration of muscle fibers, a common feature in muscular dystrophies. The majority of LGMD 2A patients require a wheelchair by their second to fourth decade of life.

An insight on what could have triggered the onset of symptoms at the above mentioned typical presenting age?

Based on existing literature, the age range of 8-16 years marks a significant period of adolescent development, encompassing physical, skeletal, hormonal, psychological, and behavioral changes. During this transitional phase, skeletal muscles undergo repeated trauma and remodeling. This remodeling process involves an intracellular protein release.In cases of calpain 3 deficiency or mutations, three main changes are expected to occur during the peak of the first and second decades of life, based on the known functions of calpain 3:

- Muscles affected by calpain 3 deficiency are unable to break down unnecessary proteins during the initial stages of repair, particularly during the initial disassembly of myofibrils at the injury site.
- Calpain 3 deficiency leads to a failure to cleave proteins such as titin, filamin C, vinexin, ezrin, and talin, resulting in an accumulation of intracellular substrates in the injured area.
- There is a failure in the effective remodeling of the sarcomeres.

Studies have indicated that during eccentric exercise, along with increased activity and strenuous exercise with age, the proteins that generate calpain 3 are downregulated. This downregulation contributes to a more aggressive expression of signs and symptoms. Supporting this notion, Duguz et al. conducted knockout studies in mice, demonstrating that muscles deficient in calpain 3 fail to regain their full weight upon maturity. Additionally, the presence of abnormal sarcomeres leads to a decrease in the force generation capacity of fibers. Furthermore, an upregulation of heat shock proteins within individual muscle fibers hinders their optimal contractility.[1,2,3,4]

2. DYSF gene: Dysferlin:

Ferlins are a class of proteins characterized by the presence of C2 domains, with a dominant DYSF domain and a specific sequenced ferlin domain. Ferlins are classified into two types, type I and type II, based on the presence or absence of these two domains. While the DYSF domain is found in various tissues such as skeletal muscle, heart, and brain, it is particularly concentrated along the invaginations of the T-tubules of the sarcolemma and within the cytoplasm. In healthy skeletal muscles, DYSF plays a crucial role in membrane trafficking and vesicle transport within the cell. The dynamic activity of DYSF makes the sarcolemmal and plasma membranes of skeletal muscle susceptible to stretch-induced injuries. Upon such injuries, an emergency plasma membrane repair (PMR) mechanism is initiated within seconds. This repair process is believed to be triggered by a surge of calcium following a stretch-induced pooling of intracytoplasmic calcium, which then recruits membrane repair endovesicles aggregated beneath the plasma membrane. In addition to its role in PMR, DYSF is also involved in membrane trafficking by localizing syntaxin 4 and SNAP-23 through binding to phosphatidylserine and phosphatidylinositol bisphosphate. DYSF is also implicated in the generation of T-tubules and the optimization of the interaction between L-type calcium channels and Ryanodine receptors.

In LGMD 2B, also known as dysferlinopathy, DYSF, CAPN3, and COL6A1 exhibit the highest number of pathogenic variants, indicating greater allelic heterogeneity in these genes compared to others in the panel. The major contributors to LGMD phenotypes are CAPN3 (17%), DYSF (16%), FKRP (9%), and ANO5 (7%). Dysferlinopathy typically presents with muscle weakness in the posterior compartments of the thighs and legs, gradually progressing to involve the upper limbs, particularly the subscapular and periscapular regions of the shoulder girdle. In later stages, it may also affect the anterior compartments of the thighs and legs, as well as the finger flexors.

Possible Pathogenic mechanisms causing failure of effective muscle contractions and weakness:

- Ineffective development of T-tubules can lead to a cessation of impulse generation and disruption of excitation-contraction mechanisms at the neuromuscular junction, resulting in the exhaustion of the intracytoplasmic calcium pool.
- Defects in membrane transport and vesicle recruitment following repeated stretch-induced micro muscle injuries can impair the plasma membrane repair (PMR) mechanism.
- An imbalance between muscle fiber degeneration and regeneration can result in increased sheer stress, leading to the deposition of fibrotic and fatty tissue in the muscle vicinity. This accumulation contributes to the pseudo-hypertrophic clinical appearance of proximal limb muscles.[5,6,7,8]

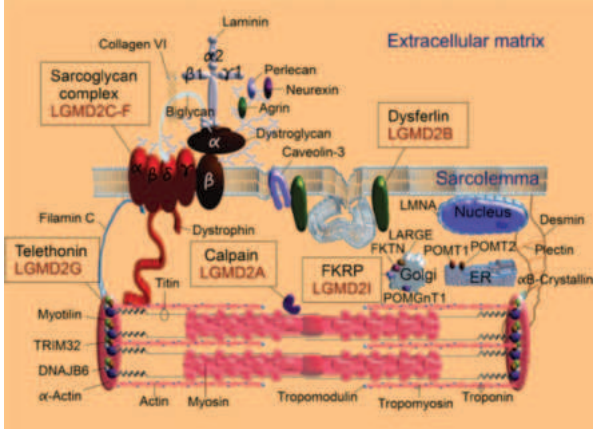

Image courtesy[43]

3. SARCOGLYCANS:

The dystrophin-glycoprotein complex (DGC) serves as a crucial link between the extracellular matrix, plasma membrane, and cytoskeleton, in addition to its role as a signal transducer. Comprising four integral proteins - α-, β-, γ-, and δ-sarcoglycan - the DGC plays a pivotal role in mechanically stabilizing the sarcolemma. These tetramers not only mutually support each other but also stabilize and reinforce the connection between dystrobrevin and syntrophin.

α-sarcoglycan:

Alpha-sarcoglycan (α-SGC) is predominantly expressed in skeletal muscle, with lower levels found in cardiac muscles. The presence of a cadherin domain in the extracellular region of α-SGC indicates its calcium-dependent binding with the dystroglycan complex. Mutations in α-SGC are responsible for causing LGMD 2D. Despite its lower expression in cardiac muscles, reported cases have shown simultaneous cardiac involvement alongside the primary clinical features of LGMD. The severity of LGMD 2D depends on the type of mutation. Nonsense mutations result in a complete loss of α-SGC, leading to a more severe form of the disease, while missense mutations present as a milder clinical form.

β-Sarcoglycan:

Beta-sarcoglycan (β-SGC) is a transmembrane component of the dystrophin-glycoprotein complex (DGC). It contains three asparagine residues that serve as N-linked glycosylation sites. Studies using gene knockout models with glycosylation-defective mutations have demonstrated that β-SGC plays a crucial role in the proper assembly and folding of the Sarcoglycan complex (SGC), as evidenced by self-aggregation and disruption of the complex. Mutations in the β-SGC gene lead to LGMD 2E.

The expression and function of β-SGC are equally important in both skeletal and cardiac muscle, which explains why patients diagnosed with LGMD 2E often have associated cardiac conditions. Unlike mutations in α-SGC, even missense mutations in β-SGC can result in more severe forms of muscular dystrophy. Studies exploring treatment strategies for LGMD 2E have shown that calcium channel blockers can lead to transient improvement in the disease, suggesting that calcium overload may be a primary molecular cause of disease progression. Verapamil is the preferred drug for treating ischemia-induced cardiomyopathy in LGMD 2E patients, as it has been shown to temporarily mitigate and restore cardiac morphology.

γ-Sarcoglycan:

Gamma-sarcoglycan (γ-SGC) is composed of two subunits that protrude extracellularly. Within γ-SGC are embedded tyrosine residues, the phosphorylation of which facilitates bidirectional signal transduction via the γ-SGC-integrins-extracellular matrix pathway. This protein is exclusively expressed in both skeletal and cardiac muscles. Frameshift mutations in γ-SGC can chemically disrupt the phosphorylation of subunits, leading to a more severe form of muscular dystrophy and cardiomyopathy. The expression of γ-SGC is directly related to the expression of α-SGC and β-SGC, but it is independent of δ-SGC. Therefore, the overall function of γ-SGC is to appropriately assemble, localize, and function the Sarcoglycan complex (SGC), which is directly influenced by the stoichiometric ratios of the tetrameric units.

δ-Sarcoglycan:

Delta-sarcoglycan (δ-SGC) primarily functions as a linker protein between α-SGC and β-SGC, strengthening their bond with other components of the dystrophin-glycoprotein complex (DGC). Alongside filamin 1, δ-SGC plays a crucial role in actin polymerization and the signaling cascade essential for cell growth, differentiation, migration, and survival. In smooth muscles, δ-SGC regulates vascular function and membrane integrity. Studies involving δ-SGC deficiency in smooth muscles have demonstrated severe cardiomyopathy, along with other clinical features of limb-girdle muscular dystrophy (LGMD).

Possible pathogenic mechanisms for sarcoglycanopathies in general:

- Deficiencies resulting in the defective assembly and localization of the Sarcoglycan complex (SGC) lead to a decrease or complete absence of the SGC at the sarcolemma. This destabilizes the muscle membrane, making it more prone to damage during contractions.
- It is important to note that in sarcoglycanopathies, the loss of one SGC subunit results in the reduction or complete loss of the other three subunits of the transmembrane tetrameric structure. This pattern resembles more closely a muscle dystrophy caused by dystrophin deficiency.[9]

4. GMPPB gene :

GMPPB is present in two isoforms, one shorter with 8 exons and one longer with 9 exons. The longer isoform, which is highly expressed in skeletal muscles and the brain, is currently a focus of researchers. GMPPB is an enzymatic intracellular protein that plays a role in the formation of GDP-mannose. This molecule, in turn, O-mannosylates alpha dystroglycan, enabling it to bind to extracellular linker proteins for anchoring. Muscular dystrophies related to GMPPB mutations account for approximately 5% of the general population. Among these cases, 2-3% are congenital muscular dystrophies and 5-7% present with a limb-girdle muscular dystrophy (LGMD) 2R phenotype.

2 possible pathogenic mechanisms acts as trigger for underlying presenting symptoms:

- GMPPB mutations result in defective O-mannosylation of alpha dystroglycan, disrupting its linkage with extracellular proteins.
- In adult-onset LGMD 2R, GMPPB is thought to be involved in the recruitment, pentameric assembly, and organization of acetylcholine receptors (AchR) at the post-junctional muscle membrane in the neuromuscular junction (NMJ). Therefore, GMPPB mutations lead to a reduced number of AchRs organized, resulting in the failure of impulse transmission at the NMJ.
- LGMD 2R, similar to other limb-girdle muscular dystrophies (LGMDs), is characterized by proximal muscle weakness, more pronounced in the lower limbs than in the upper limbs. Patients commonly report difficulties such as getting up from a sitting position, running, climbing stairs, frequent falls, and difficulties in keeping pace with their same-age peers during childhood. A notable feature specific to LGMD 2R is the involvement of axial muscles, leading to hyperlordosis in the early 20s. Due to the underlying pathology involving AchR cluster arrangement at the NMJ, treatment with pyridostigmine (an acetylcholinesterase inhibitor) and 3,4-diaminopyridine is believed to relieve weakness, improve daily activities, and delay progression by about 3-4 years.[10]

5. UTRN:

Utrophin is known as homologue of dystrophin protein since one third of the size resembles similar nucleotide sequence as that of dystrophin. It acts in conjunction with dystrophin proteoglycan complex in cytoskeleton stabilization and signal transduction. Utrophin is highly expressed in both skeletal muscles (at NMJ and MTJ- musculo tendinous junction) and cardiac muscles. Utrophin in combination with Ca/calmodulin cpmplex helps in anchoring with non-muscle F-actin with ECM. It acts as an initiating segment in agrin induced AchR clustering and stabilising at NMJ. Animal model studies have shown that uthrophin rises in expression within skeletal muscles between 11th – 17th weeks of gestation, then gradually declines till term. Henceforth, maximal presenting cases will show peak symptoms at initial months of first decade with hastening progression towards adulthood. Since utrophin resembles 80% of DMD gene and being highly pronounced at intercalated discs of cardiac muscles, LGMD patients suffering from this protein mutation are always associated with cardiac morbidities. Song Y et al. in their knockout model mice experimental studies have shown that synthetic transgene encoding a miniaturized utrophin delivered via adeno vector delayed progression till adulthood.

Possible pathogenic mechanism underlying the presenting symptoms:

- The failure of acetylcholine receptor (AchR) clustering at the post-synaptic membrane, along with destabilized AchRs at the neuromuscular junction (NMJ), leads to reduced transmission impulse and muscle contraction initiation.
- Defective anchoring of the dystrophin-glycoprotein complex (DPC) and actin in skeletal muscles results in a failed signal transduction mechanism.
- Failure to generate force at the myotendinous junction (MTJ) of the muscle leads to defective contractions, reflexes, and tone of skeletal muscle.
- Decreased action potential spread due to defective intercalated discs of cardiac membranes leads to gradual cardiac pathology, such as cardiomyopathy, heart failure, decreased ejection fraction, etc.[11,12]

6. PSAP: PROSAPOSIN:

PSAP exists in two isoforms, both intracellularly and extracellularly. Upon action by N-glycosidase F, it releases saposins A, B, C, and D, which are equally pronounced in extracellular and intracellular environments. PSAP is expressed in skeletal muscle, heart, and platelets. While its role as a neurotrophic factor and in the differentiation of cholinergic neuron cell lines is established, its role in skeletal muscle expression is not well understood. Based on current knowledge, *possible pathogenic mechanisms of PSAP mutations could include*:

- Failure to fulfill its neurotrophic role at the neuronal level due to protein mutation.
- Defective release of presynaptic neurotransmitters at cholinergic neural cell lines, leading to neurotransmission failure.

Further research focusing on PSAP as a major protein in skeletal muscle expression could elucidate its basic physiological mechanisms and provide insights into its molecular pathogenic features, which may mimic limb-girdle muscular dystrophy (LGMD) features when mutated or deficient.[13,14]

7. TNPO3: transportin 3:

Transportin 3 (TNPO3) is a member of the beta superfamily of nuclear transport proteins. It plays a crucial role in transporting serine/arginine-rich proteins such as SRSF1, SRSF2, RBM4, and CPSF6 from the cytoplasm into the nucleus via vaults. These proteins, in turn, regulate RNA metabolism, translation, splicing, and the synthesis of new proteins essential for skeletal muscle function. Mutations in the TNPO3 gene cause LGMD D2. The clinical phenotype of LGMD D2 is characterized by severe weakness, initially affecting the pelvic girdle muscles and then progressing to involve the shoulder girdle muscles. In severe cases, distal muscle weakness, winging of the scapulae, scoliosis, and occasional facial weakness may also be present. However, there is no observed cognitive impairment or cardiac involvement. Respiratory involvement has only been reported in cases of juvenile-onset severe phenotype.

Possible pathogenic mechanisms of LGMD D2 include:

- Mutation in the TNPO3 gene leading to defective transport of serine/arginine-rich proteins, resulting in impaired RNA splicing and reduced production of proteins necessary for muscle fiber stability.
- Defective nuclear membrane contributing to altered muscle fiber morphology. LGMD D2 patients often exhibit muscle fiber atrophy and increased autophagic activity due to abnormal fibrillary protein arrangement.
- These changes are reflected in histochemical findings during muscle biopsies. Missense mutations have been shown to result in more severe forms of the disease compared to nonsense and frameshift mutations.[15,16,17,18]

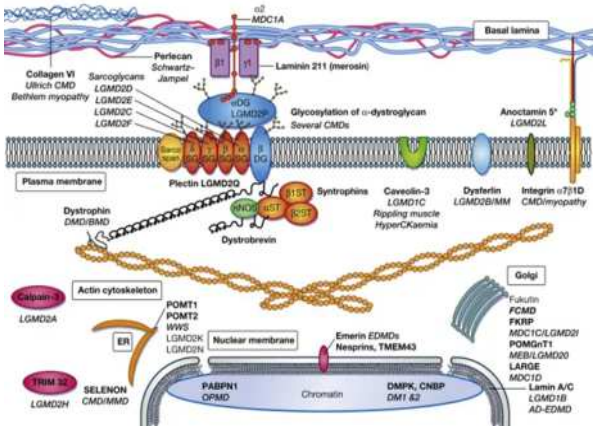

Image courtesy [45]

8. FKRP gene:

FKRP is responsible for glycosylating O-mannosylation of alpha dystroglycan by adding Ribitol-5-Phosphate, using CDP ribitol as a substrate. This process increases laminin binding GlcA-Xyl dimers. Glycosylation of alpha dystroglycan at the dimer level is crucial for stabilizing the sarcolemma and anchoring it to the extracellular matrix. Mutations in the FKRP gene lead to LGMD R, with approximately 150 mutant variants reported to date, among which LGMD R9 is more frequent.

Possible pathogenic mechanisms of FKRP mutations include:

- Hypoglycosylation of alpha dystroglycan due to FKRP mutation, resulting in an unstable sarcolemmal membrane that is more prone to degeneration with age.
- It is noteworthy that a significant number of mothers of diagnosed patients have reported a history of decreased fetal movements during the third trimester of pregnancy, as well as poor sucking postnatally. However, the accuracy of this history in contributing to the diagnostic pathway for doctors without any family history of the disease is questionable. Further research could focus on antenatal signs that may serve as warning signs for LGMDs.[19,20,21,22,23]

9. POMT1 gene:

POMT1 is a protein that, when combined with its counterpart POMT2 within the endoplasmic reticulum, functions as an enzyme. This enzyme adds a sugar molecule to alpha dystroglycan (DG), contributing to the stabilization of the sarcolemma of skeletal muscle fibers and protecting them from damage induced by stretching. Glycosylation is a crucial and ongoing biochemical process necessary to maintain alpha DG function at its optimal level in the sarcolemma. Mutations in POMT1 are more frequently reported than mutations in POMT2, leading to LGMD 2K. The severity of symptoms, unlike with other genes, depends on the phenotype of POMT and the location of the mutation on the gene.

Possible pathogenic mechanisms include:

- Defective enzymatic glycosylation of alpha DG, rendering the sarcolemmal membrane more susceptible to damage from muscle contractions.
- Improper anchoring of alpha DG with the extracellular matrix (ECM) and cytoskeleton.[24,25,26]

10. COL6A1, COL6A2, COL6A3 gene:

Type VI collagen is located in ECM and skeletal muscle cells such that it makes up the intricate lattice meshwork to provide flexibility and stability to the muscle fibres. Type VI collagen is made up of 3 different chain components. Components of each chain are provided by collagen type VI alpha 1 chain, collagen type VI alpha 2 chain and collagen type VI alpha 3 chain. These chains firmly bind together to form mature type VI collagen. Its primary function is to link the basement membrane of ECM and skeletal muscle cells and functions as basal structural framework material. Mutation of COL6A2 gene is more when compared to other counterparts as per current records available. These mutations may either present as autosomal recessive LGMDR 22 or dominant in the form of LGMD D5 (According to new ENMC classification of LGMD). Based on the phenotypic variant disease oscillates from severe congenital muscular dystrophy to milder adult onset LGMD. Unique features of these LGMD patients are-wasting of muscles is more prominent clinical feature clinically, proximal muscle weakness and joint contractures are seen more often as disease progresses with age.

Possible pathogenic mechanism:

- Defective type VI collagen leads to formation of purposeless ECM and basement membrane, which further decreases the ability of the skeletal muscle to withstand shear stress during contractions.
- Since type VI collagen forms most of the skeletal architecture of the body, its concentration and turnover is directly proportional to the age related developmental changes occurring in body. Henceforth, progression of the disease as age advances may be contributed to widespread distribution of type VI collagen in human body.[27]

11. ANO5 gene:

The ANO5 gene, a member of the anoctamin family, encodes the Anoctamin-5 protein. ANO5 plays a crucial role in the formation and maintenance of calcium-sensitive chloride channels in the muscle cell membrane. It is highly expressed in both cardiac and skeletal muscles. Chloride ions are essential for maintaining the dynamic membrane potential. ANO5 is stored in vesicles intracellularly and within the sarcoplasmic reticulum. Also known as 16E (TMEM16E), ANO5 is involved in membrane repair, utilization, and recycling of membrane phospholipids. Mutations in ANO5 lead to LGMD R12, with a prevalence of 0.27 per 100,000 population.

One distinguishing characteristic of LGMD R12 is that the disease is often diagnosed either through elevated creatine kinase levels in routine investigations or when patients report frequent exercise-

induced myalgia. Atrophy and varying creatine kinase levels help differentiate between LGMD R12 and LGMD R2. The latter has an earlier onset, while LGMD R12 typically presents in the fourth to fifth decades. Patients with LGMD R12 become ambulatory but progress very slowly.

Possible pathogenic mechanisms include:

- Intracellular and serum calcium homeostasis, regulated by calcium-sensitive chloride channels, likely plays a role in the expression of the ANO5 protein based on the age at presentation and the exacerbation of symptoms after exercise-induced myalgia.
- Mutated ANO5 may disrupt the balance between sarcolemmal repair mechanisms and phospholipid scrambling in the muscle membrane, leading to a vicious cycle of fiber regeneration and degeneration.[28,29,30]

12. DNAJB6:

DNAJ6 belongs to the evolutionary HSP40 family of proteins. It is found in two isoforms, a larger 'a' isoform and a smaller 'b' isoform. Both isoforms contain an N-terminal domain, a G/F domain with glycine and phenylalanine residues, and a C-terminal domain with serine-rich residues. The N-terminal domain is thought to be involved in detecting abnormally folded proteins, regulating chaperone activity via ATPase activity. LGMD D1 is caused by a mutation in isoform 'b' of DNAJ6, and it is inherited in a dominant manner. Patients typically exhibit more distal muscle weakness initially than proximal muscle weakness.

Possible pathogenic mechanisms include:

- Case reports, including investigations into ATPase activity, suggest that mutations in DNAJ6 lead to a failure in regulating muscle protein folding.[31,32]

13. TCAP gene [titin-cap gene] :

Limb-girdle muscular dystrophy 2G (LGMD2G) is a rare genetic muscle disorder caused by mutations in the telethonin (TCAP) gene, located on chromosome 17q11-12. Initially documented in Brazilian patients, this condition has more recently been observed in Chinese, Moldavian, and Portuguese populations. Telethonin, a specialized 19-kDa sarcomeric protein, is exclusively expressed in mature skeletal and cardiac muscle tissues, where it resides in the Z-disc structure. Functionally, telethonin acts as a substrate for the serine kinase domain of titin, a key protein involved in muscle elasticity. Titin phosphorylates the C-terminal domain of telethonin during early myocyte development. The TCAP gene, consisting of two exons and encoding 167 amino acids, is critical for proper muscle function.

Deficiencies in telethonin not only lead to LGMD type 2G but also contribute to certain forms of hypertrophic and dilated cardiomyopathies, conditions affecting the heart muscle. Molecular studies have shown that pathogenic variants disrupting telethonin function can result in cardiac complications and severe myocyte enlargement (hypertrophy). Of particular interest are TCAP mutations associated with hypertrophic cardiomyopathy (HCM), which enhance binding to other cardiac proteins like titin/connectin and calsarcin-1. These mutations augment the interaction of TCAP with titin and calsarcin-1 within the Z-disc, potentially increasing passive tension and calcium sensitivity during muscle contraction.

Telethonin plays a crucial role in sarcomeric assembly, facilitating the linking of the two antiparallel titin domains (Z1-Z2) through beta strand crossing. This interaction represents one of the strongest observed protein-protein interactions in muscle biology. Additionally, telethonin interacts with various other Z-disc proteins involved in sarcomeric assembly, including LIM protein (MLP), Ankrd2, myostatin, potassium channel B-subunit minK, protein kinase D, murine double minute2 (MDM2), and calsarcin.

LGMD2G typically presents in the second decade of life with prominent weakness in the pelvic girdle muscles and associated distal weakness leading to foot drop. Approximately 50% of individuals with LGMD2G develop calf muscle hypertrophy, and about 40% lose independent ambulation during their third or fourth decade of life. Telethonin is one of the most abundant nuclear mRNAs transcribed in skeletal muscle, underscoring its crucial role in muscle function and highlighting the significance of TCAP in sarcomeric organization.[33,34,35]

14. BETA-DYSTROGLYCAN:

β-Dystroglycan, a 43-kDa transmembrane glycoprotein associated with dystrophin, plays a crucial role in linking dystrophin to the laminin-binding α-dystroglycan. The α-/β-dystroglycan complex is encoded by a single gene located on chromosome 3p21 and is expressed widely in both muscle and nonmuscle tissues.The dystroglycan complex comprises α- and β-dystroglycan, which are initially synthesized as a single polypeptide from a highly conserved gene. This polypeptide undergoes posttranslational proteolytic cleavage to form the two closely associated subunits. These transmembrane proteins interact with laminin-2 in the extracellular matrix and bind to dystrophin on the cytoplasmic side of the cell membrane.

Dystroglycan, with a molecular weight of 156 kDa, binds to β-dystroglycan, sarcoglycans, and the α2 chain of laminin-2 or merosin. The dystroglycan complex is crucial for maintaining the structural integrity of muscle fibers. β-dystroglycan, as a transmembrane protein, binds to the C-terminal region of dystrophin inside the cell and interacts with α-dystroglycan outside the cell, forming a key part of the dystroglycan complex.[36]

15. DAG COMPLEX: Dystroglycan complex

The dystroglycan complex comprises two main components, α-dystroglycan and β-dystroglycan, which are derived from a single gene but differ significantly in size and characteristics. α-Dystroglycan, weighing 156 kDa, is an extracellular protein that noncovalently binds to β-dystroglycan within the dystroglycan complex. It also interacts with laminin and/or agrin in the extracellular matrix. On the other hand, β-dystroglycan is a 43 kDa glycoprotein that serves as an integral membrane protein. The dystroglycan complex is a large structure that includes several other proteins, such as the sarcoglycans (α-, β-, δ-, and γ-sarcoglycans), α-dystrobrevin, neuronal NO synthase (nNOS), and the syntrophins (α, β1, and β2). These proteins form a complex that plays a critical role in binding to dystrophin or utrophin, thereby contributing to the structural integrity of muscle fibers.

The dystroglycan complex, located on the intracellular side, interacts with the proteins dystrophin or utrophin. These proteins, in turn, bind to the actin cytoskeleton within the muscle cell. Utrophin, which is shorter than dystrophin, is concentrated at the neuromuscular junction. Throughout the skeletal muscle cell, the dystroglycan complex is present, with higher concentrations at the neuromuscular junction and the myotendinous junction. In patients with Duchenne muscular dystrophy, there is a significant reduction in the dystroglycan complex, leading to muscle fiber breakdown and degeneration. The dystroglycan complex plays a crucial role in connecting the intracellular actin cytoskeleton to the extracellular matrix. This connection is vital for maintaining the structural integrity of the muscle cell membrane, protecting it from damage caused by muscle contractions.

At the neuromuscular junction, the dystroglycan complex may also be involved in cell-to-cell signaling. The binding of agrin to dystroglycan could be a key factor in regulating the formation and maintenance of the postsynaptic apparatus in muscle. However, the exact role of the agrin/dystroglycan complex interaction in postsynaptic signaling is not yet fully understood.

CONCLUSION:

Once the detailed gene role in its physiological as well as pathological form is well known, this will make the pharmaceutical as well as genetic researchers on what forum should they concentrate to mimic or replace the lost or mutated gene. This review acts like a physiological reference for the knowledge of underlying pathognomic features of typical signs and symptoms of paticular type of LGMD. As mentioned in above review the basic morphopathological features genes triggering to the onset of signs and symptoms at particular age of onset might also throw light on the precautionary drug administrations as well as therapeutic interventions which can delay the morbidity.

Conflict Of Interest:

Authors show no conflict of interest.

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