근이양증의 분자적 병리학적 진단

Molecular and Pathological Diagnosis of Muscular Dystrophies

최영철
연세대학교 의과대학 신경과교실

Young-Chul Choi, M.D., Ph.D.
Department of Neurology, Yonsei University College of Medicine, Seoul, Korea

주소: 135-720 서울시 강남구 146-92
연세대학교 의과대학 영동세브란스병원 신경과
Tel: +82-2-2019-3323
Fax: +82-2-3462-5904
E-mail:ycchoi@yumc.yumc.ac.kr

Abstract

The muscular dystrophies are a diverse group of inherited muscle disorders characterized by progressive muscle weakness and wasting with characteristic histologic abnormalities such as degeneration, necrosis, and regeneration of muscle fibers. With progress in molecular genetics methods, new discoveries of dystrophin and related molecules have dramatically changed the understanding and diagnosis of a large group of muscular dystrophy patients. Dystrophin and its related molecular associates are tightly associated and form an essential cytoskeletal system (dystrophin-glycoprotein complex) at the muscle fiber surface membrane, which is critical for maintaining the integrity of the sarcolemma and muscle fibers. Deficiency of one of these sarcolemmal proteins, including dystrophin, dystroglycans, sarcoglycans, and laminin-2, leads to the breakdown and instability of muscle fibers and to clinically observed progressive muscle weakness. Identification of the molecular cause of muscular dystrophies would allow a genetic oriented classification and diagnosis using DNA or protein analysis. However, definition of the molecular pathogenesis of muscular dystrophies has not been completely possible until now. Future advances in this field should allow the exact diagnosis and treatment of muscular dystrophies.

Key words: muscular dystrophy, dystrophin-glycoprotein complex (DGC), limb-girdle muscular dystrophy (LGMD), facioscapulohemeral dystrophy, myotonic dystrophy

Introduction

Muscular dystrophies are a diverse group of inherited muscle disorders characterized by progressive muscle weakness and wasting with characteristic histologic abnormalities such as degeneration, necrosis, and regeneration of muscle fibers. Erb first introduced the term “muscular dystrophy” in 1891.[1] Hoverer, these disorders have a wide range of clinical manifestation, from a severe childhood-onset form that leads to early death, to more benign adult forms that do not affect life span and may cause only minimal disability. Until recently, it has only been possible to classify these disorders on the basis of their clinical presentation - for example, limb-girdle muscular dystrophy (LGMD), facioscapulohumeral muscular dystrophy (FSHD), Duchenne/Becker muscular dystrophy (DMD/BMD), congenital muscular dystrophy (CMD), distal myopathy, and myotonic dystrophy (DM). With the application of advanced molecular genetic techniques beginning in the 1980s, the dystrophin gene was finally identified in 1985, and its protein product (dystrophin) in 1987.[2-4] Since then, using a variety of increasingly sophisticated techniques, the genes and

gene products for many other muscle disorders have been also identified.[5]

Identification of the molecular pathogenesis of muscular dystrophies now allows for a new classification and understanding based on a genetic or pathologic process, as determined by DNA or protein analysis. Table 1 presents a classification for which the genetic cause has been identified, but not fully defined. With the discovery of dystrophin deficiency as the cause of both DMD and BMD, these forms have been classified as "dystrophinopathy", which replaced the traditional term "Duchenne/Becker muscular dystrophy".

Table 1. Classification of the Muscular Dystrophies

Disease	Inheritance	Gene Mutation	Protein
X-linked dystrophies			
Duchenne/Becker	XR	Xp21	Dystrophin
Emery-Dreifuss	XR	Xq28	Emerin
Limb-girdle muscular dystrophies(LGMD)			
LGMD 1A	AD	5q31	Myotilin
LGMD 1B	AD	1q11-1	LaminA/C
LGMD 1C	AD	3p25	Calveolin-3
LGMD 1D	AD	6q23	Calveolin-3
LGMD 1E	AD	7q	?
LGMD1F	AD	7q	Filamin
LGMD 2A	AR	15q15.1	Calapin-3
LGMD 2B	AR	2p13	Dysferin
LGMD 2C	AR	13q12	γ-sarcoglycan
LGMD 2D	AR	17q12	α-sarcoglycan
LGMD 2E	AR	4q12	β-sarcoglycan
LGMD 2F	AR	5q33	δ-sarcoglycan
LGMD 2G	AR	17q12	telethonin
LGMD 2H	AR	9p31	E3-ubiquitine ligase
LGMD 2I	AR	19q31	FKRP
LGMD 2J	AR	2q31	titin
Congenital muscular dystrophies(CMD)			
(With CNS involvement)			
Fukuyama CMD	AR	9q31-33	Fukutin
Walker-Warburg symdrome	AR	9q34	POMT1
Muscle-Eye-Brain CMD	AR	1 p3	POMGNT1
(Without CNS involvement)			
Merosin-deficient classic type	AR	6q2	Laminin-α2(merosin)
Integrin-deficient CMD	AR	12q13	Integrin-α7
Distal muscular dystrophies			
Late Adult Onset 1A(Wellander)	AD	2p13	?
Late Adult Onset 1B(Markesbery)	AD	2p	?
Early Adult Onset 1A(Nonaka)	AR	9p1-q1	GNE
Early Adult Onset 1B(Miyoshi)	AR	2q12-14	Dysferin
Early Adult Onset 1C(Laing)	AD	14q	?
Other dystrophies			
Facioscapulohumeral	AD	4q35	?
Oculopharyngeal	AD	14q11	Poly(A) binding protein2
Myotonic dystrophy type1	AD	19	DMPK gene
Myotonic dystrophy type 2	AD	3q21	ZNF9

Other protein deficiencies have also been identified in LGMD patients such as sarcoglycans, dysferlin and calpain, which are classified as "sarcoglycanopathy," "dysferlinopathy" and "calpainopathy". Despite recent advances in molecular genetics, it is difficult to assign an accurate and clinically useful classification to the various muscular dystrophies.

The Dystrophin-Glycoprotein Complex

The discovery of dystrophin and related molecules has been crucial to the understanding and diagnosis of a large group of muscular dystrophies.[6, 7] A schematic figure of the dystrophin-glycoprotein complex (DGC) is shown in Fig. 1.

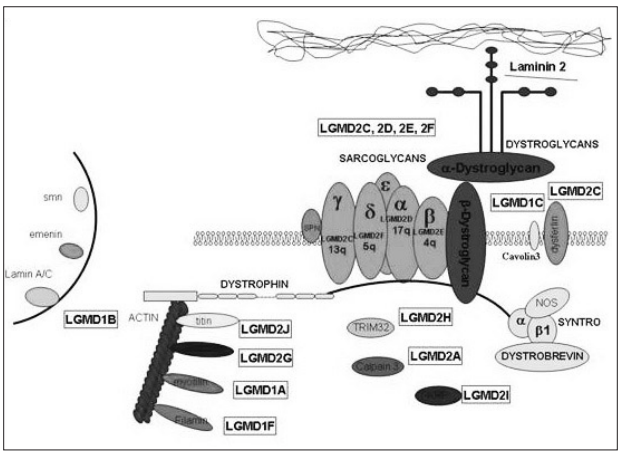

Figure 1. Dystrophin-glycogrotein complex

The proteins initially were referred to as dystrophin-associated proteins (DAPs) or dystrophin-associated glycoproteins (DAGs). The DGC include several proteins on the cytoplasmic side (e.g. dystrophin, syntrophins, and dystrobrevin) and two transmembrane glycoprotein sub-complexes (dystroglycans and sarcoglycans) (Table 2). Dystrophin is a large rod-shaped cytoskeletal protein that binds F-actin.[4] The dystroglycan complex is composed of α- and β-dystroglycan (156 and 43 kDa) derived by the proteolytic processing of a single precursor protein.[8] β-dystroglycan binds at the cysteine-rich region of dystrophin and is linked to α-dystroglycan on the external side of the sarcolemma. Outside the muscle fiber, α-dystroglycan is highly glycosylated and binds to the α_2-laminin subunit of laminin-2 (merosin). Syntrophins are intracellular proteins that directly bind to the C-terminus of dystrophin.[9] The sarcospan is a four transmembrane domain protein.[10] The sarcoglycan complex is composed of four (α, β, γ, and δ) proteins with a single transmembrane domain, a relatively small intracellular domain, and a large extracellular domain. The recently identified ε-sarcoglycan is expressed ubiquitously in many tissues, and may have a different function.[11] Only α-sarcoglycan has an extracellular N-terminus. F-actin and laminin-2 interact with the DGC, providing important continuity from the intracellular cytoskeleton to the extracellular matrix of the muscle fiber, which yields membrane stability. Dystrophin and its molecular associates are tightly associated and form an essential cytoskeletal system at the muscle fiber surface membrane.[6] The complex is critical for maintaining the integrity of the sarcolemma and muscle fiber.[12] The functions of dystrophin and its associated complex are not fully understood. In skeletal muscle, the most probable role of the complex is mechanical reinforcement of the sarcolemma and signal transduction.[13, 14] The DCG tightly connects intracellular cytoskeletal F-actin to the extracellular matrix, thus forming the following linkage: F-action-Dystrophin-Dystroglycan & Sarcoglycan complex-Lamin2-Extracellar Matrix, which serves as a "bolt" or "nut" for maintaining the integrity of the sarcolemma. Muscle fibers containing a mutation of the dystrophin gene, dystrophin-deficiency, or dystrophin-loss are vulnerable to injury during contraction and relaxation due to a loss of cytoskeletal integrity, which leads to fiber necrosis, regeneration, and clinically observed progressive muscular weakness. Not only in dystrophinopathy, but also in LGMD (sarcoglycanopathy) and CMD, the result of DCG breakdown is the membrane instability of muscle fibers and the manifestation of muscle weakness (Fig. 2).

However, many other myopathies were caused by not only sarcolemma protein loss, but also by loss of sarcomeric proteins, nuclear associated proteins, enzymes, or other mechanisms (Table 3).

Table 2. The Dystrophin-glycoprotein Complex (DGC)

I. Glycoprotein complex
A) dystroglycan complex
α-dystroglycan (156DAG): a large extra-cellular laminin-binding glycoprotein
β-dystroglycan (43DAG): a transmembrane glycoprotein
B) sarcoglycan complex (SGC)
α-sarcoglycan (50DAG, adhalin): a transmembrane glycoprotein
β-sarcoglycan (43DAG): a transmembrane glycoprotein
γ-sarcoglycan (35DAG): a transmembrane glycoprotein
δ-sarcoglycan (35DAG): a transmembrane glycoprotein
ϵ-sarcoglycan: : a transmembrane glycoprotein
II. Cytoplasmic complex
syntrophin-complex
α1-syntrophin (59DAP), an acidic intra-cellular fairly muscle-specific protein
β1-syntrophin (59DAP), a basic ubiquitously expressed protein
β2-syntrophin (59DAP), a basic protein localized at the NMJ
dystrobrevin : α-dystrobrevin, β-dystrobrevin
III. Others
dystrophin
sarcospan: (25DAP): a transmembrane protein
unclassified: a large F-actin binding intra-cellular protein

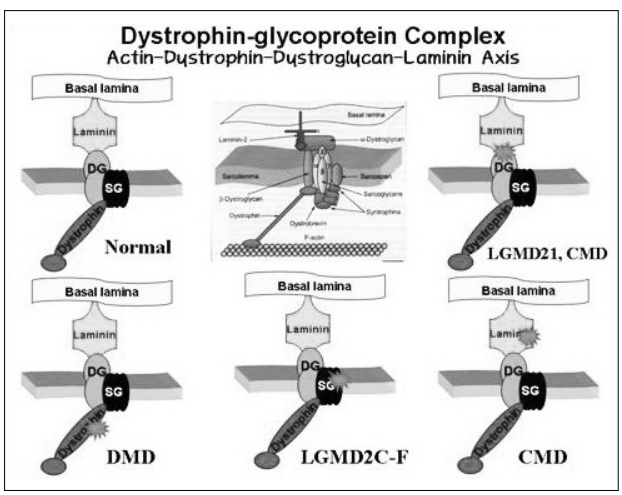

Figure 2. A schematic of the dystrphin-gylcoprtein complex (DGC). (DMD; Duchenne muscular dystrophy, LGMD; Limb-girdle muscular dystrophy, CMD; congenital muscular dystrophy)

The Dystrophinopathy: Duchenn and Becker Muscular Dystrophy

DMD and BMD are X-linked allelic myopathies caused by dystrophin deficiency, characterized by progressive muscular weakness and degeneration of skeletal muscle. DMD is the most common X-linked recessive lethal myopathy with an incidence of 1 in 3500 newborns, whereas BMD has a frequency of about 5 per 100,000, for a combined incidence of 1 in 3,000. It has been estimated that approximately one third of the cases result from new mutations.[15,16] Clinical symptoms of the DMD are observed after 2-3 years of age. Most affected boys exhibit retarded motor development, with approximately half of them failing to walk until the age of 18 months. Other early onset characteristics include an unusual waddling gait, difficulties with running and jumping, lumbar lordosis, and calf enlargement.[15,16] Weakness and wasting of muscle are progressive and symmetrical, affecting the lower limbs before the upper limbs, and the proximal muscles before the distal muscles. Joint contractures are an important clinical manifestation, and by the age of 6 years most patients have contractures at the iliotibial bands, hip joints, and heel cords. Regenerating fibers become less abundant as the disease progresses and are eventually replaced by adipose and connective tissues, accounting for the pseudohypertrophic muscles. The affected children are usually wheelchair-bound by 12 years of age. As the disease progresses, the contractures develop further, leading to asymmetrical spinal deformities. Most

Table 3. Muscular dystrophy classification with molecular defect

Muscular Dystrophy Classification with Molecular Defect	
Proteins of molecular defects	Nomenclature
Sarcolemma associated	Dystrophinopathy (DMD/BMD)
Dystorphin	LGMD2C, 2D, 2E, 2F
Sarcoglycans ($\alpha, \beta, \gamma, \delta$)	LGMD1C
Caveolin	LGMD2B
Dysferlin	ITGA7
Integrin α7	
Extracellular Matrix	
Laminin 2 chain	Congenital muscular dytrophy (MDC1A)
Collagen 6A1, A2, A3	UCMD/Bethlem Myopathy
Proteins with enzymatic activity	
Calpain 3	LGMD2A
Fukutin	Fukuyama CMD
Fukutin Reated protein	MDC1C/LGMD2I
POMGnT1	MEB
POMT1	WWS
TRIM 32	LGMD2H
GNE	HIBM/DMRV
Saromeric proteins	
Myotilin	LGMD1A
Telethonin	LGMD2G
Titin	LGMD2J
Nuclear associtated proteins	EDMD
Emerin	EDMD1/LGMD1B
Lamin A/C	Oculopharyngeal muscular dystrophy
PAB2	
Others	RSMD1
Selenoprotein N	MD + epidermolysis Bullosa
Plectin	
Repeat expansion disease	Myotonic dystrophy type 1
DMPK-CTG repeat	Myotonic dystrophy type 2
ZNF9-CCTG repeat	
Large telemetnic deletion	Fascioscapulohumeal dystrophy
Deletion of D4Z4 repeat	

patients die at approximately age 20 of pneumonia related to chronic respiratory insufficiency. Cardiac involvement is a consistent part of DMD. As many as 90% of DMD patients show electrocardiogram abnormalities.[17] The heart exhibits fibrosis in the posterobasal portion of the left ventricular wall. Defects in the intra-atrial conduction system are more common than atrioventricular and infranodal disturbances. Despite a known predisposition to cardiac disease, most patients with DMD remain surprisingly free of cardiovascular symptoms. Approximately 20% of affected patients will be mentally handicapped. The impairment of intellectual function appears to be nonprogressive and affects verbal ability more than performance.[18] BMD has a milder clinical course and a slower disease progression.[19] The majority of BMD patients initially experience difficulties between 5 and 15 years of age, although onset in the third or forth decade of age, or even later, can occur. By definition the affected patients remain ambulant until 16 years of age or later, thus allowing the clinical distinction from patients with DMD. Patients with BMD have a reduced life expectancy, but the majority of patients survive into at least the forth or fifth decade. A well-recognized subgroup of patients with an intermediate course between those typical of Duchenne and Becker dystrophies are referred to as intermediate patients or outliers.[20] These patients can usually be recognized by the age of 3 years via the relative preservation of strength in neck flexion (anti-gravity neck flexor muscles), whereas patients with DMD lack this ability throughout their entire life. Intermediate patients retain the ability to climb stairs and walk (after the age of 12, but not beyond 15 years) longer than patients with typical DMD.

The gene for DMD/BMD, dystrophin, was identified by positioning cloning in 1988.[21, 22] The dystrophin gene is one of the largest human genes identified, spanning more than 2000 kb of genomic DNA, and is composed of 79 exons that encode a 14-kb transcript.[23, 24] It is localized at the short arm of the X chromosome (Xp21.1). The dystrophin gene produces several isoforms of dystrophin.[25] The molecular weight of full-length dystrophin is 427 kd. Four distinct domains have been defined: 1) an amino terminus that associates with actin or an actin-like protein, 2) a rod domain consisting of long flexible rows of 24 αhelical repeats, 3) a cysteine-rich region, and 4) a unique carboxy terminus. Dystrophin tightly associates with a large oligomeric complex of sarcolemmal glycoproteins through its cysteine-rich domain and carboxy-terminus, whereas the amino-terminal domain interacts with actin or an actin-like protein.[26, 27] In dystrophinopathies, the absence of dystrophin leads to a secondary reduction in all components of the DGC and disruption of the DGC. Dystro phinopathies cover a wide range of clinical severity (presenting symptom, age of onset and rate of progression) as mentioned above. The simplest explanation for this phenotype variation is mutations of the "reading frame rule" in protein translation. In-frame deletions produce smaller, semi-functional proteins, while frame shifting deletions generally produce a few incorrect amino acids before a premature stop codon is generated. There are exceptions to this reading frame rule. Complete dystrophin deficiency may be associated with a relatively benign phenotype. The amount, quality, and even distribution of dystrophin and the region of exon deletion are functionally important. Mutations in the dystrophin gene are not randomly distributed, but occur at two particular hotspots with about 30% occurring at a proximal hotspot.

The pathological findings in dystrophinopathy are increased variation in fiber size, evidence of fiber necrosis with phagocytosis, hypercontracted eosinophilic hyaline fibers, some centrally nucleated fibers, and increased connective tissue (Fig. 3). Immunostaining of muscle sections with antibodies to dystrophin is essentially negative in DMD patients, whereas in BMD patients staining is decreased or interrupted (Fig. 4).[28] A patch staining, similar to that seen in BMD muscle, is also observed in other muscle disorders as well as in symptomatic carriers of dystrophinopathy (Fig. 4).[29] Western blot analysis for dystrophin is particularly useful in BMD patients

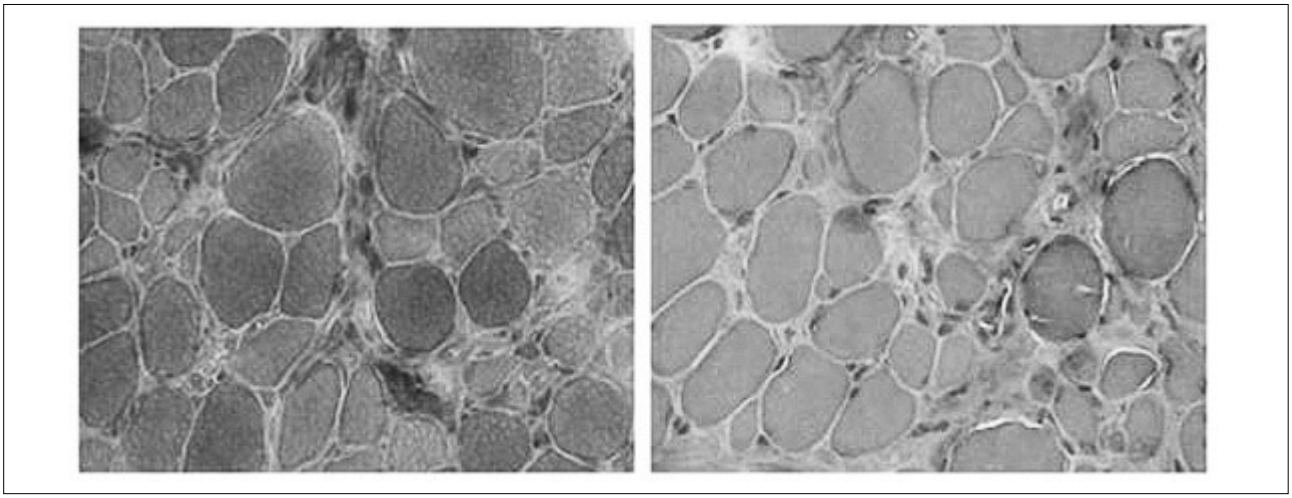

Figure 3. Section from muscle biopsies taken from patients with Dystrophinopathy (Right; Gomoritrichrome staining, left; H&E staining)

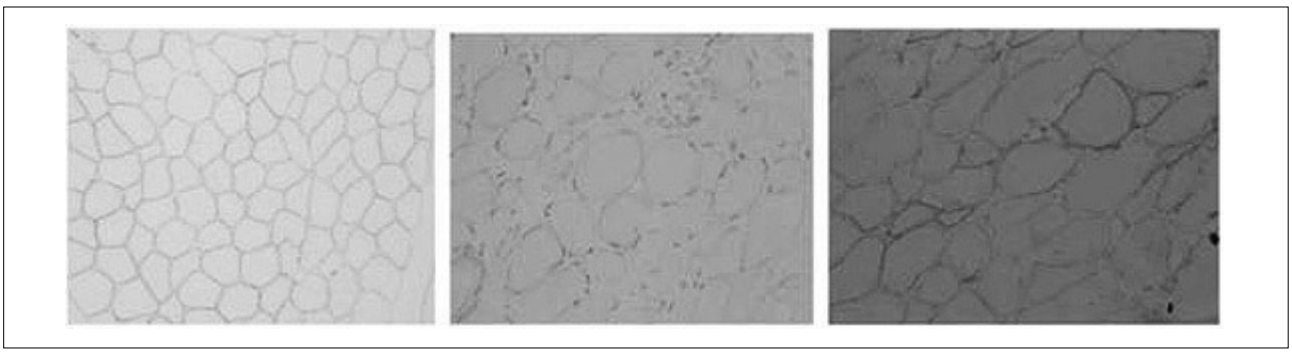

Figure 4. Immunostaining of muscle sections with antidystrophin antibodies.(right; normal, middle; dystrophinopathy, left; manifested carrier in female)

because bands of abnormal size and low amounts are easily detected.

Limb-girdle Muscular Dystrophy (LGMD)

Limb-girdle muscular dystrophies (LGMD) are clinically and genetically heterogeneous muscle disorders, characterized by a progressive weakness and muscle wasting of the pelvic and shoulder-girdle, ranging from severe forms with onset in the first decade and rapid progression to milder forms with later onset and a slower course.[30] The term 'limb-girdle dystrophy' was introduced by Walton and Nattrass in 1954.[31] From the clinical viewpoint, LGMD refers to patients not fitting into one of the defined muscle disorders with typical clinical feature such as DMD/BMD, FSHD, and myotonic dystrophy. The recent advances in understanding the molecular pathogenetic mechanism of LGMD have made possible a genetic classification based on the molecular defect from the clinical classification. By now, more than 16 of LGMD subtypes have been identified. LGMD1 refers to the autosomal mutation dominantly inherited, while LGMD2 consists of disorders with autosomal recessive transmission.

Autosomal dominant LGMD (LGMD1)

Autosomal dominant LGMD (LGMD1) is a heterogeneous group of disorders. LGMD1 is a relatively

small proportion of all LGMD patients, less than 10%. There are to date, at least six autosomal dominant forms of LGMD which have been assigned to chromosomal loci or genes (Table 4).

LGMD1A is called "myotilinopathy" and is assigned to chromosome 5q[31], the gene locus that codes for myotilin.[32] The disease is characterized by onset at the age of 42-77 years with an initial muscle weakness in distal or proximal leg muscles, eventually spreading to other muscle groups of the lower and upper extremities. Associated signs of cardiomyopathy, respiratory failure and peripheral neuropathy are present in a fraction of patients. Myopathological features of focal myofibrillar destruction resulting in intracytoplasmic deposits, strong immunoreactivity to myotilin, multiple rimmed and centrally or subsarcolemmally located non-rimmed vacuoles, and streaming Z-lines were observed in each patient studied.[33]

LGMD1B, due to LMNA gene mutations, is a relatively rare form of LGMD characterized by proximal muscle involvement associated with heart involvement comprised of atrioventricular conduction blocks and dilated cardiomyopathy.[34] Mutations in the lamin A/C gene have been reported in a variety of disorders including autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD) and LGMD1B. AD-ADMD is characterized by early contractures of elbows and Achilles tendons, and a humero-peroneal distribution of weakness combined with cardiomyopathy with conduction defects. LGMD1B and AD-EDMD are allelic disorders.[35]

LGMD1C (caveolinopathy), a third dominant form was identified by mutations in the caveolin-3 gene (CAV3) located at 3p25, encoding caveolin-3, the muscle-specific form of the principal protein compo-

Table 4. Classification of LGMD

Disease	Location	Gene / Product	Pathogenic Mechanism
Autosomal Dominant			
LGMD1A	5q31	MYOT / myotilin	?
LGMD1B	1q11-21	LNMA/C / laminA/C	?
LGMD1C	3p25	CAV3 / Caveolin-3	Abnormal oligomerization of caveolins
LGMD1D	6q23	?	?
LGMD1E	7q	?	?
LGMD1F	7q	FLNC/Filamin	Inability of mutant protein to dimerization
Autosomal Recessive			
LGMD2A	15q15	CAPN3 / Calpain-3	Loss of proteolytic activity
LGMD2B	2p13	FER-1 / dyferlin	Abnormal membrane repair
LGMD2C	13q12	SGCC / γ-saroglycan	Abnormal membrane integrity
LGMD2D	17q21-q21	SGCA / α-saroglycan	Abnormal membrane integrity
LGMD2E	4q12	SGCB / β-saroglycan	Abnormal membrane integrity
LGMD2F	5q33	SGCD / δ-saroglycan	Abnormal membrane integrity
LGMD2G	17q12	TCAP / Telethonin	Abnormal saromeric z-line assembly
LGMD2H	9q31-34	TRIM32	?
LGMD2I	19q13.3	FKRP	Defective glycosylation of γ-dystrolycan
LGMD2J	2q31	Titin	Abnormal saromeric assembly

nent of caveolae in the plasma membrane. This form is the most common in LGMD1, characterized by proximal weakness of predominantly leg muscle associated with cardiac arrhythmia and dilated cardiomyopathy. The age of onset is 4-38 years. Slow progression of weakness and hypertrophic calf muscle are noted. The sarcolemma localization of caveolin-3 is linked to a complex pathway where oligomerization occurs in the Golgi apparatus (a possible dominant negative mechanism). Immunostaining with anti-caveolin-3 was reduced 90-95%. Dystrophin and sarcoglycan staining was only slightly affected.[36]

Autosomal recessive LGMD (LGMD2)

Autosomal recessive LGMS is a heterogeneous group of disorders, which include at least ten different genetic entities (Table 4). The first LGMD2, LGMD2A, was mapped to 15q in 1991.[37] and nine additional forms of AR-LGMD have also been mapped. The protein products of these ten genes have been identified (Table 4). They are: calpain-3 for LGMD2A,[38] dysferlin for LGMD2B,[39,40] α-sarcoglycan (SG) for LGMD2D,[41,42] β-SG for LGMD2E, [43,44] γ-SG for LGMD2C,[45,46] δ-SG for LGMD2F,[47] the sarcomeric protein telethonin for LGMD2G,[48] TRIM32 for LGMD2H,[49] fukutin-related protein (FKRP) for LGMD2I,[50] and titin for LGMD2J.[51]

1) LGMD2A (Calpainopathy)

LGMD2A, calpainopathy, was the first form of limb-girdle dystrophy identified that is caused by a deficiency of a non-structural protein, the enzyme calpain 3. Calpain 3 (p94) is a calcium-dependent protease that may be implicated in the contractile process since it can bind strongly to titin.[52] Demonstration of the involvement of muscle-specific calcium-activated neutral protease 3 in LGMD2A was the first example of an enzymatic rather than a structural protein defect causing a progressive muscular dystrophy. Calpainopathy is the most frequent form of LGMD, accounting for about 30% of the identified cases. Clinically, LGMD2A is characterized by symmetrical and selective proximal atrophy with no cardiac or facial disturbance and normal intelligence. However, the course is highly variable.[53] A wide intra and interfamilial clinical variability ranging from severe to mild forms was reported.[54] The mean age at onset was 13.7 years (ranging from 2 to 40 years), and the mean age at loss of walking ability was 17.3 years (range 5-39 years) after onset with no sex difference between the age at onset or progression. Calf hypertrophy and elevated serum creatine kinase (CK) has been reported. Serum CK levels are higher than normally observed, yet not as elevated as observed in dystrophinopathies or sarcoglycanopathies. The calpain 3 gene in chromosomal region 15q15 is comprised of 24 exons and covers a genomic region of 50 kb. It is expressed as a 3.5 kb transcript, and a 94 kDa translated protein. To date, more than 140 different mutations have been described.[55] Muscle pathology shows marked variations in fiber size with necrotic and regenerating process and interstitial fibrosis. One of the striking findings in the later stages of calpainopathy is an abnormal lobulation of type 1 fibers after staining with NADH-tetrazolin reductase. Immunocytochemical studies show normal sarcolemmal labeling with dystrophin, utrophin and sarcoglycan antibodies. Because of the instability of calpain-3 in muscle, protein immunostaining is not a reliable diagnostic measure for detecting calpainopathy. Direct mutation testing must be performed in order to diagnose calpainopathy through molecular methods. Although not a definitive method, western blot detection calpain-3 protein in muscle is nevertheless an easy and useful method for the primary screening of calpainopathy.[55] The analysis of calpain-3 in other forms of muscular dystrophy revealed no apparent alteration in sarcoglycanopathy or telethoninopathy patients.[56,57] However, a secondary reduction of calpain-3 was reported in LGMD2B patients, suggesting a possible association between calpain-3 and dysferlin.[56,58,59] Subsequently, other studies have shown a secondary reduction of calpain-3 in other forms of LGMD such as LGMD2I and LGMD2J, which requires further

studies.[51,60]

2) LGMD2B (Dysferlinopathy)

LGMD type 2B (LGMD2B) is caused by mutations in the dysferlin gene (DYSF) located on chromosome 2p13.3, which induces a dysfunction of dysferlin at the protein level.[40] Dysferlin is a member of the FER-1 protein family and contains six putative C2 domains, which can bind to phospholipids, inositol polyphosphates, Ca2+, and intracellular proteins.[61] Dysferlin is expressed predominantly in the skeletal muscle and localizes to the plasma membrane of muscle fibers. In addition, it has been suggested to be involved in membrane fusion.[62,63] A recent animal study has suggested that dysferlin plays a role in the sarcolemma repair process.[64] DYSF has been also shown to cause Miyoshi myopathy, which is a rare form of distal myopathy characterized by calf muscle weakness. Both LGMD2B and Miyoshi myopathy are referred to as "dysferlinopathy" because of the deficiency of the same protein.

The clinical spectrum may present with myalgia, high CK, or difficulty walking on tip toes. The disease generally manifests during the early adulthood (average onset is noted between the age of 17-30 years), and the disease generally has a benign course, with confinement to a wheelchair occurring in approximately 10% of patients.[65,53] Despite the slow clinical evolution, an extremely high CK level (10-20 times the normal value) is characteristic of LGMD2B, being indicative of the protein's role in normal muscle homeostasis ("leaky" membrane) rather than being essential for structural stabilization of the skeletal muscle.[66,67] The proportion of LGMD2B is thought to be relatively high in Brazilian and Japanese populations (estimated to be 19-25% of all ARLGMD cases).[65,68] Moreover, the founder mutation of DYSF appears to be present in a population of Libyan Jews.[69] However, another recent study reported a relatively lower frequency of dysferlin deficiency in Caucasian patients with the LGMD phenotype (approx. 1%), indicating a frequency variation according to population ethnicity.[70] Although insufficient to estimate the frequency of LGMD2B in Korean ARLGMD patients due to their small number, the frequency of LGMD2B might be relatively high among ARLGMD patients in Korea.[71] A detailed population-based study will be needed to determine the exact frequency of LGMD2B in Korea. The dysferlin gene, DYSF, is relatively large, comprising 55 exons that span a genomic region of > 150kb.[72] Although direct gene analysis provides the most reliable diagnosis, it is costly, time-consuming, and labor intensive because of the large size of the DYSF. Moreover, defects in the dysferlin gene involve mostly single nucleotide changes with no common mutations, gross rearrangements, or mutational hotspot that could aid detection.[73] For this reason, it would be better to initiate screening for dysferlin deficiency using antibodies against this protein in muscle biopsies. Complete loss of dysferlin without deficiency of other proteins appears to be specific for primary dysferlinopathy as observed by immunocytochemistry.[73]

Muscle biopsy shows non-specific myopathic changes that include marked variation in fiber size, centrally situated nuclei, fiber splitting, scattered necrotic and regenerating fibers, and an occasional perivascular infiltrate comprised of lymphocytes and macrophages. There is increased connective tissue in the endomysium and perimysium. Fiber type distribution is normal, while rimmed vacuoles and ragged red fibers are absent.

The molecular diagnosis of dysferlinopathy by protein analysis is now easily available. The pattern of immunostaining against dysferlin antibody is either completely absent or markedly reduced. It is noteworthy that a recent study has found reduced dysferlin immunoreactivity in about half of sarcoglycanopathies and 20% of dystrophinopathies examined, indicating that diminished dysferlin expression could be due to secondary processes related to muscle cell degeneration.[74]

3) LGMD2C-F (Sarcoglycanopathy)

Mutations in the genes encoding the sarcoglycan proteins (α, β, γ, and δ) (primary sarcogly-

canopathies) have recently been shown to cause some cases of genetically heterogeneous autosomal recessive muscular dystrophies (limb-girdle muscular dystrophy types 2D, 2E, 2C, and 2F, respectively).[75] Sarcoglycans are transmembrane proteins within the DGC, and are of unknown function.[7,75-77] A fifth sarcoglycan (ε-sarcoglycan), has recently been identified through its homology to α-sarcoglycan, and its gene has been mapped to chromosome 7q21.11 It is not yet known to be involved in any muscle disorders.

The pathogenesis of the sarcoglycanopathies is likely very similar to that of dystrophinopathy, as both involve the loss of DGC integrity. Loss of the sarcoglycans leads to membrane instability, with consequent efflux of cytoplasmic components, such as serum kinase out of the myofiber, and an influx of calcium and other material into the cell.[78] As loss or deficiency of any one of the four sarcoglycans causes muscular dystrophy, these proteins must play a critical role in the maintenance of membrane integrity.

Patients with a primary sarcoglycanopathy are clinically indistinguishable from those with primary dystrophinopathies. Sarcoglycanopathies show a wide spectrum of clinical severity. α-sarcoglycanopathy patients typically show missense mutation, which can vary from Duchenne-like to asymptomatic. The other sarcoglycanopathies (β γ and δ) show a higher frequency of deletion mutation, which leads to complete loss of the corresponding proteins and severe phenotype. All patients presented with proximal weakness, elevation of serum creatine kinase, and calf hypertrophy. Cardiac involvement was variable.

The pathology of muscle in sarcoglycanopathies shows a dystrophic myopathy, indistinguishable from DMD/BMD. Light microscopic findings show degeneration and regeneration of muscle fibers, myofiber size variation, increased number of central nuclei, and endomysial fibrosis. Diagnosis was done by protein analysis of a muscle biopsy using immunohistochemistry for the sarcoglycan proteins. Sarcoglycanopathy patients show a dramatic reduction in all four sarcoglycan proteins. Loss of any one of these sarcoglycan proteins leads to the secondary reduction or absence of others. Dystrophin protein can be normal or lead to secondary reduction. Partial reduction of sarcoglycan proteins observed by immunostaining is a less specific diagnosis for primary sarcoglycanopathy. Therefore, multiple western blotting is useful for differential diagnosis in sarcoglycanopathies, DMD, and female carrier DMD.

Fascioscapulohumeral Muscular Dystrophy

Facioscapulohumeral muscular dystrophy (FSHD) has an estimated prevalence of one in 20,000 people, making it the third most common form of muscular dystrophy after Duchenne and myotonic muscular dystrophy.[79] FSHD, an autosomal dominant neuromuscular disease, was first described by Landouzy and Dejerine in 1885. The majority of cases are familial. Sporadic cases resulting from de novo mutations account for 10-30%.[80,81] Characteristics of the disease are the early involvement of facial and scapular muscles with eventual spreading to the pelvic and lower limb muscles, with the foot extensors often affected earlier and more severely than pelvic muscles. Because bulbar, respiratory, and cardiac functions are generally spared, life expectancy is normal. Eventually, 20% of patients become wheelchair-bound.[79] Another characteristic clinical feature is the asymmetry in muscle weakness found prominently in the face and shoulder girdle. Extramuscular manifestations of FSHD include high frequency hearing loss, retinal telangiectasias, and predisposition to atrial arrhythmias.[82] In 1990, linkage to a marker on chromosome 4q 35, p13E-11, was found. Subsequently, affected people were shown to carry a small EcoRI fragment detected by p13E-11 (Fig. 5).[83] Normal individuals have 11 or more repeats on chromosome 4q35, whereas individuals with FSHD have 10 or fewer repeats on one copy of 4q[35.84] The diagnosis of FSHD can be confirmed by DNA restriction fragment analysis in 95% of patients.[85] An EcoRI fragment size below 38 kb is compatible with a diagnosis of FSHD.[86] Although the size of the small fragment correlates inversely with disease severity, the exact rela-

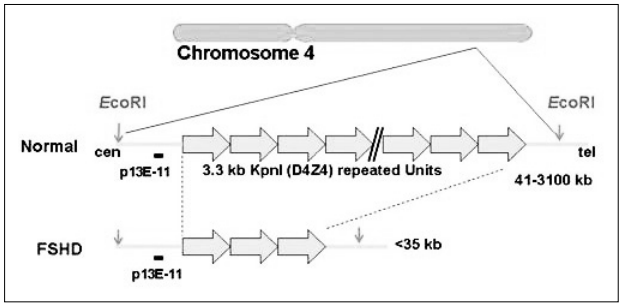

Figure 5. The FSHD region gene. In normal, the D4Z4 (KpnI unit) repeat unit contain more than 11(more than 35 kb). In FSHD, this fragment is reduced to the fewer than 10 repeat, less than 35 kb.

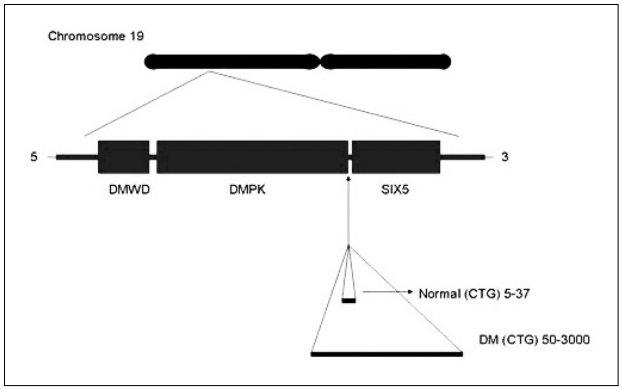

Figure 6. The DM1 locus. the DTG repeat in DM1 is increased more than 50.

tionship of the fragment to the pathogenesis of the clinical disease is unclear. FSHD is currently untreatable. Few therapeutic trials of this disorder have been conducted.

Myotonic Dystrophy

Myotonic dystrophy (dystrophia myotonia, DM) is the most common inherited muscle disorder in adults.[87] DM is an autosomal dominant degenerative disease of the skeletal muscle, heart, eyes, endocrine system, central and peripheral nervous systems, gastrointestinal organs, bone, and skin. The most characteristic feature is muscle weakness and wasting with myotonia that begins in the distal limb and cranial muscle. The prevalence is 5/100,000 and the incidence is about 13.5/100,000 live births. Based on its genetic locus and clinical features, DM is subclassified as myotonic dystrophy 1 (DM1) and myotonic dystrophy 2 (DM2, or PROMM). The genetic basis for DM1 is an expansion of an unstable cytosine-thymine-guanine (CTG) trinucleotide repeat in the 3' untranslated region of the myotonic dystrophy protein kinase (DMPK) gene in chromosome 19 (Fig. 6).[88] Normal DMPK alleles have 4 to 37 CTG repeats, whereas DM1 alleles have 50 to more than 4000 repeats. Longer expansion correlates with earlier symptom onset and a more severe disease. The size of the expanded repeat and the severity of the disease tend to increase in successive generations (anticipation). DM1 is divided into congenital and classical phenotypes according to the age of symptom onset and disease severity. The first symptom is usually grip myotonia that begins in the second, third, or fourth decade. The earliest signs of weakness can be found in the finger flexor, neck flexor, and facial muscle. Muscles of the proximal limb and limb girdle are affected later. The signs at presentation are ptosis, facial weakness, temporalis wasting, and weakness of the neck and distal muscle. The extramuscular features of DM are related to the heart (conduction defect, arrhythmia), smooth muscle (impaired intestinal motility and uterine contractility), lens (cataract), brain (mental retardation, hypersomnolence, and neuropyschiatric manifestation), and endocrine regulation (testicular atrophy, insulin resistance, and abnormal growth hormone release). The heart-related features are progressive fibrosis of the conduction system and abnormal excitation. Cardiac arrest due to heart block or ventricular tachyarrhythmia is a common cause of death. Approximately 10% of patients with congenital DM1 present in infancy with neonatal hypotonia, feeding and respiratory difficulties, and mental retardation survive into childhood. Infants with congenital DM1 have very large repeat expansions (>1000 CTG repeats). In 1994, Thornton et al.[89] described an autosomal dominant disorder similar to DM without CTG repeat expansion at the DMPK. Ricker et al.[90] named this disease “proximal myotonic myopathy” because proximal muscle weakness with-

out atrophy was observed predominantly as opposed to the distal muscle involvement seen in DM1. Because of the close phenotypic resemblance to DM, this disease was called “myotonic dystrophy type 2”. In 1998, Ranum et al.[91] assigned the DM2 locus to chromosome 3q. DM2 is caused by a CCTG expansion (mean approximately 5000 repeats) located in intron one of the zinc finger protein 9 (ZNF9) gene.[92]

The histopathology of DM1 is characterized by atrophy of muscle fibers and abnormality of the myonuclei. Furthermore, type 1 fiber atrophy with type 2 fiber hypertrophy, increased numbers of centrally located nuclei occurring as chains (chained nuclei) within muscle fibers, ringed fibers with sarcoplasmic masses, and pyknotic nuclear clump are observed. DNA testing for the diagnosis of DM has been available since the discovery of the expanded CTG or CCTA repeat as the genetic mutation. PCR based estimation of repeat number or southern blotting is now the standard approach for detecting trinucleotide expansions.[93]

References

1. Erb WH. Dystrophia muscularis progressiva: Klinische und pathologischanantomische Stuien. Dtsch Z Nervenheik 1891; 1: 13.
2. Kunkel LM, Monaco AP, Middlesworth W, Ochs HD, Latt SA. Specific cloning of DNA fragments absent form the DNA of a male patients with an X-chromsome deletion Proc Natl Acad Sci USA 1985; 82; 4778-82.
3. Ray PN, Belfall B, Duff C *et al*. Cloning of the breakpoint of an X;21 translocation associated with Dechenne muscular dystrophy. Nature 1985; 318: 672-5.
4. HoffmanEP, Bengsl PM, Udd B *et al.* Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 1987; 51: 919-28.
5. World Muscle Society. Neruomsucular disorders: gene location. Neuromuscular Disorders 2005; 15: 89-114.
6. Wotton R. Muscular dystrophies.: disease of the dystrophin-glycoprotein complex. Science 1995: 270: 755-6.
7. Ozawa E, Noguchi S, Mizuno Y, Hagiwara Y, Yoshida M. From dystrophinopathy to sarcoglycanopathy: Evolution of a concept of muscular dystrophy. Muscle Nerve 1998; 21: 421-38.
8. Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille C, Slaughter CA, Sernett SW, Campbell KP. Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. Nature 1992; 355: 696-702.
9. Suzuki A, Yoshida M, Hayashi K, Mizuno Y, Hagiwara Y, Ozawa E. Molecular organization at the glycoprotein-complex-binding site of dystrophin: Three dystrophin-associated proteins bind directly to the carboxy-terminal portion of dystrophin. Eur J Biochem 1994; 220: 283-92.
10. Crosbie RH, Heighway J, Venzke DP, Lee JC, Campbell KP. Sarcospan, the 25-kDa transmembrane component of the dystrophin-glycoprotein complex. J Biol Chem 1997; 272: 31221-4.
11. McNally EM, Ly CT, Kunkel LM. Human epsilon-sarcoglycan is highly related to γ-sarcoglycan (adhalin), the limb girdle muscular dystrophy 2D gene. FEBS Lett 1998; 422: 27-32.
12. Campbell KP. Three mudcular dystrophies: loss of cytoskeletal-extracellular matrix linkage. Cell 1995; 80: 675-9.
13. Karpati G. Recent developments in the biology of dystrophin and related molecules. Curr Opin Neurol Neurosurg 1992; 5: 615-21.
14. Bork P, Sudol M. The WW domain: A signalling site in dystrophin? Trends Biochem Sci 1994; 19: 531-3.
15. Moser H. Duchenne muscular dystrophy: pathogenetic aspects and genetic prevention. Hum Genet 1984; 66: 17-40.
16. Emery AE. Muscle histology and creatine kinase levels in the fetus in Duchenne muscular dystrophy. Nature 1977; 266: 472-3.
17. Farah MG, Evans EB, Vignos PJ Jr. Echo cardiographic evaluation of left function in Duchenne’s muscular dystrophy. Am J Med 1980; 69: 248-54.

18. Leibowitz D, Dubowitz V. Intellect and behavior in Duchenne muscular dystrophy. Dev Med Neurol 1981: 23: 577-90.
19. Blythe H, Pugh RJ. Muscular dystrophy in childhood: the genetic aspect: a field study in the Leeds region of clinical types and their inheritance. Ann Hum Genet 1958; 23: 127-63.
20. Brooke MH, Fenichel G, Griggs RC, Mendell JR, Moxley RT. Clinical investigation in Duchenne dystrophy. 2. Determination of the "power" of therapeutic trials based on the natural history. Muscle Nerve 1983; 6: 91-7.
21. Monaco AP, Kunkel LM. Cloning of the Duchenne /Becker muscular dystrophy locus. Adv Hum Genet 1988; 17: 61-98.
22. Worton RG, Burghes AH. Molecular genetics of Duchenne and Becker muscular dystrophy. Int Rev Neurobiol 1988; 29: 1-76.
23. Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 1987; 50: 509-17.
24. Hoffman EP, Brown RH, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 1987; 51: 919-28.
25. Sadoulet-Puccio HM, Kunkel LM. Dystriphin and its isoform. Brain Pathol 1996; 6: 25-35.
26. Campbell KP, Kahl SD. Association of dystrophin and an integral membrane glycoprotein. Nature 1989; 338: 259-62.
27. Yoshida M, Ozawa E. Glycoprotein complex anchoring dystrophin to sarcolemma. J Biochem 1990; 108: 748-52.
28. Hoffman EP, Fischbeck KH, Brown RH *et al.* Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. N Engl J Med 1988; 318: 1363-8.
29. Arahata K, Hoffman EP, Kunkel LM *et al.* Dystrophin diagnosis: Comparison of dystrophin abnormalities by immunofluorescence and immunoblot analyses. Proc Natl Acad Sci USA 1989; 86: 7154-8.
30. Bushby KM. the limb-girdle msuculr dystrophies-multiple gene, multiple mechanism. Hum Mol Genet 1999; 8: 1875-82.
31. Walton JN, Nattrass FJ. On the classification, natural history and treatment of the myopathies. Brain 1954; 77: 169-231.
32. Hauser MA, Horrigan SK, Salmikangeas P *et al.* Myotilin is mutated in limb girdle muscular dystrophy1A. Hum Mol Genet 2000; 9: 2141-7.
33. Olive M, Goldfarb LG, Shatunov A, Fischer D, Ferrer I. Myotilinopathy: refining the clinical and myopathological phenotype Brain. 2005; 128: 2315-26.
34. van der Kooi AJ, Ledderhof TM, de Voogt WG *et al.* A newly recognized autosomal dominant limb girdle muscular dystrophy with cardiac involvement. Ann Neurol 1996; 39: 636-42.
35. Muchir A, Bonne G, van der Kooi AJ *et al.* Identi fication of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B) Hum Mol Genet. 2000; 22: 1453-9.
36. Minetti C, Sotgia F, Bruno C *et al.* Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. Nature Genet 1998; 18: 365-8.
37. Beckmann JS, Richard I, Hillarie D *et al.* A gene for limb girdle muscular dystrophy maps to chromosome 15 by linkage. C R Acad Sci Paris 1991; 132: 141-8.
38. Richard I, Broux O, Allamand V *et al.* A novel mechanism leading to muscular dystrophy: mutations in calpain 3 cause limb girdle muscular dystrophy type 2A. Cell 1995; 81: 27-40.
39. Bashir R, Britton S, Stratchan T *et al.* A novel mammalian gene related to the C. elegans spermatogenesis factor fer-1 is mutated in patients with limb-girdle muscular dystrophy type 2B (LGMD2B). Nat Genet 1998; 20: 37-42.
40. Liu J, Aoki M, Illa I *et al.* Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. Nat Genet 1998; 20:

31-36.
41. Röberds SL, Leturcq F, Allamand V *et al.* Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. Cell 1994; 78: 625-33.
42. McNally EM, Yoshida M, Mizuno Y, Ozawa E and Kunkel LM. Human adhalin is alternatively spliced and the gene is located on chromosome 17q21. Proc Natl Acad Sci 1994; 91: 9690-4.
43. Lim LE, Duclos F, Allamand V *et al.* γ-Sarcoglycan (43 DAG): characterization and involvement in a recessive form of limb-girdle muscular dystrophy linked to chromosome 4q12. Nat Genet 1995; 11: 257-65.
44. Bonnemann CG, Modi R, Noguchi S *et al.* Mutations cause autosomal recessive muscular dystrophy with loss of the sarcoglycan complex. Nat Genet 1995; 11: 266-73.
45. Noguchi S, McNally EM, Ben Othmane K *et al.* Mutations in the dystrophin-associáted protein γ-sarcoglycan in chromosome 13 muscular dystrophy. Science 1995; 270: 819-22.
46. McNally EM, Passos-Bueno MR, Bonnemann C *et al.* Mild and severe muscular dystrophy caused by a single γ-sarcoglycan mutation. Am J Hum Genet 1996; 59: 1040-7.
47. Passos-Bueno MR, Moreira ES, Vainzof M, Marie SK, Zatz M. Linkage analysis in autosomal recessive limb-girdle muscular dystrophy (AR-LGMD) maps a sixth form to 5q33-34 (LGMD2F) and indicates that there is at least one more subtype of AR LGMD. Hum Mol Genet 1996; 5: 815-20.
48. Moreira ES, Vainzof M, Marie SK, Sertie AL, Zatz M, Passos-Bueno MR. The seventh form of autosomal recessive limb-girdle muscular dystrophy is mapped to 17q11-12. Am J Hum Genet 1997; 61: 151-9.
49. Frosk P, Weiler T, Nylen E *et al.* Limb-girdle muscular dystrophy type 2H associated with mutation in TRIM32, a putative E3-ubiquitin-ligase gene. Am J Hum Genet 2002; 703: 663-72.
50. Brockington M, Yuva Y, Prandini P *et al.* Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. Hum Mol Genet 2001; 10: 2851-9.
51. Bushby KM, Beckmann JS. The 105th ENMC sponsored workshop: pathogenesis in the non-sarcoglycan limb-girdle muscular dystrophies, Naarden, April 12-14, 2002. Neuromuscul Disord 2003; 13: 80?90.
52. Sorimachi H, Ishiura S, Suzuki K. A novel tissue-specific calpain species expressed predominantly in the stomach comprises two alternative splicing products with and without Ca(2+)-binding domain. J Biol Chem. 1993; 15: 19476-82.
53. Zatz M, Vainzof M, Passos-Bueno MR *et al.* Limb-girdle muscular dystrophy: one gene with different phenotypes, one phenotype with different genes. Curr Opin Neurol 2000; 13: 511-7.
54. Richard I, Roudaut C, Saenz A *et al.* Calpainopathy: a survey of mutations and polymorphisms. Am J Hum Genet 1999; 64: 1524?40.
55. Fanin M, Fulizio L, Nascimbeni AC *et al.* Molecular diagnosis in LGMD2A: mutation analysis or protein testing? Hum Mutat 2004; 24: 52-62.
56. Vainzof M, Anderson LV, Moreira ES *et al.* Characterization of the primary defect in LGMD2A and analysis of its secondary effect in other LGMDs. Neurology 2000; 54: A436.
57. Vainzof M, Moreira ES, Passos-Bueno MR *et al.* The effect of telethonin deficiency in LGMD-2G and its expression in other forms of muscular dystrophies and congenital myopathies. BBA-Gene Basis Dis 2002; 1588: 33-40.
58. Anderson LV, Harrison RM, Pogue R *et al.* Secondary reduction in calpain 3 expression in patients with limb girdle muscular dystrophy type 2B and miyoshi myopathy (primary dysferlinopathies). Neuromuscul Disord 2000; 10: 553-9.
59. Vainzof M, Pavanello RCM, Anderson LVB, Moreira ES, Passos-Bueno MR, Zatz M. Dysferlin analysis in autosomal recessive limb-girdle muscular dystrophies (AR-LGMD). J Mol Neurosci 2001; 17: 71-80.
60. Haravuori H, Vihola A, Straub V *et al.* Secondary calpain3 deficiency in 2q-linked muscular dystrophy: titin is the candidate gene. Neurology 2001; 56: 869-77.

61. Britton S, Freeman T, Vafiadaki E *et al.* The third human FER-1-like protein is highly similar to dysferlin. Genomics 2000; 68: 313-21.
62. Matsuda C, Aoki M, Hayashi YK, Ho MF, Arahata K, Brown RH Jr. Dysferlin is a surface membrane-associated protein that is absent in Miyoshi myopathy. Neurology 1999; 53: 1119-22.
63. Anderson LV, Davison K, Moss JA *et al.* Dysferlin is a plasma membrane protein and is expressed early in human development. Hum Mol Genet 1999; 8: 855-61.
64. Bansal D, Miyake K, Vogel SS *et al.* Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature 2003; 423: 168-72.
65. Passos-Bueno MR, Vainzof M, Moreira ES, Zatz M. Seven autosomal recessive limb-girdle muscular dystrophies in the Brazilian population: from LGMD2A to LGMD2G. Am J Med Genet 1999; 82: 392-8.
66. Bansal D, Miyake K, Vogel SS *et al.* Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature 2003; 423: 168-72.
67. Lennon NJ, Kho A, Bacskai BJ, Perlmutter SL, Hyman BT, Brown RH Jr. Dysferlin interacts with annexins A1 and A2 and mediates sarcolemmal wound-healing. J Biol Chem 2003; 278: 50466-73.
68. Tagawa K, Ogawa M, Kawabe K *et al.* Protein and gene analyses of dysferlinopathy in a large group of Japanese muscular dystrophy patients. J Neurol Sci 2003; 211: 23-8.
69. Argov Z, Sadeh M, Mazor K *et al.* Muscular dystrophy due to dysferlin deficiency in Libyan Jews. Clinical and genetic features. Brain 2000; 123: 1229-37.
70. Fanin M, Pegoraro E, Matsuda-Asada C, Brown RH Jr, Angelini C. Calpain-3 and dysferlin protein screening in patients with limb-girdle dystrophy and myopathy. Neurology 2001; 56: 660-5.
71. Oh SH, Kang SW, Lee JG, Na SJ, Kim TS, Choi YC. Clinical and Pathological Characteristics of Four Korean Patients with Limb-Girdle Muscular Dystrophy type 2B. Journal of the Korean Medical Science 2004; 19: 447-52.
72. Aoki M, Liu J, Richard I *et al.* Genomic organization of the dysferlin gene and novel mutations in Miyoshi myopathy. Neurology 2001; 57: 271-8.
73. Liu J, Aoki M, Illa I *et al.* Dysferlin, a novel skeletal muscle genes, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. Nat Genet 1998; 20: 31-6.
74. Piccolo F, Moore SA, Ford GC, Campbell KP. Intracellular accumulation and reduced sarcolemmal expression of dysferlin in limb-girdle muscular dystrophies. Ann Neurol 2000; 48: 902-12.
75. Duggan DJ, Hoffman EP. Autosomal recessive muscular dystrophy and mutations of the sarcoglycan complex. Neuromuscul Disord. 1996; 6: 475-82.
76. Worton R. Muscular dystrophies: diseases of the dystrophin-glycoprotein complex. Science 1995; 270: 755-6.
77. Straub V, Campbell KP. Muscular dystrophies and the dystrophin-glycoprotein complex. Curr Opin Neurol 1997; 10: 168-75.
78. Straub V, Dulcos F, Venzke DP *et al.* Moelcular pathogenesis of muscle degeneration in the δ -sarcoglycan deficient hamster. Am J Pathol 1998: 153: 1913-7.
79. Tawil R. Facioscapulohmeral muscular dystrophy. Currentr Neurology and Neuroscience Reports 2004; 4: 51-4.
80. Lunt PW, Harper PS. Genetic counseling in facioscapulohumeral muscular dystrophy. J Med Genet 1991; 28: 655-64.
81. Padberg GW, Frants RR, Brouwere OF, Wijmenga C, Bakker E, Sandkuijl LA. Facioscapulohumeral muscular dystrophy in the Dutch population. Muscle Nerve 1995; 2: S81-S84.
82. Padberg G, Brouwer OF, de Keizer RJ *et al.* On the significance of reinal vascular disease and hearing loss in facioscapulohumeral muscular dystrophy. Muscle Nerve 1995; 2: S73-S80.
83. Wijmenga C, Hewitt JE, Sandkuiji LA *et al.* Chromosome 4q DNA rearrangements associated with facioscapulohumeral muscular dystrophy. Nat Genet 1992; 2; 26-30.
84. Tawil R, Figlewicz DA, Griggs RC, Weiffenbach B.

Facioscapulohumeral dystrophy: a distinct regional myopathy with a novel molecular pathogenesis. Ann Neurology 1998; 43: 279-82.
85. Upadhyaya M, Maynard J, Rogers MT *et al.* Improved molecular diagnosis of facioscapulohumeral muscular dystrophy (FSHD): validation of the differential double digestion for FSHD. J Med Genet 1997; 34: 476-9.
86. Orrell RW, Tawil R, Forrester J, Kissel JT, Mendell JR, Figlewicz DA. Definite molecular diagnosis of facioscapulohumeral dystrophy. Neurology 1999; 52: 1822-6.
87. Harper PS. Myotonic dystrophy (2nd ed.), Saunders, London/Philadelphia (1989)
88. Brook JD, McCurrach ME, Harley HG *et al.* Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. Cell 1992: 68: 799-808.
89. Thornton CA, Griggs RC, Moxley RT. Myotonic dystrophy with no trinucleotide repeat expansion. Ann Neurol 1994; 35: 269-72.
90. Ricker K, Koch MC, Lehmann-Horn F. Proximal myotonic myopathy. Clinical features of a multisystem disorder similar to myotonic dystrophy. Arch Neurol 1995; 52: 25-31
91. Ranum LP, Rasmussen PF, Benzow KA, Koob MD, Day JW. Genetic mapping of a second myotonic dystrophy locus. Nat Genet 1998; 19: 196-8.
92. Liquori CL, Ricker K, Moseley ML *et al.* Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. Science 2001; 293: 864-7.
93. Warner JP, Barron LH, Kelly K, Dow D, Fitzpartick DR, Brock DJ. A general method for the detection of large CTG repeat expansions by fluorescent PCR. J Med Genet. 1996; 33: 1022-6.

국문요지

근이양증 (muscular dystrophy)는 임상적으로는 유전적인 요인으로 진행성 근력저하를 보이고, 근병리적으로는 근섬유의 괴사 및 재생을 특징으로 하는 퇴행성 근육질환이다. 근이양증 중 발병년령과 침범 부위의 특징에 따라서 선천성 근이양증 (congenital muscular dystrophy), Duchenn형 근이양증 (Duchenne muscular dystrophy, DMD), Becker형 근이양증 (Becker muscular dystrophy, BMD), 지대형 근이양증 (Limb-girdle muscular dystrophy), 안면견갑상완 근이양증(fascioscapulohumeral muscular dystrophy), 근긴장성 이양증 (myotonic dystrophy) 등으로 구분하게 되었고 이 분류가 지금까지도 사용하고 있다. 1980년대의 분자생물학의 발달로 근세포막에 존재하는 단백질 및 그 유전자의 연구가 활발하게 진행되어 1986-7년 "dystrophin 유전자"가 발견되고 이 유전자 돌연변이(mutation)에 의해서 dystrophin 단백이 소실되어 DMD 및 BMD가 발병하는 것이 밝혀졌다. 그 후 여러 연구자에 의해서 근세포막에 존재하는 다른 단백질을 발견하게 되었고, 그 유전자 변이가 또 다른 형의 근이양증의 원인이 된다는 것도 밝혀졌다. 더 나가서 같은 유전자 변이에 의해서 생긴 병이라도 표현형이 다른 근육질환이 발생할 수도 있다는 것도 발견되었다. 그 결과 근이양증은 원인 유전자와 단백질에 따라 분류가 좀더 세분화되고, 명칭도 변화되고, 새로운 원인유전자들이 발견되고 있다. 따라서 향후 근이양증에 대한 분자유전학적 병인이 더 많이 밝혀질 것이고, 근육질환의 분류도 변화할 것으로 생각된다.

중심단어: 근이양증, dystrophin-glycoprotein 복합체, 지대 형 근이양증, 안면견갑상완 근이양증, 근긴장성 이 양증