

Limb Girdle Muscular Dystrophy: Diagnosis



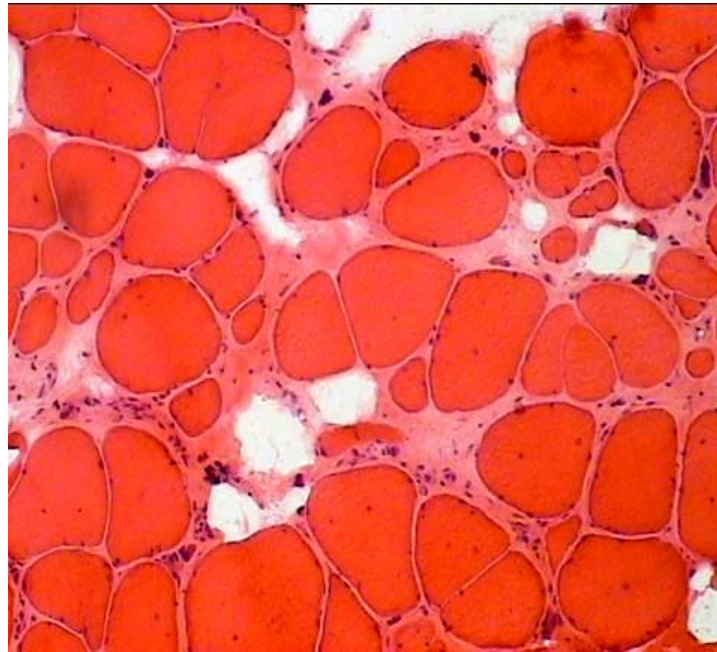
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Limb girdle muscular dystrophy (LGMD): recessive form

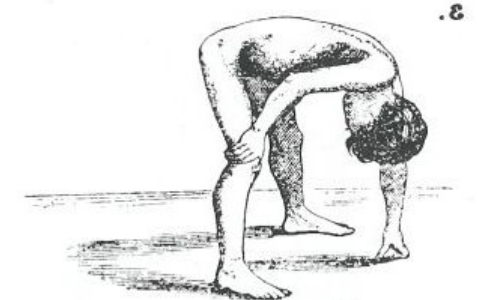
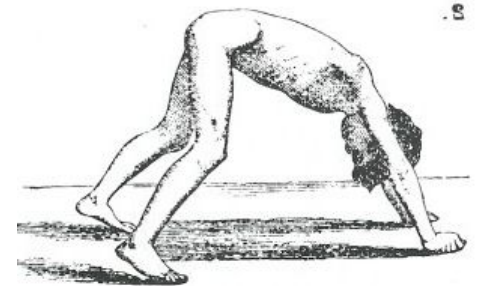
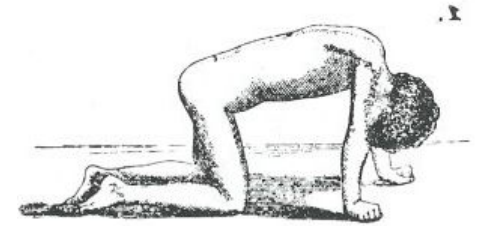
Diagnostic criteria:

- Progressive proximal weakness and atrophy of limb muscles
- Creatine kinase levels elevated (4-20x)
- Dystrophic changes in muscle



Recessive types of LGMD: Clinical phenotype

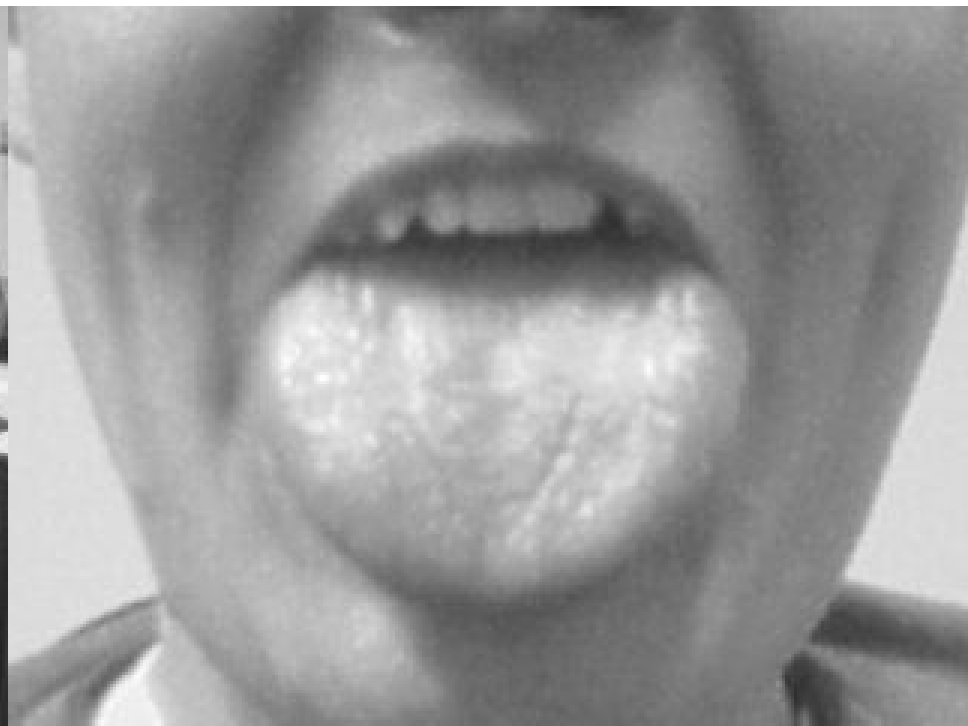
- Weakness most severe in legs
- Weakness is usually symmetric
- Waddling lordotic gait with Trendelenburg's sign
- Gower's sign on standing up
- No facial muscle involvement



Limb girdle muscular dystrophy type 2I



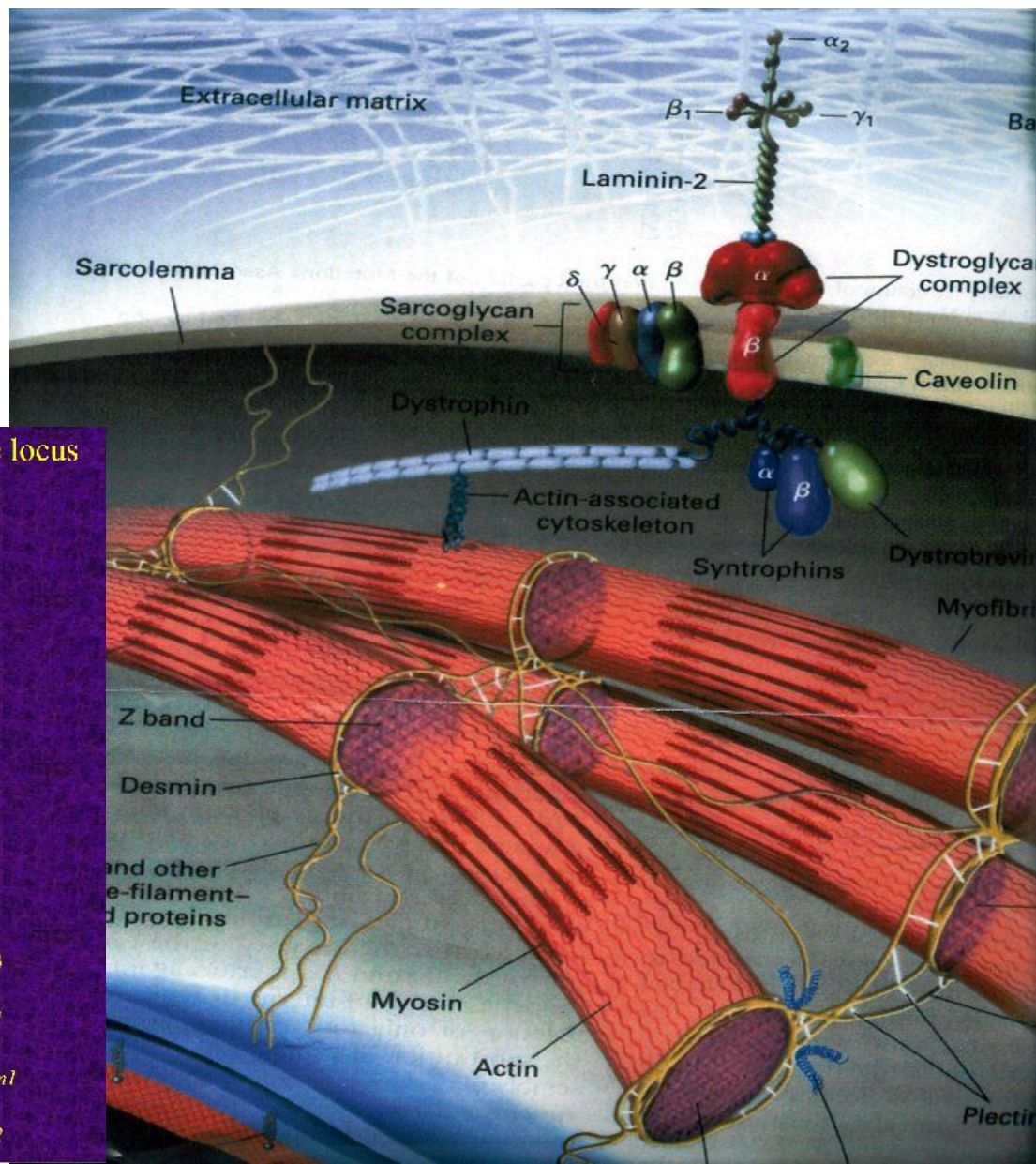
Phenotype in more severely affected LGMD



Unusual presentation
of LGMD2A
(Calpainopathy)

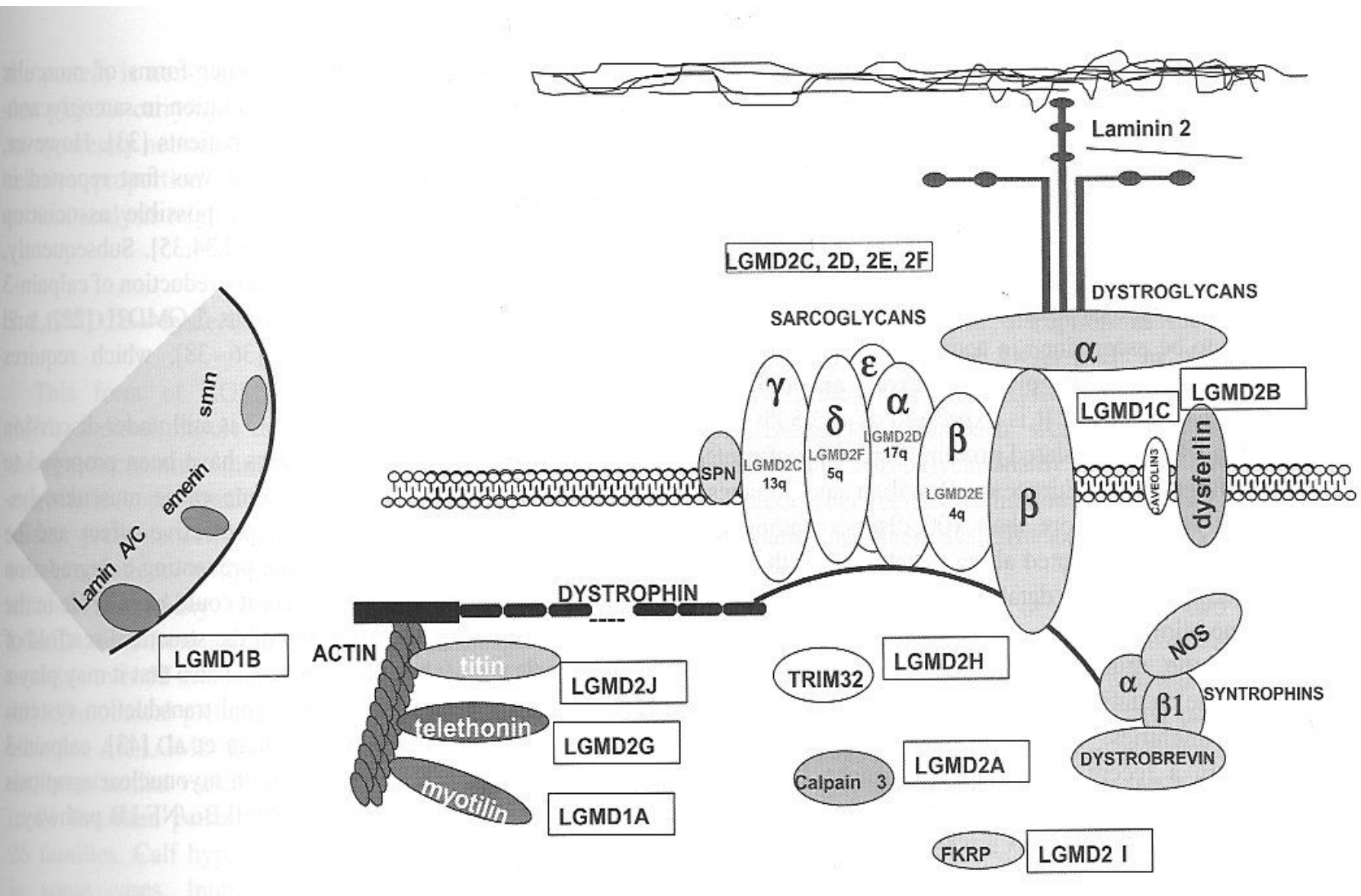


Limb girdle muscular dystrophies



	Protein defect	Gene locus
Dominant types		
LGMD 1A	Myotilin	5q22
LGMD 1B	Lamin A/C	1q11
LGMD 1C	Caveolin-3	3p25
LGMD 1D	?	6q23
LGMD 1E	?	7q
Recessive types		
LGMD 2A	Calpain-3	15q15
LGMD 2B	Dysferlin	2q13
LGMD 2C	γ-sarcoglycan	13q13
LGMD 2D	α-sarcoglycan	17q12
LGMD 2E	β-sarcoglycan	4q12
LGMD 2F	δ-sarcoglycan	5q33
LGMD 2G	Telethonin	17q11
LGMD 2H	TRIM32	9q31
LGMD 2I	Fukutin-related protein	19q13.3
LGMD 2J	Titin	3p21
LGMD2K	O-Mannosyl-transferase-1	<i>POMT1</i>
LGMD2L	Fukutin	<i>FKTN</i>
LGMD2M	O-Mannose β-1, 2-N-acetylglucosaminyl transferase	<i>POMGn1</i>
LGMD2N	O-Mannosyl-transferase-2	<i>POMT2</i>

Proteins involved in limb girdle muscular dystrophy



From Zatz et al. Neuromusc Disord 2003; 13: 532-544.

Why we should refine diagnosis of patients with LGMD?

- Molecular diagnosis is a prerequisite for specific treatment
- Persons with LGMD request a specific diagnosis
- Diagnosis facilitates estimation of prognosis and genetic counseling
- Diagnosis can guide the follow-up strategy
- Diagnosis provides an opportunity to enter clinical trials
- Understanding phenotype-genotype relationships in LGMD, will simplify future diagnostic procedures

Diagnosis: LGMD2

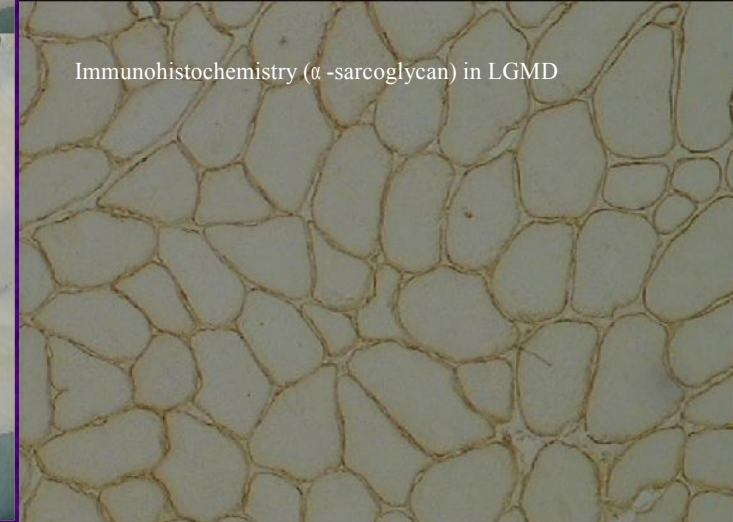
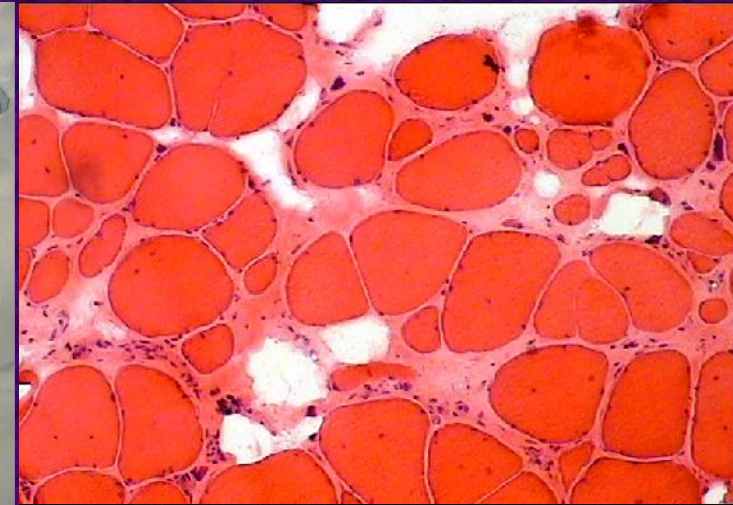
Clinical criteria and;

Before 1990

- Creatine kinase levels
- EMG
- Muscle histology

After 1990

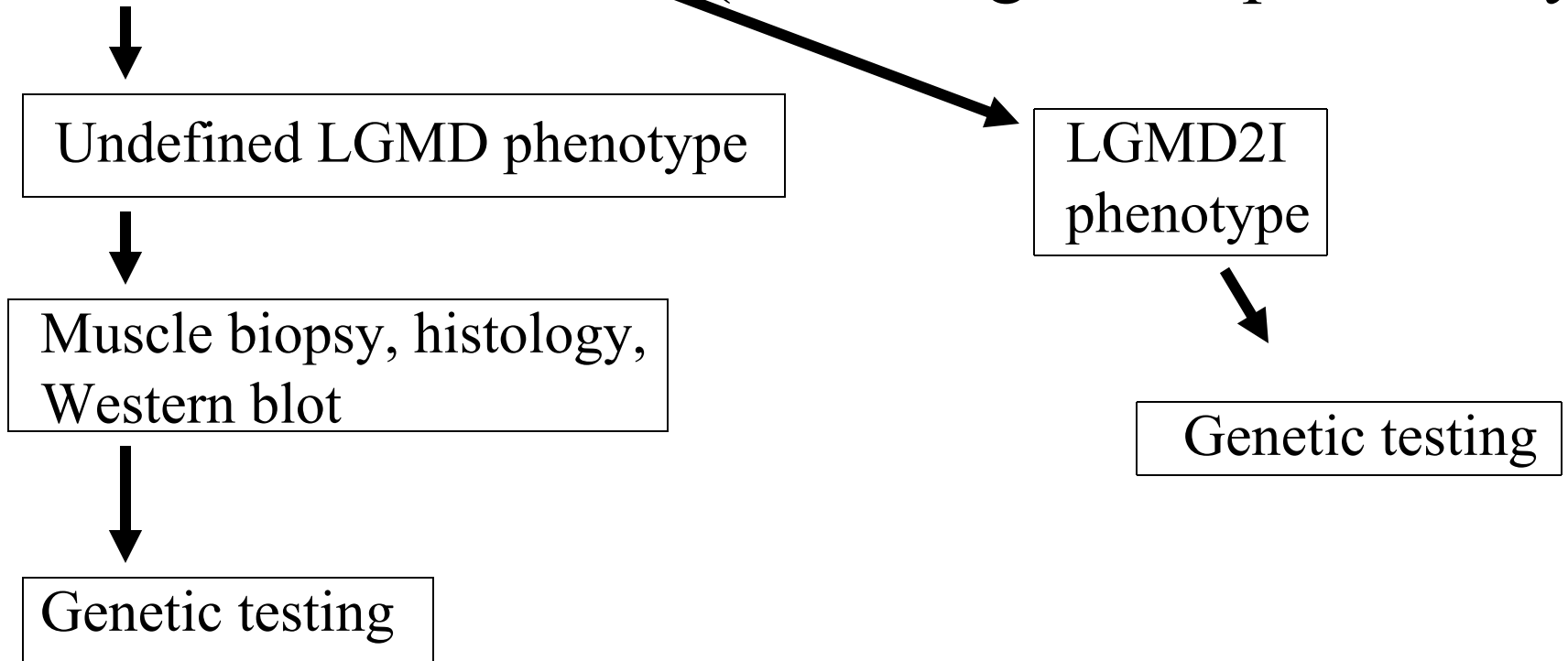
- Protein detection by immunohistochemistry, Western blot
- Genetic tests

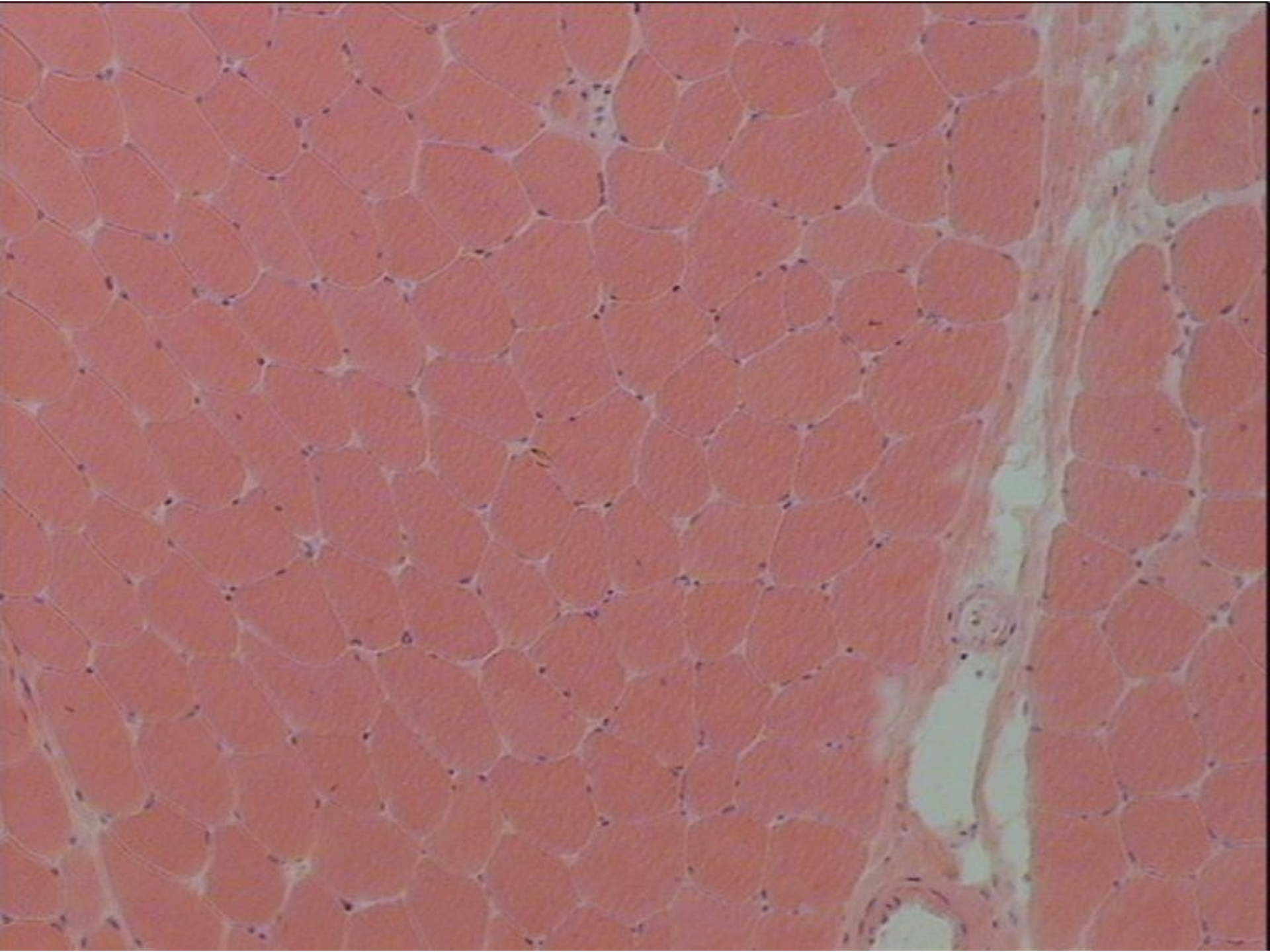


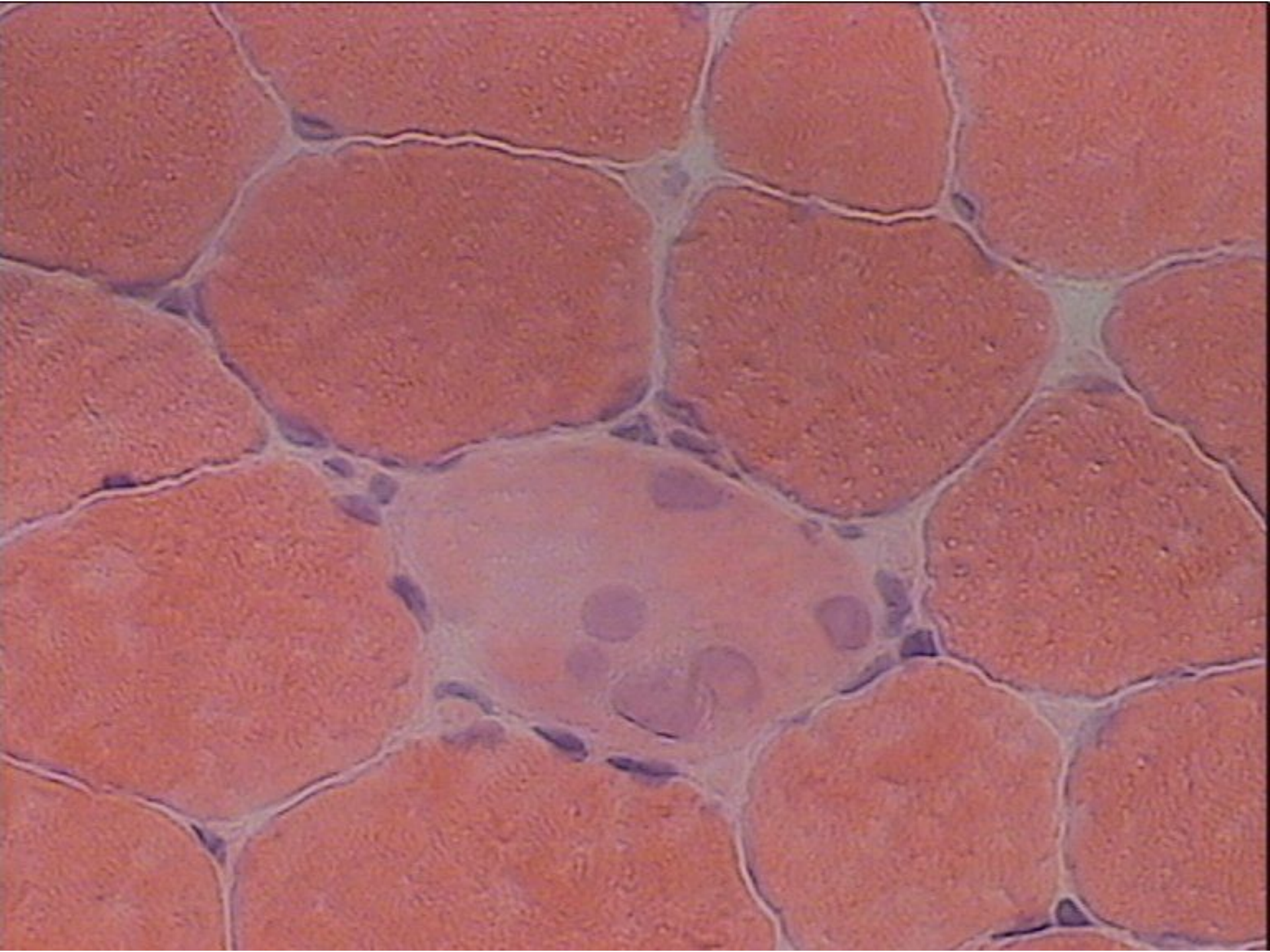
Diagnostic strategy in LGMD

EMG obsolete

Clinical assessment (including cardiopulmonary)



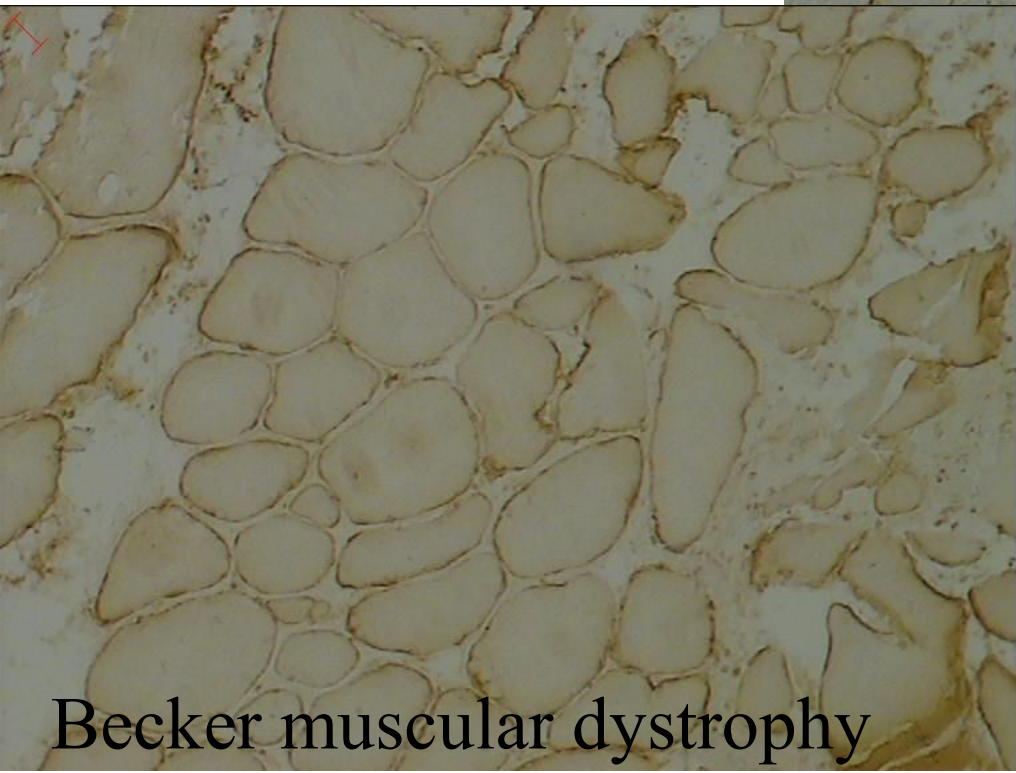




Dystrofin- farvninger

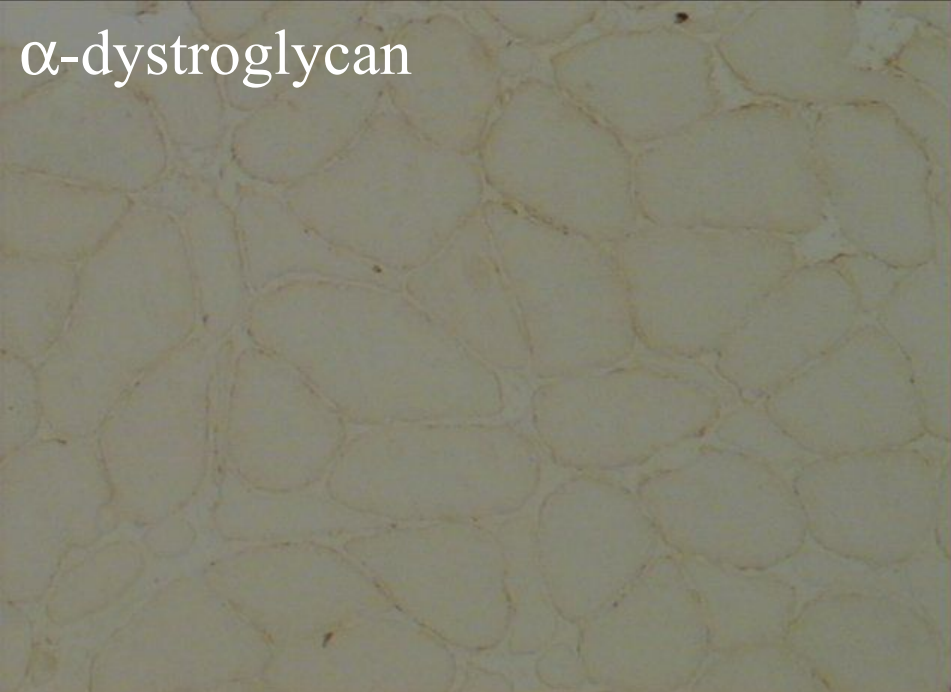


Limb girdle muscular dystrophy

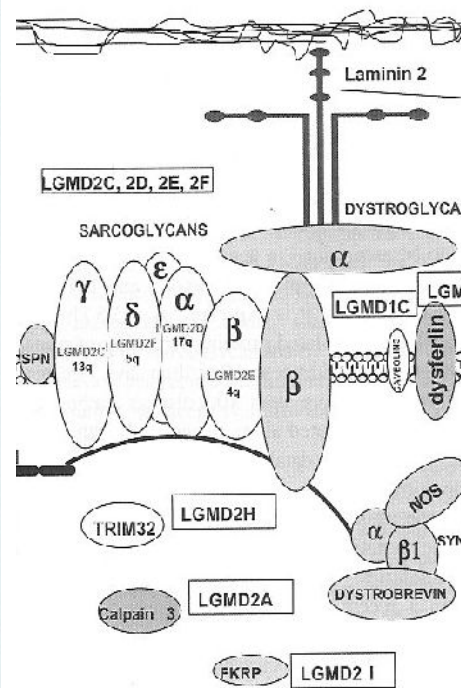
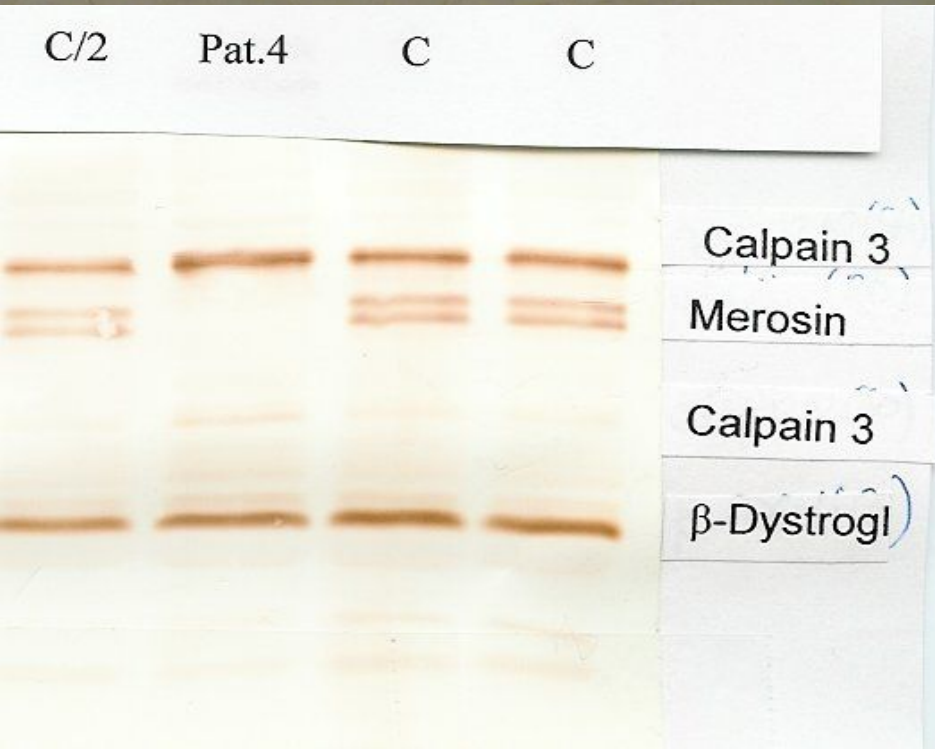
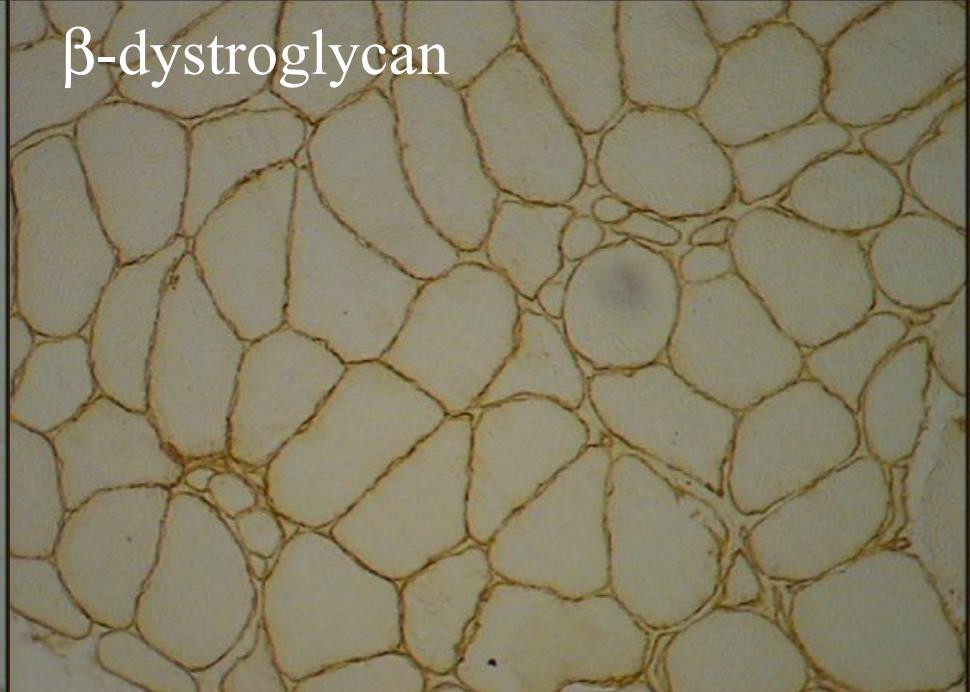


Becker muscular dystrophy

α -dystroglycan



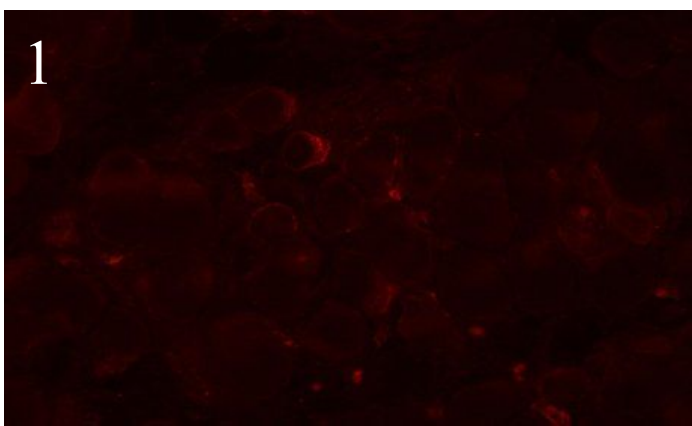
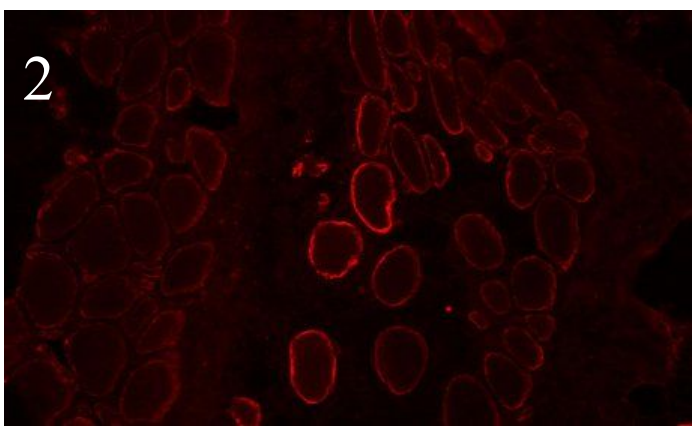
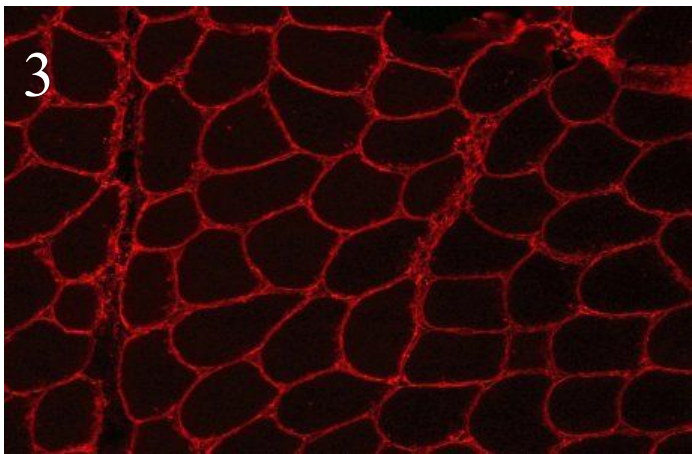
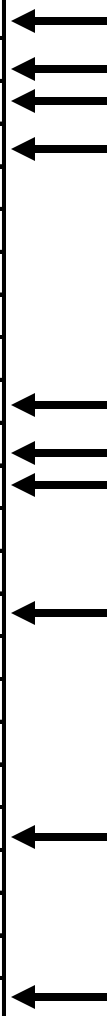
β -dystroglycan



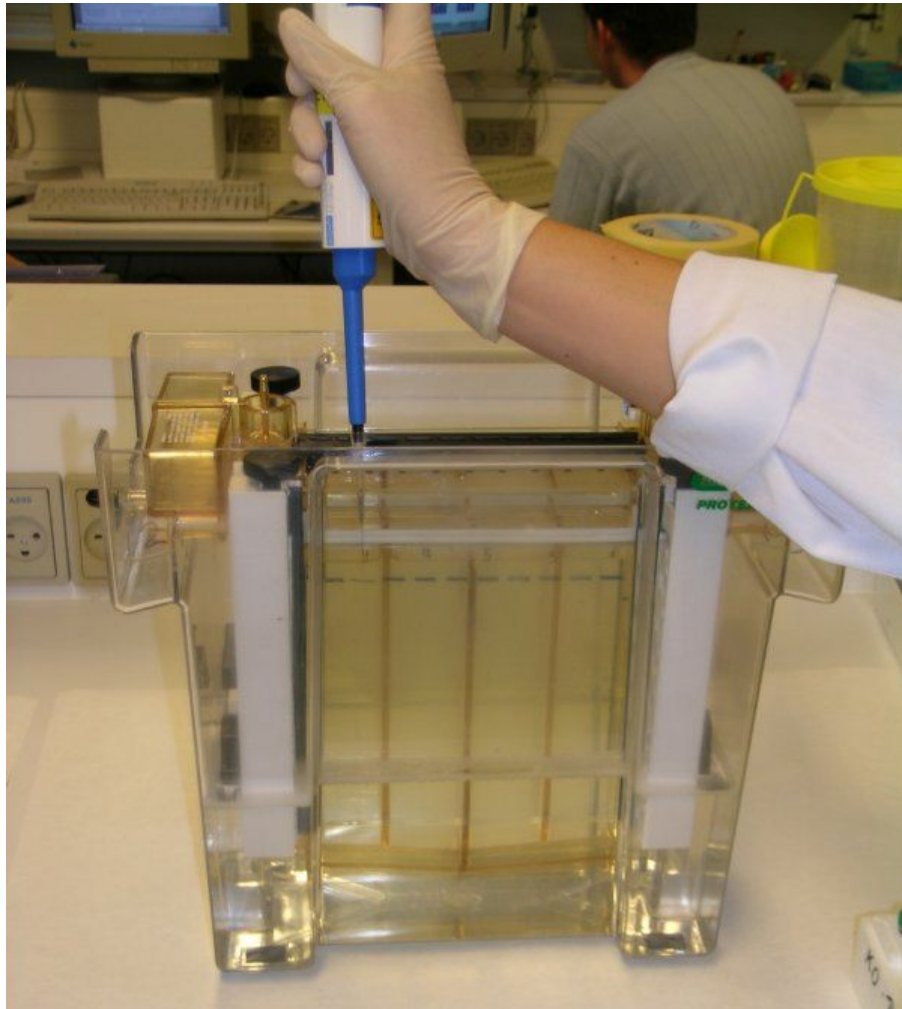
Combined α -dystroglycan and merosin deficiencies indicate fukutin-related protein defect

Glycosylation defects in the 27 unclassified LGMD2 patients

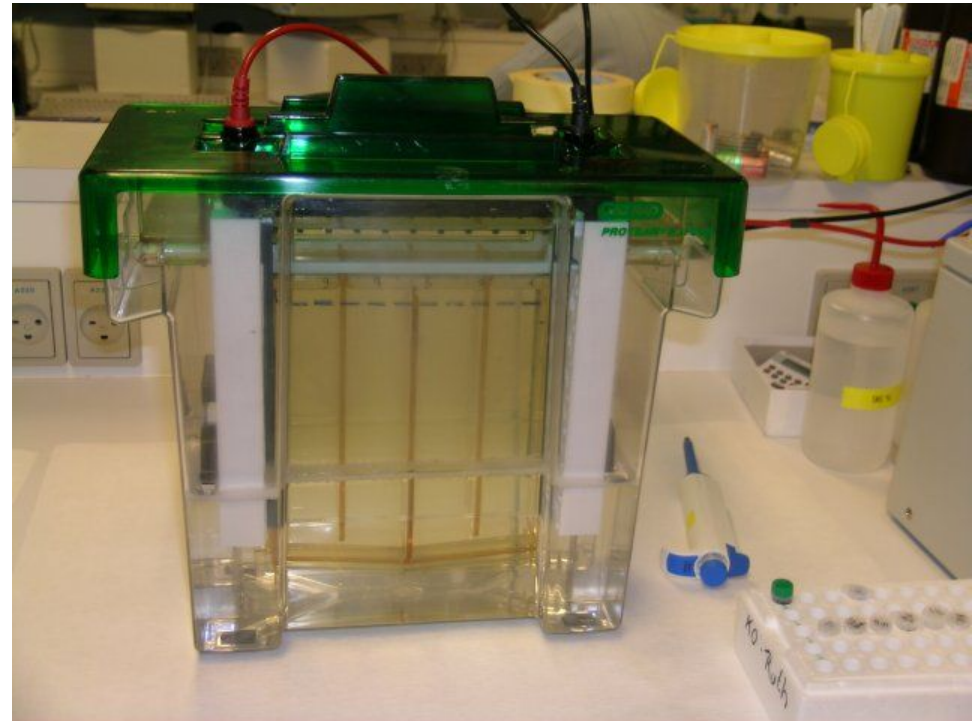
R nr	VIA4	IIH6C	Core
52-1071	3		
66-01S0	1	1	1
39-0404	3	3	3
55-1418	1	1	2
56-0393	1	2	2
45-0641	1	1;2	3
74-3189	1	1;2	3
45-0641	2	1;2	3
39-1753	2	3	3
58-0123	2	3	2
71-2755	2	2	2
79-1634	2	1;2	3
62-2192	1	2	3
45-1251	1	1	3
33-0213	1	2	2
73-1644	1	2	1
65-030	1	2	1
55-1064	1	1	3
67-1856	2	2	2
39-1079	1	3	2
62-2391	1	1	1
56-0393	2	2	3
44-0232	1	2	3
54-2646	3	3	2
71-1886	1	1	1
60-2783	2	2	3
54-2222	1	2	2



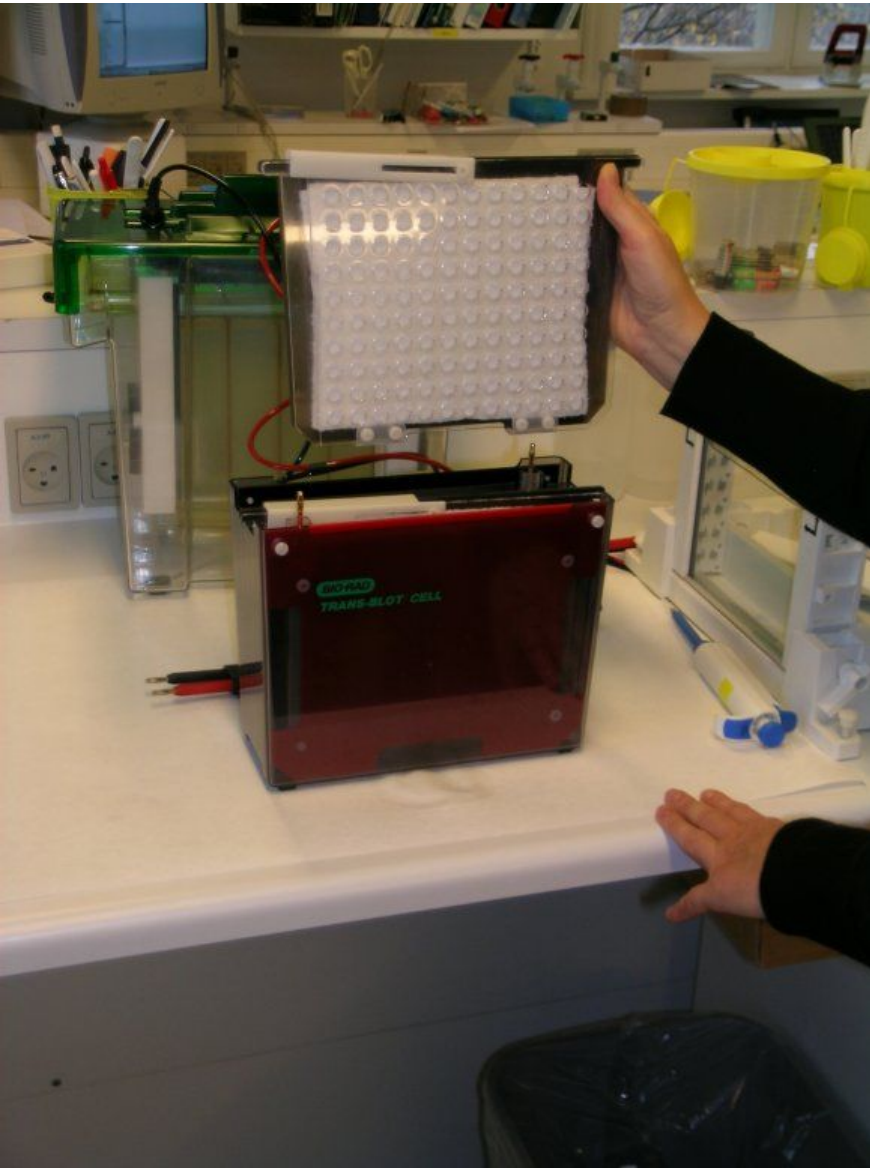
Application of homogenized muscle sample to the gel



Separation of proteins by electrophoresis



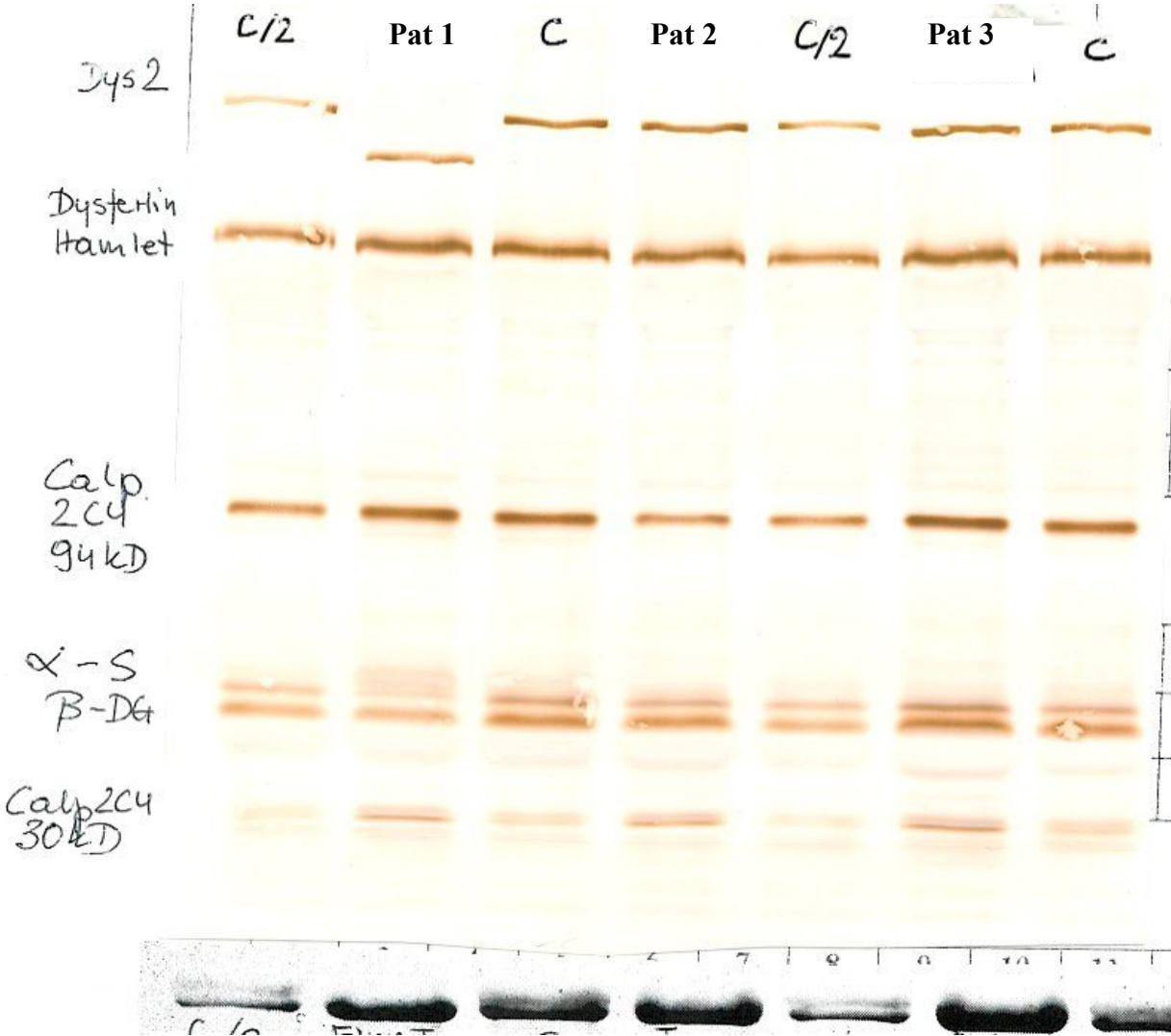
Blotting of proteins
from gel to paper



Incubation of blotted paper with
specific antibodies



Becker muscular dystrophy



Re-diagnosing recessively inherited LGMD in Denmark

Patients with a LGMD2 phenotype registered in our department or at the Danish MDA were offered evaluation for LGMD2 classification

- Clinical exam, cardiac and pulmonary tests
- Muscle biopsy (histology, Western blot)
- Molecular genetic testing

Sveen ML, Schwartz M, Vissing J. High prevalence and phenotype-genotype correlations of limb girdle muscular dystrophy type 2I in Denmark. **Ann Neurol** 2006; 59: 808-815.

Prevalence of LGMD2 sub-groups in Denmark

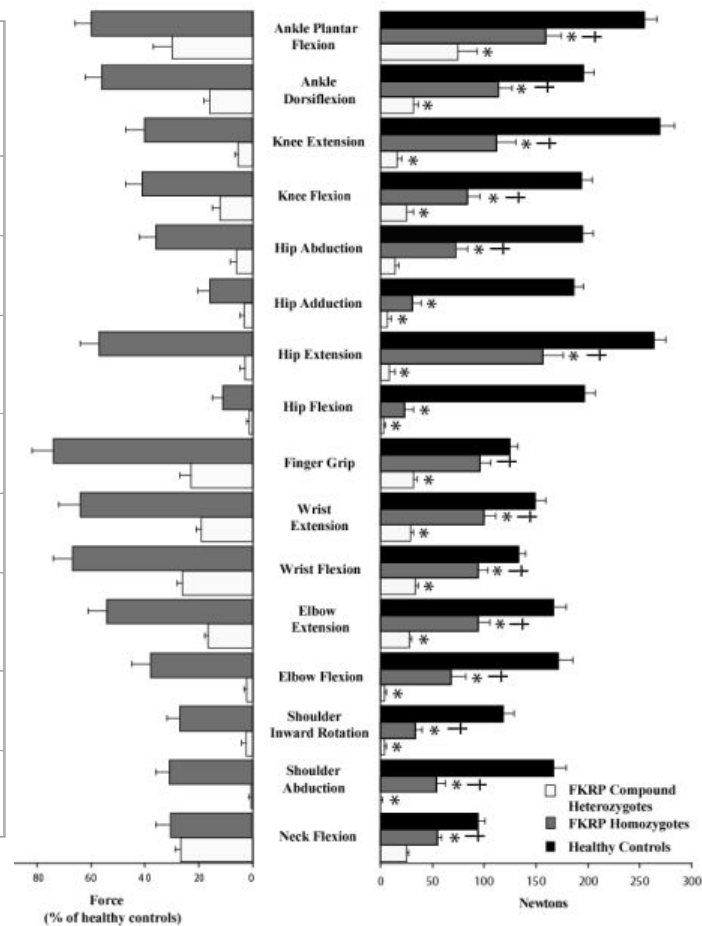
Of 136 patients investigated, 114 met LGMD diagnostic criteria

- 45 Fukutin-related protein (FKRP) deficiency (LGMD2I)
- 9 α -sarcoglycanopathy (LGMD2D)
- 6 β -sarcoglycanopathy (LGMD2E)
- 2 γ -sarcoglycanopathy (LGMD2C)
- 15 Calpainopathy (LGMD2A)
- 2 Dysferlinopathy (LGMD2B)
- 9 Becker muscular dystrophy
- 27 Unclassified

Mutations and muscle force in Danish LGMD2I patients

No. of patients	Nucleotide change on allele one	Amino acid substitutio	Nucleotide change on allele two	Amino acid substitution
28	826C>A	L276I	826C>A	L276I
4	826C>A	L276I	1384C>T	P462S
2	826C>A	L276I	1187dupA	A397GfsX15*
2	826C>A	L276I	919T>A	Y307N
1	826C>A	L276I	477G>C	A157P*
1	826C>A	L276I	158_162dup	R54_E55insCGfsX15*
1	826C>A	L276I	605T>A	L202Q

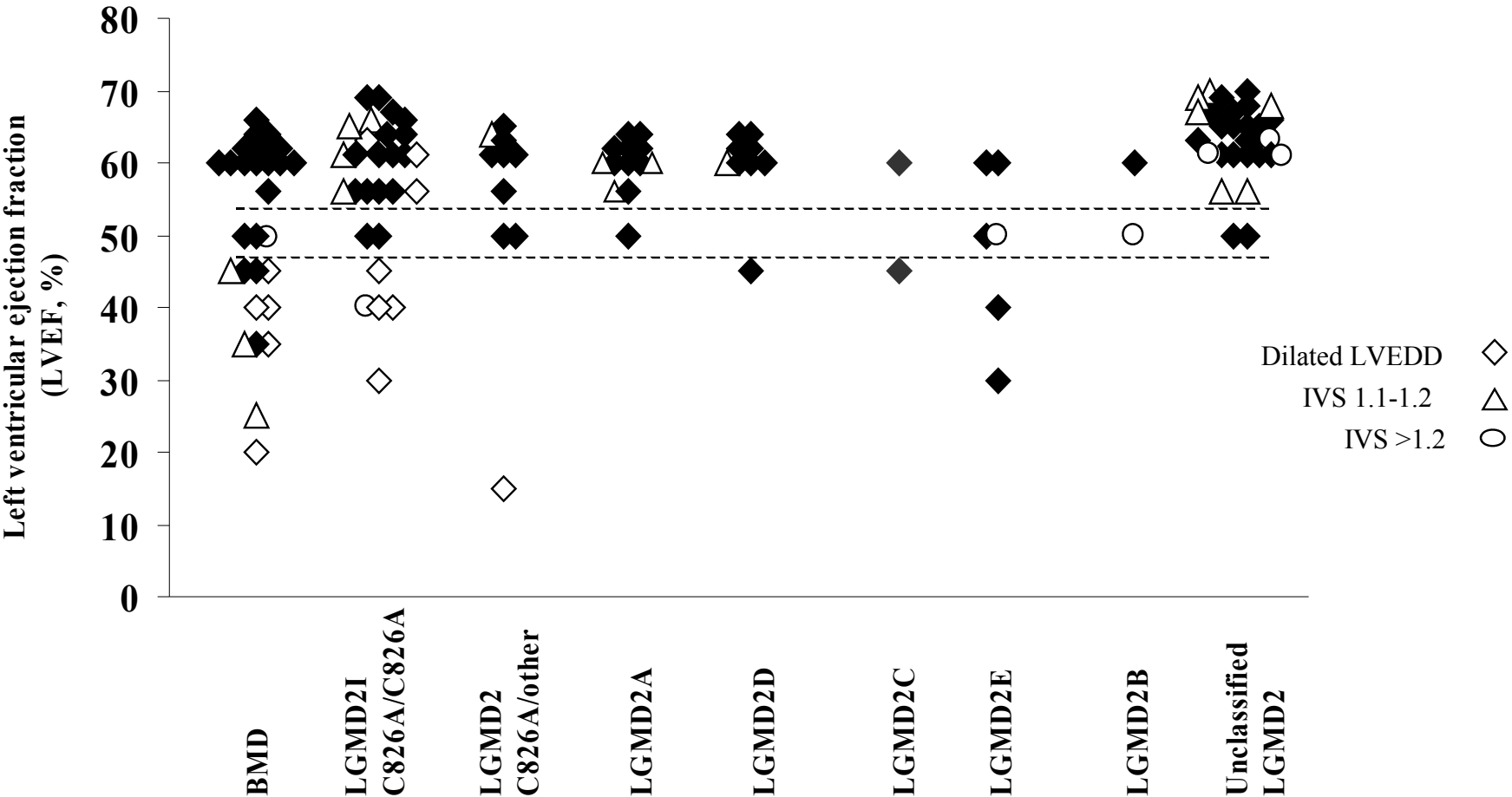
* Mutations not previously described.



Sveen ML, Schwartz M, Vissing J. High prevalence and phenotype-genotype correlations of limb girdle muscular dystrophy type 2I in Denmark. **Ann Neurol** 2006; 59: 808-815.

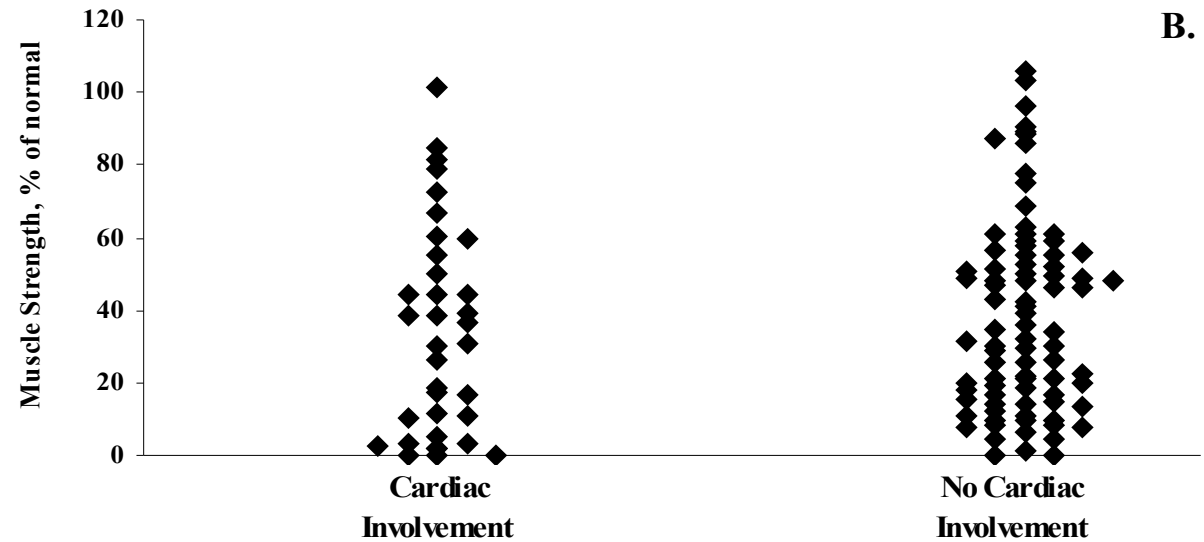
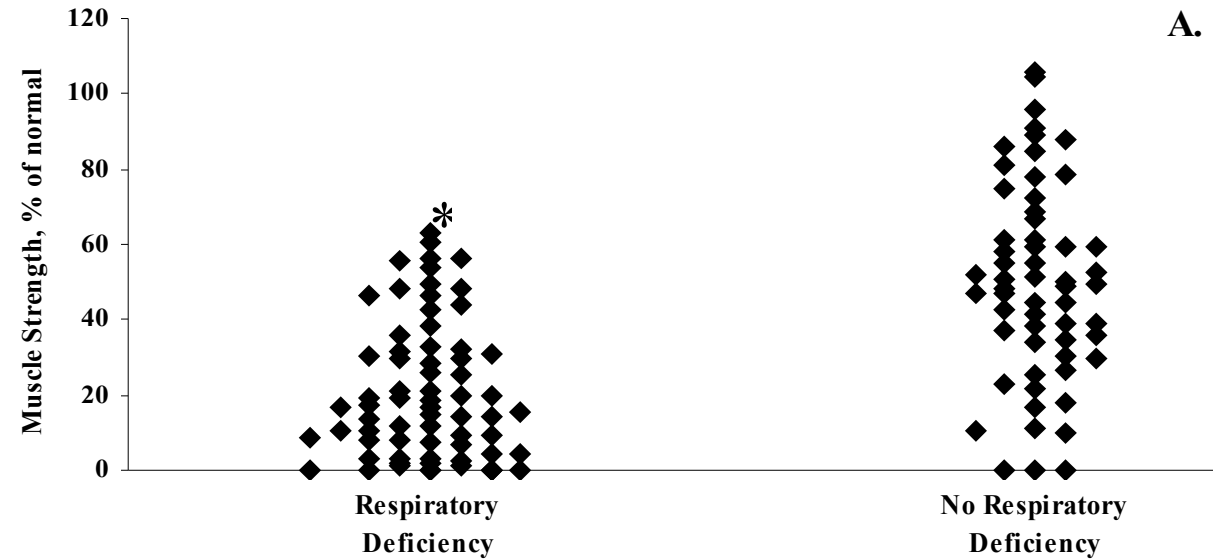
	Homozygote patients N =28	Compound heterozygote patients N =11
No. of patients	27	11
Average age (yrs)	35±3	33±2
Onset (yrs)	18±3	5±1
Diagnosis (yrs)	30±3	9±2
Gender (male/female)	15/12	5/6
Consanguinity	0/28	0/11
Creatine kinase IU/L	1683±331	1284±558
Loss of ambulation (yrs)	4/28 (44±1)	10/11 (20±2)
Scoliosis	6/28	8/11
Achilles tenotomy	2/28	5/11
Muscle pain at rest	5/28	3/11
Exertional muscle pain	11/28	5/11
Myoglobinuria	9/28	0/11
Muscle mass (kg)	8.2±1.5	2.5±0.2
Tongue hypertrophy	9/28	8/11
Calf hypertrophy	17/28	3/11

Cardiac involvement in BMD and LGMD2



Sveen, Thune, Køber, Vissing. Arch Neurol 2008;65:1196-1201.

Cardiac involvement vs. muscle strength and respiratory function in patients with BMD and LGMD2



Limb girdle muscular dystrophy

	Protein defects	Gene	Diagnostic test
Dominant types			
LGMD 1A	Myotilin	5q22	(Gene)
LGMD 1B	Lamin A/C	1q11	Gene
LGMD 1C	Caveolin-3	3p25	Gene, histo
LGMD 1D	?	6q23	Linkage
LGMD 1E	?	7q	Linkage
Recessive types			
LGMD 2A	Calpain-3	<i>CAPN3</i>	Gene, Histo
LGMD 2B	Dysferlin	<i>DYSF</i>	WB
LGMD 2C	γ -sarcoglycan	<i>SGCG</i>	WB, gene
LGMD 2D	α -sarcoglycan	<i>SGCA</i>	WB, gene
LGMD 2E	β -sarcoglycan	<i>SGCB</i>	WB, gene
LGMD 2F	δ -sarcoglycan	<i>SGCD</i>	WB, gene
LGMD 2G	Telethonin	<i>TCAP</i>	WB, gene
LGMD 2H	TRIM32	<i>TRIM32</i>	Gene
LGMD 2I	Fukutin-related protein	<i>FKRP</i>	Gene
LGMD 2J	Titin	<i>TTN</i>	(Histo, gene)
LGMD2K	O-Mannosyl-transferase-1	<i>POMT1</i>	Histo, (gene)
LGMD2L	Fukutin	<i>FKTN</i>	Histo, (gene)
LGMD2M	O-Mannose β -1, 2-N-acetylglucosaminyl transferase	<i>POMGn1</i>	Histo, (gene)
LGMD2N	O-Mannosyl-transferase-2	<i>POMT2</i>	Histo, (gene)