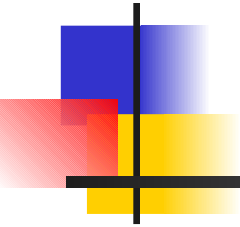


# Limb- Girdle in Norway....



**A story of FKRП (LGMD 2I)**

**The rest is assumption....**

**By Sigurd Lindal and Christoffer Jonsrud**



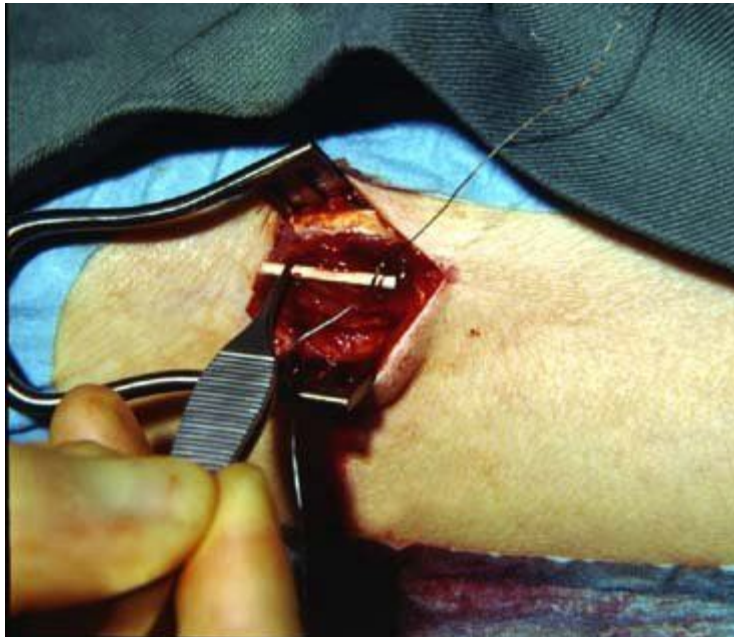
# De kliniske tall 1997-2008..

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- Fra NMK (patologi) Tromsø:
  - 1870 pasienter (2970 bopsier eller undersøkelser)
  - 391 pasienter med Limb Girdle (som hoveddiagnose eller diff.diagnose)

# Open Biopsy method

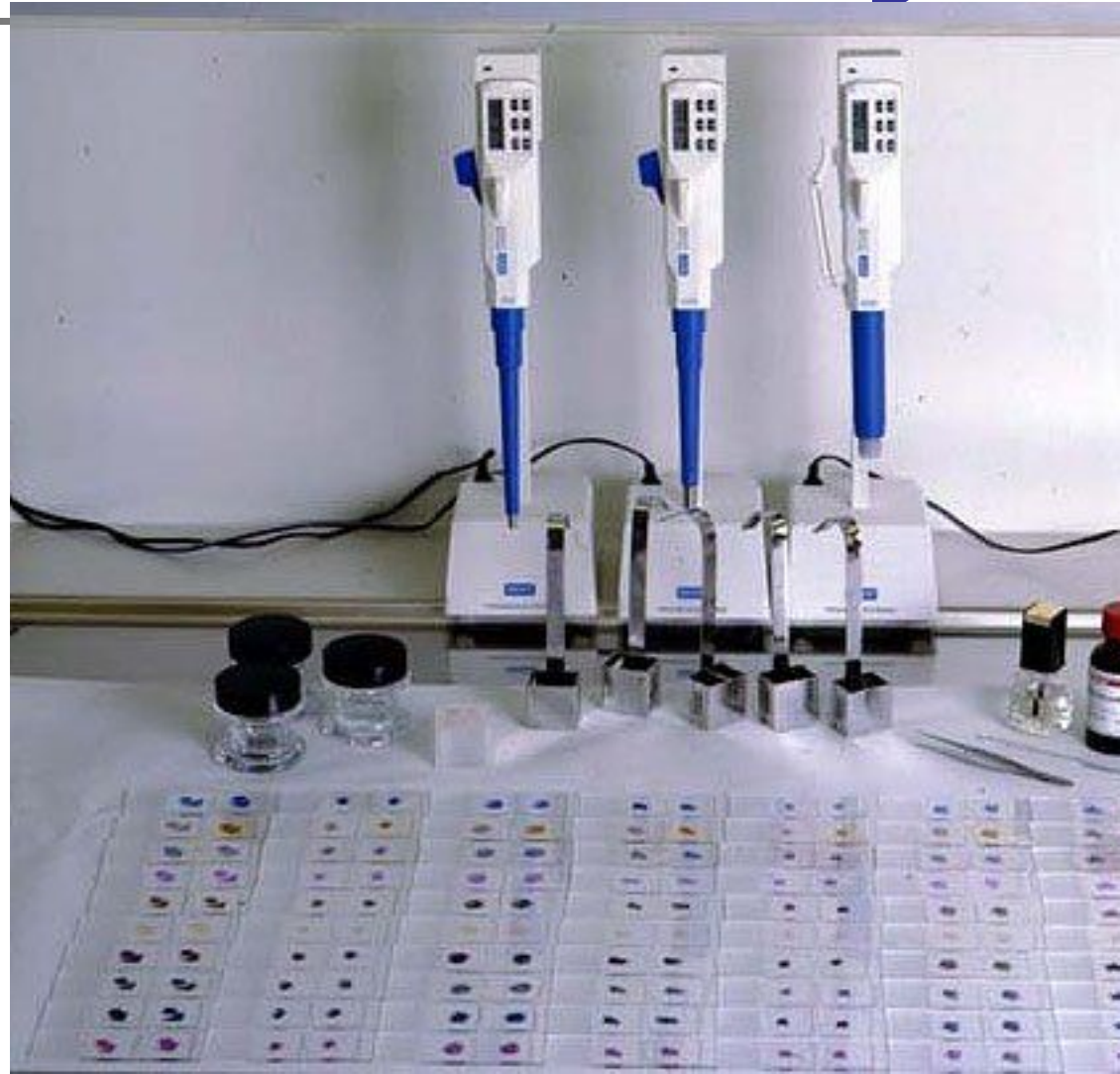
- Åpen biopsimetode
  - Mer materiale, flere muligheter
  - God kvalitet , korrekt diagnose



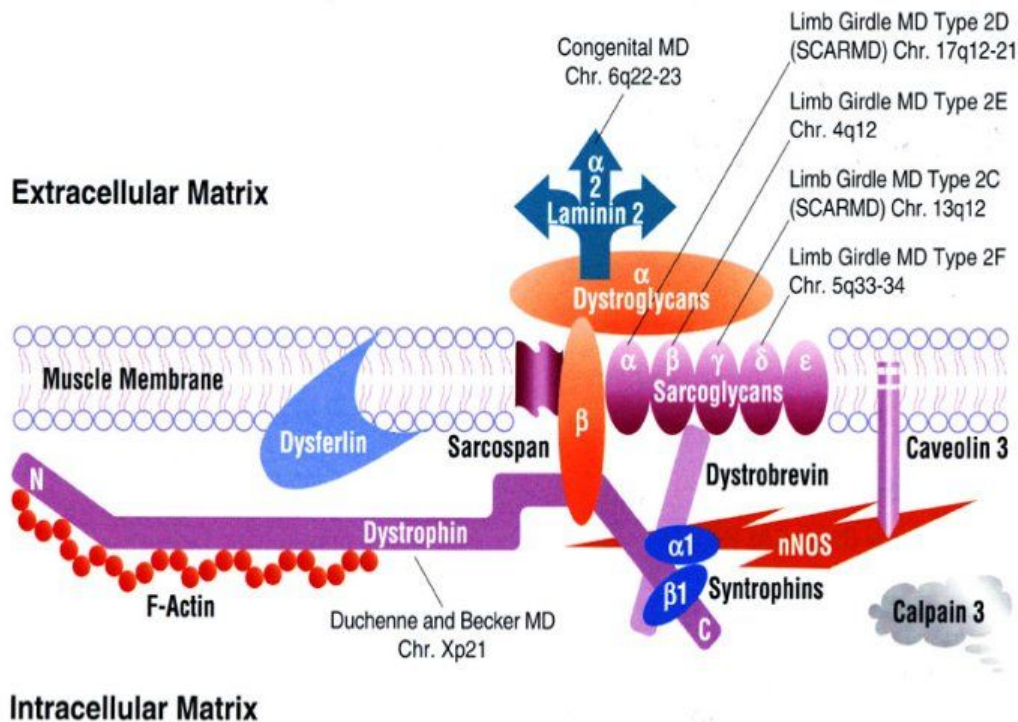
# First : 30 slides for special staining and immunostaining

H&E

- Gomori trichrome
- ATP (4.3-4.5- 9.4)
- NADH
- Sur phosfatase
- Non-spesifikk esterase
- PAS
- Oil-red O
- MHC1
- Ev. Metabolsk
  - Cox/SDH
  - PFK
  - AMP
  - Myophos



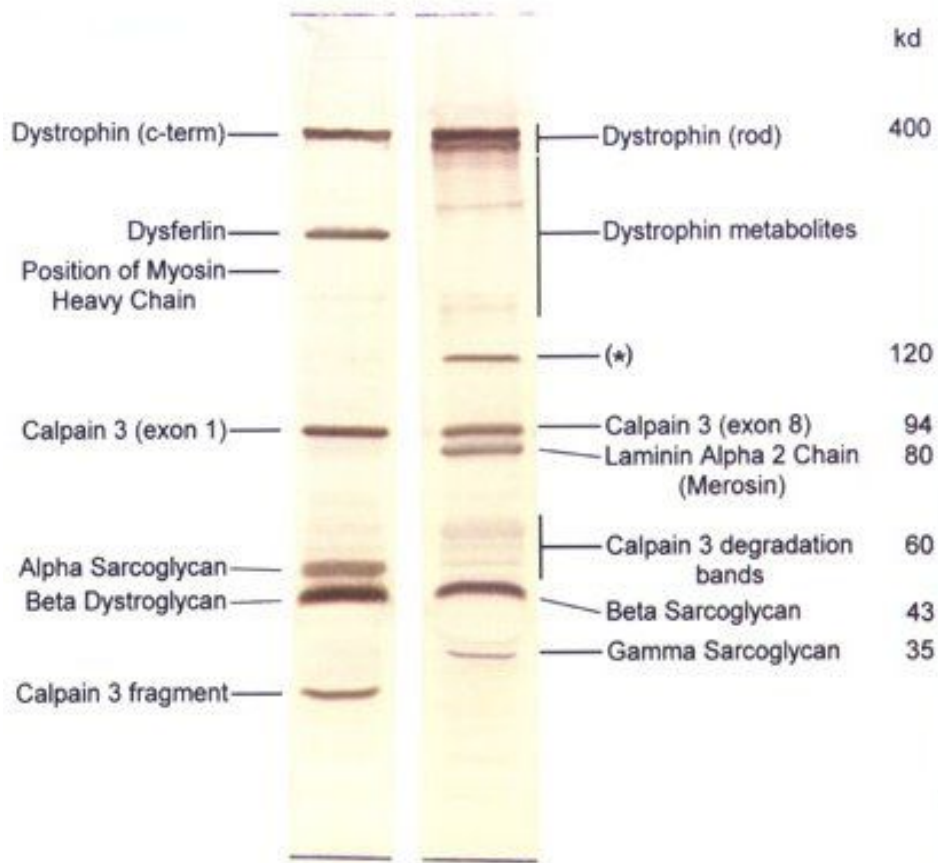
# Immunohistochemical analysis



- Dystrofin
- Alfa-dystroglycan
- Alfa-sarcoglycan
- Beta-dystroglycan
- Beta-sarcoglycan
- Delta-sarcoglycan
- Gamma-sarcoglycan
- Dysferlin
- Spectrin
- Merosin (80 and 300)
- Emerin

A schematic diagram illustrating the arrangement of dystrophin, dystrophin associated proteins and their link to different muscular dystrophies.

# Western blot



- Dystrophin
- Calpain
- Dysferlin
- Merosin
- Caveolin



# CLASSIFICATION OF LGMD

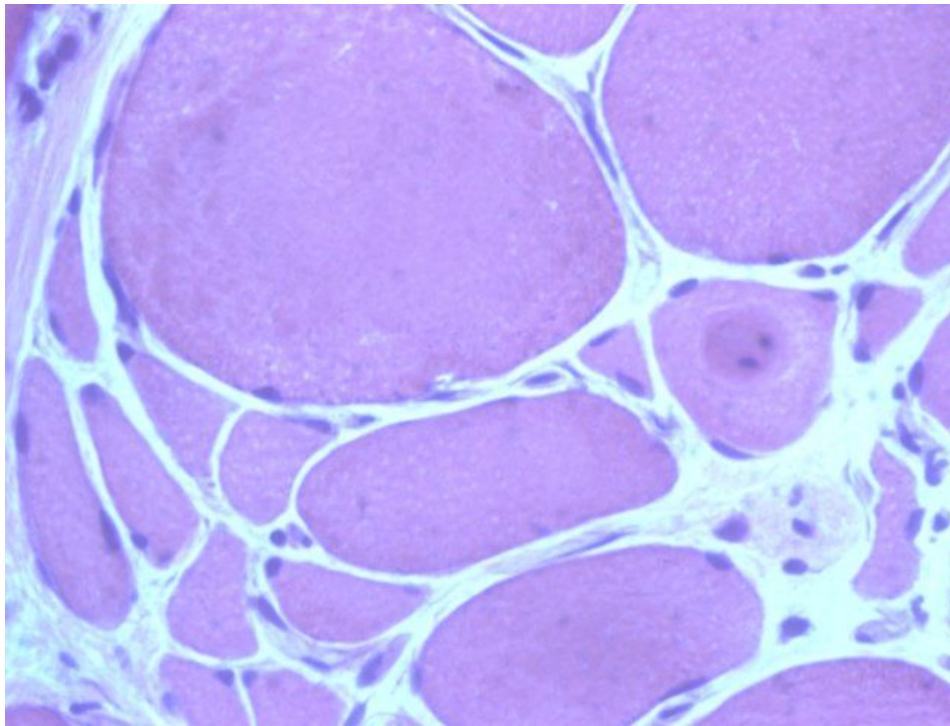
Bushby and Beckmann, 2003

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<b>DISEASE</b>	<b>GENE LOCUS</b>	<b>GENE PRODUCT</b>	<b>MW (kDa)</b>	<b>LOCALISATION</b>
<b>LGMD2A</b>	<b>15q15 (1995)</b>	<b>Calpain 3</b>	<b>94</b>	
<b>LGMD2B</b>	<b>2p13 (1994)</b>	<b>Dysferlin</b>	<b>230</b>	<b>Membrane</b>
<b>LGMD2C</b>	<b>13q12 (1992)</b>	<b><math>\alpha</math>-sarcoglycan</b>	<b>35</b>	<b>Transmembrane</b>
<b>LGMD2D</b>	<b>17q11-q12</b>	<b><math>\beta</math>-sarcoglycan</b>	<b>50</b>	<b>Transmembrane</b>
<b>LGMD2E</b>	<b>4q12 (1995)</b>	<b><math>\gamma</math>-sarcoglycan</b>	<b>43</b>	<b>Transmembrane</b>
<b>LGMD2F</b>	<b>5q33-34(1996)</b>	<b><math>\delta</math>-sarcoglycan</b>	<b>35</b>	<b>Transmembrane</b>
<b>LGMD2G</b>	<b>17q11-q12</b>	<b>(1997) Telethonin</b>	<b>19</b>	<b>Sarcomeric</b>
<b>LGMD2H</b>	<b>9q31 (1998)</b>	<b>TRIM32</b>	<b>72</b>	
<b>LGMD2I</b>	<b>19q13 (2001)</b>	<b>FKRP</b>		
<b>LGMD2J</b>	<b>2q 24-31</b>	<b>Titin</b>	<b>4200</b>	<b>Sarcomeric</b>

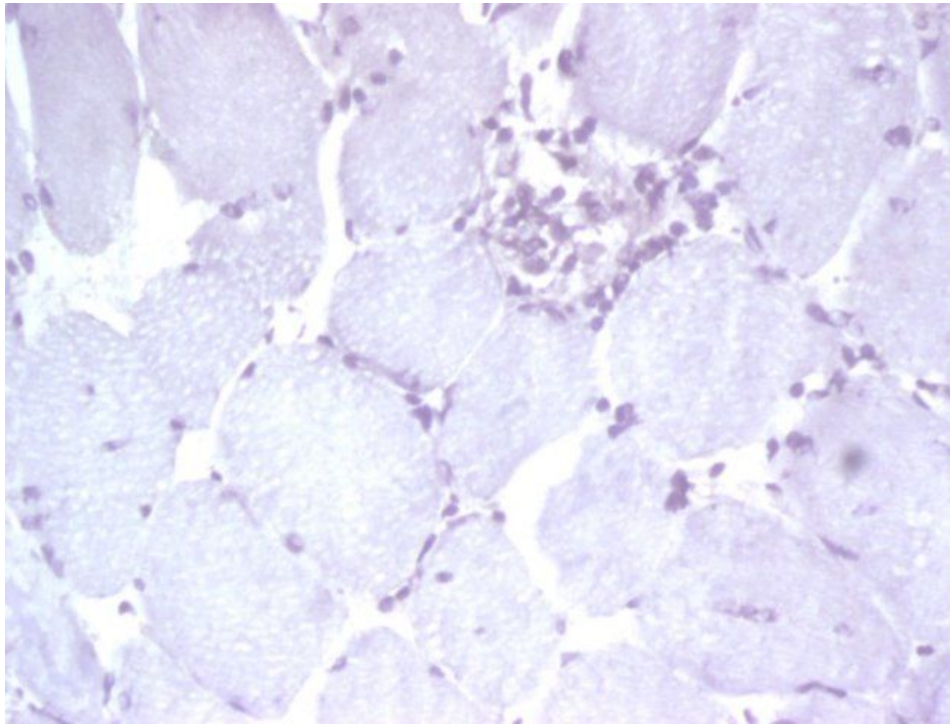
K,L , M.....

# Calpain LGMD2A



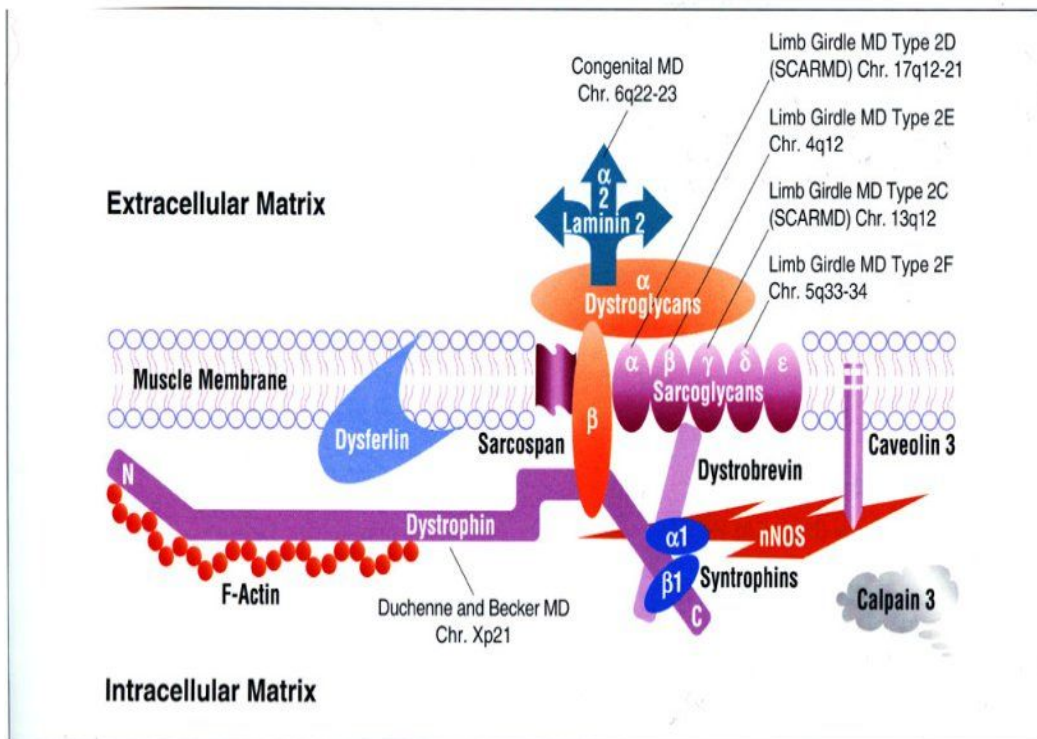
- Since 1998
- Western blot:
  - 70 Normal
  - 18 patients with complete loss calpain band ( 4 LGMD2I !)
  - 16 patients with weak calpain bands
- DNA analysis:
  - 4 patients with *CAPN3* mutation(s)

# LGMD 2B Dysferlinopathy



- 5 patients
- Dysferlin neg.

# Results (since 1994)



A schematic diagram illustrating the arrangement of dystrophin, dystrophin associated proteins and their link to different muscular dystrophies.

- Alfa-sarcoglycan (adhalin) (n=1)
- Beta-dystroglycan (n=1\*)
- Beta-sarcoglycan (2)
- Delta-sarcoglycan
- Gamma-sarcoglycan (n=1)

# Abnormal alfa- dystroglycan glycosylation... FKRP

- Abnormal glycosylation of  $\alpha$ -dystroglycan
  - Fukuyama MDC
  - Muscle-eye-brain-disease
  - *MDC 1C/LGMD 2I*
  - Walker-Warburg-syndrome
  - Others rare forms

Ullrich CMD

MDC1A

FCMD

MEB

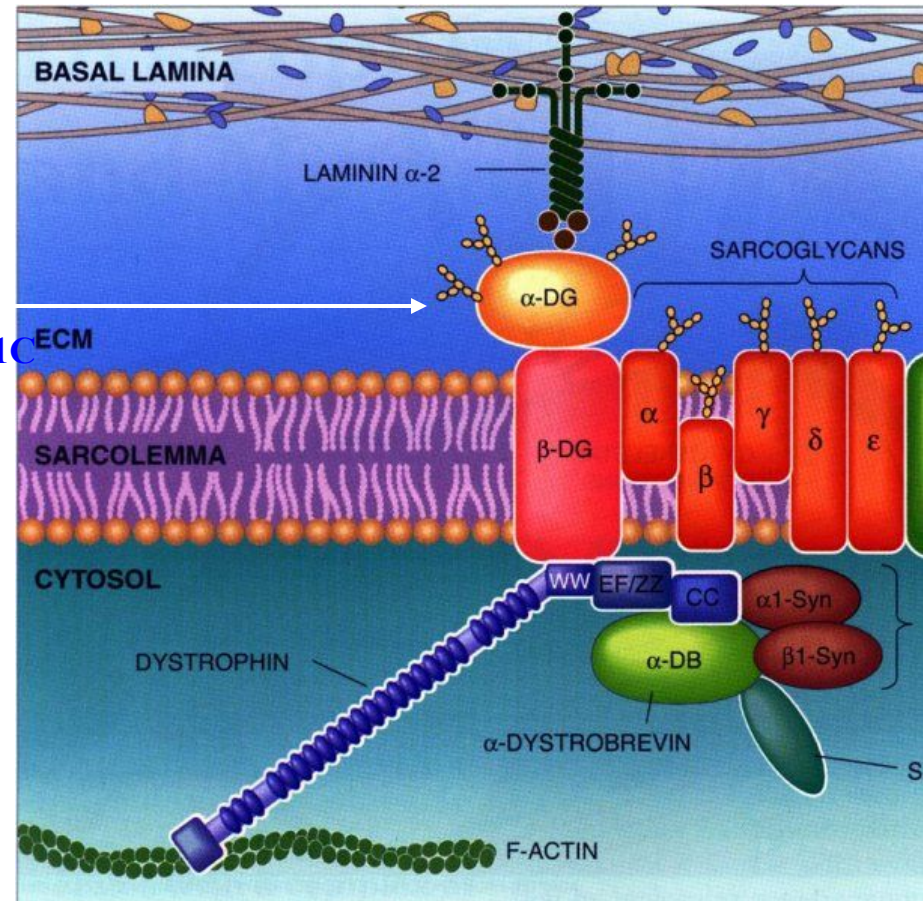
FKRP/MDC1C

MDC1B

WWS

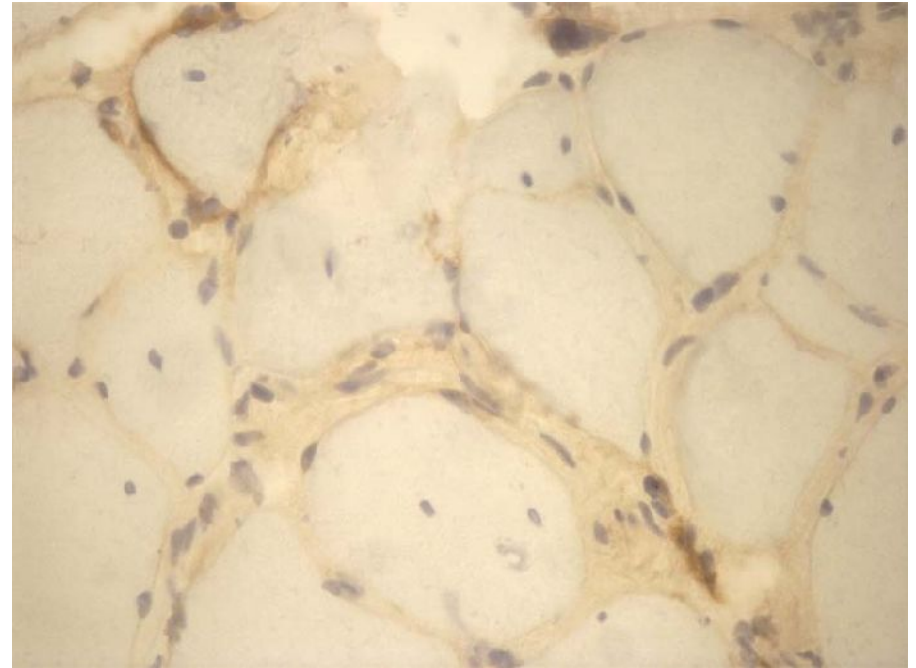
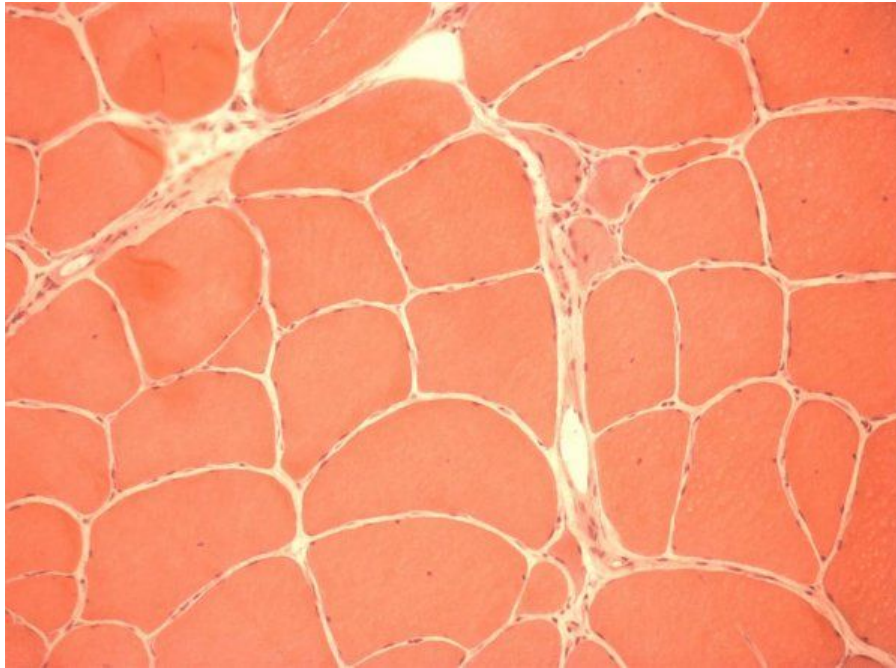
Intergrin- $\alpha 7$   
Deficiency

DMD





# Expression of $\alpha$ -Dystroglycan



$\alpha$  Dys



# FKRP analysis ( LGMD 2I)

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- DNA analysis for FKRP:
  - Loss of  $\alpha$ -Dystroglycan expression
    - All patients with weak or missing bands for calpain
    - All patients with partial merosin staining
    - Patients with non-conclusive muscle dystrophy
    - Patients with non-conclusive inflammatory myopathy



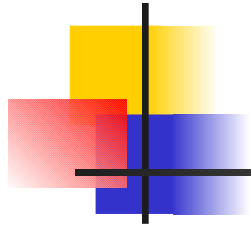
# FKRP LGMD 2I

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- Pr. 23.10.08

- Christoffer Jonsrud: Updated results from
- DNA analysis of FKRP in Norway
- Prevalence of FKRP in Norway
- Prevalence of LGMD in Norway ??

# LGMD2I: *FKRP*; status pr. 20/10-08



No#	Nucleotide change Allele 1	Amino Acid substitution	Nucleotide change Allele 2	Amino acid substitution
76	c.826C>A	p.Leu276Ile	c.826C>A	p.Leu276Ile
6	c.826C>A	p.Leu276Ile	c.962C>A	p.Ala321Glu
3	c.826C>A	p.Leu276Ile	c.899T>C	p.Val300Ala
1	c.826C>A	p.Leu276Ile	<b>c.913C&gt;T *</b>	<b>p.Pro305Ser</b>
1	c.826C>A	p.Leu276Ile	<b>c.1323T&gt;G *</b>	<b>p.Phe441Leu</b>
<b>1</b>	<b>c.170_189del20</b>	<b>p.Glu57AlafsX68</b>	<b>c.899T&gt;C</b>	<b>p.Val300Ala</b>

\* denotes novel, putative disease-causing mutations. c.913T and c.1323G were absent from 100 normal chromosomes. Both p.Pro305 and p.Phe441 are conserved in corresponding proteins from mouse, rat, cow, dog, chicken and zebrafish.



# LGMD2I: *FKRP* ; status pr. 20/10-08

## Sequencing

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- ~200 Normal sequences (diff. Diagnoses?)
- 76 homozygotes (59 families)
- 12 compound heterozygotes (10 fam)
  - 88 confirmed molecularly
  - 1/54000 with LGMD2I
  - Aprx. 1/120 heterozygous carriers
- 6 heterozygous index patients ??
  - Exon 1,2,3 ? /c.DNA analysis??/ random?

# CAPN3



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- (all? with apparent calpain defect judged by WB)
- 21 unrelated families; 9 patients in 7 families
  - Sequencing all 24 exons, and MLPA:
  - Missense mutations, 1 "in frame" deletion
  - +Several known/most probable polymorphisms
- Compound heterozygotes:
  - **c.304C>T (p.P102S)** / c.2393C>A (p.A798E) (Leiden; 3 times)
  - c.580delT (fs) / c.802-9G>A (Leiden)
  - c.1345A>G (p.Ser479Gly) / c.1355-10G>A (UV) (Leiden) (from USA)
- Heterozygotes:
  - c.319G>A (p.Gly107Lys) (Leiden; 3 times)
  - c.643-663del21 (Leiden; 5 times)
  - **c.304C>T (p.P102S)** (2 families / 3 affected) (Leiden; once)