

# New phenotypes of autophagic myopathies

Sydney Neurophysiology Workshop  
Park Hyatt

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# Two types of vacuoles in autophagic vacuolar myopathies

- Membrane bound autophagic vacuoles
  - Formed by lysosomal structures
    - contain lysosomal enzymes
  - Most lined with sarcolemmal proteins
  - LAMP2 defect Danon disease, VMA21 defect XMEA, some of the vacuoles in AMD Pompe
- Non-membrane bound autophagic vacuoles
  - Most of the rimmed vacuoles
  - No sarcolemmal features
  - Contain more autophagosomal vesicles than lysosomes
  - s-IBM, many distal myopathies, myofibrillar myopathies, some vacuoles in AMD Pompe

# X-Linked Myopathy with Excessive Autophagy: A New Hereditary Muscle Disease

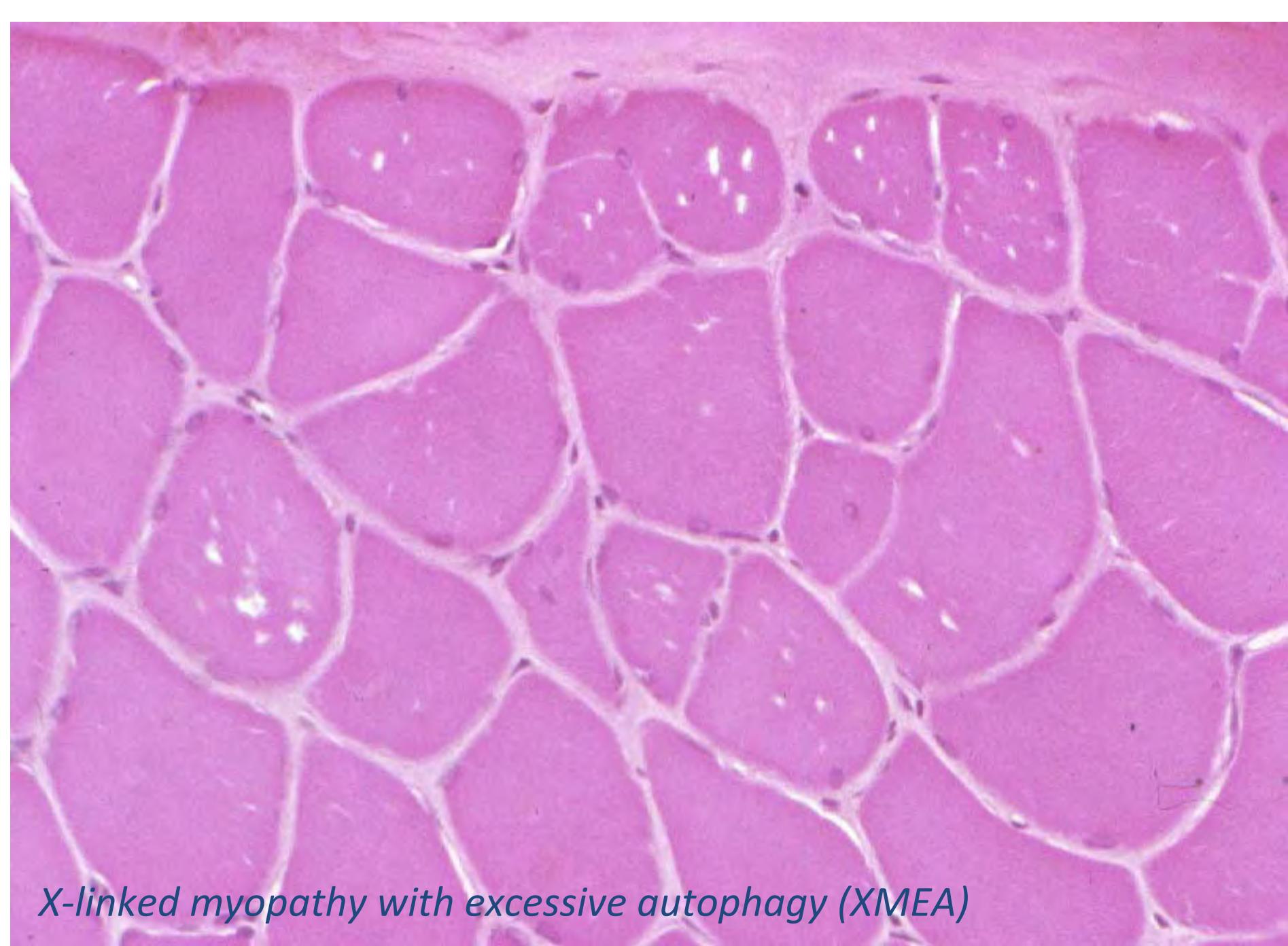
Kalimo H, Savontaus M-L, Lang H, Paljärvi L, Sonninen V, Dean PB, Katevuo K, Salminen A. X-linked myopathy with excessive autophagy: a new hereditary muscle disease. *Ann Neurol* 1988;23:258–265

ORIGINAL PAPER

Acta Neuropathologica 2013; 125(3):439-57

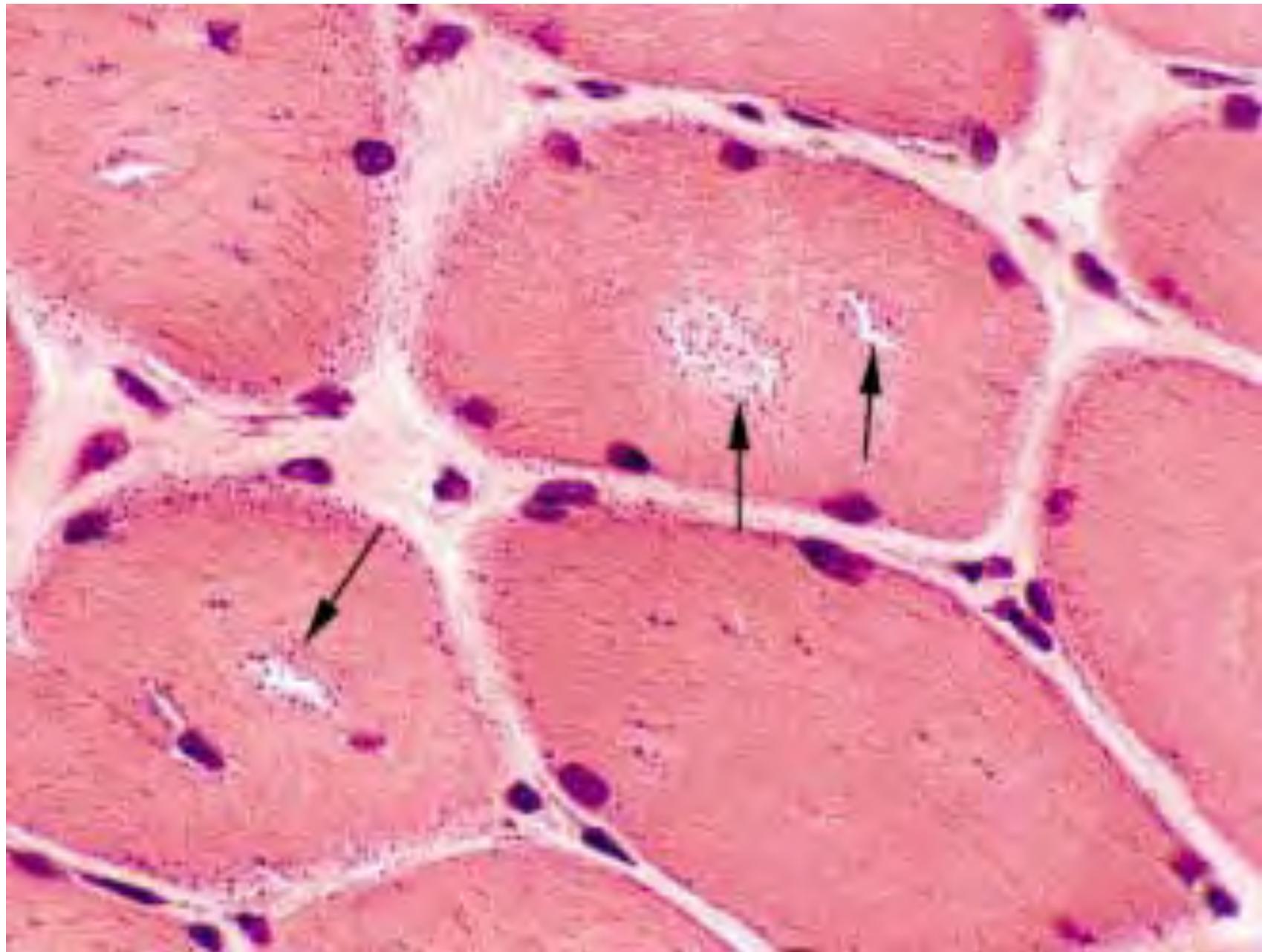
## VMA21 deficiency prevents vacuolar ATPase assembly and causes autophagic vacuolar myopathy

Nivetha Ramachandran · Iulia Munteanu · Peixiang Wang · Alessandra Ruggieri · Jennifer J. Rilstone · Nyrie Israelian · Taline Naranian · Paul Paroutis · Ray Guo · Zhi-Ping Ren · Ichizo Nishino · Brigitte Chabrol · Jean-Francois Pellissier · Carlo Minetti · Bjarne Udd · Michel Fardeau · Chetankumar S. Tailor · Don J. Mahuran · John T. Kissel · Hannu Kalimo · Nicolas Levy · Morris F. Manolson · Cameron A. Ackerley · Berge A. Minassian

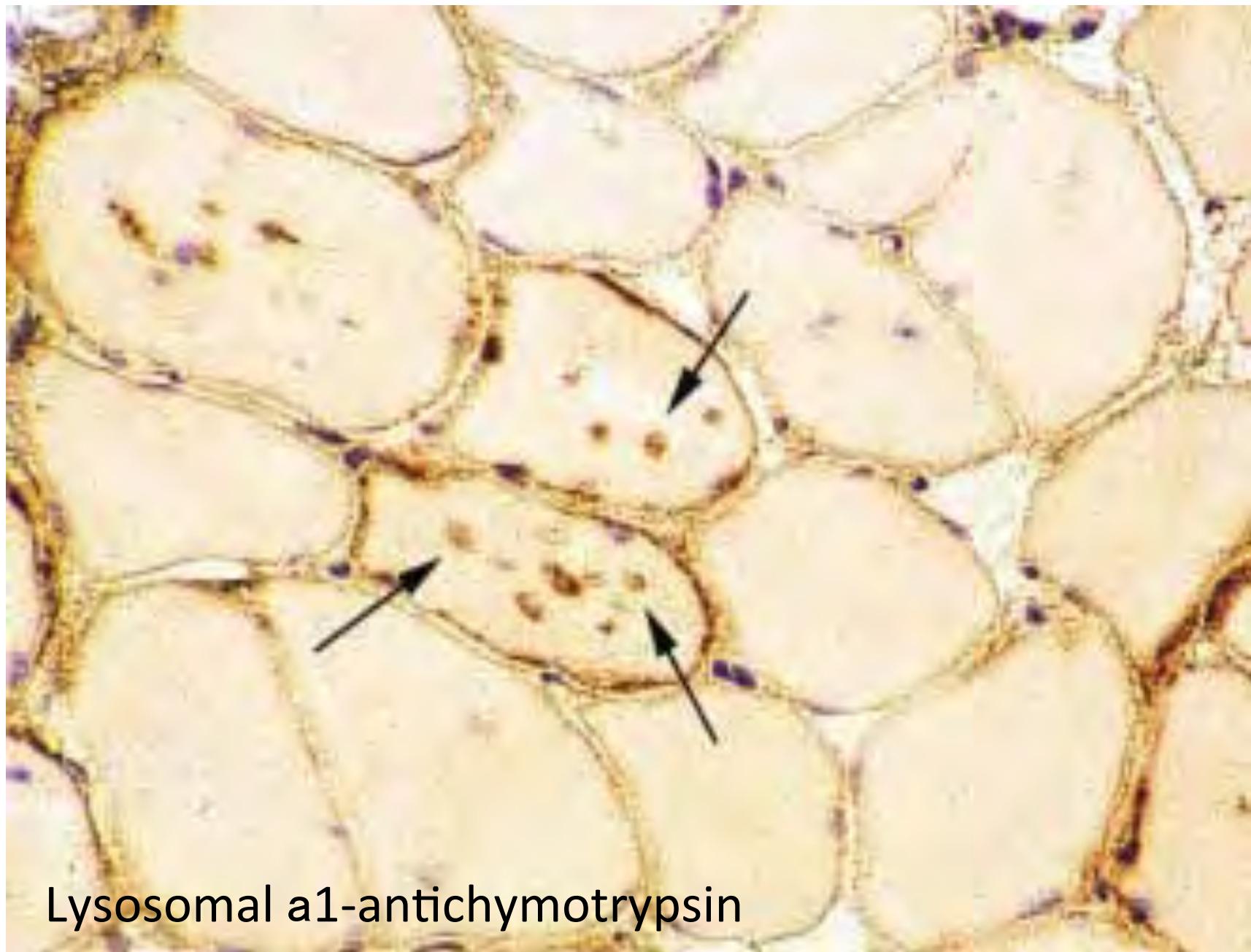


*X-linked myopathy with excessive autophagy (XMEA)*

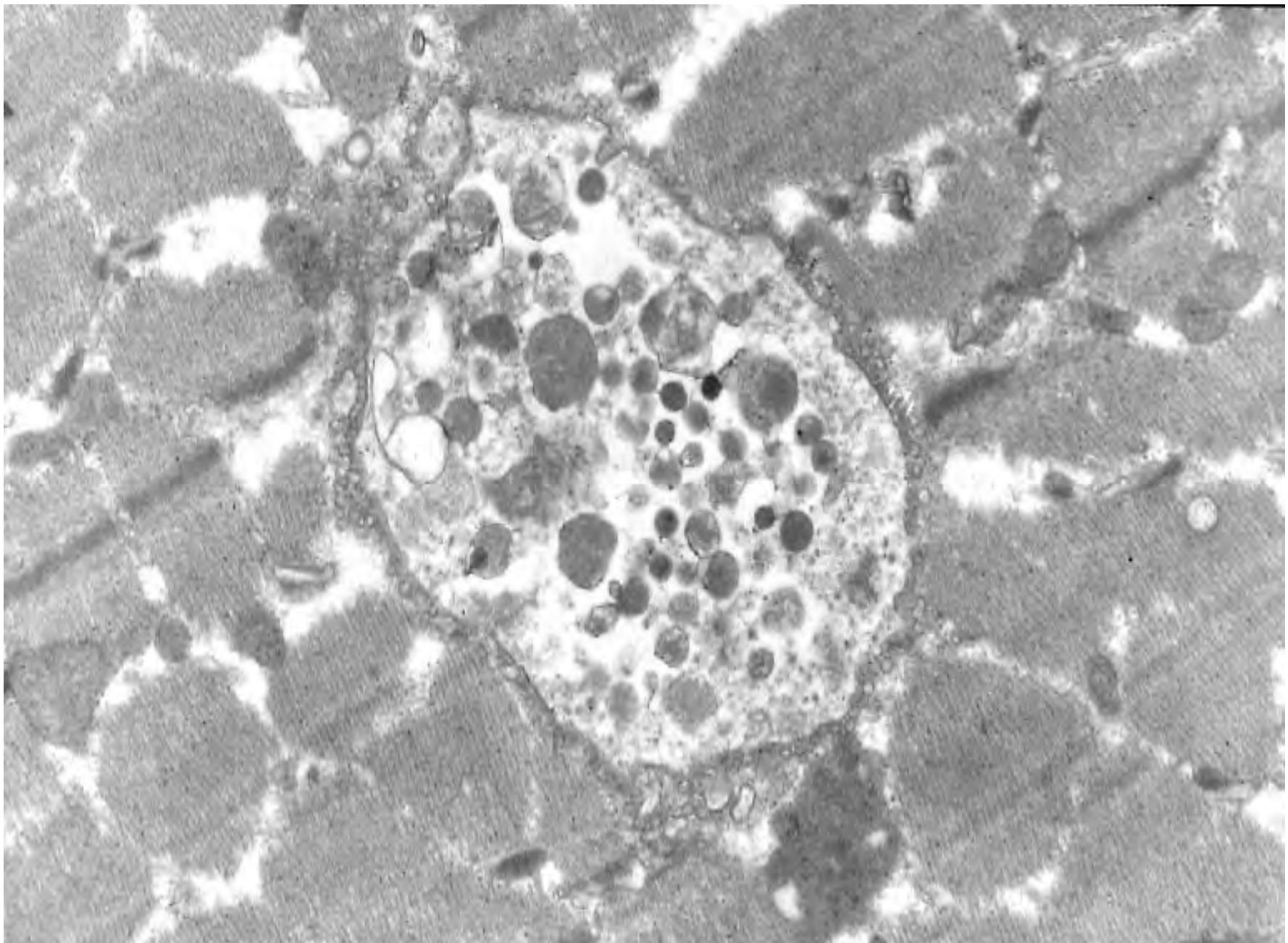
## *X-linked myopathy with excessive autophagy (XMEA)*



**XMEA**

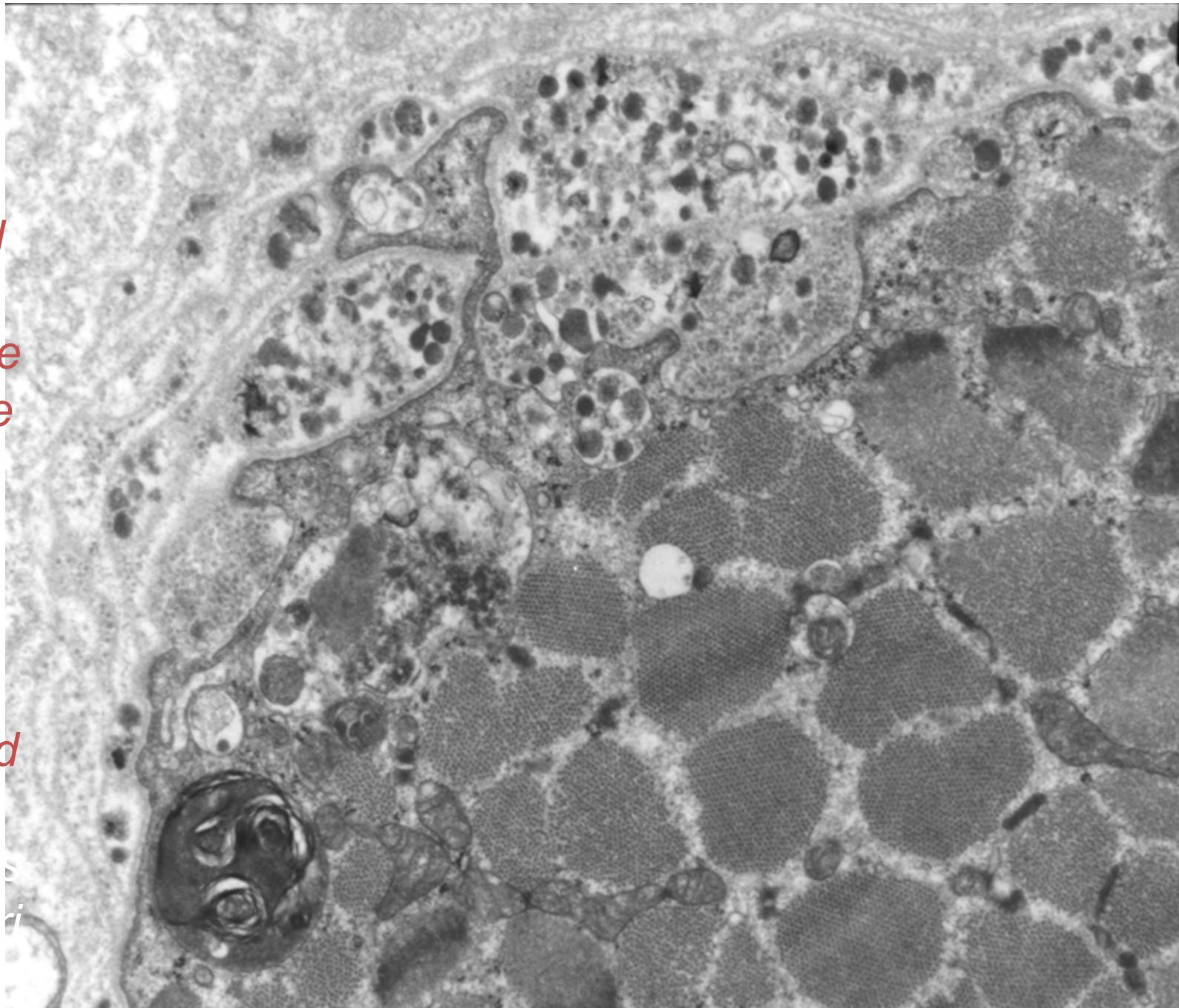


*X-linked myopathy with excessive autophagy (XMEA)  
membrane bound vacuoles*



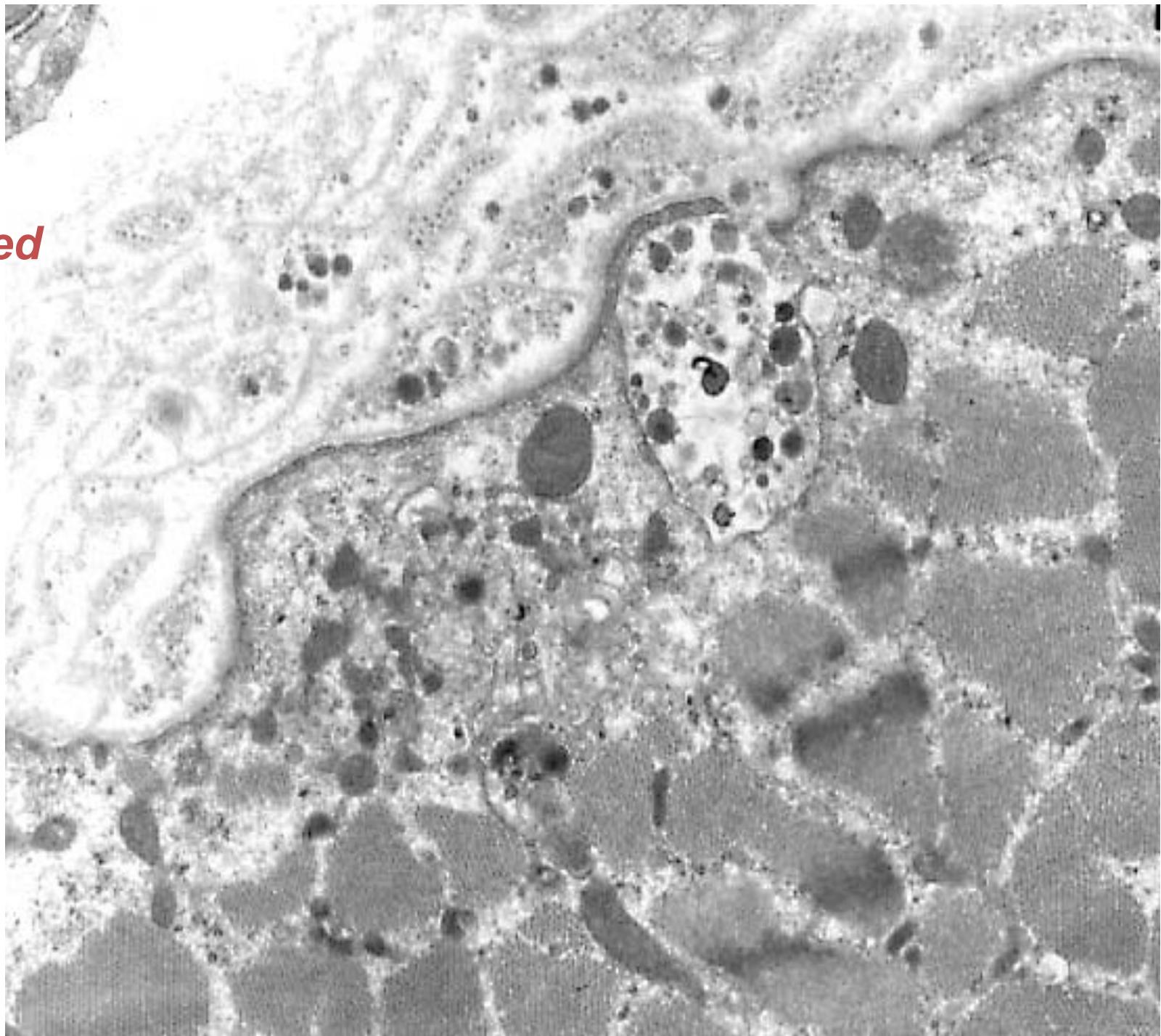
XMEA:

*Lysosomal  
vacuoles  
accumulate  
as they are  
not  
normally  
processed  
and  
instead  
exocytosed*



XMEA:

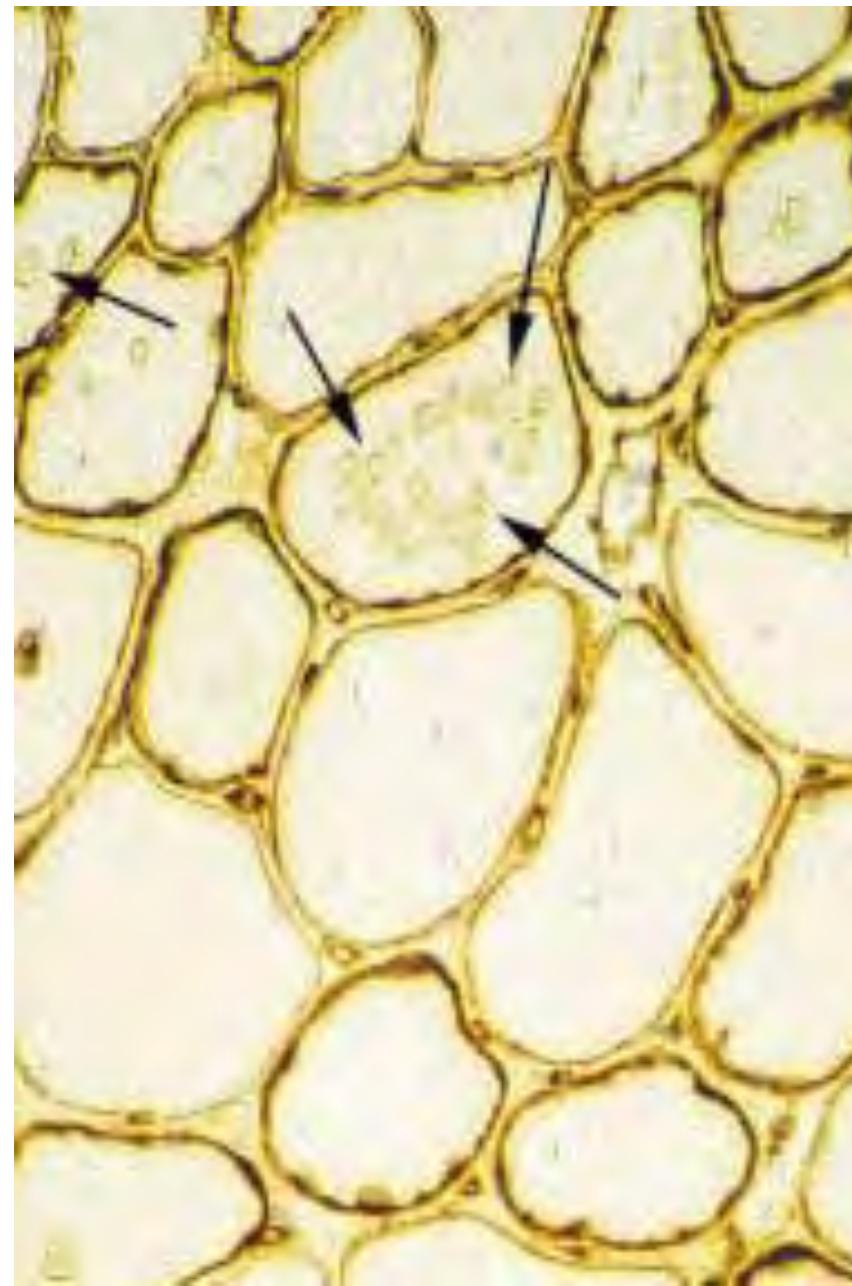
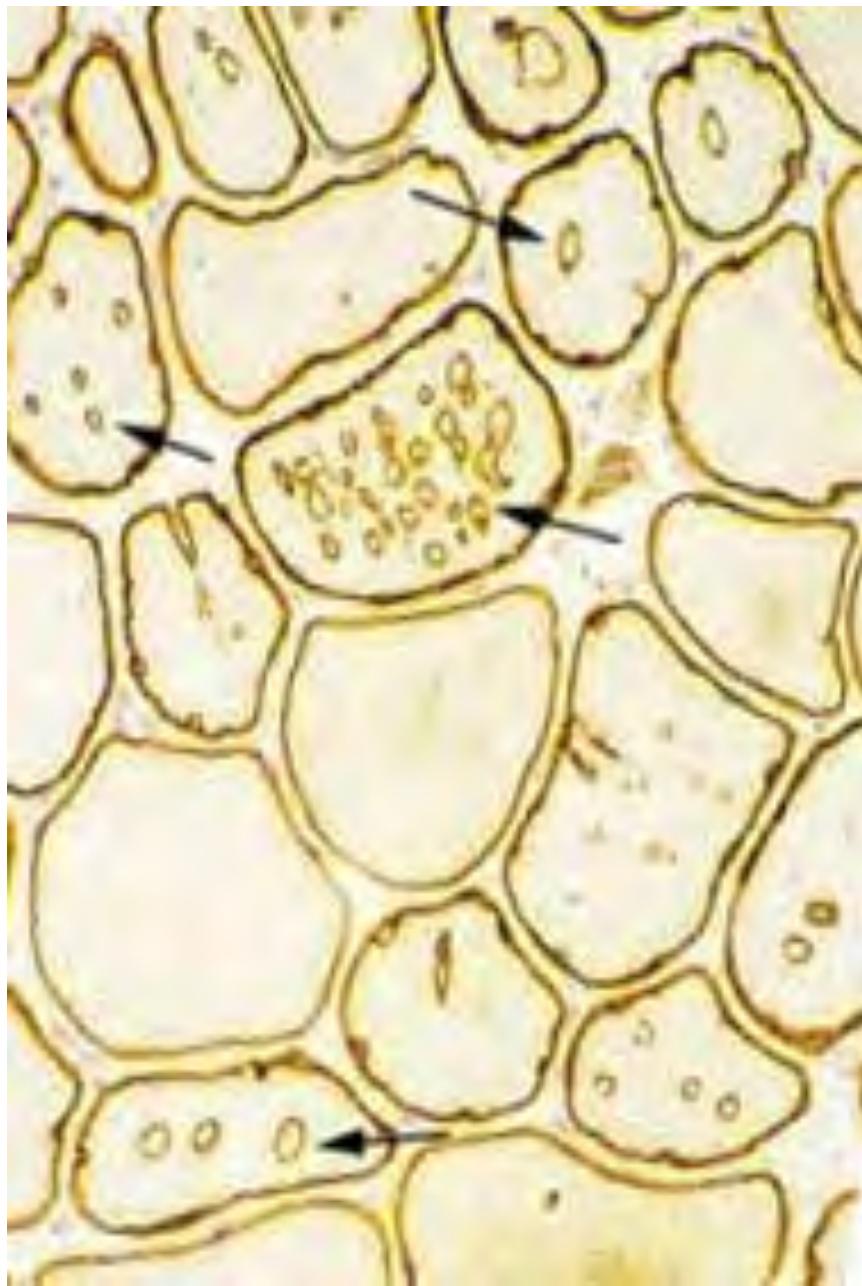
*exocytosed  
material  
induce  
multiple  
layers of  
the basal  
lamina*



*Dys-2*

*XMEA*

*merosin*



# XMEA molecular pathology

- VMA21 is an essential assembly chaperone of the vacuolar ATPase (V-ATPase), the principal mammalian proton pump complex for lysosome acidification.
- Decreased VMA21 raises lysosomal pH which reduces lysosomal degradative capacity and blocks autophagy.
- > resulting in proliferation of large and ineffective autolysosomes that engulf sections of cytoplasm, merge, and vacuolate the cell

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  - s-IBM, many distal myopathies, myofibrillar myopathies, some vacuoles in AMD Pompe

# Rimmed vacuoles in muscle pathology: signs of altered autophagy

- Acta Neuropathol. 1980;51(3):229-35. **Rimmed vacuoles.**
- Fukuhara N, Kumamoto T, Tsubaki T.
- Rimmed vacuoles (Dubowitz and Brooke 1973) have been found in 12 cases with various neuromuscular diseases and are **considered to be autophagic** in nature. They consisted of multilaminated **membranous structures** accompanied by **glycogen granules, dense bodies, and amorphous, granular, and fibrillar material.**
- The "lined vacuoles" described by Carpenter et al. (1978) in inclusion body myositis closely agree with the rimmed vacuoles in respect of histochemical and ultrastructural features.

Nonaka et al. Familial distal myopathy with rimmed vacuole and lamellar (myeloid) body formation. J Neurol Sci. 1981.

- Three cases from 2 families had muscle weakness with predilection for distal extremities, predominantly affecting the tibialis anterior muscles, and onset in early adulthood.
- The **striking finding in their muscle biopsies was the presence of "rimmed" vacuoles** which had **acid phosphatase-positive** autophagic activity and which contained numerous concentric lamellar bodies in various forms (myeloid and cabbage bodies).
- **Despite rapid clinical progression, not only necrotic fibers with phagocytosis, as seen in Duchenne dystrophy, but also evidence of regeneration were virtually absent.**
- Continuous destruction of myofibrils by activation of certain **lysosomal proteolytic enzymes might be responsible for the production of atrophic fibers.**

# Rimmed vacuoles in muscle pathology: signs of altered autophagy

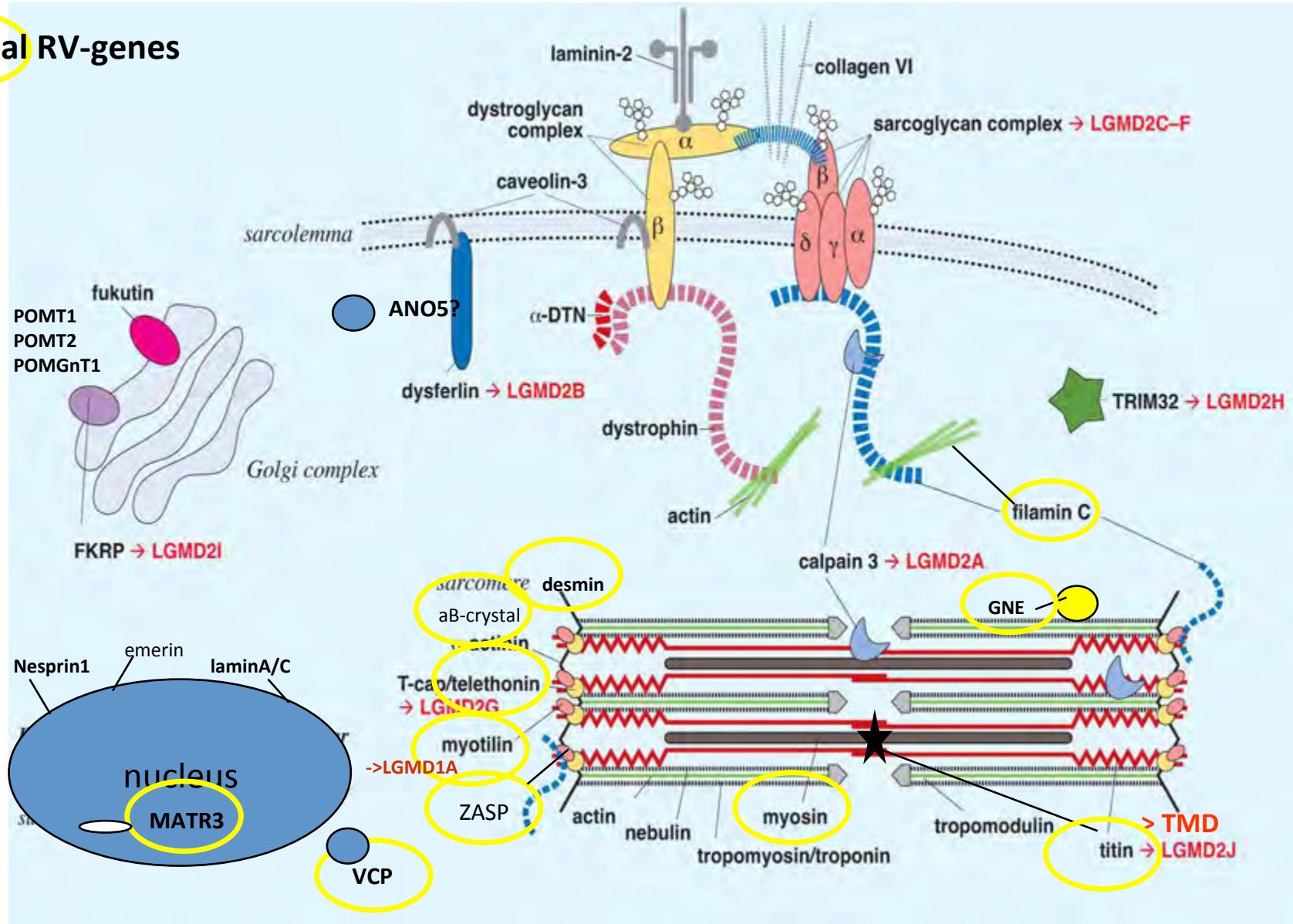
- Rimmed Vacuolar myopathies
  - Titinopathies
    - Distal TMD, HMERF (A150, Mex1), proximal (A140, Mex1)
  - Distal myopathies
    - GNE-myopathy, Welander, MPD3, MATR3, VCP, OPDM
  - Myofibrillar myopathies
    - MYOT, DES, ZASP, CRYAB, FLNC, FHL-1, PLEC1
  - Other myopathies
    - OPM, LGMD1D, VCP, Pompe, AD-MYH2, MSS, LGMD1G
- Sporadic s-IBM: remains a big enigma
  - RV- myopathy + mitochondrial+ inflammatory
- Large number of unknown diseases
  - 1-2 occasional fibers in many diseases even neurogenic

# Our interest in the rimmed vacuolar myopathies: the Distal Myopathies

- AD-late onset
    - \* Welander (TIA1 mutation)
    - \* TMD Titinopathy (Udd)
    - \* Myotilinopathy
    - \* ZASPopathy (Markesberry-Griggs)
    - \* Matrin3 defect VCPDM (Feit)
    - \* VCP-mutated distal myopathy
    - \* Thenar distal myopathy (Edström)
    - \* Oculopharyngeal distal OPDM
  - AD-adult onset
    - \* Desminopathy
    - \* Finnish-MPD3 (Mahjneh 2003)
    - \* Italian 19p13 (Servidei 1999)
    - Distal ABD-Filaminopathy (Duff 2010)
  - AD-early onset
    - Myosinopathy MYH7 (Laing)
    - KLHL9 mutated distal myopathy
  - AR-early onset
    - Distal nebulin myopathy
    - \* Oculopharyngeal distal myopathy OPDM
  - AR-early adult onset
    - Dysferlinopathy (Miyoshi)
    - Distal Anoctaminopathy
    - \* GNE-myopathy DMRV (Nonaka)
    - \* Distal Titinopathy (Udd)
  - AR-adult onset
    - Miyoshi-like non-DYSF/ANO5
    - \* Distal Titinopathy (Udd)
- \* Rimmed vacuolar pathology

# Proteins in distal dystrophies with RV-pathology

## Distal RV-genes



# The example of DNAJB6 mutated LGMD1D

LETTERS

nature  
genetics

## Mutations affecting the cytoplasmic functions of the co-chaperone DNAJB6 cause limb-girdle muscular dystrophy

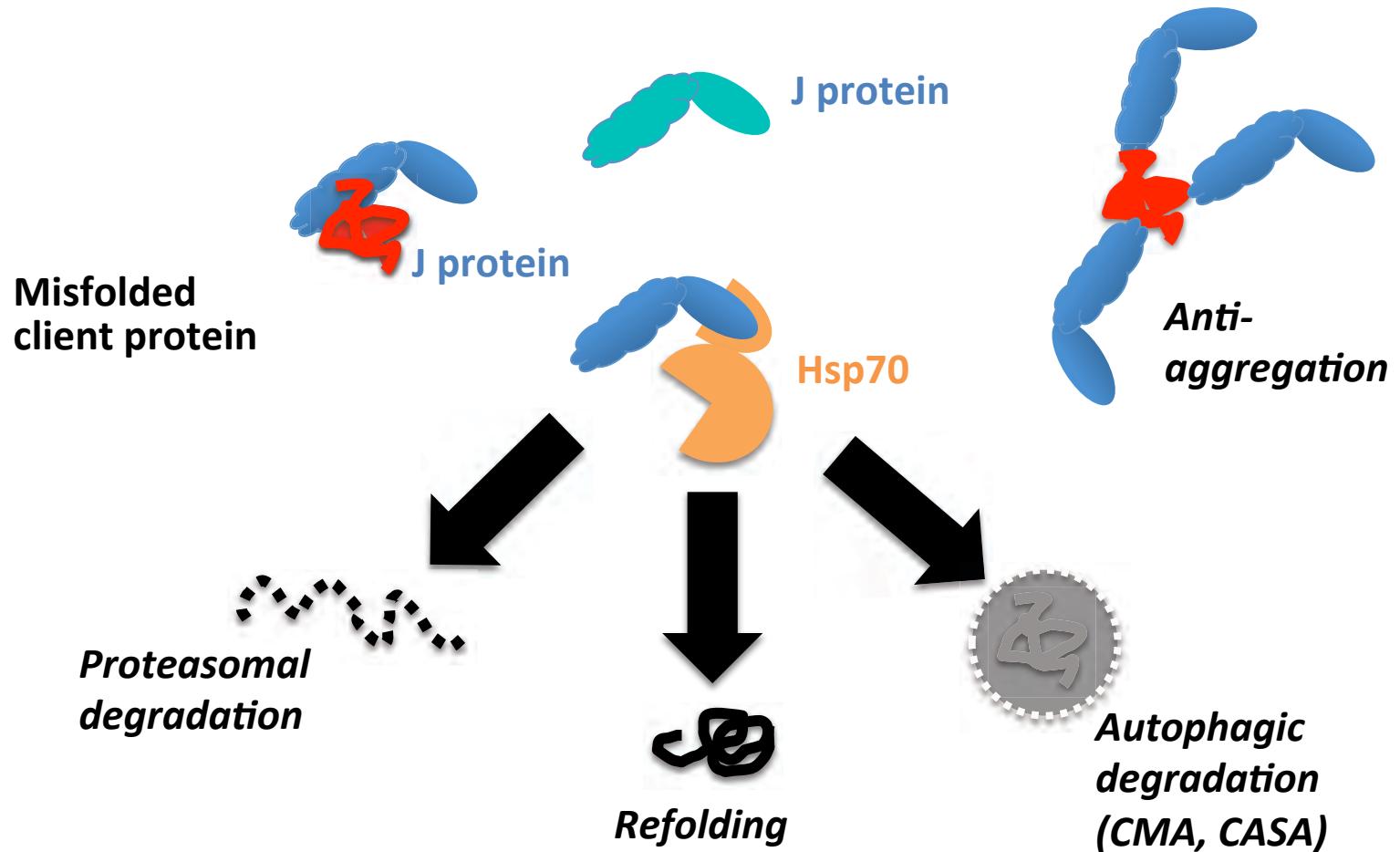
Jaakko Sarparanta<sup>1,13</sup>, Per Harald Jonson<sup>1,13</sup>, Christelle Golzio<sup>2,13</sup>, Satu Sandell<sup>3,4</sup>, Helena Luque<sup>1</sup>, Mark Screen<sup>1</sup>, Kristin McDonald<sup>5,6</sup>, Jeffrey M Stajich<sup>5</sup>, Ibrahim Mahjneh<sup>7,8</sup>, Anna Vihola<sup>1</sup>, Olayinka Raheem<sup>3</sup>, Sini Penttilä<sup>3</sup>, Sara Lehtinen<sup>1</sup>, Sanna Huovinen<sup>3,9</sup>, Johanna Palmio<sup>3</sup>, Giorgio Tasca<sup>10</sup>, Enzo Ricci<sup>11</sup>, Peter Hackman<sup>1</sup>, Michael Hauser<sup>5,6</sup>, Nicholas Katsanis<sup>2</sup> & Bjarne Udd<sup>1,3,12</sup>

Nat Genet 2012; 44: 450-5, S1-2.

# DNAJB6 functions

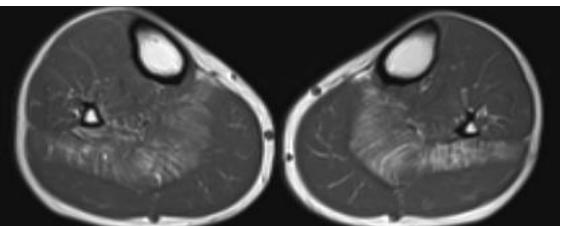
- Brain enriched co-chaperone of the J protein (Hsp40) family
  - >> no CNS phenotype in LGMD1D?
- J proteins interact with the Hsp70 (HSPA) chaperones
  - stabilize Hsp70–substrate interactions and stimulate ATPase activity
  - provide the Hsp70 machinery with functional specificity
  - DNAJB6 known ligand of HSPA8 (Hsc70)
- Muscle expression low, not previously detailed
- KO -/- lethal E8,5 ; chorioallantoic fusion defect
- Short isoform cytoplasmic, long isoform nuclear
  - Long isoform lost in breast cancer
  - Short isoform shown to reduce Huntingtin aggregates

# Functions of J proteins and Hsp70s

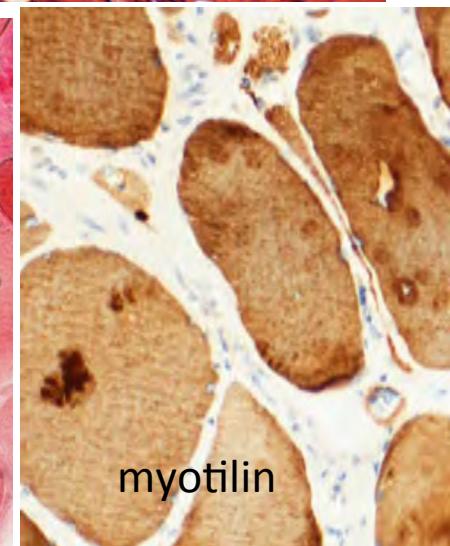
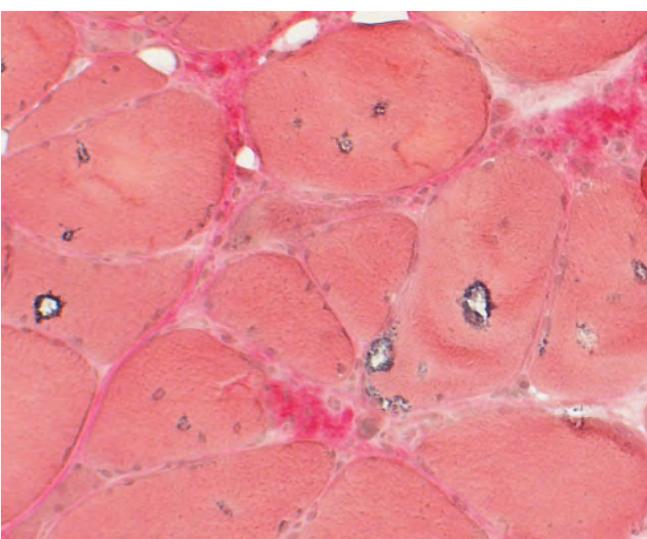
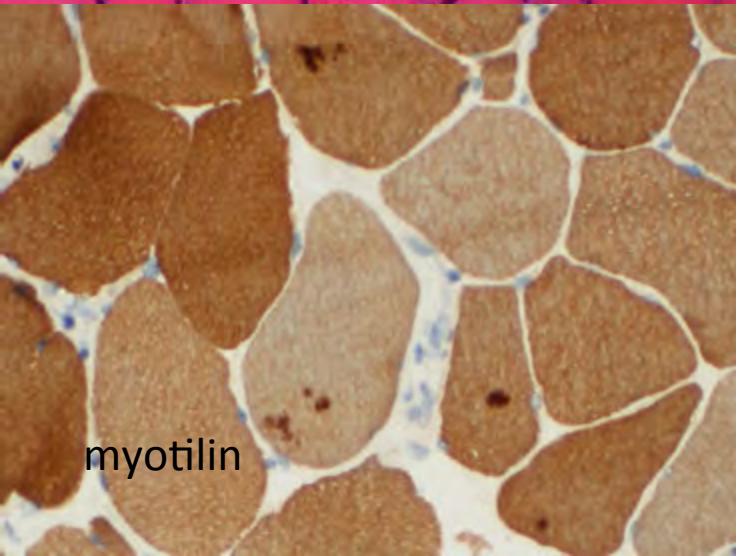
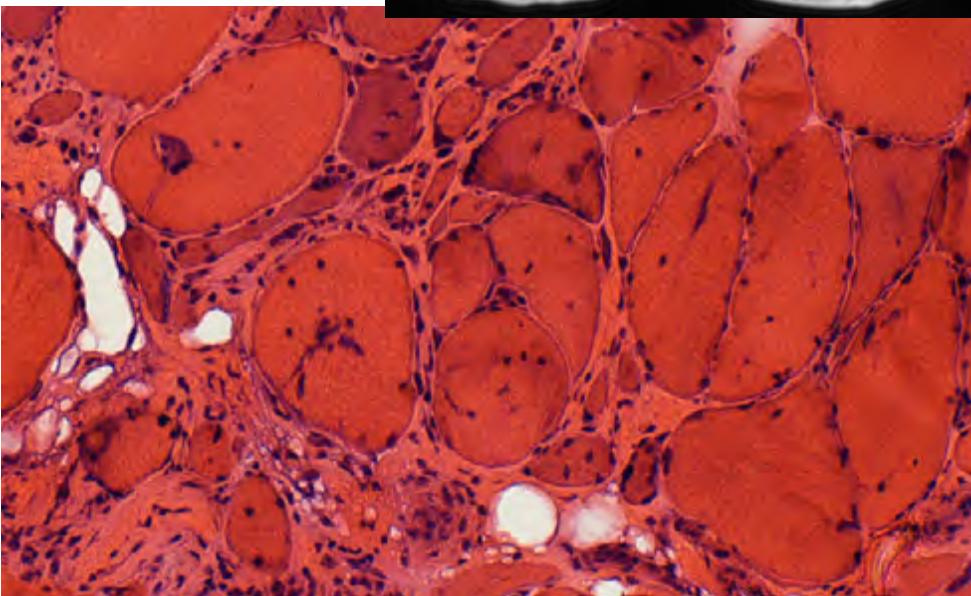
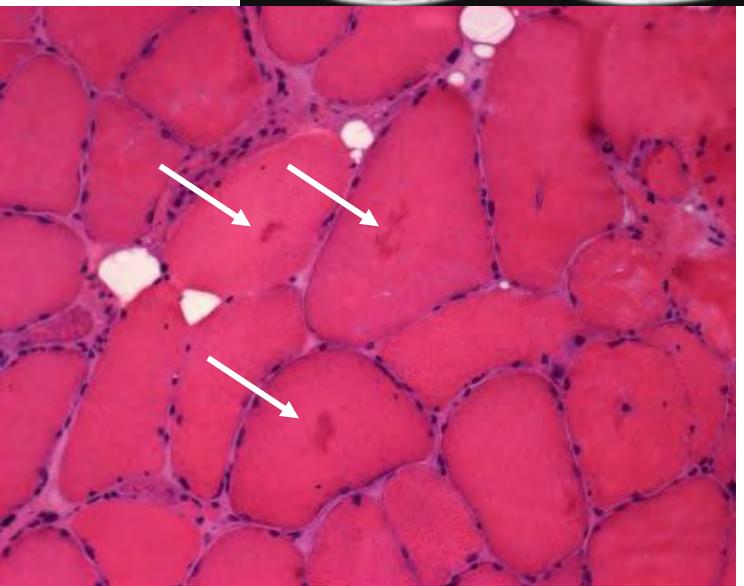
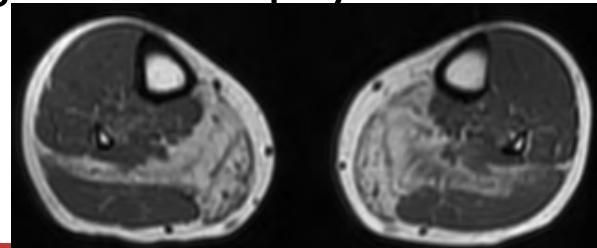


# LGMD1D muscle pathology: 2 brothers medial gastroc biopsy

age 40  
early  
symptoms



the  
brother at  
age 50

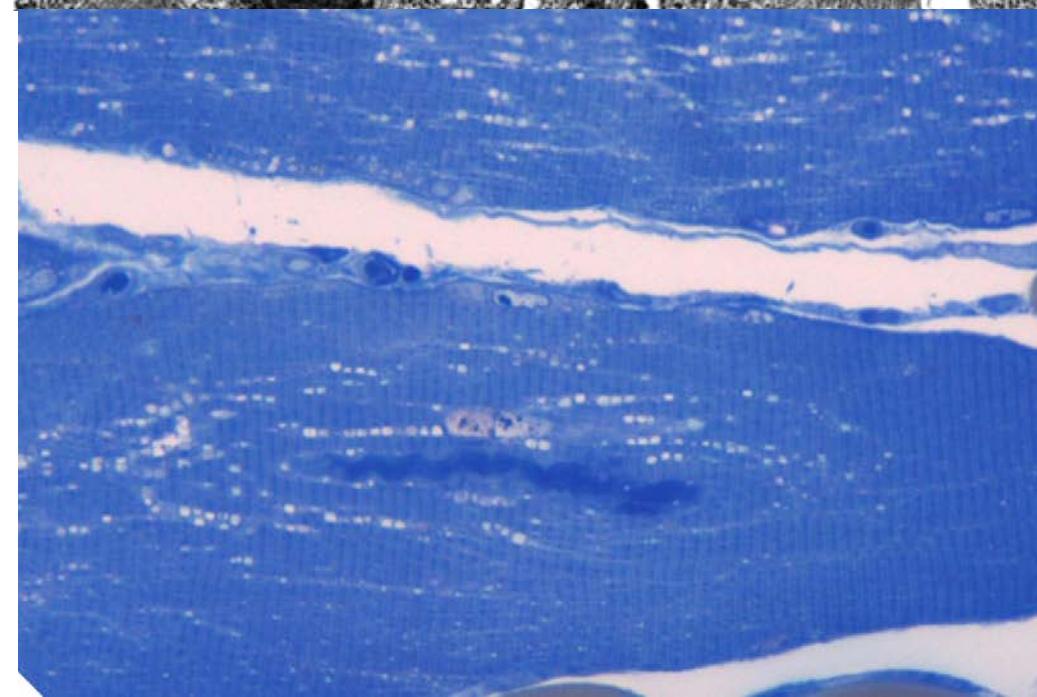
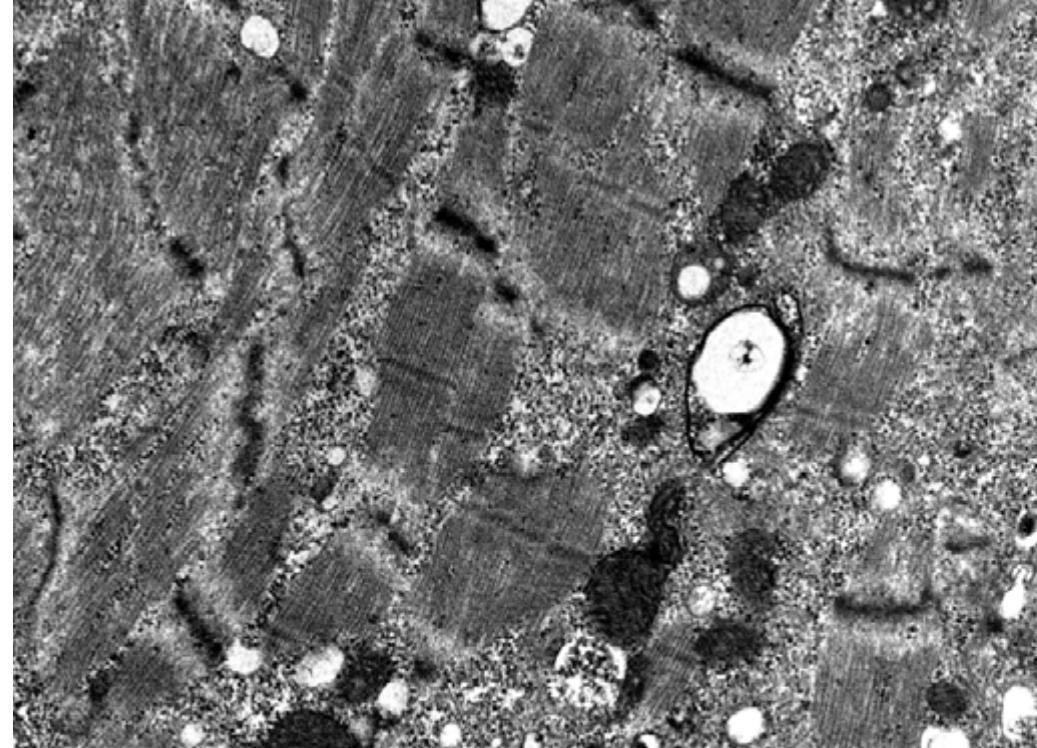
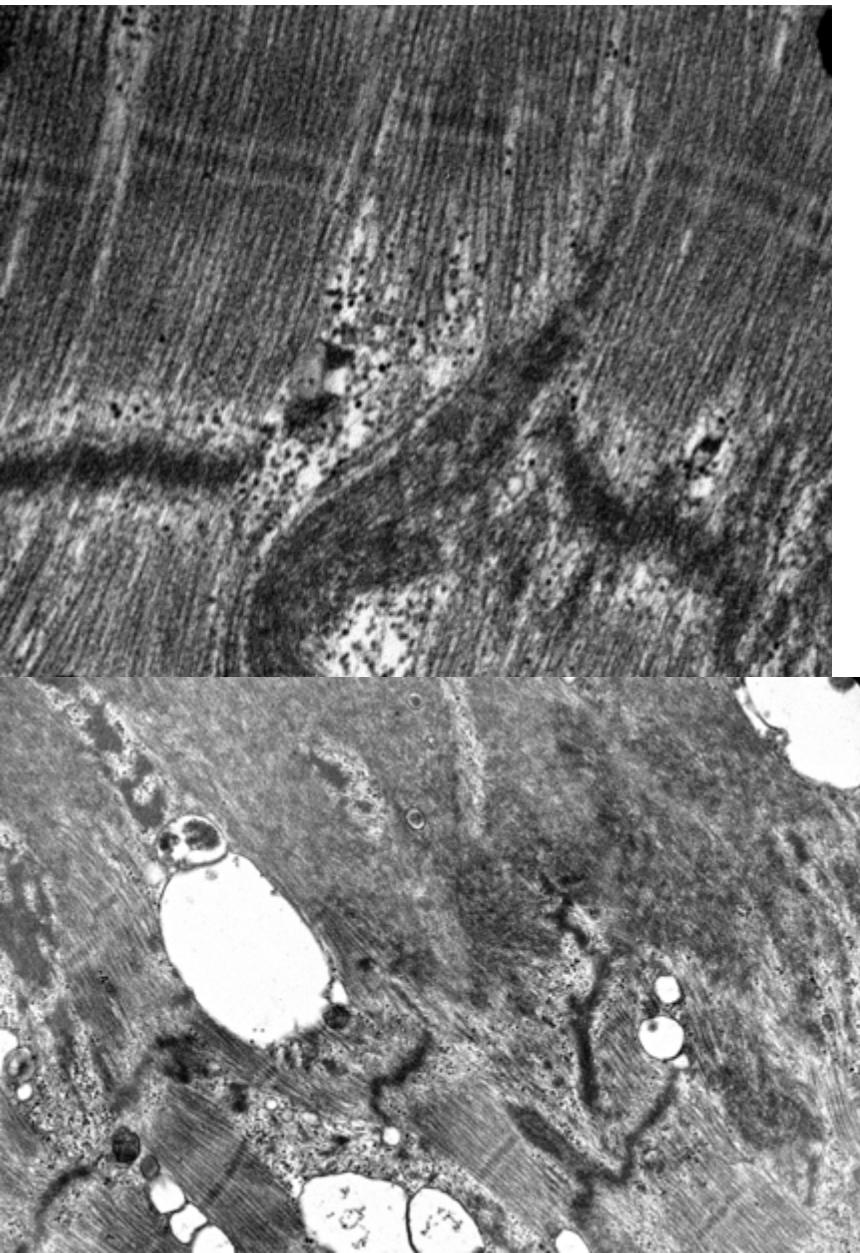


myotilin

myotilin

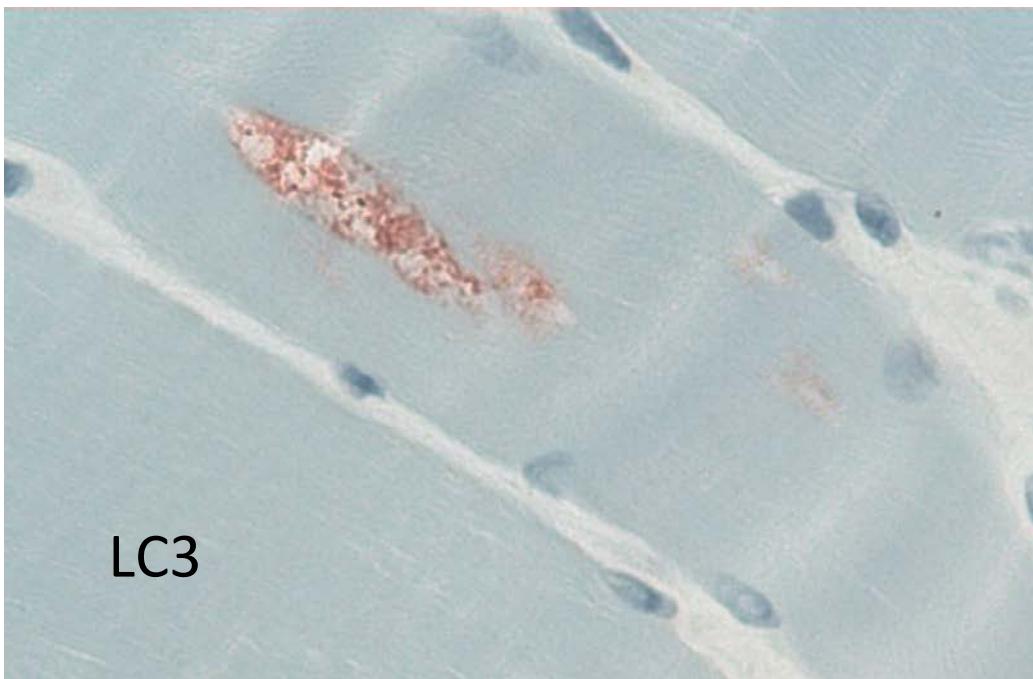
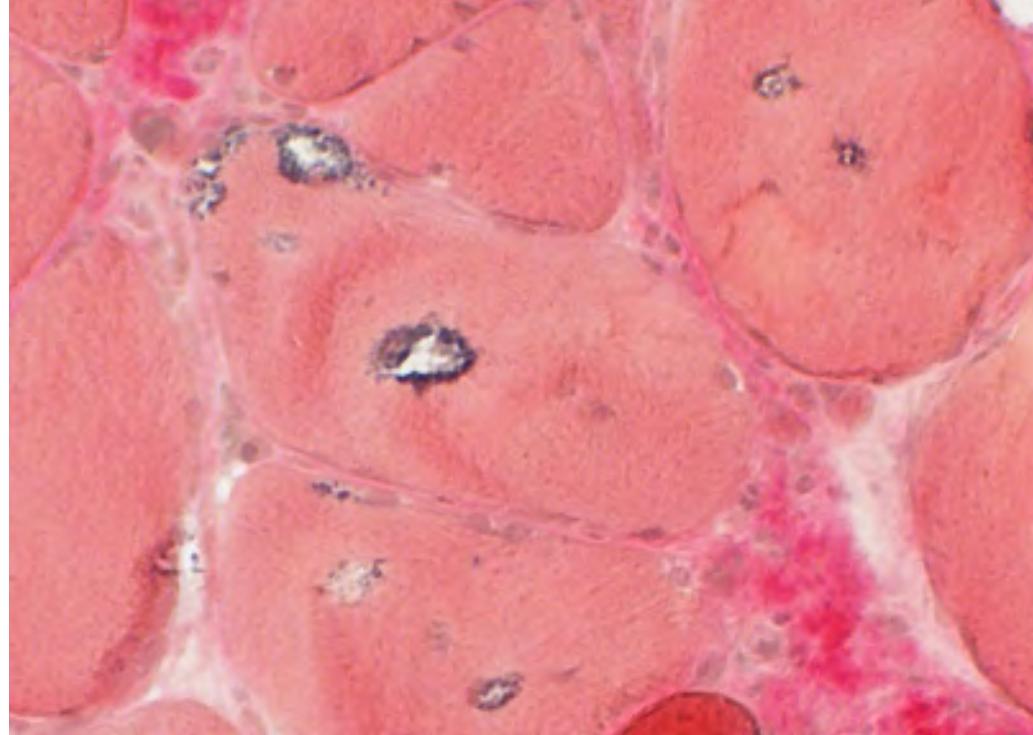
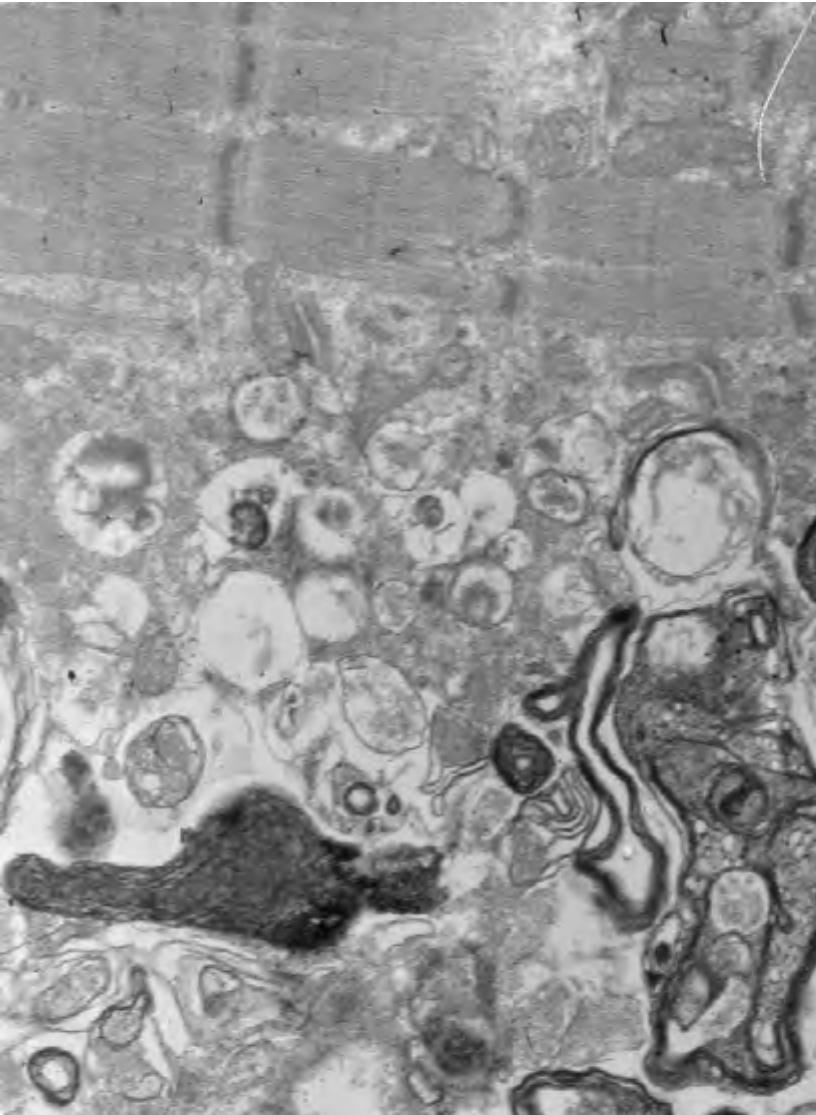
# LGMD1D

- Myofibrillar aggregates



# LGMD1D

- Rimmed vacuoles with LC3, SMI-31, p62, TDP-43



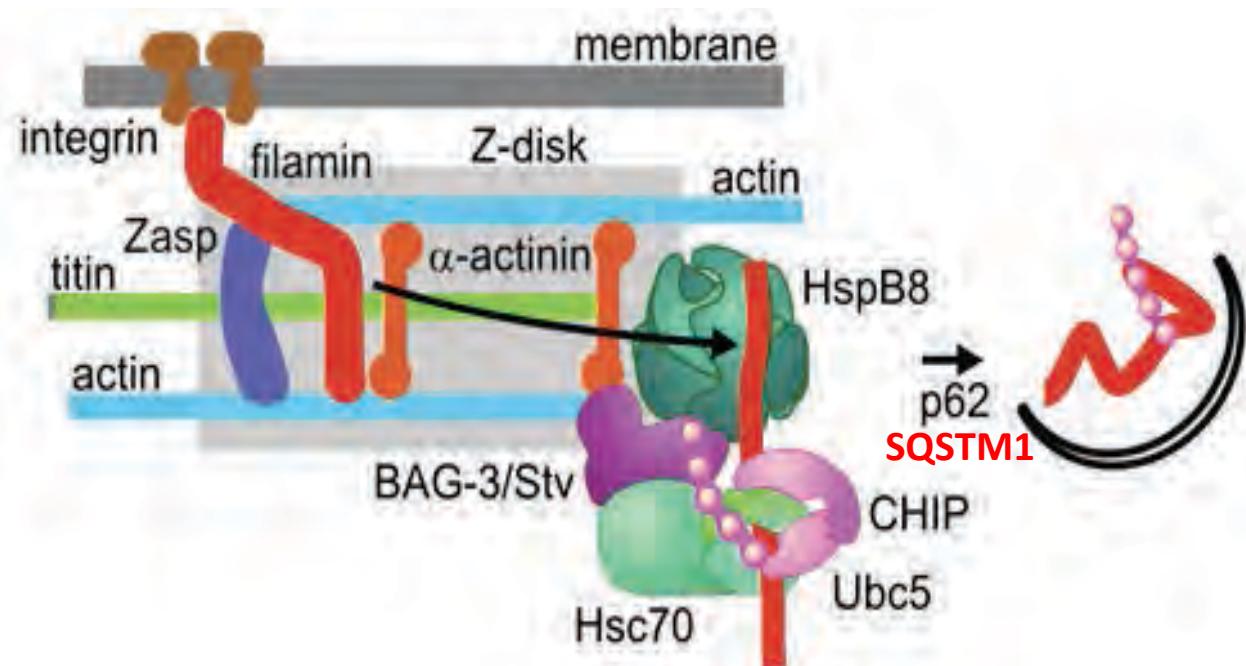
LC3

# Association with CASA complex:

- DNAJB6 ligand of all partners

- Chaperone-assisted selective autophagy (CASA) was recently reported to be important for the maintenance of the sarcomeric structure.
- Mediated by a Z-disc associated protein complex

- HSPA8 (Hsc70)
- BAG3
- HSPB8 (Hsp22)
- STUB1 (CHIP)



# Lessons from LGMD1D

1. DNAJB6 interacts with the co-chaperone BAG3 and other components of the CASA autophagy pathway
  2. Defect chaperonal function/antiaggregation of DNAJB6 and CASA complex cause insufficient maintenance of sarcomeric proteins over the years
    - Defect proteins are not correctly refolded or delivered to degradation
    - misfolded proteins aggregate -> defect maintenance of normal Z-disk and myofibrillar structure
  3. -> autophagy is induced by heavy increase of LC3- and p62 positive autophagosomes >> fiber atrophy
    - > all is not cleared >> rimmed vacuolar pathology
- >> The same process happens with primary mutant sarcomeric proteins: myotilin, desmin,....
- 27/11/14

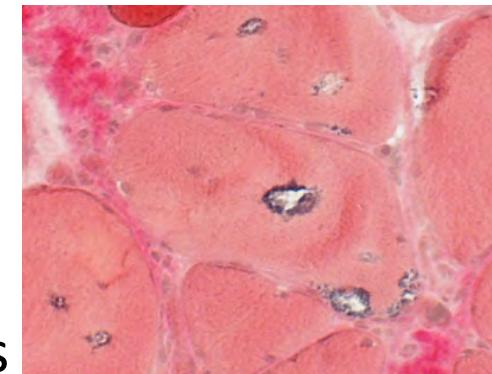
# Welander distal myopathy

(PhD thesis 1951: 249 patients)



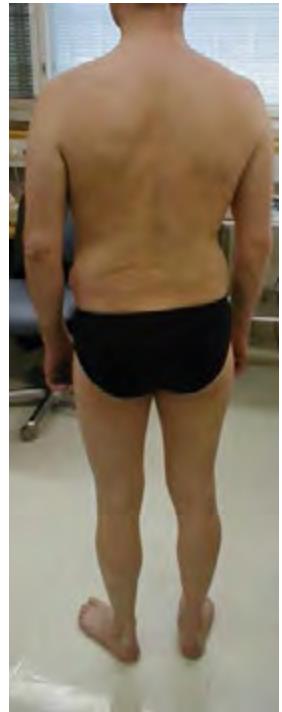
Dr Lisa Welander

- Late onset after age 40-60
  - 2/3 present with extension weakness of index fingers
  - ¼ present with mild drop foot
  - Slow evolution – difficulties with manual finger skills
  - Some complain of cold fingers
- AD inheritance
- CK normal or 1-3x UNL
- Pathology: **rimmed vacuoles** in affected muscles
- Molecular genetics: **TIA1 gene mutation**
  - TIA1 involved in mRNA processing and turnover by stress granules
  - Mutation causes altered stress granule dynamics
  - > leads to defect re-cycling > autophagic pathology
- Epidemiology:
  - 500-800 patients in Sweden, some 100 patients in Finland, 1 UK family



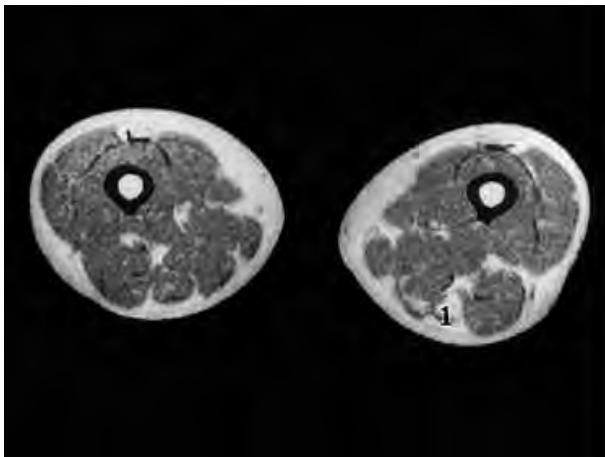
(Hackman et al 2013)

# Welander distal myopathy

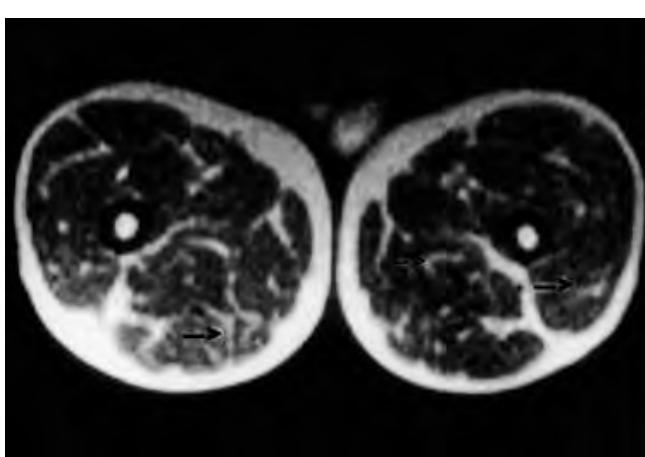


# Muscle-MRI in WDM

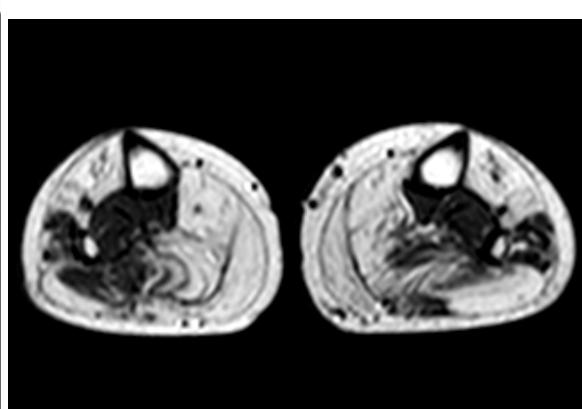
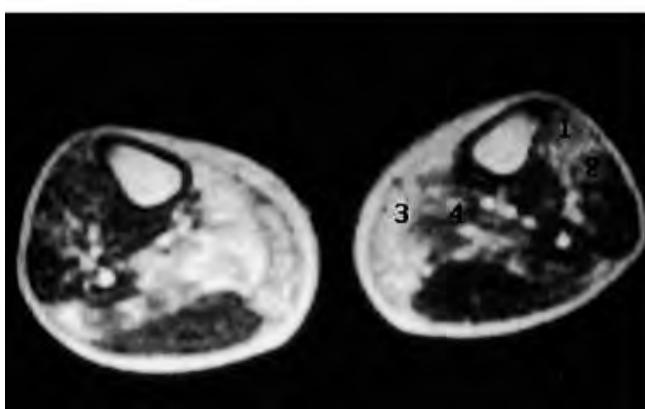
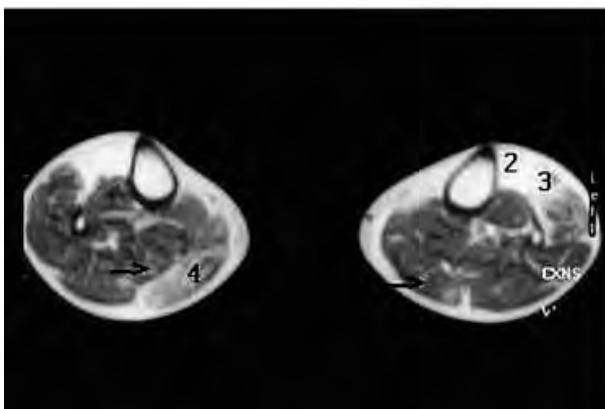
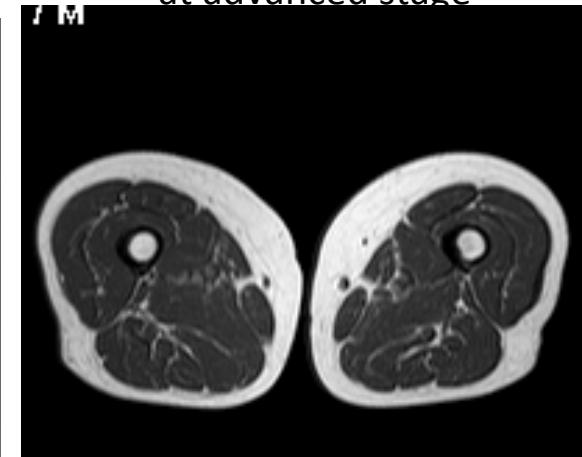
Onset in lower legs



Onset in finger extension

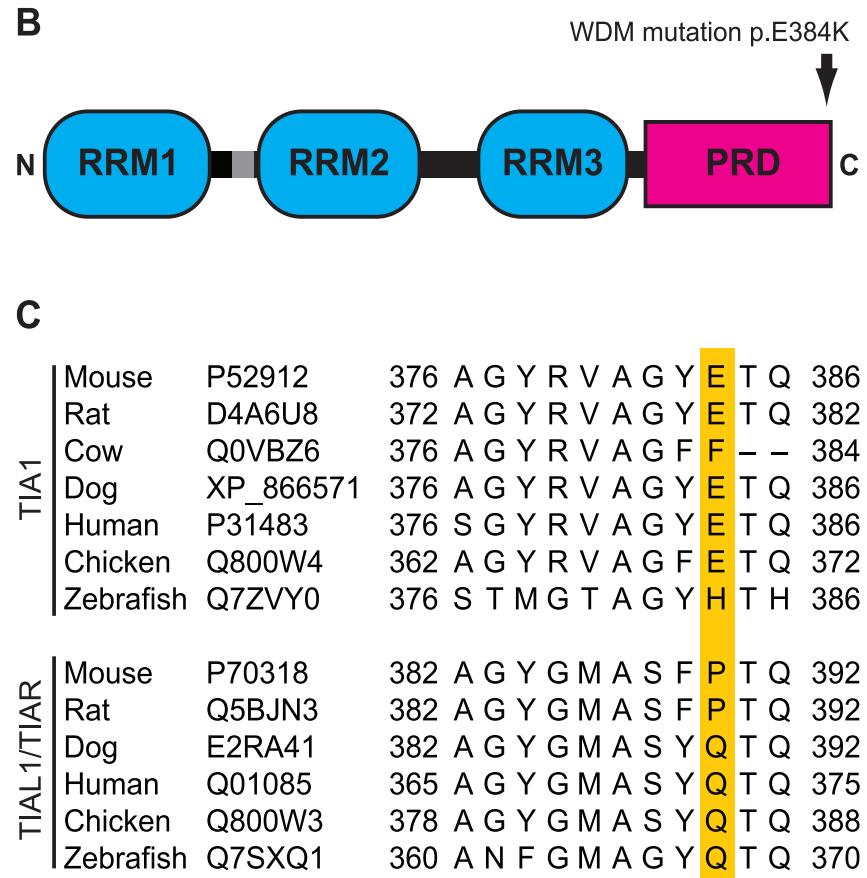


No proximal even at advanced stage



# Welander Distal Myopathy: mutation in TIA1

- “T-cell restricted intracellular antigen” (based on erroneous identification of GMP-17 as a TIA1 isoform)
- stress granule-associated RNA-binding protein**
- Splicing regulator**
- Identified mutation p.E384K
- Located in the glutamine-rich prion-like domain (PrLD)
- Mutated residue conserved in most tetrapod TIA1 orthologs



# TIA1 – stress granule dynamics

- Stress granules (SG) are cytoplasmic protein complexes that form upon different types of stress (oxidative stress, heat shock etc.) and sequester untranslated mRNAs
  - > regulation of translation activity by keeping mRNA from access to ribosomes
- TIA1 is a central regulator of stress granules
- Increased SG number and decreased recovery rate induced by mutant TIA1 >> decreased SG dynamics caused by slightly increased aggregation propensity of the prion-related domain
  - > late onset proteinopathy with induction of the autophagic machinery

# MATR3 defect VCPDM

## Vocal cord and pharyngeal distal myopathy (Feit 1998)

- Onset age 35-57, frequently asymmetric
- Ankle dorsiflexion, some have onset in hands (finger ext or abd pollicis), or dysphonia, dysphagia
  - > slow progression: foot drop and proximal lower limb weakness
- Shoulder weakness infrequent and ptosis rarely
- CK: mildly elevated, 2-4x fold UNL
- EMG: myopathic – neurogenic, NCV borderline
- Biopsy: rimmed vacuoles
- Original US-family, one Bulgarian and 6 German families (Halle)
- All have exactly the same mutation: p.S85C
  - > Independently on different haplotypes
- Pathomechanism?
  - > less protein in patient muscle nuclei??

- Clinically similar to Welander

(Senderek et al. AJHG 2009  
Müller et al J Neurol 2014)

This is now more complicated:

BRIEF COMMUNICATIONS

Nature Neuroscience, March 31, 2014

- 1 ALS family with a new mutation
- > revision of the VCPDM family to ALS

## Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis

Janel O Johnson<sup>1,28</sup>, Erik P Plioro<sup>2,28</sup>, Ashley Boehringer<sup>3,28</sup>, Ruth Chia<sup>4,28</sup>, Howard Feit<sup>5</sup>, Alan E Renton<sup>1</sup>, Hannah A Pliner<sup>1</sup>, Yevgeniya Abramzon<sup>1</sup>, Giuseppe Marangi<sup>1,6</sup>, Brett J Winborn<sup>7</sup>, J Raphael Gibbs<sup>8,9</sup>, Michael A Nalls<sup>10</sup>, Sarah Morgan<sup>9</sup>, Maryam Shoai<sup>9</sup>, John Hardy<sup>9</sup>, Alan Pittman<sup>9</sup>, Richard W Orrell<sup>11</sup>, Andrea Malaspina<sup>12</sup>, Katie C Sidle<sup>9</sup>, Pietro Fratta<sup>13</sup>, Matthew B Harms<sup>14</sup>, Robert H Baloh<sup>15</sup>, Alan Pestronk<sup>14</sup>, Conrad C Weihl<sup>14</sup>, Ekaterina Rogova<sup>16</sup>, Lorne Zinman<sup>17</sup>, Vivian E Drory<sup>18</sup>, Giuseppe Borghero<sup>19</sup>, Gabriele Mora<sup>20</sup>, Andrea Calvo<sup>21</sup>, Jeffrey D Rothstein<sup>22</sup>, ITALSGEN<sup>23</sup>, Carsten Drepper<sup>24,25</sup>, Michael Sendtner<sup>24</sup>, Andrew B Singleton<sup>10</sup>, J Paul Taylor<sup>7</sup>, Mark R Cookson<sup>4</sup>, Gabriella Restagno<sup>26,29</sup>, Mario Sabatelli<sup>27,29</sup>, Robert Bowser<sup>3,29</sup>, Adriano Chiò<sup>21,29</sup> & Bryan J Traynor<sup>1,22,29</sup>

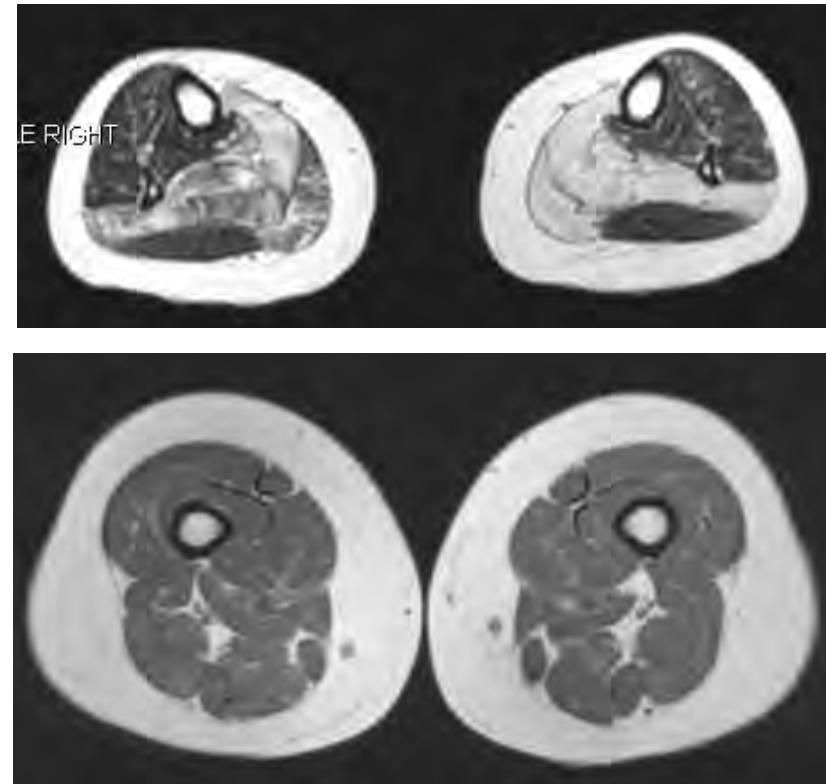
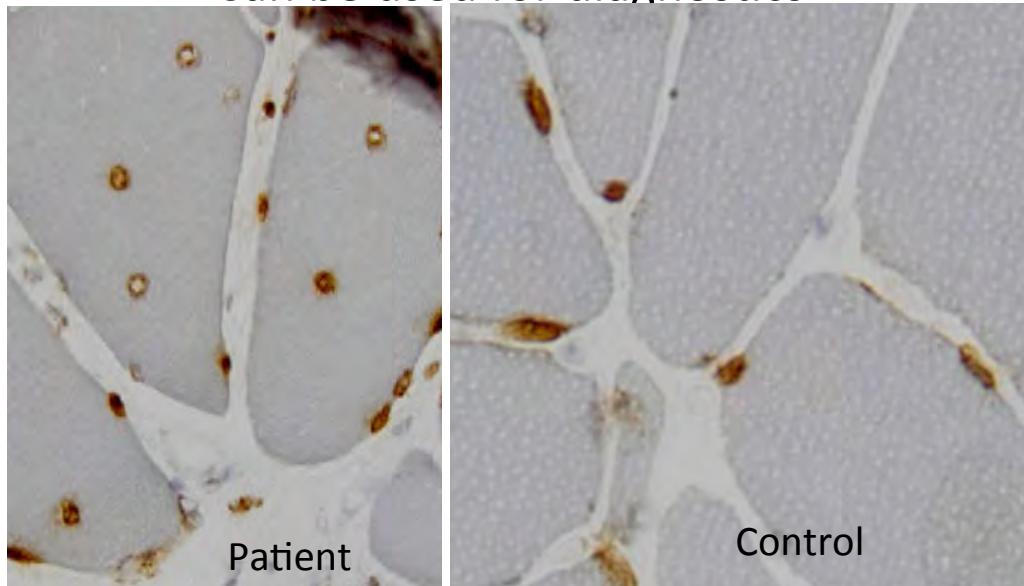
etiology of two-thirds of the familial form of ALS and 11% of the more common sporadic form of the disease are now known<sup>1</sup>. Nevertheless, the discovery of more genes would allow more complete mapping of the cellular pathways underlying this condition.

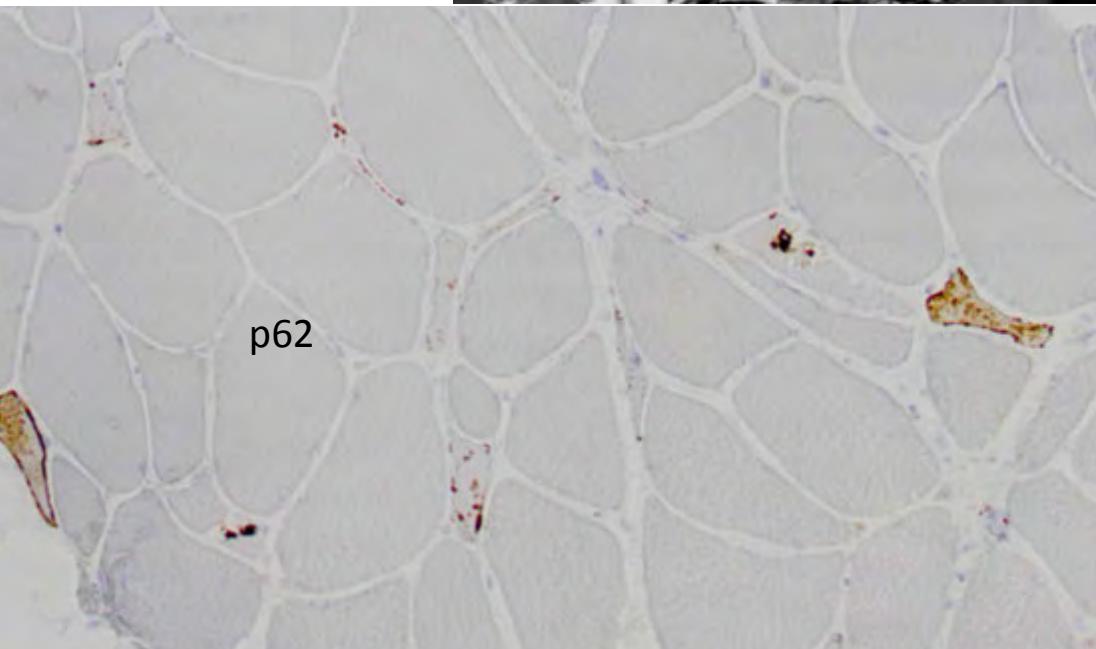
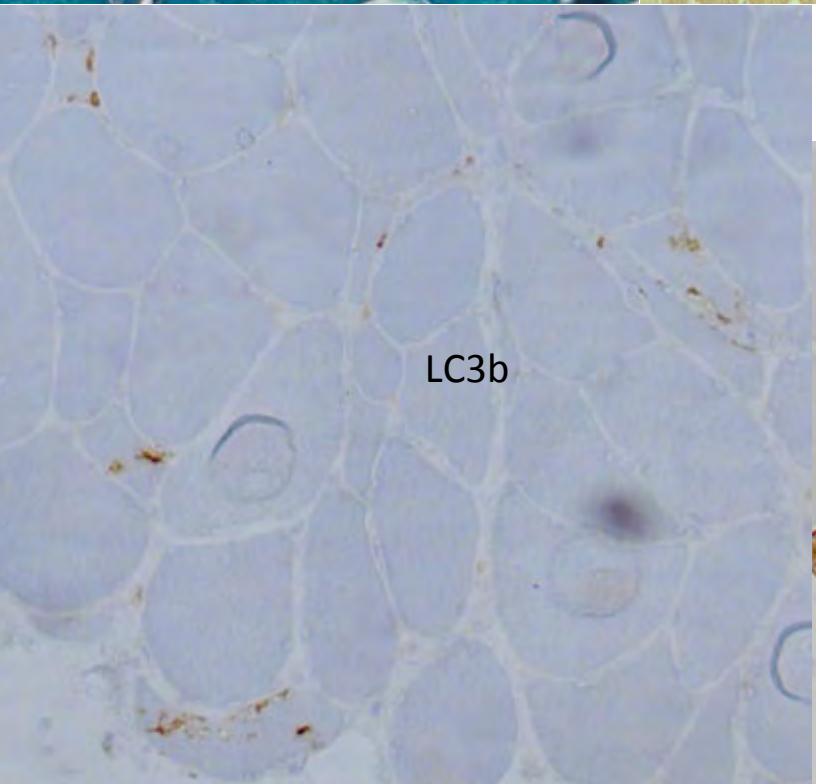
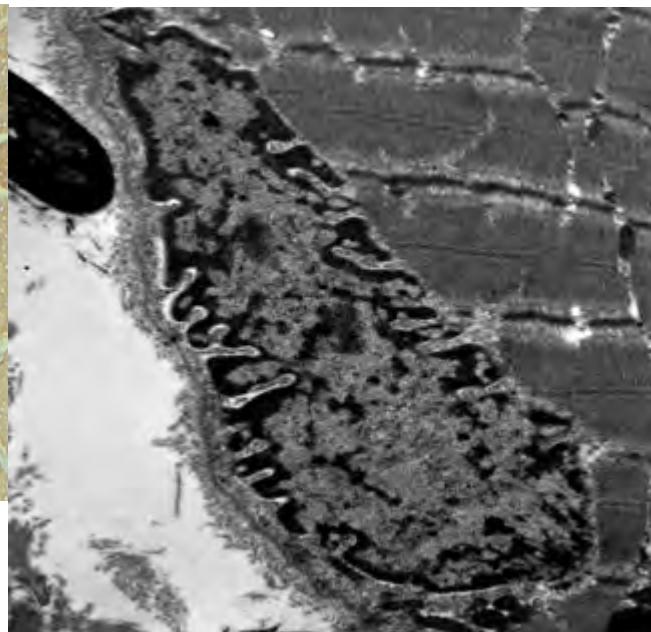
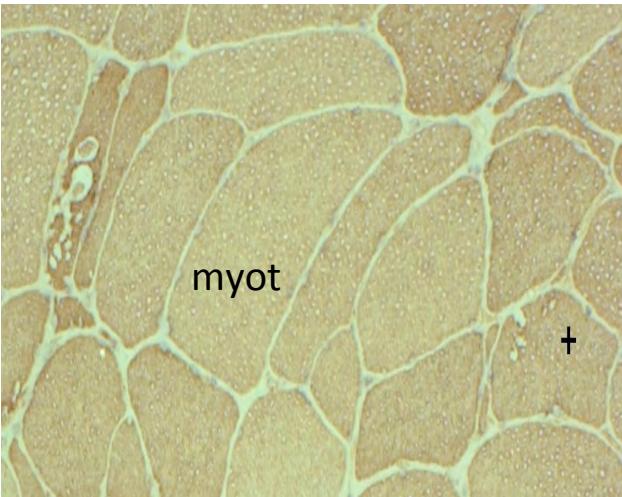
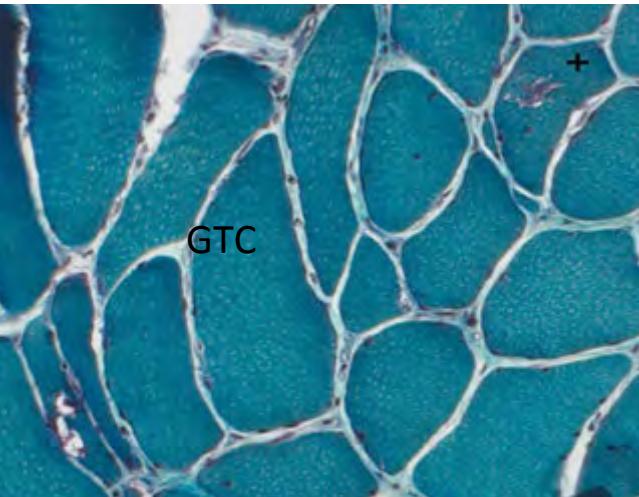
Here we applied exome sequencing to a family of European ancestry in which several individuals had been diagnosed with ALS and dementia (Fig. 1a) with the aim of identifying the causative mutation. We found two previously unknown, heterozygous missense variants that segregated with disease in this kindred, namely Ala436Val (chr5:126156748, C>T) in *LMNB1* and Phe115Cys (chr5:138643448, T>G) in *MATR3*. Neither variant was present in population polymorphism databases (including the Exome Sequencing Project ( $n = 13,000$  control chromosomes), the 1000 Genomes Project ( $n = 2,184$  chromosomes) and dbSNP) or in the Human Gene Diversity Panel ( $n = 2,102$  chromosomes screened in our laboratory). The *MATR3* variant was also not present in another 5,190 neurologically normal subjects genotyped in our laboratory, bringing the total number of control chromosomes that did not carry this transversion to 27,666.

A Ser85Cys (chr5:138643358, C>G) mutation in *MATR3* was previously reported as the cause of autosomal dominant distal amygda-

# MATR3 S85C-mutation in a new US-UK Family

- Late onset: Ankle weakness but **no bulbar symptoms**
- 2/4 developed respiratory failure
- Atrophy of hand muscles and finger extensor weakness. MRI: posterior> anterior
- MATR3 immunohistochemistry:
  - Yes, central nuclear reduction
  - > Can be used for diagnostics





MATR3 pathology

## MATR3-defect VCPDM

- also motor neuron disease ??

- New evaluation of motor nerve disease component in our family and in patients in the Halle-families:
- A number of patients have remarkably brisk reflexes early in the disease and in the upper limbs even after 10 years of disease duration, although no Babinski or other pathological UMN signs
- However:
  - no fasciculations
  - No bulbar findings towards lower motor neuron bulbar involvement
  - No high amplitude MUPs at any stage of the disease
  - No fiber type grouping in the pathology
- >>> a milder component of UMN involvement cannot be excluded, but the muscle atrophy is only caused by the myopathic loss of muscle

DMRV/hIBM

GNE

RV-myopathy

TMD TTN

HMERF TTN

MFM MYOT

MFM ZASP

AD MYH2

MFM FLNC

MFM DES

RB, MFM FHL1

DMRV/hIBM  
GNE

RV-myopathy

TMD	TTN	DNAJB6	LGMD1D
HMERF	TTN	CRYAB	MFM
MFM	MYOT	BAG3	MFM
MFM	ZASP	SIL1	MSS
AD	MYH2		
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DNAJB6 LGMD1D  
CRYAB MFM  
BAG3 MFM  
SIL1 MSS  
  
VCP  
  
PABP1 OPM  
TIA1 Welander  
MATR3 VCPDM

DMRV/hIBM  
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PABP1 OPM  
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Acid a-glucosidase  
Pompe  
LAMP2  
Danon  
VMA21  
XMEA  
Chloroquin tox  
experimental

DMRV/hIBM  
GNE

s-IBM

RV-myopathy

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Acid a-glucosidase LAMP2 VMA21 Chloroquin  
Pompe Danon XMEA experimental

## S-IBM

Nbr1  
p62 (*SQSTM1*)

- > cargo receptors
- > mono/poly Ub-binder
- > non-Ub autophagy
- > shell on aggregates
- > degraded by autophagy
- > increased if AP ↓

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DMRV/hIBM  
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RV-myopathy  
Defect autophagy

Autophagosome – lysosome fusion ↓  
 ->>> accumulation of LC3 cargo  
 - >>> LC3 vesicles lack LAMP1  
 ER-phagy ↓ -> myeloid bodies  
 Ribophagy (Ubp3p) ↓ -> mRNA acc  
 Translation (eIF2a-P+) ↓ -> mRNA acc  
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VCP

PABP1	OPM
TIA1	Welander
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Acid α-glucosidase  
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LAMP2      VMA21      Chloroquin  
Danon      XMEA      experimental

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Defect autophagy**

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**Primary defects**

of sarcomeric proteins

-> missfolded proteins and aggregates

->> proteasome incapable

->> induced macroautophagy overloaded?

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CRYAB	MFM
BAG3	MFM
SIL1	MSS

VCP

PABP1	OPM
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Acid a-glucosidase

Pompe

LAMP2

Danon

VMA21

XMEA

Chloroquin

experimental

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 + mitophagy ↓ -> acc of defect Mt

**Primary defects**  
**of chaperone machinery**  
 -> aggregates of misfolded proteins  
 ->> proteasome incapable  
 ->> induced macroautophagy overloaded?

PABP1	OPM
TIA1	Welander
MATR3	VCPDM

VCP

DNAJB6	LGMD1D
CRYAB	MFM
BAG3	MFM
SIL1	MSS

Acid a-glucosidase  
Pompe

LAMP2	VMA21	Chloroquin
Danon	XMEA	experimental

## S-IBM

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SQSTM1

DMRV/hIBM  
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CRYAB	MFM
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VCP

PABP1	OPM
TIA1	Welander
MATR3	VCPDM

Stress granule  
dynamics

Acid a-glucosidase	LAMP2	VMA21	Chloroquin
Pompe	Danon	XMEA	experimental

## S-IBM

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p62 (SQSTM1)

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 Translation (eIF2a-P+) ↓ -> mRNA acc  
 + mitophagy ↓ -> acc of defect Mt

LC3=MAP1  
 HDAC6  
 > polyUb substr

Microtubules

TDP-43  
FUS1  
Tob1

Stress granule dynamics

Acid a-glucosidase  
Pompe

LAMP2  
Danon

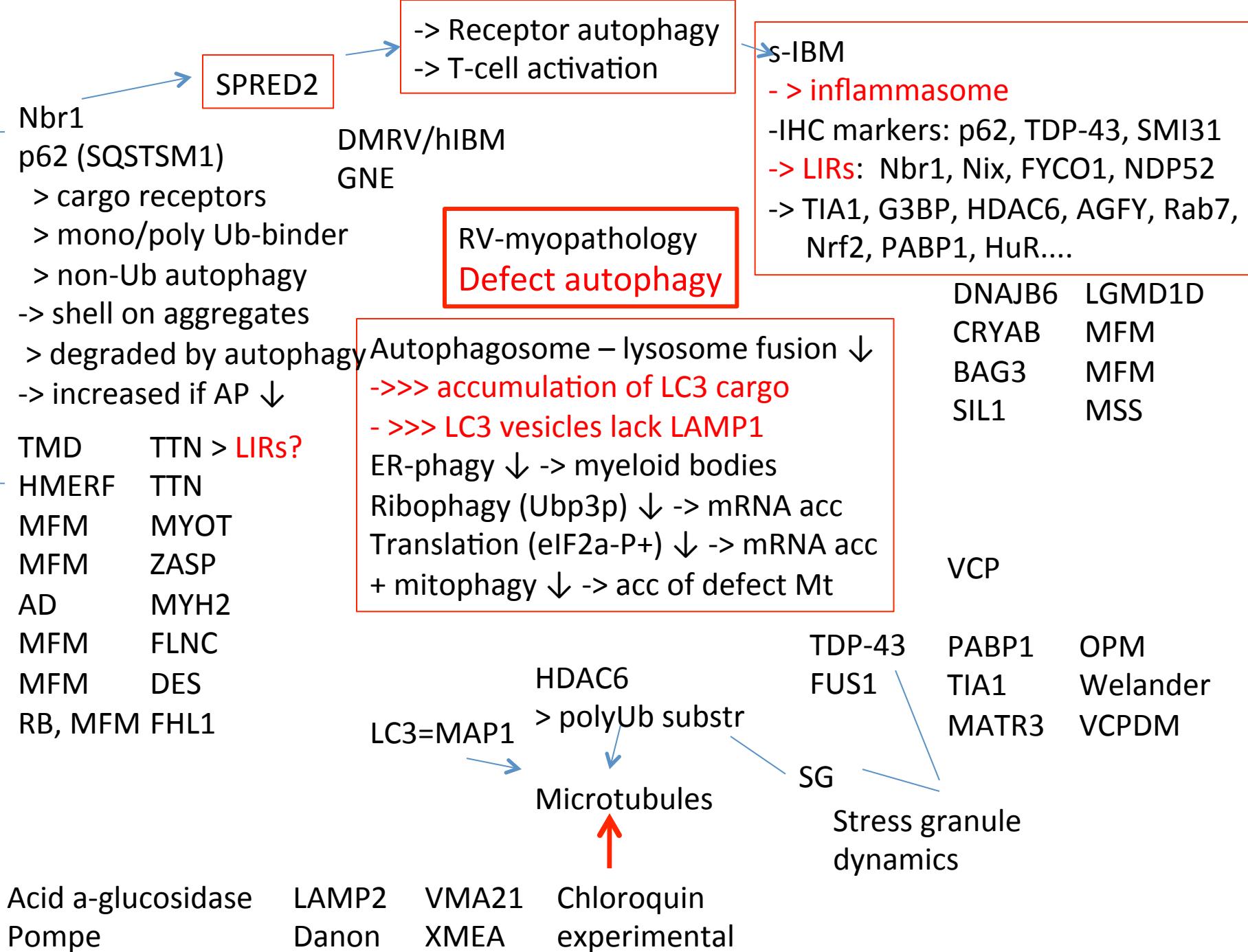
VMA21  
XMEA

Chloroquin  
experimental

DNAJB6	LGMD1D
CRYAB	MFM
BAG3	MFM
SIL1	MSS

VCP

PABP1	OPM
TIA1	Welander
MATR3	VCPDM



# Rimmed vacuoles

Imbalance in autophagic re-cycling/turnover:

1. Increase of defect and misfolded proteins:
  - primary mutated sarcomeric proteins : myotilin, desmin, filaminC, ...
  - Defect maintenance of normal proteins: chaperonal defects: CRYAB, BAG3, DNAJB6, HSPB8 ...
  - Defect ER-quality control: SIL1, VCP
  - Defect cargo delivery: SQSTM1, TDP-43
2. Decreased autophagic capacity - lysosomal defects  
LAMP2, XMEA, Pompe
3. Altered mRNA handling and translation: TIA1,MATR3  
-> secondary defect of protein turnover

# Collaborations

## Our Helsinki group

Peter Hackman  
Per Harald Jonson  
Anna Vihola  
Jaakko Sarparanta  
Helena Luque  
Anni Evilä  
Mridul Johari

## Our Tampere group

Merja Soininen  
Tiina Suominen  
Olayinka Raheem  
Satu Luhtasela  
Henna Koskinen  
Sanna Huovinen  
Hannu Haapasalo  
Sini Penttilä  
Satu Sandell  
Johanna Palmio  
Manu Jokela

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Hannu Kalimo  
Carina Wallgren-Petterson  
Anders Paetau  
Olli Carpen

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Anders Oldfors  
Fengqing Xiang  
Thomas Sejersen

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Francoise Chapon  
Bruno Eymard  
CHRU Lille, Strasbourg  
Ana Ferreiro  
Jean Pouget

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David Hilton-Jones  
Caroline Sewry  
Mathias Gautel, Elisabeth Ehler  
Gonzalo Blanco, Derek Blake

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C Sue, N Clarke, M Needham

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Ichizo Nishino

## Israel

Zohar Argov  
Stella Mitrani-Rosenbaum

## Norway

Arve Dahl  
Thorberg Thorbergsen

## Spain

Isabel Illa  
Juan Vilchez/Nuria Muelas Gomez  
Montse Olive  
Jose Fernandez-Pardal

## Portugal

Luis Negrao

## Italy

Giorgo Tasca/Enzo Ricci  
Alessandro Malandrini  
Luciano Merlini  
Enrico Bertini  
Geraldine Faulkner  
Marzia Pollazzon

## Germany

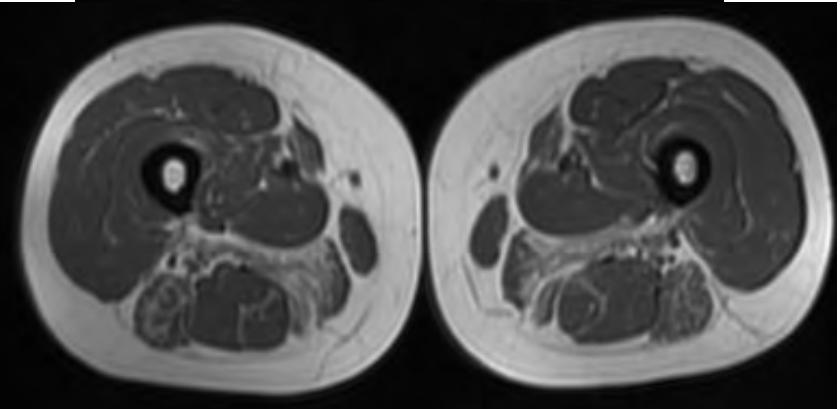
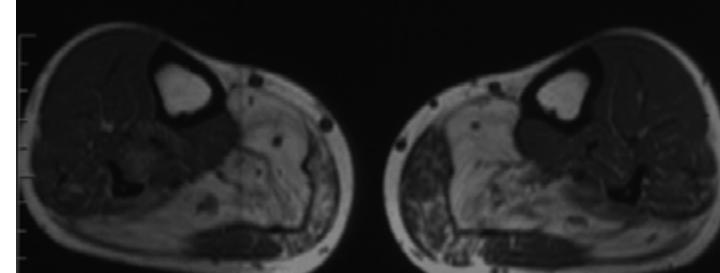
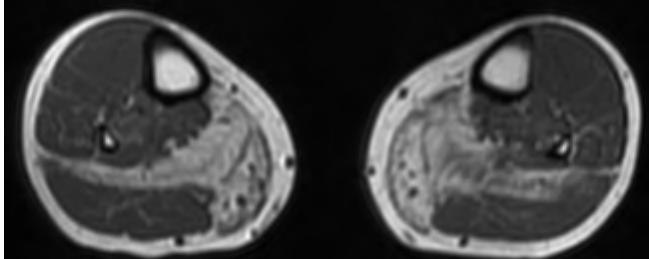
Rolf Schröder  
Dieter Fürst  
Dirk Fischer >> Switzerland  
Thomas Voit >> Paris

## USA

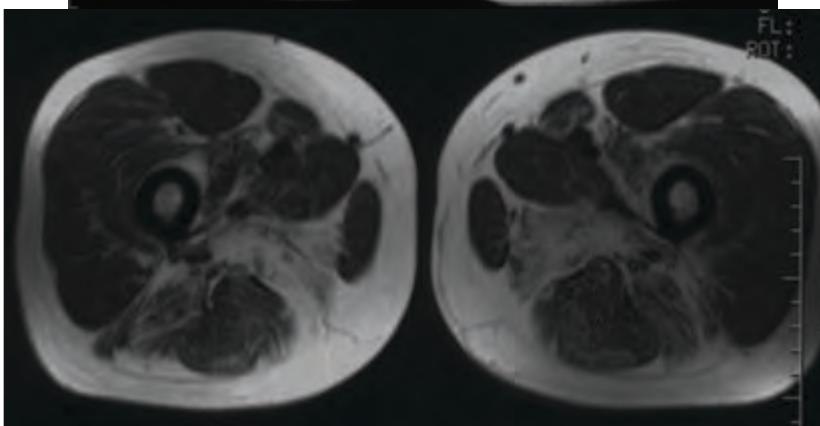
Robert C Griggs  
Judith Miller  
Andrew Engel

# Muscle MRI

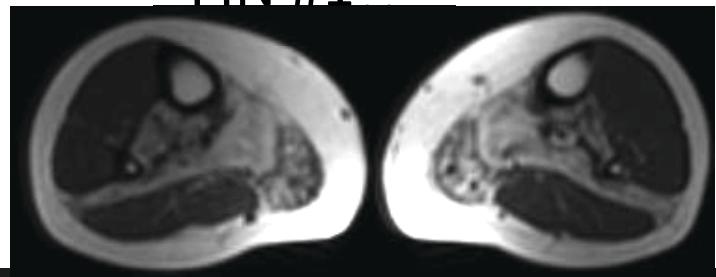
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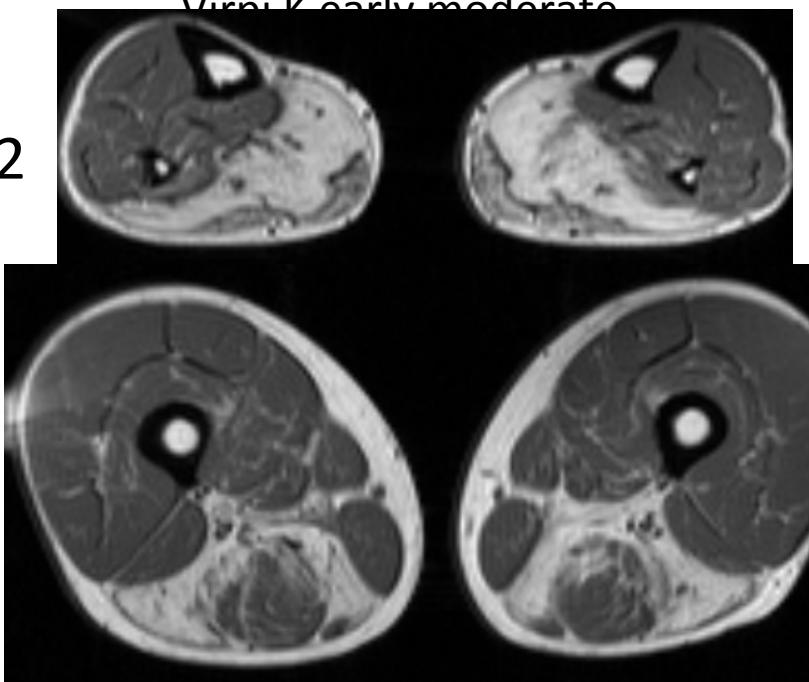
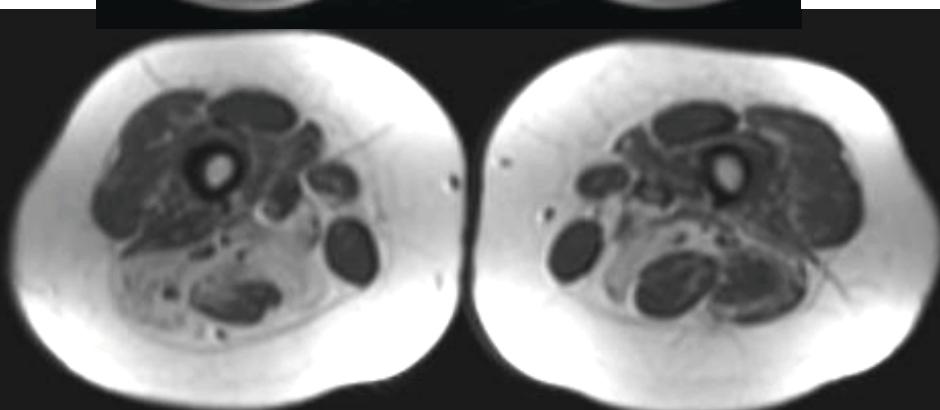
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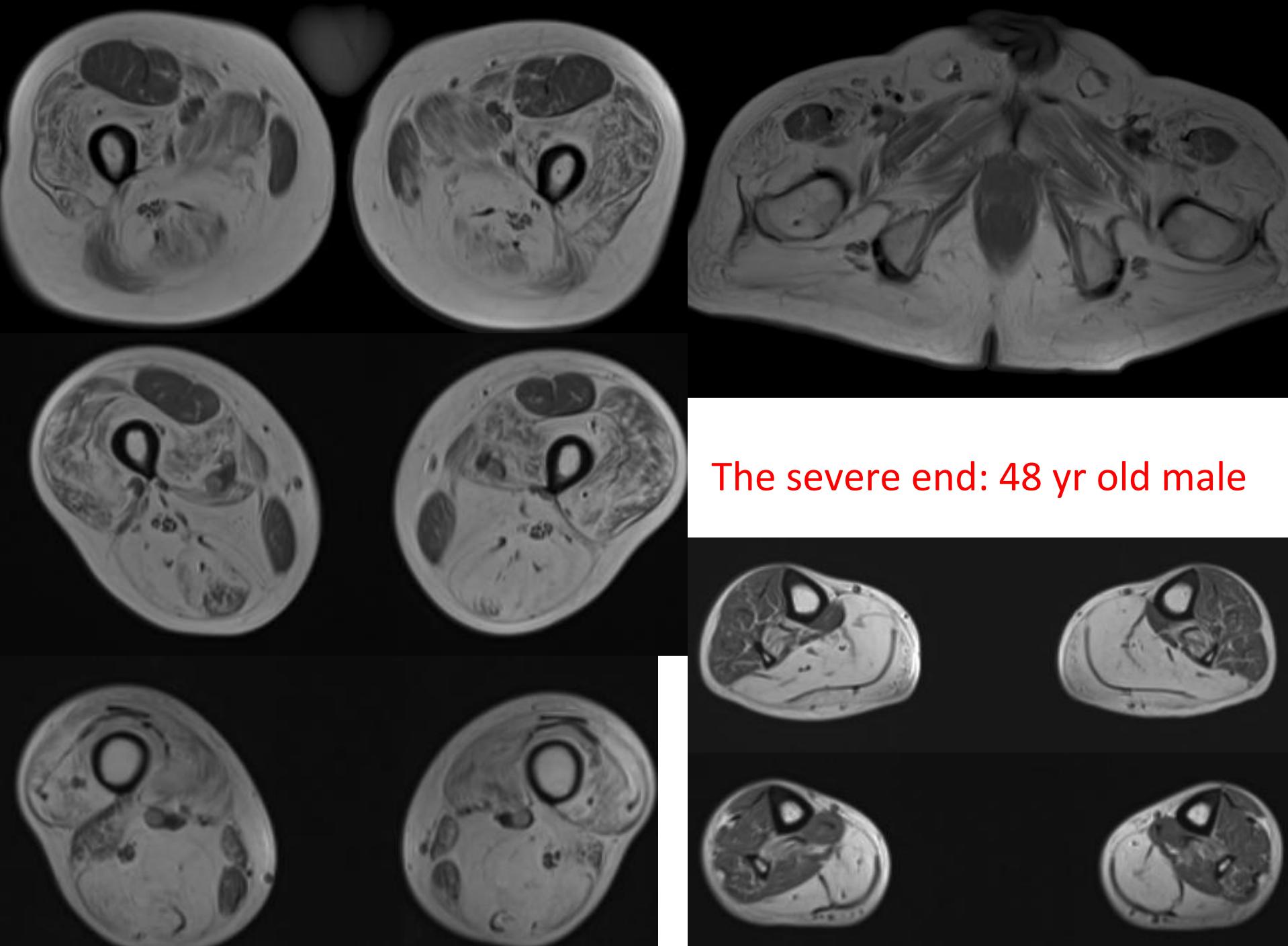


Virni K early moderate



IT #2



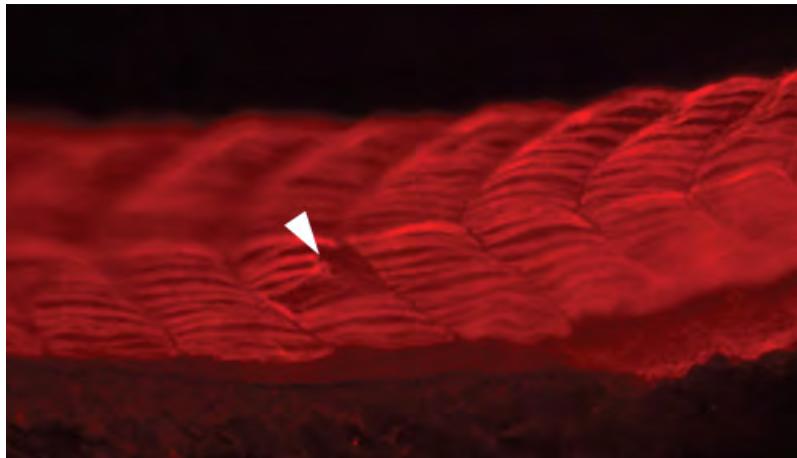


The severe end: 48 yr old male

# Pathogenicity of the mutants

## Zebrafish studies (at Duke)

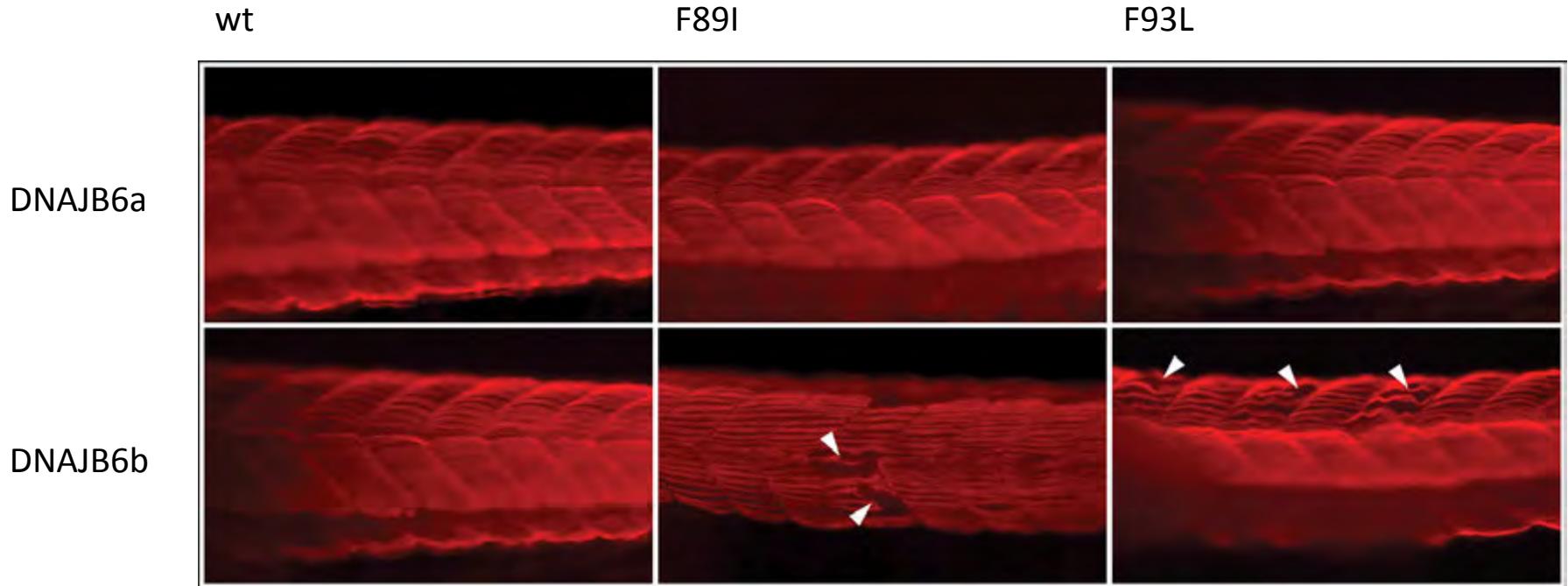
- Effects of DNAJB6 knockdown and LGMD1D mutations studied *in vivo* in zebrafish
- Splice-blocking morpholino or DNAJB6 mRNA injected to fish embryos at 2-cell stage
- Embryos scored for muscle phenotype after 2 days

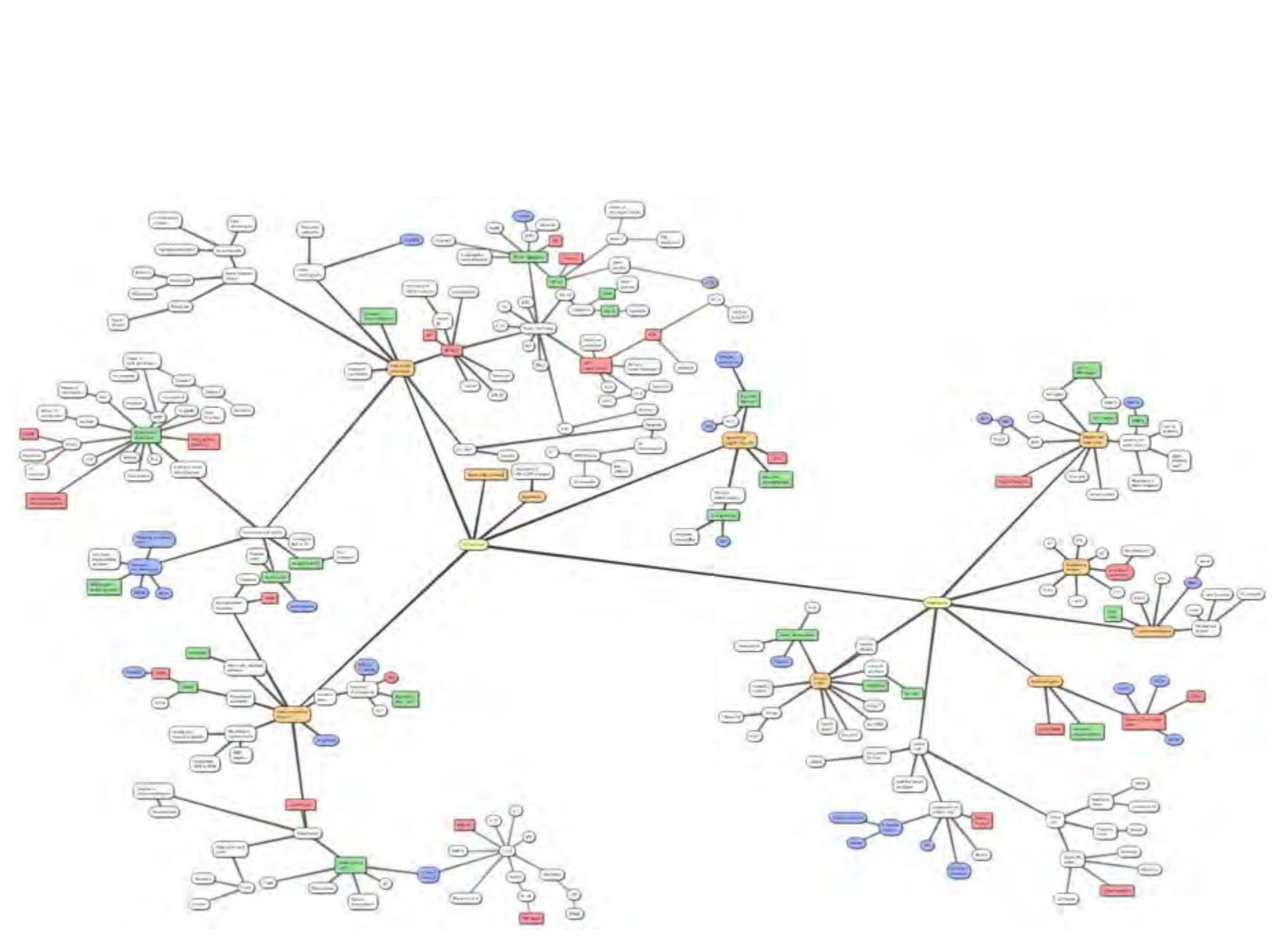


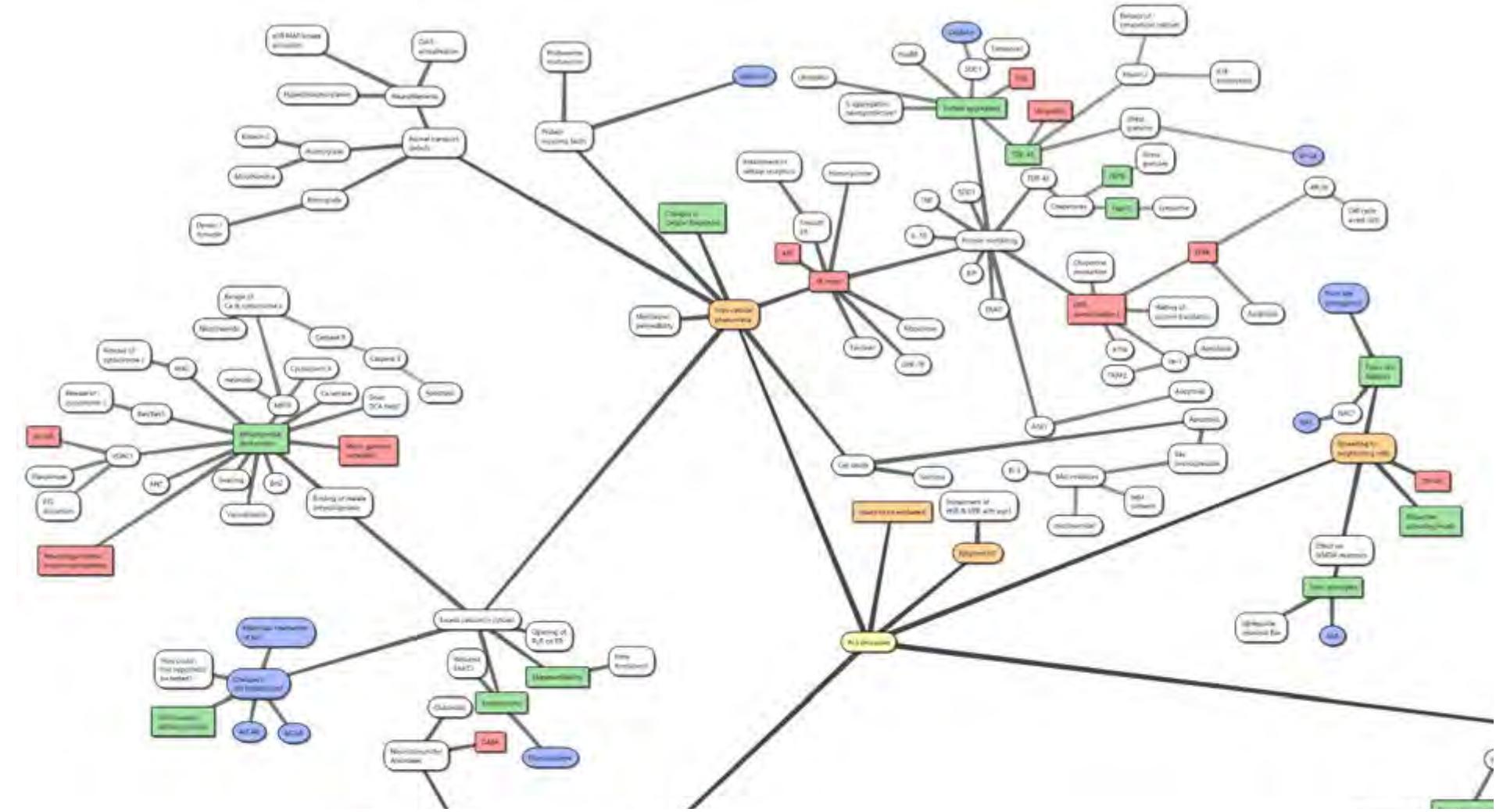
DNAJB6 knockdown  
(splice-blocking mo)

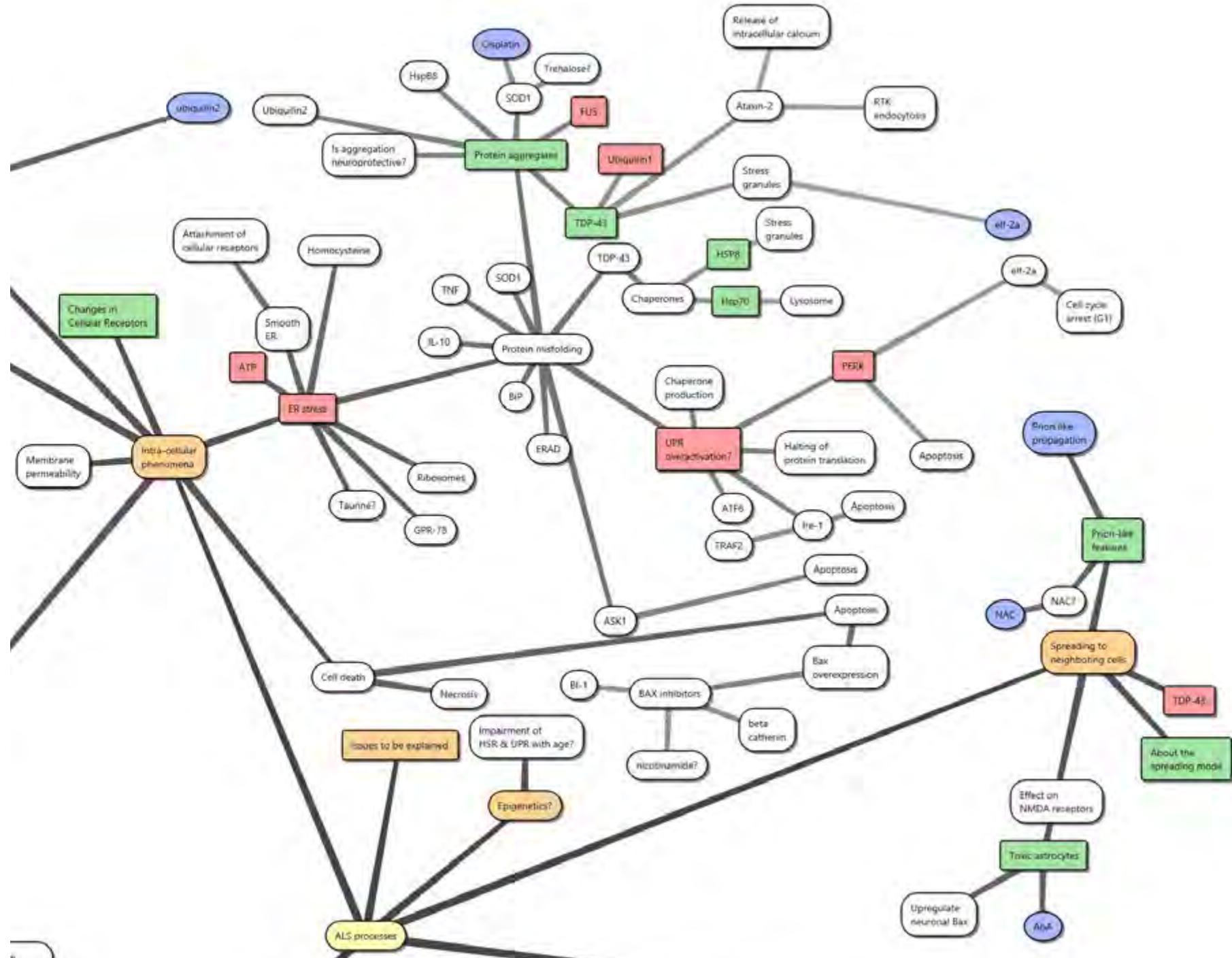
# Zebrafish studies

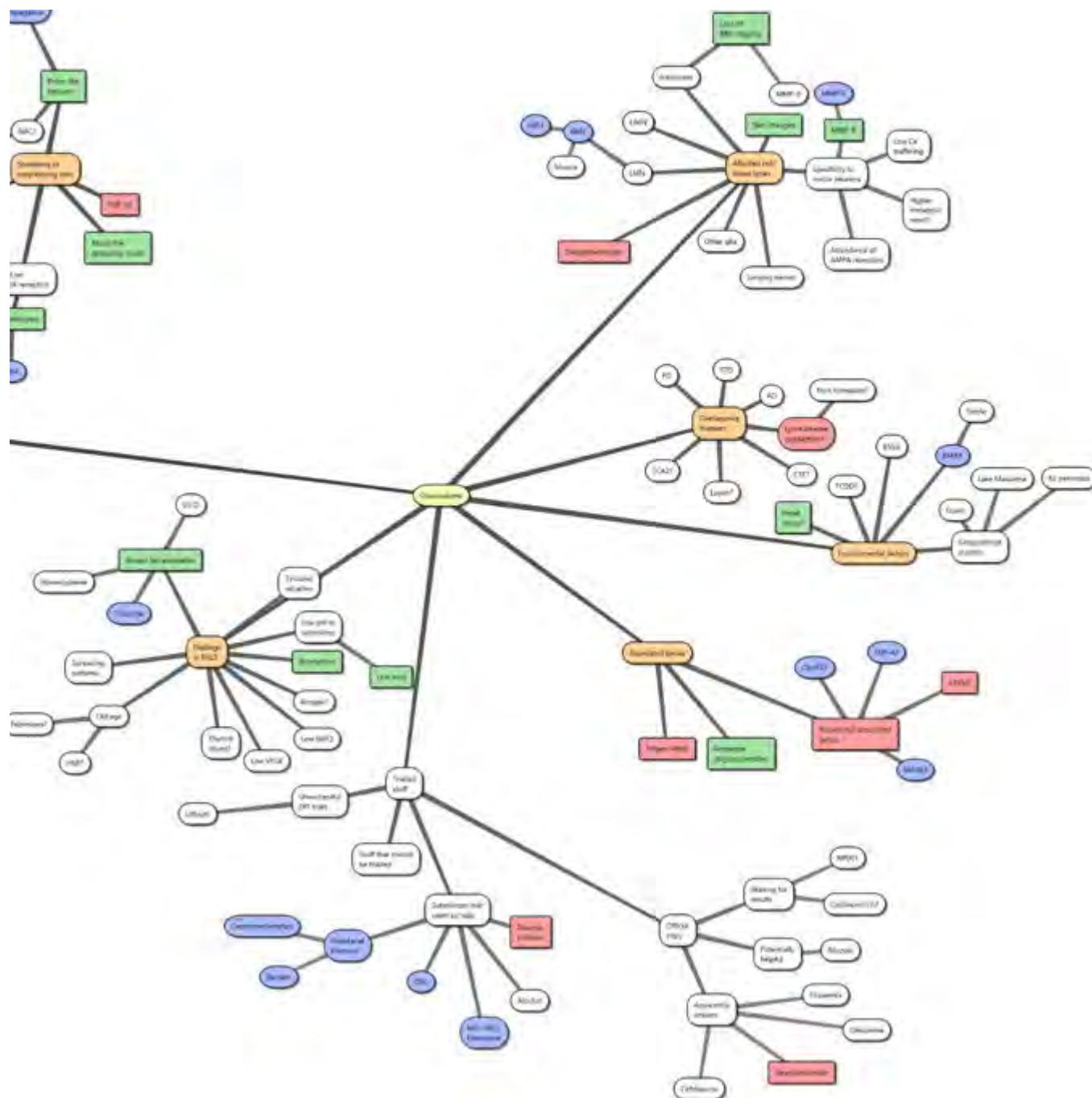
- Muscle phenotype caused by expression of mutant cytoplasmic isoform

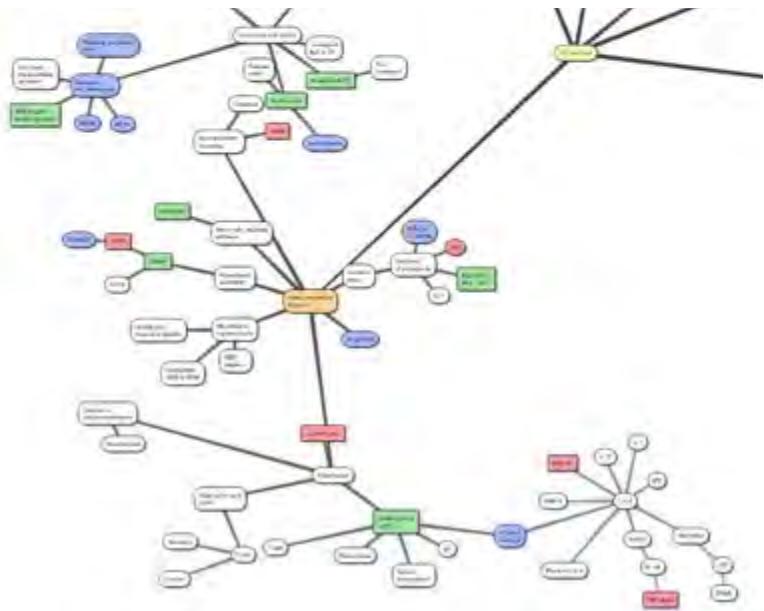




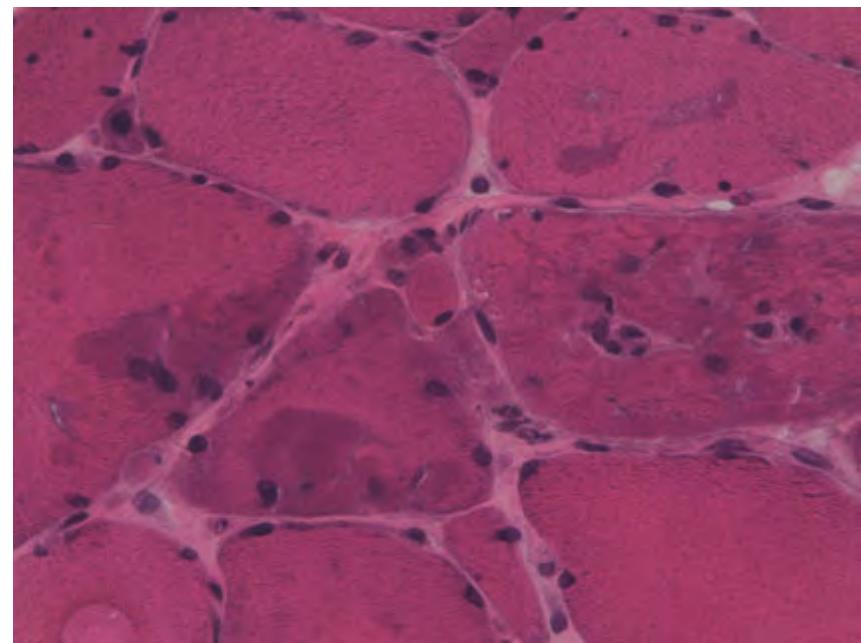
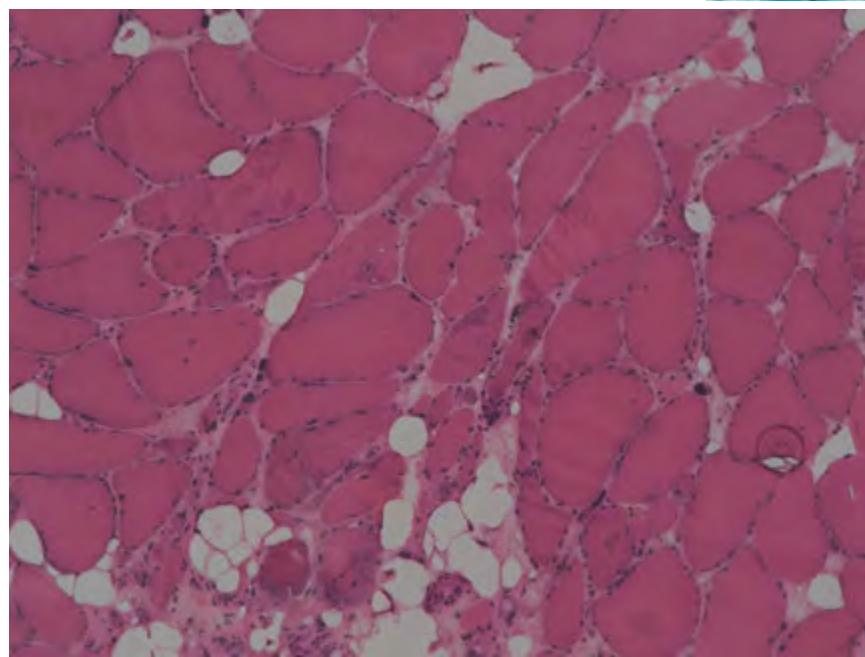
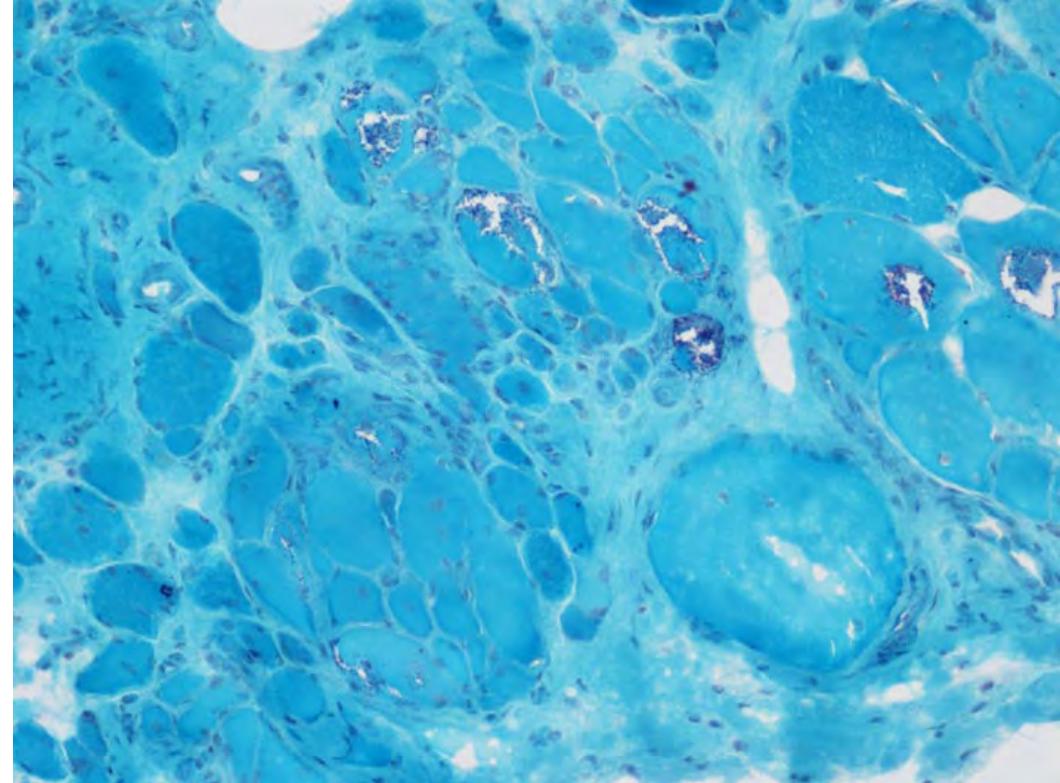






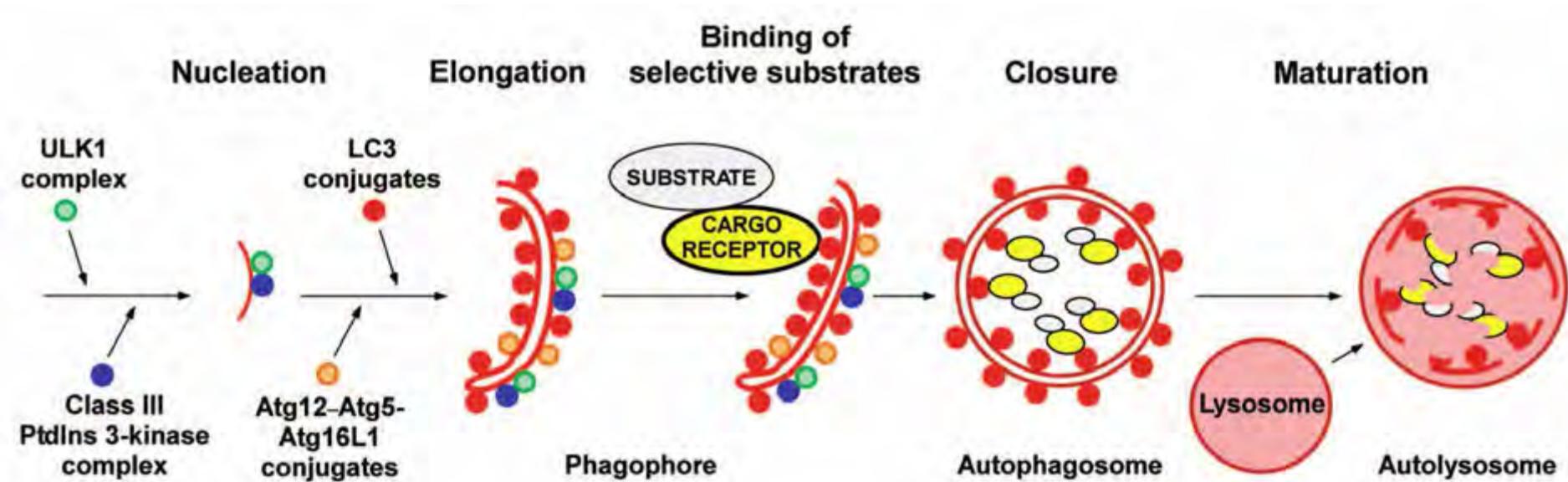


Can be:  
severe rimmed vacuolar  
with less obvious  
myofibrillar  
Or rarely:  
Severe myofibrillar with  
less rimmed vacuolar



# Autophagic machinery

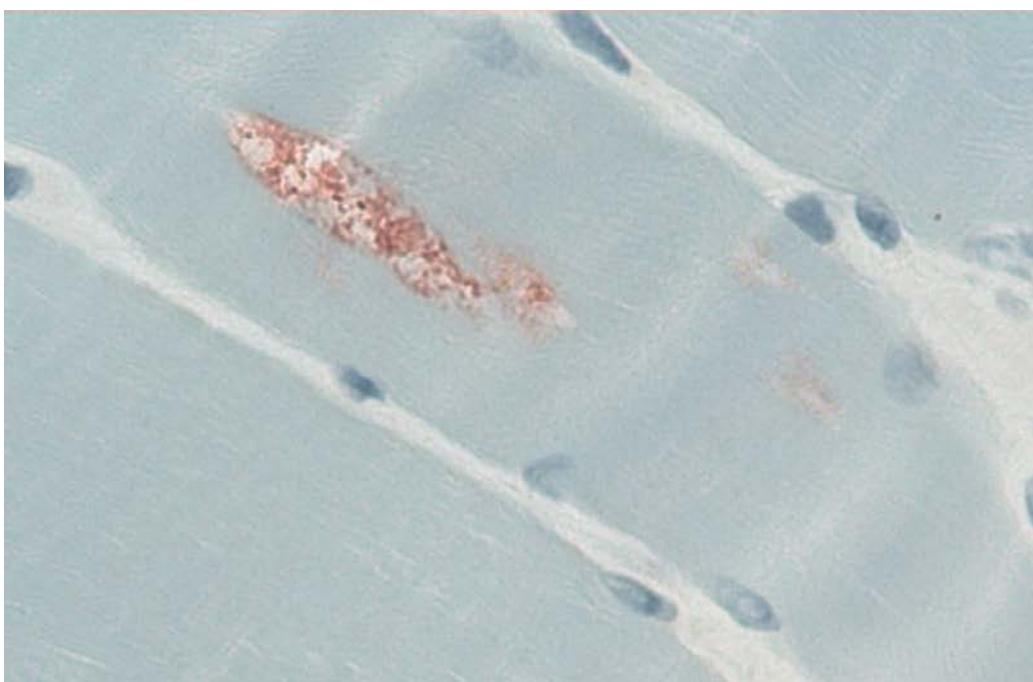
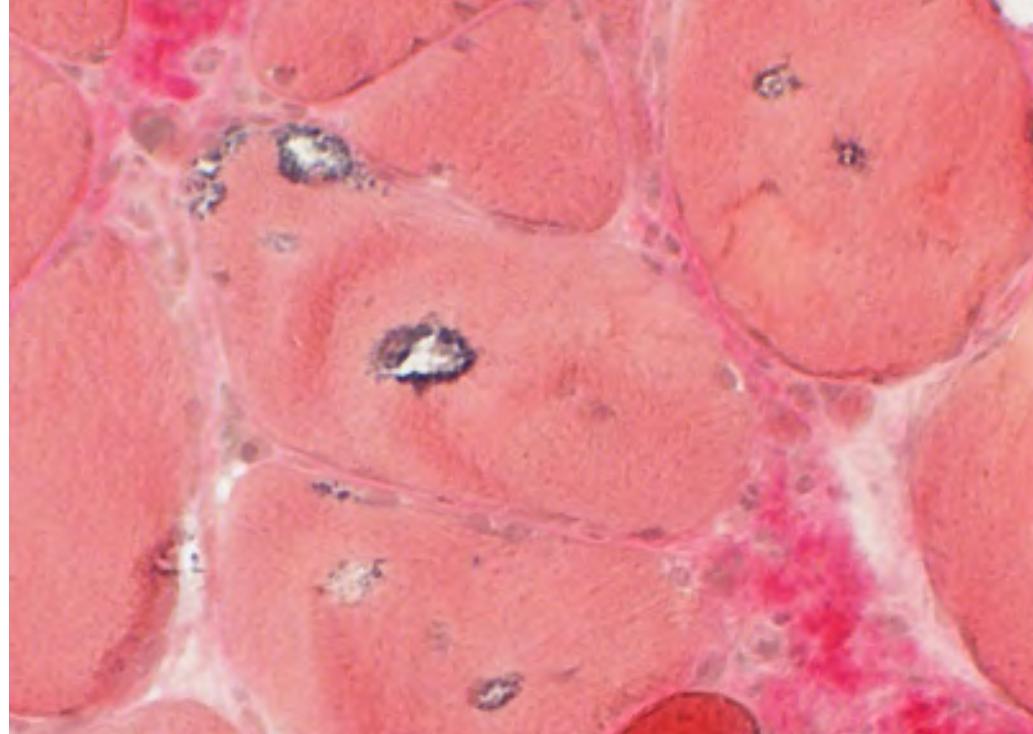
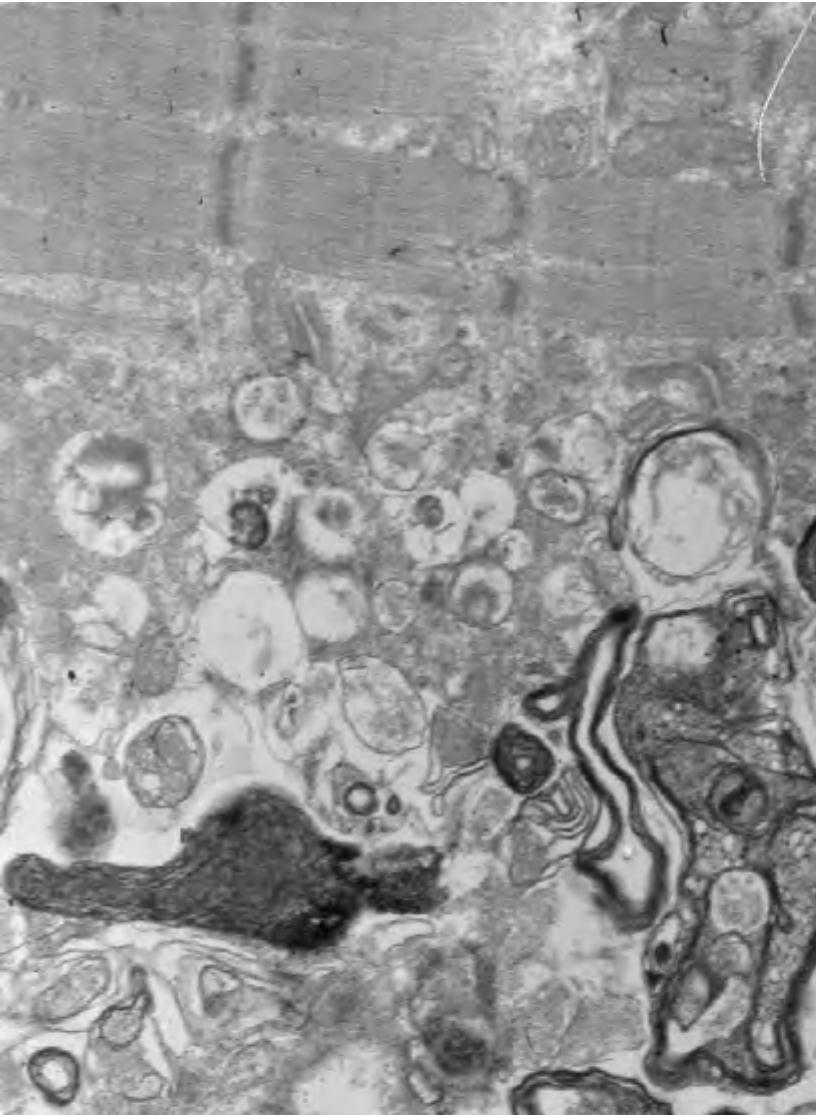
## LC3s and GABARAPs needed



Autophagosomes double membraned

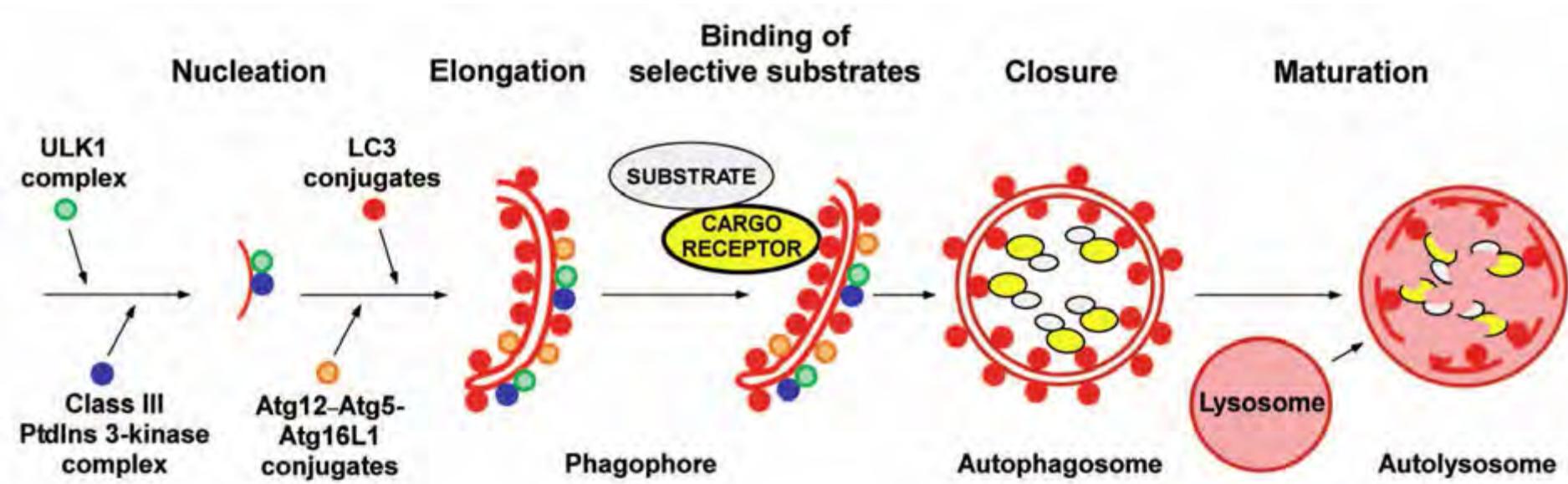
# LGMD1D

- Rimmed vacuoles with LC3, SMI-31, p62, TDP-43

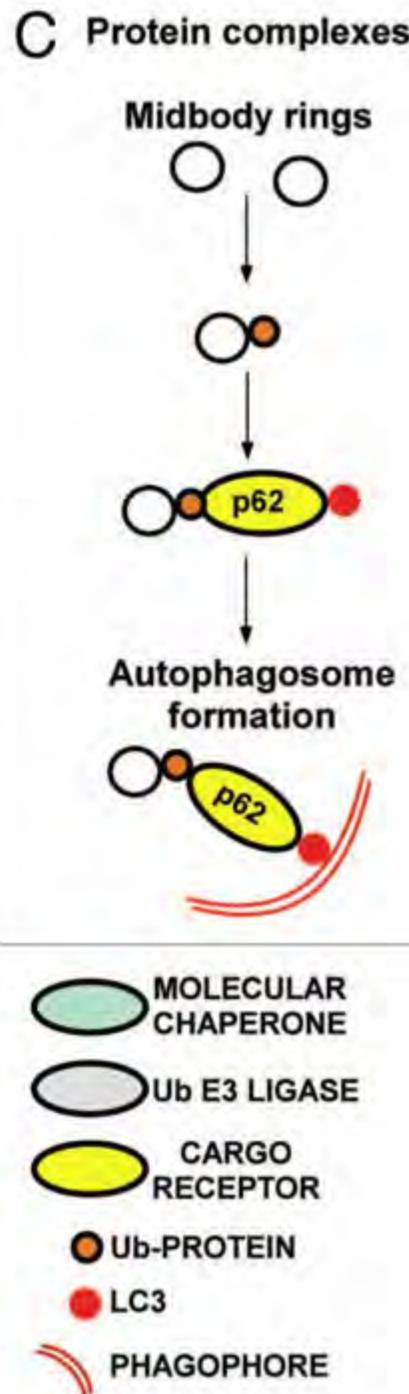
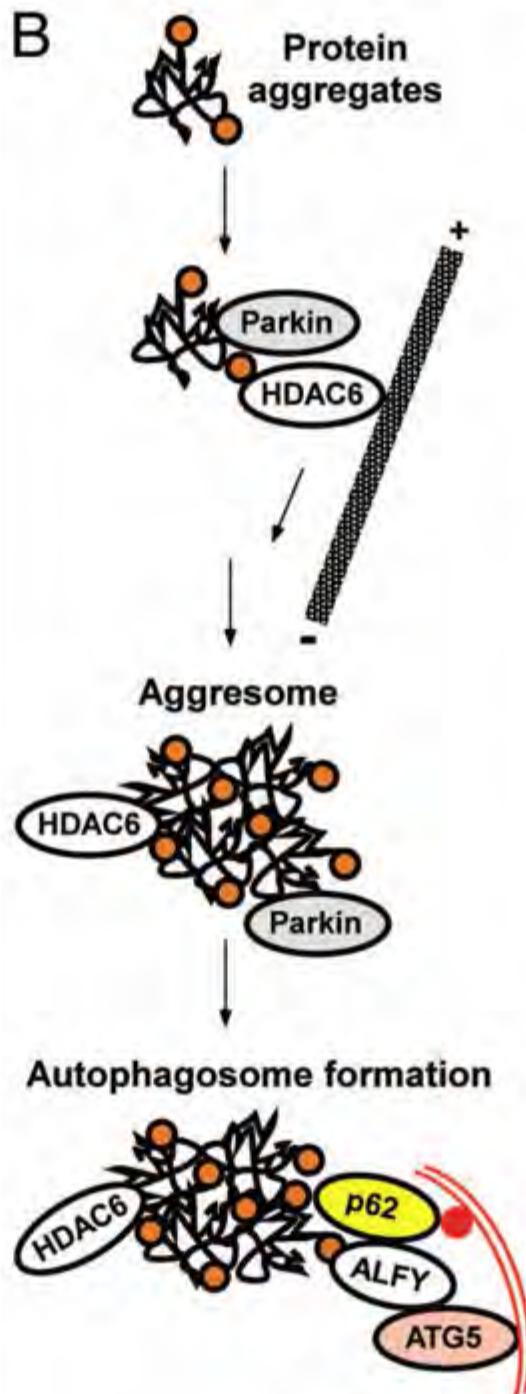
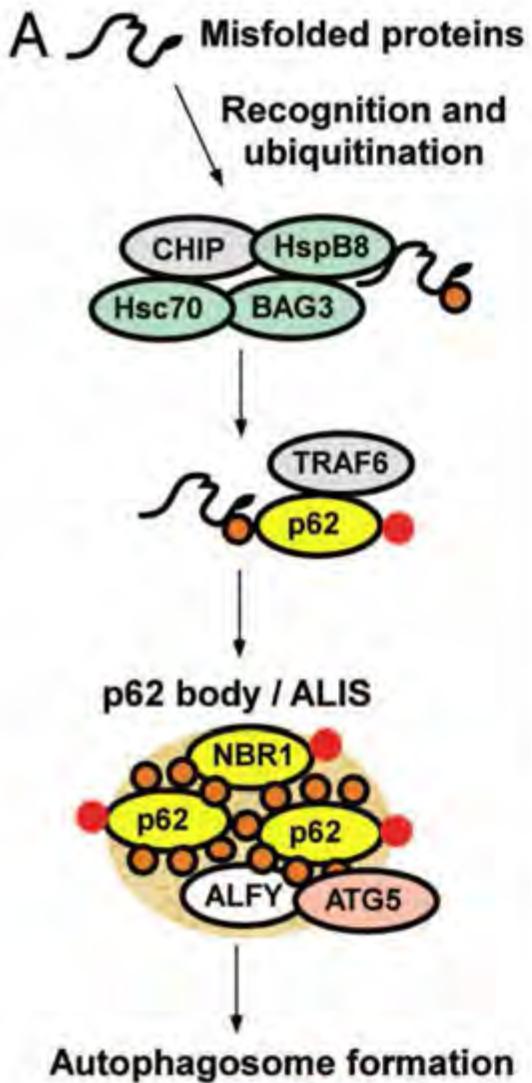


# Autophagic machinery

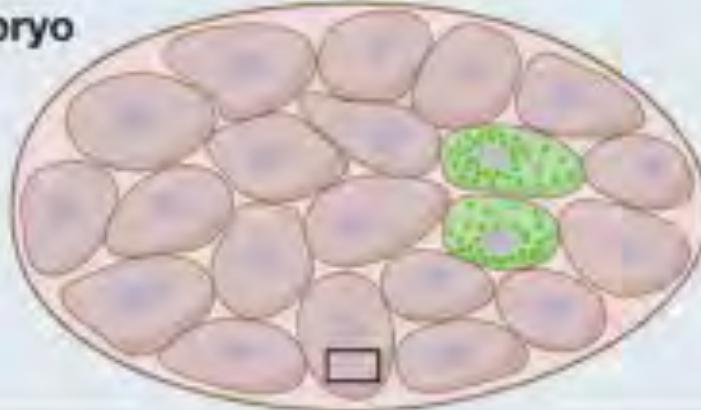
## LC3s and GABARAPs needed



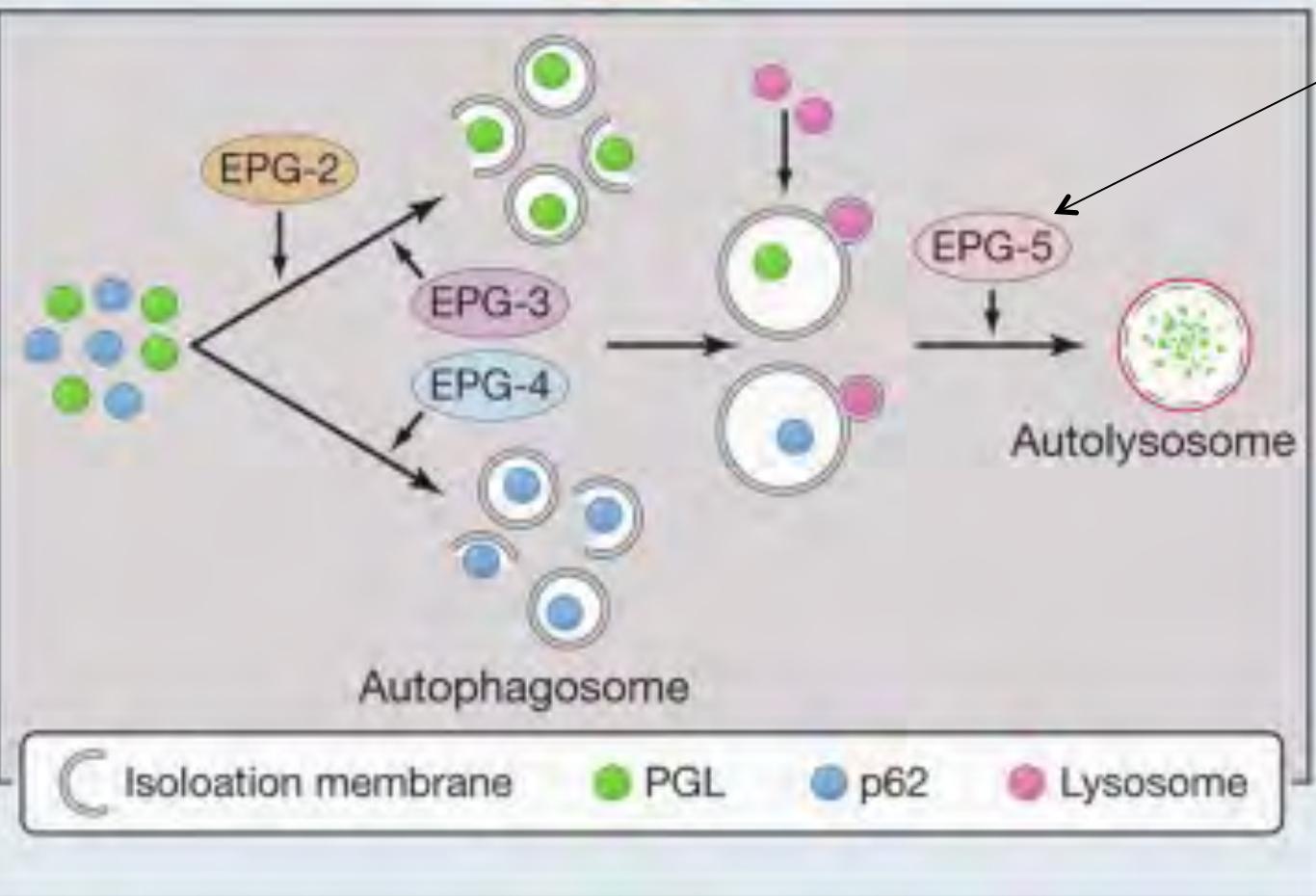
Normal autophagosome double membraned  
➤ Vesicles in RVs are usually single membrane



*C. elegans* embryo



Vici syndrome



DMRV/hIBM  
GNE

S-IBM

RV-myopathy

TMD TTN  
HMERF TTN  
MFM MYOT  
MFM ZASP  
AD MYH2  
MFM FLNC  
MFM DES  
RB, MFM FHL1

But there will be many more  
RV-myopathies waiting for  
definition

PABP1 OPM  
MATR3 VCPDM  
VCP  
(also Ub-proteasome)

SIL1  
MSS

DNAJB6 LGMD1D  
CRYAB MFM  
BAG3 MFM

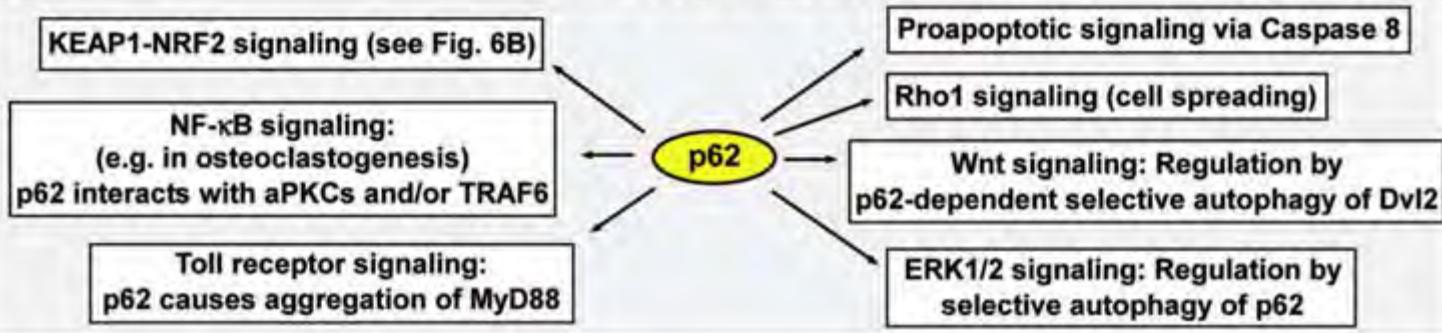
-----  
TIA1 WDM

Acid a-glucosidase  
Pompe

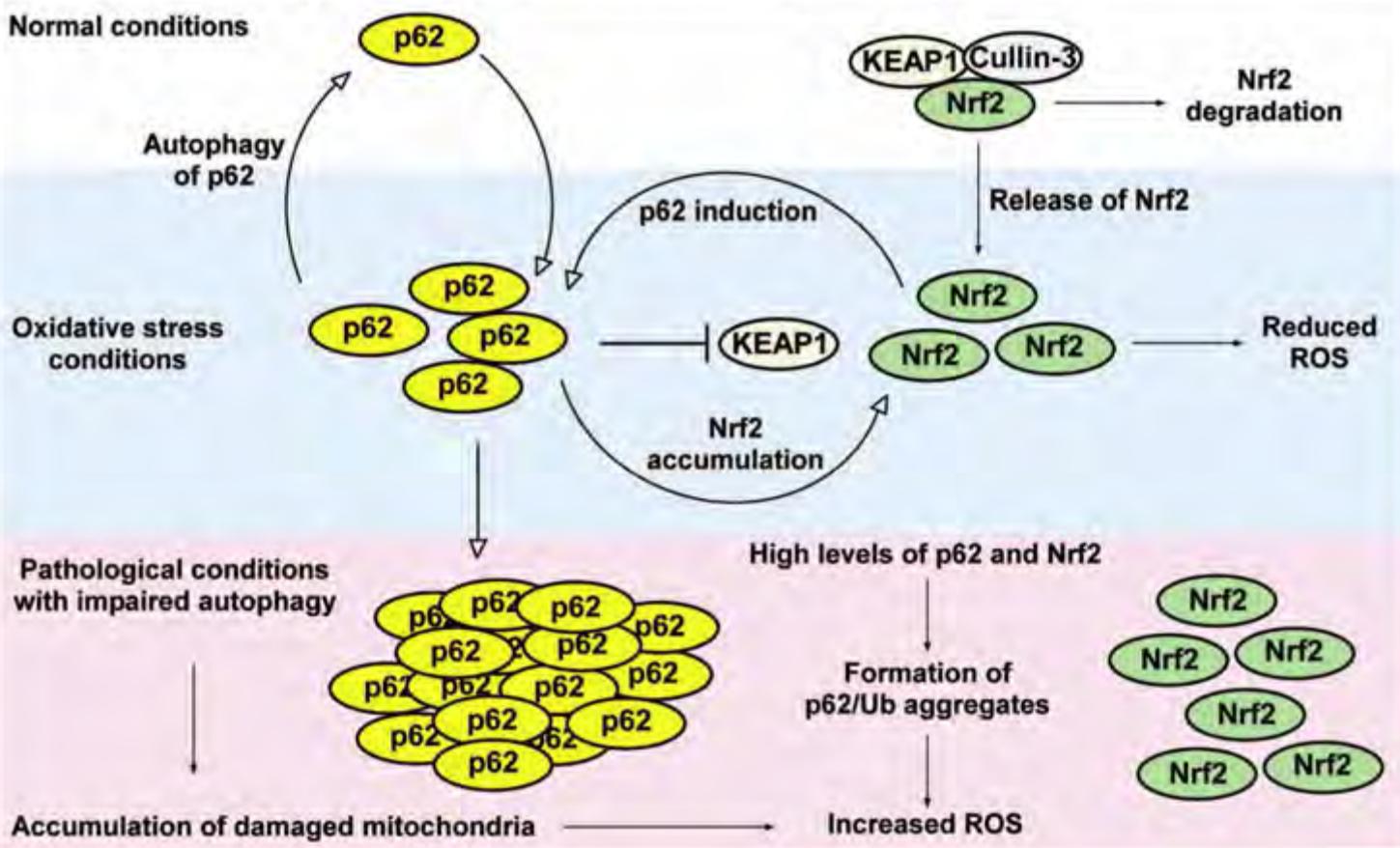
LAMP2 VMA21 Chloroquin  
Danon XMEA experimental

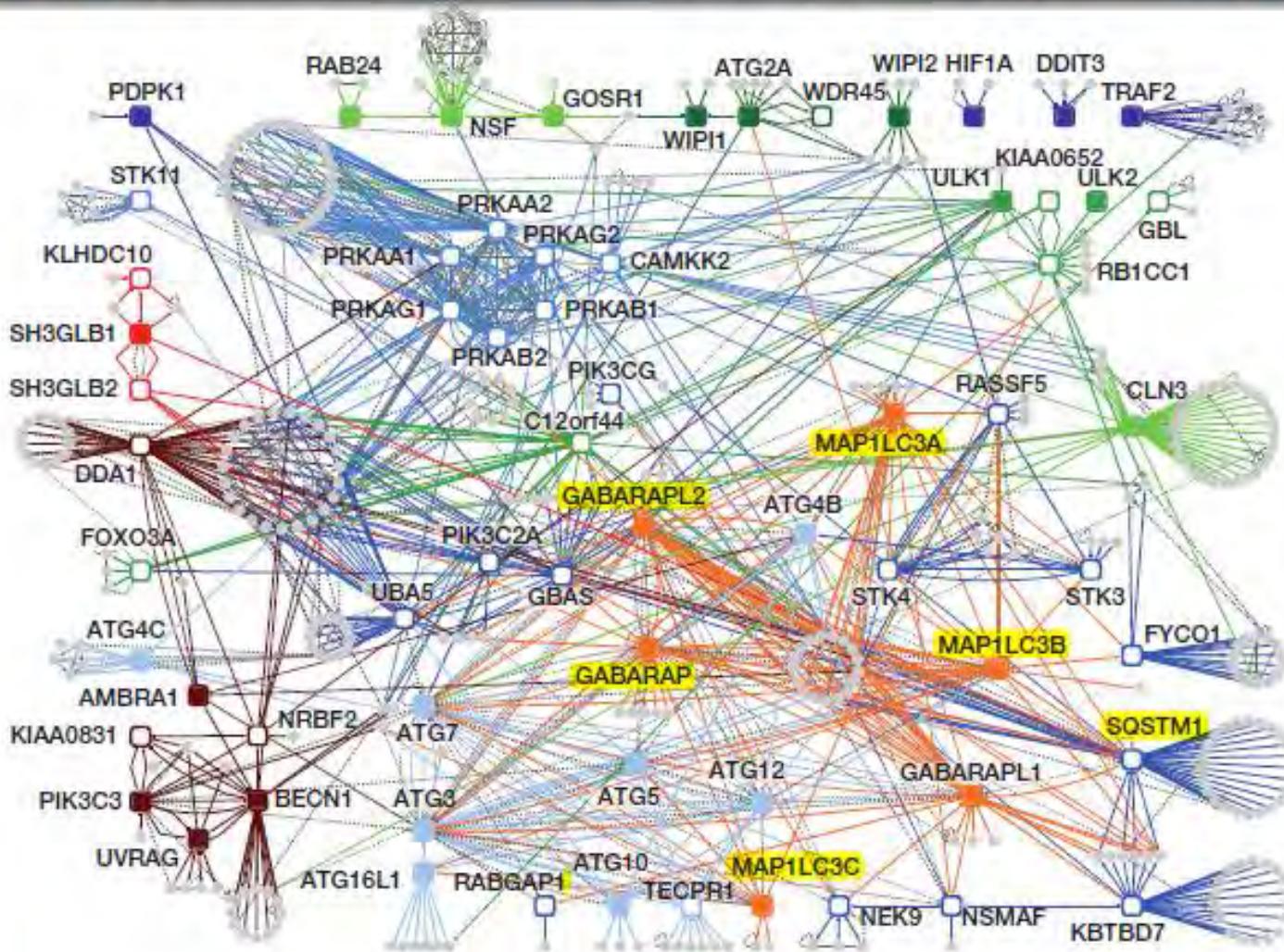
**A**

## Signaling roles of p62

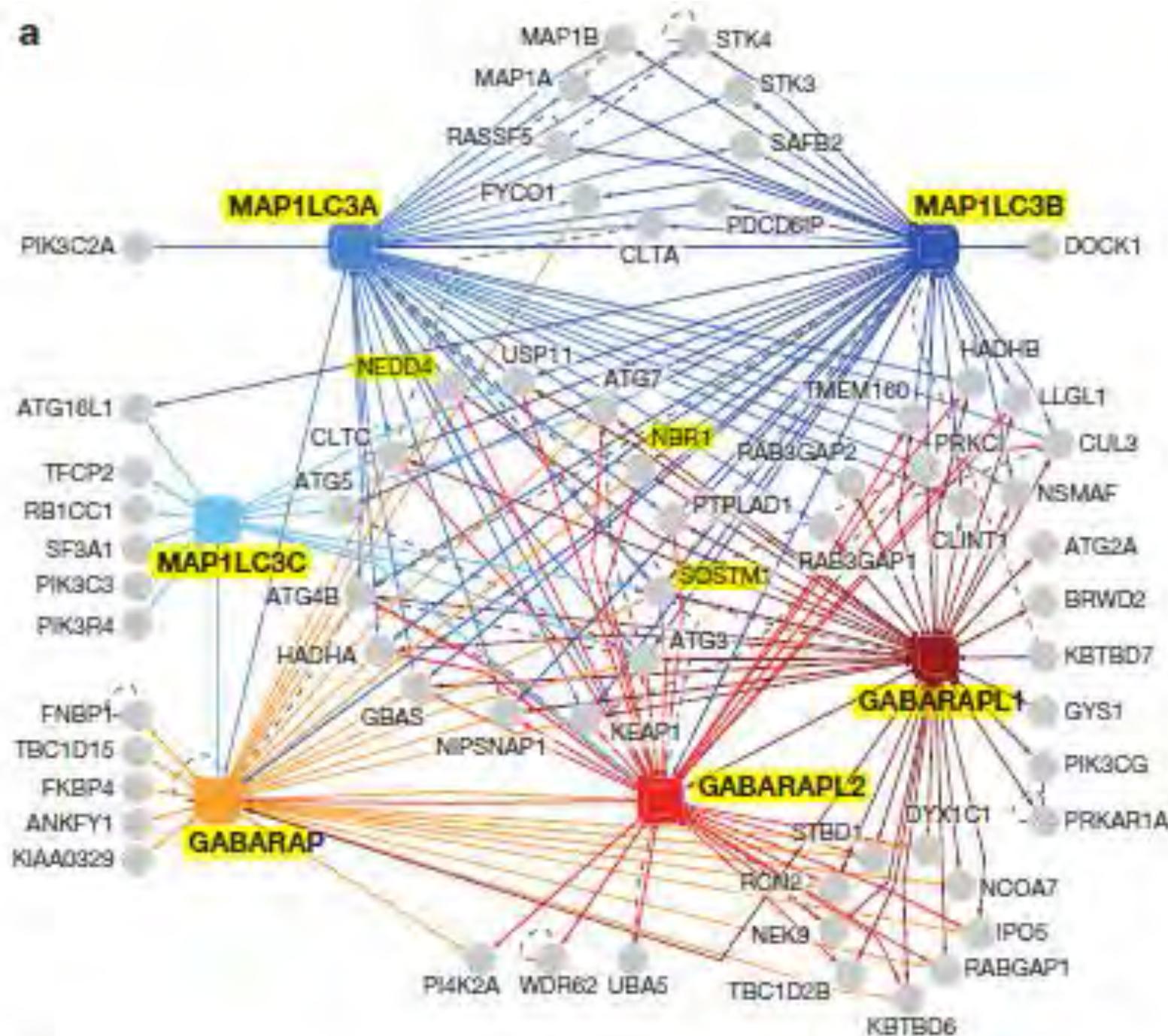
**B**

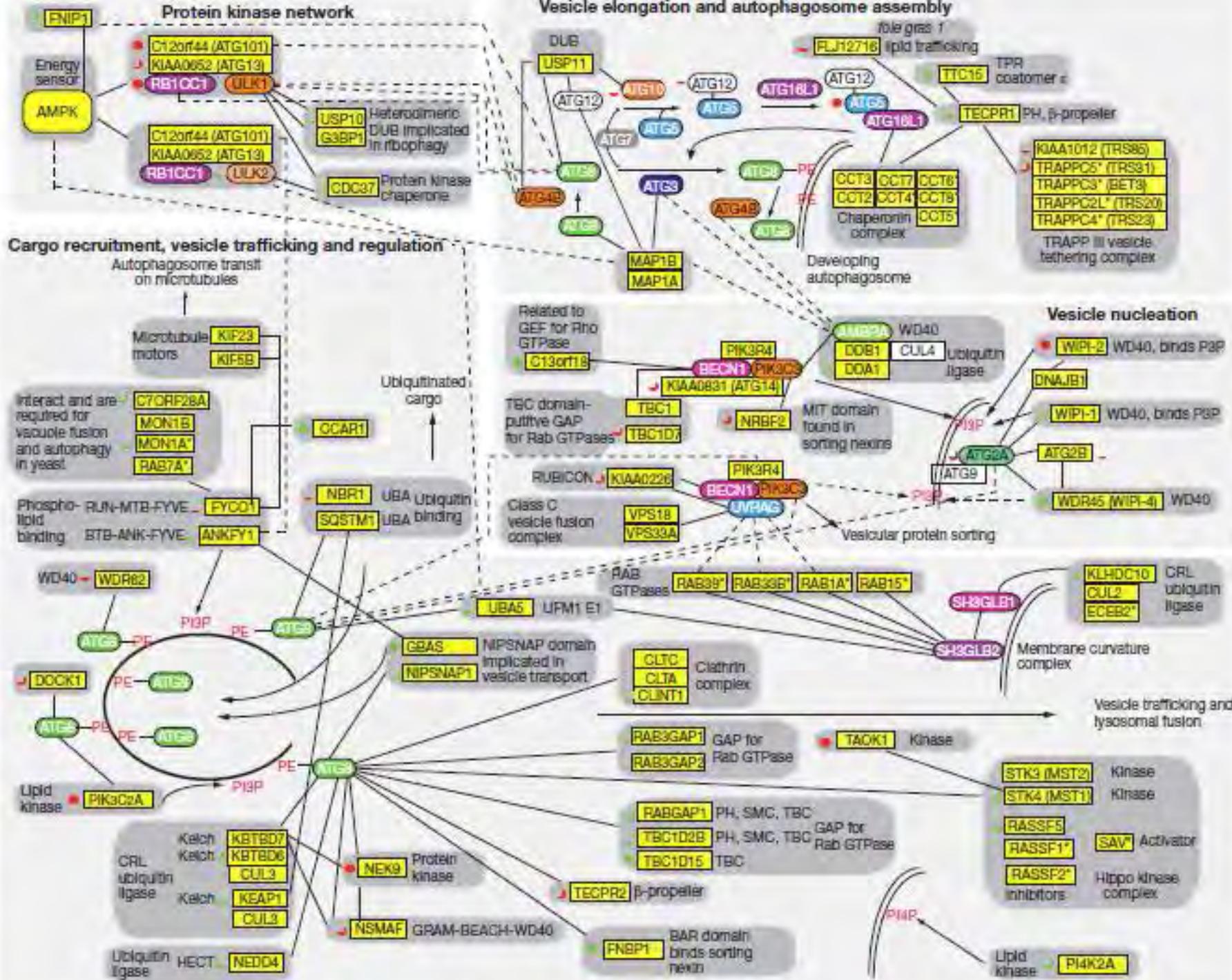
## p62 and Nrf2 in oxidative stress response





- **ULK1 kinase network**
- **UBL conjugation system**
- **Vesicle trafficking components**
- **ULK1, ULK2, RB1CC1, KIAA0652, C12orf44 GBL, FOXO3A**
- **ATG3, ATG4B, ATG4C, ATG5, ATG7, ATG10, ATG12, ATG16L1, TECPR1**
- **NSF, RAB24, GOSR1, CLN3**
- **PIK3C3-BECN1 network**
- **Human ATG8s**
- **Human ATG8s interacting proteins**
- **SH3GLB1 network**
- **MAP1LC3A, MAP1LC3B, MAP1LC3C, GABARAP, GABARAPL1, GABARAPL2**
- **SQSTM1, RASSF5, FYCO1, UBA5, KBTBD7, PIK3C2A, NSMAF, PIK3CG, STK4, STK3, RABGAP1, NEK9, GBAS**
- **ATG2-WIPI network**
- **ATG2A, WIPI1, WIPI2, WDR45**
- **SH3GLB1, SH3GLB2, KLHDC10**
- **TRAF2, HIF1A, DDIT3, PDPK1**

**a**



# Patients of the Finnish LGMD1D FF1-family

Proband age 76



daughter age 40



Brother age 71

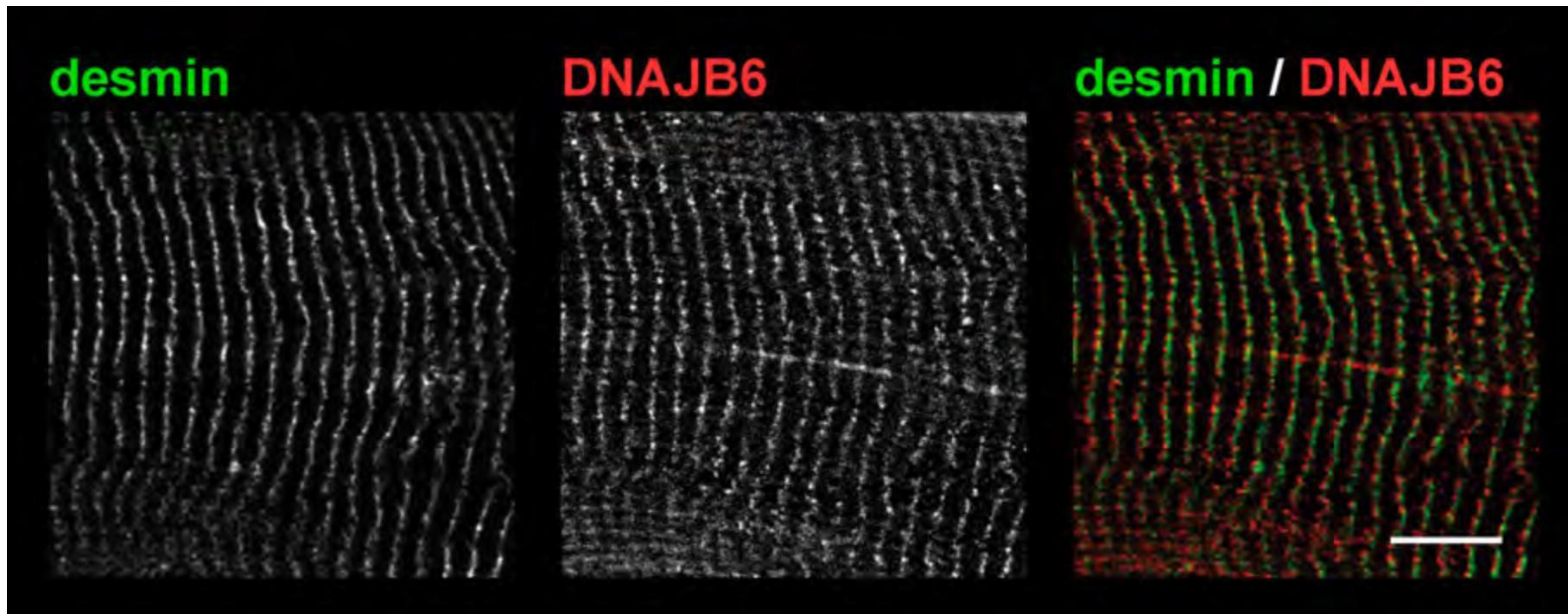


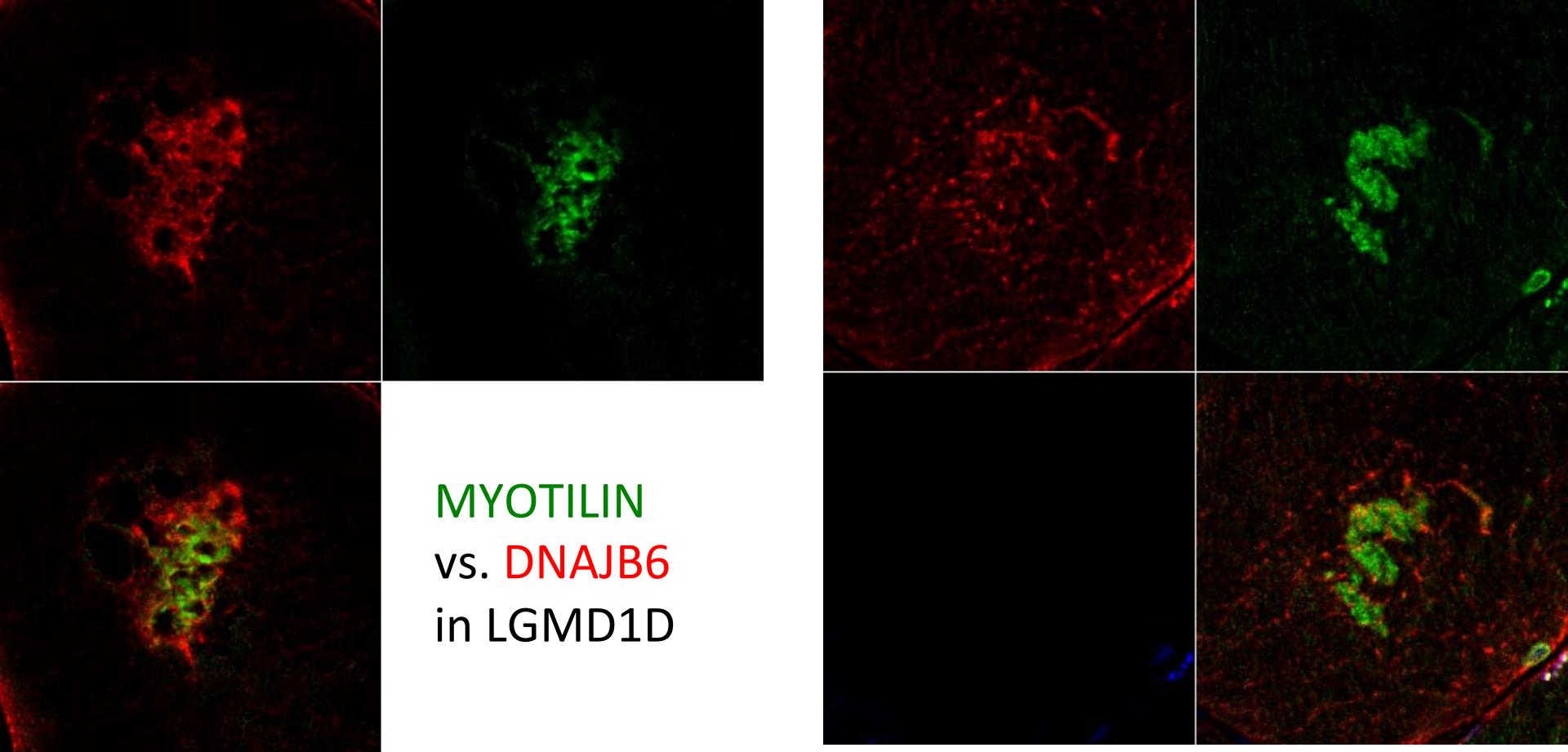
Cousin age 81



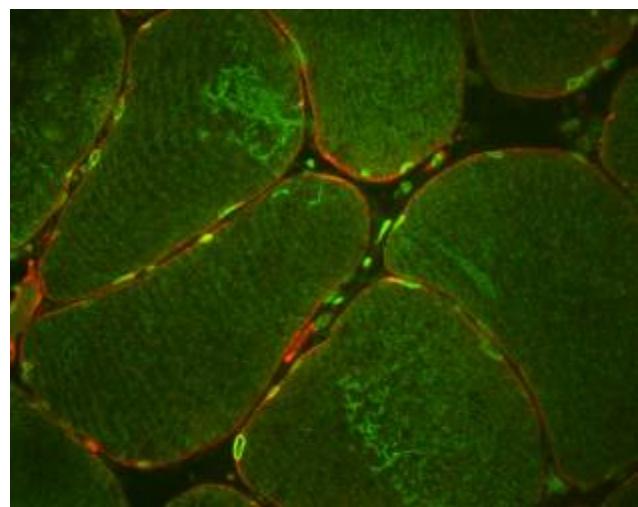
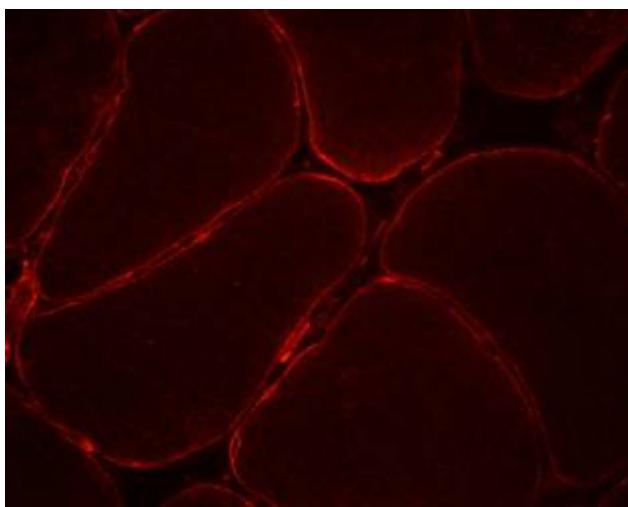
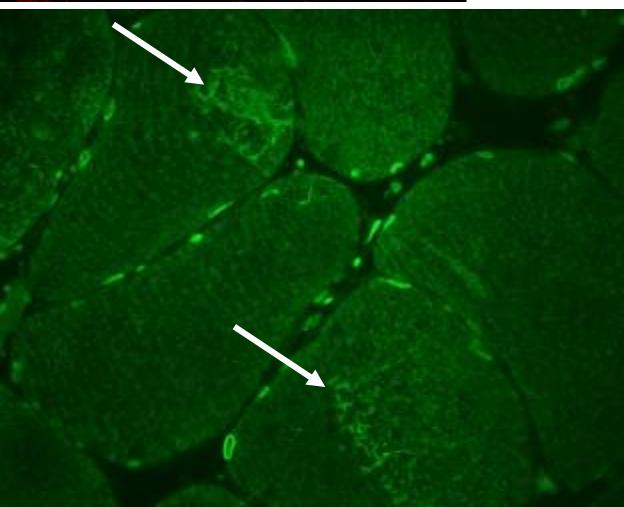
# DNAJB6: expression in LGMD1D patient muscle

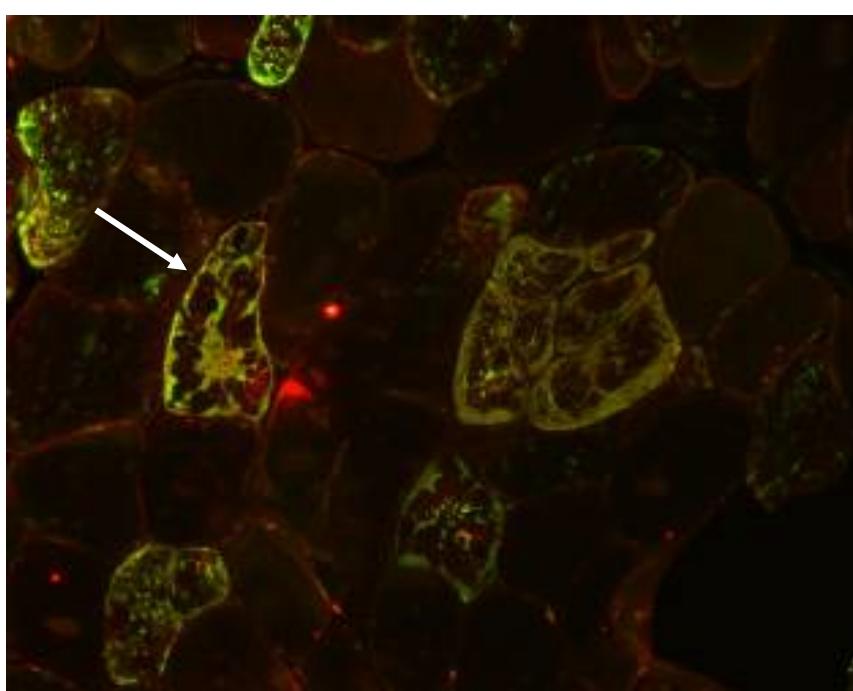
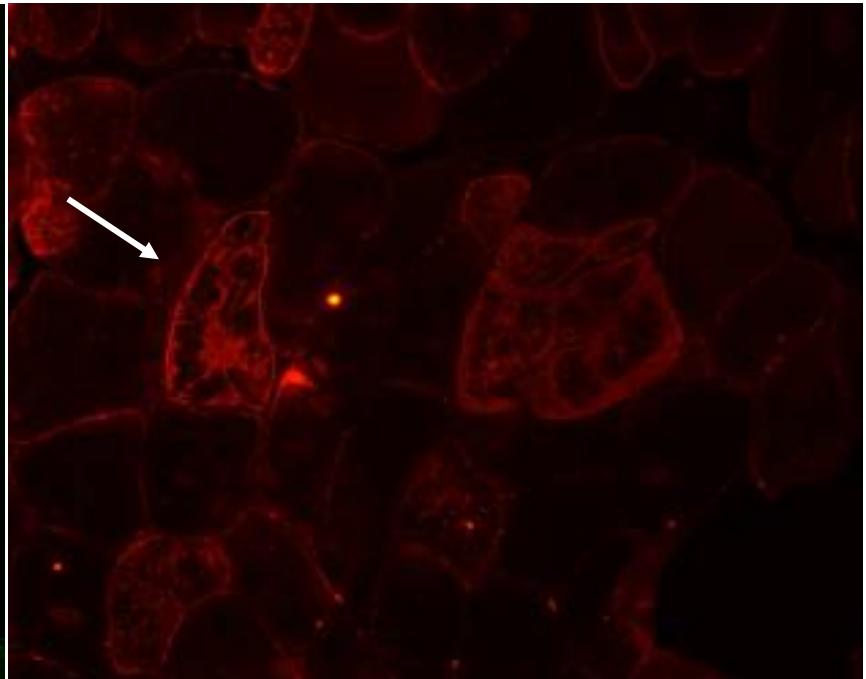
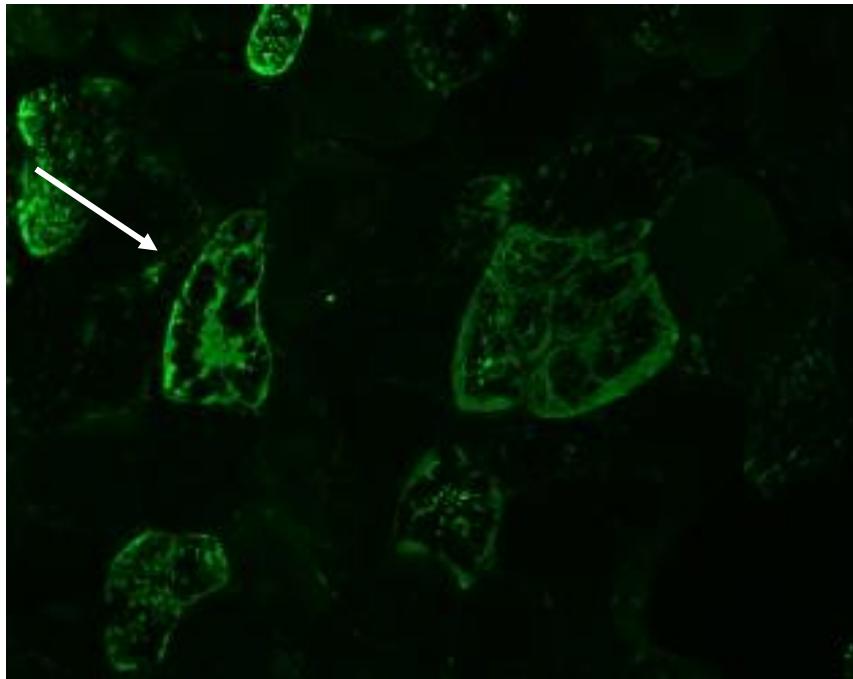
- mRNA transcripts show normal levels
  - ~ 50:50 mutant vs. wt
- DNAJB6 protein is located in the Z-disk
  - No abnormal location in unaltered myofibers of patients





MYOTILIN  
vs. DNAJB6  
in LGMD1D





Myotilinopathy S60C:  
Co-localization of  
**MYOT** and **DNAJB6**