

Muscle biopsy analysis-role in clinical practice

Sydney Neurophysiology Workshop
in association with ANZAN
15^t - 16th, November 2014

Bjarne Udd

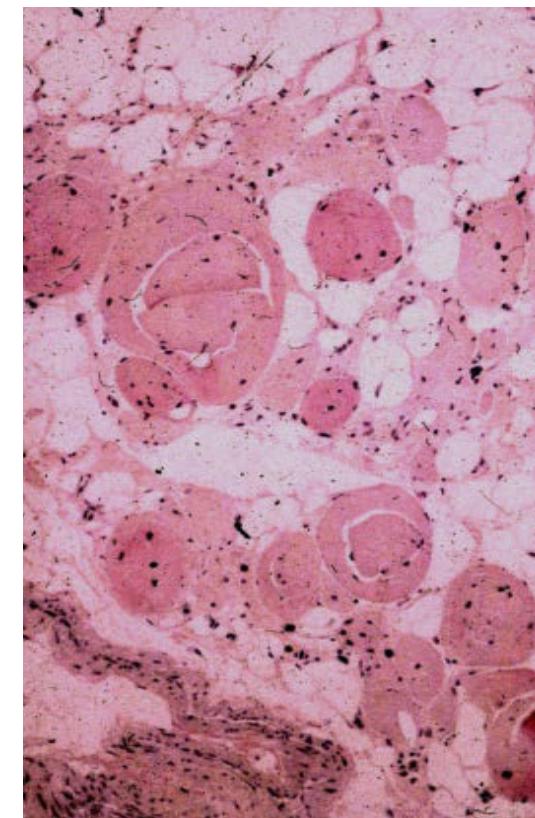
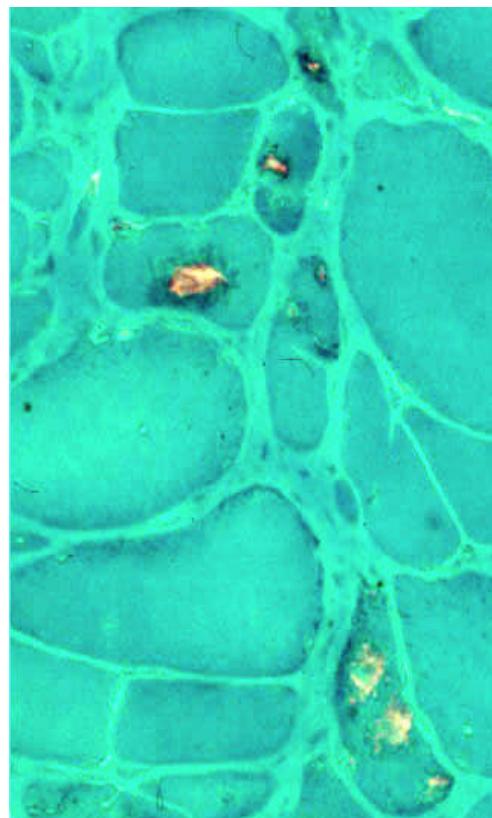
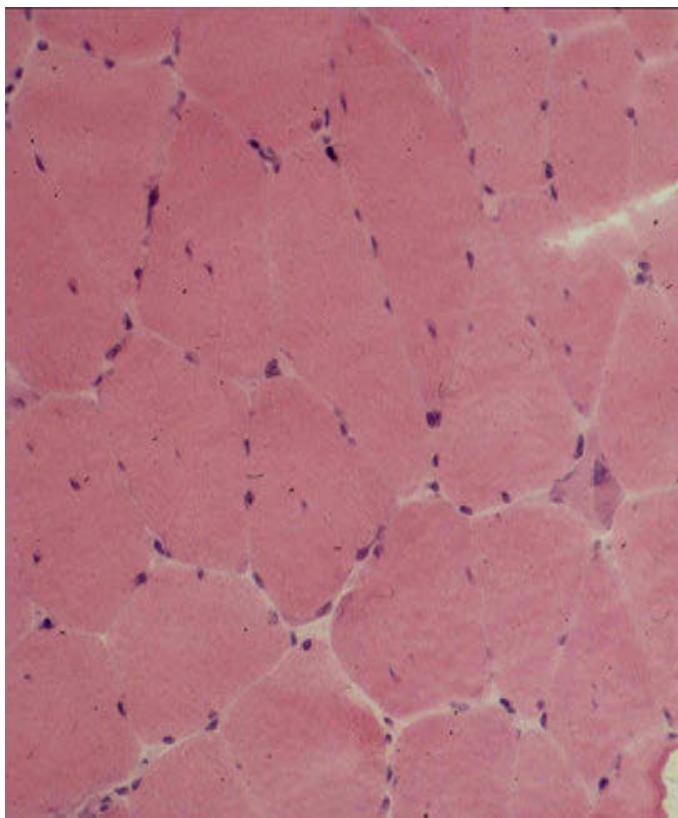
Neuromuscular Research Center, Tampere University Hospital
Folkhälsan Institute of Genetics, University of Helsinki
Department of Neurology, Vasa Central Hospital

Old truths and new opportunities

- Muscle biopsy is and will stay a corner stone of diagnostics
 - the role will not diminish with Next Gen Sequencing
- Aspects on how to increase the diagnostic yield
 - A large proportion of biopsies in the first US Duchenne trial were useless
 - New insight in the role of correct site of biopsy
 - Mainly derived from extensive use of muscle MRI
 - Some examples of molecular pathology methods and possibilities
 - New molecular pathology methods need centers of expertise
- Reluctant use because open surgical biopsy is highly invasive?
 - Not necessary with alternative technique

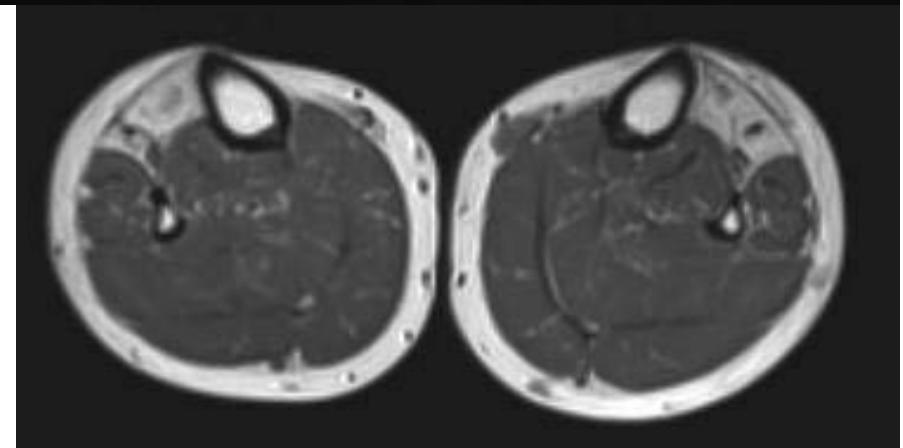
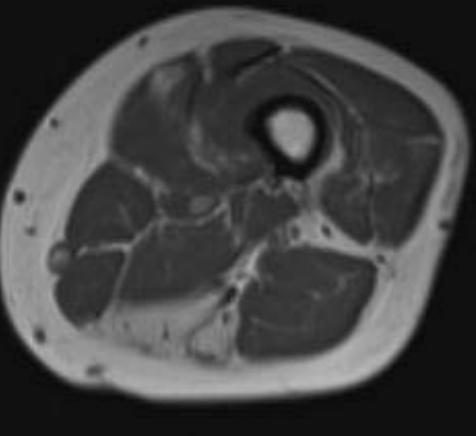
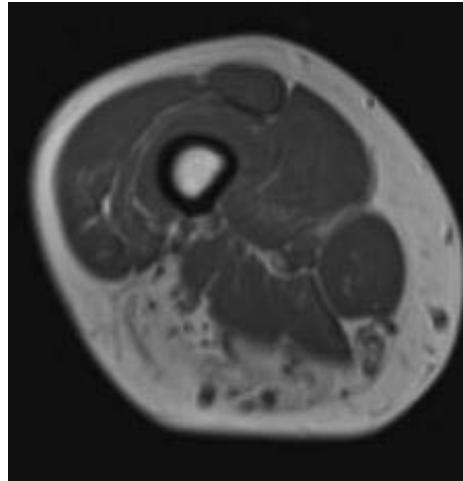
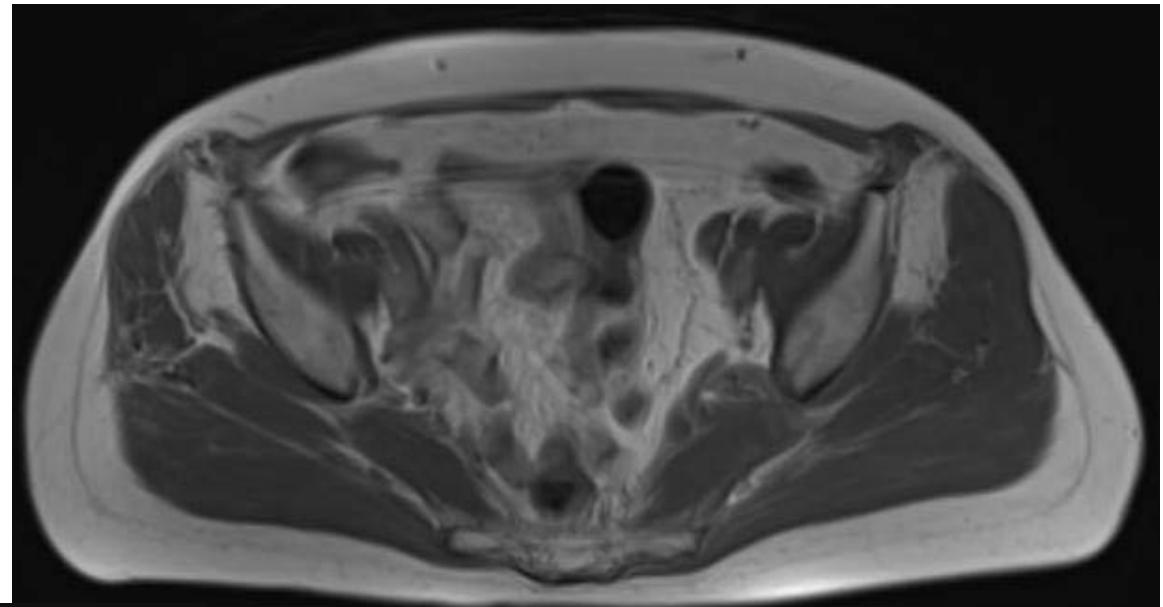
New insight in the role of correct site of biopsy

> the interest of muscle imaging – experience with distal myopathies



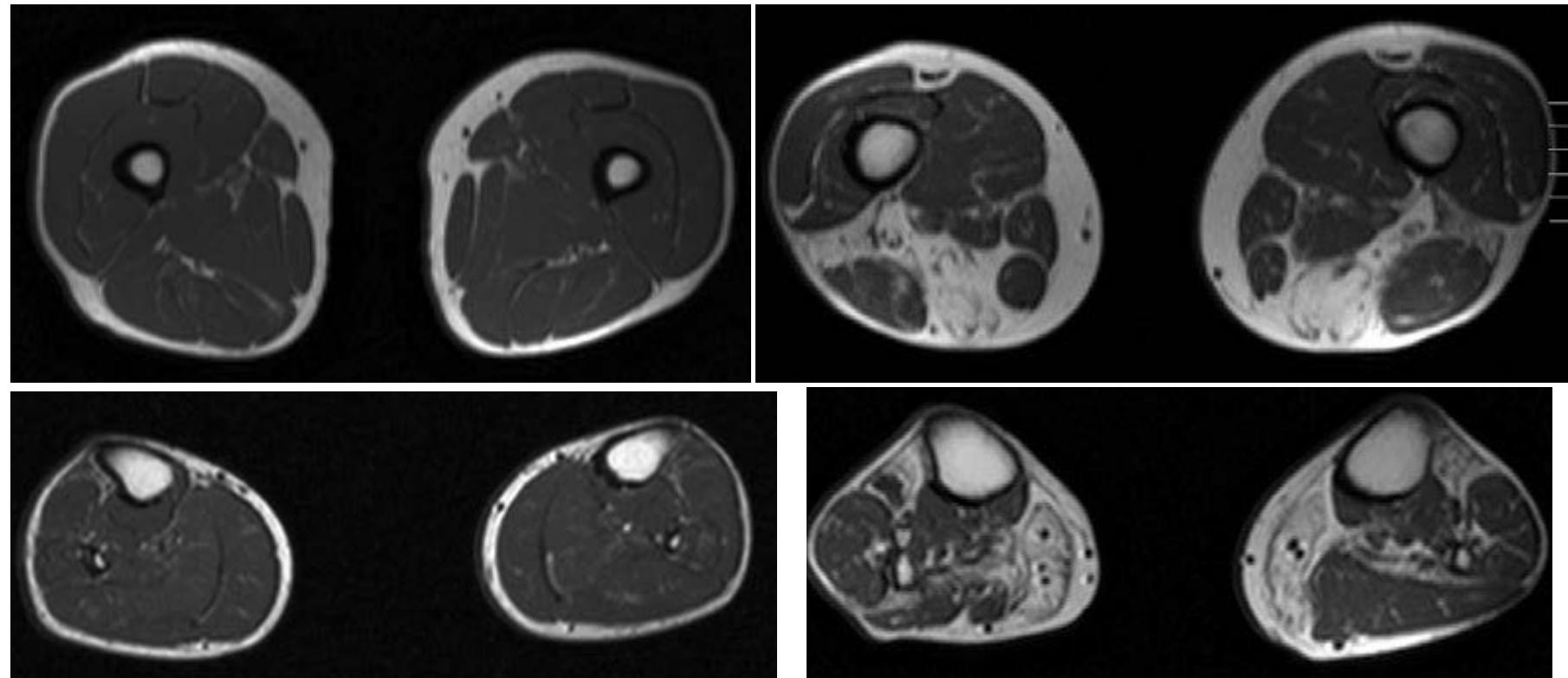
Distal titinopathy
-Tibial muscular dystrophy (TMD)

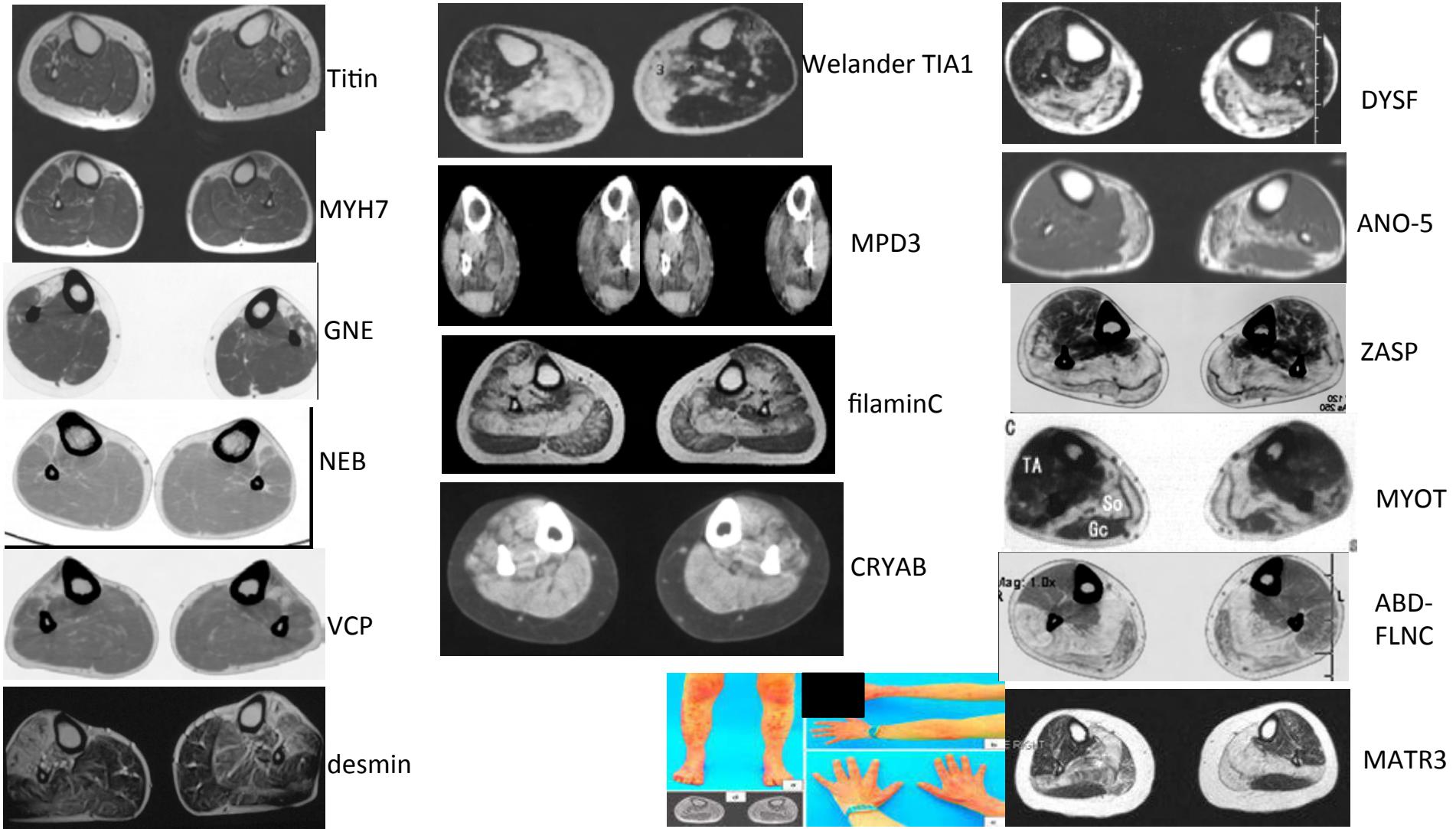
- male age 60 yrs



Variations of severity in AD distal titinopathy TMD

2 males both 55 years old with identical mutation

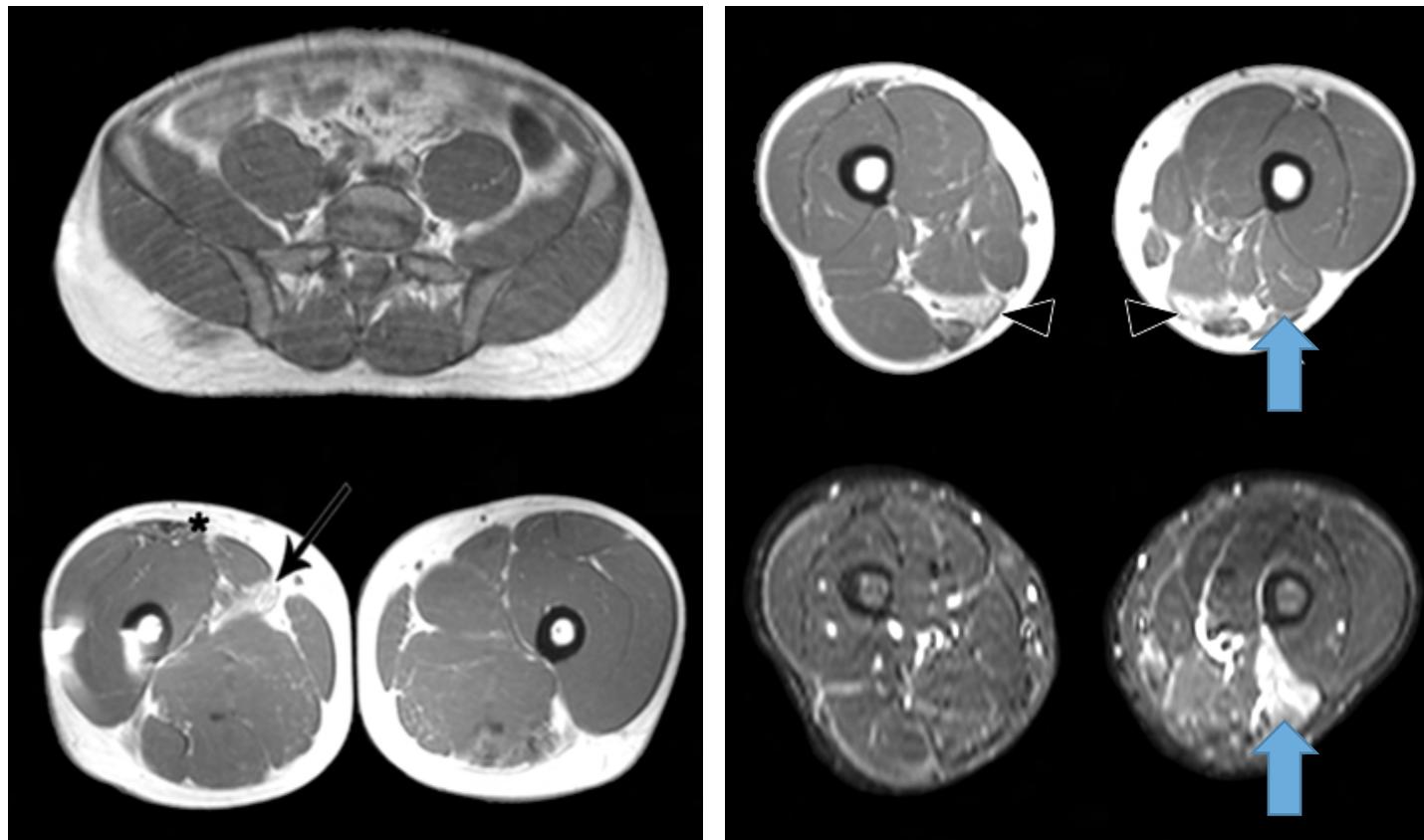




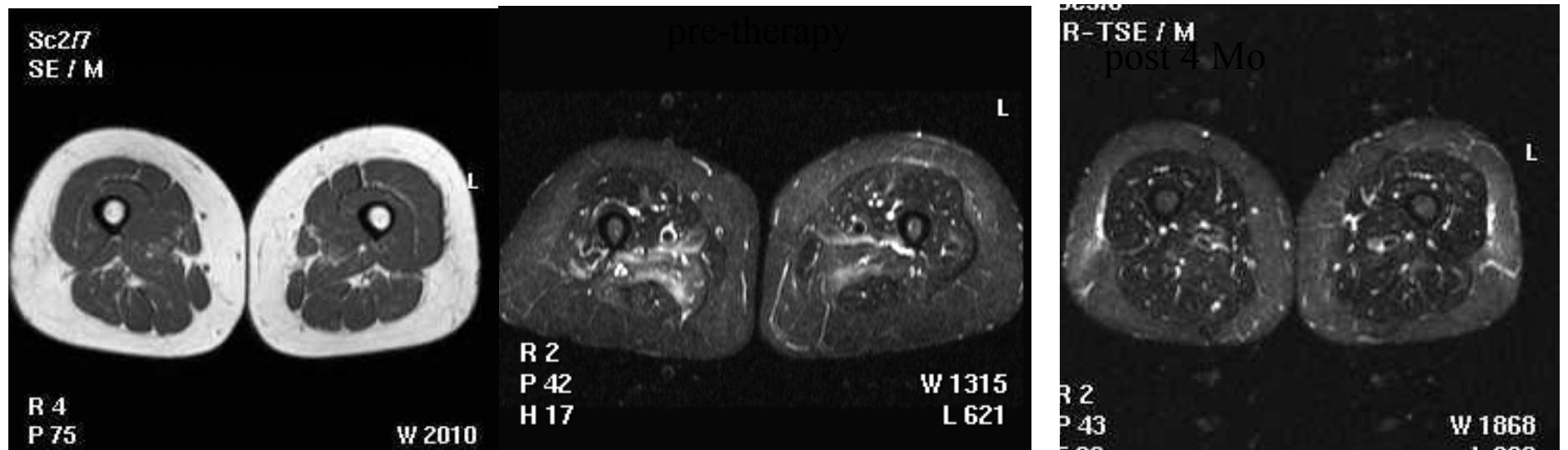
FSH: asymmetry and edema

>MRI can provide insight of the pathomechanism

Courtesy E Ricci/G Tasca



Myositis: subacute Jo-1 pos antisynthetase syndrome

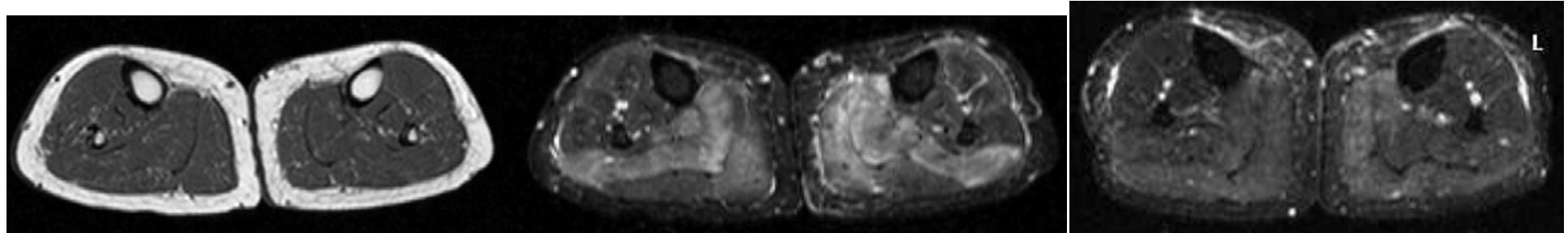


Before treatment:

No degenerative changes

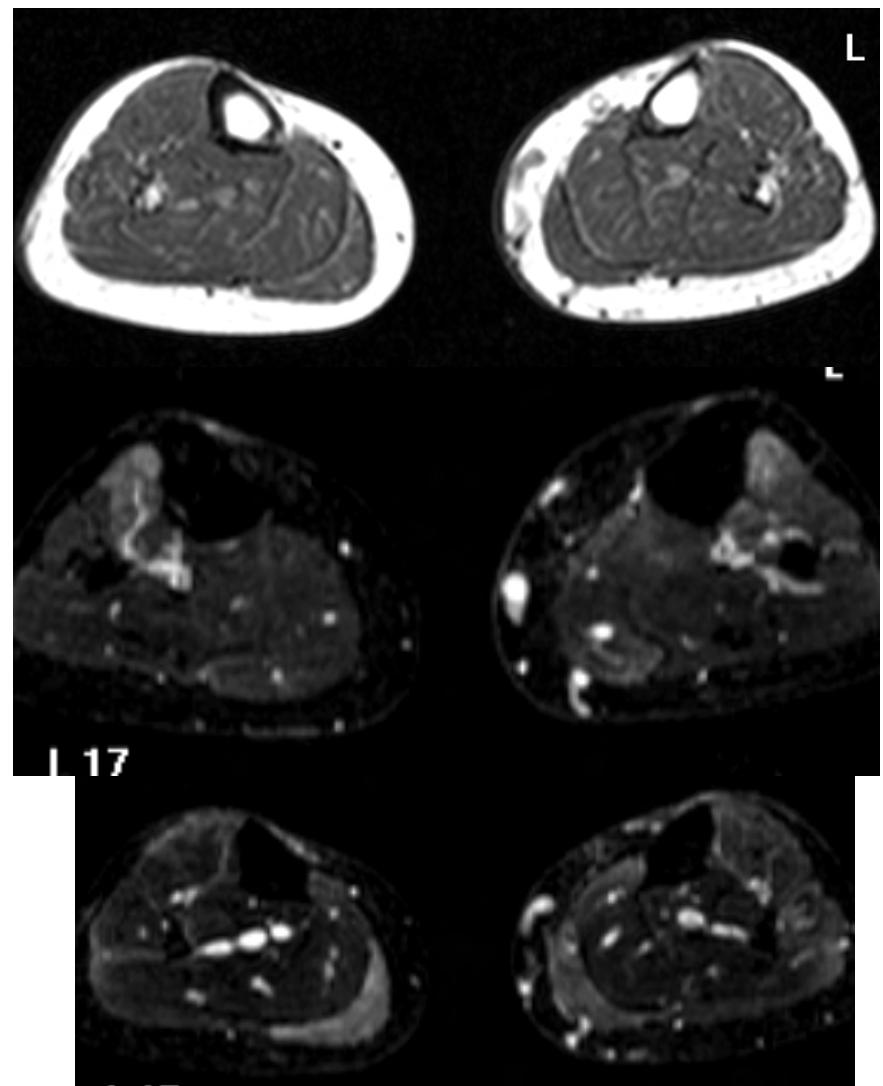
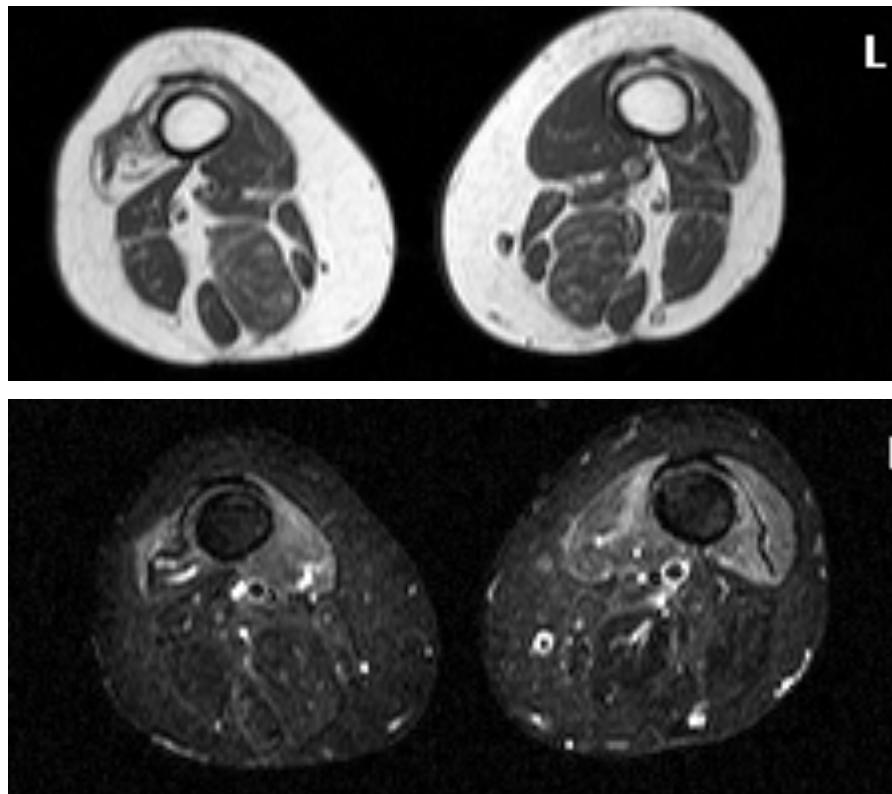
Edema in AM, AL, BF, calf

3 Mo after steroids
incomplete response



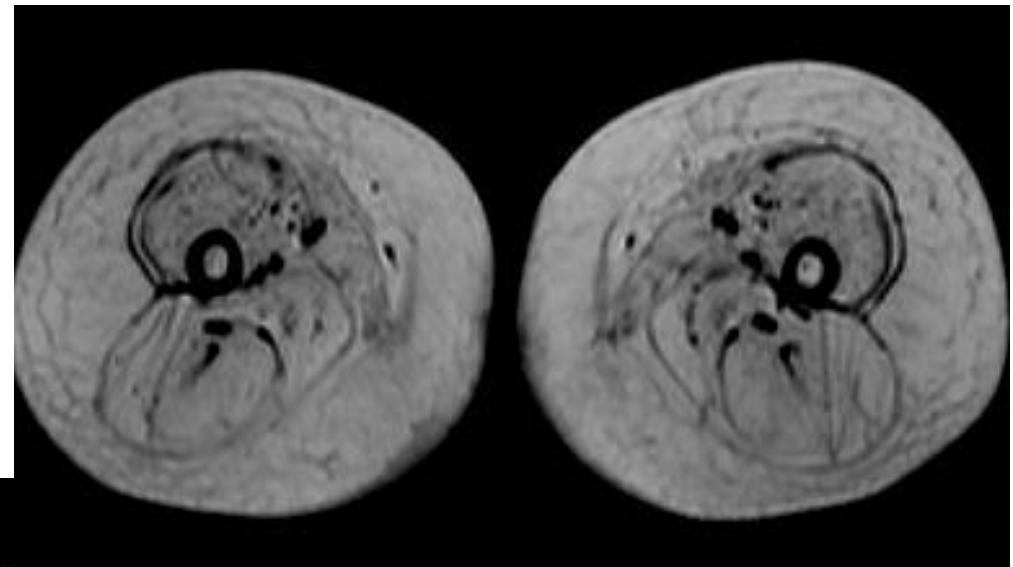
Sporadic inclusion body myositis (s-IBM) MRI imaging

- T1 and STIR fs sequences



Or you may need muscle for diagnostics at the very advanced stage of dystrophy

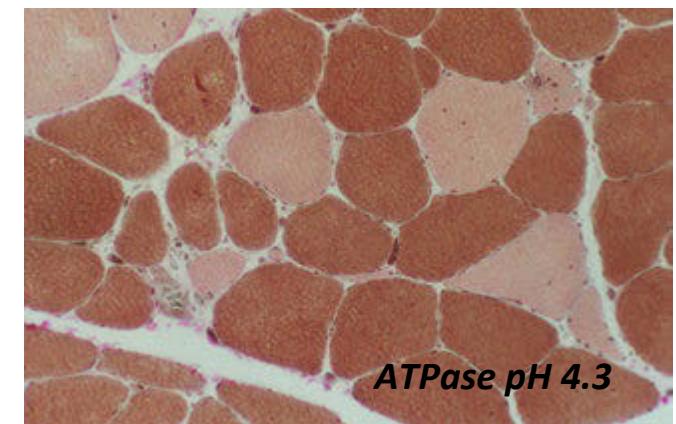
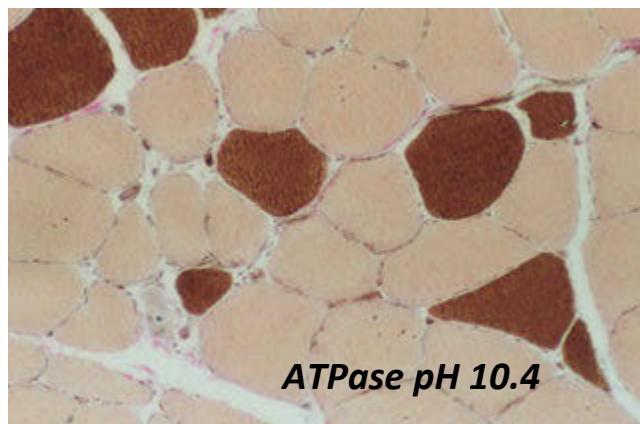
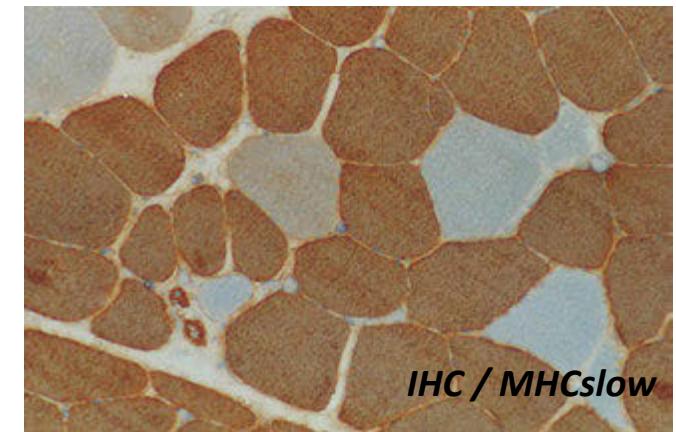
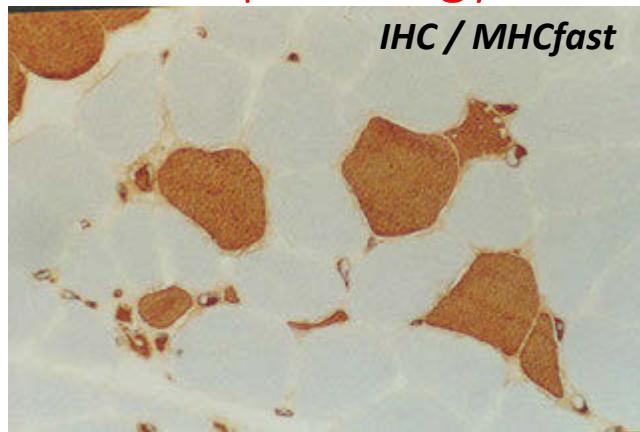
- With unproven mutations:
- to check the protein expression
- or mRNA of a potential splice site
- The case: LGMD2D, age 18



Aspects on how to increase the diagnostic yield

Some examples of molecular pathology methods

- Our extended work with myotonic dystrophy type 2 showed a severe atrophy of some type 2 fibers
- easily overlooked by ATPase fiber typing



Neurology 2003:

Histopathological differences of myotonic dystrophy type 1 (DM1) and PROMM/DM2

A. Vihoia, MSc; G. Bassot, MD; G. Moolia, MD, PhD; S. Zhang, PhD; H. Haapavesi, MD, PhD;

A. Pastan, MD, PhD; K. Mancinelli, PhD; A. Rouche, MSc; J.Y. Hogrel, PhD; P. Laloriti, MD;

T. Matsunaga, MD; J.F. Pollicino, MD, PhD; R. Krahe, PhD; B. Eymard, MD, PhD; and B. Udd, MD, PhD

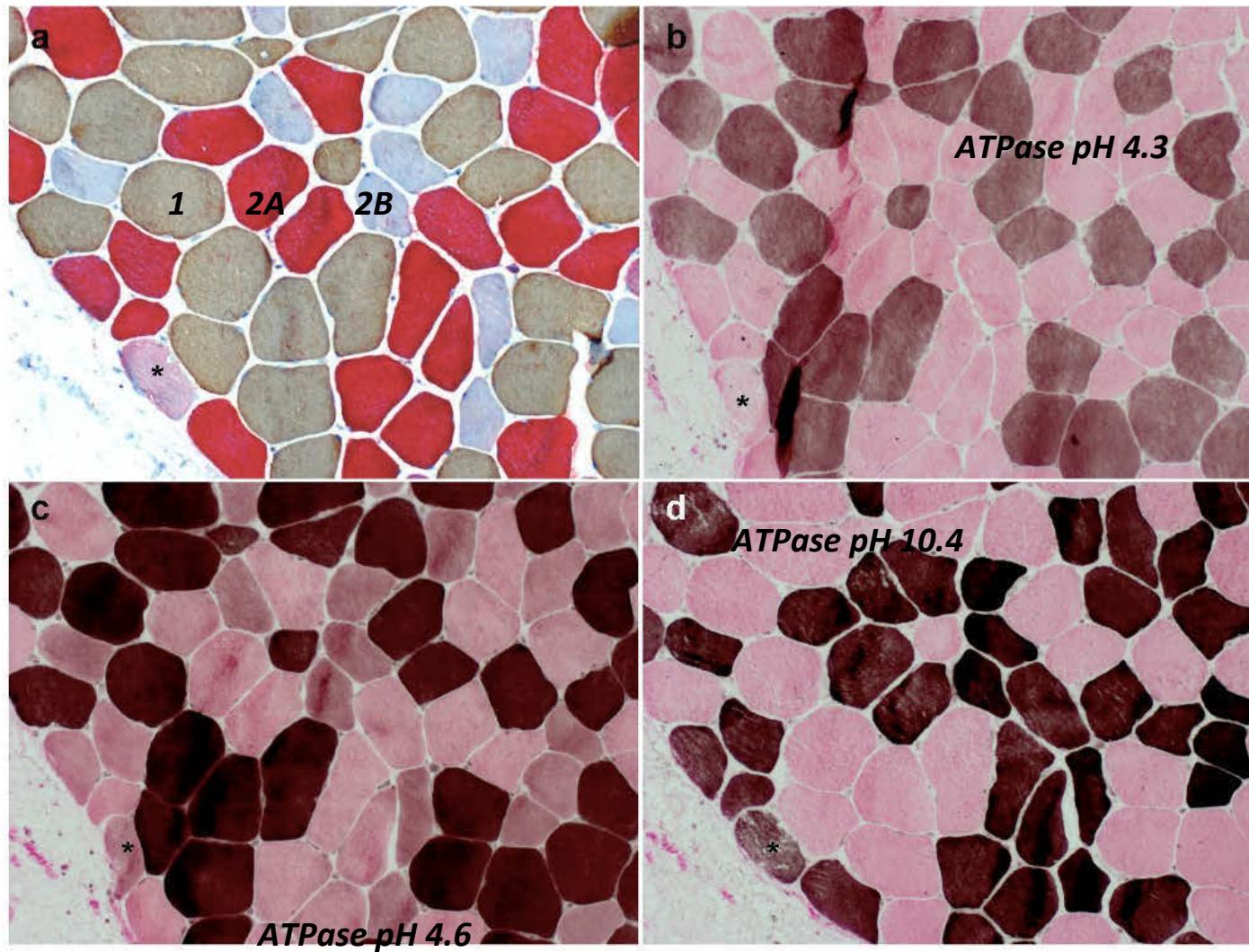
Abstract—Muscle biopsy findings in DM2 have been reported to be similar to those in DM1. The authors used myosin heavy chain immunohistochemistry and enzymes histochemistry for fiber type differentiation on muscle biopsies. Their results show that DM2 patients display a subpopulation of type 2 nuclear clump and other very small fibers and, hence, preferential type 2 fiber atrophy in contrast to type 1 fiber atrophy in DM1 patients.

NEUROLOGY 2003;60:1854–1857

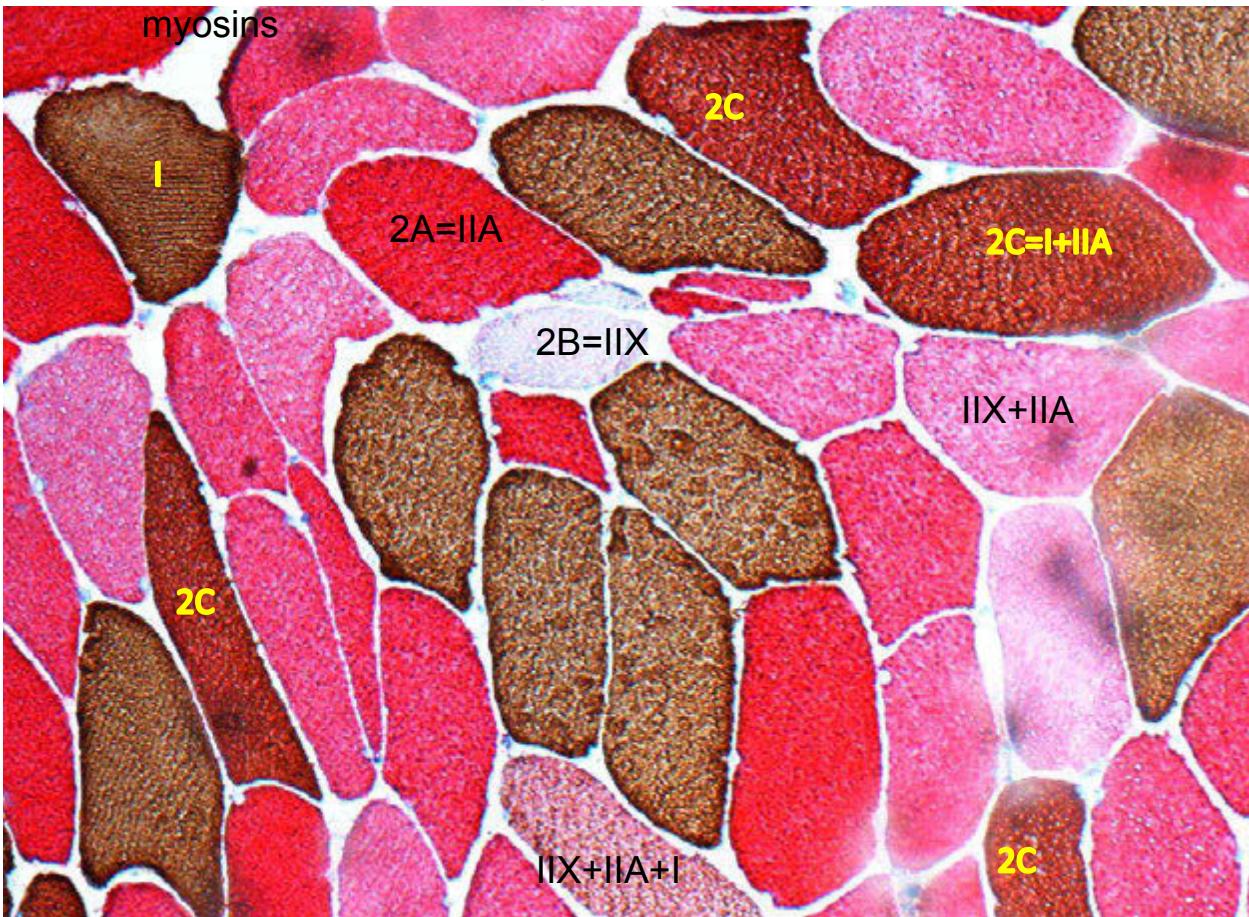
Myotonic dystrophies are categorized as DM1 (myotonic dystrophy, Steinert's disease; OMIM 160900), caused by a (CTG)_n expansion mutation in 19q13,

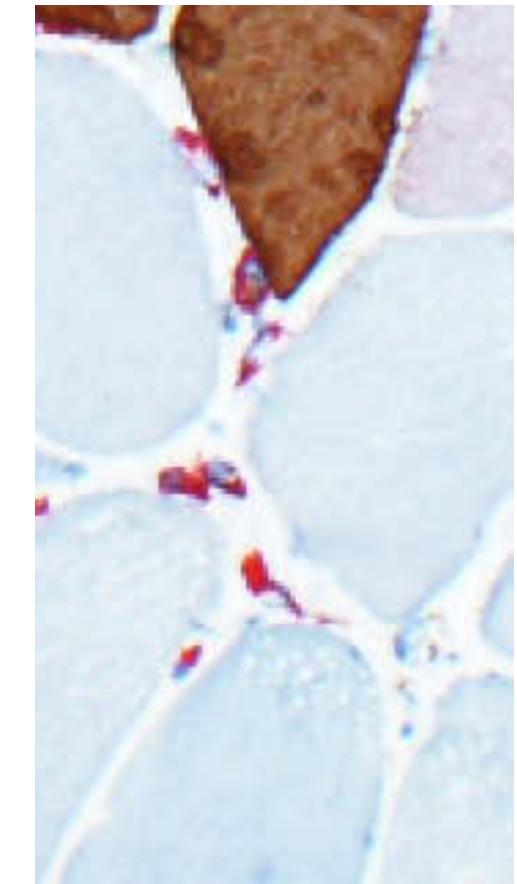
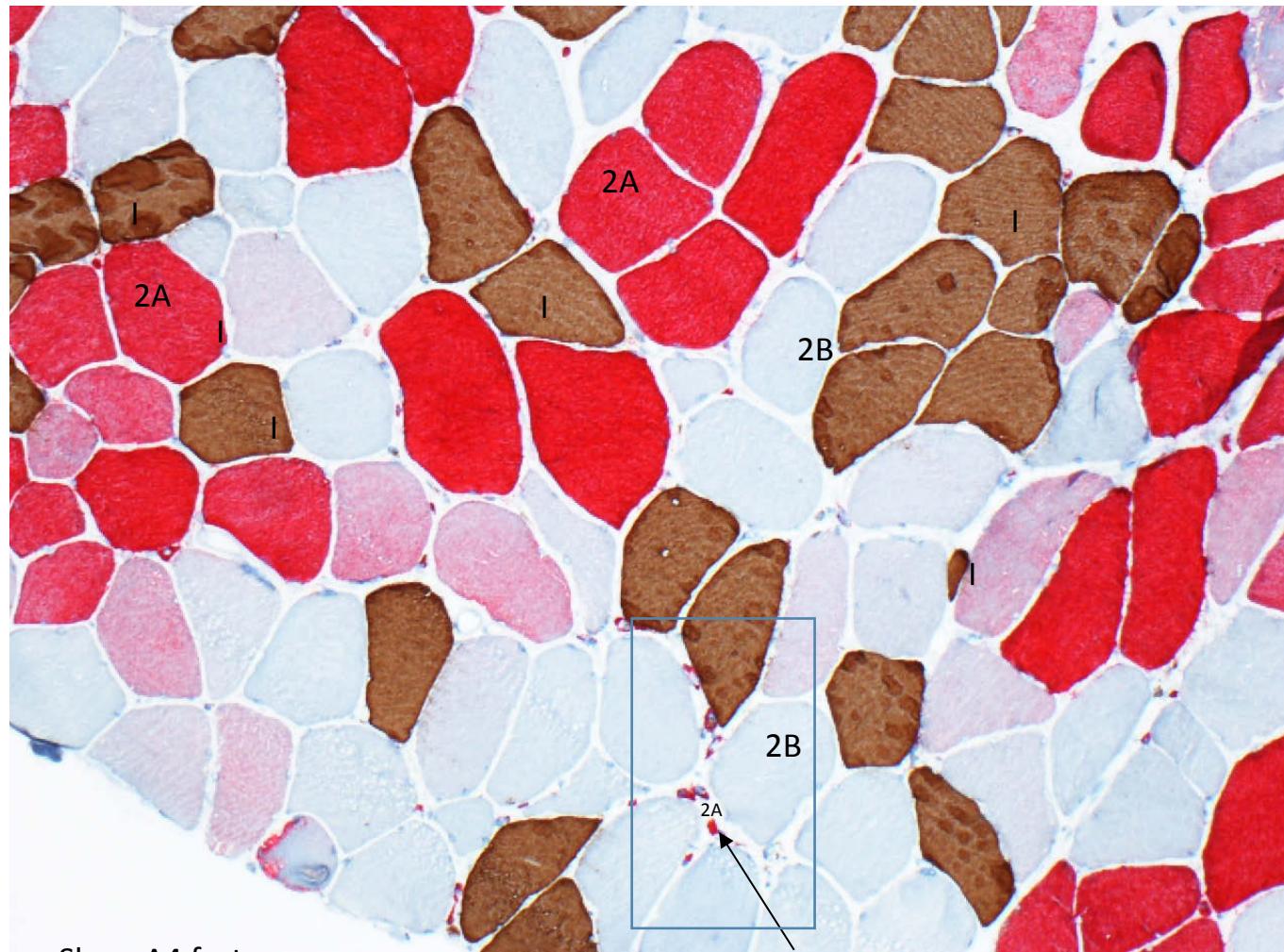
Muscle biopsies. Deltoid muscle biopsies were obtained from three French DM2 patients, two of whom originated from France and one from Serbia. Detailed clinical features of one patient (P3)

>>> improved technique by double immunostaining of the fiber types

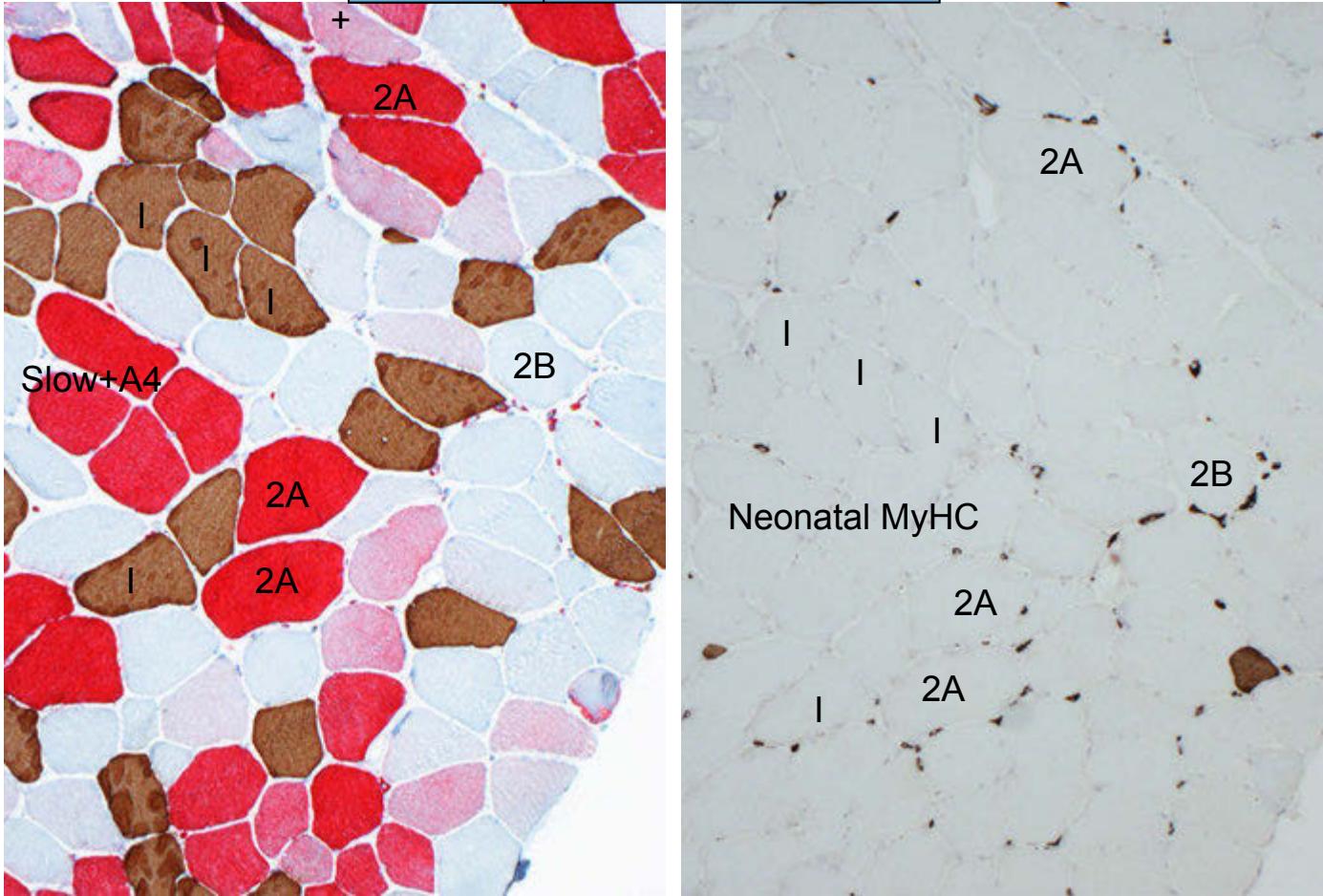


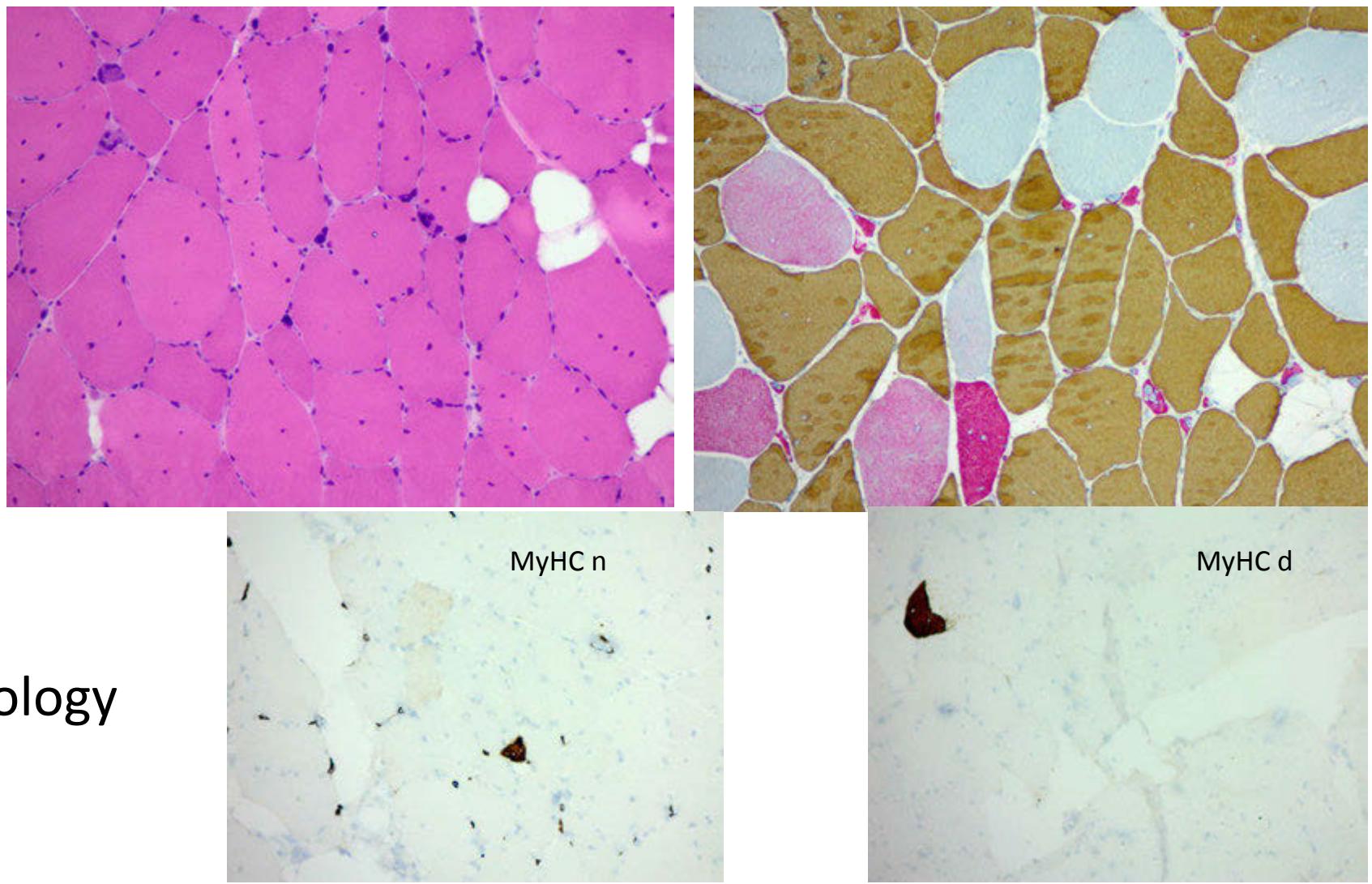
Hybrids: slow + fast: reddish brown: type 2C fibers,
pink: 2A + 2B hybrid expression of IIA and IIX



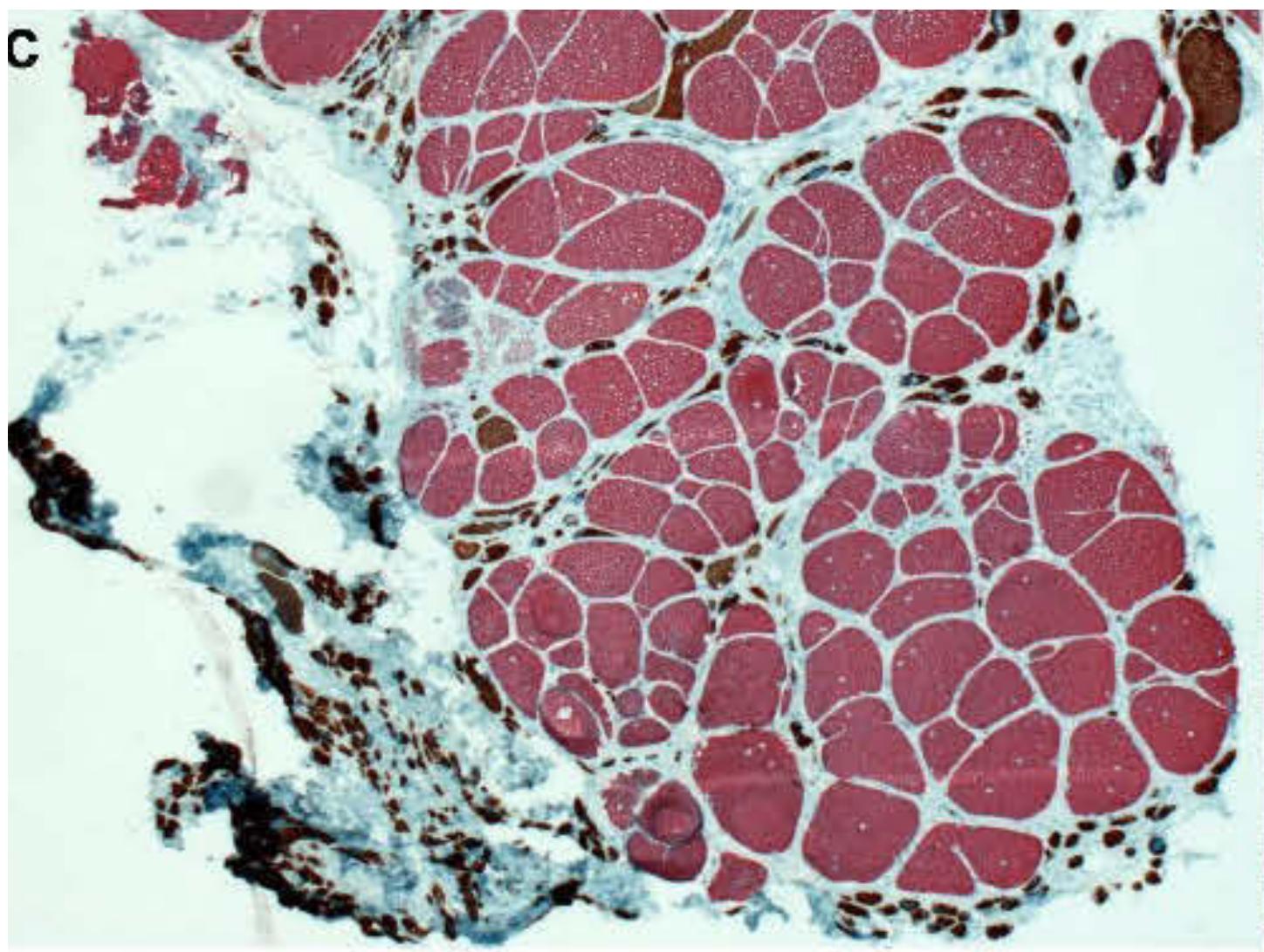


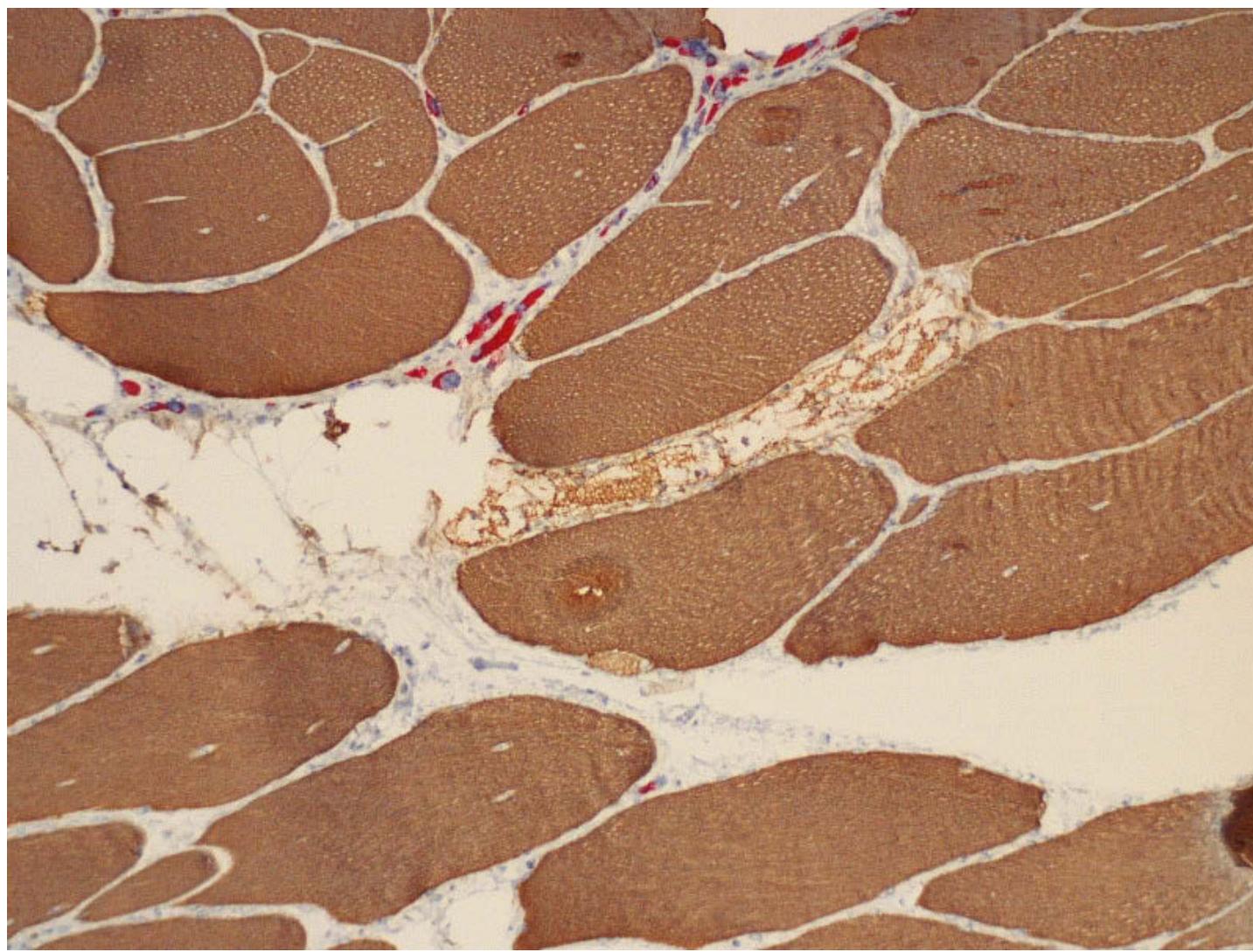
Slow brow	I	2A /IIA	2B/IIX
fast A4 red:	brown	red+++	-/





DM2
pathology





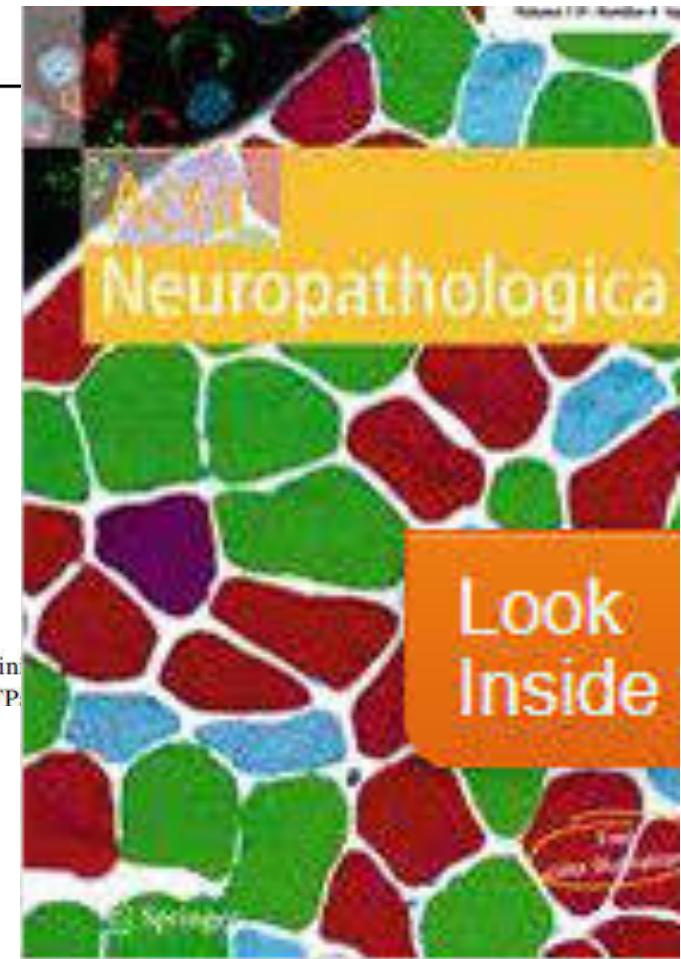
Novel myosin heavy chain immunohistochemical double staining developed for the routine diagnostic separation of I, IIA and IIX fibers

Olayinka Raheem · Sanna Huovinen ·
Tiina Suominen · Hannu Haapasalo ·
Bjarne Udd

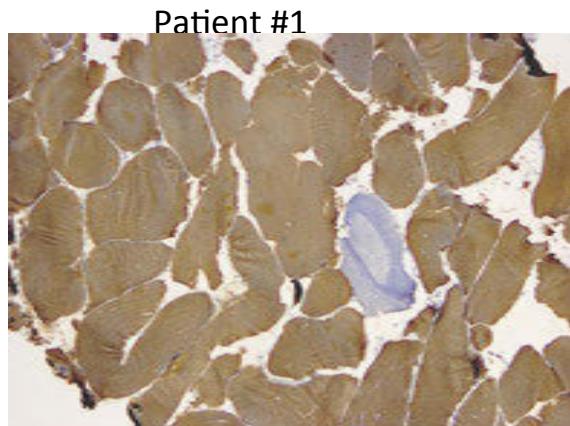
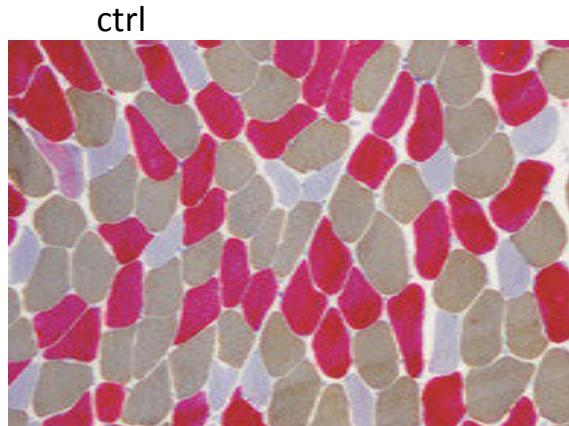
Received: 27 November 2009 / Revised: 14 January 2010 / Accepted: 17 January 2010
© Springer-Verlag 2010

Abstract The different histochemical ATPase properties of myosins separating the muscle fiber types have been

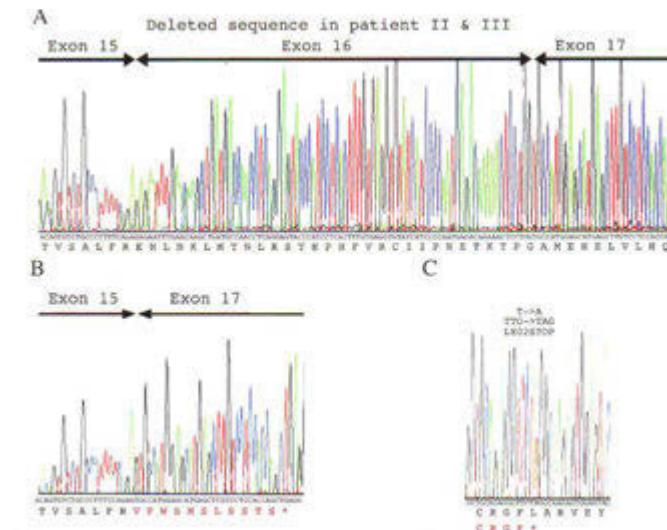
fibers in routine diagnostics. With this double staining method, we are able to distinguish among type I (ATP-



**Routine diagnostic use of double IHC staining of myosins for fiber typing
> direct tools to identify a new disease**



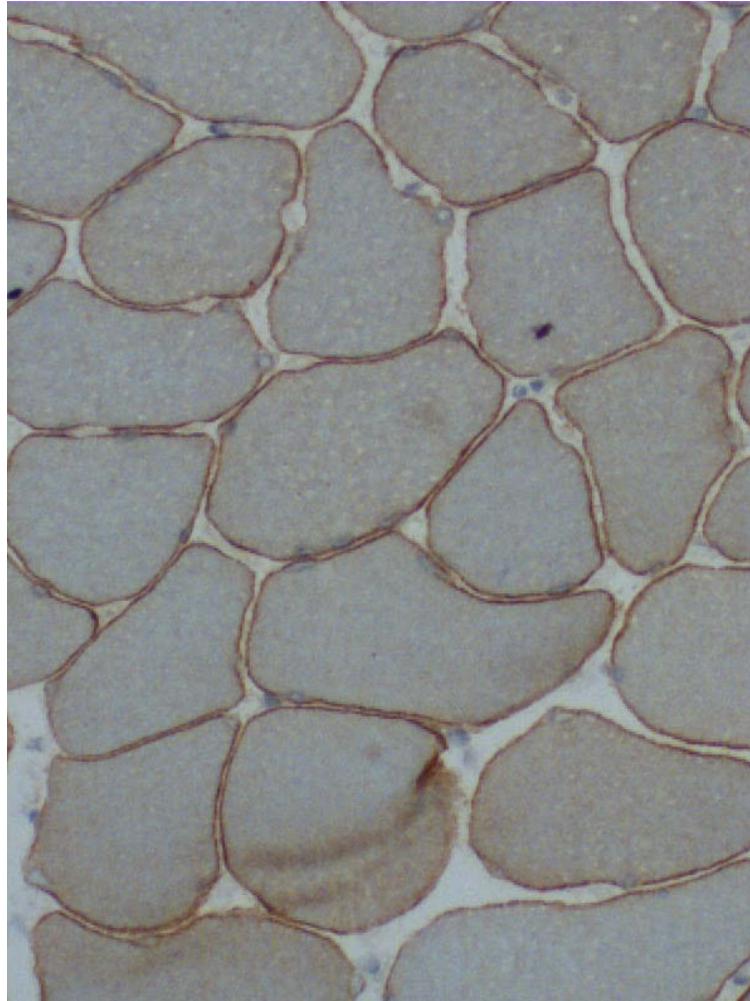
>> lack of fast IIA myosin (MYH2)



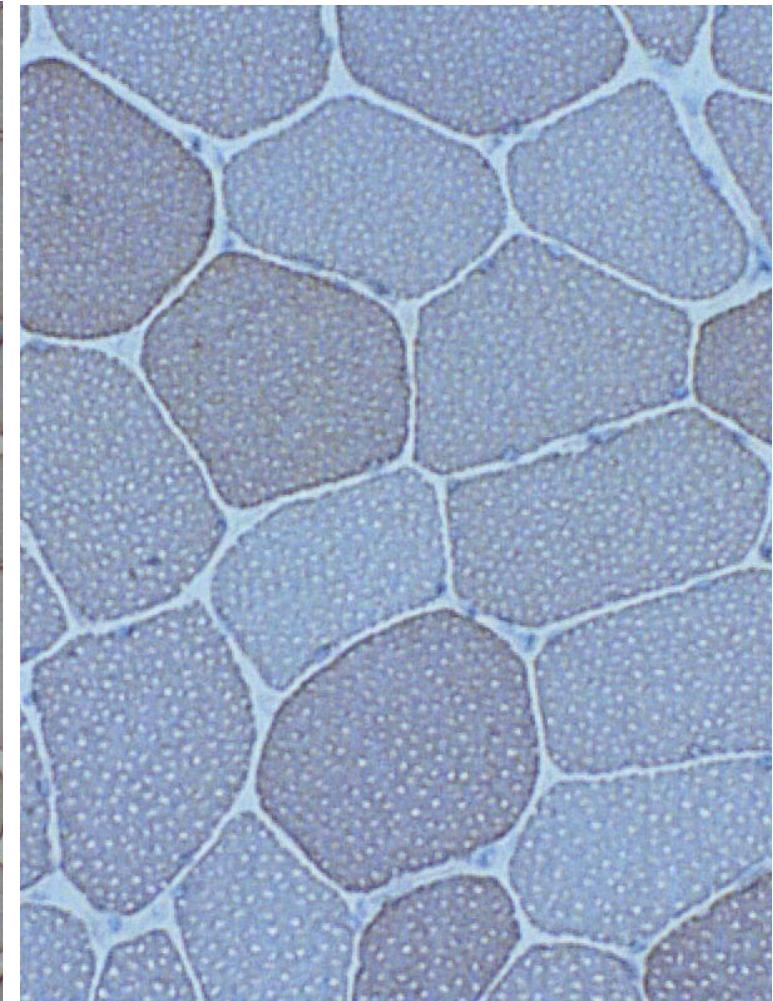
Finnish MYH2 patients: compound heterozygous nonsense mutations
→ splicing Ex16 del f11X + TTG>TAG p.L802X

Tajsharghi H, Hilton-Jones D, Raheem O, Saukkonen AM, Oldfors A, and Udd B. (**Brain 2010**).
Human disease caused by total loss of fast Ila myosin heavy chain due to MYH2 mutations.

- More spin-off from work with myotonic dystrophy type 2
- Carriers of het CLCN1 rec mutations have a more severe myotonia
- Can the loss of chloride channel be visualized?
- > no success
- Instead we introduced a method for non-dystrophic



CLC-1 IHC control



Becker cong myotonia patient R894X

New immunohistochemical method for improved myotonia and chloride channel mutation diagnostics

Olayinka Raheem, MSc

Sini Penttilä, MSc

Tiina Suominen, MSc

Mika Kaakinen, PhD

James Burge, MRCP

Andrea Haworth, PhD

Richa Sud, PhD

Stephanie Schorge, PhD

Hannu Haapasalo, MD,
PhD

Satu Sandell, MD

Kalervo Metsikkö, PhD

Michael Hanna, FCRP

Bjarne Udd, MD, PhD

ABSTRACT

Objective: The objective of this study was to validate the immunohistochemical assay for the diagnosis of nondystrophic myotonia and to provide full clarification of clinical disease to patients in whom basic genetic testing has failed to do so.

Methods: An immunohistochemical assay of sarcolemmal chloride channel abundance using 2 different CIC1-specific antibodies.

Results: This method led to the identification of new mutations, to the reclassification of W118G in *CLCN1* as a moderately pathogenic mutation, and to confirmation of recessive (Becker) myotonia congenita in cases when only one recessive *CLCN1* mutation had been identified by genetic testing.

Conclusions: We have developed a robust immunohistochemical assay that can detect loss of sarcolemmal CIC-1 protein on muscle sections. This in combination with gene sequencing is a powerful approach to achieving a final diagnosis of nondystrophic myotonia. *Neurology*® 2012;79:2194–2200

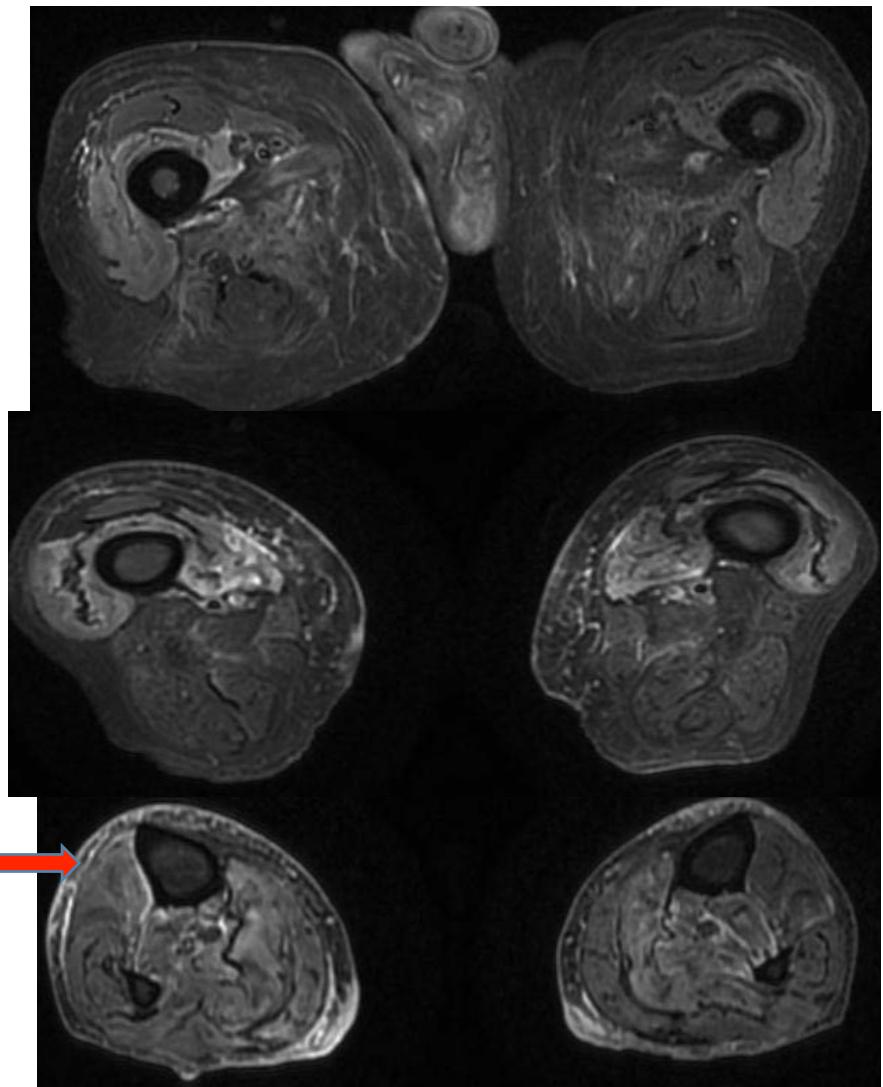
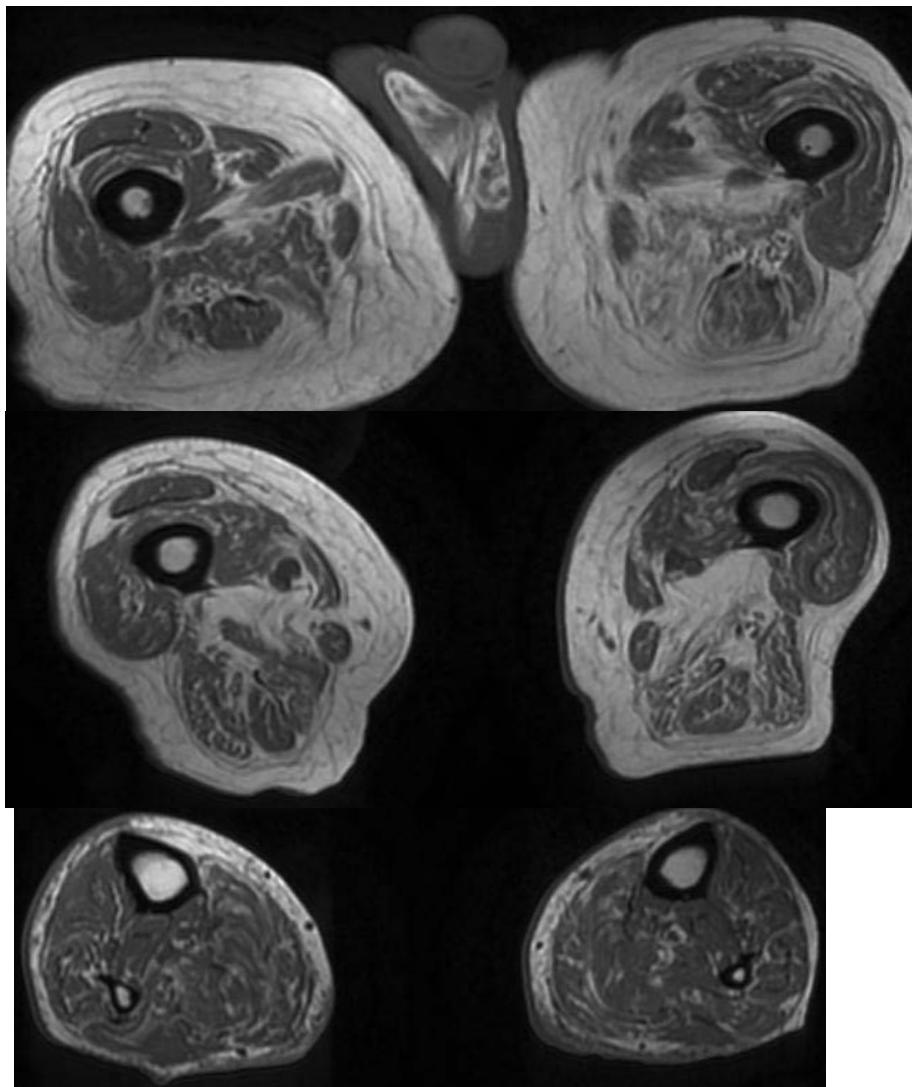
Old truths and new opportunities

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 - New insight in the role of correct site of biopsy
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 - Some examples of molecular pathology methods
- New methods need centers of expertise
- Reluctant use because open surgical biopsy is highly invasive?
 - Not necessary with alternative technique

The alternative biopsy technique

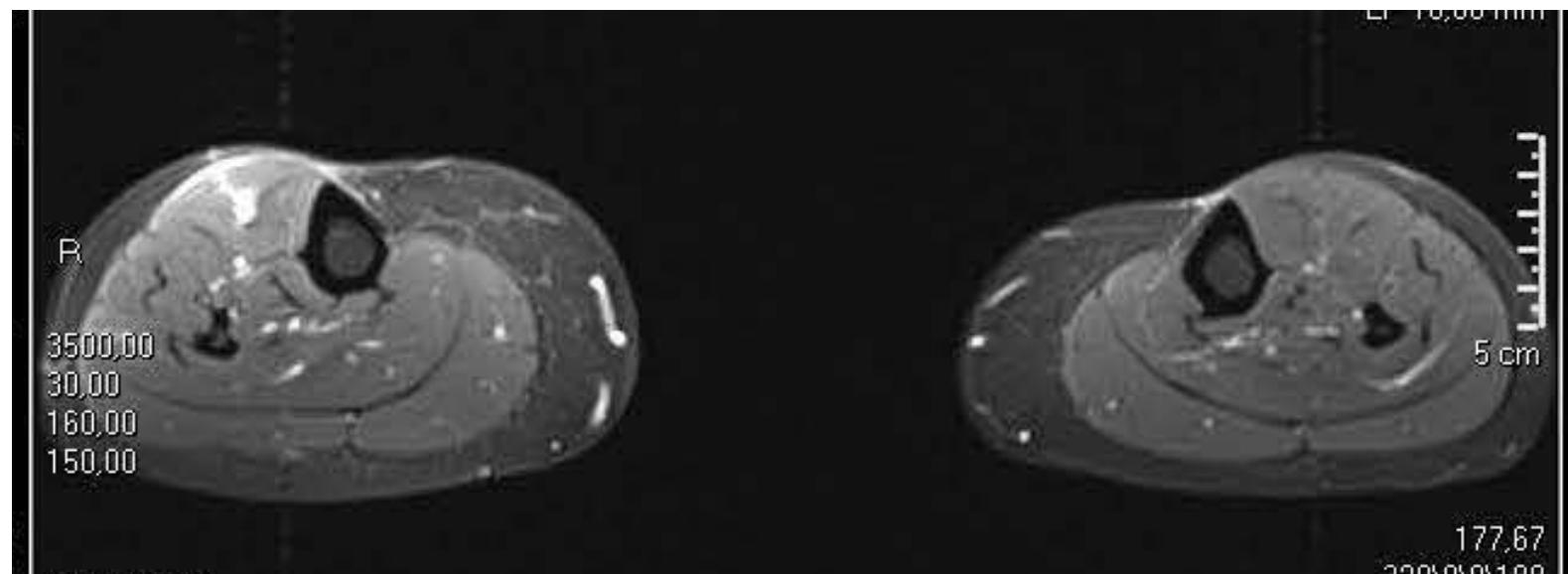
Case history

- 64 yrs male, severe pemphigus in 2012 treated with steroid
- Lower limb muscle weakness started 1-2 yrs before
- Very slow progression
- CK normal
- EMG: myopathic
- Vastus lateralis biopsy in 2012: mild neurogenic changes, 1 RV-fiber
- New assessment 2014: pemphigus resolved, muscle weakness not
- MRI:



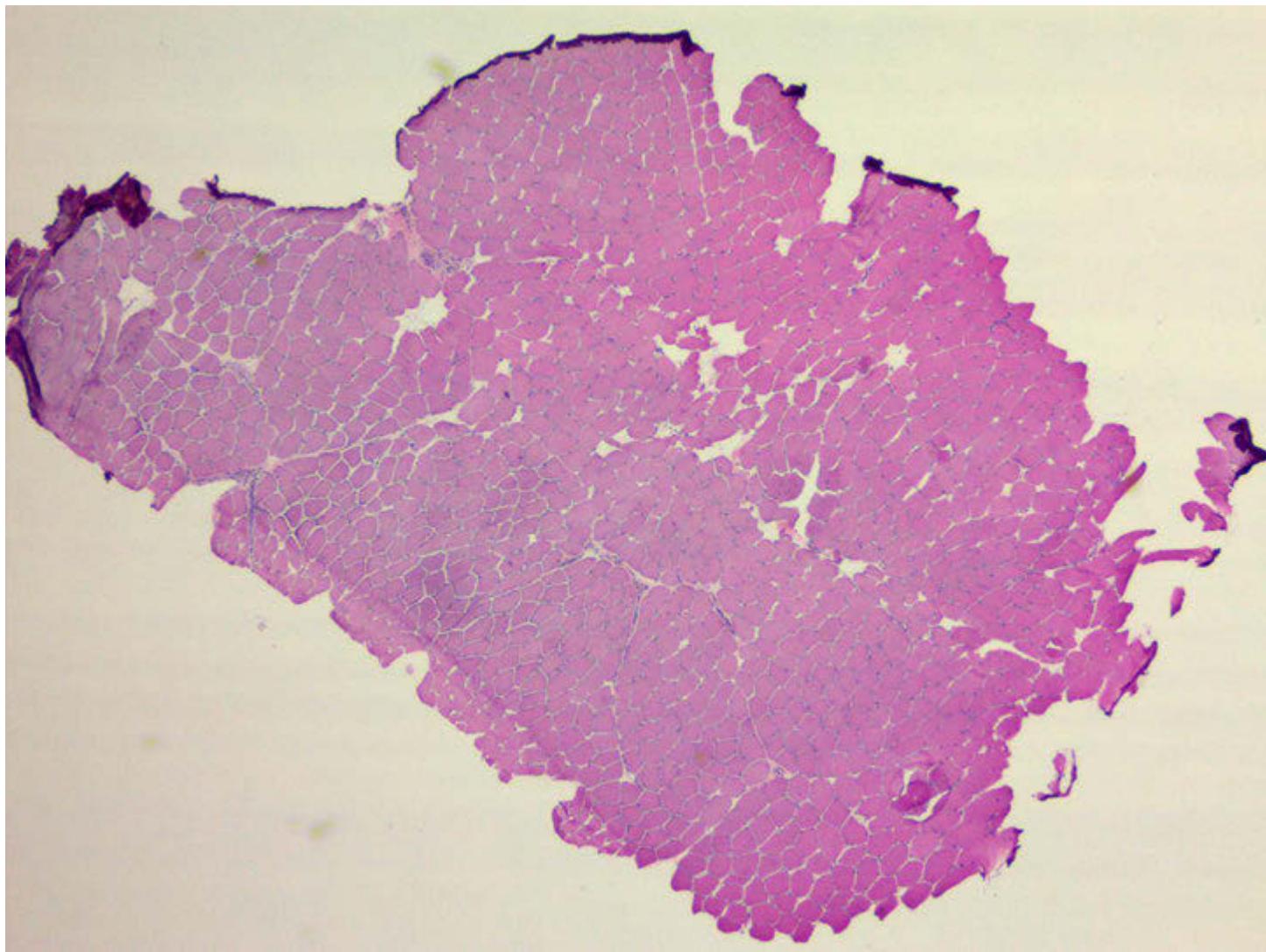
Bedside biopsy in the outpatient clinic





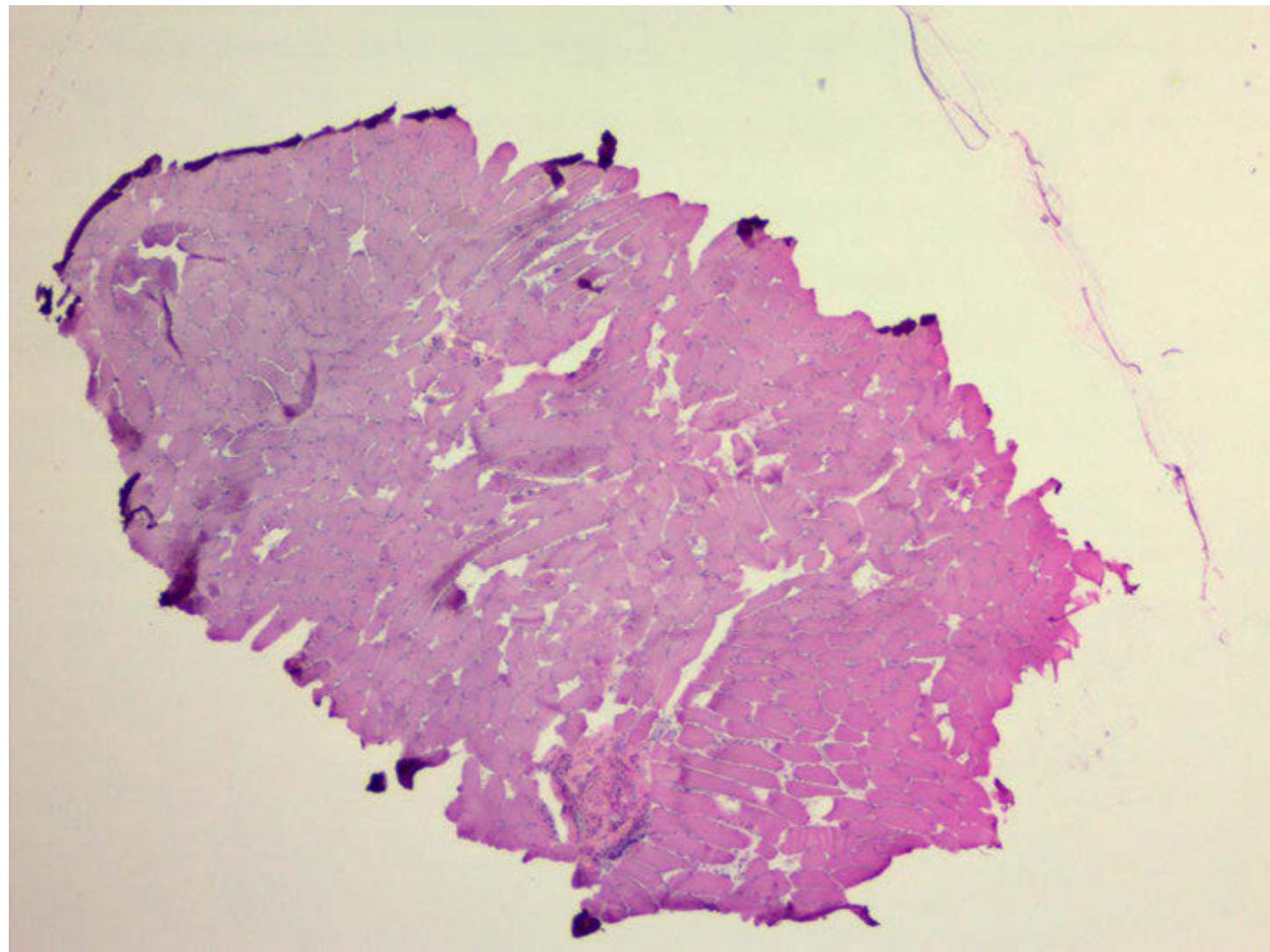
11 yrs old girl

- toe walker
- mild ankle contractures
- no weakness
- CK normal
- EMG normal
- Bx: medial gastrocnemius



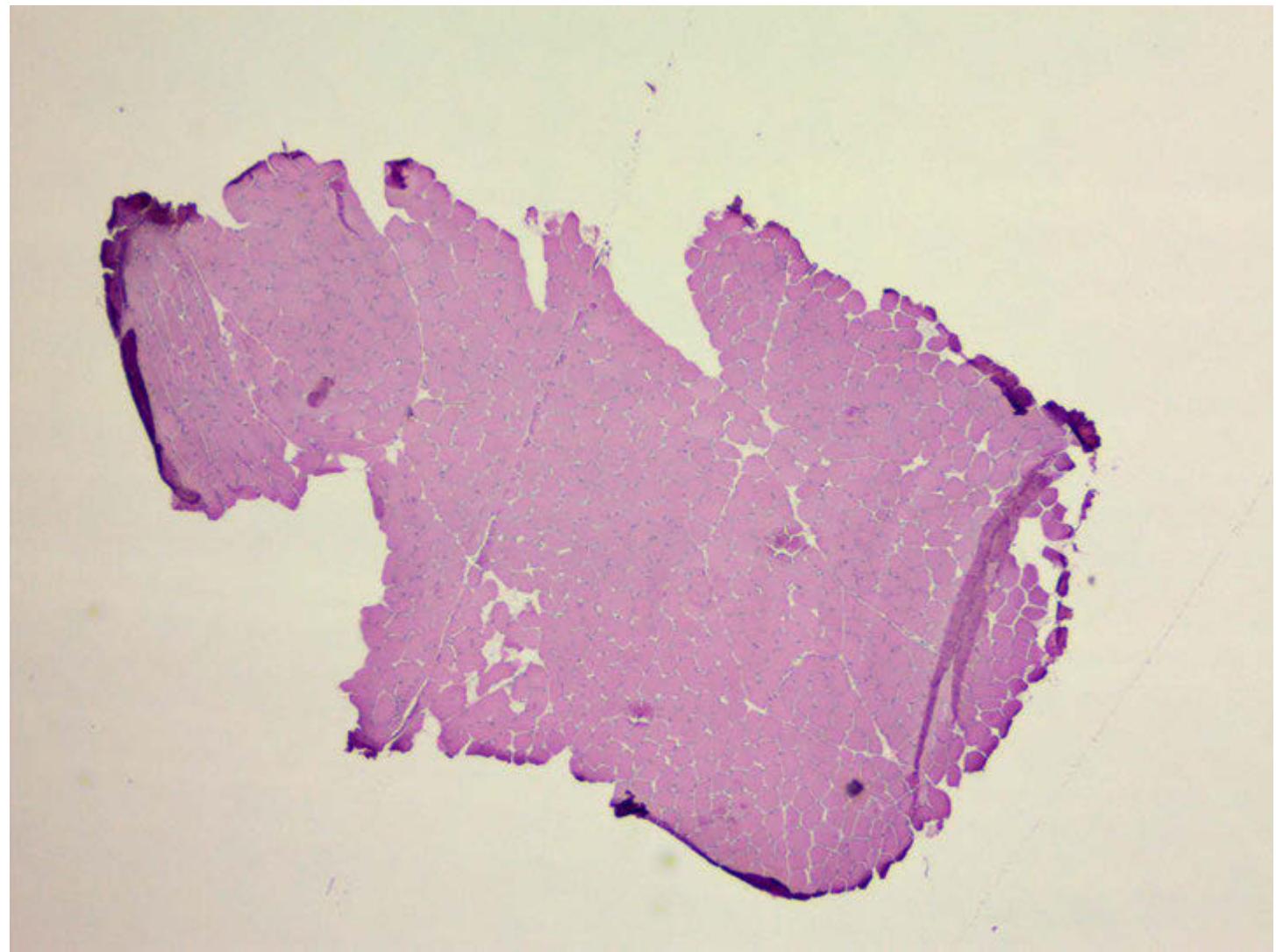
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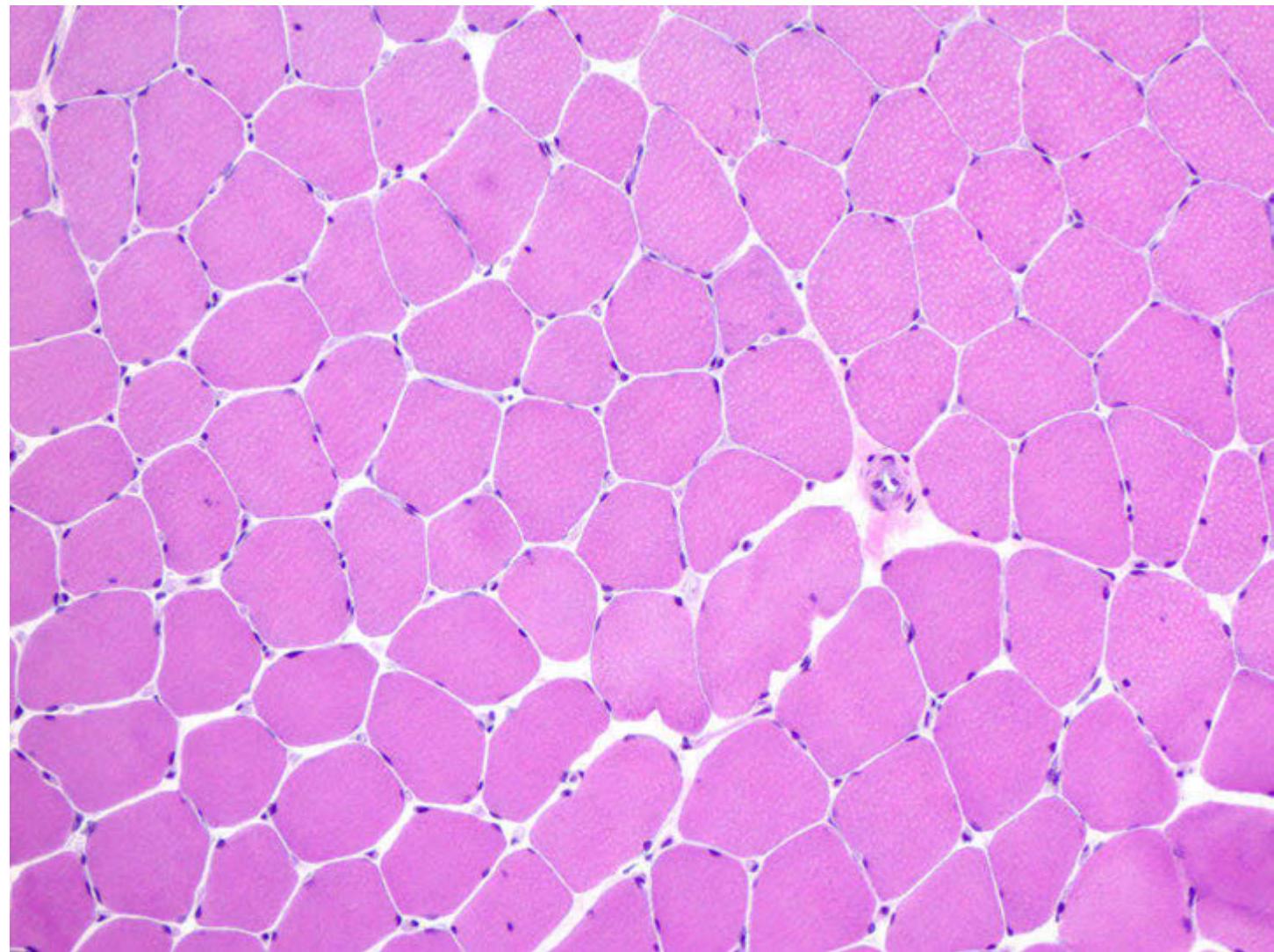
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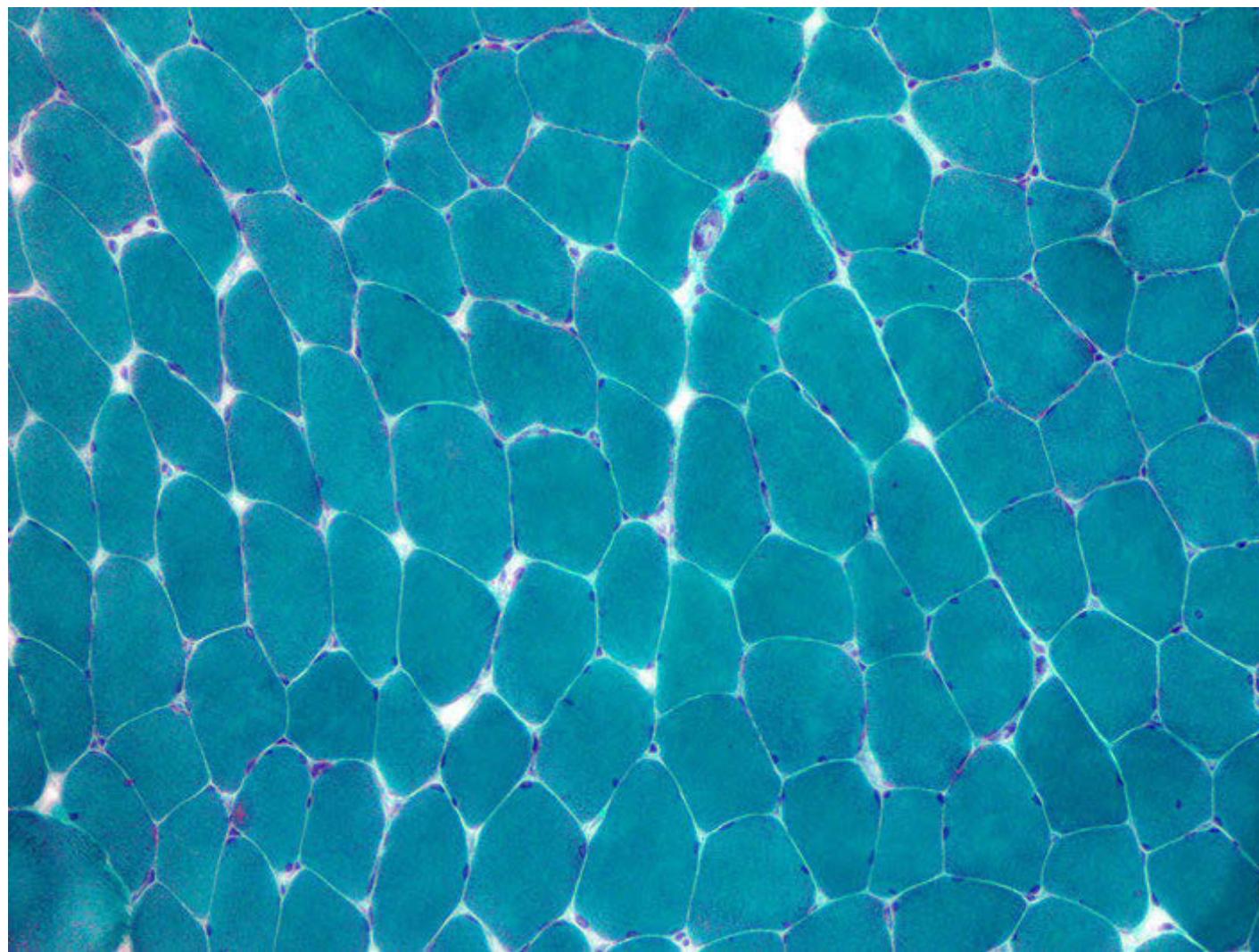
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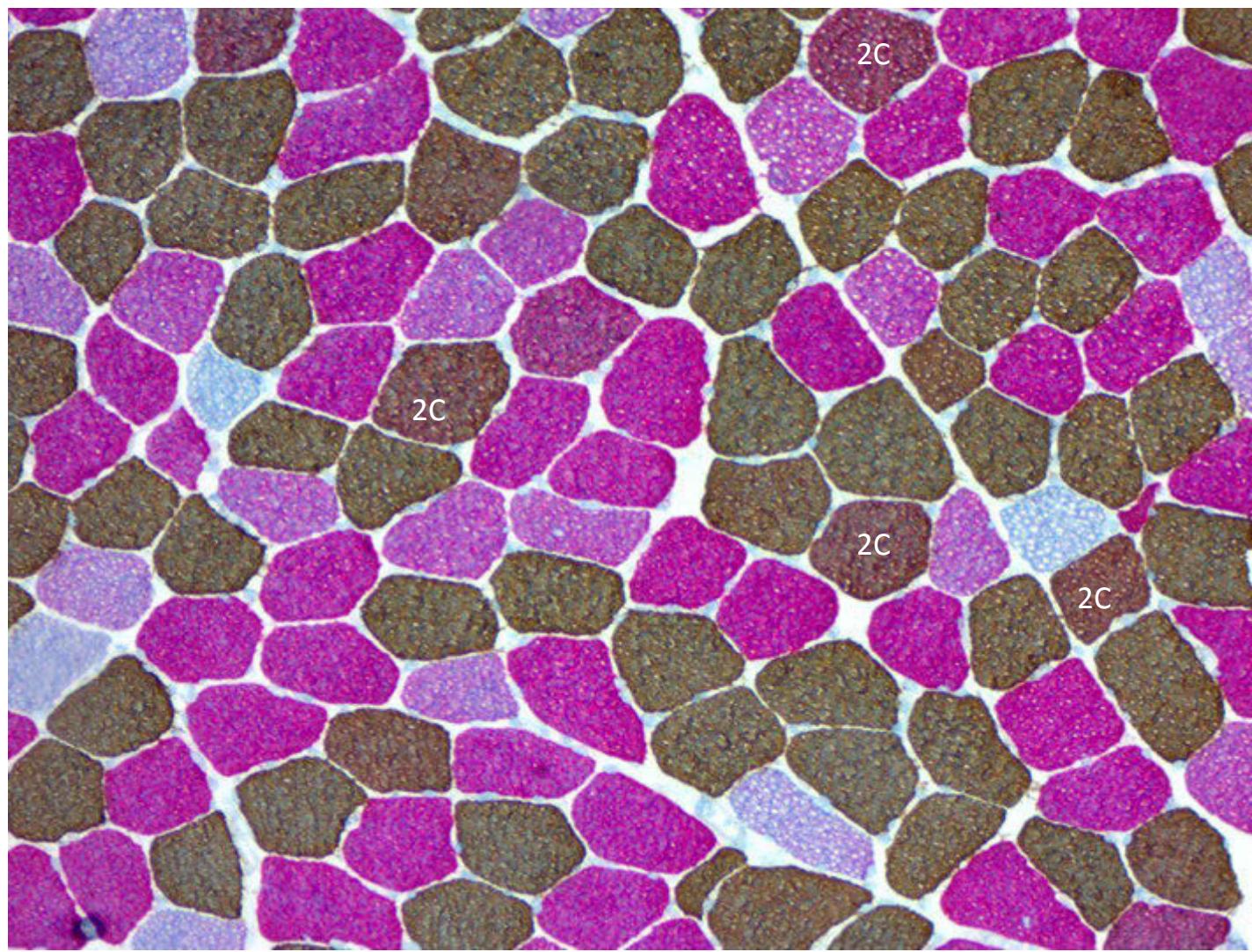


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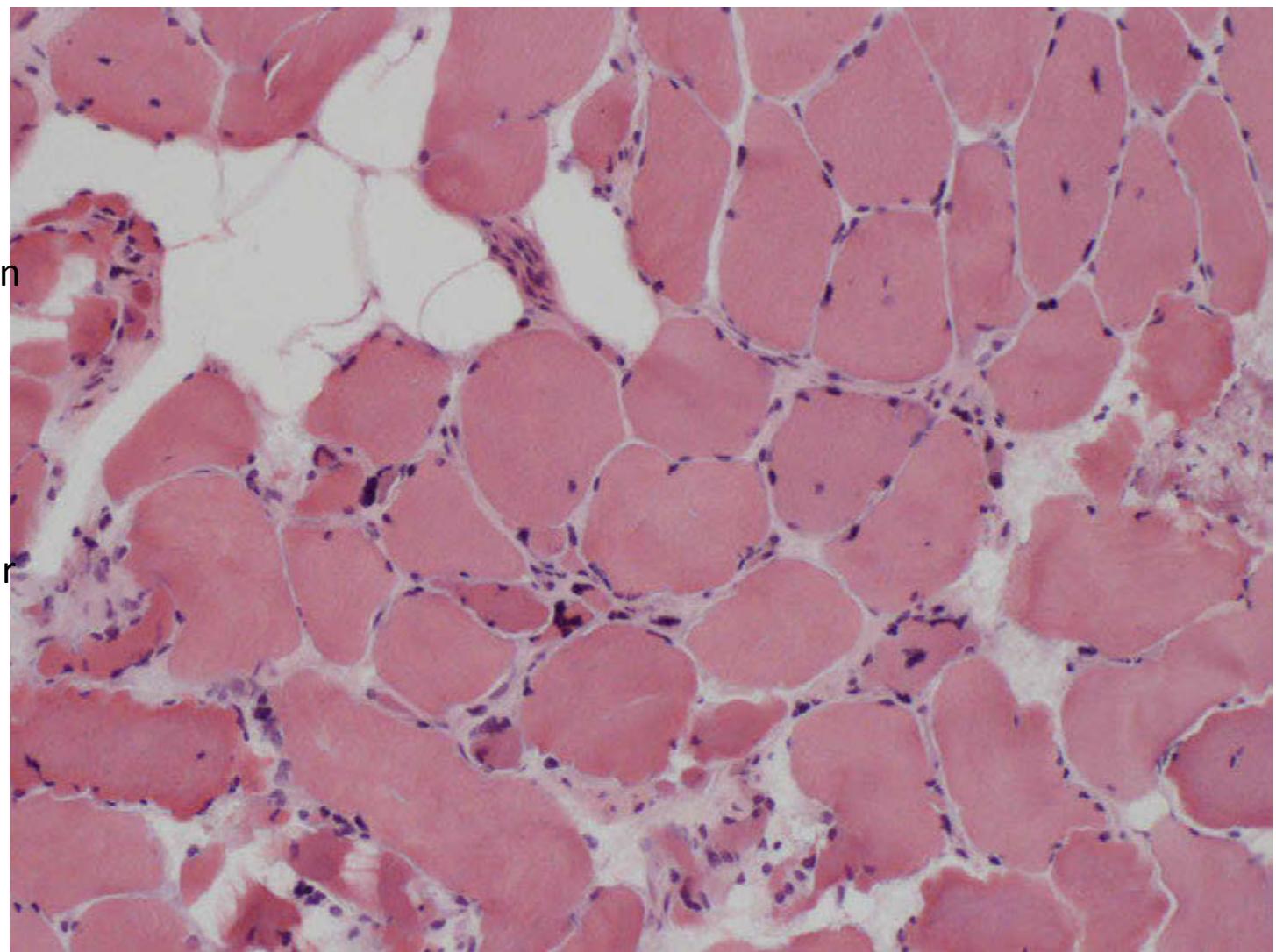
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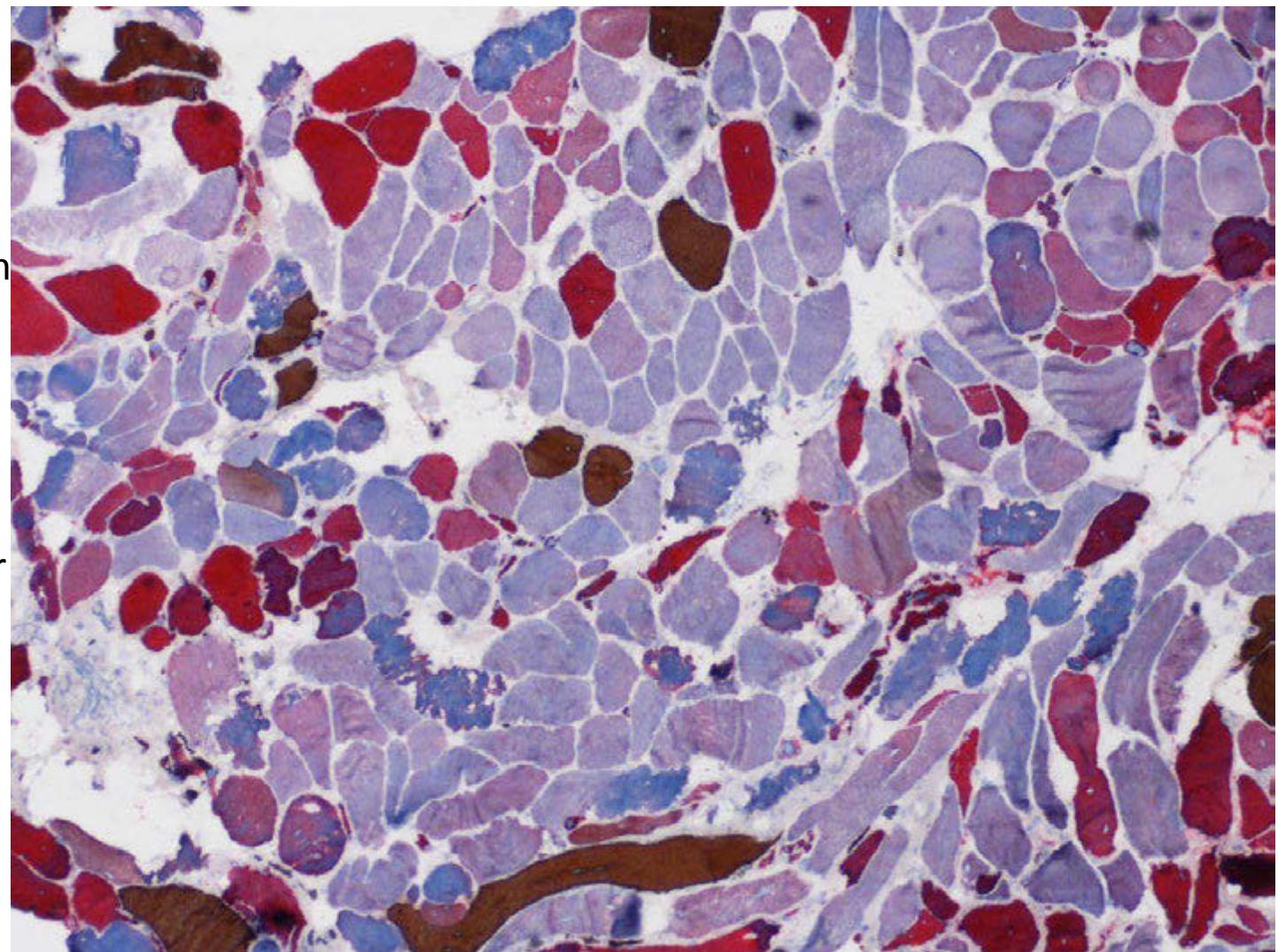




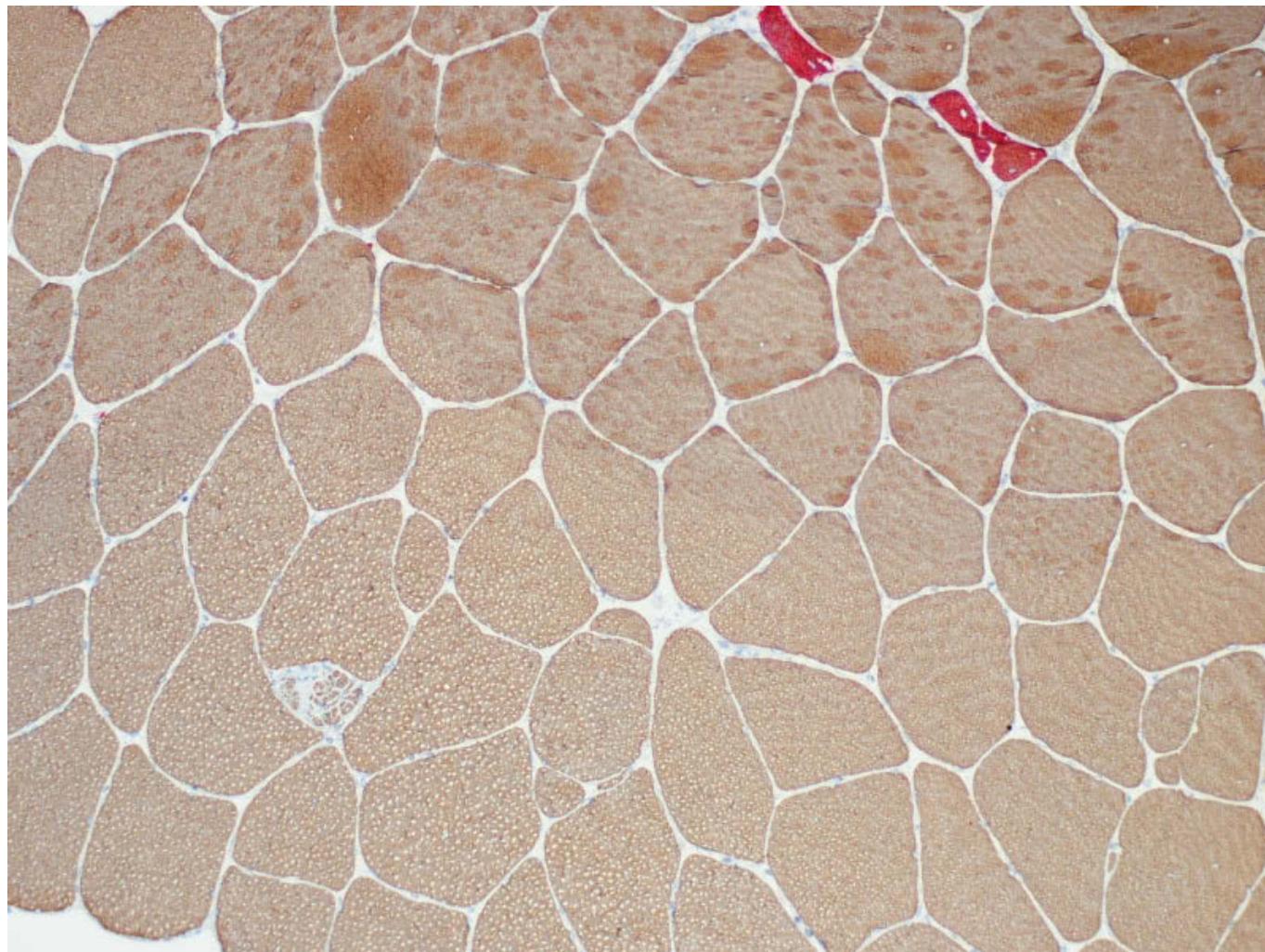
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- **New Bx: TibAnt sin**



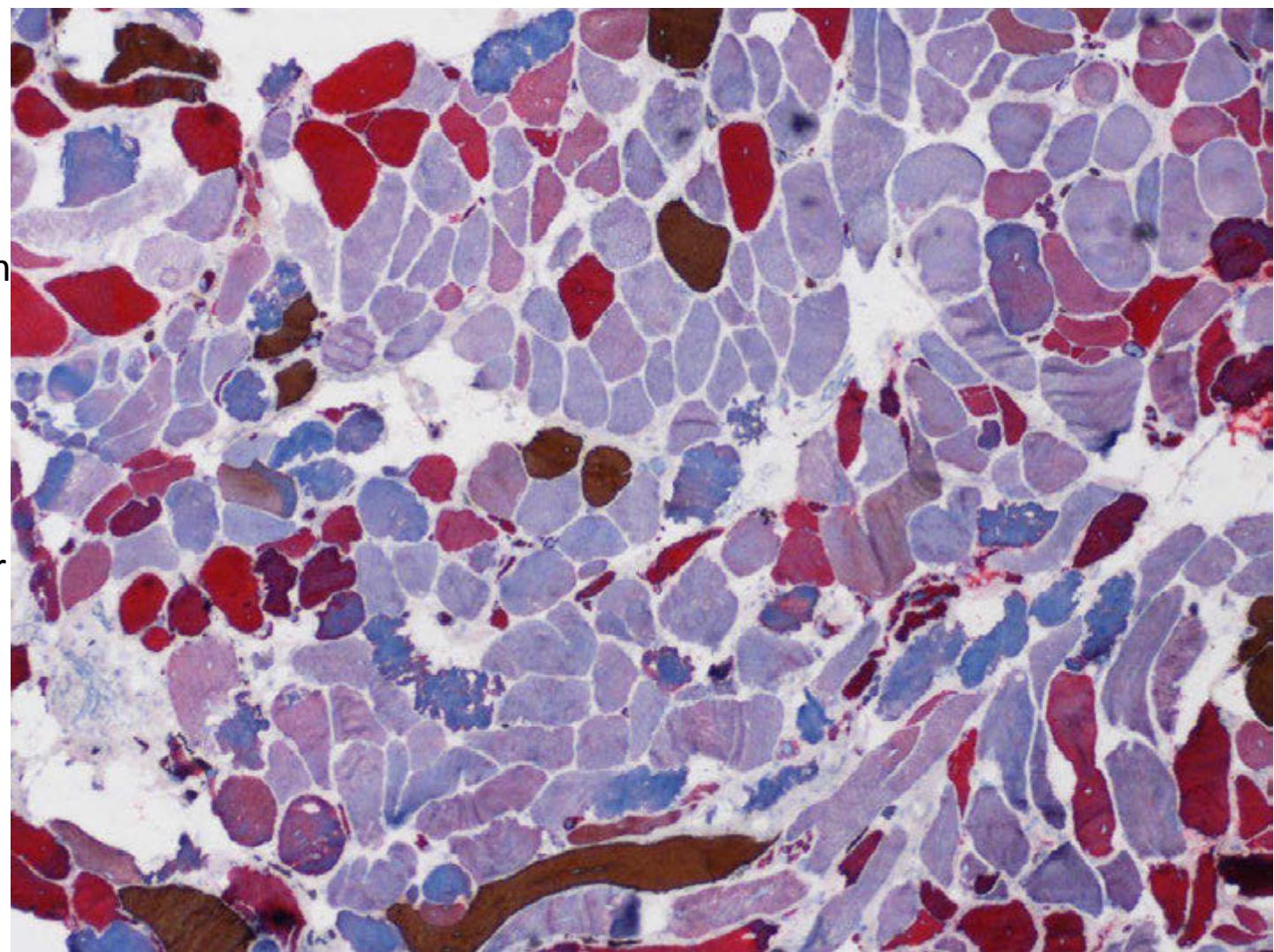
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The more
normal
fiber type
distribution
Tibialis
anterior



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 - New insight in the role of correct site of biopsy
 - Mainly derived from extensive use of muscle MRI
 - Some examples of molecular pathology methods
- **New molecular pathology methods need centers of expertise**
 - 1 full time muscle pathologist and cell biologist per 500 samples/year
- Reluctant use because open surgical biopsy is highly invasive?

