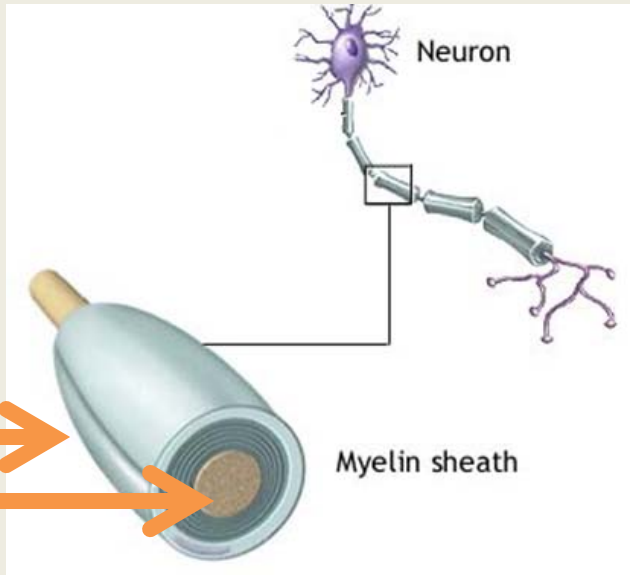
DISCLOSURES

- GRANT SUPPORT
  - NINDS/NCATS
  - Muscular Dystrophy Association
  - Charcot Marie tooth Association
- CONSULTING
  - AlnylamPharmaceuticals
  - Acceleron Pharma
  - FLEX Pharma

What is CMT?

- Neuropathies: Primary diseases of nerve
- Peripheral Nerves
  - Demyelinating Neuropathy
  - Axonal Neuropathy
- Clinical Hallmarks
  - Distal muscle weakness and atrophy
  - Loss of proprioception and sensation
  - Classical steppage gait, pes plannus or pes cavus
  - Fatigue and depression can often accompany disease
- Genetic heterogeneity
  - Over 50 known genetic causes

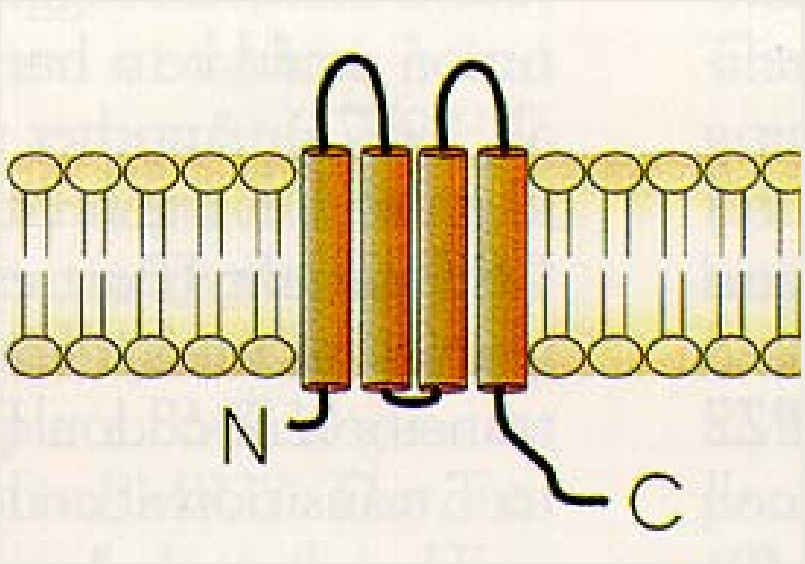


Inherited neuropathies: chromosomes and genes (December 2016)

> 90 GENES

30-50 available commercially

Peripheral Myelin Protein 22 (PMP22)



From: Nelis et al. 1999

PMP22 functions

- Structural protein of peripheral myelin
- Unknown function

Duplication of PMP22 ☞ CMT1A (overexpression of the protein)

50 % of all CMT

Deletion of PMP22 ☞ HNPP (underexpression of the protein)

1A: Charcot-Marie-Tooth Neuropathy Score (CMTNS)

Parameter	0	1	2	3	4	Score
Sensory symptoms	None	Limited to toes	Extend up to and may include ankle	Extend up to and may include knee	Extends above knees	
Motor symptoms legs	None	Trips, catches toes, slaps feet	AFO on at least 1 leg or ankle support	Cane, walker, ankle surgery	Wheelchair most of the time	
Motor symptoms arms	None	Difficulty with buttons/zippers	Unable to do buttons or zippers, but can write	Can't write or use keyboard	Proximal arms	
Pin sensibility	Normal	Reduced in fingers/toes	Reduced up to and may include wrist/ankle	Reduced up to and may include elbow/knee	Reduced above elbow/knee	
Vibration	Normal	Reduced at fingers/toes	Reduced at wrist/ankle	Reduced at elbow/knee	Reduced above elbow/knee	
Strength legs	Normal	4+,4 or 4- on foot dorsiflexion	≤3 foot dorsiflexion	≤3 dorsi and plantar flexion	Proximal weakness	
Strength arms	Normal	4+,4 or 4- on intrinsics or finger ext	≤3 intrinsics or finger ext	<5 wrist extensors	Weak above elbow	
Ulnar CMAP (Median)	>6 mV (>4 mV)	4-5.9 mV (2.8 – 3.9)	2-3.9 mV (1.2 –2.7)	0.1-1.9 mV (0.1 – 1.1)	Absent (Absent)	
UlnarSNAP (Median)	>9 µV (>22µV)	6-8.9 µV (14 – 21.9)	3-5.9 µV (7 – 13.9)	0.1-2.9 µV (0.1 1 6.9)	Absent (Absent)	
Total						**36 MAX**

Table I: Demographics and CMT Scores

	CMTES-R	CMTNS-R
Age, years (mean +/-SD)	41.1 +/- 18.0	43.1 +/- 16.7
Sex (M/F)	530 (56%)/678 (44%)	316 (58%)/231 (42%)
Race (white yes/no)	1100 (91%)/109 (9%)	480 (88%)/68 (12%)

	Baseline	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
CMTES	9.8 +/- 4.9 (1177)	9.8 +/-4.6 (377)	10.2 +/-4.8 (321)	10.5 +/-5.2 (244)	10.6 +/-4.9 (208)	10.7 +/-5.3 (160)	11.4 +/-5.0 (119)
CMTES-R	13.3 +/- 6.4 (1177)	13.4 +/- 6.9 (377)	14.1 +/-6.2 (321)	14.4 +/-6.7 (244)	14.5 +/- 6.3 (208)	14.7 +/-6.8 (160)	15.6 +/-6.4 (119)
CMTNS	15.4 +/-5.7 (517)	15.5 +/-5.6 (54)	17.6 +/-5.9 (32)	17.4 +/-6.6 (18)	17.2 +/-5.4 (17)	21.5 +/-7.0 (15)	18.2 +/-5.1 (11)
CMTNS-R	19.1 +/- 7.1 (517)	19.0+/-7.0 (54)	22.1 +/- 7.5 (32)	21.8 +/-8.3 (18)	21.1 +/-6.9 (17)	26.4 +/-8.4 (15)	22.2 +/-6.7 (11)

CMTNS = Charcot-Marie-Tooth Neuropathy Score version 2
CMTNS-R = Rasch Charcot-Marie-Tooth Neuropathy Score version 2
CMTES = Charcot-Marie-Tooth Examination Score
CMTES-R = Rasch Charcot-Marie-Tooth Examination Score
Data are +/- SD (n)

BIOMARKERS
Measurable Indicator of Biological or Pathogenic Process

- TARGET ENGAGEMENT FOR A TREATMENT
- TREATMENT RESPONSIVENESS
  - MAY NORMALIZE WITH THERAPY
  - MAY BE MORE SENSITIVE TO CHANGE THAN COA
- MARKERS OF DISEASE BIOLOGY
  - SHOULD CORRELATE WITH WHAT IS KNOWN ABOUT THE DISEASE BIOLOGY

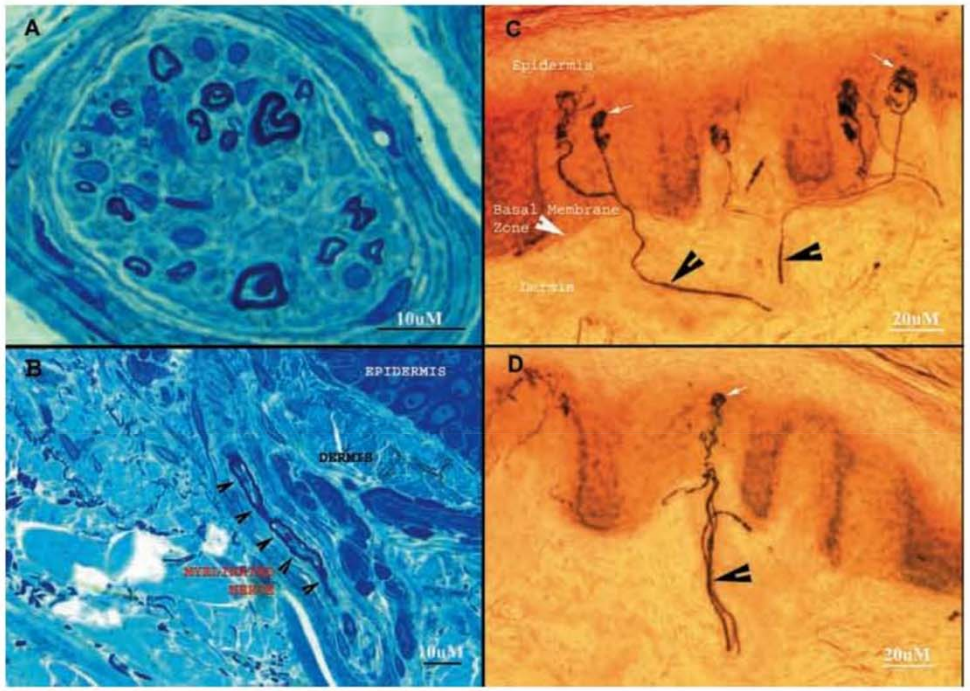
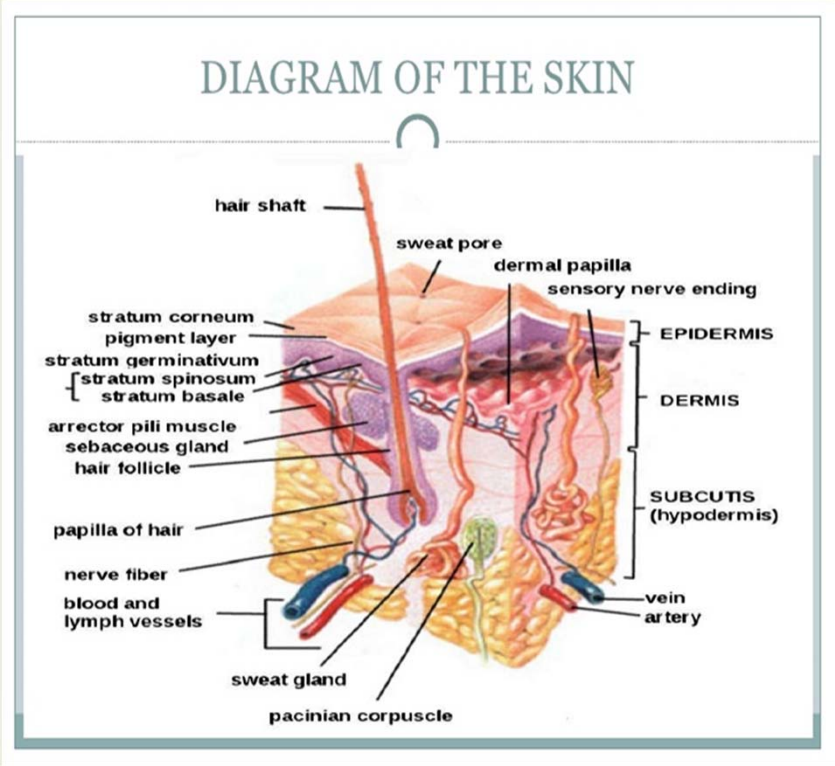


Fig. 1 Dermal myelinated nerves are identifiable in the glabrous skin. Glabrous skin from the lateral aspect of the finger was taken from a control subject and stained with toluidine blue. The dermal myelinated nerve fibres within a small nerve bundle are shown (**A**). These small nerve bundles run parallel to the surface of skin. In **B**, a longitudinal section of myelinated nerve fibre is shown (arrowheads). These nerve fibres can also be identified with immunohistochemistry by using polyclonal antibodies to neuron-specific ubiquitin hydrolase (PGP9.5), a pan-axonal marker (black arrowheads in **C** and **D**). These nerves innervate dermal mechanical receptors, Meissner receptors are indicated by white arrowheads in **C** and **D**. Notice that these myelinated nerve fibres run in the vertical direction and often in a one-to-one relationship with individual Meissner receptors. Thus, they behave as 'naturally occurring teased nerve fibres'. This is particularly suitable for studying the molecular architecture of myelinated nerve fibres.

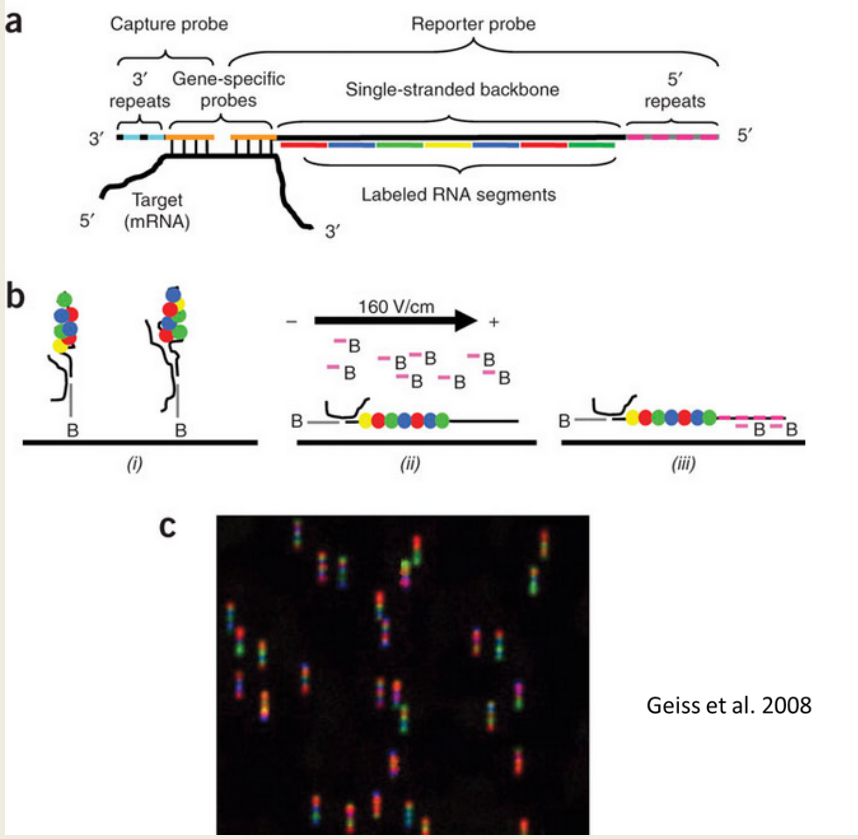
Schwann Cell Profiling in Human Skin Biopsies



- Skin Biopsies contain dermal nerves and associated Schwann cells
- The ability to accurately measure PMP22 levels in patient samples could provide a way to assess treatment outcome
- To date, qPCR methods have not reliably detected differences between CMT1A patients and controls
  - qPCR has limited precision
  - Schwann cell content in skin can vary

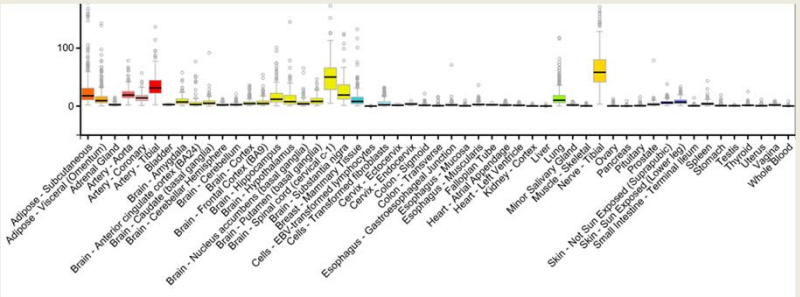
Nanostring Analysis of Human Skin Samples

- Digital Nanostring Analysis employs barcoded probes that directly detect target RNA's (Geiss et al, 2008)
  - High sensitivity (< 1 copy per cell)
  - No enzymes required to perform assay
- Custom panels can multiplex hundreds of gene targets in a single reaction
  - Developed custom panel of 50+ genes, most of which are SC-specific genes, including PMP22 and SC-specific normalizing genes, such as *GLDN*, *CADM4*

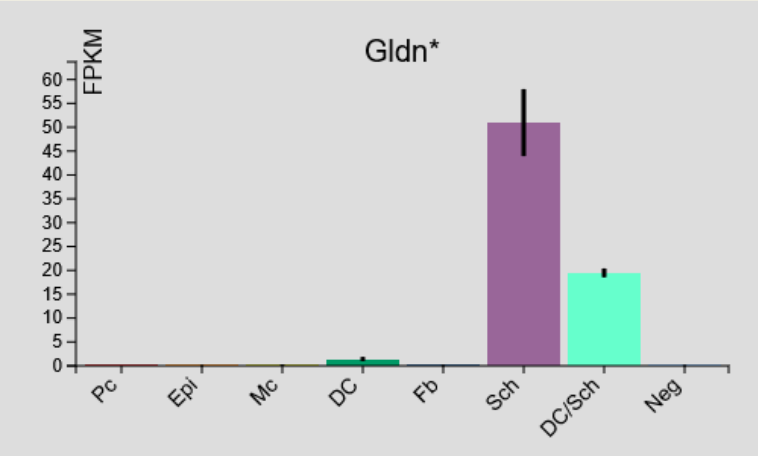


Geiss et al. 2008

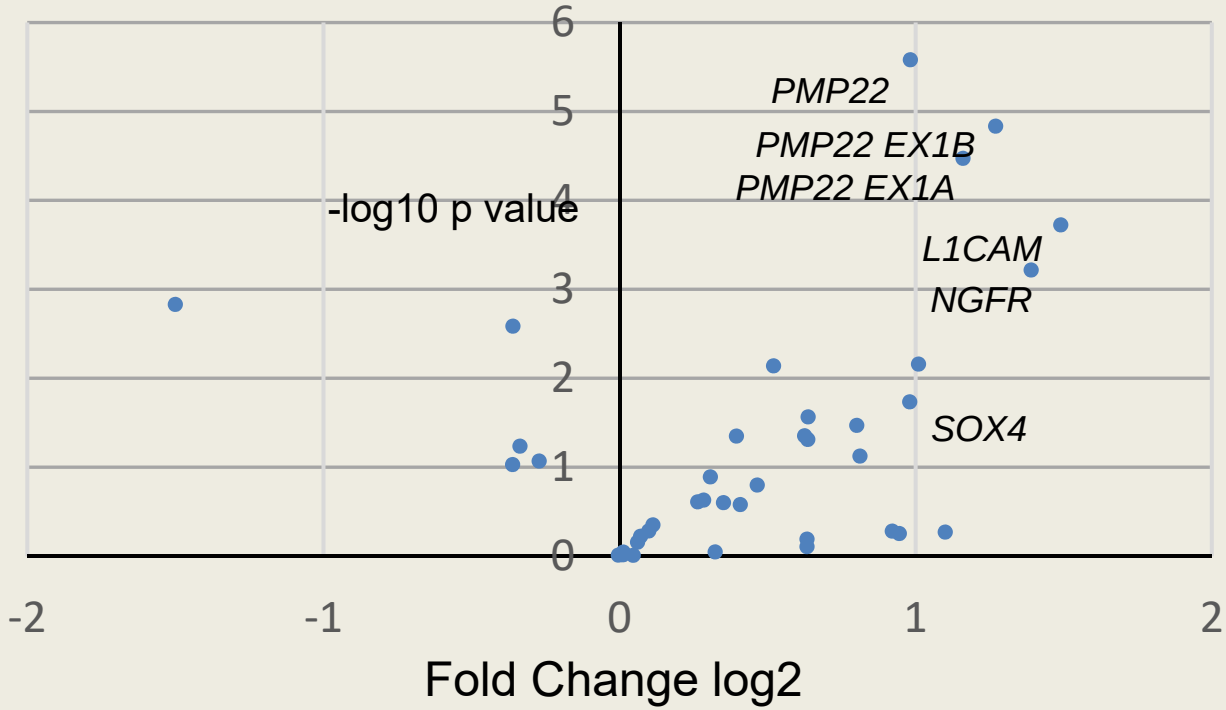
Human Peripheral Nerve Profiling: tibial nerve at Broad Institute (gtexportal.org)

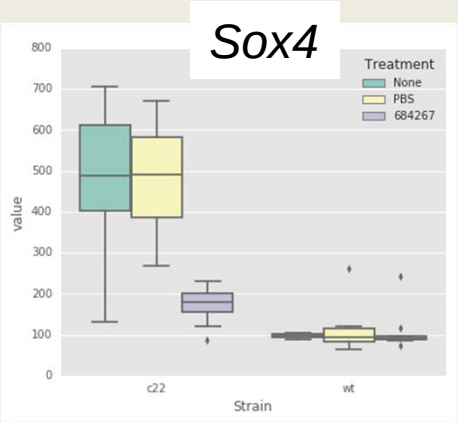


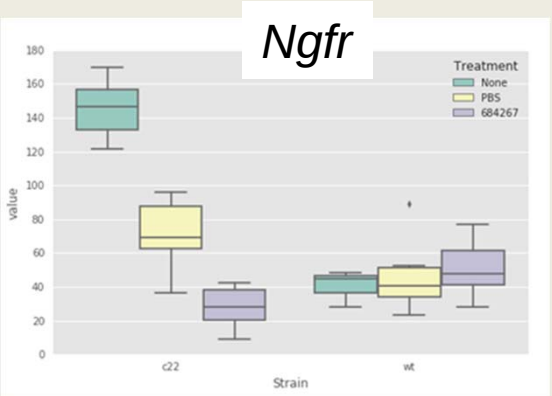
Expression profiles of separated skin types in mouse embryonic skin (hair-gel.net)

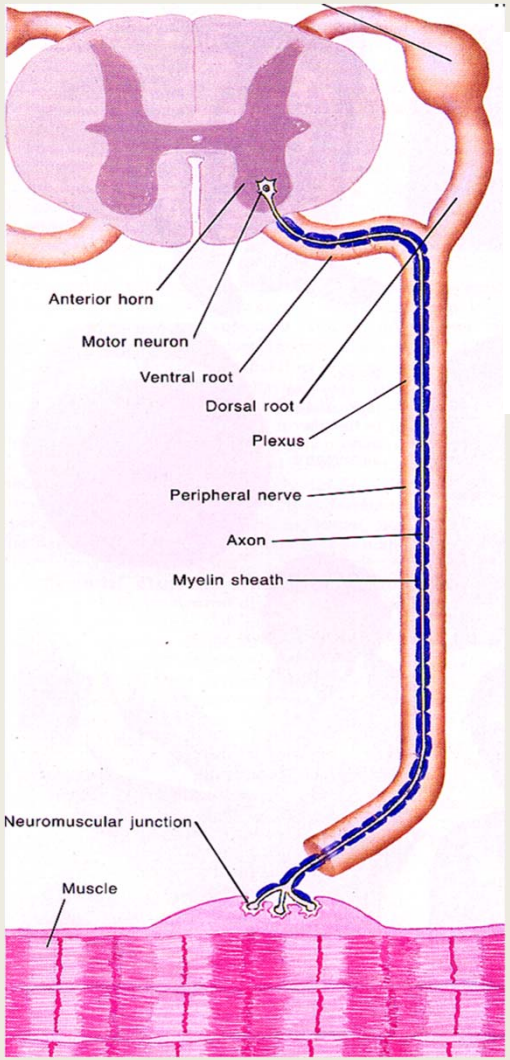


Pilot Nanostring Analysis of Skin Biopsies
Normalized to 6 control genes









ARTICLE OPEN ACCESS

Plasma neurofilament light chain concentration in the inherited peripheral neuropathies

Åsa Sandelius, PhD, Henrik Zetterberg, PhD, Kaj Blennow, PhD, Rocco Adiutori, Andrea Malaspina, PhD, FRCP, Matilde Laura, PhD, Mary M. Reilly, MD, FRCP, FRCPI,* and Alexander M. Rossor, PhD, MRCP*

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Neurology® 2018;90:e518-e524. doi:10.1212/WNL.0000000000004932

EDITORIAL

Neurofilament light, biomarkers, and Charcot-Marie-Tooth disease

Davide Pareyson, MD, and Michael E. Shy, MD

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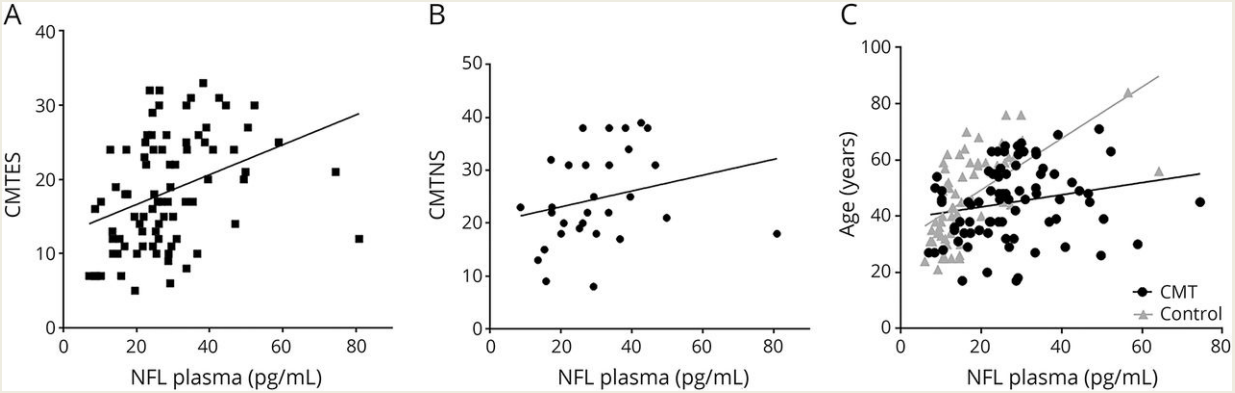
Neurology® 2018;90:257-259. doi:10.1212/WNL.0000000000004936

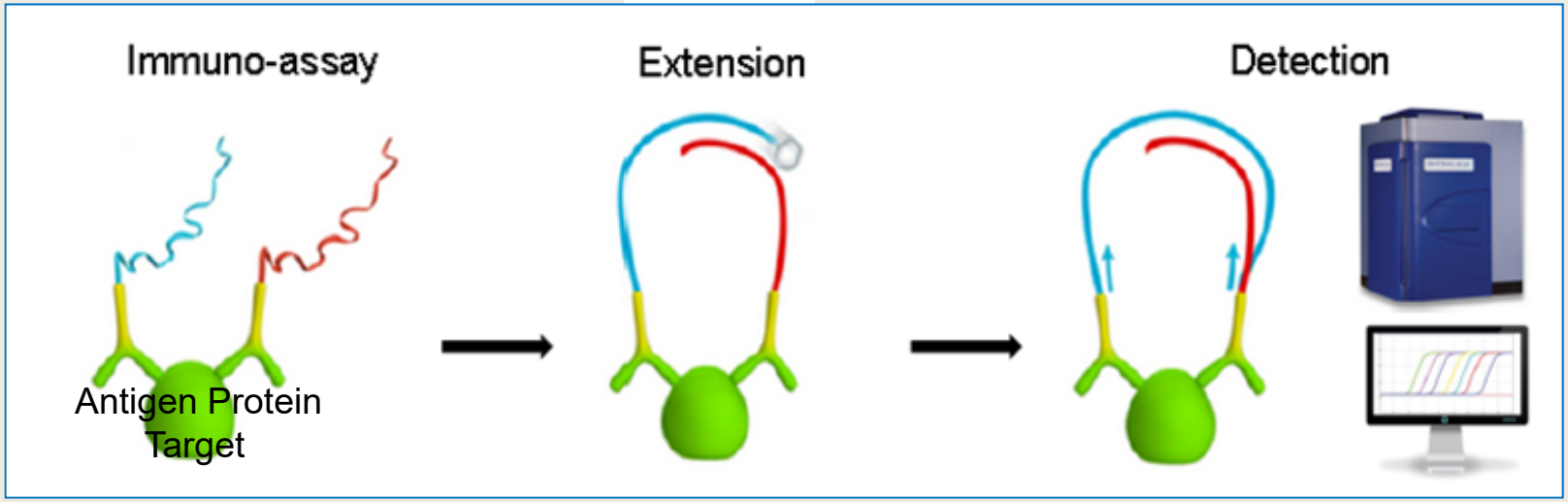
Figure 2 Plasma neurofilament light (NfL) concentration and Charcot-Marie-Tooth disease (CMT) severity



Åsa Sandelius et al. Neurology 2018;90:e518-e524

Olink Immuno PCR: Broader Protein Profiling

High Multiplex Immunoassays

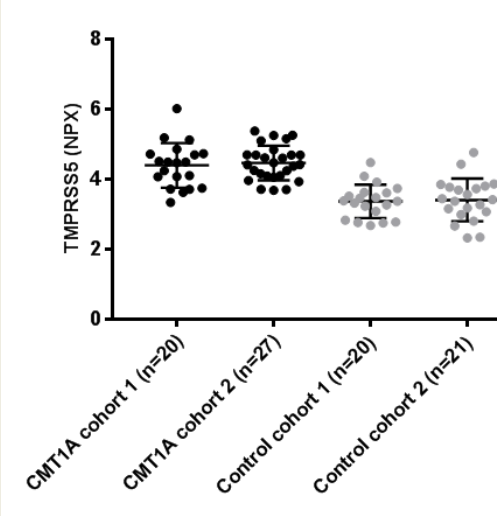


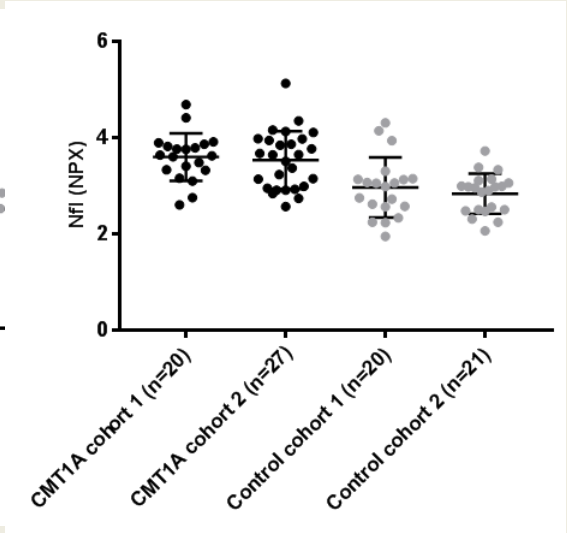
Key Advantages:

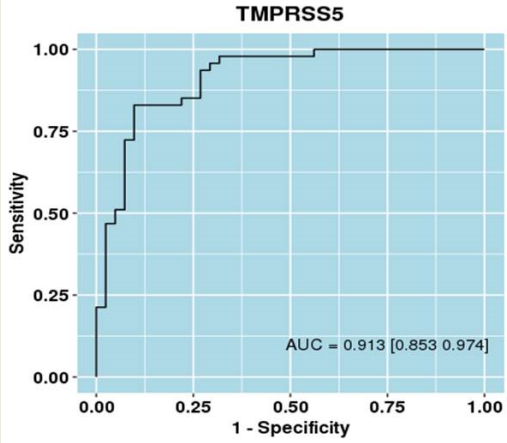
- high multiplex 92 different protein assays
- 88 biological samples run per plate, takes only 2 days
- only 1ul biological sample used per panel
- choice 14 different fixed protein panels each for 92 different targets
- total menu ~ 1200 biologically relevant proteins
- sensitivity ~ X5 ELISA
- once detect candidate biomarker translate into a cost effective singleplex ELISA assay

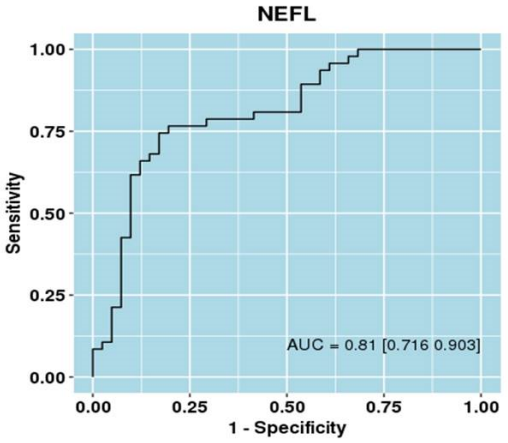
Sample types:
Plasma/serum, cell culture sups, cell lysates

TMPRSS5 NEFL







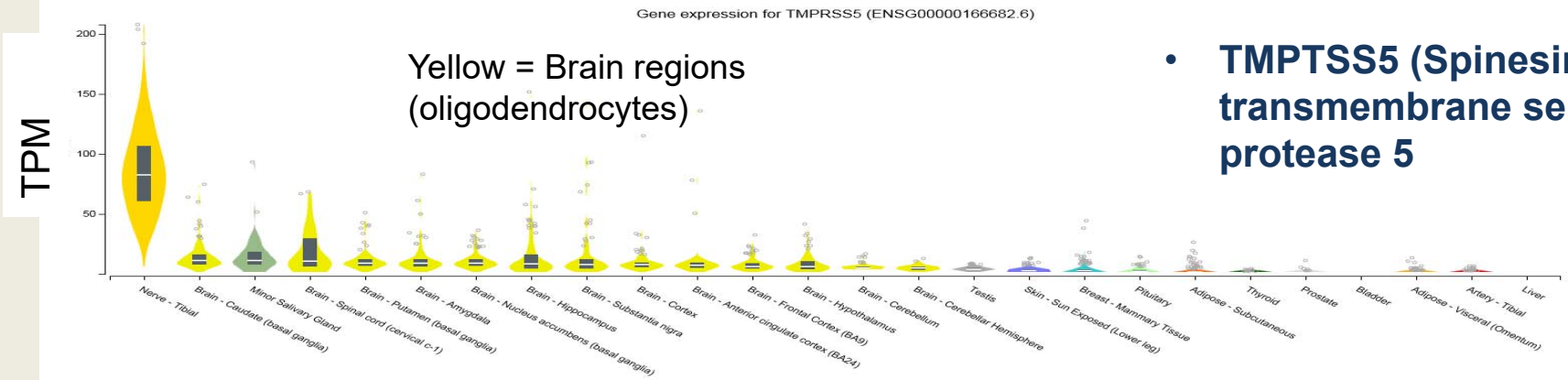


Biology & Link to Disease Mechanism: TMPRSS5

A.

TMPRSS5 expression in human tissues: Broad institute

Tibial Nerve

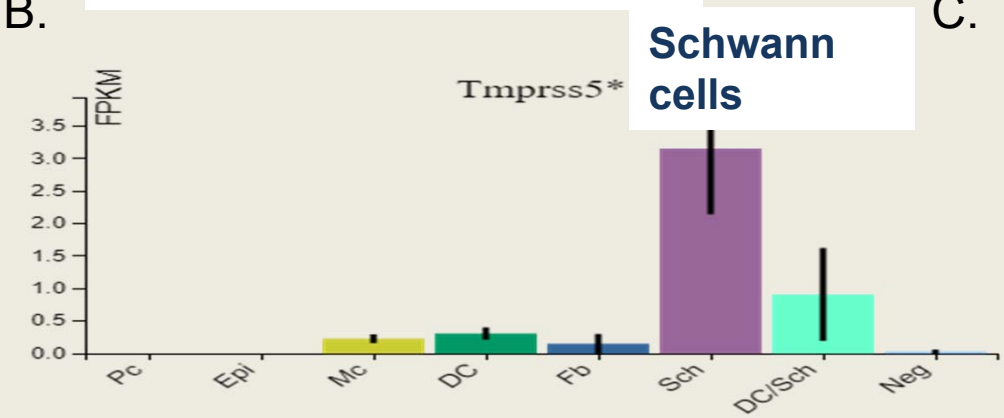


Yellow = Brain regions (oligodendrocytes)

- **TMPTSS5 (Spinesin) is transmembrane serine protease 5**

B.

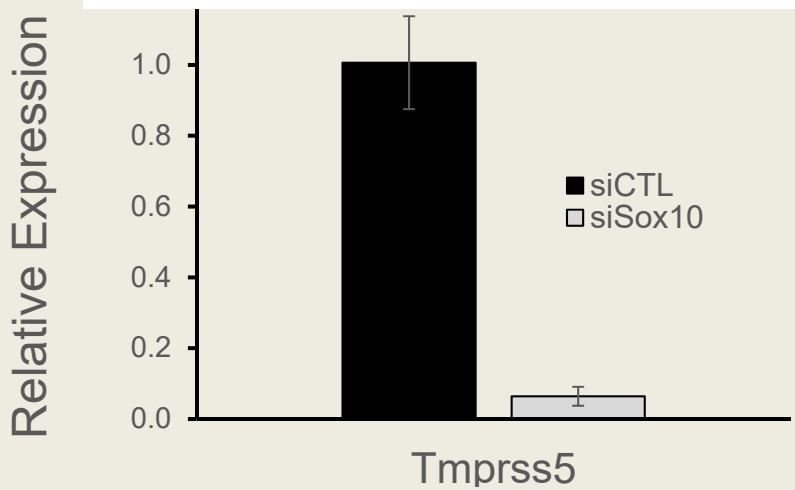
Mouse skin cell types: Hairgel



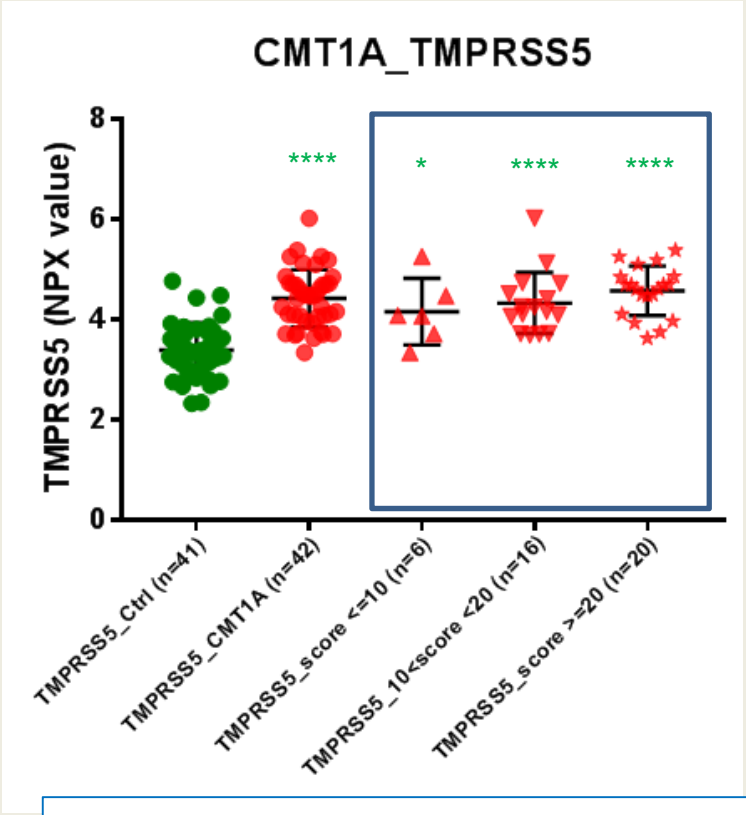
Schwann cells

C.

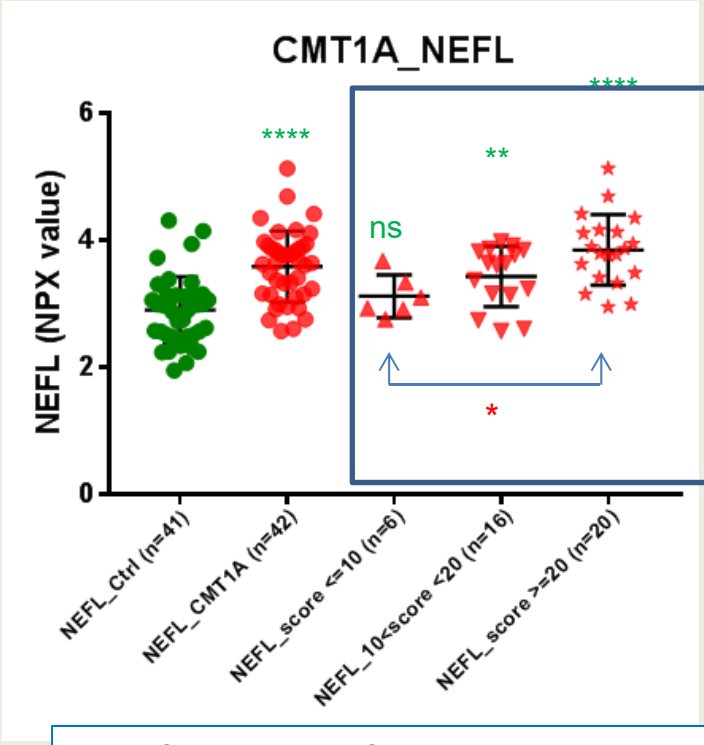
siRNA **KD TMPRSS5 regulated by Sox 10** S16 cell line



TMPRSS5 & NfL: Correlation to RASCH NS Disease Score

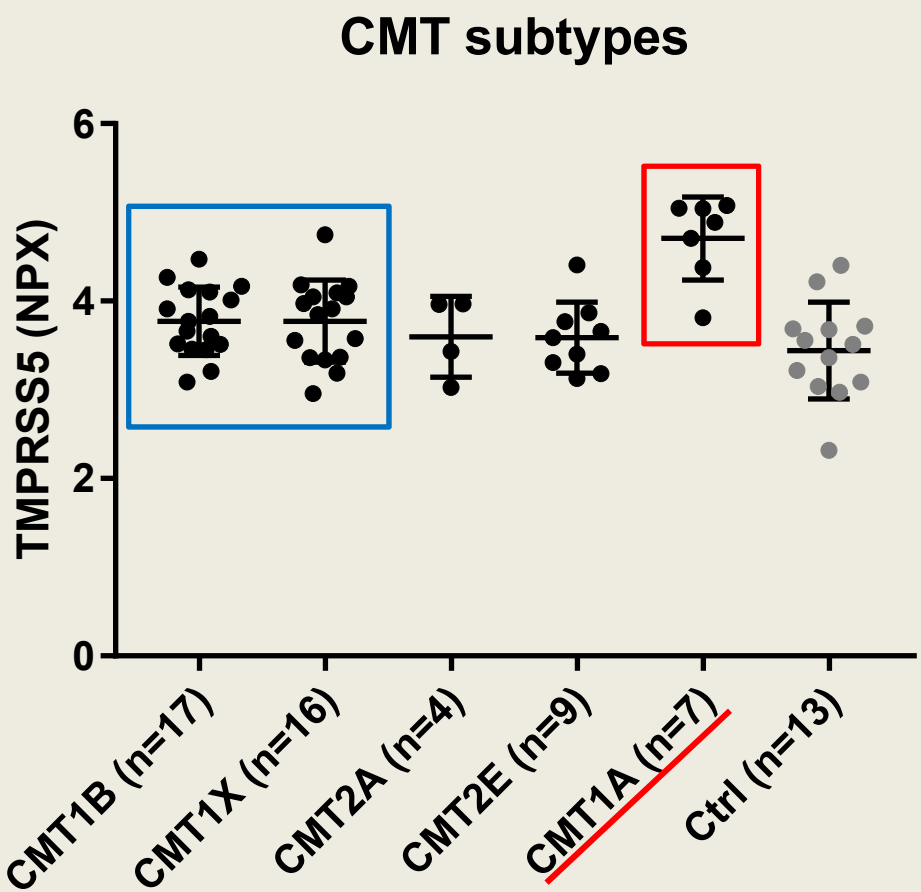


- TMPRSS5 about 2 fold increase over controls in all 3 score bins
- All 3 score bins significantly p<0.05 above controls (green)



- NfL about 1.6 fold increase maximum in top score bin, increases across bins
- Top two score bins significantly p<0.05 above controls
- Score < 10 bin not significantly above controls

TMPRSS5 Specificity to CMT1 Sub Types



Subtype	CMT1B (n=17)	CMT1X (n=16	CMT2A (n=4)	CMT2E (n=9)	CMT1A (n=7)	Ctrl (n=13)
mean NPX	3.774	3.773	3.597	3.589	4.706	3.443
SEM	0.096	0.119	0.126	0.094	0.117	0.052
FC	1.26	1.26	1.11	1.11	2.40	1.00
p value	0.1876	0.2003	0.9647	0.9156	0.0001	

- CMT1A significantly up regulated p=0.001 2.4 fold
- Not statistically significantly up in the other subtypes demyelinating or axonal damage
- Possibly a modest trend of up regulation in CMT1B and CMT1X FC 1.28 (demyelinating forms)
  - Test additional samples - length disease duration /severity

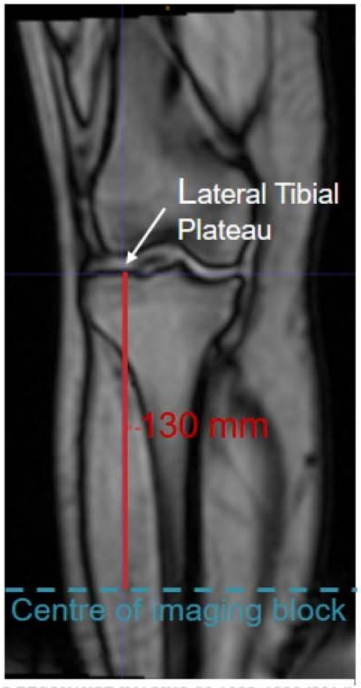
BIOMARKERS AND CMT1A: MRI LONDON AND IOWA CITY

UCL

MRC Centre for Neuromuscular Diseases

MRI protocol

- Improved scanning block positioning method
- T1-weighted and STIR
  - thighs and calves
- 2D 3-point Dixon fat quantification
  - thighs and calves
- 3D 3-point Dixon fat quantification
  - distal thighs to feet



JOURNAL OF MAGNETIC RESONANCE IMAGING 39:1033–1038 (2014)

Improved Anatomical Reproducibility in Quantitative Lower-Limb Muscle MRI

Arne Fischmann, MD, MHBA,[1,2,3*] Jasper M. Morrow, MBChB, FRACP,[3] Christopher D.J. Sinclair, PhD,[3,4] Mary M. Reilly, MD, PhD,[3] Michael G. Hanna, MD, PhD,[3] Tarek Yousry, MD, Dr. med habil,[3,4] and John S. Thornton, PhD[3,4]

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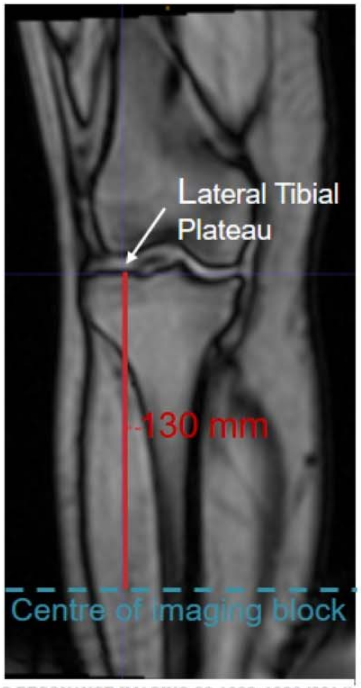

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Results

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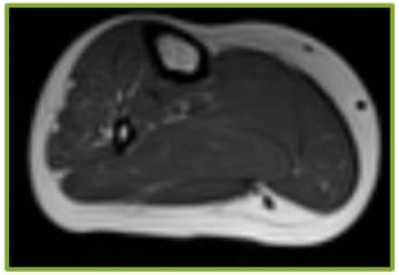
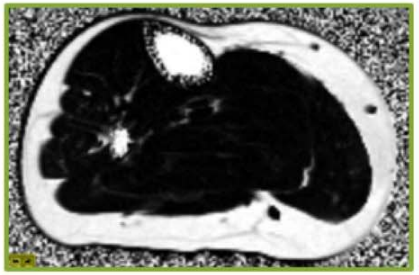
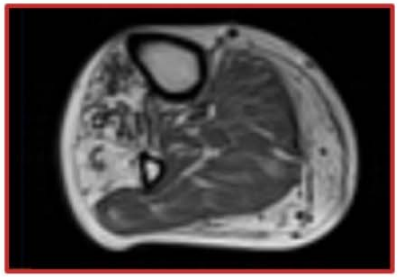
Baseline assessment – disease spectrum

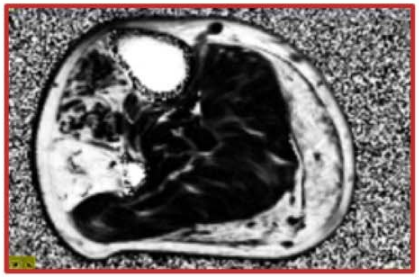
35 year old
Female
CMTNS 5



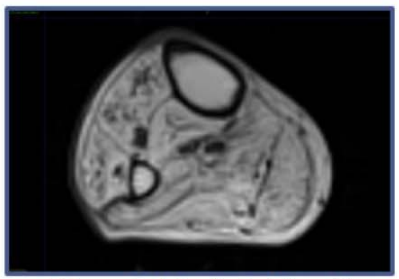


43 year old
Male
CMTNS 19

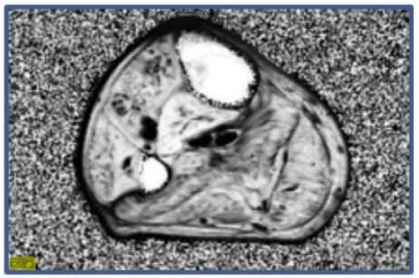




64 year old
Male
CMTNS 23



T1-weighted right calf



Fat fraction map right calf

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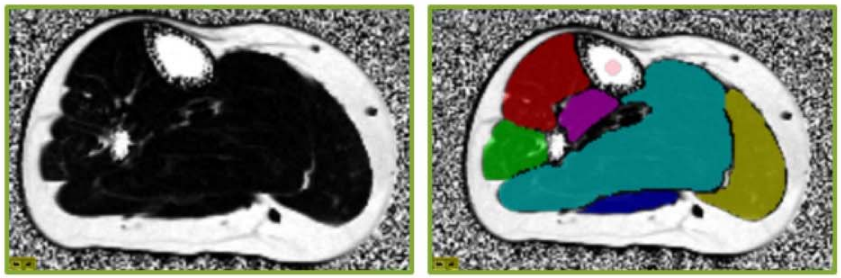
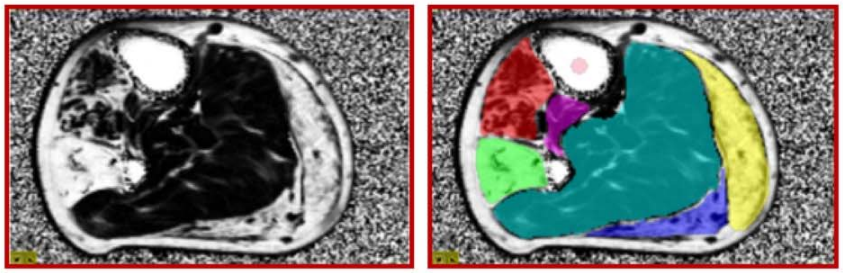
Baseline quantitative MRI assessment

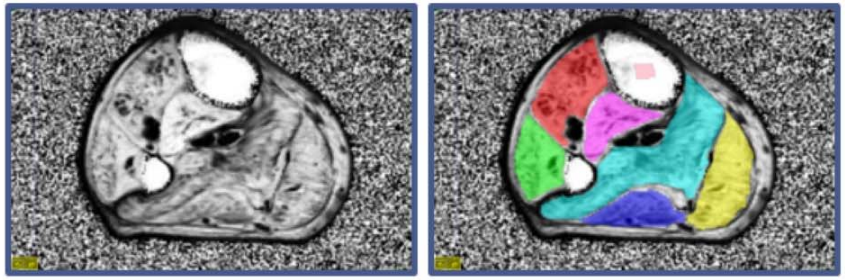
35 year old
Female
CMTNS 5



43 year old
Male
CMTNS 19



64 year old
Male
CMTNS 23

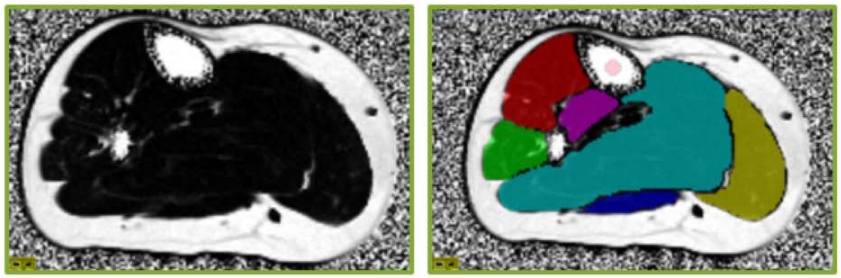


UCL

MRC Centre for Neuromuscular Diseases

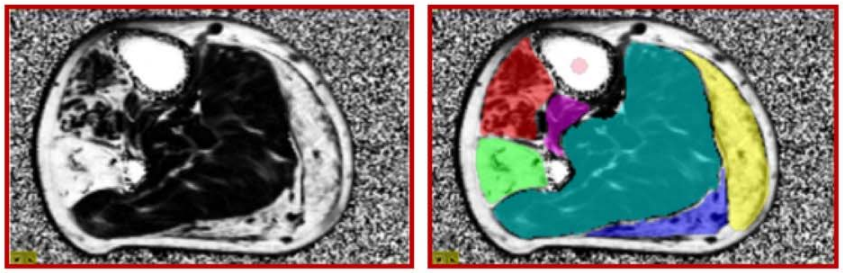
Baseline quantitative MRI assessment

35 year old
Female
CMTNS 5



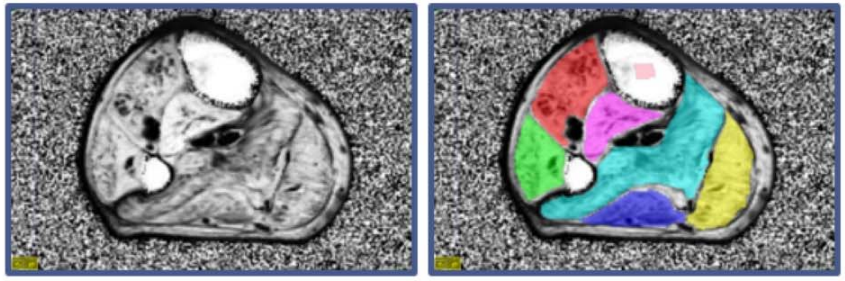
Tibialis Anterior	3.7%
Peroneus Longus	9.8%
Lateral Gastrocnemius	3.6%
Medial Gastrocnemius	4.3%
Soleus	2.6%
Tibialis Posterior	2.1%
Overall	3.6%

43 year old
Male
CMTNS 19



Tibialis Anterior	34.2%
Peroneus Longus	87.7%
Lateral Gastrocnemius	62.5%
Medial Gastrocnemius	70.5%
Soleus	6.3%
Tibialis Posterior	19.4%
Overall	30.1%

64 year old
Male
CMTNS 23



Tibialis Anterior	64.2%
Peroneus Longus	71.9%
Lateral Gastrocnemius	60.2%
Medial Gastrocnemius	68.2%
Soleus	54.9%
Tibialis Posterior	84.5%
Overall	64.1%

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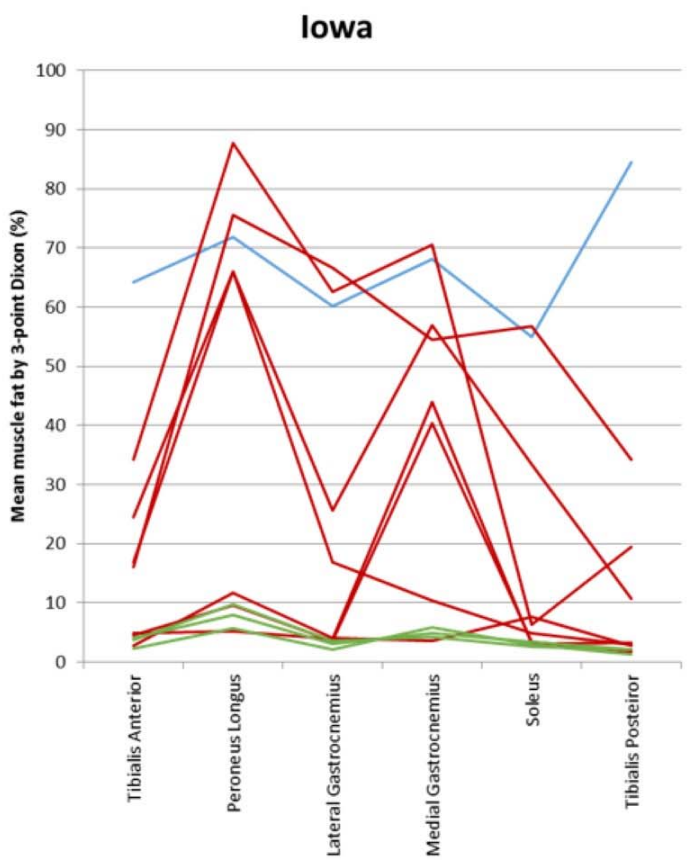
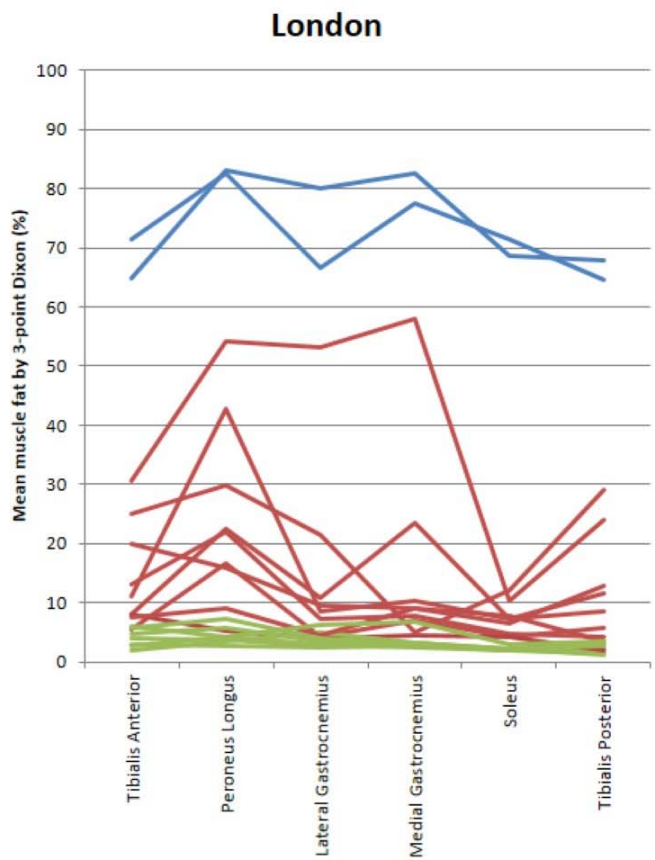
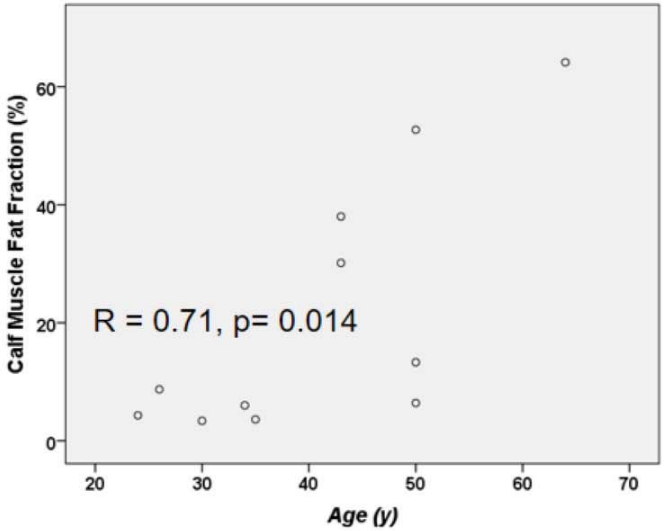
Disease distribution in CMT1A



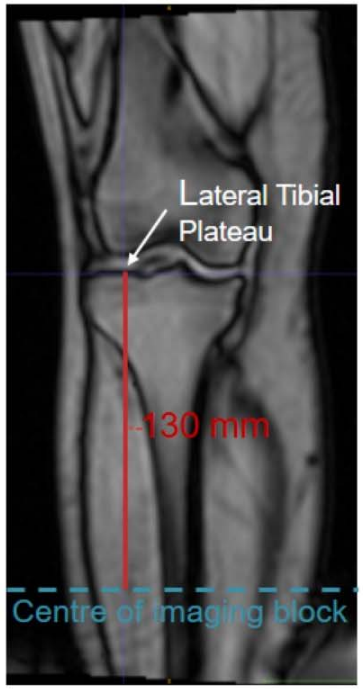


MRI correlation with age



Factor	rho	p
Age	0.71	0.014
Disease Duration	0.80	0.003
CMTNS	0.70	0.026
Rasch NS	0.81	0.004

Clinical correlations of overall calf muscle fat fraction



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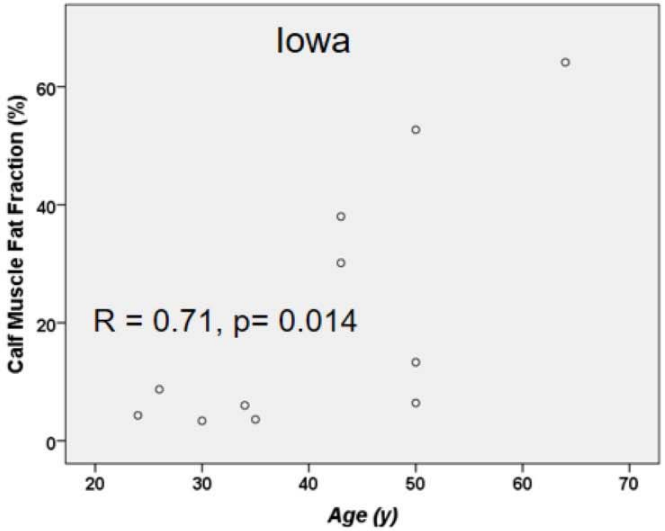

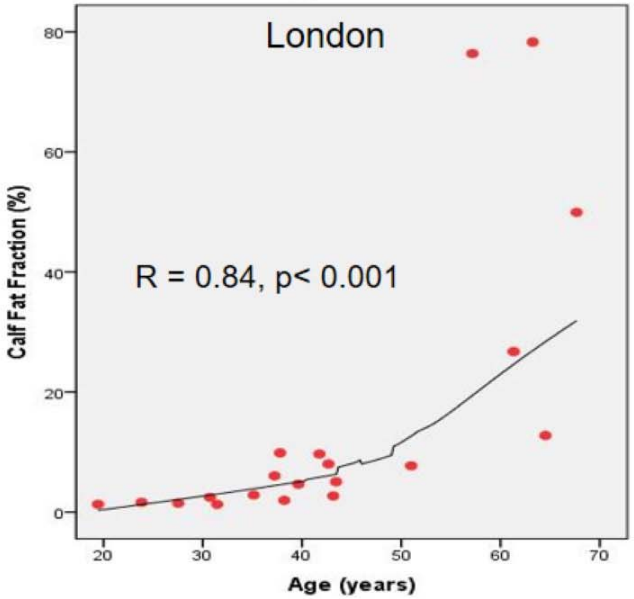

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Clinical correlations of overall calf muscle fat fraction

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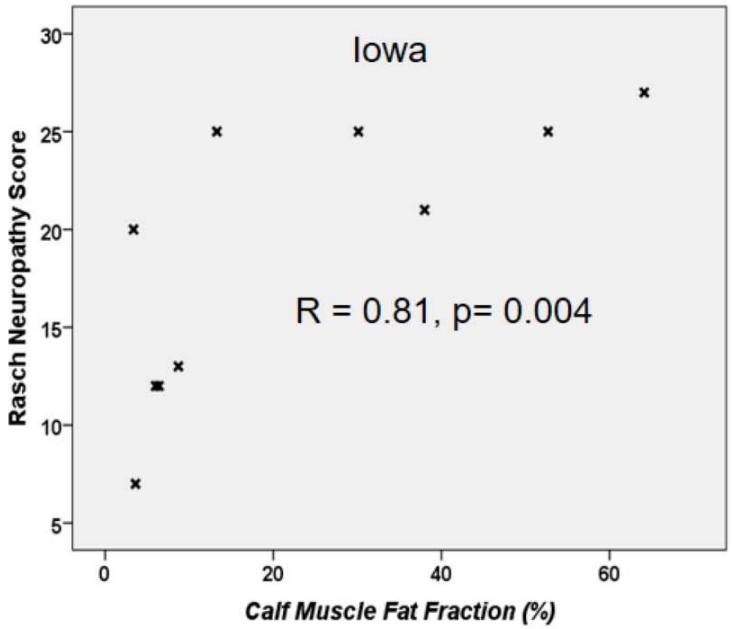
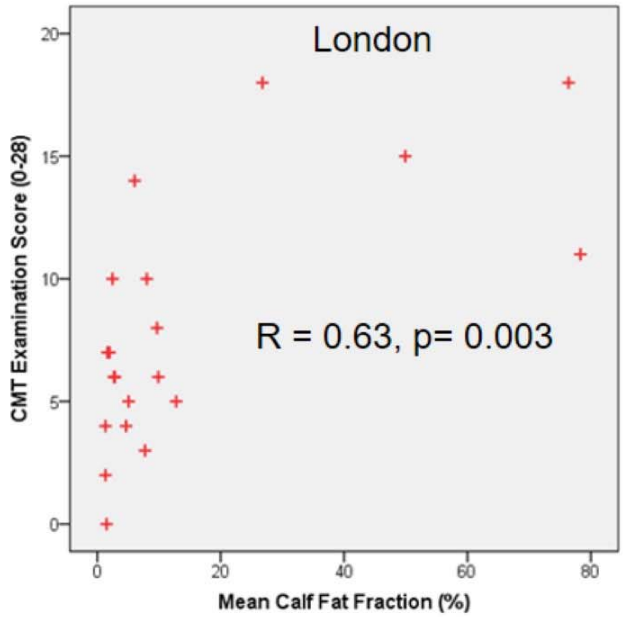
MRI correlation with severity





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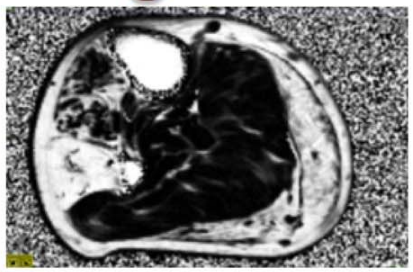
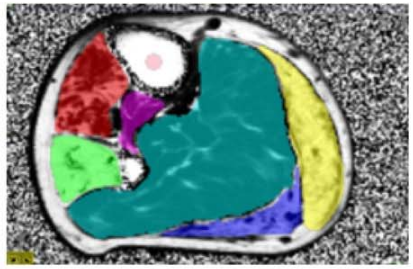
Longitudinal data

43 year old
CMTNS 19
Baseline





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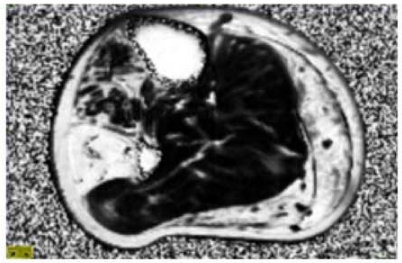
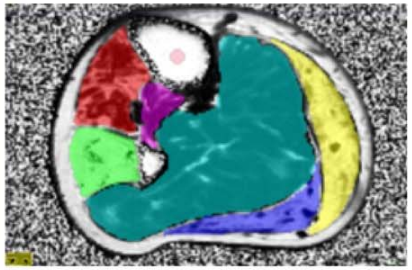
43 year old
CMTNS 19
1 year





Tibialis Anterior	36.0%
Peroneus Longus	87.0%
Lateral Gastrocnemius	69.2%
Medial Gastrocnemius	74.1%
Soleus	7.8%
Tibialis Posterior	22.6%
Overall	32.5%

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Longitudinal data

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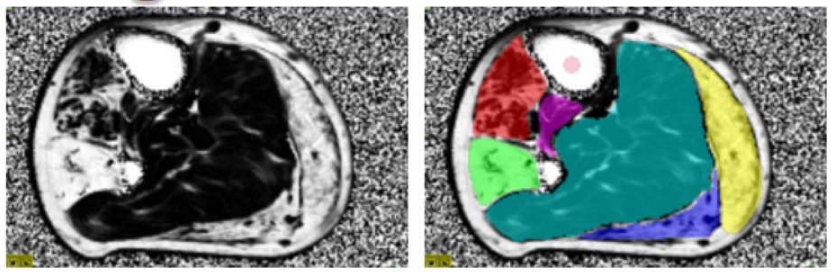

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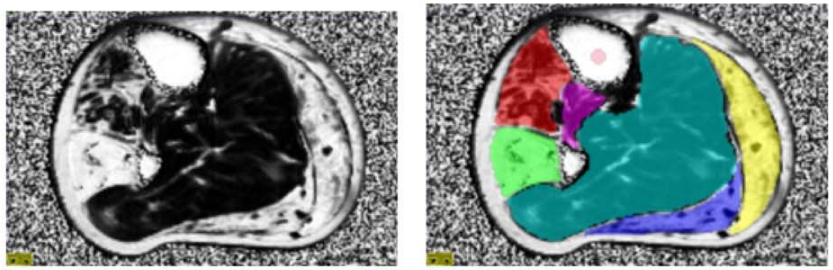

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Muscle	Mean change	s.d.	p
Tibialis Anterior	0.8%	1.9%	0.19
Peroneus Longus	1.6%	2.1%	0.04
Lateral Gastrocnemius	3.7%	3.1%	0.005
Medial Gastrocnemius	3.0%	4.8%	0.08
Soleus	1.7%	2.6%	0.07
Tibialis Posterior	0.9%	2.4%	0.25
Overall	1.8%	1.7%	0.009

SRM
1.04

SRM	Responsiveness
< 0.2	Minimal
0.2 – 0.5	Small
0.5 – 0.8	Moderate
> 0.8	Large

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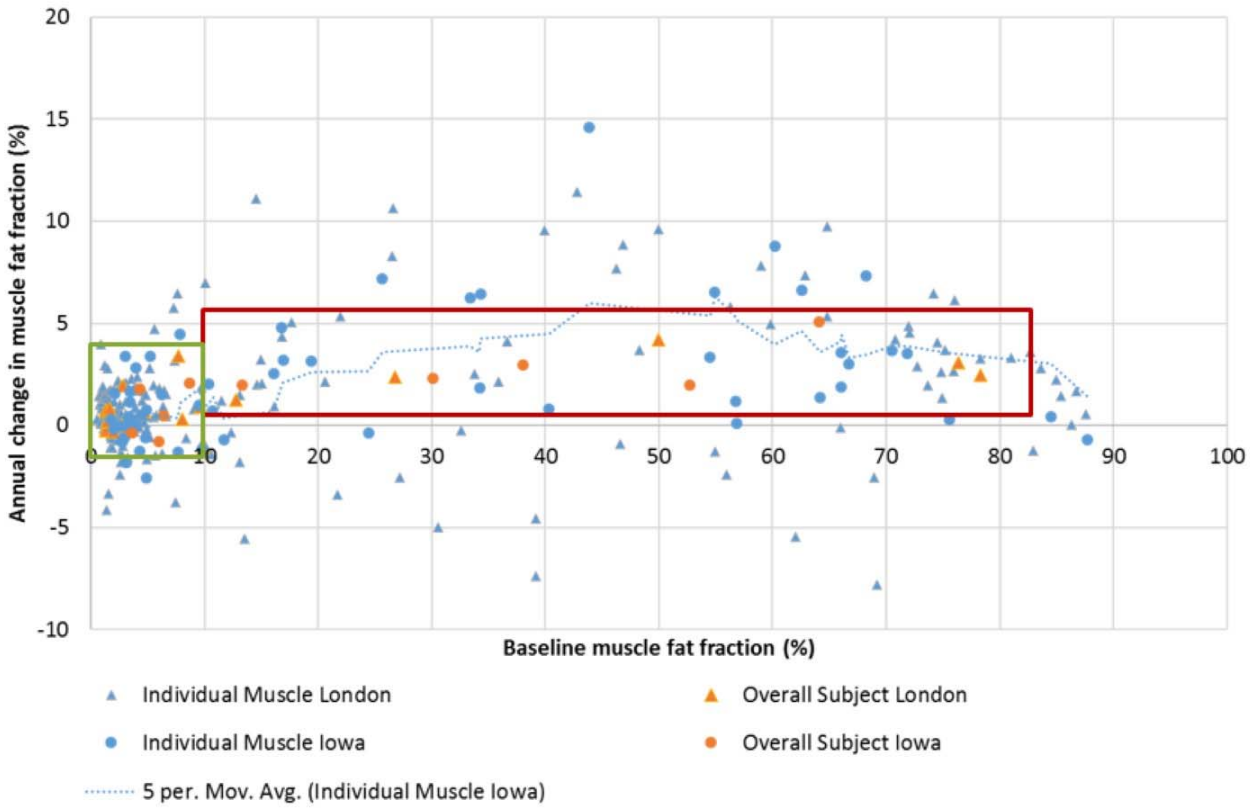
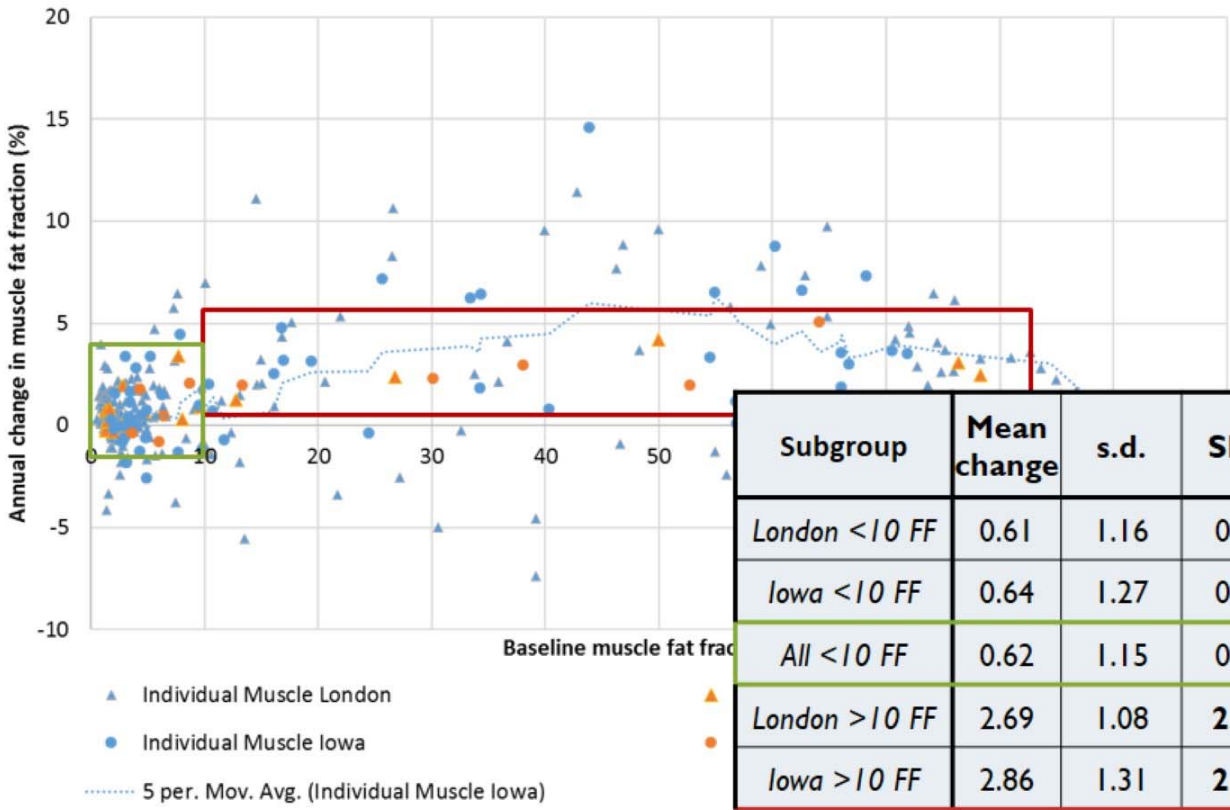
Maximising responsiveness



Maximising responsiveness



Subgroup	Mean change	s.d.	SRM
London <10 FF	0.61	1.16	0.53
Iowa <10 FF	0.64	1.27	0.51
All <10 FF	0.62	1.15	0.54
London >10 FF	2.69	1.08	**2.49**
Iowa >10 FF	2.86	1.31	**2.19**
All >10 FF	2.77	1.14	**2.44**

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Conclusions

1. Establish a quantitative MRI imaging protocol to be used across multiple sites and countries
   Excellent quality MRI measures RELIABLITY ✓
2. Apply this protocol to a cross-sectional group of CMT1A patients
3. Assess sensitivity of MRI fat quantification as an outcome measure through repeat assessment after 12 months

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1. Establish a quantitative MRI imaging protocol to be used across multiple sites and countries
   Excellent quality MRI measures RELIABLITY ✓
2. Apply this protocol to a cross-sectional group of CMT1A patients
   Excellent clinical correlations VALIDITY ✓
3. Assess sensitivity of MRI fat quantification as an outcome measure through repeat assessment after 12 months
   Excellent sensitivity, SRM 2.4 in >10%FF
   RESPONSIVENESS ✓

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MDA, CMTA

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