Personalised medicine in the face of multi-scale heterogeneity

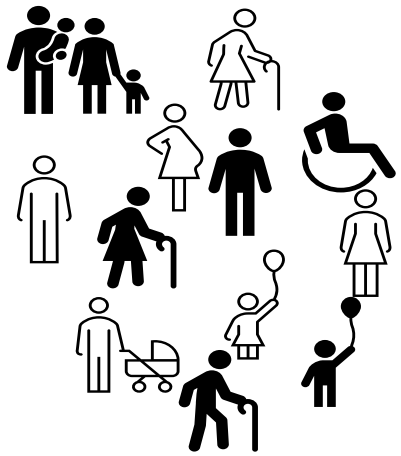
Turing-Roche Knowledge Share

27th June 2023

Dr Chris Banerji, PhD, MBBS

Email: cbanerji@turing.ac.uk

What is Multi-scale Heterogeneity in systemic pathology?



Population Scale

Even though they have the same disease: people are all different (genetics, risk factors, etc.,)

Inter-patient heterogeneity

Individual Scale

R Leg: Low disease burden

L Leg: High disease burden

Within an individual patient different tissues may have different levels of activity (inflammation, tumour burden, etc.,)

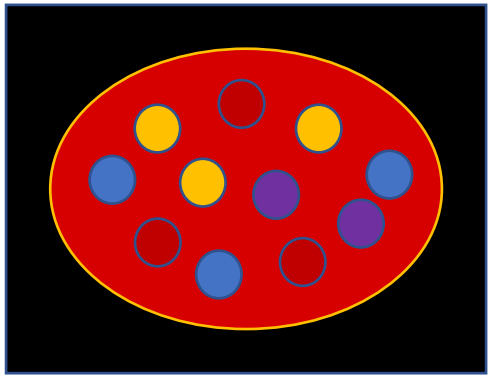
Intra-patient heterogeneity

<u>Understanding multi-scale heterogeneity leads to:</u>

1. Better patient stratification for clinical trials
2. Better tissue and imaging biomarkers
3. Better understanding of residual disease burden post therapy

To name a few benefits!

Tissue Scale

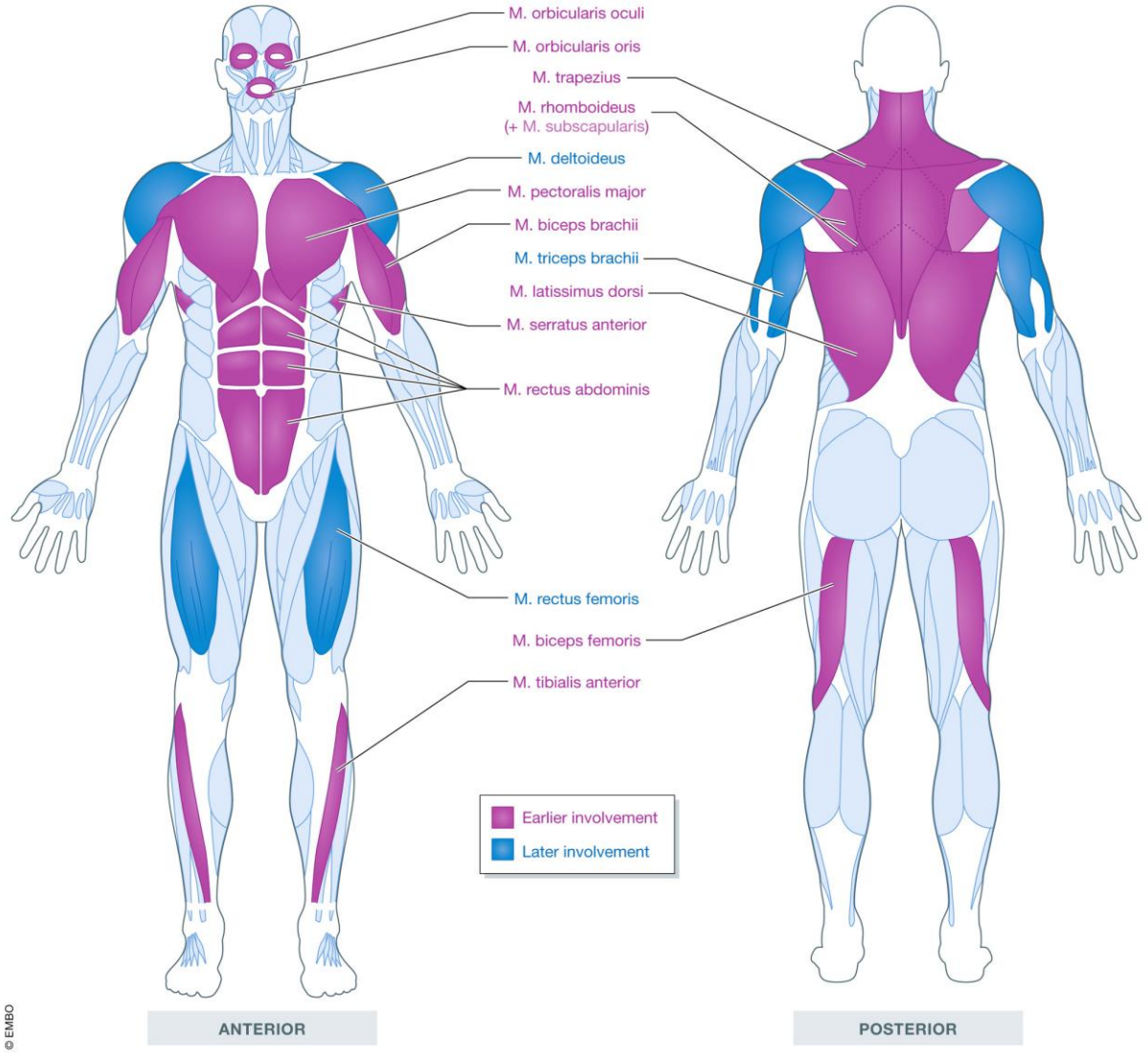


Within an affected tissue, different patient cells may have different levels of disease activity (molecular markers, treatment resistance, etc.,)

Inter-cellular heterogeneity

FSHD in two words: Multi-scale Heterogeneity!

Muscle weakness and wasting of specific groups



Banerji and Zammit, *EMBO Mol. Med.* **13**(8), 2021.

What is FSHD?

Autosomal dominant (ch4 linked)

Prevalence ~1/7500 (and increasing as diagnosis improves)

Linked to mis-expression of DUX4 TF

No treatment (this takes time!)

BUT ALSO No health advice at present

Extra-muscular symptoms:

- Retinal telangiectasia (Coat's-like) – messy blood vessels in eye
- High frequency hearing loss – unclear aetiology

Highly Heterogeneous Clinical Presentation:

- 7 distinct clinical subtypes (Banerji et al., Neuromusc. Disord., **30**(4), 2020) – order of muscle involvement varies
- Age of onset varies: some children are wheelchair bound, some octogenarians climb mountains
- Identical twins can have dramatically different trajectories

WHAT CAUSES INTER-PERSONAL HETEROGENEITY?

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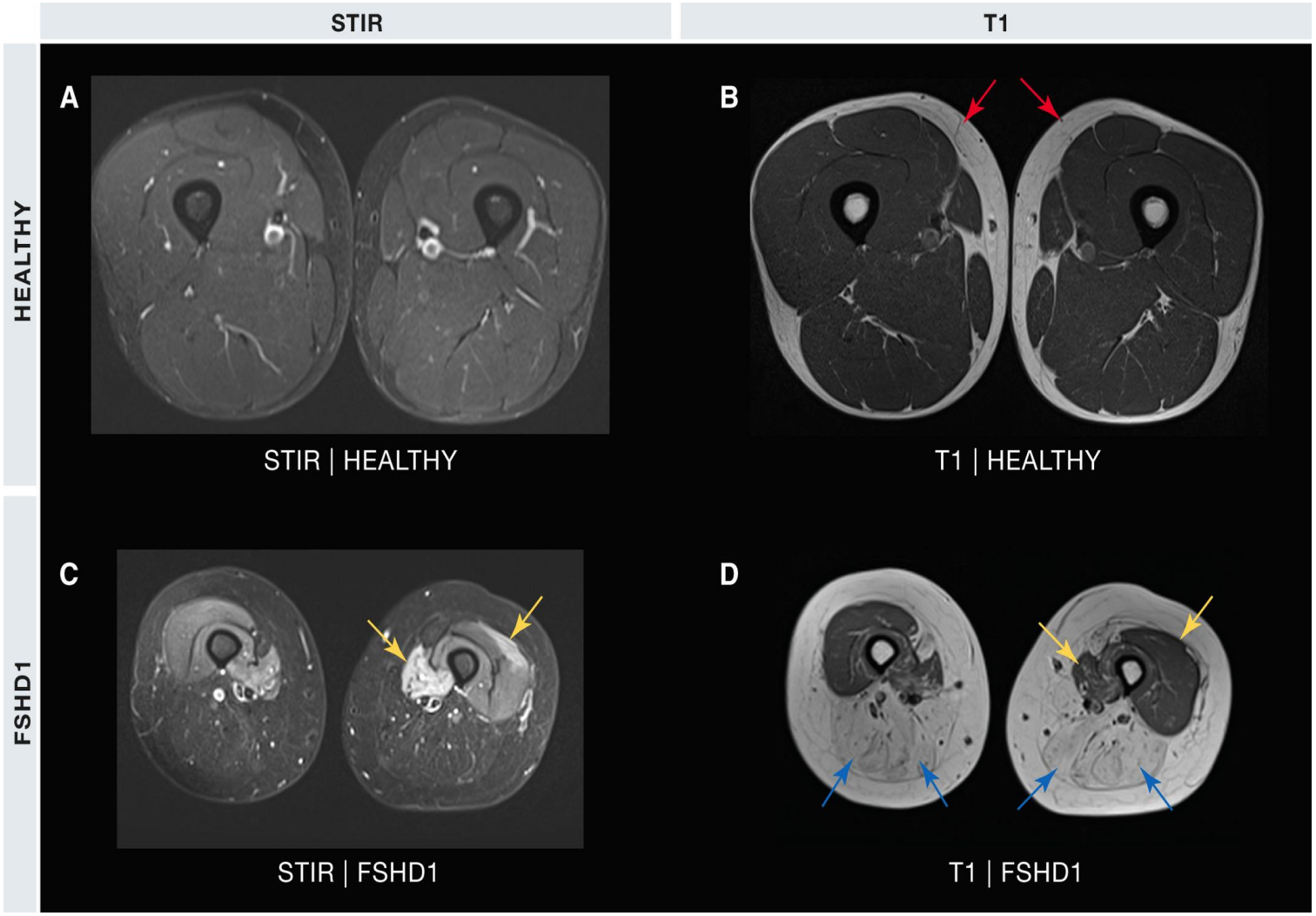
FSHD in two words: Multi-scale Heterogeneity!

MRI scale: muscles show inflammation (STIR) and fatty replacement (T1)



Heterogenous Muscle MRI Findings

- **Inflammation:** 11-16% of FSHD muscles show STIR+ on MRI. No consensus distribution. Triggers unknown.
- **Fatty replacement**: correlates with clinical weakness, progresses as disease gets worse

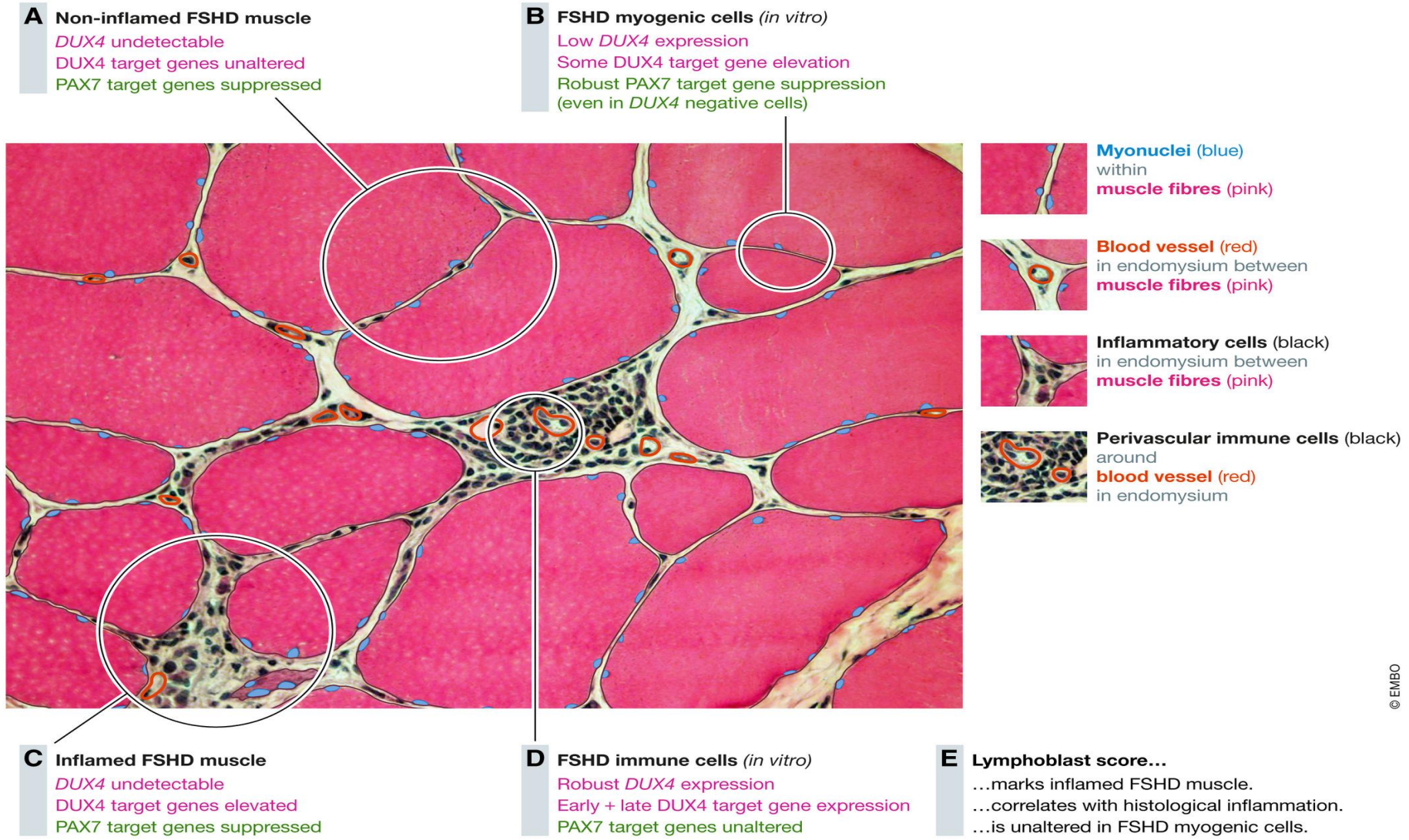
Inflamed muscle is replaced by fat 7x faster BUT fatty replacement still happens in absence of inflammation

WHAT DRIVES THIS INTRA-PERSONAL HETEROGENEITY?

Banerji and Zammit, *EMBO Mol. Med.* **13**(8), 2021.

FSHD in two words: Multi-scale Heterogeneity!

Cellular scale: DUX4 expression is associated with cell death



Banerji and Zammit, *EMBO Mol. Med.* **13**(8), 2021.

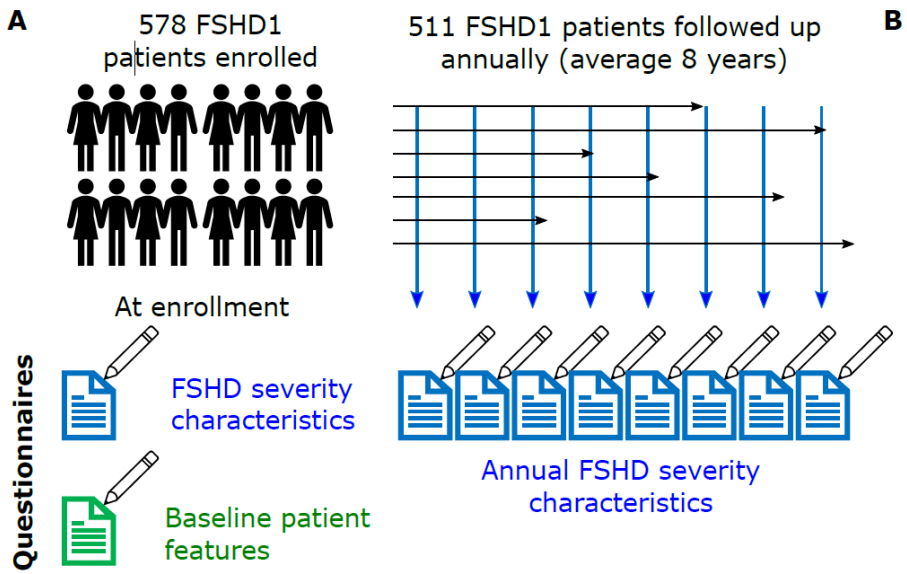
Heterogenous single cell gene expression

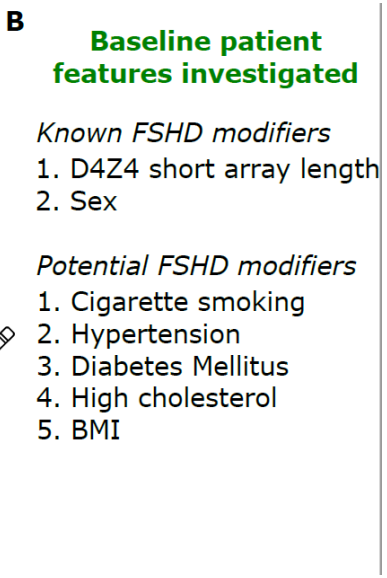
- DUX4 is believed to drive pathology but its only expressed in 1/1000 single muscle cells
- It is also expressed in immune cell types (Banerji, Hum. Mol Genet, **29**(13) 2020)

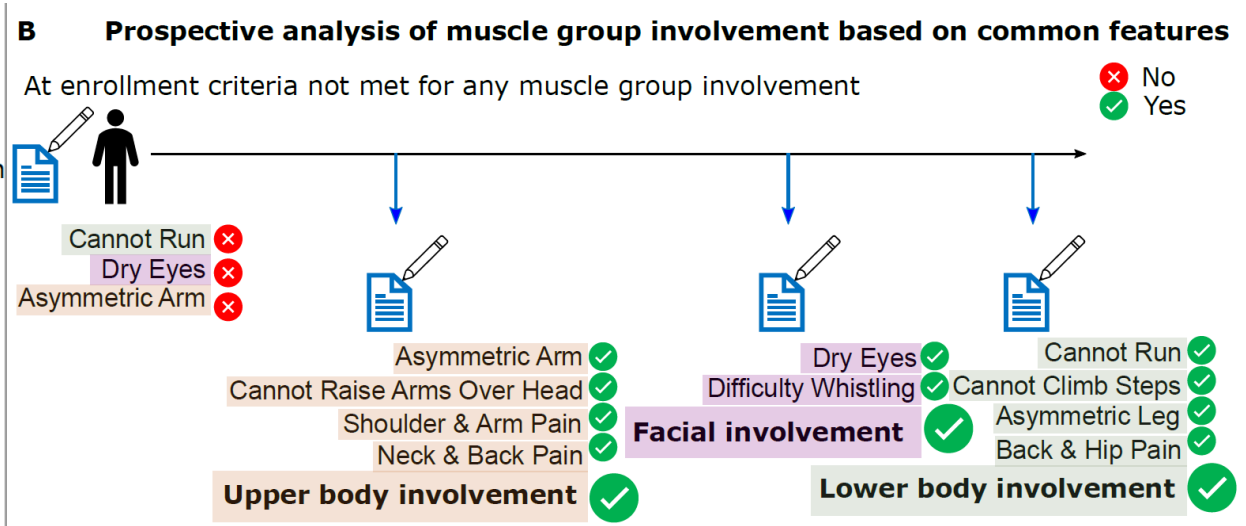
WHY IS DUX4 EXPRESSION SO DIFFERENT BETWEEN SINGLE CELLS?

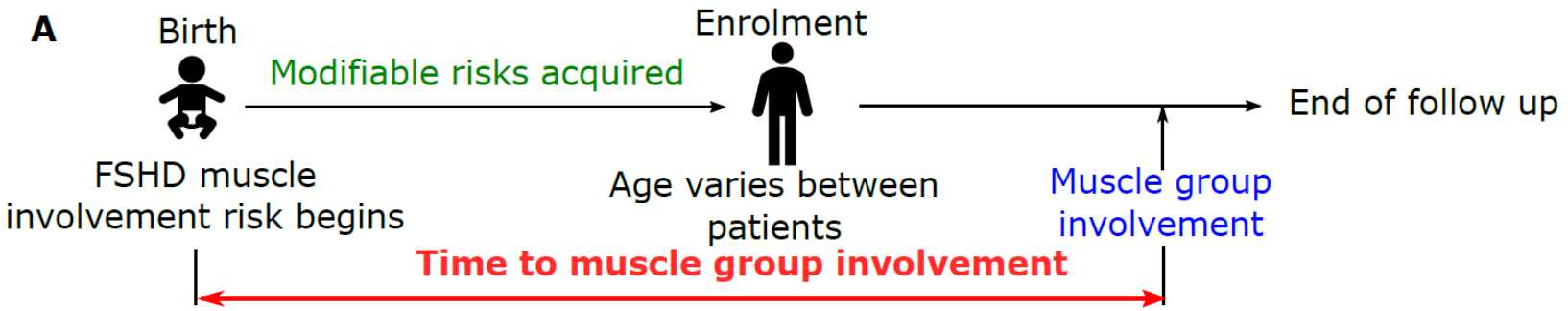
Characterising inter-personal heterogeneity in FSHD

USA FSHD patient registry – complex multimodal dataset, manually curated with clinical experience







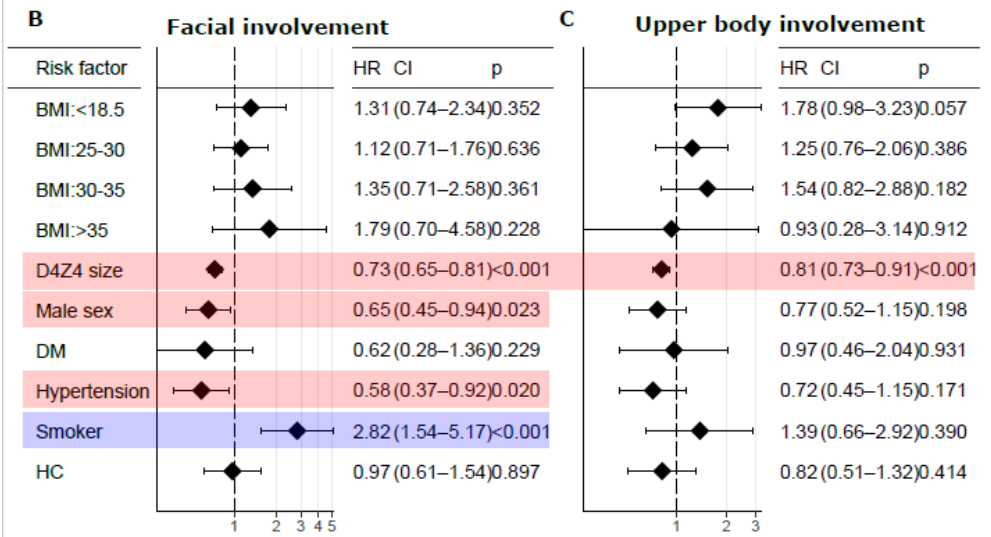


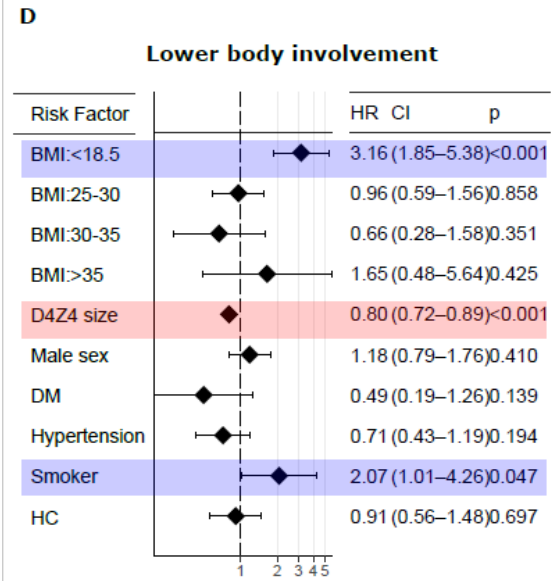
First prospective time-to-event analysis of how common risks impact FSHD symptom onset

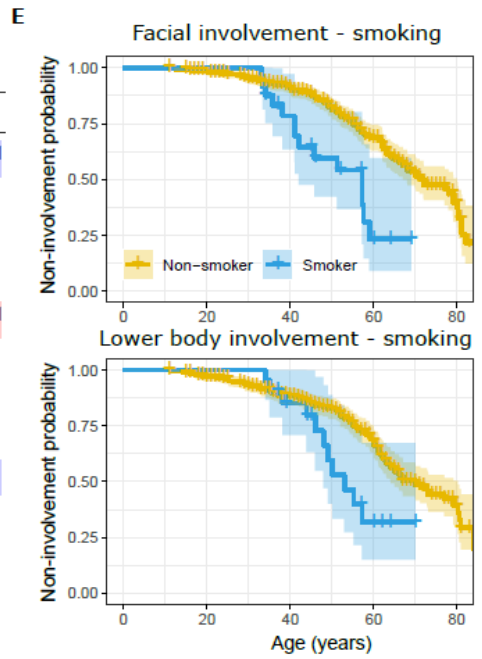
Risk of muscle group involvement over time ~ D4Z4 short allele length + sex + BMI + hypertension + high cholesterol + cigarette smoking + diabetes mellitus

Banerji et al., 2023a, *submitted:* *https://www.medrxiv.org/content/10.1101/2023.05.17.23290091v1*

Smoking drives a 2 fold acceleration of FSHD symptom onset

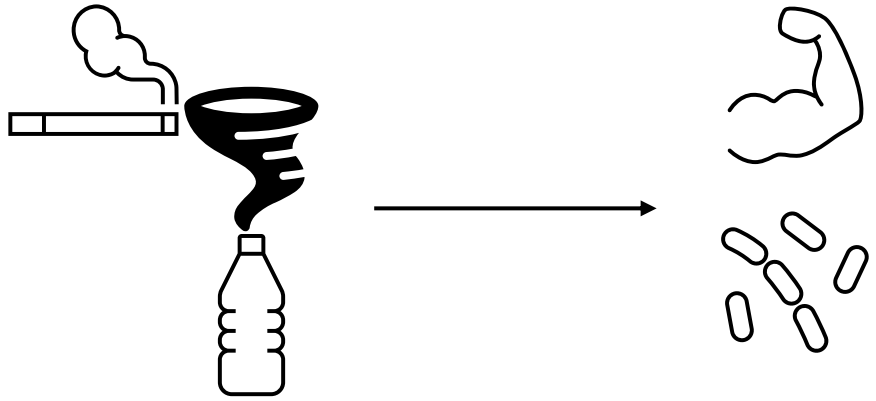




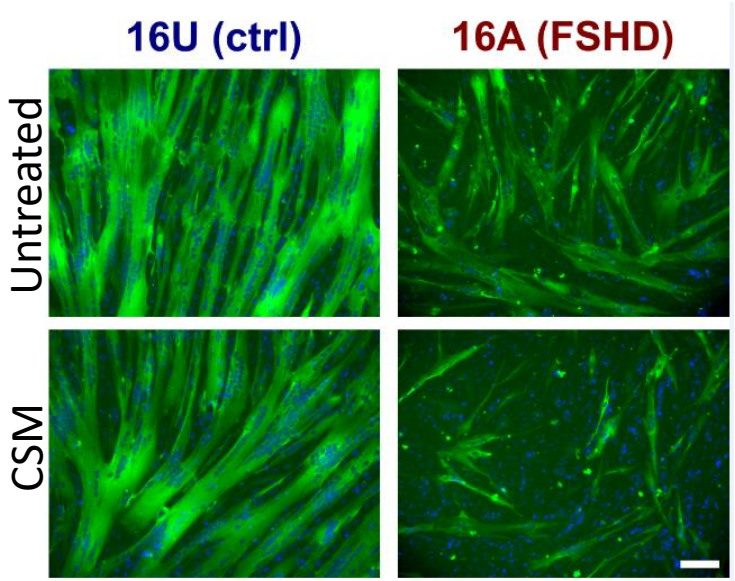


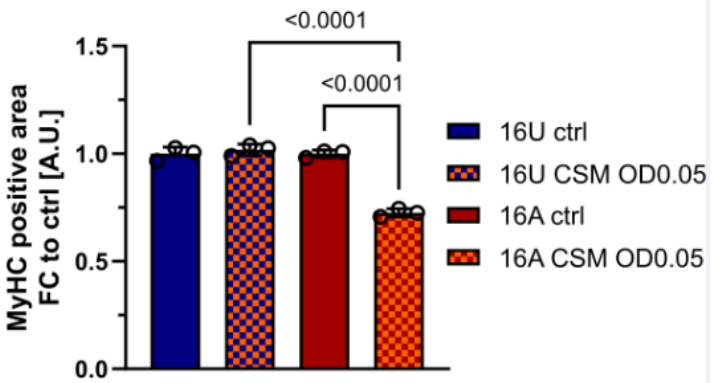
Back to the lab!

Cigarette smoke media (CSM)



FSHD and healthy muscle cells





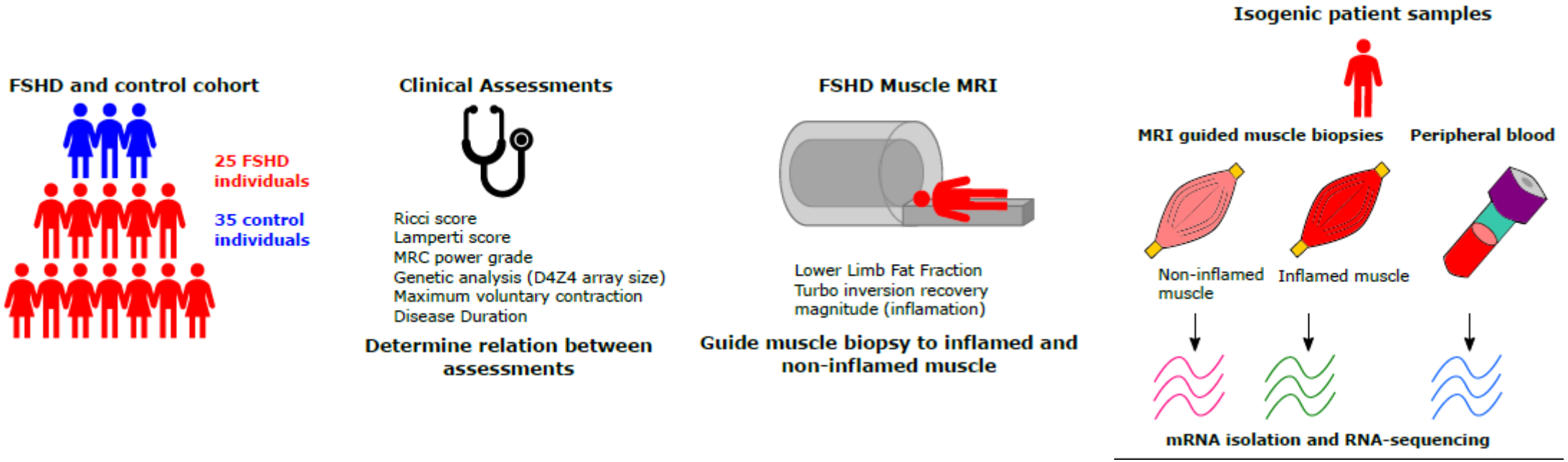
CSM disproportionately inhibits FSHD muscle regeneration in vitro

We have shown this is due to deficient FSHD mitochondria

Banerji et al., 2023a, *submitted:* https://www.medrxiv.org/content/10.1101/2023.05.17.23290091v1

Understanding intra-personal heterogeneity in FSHD

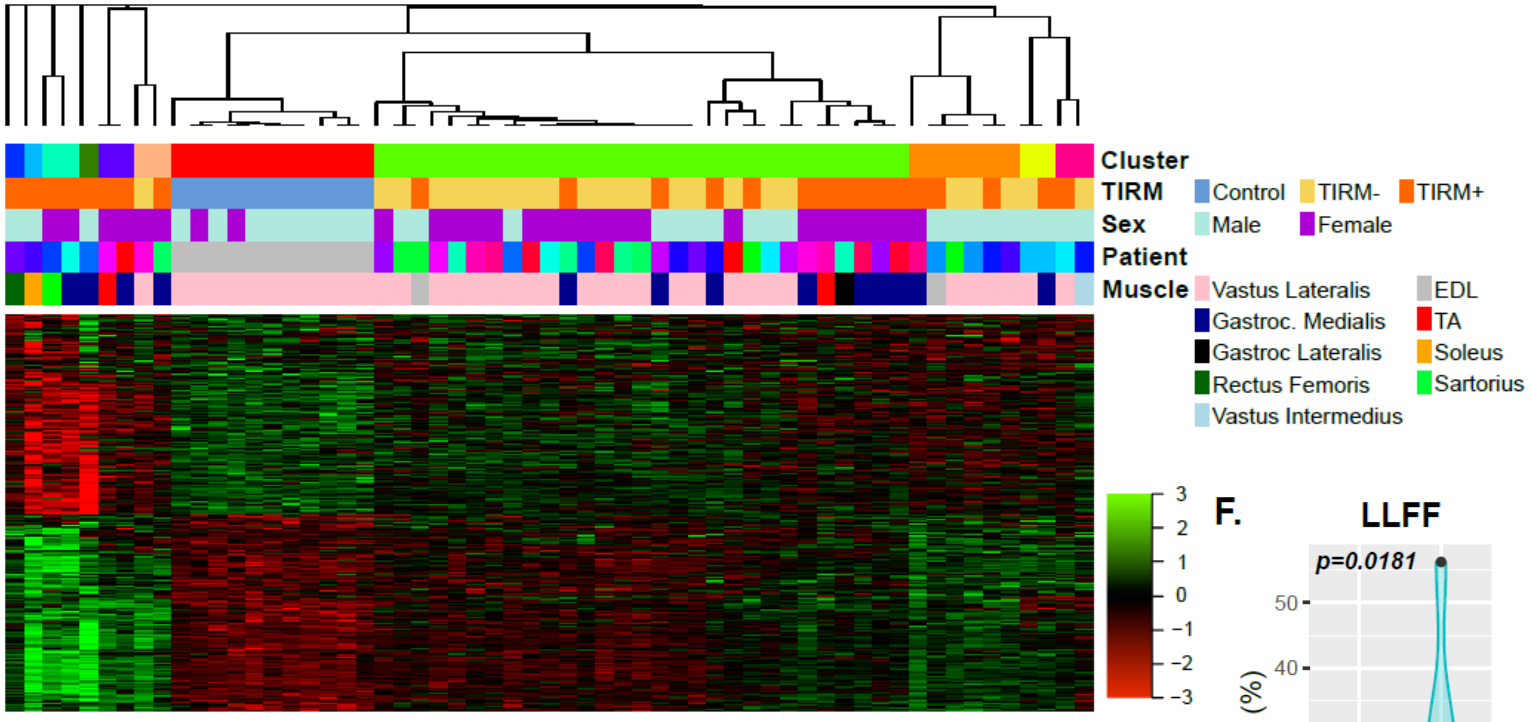
Dutch FSHD cohort – characterised clinically, by MRI and from **each patient** took 3 tissue samples for transcriptomics

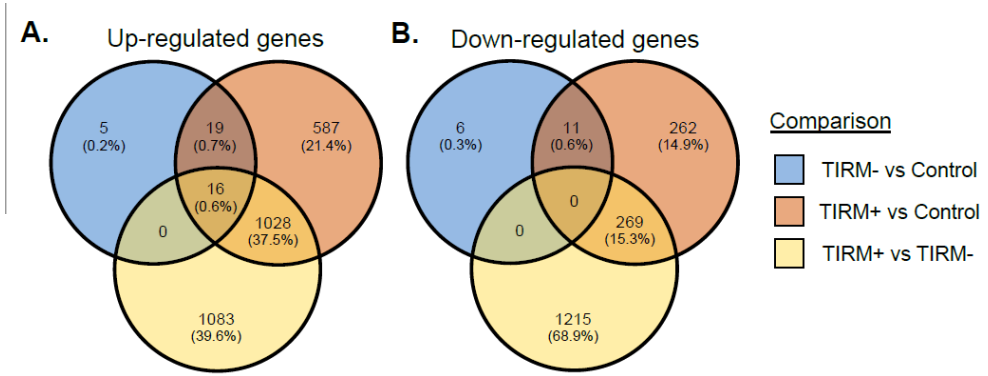


Banerji et al., 2023, *Brain Comms. in press;* Engquist … & Banerji, 2023, *Human Molecular Genetics, under review*

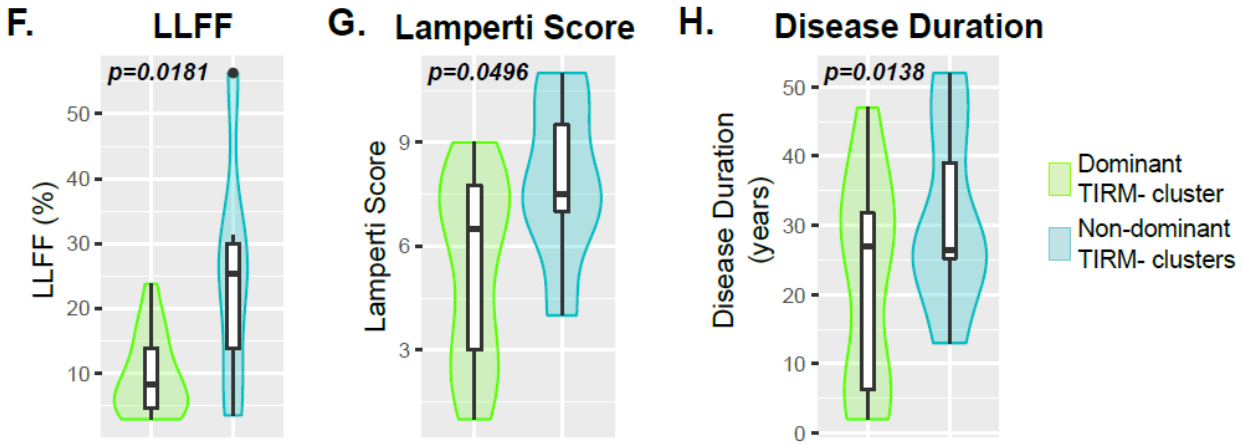
Understanding intra-personal heterogeneity in FSHD

Differential expression analysis identified genes associated with FSHD independently of muscle inflammation on MRI (TIRM+)
i.e. molecular heterogeneity indep. of MRI heterogeneity





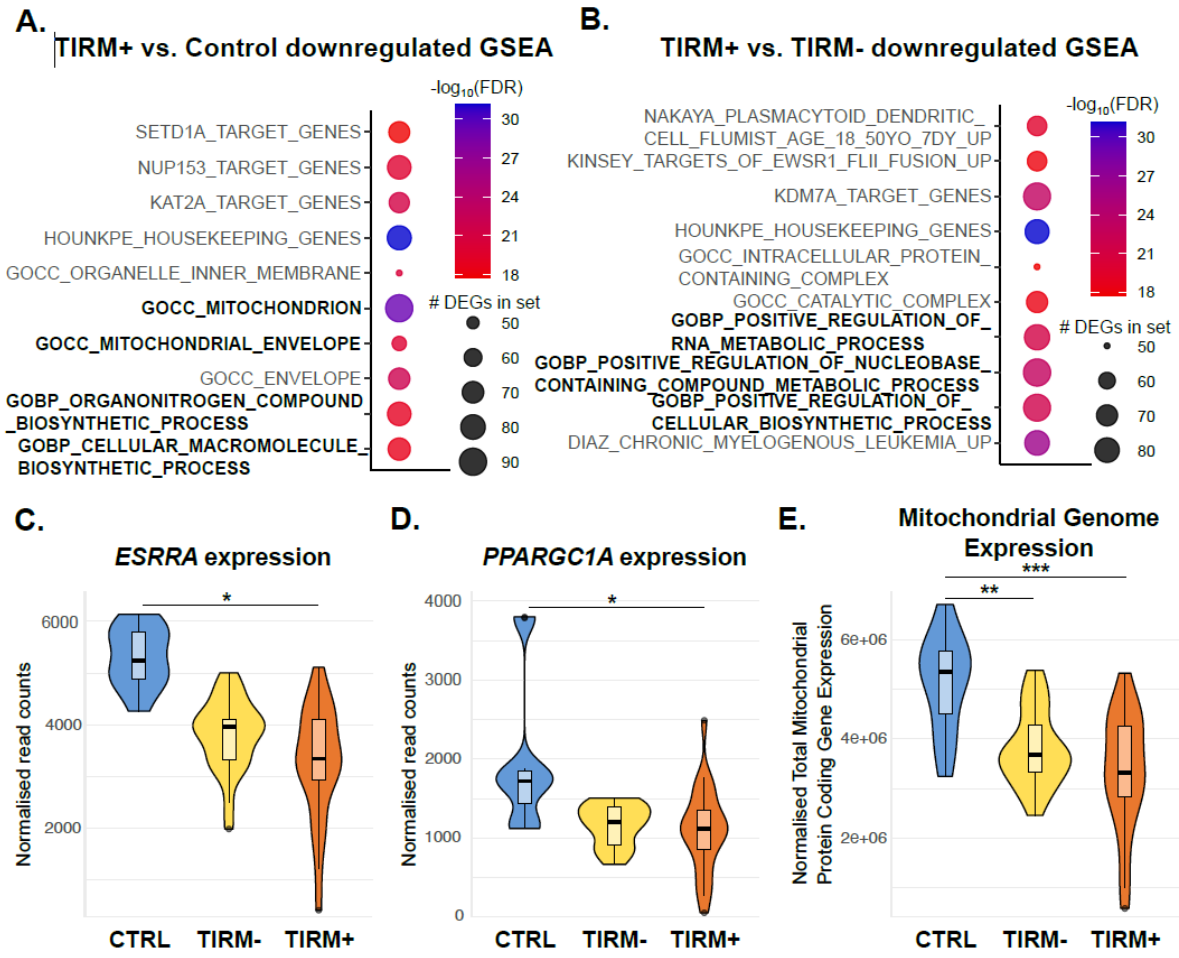
K-mediods clustering of samples on these genes identified a molecular subtype of patients with milder disease



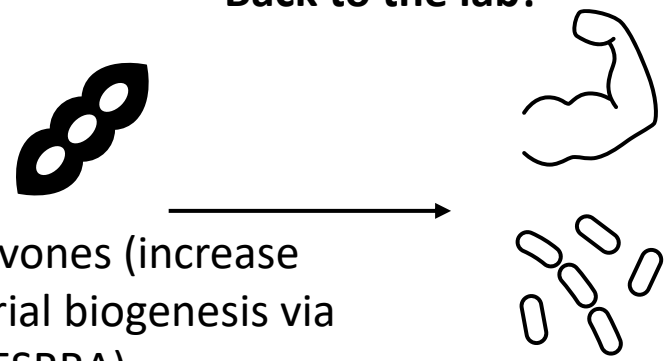
Engquist … & Banerji, 2023, *Human Molecular Genetics, under review*

Understanding intra-personal heterogeneity in FSHD

Differential gene expression identified deficient mitochondrial biogenesis in FSHD muscle, which worsens with disease activity

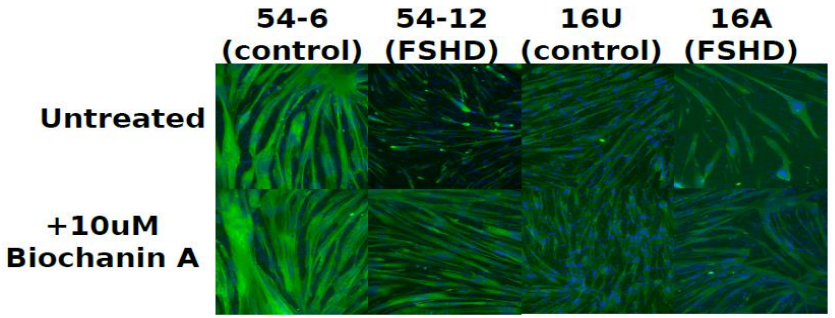


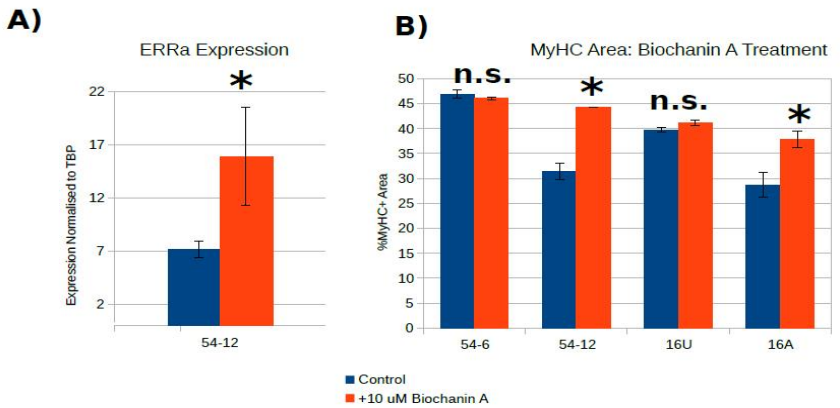
Back to the lab!



FSHD and healthy muscle cells

Soy isoflavones (increase mitochondrial biogenesis via ESRRA)





Engquist … & Banerji, 2023, *Human Molecular Genetics, under review;* Banerji et al., 2019, *Hum. Mol. Genet.*, **28**(8), 1244-1259

The Alan Turing Institute

How do we monitor FSHD?

Maybe patients should stop smoking and eat soy… but how do we know if this is helping?

Over the years published on muscle biopsy transcriptomic biomarker for progression/severity (PAX7 score)

BUT these are invasive!

PAX7 target gene repression is a superior FSHD biomarker than DUX4 target gene activation, associating with pathological severity and identifying FSHD at the single-cell level

Christopher R S Banerji, Peter S Zammit

Human Molecular Genetics, Volume 28, Issue 13, 1 July 2019, Pages 2224–2236,

PAX7 target genes are globally repressed in facioscapulohumeral muscular dystrophy skeletal muscle

Christopher R. S. Banerji, Maryna Panamarova, Husam Hebaishi, Robert B. White, Frédéric Relaix, Simone Severini & Peter S. Zammit

Nature Communications **8**, Article number: 2152 (2017) | Cite this article

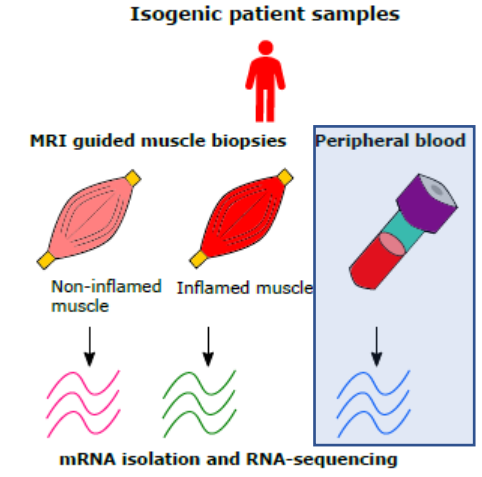
PAX7 target gene repression associates with FSHD progression and pathology over 1 year

Christopher R S Banerji

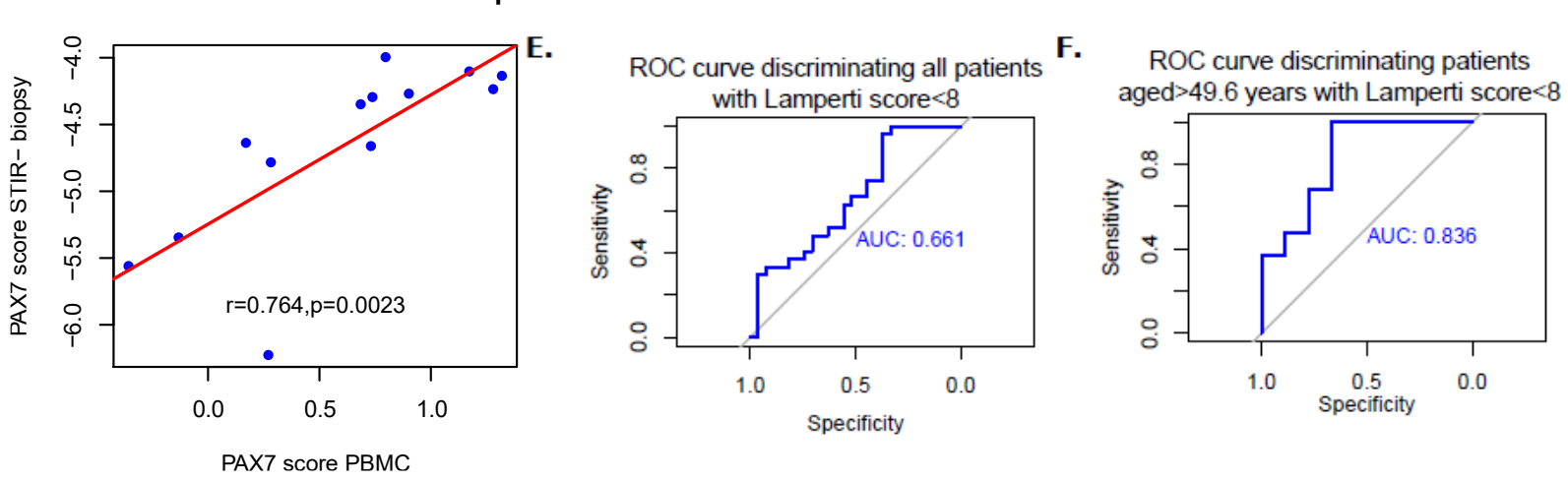
Human Molecular Genetics, Volume 29, Issue 13, 1 July 2020, Pages 2124–2133,

Banerji et al., 2023, *Brain Comms. in press;*

New data – minimally invasive



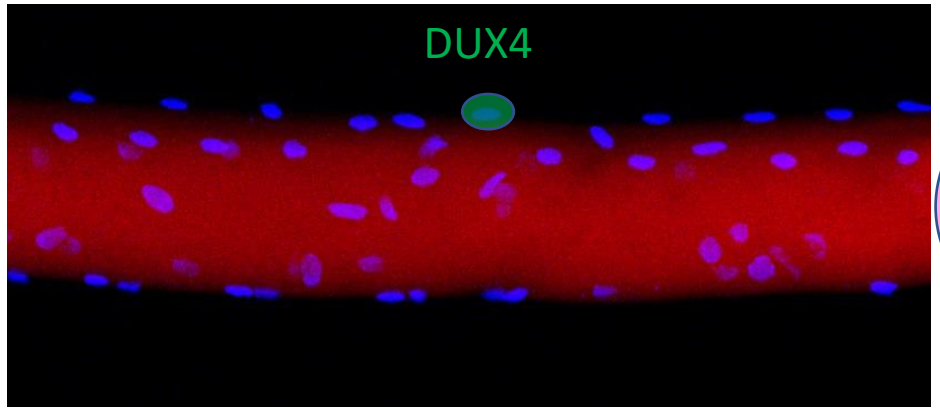
Validation in independent cohort of 54 FSHD patient blood samples – severity biomarker accuracy improves as patients age…**predictive power?**

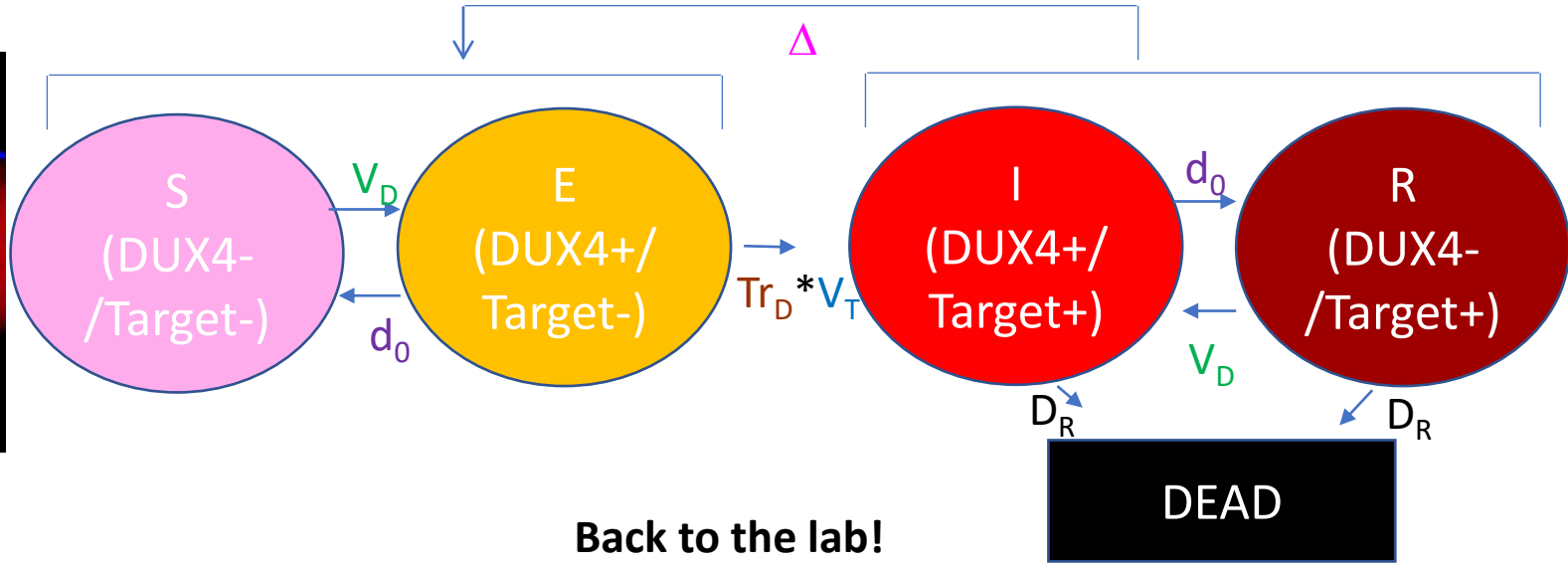


Biomarker of severity correlates between muscle and blood

Cellular heterogeneity in FSHD

DUX4 is a protein that causes cell death and is a **key therapeutic target in FSHD**
BUT its only expressed in 1/1000 myonuclei in a muscle fibre





$$\frac{dS}{dt} = \delta_0 E - v_D S - \Delta(I+R)S$$
$$\frac{dE}{dt} = v_D S - E(\delta_0 + Tr_D v_T) - \Delta(I+R)E$$
$$\frac{dI}{dt} = Tr_D v_T E + v_D R - \delta_0 I - D_R I + \Delta(I+R)E$$
$$\frac{dR}{dt} = \delta_0 I - v_D R - D_R R + \Delta(I+R)S$$
$$\frac{dD}{dt} = D_R(R+I)$$

Cowley … &Banerji, 2023, *eLife,* **12**:e88345

Back to the lab!

6 Parameters all experimentally estimated:

d_0= Degradation rate of DUX4 mRNA

V_D=Transcription rate of DUX4

V_T=Transcription rate of DUX4 Targets

Tr_D= Transition rate from DUX4 mRNA ->active DUX4 protein

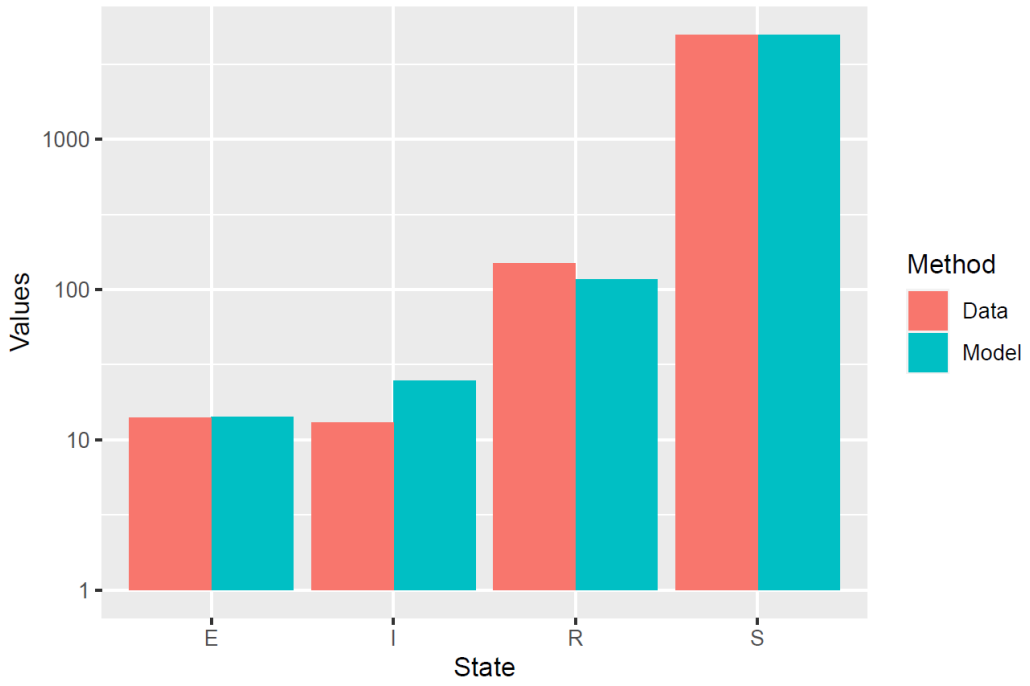
D_R = Death rate of Target+ cells

Δ = Rate at which a cell expressing DUX4 protein can 'infect' a protein negative cell

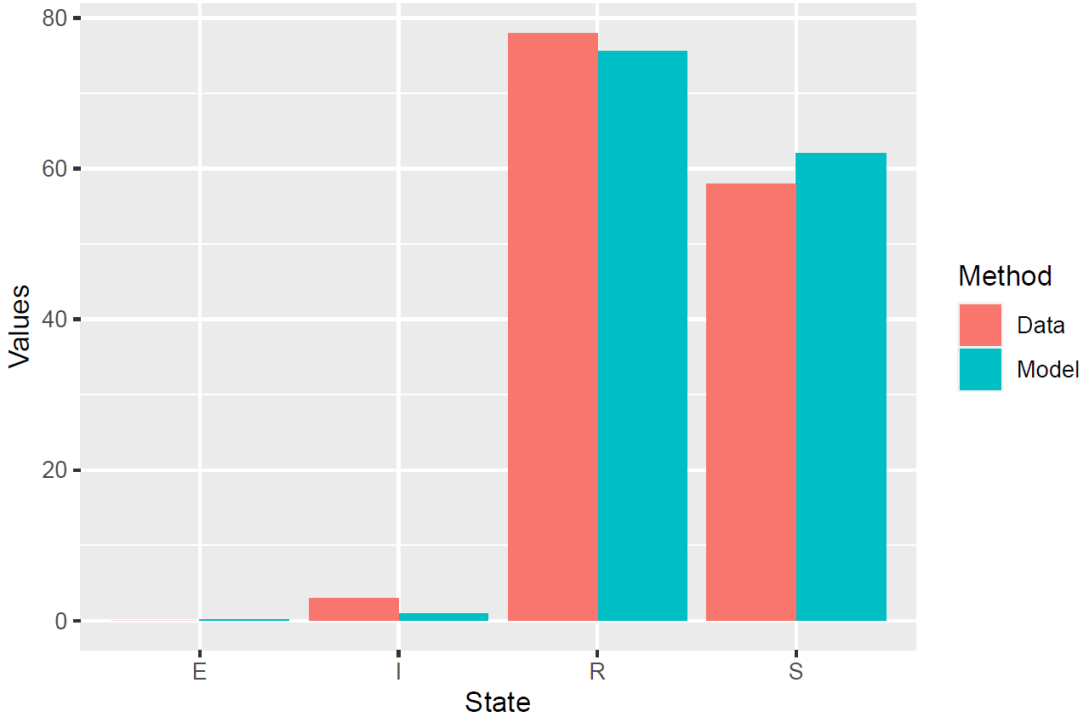
Cellular heterogeneity in FSHD

Model fits very well to real world data and predicts that only a small number of cells expressing DUX4 at any one time drives significant cell death

Model vs data comparison on scRNAseq of FSHD patient muscle cells



Model vs data comparison on snRNAseq of FSHD patient muscle fibres

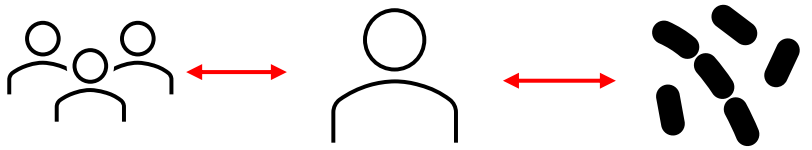


Cowley … &Banerji, 2023, *eLife,* **12**:e88345

Conclusions

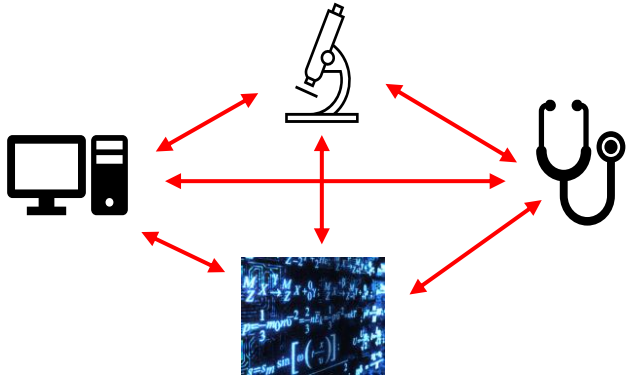
Understanding multi-scale heterogeneity drives great insights in complex pathology

BUT:



Relevant data is key:

- Large cohort studies (population level)
- Multimodal assessments of patients (individual level)
- Single cell and nucleus technologies (tissue level)



Validation and inter-disciplinary cross talk is essential:

- Bench to bedside and bedside to bench
- Biology to mathematics and mathematics to biology
- Requires a strong network of collaboration

Methodological challenges remain:

- Integration of data across scales remains challenging

Acknowledgements

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Dr Jeffrey Statland

University of Bath
Matt Cowley

My contact details:
Chris Banerji:
cbanerji@turing.ac.uk