FSHD Disease Mechanisms and Models

Silvère M. van der Maarel, Ph.D.
Leiden University Medical Center

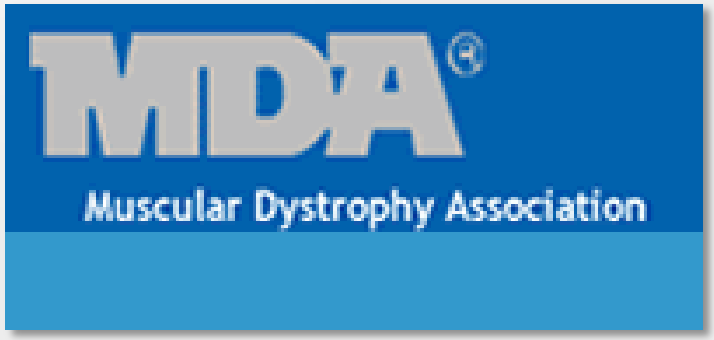
An Integrative Approach

Modern Research is Teamwork!

FSHD AND CHROMATIN DISEASE

Richard Lemmers
Judit Balog
Yvonne Krom
Peter Thijssen
Lucia Clemens-Daxinger
Amanda Mason
Marlinde van den Boogaard
Bianca den Hamer
Kirsten Straasheijm
Patrick van der Vliet

Modern Research is Teamwork!



Geraldi Norton Foundation & the Eklund Family

George & Jack Shaw & the Shaw Family Foundation

FSHD and the Fields Center

- Fields Center was established in 2007:
  - Strategic Alliance to create a clinical/scientific network between Rochester-Leiden-Seattle-Nijmegen-Nice
  - Expedite Research and Therapy Development
  - Non-exclusive

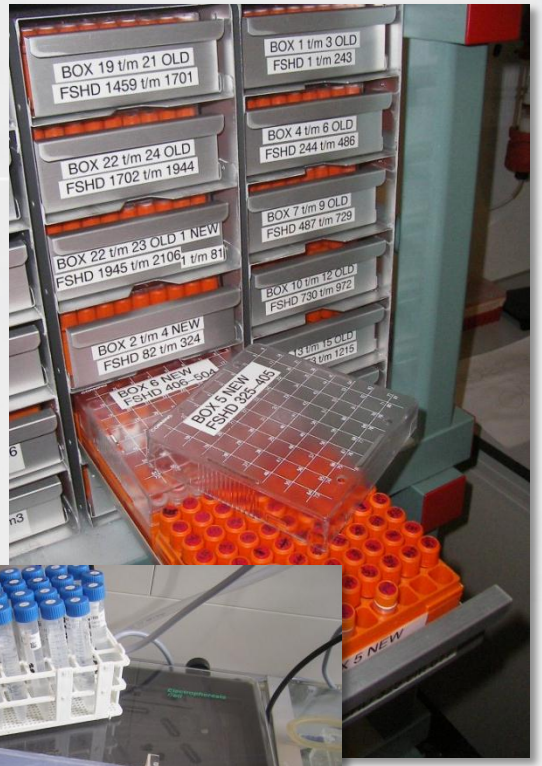
  - Protocols freely available
  - Sharing resources
  - Standards for Registries
  - Standards of care, diagnosis
  - 50+ publications

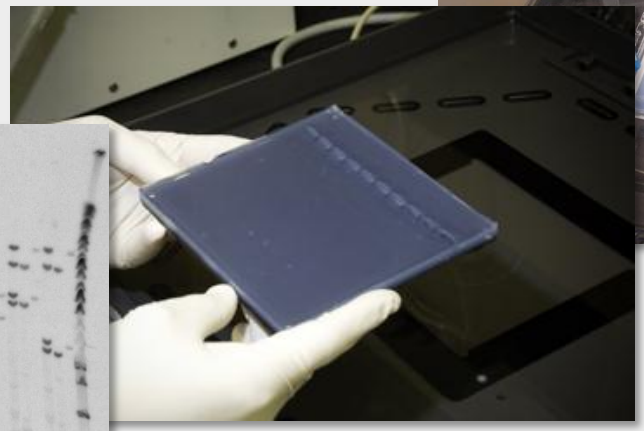
  - www.urmc.rochester.edu/fields-center/

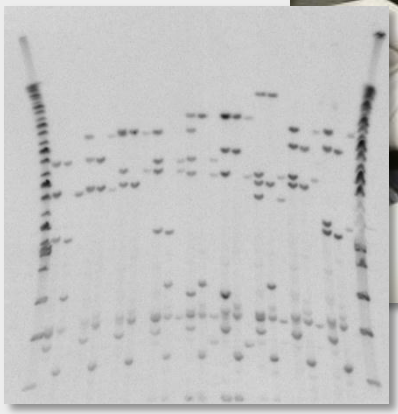
FSHD at the LUMC

- Long tradition of:
  - Genetic research
  - Molecular and cellular biology

  - DNA diagnosis
  - Assistance in diagnosis





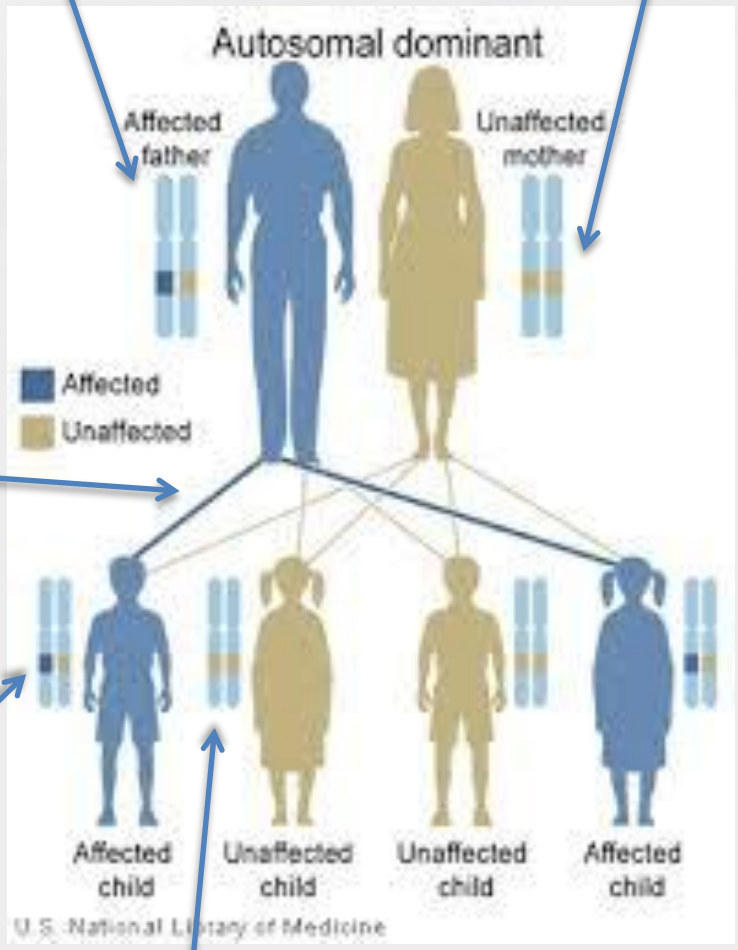


FSHD Genetics

For most families, FSHD is an autosomal dominant disorder with incomplete penetrance

23 pairs

25,000 genes

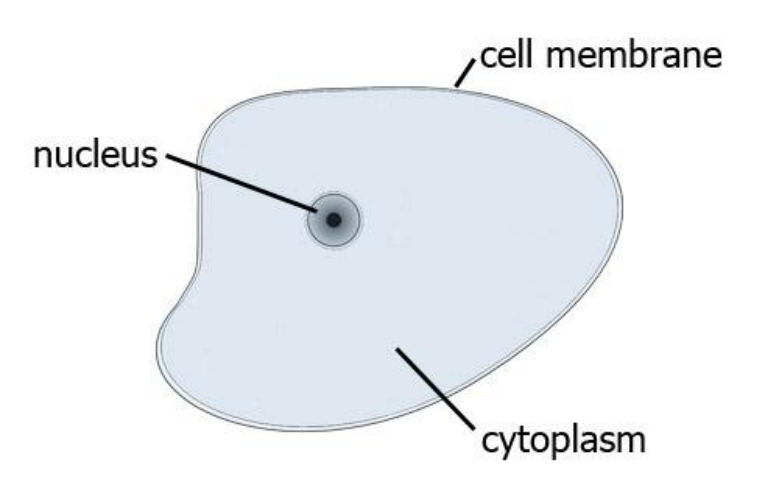


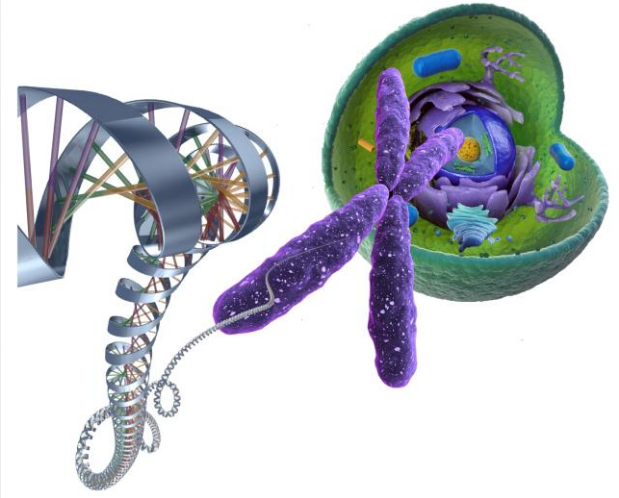
transmission

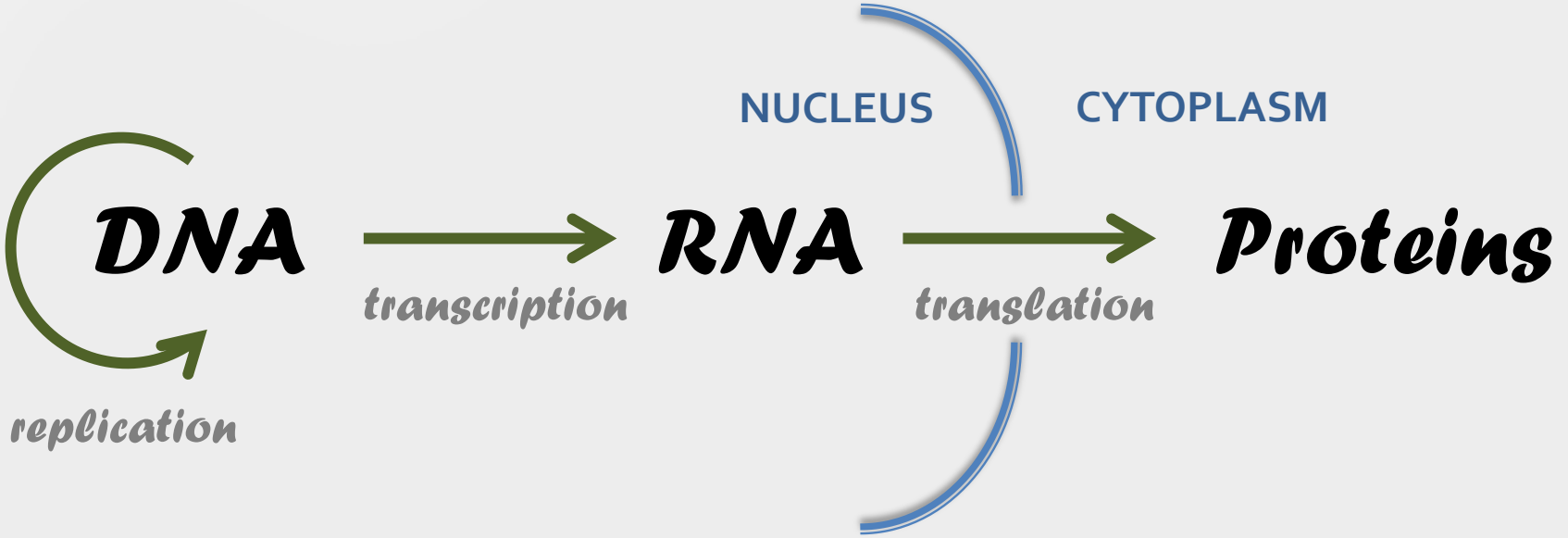
Genetic error

3.2 billion elements

The Central Dogma of Biology

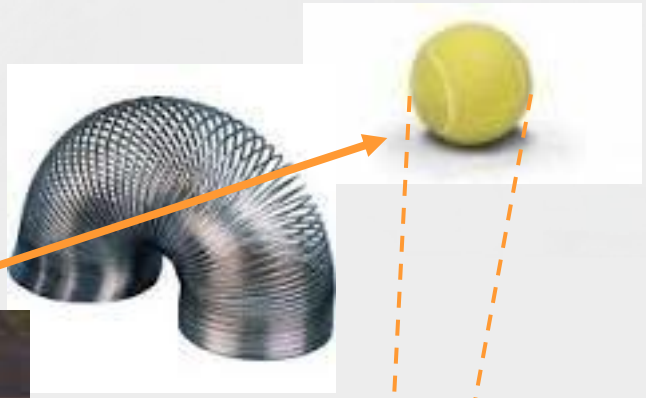


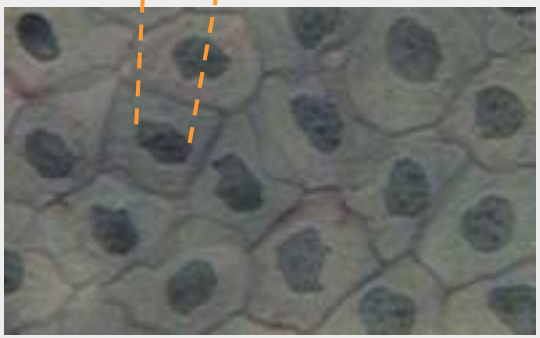




How much DNA?

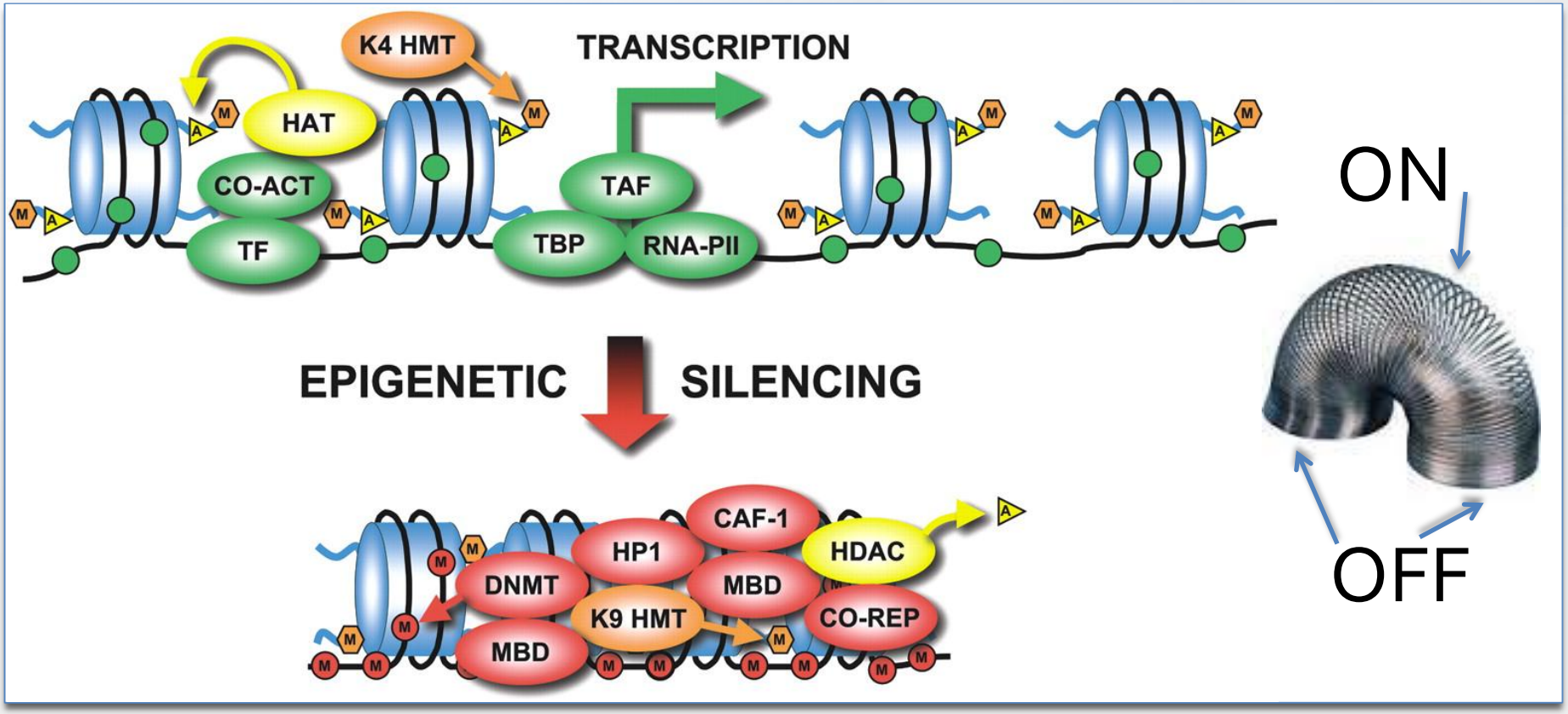
Each cell contains DNA, how much?





6.5 ft of DNA in each nucleus !

Gene regulation: on/off switch



Breakthrough in 2010

- FSHD is caused by the inappropriate production of the DUX4 protein in muscle of FSHD individuals (Lemmers *et al.*, Science 2010)

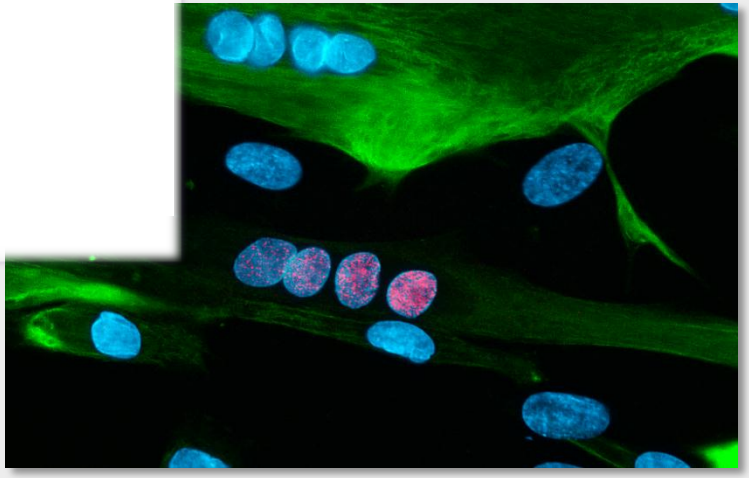
The New York Times

Reanimated 'Junk' DNA Is Found to Cause Disease

By GINA KOLATA
Published: August 19, 2010

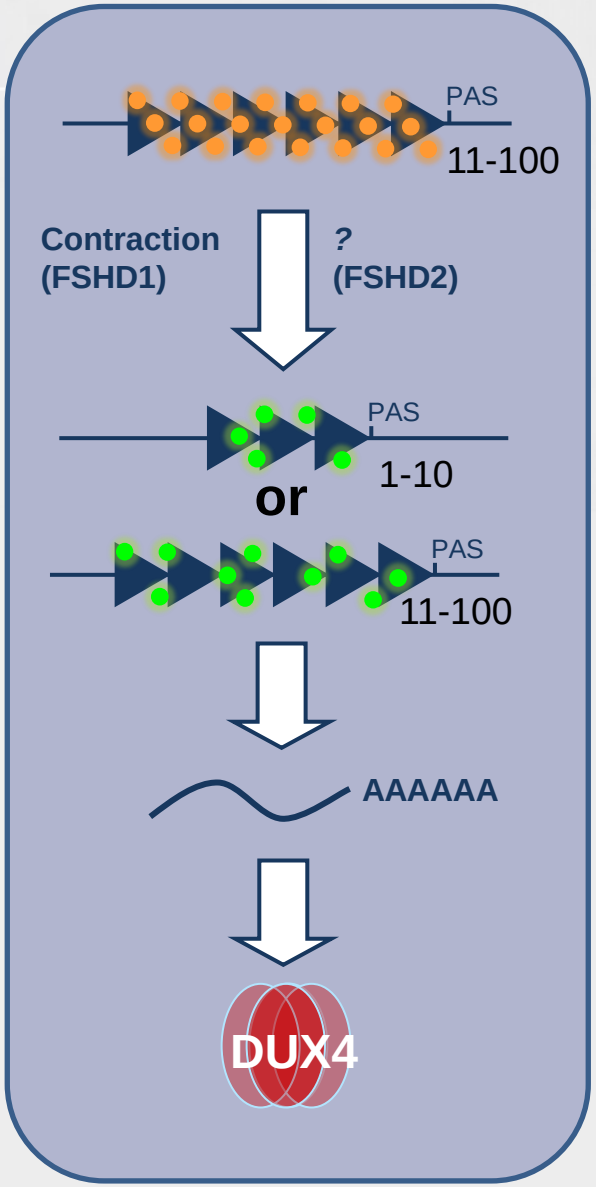
…"If we were thinking of a collection of the genome's greatest hits, this would go on the list," said Dr.Francis Collins, a human geneticist and director of the National Institutes of Health.

THE FRONT PAGE

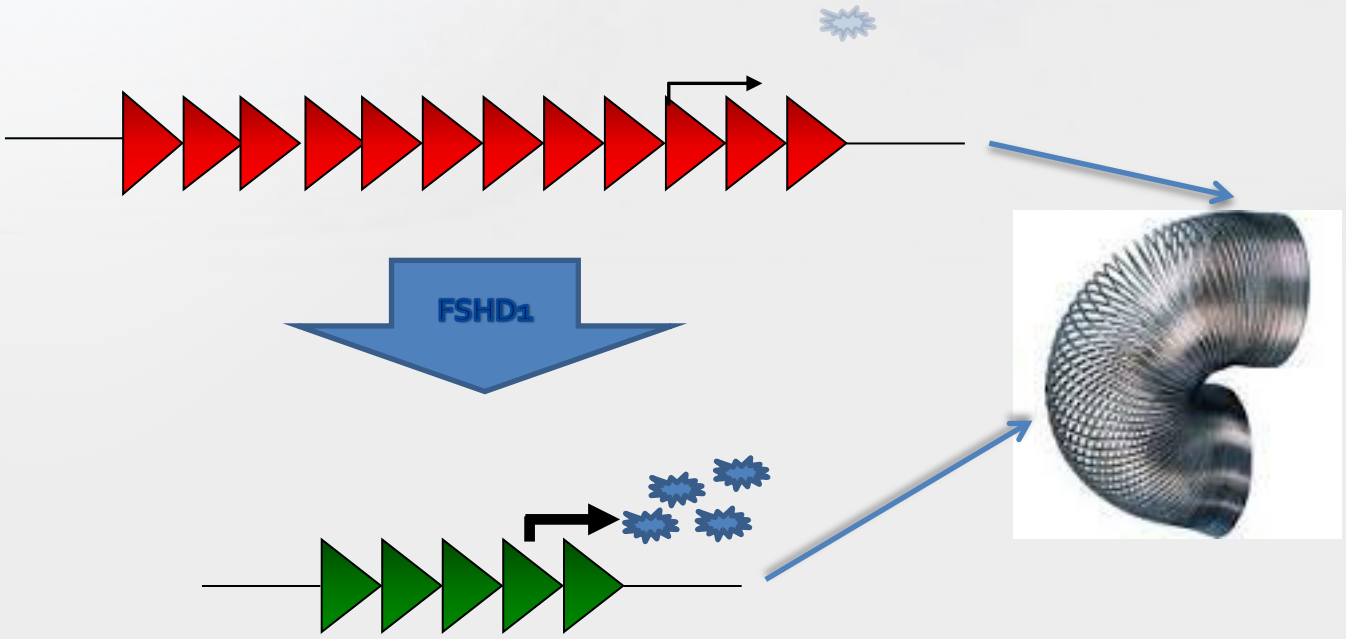


D4Z4, at the heart of FSHD

- Most individuals with FSHD have a contraction of a repeated DNA structure on chromosome 4
- This structure is called D4Z4
- Contraction leads to a change in the 3D organization and regulation of D4Z4
- Some patients have a similar change in 3D structure and regulation of D4Z4 in the absence of contraction (FSHD2)
- These changes lead to the production of a protein called DUX4 which should not be expressed in skeletal muscle.

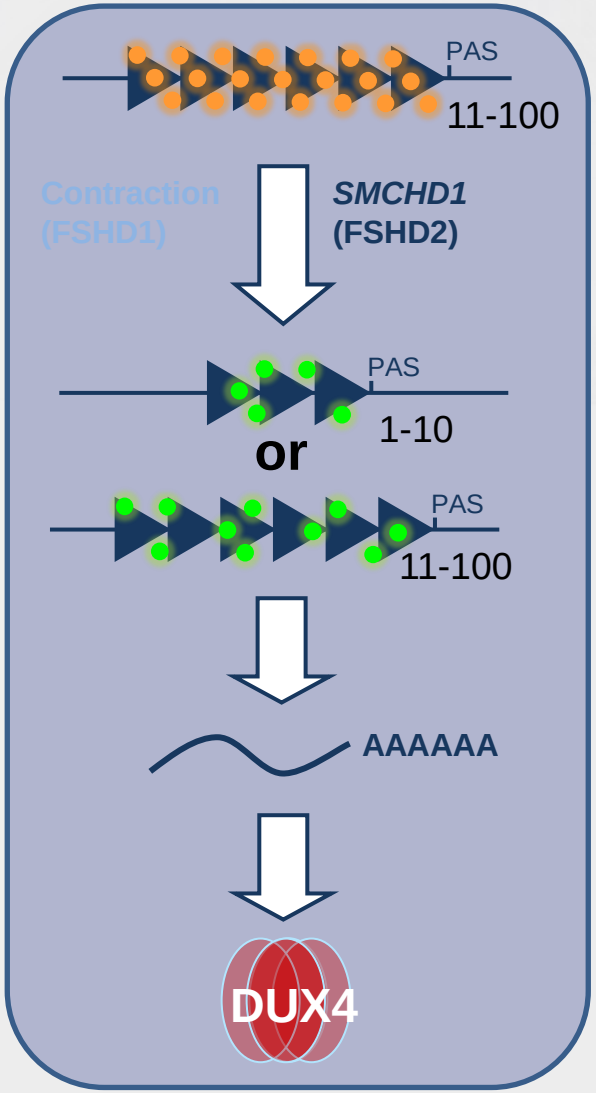


Primary disease mechanism in FSHD1

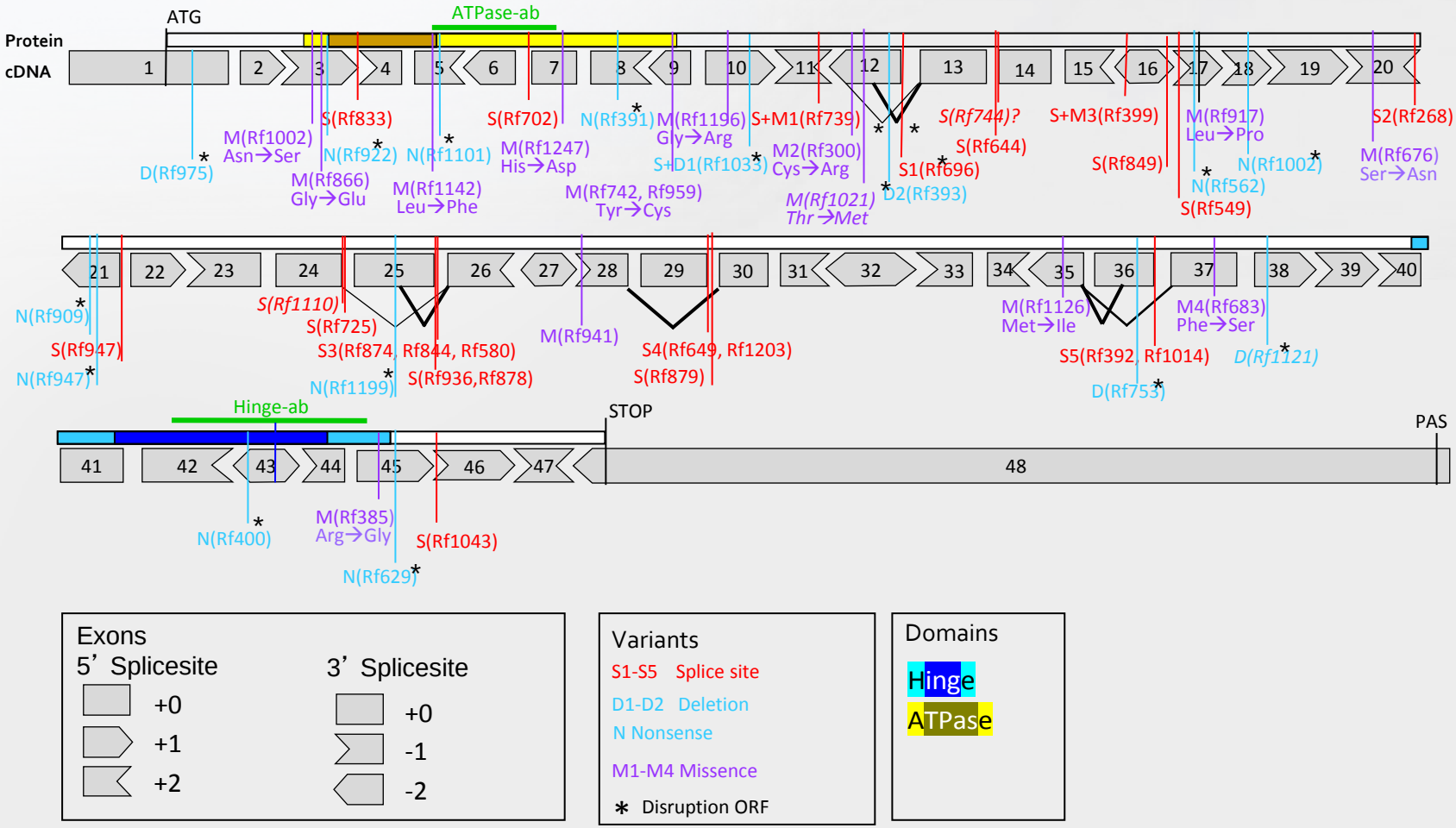


Mutations in *SMCHD1* cause FSHD2

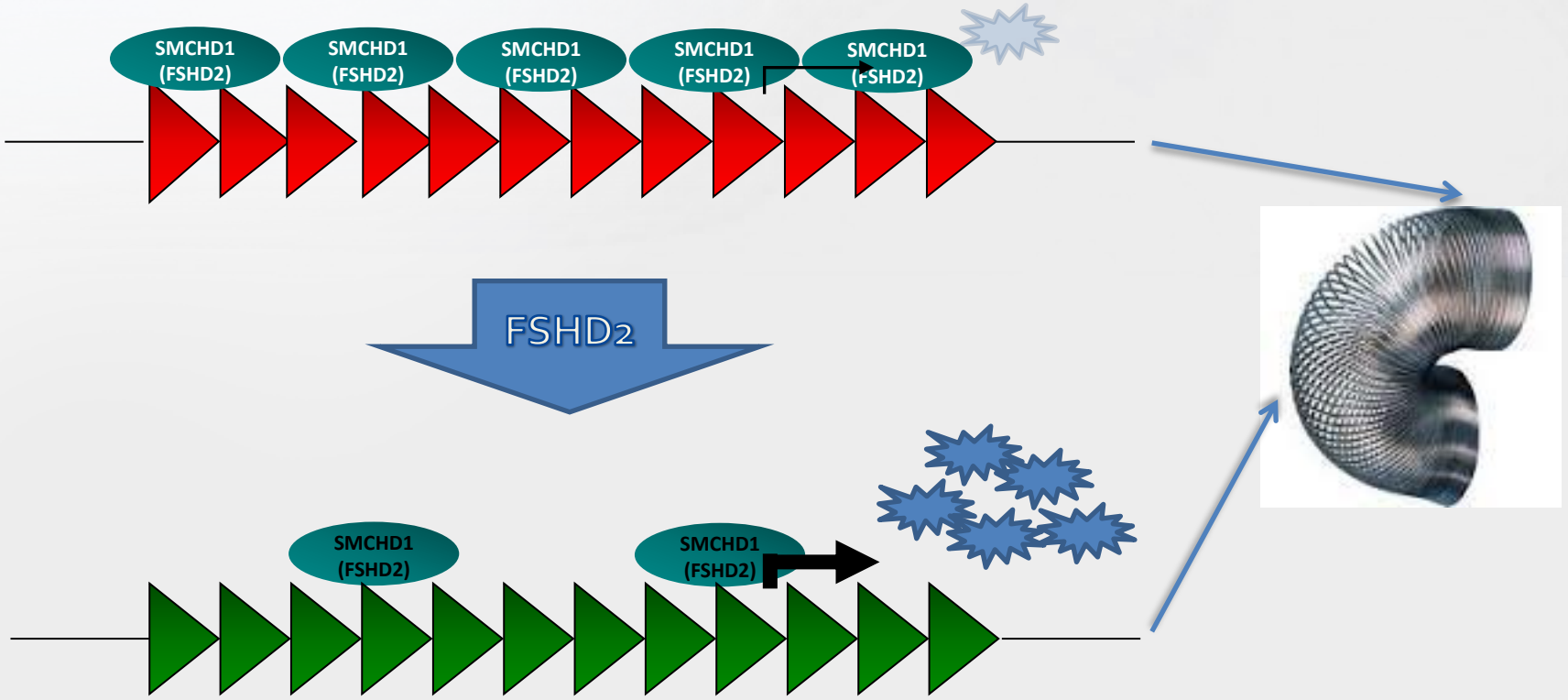
- For a long time the existence of contraction-independent FSHD was questioned
- We showed that changes in 3D chromatin structure of D4Z4 seen in FSHD1 patients can segregate in FSHD2 families
- This led to the identification of mutations in *SMCHD1* underlying 85% of FSHD2



Mutations in *SMCHD1* explain 80% of FSHD2



SMCHD1 binds to D4Z4 and represses DUX4



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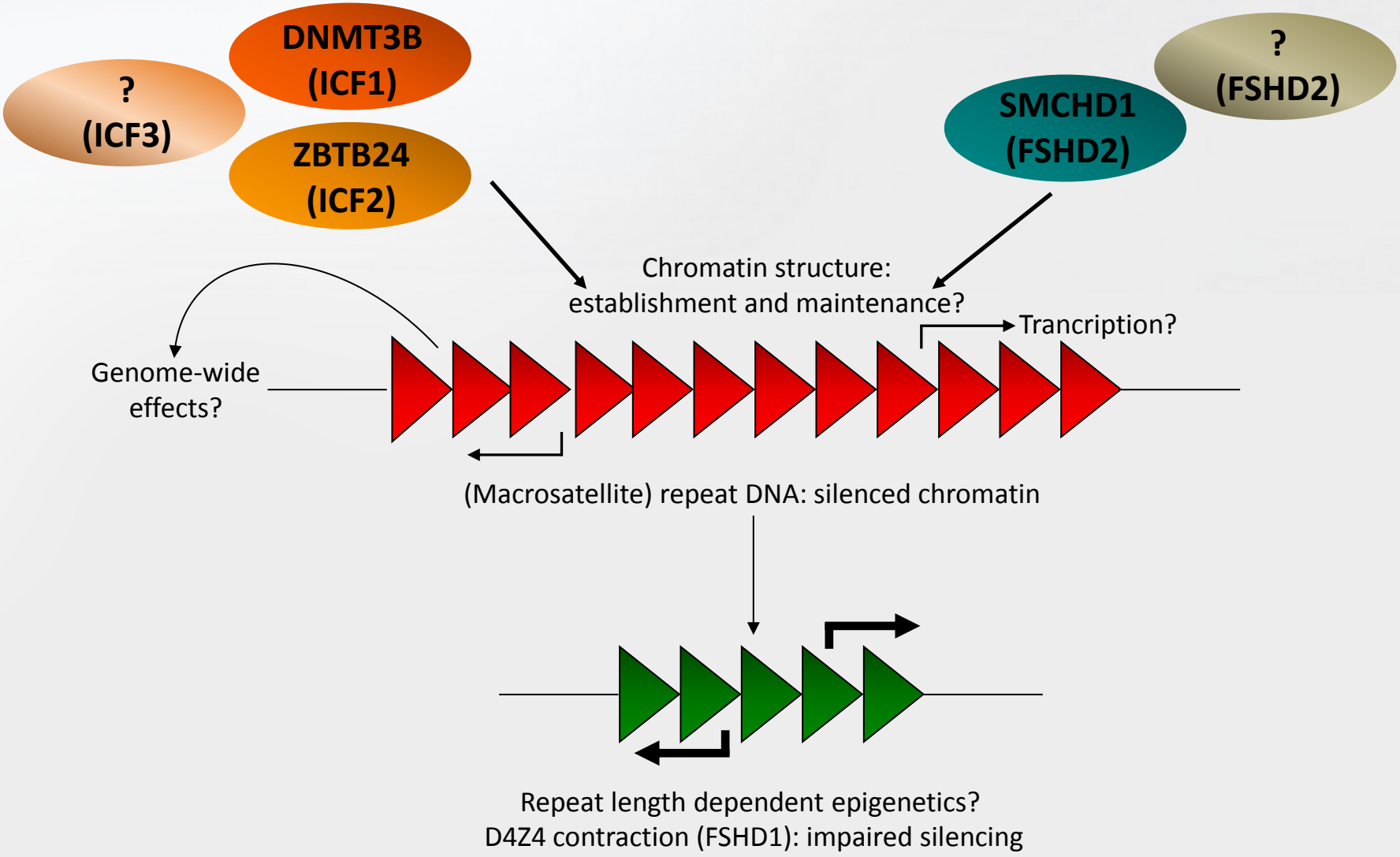
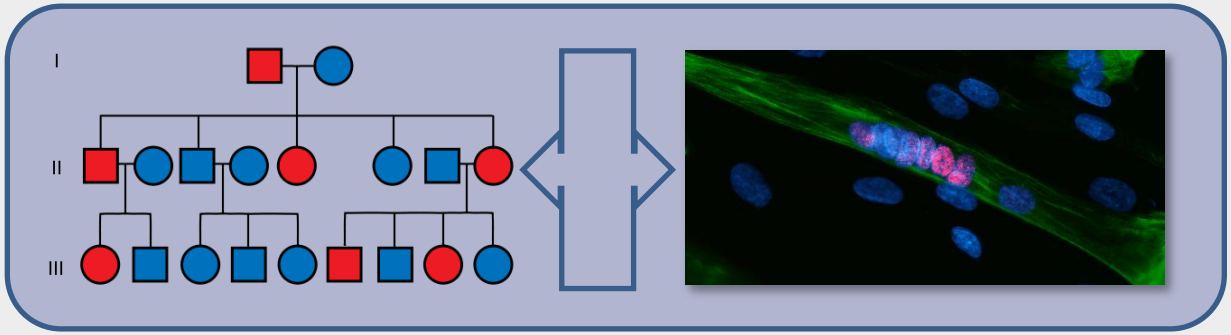
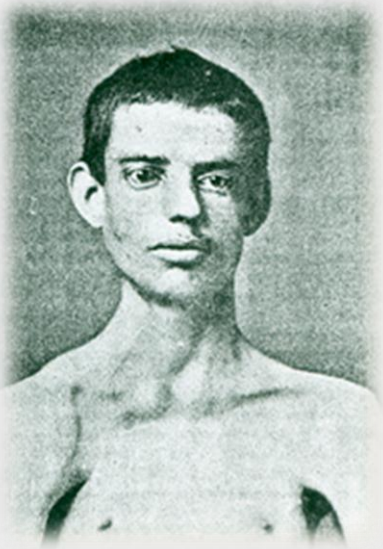


Clinical Variability

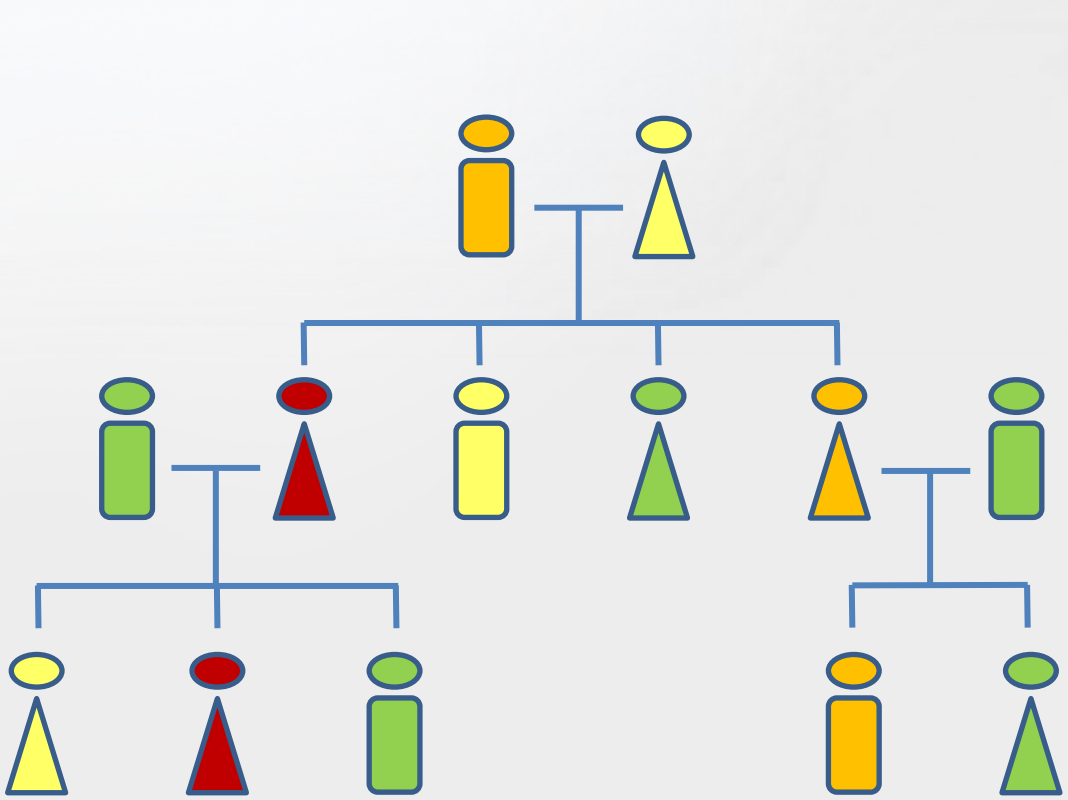
- Large variability in onset, progression and severity;
- Between families and within families;
- What protects gene-carriers from becoming affected?;
  - Environmental factors?
  - *Genetic modifiers of D4Z4?*
  - *Role for SMCHD1?*

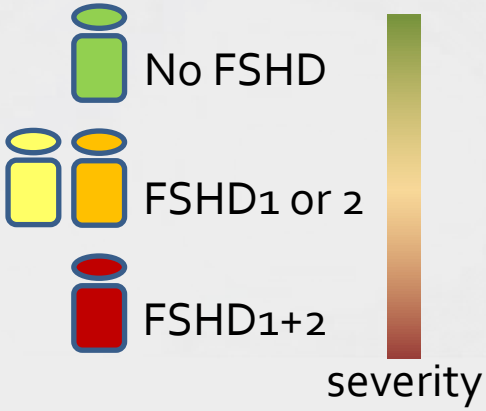




Landouzy and Dejerine

The FSHD2 gene is a modifier for FSHD1

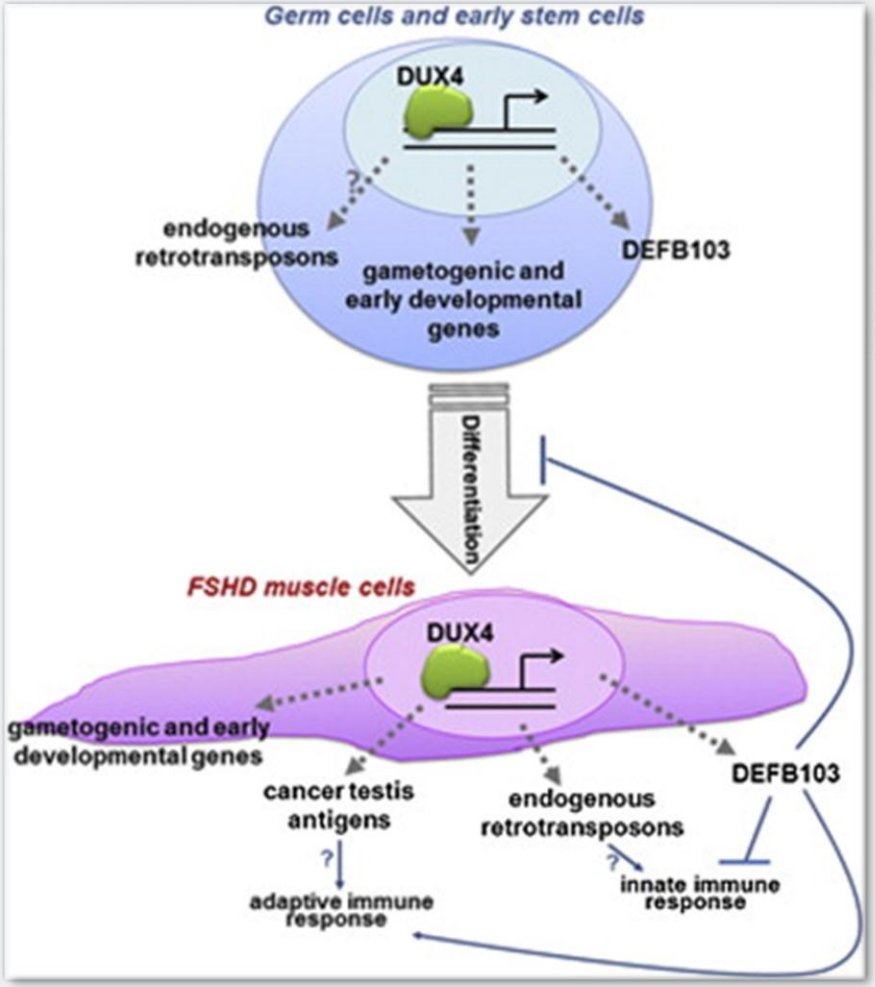




Families with FSHD1 and FSHD2
Sacconi *et al.*, Am J. Hum. Genet. 2013

Consequences of DUX4 in muscle

- DUX4 activates germline and early stem cell programs in skeletal muscle;
- DUX4 induces elements that create an inflammatory reaction to muscle;
- At the same time, DUX4 suppresses some patways of our immune system;
- These pathways and programs lead to muscle atrophy and cell death.

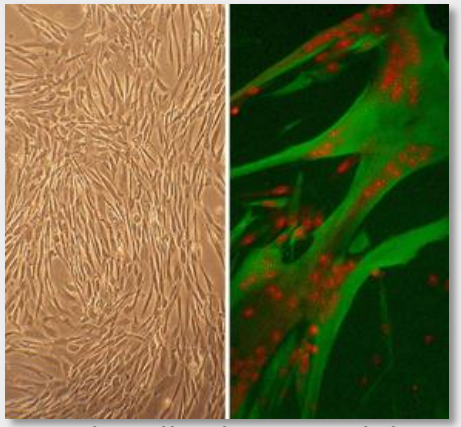


Geng *et al.*, Dev. Cell 2012

What is next?

- Translational research:
  - Increase our understanding of disease mechanism;
  - Translate our findings to models that allow validation of the mechanism;
  - Identify potential targets for therapy;
  - Apply disease models for drug screens;
  - Validate hits from drug screens;
  - Clinical trials

Yeast models

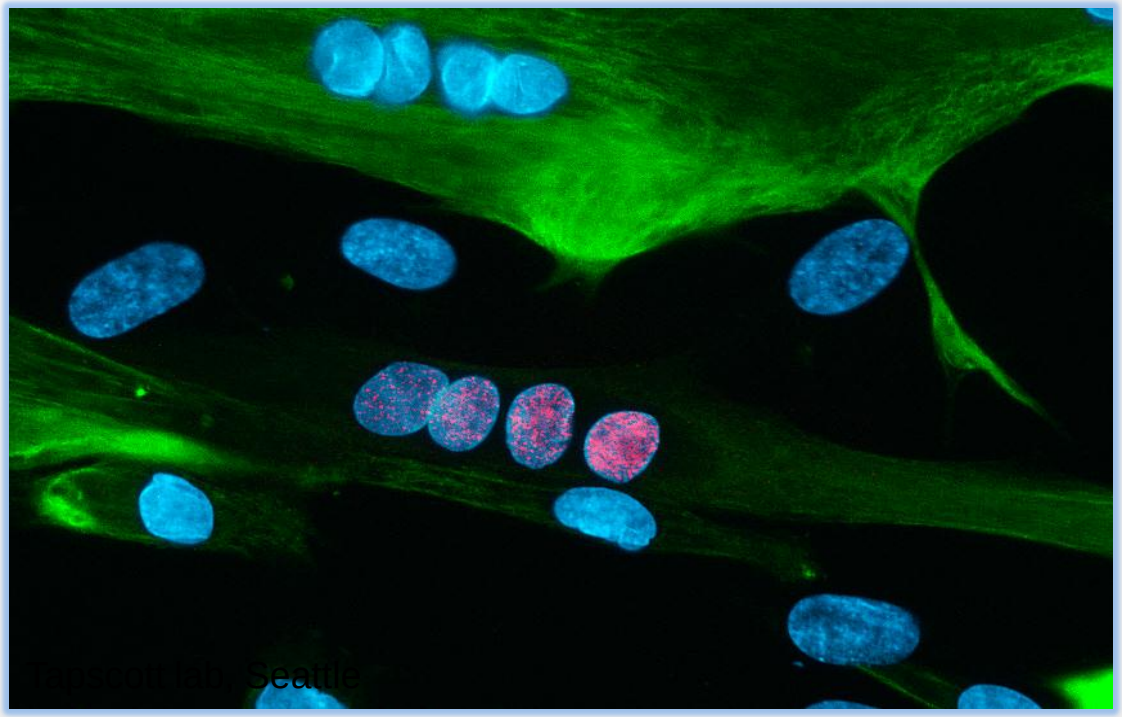


Muscle cell culture models

Mouse models

Disease Models for FSHD

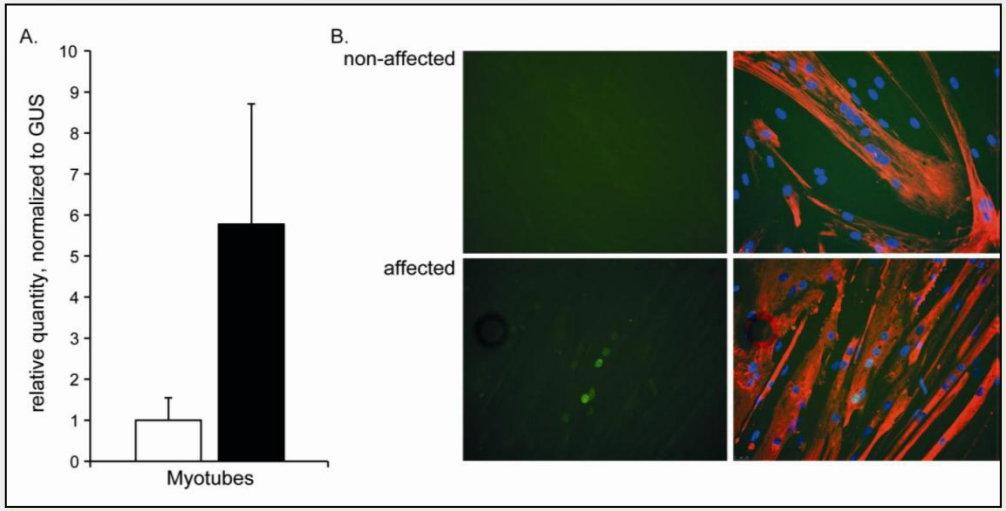
Any disease model for FSHD should take into account the bursts of expression pattern of DUX4



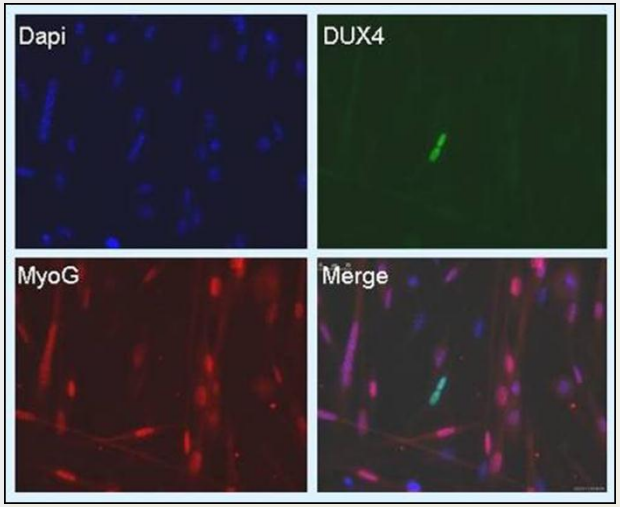
DUX4 at the tipping point

Models for Translational Research

- Cellular and Animal Models:
  - Isogenic myoblast clones with or without mutation (coll. G. Butler-Browne and V. Mouly);
  - Mouse models with normal-sized and FSHD-sized D4Z4 arrays;



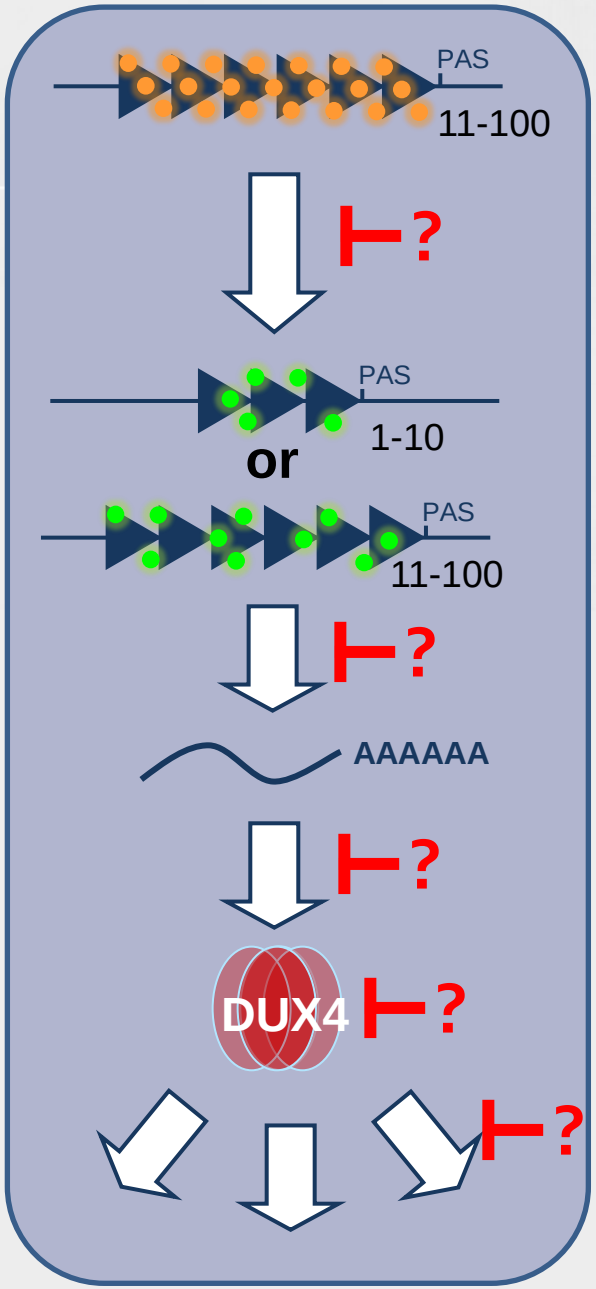
DUX4-positive nuclei in affected clones only
(De Krom *et al.*, Am J. Path. 2012)



DUX4-positive nuclei in FSHD mouse
(De Krom *et al.*, PLoS Genet, in press)

Towards Therapy

- Current knowledge of disease mechanism already gives leads to intervention:
  - Can we prevent the change in 3D structure and regulation of D4Z4?
  - Can we prevent the production of DUX4 at RNA or protein level?
  - Can we prevent the action of DUX4?
  - Can we treat the downstream pathways of DUX4?

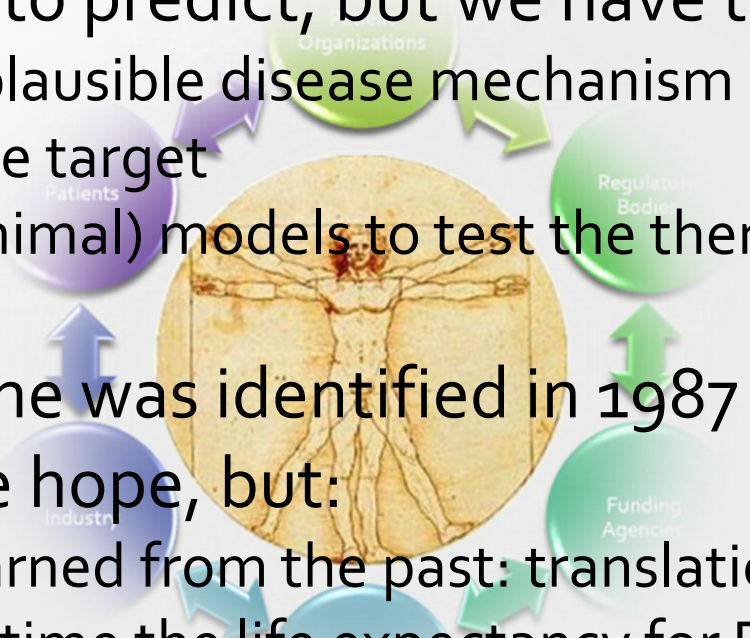


Take home messages

- There are at least two genetic forms of FSHD
  - The common form FSHD1 (1-10 D4Z4 units)
  - The rare form FSHD2 (mostly mutations in SMCHD1)
- Both forms can be genetically confirmed with great accuracy
- Both forms have an identical disease mechanism
  - Expression of DUX4 in skeletal muscle
- Some individuals have FSHD1 and FSHD2
  - Individuals have more variable disease severity
- We have uncovered the mechanistic basis of FSHD

How much longer?

- Not possible to predict, but we have the essentials:
  - We have a plausible disease mechanism
  - We know the target
  - We have (animal) models to test the therapeutic molecules
- The DMD gene was identified in 1987 and only now there is some hope, but:
  - We have learned from the past: translational research
  - In the meantime the life expectancy for DMD has dramatically increased: quality of care



THANK YOU!