University of Massachusetts Medical School

&

SEN. PAUL D. WELLSTONE MD COOPERATIVE RESEARCH CENTER For FSHD Research

# Genetically Similar, Epigenetically Different

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**Principal Investigators**

**The Wellstone Program**
**Departments of Cell and Developmental Biology & Neurology**

# Epigenetics is Increasingly a Focal Area in Human Health and Disease Research

TIME msnbc SEARCH TIME.COM
IN PARTNERSHIP WITH CNN **Health & Science**
Main • Ecocentric Blog • Going Green • Environment • Ocea

## Why Your DNA Isn't Your Destiny

By **JOHN CLOUD** Wednesday, Jan. 06, 2010

THE WALL STREET JOURNAL.

WSJ's blog on health and the business of health.

NOVEMBER 17, 2011, 1:38 PM

## Rats That Get No Kick From Cocaine

Cocaine can change the brain in ways that can be passed on to male offspring making them less likely to find the drug rewarding or work hard to get it.

The New York Times **February 24, 2009**

## The Epigenome: Guiding Cells to Their Specialized Roles

Researchers are finding that a complex layer of proteins and markers called the epigenome controls access to genetic information, allowing each cell to read the genes necessary for cell-specific functions but blocking off most of the rest of the genome.

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**Mind & Brain / Genes & Health**

**The Brain** The Switches That Can Turn Mental Illness On and Off

The difference between one personality and another is not determined by genes alone. Love's got something to do with it too.

**by Carl Zimmer**
**From the June 2010 issue; published online June 16, 2010**


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Epigenomics

# Complexity of an organism


**$3 \times 10^{13}$ Cells/human**

**$2 \times 10^{12}$ Proteins/cell**

**~22,000 Protein-coding genes**
**>90% have alternative splicing**
**>40,000 noncoding RNAs**
**>400,000 regulatory regions**
**1 Genome**

**Each cell requires a specific complement of gene products**

**Expression of necessary genes**
**Repression of unwanted genes**

# Complexity of an organism


**3 x $10^{13}$ Cells/human**

**2 x $10^{12}$ Proteins/cell**

**~22,000 Protein-coding genes**
**>90% have alternative splicing**
**>40,000 noncoding RNAs**
**>400,000 regulatory regions**
**1 Genome + Epigenome =**

**Expression of necessary genes**
**Repression of unwanted genes**

- ⇨ **Organize the nucleus**
- ⇨ **Affect mRNA content**
- ⇨ **Integrate the environment**
- ⇨ **Cellular memory**

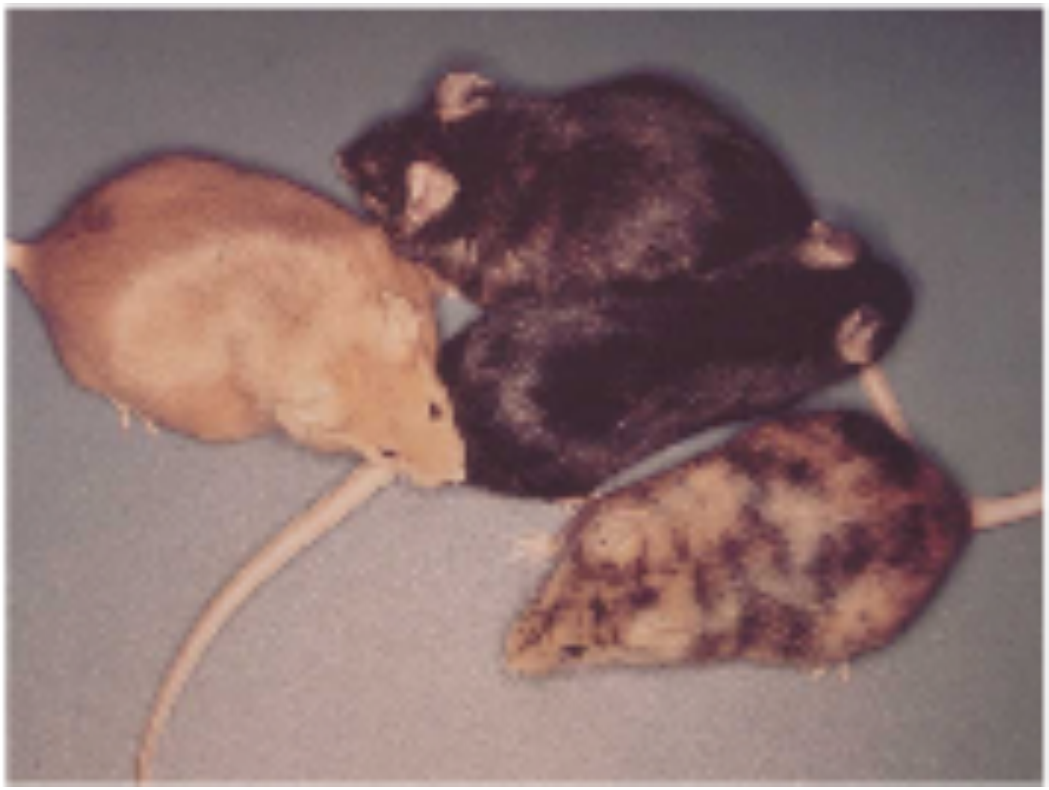
# Epigenetic Gene and Genome Regulation

**"The structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" - Adrian Bird**

**Keys:** DNA sequence independent
Context dependent
Stable/Heritable
Dynamic/reversible
Responsive

**Chromatin: DNA, histones, non-histone proteins, RNA**

# These mice are genetically identical yet epigenetically different

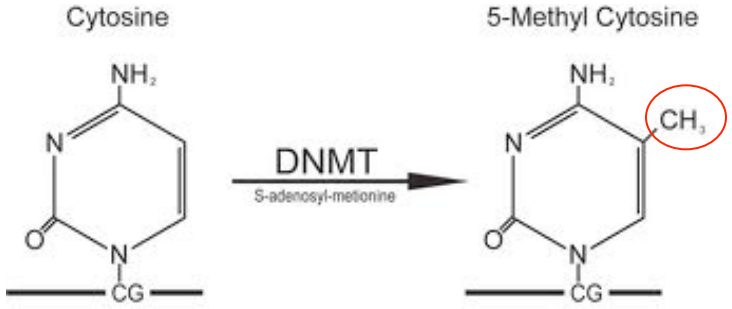


**"Epialleles"**

Waterland and Jirtle (2003) *MCB* 23:5293

# DNA methylation is the classic epigenetic mark

## Human DNA methylation is exclusively on CpGs




**Epialleles**

```
5′-CATATGCGTGCGCAA
   |||||||||||||||
   GTATACGCACGCGTT-5′
```

```
          M     M
5′-CATATGCGTGCGCAA
   |||||||||||||||
   GTATACGCACGCGTT-5′
          M     M
```

**CpG is symmetrical**

## Art Riggs (1975) CpG methylation as a mechanism for memory

5’ -C$^{m}$pG
Gp$^{m}$C-5’

DNA replication

5’ -C$^{m}$pG
G p C-5’

Methylation

5’ -C$^{m}$pG
Gp$^{m}$C-5’

5’ -C pG
Gp$^{m}$C-5’

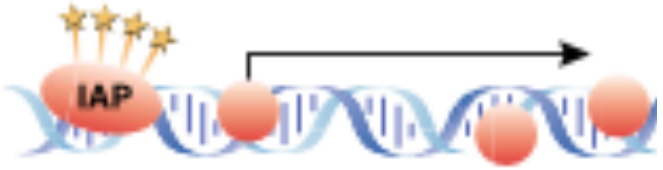
Methylation

5’ -C$^{m}$pG
Gp$^{m}$C-5’

# Epigenetic differences can have profound long-term health consequences

Epialleles


**$A^{IAP}$ allele, methylated**




**Brown, normal**

**$A^{IAP}$ allele, unmethylated**


**Yellow, obese, spontaneous tumors**

**Genetically identical**
**Epigenetically different**

→ **Affects long-term health**
**→ heritable?**

Waterland and Jirtle, *Mol Cell Biol*, 2003

# Epigenetics: Nurture (vs. Nature)

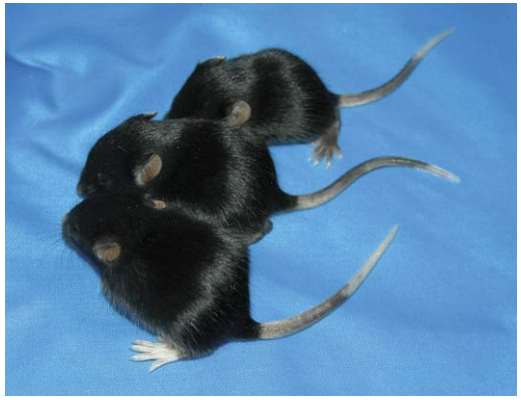
***Heritable changes in gene activity that do not involve alterations to the genetic code***

**Nature: Genetics**

**Passed on by Mom and Dad**

**Affected by:**

- **Mutagens**
- **Mistakes**



***Genetically identical***

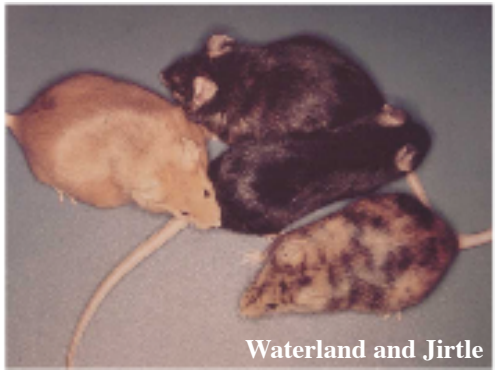
*vs.*

**Nurture: Epigenetics**

**Passed on by Mom and Dad**

**Affected by:**

- **Stress**
- **Sleep**
- **Diet**
- **Prenatal care**
- **Environment**



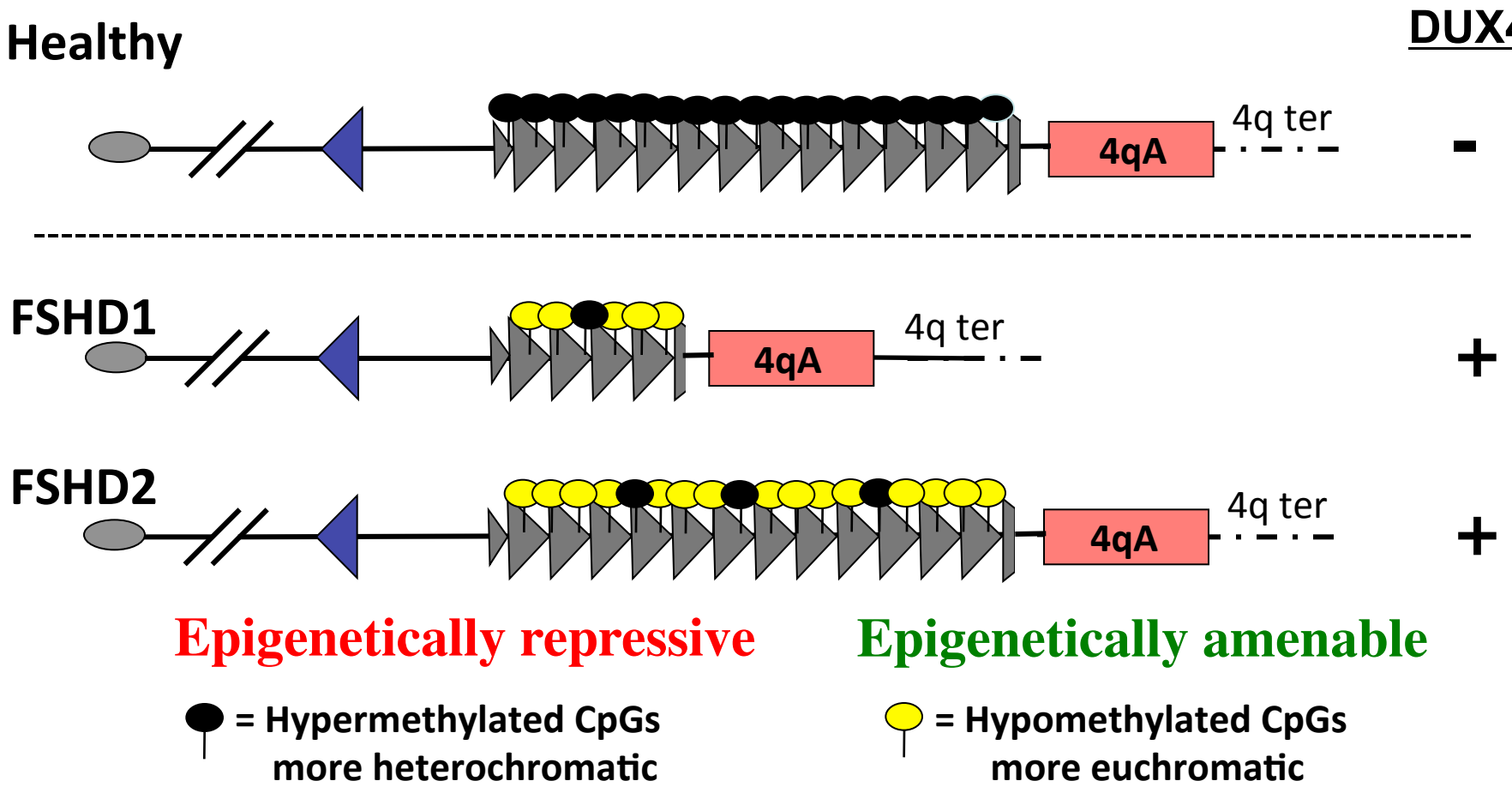

***Genetically identical, epigenetically different***

**Epigenetic differences in genetically ~ equivalent people (e.g., identical twins) can have profound effects**

# Epigenetic Diseases

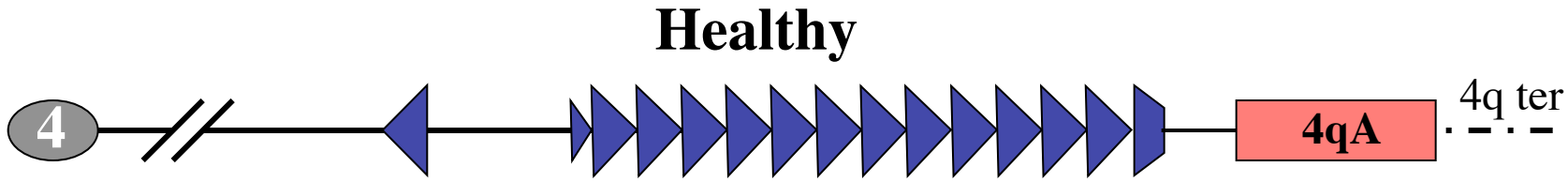
| Disease | Epigenetics | Manifestation |
|---|---|---|
| FSHD | Chromatin structure | Progressive skeletal muscle loss |
| Rett Syndrome | MeCP2 | Intellectual disabilities |
| ATR-X | Snf2 remodeling | Intellectual disabilities, α-thalassaemia |
| Fragile X Syndrome | DNA methylation | Intellectual disabilities |
| ICF Syndrome | DNA methylation | Immunodeficiency |
| Angelman's Syndrome | LOI | Intellectual disabilities |
| Prader-Willi Syndrome | LOI | Obesity, intellectual disabilities |
| Beckwith-Wiedemann | LOI | Organ overgrowth |
| Leukemia | DNA methylation | Disrupted haematopoiesis |
| Lupus | DNA methylation | Chronic inflammation in joints, skin |
| Cancer | DNA methylation | Uncontrolled cell cycle |
| Rubinstein-Taybi | CBP (HAT) | Intellectual disabilities |
| Multiple sclerosis | HDAC? | Autoimmune CNS degeneration |
| Spinal muscular atrophy | HDAC? | Motor neuron disease |
| Osteoarthritis | DNA methylation? | Destruction of articular cartilage ECM |
| Obesity | DNA methylation | |
| Diabetes | DNA methylation | |
| Bipolar Disorder | DNA methylation | |

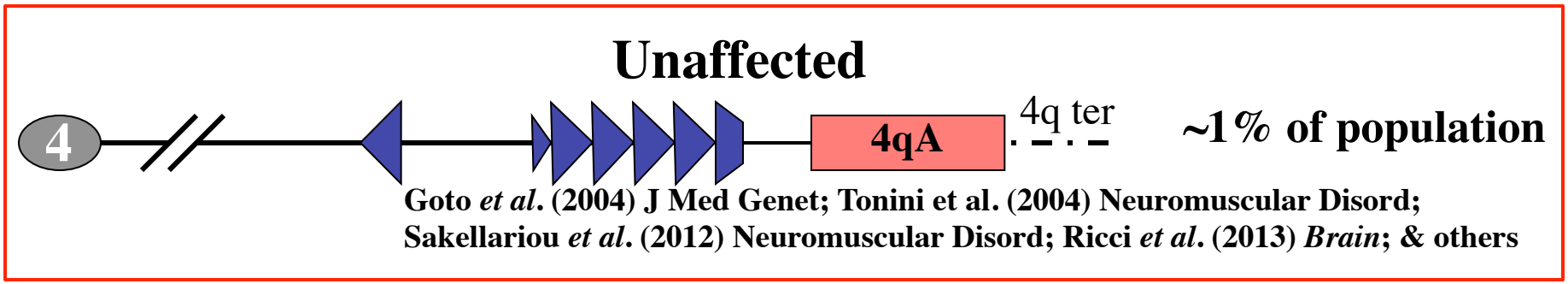
# FSHD is linked to D4Z4, the A type subtelomere and the epigenetic status of the 4q35 D4Z4 repeat

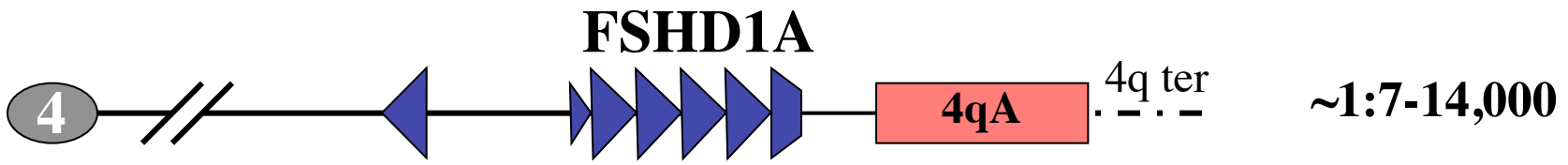



van Overveld *et al*. (2003) *Nat Genet*; De Greef *et al*. (2007) *Neurology*; De Greef *et al*. (2009) *Hum Mutat*

# A putatively pathogenic FSHD1 deletion shows very low penetrance



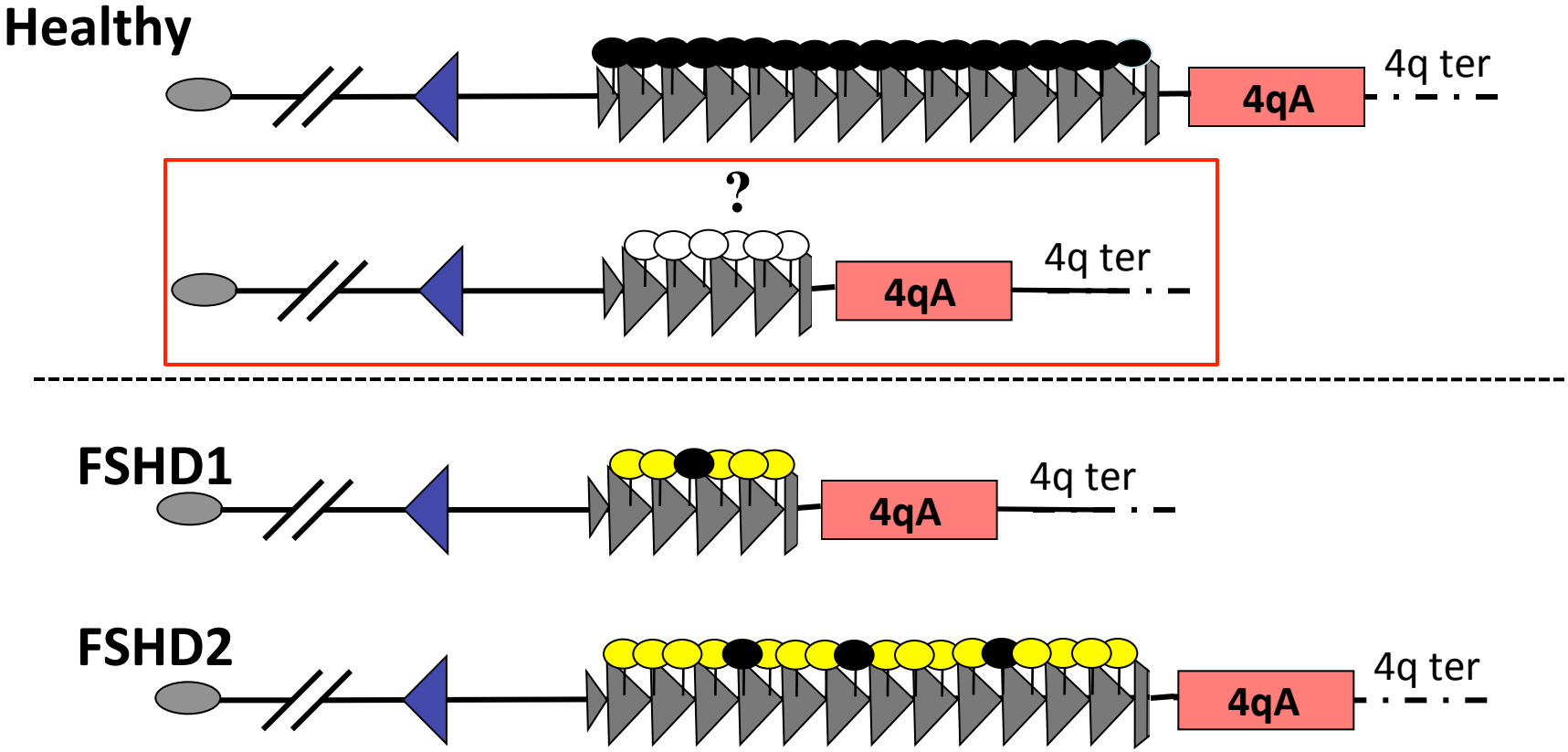




**The deletion itself is not pathogenic**

**The 4qA sub-telomere is not pathogenic**

**The genetics are permissive, not pathogenic**

# FSHD is linked to the A type subtelomere and the epigenetic status of the 4q35 D4Z4 repeat



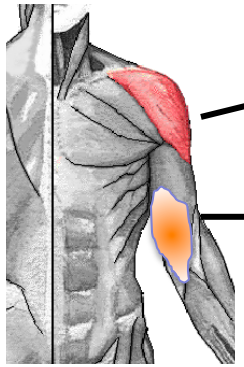

**Epigenetically repressive**

= Hypermethylated CpGs
more heterochromatic

**Epigenetically amenable**

= Hypomethylated CpGs
more euchromatic

# Wellstone family cohorts of muscle biopsies and myogenic cell cultures from FSHD1-affected and 1st degree relatives



**Deltoid – Expect less pathology**

**Biceps – Expect more pathology**

**Subjects screened for FSHD clinically and genetically (Blood)**

Genotyping (Iowa Wellstone)

**Biopsies (KKI-JHU)** (Wagner)

Animal models (Emerson, Wagner & Kunkel)

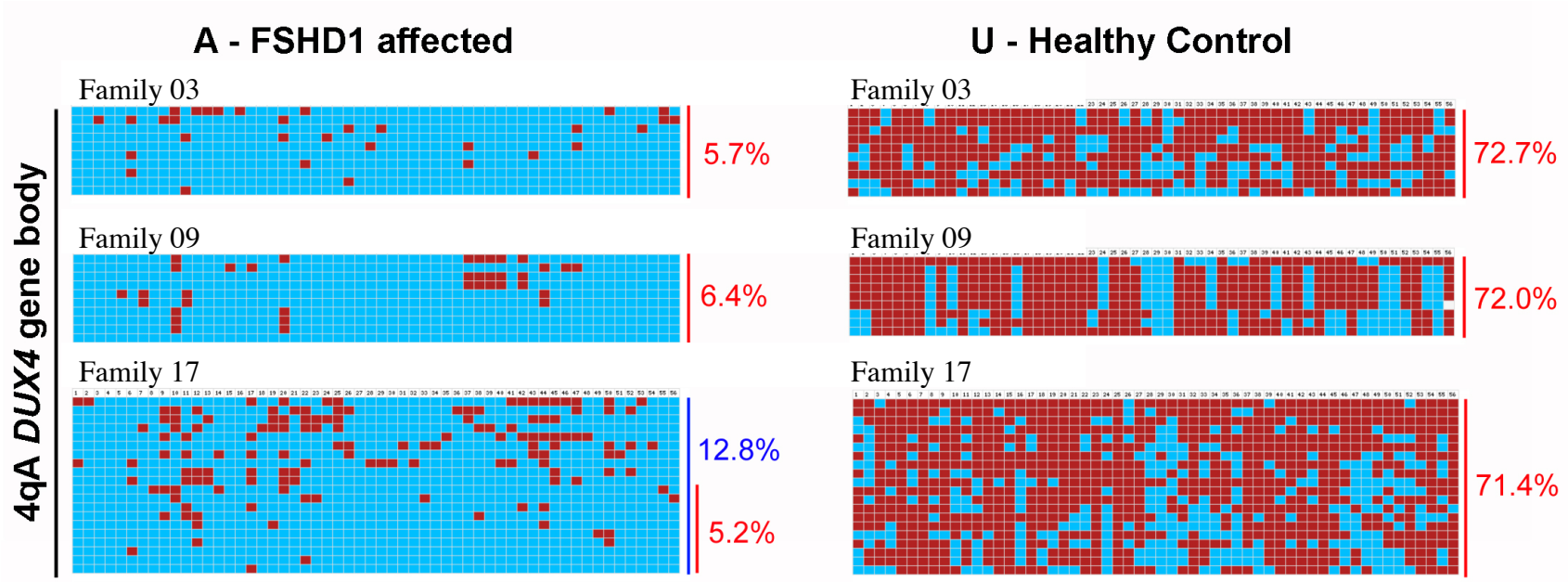
Cell Culture (UMMS)

mRNA & protein analysis (Emerson, King & Kunkel)

**Epigenetic analysis (Jones and Jones)**

Genetic analysis (Emerson, King, Kunkel, Wagner & Tupler)

# The pathogenic *DUX4* gene is epigenetically "ON" in FSHD1 affected subjects




**Epigenetically amenable**

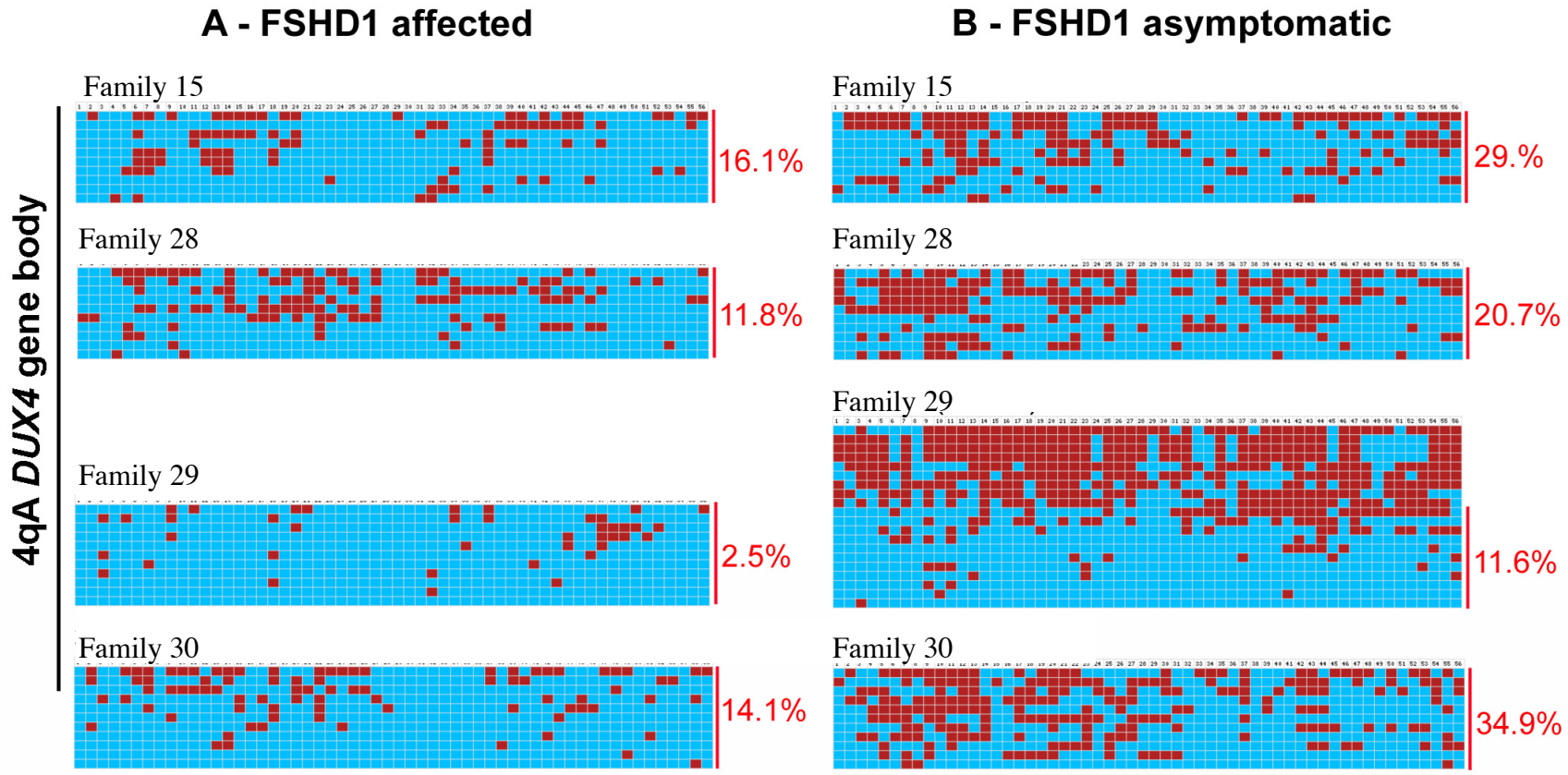
**Epigenetically repressive**

**Unmethylated CpG**

**Methylated CpG**

T. Jones *et al*. (2014)

# FSHD1-asymptomatic subjects are epigenetically more OFF than FSHD1-affected subjects




**Epigenetically amenable**

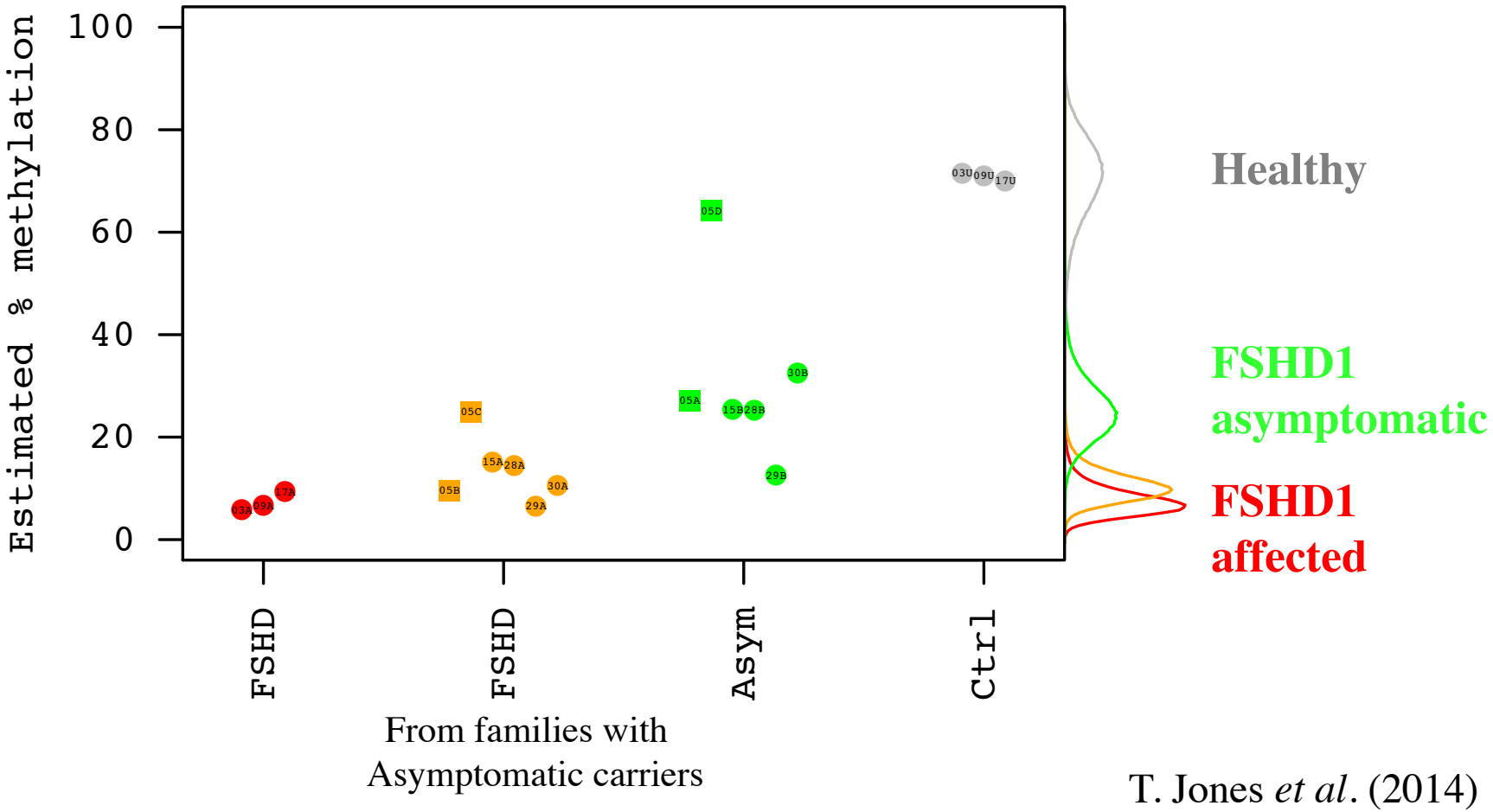
**Epigenetically repressive**

**Unmethylated CpG**

**Methylated CpG**

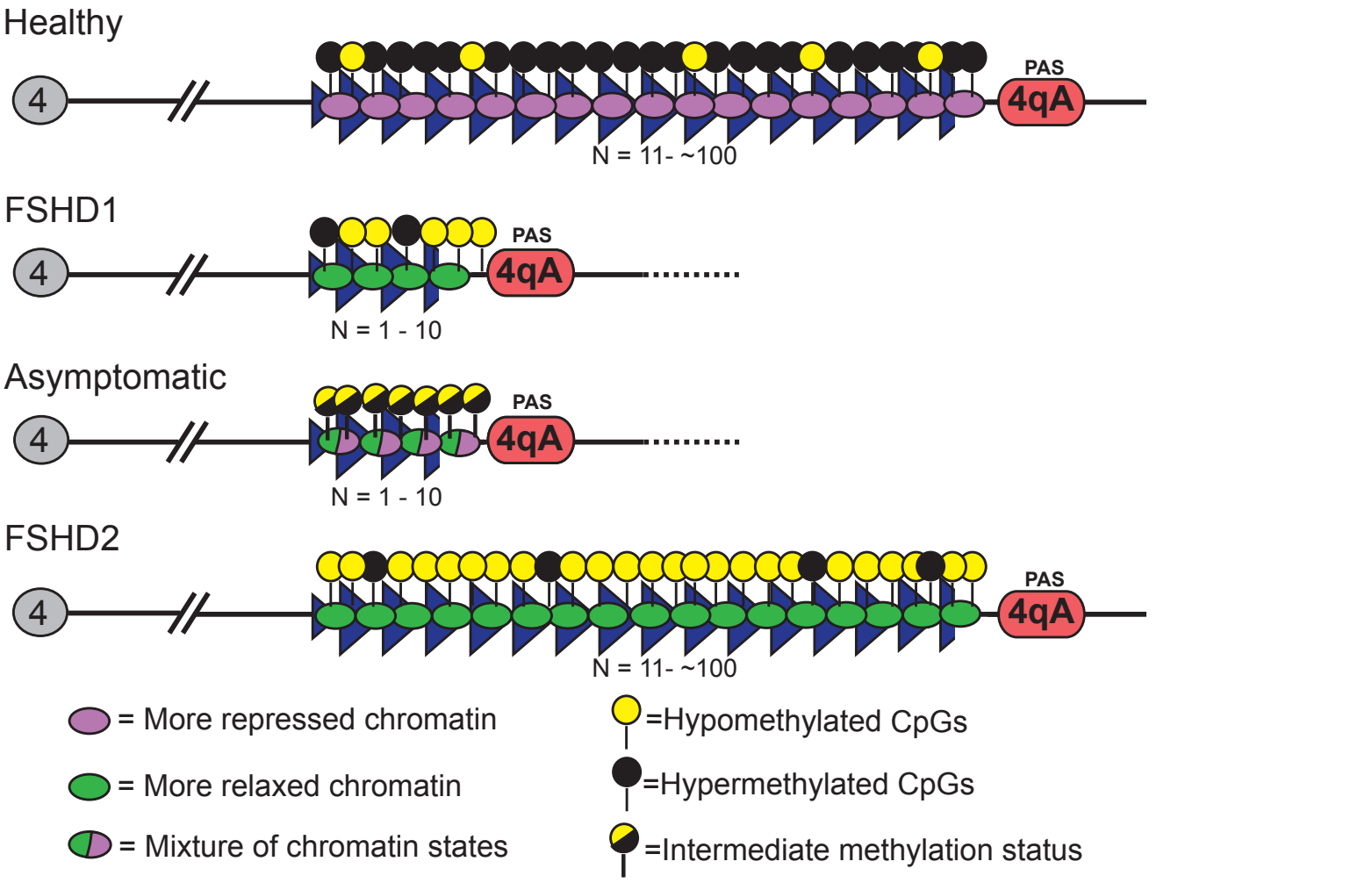
T. Jones *et al*. (2014)

# DNA methylation profiles of asymptomatic subjects show intermediate levels of DNA methylation




T. Jones *et al*. (2014)

# The overall chromatin state of asymptomatic subjects is more epigenetically stable and refractory to gene expression




Himeda *et al*. (2014) *Antiox Redox Signaling*

# FSHD is an epigenetic disease

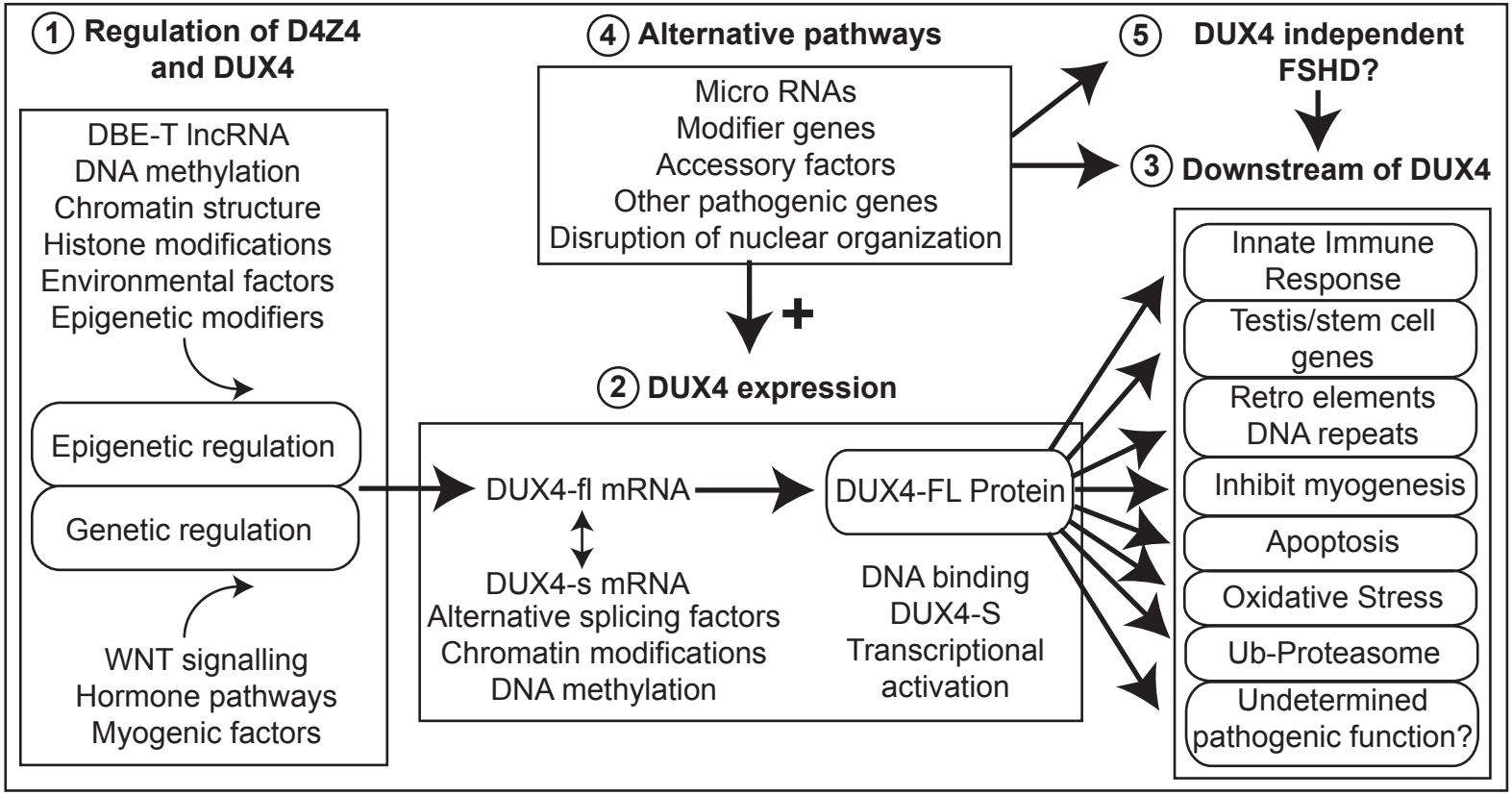
**What are the implications for therapeutic development?**

# Epigenetic Gene and Genome Regulation

**"The structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" - Adrian Bird**

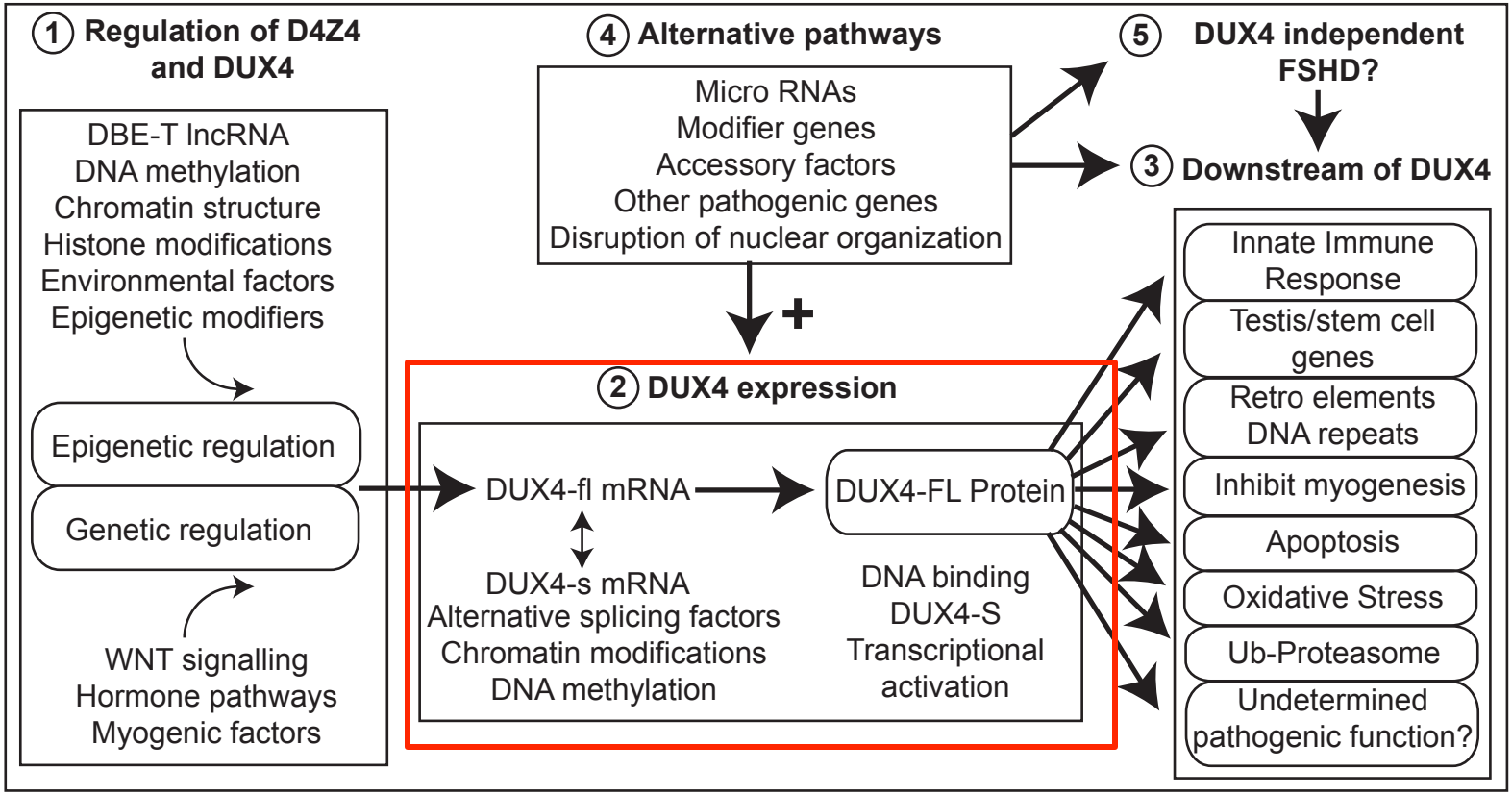
**Keys:** DNA sequence independent
Context dependent
Stable/Heritable
Dynamic/reversible → Druggable
Responsive

# Numerous therapeutic targets for FSHD



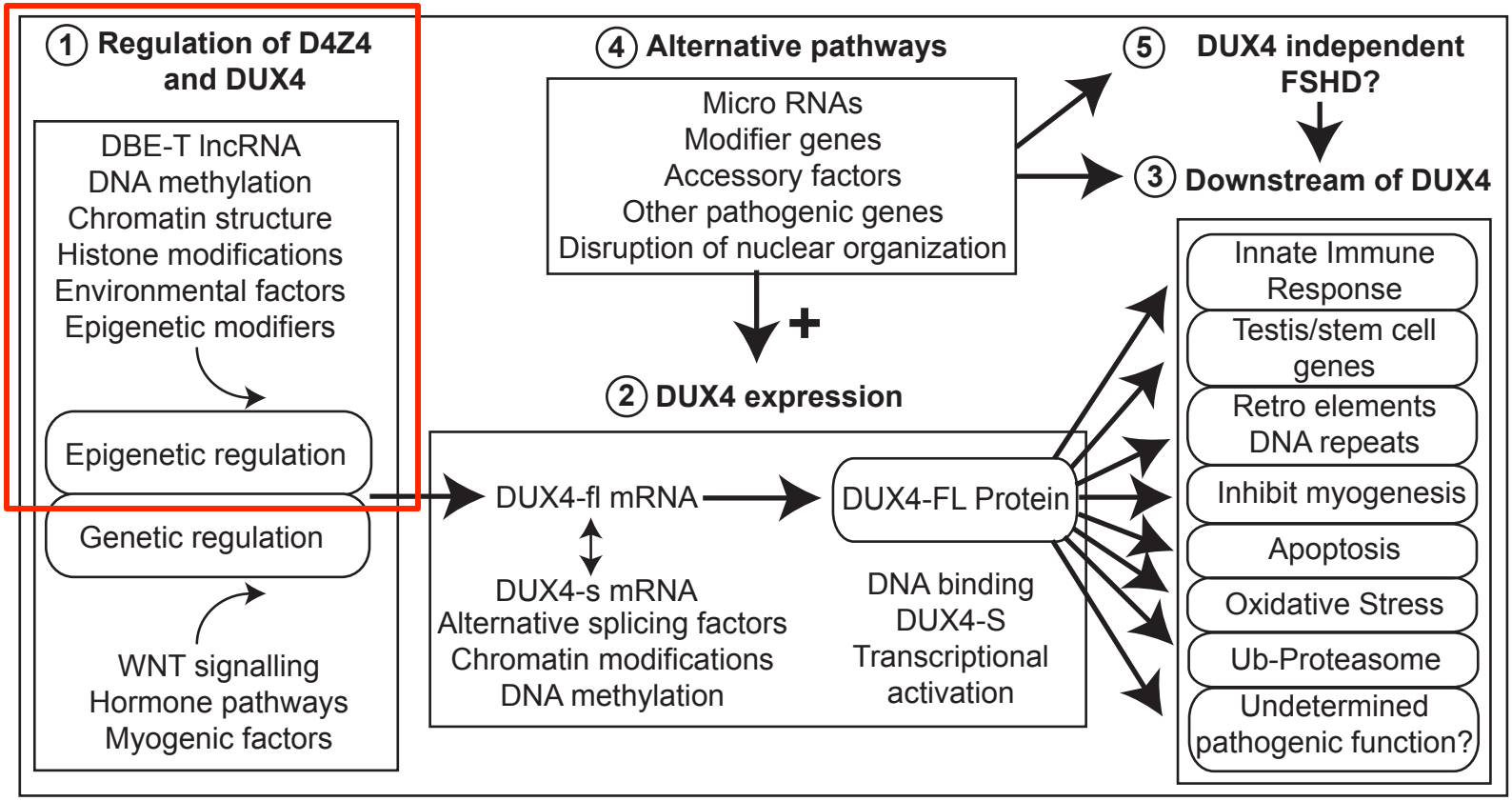

Himeda *et al*. (2014) *Antiox Redox Signaling*

# Antisense targeting of the cytoplasmic *DUX4* mRNA; blocking DUX4 protein function




Himeda *et al*. (2014) *Antiox Redox Signaling*

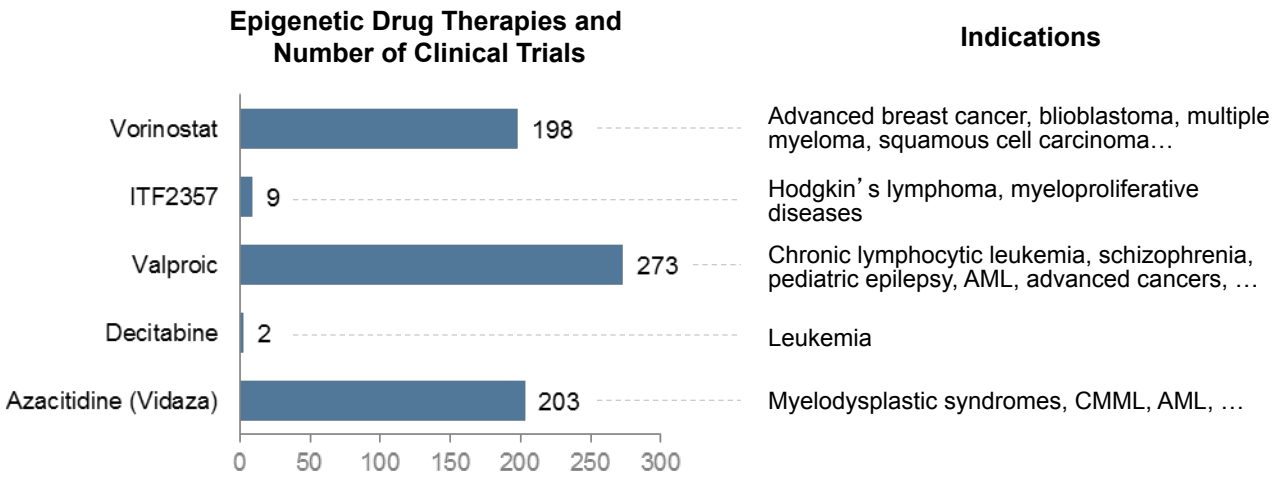
# Epigenetic regulation of *D4Z4/DUX4* is a viable therapeutic target for FSHD




Himeda *et al*. (2014) *Antiox Redox Signaling*

# Drug Therapies Can Also Target Epigenetics to Treat Disease Directly and Specifically

**Clinical Trials**




**Pre-Clinical Development**

*"Almost every big pharmaceutical company has a robust program in epigenetics. It's quite a change in the past few years."*

Yang Shi - JNCI, 2012

- New classes of epigenetic drugs target individuals with specific genetic defects in their tumors; toxicity is minimized
  - Targeting: HMTs (EZH2, LSD1, IDH1&2)
- Large area of drug development
  - Epizyme, Inc., Constellation Pharmaceuticals, EpiTherapeutics, Agios Therapeutics, GSK, AstraZeneca, Novartis, among others
- Challenge: Identify subset of patients that can benefit from the treatments

# Summary

- **Epigenetic regulation is context dependent, sequence independent; stable, heritable yet reversible**
- **FSHD is an epigenetic disease**
  **Overall epigenetic status of the D4Z4 correlates with clinical FSHD**
- **Small but significant epigenetic differences between being asymptomatic and FSHD-affected**
- **Epigenetic status of the D4Z4 is a viable FSHD therapeutic target**
- **Many drugs targeting epigenetic modifications are being developed for many diseases and may be applicable to FSHD**

# Individual epigenetic status of the FSHD-associated D4Z4 macrosatellite correlates with disease


**At University of Massachusetts Medical School (and formerly BBRI)**

Takako Jones, Charis Himeda, Chi Yan, Jennifer Chen, Oliver King, Charles P. Emerson Jr., and Peter L. Jones

**At Kennedy Krieger Institute and Johns Hopkins University**

Kathryn R. Wagner

**At Boston University School of Medicine**

Sachiko Homma, Mary Lou Beerman and Jeffrey Boone Miller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Eunice Kennedy Shriver NICHD National Institute of Child Health & Human Development

NIAMS National Institute of Arthritis and Musculoskeletal and Skin Diseases

University of Massachusetts Medical School

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FSH SOCIETY FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

AFM Association Française contre les Myopathies

MDA Muscular Dystrophy Association Fighting Muscle Disease