Introduction to FSHD Diagnostics (Pt 1)

A proper diagnosis for any genetic disease should involve a combination of clinical evaluation, family history, genetic analysis and genetic counseling.

FSHD is a complicated disease.
It can be difficult to diagnose both clinically and genetically.

Clinical evaluations should only be performed by qualified medical professionals, preferably a neurologist, when available.

Genetic counseling should be considered.
https://www.nsgc.org/page/find-a-genetic-counselor

Important for FSHD (covered in this section):

Clinical-grade genetic testing vs research genetic testing

Types of genetic testing:

FSHD1 deletion testing*
- **Pulsed-field gel electrophoresis (PFGE) and Southern blot**
- **Molecular combing**
- **Optical mapping**
- **Single molecule long-read sequencing**

FSHD2 testing**
- **Candidate gene sequencing**
- **Restriction enzyme DNA methylation analysis**

Epigenetic testing**
- **Combined FSHD1 and FSHD2, based on bisulfite PCR**

* Requires high molecular weight genomic DNA, and thus typically a blood draw.
** Can be performed on any source of genomic DNA, including saliva.

Introduction to FSHD Diagnostics (Pt 2)

Clinical testing vs Research testing

In the USA, all genetic testing in which the results will be used for clinical purposes (i.e., to make decisions about one's healthcare or for family planning) must be performed in a CLIA-certified laboratory.

Clinically relevant genetic testing.

CLIA is the acronym for the Clinical Laboratory Improvement Amendments of 1988. This law requires any facility performing examinations of human specimens (e.g., tissue, blood, urine, saliva, etc.) for diagnosis, prevention, or treatment purposes to be certified by the Secretary of the Department of Health and Human Services.

The purpose for CLIA regulations is to ensure the accuracy of the clinical testing.

The reason CLIA-certification is important is that the results of the clinical testing have significant impact on the life and health of the person being tested.

However, CLIA-certification does not address the relevance of the testing to the particular indication. For example, a CLIA-certified test for CEA (a colorectal cancer marker) only guarantees that the reported number is correct, not whether or not the level found is indicative of colon cancer. CLIA regulates the testing, not the interpretation.

Some tests are considered by the CDC or FDA to be so simple that there is little risk of error and thus the CLIA requirements are waived. These tests have a certificate of waiver (COW) CLIA status and include some glucose tests and pregnancy tests.

Genetic testing does not have COW status and must be performed in a CLIA-certified lab.

CMS.gov
Centers for Medicare & Medicaid Services

The Centers for Medicare & Medicaid Services (CMS) regulate all laboratory testing (except research testing) performed on humans in the U.S. through CLIA.

For more information, see:
https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA

Introduction to FSHD Diagnostics (Pt 3)

Clinical testing vs Research testing

In the USA, all genetic testing in which the results will be used for clinical purposes (i.e., to make decisions about one's healthcare or for family planning) must be performed in a CLIA-certified laboratory.

Research genetic testing.

Research genetic testing is typically performed in a research laboratory by research staff as part of a research project, and thus, it is not required to be performed under the same conditions found in a CLIA-certified laboratory.

However, this does not imply the accuracy of research testing is lower; in fact, research testing may be more accurate.

Remember, researchers have an intellectual interest in the accuracy of the results and are likely experts in the particular test procedure being performed and the specific disease condition being assayed. It is not being performed as a for-fee service; the value to the researchers is in the data obtained and the help they have provided.

Regardless, the results of research testing should not be used for making healthcare-related decisions, and they will not get you into a clinical trial.

FSHD research genetic testing.

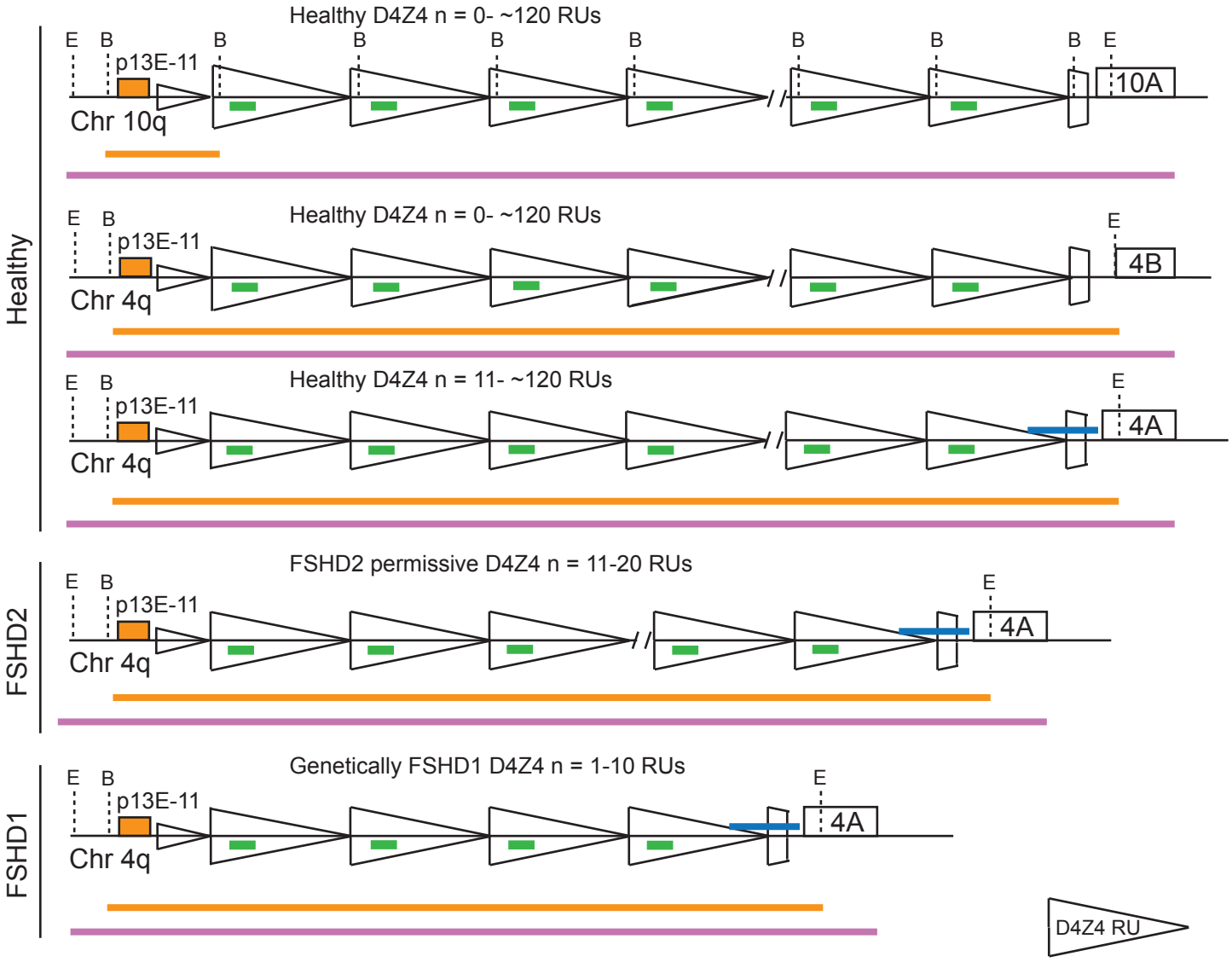
Genetic testing for FSHD started as research testing at Leiden University in The Netherlands, where the FSHD1 mutation was first discovered and characterized.

Traditionally, the FSHD field (including patients, clinicians, and researchers) has relied heavily on research genetic testing since the procedure is complicated, expensive, and requires specialized equipment.

Traditional research testing in FSHD has proven to be extremely reliable and accurate. Today, many people in the FSHD community still have only a research test result confirming their status.

Introduction to FSHD Diagnostics (Pt 4)

Comparison of FSHD diagnostic techniques: specificity and detection.



FSHD1 deletion analysis by PFGE and Southern blotting

- p13E-11 is the DNA sequence used to probe Southern blots specifically for the 4q and 10q fragments.
- The EcoRI/BlnI cut DNA fragment specific for the chromosome 4q35 D4Z4 array.
- E = EcoRI restriction enzyme cut sites.
- B = BlnI restriction enzyme cut sites.

Epigenetic analysis

- BSSA assay analyzes the distal most D4Z4 RU when it is 4qA.
- BSSX assay analyzes all 4q and 10q D4Z4 RUs regardless of haplotype.

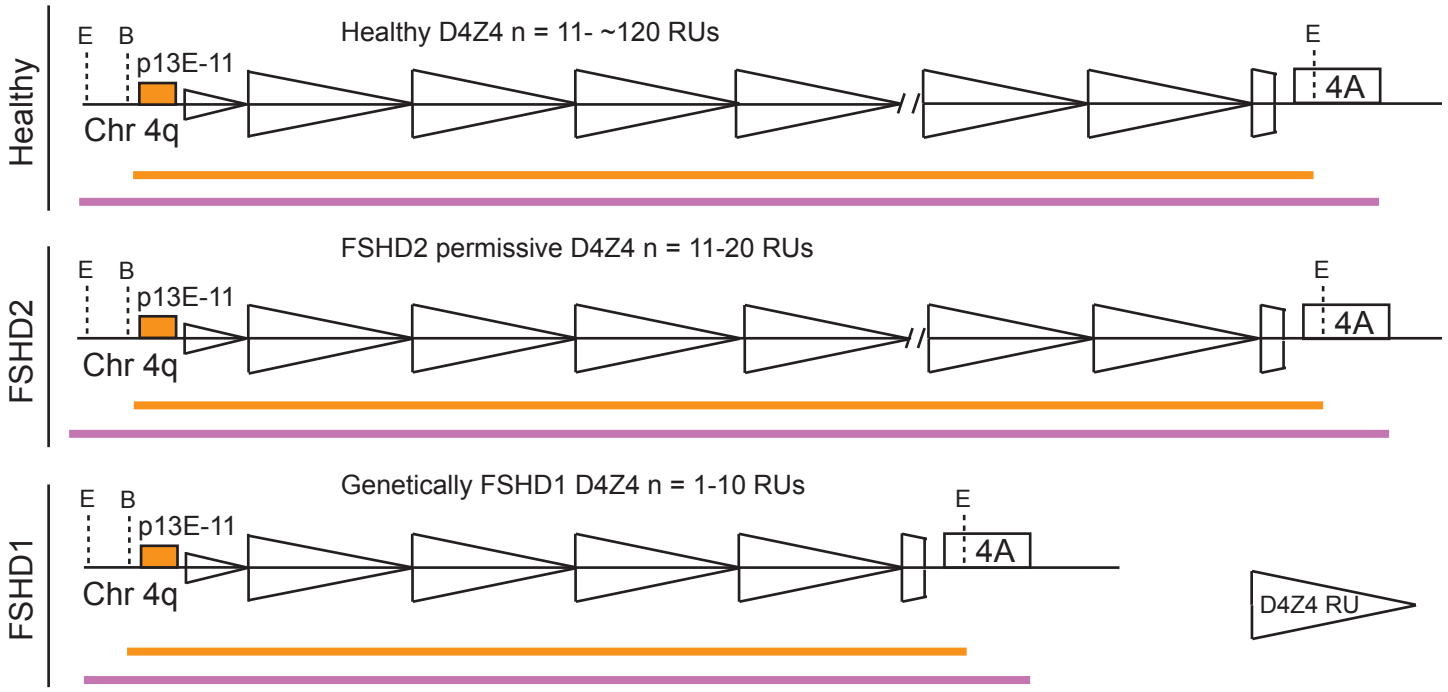
Direct visualization of D4Z4 repeat size

- Molecular combing and optical mapping detect and distinguish the entire chromosome 4 and 10 D4Z4 arrays as well as the A or B subtelomeres.

Introduction to FSHD Diagnostics (Pt 5)

FSHD1 vs FSHD2 testing

Techniques measuring the 4q35 D4Z4 array can identify FSHD1; however, they cannot distinguish FSHD2 from healthy.



FSHD1 deletion analysis by PFGE and Southern blotting

The EcoRI/BlnI cut DNA fragment specific for the chromosome 4q35 D4Z4 array.

Direct visualization of D4Z4 repeat size

The molecular combing and optical mapping techniques detect and distinguish the entire chromosome 4q35 and 10q26 D4Z4 arrays as well as their associated A or B subtelomeres.

FSHD2 diagnostic genetic testing

FSHD1 D4Z4 deletion testing does not identify FSHD2.

FSHD2 is distinguished from healthy and FSHD1 by:

1) decreased DNA methylation on both 4q and 10q D4Z4 arrays.
2) mutation in one of the FSHD2 genes (*SMCHD1*, *DNMT3B*, or *LRIF1*).

Therefore:

Genetic diagnosis of FSHD2 involves assaying the DNA methylation levels on all four D4Z4 arrays and/or identifying potential FSHD2 gene mutations.

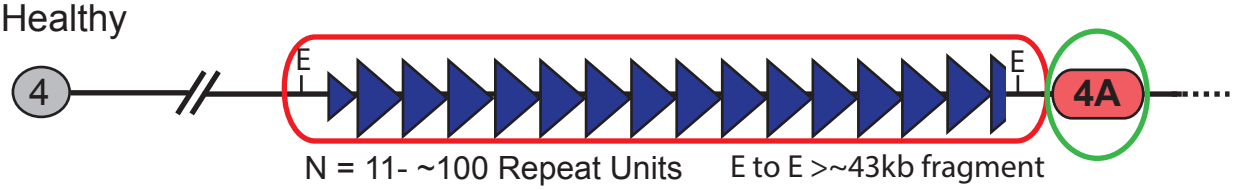
Exome sequencing or targeted candidate gene sequencing may identify FSHD2 pathogenic mutations in the *SMCHD1*, *DNMT3B*, or *LRIF1* genes.

Introduction to FSHD Diagnostics (Pt 6)

FSHD genetic testing vs epigenetic testing

FSHD genetic testing identifies the size of the chromosome 4q D4Z4 array (red) and permissive allele (green) either by 1) restriction enzyme digestion, PFGE and Southern blot, or 2) by visualization using molecular combing or single-molecule optical mapping. These all require very large pieces of DNA and thus typically require a blood draw.

Healthy

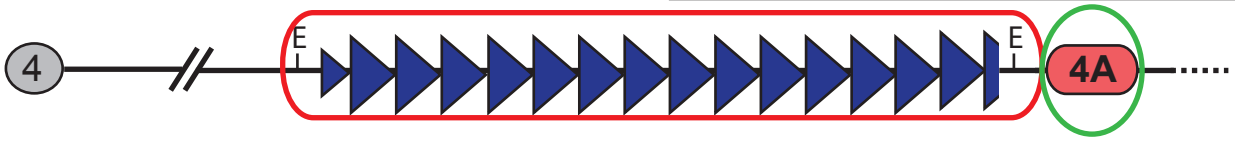


N = 11- ~100 Repeat Units E to E >~43kb fragment

FSHD1: D4Z4 Contraction

N = 1-10 Repeat Units

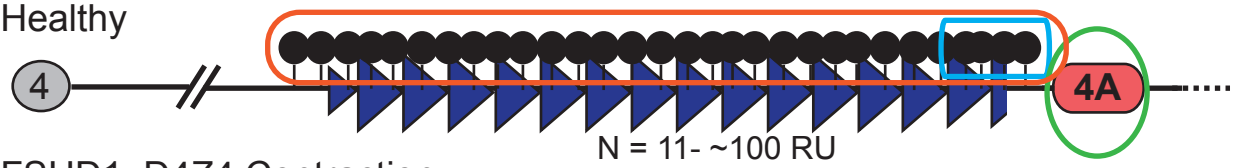
FSHD2: D4Z4 Contraction-independent, thus not identified by these techniques.



N = 8- ~20 Repeat Units (Ave 12-16 RU)

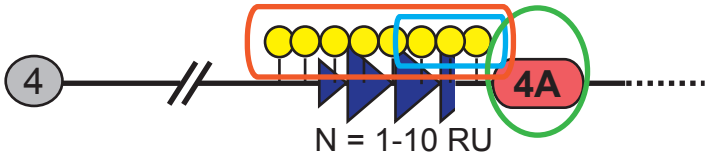
FSHD epigenetic testing identifies the methylation state of the distal most D4Z4 repeat unit on chromosome 4q (blue) to identify FSHD1, and the methylation state of the entire D4Z4 array (orange) to identify FSHD2, as well as assaying for the presence of an FSHD permissive allele (green). This is based on bisulfite sequencing and thus can be done on low molecular weight and fragmented DNA and from any source, including saliva.

Healthy



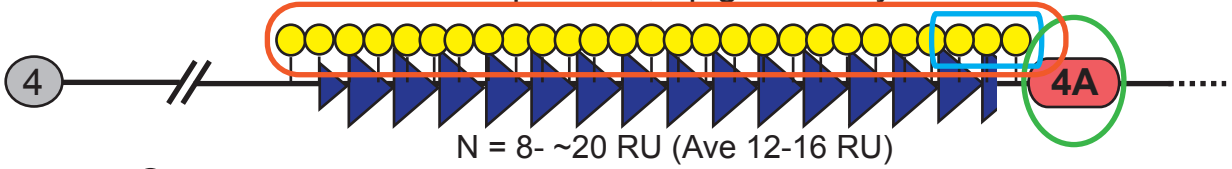
N = 11- ~100 RU

FSHD1: D4Z4 Contraction



N = 1-10 RU

FSHD2: D4Z4 Contraction-independent, epigenetically distinct and identified.



N = 8- ~20 RU (Ave 12-16 RU)

= Hypermethylated CpGs = Hypomethylated CpGs

Introduction to FSHD Diagnostics (Pt 7)

Comparison of FSHD diagnostic technique workflows and cost.

Standard FSHD1 (PFGE/Southern) and FSHD2 testing

Visit healthcare professional for fresh blood collection (15ml)
Venipuncture
requires phlebotomist visit

↓

Ship overnight ambient temp

↓

HMW genomic DNA prep

↓

Agarose embedding
Multiple RE digestions

↓

PFGE

↓

Southern blotting p13E11
Haplotyping

↓

Analysis:
4qA deletion = FSHD1
If no 4qA-linked deletion:

↓

DNA methylation assay or
SMCHD1 sequencing
FSHD2 or not FSHD

~$1-2,000 FSHD1* + ~$900 FSHD2*

VS

Epigenetic testing for FSHD1 and FSHD2

Saliva sample (2ml) collected anywhere at any time (stable for >1yr)
Non-invasive

↓

Ship regular mail ambient temp
domestic or international

↓

Standard genomic DNA prep

↓

PCR for haplotyping

↓

BS conversion

↓

BS-PCR

↓

Sequencing and analysis
FSHD1, FSHD2,
not FSHD

<$200 FSHD1 & FSHD2

More accessible, less expensive, still highly accurate

VS

"New" FSHD testing (Molecular combing & Single-molecule optical mapping)

Visit healthcare professional for fresh blood collection (15ml)
Venipuncture
requires phlebotomist visit

↓

Ship overnight ambient temp

↓

Specialized DNA preps

↓

Automated systems

↓

Analysis: FSHD1 or not FSHD1

In addition, provides size of all 4 chromosomes and can ID recombination events

↓

If no 4qA-linked deletion:

↓

SMCHD1 sequencing
or NMD gene panel
FSHD2 or other NMD

~$750 FSHD1* + ~$500 FSHD2 / NMD*

* These costs are estimates from information available at this time. Costs vary depending on company and technique and can change. Please see the individual testing providers for up-to-date pricing.