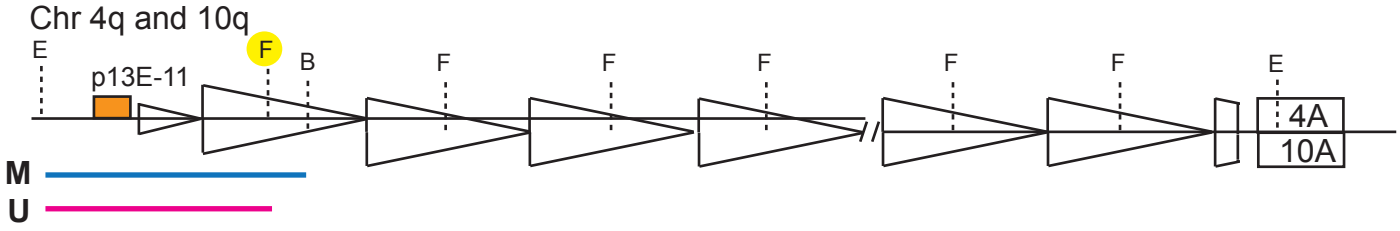
FSHD2 Diagnostics (Pt 1)

FSHD1 vs FSHD2 testing

DNA methylation can distinguish FSHD2 from healthy and FSHD1.

There are two methods used to assay DNA methylation for FSHD2: 1) methyl-sensitive restriction enzyme (MSRE) digestion and 2) bisulfite genomic sequencing.

1) MSRE digestion for FSHD2.

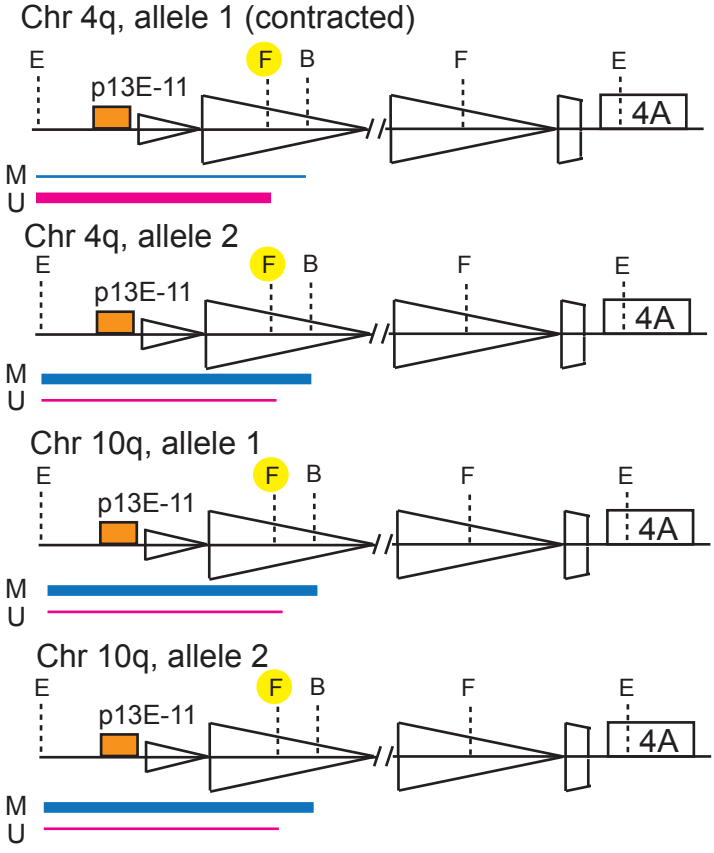


The restriction enzyme FseI has a recognition site of GGCCGGCC; however, if any of the CGs are methylated, the enzyme cannot cut the DNA.

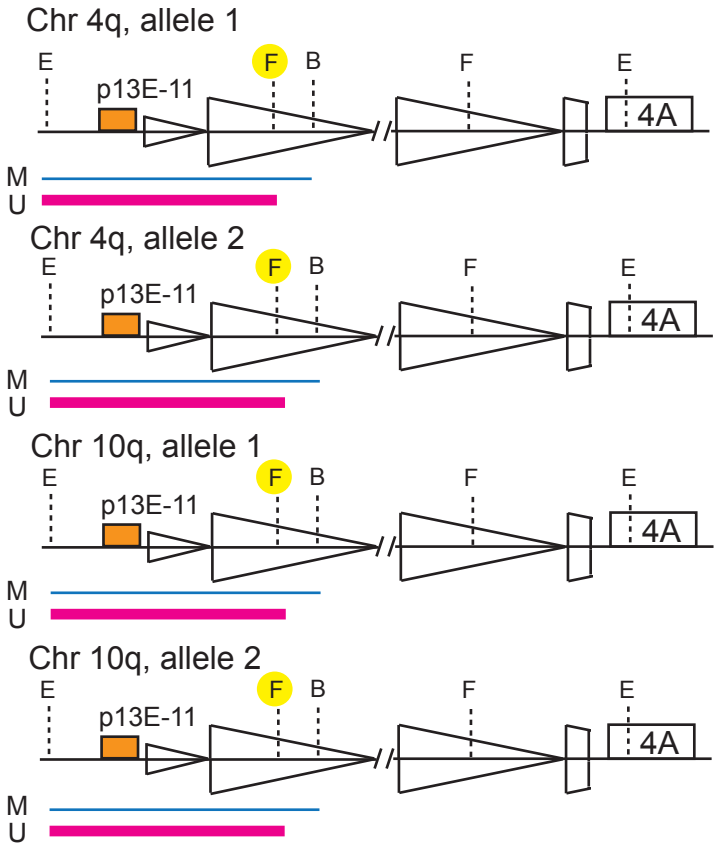
There is a single FseI restriction site (F) in each D4Z4 RU on chromosomes 4 and 10. In healthy individuals, this site is usually methylated. Therefore, FseI cannot cut the healthy DNA.

Genomic DNA is first digested with EcoRI (E) and BglII (B), which cut independent of DNA methylation status. Then the DNA is digested with FseI, and the ratio of FseI cut to uncut is determined, which corresponds to the percent methylation of the site (<30% = FSHD2).

FSHD1: only contracted is hypomethylated



FSHD2: all 4 are hypomethylated



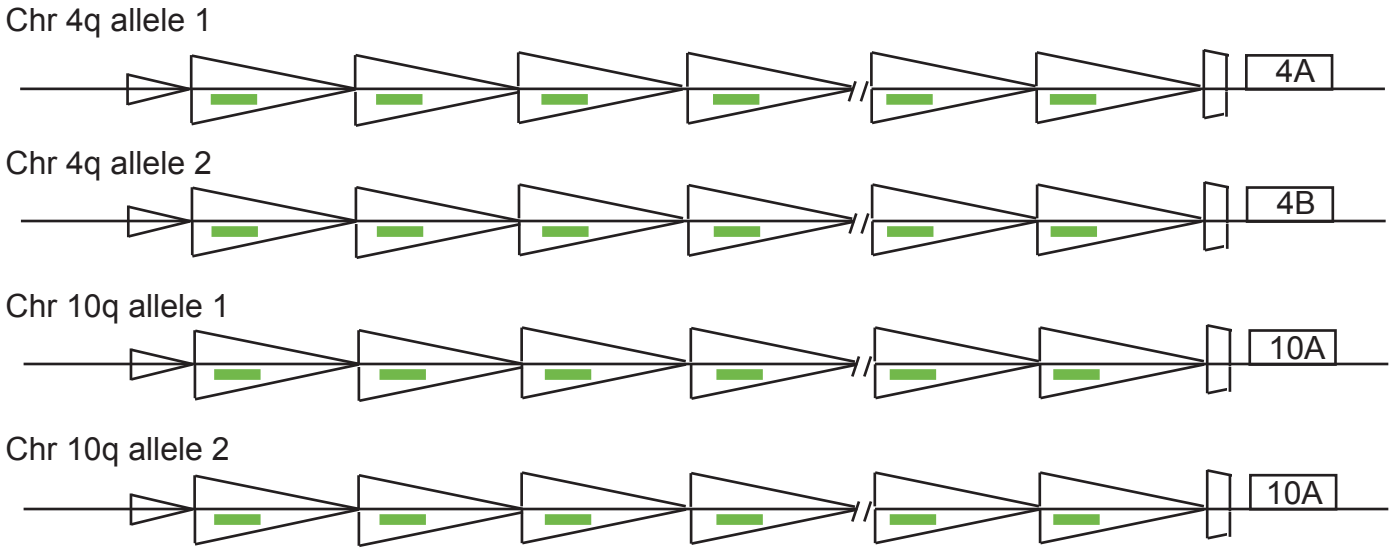
FSHD2 Diagnostics (Pt 2)

FSHD1 vs FSHD2 testing

DNA methylation can distinguish FSHD2 from healthy and FSHD1.

There are two methods used to assay DNA methylation for FSHD2: 1) methyl-sensitive restriction enzyme (MSRE) digestion and 2) bisulfite genomic sequencing (BSS).

2) The BSS assay determines the DNA methylation level of all chromosome 4q35 and chromosome 10q26 D4Z4 repeat units.



Region assayed by the BSS Epigenetic Assay 2 analyzes 59 CpGs in each D4Z4 repeat unit.

FSHD1: only the contracted 4q35 D4Z4 array is hypomethylated. Therefore, the majority of D4Z4 repeat units are methylated.

FSHD2: all 4 D4Z4 arrays (both on 4q35 and both on 10q26) are hypomethylated. Therefore, the majority of D4Z4 repeat units are unmethylated.

If the average DNA methylation for the BSS assay is <30%, AND the individual has at least one FSHD permissive 4qA chromosome, the subject is FSHD2.

FSHD2 Diagnostics (Pt 3)

FSHD1 vs FSHD2 genetic testing

DNA sequencing can identify FSHD2.

There are three DNA sequencing methods used to assay for FSHD2: 1) candidate gene sequencing for known FSHD2 genes (*SMCHD1*, *DNMT3B*, or *LRIF1*), 2) gene panel arrays for neuromuscular disease genes, and 3) whole exome sequencing. Any diagnostic testing of this sort must still be performed in a CLIA-certified lab.

1) Candidate gene sequencing; very specific for FSHD2, ID new *SMCHD1* mutations.

There are more than 100 different FSHD2 pathogenic mutations identified so far in the *SMCHD1* gene. These are dominant mutations.

Lemmers *et al*., 2019, *Journal of Medical Genetics* 56:693-700.

There have been 2 different FSHD2 pathogenic mutations reported so far in the *DNMT3B* gene. These are dominant mutations.

van den Boogaard *et al*., 2016, *American J of Human Genetics* 98:1020-9.

There has been 1 homozygous pathogenic mutation for FSHD2 reported so far in the *LRIF1* gene. This is a recessive mutation.

Hamanaka et al., 2020, *Neurology* 94:e2441-7.

As described above, most cases of FSHD2 result from pathogenic dominant mutations in the *SMCHD1* gene. Thus, direct sequencing of *SMCHD1* is typical for FSHD2 diagnostics. Remember, FSHD2 still requires an FSHD permissive 4qA allele on an intermediate sized chromosome 4q35 D4Z4 array (<21RUs).

SMCHD1 deep gene sequencing is available at:

University of Iowa Diagnostics Laboratory
Leiden University Medical Center

2) Neuromuscular disease (NMD) gene array panels: very specific, high depth.

There are at least 255 genes (by our count) that are known to give a neuromuscular disease clinical presentation when mutated. These are grouped based on the class of NMD and are available for targeted high-depth sequencing. *SMCHD1* is present on some of the newer muscular dystrophy focused arrays. In addition, using these may identify mutations in other NMD genes that could modify FSHD severity or provide an alternative genetic diagnosis.

FSHD2 (*SMCHD1*) gene panel testing can be performed at:

Invitae (add on to Limb-Girdle Muscular Dystrophy Panel)

FSHD2 Diagnostics (Pt 4)

FSHD1 vs FSHD2 genetic testing

DNA sequencing can identify FSHD2.

There are three DNA sequencing methods used to assay for FSHD2: 1) candidate gene sequencing for known FSHD2 genes (*SMCHD1*, *DNMT3B*, or *LRIF1*), 2) gene panel arrays for neuromuscular disease genes, and 3) whole exome sequencing. Any diagnostic testing of this sort must still be performed in a CLIA-certified lab.

3) Whole exome sequencing (WES): more information, expensive, less depth.

Mutations in *SMCHD1*, *DNMT3B* and *LRIF1* account for ~85% of FSHD2 cases. Thus, there are other FSHD2 disease genes present. In addition, a clinical FSHD evaluation may be incorrect. WES will identify mutations in ~all protein coding genes and therefore can be used to identify mutations in both the known FSHD2 genes and to identify new FSHD2 genes, or, alternatively, find mutations in other NMD genes indicative of a different myopathy. Still, many NMD cases (as high as 40%) still go undiagnosed.

Often performed as a research test.

Available as a CLIA-certified test at a number of next-generation sequencing facilities, including:

- Novagene
- Genewiz
- Illumina

Whole exome sequencing selectively sequences the protein-coding regions of genes (~1% of the human genome or ~30,000,000 bp of DNA). Humans have about 180,000 exons.