Understanding your report (Pt 1)

If you participated in the epigenetic FSHD research testing performed by the Jones lab at UNR, and you indicated you were interested in receiving the results, then you will have received an email with an attached PDF file with the research testing results similar to what is shown below.

There are five sections to your report and several figures at the end.

FSHD1/2 DNA Methylation Research Analysis – Not for Clinical Use May 20, 2021

Name: Peter

Your results are consistent with: FSHD1

This is the overall conclusion of your analysis.

Section 1: Explanation of results

Below is a narrative explaining our testing and your analysis. More information can be found at https://myfshd.org/. Experimental details and a scientific narrative follow in the next section, if interested.

The conclusion from the analysis, shown in detail below in sections 3 and 4, indicates that you are genetically FSHD1 (Landouzy-Dejerine myopathy type 1). This report represents a research test analysis for you. This conclusion was determined based on comparisons of the results of your analysis with the results obtained from performing this same research testing procedure on numerous samples obtained from individuals confirmed to be genetically FSHD1, FSHD2, and those confirmed to be genetically not FSHD1 or not FSHD2. Your results clearly place you in the FSHD1 category. There is a wide variability in the clinical manifestation of FSHD among individuals and within families, and many individuals known to be genetically FSHD are mild or asymptomatic and may not show clinical signs of FSHD until later in life, if ever.

Unlike other genetic testing for FSHD, this epigenetic test does not measure the size of the FSHD mutation in the chromosome 4q D4Z4 array; instead it measures the level of DNA methylation associated with this D4Z4 array. DNA methylation is a modification of your DNA that is indicative of gene activity. Low levels of DNA methylation (<30%) of the D4Z4 region are associated with FSHD (the pathogenic gene is on); high levels (>35%) are not associated with FSHD (the pathogenic gene is OFF). We have previously determined the methylation levels that distinguish FSHD1 from other individuals. Your methylation levels are clearly in the FSHD1 ranges. Please note that we have included some figures at the end of the report to help you with understanding your analysis. In addition, there is more information on understanding your report on the MyFSHD.org website https://myfshd.org/test-for-fshd/.

Please remember that this is a research test result, and while it is highly accurate, it does not constitute a medical diagnosis or official genetic diagnosis of FSHD.

Please be aware that having the genetic and epigenetic characteristics for FSHD does not mean that you have clinical FSHD or that you will develop clinical FSHD symptoms in the future. There is great variability in the age of onset and clinical presentation of FSHD among individuals and even within families, with some people being asymptomatic most or all of their lives.

Please feel free to reach out to peterjones@med.unr.edu with any questions about this research testing result. We are happy to set up a phone call or a Zoom or Skype meeting to go over your and your family's reports.

Here, we will walk you through your results. In addition, you can view the videos on the MyFSHD.org website. If you still have questions, please contact Dr. Peter Jones at peterjones@med.unr.edu, and we will arrange to go over the report with you by phone, Skype or Zoom meeting.

Understanding your report (Pt 2)

The first section of your report is a summary of the results. If all you want to know is FSHD or not FSHD (healthy), this is as far as you need to go.

This is the first name for the subject in the report.

This is the overall result of this research test for this individual. This is not a medical diagnosis and, as a research result, is not clinically relevant. The rest of the report explains how we arrived at this conclusion.

This is a brief overview of your results and the testing that was performed. This testing does not provide the size of the D4Z4 repeat array deletion. Alternatively, this indirectly detects the presence of FSHD1 or FSHD2.

Name: Peter

Your results are consistent with: FSHD1

This is the overall conclusion of your analysis.

Section 1: Explanation of results

Below is a narrative explaining our testing and your analysis. More information can be found at https://myfshd.org/. Experimental details and a scientific narrative follow in the next section, if interested.

The conclusion from the analysis, shown in detail below in sections 3 and 4, indicates that you are genetically FSHD1 (Landouzy-Dejerine myopathy type 1). This report represents a research test analysis for you. This conclusion was determined based on comparisons of the results of your analysis with the results obtained from performing this same research testing procedure on numerous samples obtained from individuals confirmed to be genetically FSHD1, FSHD2, and those confirmed to be genetically not FSHD1 or not FSHD2. Your results clearly place you in the FSHD1 category. There is a wide variability in the clinical manifestation of FSHD among individuals and within families, and many individuals known to be genetically FSHD are mild or asymptomatic and may not show clinical signs of FSHD until later in life, if ever.

Unlike other genetic testing for FSHD, this epigenetic test does not measure the size of the FSHD mutation in the chromosome 4q D4Z4 array; instead it measures the level of DNA methylation associated with this D4Z4 array. DNA methylation is a modification of your DNA that is indicative of gene activity. Low levels of DNA methylation (<30%) of the D4Z4 region are associated with FSHD (the pathogenic gene is on); high levels (>35%) are not associated with FSHD (the pathogenic gene is OFF). We have previously determined the methylation levels that distinguish FSHD1 from other individuals. Your methylation levels are clearly in the FSHD1 ranges. Please note that we have included some figures at the end of the report to help you with understanding your analysis. In addition, there is more information on understanding your report on the MyFSHD.org website https://myfshd.org/test-for-fshd/.

Please remember that this is a research test result, and while it is highly accurate, it does not constitute a medical diagnosis or official genetic diagnosis of FSHD.

Understanding your report (Pt 3)

Section 2 is a genetic analysis to determine if you have at least one FSHD permissive chromosome 4A or 4AL. This involves identifying DNA sequences on both sides of the D4Z4 array. Together, this is called your haplotype. This helps to decide which assays to run and how to interpret them. If you do not have a 4A or 4AL chromosome (4B/4B), you cannot have FSHD.

Experimental details for your analysis:

Section 2: Initial analysis.

This individual has 1 FSHD permissive chromosome. This does not mean they have FSHD.

Genotyping Result. This determines if you have an FSHD permissive chromosome 4A or 4AL.

Chromosome 4A/4AL/B	4A	4B
4A161/4B163	FSHD Permissive	FSHD Non-permissive

Having either one or two FSHD permissive chromosomes does not mean that you have FSHD (~75% of people have at least one FSHD permissive chromosome 4). However, if you are one of the ~25% of the people in the population who have two non-permissive chromosomes (4B/4B), then you cannot have FSHD and cannot develop FSHD.

This is your FSHD region haplotype and tells us if you likely have an FSHD permissive chromosome 4A or not. The 4A161 is the most common FSHD permissive haplotype.

This individual has 1 nonpermissive chromosome 4B; they could still be FSHD but not linked to this chromosome.

The 4A161L is a sequence variation of 4A161 and is a less common FSHD permissive haplotype. It requires a different assay for the epigenetic analysis in the next section.

Chromosome 4A/4AL/B	4AL	4B
4A161L/4B163	FSHD Permissive	FSHD Non-permissive

It is very important to understand:

Having either one or two FSHD permissive chromosomes does not mean that you have FSHD; however, if you have two nonpermissive chromosomes (4B/4B) you cannot have FSHD. This step merely informs us as to which assays to perform for the analysis.

Understanding your report (Pt 4)

Section 3 is the first epigenetic analysis. If you have at least one 4A or 4AL chromosome, you will have a result here. This determines if you are FSHD or not FSHD. If your haplotype is 4B/4B, you cannot have FSHD and this assay will not produce a result and will state "NA" for "not applicable".

We perform the first assay (BSSA) on everyone, just to be sure. If you have at least one 4A chromosome, you will get a result for this assay. If you are 4AL, you will have a result under the BSSL assay. This assay determines if you have the methylation characteristics associated with FSHD.

If you are 4A/4B (or otherwise have only one 4A), then your relevant quartile (RQ) for FSHD determination is Q2. If you are 4A/4A, then your RQ is Q1. If your RQ is <25%, your DNA methylation levels are consistent with those who are known to be genetically FSHD. If your RQ is >35%, then your methylation levels are consistent with those known to be genetically healthy in respect to FSHD.

Section 3: FSHD or not FSHD?

Epigenetic Research Assay 1 (BSSA): If you have at least one permissive chromosome 4A, you will have a result here. If you are 4A/4A, your relevant quartile (RQ) point of measurement will be Q1. If you only have one 4A, then your RQ will be Q2. If you do not have any 4A chromosomes, it will say "NA" here. In addition, while the RQ normally gives us a clear answer, we may also take into account the range of methylation to be more precise.

DNA Methylation of 4A D4Z4 Distal Repeat	RQ: 22.3 %	FSHD
RQ <25% = FSHD, RQ = 25 - 35% = potentially FSHD, RQ >35% = Healthy		
Range: 1.8 – 44.6 %	Q1: 8.9 %	Q2: 22.3 %	Q3: 38 %

The result is either FSHD, Not FSHD, Mosaic FSHD, or Borderline.

The DNA in your saliva came from thousands of individual cells. The DNA sequence is the same for all of them; however, the specific DNA methylation state is different for each one. We typically analyze 16 - 24+ independent chromosomes to get an accurate determination of your overall methylation status. As you can see, this individual has one chromosome 4A in each cell; however, the range of DNA methylation is from 1.8% to 46.1%. Thus, some cells have chromosomes that have very low methylation levels, in the FSHD range, and some cells have chromosomes with methylation levels in the healthy (not FSHD) range. The RQ tells us that, on average, the methylation state is consistent with FSHD, yet there are still some with healthy levels. This variation is different among individuals and may explain some differences in severity in families. The range values are useful for borderline cases (RQ 25-25%) and for supporting the FSHD1 vs. FSHD2 determination later.

These are the quartile measurements. In addition to providing the RQ, they give an idea of how representative the range may be. Since Q3 is still near the FSHD range, likely there are very few chromosomes with "healthy" levels of methylation.

Understanding your report (Pt 5)

Section 4 reports the results of Epigenetic Research Assay 2, which determines if you have DNA methylation levels characteristic of FSHD1 or FSHD2.

We perform the second assay on everyone as well. It ensures the bisulfite conversion went OK, and the assay is not dependent upon being FSHD permissive or not. Thus, everyone gets a result here. However, the goal of this assay is to determine if you are FSHD1 or FSHD2.

This assay checks the methylation status of all of your D4Z4 repeats on chromosomes 4 and 10. FSHD1 is caused by a contraction on a single 4A chromosome; therefore, most of the D4Z4 repeats have healthy methylation. FSHD2 is caused by mutations in the genes encoding the proteins required for methylation; therefore, all of the D4Z4 repeats will have low levels of DNA methylation.

Section 4: FSHD1 or FSHD2?

Epigenetic Research Assay 2: **This test analyzes all of your D4Z4 repeats on chromosomes 4q and 10q and lets us know that our analysis is working. Everyone (including those of you who are 4B/4B) will have a result here. If you are positive for FSHD in Section 3, this test will determine if you are FSHD1 or FSHD2. If you are not FSHD in Section 3, you will be healthy or not FSHD here.**

DNA Methylation of D4Z4 Repeats	Mean: 45 %	FSHD1
If BSSA or BSSL predicts FSHD, and the mean is <30% = potentially FSHD2 If BSSA Q2 or Q3 is >35%, not likely FSHD2		
Range: 12.1 – 71.4 %	Q1: 23.7 %	Q2: 38.6 %	Q3: 63.5 %

This will report FSHD1, FSHD2, healthy or not FSHD.

If the average methylation is <30%, this is typically consistent with FSHD2. However, it is also possible to have multiple short D4Z4 arrays, such as on both chromosome 4s, which could give a low average methylation without an FSHD2 mutation. It is also possible to be FSHD1 + 2, which is rare, so sometimes it is difficult to definitively state one is FSHD2. However, if you are negative for FSHD1 by standard genetic testing, this result is very clear. Regardless, if methylation is >35%, you are clearly not FSHD2. When combined with a result from assay 1 of "FSHD", >35% = FSHD1. When combined with a result from assay 1 of "FSHD", <30% = FSHD2. When combined with a result from assay 1 of "healthy", the results of this assay always are "healthy" or "not FSHD2".

These are the quartile measurements. They give an idea of how representative the range may be. The Q2 and Q3 are useful on borderline FSHD2 determinations since there should be very few reads >30% overall in FSHD2, as opposed to FSHD1 with multiple short chromosomes, which could give a low average methylation, too.

Understanding your report (Pt 6)

Finally there is another commentary explaining in detail how we came to the overall conclusion shown in Section 1.

This is a narrative to explain how we came to the conclusions found in the report. The relevant methylation levels are discussed for each assay. In addition, it may include comments about family history and any anomalies taken into consideration. Again, please note this is a research evaluation based on our extensive work analyzing the DNA methylation of documented FSHD1, FSHD2, and non-FSHD subjects. While our analysis is highly accurate, there are always exceptions, and results should be confirmed in a CLIA-approved lab with a clinically valid assay.

Section 5: Detailed explanation of experimental results.

Additional information for those of you interested in knowing more about your experimental analysis and how we arrived at our conclusions.

Genetic analysis indicates that you have one FSHD permissive 4A161 chromosome and one nonpermissive chromosome 4B. Since you have at least one FSHD permissive chromosome, we proceeded with the methylation analysis. Low levels of DNA methylation (<25%) of the D4Z4 region are associated with FSHD; high levels (>35%) are not typically associated with FSHD. For reference, the RQ is the important number.

The results of the Epigenetic Assay 1 (BSSA) show a relevant quartile (Q2) DNA methylation level of 23.2% on the permissive 4A chromosome, which is in the range (RQ<25%) typically found for individuals known to have genetic FSHD. Thus, this result supports that you have genetic FSHD. The Epigenetic Assay 2 shows a mean DNA methylation level of 45% across all D4Z4 repeat units, which is outside of the range typically found in individuals known to have FSHD2 (mean <30%). Taken together, this research analysis is consistent with what is typically found for individuals known to be genetically FSHD1.

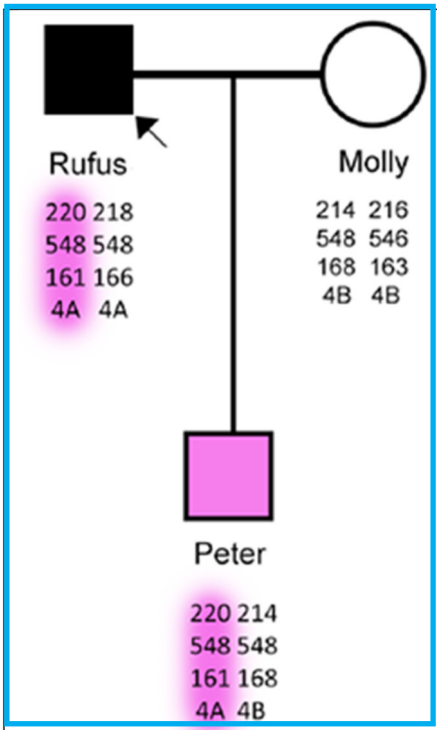
This interpretation of the research test results is based upon our current understanding of the supporting science at the time this analysis was performed and subject to change if our understanding of FSHD genetics and epigenetics changes. While this research testing is highly accurate, it is still recommended that all participants undergo CLIA-approved testing for confirmation of this result. This research result will not qualify you for a clinical trial.

Understanding your report (Pt 7)

Some reports, particularly those with children being analyzed, will include family pedigrees. This helps provide additional confirmation of inheritance of an FSHD1 or healthy chromosome.

Section 5: Detailed explanation of experimental results.

Additional information for those of you interested in knowing more about your experimental analysis and how we arrived at our conclusions.



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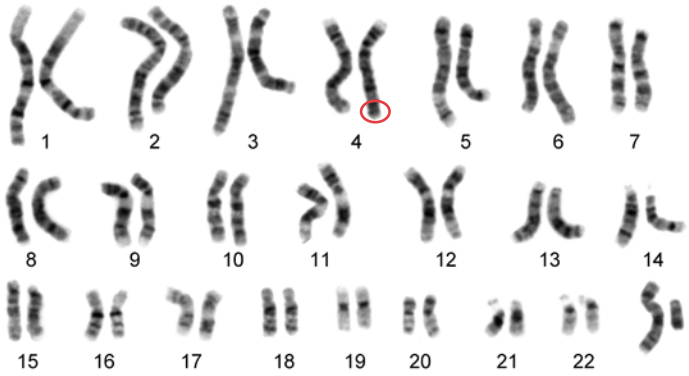
The results of the Epigenetic Assay 1 (BSSA) show a relevant quartile (Q2) DNA methylation level of 23.2% on the permissive 4A chromosome, which is in the range (RQ<25%) typically found for individuals known to have genetic FSHD. Thus, this result supports that you have genetic FSHD. The Epigenetic Assay 2 shows a mean DNA methylation level of 45% across all D4Z4 repeat units, which is outside of the range typically found in individuals known to have FSHD2 (mean <30%). In addition, there is genetic FSHD1 in your family. Genetic analysis confirmed that you did inherit the familial pathogenic FSHD chromosome 4A (220, 548, 161, 4A; pink) from your father. Taken together, this research analysis is consistent with what is typically found for individuals known to be genetically FSHD1.

This interpretation of the research test results is based upon our current understanding of the supporting science at the time this analysis was performed and subject to change if our understanding of FSHD genetics and epigenetics changes. While this research testing is highly accurate, it is still recommended that all participants undergo CLIA-approved testing for confirmation of this result. This research result will not qualify you for a clinical trial.

If multiple family members participated and provided consent, we may include a family pedigree. You may also ask for this if not included, on the condition that all members agree to reveal their status. A black filled-in square or circle denotes that individual had prior genetic confirmation of FSHD. A half-filled square or circle denotes that individual self-reports symptoms of FSHD. Open squares and circles either indicate healthy or unknown health status. The pink shading of the numbers indicates genetic tracking of an FSHD1 chromosome within the family. The numbers are used for this tracking. A pink box or circle indicates a result consistent with FSHD from this research testing.

Understanding your report: Chromosomes 4 and 10

You have 23 pairs of chromosomes (46 total); one from Mom and one from Dad



The FSHD region is on chromosome 4
It is called a D4Z4 repeat and encodes the pathogenic DUX4 gene.
There is a similar region on chromosome 10 that is not pathogenic.
You only need 1 of your chromosome 4s to have a mutation to develop FSHD1.
The first part of your report analyzes the genetics of your two chromosome 4s and 10s

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The critical question being answered in this initial analysis:

Do you have a chromosome 4 that is FSHD-permissive?

There are two types of FSHD permissive chromosome 4s: 4A and 4AL
Chromosome 4B types are FSHD non-permissive

Because chromosome 4 and 10 are very similar, we have to assay all 4 chromosomes (both 4s and both 10s) to answer this question. The important part for FSHD is the chromosome 4 analysis

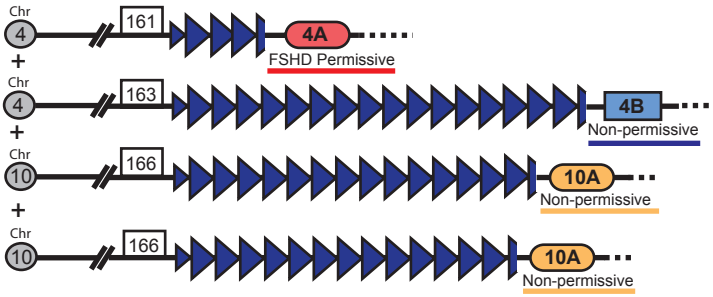
There are three possibilities for your two chromosome 4s:

Permissive/Permissive: 4A/4A or 4A/4AL or 4AL/4AL
Permissive/non-permissive: 4A/4B or 4AL/4B
Non-permissive/Non-permissive = 4B/4B, and you cannot develop FSHD
The 4A166 is a special case of an FSHD permissive chromosome that is not associated with FSHD

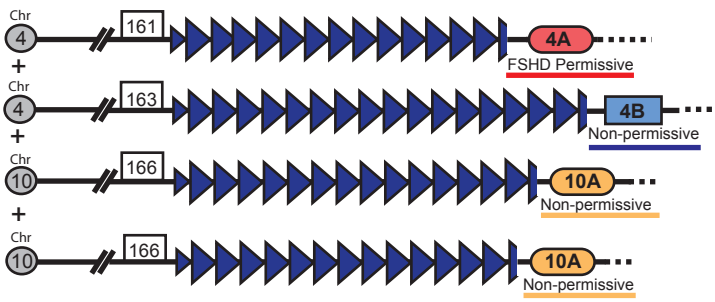
The number associated with your 4 and 10 (e.g.161, 163, 166) refers to a sequence that helps inform us about a chromosome being permissive or non-permissive (e.g. 4A161 is permissive).

Below are two examples of someone who is 4A161/4B163, 10A166/10A166. This does NOT determine if you have FSHD or not, it merely indicates if you are FSHD-permissive
Just because you are FSHD-permissive does not mean you have FSHD!

FSHD1: 4A161/4B163



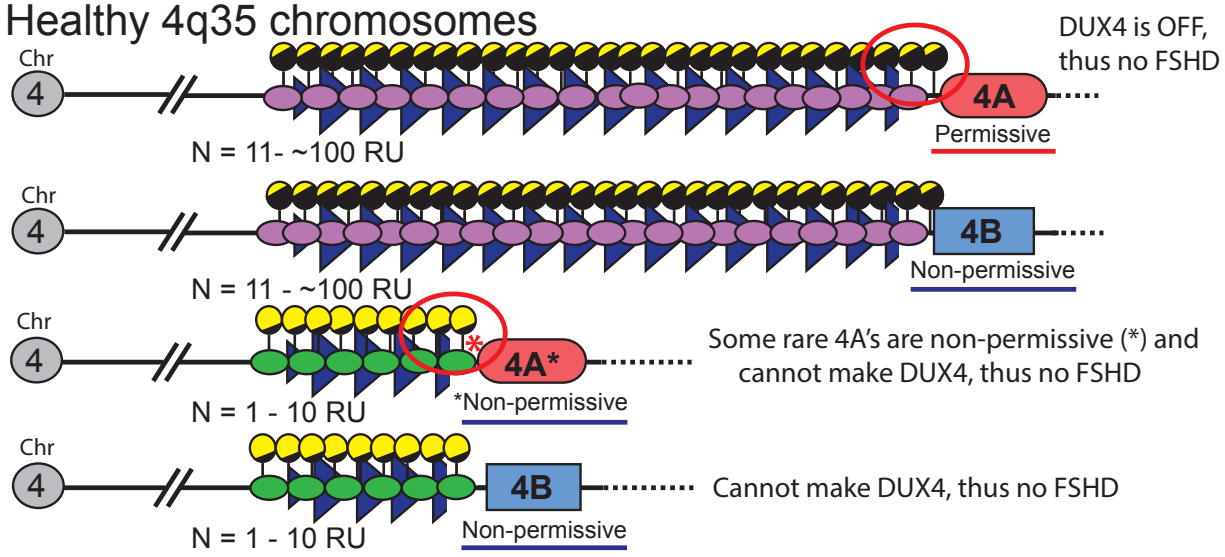
Healthy: 4A161/4B163



= D4Z4 repeat unit (RU)

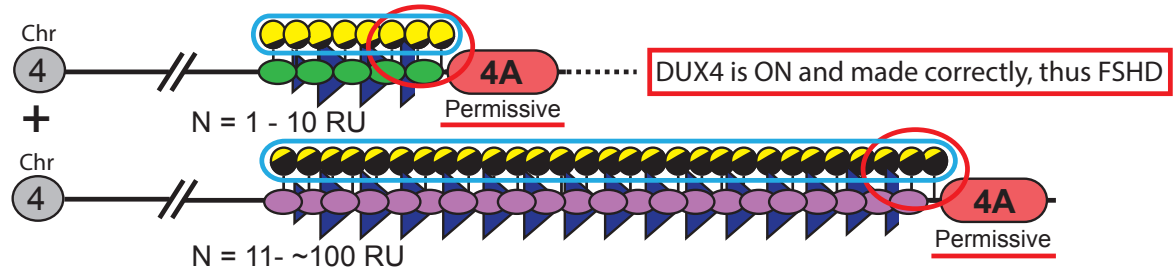
Testing for FSHD using epigenetics

Healthy 4q35 chromosomes

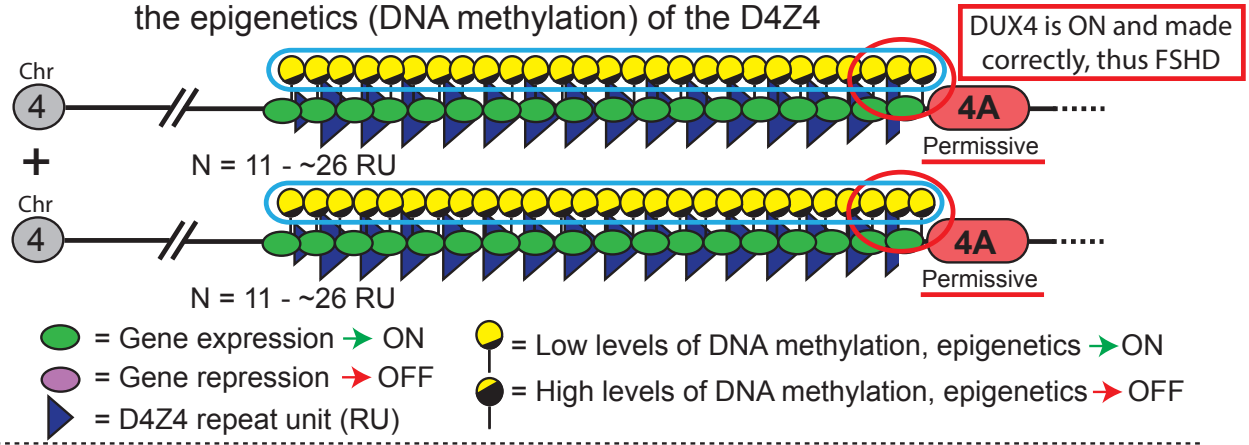


FSHD 4q35 chromosomes

FSHD1: DNA deletions alter the epigenetics (DNA methylation) of the D4Z4



FSHD2: Mutations in genes responsible for epigenetic repression alter the epigenetics (DNA methylation) of the D4Z4



Epigenetic testing for FSHD: Step 1, do you have a permissive chr 4A?

Step 2, do you have FSHD epigenetics?

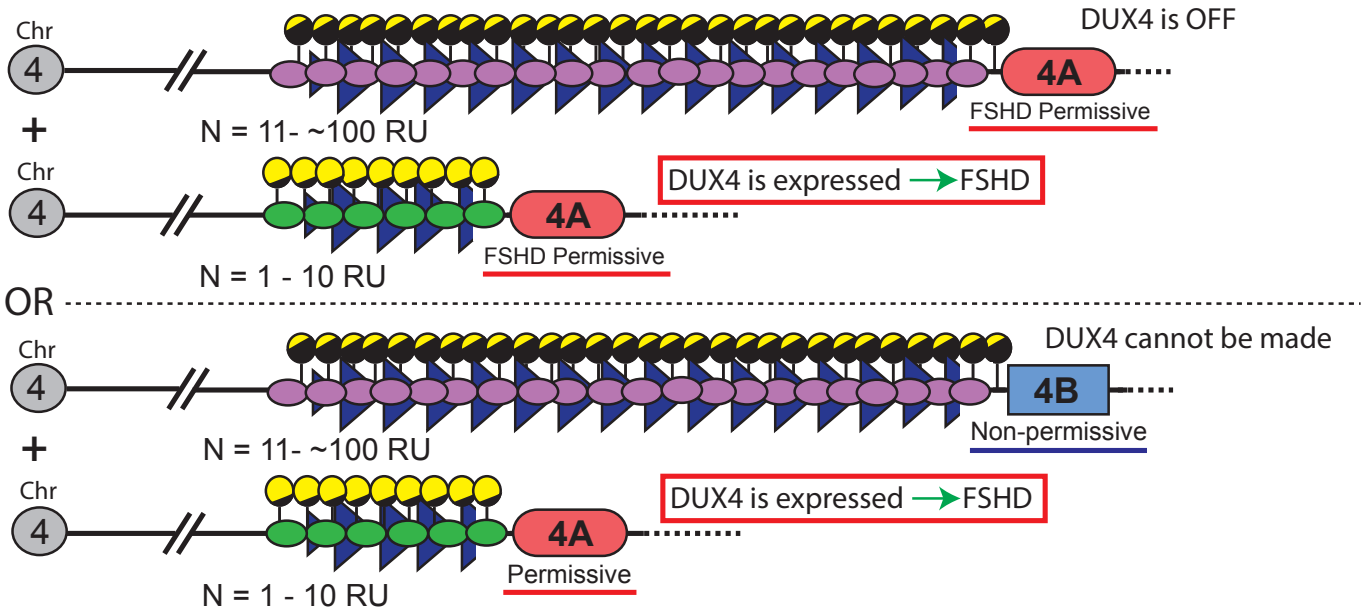
FSHD BSS Assay 1: Are you healthy or FSHD?

Step 3, do you have FSHD1 or FSHD2 epigenetics?

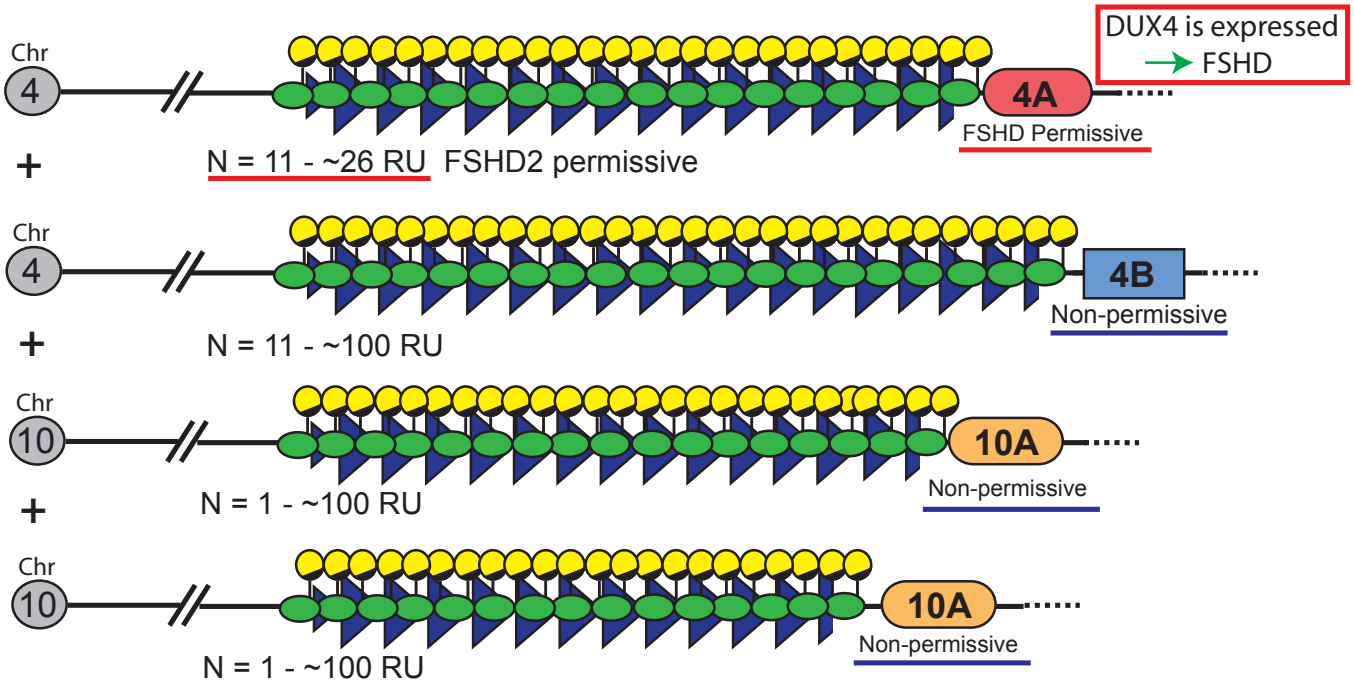
FSHD BSS Assay 2: If FSHD, is it FSHD1 or 2?

FSHD1 vs FSHD2

FSHD1: One FSHD permissive chr. 4 is contracted to between 1-10 RUs

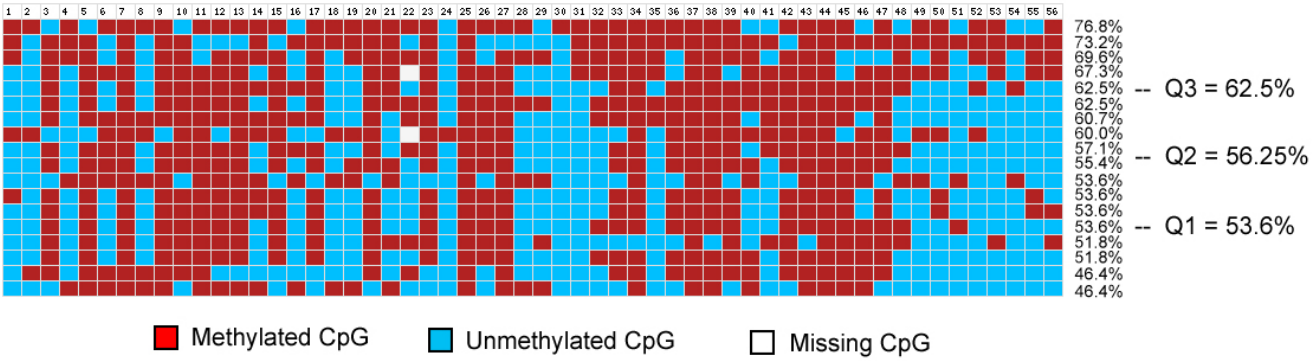


FSHD2: Both chr. 4s and both chr. 10s are altered at the same time. One chr 4 needs to be FSHD permissive AND between 11-26 D4Z4 RUs long

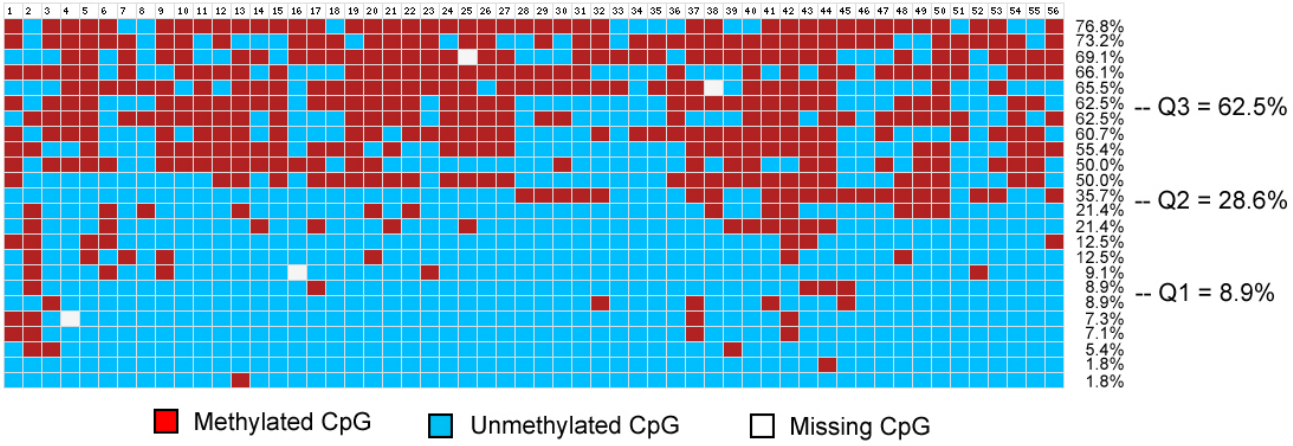


= Gene expression → ON
= Gene repression → OFF
= D4Z4 repeat unit (RU)
= Low levels of DNA methylation, epigenetics → ON
= High levels of DNA methylation, epigenetics → OFF

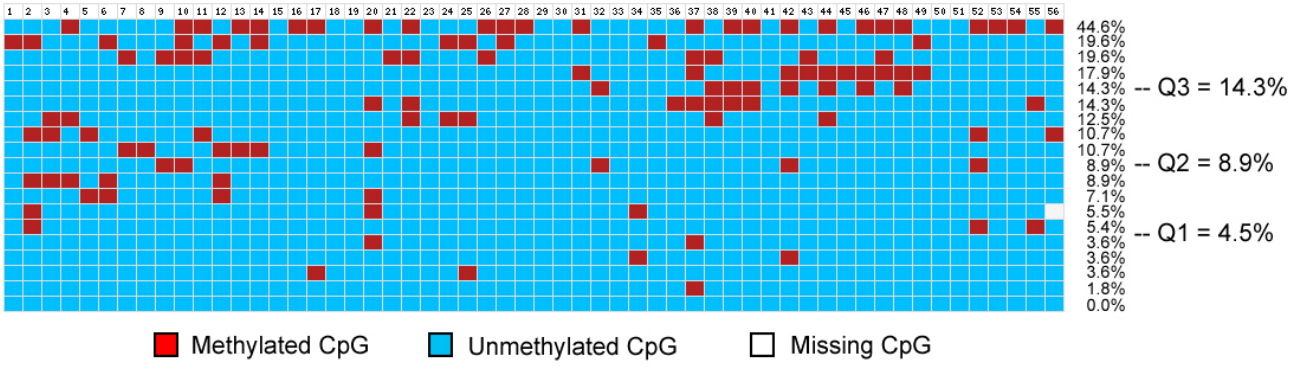
Example DNA methylation analysis of an individual that does not have genetic FSHD.



Example DNA methylation analysis of an individual with 4A/4A genetic FSHD1.

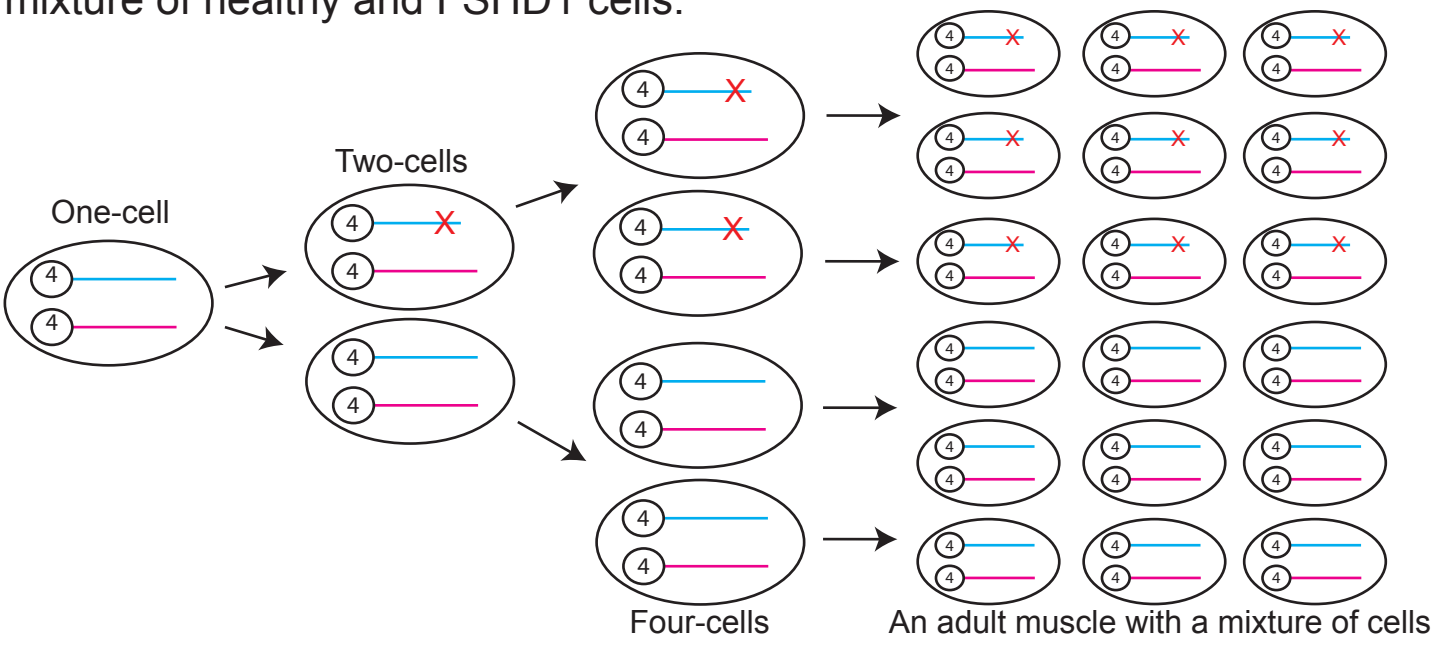


Example DNA methylation analysis of an individual with 4A/4B genetic FSHD1.



Mosaic for FSHD1

Mosaic FSHD1: A spontaneous contraction occurs after fertilization such that only some cells have a contracted chromosome 4. Thus, one is a mixture of healthy and FSHD1 cells.



Mosaic FSHD1: An individual with two populations of cells, one with two healthy chromosome 4s and one with FSHD1 mutation