Understanding the report:
My methylation numbers.

The results of your epigenetic research testing for FSHD are reported in % methylation and % methylation ranges and quartiles of methylation. This raises some questions.

Where do these numbers come from?
What do these numbers mean?
Why are there ranges if my genetics are all the same?
What do the quartiles (Q1, Q2, Q3) mean?
Can I learn anything about my disease progression?

Epigenetic Research Assay 1 (BSSA):

DNA Methylation of 4A D4Z4 Distal Repeat		**RQ: 22.3 %**	**FSHD**
RQ is <25% = FSHD, 25 - 35% = Borderline, >35% = Healthy			
Range: 1.8 – 46.1 %	**Q1: 8.9 %**	**Q2: 22.3 %**	**Q3: 29.5 %**

Shouldn't I have just one or two levels of methylation for my two chromosome 4s?

Keep in mind that although your DNA sequence (genetics) is the same in all of your cells, the methylation state (epigenetics) and pattern of a sequence can vary.

Also, a DNA sample from saliva (or blood or tissue) contains the DNA from tens of thousands of cells, again, all with the same DNA sequence, but with potentially different methylation patterns.

But if all of the methylation is different in each cell, how can it mean anything?

In the FSHD region, the methylation pattern may be different and the exact levels may be different, but what matters is the overall methylation levels across the FSHD region of your two chromosome 4s.

In the following examples, you will see that each chromosome 4 from a cell has its own methylation pattern and level of methylation. On average, you may have 25% methylation; however, there are different ways to get that number. Do you have a wide range or a narrow range? This may affect severity.

On the following pages, we will take you through some examples to address these questions and more.

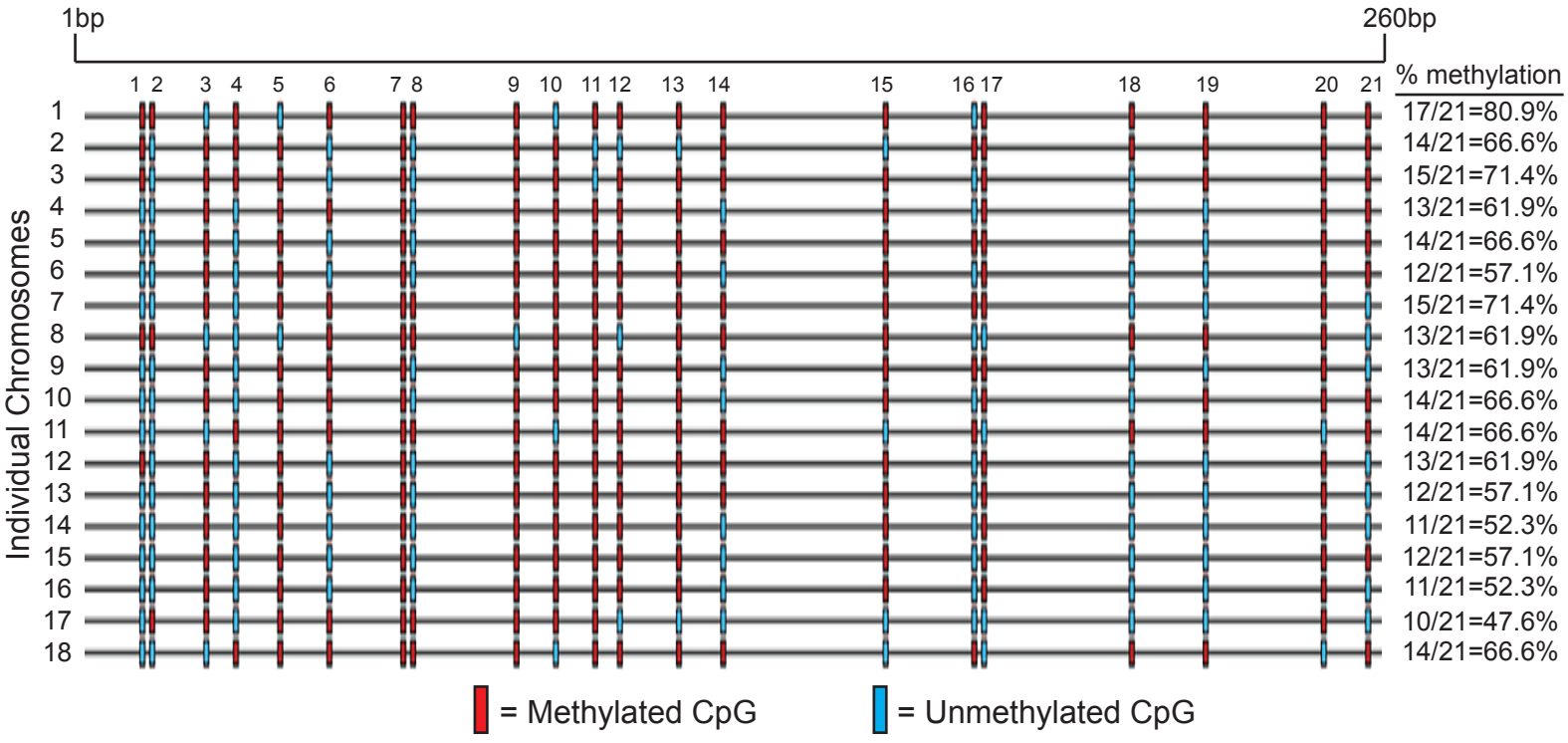
Understanding the report: Where do my methylation numbers come from? (Pt 1)

The results of your epigenetic research testing for FSHD are reported in % methylation. Remember, your saliva sample has DNA from thousands of cells and although the DNA sequence in the FSHD region is the same in each cell, the methylation patterns can be different, but not that much different. The analysis assays a randomly selected set of your chromosome 4s, all from different cells, at each individual methylation site (CpG dinucleotide) in the FSHD region. The results show the variability of the methylation in each cell. However, on average, the healthy, non-FSHD chromosomes will be more methylated and any contracted, FSHD1 chromosomes will be significantly less methylated. If you are 4A/4A, there will be two populations of chromosomes assayed; if you are 4A/4B, there is only one population of chromosomes assayed (the 4As).

The epigenetic FSHD research testing assay analyzes 570bp of DNA with 56 CpGs. There will be examples of what that looks like on pages 4-6. However, to more easily explain the assay, here we will show a smaller example of only 260bp of DNA. There are 21 CpG sites that can be either methylated (red) or unmethylated (blue), indicated by colored rectangles. In these examples, there are 18 chromosomes analyzed (arranged vertically #1-18). The percent methylation for each linear chromosome is listed at the right.

See the example below for a healthy subject:

Haplotype 4A/4A: methylation ranges from 47.6-80.9%; Q1=57.1%, Q2=61.9%, Q3=66.6%
Q1 is the relevant quartile for A/A. Q1 is >35% → Healthy.



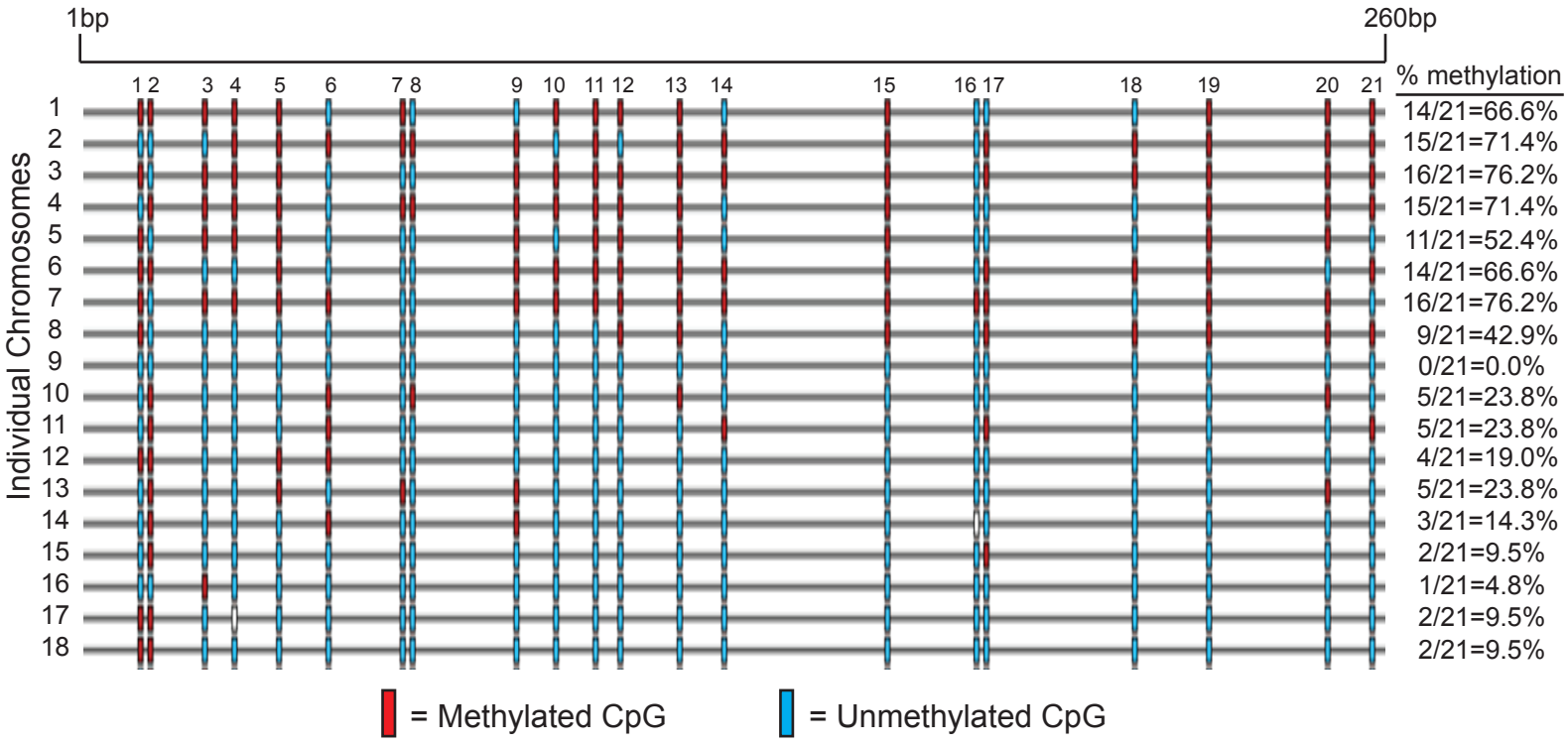
Understanding the report: Where do my methylation numbers come from? (Pt 2)

The analysis assays a randomly selected set of your chromosome 4s, all from different cells, at each individual methylation site (CpG dinucleotide) in the FSHD region. On average, the healthy, non-FSHD chromosomes will be more methylated, and the contracted, FSHD1 chromosomes will be significantly less methylated. If you are 4A/4A and FSHD1, both the contracted and non-contracted chromosomes are assayed, and there will be two populations of methylation. If you are 4A/4B, only the 4A is assayed, and there is one population of methylation.

The epigenetic FSHD research testing assay analyzes 570bp of DNA with 56 CpGs. There will be examples of what that looks like on pages 4-6. However, to more easily explain the assay, here we will show a smaller example of only 260bp of DNA. There are 21 CpG sites that can be either methylated (red) or unmethylated (blue), indicated by colored rectangles. In these examples, there are 18 chromosomes analyzed (arranged vertically #1-18). The percent methylation for each linear chromosome is listed at the right.

See the example below for an FSHD1 subject:

Haplotype 4A/4A: methylation ranges from 0-76.2%; Q1=9.5%, Q2=23.8%, Q3=66.6%
Q1 is the relevant quartile for A/A. Q1 is <25% → FSHD.

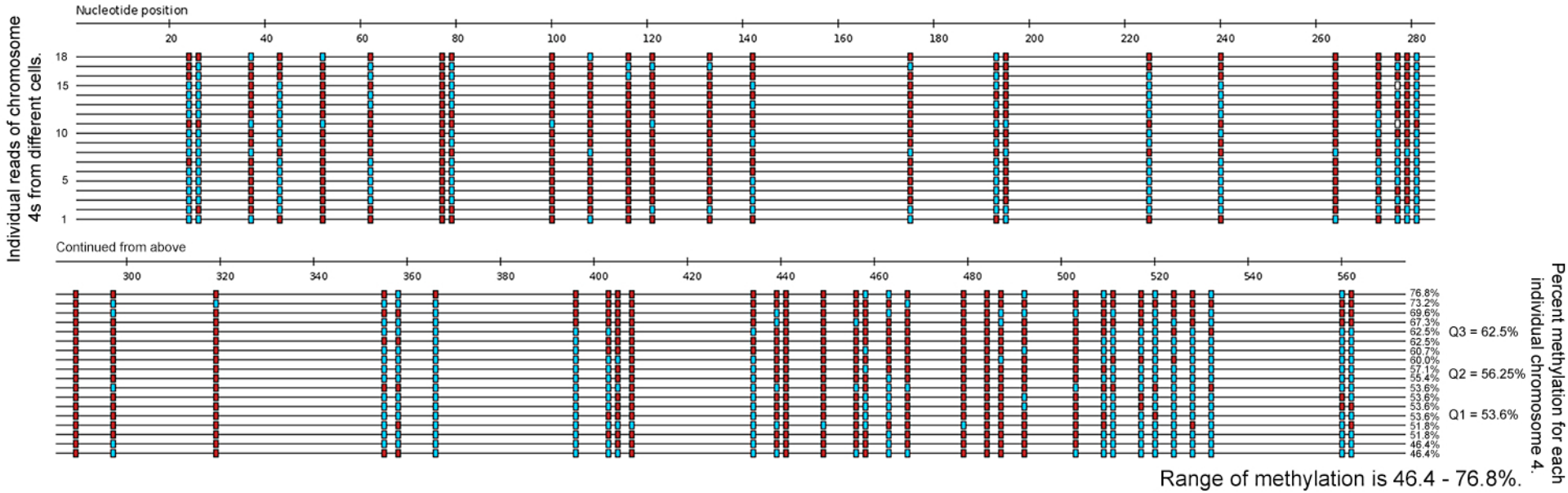


Notice that chromosomes #10, 11, and 13 all have 23.8% methylation, but the specific methylated CpGs are different. In FSHD, it is the level of methylation that is important, not the specific sites.

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

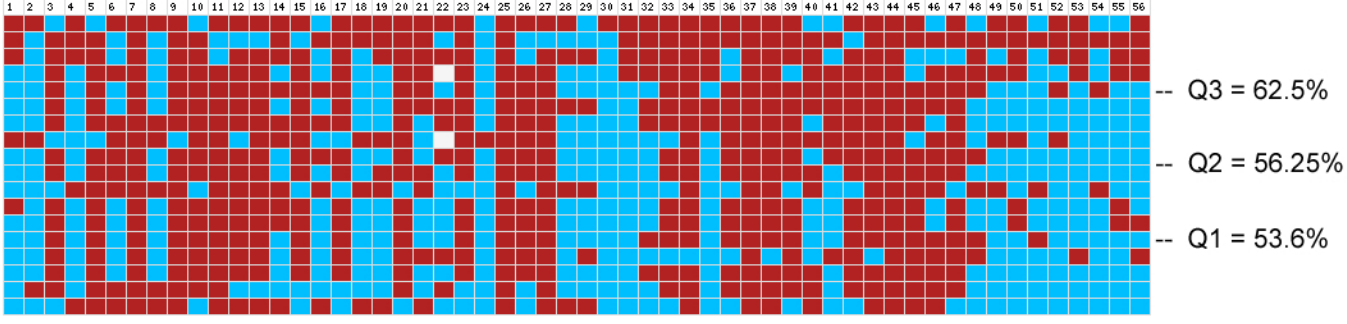
There are 56 CpGs analyzed in the same 570 base pair region of chromosome 4 on DNA from different cells.

Individual chromosome 4s, either from different cells or from the same cell, have different methylation patterns but the same DNA sequence.



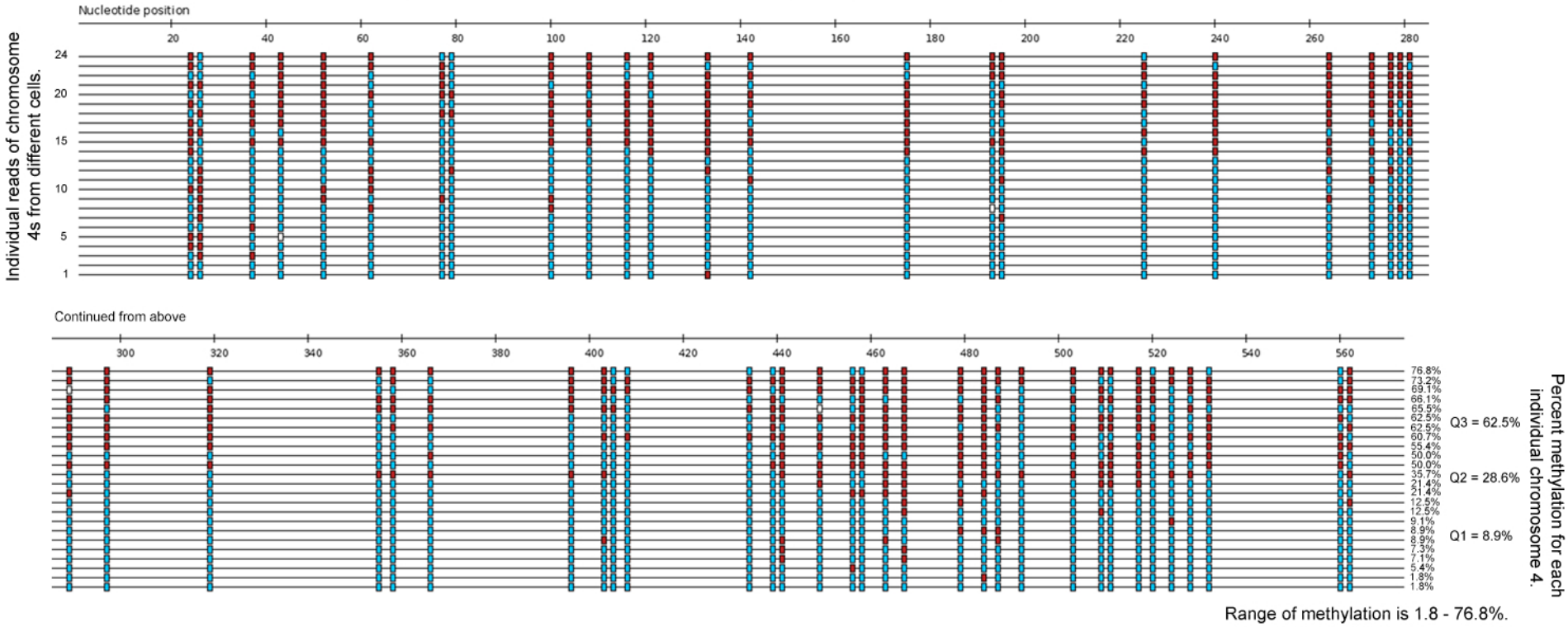
Range of methylation is 46.4 - 76.8%.

Graphic summary of the above data only showing sites of methylation and their status.



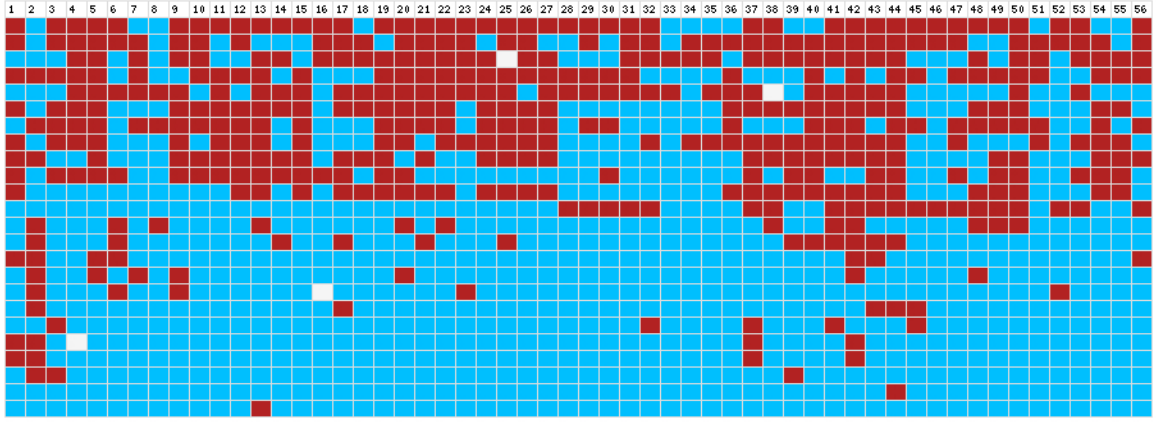
Example DNA methylation analysis of an individual with FSHD1 and a 4A/4A haplotype.

There are 56 CpGs analyzed in the same 570 base pair region of chromosome 4 on DNA from different cells.
Every chromosome 4, either from the same cell or different cells, has a different methylation pattern but the same DNA sequence.
Since this individual is 4A/4A, both of their chromosome 4s are being analyzed. Since they are FSHD1, one 4A is short and one 4A is long.



Range of methylation is 1.8 - 76.8%.

Graphic summary of the above data only showing sites of methylation and their status.



Methylated CpG

Unmethylated CpG

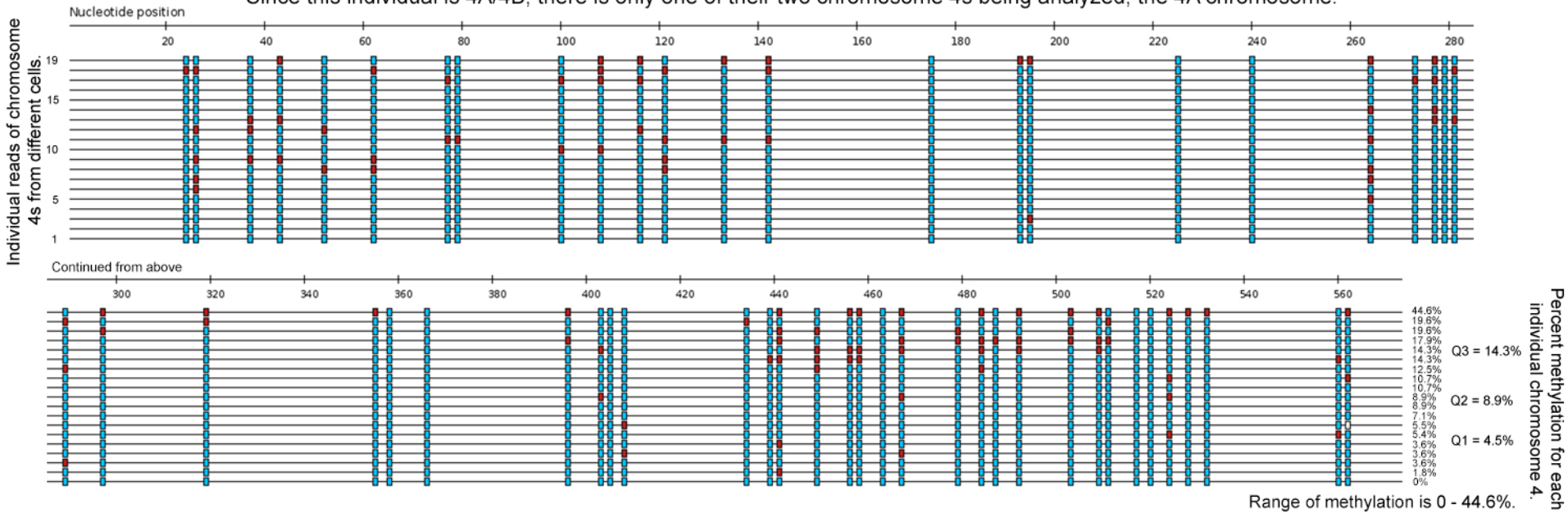
Missing CpG

For 4A/4A, RQ is Q1
<25% = FSHD

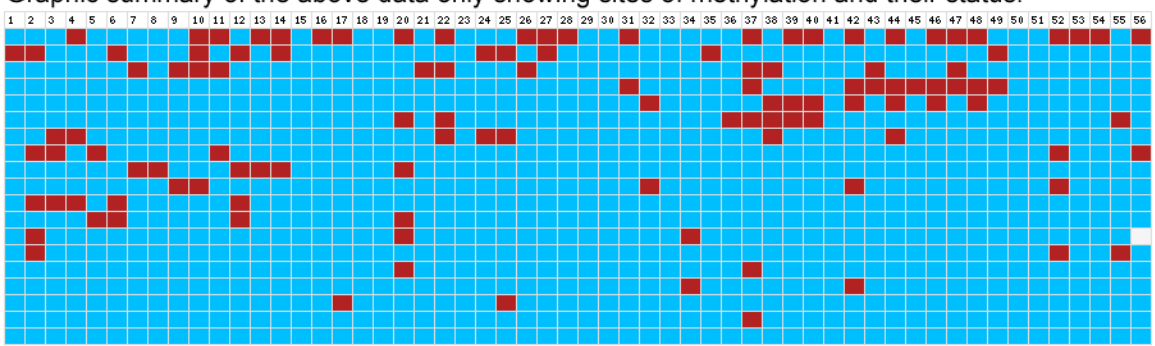
You can readily see that there are two epigenetic populations, one with low methylation and one with high methylation.

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

There are 56 CpGs analyzed in the same 570 base pair region of chromosome 4 on DNA from different cells.
Every chromosome 4, either from the same cell or different cells, has a slightly different methylation pattern but same DNA sequence.
Since this individual is 4A/4B, there is only one of their two chromosome 4s being analyzed, the 4A chromosome.



Graphic summary of the above data only showing sites of methylation and their status.



Methylated CpG

Unmethylated CpG

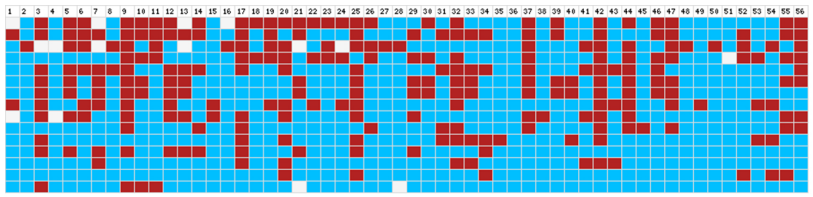
Missing CpG

For 4A/4B, RQ is Q2
<25% = FSHD

You can readily see that there is only one epigenetic population of chromosomes, all with low methylation.

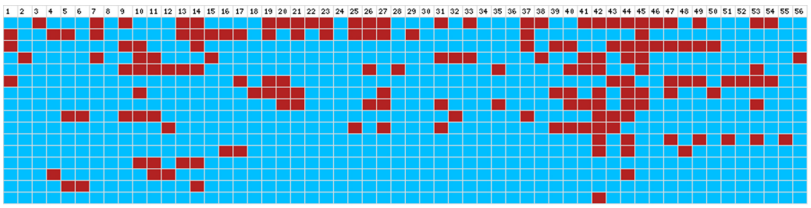
Understanding the report:
What do my methylation numbers mean? (Pt 1)

Below are the methylation analyses for six FSHD1 subjects with a 4A/4B haplotype.
All subjects are genetically confirmed for a 22kb deletion (6RUs).
Thus, only the contracted 4A is being analyzed for an easy comparison.



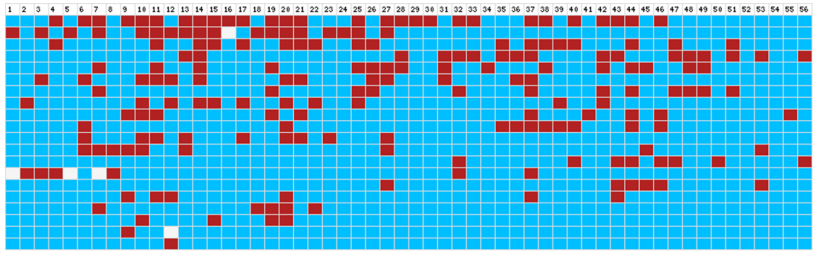
Subject 1
Q2=33.3%

Range
53.8
50.0
50.0
41.8
41.1
39.3
35.7
35.7
33.3
23.2
23.2
21.4
14.3
10.7
7.4



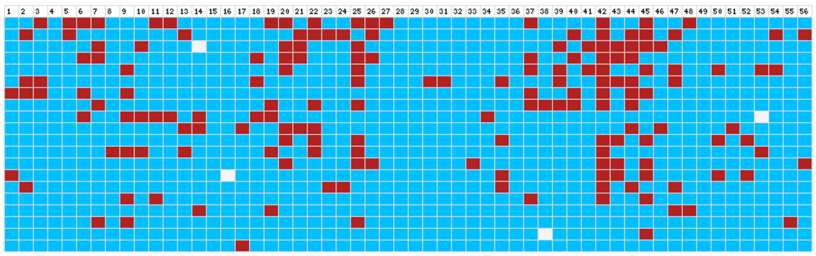
Subject 2
Q2=19.7%

Range
48.2
32.1
26.8
25.0
25.0
23.2
23.2
21.4
17.9
16.1
12.5
8.9
7.1
7.1
5.4
1.8



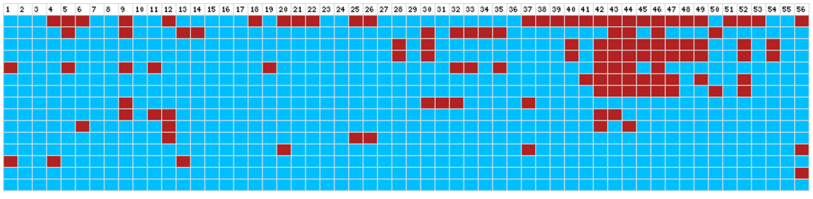
Subject 3
Q2=17.0%

Range
50.0
32.7
32.1
30.4
28.6
23.2
21.4
19.6
17.9
17.9
16.1
16.1
14.3
11.3
10.7
10.7
8.9
7.1
1.8
1.8



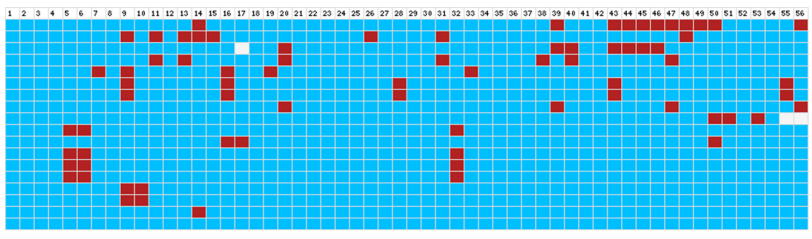
Subject 4
Q2=16.1%

Range
28.6
26.8
21.8
21.4
21.4
21.4
17.9
17.9
16.4
16.1
16.1
16.1
14.3
12.7
12.5
8.9
7.1
7.1
1.8
1.8



Subject 5
Q2=8.9%

Range
50.0
23.2
23.2
23.2
21.4
16.1
14.3
8.9
8.9
7.1
5.4
5.4
5.4
1.8
0



Subject 6
Q2=5.5%

Range
19.6
14.3
12.7
12.5
8.9
8.9
8.9
7.1
5.6
5.4
5.4
5.4
5.4
5.4
3.6
3.6
1.8
0

Understanding the report: What do my methylation numbers mean? (Pt 2)

What does this mean? The verdict is still out.

Analyses for six FSHD1 subjects with a 4A/4B haplotype and contracted 4A161 measuring 22kb or 6RUs.

There is great heterogeneity in each subject and between subjects.

You will notice that there are two ways to get 16-17%, a narrow range (1.8 - 28.9%) and a broad range (1.8 - 50.0%), the latter with more chromosomes with “healthy” methylation levels.

This may (or may not) impact upon or correlate with disease progression. This is an active area of research in several labs.

Summary of six subjects with 6RUs
Subject 1: Q2 = 33.3%; range = 7.4 - 53.8%
Subject 2: Q2 = 19.7%; range = 1.8 - 48.2%
Subject 3: Q2 = 17.0%; range = 1.8 - 50.0%
Subject 4: Q2 = 16.1%; range = 1.8 - 28.6%
Subject 5: Q2 = 8.9%; range = 0.0 - 50.0%
Subject 6: Q2 = 5.5%; range = 0.0 - 19.6%

In general, the larger deletions (i.e., shorter arrays) typically in the 1-3RU range, tend to have a more severe clinical presentation of FSHD.

In general, the smaller deletions (i.e., the longer arrays) typically in the 7-10RU range, tend to be less severe to even asymptomatic for clinical FSHD.

Some observations so far from this study:

We, and others, have found that in general:

- **The shorter D4Z4 arrays have less methylation.**
- **The longer D4Z4 arrays have more methylation.**
- **In families with the same size deletion, the less affected individuals typically, but not always, have more methylation than the more severely affected family members.**

There tends to be wider epigenetic variability in the midsized deletions (4-7RU range).

These are generalities and there are always going to be exceptions!

We are currently doing a large-scale study to investigate these preliminary correlations. Please do not read too much into your research test results!