Update to the CLIA diagnostic procedure using optical genome mapping for the diagnosis of facioscapulohumeral dystrophy

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Aim of the study

- Evaluate the diagnostic value of OGM for complex FSHD
- Suggest the improved laboratory procedure to diagnose FSHD

Background

Facioscapulohumeral muscular dystrophy (FSHD) is a rare progressive muscular dystrophy. The expression of the causative gene, *DUX4* relies on the contraction or hypomethylation of the D4Z4 repeat sequence on chromosome 4q35 and a permissive haplotype.

Cases with complex mechanisms such as hybrid or, mosaicism is undiagnosable due to limitations in methods. Current diagnostic methods include restriction enzyme digestion, pulsed field gel electrophoresis (PFGE), simple sequence length polymorphism (SSLP) and Southern blot (SB) to identify chromosomes, the number of repeats of the D4Z4 sequence, and the type of haploidy. These results are combined to determine the affected and unaffected.

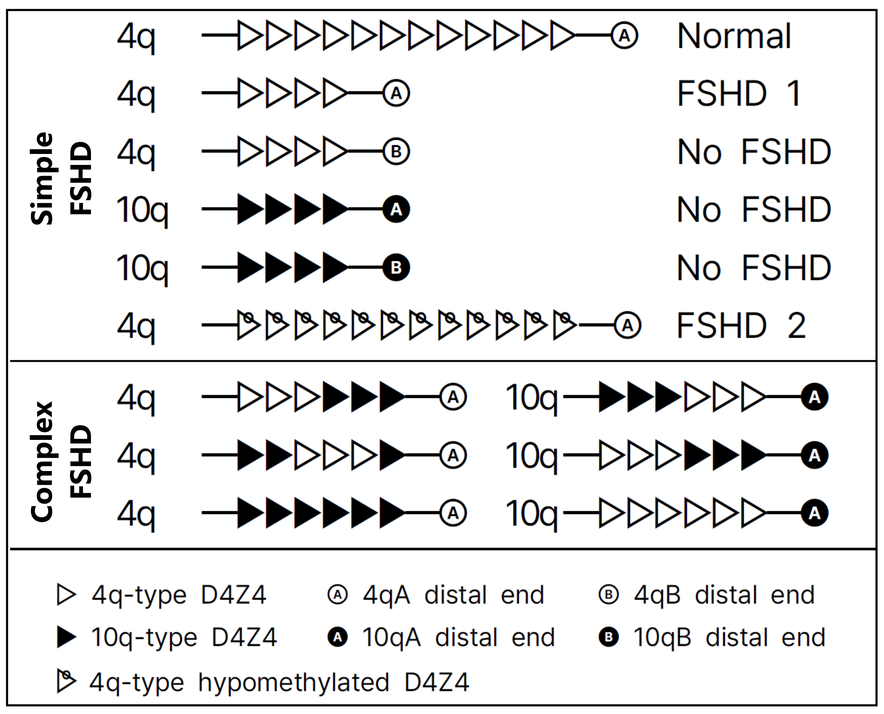


Figure 1. Known molecular pathogenesis of FSHD. Expression of *DUX4* requires a permissive haplotype (4qA) on chromosome 4 containing a poly (A) signal required for stabilization of mRNA. A sequence homologous to the 4q35 D4Z4 repeat is located on chromosome 10q26, requiring differentiation techniques in diagnostic testing. Type 2 FSHD is driven by hypomethylation of the D4Z4 repeat sequence, induced by pathogenic variants in the methylation-related genes SMCHD1 or DNMT3B, resulting in chromatin opening and expression of *DUX4* and, like type 1 FSHD, requires a permissive haplotype.

Optical Genome Mapping (OGM)

- Detects structural variants (SV) across a whole genome
- Capture images of base-specific fluorescent-labelled DNA
- Pattern of fluorescence is compared to reference genome
- SVs are detected by the difference in patterns
- Can detects contractions in repetitive sequences
- Performance of OGM in detection of simple repeat contraction is formerly established

RESULT

- The discrepancy in the number of D4Z4 repeats was relatively pronounced in 4q
- OGM has a better ability to distinguish between chromosomes compared to SB
- Repeat counts are more accurate for SB compared to OGM
- For hybrid types, a discrepancy was identified between the two methods
- This is due to the differences in the characteristics of each method mentioned above

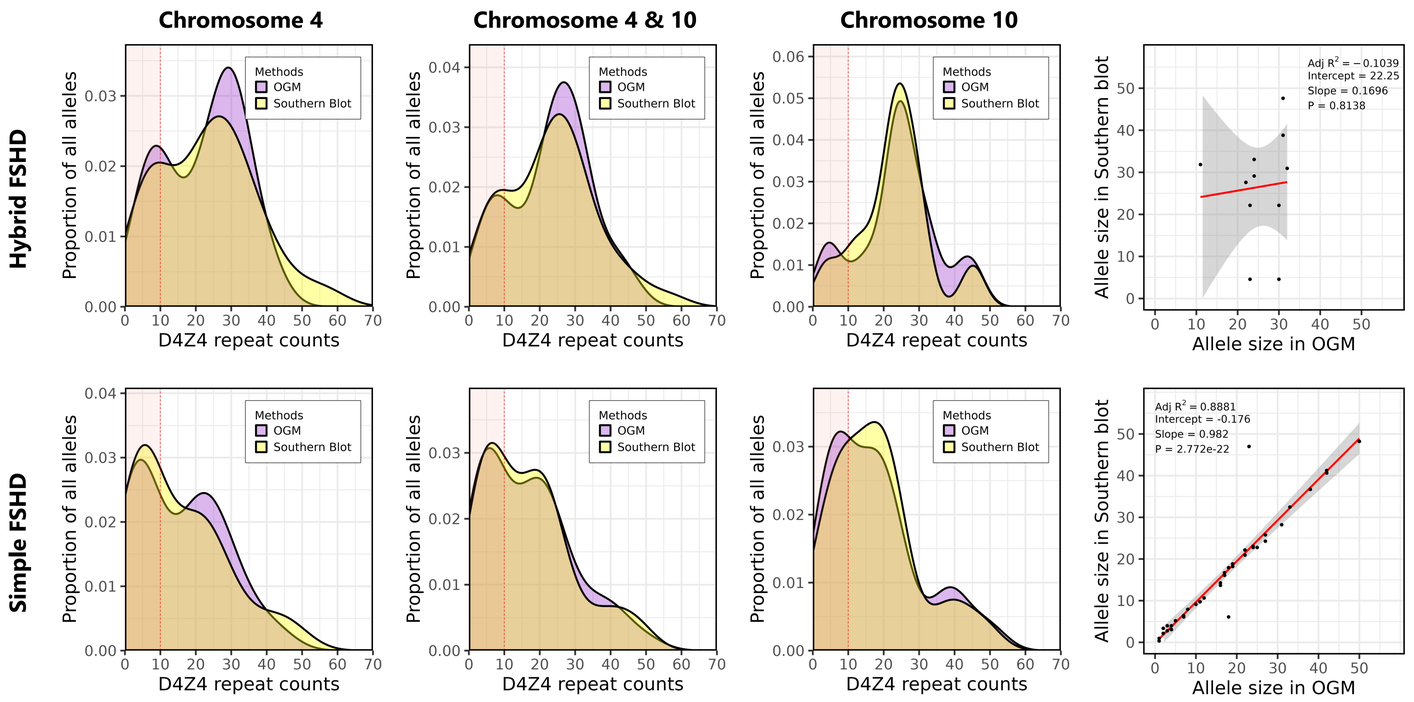
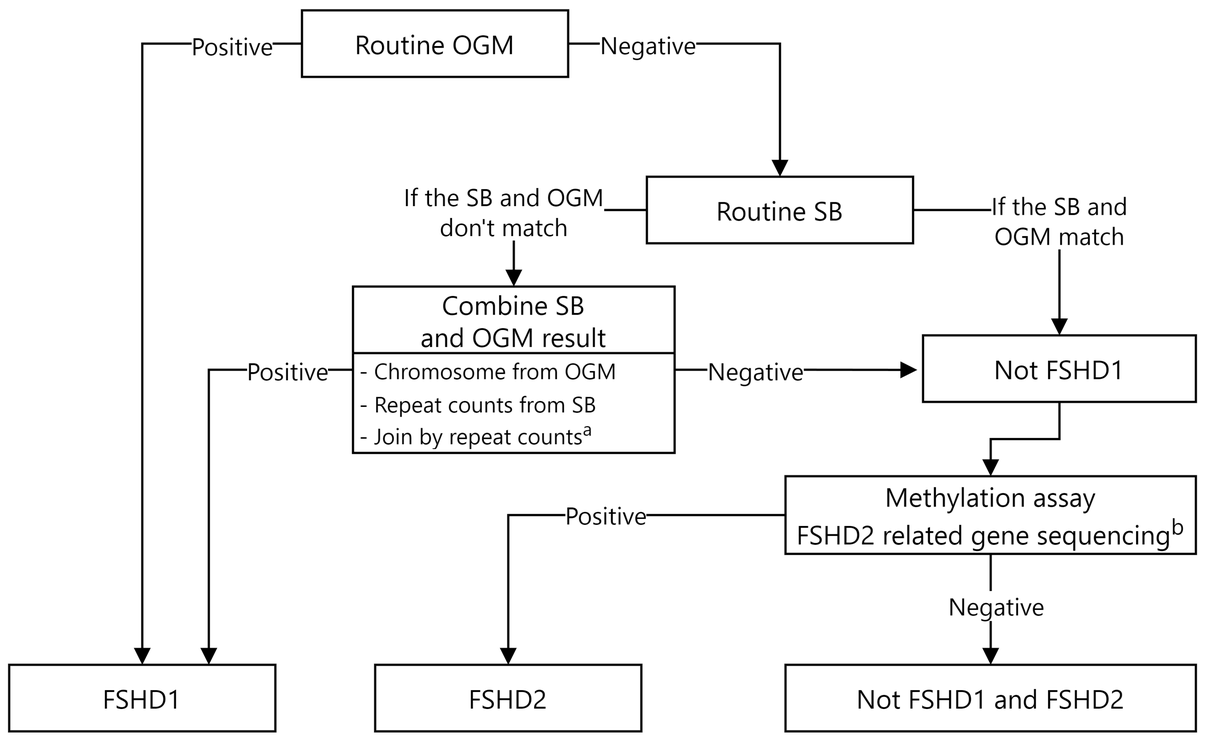


Figure 2. A total of 226 allele counts were identified for the 26 patients, and density histogram analysis revealed the greatest discrepancy in D4Z4 repeat counts between the two methods for the 10 hybrid cases. Subsequent linear regression analysis discovered that patients with simple FSHD had adjusted $R^2$and *p*-values of 0.8881 and <0.01 respectively, while hybrid patients had values of -0.1039 and 0.8138, indicating a substantial difference in results.



[a]Based on chromosome-independent repeat counts, select repeat counts from SB, and chromosome information from OGM.
[b]This step utilizes long-read sequencing, such as ONT, which can additionally determine methylation status.

Figure 3. Suggested laboratory workflow for diagnosis of FSHD.

Conclusion

- Neither SB nor OGM alone could diagnose hybrid FSHD, and the combination of both was required
- For a rapid and reliable FSHD diagnosis, we suggest performing OGM first, followed by SB based on additional criteria, as shown in the results.

Method

We reviewed the laboratory results of 218 patients with suspected FSHD at Seoul National University Hospital from 2017 to 2021, including 30 patients with complex FSHD. Complex FSHD includes hybrid, mosaic and 4 on 10 that is not diagnosed by D4Z4 repeat contractions. OGM was performed in 26 patients, and the results were compared with SB using linear regression and the Bland-Altman analysis.