Molecular Diagnosis of Facioscapulohumeral Muscular Dystrophy (FSHD) using Optical Genome Mapping

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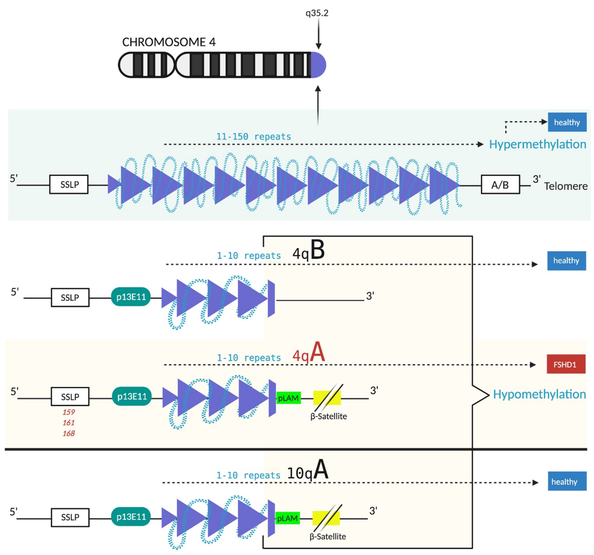
BACKGROUND

- Facioscapulohumeral muscular dystrophy (FSHD) is characterized by progressive and often asymmetric muscle weakness of the face, scapular stabilizers, shoulders, arms, lower leg, and hip girdle. Around 20% of patients are wheelchair-bound, and some also present with sensory, cardiac or neurological manifestations. The age of onset is variable and ranges from infancy to adult.
- Two genetically distinct FSHD subtypes have been identified FSHD1 and FSHD2. FSHD1 is observed in 95% of patients.
- The number of D4Z4 repeats on chromosome 4q generally varies from 11 to 150 copies in healthy individuals, Contraction of repeats to 1-10 in the permissive allele 4qA causes FSHD1. Telomeric region on the chromosome 10 (q26) is 99% identical to 4qA region that is not associated with FSHD.
- FSHD2 is clinically identical to FSHD1 but has a different genetic cause. In FSHD2, individuals have D4Z4 ranges from 11 and above repeats (normal repeats) on the permissive chromosome 4 haplotype (4qA) is caused by hypomethylation of the D4Z4 region due to pathogenic variants in either the SMCHD1 (80% cases) and other genes.

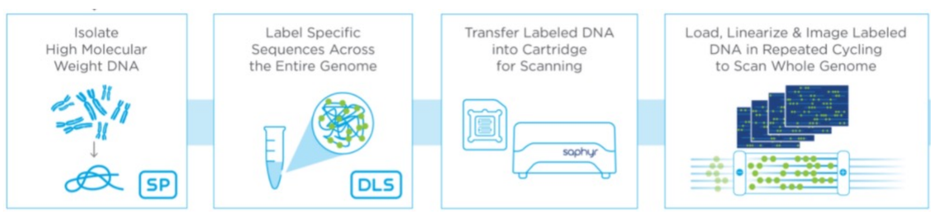


Schätzl, T., Kaiser, L. & Deigner, HP. *Orphanet J Rare Dis* **16,** 129 (2021).

METHODS

- We performed whole-genome optical mapping using the Bionano Genomics Saphyr with subsequent analysis by Bionano Enfocus FSHD analysis software (Bionano, San Diego, CA) to identify FSHD haplotype and D4Z4 repeat number.
- Molecules aligning the D4Z4 repeat regions on chromosome 4 using human genome reference build GRCh38 are distinguished from regions of high homology on chromosome 10.
- The permissive (4qA) and non-permissive alleles (4qB) were assigned using the dynamic-programming algorithm included in the Enfocus FSHD analysis pipeline.
- The D4Z4 repeat size is determined based upon the measurement of the interval distance between labels flanking the D4Z4 arrays.

BIONANO SINGLE-MOLECULE MAPPING



https://bionanogenomics.com/clinical/enfocus-fshd-analysis/

- FSHD testing using optical mapping was performed on 196 patients including 111 males (56 %) and 85 females (44%).
- The median patient age at time of testing was 42 years (ranging from 3.5 to 66 years).
- Clinicians ordered one of the following FSHD panel tests:
  - FSHD1 stand-alone test (106 or 54% of patients), 94 patients showed contraction of D4Z4 allele on 4qA and 38 individuals were negative
  - FSHD1 and 2 panel (56 or 28% of patients)
  - FSHD plus neuromuscular dystrophy panel (30 or 15% of patients)
  - FSHD1 plus WES panel (3 patients)
  - 1 patient each received FSHD2 stand-alone or FSHD1 plus WGS.

FSHD IDENTIFIES PATIENT RESULT SUMMARY

- 96/196 patients referred were positive for a D4Z4 contraction resulting in diagnosis of FSHD1. 4 of these 96 also had a VOUS in SMCHD1 (unknown these VOUS act as modifier in FSHD1).
- 1/89 patients tested for FSHD 1 & 2 were positive for FSHD2 (pathogenic SMCHD1 variant with a 4qA allele)
- 39/107 tested for only FSHD 1 were negative for D4Z4 contraction but carried a 4qA allele, however they did not follow up with FSHD 2 testing so it is unknown if they may have FSHD 2.

Disease Association	Haplotype	D4Z4 Number of Repeats	SMCHD1	196 Total Patients tested (overall diagnostic yield = 48%
FSHD Type 1 (~95%)	4qA (Permissive)	1-10	Not analysed	94 patients
FSHD Type 2 (<5%)	4qA (Permissive)	>11	Pathogenic variant	1 patient
FSHD 1 and 2	4qA (Permissive)	1-10	Pathogenic variant	1 patient (see pt. 1 below)
Mosaic FSHD1	4qA (Permissive)	1-10	Not analysed	2 patients

BIONANO PLUS NGS IDENTIFIES FSHD 1 & 2

Patient 1: 40 yr old female with family history of FSHD, weakness, muscle loss, and scapular winging

- BioNano result: 4qA with 8 repeats and 4qA with 55 repeats
- NGS result: SMCHD1 c.3938C>G (p.Ser1313Ter) = Likely Pathogenic
- Variants in SMCHD1 may lead to a more severe phenotype in patients with a D4Z4 repeat contraction (PMID: 25370034, 24075187).

BIONANO IDENTIFIES MOSAIC FSHD 1

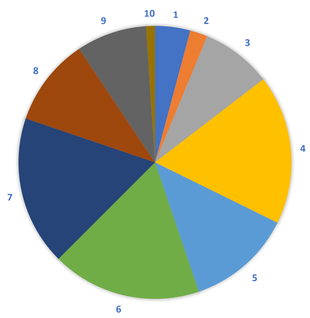
Patient 2: 53 yr old female with muscular dystrophy

- BioNano result: three 4qA alleles: 4qA with 7 repeats, 4qA with 28 repeats and 4qA with 57 repeats

Patient 3: 16 yr old male with clinical features suggestive of FSHD, similar clinical presentation in mother

- BioNano result: three 4qA alleles: 4qA with 5 repeats, 4qA with 17 repeats and 4qA with 18 repeats

Figure 1. 4qA allele size distribution among 96 patients positive for FSHD1.



CONCLUSION

- Southern blotting is gold standard method in the diagnosis of FSHD, however; it is labour-intensive, time consuming , radioactive method and needs a large quantity of high-quality DNA.
- Our results demonstrate that whole genome optical mapping offers a promising alternative method for FSHD1 diagnosis that is less time consuming and more accurate in estimate the number of D4Z4 units than the conventional southern blotting technique, Optical mapping could not detect rearrangements between 4q35 and 10q26 D4Z4 repeats.
- Combined with next generation sequencing (NGS) technology to detect the sequence and copy number variants in SMCHD1 or DNMT3B, and genes associated with neuromuscular disorders, comprehensive neuromuscular disorder testing including FSHD can be an option for providers to test patients with undiagnosed neuromuscular diseases.

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