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

Madhuri Hegde, Naga Guruju, Babi Nallamilli, Vanessa Jump, Ephrem Chin, Taraka E. Donti, Suresh Shenoy, Ryan Nara, Zhiwen Luo, Christin Collins

PerkinElmer For the Better

PerkinElmer GENOMICS

BACKGROUND

Fascioscapulohumeral Muscular Dystrophy (FSHD):

- Autosomal dominant genetic condition
- Slowly progressive muscle weakness, scapular winging, atrophy of upper extremities
- Foot drop, lower extremity weakness
- Chronic fatigue and pain
- Curved spine
- Age of onset: infancy to adulthood, typically in teens.
- Variable severity and muscle distribution

- FSHD1
  - Accounts for approximately 95% of the FSHD individuals
  - Pathogenic contraction of the D4Z4 repeat (1-10 repeats) with permissive chromosome 4 haplotype (4qA)
  - Phenotypic severity of FSHD1 correlates with the D4Z4 repeat size. The shorter of the D4Z4 repeats is usually associated with earlier onset, more severe clinical presentations and higher penetrance.
- FSHD2
  - Account for about 5% of the FSHD patients
  - Hypomethylation of the normal D4Z4 repeat allele (11 or above repeats) with permissive 4qA haplotype resulting from a heterozygous pathogenic variant in either SMCHD1 or DNMT3B.
- Testing of FSHD
  - ✓ Southern blotting has been traditionally the only clinical diagnostic method commercially available to interrogate the D4Z4 region for haplotype and repeat size.
  - ✓ 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 has been recently implemented.
  - Molecules aligning of the D4Z4 repeat regions on chromosomes 4 using human genome reference build GRCh38 are distinguished from regions of high homology on chromosome 10.
  - The 4qA and 4qB are assigned using the dynamic-programming algorithm including in the Enfocus FSHD analysis pipeline.
  - The D4Z4 repeat size is determined based on the measurement of the interval distance between labels flanking the D4Z4 arrays.

CONCLUSION

- Optical mapping offers a promising alternative method for FSHD diagnostic testing due to lower DNA input needed for analysis and non-radioactive method.
- 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.

RESULTS

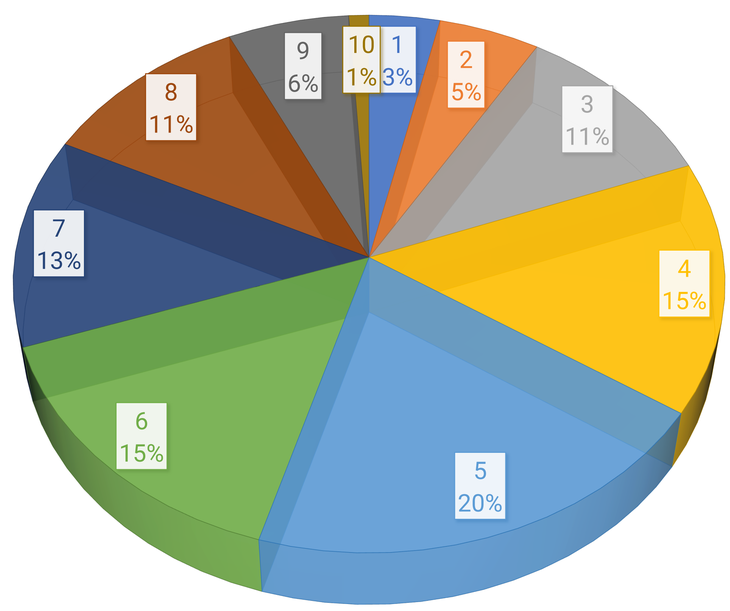
1. Patient cohort:
- Total 380 patients including 207 males (54 %) and 173 females (46%).
- The median patient age at time of testing was 43 years (ranging from 4 month to 83 years).

2. Test categories:
- FSHD1 stand alone test
- FSHD 1 and 2 panel (FSHD 1 & 2)
- FSHD and Neuromuscular disease panel (FSHD+NMD)

3. Diagnostic yield
- The general diagnostic yield for FSHD1 & 2 is about 53% in our patient cohort.
- 198/380 patients referred were positive for a D4Z4 contraction resulting in diagnosis of FSHD1.
- 5/184 patients tested for FSHD 1 & 2 were positive for FSHD2 (pathogenic/likely pathogenic SMCHD1 variant with normal 4qA alleles. One patient had both D4Z4 contractions (8 repeats) and a variant in the SMCHD1 gene (FSHD2).

Disease Association	# of D4Z4 repeats on 4qA permissive haplotype	SMCHD1	# of patient
FSHD Type 1	1-10	Not Applicable(NA)	198
FSHD Type 2	>11	Pathogenic or likely pathogenic variant	5
FSHD 1 and 2	1-10	Pathogenic variant	1
Mosaic FSHD1	1-10	Not Applicable(NA)	4
Non-Dx	-	-	177

D4Z4 repeat size distribution among 198 patients positive for FSHD 1



Scan to Download