

Fascioscapulohumeral Muscular Dystrophy Genetic Testing by Optic Mapping

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BACKGROUND

- **Fascioscapulohumeral Muscular Dystrophy (FSHD):**
 - autosomal dominant genetic condition
 - slowly progressive muscle weakness
 - Scapular winging
 - Atrophy of upper extremities
 - Foot drop due to lower extremity weakness
 - Chronic fatigue and pain
 - Curved spine
 - age of onset: infancy to adulthood, typically in teens.
 - Variable severity and muscle distribution
 - genetically classified as FSHD1 and FSHD2
- **FSHD1**
 - account 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 roughly 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 chromosome 4 haplotype (4qA) due to a heterozygous pathogenic variant in either *SMCHD1* or *DNMT3B*.
- 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 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.

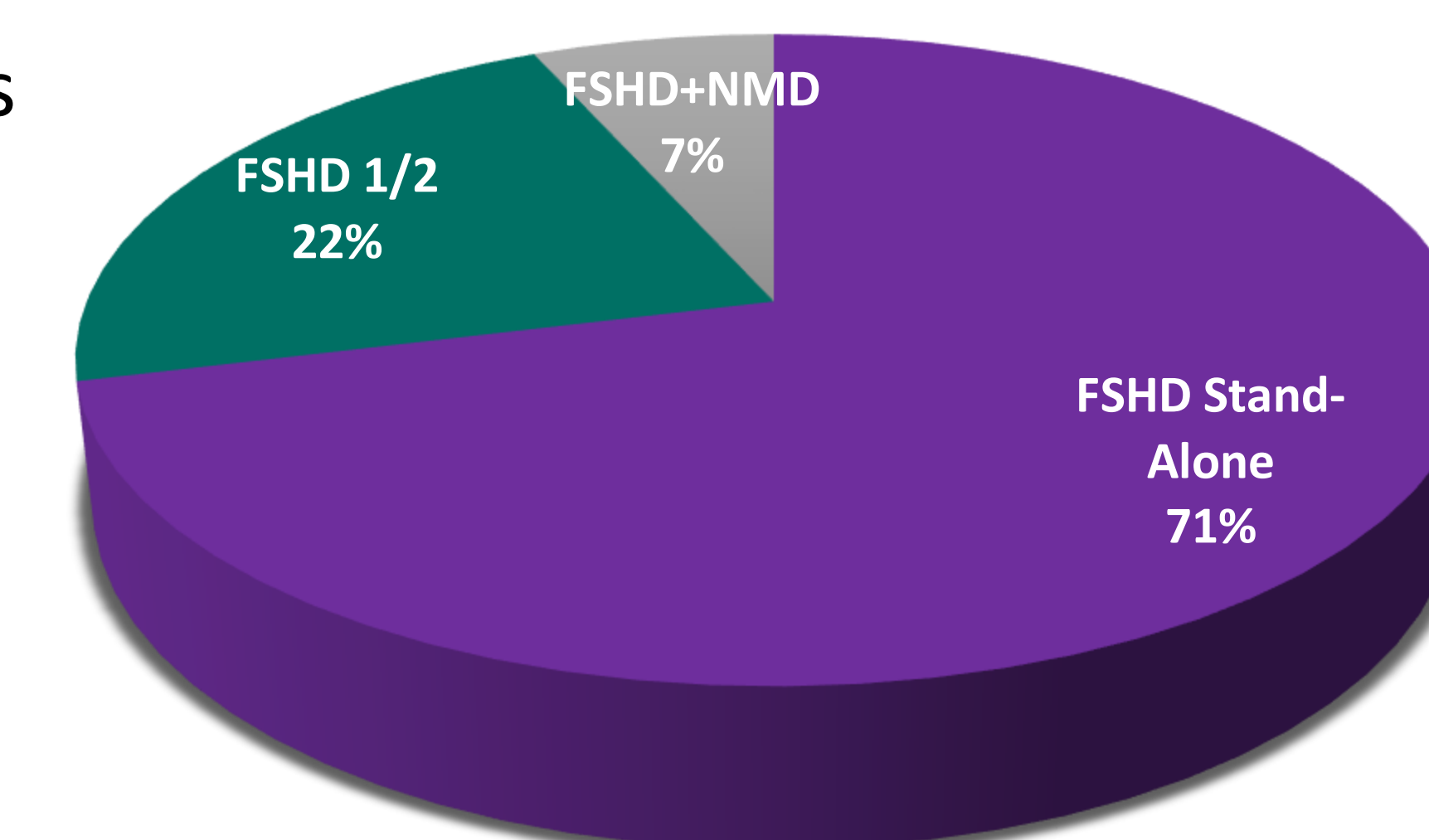
RESULTS

1. Patient cohort:

- Total 45 including 30 male and 15 females
- Age ranges 6-72 years old.

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

- 27 of 45 cases resulted in a D4Z4 contraction repeat on a permissive haplotype, consistent with FSHD1
- 18 of 45 were negative.
 - 14 negative carried 4qA; 4 with 4qB
- 1 case with heterozygous *SMCHD1* VOUS on 4qB
- 6 cases with negative *SMCHD1* / 4qA

Diagnostic yield	Number of cases	% of cases
D4Z4 contraction/4qA	27	60
Negative	18	40
total	45	100

4. Interpretation Guide

Disease Association	Haplotype	D4Z4 Number of Repeats	<i>SMCHD1</i>
FSHD Type 1 (~95%)	4qA (Permissive)	1-10	
FSHD Type 2 (<5%)	4qA (Permissive)		Pathogenic variant
Uncertain FSHD Type 2 disease association	4qA (Permissive)	11 or above	VUS
No disease association	4qA (Permissive)	0*	
	4qA (Permissive)	11 or above	
	4qB (Non-permissive)	Any number of repeats	
	2 copies of 4qB (Non-permissive)	Any number of repeats	Pathogenic variant or VUS

VUS = Variant of Uncertain Significance

*Deletion of the D4Z4 array does not cause FSHD

CONCLUSION

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