GENETIC TESTING RESULTS
Facioscapulohumeral Muscular Dystrophy Testing

bionano laboratories

Patient Name: Sample Report	**Provider Name:** Dr. XXX XXXXXX	**Sample Type:** Blood
Patient Date of Birth: XX/XX/XXXX	**Provider Phone:** XXX-XXX-XXXX	**Sample Collected:** XX/XX/XXXX
Patient Sex: XXXX	**Provider Fax:** XXX-XXX-XXXX	**Sample Received:** XX/XX/XXXX
Patient ICD-10 code(s): XX.XX	**Provider Institution:** XXXXXXXXXX	**Order Date:** XX/XX/XXXX
Accession Number: XXXXXXXXXX		**Report Issued:** XX/XX/XXXX

Test Result Summary: Positive

The results of Facioscapulohumeral Muscular Dystrophy 1 (FSHD1) Testing are: Positive

Chromosome 4

Region	Haplotype	Repeat Count (Units)	Normal repeat range	Classification
4q35	4qA	**3**	11-150	Pathogenic
4q35	4qB	28	1-150	Not disease causing

Chromosome 10

Region	Haplotype	Repeat Count (Units)
10q26	10qA	6
10q26	10qA	29

The 4q and 10q subtelomeric regions are highly homogenous, including D4Z4 repeat arrays. This test assesses the benign 10q26 arrays to allow for the discernment of the chromosome 4 D4Z4 repeat region.

Informational note: No copy number loss overlapping the SMCHD1 gene was observed.

Please note that the current size limitation for detection of copy number changes overlapping *SMCHD1* is ≥ 500 kbp

Clinical Interpretation and Discussion:

This test result shows D4Z4 contraction on the 4qA permissive haplotype, which is consistent with a genetic diagnosis of facioscapulohumeral muscular dystrophy Type 1 (FSHD1). These results must be interpreted in the context of this individual's personal and family history. Testing is available for family members of this individual at risk or suspected of having FSHD.

FSHD is a neuromuscular disorder causing skeletal muscle weakness which can manifest in a variety of ways (PMID: 20301616):

- Facial weakness: difficulty whistling, drinking through a straw, turning up the corners of the mouth when smiling, or fully closing the eyes when sleeping
- Shoulder weakness: shoulder blades stick out rather than resting flat against the back of the chest wall (scapular winging); this is the most common initial symptom
- Upper arm weakness: difficulty raising arms above the head or throwing a ball
- Abdominal weakness: abnormal curvature of the lower back (lordosis)
- Hip girdle weakness: difficulty climbing stairs or walking long distances; about 20% of individuals with FSHD need a wheelchair by age 50
- Lower leg weakness: difficulty lifting the front of the foot causing it to drag when walking (foot drop)

This weakness can be asymmetric, meaning one side may be more affected than the other. It is also progressive, slowly worsening and impacting other parts of the body over time. It is estimated that 70% of individuals with FSHD have chronic pain or fatigue. Some individuals have additional symptoms including mild high-tone hearing loss, anomalies of the light-sensitive region at the back of the eye (retina), and respiratory dysfunction.

Symptoms of FSHD can vary widely between individuals, including those in the same family. The onset of symptoms is typically during adolescence, although it can range from infancy to later adulthood, and life expectancy is typical. Standards of care and management for individuals with FSHD have been established (PMIDs: 20554202, 20301616).

While the symptoms of FSHD type 1 and 2 are indistinguishable, they have different genetic causes. FSHD1 is caused by the presence of two genetic factors on the same copy of chromosome 4: a shortened number of DNA repeats (less than 11) in the D4Z4 region causing hypomethylation and the presence of a polyadenylation sequence (4qA haplotype; also called the 4qA permissive haplotype). Either of these findings alone or on opposite copies of chromosome 4 will not cause FSHD1.

70-90% of individuals with FSHD1 inherited the genetic change from a parent; for the other 10-30%, the genetic change is not inherited and is new in that individual (de novo). Each child of a person with FSHD1 has a 50% chance of inheriting the copy of chromosome 4 with the genetic variants and a 50% chance of inheriting the copy of chromosome 4 without the genetic variants. Consultation with a genetics professional may be helpful to discuss recurrence risk and testing for other family members.

Of note, there was no copy number loss (≥ 500 kbp) overlapping the *SMCHD1* gene observed; however, a loss affecting this gene might still be present due to the limitations of this test. Additional testing may be indicated based on the clinical

Genetic Counseling and Family Resources

Genetic counseling is recommended. A genetic counselor can review these results with an individual and family members, can provide background information on genetics, and discuss additional resources or next steps that may be useful. Additionally, the genetic counselor may review medical and family history of the individual tested. This may help the genetic counselor better answer questions about the impact of specific results. To access genetic counseling services, please visit the National Society of Genetic Counselors (NSGC) website (https://findageneticcounselor.nsgc.org/) or call 312-321-6834 for further assistance.

To best prepare for a session with a genetic counselor, it may be helpful to review the following resources and create a list of questions:

FSHD Society: https://www.fshdsociety.org/
FSH-Muscular Dystrophy Support Group: https://fsh-group.org/
Muscular Dystrophy Association: https://www.mda.org/disease/facioscapulohumeral-muscular-dystrophy
FSHD Global Research Foundation LTD: https://fshdglobal.org/

MedlinePlus: This website can be used to find information about genetic topics, genes, specific genetic conditions, and broad topics like autism spectrum disorder. It also provides links to other websites for more in-depth information about genetic conditions, patient support and advocacy resources, and relevant clinical trials. https://medlineplus.gov/genetics

Methodology, References & Limitations

Methodology: The Bionano EnFocus™ FSHD Analysis is performed using an optical genome mapping system. Based on specific labeling and mapping of ultra-high molecular weight DNA in nanochannel arrays, optical genome mapping enables high resolution analysis of the D4Z4 repeat array. Molecules aligning to regions of interest in chr4 and chr10 are extracted and assembled. The resulting consensus maps are used for the Bionano EnFocus™ FSHD Analysis. The repeat arrays are sized, and the permissive and non-permissive alleles (4qA and 4qB) assigned. The haplotype must have a minimum of 4x repeat-spanning coverage. Additional copy number losses of the *SMCHD1* gene, or structural variants in the proximity of the D4Z4 repeat array on chr4 or chr10 are noted for informational purposes only, and follow-up testing to confirm potential pathogenic variants is recommended. The result data are imported into Bionano Access, a graphical user interface tool for visualization and curation. Genomic assessment is based on GRCh38/hg38.

Analytical validation of the Bionano EnFocus™ FSHD Analysis included comprehensive assessment of performance characteristics in FSHD1 positive (contracted/permissive 4qA) samples and FSHD1 negative (non-contracted/4qA or 4qB) samples. Overall accuracy, Positive Predictive value, Negative Predictive Value was >99%. Repeatability of the assay was 100%. Reproducibility of the assay was 100%. Limit of detection of pathogenic contracted 4qA allele was achieved at 17% Variant Allele Fraction (VAF).

References: PMID: 11761483, 1363881, 8733043, 30666819, 20724583, 34384893.

Limitations: The current size limitation for detection of copy number changes overlapping *SMCHD1* is ≥ 500 kbp. The interpretation of the D4Z4 repeats is based on our current understanding of the genome. These interpretations may change over time as more information about this gene becomes available. Possible sources of testing error include rare and novel genetic variants that interfere with analysis, sample misidentification, and other sources. Rare rearrangements involving 4qter and 10qter, that could impact diagnosis, are unlikely to be identified by this assay. This method cannot detect single-nucleotide variants or other small variants with potential functional impacts.

This test was developed and performed by Bionano Laboratories (9540 Towne Centre Drive, Suite 155, San Diego, CA 92121). CA License Number: CLR-90004882, CLIA ID: 05D2235036, CAP ID: 9336423.

Pursuant to the requirements of CLIA '88, this technical component and the professional component was performed by Bionano Laboratories. This test is used for clinical purposes. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). This test was developed and its performance characteristics determined by Bionano Laboratories. It has not been cleared or approved by the US Food and Drug Administration.

XXXXXXXXXX, PhD, FACMG – Clinical Laboratory Director, Bionano Laboratories

Report Electronically Signed By: XXXXXXXXXX

Remote Site Location: XX-XXXXX-XX