LABORATORY REPORT

Name	:Master SAFFAN KHAN	Sex/Age	: Male/3 Years 6 Months
Ref By	:		:

DUCHENNE MUSCULAR DYSTROPHY(DMD) ANALYSIS BY MLPA

Clinical History Available:

Referred for DMD testing

Test Characteristics

Copy number changes in targeted regions of the *DMD* gene are identified by hybridizing with MLPA (Multiplex Ligation dependent Probe Amplification) probes.

TEST RESULTS AND INTERPRETATION

NO DELETIONS OR DUPLICATIONS DETECTED IN *DMD* GENE

(Fig:1&2 in Appendix 1)

Summary-

Duchenne muscular dystrophy (DMD) (OMIM*300377) is a recessive, X-linked disorder caused by mutations in the dystrophin (*DMD*) gene located on the human X chromosome (Xp21.2). The gene encodes a protein dystrophin that is a part of the dystroglycan complex (DGC), which provides structural stability to the muscle tissue. The disorder, characterized by progressive muscle weakness and atrophy, occurs at a frequency of about 1 in 3,500 almost exclusively in males. More than 1,000 mutations in the *DMD* gene have been identified in people with the Duchenne and Becker forms of Muscular Dystrophy. Most of the mutations that delete or duplicate part of the *DMD* gene will prevent any functional dystrophin protein from being produced, leading to pathogenicity.

For this patient, no deletions or duplications were detected in abnormal range, within the detection limits of MLPA, in the *DMD* gene (Fig: 1&2).

Recommendations:

- Genetic counseling is advised.
- A clinical correlation is recommended.
- *DMD* gene sequencing is recommended.

Dr. Mehul Mistri
Ph. D. (Scientist)

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Technical Note:

Methodology:

Copy number changes in targeted regions of the *DMD* gene were identified by hybridizing with MLPA (Multiplex Ligation dependent Probe Amplification) probes. Each MLPA probe consists of two hemi-probes that bind to adjacent sites on the target sequence. Upon ligation and subsequent PCR amplification, each distinct MLPA probe (specific to distinct target regions) generates an amplicon with a unique length which are separated and quantified by capillary electrophoresis. Deletions of a probe's recognition sequence on the X-chromosome will lead to a complete absence of the corresponding probe amplification product in males. Heterozygous deletions within target sequences will prevent efficient probe binding and give a 35-50% reduced relative peak area of the amplification product specific to that probe set. Copy number differences of various exons between test and control DNA samples can be detected by analyzing the MLPA peak patterns.

LIMITATIONS:

- The MLPA test will not detect the point mutations in the *DMD* gene, which are the second most common cause of genetic defects in the *DMD* gene. It is therefore recommended to use MLPA in combination with sequence analysis.
- A point mutation or polymorphism in the sequence detected by a probe, which results in reduced probe binding efficiency, can also cause a reduction in relative peak area. Therefore, single exon deletions detected by MLPA should always be confirmed by other methods like multiplex PCR or sequencing.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect most inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region do exist but remain undetected.
- Although all precautions are taken during Molecular Genetic testing the currently available data indicate that the technical error rate for all types of Molecular DNA analysis is approximately 2%.
- This test has not been validated by the FDA. This report is for research purposes only, not for use in clinical diagnostic or therapeutic applications.

For specimens received from non NCGM locations, it is presumed that it belongsto the patient as identified on the labels of the container/Test Requisition Formand it has been verified as per GCLP (Good Clinical Lab Practices) by the referrer atthe time of collection of the specimen. NCGM's responsibility is limited to theanalytical part of the assay performed.

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APPENDIX-1

DMD-MLPA Result Figures

Fig-1: 51122803488_SAFFAN_RATIO CHART: DMD probemix 1

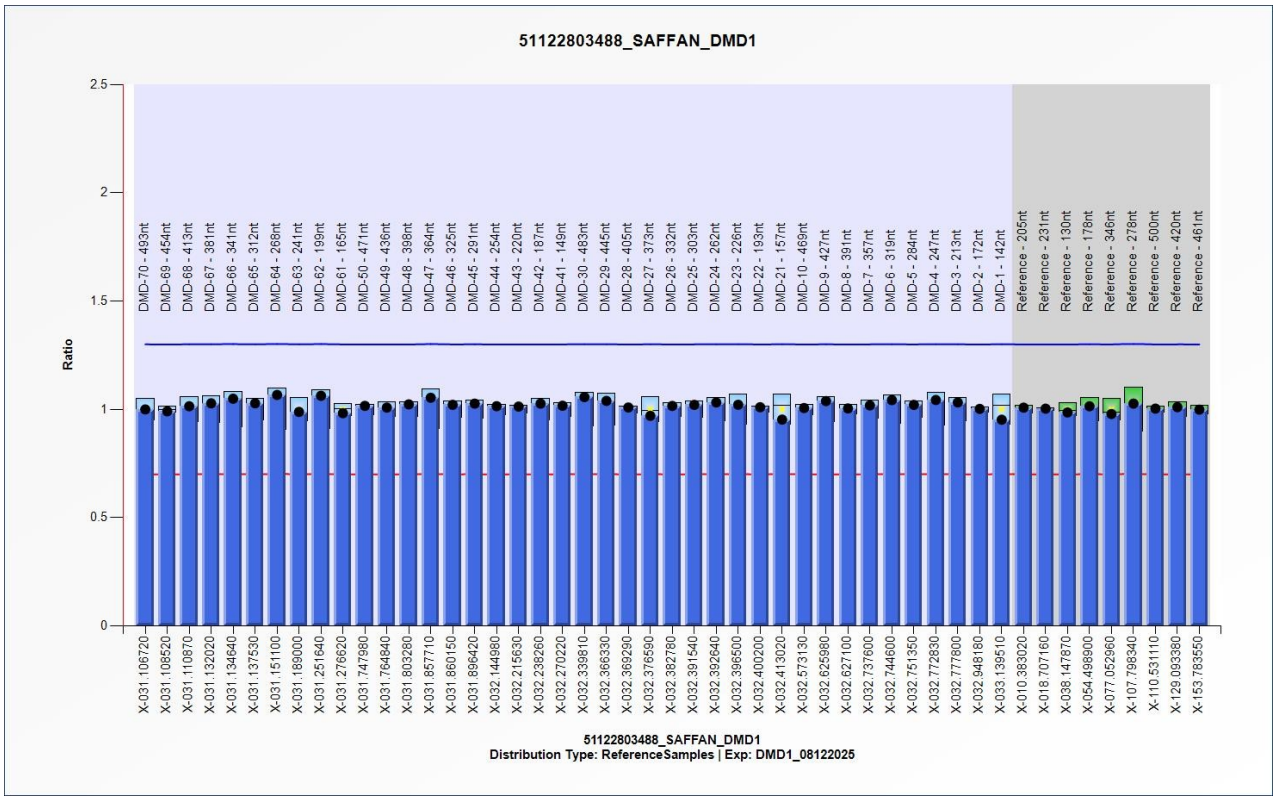

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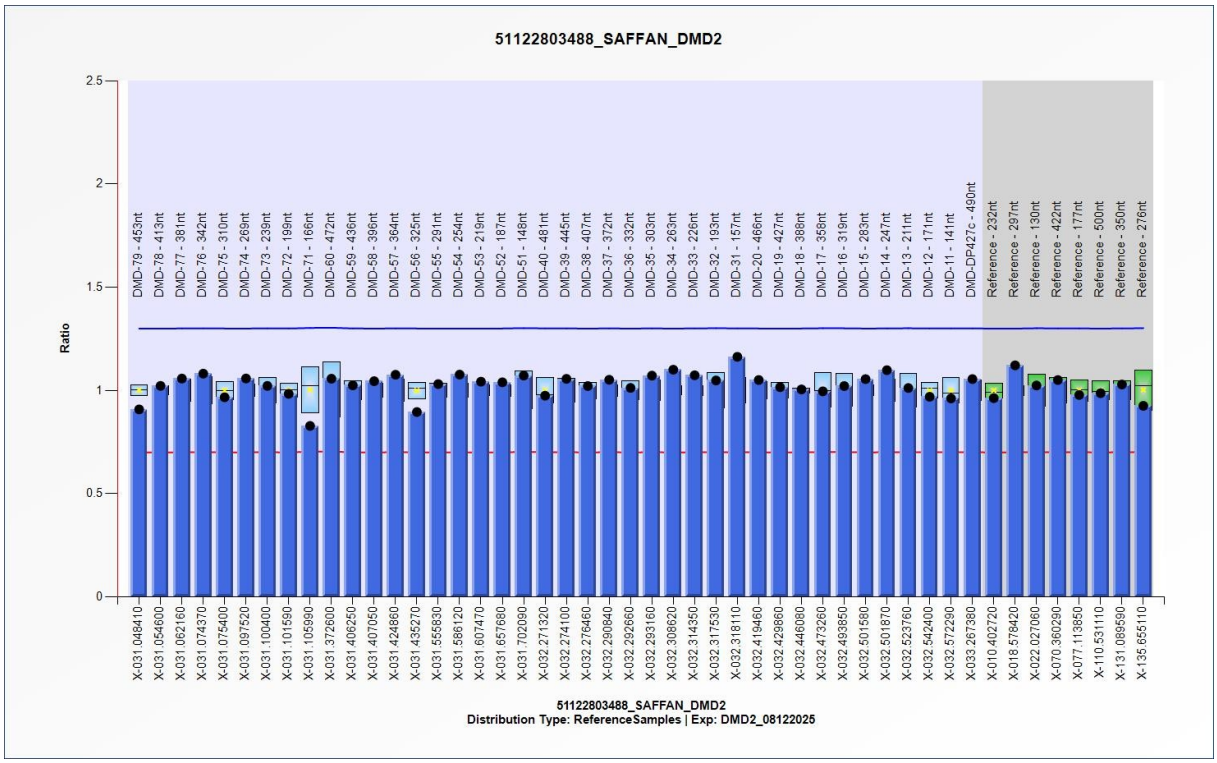
Fig-2: 51122803488_SAFFAN_RATIO CHART: DMD probemix 2



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