DEPARTMENT OF GENOMICS AND MOLECULAR DIAGNOSTICS

DMD ANALYSIS BY MLPA

Referral Reason

Master Shreyansh born of non consanguineous marriage reported with undescended testis,neonatal hypoglycemia with macroglossia ,facial dysmorphism with frontal bossing,thin eyebrowline,epicanthic folds,depressed nasal bridge,long smooth philthrum,fleshy ear lobes,micrognathia,mild clinodactyly,mild braccydatyly of toes.He was referred for genetic testing for ***DUCHENNE MUSCULAR DYSTROPHY (DMD).***

Test Result

No deletion/duplications detected in the *DMD* gene of this individual.

Interpretation

This individual is found to be normal for the deletions/duplications for all the 79 exons tested for DMD gene.

NOTE:Negative result does not rule out the DMD.Mutations detectable by sequence analysis(SNPs) are found in approximately one-third of DMD cases and in approximately 20% of BMD cases. In DMD cases, the vast majority of these mutations result in premature protein termination whereas missense mutations are rare (http://www.LOVD.nl/DMD).

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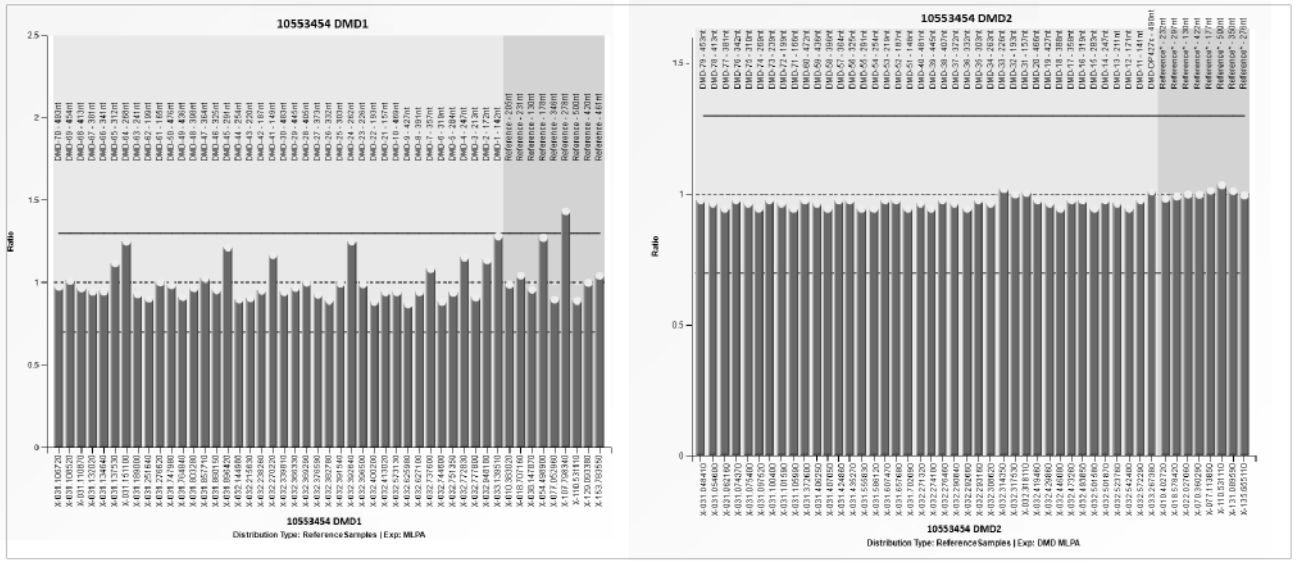

Fig1: Probe distribution ratio for 10553454

Recommendations

- Genetic Counselling is recommended.
- Genetic evaluation (WES and CMA) is recommended

Methodology

Multiplex Ligation-dependent probe amplification (MLPA) method is used for detection of deletion and duplication in 79 exons on DMD gene using SALSA MLPA probe mix available from MRC (Holland). Coffalyser.Net software is used for data analysis. The exon numbering used in this P034-B2 DMD-1 and P035-B1 DMD-2 product description is the exon numbering from the RefSeq transcript NM_004006.2, which is identical to the LRG_199 sequence.

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Clinical features

The dystrophinopathies include Duchenne muscular dystrophy (DMD, OMIM 310200), Becker muscular dystrophy (BMD, OMIM 300376), and dilated cardiomyopathy, type 3B (CMD3B, OMIM 302045). Duchenne muscular dystrophy is the most common form of congenital muscular dystrophy in all ethnic groups. DMD presents in affected boys in early childhood with difficulty walking and climbing stairs, progressive proximal weakness, pseudohypertrophy of the calves, and greatly elevated serum creatine phosphokinase (CK) levels (Darras et al. 2011). Patients become wheelchair dependent by age 13. Dilated cardiomyopathy and congestive heart failure occur in nearly all patients by their late teens or early 20s. DMD muscle biopsies show a dystrophic process with fibrosis and fatty infiltration. Dystrophin protein content in DMD patients is less than 5% of controls when measured by Western blot or immunohistochemistry (Hoffman et al. 1988).

Becker muscular dystrophy is a relatively mild form of dystrophinopthy with later onset of proximal weakness and preservation of ambulation into the third decade of life. The minimum age for wheelchair dependency in BMD is age 16. As in DMD, cardiomyopathy is a significant cause of morbidity, however, serum CK levels are less elevated than in DMD (Zatz et al. 1991). Dystrophin protein content in BMD patients varies from 20% of control levels to normal levels when measured by Western blot or immunohistochemistry (Hoffman et al. 1988). Both DMD and BMD can be associated with intellectual deficits, involving working memory and executive function (Darras et al. 2011).

DMD-related cardiomyopathy in the absence of skeletal muscle disease is a less common form of dystrophinopathy which presents in affected males between 20 and 40 years of age and later in carrier females (Beggs 1997). In some mild BMD patients, cardiomyopathy can be the presenting feature (Towbin 1998). Females who are heterozygous for a DMD causative mutation are at risk for dilated cardiomyopathy and 70% have slightly elevated CK (Schade van Westrum et al. 2011). Carriers may also present with a myopathy resembling limb-girdle muscular dystrophy (Moser and Emery 1974).

The dystrophinopathies are inherited as X-linked recessive disorders caused by DMD gene located at Xp21 which encodes dystrophin. Heterozygous female carriers are at increased risk for dilated cardiomyopathy. Approximately two-thirds of the mutations in DMD patients are deletions of one or more exons in the DMD gene. The occurrence of deletions is slightly higher in BMD patients. Duplications are found in approximately 10% of DMD patients and 20% of BMD patients. In general, in-frame deletions, which preserve partial functional dystrophin protein, are correlated with a milder (BMD) clinical phenotype (Monaco et al. 1988; Aartsma-Rus et al. 2006).

Limitations: DMD base pair substitutions, small deletions/duplications, deep intronic, and regulatory region mutations will not be detected. Breakpoints for large deletions/duplications will not be determined. Diagnostic errors can occur due to rare sequence variation.

Disclaimer

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- Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Errors in our interpretation of results may occur if information given is inaccurate or incomplete.
- This test doesn't detect SNPS in the DMD gene.
- Although all precautions are taken while conducting these tests, there is a standard error rate of approximate 1% in all genetic tests and this should be taken into consideration before any clinical decision.
- It is presumed that the specimen used to perform the test belongs to the patient specified above, such verification having been carried out at the collection level of sample.
- Diagnostic errors can occur due to rare sequence variations. Regulatory region mutations, large gene deletions/duplications and some deep intronic mutations will not be detected.

References

1. Katharina J Hoff (2009): The effect of sequencing errors on metagenomic gene prediction. BMCGenomics, 10:520.
2. Hoffman, E. P. et al. Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. N. Engl. J. Med. (1988). doi:10.1056/NEJM198805263182104
3. Muntoni F. et al. (1993). Deletion of the Dystrophin Muscle-Promotor region associated with X- linked dilated cardiomyopathy. N EnglJ Med 329:921-925.
4. Dastur RS et al. (2011). Identification of deletions and duplications in the DMD gene and female carrier status in western India using combined methods of multiplex polymerase chain reaction and multiplex ligationdependent probe amplification. Neurol India 59:803-809.
5. McKusick V.A., Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders. Baltimore: Johns Hopkins University Press (12th edition), 1998

***** End Of Report *****

Suggested clinical correlation & follow up

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