Clinical Exome Sequencing Analysis

Patient name	:	**Master. XXX**	**PIN**	:	**AND24400007852**
Gender/ Age	:	**Male/ 9 years**	**Sample number**	:	**402406914**
Hospital/Clinic	:	**ESIC Hospital - Tirunelveli**	**Sample collection date**	:	**14.06.2024**
Specimen	:	**Peripheral Blood**	**Sample receipt date**	:	**14.06.2024**
			Report date	:	**10.07.2024**

Clinical history

Proband, Master. XXX was born to non-consanguineous parents. He is presented with delayed milestones. His CPK levels are elevated ≈2000 IU/l. He has an asymptomatic brother. Proband, Master XXX is suspected to be affected with Duchenne Muscular Dystrophy/ Becker Muscular Dystrophy/ Congenital muscular dystrophy and has been evaluated for pathogenic variations.

Results

Pathogenic Copy Number Variant was identified

List of significant Copy Number Variant identified related to phenotype:

Chromosome	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
Chromosome X	chrX:g.(?_32287529)_(32595876_?)del	Deletion	Hemizygous	Becker muscular dystrophy **(OMIM#300376)** Duchenne muscular dystrophy **(OMIM#310200)**	**Pathogenic**	X linked Recessive

*Genetic test results are based on the recommendation of American college of Medical Genetics [1].
No other variant that warrants to be reported for the given clinical indication was identified.

NGS based assays to detect large duplication is low and an alternate method of testing like MLPA/Microarray/PCR is recommended to confirm the same.

Interpretation

CNV deletion chrX:g.(?_32287529)_(32595876_?)del

CNV summary: On *in silico* CNV analysis, a hemizygous deletion of size [~308.3Kb], on chromosome X chrX:g.(?_32287529)_(32595876_?)del encompassing exons 13 to 43 of the *DMD* gene suggestive of a copy number variant was detected. The precise breakpoint cannot be determined by this methodology.

CNV score: *In-silico* analysis identified Z score of ~-5.1, which suggestive of a hemizygous deletion of this region. The coverage and depth of these regions are sufficiently targeted in this assay.

Literature Evidence: The hemizygous deletion of *DMD* exons 13–37 has been previously reported in patients affected with Duchenne/Becker muscular dystrophy [3].

OMIM phenotype: Becker muscular dystrophy (OMIM#300376) and Duchenne muscular dystrophy (OMIM#310200) are caused by mutation in the *DMD* gene (OMIM*300377). These diseases follow X linked recessive pattern of inheritance [2]. The muscular dystrophy that carries the Becker eponym is similar to Duchenne muscular dystrophy in the distribution of muscle wasting and weakness, which is mainly proximal, but the course is more benign, with age of onset around 12 years; some patients have no symptoms until much later in life. Loss of ambulation also varies from adolescence onward, with death usually in the fourth or fifth decade. In some cases, as in Duchenne muscular dystrophy, a degree of mental impairment is present.

Variant classification: Based on the above evidence, this copy number variant is classified as a pathogenic and has to be carefully correlated with the clinical symptoms.

The specificity of NGS based assays to detect large deletion is low and an alternate method of testing like MLPA /Microarray/PCR is recommended to confirm the same.

Recommendations

- **NGS based assays to detect large duplication is low and an alternate method of testing like MLPA/Microarray/PCR is recommended to confirm the same.**
- Sequencing the variant(s) in the parents and the other affected and unaffected members of the family is recommended to confirm the significance.
- Genetic counselling is advised

Methodology

DNA extracted from the blood, was used to perform targeted gene capture using a custom capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using Illumina sequencing platform. This kit has deep exonic coverage of all the coding regions including the difficult to cover regions. The sequences obtained are aligned to human reference genome (GRCh38.p13) using Sentieon aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon DNAscope has been used to call the variants. Detected variants were annotated and filtered using the VarSeq software with the workflow implementing the ACMG guidelines for variant classification. The variants were annotated using 1000 genomes (V2), gnomAD (v3.1,2.1.1), ClinVar, OMIM, dbSNP, NCBI RefSeq Genes. In-silico predictions of the variant was carried out using VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

Sequence data attributes

Total reads generated	6.04 Gb
Data ≥ Q30	97.36%

Genetic test results are reported based on the recommendations of American College of Medical Genetics [1], as described below:

Classification	Interpretation
Pathogenic	A disease-causing variation in a gene which can explain the patients' symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed
Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non- disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

Disclaimer

- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants are not assessed using this assay.
- Certain genes may not be covered completely, and few mutations could be missed. Variants not detected by this assay may impact the phenotype.
- The variations have not been validated by Sanger sequencing.
- The above findings and result interpretation was done based on the clinical indication provided at the time of reporting.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.
- Incidental or secondary findings that meet the ACMG guidelines can be given upon request [4].

References

1. Richards, S, et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine: official journal of the American College of Medical Genetics. 17.5 (2015): 405-424.
2. Amberger J, Bocchini CA, Scott AF, Hamosh A. McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic Acids Res. 2009 Jan;37(Database issue):D793-6. doi: 10.1093/nar/gkn665. Epub 2008 Oct 8.
3. Lee BL, et al. Genetic analysis of dystrophin gene for affected male and female carriers with Duchenne/Becker muscular dystrophy in Korea. J Korean Med Sci. 2012 Mar;27(3):274-80. doi: 10.3346/jkms.2012.27.3.274. Epub 2012 Feb 23. PMID: 22379338; PMCID: PMC3286774.
4. Kalia S.S. et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med., 19(2):249-255, 2017.

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