Clinical Exome Sequencing Analysis

Patient name	:	**Master. XXX**	**PIN**	:	**XX**
Gender/ Age	:	**XX**	**Sample number**	:	**XX**
Referring clinician	:	**XX**	**Sample collection date**	:	**XX**
Hospital/Clinic	:	**XX**	**Sample receipt date**	:	**XX**
Specimen	:	**Peripheral Blood**	**Report date**	:	**XX**

Clinical history

Proband Master. XXX was first born male child to non-consanguineous parents. He was presented with chief complaints of macrocephaly (head circumference - 53cm), walking difficulty from 4.5 years of age, frequent falls, difficulty in climbing stairs, slurry speech of certain syllables, calf muscle hypertrophy of both legs, tip toe walking, broad based gait and deep tendon reflexes. His serum CPK was indicative of 400 mcg/l. Proband parents had a bad obstetric history of G2: IUD of female fetus and G3: Miscarriage at 8 weeks GA. He is suspected to be affected with limb girdle muscular dystrophy. Proband Master. XXX has been evaluated for pathogenic variations.

Results

Likely pathogenic variant causative of the reported phenotype was detected

List of significant variant identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
DMD (-)	Exon 21	c.2646delT **(p. Leu883PhefsTer8)**	Hemizygous	Duchenne muscular dystrophy **(OMIM#310200)**	**Likely 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.

Interpretation

DMD: c.2646delT

Variant summary: A hemizygous single base pair deletion in exon 21 of the *DMD* gene (chrX:g.32485076delA, NM_004006.3, Depth: 93x) that results in a frameshift and premature truncation of the protein 8 amino acids downstream to codon 883 (p. Leu883PhefsTer8) was detected. This variant is a frameshift variant which occurs in an exon of *DMD* upstream where nonsense mediated decay is predicted to occur. This variant is predicted to cause loss of normal protein function through protein truncation.

Population frequency: This variant has not been reported in gnomAD database and 1000 genomes database.

***In-silico* prediction:** The *in-silico* predictions of the variant are damaging by MutationTaster2.

OMIM phenotype: Duchenne muscular dystrophy (OMIM#310200) is caused by mutations in the *DMD* gene (OMIM*300377). Dystrophin-associated muscular dystrophies range from the severe Duchenne muscular dystrophy (DMD) to the milder Becker muscular dystrophy. Mapping and molecular genetic studies showed that both are the result of mutations in the huge gene that encodes dystrophin, also symbolized DMD. Approximately two-thirds of the mutations in both forms are deletions of one or many exons in the dystrophin gene. Although there is no clear correlation found between the extent of the deletion and the severity of the disorder, *DMD* deletions usually result in frameshift. This disease follows X-Linked recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a likely pathogenic variant. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed**.

Recommendations

- Sequencing the variant(s) in the parents and the other affected and unaffected members of the family is recommended to confirm the significance.
- Alternative test is strongly recommended to rule out the deletion/duplication.
- 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 GenoLab M 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 (3.1.2,2.1.1), 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	10.94 Gb
Data ≥ Q30	97.70%

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 and CNV 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 [3].

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. 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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