Whole Exome Sequencing Analysis

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

Clinical history

Proband, XX is first born to non-consanguineous parents. He is a term baby presented with chief complaints of gower's sign, difficulty in climbing stairs and jumping, from 7 years of age. His MLPA analysis in *DMD* gene indicative of no deletions or duplications. He has a younger brother who is alive and well. Proband, XX is suspected to be affected with Duchenne Muscular Dystrophy and has been evaluated for pathogenic variations.

Results

Likely compound heterozygous variants were identified in *CAPN3* gene

List of uncertain significant variants identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
CAPN3 (+)	Exon 21	c.2243G>T **(p.Arg748Leu)**	Heterozygous	Muscular dystrophy, limb-girdle, autosomal recessive 1 **(OMIM#253600)**	**Likely Pathogenic**	Autosomal Recessive
	Exon 24	c.2443C>G **(p.Leu815Val)**	Heterozygous		**Uncertain Significance**	

*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

Likely compound heterozygous variants were identified in *CAPN3* gene

Variant 1: *CAPN3*: c.2243G>T

Variant summary: A heterozygous missense variation in exon 21 of the *CAPN3* gene (chr15:g.42410646G>T, NM_000070.3, Depth: 125x) that results in the amino acid substitution of Leucine for Arginine at codon 748 (p.Arg748Leu) was detected.

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

Literature evidence: A missense variant in the same amino acid position (c.2243G>A; p.Arg748Gln) has been previously classified as Pathogenic significance in ClinVar database [3]. A missense variant in the same amino acid position (c.2243G>A; p.Arg748Gln) has been previously reported in a patient affected with Limb Girdle Muscular Dystrophy Type 2A in homozygous state [4].

***In silico* predictions:** The *in-silico* predictions of the variant are Deleterious by CADD. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

Variant classification: Based on the evidence, this variant is classified as 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.**

Variant 2: *CAPN3*: c.2443C>G

Variant summary: A heterozygous missense variation in exon 24 of the *CAPN3* gene (chr15:g.42411750C>G, NM_000070.3, Depth: 72x) that results in the amino acid substitution of valine for leucine at codon 28583 (p.Ser28583Pro) was detected.

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

Clinical evidence: This variant has been previously classified as uncertain significance in ClinVar database [5].

***In silico* predictions:** The *in-silico* predictions of the variant are damaging by SIFT, Polyphen-2 (HumDiv) and MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

Variant classification: Based on the evidence, this variant is classified as variant of uncertain significance. **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.**

OMIM phenotype: Muscular dystrophy, limb-girdle, autosomal recessive 1 (OMIM#253600) is caused by homozygous or compound heterozygous mutation in the *CAPN3* gene (OMIM*114240). Autosomal recessive limb-girdle muscular dystrophy-1 affects primarily the proximal muscles, resulting in difficulty walking. The age at onset varies, but most patients show onset in childhood, and the disorder is progressive. Other features may include scapular winging, calf pseudohypertrophy, and contractures .This disease follows autosomal recessive pattern of inheritance [2].

The variants are reported to be in likely compound heterozygous state. These likely compound heterozygous variants are strongly recommended to confirm the cis or trans status by parental segregation analysis.

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 recommended.

Methodology

DNA extracted from the blood was used to perform whole exome using whole exome 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 (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	10.82Gb
Data ≥ Q30	93.04%

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 [6].

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. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000128570.63
4. Fadaee M, et al. Report of limb girdle muscular dystrophy type 2a in 6 Iranian patients, one with a novel deletion in *CAPN3* gene. Neuromuscul Disord. 2016 Apr-May;26(4-5):277-82. doi: 10.1016/j.nmd.2016.02.003. Epub 2016 Feb 15. PMID: 27020652.
5. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV002506052.1.
6. 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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