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 was born to endogamous parents. He is presented with chief complaints of difficulty in climbing stairs from 21 years of age, gower's sign, difficulty in walking, frequent falls and has proximal hand muscle weakness and distal leg muscle weakness. He has a history of fall in 2021. His MRI spine indicative of inflammatory myositis sequel. His EMG suggestive of myopathic changes. His MRI bilateral thighs indicative of marked fatty atrophy in bilateral hamstring and adductor muscles. He had elevated serum CPK levels, 6,900 U/L. His MLPA analysis in *DMD* gene revealed no deletions/duplications. Bilateral OC and CC suggestive of Sensorimotor Polyradiculoneuropathy. He has a younger brother, alive and well. Proband, XX is suspected to be affected with muscular dystrophy and has been evaluated for pathogenic variations.

Results

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
CAPN3 (+)	Exon 10	c.1319G>A **(p.Arg440Gln)**	Homozygous	Muscular dystrophy, limb-girdle, autosomal recessive 1 **(OMIM#253600)**	**Pathogenic** (PM2, PP5, PP3, PS3, PM1, PM5, PP2)	Autosomal 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

***CAPN3*: c.1319G>A**

Variant summary: A homozygous missense variation in exon 10 of the *CAPN3* gene (chr15:g.42399617G>A, NM_000070.3, Depth: 184x) that results in the amino acid substitution of Glutamine for Arginine at codon 440 (p.Arg440Gln) was detected.

Population frequency: This variant has a minor allele frequency of 0.0164% in gnomAD database and has not been reported in 1000 genomes databases.

Clinical and Literature evidence: This variant has been previously classified as pathogenic in ClinVar database [3]. This variant has been previously reported in patient affected with limb-girdle muscular dystrophy 2A in compound heterozygous state [4].

***In-silico* prediction**: 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.

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].

Variant classification: Based on the evidence, this variant is classified as a 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.
- Sanger sequencing is strongly recommended to rule out false positives.
- Genetic counselling is advised.

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 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 (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	15.66 Gb
Data ≥ Q30	91.14%

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 [5].

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/VCV000217147.43
4. Fanin M, et al. How to tackle the diagnosis of limb-girdle muscular dystrophy 2A. Eur J Hum Genet. 2009 May;17(5):598-603. doi: 10.1038/ejhg.2008.193. Epub 2008 Oct 15. PMID: 18854869; PMCID: PMC2986267.
5. 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.

This report has been reviewed and approved by:

Sivasankar.S, Ph.D Molecular Biologist	Muthukumaran. S, Ph.D Clinical Bioinformatician	Sachin. D.Honguntikar, Ph.D, Molecular Geneticist	Dr. G. Suriyakumar Director

Appendix I

Gene list based on phenotypes used for screening of pathogenic and likely pathogenic variants:

ANO5, B4GAT1, CAPN3, CAV3, CAVIN1, CHKB, DAG1, DES, DMD, DNAJB6, DPM1, DPM3, DYSF, EMD, FHL1, FKRP, FKTN, GOSR2, ITGA7, LAMA2, LARGE1, LMNA, MYOT, PNPLA2, POMGNT1, POMGNT2, POMT1, POMT2, SGCA, SGCB, SGCD, SGCG, SMN1, SMN2, SYNE1, SYNE2, TCAP, TMEM43, TRIM32, TTN