Clinical Exome Sequencing Analysis

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

Clinical history

Proband, XXX presented with history of weakness in both upper and lower limbs, vision problem since childhood around 10 years of age. He is suspected to be affected with Peripheral Neuropathy, Charcot-Marie-Tooth Hereditary Neuropathy or Spinal muscular atrophy. Proband, XXX has been evaluated for pathogenic variations.

Results

Likely pathogenic variant was identified in *MFN2* gene

List of significant variant identified related to phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
MFN2 (+)	Exon 7	c.637A>T **(p.Ile213Phe)**	Heterozygous	Charcot-Marie-Tooth disease, axonal, type 2A2A **(OMIM#609260)** Hereditary motor and sensory neuropathy VIA **(OMIM#601152)**	**Likely pathogenic**	Autosomal Dominant

				Charcot-Marie-Tooth disease, axonal, type 2A2B **(OMIM#617087)** Lipomatosis, multiple symmetric, with or without peripheral neuropathy **(OMIM#151800)**		Autosomal Recessive

List of significant carrier variants identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern	Literature
RNASEH2B (+)	Exon 7	c.529G>A **(p.Ala177Thr)**	Heterozygous	Aicardi-Goutieres syndrome 2 **(OMIM#610181)**	**Likely Pathogenic**	Autosomal Recessive	Clinvar - 1262 Pubmed ID 34042169
CFTR (+)	Exon 20	c.3197G>A **(p.Arg1066His)**	Heterozygous	Cystic fibrosis **(OMIM#219700)** Congenital bilateral absence of vas deferens **(OMIM#277180)**	**Likely Pathogenic**	Autosomal Recessive	Clinvar- 7158 Pubmed ID 10922396

*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

***MFN2:* c.637A>T**

Variant summary: A heterozygous missense variation in exon 7 of the *MFN2* gene (chr1:11998807A>T, NM_014874.4, Depth: 139x) that results in the amino acid substitution of Phenylalanine for Isoleucine at codon 213 (p.Ile213Phe) was detected.

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

Clinical and Literature evidence: A missense variant in the same amino acid position (p.Ile213Thr) has been previously reported as pathogenic in ClinVar database for the Charcot-Marie-Tooth disease type 2 condition [3]. A missense variant in the same amino acid position (p.Ile213Thr) has been previously reported in patient affected with Hereditary motor and sensory neuropathy in heterozygous state [4].

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

OMIM phenotype: Charcot-Marie-Tooth disease, axonal, type 2A2A (OMIM#609260) and Hereditary motor and sensory neuropathy VIA (OMIM#269880) are caused by heterozygous mutation in the *MFN2* gene (OMIM*608507). These diseases follow autosomal dominant pattern of inheritance [2]. Charcot-Marie-Tooth disease, axonal, type 2A2B (OMIM#617087) and Lipomatosis, multiple symmetric, with or without peripheral neuropathy (OMIM#151800) is caused by homozygous or compound heterozygous mutation in the *MFN2* gene (OMIM*608507). These diseases follow autosomal 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.**

Additional Variant(s)

The additional variants identified may or may not be related to patient's phenotype. Phenotype – genotype correlation is recommended.

List of additional variants identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
PDE11A (-)	Exon 4	c.1192G>T **(p.Glu398Ter)**	Heterozygous	Pigmented nodular adrenocortical disease, primary, 2 **(OMIM#610181)**	**Likely Pathogenic**	Autosomal Dominant

Recommendations

- The *CFTR* gene has pseudogene in the human genome. Validation of the variant(s) by Sanger sequencing is strongly recommended to rule out false positives.
- Sequencing the variant(s) in the partner 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 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	5.01 Gb
Data ≥ Q30	95.74%

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/VCV000575481.8
4. Yang, Kai, et al. "Metabolic and biophysical study of the *MFN2*^{Ile213Thr} mutant causing Hereditary Motor and Sensory Neuropathy (HMSN)." American Journal of Translational Research 13.10 (2021): 11501. PMID: 34786076.

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.

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