Whole Genome Sequencing Analysis

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

Clinical history

Proband, XXX is born of endogamous marriage. She is presented with chief complaints of left-hand tremors and slowness of movements for past 8 months, dopamine deficiency and hypercobalaminemia. She has a history of Dengue two years back. Her TRODAT Brain SPECT/MRI Hybrid fusion study revealed presynaptic dopaminergic dysfunction on both sides (R>L), features consistent with presynaptic Parkinsonian syndrome (Young onset Parkinsonism). Her EEG revealed abnormal findings, shows potentially epileptogenic activity. Her NCS study revealed distal latencies prolonged in both peroneal and tibial nerves, CMAP amplitude are reduced in both peroneal nerves, F wave latencies are prolonged in both lower limbs, SNAPs are within normal limits in both sural nerves, findings suggestive of motor radiculo neuropathy. She has a brother and two children, alive and well. Proband, XXX has been evaluated for pathogenic variations.

Results

Heterozygous likely pathogenic variant was detected

List of significant variant identified related to phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
#*PINK1* (+)	Exon 5	c.1000_1004delinsACCAAC **(p.Pro334ThrfsTer58)**	Heterozygous**	Parkinson disease 6, early onset **(OMIM#605909)**	**Likely pathogenic**	Autosomal Recessive**

#Sanger sequencing is strongly recommended to rule out false positives.

****This is an autosomal recessive disorder caused by bi-allelic (homozygous or compound heterozygous) pathogenic/likely pathogenic variants in the *PINK1* gene. The assay has detected a single heterozygous Likely Pathogenic variant in *PINK1* gene mentioned in the table above. No other clinically relevant variant is detected in this gene. Kindly correlate clinically.**

No significant CNVs for the given clinical indications that warrants to be reported was detected.

*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

***PINK1*: c.1000_1004delinsACCAAC**

Variant summary: A heterozygous indel variation in exon 5 of the *PINK1* gene (chr1:g.20645600_20645604delinsACCAAC, NM_032409.3, Depth: 35x) that results in a frameshift and premature truncation of the protein 58 amino acids downstream to codon 334 (p.Pro334ThrfsTer58) was detected.

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 deleterious by MutationTaster2. The reference codon is conserved across mammals in PhyloP and GERP++ tools.

OMIM phenotype: Parkinson disease 6, early onset (OMIM#605909) is caused by homozygous mutation in the *PINK1* gene (OMIM*608309). This disorder is characterized by rigidity, bradykinesia, asymmetric onset, postural instability, and favorable response to levodopa. Other variable features included resting tremor, frozen gait, sleep benefit, dystonia at onset (in 2 patients), hyperreflexia, and levodopa-induced dyskinesias. Progression was generally slow. Although patients with Parkinson disease-6 were originally found to have homozygous mutations in the *PINK1* gene, **a subset of patients have been reported with heterozygous mutations in the *PINK1* gene, suggesting that heterozygous mutations may also contribute to disease development.** This disease follows 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.**

****Autosomal recessive disorder is caused by biallelic (homozygous or compound heterozygous) pathogenic/likely pathogenic variant in the *PINK1* gene. We have detected a single heterozygous variant mentioned in the results table above. However, a second significant heterozygous variant in the *PINK1* gene was not detected. The single heterozygous variant in presence of partially matching phenotype needs to be carefully correlated. The sensitivity of NGS based assays to detect large heterozygous deletions/duplications is low and an alternate method is recommended.**

Additional Variant(s)

List of uncertain significant variant identified:

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

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
HTRA1 (PRSS11) (+)	Exon 7	c.1164G>A **(p.Met388Ile)**	Heterozygous	Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy type 2 **(OMIM#616779)**	**Uncertain Significance**	Autosomal Dominant

List of significant carrier variant identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
SPINK5 (+)	Exon 29	c.2832_2835inv **(p.Phe944_Tyr945delinsLeuTer)**	Heterozygous	Netherton syndrome **(OMIM#256500)**	**Likely Pathogenic**	Autosomal Recessive

Recommendations

- **Sanger sequencing is strongly recommended to rule out false positives.**
- 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 blood was used to perform whole genome sequencing. The sample is sequenced at a mean ~30x depth on Illumina sequencing platform. We follow the GATK best practices framework implemented in Illumina DRAGEN® to perform the variant calling. Briefly, the sequences obtained from sequencer is pre-processed and then aligned to the Human reference genome (GRCh38) using BWA aligner. The aligned reads are analyzed using DRAGEN for removing duplicates, recalibration and re-alignment of indels. Single nucleotide variants (SNVs) and small Indels identified in the sample is

annotated using VarSeq pipeline. Gene annotation of the variants is performed using VEP program against the Ensembl release 99 human gene model. A copy number algorithm is used to detect copy number variations (CNVs) in the genome and identify regions with an unexpected number of reads. Regions with number of alignments that are fewer or higher than the expected number are classified as losses or gains, respectively. Furthermore, structural variants of ~50 bases or larger are identified using the DRAGEN Structural Variant (SV) Caller.

The mitochondrial genome contains 37 genes, all of which are essential for normal mitochondrial functioning. Therefore, sequences are additionally aligned to the revised Cambridge mitochondrial reference genome (rCRS) to identify SNVs/Indel variants within the mitochondrial genome (NC_012920.1).

This assay involves the evaluation of SMN1 deletions/duplications involving exon7 and 8 in addition to pseudogenes such as *GBA* and *CYP21A2*. Repeat-expansions in 57 genes, including *AR*, *ATN1*, *ATXN1*, *ATXN2, ATXN3, ATXN7, ATXN8OS, ATXN10, CACNA1A, CNBP, CSTB, C9ORF72, DMPK, FMR1, FXN, HTT, JPH3, NOP56, PABPN1, PHOX2B, PPP2R2B, PRNP* and *TBP* are detected using the Expansion Hunter tool. Genes with long repeats (>80 repeats) detected at premutation level are recommended to be validated using an alternate method. (**Internal confirmatory testing using an orthogonal method will be done for repeats reported *FMR1, HTT, DMPK, FXN, SCA* (1,2,3, 6, 7, 12), *GBA*, *CYP21A2* genes and *SMN1* deletion).

Clinically relevant variants in both coding and non-coding regions are annotated using variants published in literature and a set of diseases databases : ClinVar, OMIM, HGMD, LOVD, DECIPHER (population CNV) and SwissVar. Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v3.1 & 2.1.1), dbSNP (GCF_000001405.38), 1000 Japanese Genome, TOPMed (Freeze_8), and Genome Asia. The effect of non-synonymous variants is predicted using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Clinically significant variants are used for interpretation and reporting.

Sequence data attributes

Total reads generated	:	142.74 Gb
Total Reads aligned	:	98.05%
Data ≥ Q30	:	97.61%

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.

The transcript used for clinical reporting generally represents the canonical transcript (MANE Select), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.

Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.

The *in-silico* predictions are based on Variant Effect Predictor (v104), [SIFT version - 5.2.2; PolyPhen - 2.2.2; LRT version (November, 2009); CADD (v1.6); Splice AI; dbNSFPv4.2] and MutationTaster2 predictions are based on NCBI/Ensembl 66 build (GRCh38 genomic coordinates are converted to hg19 using UCSC LiftOver and mapped to MT2).

Diseases databases used for annotation includes ClinVar (updated on 5082021), OMIM (updated on 5082021), HGMD (v2021.3), LOVD (Nov-18), DECIPHER (population CNV) and SwissVar.

Disclaimer

- The classification of variants of unknown significance can change over time, Anderson Diagnostics and Labs cannot be held responsible for it.
- The sensitivity of this assay to detect large deletions/duplications of more than 10 bp or copy number variants (CNV) is 70-75%. The CNVs detected have to be confirmed by alternate method.
- 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.

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