Whole Exome Sequencing Analysis

Patient name	:	**Miss. XXX**	**PIN**	:	**XX**
Gender/ Age	:	**Female / 23 Years**	**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, Miss. XXX presented with recurrent episodes of syncope/seizure. She was diagnosed with Long QT syndrome, on medication propranolol and AED's. Proband, Miss. XXX has been evaluated for pathogenic variations.

Results

Variant of uncertain significance related to the given phenotype was detected

List of uncertain significant variant identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
RYR2 (+)	Exon 8	c.490C>T **(p.Pro164Ser)**	Heterozygous	Ventricular arrhythmias due to cardiac ryanodine receptor calcium release deficiency syndrome **(OMIM#115000)** Ventricular tachycardia, catecholaminergic polymorphic, 1 **(OMIM#604772)**	**Uncertain Significance**	Autosomal Dominant

*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

***RYR2*: c.490C>T**

Variant summary: A heterozygous missense variation in exon 8 of the *RYR2* gene (chr1:g.237377349C>T, NM_001035.3, Depth: 80x) that results in the amino acid substitution of Serine for Proline at codon 164 (p.Pro164Ser) was detected.

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

Clinical and Literature evidence: This variant has been previously classified as pathogenic in ClinVar database [3]. The observed variant has previously been reported in patients affected with catecholaminergic polymorphic ventricular tachycardia [4]. This variant lies in the "Inositol 1,4,5-trisphosphate/ryanodine receptor" domain of the *RYR2* protein [5]. *RYR2* has been reported to be associated with several heart diseases including long QT syndrome (LQTS), however, the clinical phenotypes or genetic characteristics of LQTS patients with *RYR2* mutations were not clarified yet [6].

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

OMIM phenotype: Catecholaminergic polymorphic ventricular tachycardia-1 (OMIM#604772) and ventricular arrhythmias due to cardiac ryanodine receptor calcium release deficiency syndrome (OMIM#115000) are caused by heterozygous mutations in the *RYR2* gene (OMIM*180902). Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmogenic disorder of the heart characterized by a reproducible form of polymorphic ventricular tachycardia induced by physical activity, stress, or catecholamine infusion, which can deteriorate into ventricular fibrillation. Patients present with recurrent syncope, seizures after physical activity or emotional stress. Ventricular arrhythmias due to cardiac ryanodine receptor calcium release deficiency syndrome (VACRDS) is characterized by syncope and cardiac arrest. Polymorphic ventricular tachycardia and ventricular fibrillation have been documented in these patients. Symptoms generally occur with physical activity or emotional stress. These diseases follow autosomal dominant pattern of inheritance [2].

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

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 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 using1000 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	9.97 Gb
Data ≥ Q30	96.18%

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

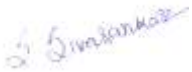
- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.
- Certain genes may not be covered completely, and few mutations 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 [7].

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/VCV002203006.1
4. Lin Y, et al. Whole exome sequencing identified a pathogenic mutation in *RYR2* in a Chinese family with unexplained sudden death. J Electrocardiol. 2018 Mar-Apr;51(2):309-315. doi: 10.1016/j.jelectrocard.2017.10.002. Epub 2017 Oct 10. PMID: 29132927.
5. https://www.ebi.ac.uk/interpro/search/text/PF08709/#table
6. Fukuyama, M., et al. "Novel *RYR2* mutations causative for long QT syndromes." *EUROPEAN HEART JOURNAL*. Vol. 38. GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND: OXFORD UNIV PRESS, 2017.

7. 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