Name: Fetus of Pooja Avinash Gongavi	**Age/Gender:** --/--
Referring Physician: Dr. Seema Amrut Sultane	**Referring Centre:** Sultanes Fetal Care Clinic-Kolhapur
Specimen Type: Amniotic Fluid	**Date of Collection:** 06.05.2022
Sample ID: 2200053717	**Date Received:** 07.05.2022
Patient ID: 1002218691	**Report Date:** 09.06.2022

SINGLE VARIANT ANALYSIS TEST REPORT

Clinical History:

The index child, Baby of Pooja Gongane, presented with refractory epilepsy, tachycardia, fever, lethargy and convulsion. His EEG showed multifocal epileptiform activity over both hemispheres with burst-suppression pattern and disorganized background back ground activity. His Clinical exome sequencing analysis revealed the presence of two heterozygous missense variant of uncertain significance in the *GABBR2* and *SCN2A* genes respectively, detailed below:

Variant 1: A heterozygous missense variant of uncertain significance in the exon 19 of the *GABBR*2 gene (c.2667G>T; hg19). Variations in the *GABBR2* gene are known to cause early infantile epileptic encephalopathy 59 and are inherited in an autosomal dominant manner. This variant is identified in Mrs. Pooja Avinash Gongavi and not detected in Mr. Avinash Gongavi.

Variant 2: A heterozygous missense variant of uncertain significance in the exon 10 of the *SCN2A* gene (c.1283A>T; hg19). Variations in the *SCN2A* gene are known to cause early infantile epileptic encephalopathy 11 and benign familial neonatal infantile seizures 3 and are inherited in an autosomal dominant manner. This variant is not detected in the Mrs. Pooja Avinash Gongavi not detected in Mr. Avinash Gongavi.

Mrs. Pooja Avinash Gongavi is currently pregnant and the Fetus of Pooja Avinash Gongavi is now being evaluated for the above gene variants using Sanger sequencing.

Results:

Gene	Clinical condition	Location	Variant detected in the index child	Status of variant tested in Fetus of Pooja Avinash Gongavi	Interpretation
GABBR2 chr9	Early Infantile epileptic encephalopathy 59	Exon 19	c.2667G>T, p.Gln889His	**Detected (Heterozygous)**	**Detailed in impression**
SCN2A chr2	Epileptic encephalopathy-11; Benign familial neonatal-infantile seizures-3	Exon 10	c.1283A>T p.Tyr428Phe	**Not Detected**	**Unaffected**

The variant analysis in Sanger sequencing is based on *GABBR2*, *SCN2A* gene reference sequences ENST00000259455.2; ENST00000357398.3; hg19 [1]. The exon number and nucleotide numbers will differ based on the reference sequence and the database used.

Note: No significant maternal contamination was detected on PCR-based VNTR analysis with a lower limitof detection of 10%.

Additional Note: *GABBR2* gene variants (c.2667G>T, p.Gln889His) were classified as Variants of Uncertain Significance (VUS) based on the NGS report. Therefore the presentation of the associated condition cannot be determined on the basis of the presence and zygosity of the variant alone and requires detailed clinical correlation.

Gene Disease association:

Developmental and epileptic encephalopathy-59 (DEE59) is caused by heterozygous mutation in the GABBR2 gene (607340) on chromosome 9q22. Developmental and epileptic encephalopathy-59 (DEE59) is characterized by severe global developmental delay apparent in infancy with onset of various types of seizures in the first months of life (range 3 to 11 months). The seizures are usually refractory and are often associated with hypsarrhythmia on EEG, although brain imaging is usually normal. More severely affected individuals may be unable to speak or walk, have poor interaction, and require a feeding tube [2].

Developmental and epileptic encephalopathy-11 (DEE11) and Benign familial neonatal-infantile seizures-3 (BFIS3) are caused by heterozygous mutation in the *SCN2A* gene (182390) on chromosome 2q24.

Developmental and epileptic encephalopathy-11 (DEE11) is a neurologic disorder characterized by onset of seizures in the first days, weeks, or months of life. Some patients may have later onset. Seizures comprise multiple types, including tonic, generalized, and myoclonic, and tend to be refractory to medication. However, some patients with onset of seizures before 3 months of age may respond to sodium channel blockers, particularly phenytoin. About half of patients become seizure-free in childhood. Affected individuals have global developmental delay, usually with severely impaired intellectual development, although some may be less severely affected and show autism spectrum disorder. Additional common features include microcephaly, hypotonia, and abnormal movements, such as dystonia, dyskinesias, and choreoathetotic movements. Brain imaging may show white matter defects. The phenotype is highly variable, even in patients with the same mutation [5].

Benign familial neonatal-infantile seizures is an autosomal dominant disorder in which afebrile seizures occur in clusters during the first year of life, without neurologic sequelae [6].

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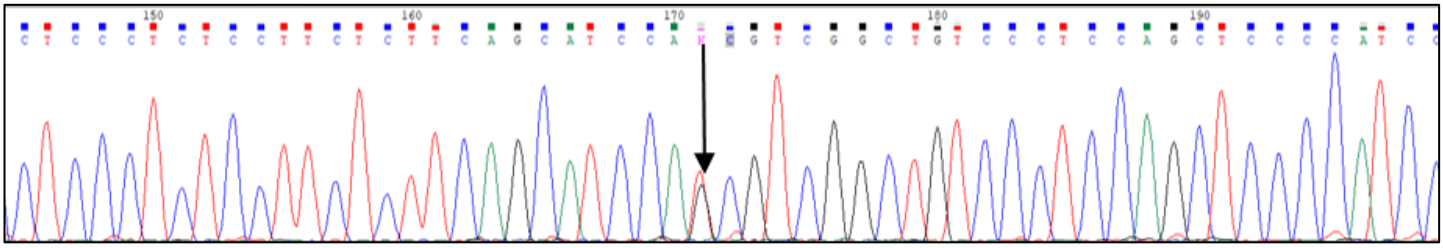
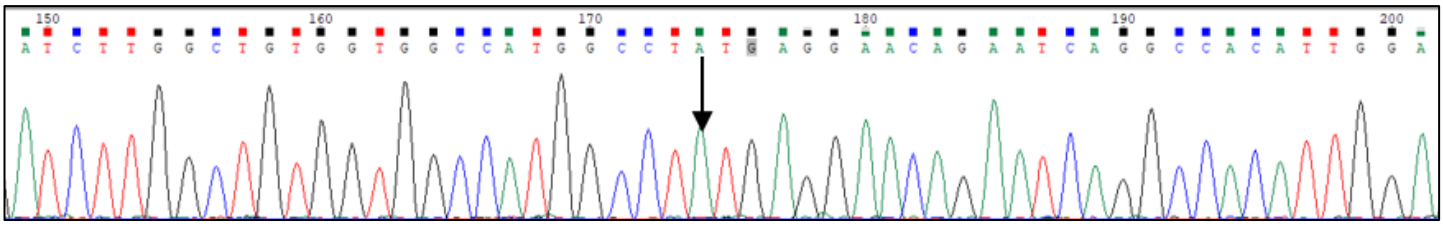
Sequence chromatogram for variant 1 (*GABBR2*; c.2667G>T) of the Fetus of Pooja Avinash Gongavi:



Sequence chromatogram for variant 2 (*SCN2A*; c.1283A>T) of the Fetus of Pooja Avinash Gongavi:



Interpretation

Variant 1: A heterozygous missense variant in exon 19 of the *GABBR2* gene (Chr9: 101053025; c.2667G>T; hg19) that results in the amino acid substitution of Histidine for Glutamine at codon 889 (p.Gln889His) was detected in index child by NGS and mother by Sanger.

The familial *GABBR2* gene variant (c.2667G>T) has been detected in heterozygous state in the Fetus of Pooja Avinash Gongavi.

Variant 2: A heterozygous missense variant in exon 10 of the *SCN2A* gene (chr2:166170518; c.1283A>T; hg19) that results in the amino acid substitution of Phenylalanine for Tyrosine at codon 428 (p.Tyr428Phe) was detected in index child by NGS.

The familial *SCN2A* gene variant (c.1283A>T) has not been detected in the Fetus of Pooja Avinash Gongavi.

Impression:

The familial *GABBR2* gene variant (c.2667G>T) has been detected in heterozygous state in the Fetus of Pooja Avinash Gongavi.

However, the heterozygous variants detected in *GABBR2* gene are Variants of Uncertain Significance (VUS), due to this it is strongly recommended that no decision on this pregnancy be taken on the basis of the reported finding.

The above result suggests that the fetus is less likely to be affected with the *SCN2A* associated phenotype (Epileptic encephalopathy-11; Benign familial neonatal-infantile seizures-3).

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Recommendations:

1. **<u>Urgent Genetic counseling</u>** and clinical correlation

Pallavi Kadam
Scientific Officer

Dr. Sam Balu
Deputy General Manager-Genomics

PRECAUTIONS: Although all precautions are taken during DNA tests, the currently available data indicate that the technical error rate of all types of DNA analysis is approximately 2%. It is important that all clinicians or persons requesting DNA diagnostic tests are aware of these data before acting upon these results.

Test Methodology:

Exon 19 of the *GABBR2* and exon 10 of the *SCN2A* gene were PCR-amplified and the product was sequenced using Sanger sequencing. In case of mosaicism in leucocytes, the detection limits of Sanger sequencing for presence of variation is ~12%. The sequence was aligned to available reference sequences ENST00000259455.2; ENST00000357398.3; hg19 [1], to detect variation using variant analysis software programs. Variant classification follows the tenets of American College of Medical Genetics (ACMG) guidelines [3].

Disclaimer:

- **In accordance to the Pre-Conception and Pre-Natal Diagnostic Testing (PCPNDT) Act, 2003- Govt. of India;Lab does not disclose the gender of the fetus.**
- About 0.44% of total cases are susceptible to allele dropout/dropin phenomenon, which can lead to misdiagnosis [6].
- The potential presence of maternal cell contamination (MCC) in this fetal sample has been ruled out.

References:

1. ENSEMBL: http://www.ensembl.org
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