MUTATION CONFIRMATION BY SANGER SEQUENCING

Details	Remarks
Sample Type	EDTA Whole Blood
Quality of Sample	Acceptable
Clinical Indication	[redacted] a 10 months 16 days male child was presented with USG scan suggestive of milticystic dysplastic kidney with normal right kidney and urinary bladder. Whole Exome Sequencing results showed the presence of variants **c.413C>A, (p.Ala138Glu)** in ***TMPRSS3*** gene in **Heterozygous (Pathogenic)** and **c.293-13C>G** in ***CYP21A2*** gene in **Heterozygous (Pathogenic)** state. Proband EDTA Blood sample is being evaluated for these variants.
Test Requested	Sanger Sequencing

RESULTS

Fig. No.	Sample Name	Gene Name	Variant Tested	Variant Status	Inheritance
1.	[redacted]	***TMPRSS3***	chr21:c.413C>A, (p.Ala138Glu)	Present (Heterozygous)	Autosomal Recessive
2.		***CYP21A2***	chr6:c.293-13C>G	-	Autosomal Recessive

INTERPRETATION

A **heterozygous** variant **chr21:c.413C>A, (p.Ala138Glu)** in the ***TMPRSS3*** gene is **detected** in the provided sample.

Variant **chr6:c.293-13C>G** in the ***CYP21A2*** gene is **not detected** in the provided sample.

Note: CYP21A2 MLPA was performed for this sample and found heterozygous variant c.-113G>A (The 113bp SNP is a promoter variant which is present at -113 bp (before start codon) of CYP21A2 gene, which is also pseudogene derived sequence, reported to reduce 20 percent of transcriptional activity) and Sanger sequencing results showed a wild homozygous nucleotide in the position of chr6:c.293-13C>G which might be due to the deletion of one copy of CYP21A2 gene.

TEST INFORMATION

Fig 1: This assay tests for the confirmation of variant in the provided sample which has been detected in ***TMPRSS3*** gene in Index Patient. Analysis is performed only for variant at **c.413C>A, (p.Ala138Glu)** in ***TMPRSS3*** gene.

Fig 2: This assay tests for the confirmation of variant in the provided sample which has been detected in ***CYP21A2*** gene in Index Patient. Analysis is performed only for variant at **c.293-13C>G** in ***CYP21A2*** gene.

RECOMMENDATION

Please correlate clinically and genetic counselling is recommended.

METHODOLOGY

Targeted sequencing and mutation analysis was performed by Polymerase Chain Reaction (PCR) followed by automated DNA sequencing of the amplicon using BigDye ABI Genetic Analyzer 3500DX platform. The raw data obtained is subsequently analyzed for the nucleotide variants.

DISCLAIMER

- The results specifically pertain to the examined sample and may not accurately represent the fetal chromosome constitution in instances of confined placental mosaicism or when the sample is contaminated with maternal cells.
- Despite taking all necessary precautions during DNA-based tests, the technical error rate for all types of DNA analysis is approximately 2%. Therefore, it is crucial to interpret all results within this context before taking any action based on these results.
- The report is generated within a specific timeframe known as the turnaround time (TAT) once received the sample received at the lab. However, the actual TAT may differ based on the complexity of the requested test(s) and information provided along with the sample. Lifecell cannot be held responsible for any delays that occur beyond the mentioned TAT.
- In certain rare cases, genetic tests may not provide accurate results, for example, when the quality of the sample given to Lifecell is not optimal. If a test performed by Lifecell fails due to unforeseen or unknown reasons beyond their control, Lifecell cannot be held responsible for any incomplete, potentially misleading, or incorrect results that were not foreseeable beforehand.
- Lifecell Pvt. Ltd has validated the test and determined its performance characteristics in accordance with the CAP/ACMG and NABL guidelines. All investigations have their limitations which are imposed by the limits of sensitivity & specificity of individual assay procedures as well as the quality of the specimen received by the laboratory.
- Clinical interpretation of given test result should be evaluated within the context of the patients medical history and other diagnostic laboratory test results.
- The present report comprises genetic analysis of the sample provided. It is important to note that this

report cannot be accurately interpreted without information regarding clinical features and other laboratory reports, investigations. The information contained herein should not be considered a substitute for professional medical advice or diagnosis. It is highly recommended to consult with a qualified healthcare professional or medical specialist for a comprehensive evaluation and interpretation of your specific medical condition.

- This test is designed to detect mutations in the above-mentioned regions only. Sequences surrounding the regions of interest are analyzed but not reported. In rare cases, allele dropout can cause heterozygosity to be reported as homozygosity. This phenomenon occurs when one of the alleles fails to amplify during the genetic testing process,
- This assay is unable to differentiate between cis and trans mutations. Though oligos are designed specifically to parent gene using bioinformatics tool, Interference of pseudogene sequence cannot be ruled out completely. Any change in primer binding site can result and interfere with the results and allele dropout cannot be ruled out using this experiment.
- Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations caused by the presence of pseudogenes, various transcript ID, repetitive, or homologous regions.
- This document is for clinical interpretation and not for medico-legal purpose

ANNEXURE

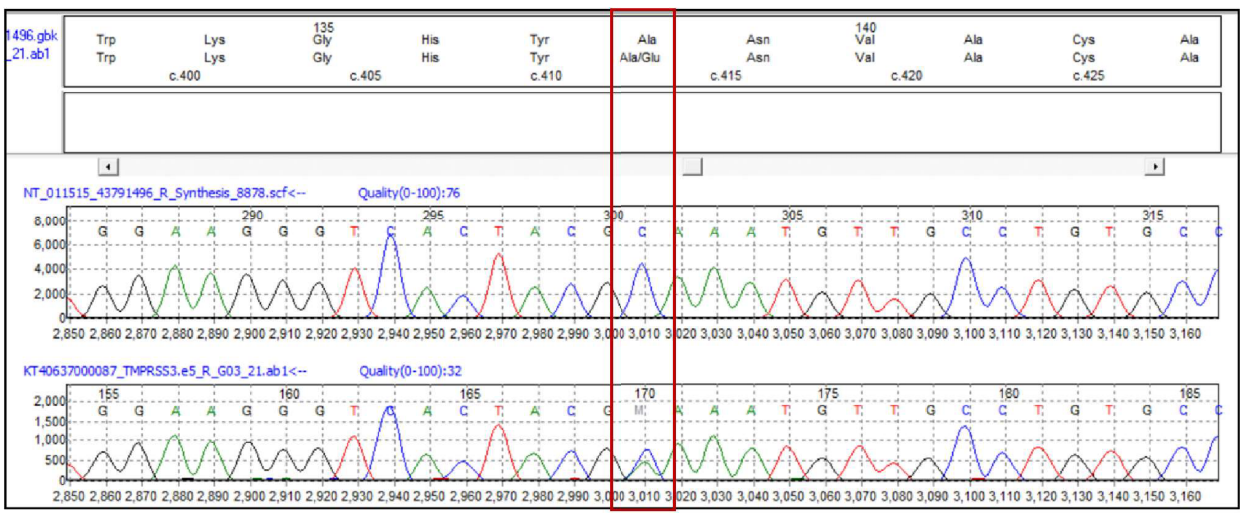

Fig 1: Sanger sequencing data (electropherogram) for the provided sample showing **nucleotide change** at **chr21: c.413C>A,, (p.Ala138Glu)** in ***TMPRSS3*** gene.

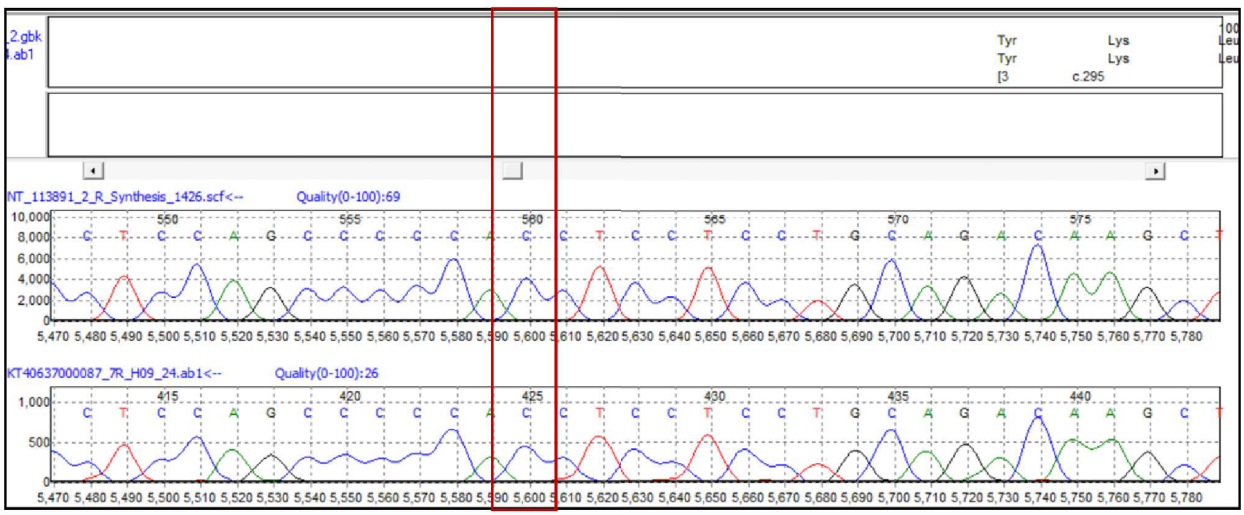

Fig 2: Sanger sequencing data (electropherogram) for the provided sample showing **no nucleotide change** at **chr6: c.293-13C>G** in ***CYP21A2*** gene.

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