CASE STUDY

Differential Diagnosis Between 3-MCC Deficiency and Holocarboxylase Synthetase Deficiency Facilitated by the Strand Clinical Exome Test

Introduction

Inborn errors of metabolism (IEM) are congenital disorders that are caused by mutations within a single gene. Some IEM disorders are characterized by inability to breakdown and absorb nutrients, resulting in delayed development and malnutrition. One such IEM disorder is 3-methylcrotonyl-CoA carboxylase deficiency (also known as 3-MCC deficiency), wherein the breakdown of proteins containing leucine is impaired.

Symptoms of 3-MCC deficiency include vomiting and diarrhea in infants, weak muscles and feeding problems in children. Sometimes, the deficiency can be fatal as well (Grünert et al. 2012).

A deficiency in the holocarboxylase synthetase enzyme is evident if mutations in the *HLCS* gene are present in a person's genome. This enzyme is required for the regulation of other enzymes involved in the catabolism of fats and proteins. Another important function of this enzyme is regulation of expression of other genes (Bao et al. 2011; Liu & Zempleni 2014). Symptoms of the deficiency of this enzyme include feeding problems in infants, breathing difficulties, skin rashes and alopecia. In some cases, a deficiency of this enzyme can be fatal to infants.

Recently, a couple was referred to Strand Life Sciences for genetic diagnosis. The unfortunate couple had lost two children, one at the age of 3 years and the other just 4 days old. Unable to conceive, they consulted a renowned geneticist in Hyderabad and discussed the symptoms that their young son (age 3 years) had suffered from. The geneticist- pediatrician suspected the incidence of a genetic mutation causing either a 3-MCC deficiency or a holocarboxylase synthetase deficiency, in the lost children (Tammachote et al. 2009; Fonseca et al. 2016). She recommended that the young boy's DNA sample be analyzed to understand the reason for his symptoms and the resultant fatality. The Strand Clinical Exome test was used to differentiate between these two possibilities in order to provide an accurate diagnosis.

Gender: Male

Age: 3 years

Location: Hyderabad, Andhra Pradesh

Diagnosis: Holocarboxylase synthetase deficiency

Strand Test: Clinical Exome

Conclusion:

- A VUSD in the *HLCS* gene identified in the proband.
- The proband is homozygous for this mutation.
- Parents are heterozygous for the same mutation.
- The chances of them transmitting this mutation to future progeny in a homozygous manner are 25%.

Patient Profile

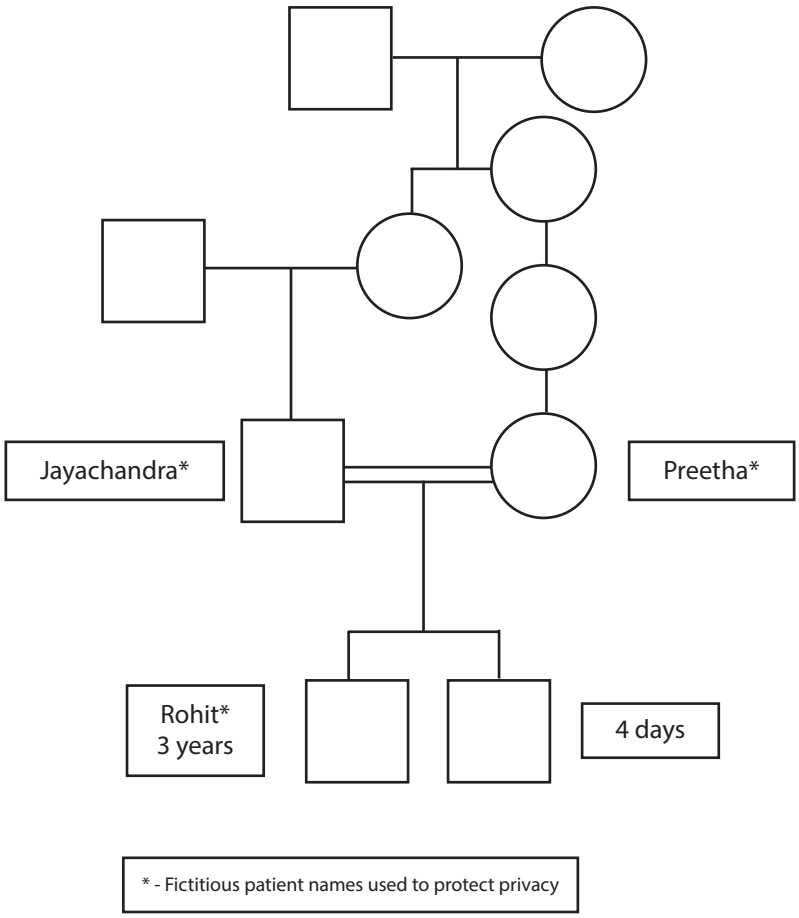
Rohit*, a 3-year-old boy, suffered from issues like feeding problems, malnutrition, vomiting and diarrhea. He remained sickly throughout his infancy and finally succumbed to his health issues at the age of 3 years. Medical investigations to understand the causes of his problems had been inconclusive. However, a dried blood spot from this child had been obtained and preserved during these investigations. The same was sent to Strand Life Sciences to extract DNA for genetic analysis.

* *Name changed to protect patient privacy*

Family History

Rohit's parents, Preetha* and Jayachandra*, are a consanguineous couple. Preetha's grandmother and Jayachandra's mother are sisters. The couple had lost their first child at the age of 4 days and Rohit at the age of 3 years.

Family Tree - Pre-Test Genetic Counselling



Considering the consanguinity in the family, the Strand Clinical Exome test was prescribed to understand whether inheritable mutations that could cause an IEM were present in the family.

DNA extracted from the dried blood sample from Rohit was used to understand if the child had 3-MCC deficiency or holocarboxylase synthetase deficiency.

Results of Genetic Testing (Rohit)

RESULT Rohit is **homozygous** for a 'Variant of Unknown Significance with probable Damaging Effect (VUSD)' in exon 11 of the *HLCS* gene.

A new mutation- a Variant of Unknown Significance – was found in exon 11 of the *HLCS* gene in Rohit's DNA. The *HLCS* gene codes for an enzyme – holocarboxylase synthetase- that adds a molecular tag- biotin- to various enzymes that are engaged in the breakdown of fats and proteins. When the *HLCS* gene is mutated, this tagging function is inefficient and results in reduced breakdown of fats and proteins for production of energy.

Analysis of the mutant gene sequence using bioinformatics tools suggested that the mutation in Rohit's DNA is likely to alter some amino acids that are crucial for the functions of the holocarboxylase synthetase protein. Therefore, the variant has been re-classified as 'Variant of Unknown Significance with a Probable Damaging Effect (VUSD)'.
Rohit was homozygous for this new VUSD.

In order to understand the chances of transmitting the same mutation to other progeny of Preetha and Jayachandra, genetic analysis of their DNA was suggested.

Results of Genetic Testing (Preetha)

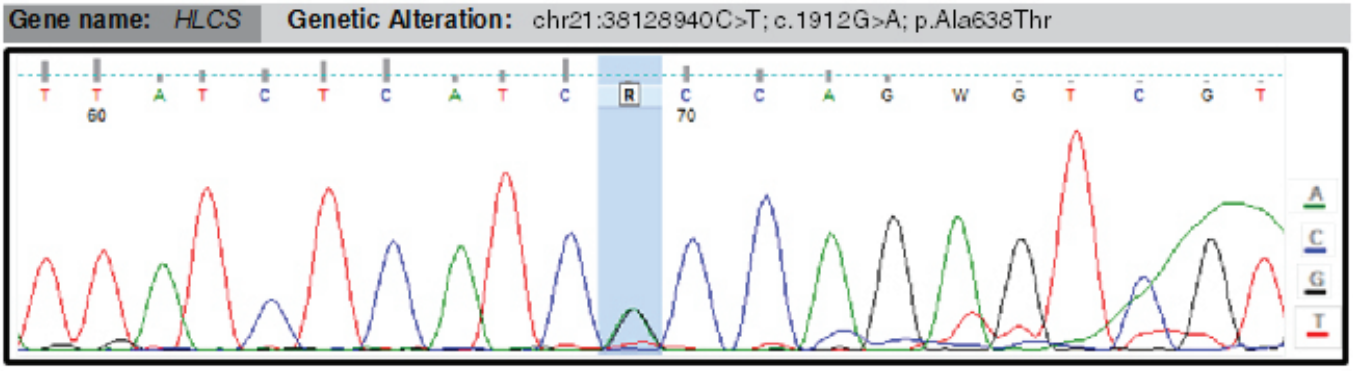


Figure 1. Electropherogram of Sanger sequencing data from the individual showing a heterozygous nucleotide change 'G>A' at position c.1912 in the HLCS gene. This variation was confirmed by sequencing with reverse primer in two independent experiments.

Strand offers a Mutation-Specific Test* (MST) which is designed to identify specific mutations in the genomes of family members of probands (index patients). These tests are fast and highly specific.

An MST for the identified VUSD mutation in the *HLCS* gene showed that Preetha is heterozygous for this mutation. Essentially, she has one normal copy of this gene and one mutant copy.

Results of Genetic Testing (Jayachandra)

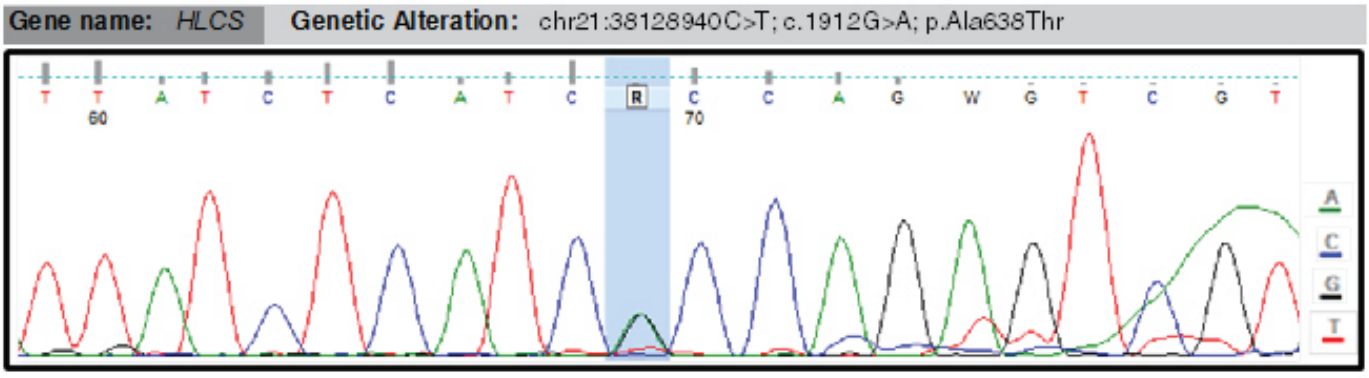


Figure 2. Electropherogram of Sanger sequencing data from the individual showing a heterozygous nucleotide change 'G>A' at position c.1912 in the HLCS gene. This variation was confirmed by sequencing with reverse primer in two independent experiments.

Likewise, Jayachandra is also heterozygous for this mutation in the *HLCS* gene.

The parameter marked with an * are not accredited by NABL and CAP.

Counselling Post Genetic Analysis

Preetha and Jayachandra are heterozygous for the VUSD mutation in exon 11 of the *HLCS* gene identified in their son. They were advised that their chances of having another child with a similar homozygous inheritance as Rohit were 25%.

Conclusions

- A consanguineous couple with a history of infant mortality was referred to Strand Life Sciences for genetic analysis.
- Symptoms presented by the deceased 3- year-old child of the couple indicated the presence of 3-MCC deficiency or holocarboxylase synthetase deficiency.
- The Strand Clinical Exome Test was leveraged to arrive at a differential diagnosis between these two IEM disorders.
- The proband was found to be homozygous for a VUSD mutation in exon 11 of the *HLCS* gene.
- The parents were offered an MST and were found to be heterozygous for the same mutation.
- They were counselled about their chances of having one or more children who would also be homozygous for this VUSD.

Strand Clinical Exome Test

The Strand® Clinical Exome test is a Laboratory Developed Test (LDT) that was developed and its performance characteristics determined by Strand Center for Genomics and Personalized Medicine at Strand Life Sciences. The test covers ~4500 genes and is a comprehensive test for IEM disorders as well as neuromuscular and neurocognitive developmental disorders.

References

Bao, B. et al., 2011. Human holocarboxylase synthetase with a start site at methionine-58 is the predominant nuclear variant of this protein and has catalytic activity. Biochemical and biophysical research communications, 412(1), pp.115–20. Available at:http://www.ncbi.nlm.nih.gov/pubmed/21802411 [Accessed November 13, 2017].

Fonseca, H. et al., 2016. 3-Methylcrotonyl-CoA carboxylase deficiency: Mutational spectrum derived from comprehensive newborn screening. Gene, 594(2), pp.203–210. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27601257 [Accessed November 13, 2017].

Grünert, S.C. et al., 2012. 3-methylcrotonyl-CoA carboxylase deficiency: Clinical, biochemical, enzymatic and molecular studies in 88 individuals. Orphanet Journal of Rare Diseases, 7(1), p.31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22642865 [Accessed November 13, 2017].

Liu, D. & Zempleni, J., 2014. Holocarboxylase synthetase interacts physically with nuclear receptor co-repressor, histone deacetylase 1 and a novel splicing variant of histone deacetylase 1 to repress repeats. Biochemical Journal, 461(3), pp.477–486. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24840043 [Accessed November 13, 2017].

Tammachote, R. et al., 2009. Holocarboxylase synthetase deficiency: novel clinical and molecular findings. Clinical Genetics, 78(1), pp.88–93. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20095979 [Accessed November 13, 2017].

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