Molecular Genetics Report – Genomic DNA

Test Requested: Whole Exome Sequencing to confirm the diagnosis Treacher Collins Syndrome

Clinical Information: The child of and was previously diagnosed with Treacher Collins Syndrome. No previous testing has been completed.

Summary of Result

Pathogenic Mutations Associated with Reported Phenotype:

<table>
<thead>
<tr>
<th>Variant</th>
<th>Gene</th>
<th>Condition</th>
<th>Mode of Inheritance</th>
<th>Pathogenic Status of Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM_001135243.1:c.1978C&gt;T; p.Arg660Ter</td>
<td>TCOF1</td>
<td>Treacher Collins Syndrome</td>
<td>Autosomal Dominant</td>
<td>Established</td>
</tr>
</tbody>
</table>

The mother of Ms. is heterozygous for the same mutation.

Carrier Screening Secondary Findings:

<table>
<thead>
<tr>
<th>Mother:</th>
<th>Father:</th>
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<tr>
<td>Heterozygous</td>
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<td>NM_014625.2:c.59C&gt;T; p.Pro20Leu</td>
<td>NPHS2</td>
<td>Nephrotic syndrome, steroid resistant</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>Non-Carrier</td>
<td>Heterozygous</td>
<td>NM_000277.1:c.1208C&gt;T; p.Ala403Val</td>
<td>PAH</td>
<td>Hyperphenylalaninemia</td>
<td>Autosomal Recessive</td>
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<td>Non-Carrier</td>
<td>Heterozygous</td>
<td>NM_001003841.2:c.517G&gt;A; p.Asp173Asn</td>
<td>SLC6A19</td>
<td>Hartnup Disorder</td>
<td>Autosomal Recessive</td>
</tr>
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<td>Non-Carrier</td>
<td>Heterozygous</td>
<td>NM_015102.3:c.1024C&gt;T; p.Arg342Cys</td>
<td>NPHP4</td>
<td>Nephronophthsis 4</td>
<td>Autosomal Recessive</td>
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Result and Interpretation

Pathogenic Mutations Associated with Reported Phenotype

1. **Heterozygous for NM_001135243.1: c.1978C>T; p.Arg660Ter in the TCOF1 gene.**

   Treacher Collins Syndrome: Treacher Collins Syndrome is caused by heterozygous mutations in the TCOF1 gene (OMIM# 154500). Treacher Collins Syndrome is an autosomal dominant disorder of craniofacial development. Most affected individuals have underdeveloped facial bones, particularly the cheek bones, and a very small jaw and chin (micrognathia). Some people with this condition are also born with an opening in the roof of the mouth called a cleft palate. People with Treacher Collins syndrome often have eyes that slant downward, sparse eyelashes, and a notch in the lower eyelids called an eyelid coloboma. Some affected individuals have additional eye abnormalities that can lead to vision loss. This condition is also characterized by absent, small, or unusually formed ears.


   This mutation, when inherited as heterozygous, causes Treacher Collins Syndrome. Ms. [Name Redacted] and her mother [Name Redacted] are heterozygous for the mutation.

Carrier Screening Secondary Findings

1. **Heterozygous for NM_014625.2: c.59C>T; p.Pro20Leu in the NPHS2 gene.**

   Nephrotic Syndrome, steroid resistant: Nephrotic syndrome is caused by compound heterozygous or homozygous mutations in the NPHS2 gene (OMIM# 600995). Nephrotic syndrome is a renal disease clinically characterized by severe proteinuria, resulting in complications such as hypoalbuminemia, hyperlipidemia and edema. The disorder is resistant to steroid treatment and progresses to end-stage renal failure in the first or second decades. Some patients show later onset of the disorder.

   NPHS2 NM_014625.2: c.59C>T; p.Pro20Leu: This mutation is previously reported to cause autosomal recessive Nephrotic Syndrome (Boute et al. (2000) Nat Genet 24,349).

   This mutation, when inherited as compound heterozygous or homozygous, causes autosomal recessive Nephrotic Syndrome. [Name Redacted] is heterozygous for the mutation. Both of her parents [Name Redacted] are heterozygous for this mutation.

2. **Heterozygous for NM_000277.1: c.1208C>T; p.Ala403Val in the PAH gene.**

   Hyperphenylalaninemia: Hyperphenylalaninemia is caused by compound heterozygous or homozygous mutations in the PAH gene (OMIM# 261600). Hyperphenylalaninemia is referred to as “Non- Phenylketonuria” disease (Non-PKU). Phenylketonuria is an inherited disorder that increases the levels of a substance called phenylalanine in the blood. Phenylalanine is a building block of proteins (an amino acid) that is obtained through the diet. It is found in all proteins and in some artificial sweeteners. If PKU is not treated, phenylalanine can build up to harmful levels in the body, causing intellectual disability and other serious health problems. Non-PKU hyperphenylalaninemia is a milder form of the disease and individuals are at a smaller risk of brain damage.
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PAH NM_000277:1: c.1208C>T; p.Ala403Val: This mutation is previously reported to cause autosomal recessive Hyperphenylalaninemia (Réblová et al. (2013) Clin Chim Actaepub, epub).

This mutation, when inherited as compound heterozygous or homozygous, causes autosomal recessive Hyperphenylalaninemia. [Redacted] is heterozygous for the mutation. Her father, [Redacted], is heterozygous for the same mutation.


Hartnup Disorder: Hartnup disorder is caused by compound heterozygous and homozygous mutations in the SLC6A19 gene (OMIM# 234500). Hartnup disorder is caused by an abnormality of renal and gastrointestinal neutral amino acid transport and is noted for its clinical variability. First described in 1956, HND is characterized by increases in the urinary and intestinal excretion of neutral amino acids. Individuals with typical Hartnup aminoaciduria may be asymptomatic, some develop a photosensitive pellagra-like rash, attacks of cerebellar ataxia and other neurological or psychiatric features.

SLC6A19 NM_001003841:2: c.517G>A; p.As173Asn: This mutation is previously reported to cause autosomal recessive Hartnup Disorder (Seow et al. (2004) Nat Genet 36,1003).

This mutation, when inherited as compound heterozygous or homozygous, causes autosomal recessive Hartnup Disorder. [Redacted] is heterozygous for the mutation. Her father, [Redacted], is heterozygous for the same mutation.


Nephronophthisis 4: Nephronophthisis is caused by compound heterozygous and homozygous mutations in the NPHP4 gene (OMIM# 606966). It is a disorder that affects the kidneys. It is characterized by inflammation and scarring (fibrosis) that impairs kidney function. These abnormalities lead to increased urine production (polyuria), excessive thirst (polydipsia), general weakness, and extreme tiredness (fatigue). In addition, affected individuals develop fluid-filled cysts in the kidneys, usually in an area known as the corticomedullary region. Another feature of nephronophthisis is a shortage of red blood cells, a condition known as anemia. Nephronophthisis eventually leads to end-stage renal disease (ESRD), a life-threatening failure of kidney function that occurs when the kidneys are no longer able to filter fluids and waste products from the body effectively.

NPHP4 NM_015102:3: c.1024C>T; p.Arg342Cys: This mutation is previously reported to cause autosomal recessive Nephronophthisis 4 (Hoefele et al. (2005) Hum Mutat 25,411).

This mutation, when inherited as compound heterozygous or homozygous, causes autosomal recessive Nephronophthisis 4. [Redacted] is heterozygous for this mutation.

Methods: Exome Sequencing was carried out on DNA extracted from all patients. The Nextera Rapid Capture Exome kit from Illumina allows the identification of coding variants within 37Mb of selected exonic content. The design of the target probes has been optimized to provide uniform and specific coverage within the exonic content and provides comprehensive sequencing data with reliable identification of known as well as unknown coding variants. Nomenclature utilized in this report follows that of HGVS. Our method of analysis included the following:

1. Analyze the genes reported in association with Treacher Collins Syndrome.
2. The following variants were analyzed:
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I. Variant(s) present in one or both parents as heterozygous and the proband as homozygous or heterozygous

II. Either the gene or the variant itself is relevant to the phenotype as a whole or part of the symptoms associated with the phenotype present in the affected individual(s).

3. Variants’ pathogenicity was determined by referencing those documented in the Human Genome Mutation Database (HGMD). Unreported mutations with potentially deleterious effects on the protein (nonsense mutations, frameshift deletions) were also analyzed. All potentially pathogenic variants were further compared to our patient database to incorporate allele frequency in the determination of pathogenicity.

4. Carrier Screening was also completed for all samples submitted for testing. For a list of genes analyzed as part of Viafet’s Carrier Screening, please see Appendix A

Limitations of Test: Whole Exome Sequencing does not analyze all genes in the human genome. For this reason, it is possible that a patient can have a gene mutation that cannot be detected. The following cannot be examined due to technological restraints:

a) Current technology does not cover 5-10% of the exome
b) Triplet repeats (Fragile X syndrome, Huntington syndrome)

c) Copy number variants (chromosomal abnormalities—other testing methods are available at Viafet)

d) Abnormal methylation

e) Mitochondrial genome sequencing (other testing methods are available at Viafet)

Recommendation: Genetic counseling is recommended to explain the result and potential reproductive options to the family. A reported heterozygous mutation in the TCOF1 gene was identified in [redacted] and her mother, [redacted], genetically confirming the diagnosis of Treacher Collins Syndrome.

Pre-Implantation Genetic Diagnosis (PGD) is available at Viafet Laboratory for the parents of [redacted] should they wish to plan a pregnancy in the future free of this condition. If the parents are to pursue natural pregnancy, future offspring are at 50% risk of being affected, 50% (1/2) will be unaffected non-carriers.

Mutation screening is available for other family members upon request.

As part of Viafet’s Carrier Screening, a pathogenic mutation causing autosomal recessive Nephrotic Syndrome was found in both parents, [redacted] and [redacted]. Pre-Implantation Genetic Diagnosis (PGD) is also available for this mutation at Viafet Laboratory. If the parents are to pursue natural pregnancy, future offspring are at 25% risk of being affected, 50% (1/2) will be unaffected carriers and 25% (1/4) will be unaffected non-carriers.

Report electronically signed by:
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Clinical Molecular Genetics