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PATHOGENIC PARTIAL DUPLICATIONS IN THE TCOF1 GENE IDENTIFIED IN A TREACHER COLLINS SYNDROME: CASE REPORT AND REVIEW OF LITERATURE

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ABSTRACT

Severe craniofacial deformities are the hallmark of the uncommon autosomal dominant condition Treacher Collins Syndrome (TCS). Mutations in the TCOF1 gene, which codes for the nucleolar phophophoprotein treacle, are linked to the syndrome. We discuss the case of an 8-month-old boy who has bilateral microtia, missing cheekbones, micrognathia, a small eye globe with a downslanting palpebral fissure, low-set ears, a short neck, and a depressed nasal bridge. Her heterozygous status for c.2860-2781+149dup (tandem duplication of exons 18 through 23) partial gene duplication in the TCOF1 gene, which is probably harmful, was revealed by sequencing testing. This is in line with TCS's clinical diagnosis. It is hoped that this case report would aid in future cases' diagnosis and provide crucial information regarding prognosis and best practices in care.

Key-words: Treacher Collins Syndrome (TCS),

BACKGROUND

Treacher Collins Syndrome (MIM #154500) is an uncommon congenital autosomal dominant condition. Bilaterally symmetrical craniofacial deformities are its defining feature. The estimated prevalence of TCS is 1/50,000 live births. Forty percent of instances have a family history, while de novo mutations account for sixty percent of cases (Bowman *et al.,* 2012; Wang *et al.,* 2014; Jones *et al.*,1975). The main clinical characteristics include mandibular hypoplasia, downslanting palpebral fissures, colobomas of the lower eyelid with absent eyelashes medial to the defect, ear malformations frequently linked to bilateral conductive hearing loss and atresia of the external ear canal, macrostomia, and micrognathia. Malar hypoplasia is brought on by hypoplasia of the zygomatic complex.

There is frequently cleft palate present (Bowman *et al.,* 2012; Wang *et al.,* 2014; Teber *et al.,* 2004). It is believed that during the fifth and eighth week of embryonic development, a complex pattern of abnormalities is brought about by defective development of structures derived from the first and second branchial arches. Wide variations exist both within and between families in TCS. It includes anything from neonatal deaths brought on by obstructed airways to phenotypes that are missed by medical tests (Poswillo, 1975; Splendore *et al.,* 2002). In TCS patients, mutations in the TCOF1 gene (OMIM, # 606847), which is situated on chromosome 5q32–5q33.1, were found to be heterozygous. 26 exons make up the longest transcription of TCOF1, including the alternatively spliced exons 6A and 19 (So *et al.*,2004). This gene produces the slow-complexity protein Treacles, which has 1411 amino acids. This protein, which is rich in serine and alanine, has distinct N and C termini and a sizable central repeat domain that shares patterns with other nucleolar trafficking proteins. Treacles is a nucleolar phophoprotein that is consequently implicated in the transcription of ribosomal DNA genes. While the LIS1 homology motif found in the N-terminal region of Treacles may help regulate the dynamics of microtubules by mediating dimerization or by directly binding cytoplasmic dynein heavy chain or microtubules, the C-terminal region of Treacles is crucial for localization to the nucleous (So *et al.*,2004; Marszalek *et al.,* 2003). Inhibition of the production of correctly modified mature ribosomal RNA and inhibition of rRNA gene transcription in the prefusion neural folds during the early stages of embryogenesis may be the cause of the abnormal development due to treacle haploinsufficiency, which will affect the proliferation and proper differentiation of these embryonic cells (Gonzales *et al.,* 2005).

The spectrum of phenotypic expression among carriers of TCOF1 gene mutations is, however, poorly understood. This study presents the clinical, biochemical, and genetic conditions of related married family whose infant had low-set ears, a short neck, a depressed nasal bridge, micrognathia, small eye globe with down-slanting palpebral fissure, and dysmorphic bilateral microtia.

Case Representation:

The pediatric genetics and metabolic counseling services at Alnoor Hospital in Makkah, Saudi Arabia, received a referral for an 8-month-old boy. His parents were connected (second degree relatives) when he was born. There has never been an abortion or a death in the family. Because of the fetal discomfort, his 37-year-old mother had to have a cesarean section to birth him. Premature Rapture of Membrane (PROM), Polyhydramnios, and EBV infection were all in her past.

The newborn was dysmorphic, with bilateral microtia, nonexistent cheekbones, a small eye globe with a downslanting palpebral fissure, micrognathia, low-set ears, a short neck, and a depressed nasal bridge, according to clinical examination results. At eight months old, the patient's weight was 7.12 kg, height was 69 cm, head circumference was 44.5 cm, and he had a clear throat. He appeared well and was not in any pain. A chest examination revealed no deformities and a typical, symmetrical chest shape. Normal bilateral vesicular breathing sounds were auscultated; no additional noises were detected. Cardiovascular exams revealed no murmurs and audible first and second heart sounds.

An examination of the abdomen showed no organomegaly and a soft, loose abdomen. Examination of the central nervous system revealed normal power, tone reflex, and gait. A small Subependymal cyst was discovered via brain ultrasonography. A nasal cavity examination revealed left-sided choanal atresia. On paranasal CT scans, a minor ethmoidal myelocele was observed. The results of the abdominal ultrasound, ECHO, and brain CT and MRI were normal. A skeletal survey revealed no obvious anomalies. A suspected cribriform plate defect causing a minor ethmoidal myelocele, encephalocel, and flattening of the mandibular anterior arch is visible on a CT scan of the maxillofacial bone. The patient provided a blood sample, which was submitted to the genetic lab to look for mutations linked to Treacher Collins Syndrome.

MATERIAL AND METHODS

This study was authorized by the Umm Al-Qura University School of Medicine's Institutional Review Board and Research Ethics Committee in Saudi Arabia. Following the patient's parents' informed written agreement, blood samples in EDTA were taken from the patient. The coding areas of TCOF1, POLR1C, and POLR1D were sequenced and subjected to deletion/duplication analysis by the John Hopkins DNA Diagnostic Laboratory (Baltimore, MD, USA). The ultrasonic technique was used to extract and fragment DNA. The exon/exon boundaries and coding sections of the aforementioned genes were captured using Illumina MiSeq Next Generation Sequencing (NGS) equipment. The Burrow-wheeler Aligner (BWA) was used for alignments to the human reference genome (GRCh37/hg19), and GATK was used for variation calling.

Sanger sequencing was done to verify newly discovered mutations and/or make up for NSG coverage that was less than 50X at more than 1% of the targeted nucleotides. To read depth, dosage analysis with NSG normalization was utilized. The sequencing data was evaluated by several employees. The DDL Clusterpipeline.v140309.sh DDL Pipeline CT12.001a was used for bioinformatics analysis. 99% for inherited single nucleotides and small insertion/deletion variants for the nucleotides evaluated, 99% for multi-exon deletions and 98 % for single exon deletions, 90% for multi-exon duplications and 75 % for single exon duplications, according to the analytical sensitivity. Lower bounds on single exon duplication detection; lower bounds on single nucleotide variant detection; 25% allele frequency and greater than 99% sensitivity.

RESULTS AND DISCUSSION

The results of the sequencing and deletion/duplication analysis of the coding areas of the POLR1C, POLR1D, and TCOF tests showed that the TCOF1 gene is heterozygous for c.2860-2781+149dup, a partial gene duplication that is likely pathogenic and involves tandem duplication of exons 18 through 23. By using sequencing and deletion/duplication testing on the TCOF, POLR1C, and POLR1D genes that are linked to TCS, no additional reportable variants were found. Treacher Collins Syndrome's clinical diagnosis criteria are linked to a partial gene duplication in the TCOF1 gene, making it the molecular etiology of the condition.

Treacher Collins Syndrome is linked to TCOF1 gene mutations. Although there have been several variants found throughout the TCOF1 gene, this is the first case report of this kind of mutation that we are aware of. Variations in bilateral downward slanting of the palpebral fissures, colobomas of the lower eyelids with a paucity of lashes medial to the defect, cleft palate, hypoplasia of the facial bones, malformation of the external ears, atresia of the external auditory canals, and bilateral conductive hearing loss are typically associated with these features (Teber *et al.*, 2004; Splendore *et al.*, 2000; Edwards *et al.*, 1997; Dauwerse *et al.*, 2011). Regrettably, there is a great deal of variation in the phenotypes found within and within families. There is no link between genotype and phenotype,

according to several recent researches. As a result, neither the kind nor the position of mutations was able to explain the origin of the significant clinical diversity seen in TCS patients, both within and between families. Environmental factors, gene mutations in related or similar developmental pathways, and differences in chromosomal backgrounds, such as polymorphisms in the wild-type allele of the TCOF1 gene, are among the theories put out to explain this heterogeneity (Splendore *et al.*, 2000; Edwards *et al.*, 1997).

As with other autosomal dominant disorders, clinical severity appears to defy the traditional link between the inherited mutation and the disease. Clinical diagnosis is typically based on the discovery of a particular aberrant phenotypic pattern. For several of these developmental diseases, the underlying genetic reasons have been determined. Because patients with non-classical phenotypes can be included in the spectrum if they exhibit a pathological genotype that has also been seen in patients with regular phenotypic expression, the range of phenotypic expression of a given syndrome can be determined with less bias thanks to the possibilities of molecular diagnosis. Anticipatedly, the examination of genotype-phenotype correlations will contribute to the development of more precise standards for clinical diagnosis (Edwards *et al.*, 1997; Wise *et al.*, 1997).

Genetic counseling is important, especially for situations with ambiguous clinical characteristics. Unquestionably, one of the biggest challenges of our day is understanding unpredictability. Sequencing the areas that might be in charge of some of the phenotypic variance seen in TCS individuals is a possibility. To resolve the issue of genetic heterogeneity and to gain a deeper understanding of the disease, the TCOF1 gene must be characterized.

In summary, Treacher Collins Syndrome (TCS) is linked to a TCOF1 gene deficiency. This gene's disruptive mutation exhibits significant intra- and inter-family heterogeneity. Through early detection and counseling, we hope to stop future diagnoses of Treacher Collins Syndrome and other genetic illnesses. Furthermore, we highly advise prenatal diagnostic for the baby's parents and sequencing analysis for family members.

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