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Short Communication

Mutation in exon 13 of the *TCOF1* gene in patient with Treacher Collins syndrome

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Treacher Collins Syndrome (TCS) is associated with an abnormal differentiation of the first and the second pharyngeal arch during fetal development. This causes mostly craniofacial deformities, which require numerous corrective surgeries. TCS is an autosomal dominant disorder and it occurs in general population with the frequency of in 1 in 50,000 live births. TCS is caused by mutations in the *TCOF1* gene. This gene encodes the serine/alanine-rich protein named Treacle. TCS can also be caused by mutations in the POLR1C and POLR1D genes. About 70 % of recognized mutations of the *TCOF1* gene are deletions, which lead to a frame shift, formation of termination codon and shortening of the protein product of the gene. A heterozygous insertion c.2013_2014insG was described in one patient with TCS. The insertion causes premature termination of translation at 680aa.

Keywords: TCOF1, insertion, premature termination, Treacher Collins syndrome

1 Introduction

Treacher Collins syndrome (TCS) [OMIM 154500] is an autosomal dominant disorder affecting differentiation of the first and the second pharyngeal arches (Gorlin et al. 2001). This syndrome occurs with an estimated prevalence of 1 in 50,000 live births. In 60 % of cases the disease is caused by *de novo* mutations; family history was confirmed in 40 % of patients.

Patients with Treacher Collins syndrome are characterised by a typical phenotype: downward slanting palpebral fissures, coloboma of the lateral part of the lower lid, hypoplasia of the facial bones, external and middle ear defects, cleft lip and palate (Dixon MJ 1996). Treacher Collins syndrome is caused mainly by mutations in the TCOF1 gene, which encodes a low complexity serine/alanine-rich nucleolar phosphoprotein called Treacle. Over 90 % of patients with TCS have a mutation in the TCOF1 gene. Dauwerse et al. (2011) detected mutations in genes encoding subunits of RNA polymerases I and III (POLR1C and POLR1D) in patients with Treacher Collins syndrome.

Most mutations identified in the TCOF1 gene are deletions resulting in a truncated protein (Gladwin et al. 1996; Splendore et al. 2000; Marszalek et al. 2003). No genotype/phenotype correlations have been found.

The aim of the paper was to identify a mutation in exon 13 of the TCOF1 gene in a patient with Treacher Collins syndrome.

2 Material and methods

DNA was isolated from peripheral blood leukocytes of the patient with TCS. Exons 1 through 27 of *TCOF1*, including exon-intron borders, were amplified by PCR under optimal conditions, using specific primers. The PCR products were subjected to multitemperature single-stranded conformation polymorphism (MSSCP) analysis at 5°C, 15°C and 25°C, using the DNA Pointer Mutation Detection System. The electrophoresis was followed by silver staining.

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The PCR products were purified on the DNA GelOut columns followed by direct sequencing with the use of a BigDye ver.3.0 dye terminator cycle sequencing kit and specific primers. The dideoxy-terminated fragments were identified by capillary gel-electrophoresis based on the ABI 310 DNA Analysis System.

The MSSCP analysis of the amplified fragments of exon 13 of the TCOF1 gene demonstrated changes in the electrophoretic mobility in this patient. In order to confirm the results obtained in MSSCP analysis a direct sequence analysis was performed.

3 Results and discussion

Sequence analysis demonstrated a heterozygotic c.2013_2014insG mutation in exon 13 of TCOF1 (Figure 1).

A majority of mutations responsible for Treacher Collins syndrome are localized in the hot spots in exons 10, 13, 15, 16, 23 and 24 (Splendore et al. 2000). The most commonly occurring mutations of the TCOF1 gene include deletions, which cause a shift of the reading frame, formation of the termination codon and shortening of the protein product. The next most common mutations of the TCOF1 gene are insertions, the longest insertion localized on exon 5 (Marszalek-Kruk et al. 2012).

Analysis of the c.2013_2014insG indicates that it causes a premature termination of translation at 680aa. The table indicate location of the G insertion and position of STOP codon (Table 1).

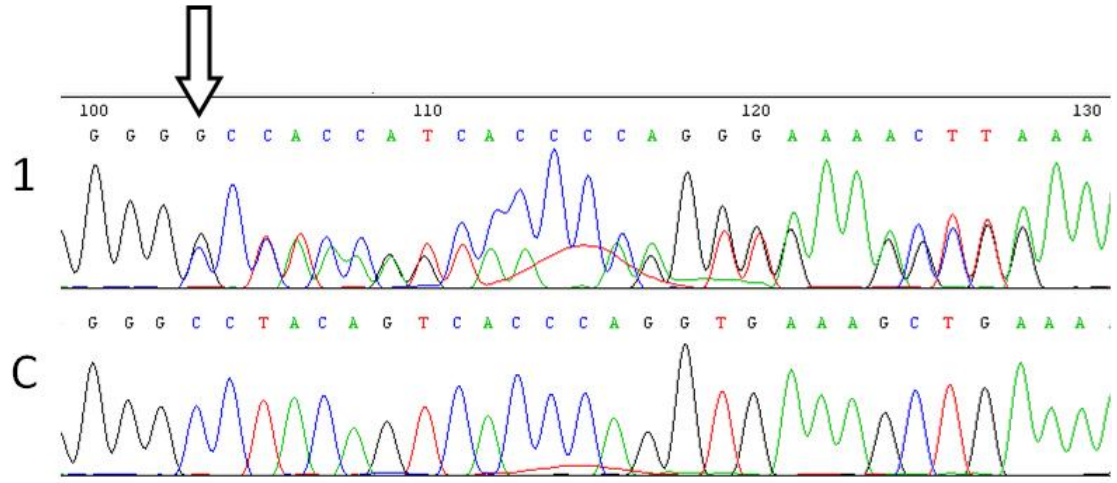

Figure 1 Sequence analysis of the amplified fragment of exon 13 of the TCOF1 gene: 1-patient; C-control. Arrow indicates location of the G insertion

Table 1 Position of the G insertion and STOP codon

G	**G**	**G**	**C**	**C**	**T**	**A**	**C**	**A**	**G**	**T**	**C**	**A**	**C**	**C**	**C**	**A**	**G**	**G**	**T**	**G**	**A**	**A**	**A**	**G**	**C**	**T**	**G**	**A**	**A**
Gly			Pro			Thr			Val			Thr			Gln			Val			Lys			Ala			Glu		
G	**G**	**G**	**G**	**C**	**C**	**T**	**A**	**C**	**A**	**G**	**T**	**C**	**A**	**C**	**C**	**C**	**A**	**G**	**G**	**T**	**G**	**A**	**A**	**A**	**G**	**C**	**T**	**G**	**A**
Gly			Ala			Tyr			Ser			His			Pro			Gly			Glu			Ser			**STOP**		

4 Conclusions

We believe that these findings will facilitate precise diagnosis of the patient and will extend our knowledge on the pathogenesis of TCS. Molecular diagnosis of TCS is essential in prenatal and postnatal screening, being of great importance for genetic counseling, as well.

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