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Case Report Open Access

# Study of a Family Presenting Novel Mutation of the TCOF1 Gene Associated with Treacher Collins Syndrome

**Dell'Edera Domenico[1]*, Tinelli Andrea[3], Pacella Elena[6], Malvasi Antonio[2], Novelli Antonio[4], Conte Chiara[4], Bertoli Marta[4], Alesi Viola[4], Monti Condesnitt Vito[5] and Epifania Annunziata Anna[1]**

[1]*Unit of Cytogenetic and Molecular Genetics, Madonna delle Grazie Hospital, Matera, Italy*
[2]*Obstetric and Gynecology, Department Santa Maria Hospital, Bari, Italy*
[3]*Obstetric and Gynecology, Department V. Fazzi Hospital, Lecce, Italy*
[4]*Unit of Medical Genetics, San Pietro FBF Hospital, Rome, Italy*
[5]*Local Public Health, Taranto, Italy*
[6]*Department of Ophthalmology, University of Rome, Rome, Italy*

## Abstract

Treacher Collins syndrome (TCS), due to a mutation in the treacle gene (5q31-32), is the most common type of Mandibulofacial Dysostosis (MDF). The most important features of the considered diseases are hypoplasia, micrognathia, microtia, conductive hearing loss, and cleft palate. In this paper molecular and clinical analysis in a family with several members affected by MFD are reported. Clinical signs as well as inheriting pattern have been considered to reach a correct diagnosis.

As genealogic tree showed Autosomic Dominant pattern (AD), Autosomic recessive diseases were not considered in different diagnosis. Furthermore, pathognomonic signs drew us to focus the attention on the possibility that Treacher Collins Syndrome occurred.The molecular research of gene TCOF1 confirmed the presence of a mutation that have never been described in literature before now (c.599delG.). MFD occurs in clinical and genetic different typologies of diseases, and in most cases a certain diagnosis can be reached by means of molecular genetics analysis.

**Keywords:** Mandibulofacial Dysostosis (MFD); Treacher Collins Syndrome (TCS); Orofacial features

## Introduction

Mandibulofacial Dysostosis (MDF) concerns a genetically heterogeneous group of disorders characterized by abnormal craniofacial development that is not associated with any limb anomalies [1].

Treacher-Collins syndrome (TCS; OMIM#154500) or Franceschetti-Klein Syndrome is the most common form of MFD [2]. The incidence of TCS is about 1 in 50.000 newborns [3], it has autosomal dominant inheritance and high penetrance (90%).

TCOF1 are a de novo event in about 50-60% of cases, while in 40-50% of cases are maternally or paternally inherited [4]. Clinical and molecular analysis developed on a family where three members have been affected by TCOF1 are reported. Clinical examination on members of the above mentioned family suggested TCS. TCOF1 molecular analysis highlighted a mutation never described in literature, confirming clinical diagnosis.

## Materials and Methods

After a gynaecologic request, a 22 years old pregnant woman (V.S. at 11 week), has been investigated for the prenatal screening of the first trimester (Down and Edwards syndromes, through bitest).

Clinical examination and personal as well as family Y anamnesis highlighted some specific signs, suggesting clinical diagnosis of MFD (Figure 1).

Mister V.F. (Figure1, I1), Mister V.D. (Figure 1, II2) and Mrs V.S. (Figure 1, II3) showed the following dysmorphic signs: zygomatic bones hypoplasia, mandibular hypoplasia, inferior eyelids coloboma and partial cilia absence, downslanting palpebral fissures, microtia and partial Artesia of external ear duct (Figure 2). They did not show mental retardation or any intellectual disability.

V.S. was hospitalised at the age of 9 for urgent surgery for a left

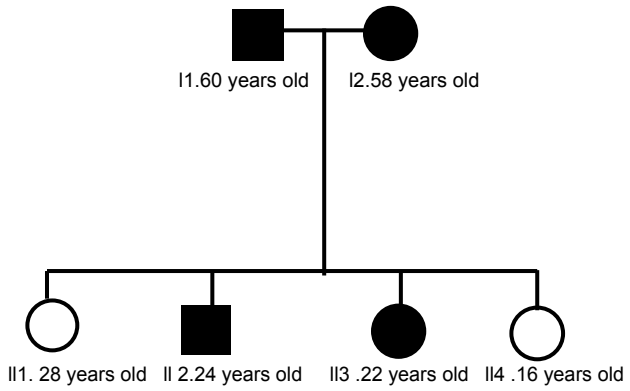


**Figure 1:** Pedigee of the family with novel mutation of the TCOF1 gene.

***Corresponding author:** Domenico Dell'Edera, Unit of Cytogenetic and Molecular Genetics, Madonna delle Grazie Hospital, Matera, 75100 Matera, Italy, Tel: +39 0835253439; Fax: +39 0835253863; E-mail: ducati98@libero.it

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ear abscess coming from apreauricular fistula; at the age of 20 he had been affected by a miscarriage. Others family members examined in the same setting did not show signs of MFD (Figure 1, II1 and II4).The clinical signs highlighted that the members under investigation showed the clinical features associated to Treacher Collins syndrome.

After informed consent has been obtained from patients, blood samples were collected. Genomic DNA was prepared from peripheral blood samples using purification kit (Qiagen, GmbH, Germany). Coding regions and intron/exon boundaries of the TCOF1 gene were amplified in 28 reactions using specific primers [5]. For exons 6A, 10, 16A, 24, and 25 specific primers were self-designed (Table 1).

PCR amplification was performed in a 25 µl reaction volume containing 2.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA), 1X reaction buffer (10 mM TrisHCl pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$), 200 µM of each deoxyribonucleoside triphosphate (dNTPs) and 0.2 mM each of primers using a PTC 100 thermocycler (MJ Research, Inc. Waltham, MA, USA).

A 10 minute denaturation step at 94°C was followed by 30 cycles at 94°C for 30 seconds, annealing temperature was performed for each primer for 30 seconds at 52.5-62°C, and extending for 30 sec at 72°C; the reaction was completed by a final extension for 7 minutes at 72°C. Amplicons were checked by agarose gel electrophoresis before sequencing analysis, to make sure that only the specific product was amplified.

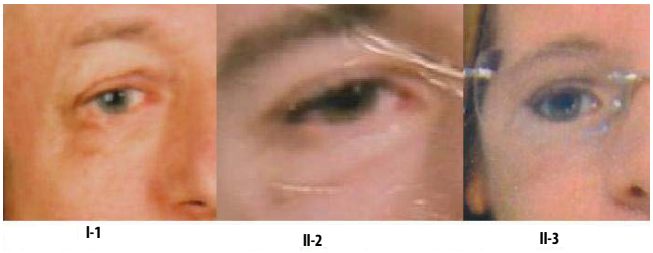


**Figure 2:** These pictures are showing the antimongoloid slants of the palpebral fissure, mandibular and zygomatic hypoplasia, coloboma of the lower lid, and absence of lower eyelid cilia.

| | FORWARD | REVERSE |
|---|---|---|
| Ex 6A | TTTATCAACTGCTGAAGCCCC | ATAGTCCTCCCTCTCCCCAAC |
| Ex 10 | CTGAACCTAGAGCCCTGTGGG | AGACAGAGTCCCAGAGTGAGG |
| Ex 16A | TGGAAACCAGAGTGCCTGAG | TGATCCTGCAGCATCTGCAG |
| Ex 24 | GCACCTCCCAACATTGAC | GAACCAGGTCTGGGTGT |
| Ex 25 | TCACTAGTCCTCAGGAGGT | CTGCCTGGCTCTCTGGGA |

**Table 1:** Primers self-designed for TCOF1 gene amplification and sequencing.

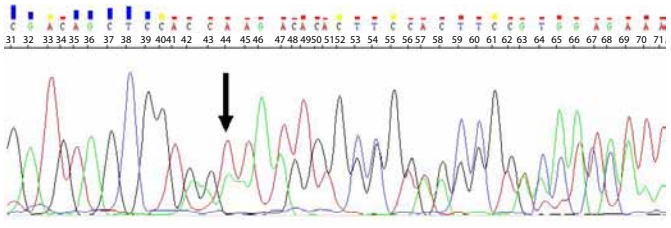

**Figure 3:** One bp deletion c.599delG. It was located in exon 6 and it caused a stop codon 19 codons later.

PCR products were purified either by digestion with Antartic Phosphotase and Exonuclease I (New England BioLabs Inc.) and were sequenced in both directions using the Applied Biosystem Big Dye Terminator v3.1 Cycle sequencing kit. The new mutation was confirmed on more than 100 controls chromosomes by sequencing.

The mutation was named according to the genomic reference (NT_029289) and the cDNA that corresponds to the major treacle isoform (NM_001135243.1) [6]. Mutation nomenclature is based on Human Genome Variation Society (H.G.V.S.) nomenclature guidelines (http://www.hgvs.org/mutnomen) [7].

## Results

Molecular analysis of TCOF1 gene confirmed clinical diagnosis of V.F., V.D. and V.S., highlighting the mutation c.599delG (Figure 3).

It was located in exon 6 and it produces a stop codon 19 codons later. Such mutation has not been previously described, and results in TCS clinical phenotype.

Mrs V.S. asked for genetic counselling: she was informed about the 50% risk of conceiving a child carrying her TCOF1 mutation and about the changeable clinical expression of the disease. After reflection, she refused to undergo genetic prenatal diagnosis. At twenty weeks ultrasound screening did not evidence any sign of TCS. A healthy male was born after uneventful physiological pregnancy.

## Discussion

Mandibular facial Dysostosis (MDF) are to be considered as variable clinical phenotypes. In several cases molecular genetics analysis can be helpful to define the correct diagnosis. In the investigated family, clinical suspicion was confirmed by TCOF1 analysis.

Accurate etiological definition and molecular mechanisms insights allow defining prenatal diagnosis strategies or, in postnatal diagnosis. Early diagnosis in TCS patients allows planning early and accurate interventions from clinical as well as psychological point of view. A multidisciplinary approach, including maxillofacial surgery and orthodontist, allows treating anesthetic and functional disabilities and improving quality of life.

### Acknowledgements

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