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# Mutational Analysis of *TCOF1*, *GSC*, and *HOXA2* in Patients With Treacher Collins Syndrome

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**Abstract:** Treacher Collins syndrome is an autosomal dominant craniofacial malformation mainly caused by mutations in the *TCOF1* gene. Few cases have been observed in the Chinese population. Herein, the authors report the mutational analysis of *TCOF1*, *GSC*, and *HOXA2* to determine the mutational features of the 3 genes in Chinese patients with Treacher Collins syndrome. Genomic DNA of the patients and their parents was extracted from peripheral blood following a standard protocol. DNA sequencing analysis was performed on all exons and the exon-intron borders of *TCOF1*, *GSC*, and *HOXA2* in addition to the 1200-bp upstream of *TCOF1*. Four novel single nucleotide polymorphisms were detected in *TCOF1*, one of which was in the promoter region. Mutations in *GSC* and *HOXA2* were not found in the 3 patients. Our results suggest the possibility of genetic heterogeneity or different mechanisms leading to the disease. Further functional study of the alteration is necessary to obtain more definitive information.

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**Key Words:** Chinese, single nucleotide polymorphisms, *TCOF1* gene, Treacher Collins syndrome

Treacher Collins syndrome (TCS, OMIM 154500) is a craniofacial development disorder. Although an autosomal recessive form of the syndrome has been reported,[1,2] most cases are inherited as an autosomal dominant trait. High intra- and interfamilial phenotypic variations have been identified, and no genotype-phenotype correlation in the syndrome has been found based on the evaluation of the clinical variability in TCS.[3–5] Anticipation is often observed in TCS families due to ascertainment bias.[4,5] TCS is likely in patients who are symmetrically affected, usually characterized by downward slanting palpebral fissures with lower eyelid coloboma, hypoplasia of the mandible and zygomatic, malformed ears, and conductive hearing loss due to atresia of the external ear canal.

The gene associated with the syndrome is *TCOF1*, which is located in 5q32-q33.1.[6] The gene contains 28 exons and several alternative splice sites.[3,6,7] To date, >150 different pathogenic mutations have been reported in the coding region of *TCOF1*, most of which are novel deletions or duplications leading to a premature stop codon.[8] Mutations in the promoter region have been proven to be functional,[9] representing a different mechanism causing the syndrome. In mice, haploinsufficiency of *TCOF1* results in the depletion of neural crest cell precursors as the result of high levels of cell death in the neuroepithelium, which can lead to a reduced number of neural crest cells migrating into the developing craniofacial complex.[10] Recent research on *TCOF1* mutant mice has demonstrated that effective cavitation of the middle ear is intimately linked to the growth of the auditory bulla, a neural crest cell-derived structure that encapsulates all middle ear components, and that defects in those processes have a profoundly detrimental effect on hearing.[11]

TCS is thought to represent defective structures derived from the embryonic first and second branchial arches, which are involved in a number of syndromic microtia,[12] including Goldenhar syndrome, Nager syndrome, and Miller syndrome. The overlap of patients with these syndromes may suggest a common genetic cause. Among the genes implicated in these syndromic microtia are *GSC* and *HOXA2*. A missense mutation in *HOXA2* has been shown to cause autosomal recessive microtia with cleft palate.[13] Similarly, a same-sense mutation and a missense mutation have been documented in 8 patients with isolated microtia.[14] We therefore report a mutational analysis of *TCOF1, GSC*, and *HOXA2* in 3 Chinese patients with TCS.

## PATIENTS AND METHODS

### Subjects

All patients with a clinical diagnosis of TCS were recruited through the Eye and ENT Hospital of Fudan University in China. The study protocols were previously approved by the Ethics Committee of the hospital, and blood samples were collected after informed consent was obtained from patients and their legal guardians. The parents also provided written permission to publish the family photos shown in Figure 1. The parents of these patients were all nonconsanguineous, and none of the patients had any family history of TCS.

### Patient 1

The patient was a 10-year-old boy. His mother became pregnant at 19 years of age, and the pregnancy was complicated by trauma at day 40, which was treated with antibiotics. After birth,

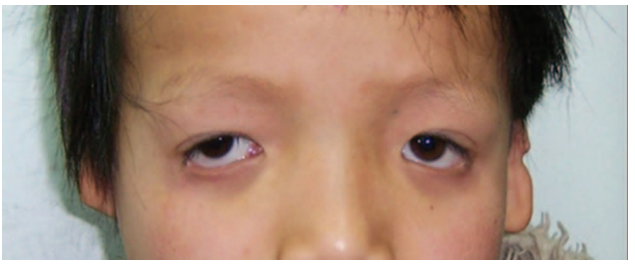

**FIGURE 1.** Patient 1 had slanting palpebral fissures, coloboma of the eyelid, bilateral microtia, atresia of the external ear canal, hearing loss, and hypoplastic zygomatic arches.

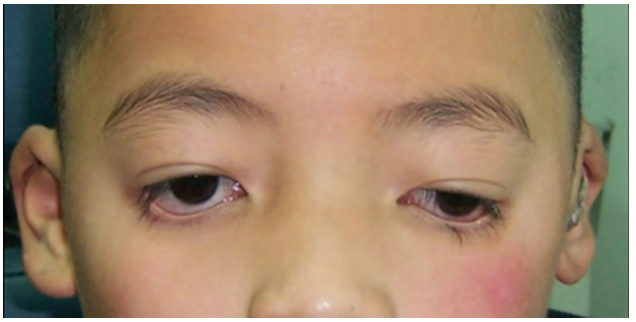

**FIGURE 2.** Patient 2 with slanting palpebral fissures, coloboma of the lid, mild deformity of the left ear, atresia of the left external ear canal, stenosis of the right external ear canal, hearing loss, hypoplastic zygomatic arches, and deformation of both forearm.

slanting palpebral fissures with coloboma of the eyelid, hypoplastic zygomatic arches, bilateral severe microtia and atresia of the external ear canal were observed. Objective audiometry confirmed bilateral conductive hearing loss with a hearing threshold of approximately 70 dB; there were no further abnormal clinical findings (Fig. 1).

## Patient 2

This patient was an 11-year-old boy, and there was no history of exposure to teratogenic substances or the presence of illness of the mother. He was born with slanting palpebral fissures, coloboma of the lid, hypoplastic zygomatic arches, mild deformity of the left ear, atresia of the left external ear canal, and stenosis of the right ear canal. His hearing loss of approximately 50 dB was successfully treated by surgery, and no further physical abnormalities were apparent (Fig. 2).

## Patient 3

Patient 3 was a 14-year-old girl born to a healthy mother without remarkable pregnancy history. She had slanting palpebral fissures, coloboma of the lid, hypoplastic zygomatic arches and mandible, and stenosis of bilateral external ear canal with conductive hearing loss of 55 dB (Fig. 3).

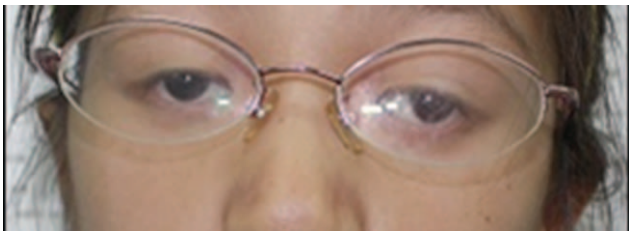

**FIGURE 3.** Patient 3 had slanting palpebral fissures, coloboma of the lid, stenosis of bilateral external ear canal, hearing loss, hypoplastic zygomatic arches, hypoplasia of the mandible, and deformation of both forearm.

**TABLE 1.** PCR Primers for the Amplification of TCOF1, GSC, and HOXA2

| PCR ID | Forward Primer | Reverse Primer |
|---|---|---|
| *TCOF1*-Promotor1 | gagggcaactgccatgtatt | tggtggtagatcaggggaag |
| *TCOF1*-Promotor2 | agactcatgcagtgccctct | gatcaacaatacgcccatt |
| *TCOF1*-exon2 | gcgccaatgggcggtattgttg | ggaaggcagagattgcggctcctc |
| *TCOF1*-exon3 | catgagtttggggagatctgg | atgtgagctggctttctggag |
| *TCOF1*-exon4 | gcatgggtcagctcctatcac | tctcctccccagggtctttta |
| *TCOF1*-exon5 | aagaatgtgggccagtccttt | gcagcaggcatcatccttatc |
| *TCOF1*-exon6 | catgtcccaagaactgggatt | ctgaccctccctcgtctaggt |
| *TCOF1*-exon7 | gcctcagagatgtgtgggaac | ctcctggtcaccctaccacag |
| *TCOF1*-exon8 | ccaggtgggagatgaacgtaa | agaggtgctcatggcagagtg |
| *TCOF1*-exon9 | ggaggctccaggagtgagagt | atagggcaaatgatgccacac |
| *TCOF1*-exon10–11 | acgtggtgtcctgtgtctcct | caaaaccacaggaggtcttttga |
| *TCOF1*-exon12–13 | ctctcctcccctcactcacat | ggggtgctgactgtggtgt |
| *TCOF1*-exon14–15 | cagaacagatgggggactctg | caacactagcccccagtcaag |
| *TCOF1*-exon16 | gaaggcacgcacaatgagttt | tccccactatggcacaactct |
| *TCOF1*-exon17–18 | gttgtgccatagtggggagtg | accacgcccagccctatac |
| *TCOF1*-exon19 | agtgctgtgctgggtcttagc | ccacaaaaaagctctggcaac |
| *TCOF1*-exon20 | caggccggtaaattgggttat | aggtgatttgggggggttgtag |
| *TCOF1*-exon21 | accagttttgccccttttgact | acttgttgcagggagtgttcc |
| *TCOF1*-exon22 | tgtgtgccccatctaacacag | aaacatggccctggagttttt |
| *TCOF1*-exon23 | aatagaaatggggcctcagga | gacctgagggatcgggtagac |
| *TCOF1*-exon24 | tgatagggcagggtgatccta | tctcacttggagaggctctgg |
| *TCOF1*-exon25–26 | gcaccctcttcgctcttaggt | gaggaatgagaccaggtgctg |
| *TCOF1*-exon27 | gttgtgatggcttctggtggt | ttccccaacaccctctacct |
| *TCOF1*-exon28 | ggtagaagggtgttggggaag | tttccccttagagcccaccta |
| *GSC*-exon1 | tggtctgagctccgtcctac | aattaaccaaccggctccat |
| *GSC*-exon2 | caattctcagcatccccttg | ttcaacttcctgggcctaaa |
| *GSC*-exon3 | gcccaggaagttgaatgaaa | cttggctccagactgatggt |
| *HOXA2*-exon1–1 | tcccaggcacacacactaga | cgtaattcatggccttctcc |
| *HOXA2*-exon1–2 | ccccatacggctgtaatcag | gaaggaagagggtcccagag |
| *HOXA2*-exon2 | ccaactgtgcgtgtgtctct | ttggtgatgctttgttttgc |

PCR, polymerase chain reaction.

## DNA Sequencing

Genomic DNA of the patients and their parents was extracted from peripheral blood following a standard protocol. All exons and the exon-intron borders of *TCOF1*, *GSC*, and *HOXA2* were amplified by PCR under optimal conditions using specific primers (Table 1); the 1200-bp upstream of *TCOF1* were also amplified.

A mixture with a total volume of 20 μL was prepared for each reaction including 1× HotStarTaq buffer, 2.0 mM $Mg^{2+}$, 0.2-mM dNTP, 0.2 μM of each primer, 1 U HotStarTaq polymerase (Qiagen Inc), and 1-μL template DNA. The cycling program was 95°C for 15 minutes; 11 cycles of 94°C for 15 seconds, 62°C to 0.5°C per cycle for 40 seconds, and 72°C for 1 minute; 24 cycles of 94°C for 15 seconds, 54°C to 58°C for 30 seconds, and 72°C for 1 minute; and 72°C for 2 minutes. The PCR products were purified using SAP and ExoI. A mixture of 1 U SAP, 6 U ExoI, and 8-μL PCR products was incubated at 37°C for 60 minutes, followed by incubation at 70°C for 10 minutes. The reaction mixture included 2-μL BigDye 3.1 mix, 2-μL sequencing primer (0.4 μM), and 1- to 2-μL purified PCR product. The cycling program was 96°C for 1 minute followed by 28 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes.

The final products were then analyzed using a capillary sequencer (ABI Prism 3730xl sequencing).

## RESULTS

We identified 12 different variations in *TCOF1*, 1 previously reported SNP in *GSC*, and no alterations in *HOXA2*. Among the 12 variations in TCOF1, −26T>A, 17693G>A, 21761–21765delCTCTC and 21968G>T have not been previously reported as a *TCOF1* mutation or polymorphism and were not in the dbSNP. All the 4 variations were also identified in healthy unaffected controls in the form of compound heterozygosity.

Analysis of patients' normal parents showed that there was no parental origin of the −26T→A or 17693G→A mutations. Interestingly, only the father of patient 1 and mother of patient 2 possessed the 21968G→T alteration in the homozygous form, explaining the heterozygous genotype of their children. Analysis of patient 2's normal parents showed that the father possessed the 5-bp deletion, whereas his mother did not, indicating that the 5-bp deletion was inherited from the father. All of the alterations found within our subjects are described in Table 2.

## DISCUSSION

The molecular mechanism responsible for TCS is still not defined; however, there has been remarkable work done in this area. First, haploinsufficiency has been proposed as one of the molecular mechanism underlying the disorder because deletion or insertion mutations in *TCOF1* were the most likely to result in the creation of a premature termination codon and a truncated protein. Second, as mutations that do not alter canonical splice signals but influence splicing have been recognized as a novel form of mutation,[15] synonymous alterations in *TCOF1* should be further investigated with functional assays before excluding pathogenicity. And last, mutations in the promoter region of *TCOF1* could impair the DNA-binding to the YY1 transcription factor.[9] It suggests a possibility that changes outside of the coding region might alter expression level of functionally normal protein.

As previous studies mainly focused on the coding region of *TCOF1*, the current study detected all the exons and the exon-intron borders of *TCOF1* in addition to the 1200-bp upstream of *TCOF1*. We identified 1 novel SNP −26T→A in the promoter region of *TCOF1*; however, functional study of this abnormality is necessary to obtain more definitive information. It is interesting to note that only the father of patient 1 and mother of patient 2 possessed the 21968G→T alteration in the homozygous form, explaining the heterozygous genotype of their children. Also interestingly, patient 2's father possessed the 5-bp deletion, whereas his mother did not, indicating that the 5-bp deletion was inherited from the father. All of the novel variations could also be found in the control samples. Therefore, no pathogenic mutations were identified in our patients. A lack of mutations in *TCOF1* suggests that other novel genes or complex changes in gene regulatory networks might be responsible for TCS in the subjects analyzed.

Mutations in *TCOF1* seem to be extremely variable, and TCS demonstrates great phenotypic variability.[3,4] Due to the clinical overlap, *TCOF1* was analyzed and excluded in patients with Goldenhar, Nager, and Miller syndromes.[16,17] Therefore, it is meaningful to clarify whether the patients with TCS have alterations in those genes related with other syndromes.[14,18] HoxA2 is a key transcription factor during development of the second branchial arch that has a main contribution in development of the external and middle ear in mouse.[19] GSC is a transcription factor that plays an essential role during the process of gastrulation in early embryonic development.[20] Mice with a homozygous disruption of *gsc* revealed multiple defects containing the lower mandible as well as components of the inner ear and the external auditory meatus.[21] Both of the genes have been identified responsible for the syndromic microtia.[13,14] We therefore sequenced all the exons of *GSC* and *HOXA2*; however, we did not find any mutations in these genes except one previously reported SNP 1244G→T in *GSC*. Although these syndromes all derived from first and second branchial arches in embryonic period, they might be altered by different genes and regulated by complex gene networks.

In the present study, we performed *TCOF1*, *GSC*, and *HOXA2* mutation analysis in 3 Chinese patients with TCS. We detected 12 polymorphic changes in *TCOF1*, 4 of which were novel. And we also excluded mutations of *GSC* and *HOXA2* in the 3 patients. Mutations in the *TCOF1* gene are not always found in patients with TCS.[8,17,22] These results suggest the possibility of genetic heterogeneity or the existence of different mechanisms leading to the syndrome. We hypothesized several possibilities to explain the undetected *TCOF1* mutations in these TCS patients. First, there may be another gene that might be located near *TCOF1* that is responsible for TCS. Second, nonsequential factors that can modulate the expression of *TCOF1*, for instance, the methylation of the gene or the mi-RNA regulation, may be involved. Further study is needed to explore the potential mechanism of these alterations in the occurrence of TCS.

**TABLE 2.** Polymorphisms Found in This Study

| Gene Tested | Exon or Intron | Patient | Position in Cutted Sequence | SNP Property | Functional Change | dbSNP Identifier |
|---|---|---|---|---|---|---|
| TCOF1 | 5'UTR | 1, 2, 3 | −89T→G | None | Unkown | rs4565199 |
| | 5'UTR | 3 | −26T→A | None | Unkown | Unkown |
| | Intron6 | 3 | 14036G→A | None | Unkown | rs2255796 |
| | Exon11 | 2 | 17681C→T | Synonymous | Pro526Pro | rs2071238 |
| | Exon11 | 3 | 17693G→A | Synonymous | Gly530Gly | Unkown |
| | Exon12 | 2 | 18111A→G | Synonymous | Ser614Ser | rs2071239 |
| | Exon13 | 2 | 18434G→C | Missense | Ala665Pro | rs2071240 |
| | Intron16 | 2 | 21761-21765delCTCTC | None | Unkown | Unkown |
| | Exon17 | 2 | 21786T→C | Missense | Val887Ala | rs7713638 |
| | Exon17 | 1, 2 | 21968G→T | Missense | Ala948Ser | Unkown |
| | Exon24 | 3 | 38922C→T | Missense | Ala1390Val | rs15251 |
| | Intron25 | 3 | 41054G→C | None | Unkown | rs2569062 |
| GSC | Intron2 | 1 | 1244G→T | None | Unkown | rs3905049 |

SNP, single nucleotide polymorphism.

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# Successful Treatment of Silent Sinus Syndrome With Combined Endoscopic Sinus Surgery and Blepharoplasty Without Orbital Floor Reconstruction

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**Abstract:** Silent sinus syndrome (SSS) is a rare clinical syndrome associated with characteristic spontaneous and gradual downward bowing of the orbital floor caused by impaired sinus ventilation. The author experienced a rare case of SSS in a 35-year-old woman patient. She was referred for evaluation of a spontaneous orbital asymmetry with right enophthalmos and hypoglobus. She underwent functional endoscopic sinus surgery to open obstructed maxillary sinus ostium and aesthetic eyelid surgery to enhance the appearance of her orbital asymmetry. These surgical treatments brought about the effect of making her eyes look more symmetric, refreshed, and alert. Here, the author reports a good treatment result of SSS without orbital floor reconstruction along with review of literatures.

**Key Words:** Blepharoplasty, endoscopic surgery, silent sinus syndrome

Silent sinus syndrome (SSS) is a rare clinical syndrome that can pose a diagnostic challenge. The patient may present with unilateral, painless, and spontaneous enophthalmos and hypoglobus. Its exact pathogenesis is unknown. It is associated with characteristic radiologic features including an opacified maxillary sinus, obstructed natural ostium, and downward bowing of the orbital floor caused by ipsilateral maxillary sinus atelectasis.[1,2] Given its rarity and peculiar pathogenesis, optimal treatment guidelines have not been clearly established.[3] Thus far, SSS is usually managed in a two-step manner. First, endoscopic sinus surgery (ESS) is favored to resolve the negative antral pressure.[4,5] The second step, repair of the orbital floor, is required to improve the facial asymmetry. In this clinical report, the author demonstrates a good surgical outcome for SSS after combined ESS and cosmetic eyelid surgery without the need for a two-step surgical approach.

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