Review Article

Treacher Collins Syndrome: The genetics of a craniofacial disease

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1. Introduction

Treacher Collins Syndrome (TCS; OMIM #154500) is a rare autosomal dominant (AD) mandibulofacial dysostosis occurring in 1 in 10,000–50,000 births, that ensues subsequent to mutations in
studies have illustrated that proper expression is essential for craniofacial development, and additionally disordered craniofacial development seen in TCS has implicated ear cavity, inner ear structures are typically unaffected [4,11]. In a large cohort, downward slanting palpebral fissures and a hypoplastic zygomatic complex where observed to be the most commonly-occurring features [2].

TCS patients have normal intelligence. As a result of distorted physical appearance, patients often experience significant psycho-social challenges and social stigma [9]. TCS has a variable phenotype with major clinical characteristics including bimaxillary micrognathia and retrorollia (78% of patients), coloboma of the lower eyelids (69%) with associated loss of medial eyelashes (53%), external ear aplasia or microtia (78%), downward slant of palpebral fissure secondary to hypoplasia of the lateral orbit, a large or protruding nose, and zygomatic bone hypoplasia (89%) [4,10]. Minor clinical characteristics include cleft lip with or without concomitant cleft palate (28%), hair displacement anterior to the auricle (26%), airway dysfunction such as tracheostoma or chonial stenosis/atresia, external ear atresia, stenosis of the external auditory canal (35%) and approximately 50% of patients have conductive hearing loss due to ear malformations including microtia, ossicular chain malformation, ankylosis, and meatal atresia [4,10,11]. In a large cohort, downward slanting palpebral fissures and a hypoplastic zygomatic complex where observed to be the most commonly-occurring features [2].

Despite extensive malformation and hypoplasia of the middle ear cavity, inner ear structures are typically unaffected [4,11]. Interestingly, conductive hearing loss occurs at a significantly lower frequency in patients with mutations of the 3’ open reading frame of TCOFI [2]. Other minor characteristics include ophthalmologic complaints including refraction error (58%), strabismus (37%), amblyopia (33%), and anisometropia (17%). In one sample, dental anomalies were identified in 60% of TCS patients, with an average of 1–8 anomalies per patient. The most frequent dental anomalies were tooth agenesis (33.3%) predominantly affecting the mandibular second premolars, and enamel opacities (20%) [12]. Together, these features contribute to speech and language difficulties, visual impairment or loss (37%), conductive hearing loss, breathing difficulty, and obstructive sleep apnea [11,13].

1.1. Diagnosis

Diagnosis of TCS is usually made at birth and is primarily a clinical diagnosis supported by radiographic data and molecular studies. However, diagnosis can also occur prenatally if molecular analysis is performed by amniocentesis (~15–18 weeks gestation) or chorionic villous sampling (~10–12 weeks gestation) [14]. In affected families, subsequent pregnancies can be monitored with transvaginal and abdominal ultrasound. Sonographic imaging combined with linkage analysis can identify the disease as early as the first trimester [15]. Anomalies commonly observed by ultrasound include polyhydramnios, abnormal fetal swallowing, microcephaly, distorted facial characteristics such as antimongoloid slanting palpebral fissures, malformation of the auricles, microphthalmos, micrognathia [16–18]. Ophthalmologic radiographs are useful in identifying aplasia or hypoplasia of the zygomatic arch, and Ophthalmologic X-ray view (Water’s view) and orthopantomogram films are helpful in identifying mandibular hypoplasia. Computed tomography (CT) scans can establish malar hypoplasia and cephalometric radiographs can determine the extent of mandibular retrognathia [19].

Genetic testing, which should be considered in patients with at least two of the major features or three minor features, utilizes a broad range of diagnostic modalities to determine mutations in TCOFI, POLR1C and POLR1D [4]. In general, whole gene or whole exon deletions are not detected in TCS. The strategy for testing first utilizes sequence and deletion/duplication analysis in patients with a family history consistent with AD inheritance (40% of TCS cases have a positive family history) and in cases that are the first occurrence of TCS in a family. If this approach is not effective, POLR1D should be sequenced. POLR1C sequence analysis should be utilized in families with multiple affected siblings, consanguinity, or in patients in whom TCOFI and POLR1D testing was negative. Families with a known history of TCS may opt for pre-implantation genetic diagnosis or in utero testing.

Although penetrance of the disorder is high, there is a considerable amount of phenotypic variation ranging from perinatal airway obstruction to milder variants with minimal dysmorphism [20–22]. For example, the common TCOFI 4369_4373delAAGAA mutation is known for its incomplete penetrance and patients should be counseled regarding the inability to provide comprehensive insight into the extent of facial malformation or disease severity [4]. Therefore, despite the availability of genetic testing modalities, the phenotype cannot be readily predicted by the patient’s genotype [2]. The differential diagnosis for TCS is broad and includes other mandibulofacial dysostoses including Toriello syndrome, Bauru syndrome, Heder-Toriello-Petty syndrome, and Guion-Almeida syndrome. There are phenotypic features of TCS in addition to mandibular dysostosis that are seen in other diseases. Limb deformities are found within Nagar and Miller syndrome. Colobomas, which in TCS occur symmetrically on the lower eyelids, are found also in Goldenhar syndrome – albeit asymmetrically on the upper lids. Some permanent features of TCS, namely micrognathia, glossoptosis, and cleft palate, are found to self-correct without intervention in Pierre Robin syndrome. Finally patients with nonsyndromic mandibular hypoplasia also have similar mandibular deformities such as temporomandibular joint ankylosis, aglossia, and microglossia [4].

1.2. Management

An extensive range of surgical interventions is utilized to secure respiratory function, ensure proper feeding, improve hearing, and reconstruct profound periorbital and craniofacial defects. Extent of airway compromise is diverse, with some infants requiring immediate tracheostomy. TCS management is a multidisciplinary affair, as interventions range from reconstructive to psychosocial. Satisfaction with reconstructive surgery varies, and a recently published cross-sectional cohort study of 58 TCS patients illustrated patient dissatisfaction with physical characteristics of their ears, facial profile, eyelids, chin, and teeth. Patients were also distressed by functional challenges of hearing, nasal patency, eye tearing, altered smell, and eyelid closure. Despite challenges in the surgical management of TCS, there is a relatively extensive armamentarium to address the profound dysmorphism seen in this disease.

Complaints concerning the eyelids were most frequently managed by lateral canthopexy and lower eyelid reconstruction.
Ear malformations are corrected by external ear reconstruction, ear epithesis, and tympanoplasty. Nasal reconstruction utilizes nasal hump reduction, rhinoplasty, septoplasty, and nasal tip reconstruction. Deformities of the facial profile and chin require complex interventions including Le Fort I osteotomy, genioplasty, and mandibular distraction osteogenesis. Sleep endoscopy [13], speech and swallow therapy, and implantation of bone-anchored hearing aids [BAHA] are among other supplemental interventions [10]. Despite extensive operative intervention, 25% of patients desire further treatment with the majority requesting further eyelid reconstruction. Of note, in a study by Arndt et al., surgical improvements of facial appearance appears to have a positive influence on psychosocial and social factors [23].

The precise timeline of surgical management of TCS is complex, and depends in part on phenotypic severity. Early neonatal management revolves around airway maintenance, ensuring proper nutrition, and closely monitoring infant growth parameters and depends in part on phenotypic severity. Early neonatal improvements of facial appearance appears to have a positive influence on psychosocial and social factors [23].

The comprehensive surgical management of TCS is multifaceted and falls outside of the purview of this paper. However, salient aspects of treatment will be highlighted in the broad scope of the syndrome. In particular, Chang and Steinbacher provide an excellent summary of the timeline for reconstructive intervention in TCS patients. The hypoplastic mandible is managed early in the neonate to offset airway obstruction, with treatment generally consisting of distraction osteogenesis [25] or, less frequently, en bloc mandibular advancement when the neonatal bone or anatomy precludes distraction. In the setting of a stable airway, mandibular distraction can be delayed until early childhood or until the arrival of the permanent teeth, allowing for improved bone quality. Management of significant malocclusion occurs during late adolescence, when bone maturity has been established. In addition, cautious and detailed preoperative planning as a multidisciplinary team coupled with appropriate computed tomography and/or computer-aided imaging are essential to surgical success. Point-based geometric morphometrics have recently been used by Nikkhah et al. to measure and characterize TCS deformities and may have utility in planning surgical reconstruction [26]. TCS is of particular significance to the anesthesiologist, as this cohort of patients is particularly difficult to intubate and extubate. In this way, a range of techniques is utilized, including the use of the laryngeal mask airway (LMA), fiberoptic intubation (using either fiberoptic bronchoscopy or through the LMA), or tracheostomy as the last resort [27].

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2.2. The role of treacle

(1) Regulating transcription of rDNA
(2) Pre-processing of the ribosomal-RNA transcript

Treacle is a 144 kDa protein encoded by TCOF1 consisting of at least three distinct functional domains [7,39]. Treacle's C-terminal domain has been implicated in nuclear import and nucleolar localization while the N-terminus is putatively responsible for nuclear export [7]. Through anti-Treacle antibody targeting of these two domains, the endogenous protein has been localized to the dense fibrillar region of the nucleolus. The majority of mutations occur at the nuclear import domain leading to a loss of Treacle's import signal, suggesting that absent nuclear and nucleolar localization may underlie TCS pathogenesis [7]. Treacle's central domain contains a number of protein kinase C and casein kinase II phosphorylation site repeats, a finding that correlates well with in vitro studies illustrating that Treacle is highly phosphorylated and associated with casein kinase II [39]. This phosphorylation status may have a role in normal Treacle functionality. While Treacle's exact molecular function within the nucleolus is unclear, previous studies have identified two key roles that support its function in ribosomal biogenesis. In vitro studies have demonstrated that Treacle colocalizes to the same regions of ribosomal DNA as Upstream Binding Factor (UBF), a transcription factor involved in ribosomal DNA transcription. Associated knockout studies demonstrate that Treacle is also a regulator of ribosomal DNA gene transcription.

Treacle has been shown to interact with Nop56-associated pre-ribosomal ribonucleoprotein (pre-rRNPs) complexes, which are known to methylate 2'-O ribose moieties on pre-rRNA. This finding suggests that Treacle has an additional role in the pre-processing of a ribosomal RNA [40]. As discussed previously, Treacle has multiple constituent domains with different molecular functions. Treacle's central domain binds with RNA Polymerase I (polI) and is essential for the nucleolar recruitment of Poll [41]. The C-terminal domain is responsible for rDNA promoter recognition, UBF recruitment, and nucleolar targeting [37]. In vitro siRNA-knockdown studies and in vivo studies of TCOF1 haploinsufficient mice have further implicated Treacle's role in the transcription of rDNA and its association with human Nop56-associated pre-rRNPs complexes [42]. Inhibiting this function reduces the quantity of mature ribosomes, specifically the 28S subunit, which subsequently impairs proliferative ability of neural crest cells [6]. Dixon et al. illustrated tissue-specificity in neural ectoderm and neural crest tissues for diminished mature ribosome production in TCOF1 haploinsufficient mice [43]. The authors subsequently postulated that diminished ribosome biogenesis could not meet metabolic demands of the proliferating cells and led to high rates of cell death in neural crest cell formation.

Finally, it has been established that Treacle contains a nuclear export signal between amino acids 40 and 49 [44]. Treacle additionally contains a L11-homologous motif (LisH). LisH is associated with regulation of microtubule (MT) dynamics and may have a role in cell migration and chromosome segregation, either by means of mediating MT dimerization, by binding the cytoplasmic dynein heavy chain, or microtubule directly [45]. Mutations in mammalian Lis1 (Pahab1b1) result in neuronal migration defects [46]. However, there is no data that illustrates Treacle nuclear export [6].

2.3. The role of P53

Mutations contributing to the TCS phenotype have been compiled in an online registry of TCS-associated mutations [47]. In a recent study by Jones et al., it was elucidated that activation of P53 and associated pro-apoptotic proteins in mutant embryos mediated cellular death in neuroepithelium. The P53 protein, derived from the \textit{Trp53} gene, responds to cellular stress by arresting cells at the G1 phase of the cell cycle. P53 acts to maintain the balance between cell proliferation and apoptosis within all cells of the body and sits at the epicenter of a number of similar regulatory pathways. TCOF1 heterozygotes were found to have P53 levels over two-fold higher than their wild-type counterparts. The authors illustrated that \textit{Trp53} expression was not upregulated, suggesting that increased levels of P53 in TCOF1 heterozygotes resulted from post-translational stabilization of this traditionally labile molecule [48]. While TCOF1 and its action on ribosomal maturation were thought to be the key molecular factor underlying TCS, P53 may have a vital role in TCS pathophysiology. Indeed, P53 partial knockouts in TCOF1−/− mice protected against a TCS phenotype and improved post-natal viability by up to six months, whereas TCOF1 heterozygotes expire following delivery due to an inability to feed. The authors observed that complete P53 knockouts (Trp53−/−) lead to profound tumorigenesis.

Jones et al. illustrated that chemical or genetic inhibition \textit{in utero} with pifithrin—a effectively blocked neuroepithelial apoptosis and restored the migrating neural crest cell population in mutant TCOF heterozygote embryos [48]. This return to function is especially salient as it occurred without altering or restoring ribosome synthesis [43]. This finding suggests that TCOF1 haploinsufficiency causes sufficient cellular stress to activate P53 and concomitant cell-death pathways and further implicates P53 as a putative therapeutic target. In a recent article by Sloan et al. illustrates that impaired ribosome production directly activates p53. Specifically, 55 ribonucleoprotein particle (5S RNP) amasses in the nucleoplasm if ribosome synthesis is blocked. 5S RNP subsequently associates with mouse double minute 2 (MDM2), which, by a variety of mechanisms, ultimately activates p53. Taken together, this recent study further implicates the downstream role tumor suppressor p53 during failed ribosome biogenesis [49].

2.4. Genetic features and recently identified novel mutations underlying TCS pathogenesis

The TCS phenotype occurs subsequent to significant genetic heterogeneity, with well over 100 TCOF1 mutations yielding TCS [2]. The features of the mutations are diverse, and some of the most recent and salient examples will be explored. In a study by Su and colleagues, genomic PCR analysis of a neonate with classic TCS illustrated five silent mutations as well as a missense mutation as a result of an alanine to valine substitution (A810V). The study also demonstrated a novel 5-bp deletion of exon 22 (3469del) leading to a premature stop codon [8]. Deletion and insertion mutations vary in size and frequency. In a recent study utilizing multi-temperature single-strand conformation polymorphism (MSSCP) analysis of two monozygotic twins with classic TCS illustrated five silent mutations as well as a missense mutation as a result of an alanine to valine substitution (A810V). The study also demonstrated a novel 5-bp deletion of exon 22 (3469del ACTCT) leading to a premature stop codon [8]. Deletion and insertion mutations vary in size and frequency. In a recent study utilizing multi-temperature single-strand conformation polymorphism (MSSCP) analysis of two monozygotic twins with classic TCS, a de novo insertion mutation was discovered within exon 5 of TCOF1. This mutation produced termination of translation at 167aa, eliminating the nuclear localization signal. This is a unique finding for multiple reasons; common mutations of TCOF1 are deletions, most being found in other exons. In this particular study, c.484 668ins is the longest insertion mutation ever to be localized to TCOF1, at 185 bp in size [50]. The TCS phenotype can also occur via TCOF1 gene rearrangement. Bowman, et al. conducted a large-scale study that screened TCS patients by DNA sequence and dosage analysis, demonstrating the presence of partial deletions in exons of the TCOF1 gene. Among this cohort, five patients were identified as having partial deletions that occurred via gene rearrangement, accounting for 5.2% of all
pathogenic mutations. As noted earlier, the TCS phenotype is predominantly the result of premature stop codons yielding non-functional protein product. Bowman’s study is the first report of gene rearrangement causing TCS, which in their study accounted for a significant percent of TCS cases. Taken together, the authors contend that diagnosis of TCS should utilize dual sequencing and additional dosage analysis to identify novel mutations that may not otherwise be identifiable by conventional means [3]. In a study of 46 patients with a tentative diagnosis of TCS, Teber et al. observed that mutations occurred sporadically throughout the TCOF1 gene, with no focal clustering of mutations, and the majority of mutations led to premature termination due to frameshift length mutation or nonsense substitutions. Interesting, the article observed that the rates of mutation detection, the proportion of splice site mutations, and mutations predicted to result in nonsense mutation are similar between patients with mild and severe disease. They differ, however, in that missense mutations occur more commonly in phenotypically severe patients. Finally, there was no difference in the location of mutation when comparing severe versus mild TCS [2].

Gene deletions in POLR1D and POLR1C have been discovered in TCS patients without a TCOF1 mutation. The POLR1D gene, located on chromosome 13q12.2 codes for three exons that contribute to subunits of RNA polymerase I and III. Both of these polymerases have a role in ribosomal RNA and small RNA transcription, respectively. POLR1C, located on chromosome 6p21.1, codes for nine exons and additionally contributes subunits to RNA polymerase I and III. A range of mutations in these genes has been recently determined, and it was hypothesized that reductions in functional Poll or PolIII results in inadequate quantities of mature ribosomes in the neuroepithelium and neural crest cells at a critical juncture in embryogenesis, leading to subsequent activation of cell death pathways [5].

2.5. Role of the zebrafish model in TCS genetic research

A murine model has predominantly been used to study TCS. Haploinsufficiency of TCOF1 in mice results in comparable facial dysmorphism as seen in TCS. A recent study has potential to open a new venue of genetic exploration through the use of a zebrafish model. Weiner et al., using the zebrafish ortholog of human TCOF1 “BjHy2”, illustrated that a BjHy2 loss-of-function mutation attenuated cellular proliferation and led to craniofacial malformation with a TCS-like phenotype. The authors additionally elucidated a role for TCOF1 outside of ribosome synthesis [30]. In particular, TCOF1 interacts with multiple non-ribosomal targets including ndrg1, a molecular target that is found to induce apoptosis in neural crest cells, and Tbx2b, a transcription factor implicated in suppression of P53 function. This study demonstrated the first use of zebrafish as a model to study TCS and may have utility in future research [29].

3. Discussion

The majority of TCS occurs as the result of a mutation in TCOF1. These mutations, as well as novel mutations affecting other genes, have occupied much of the recent TCS genetic literature. Our study collated these recent discoveries and highlighted gaps in current knowledge of disease pathogenesis. Despite the promise of therapeutic intervention, repair of mutant genotypes seems unlikely in the current day and genetic screening in predisposed families is a fundamental strategy in the prevention of inherited TCS. Future studies should utilize recently established genetic and molecular data of TCS and correlate mutation type with phenotypic severity. This is particularly useful for the reconstructive surgeon, as patients with TCS often need a multi-stage reconstructive effort.

Knowledge of a patient’s TCS status can allow the surgeon to accordingly plan for intervention and ensure higher patient compliance. It would be additionally helpful to establish the genotype of surgically reconstructed patients and associate mutation status with success or challenges of reconstructive surgery, thus improving pre-operative planning. While a number of authors have contributed data illustrating that certain mutations are associated with phenotypic elements, there is a paucity of data describing these relationships, and this remains an area of study that can continue to be clarified.

While recent studies of novel mutations contributing to TCS phenotype may have utility in establishing in utero diagnosis of TCS, future studies should determine the role of P53 and its downstream effectors in TCS. The role of P53 in TCS pathophysiology is especially striking, as it suggests a distinguishable role of mature ribosome biogenesis and the P53 pathway in TCOF1 mutants. That being said, the ubiquitously expressed P53 is an unlikely therapeutic target. Future research should clarify the role of downstream complicated pathways, and should further specify which effectors, downstream of P53, are most closely correlated with TCS phenotype.

The association of Lish domain mutations in Tracele and TCS phenotype strongly suggest a role for Treacle outside of the nucleus. There is a scarcity of data regarding the means by which Treacle transports from the nucleolus to the cytoplasm, and further research describing this process may give insight into Treacle function beyond its role in ribosomal biosynthesis.

Trainor et al. suggested that stem cells might play a role in improving surgical outcomes. The author suggests the possibility of introducing stem cells in utero, which although controversial, is nonetheless interesting [6]. While this has incumbent dangers, the idea of directed therapy utilizing stem cells should continue to be explored.

Recent genetic insights into TCS have identified a range of new TCOF1, POLR1C, POLR1D mutations, suggested a broadened role for Treacle outside of the nucleus, have implicated P53 as a key player in TCS pathogenesis and a potential therapeutic target, and have identified zebrafish as a potentially high throughput animal model. Our literature review elaborates the genotypic and phenotypic descriptions of TCS and highlights the research to date on the relationship of Treacle, P53, and the equivocal role of the ribosome on TCS pathogenesis. While much of this data is scientifically fascinating, there is no clear therapeutic mechanism that could potentially stabilize neuroepithelium and neural crest cells in TCOF1 mutants. Even if such a mechanism did exist, its utility is questionable. Indeed, by the time that a patient with TCS is diagnosed, the disease process has begun and the time to intervene has likely passed. Therefore, the therapeutic utility of amassing genetic data is in genetic screening of high-risk families or pregnancies suspected of harboring a TCS-inducing mutation. It is our hope that continued research of the molecular underpinning of TCS will identify a distal element of the highly implicated P53 pathway that could potentially be targeted therapeutically without the risk of tumorigenesis.

References


