Chapter 13
Etiology and Anatomical Variation in Treacher Collins Syndrome

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A Personal Perspective on Treacher Collins Syndrome by the Author

My life with Treacher Collins syndrome has led me into a unique career in craniofacial biology and craniofacial anomaly research and global public outreach for

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awareness and research of craniofacial differences. I was born with severe Treacher Collins syndrome and needed a tracheostomy to save my life at birth. I have had over 20 facial reconstructive surgeries, speech therapy, and bone conduction hearing aids. My challenges were not only medical but also social and emotional. I faced years of bullying in school, along with stares and questions in public, and discrimination. Despite these challenges over my lifetime, my faith, my adoptive family, my friends, and my colleagues have all been an encouragement to me to persevere and pursue the unique goals of my life. After sensing the call to enter the craniofacial medical field while in high school, I studied biology at Purdue University for my bachelor and master of science degrees. After unsuccessful attempts at applying to medical school, I furthered my education in biomedical science at King's College London in the United Kingdom and received my first education and research experience in craniofacial embryology and genetics there. My mentors at King's College London saw potential in me as a craniofacial research scientist and encouraged me to pursue a PhD. This I achieved at the University of California San Francisco in 2012. My first postdoctoral fellowship, at the University of Calgary Faculty of Medicine, introduced me to the exciting field of three-dimensional (3-D) imaging and morphometrics (morphological or shape analysis) of the craniofacial region in animal models and in humans. My work in craniofacial 3-D imaging and morphometric analysis led to the majority of my published papers and subsequent opportunities in 3-D craniofacial imaging and analysis in clinical situations including airway imaging and volume assessment in Brazil and planned future collaborative research in craniofacial and airway 3-D imaging and analysis. My second postdoctoral fellowship, at the University of Colorado Anschutz Medical Campus, focused on craniofacial gene expression analysis. I continue to engage in public and educational outreach for craniofacial anomaly awareness and research, by speaking and guest-lecturing worldwide as well as through media interviews and even a recently published memoir, *Wonderfully Made: The Dr. Francis Joel Smith, PhD Story.*

Introduction to Treacher Collins Syndrome

Treacher Collins syndrome (mandibulofacial dysostosis, Treacher Collins-Franceschetti syndrome, Franceschetti-Zwahlen-Klein syndrome) (OMIM 154500) is a rare disorder of craniofacial development (1:50,000 live births), and 60% of cases arise as de novo mutations [1, 2]. The syndrome is named after nineteenth-century British surgeon and ophthalmologist Edward Treacher Collins, who first described the essential traits of the condition in 1900, particularly the outer notching of the eyelids and malar bone defects [3]. Genetically, it is of variable genotype and penetrance, and in terms of severity, it has a wide phenotypic variation from extremely mild to severe malformations. It is a disorder of development of the first and second branchial arches in the embryo. Defects of bone and tissues derived from these arches include malar and zygomatic hypoplasia, maxillary and mandibular defects including micrognathia and retrognathia, cleft palate, and ear defects

including auditory canal stenosis or atresia, microtia or anotia, and incomplete or missing middle ear ossicles. External characteristics include not only the aforementioned outer ear defects (microtia or anotia) but also downward slanting of the eyes due to the incomplete orbital rims, sunken cheeks for lack of zygomatic arches, and retruded chin due to the micrognathia and retrognathia [1, 2]. Figure 13.1 shows the characteristic facial features of a severe manifestation of Treacher Collins syndrome. The inheritance pattern of Treacher Collins syndrome is autosomal dominant [1, 2].

Part I: Etiology of Treacher Collins Syndrome

TCOF1*: The Master Gene of Treacher Collins Syndrome*

The gene responsible for the majority of cases of Treacher Collins syndrome is *TCOF1*. Through studies of families with Treacher Collins syndrome, their pedigrees, and candidate gene analysis, Dixon and colleagues [1, 2] pinpointed the true chromosomal location of the gene. After eliminating other putative chromosomal sites for the gene [1], Dixon and colleagues further narrowed the candidate region to a region on the long arm of chromosome 5, at locus 5q31–34 [2]. The locus was eventually more precisely pinpointed to 5q32–33.1 [4].

To understand the potential function of the gene *TCOF1*, Dixon and colleagues [5] isolated the mouse homologue of *TCOF1*, designated as *tcof1*. *Tcof1*, on mouse chromosome 18, was found to encode a low-complexity protein 133 kDa in size and rich in the amino acids serine and alanine; this protein was found to have 61.5% identity with human and other species' homologues [5]. This protein is conserved among species especially in regions of amino acid repeats and signals for nucleolar localization [5]. The nucleolus, located within the cell's nucleus, is where

Fig. 13.1 The classic facial features of Treacher Collins syndrome, including microtia, downward slanting eyes, sunken cheeks, and micrognathia. Note tracheostomy tube. (Photo of author at age 2, from author's personal archive)

ribosomes (the protein machinery of translation of messenger RNA into proteins) are made, so the localization of the *tcof1* protein product to the nucleolus suggested to Dixon et al. [5] that this protein would be involved in ribosome biogenesis and nucleolar-cytoplasmic transport. *Tcof1* was shown to be expressed in a wide variety of mouse embryonic and adult tissues and regions, with peak expression in the embryonic neural folds immediately prior to fusion [5], and also in the developing branchial or pharyngeal arches at critical morphogenetic stages, all derived from the progenitor cell population known as the neural crest. Craniofacial tissues and structures are derived from cranial neural crest cells. Wise et al. [6] confirmed that human *TCOF1* encodes a nucleolar phosphoprotein (Treacle), and mutations within *TCOF1* (as seen in the families they studied) cause defects in this nucleolar trafficking and ribosomal biogenetic protein that is critical to craniofacial development. A study of the mutational spectrum of *TCOF1* in Treacher Collins syndrome by Edwards et al. [7] revealed a premature termination codon which leads to formation of a truncated Treacle protein; this malformed protein cannot carry out its ribosomal biogenetic and nucleolar trafficking functions; *TCOF1* mutations appear to be family-specific. Edwards and colleagues [7] concluded that Treacher Collins syndrome results from haploinsufficiency of *TCOF1* and, in turn, a defective Treacle protein. Marsh and colleagues [8] confirmed the requirement for Treacle to localize specifically to the nucleolus, via induced mutations that caused the defective Treacle to localize to random locations in the cell; these mutations deleted putative nucleolar localization signals in the gene. These findings supported the hypothesis that part of Treacle's function is shuttling between the nucleolus and cytoplasm [8]. Jones et al. [9] found that Treacle protein contains kinase phosphorylation sites in its sequence, which might be critical to its function; they found kinase activity in embryonic branchial arches 1 and 2 coinciding with expression of Treacle at its peak in these arches. Hot spots for mutations in *TCOF1* were found to be clustered in five exons (exons 10, 15, 16, 23, and 24), which are responsible for 50% of all pathogenic mutations [10]. In these hot spots, mutations in exons 10, 15, and 16 occur in a region of repetitive sequences that are normally phosphorylated by casein kinase II, and mutations in exons 23 and 24 occur in the nucleolar localization signal sequence [10] that demonstrate critical functions in Treacle involving nucleolar localization, shuttling, and kinase activity.

The function of Treacle was finally pinpointed in the mid to late 2000s. Treacle was found to have a critical function in transcription of ribosomal DNA (rDNA) by binding with upstream binding factor (UBF), in the process of ribosome biogenesis in the nucleolus [11]; Treacle and UBF were found to be co-localized in the nucleolus. Treacle and UBF interact together with rDNA chromatin during rDNA transcription; this was shown to be a critical interaction when Treacle expression was downregulated by the use of specific targeted short interfering RNA, preventing transcription of rDNA and cell growth [11]. Gonzales et al. [12] observed that Treacle, through its interaction with UBF and RNA polymerase I, controls transcription of the ribosomal DNA gene and methylates the pre-rRNA.

Further, since Treacle is most actively expressed in the embryonic neural crest and later on in the branchial arches (specifically arches 1 and 2), Dixon et al. [13]

discovered in the mouse model they created for *tcof1*/Treacher Collins syndrome via germline mutations that these mutations (and the resulting defective Treacle protein) lead to deficiency in formation and proliferation and, at the same time, mass apoptosis (death) of neural crest cells—those embryonic migratory progenitor cells that give rise to craniofacial bones, tissues, and structures—and result in craniofacial hypoplasia characteristic of Treacher Collins syndrome. This demonstrated the critical function of Treacle as a spatiotemporal regulator of mature ribosome biogenesis, and deficiency of Treacle results in disrupted neural crest cell formation and proliferation via mass apoptosis in the neural crest in response to deficient ribosome production [13]. However, these defects can be experimentally reversed, as Jones and colleagues [14] discovered through their targeted inhibition of p53 (a molecule involved in apoptosis) in neural crest cells, resulting in reduced neuroapoptosis (neural crest-specific mass apoptosis) and rescue of craniofacial defects in mouse embryos, a potential therapeutic avenue. Another role for Treacle is to protect the neuroepithelium and its daughter neural crest cells from reactive oxygen species (ROS), the oxygen radicals that cause metabolic oxidative stress that can be toxic to these cells and cause the neuroapoptosis leading to the craniofacial hypoplasia of Treacher Collins syndrome [15].

POLR1C and POLR1D Cause Rare Forms of Treacher Collins Syndrome

While mutations in *TCOF1* and defects in its protein product Treacle are responsible for most cases of Treacher Collins syndrome, there are two other genes more recently implicated in rarer forms of the syndrome including an autosomal recessive inherited form of TCS. These two additional genes, *POLR1C* and *POLR1D*, encode subunits of RNA polymerases I and III, which transcribe rRNA [16, 17]. Mutations in *POLR1C* cause an autosomal recessive form of Treacher Collins syndrome, while mutations in *POLR1D* can be either autosomal recessive or dominant [16–18]. In their zebrafish model for Treacher Collins syndrome, Watt and colleagues [17] observed that *Polr1c* and *Polr1d* are expressed spatiotemporally and dynamically at first ubiquitously throughout the embryo in the earliest stages, then more prevalently around the eyes and at the midbrain/hindbrain border, and especially in the craniofacial pharyngeal arches. Mutations in these two genes lead to craniofacial cartilage hypoplasia and cranioskeletal defects characteristic of Treacher Collins syndrome. On a mechanistic basis, these mutations cause a loss of function of the RNA polymerase I and III subunits, leading to a deficiency of mature ribosome production [17]. This in turn causes neuroepithelial cell death (neuroapoptosis) dependent on Tp53 and in turn a deficiency of migratory neural crest cells. Research on *TCOF1* and *POLR1C* and *POLR1D* demonstrates that Treacher Collins syndrome is both a ribosomopathy (disorder of ribosome production) and a neurocristopathy (deficiency of neural crest cells). Watt et al. [17] also found that the Tp53-dependent neuroapoptosis can be genetically inhibited, and this could be a

potential avenue for prevention of Treacher Collins syndrome, as much as the research by Jones and colleagues [14] on *Tcof1* p53-dependent neuroapoptosis in mice shows that p53 can be inhibited leading to rescue of Treacher Collins syndrome anomalies. The research by Watt et al. [17] on mutations in *POLR1C* and *POLR1D* demonstrates the necessity for, and function of, these RNA polymerase I and III subunits in transcription of rRNA, mature ribosome biogenesis, proliferation of neural crest cells, and craniofacial development.

Part II: Anatomical Variation in Treacher Collins Syndrome

Treacher Collins Syndrome Is a Neurocristopathy

Craniofacial development begins in the neuroepithelium, the early embryonic tissue that gives rise to the neural crest. The neural crest forms when the neural plate invaginates to form parallel neural folds, which in turn close dorsally to form a tube (the neural tube) [19]. In the neural crest, neural crest cell formation is induced by signals from surrounding tissues, including Wnt signaling across the ectoderm-neural plate border, fibroblast growth factor (Fgf) signaling from the paraxial mesoderm surrounding the neural tube, and bone morphogenetic proteins (BMP) within the neural crest itself. During this process, neural crest cells are encouraged to begin migrating in a dorsoventral path away from the neural crest [19]. Induction of the neural crest is illustrated in Fig. 13.2.

Neural crest cells migrate along defined pathways leading from specific regions of the neural crest in the forebrain, midbrain, and hindbrain, and along the hindbrain there are rhombomeres r1 through r7 [20]. These pathways lead to particular destination regions including the anterior portions of the embryonic head as well as branchial arches (numbered 1–4). The craniofacial region of particular interest regarding Treacher Collins syndrome encompasses the region formed from arches 1 and 2 [20]. Neural crest cell migration along these defined pathways to particular destination regions is illustrated in Fig. 13.3.

The skull and its associated tissues originate from two progenitor populations—the paraxial mesoderm from somites and somitomeres and the neural crest. The frontal, nasal, zygomatic, lacrimal, ethmoid, sphenoid, anterior (squamous) temporal bones, maxilla, mandible, and hyoid originate from neural crest-derived cartilage, while the parietal, occipital, and posterior (petrosal) temporal bones originate from the paraxial mesoderm [21, 22]. The first arch neural crest-derived bones are the maxilla, mandible, zygoma, and the lower portion of the squamous temporal bone and, in the middle ear, the malleus and incus. Derived from the second arch are the hyoid and, in the middle ear, the stapes [21, 22]. Figure 13.4 illustrates the origins of skull bones from the paraxial mesoderm and neural crest.

However, as reviewed earlier, in Treacher Collins syndrome, faulty Treacle and RNA polymerase activity lead to metabolic oxidative stress, and this results in

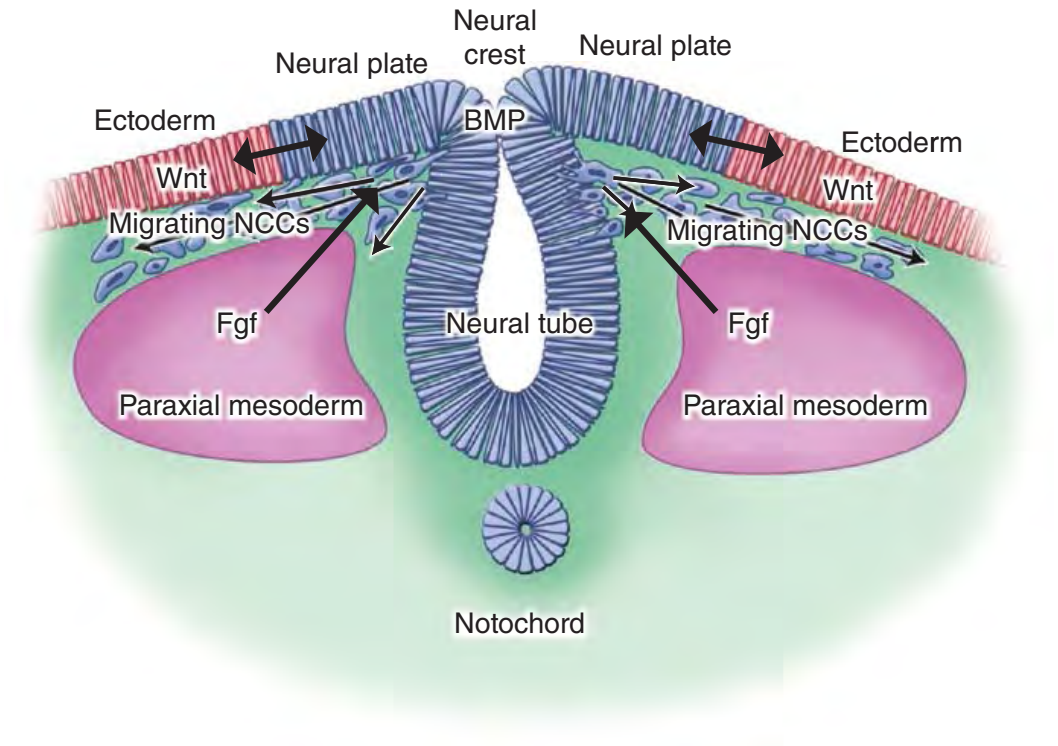

Fig. 13.2 Induction of the neural crest by morphogenetic signals from surrounding embryonic tissues and beginning of neural crest cell migration. Wnt signaling across the ectoderm-neural plate border, Fgf signaling from the paraxial mesoderm, and BMP signaling within the neural crest induce formation of neural crest cells, and these begin migrating away from the neural crest in a dorsoventral direction. (Hand-drawn by author)

massive neuroepithelial apoptosis, in turn causing a significant deficiency of neural crest cells. There are insufficient numbers of migrating neural crest cells available for complete formation of the cartilage and other tissues necessary to form the branchial arch 1 and 2 derivative craniofacial bones and tissues [13, 14].

Anatomy of Treacher Collins Syndrome: Craniofacial Malformations and Airway Obstruction

The anatomical abnormalities present in Treacher Collins syndrome are the result of hypoplasia of neural crest-derived branchial arch 1 and 2 cartilages which in turn cause hypoplastic cranioskeletal defects. These include hypoplasia or absence of the middle ear ossicles and stenosis or atresia of the auditory canals, as well as cleft palate, malar and maxillary hypoplasia, incomplete formation of the zygomatic arches and orbits, and mandibular defects including micrognathia (small mandible) and retrognathia (retruded mandible) and temporomandibular joint (TMJ) defects [1, 2]. Figure 13.5 illustrates the cranioskeletal defects of Treacher Collins syndrome.

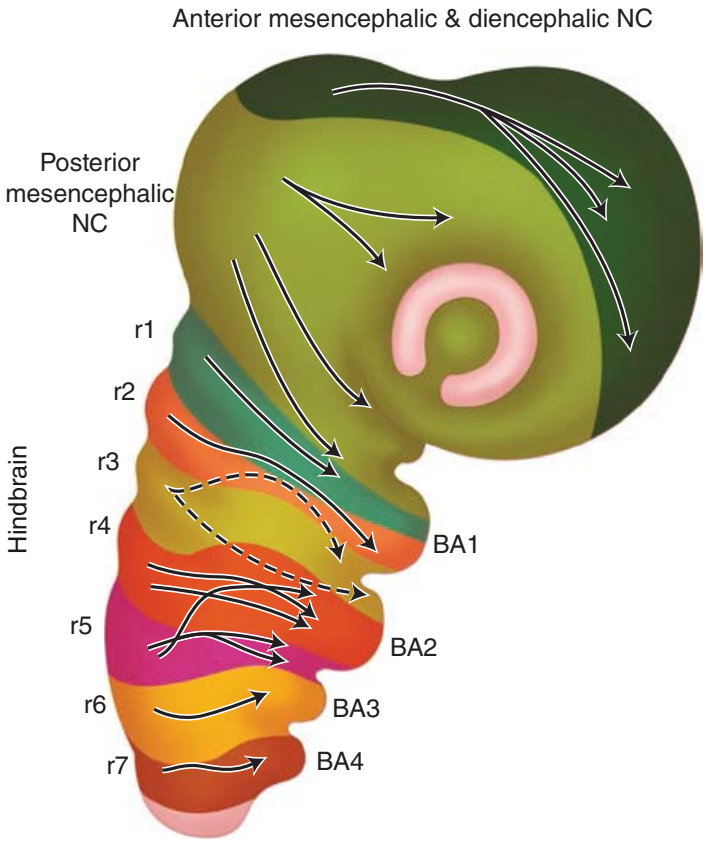

Fig. 13.3 Migration of neural crest cells along specific defined pathways from specific forebrain, midbrain, and hindbrain regions (r1–r7 are hindbrain rhombomeres) to specific destinations in anterior head regions and branchial arches (BA1–4). (Hand-drawn by author)

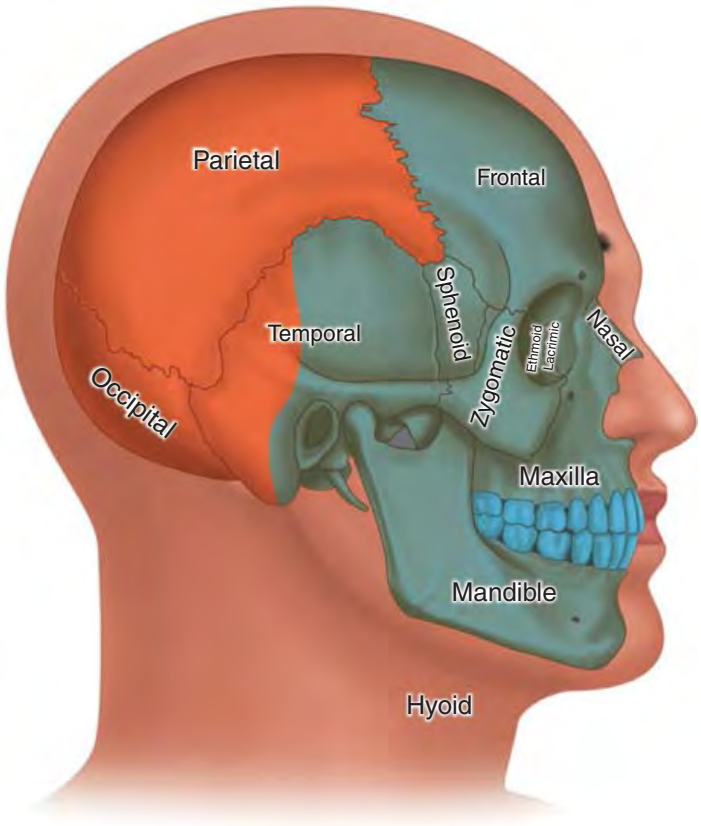

Fig. 13.4 Origins of skull bones from the paraxial mesoderm (red) and neural crest (blue). (Hand-drawn by author)

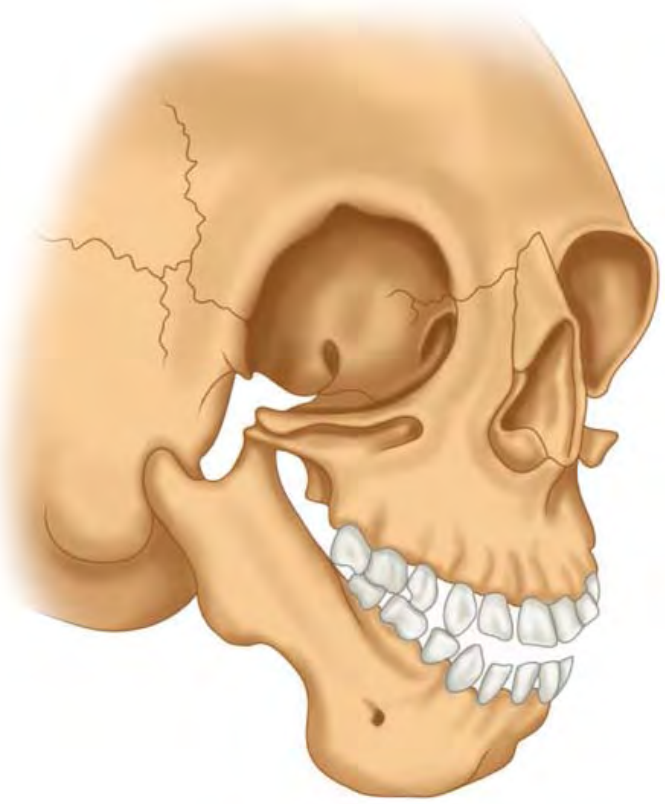

Fig. 13.5 The cranioskeletal defects characteristic of Treacher Collins syndrome. Mandibular micrognathia, retrognathia, maxillary and malar hypoplasia, absent TMJ, incomplete zygomatic arches and orbits. (Hand drawn by author, from personal medical history)

These cranioskeletal defects influence the development of overlying tissues and structures, previously illustrated in Fig. 13.1. The incomplete or absent zygomatic arches coupled with the open lower orbital rims give rise to a downward slanting of the eyes, colobomata of the eyelids, and a triangular appearance to the maxillary and malar region of the face (sunken cheeks). The mandibular micrognathia and retrognathia present themselves in a visible lack of a chin as well as an anterior open bite and lips widely parted at rest. The outer ear defects are either microtia (small outer ears) or anotia (total absence of outer ears), either with a visible stenotic ear canal opening or absent ear canal opening. These external defects vary with the severity of presentation of Treacher Collins syndrome.

Internal defects include a cleft palate (and even when repaired, short palate and velopharyngeal insufficiency) which can impair speech, as well as a stenosis or atresia of the auditory canals and incomplete or absent ossicles (malleus, incus, and stapes), which can cause conductive hearing loss or deafness. The nasal cavity and airway can be abnormally small, and in some severe cases, there is choanal atresia (blockage of the inferior openings of the nasal cavity, or choanae). The oral cavity is also abnormally small, with limited jaw opening and overcrowding of teeth due to the mandibular hypoplasia. The mandibular micrognathia and retrognathia also cause oropharyngeal airway obstruction, via glossoptosis (blockage of the oropharyngeal airway by the tongue forced posteriorly by mandibular hypoplasia). The jaw deformities, small mouth, cleft palate, and constricted throat create difficulties for mastication and swallowing, speech, and breathing.

Upper airway obstruction is the most serious, and potentially fatal, complication of Treacher Collins syndrome. Complications resulting from the upper airway obstruction include choking, impaired respiration, difficult intubation and airway placement, and sleep-disordered breathing and can lead to early death perinatally, postnatally, and any other time throughout life. Polysomnography (PSG), or sleep study, is a valuable diagnostic tool for sleep-disordered breathing including obstructive sleep apnea (OSA), which occurs with a higher prevalence in Treacher Collins syndrome [23]. Three-dimensional oronasopharyngeal imaging by computed tomography (CT) scanning for analysis of airway morphology and volume has also been used for studies on the oronasopharyngeal airway obstruction in Treacher Collins syndrome [24–26]. The studies by Ma et al. [24, 25] and Ribeiro et al. [26] have found reduced nasal, oral, and pharyngeal airway volume, which they have connected to skeletal dysmorphology in those regions including micrognathia, retrognathia, and skeletal hypoplasia in the nasal, oral, and pharyngeal regions. Figure 13.6 illustrates the upper

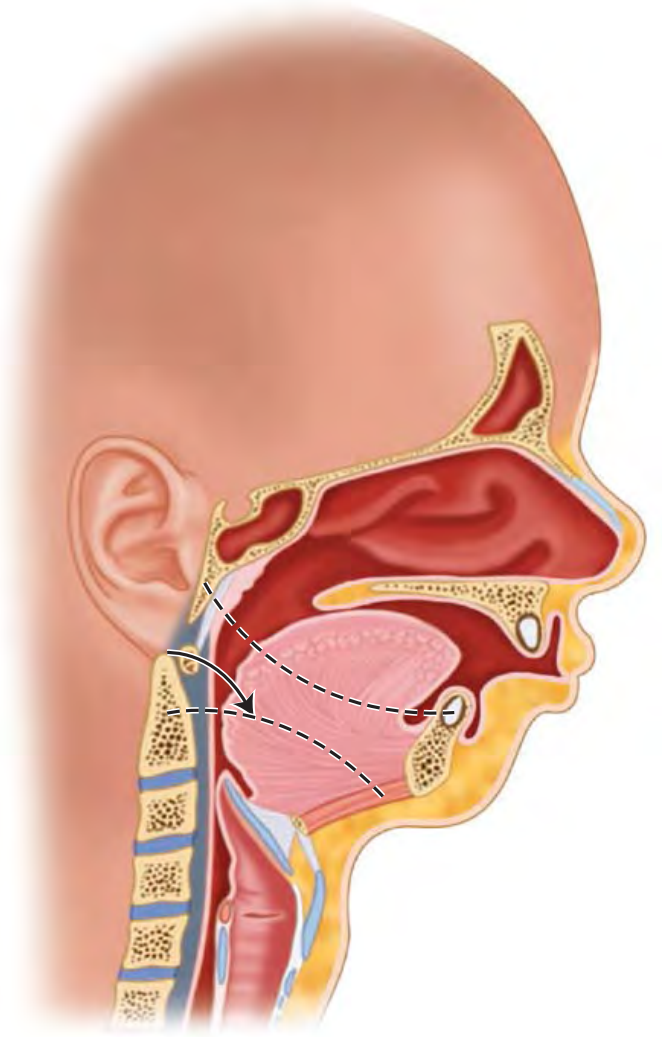

Fig. 13.6 Upper airway obstruction in Treacher Collins syndrome is caused by retrognathia (the mandibular border is drawn in dashed lines) pushing the tongue posteriorly into the oropharyngeal airway; this phenomenon is known as glossoptosis (Hand-drawn by author)

airway obstruction in Treacher Collins syndrome due to retrognathia that forces the tongue posteriorly into the oropharynx, occluding the airway (glossoptosis).

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