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Treacher Collins syndrome: A novel *TCOF1* mutation and monopodial stapes

Piranit Nik Kantaputra[1,2,3] | **Kanich Tripuwabhrut**[4] | **Worrachet Intachai**[1] | **Bruce M. Carlson**[5] | **Natalina Quarto**[6] | **Chumpol Ngamphiw**[7] | **Sissades Tongsima**[7] | **Nuntigar Sonsuwan**[8]

[1]Center of Excellence in Medical Genetics Research, Chiang Mai University, Chiang Mai, Thailand

[2]Division of Pediatric Dentistry, Department of Orthodontics and Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand

[3]Dentaland Clinic, Chiang Mai, Thailand

[4]Division of Orthodontics, Department of Orthodontics and Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand

[5]Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI, USA

[6]Department of Surgery, Division of Plastic and Reconstructive Surgery, Stanford University School of Medicine, Stanford, CA, USA

[7]National Biobank of Thailand, National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Pathum Thani, Thailand

[8]Department of Otolaryngology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Correspondence
Piranit Nik Kantaputra, Division of Pediatric Dentistry, Department of Orthodontics and Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai 50200, Thailand.
Email: dentaland17@gmail.com

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Abstract

Treacher Collins syndrome (TCS: OMIM 154500) is an autosomal dominant craniofacial disorder belonging to the heterogeneous group of mandibulofacial dysostoses.

Objective: To investigate four Treacher Collins syndrome patients of the Sgaw Karen family living in Thailand.

Method: Clinical examination, hearing tests, lateral cephalometric analyses, Computed tomography, whole exome sequencing and Sanger direct sequencing were performed.

Results: All of the patients affected with Treacher Collins syndrome carried a novel *TCOF1* mutation (c.4138_4142del; p.Lys1380GlufsTer12), but clinically they did not have the typical facial gestalt of Treacher Collins syndrome, which includes downward-slanting palpebral fissures, colobomas of the lower eyelids, absence of eyelashes medial to the colobomas, malformed pinnae, hypoplastic zygomatic bones and mandibular hypoplasia. Lateral cephalometric analyses identified short anterior and posterior cranial bases, and hypoplastic maxilla and mandible. Computed tomography showed fusion of malleus and incus, sclerotic mastoid, hypoplastic middle ear space with a soft tissue remnant, dehiscence of facial nerve and monopodial stapes.

Conclusion: Treacher Collins syndrome in Sgaw Karen patients has not been previously documented. This is the first report of monopodial stapes in a TCS patient who had a *TCOF1* mutation. The absence of a common facial phenotype and/or the presence of monopodial stapes may be the effects of this novel *TCOF1* mutation.

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1 | INTRODUCTION

Treacher Collins syndrome (TCS: OMIM 154500) is a craniofacial disorder belonging to the heterogeneous group of mandibulofacial dysostoses. It is characterised by a number of developmental anomalies restricted to the head and neck, including downward-slanting palpebral fissures, colobomas of the lower eyelids, absence of eyelashes medial to the colobomas, hypoplastic zygoma, mandibular hypoplasia, pre-auricular hair displacement and airway dysfunction. Malformations of the pinnae, external auditory canals and middle ears, leading to conductive hearing loss, are common. Abnormalities are usually bilateral and symmetric. Patients usually have normal intelligence.[1-4] Treacher Collins syndrome has a high degree of intrafamilial and interfamilial variability. The clinical variability of patients with TCS does not depend on either the type or location of the underlying mutations or gender of the patients.[1-4] Treacher Collins syndrome is a genetically heterogeneous disorder. An autosomal dominant mode of inheritance has been reported to be caused by mutations in *TCOF1* and *POLR1D*.Mutations in *POLR1C* and *POLR1D* have been reported to be associated with very rare cases involving autosomal recessive inheritance. Most patients (93%) affected with Treacher Collins syndrome have been reported to be associated with mutations in *TCOF1*, of which 60% of them are de novo mutations.[1-4] TCOF1 encodes the 1411-amino acid nucleolar phosphoprotein treacle that shuttles between the nucleolus and cytoplasm.[1-4] Treacle interacts with an upstream binding factor and RNA polymerase I during the process of ribosomal RNA transcription. Loss of function mutations in *TCOF1*, *POLR1C* and *POLR1D* disrupt ribosome biosynthesis in neural crest cells and the neuroepithelium, leading to the absence of nuclear and nucleolar localisation of treacle, and subsequent apoptosis.[2,5] Treacle is specifically crucial for the survival of craniofacial neural crest cells during the fifth and eighth week of fetal development; thus, malformations of TCS are restricted to the face and neck.[1,5,6] In mutants, increased apoptosis of both neuroepithelial and neural crest cells, which is the pathogenetic mechanism of TCS, is related to the activation of the p53 tumour suppressor.[7] Although the migration of first and second arch neural crest cells appears to be normal, the attenuated migratory stream due to both apoptosis and mitotic suppression reduces the cellular building blocks for the development of first and second arch structures.[7]

Anomalies of the external ears in patients with TCS have been reported to consist of microtia, atresia of the external auditory canals and abnormalities of middle ear ossicles, including irregular or absent auditory ossicles, fusion of malleus and incus, partial absence of stapes and oval window and complete absence of middle ear and epitympanic space. Inner ear structures are normal.[7]

Here, we report four members of a three-generation Sgaw Karen tribe family living in Thailand. All carried a novel frameshift mutation in *TCOF1* and did not show the typical facial phenotype of TCS. This is the first report of monopodial stapes in a patient with *TCOF1* mutation. Development and maldevelopment of the ear and its related structures as a result of *TCOF1* mutations are discussed.

KEYPOINTS

- A novel *TCOF1* mutation is associated with monopodial stapes, fusion of malleus and incus, absence of common facial phenotype, sclerotic mastoid, hypoplastic middle ear space with a soft tissue remnant and dehiscence of facial nerve.

2 | METHOD

2.1 | Patients

Clinical, radiographic and audiometric examinations were performed (Table 1). Four members of a three-generation Sgaw Karen tribe family were affected with Treacher Collins syndrome (Figure S1). Clinically, none of them had the typical facial gestalt of TCS, including downward-slanting palpebral fissures, colobomas of lower eyelids, absence of eyelashes medial to the colobomas, malformed pinnae, hypoplastic zygoma and mandibular hypoplasia (Figures 1A,D and 2A,D; Table 1). Patient III-3 had a tracheostomy as a result of an upper airway obstruction(Figure 2D). Lateral cephalograms of all of the patients who carried the *TCOF1* mutation (I-2, II-2, III-2, III-3) showed a short anterior cranial base length (S-N), short posterior cranial base length (S-Ar), short maxillary length (Co-A), short mandibular length (Co-Gn), short mandibular body length (Go-Gn) and short ramus length (Ar-Go) (Figures 1B,E and 2B,E) (Table S1). Posteroanteriorskullradiographs and computed tomograms showed the absence of downward-slanting orbital bones (Figures 1C,F, 2C,F, 3A and 4A), suggesting the absence of a hypoplastic temporal portion of the zygomatic bones, a consistent feature of TCS. Patient III-1 had cleft lip and palate, hypoplastic maxilla, retrognathic maxilla and mandible and a supernumerary incisor (Figure S2). Computed tomography in patients II-2 and III-3 showed fusion of malleus and incus, sclerotic mastoid, dehiscence of facial nerve, hypoplastic middle ear space with soft tissue remnant and a monopodial stapes (Figures 3 and 4). Patient III-3 had mildly narrow zygomatic bones (Figure 4A).

2.2 | Whole exome and Sanger direct sequencing

Genomic DNA was extracted from the saliva according to the prepIT® L2P protocol for the purification of genomic DNA from the Oragene® kit (DNA Genotek Inc). DNA samples of all family members were analysed by whole exome sequencing (WES). All variants were validated by Sanger direct sequencing.

TABLE 1 Clinical and molecular findings of patients

Pt	*TCOF1* mutations	Downward-slanting palpebral fissures	Overhanging upper eyelid	Colobomas of lower eyelids	Abnormal pinnae	Type of hearing loss	Severity of hearing loss	Air-Bone Gap (dB)	Pharyngeal hypoplasia	Abnormal TMJ	Cleft lip and palate
I-2	Yes	No	Yes	No	No	Mixed HL	Moderate	25	No	No	No
II-2	Yes	Mild	Yes	No	No	CHL	Moderate	30	No	No	No
III-1	No	No	No	No	No	CHL	Mild	12	No	No	Yes
III-2	Yes	Mild	Yes	No	No	CHL	Moderate	22	No	No	No
III-3	Yes	No	No	No	No	CHL	Severe	48	Yes	No	No

Note: HL, Hearing loss; CHL, Conductive hearing loss.

3 | RESULTS

The heterozygous five-base pair deletion in exon 24 of *TCOF1* (c.4138_4142del; NM_000356.3) was identified in I-2, II-2, III-2 and III-3 but not in III-1 (Figure S1).To the best of our knowledge, the c.4138_4142del mutation is novel, as it was not found in our exome data of 300 people who did not have TCS and was not present in the ExAC and gnomAD database. The *TCOF1* mutation is predicted to cause a change in sequence, premature stop codon 12 amino acid later and subsequent truncation of treacle protein (p.Lys1380GlufsTer12; NP_000347.2).Eliminating the rest of the polypeptide chain after the change is expected to be highly detrimental to the protein's function. The clinical and molecular findings of the five patients are presented in Table 1. Interestingly, patient III-1 did not have the*TCOF1* mutation but was homozygous for the rare variants in chromodomain helicase DNA-binding protein 7 (*CHD7*) (c.8250T > G; p.Phe2750Leu) (Figure S1; Table 1). Other members of the family were heterozygous for this variant.

4 | DISCUSSION

We report four Sgaw Karen patients of the same family affected with TCS who carried a novel *TCOF1* mutation. Clinically, they did not have the typical facial gestalt of TCS, which includes downward-slanting palpebral fissures, colobomas of the lower eyelids, absence of eyelashes medial to the colobomas, malformed pinnae, hypoplastic zygoma and mandibular hypoplasia. The overhanging lateral half of the upper eyelids made their eyes appear downward-slanting.

Patient III-3, who had pharyngeal hypoplasia, evidently had a more severe phenotype than her brother, father and grandmother. The more severe phenotype in successive generations, as we found in our patients, has been reported in a number of families with TCS.[1] The ethnic background of our patients might have an influence on the phenotypes observed in this family. Sgaw Karen is the largest hill-tribe group living in Thailand. Their original homeland is believed to be in the Mongolia and Tibet autonomous regions. They migrated to different parts of northern Thailand about 1000 years ago.

4.1 | Ear and auditory findings

All of our patients had conductive hearing loss with unremarkable external ears and lacked the typical facial phenotype of TCS. The I-2 patient also had sensorineural hearing loss, which was likely a result of ageing. The severity of conductive hearing loss, a common finding in patients with TCS, differed among the family members of our patients. The values of air-bone gap, the difference between the threshold for hearing acuity by bone conduction and air conduction, showed that the III-3, who had pharyngeal hypoplasia and upper airway obstruction, also had more severe conductive hearing loss (48 dB) (Table 1).

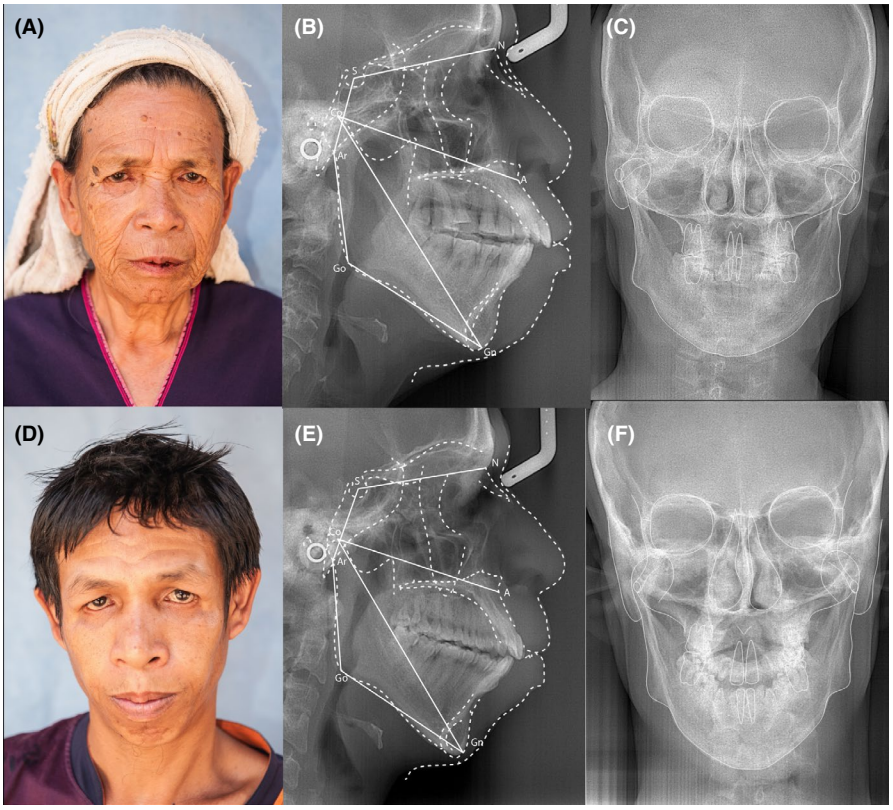

FIGURE 1 (A) Patient I-2 (D) Patient II-2. Neither has the facial phenotype of Treacher Collins syndrome. Overhanging lateral half of the upper eyelids makes the palpebral fissures look downward-slanting. Lateral cephalograms of (B) Patient I-2 and (E) patient II-2 show short anterior cranial base length, short posterior cranial base length, short maxillary length, short mandibular length, short mandibular body length and short ramus length. Postero-anterior cephalograms of (C) Patient I-2 and (F) patient II-2 show unremarkable orbital bones and zygomatic bones

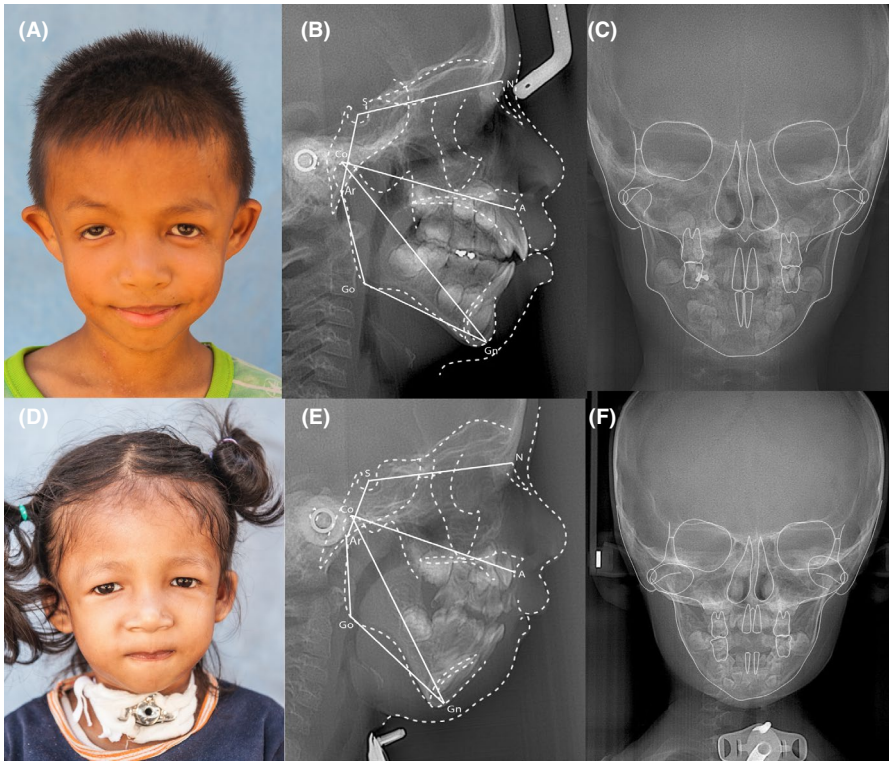

FIGURE 2 (A) Patient III-2 (D) Patient III-3. Both have minimal facial phenotype of Treacher Collins syndrome. Overhanging lateral half of the upper eyelids makes the palpebral fissures look downward-slanting. Patient III-3 has tracheostoma as a result of upper airway obstruction. Lateral cephalograms of (B) Patient III-2 and (E) Patient III-3 show short anterior cranial base length, short posterior cranial base length, short maxillary length, short mandibular length, short mandibular body length and short ramus length. Postero-anterior cephalograms of (C) Patient III-2 and (F) Patient III-3 show unremarkable orbital bones and zygomatic bones

We used computed tomography to investigate the malformations in the middle ears including ossicular malformations and middle ear cavitation defects. However, there are limitations for the use of computed tomography because it can be complicated by the anatomical involvement of facial nerve and the persistent stapedial artery.[8] The fusion of malleus and incus in patients II-2 and III-3 is frequently found in patients with TCS.[9] The malleus and incus form from the continuous rod of Meckel's cartilage that extends throughout the lower jaw and ends in the incus. Its caudal end subsequently divides into two separate ossicles, malleus and incus. Meckel's cartilage then breaks

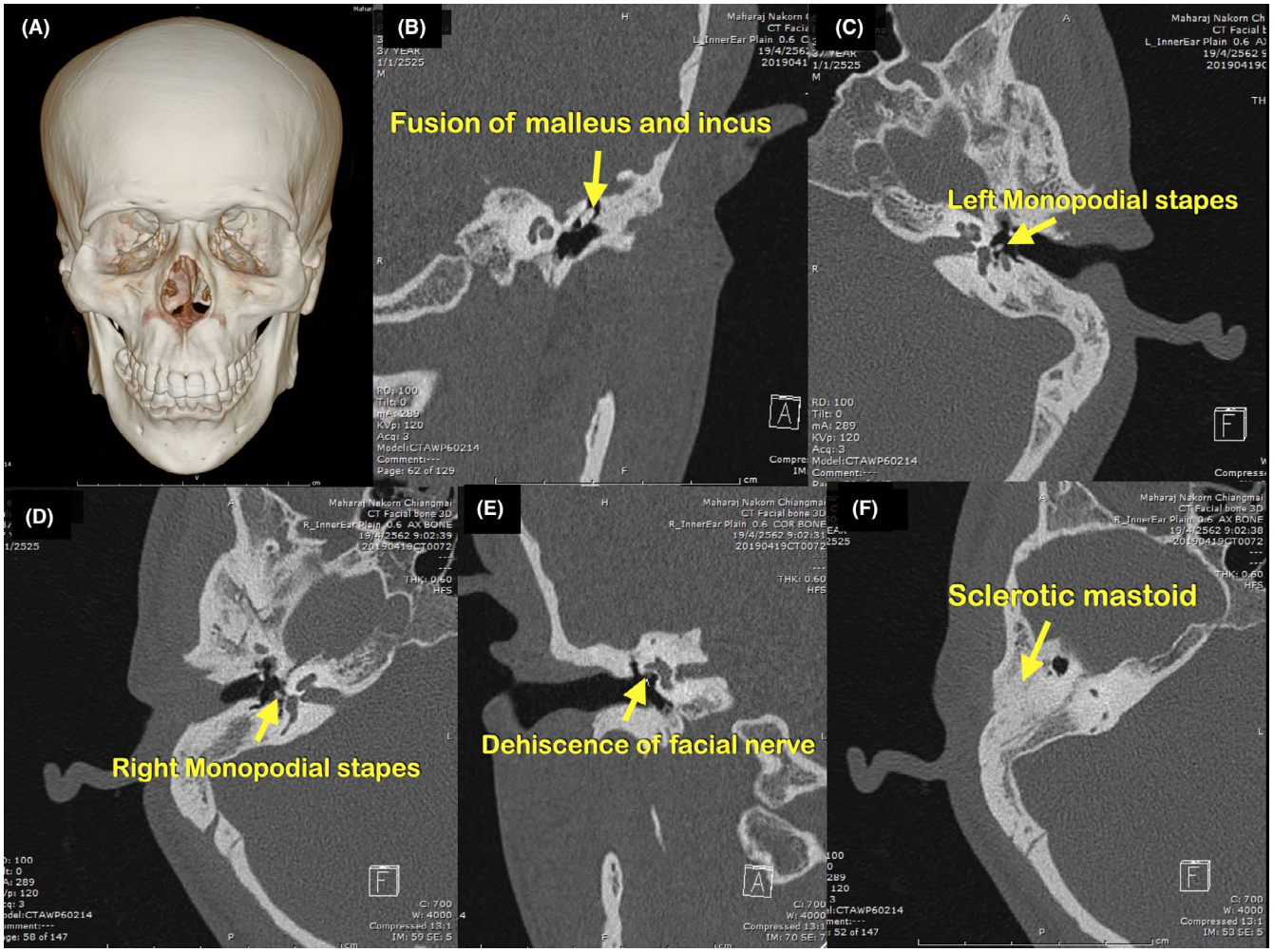

FIGURE 3 Computed tomograms of Patient II-2. (A) Unremarkable skull. (B) Fusion of right malleus and incus. (C) Monopodial stapes of left ear. (D) Monopodial stapes of right ear. (E) Dehiscence of tympanic part of right facial nerve. (F) Non-pneumatised mastoid air cells of right ear

down by an apoptosis-independent mechanism. Fused malleus and incus or malleal-incudo complex is developmentally interesting. It is a normal morphological and functional unit for most members of the rodent suborder Ctenohystrica, which includes guinea pigs. The joint is initially separated, but subsequently fuses in late development, with a suture between the two ossicles. Even though they have a fusion between malleus and incus, they do not have a problem with hearing because the movable attachment between the malleus and tympanic ring leads to a freely mobile middle ear. In mice, the malleus and incus develop from a single condensation that expresses the early cartilage markers,*Sox9* and *type II collagen* and subsequently splits to form two separate ossicles.[10,11] Fusion of the incus and stapes has not been reported in patients affected with TCS. This may be because malleus is derived from the first branchial arch, but stapes is derived from the second branchial arch.

Having three endochondral ossicles (malleus, incus and stapes) is a characteristic of mammals. During the process of evolution, the primary jaw articulation of non-mammalian vertebrates was replaced by the second articulation of membranous bones, the squamosal and dentary. The primary jaw articulation and the hypomandibular were subsequently incorporated into the middle ear to form the three-ossicle chain, with joints between them. The malleus is homologous to the articular part of Meckel's cartilage; the incus is homologous to the quadrate/palatoquadrate;the stapes is homologous to the hypomandibular.[12] Therefore the malleo-incudal joint is homologous to the primary jaw joint. Upregulation of cartilage marker genes in the presumptive joint region is subsequently replaced by upregulation of joint marker genes such as *Tgf-beta* super family members *Gdf5* and *Gdf6*. This process is essential for malleo-incudal joint formation, and dysregulation of any of these genes can lead to joint fusion. The fusion of malleus and incus in patients with TCS might happen because the presumptive joint cells fail either to downregulate the expression of chondrogenic genes or toupregulate the joint marker genes.

Having three ossicles is believed to improve the transmission of high-frequencies, allowing the ultrasonic hearing typical of many mammals.[13] However, fusion of malleus and incus in our patients led to low-to-mid frequency hearing loss. Their high-frequency hearing was unremarkable. This suggests that having three ossicles is not directly involved in transmission of the high-frequency sound as previously suggested. Rodents with physiological fusion of malleus and

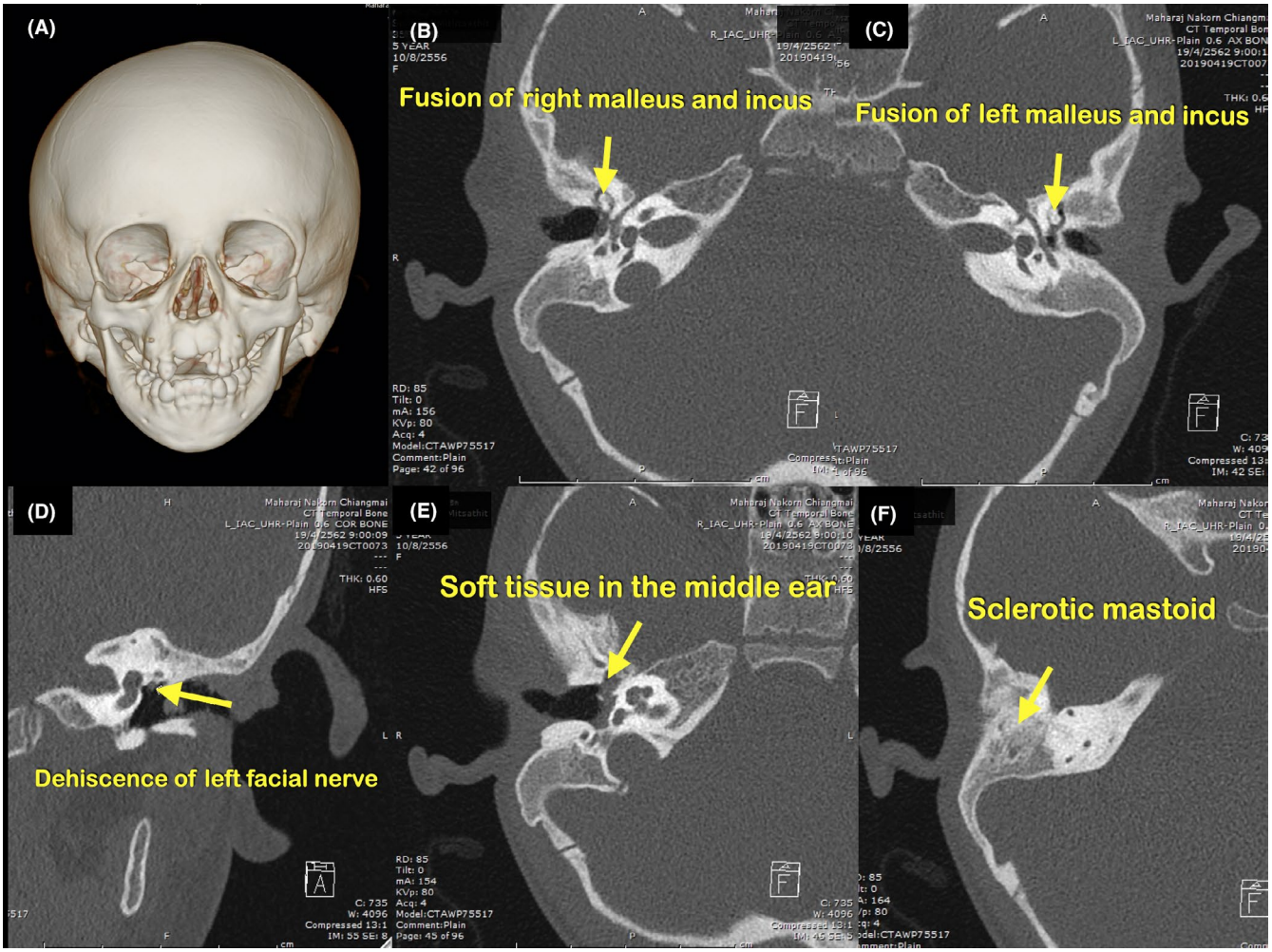

FIGURE 4 Computed tomograms of Patient III-3 at age 4 years. (A) Skull with mildly narrow zygomatic bones (B) Fusion of right malleus and incus. (C) Fusion of left malleus and incus. (D) Dehiscence of tympanic part of left facial nerve. (E) Hypoplasia of right middle ear cavity with partial opacification. Soft tissue remnant in the right middle ear. (F) Non-pneumatised mastoid air cells of right ear

incus or reptiles and birds with a single suspended ossicle (stapes/columella) do not have problem with high-frequency sound hearing.[13] Mobility of the middle ear ossicles seems to be the crucial factor in transmitting sound.

4.2 | Middle ear cavitation defects

The presence of soft tissue in the middle ear in our patients is most likely the result of incomplete removal of mesenchyme by programmed cell death (apoptosis) from the developing middle ear space or improper distribution of mesenchyme over the increasing surface area of the growing middle ear cavity, a process of creating an air-filled middle ear in mammals.[13] The middle ear cavitation process or clearance of embryonic mesenchyme, in which the embedded middle ear ossicles develop, is crucially required to generate an air-filled middle ear space across which sound vibrations can be transmitted. This middle ear cavitation process takes places in conjunction with development of the middle ear ossicles.[14]

Tcof1 is highly expressed in developing and migrating neural crest cells at embryonic day E8.5 and neural crest-derived craniofacial tissues at E9.5. Its expression is reduced as the pharyngeal arches continue to form, with expression becoming undetectable after E12.5. However, its expression in the middle ear is not observed after E9.5.[14] This suggests that the problems in the middle ear of patients affected with TCS might be the result of a deficit of neural crest cells in the developing middle ear region. This hypothesis is based on experiments involving *Tcof*$^{+}$heterozygous mice. These mice have an increase in apoptosis and a decrease in proliferation of cranial neural crest cells at the early stages of initiation and migration. This results in a reduced number of neural crest cells populating the developing face.[2,14] However, the defects in the middle ear could have been due to dysregulation of genes related to joint formation. This is because *Tcof1* is involved in malleo-incudal joint formation prior to E9.5, and mutations of this gene may lead to dysregulation of chondrogenic genes or joint marker genes and subsequent fusion of malleus and incus. Similar to *Tcof*$^{+}$heterozygous mice, our patients also exhibited a hypoplastic middle ear space.[14]

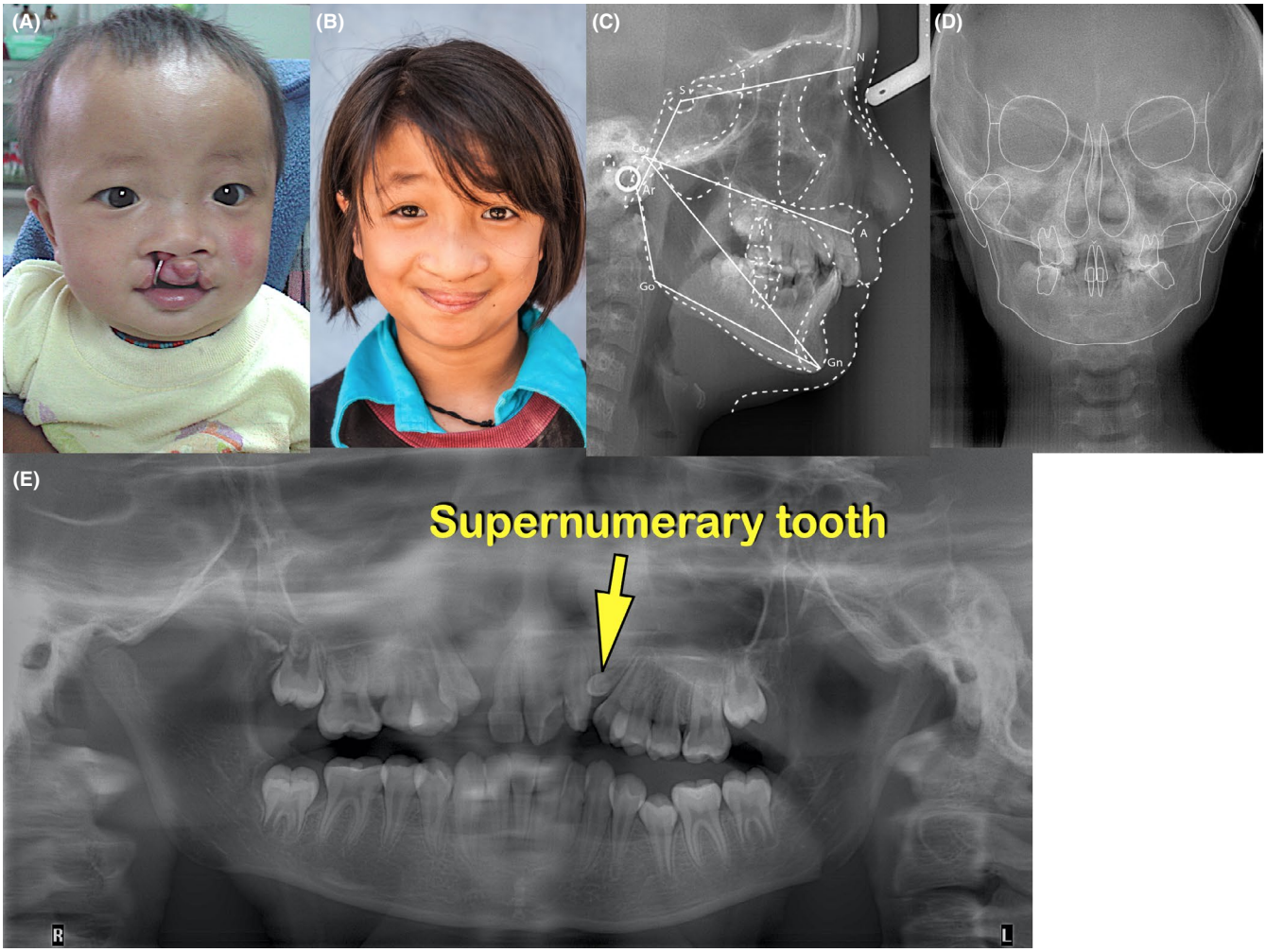

FIGURE 5 Patient III-1 at ages (A) 8 months. Note bilateral cleft lip and palate with nasoalveolar moulding device. (B) 11 years. (C) Lateral cephalogram at age 11 years show short anterior cranial base length, short posterior cranial base length, short maxillary length, short mandibular length, short mandibular body length and short ramus length. (D) Postero-anterior cephalogram at age 11 years. (E) Panoramic radiograph at age 11 years shows supernumerary tooth in the left cleft area

4.3 | Sclerotic mastoid

The mastoid is developmentally an extension of the middle ear. Effective cavitation of the middle ear is closely associated with growth of the neural crest-derived structure, the auditory bulla, which encapsulates all middle ear structures. It is a complex structure that forms from the fusion of a number of intramembranous ossifications.[15] Developmentally, it becomes pneumatised or hollowed in order to form a number of spaces, the mastoid air cells. The air cell configuration varies greatly in size and number. Air cells are important for sound reception, resonance, insulation, air reservoir action, humidification, acoustic dissipation and protection from external violence. Pneumatisation is the result of epithelium-lined evaginations arising from the lining of the middle ear and its extension. The gradual pneumatisation of air cells is achieved by positive pressure on the nasopharynx through the opening of the eustachian tube.[15] Lack of pneumatisation of mastoid or a sclerotic mastoid in our patients and other patients with TCS suggests that *TCOF1* has important role in pneumatisation of mastoid.

Patient III-1 had bilateral cleft lip and palate, conductive hearing loss, retrognathic maxilla and mandible and a supernumerary tooth in the cleft area (Figure 5A-E; Table S1). A supernumerary tooth in the cleft area has been reported to be quite common (30%) in patients with cleft lip. Her mild conductive hearing loss was most likely the result of chronic middle ear infection secondary to cleft lip and palate.[16] The variant in *CHD7* found in patient III-1 (c.8250T > G; p.Phe2750Leu) is rare. The allele frequency of the heterozygous carriers according to ExAC is 0.0002867. This variant is predicted to be disease-causing by Mutation Taster. Mutations in *CHD7* are known to cause CHARGE syndrome (MIM 214 800). Cleft lip with/without cleft palate (CLP) has been reported in 15%-36% of cases of CHARGE syndrome patients who carried *CHD7* mutations.[17] However, there is not enough support for the association of cleft lip and palate in patient III-1 and the *CHD7* variant.

5 | CONCLUSIONS

We report a novel *TCOF1* mutation in four members of a three-generation Sgaw Karen family who had a minimal facial phenotype of TCS. Our study has demonstrated that craniofacial structures of patients with TCS may be clinically normal but radiographically abnormal. Fusion of the malleus and incus in our patients is associated with low-to-mid frequency hearing loss, but not high-frequency hearing loss. The findings in our patients confirm important roles of *TCOF1* in later developmental events, including pneumatisation of mastoid, middle ear cavitation process and removal of mesenchyme by programmed cell death from the developing middle ear space. This is the first report of the association between *TCOF1* mutation and monopodial stapes.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

ETHICAL APPROVAL

The study was conducted in accordance with the Declaration of Helsinki and national guidelines. Informed consent was obtained from participants or their parents in accordance with the regulations of the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University, Thailand.

ORCID

Piranit Nik Kantaputra https://orcid.org/0000-0001-9841-0881

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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