Short Report

Facial asymmetry and clinical manifestations in patients with novel insertion of the TCOF1 gene


This study explored the role of TCOF1 insertion mutations in Taiwanese patients with craniofacial anomalies. Twelve patients with single or multiple, asymmetrical congenital craniofacial anomalies were enrolled. Genomic DNA was prepared from leukocytes; the coding regions of TCOF1 were analyzed by polymerase chain reaction and direct sequencing. Clinical manifestations were correlated to the TCOF1 mutation. Six of 12 patients diagnosed with hemifacial microsomia exhibited a novel insertion mutation 4127 ins G (frameshift) in exon 24 in the TCOF1 gene. All six patients were diagnosed with anomalies on the left side. In addition, four of these six patients had hearing impairment; three had other major anomalies; and two had developmental delay. The insertion caused a frameshift, an early truncation, the loss of two putative nuclear localization signals (residues 1404–1420 and 1424–1440), and the loss of coiled coil domain (1406–1426) in treacle protein. These findings support the existence of two regulators of growth of the mandibular condyles.

Conflict of interest
Nothing to declare

Facial asymmetry is observed in patients diagnosed with hemifacial microsomia (HFM; OMIM: 164210), which is the second most common congenital asymmetry of the lower face (1, 2). The incidences of right- and left-sided HFM are similar and that of bilateral deformity ranges from 15% to 22.5% (3, 4). The cause of facial asymmetry affects surgical outcomes (5), which supports its significance. Genetic mutations associated with HFM include to trisomy 10p14 (6), 22q11.2 microdeletion (7), 12p13.33→pter deletion (8), 10.7 cM on chromosome 14q32 (9), large 5p deletion (10), and gene mutations in SALL4 in a Goldenhar patient (11), and the TCOF1 gene (12). Mutations in the TCOF1 gene (chr5) were identified in 53–93% patients with the Treacher–Collins syndrome (TCS; OMIM: 154500) (12, 13). TCS also involves anomalies in the first and second branchial arches such as hypoplasia of the mandibular and zygomatic bones (14).

TCOF1 has 26 exons and encodes Treacle, a putative 1450 amino acids nucleolar phosphoprotein (isoform a, NP_001008656.1) (15–17) with three functional domains. The N-terminus contains the consensus nuclear export signal (13). The central treacle domain from residues 1404–1420 and 1424–1440, and the loss of coiled coil domain (1406–1426) in treacle protein. These findings support the existence of two regulators of growth of the mandibular condyles.
mutations may play a role in a subset of patients with HFM. To test our hypothesis, we examined the genotype of the TCOF1 gene in 12 patients with HFM, and assessed the incidence of TCOF1 mutations with their characteristics.

Materials and methods

Participants and controls

Twelve patients with asymmetric face anomalies were diagnosed and treated at Chung Shan Medical University Hospital from 2006 to 2010. Their major manifestations of HFM were graded from 0 to 3 severity (0: none; 3: worse) for orbital asymmetry, mandibular hypoplasia, auricular deformity, nerve involvement, and soft tissue deficiency according to the OMENS classification system (20–22) by using physical examination findings, photographs, and radiographs including posteroanterior, lateral, and cephalometry. The OMENS score was the sum of the five scores (20, 22). HFM diagnosis was distinct from a diagnosis of TCS by malar hypoplasia with downsloping palpebral fissures, defect of lower lid, and malformation of external ear. The control group of 100 people had normal facial features. This study was approved by the ethics review committee of Chung Shan Medical University Hospital, and written parental consent was obtained for all participants.

DNA purification

DNA was extracted from each blood sample (3–5 ml, ethylenediaminetetraacetic acid-containing tubes) individually using a genomic DNA Purification Kit (Gentra Systems, Minneapolis, MN). Average DNA yield was 50–100 mg per 3 ml of whole blood.

Polymerase chain reaction, PCR product purification and sequencing

DNA was amplified by PCR following an established protocol (13), except for the redesign of the primers for exons 9, 22, and 23. After DNA was denatured at 95°C for 5 min, each exon was amplified with 100 ng genomic DNA, 0.5 mmol/l per each primer and 1 unit Taq DNA polymerase (Protech Technology Enterprise Co., Taipei, Taiwan) for 30 cycles (denaturation 94°C, 30 s; annealing for 30 s; extension 72°C, 30 s). Primer sequences can be obtained from the authors upon request. Each PCR product was purified using a PCR-M Clean Up System (Viogene, Sunnyvale, CA). All PCR products were sequenced by using an Applied Biosystems (Foster City, CA) 3100 automated DNA sequencer.

Results

We screened 12 patients with HFM for mutations in the TCOF1 gene by sequencing analysis. Five cases had no TCOF1 mutation. Seven patients with asymmetric face anomalies had nucleotide changes in the TCOF1 gene. One HFM case had an unbalanced translocation of chromosome 6 and a previously reported TCOF1 single nucleotide polymorphism at c. 3938 C>T (p. A1313 V, rs15251). Six patients had a novel single base insertion (TCOF1 4127 ins G) that produced a frame shift mutation and early truncation (Fig. 1a). The insertion at 4127 was not found in the 100 normal controls (Fig. 1b) or in the parents (data not shown). The novel insertion mutation at 4127 in exon 24 affected amino acid residue 1375, altered the downstream amino acid sequence, truncated the protein at amino acid residue 1392 (Fig. 1c) and destroyed the two functional bipartite NLSs in the normal treacle protein located at residues 1365–1381and 1385–1401. Figure 1c has depicted the C-terminal region of the TCOF1 encoded protein, treacle, beginning at residue 1404.

The OMENS scoring system (Table 1) showed no significant differences in clinical manifestations of HFM between the patients with the TCOF1 4127 ins G insertion and those without this insertion mutation (Table 1). Birth characteristics and health of cardiovascular, gastroenteral, and central nervous systems were listed in Table 1. The health of heart, liver, spleen and kidneys revealed by ultrasonography were normal unless indicated. Three-dimensional computed tomography (3D-CT) of the facial bones revealed the side of hypoplasia of the mandible and was noted on Fig. 2. Surprisingly, all six patients with the novel TCOF1 gene variant exhibited left face microsoma. The parents of these six cases had not indicated any teratogen exposure. Cases 1–6 exhibited the novel base insertion (4127 ins G) in the TCOF1 gene.

Case 1

An infant girl was born to non-consanguineous 35-year-old father and 32-year-old primigravida mother who reported no history of maternal diabetes mellitus, use of herbs, or abuse of alcohol, tobacco or drugs. At birth, the girl had mild asymmetric face, mild HFM, umbilical hernia, and imperforate anus. Lower gastrointestinal series with contrast revealed imperforate anus with anopereanal fistula. Brain stem auditory-evoked potentials (BAEP) confirmed normal conductive hearing. A 3D-CT scan of the facial bone showed mild small mandible bone (Fig. 2a) and mild asymmetric face (Fig. 2b). Electroencephalograms (EEGs) revealed cerebral dysfunction with paroxymal disorder. Magnetic resonance imaging (MRI) of brain showed a persistent fifth ventricle.

Case 2

A 4-month-old boy baby had macrocephaly, micrognathia, coloboma in left eye, left crumple ear, small mandible, high arch palate, tongue tie, narrow shoulder, widely spaced nipples (atrial septal defect type 2), umbilical hernia, inguinal hernia, hydromecele, simian crease, and abnormal plantar crease. A 3D-CT scan of the facial bone showed small mandible bone (Fig. 2c,d).
Fig. 1. Cases 1–6 exhibited the novel base insertion (4127 ins G) in the TCOF1 gene. (a) The sequence of the TCOF1 gene through the mutated region (nt.4108–nt.4136) of cases 1–6. (b) The sequence of the TCOF1 gene from nt.4108 to nt.4136 of a control subject. (c) Schematic representation of isoform A and 4127 ins G frameshift mutation of human TCOF1 protein domains. The sequences show the regions of alternative C-terminal domain in the isoform A and 4127 ins G frameshift mutation of human TCOF1 (domains are not drawn to scale). Gray boxes: nuclear export signal sequence consensus. Green boxes: Treacle domain predicted by Pfam database. Blue boxes: coiled coil domains defined by n coils server. Black boxes: bipartite NLS sequence consensus fit the PSORT II prediction. Underline: two clusters of basic amino acids comprising the basic bipartite of the NLSs. Position of amino acid residues are above the sequence. The novel insertion mutation at 4127 in located in exon 24, affected amino acid residue 1375, altered the downstream amino acid sequence, and truncated the protein at amino acid residue 1392. Red: C-terminal sequence of mutant.

A cardiac ultrasonogram revealed an atrial septal defect. Ophthalmic examination revealed bilateral cataract, retinal scar and persistent hyperplastic primary vitreous in left side. Visual-evoked potentials confirmed no pick up over p100 by flash method. MRI of brain showed benign enlargement of the subarachnoid space.

Case 3
A 10-year-old girl was the first daughter of non-consanguineous parents. She was diagnosed with central precocious puberty at the age of 5 because of progressive breast enlargement and brought to our genetic clinic for asymmetric face and unilateral hearing loss. She also has torticollis and microsomia. A 3D-CT scan of the facial bone showed hypoplasia of the left mandibular bone (Fig. 2e). MRI of the brain showed a small pituitary gland (0.3 cm in height) compared to normal (range: 2.5–9.0 mm in height.) (23).

Case 4
A 15-day-old infant boy had an asymmetric face with a crumple ear and microtia on left side (Fig. 3). BAEP confirmed abnormal conductive hearing (>95 dB, bilateral). MRI of brain showed congenital aural stenosis in left side.

Case 5
The 15-day-old infant girl was the first daughter of non-consanguineous parents. The G1P1 mother noted no prenatal insult. She exhibited an asymmetric face and HFM (Fig. 2f,g). There was no major or minor abnormality.

Case 6
The proband, a 25-year-old male, was referred from local medical department because of his asymmetric face on the left side. No history of exposure to pertinent maternal illness was reported. He had no other major or minor abnormality in the past.

Among cases 7–12 who did not have the novel base insertion mutation in the TCOF1 gene, three patients had right HFM, one patient had left HFM, and two patients had bilateral microtia (Table 1).

Case 7
The 1-day-old newborn girl was the second daughter of non-consanguineous parents and exhibited an asymmetric face and HFM on right side: premature closure of anterior fontanels, bilateral crumple ears, and retinal detachment in left eye. Anterior displacement of
Table 1. Clinical characteristics of 12 infants at birth

<table>
<thead>
<tr>
<th>Case No. (sex/GA)</th>
<th>Maternal age at birth (years)</th>
<th>Weight (g)</th>
<th>Height (cm)</th>
<th>Head circumference (cm)</th>
<th>APGAR score 1</th>
<th>APGAR score 5</th>
<th>OMENSa classification+</th>
<th>Total OMENS score</th>
<th>Cardiovascular system</th>
<th>Gastroenteral system</th>
<th>Central nervous system</th>
<th>Clinical diagnosis</th>
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<tbody>
<tr>
<td>Patients with novel base insertion</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1 (F/39 weeks)</td>
<td>32</td>
<td>2720</td>
<td>49</td>
<td>33.5</td>
<td>9</td>
<td>10</td>
<td>O0 M2a E0 N0 S1</td>
<td>3</td>
<td>—</td>
<td>Imperforate anus with perineal–anal fistula</td>
<td>Left HFM</td>
<td></td>
</tr>
<tr>
<td>2 (M/38 weeks)</td>
<td>26</td>
<td>3230</td>
<td>50</td>
<td>34</td>
<td>9</td>
<td>10</td>
<td>O0 M2a E1 N0 S1</td>
<td>4</td>
<td>ASD</td>
<td>Persistent fifth ventricle</td>
<td>Macrocephaly, benign enlargement of the subarachnoid space</td>
<td>Left HFM, micrognathia, coloboma</td>
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<tr>
<td>3 (F/39 weeks)</td>
<td>29</td>
<td>3000</td>
<td>48</td>
<td>34</td>
<td>ND</td>
<td>ND</td>
<td>O0 M2a E1 N0 S1</td>
<td>4</td>
<td>—</td>
<td>Small pituitary gland (0.3 cm in height)</td>
<td>Left HFM, Hearing loss</td>
<td></td>
</tr>
<tr>
<td>4 (M/37 weeks)</td>
<td>30</td>
<td>2840</td>
<td>50.5</td>
<td>33.5</td>
<td>9</td>
<td>10</td>
<td>O0 M2a E2 N0 S1</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Left HFM bilateral severe hearing impairment</td>
</tr>
<tr>
<td>5 (F/39 weeks)</td>
<td>28</td>
<td>2900</td>
<td>50</td>
<td>34</td>
<td>9</td>
<td>10</td>
<td>O0 M1 E1 N0 S1</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Left HFM</td>
</tr>
<tr>
<td>6 (F/ND weeks)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>O0 M1 E1 N0 S1</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Left HFM</td>
</tr>
<tr>
<td>Patients without insertion mutated</td>
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<tr>
<td>7 (F/38 weeks)</td>
<td>29</td>
<td>2595</td>
<td>48</td>
<td>32</td>
<td>8</td>
<td>10</td>
<td>O3 M1 E1 N0 S1</td>
<td>6</td>
<td>—</td>
<td>Premature close of anterior fontanel</td>
<td>Right HFM</td>
<td></td>
</tr>
<tr>
<td>8 (F/39 weeks)</td>
<td>25</td>
<td>3065</td>
<td>50.5</td>
<td>34.5</td>
<td>9</td>
<td>10</td>
<td>O0 M2a E2 N1 S2</td>
<td>7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Right HFM severe hearing impairment</td>
</tr>
<tr>
<td>9 (M/40 weeks)</td>
<td>27</td>
<td>3250</td>
<td>51</td>
<td>34</td>
<td>ND</td>
<td>ND</td>
<td>O0 M1 E2 N0 S1</td>
<td>4</td>
<td>—</td>
<td>Hydrocephalus of lateral and third ventricles premature close of coronal suture</td>
<td>Left HFM</td>
<td></td>
</tr>
<tr>
<td>10 (M/39 weeks)</td>
<td>30</td>
<td>2490</td>
<td>50</td>
<td>32</td>
<td>8</td>
<td>10</td>
<td>O0 M1 E0 N3 S1</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Right HFM</td>
</tr>
<tr>
<td>11 (F/37 weeks)</td>
<td>36</td>
<td>2168</td>
<td>41</td>
<td>31</td>
<td>3</td>
<td>6</td>
<td>O0 M2a E2 N0 S1</td>
<td>5</td>
<td>PDA</td>
<td>EA, T-E fistula</td>
<td>—</td>
<td>Bilateral microtia</td>
</tr>
<tr>
<td>12 (M/40 weeks)</td>
<td>29</td>
<td>3080</td>
<td>49</td>
<td>35</td>
<td>ND</td>
<td>ND</td>
<td>O1 M1 E2 N0 S1</td>
<td>5</td>
<td>ASD</td>
<td>—</td>
<td>—</td>
<td>Bilateral microtia</td>
</tr>
</tbody>
</table>

GA, gestational age; M, male; F, female; B, boy; ASD, atrial septal defect; PDA, patent ductus arteriosus; EA, esophageal atresia; T-E fistula, tracheoesophageal fistula; ND, not detected; HFM, hemifacial microsomia.

aOMENS indicates the following: O, orbital asymmetry; M, mandible hypoplasia; E, auricular deformity; N, facial nerve involvement; S, soft tissue deficiency. 2a, glenoid fossa in anatomically acceptable position.
Fig. 2. Three-dimensional computed tomography of facial bones of patients with facial asymmetry [(a)–(i) with insertion of TCOF1 gene mutation; (j)–(l) without TCOF1 gene mutation]. (a) case 1, left view, mild small mandible bone; (b) case 1, front view, mild asymmetric face; (c) case 2, left view; (d) case 2, front view, small mandible bone; (e) case 3, front view, hypoplasia of the left mandibular bone; (f) case 5, left view; (g) case 5, front view, significant asymmetric face and left hemifacial microsomia; (h) case 8, right view; (i) case 8, front view, asymmetric face on the right side; (j) case 11, front view, significant micrognathia; (k) case 12, left view; (l) case 12, front view, malar hypoplasia and left mandible hypoplasia.

Fig. 3. Case 4 had an asymmetric face with a crumple ear and microtia on left side.

Case 8
This 4-year-old girl was the first daughter of non-consanguineous parents. Referred for cleft palate, she has an asymmetric face, HFM, preauricular tag, protruding earlobes, and microtia on the right side. 3D-CT revealed malar dysplasia, mandible hypoplasia, atlanto-occipital fusion and atlas deficiency (Fig. 2h,i). BAEP revealed severe hearing impairment.

Case 9
This male newborn was referred because of cleft palate. He has mild HFM and cupping ear on the left side. BAEP revealed mild hearing impairment.

Case 10
This male newborn was referred for asymmetrical face and small size. He has HFM and micrognathia on right side.

Case 11
The girl was the second child of non-consanguineous parents and was born after a pregnancy complicated by polyhydramnios. Intrauterine growth restriction and the absence of the gastric bubble were found at 25 weeks of gestation. APGAR scores were 3 and 6 at 1 and 5 min, respectively. She presented with short stature, dystrophy, microcephaly, large fontanel, downslanted palpebral fissures, bilateral microtia, low-set ears, high arch cleft palate, micrognathia, and continuous murmur grade II over left upper sterna border, enlarged clitoris, petechiae over the body, and overlapping of second, third, and fourth bilateral toes. A 3D-CT scan of the facial bone showed significant micrognathia (Fig. 2j). Echocardiogram revealed patent ducutus arteriosus with left to right shunt, patent foramen ovale, and narrow-angle between innominate artery and left coronary artery.

Case 12
This male newborn was referred for microtia and had small eyes, preauricular tag, over folded helix, cleft palate and micrognathia. 3D-CT showed malar hypoplasia and left mandible hypoplasia and atresia of external auditory meatus in both sides (Fig. 2k,l).

Discussion
Multiple genes contribute to the wide phenotypic spectra of the first and second branchial arch syndromes that include facial asymmetries (6, 7, 10–12, 24–26) and family members with the same mutation can exhibit distinct penetrance of the phenotype (2). Here, 6 of 12 patients diagnosed with HFM exhibited a novel frameshift mutation c. 4127 ins G in exon 24 in the TCOF1 gene. This mutation has not been reported in...
TCS patients, was not detected in 100 normal subjects or the parents of the cases, and was not an SNP (27). Although previous studies on HFM observed approximately equal numbers of left- and right-sided anomalies (3, 4), the asymmetrical facial anomalies in the five of six patients carrying the TCOF1 nt.4127 ins G mutation were on the left side. As case 9 had facial asymmetry but did not contain the TCOF1 nt.4127 G mutation, other mutations might also contribute or cause HFM (6–12). For example, screening of cases with asymmetric crying faces or micrognathia may detect abnormality in chr22q11 (28) and chr16q22 (26), with asymmetric crying faces or micrognathia may cause HFM (6–12). For example, screening of cases with asymmetric crying faces or micrognathia may detect abnormality in chr22q11 (28) and chr16q22 (26), respectively.

This TCOF1 nt.4127 G frameshift mutation destroyed the two NLS regions in the C terminus and the coiled coil domain. The mutant treacle probably exhibits defects in nuclear trafficking and appears to affect the development of the left side of the face (6/6 cases) more than the right side of the face (0/6). In addition to further studies of a larger human HFM cohort for validation, the effects of the TCOF1 nt.4127 ins G mutant on facial development may be assessed in a transgenic mouse model analogous to the transgenic TCOF1 mutant mouse model of TCS syndrome (29, 30). The skewed pattern of the mutant-bearing HFM cases supports the recent proposal by Obwegeser et al. of the existence of two regulators of growth of the mandibular condyles (31).

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References


Insertion of the TCOF1 gene