incisor. Panoramic radiography showed normal dental development with a pulp stone in the maxillary left first permanent molar. The left zygomatic arch was very narrow (Fig. 1a). Physical examination revealed short fingers with dysplastic fingernails. The right third and both fourth fingernails were absent (Fig. 1b). The dorsal skin covering the distal one-third of these fingers was smooth and shiny. Left fingernails 2, 3, and 5 were hypoplastic. The second fingers were most severely affected. The shortness of fingers did not limit him in doing his regular routine. Each finger had only one flexion crease. Toes 2–5 were also short with normal appearing toenails, and mild cutaneous syndactyly of toes 2 and 3 was observed bilaterally. Hand radiography demonstrated very small ossification centers of the right scaphoid and trapezoid, and the left trapezium, with the absence of the right trapezium, left trapezoid, and both pisiform bones. Both thumbs and their metacarpals were normal. Fusions between middle and distal phalanges of left fingers 2 and 3 and right fingers 2 and 5 were observed, and distal phalanges of right finger 3 and bilateral fingers 4 were absent (Fig. 1c). The right second and the left second and fourth middle phalanges showed cone-shaped epiphyses. Foot radiography showed very short middle and distal phalanges of toes 3–5. The phalanges of the second toes appeared normal. Fusion was observed between middle and distal phalanges of bilateral toes 3 without fusion lines between them. All proximal phalanges and great toes were normal.

Patient 2, a 37-year-old woman, was the mother of patient 1. Her face was asymmetric (left < right) and had a prominent nose with a nasal septum extending below nasal alae and a short philtrum, but was otherwise normal. Her height was 151 cm (50th centile) and OFC 51 cm (3rd centile). She was the second child in a non-consanguineous marriage. Her parents, brother, and sister were all unremarkable. The shortness of fingers was less remarkable than that in patient 1. Like in her son, second fingers were very short with bulbous tips, and most severely affected. Fingernails 3, 2, 4, and 5 were dysplastic in the order of decreasing severity (Fig. 2a). Single flexion crease was observed at the right second and both third fingers. Her thumbs and their nails appeared normal. Toes 2–5 were also short, but toenails were unremarkable. Panoramic radiography showed normal dental development with the absence of all third permanent molars. Narrowing of the right zygomatic arch was evident (Fig. 2b). Pulp stones were observed in all first permanent molars. Distal phalanges of fingers 2–5 were short and appeared fused, with short corresponding middle phalanges (Fig. 2c). Foot radiography showed fusion of the short middle and distal phalanges of toes 2–5 with no fusion lines. A middle part of proximal phalanges 2–4 were narrow.

### Mutation Analysis of *NOG* and *ROR2*

Informed consent was obtained from both patients. DNA was extracted from peripheral blood leukocytes by standard procedures. Primer sequences and PCR condition for *NOG* analysis have been described previously [Gong et al., 1999]. Primers were designed in the flanking intron sequences to amplify exons 2–8 of *ROR2*. *ROR2*-exon 9 was amplified in overlapping segments [Schwabe et al., 2000]. PCR was for *ROR2* performed at 94°C for 10 min, followed by 40 cycles at 94°C for 30 sec, 50°C for 30 sec, 72°C for 45 sec, and final extension at 72°C for 10 min. PCR products were purified by QIA quick purification kit (Qiagen, Chataworth, CA), then sequenced with BigDye terminator kit (PE Applied Biosystems, Foster City, CA) and analyzed by an ABI 377 automated sequencer (PE Applied Biosystems). As a result, no mutation in either gene was observed in the two patients.

## DISCUSSION

Both patients we have described were clinically diagnosed to have DS because they had brachydactyly, especially at the distal segment of digits, fusion between the distal interphalangeal joints, and the absence of distal phalanges 3 and 4. They also had additional, hitherto undescribed abnormalities, such as cone-shaped epiphyses of middle phalanges 2 and 4, radial-ray carpal-bone anomalies, narrowing of the zygomatic arch, and dental pulp stones. The second fingers were predominantly affected, as reported previously [Inman, 1924; Steinberg and Reynolds, 1948; Poush, 1991], while the thumbs and great toes were intact in both patients, unlike in patients described by Matthews et al. [1987]. Proximal symphalangism (PS) was ruled out, because symphalangism patterns seen in our patients were absolutely different from PS and there was no *NOG* mutation in the patients.

DS shares many manifestations with BDB, including aplasia/hypoplasia of the middle and distal phalanges of fingers 2–5, and fingernail dysplasia [Sillence, 1978; Halpern et al., 1979; Poush, 1991; Oldridge et al., 1999; Oldridge et al., 2000]. As seen in our patient 1, BDB has been reported to have DS and fourth digit hypophalangism [Comings, 1965; Zavala et al., 1975; Temtamy and McKusick, 1978]. In addition to the absence of mutation in *ROR2*, hypoplasia of the distal phalanges confined to the second fingers in patient 2 distinguished the condition in this family from BDB [Inman, 1924; Steinberg and Reynolds, 1948]. When BDB and DS are present together in a patient, it is sometimes confusing to describe the condition as DS with BDB [Comings, 1965] or BDB with DS [Zavala et al., 1975; Temtamy and McKusick, 1978]. Unequivocally differentiating diagnostic features between the two conditions, other than *ROR2* gene analysis, would be worth studying.

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*Clinical Report*

# A Dominantly Inherited Malformation Syndrome With Short Stature, Upper Limb Anomaly, Minor Craniofacial Anomalies, and Absence of *TBX5* Mutations: Report of a Thai Family

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**We report on a Thai family with dominantly inherited malformation syndrome with upper limb anomalies, short stature, quadricuspid aortic valve, and minor craniofacial anomalies. The affected individuals comprised a mildly affected mother, a moderately affected daughter, and a most severely affected son. The daughter and son had short stature. The craniofacial abnormalities comprised frontal bossing, hypoplastic nasal bones, depressed nasal bridge, and broad nasal alae. The upper limb defects varies among the patients, ranging from radial ray defects in the mother through radial and ulnar ray defects with unilateral humeral hypoplasia in the daughter to radial ray defects with severe oligodactyly and bilateral humeral hypoplasia in the son. All patients in this family had hypoplasia of the shoulder girdle and resembled what is observed in many families with Holt-Oram syndrome. Moreover, the son showed quadricuspid aortic valve with mild aortic regurgitation. However, the present family did not show any mutation of the *TBX5* gene, a disease-causing gene of Holt-Oram syndrome. The present family deserves further investigation on other genes that play a role in the development of the upper limbs, particularly of radial rays.** © 2002 Wiley-Liss, Inc.

KEY WORDS: **absent thumb; Holt-Oram syndrome; hypoplastic clavicle; hypoplastic humerus; hypoplastic ulna; oligodactyly; short stature**

## INTRODUCTION

The biological role of several genes in limb development has been elucidated, based on recent discoveries of disease-causing genes in several limb malformation syndromes, such as the *TBX5* gene in Holt-Oram syndrome (HOS) [Basson et al., 1997, 1999; Li et al., 1997]. We describe here a Thai family with a dominantly inherited malformation syndrome, characterized by radial and ulnar ray defects, short stature, and minor craniofacial malformations. The upper limb and shoulder girdle defects in the present family are more severe than those of HOS, and the molecular studies did not reveal any mutations in the *TBX5* gene.

## CLINICAL REPORT

### Patient 1

The proband was a 51-year-old Thai woman born to healthy nonconsanguineous parents (Fig. 1A). She had three affected offspring, a daughter (patient 2; Fig. 2A), a son (patient 3; Fig. 3A), and another son who died a few days after birth and was reported to be absent of thumbs and syndactylous fingers. The proband was healthy with

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normal intelligence. Height and OFC were 153 cm (50th centile) and 53 cm (3rd centile), respectively. She had minor facial dysmorphism, comprising frontal bossing, depressed nasal bridge, and broad nasal alae (Fig. 1A). Medical history and physical examination did not reveal evidence of heart disease. She had slender upper arms, short forearms with clubbed hands, absent thumbs, hypoplasia with flexion contracture of the left second finger, partial cutaneous syndactyly between the left second and third fingers, and camptodactyly of all proximal interphalangeal (PIP) joints predominantly of the right (Fig. 1A). Radiologic examination revealed slenderness of the left clavicle, hypoplastic scapulae with flat glenoid fossae particularly of the left, mild hypoplasia of the left humeral head, bowed ulnae particularly of the left, and radial ray defects, comprising the absence of the radii and thumbs, dysplastic radial carpal bones, brachymesophalongy of fingers 3, 4, and 5, and hypoplasia of the left second metacarpal and phalanges (Fig. 1B). Lateral cephalogram showed underdeveloped nasal bones.

### Patient 2

The patient was a 25-year-old woman (Fig. 2A). She was intelligent and healthy. Medical history and physical examination did not show evidence of cardiac problems. The patient refused to have echocardiography performed. She was proportionately short. Height and OFC were 145 cm (5th centile) and 53 cm (3rd centile), respectively. Frontal bossing, depressed nasal bridge, and broad nasal alae were observed. The thorax was narrow with short clavicles (Fig. 2A). The left upper limb was short with dislocation of the left shoulder joint. The right upper limb showed only shortening of the forearm. The right hand was clubbed. Both thumbs were absent. The left second finger was small. The left fourth finger was longer than the third one. Camptodactyly was evident, especially on the right (Fig. 2B). Radiologic examination demonstrated hypoplastic scapulae significantly of the left and severe left humeral hypoplasia with humeroulnar synostosis (Fig. 2C). The radiologic findings of the forearm and hand were similar to those of the proband (Fig. 2D). Lateral cephalogram showed underdeveloped nasal bones.

### Patient 3

The patient was a 23-year-old man (Fig. 3A). He was healthy and intelligent. Although clinically asymptomatic, echocardiography showed quadricuspid aortic valve with aortic regurgitation of the mild degree. Height and OFC were 150 cm (<3rd centile) and 53 cm (<3 centile) respectively. Frontal bossing, depressed nasal bridge, and broad nasal alae were evident. The shoulder was narrow and slightly drooped. Dislocation of the shoulder joints with prominent acromial processes was found. The thorax was narrow with short clavicles. The upper limbs were significantly short with clubbed hands (Fig. 3A). Only two fingers with a wide gap between them were present bilaterally. Camptodactyly was seen in the right two fingers and the left ulnar finger


Fig. 1. Patient 1. **A:** Frontal bossing, broad alar nasi, and depressed nasal bridge. Note clubbed hands and flexion contracture of fingers. **B:** Absent radii, hypoplastic and bowed right ulna, and malformed carpal bones. Note brachymesophalangy and interphalangeal joint abnormalities.

(Fig. 3B and D). Radiologic examination showed hypoplasia of the scapulae especially of the left, rudimentary humeri, right humeroulnar synostosis, bowed ulnae, absent radii, and oligodactyly (Fig. 3C and E). Three

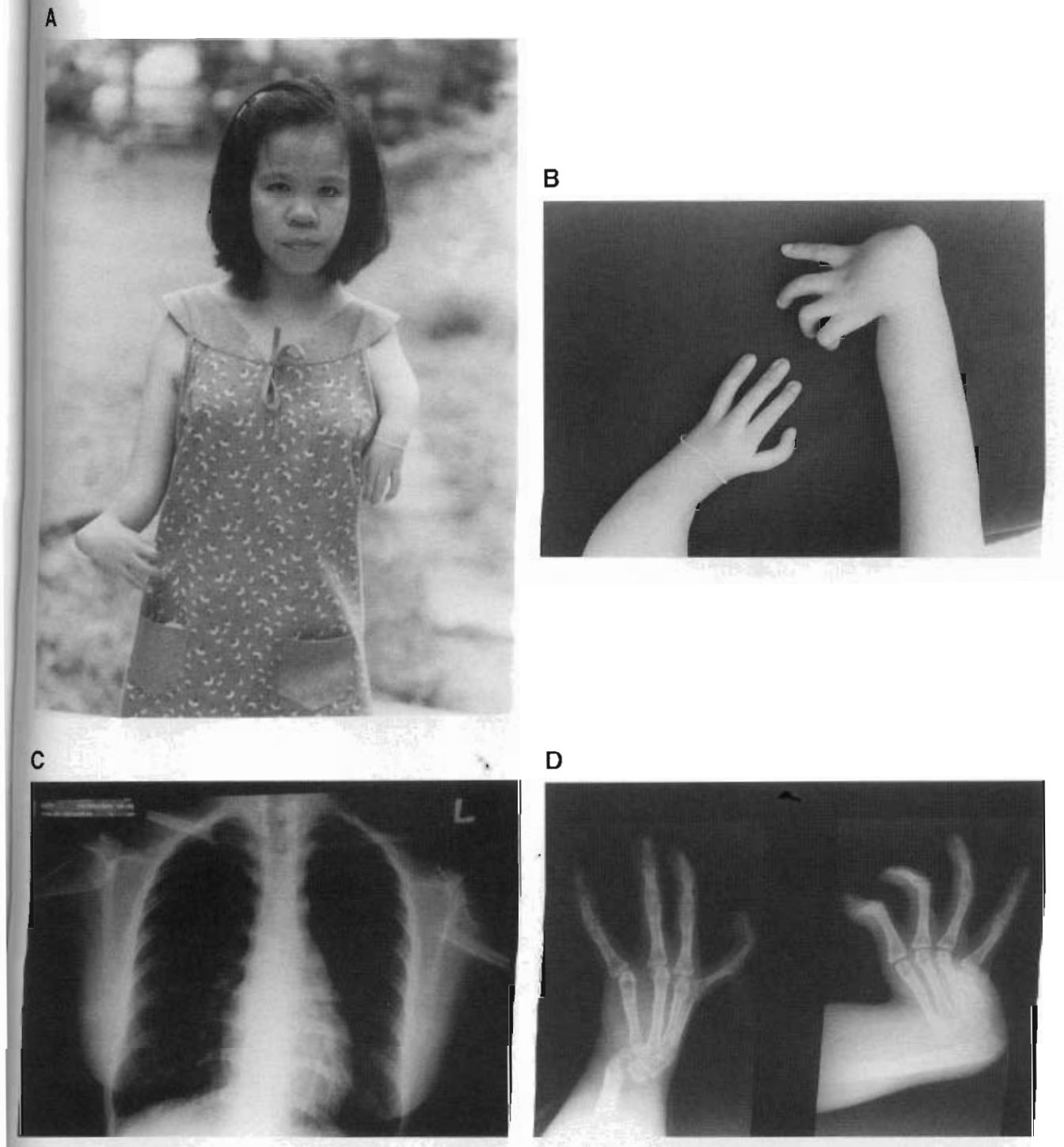


Fig. 2. Patient 2. **A:** Frontal bossing, broad alar nasi, and depressed nasal bridge. Short clavicles. **B:** Very short left arm. Right hand in clubbed position. **C:** Hypoplastic scapulae. Dislocated left shoulder joint. Left humeral hypoplasia with humeroulnar synostosis. **D:** Absence of radii, and thumbs, malformed carpal bones, and hypoplastic ulnae.

metacarpals were present bilaterally, and osseous syndactyly was noted in the proximal ends between the right ulnar metacarpals and distal ends between the left radial metacarpals. The radial fingers consisted of partially duplicated phalanges bilaterally (Fig. 3E). Lateral cephalogram showed underdeveloped nasal bones (Fig. 4).

## DNA Isolation

Peripheral blood (10 ml) was obtained from each patient. Epstein-Barr virus–transformed cell lines were established. DNA was isolated from lymphoblastoid cell lines by digestion with proteinase K and phenol-chloroform purification [Sambrook et al., 1989]

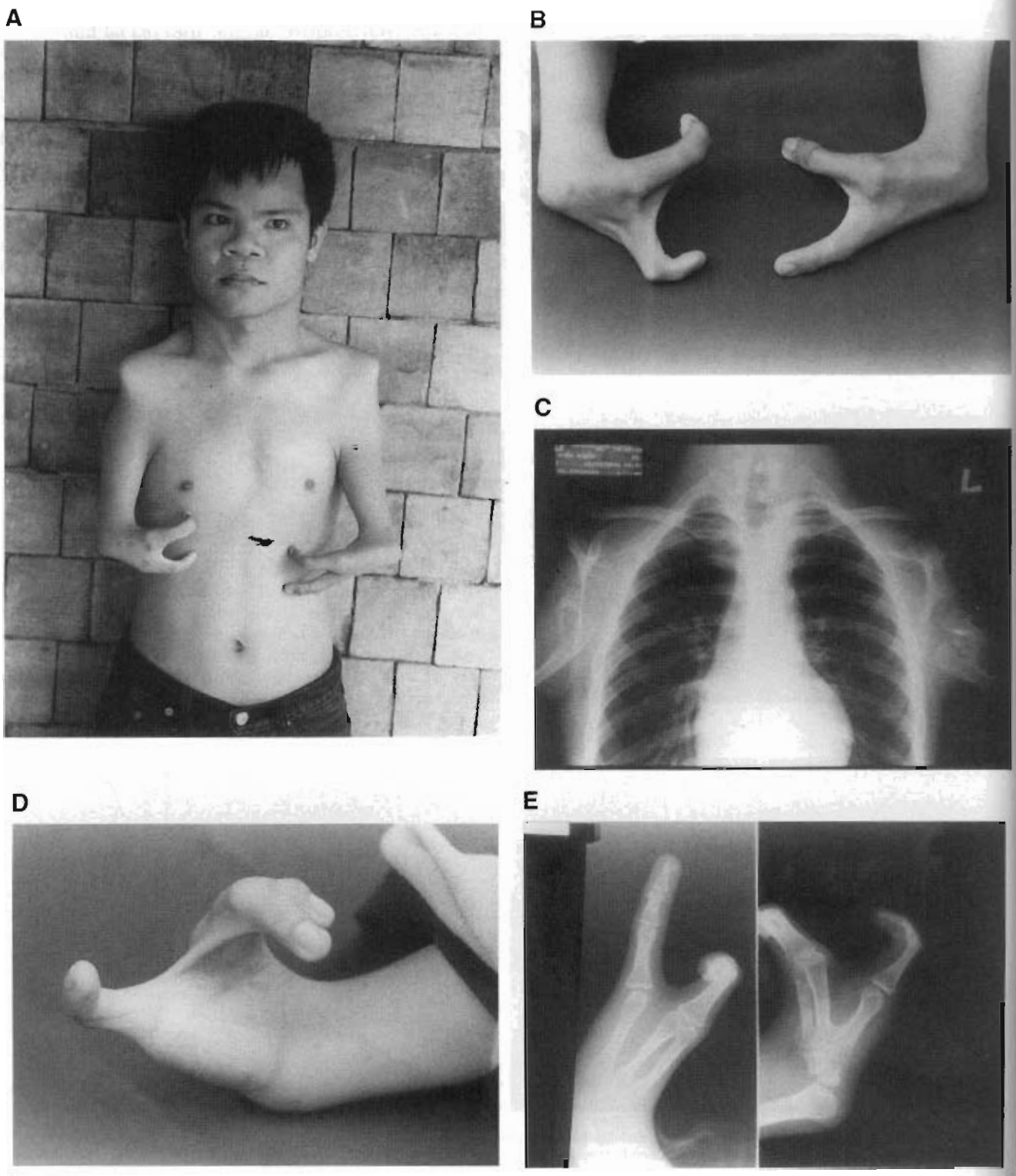


Fig. 3. Patient 3. **A:** Frontal bossing, broad alar nasi, and depressed nasal bridge. Note short clavicles with drooped shoulder, prominent acromions, and very short arms. **B:** Wide gap between syndactylous fingers. Note camptodactyly. **C:** Hypoplastic scapulae. Malformed and dislocated humeri. Right humerus is fused with proximal ulna. **D:** Syndactylous finger. **E:** Two malformed carpal bones. Three metacarpals are present in each hand. Osseous syndactyly of metacarpals. Note partially duplicated phalanges.

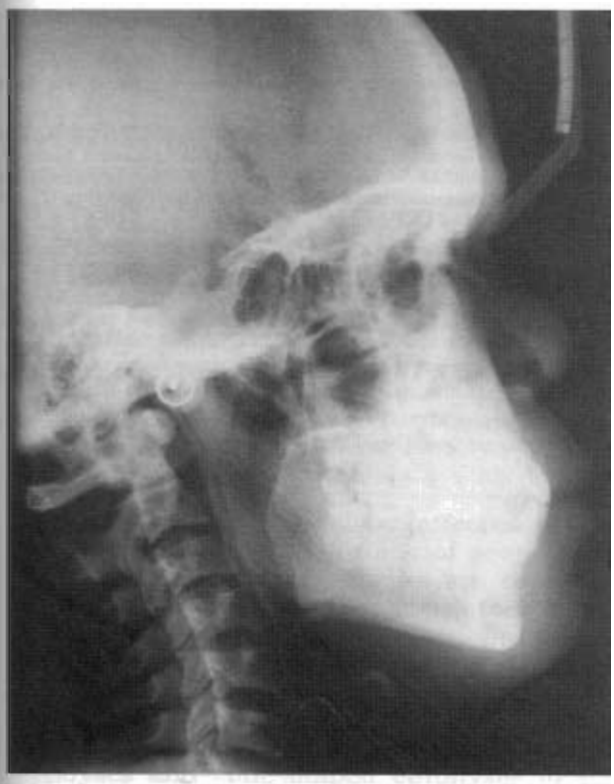

Fig. 4. Lateral cephalogram of Patient 3 shows underdeveloped nasal bones.

### *TBX5* Gene Mutation Analysis

Exons 2 through 9 of *TBX5* were PCR-amplified from genomic DNA of patient 3 using primers derived from intron sequences flanking each exon (5′ → 3′): exon 2 (bp 1–146)-2GF,R: GCTTCTTGTCCTCAGAGCAGAACCT/CAAGAGAAGCCGAGCAGGAAAGCCA; exon 3 (bp 147–242)-3GF,R: TTCTCCTCGTCCCTCTCTCTACACA/AGTTTGGGGAAGGAATGCCCACTAC; exon 4 (bp 243–361)-4GF,R: AACGGGGCTAGTTTCCGCTTCCACG/CTTTTTGGGAGAAGGTTCCACTTTT; exon 5 (bp 362–509)-5GF,R: CTTGGTGCGTGAACTGAAGCACGC/GAGGGAGACAAGGCTGGGGAATCCAG; exon 6 (bp 510–663)-6GF,R: ACGGCCCAGGCACTGGTTCCTGGG/CAGGGTTTTATCTGGAGACAAAGGG; exon 7 (bp 664–754)-7GF,R: ATTAGCTCATGTCCTGAGGTGGTCT/GTGGGGAGGAGAAAGTTGAGGAATC; exon 8 (bp 755–1098)-8GF,R: CTTTTCTGGTGGATTCTCTCACACC/GGGTAGGAACATGTCAAGGGAACT; exon 9-9GF,R: TACTTTGGACAATATACTGTCTCC/CGACCTTGAGTGCAGAATGTGAAC. PCR conditions used were 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, for 30 cycles, then kept at 72°C for 10 min. PCR products were then purified by using QIA quick columns per the manufacturer's instructions (Qiagen, Chataworth, CA). Templates were subjected to cycle sequencing by using fluoresceinated dideoxynucleotides and analyzed by Applied Biosystems 377 automated DNA sequencer.

## RESULTS

Using the genomic DNA of patient 3, direct sequencing did not show mutations in exons 2–9 of *TBX5*.

## DISCUSSION

The pattern of the upper limb defect and its left-sided predominance and possible genetic anticipation in the present family mimicked those reported in some families with HOS [Rybak et al., 1971; Newbury-Ecob et al., 1996; Li et al., 1997]. Short stature found in the present family is seldom found in HOS [Rybak et al., 1971]. Cardiovascular anomalies of HOS are varied. ASD and VSD are common, but, though rare, hypoplastic left heart and hypoplastic peripheral vasculature are described; thus, the quadricuspid aortic valve found in our patient 3 may have been a syndromic constituent, but minor craniofacial changes as in the present family might have been ignored in HOS patients in the context of the presence of other major malformations [Newbury-Ecob et al., 1996; Sletten and Pierpont, 1996]. For these reasons, we were engaged in the work of molecular analysis on the *TBX5* gene in the present family, which ultimately yielded a negative result.

The upper limb anomalies of this family were similar to those reported in a Polish family affected with HOS. Actually, the upper limb anomalies found in that Polish family and in ours were more severe than those usually found in HOS [Rybak et al., 1971; Newbury-Ecob et al., 1996]. Without the presence of short stature and craniofacial features, the diagnosis HOS might have been entertained since HOS may not have congenital heart defects [Rybak et al., 1971; Basson et al., 1999; Cross et al., 2000]. Interestingly, cranial deformity has been mentioned in that Polish family but unfortunately it was not fully described [Rybak et al., 1971]. The short stature and minor craniofacial features reported in the present family were not of their familial traits, since other members of the family did not have them.

As far as the negative molecular findings are concerned, we are aware that approximately two-thirds of HOS patients have negative molecular *TBX5* findings [Cross et al., 2000]. It may not be possible to tell whether the disorder in the present family represents genetic heterogeneity of HOS or a previously undescribed malformation syndrome. There is a possibility that the present family and the one reported by Rybak et al. [1971] had the same disorder, not related to *TBX5* mutations. The presumed gene that causes the present disorder would be expected to be functionally expressed in a multiplicity of tissues, including the upper limbs, heart, and craniofacial regions, as is the *TBX5* gene with expression in the developing heart, forelimbs, trachea, thoracic wall, retina, and telecephalon.

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American Journal of Medical Genetics 111:420–428 (2002)

*Clinical Report*

# Apparently New Osteodysplastic and Primordial Short Stature With Severe Microdontia, Opalescent Teeth, and Rootless Molars in Two Siblings

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**A Thai man and his sister affected with a newly recognized syndrome of proportionate primordial short stature are reported. The patients had severe intrauterine and postnatal growth retardation, prominent nose and nasal bridge, small pinnae, large sella turcica, areas of hypo- and hyperpigmentation of skin, dry and thin scalp hair, and long and straight clavicles. Ivory epiphyses and cone-shaped epiphyses of the hands were found when they were young, but most of them disappeared as they grew up. Scaphoid and trapezium had angular appearance. The second toes were unusually long. Distal symphalangism of toes and barchymesophalangy of fingers were noted. The findings that appear to distinguish this syndrome from the previously reported syndromes are long second toes, opalescent and rootless teeth, severe microdontia, severely hypoplastic alveolar process, and unerupted tooth. The mode of inheritance is suspected to be autosomal recessive.**



**KEY WORDS:** **dentinogenesis imperfecta; large nose; microcephaly; microdontia; opalescent teeth; osteodysplasia; primordial dwarfism; rootless teeth; Seckel syndrome**

## INTRODUCTION

Primordial "dwarfism" is used to describe patients who have severe intrauterine and postnatal growth retardation. It is a very heterogeneous group of disorder that has been classified into three main types: Seckel syndrome, microcephalic osteodysplastic primordial dwarfism (MOPD) type I/III and type II [Meinecke and Passarge, 1991; Majewski and Goecke, 1998]. Variants of MOPD or Seckel-like syndrome have been described [Shebib et al., 1991; Buebel et al., 1996]. Seckel syndrome, the best known one, is characterized by severe intrauterine and postnatal proportional short stature, severe microcephaly, mild to moderate mental retardation, large and prominent nose, and dislocation of the head of radius [Seckel, 1960]. Patients affected with MOPD can be easily distinguished from those with Seckel syndrome by being disproportionately short and having distinct radiological features [Majewski et al., 1982a, 1982b; Poznanski et al., 1983; Majewski and Goecke, 1998].

Primordial short stature with dental anomalies has been described [Seckel, 1960; Majewski and Goecke, 1982; Shebib et al., 1991; Majewski, 1992; Lin et al., 1995]. However, none, especially anyone with proportionate primordial short stature, has been reported with severe microdontia, opalescent teeth, and rootless molars. A Thai brother and sister affected with a new syndrome of this group of short stature are described. A newly recognized features comprising a newly recognized syndrome include long second toes, long and straight clavicles, distal symphalangism of toes, severe microdontia and malformation of mandibular premolars, severely hypoplastic alveolar processes, opalescent teeth, and rootless molars. The mode of inheritance is suspected to be autosomal recessive.

## CLINICAL REPORT

### Patient 1

Patient 1 was an 18-year-old man. He and his younger sister (patient 2) were born from healthy and nonconsanguineous parents (Figs. 1A and 2A and B). The older

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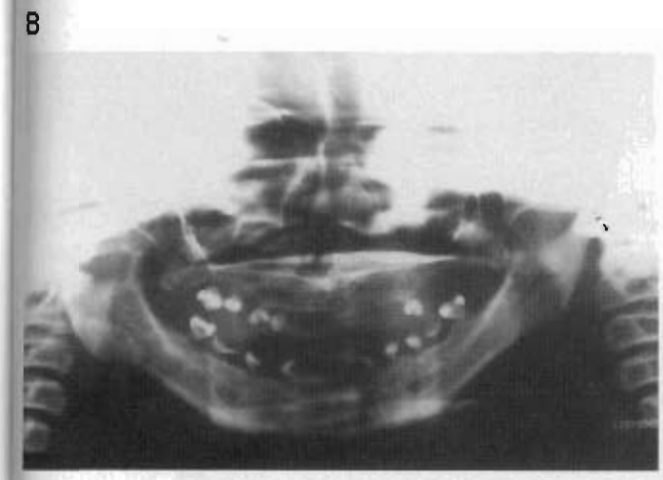


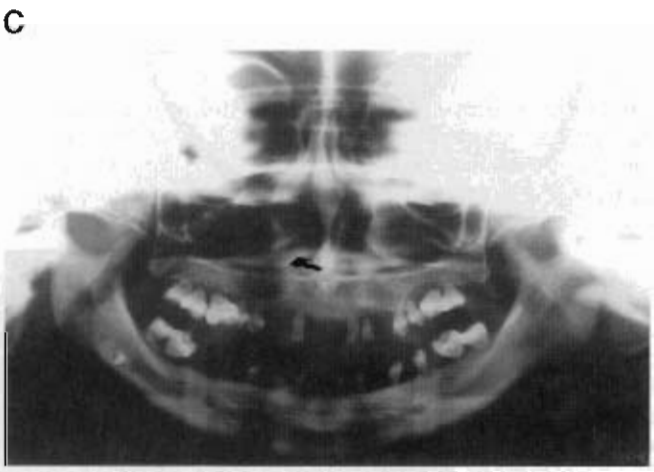




Fig. 1. **A:** Patients 1 and 2 at ages 18 and 16 years old, respectively, and their parents. Proportionate short stature is noted. **B** and **C:** Panoramic radiographs of patients 1 and 2, respectively. Note rootless molars. Dentin of incisors is less radiopaque than that of molars. Lack of cortical bone, and hypoplastic alveolar process. Mandibular premolars are very small.

sister was normal. The patient was born at term. Birth weight was 1,000 g. He started having pubic hair at age 14 years. At age 18 years, axillary hair was not apparent. Intelligence was normal.

On examination, he was proportionately short. Height, weight, and OFC were 102 cm (<3rd centile), 14 k (<3rd centile), and 43 cm (<3rd centile), respectively. Scalp hair was dry and thin. Large nose with prominent nasal bridge was observed. Pinnae were small. Hearing was normal. Like his sister, there were areas of hypo- and hyperpigmentation of the skin that did not follow lines of Blaschko lines. In some areas, the skin appeared poikilodermatous (Fig. 2A and B).

Oral examination revealed generalized microdontia and opalescent teeth. All teeth were small but the mandibular premolars were unusually small and malformed, comprising of many cusps. The mandibular left premolars had the mesio-distal dimension of 4 mm. All teeth were loose and wore off easily. The parents reported that both children had all primary and permanent teeth, but they kept falling out painlessly. The loss of teeth was not a result of dental caries. There was no history of recurrent infections. Chest was narrow. The fingers and thumbs were short, and the elbows bent (Fig. 3C). The second toes were unusually long and both great and second toes were laterally curved. Flexion

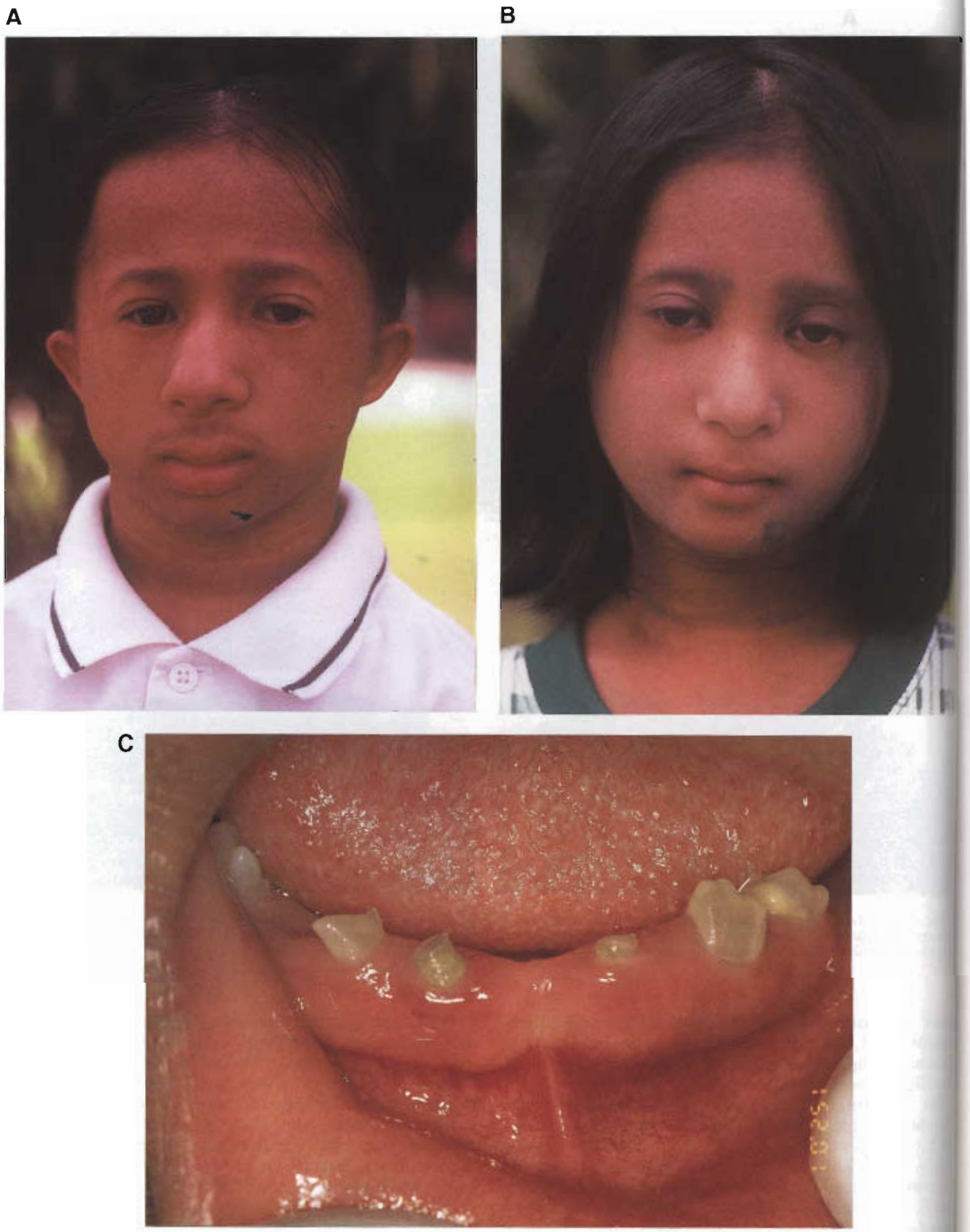



Fig. 2. **A:** Patient 1. **B:** Patient 2. Note large and prominent noses and small pinnae. Scalp hair is thin. **C:** Small and opalescent incisors with severe attrition. **D:** Opalescent teeth. Severe microdontia and malformation of mandibular premolar. Mandibular canine has prominent cingulum.

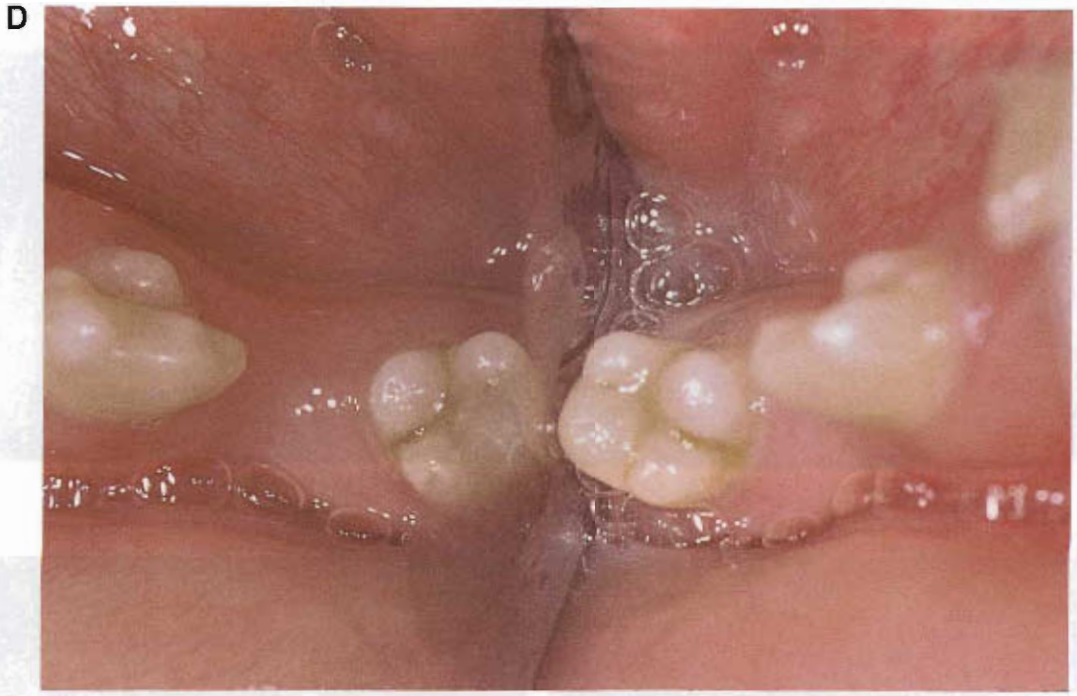



Fig. 2. (*Continued*)

contracture of the distal interphalangeal joints (IPJ) of the toes was observed (Fig. 4A). Flexion contracture of all distal IPJ was observed.

Radiographic examination showed delayed dental development, rootless molars, short-rooted incisors, and narrow root canals of incisors. Like that of his sister, the dentin of the incisors was less radiopaque than those of molars. The crowns of the molars appeared tapered occluso-cervically. Cortical bone of the alveolar process was not evident. Severe hypoplasia of the alveolar process was observed. There was hardly any bone surrounding the roots of the teeth (Fig. 1B and C). Lateral cephalographs at age 14 and 18 years showed digital marking of the skull, progressive alveolar bone loss, progressive loss of teeth, large sella turcica, and long posterior clinoid process (Fig. 5A and B).

Radiographs showed long, straight, and slender clavicles, and hypoplastic scapulae. There were 12 ribs on each side (Fig. 6A). The radius and ulna were slender. The ulna was slightly bowed. Hand radiographs taken at ages 14 and 18 years showed slender fourth and fifth metacarpals (Fig. 3A and B). The trapezium and scaphoid had angular appearance. The radiograph taken at age 14 years showed ivory epiphyses at the middle and distal phalanges of fingers 3 and 4, the distal phalanges of fingers 5, and the proximal phalanx of the right thumb. Cone-shaped epiphyses were observed at the proximal phalanges of fingers 2–5 (Fig. 3A). Ivory epiphyses and cone-shaped epiphyses were not apparent in the radiograph taken at age 18 years (Fig. 3B). Brachymesophalangy was observed. The distal phalanges of the second fingers appeared short (Fig. 3A and B). Foot radiograph showed abnormally long metatarsals and proximal phalanges of toes 2. Distal symphalangism of the fifth toes was noted (Fig. 4B). Pelvic radiograph showed tall and slender pelvic bone with shallow acetabula (Fig. 4D).

## Patient 2

Patient 2 was a 16-year-old girl. She was the younger sister of patient 1 (Figs. 1A and 2A and B). Her birth weight was 1,000 g. She started menstruation and pubic hair at ages 13 and 15 years, respectively. Menstruation was reported to be irregular. Like her brother, she had normal intellectual development. On examination, she was proportionately short. Her height, weight, and OFC were 96 cm (<3rd centile), 12 k (<3rd centile), and 41 cm (<3rd centile), respectively. Like that of her brother, the scalp hair, especially at the vertex, was dry and thin. The nose was large with prominent nasal bridge. The pinnae were small. Hearing was normal. One her left thigh and chin there were large areas of café-au-lait spots (Fig. 2B). The chest was narrow and the elbows bent. Fingers were proportionately short. There was limitation of abduction of the left leg. Toes were unremarkable. Like those of her brother, all teeth were small and opalescent. Mandibular premolars were severely small and malformed (Fig. 1B and C).

Radiographs showed narrow root canals of the incisors, short-rooted incisors, rootless molars, and an unerupted right mandibular premolar. The body of mandible was narrow. The crowns of the molars appeared tapered occluso-cervically (Fig. 1C). Lateral cephalograph showed

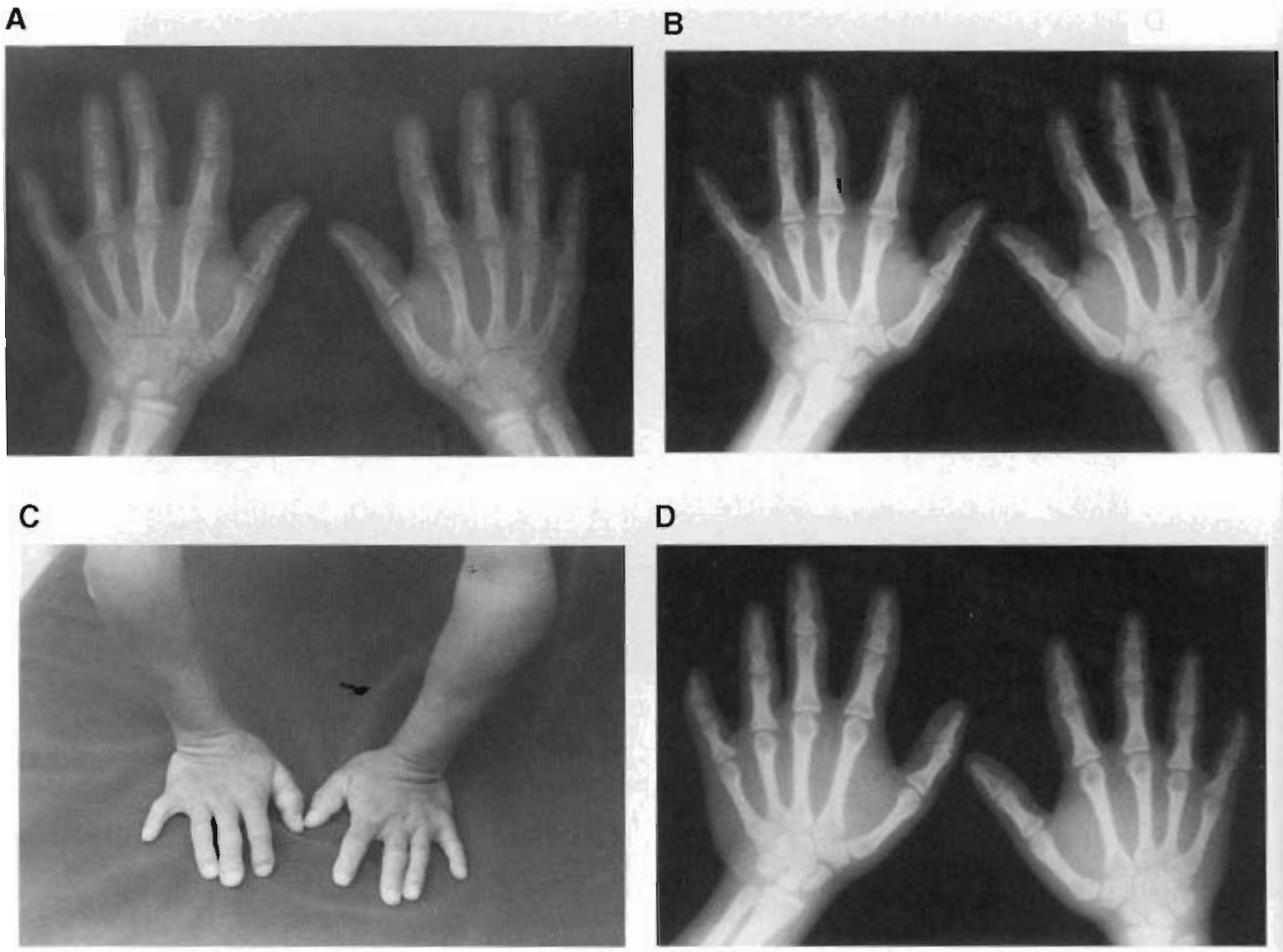



Fig. 3. **A:** Hand radiograph of patient 1 at age 14. Note ivory and cone-shaped epiphyses. Trapezium and scaphoid have angular appearance. **B:** Hand radiograph of patient 1 at age 18. Absence of ivory and cone-shaped epiphyses. Brachymesophalangy and short distal phalanx of the right second finger. Metacarpals 4 and 5 are slender. **C:** Patient 1. Brachydactyly and bent elbows. **D:** Patient 2. Hand radiograph at age 16. Brachymesophalangy is noted. Trapezium and scaphoid have angular appearance. Metacarpals 4 and 5 are slender.

large sella turcica, long posterior clinoid process, and progressive alveolar bone loss. Digital marking of the skull was observed (Fig. 5C and D). Chest radiograph showed long straight and slender clavicles, and hypoplastic scapulae. There were 12 ribs on each side (Fig. 6B). Radiograph of the elbows was unremarkable.

Hand radiographs were taken at ages 12 and 16 years. At age 12, brachymesophalangy of fingers 2–5 and ivory epiphyses at the proximal phalanges of thumbs were observed. Cone-shaped epiphyses were seen at the proximal phalanges of fingers 2 and 3 bilaterally. Like those of her brother, scaphoid and trapezium were angular in shape and metacarpals 4 and 5 appeared slender (Fig. 3D). Pelvic radiograph showed superiorly dislocated left proximal femur and slender femoral shafts (Fig. 4D). Foot radiograph showed short proximal phalanges of the great toes, long metatarsals of the second toes, and flexion contracture of toes 3–5. Brachymesophalangy of toes 2 and 3 was observed. Distal symphalangism of toes 4 and 5 was noted.

## DISCUSSION

The major manifestations found in the affected brother and sister were proportionate primordial short stature, microcephaly, severe microdontia, opalescent teeth, rootless molars, severely hypoplastic alveolar bone, distal symphalangism of toes, long second toes, and long, slender, and straight clavicles. Anomaly of tooth size has been described in patients with primordial short stature. Microdontia has been reported in Seckel syndrome [Cervenka et al., 1979] and MOPD [Majewski and Goecke, 1998]. Some patients with those syndromes have been reported to have large teeth [Seckel, 1960; Majewski and Goecke, 1982; Majewski, 1992]. The combination of microdontia, shortness of roots, delayed dental development, and hypoplastic alveolar processes has been described in MOPD [Lin et al., 1995]. The presence of opalescent teeth, severe microdontia, and malformation of mandibular premolars distinguish the current patients from having that particular syndrome.

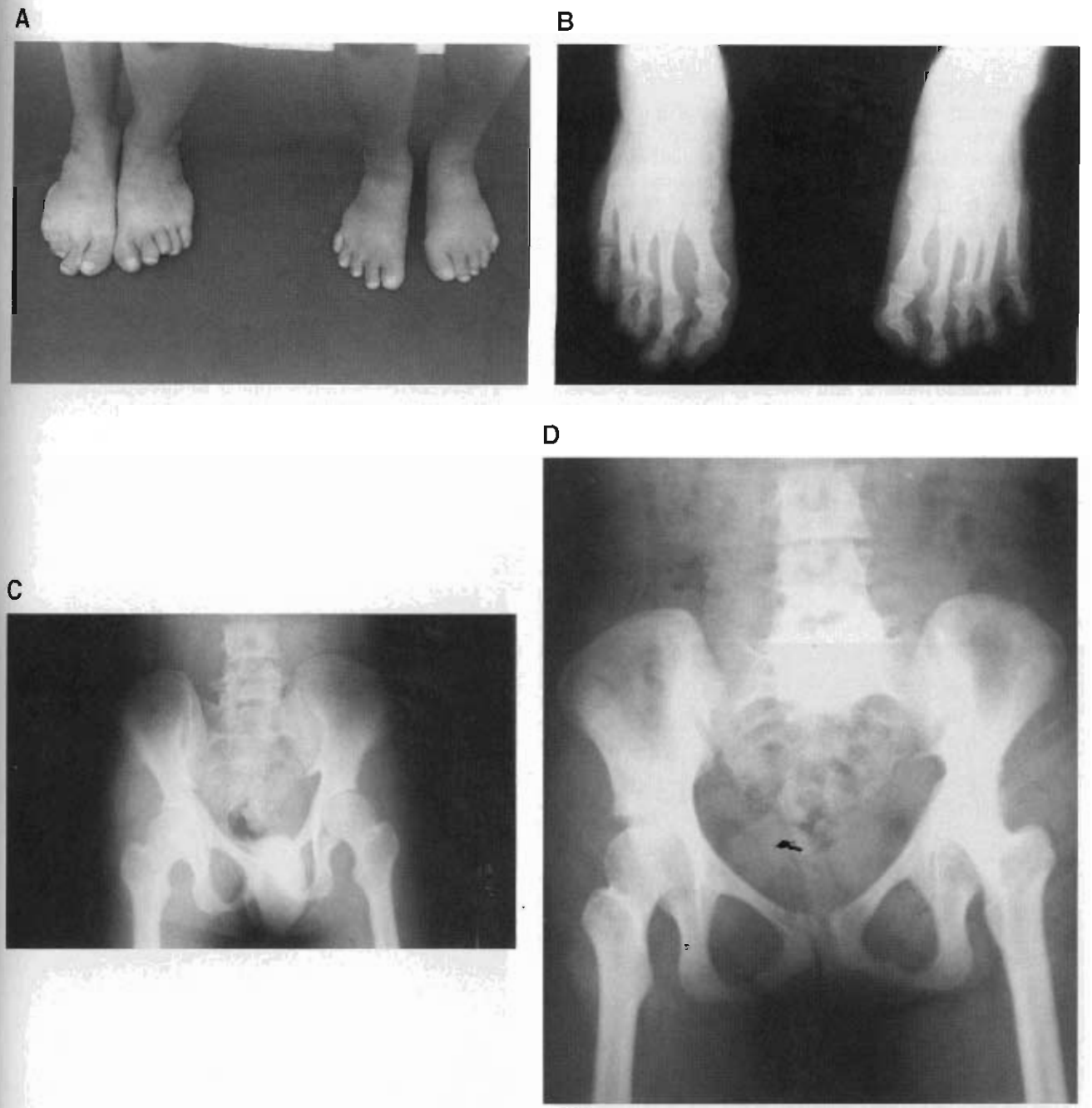



Fig. 4. **A:** From left to right, feet of patients 1 and 2. Feet of patient 1 shows long second toes and lateral deviation of toes 1 and 2. **B:** Patient 1. Very long metatarsals 2 and proximal phalanges 2 of patient 1. **C:** Patient 1. Slender pelvic bone, shallow acetabula, and femoral shafts. **D:** Patient 2. Tall and slender iliac bones and slender ischium and pubic bones, and shallow acetabula. Superiorly dislocated left femoral head.

Opalescent teeth refers to teeth with a blue-gray to amber-brown discoloration. The crowns of the teeth are unusually translucent. It can be seen as an isolated anomaly in hereditary opalescent dentin or dentinogenesis imperfecta type 2 (DI2) and dentin dysplasia type 2 (DTDP2) [Ranta et al., 1993]. Syndromic opalescent teeth have been reported in many syndromes [Kantaputra, 2001a], including osteogenesis imperfectas [O'Connell and Marini, 1999], Goldblatt syndrome [Goldblatt et al., 1991], Schimke-immunoosseous dysplasia [Fonseca, 2000], and a type of Ehlers-Danlos syndrome [Komorowska et al., 1989]. Opalescent and rootless incisors and molars have been described in a girl with disproportionate short stature, delayed growth and development, short neck, protruded abdomen, kyphosis, platyspondyly, hypoplastic acetabulum, broad nasal bridge, and hypodontia [Kantaputra, 2001b]. None of the reported patients either with proportionate or disproportionate primordial short stature had opalescent or rootless teeth. Rootless teeth have been reported

in dentin dysplasia type 1 (DTDP1). Tooth crowns of teeth with DTDP1 are not opalescent [Ranta et al., 1993]. This can distinguish teeth of the reported patient from having DTDP1.

Having ivory epiphyses and cone-shaped epiphyses in both patients was an interesting combination. Hand radiographs taken 4 years apart demonstrated that most of ivory epiphyses disappear as the patients aged. These age-related phenomena are similar to those of Seckel syndrome [Poznanski et al., 1983]. The reported patients share many features with an MOPD patient who was reported to have long and slender clavicles, ivory epiphyses, cone-shaped epiphyses, dislocated hip, with abnormal dental development [Shebib et al., 1991]. The panoramic radiograph of that particular patient and ours showed similar features consisting of microdontia, rootless molars, and severely hypoplastic alveolar process [Shebib et al., 1991]. Disproportionate short stature, synostosis of sagittal suture, short neck, short and bowed forearms, flared distal metaphyses of the femora, hypoplastic pelvis, and delayed intellectual development distinguish that patient from the current patients. Large sella turcica found in the current patients have been seen in MOPD, type Caroline Cachami [Majewski, 1992; Boscherini et al., 1996]. This leaves the question whether the basic defect of this new syndrome involves the pituitary gland. Digital marking of the skulls in both patients is frequently found in patients with craniosynostosis, which they did not appear to have [Howard et al., 1997]. Hypo- and hyperpigmentation of skin found in both patients might reflect mosaic variegated aneuploidy (MVA). However, since they did not follow Blanchko lines and the lack of hypoplasia of brain, Dandy-Walker malformations, seizures, hypotonia, and Wilms tumor in the current patients, the possibility of causing by MVA is not likely [Chitayat et al., 1990; Kajii et al., 1998; Kawame et al., 1999].

The reported patients appear to have a newly recognized syndrome. The features that make this syndrome distinct from the previously described syndromes include hypo- and hyperpigmentation of skin, opalescent teeth, rootless molars, severely hypoplastic alveolar processes, long second toes, and distal symphalangism of toes.

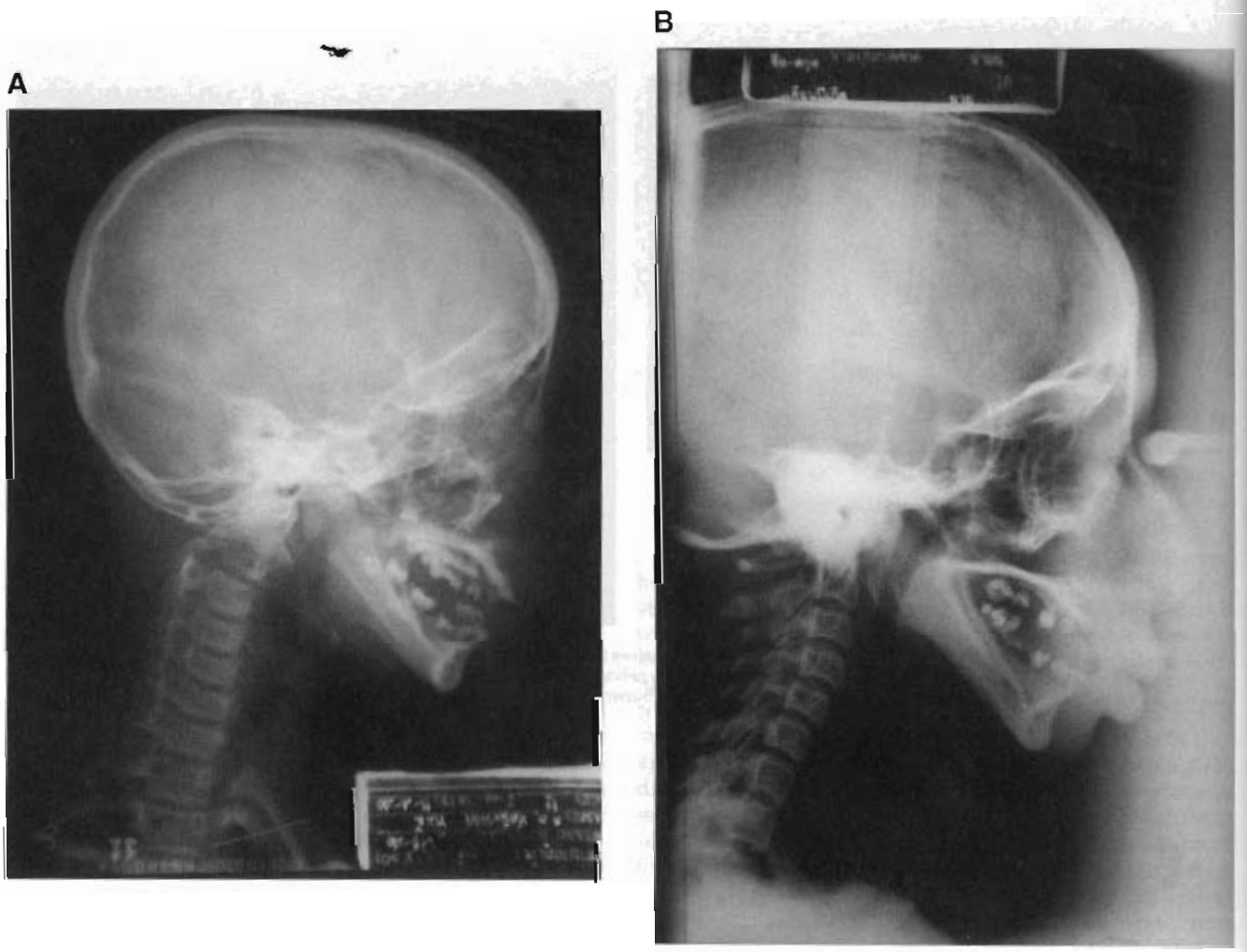
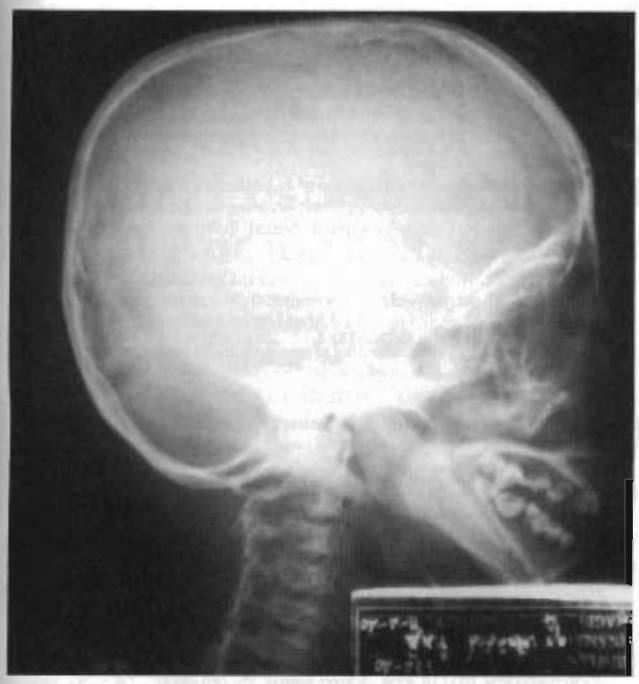
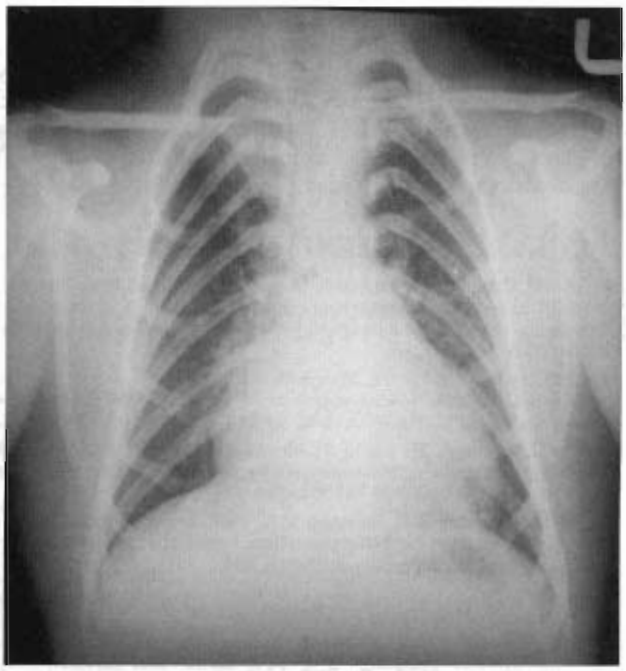
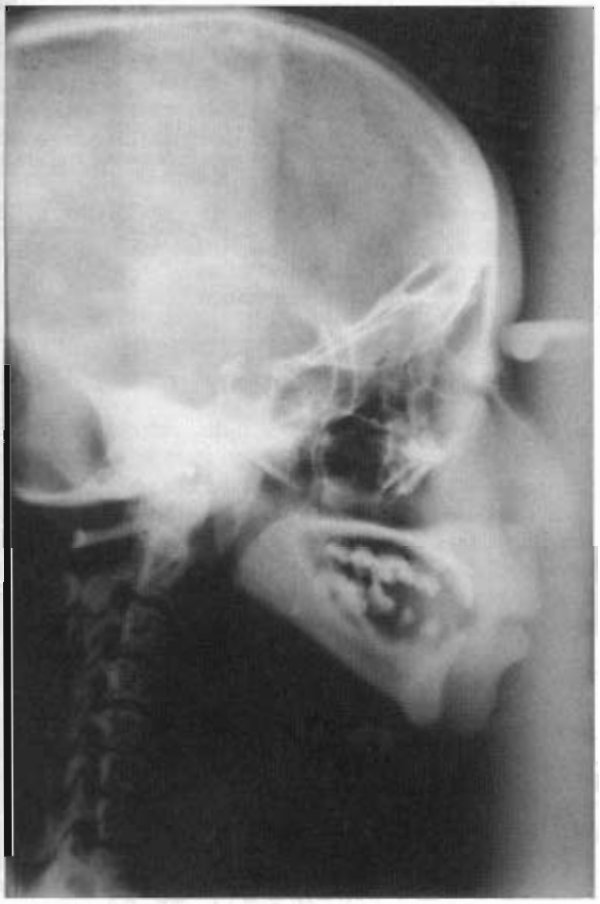



Fig. 5. **A** and **B:** Lateral radiographs of patient 1 at ages 14 and 18, respectively. **C** and **D:** Lateral radiographs of patient 2 at ages 12 and 16, respectively. Note short-rooted incisors, rootless molars, and large sella turcica with long posterior clinoid processes. Progressive alveolar bone loss is seen.







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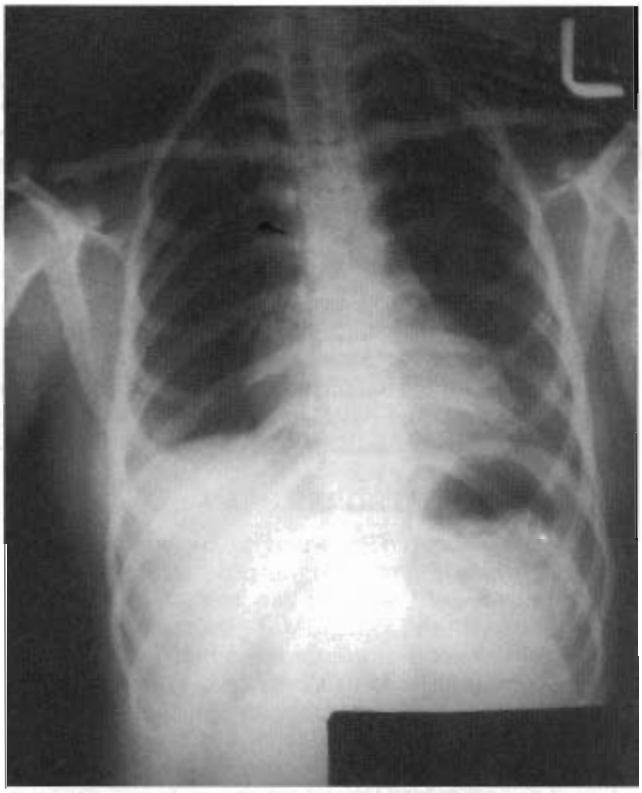

Fig. 6. A and B: Chest radiographs of patients 1 and 2, respectively. Note narrow chests, long, straight and slender clavicles, and hypoplastic scapulae.

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# A New Syndrome of Symphalangism, Multiple Frenula, Postaxial Polydactyly, Dysplastic Ears, Dental Anomalies, and Exclusion of *NOG* and *GDF5*

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**A Thai girl with a unique combination of limb and craniofacial anomalies is reported. Manifestations include blepharoptosis; prominent nose; hypodontia; multiple, hyperplastic frenula; and dysplastic ears. Limb anomalies include short stature, postaxial polydactyly of both hands and the left foot, proximal and distal symphalangism of fingers, and congenital absence of the distal phalanges of toes 2–5. Mutation analyses of *NOG* and *GDF5*, the genes responsible for symphalangism-related syndromes, were negative.** © 2003 Wiley-Liss, Inc.

**KEY WORDS:** absent distal phalanges; blepharoptosis; hypodontia; microdontia; one-rooted molar; short stature

## INTRODUCTION

Symphalangism, characterized by fusion of the phalanges, is a rare anomaly. Proximal and distal symphalangism are defined as fusion between the proximal and middle phalanges and between the middle and distal phalanges, respectively. Proximal symphalangism is more common than distal and both have been reported in association with many syndromes such as proximal symphalangism (SYM1; OMIM 185800), facio-audio-symphalangism syndrome, tarsal-carpal coalition syndrome (TCCS; OMIM 186570), and multiple synostosis syndrome (SYMS1; OMIM 186500). Heterozygous mutations of the *NOG* gene are known to cause these proximal symphalangism-associated syndromes [Gong et al., 1999; Dixon et al., 2001]. Multiple synostosis, type 2, (SYNS2) is caused by a missense mutation in growth/differentiation factor 5 (*GDF5*) or cartilage-derived morphogenetic protein-1 (*CDMP1*) [Akarsu et al., 1999].

Here, we describe a Thai patient with a unique combination of limb and craniofacial anomalies. Manifestations include blepharoptosis; prominent nose; hypodontia; multiple, hyperplastic frenula; and dysplastic ears. Limb anomalies include short stature, postaxial polydactyly of both hands and the left foot, and proximal and distal symphalangism of the fingers. *NOG* and *GDF5* are known to play crucial roles in normal joint development [Akarsu et al., 1999; Gong et al., 1999; Tsumaki et al., 2002]. Because our patient had symphalangism, we thought that the phenotype might be caused by a mutation in *NOG* or *GDF5*. However, molecular studies did not identify any mutations in those genes.

## CASE REPORT

The patient was a 12-year-old Thai girl, who was the first child of non-consanguineous, healthy parents (Fig. 1A). The patient was noted to have disproportionately short stature. Intelligence was normal. Her height, weight, and head circumference were 138 cm (3rd–10th centile), 36 kg (50th centile), and 51.5 cm (50th–75th centile), respectively. Hair and skin appeared normal. She had dolichocephaly, bilateral blepharoptosis, highly arched eyebrows, and sloping shoulders. Broad, prominent nasal bridge was observed. The philtrum was broad. The lower part of helices and lobules were hypoplastic (Fig. 1A). Hearing and echocardiography were unremarkable.

Oral examination showed multiple, hyperplastic frenula and highly arched palate (Fig. 1B). The primary

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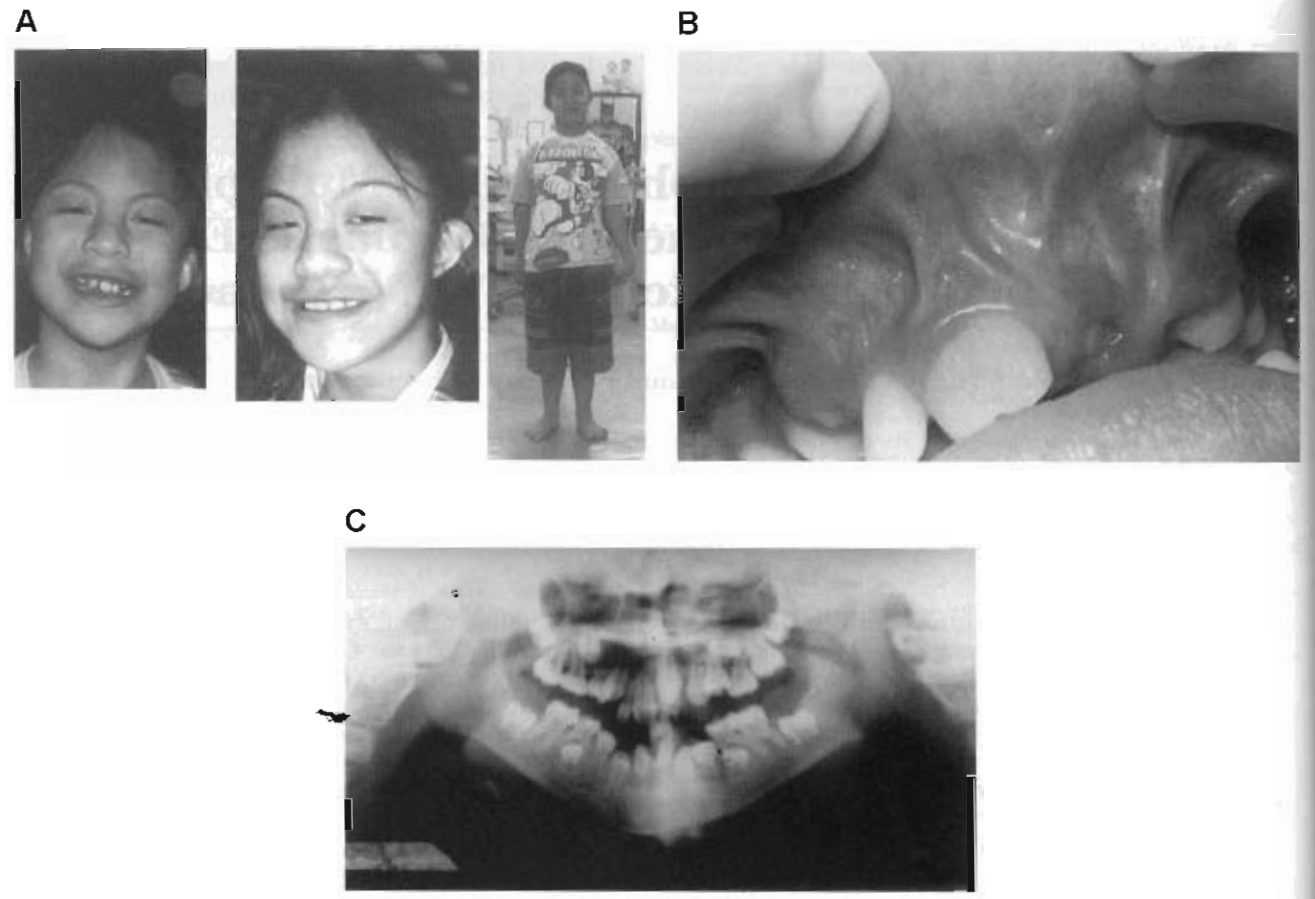


Fig. 1. A: Patient at the ages of 8, 11, and 12 years. B: Large maxillary permanent central incisor. Multiple, hyperplastic frenula. C: Panoramic radiograph at the age of 8 years showed hypodontia, short-rooted incisors, and premolars.

and permanent molars were small. The shape of the mandibular second premolar and first permanent molar resembled that of the mandibular second primary molar and mandibular second permanent molar, respectively. The maxillary first permanent molars and all second permanent molars had one root. Congenital absence of the maxillary permanent lateral incisors, and mandibular permanent central incisors were noted (Fig. 1C).

The hands were small with bilateral postaxial polydactyly. The polydactylous fingers could not be moved voluntarily. Brachydactyly and tapering of all fingers were noted (Fig. 2A). Flexion at the interphalangeal joint (IPJ) was possible only at the distal interphalangeal joint (DIPJ) of the third fingers, making it impossible for her to make a fist. No cutaneous creases were observed over the dorsum of the affected joints. Fingernails were unremarkable (Fig. 2A). Roentgenograms of the hands at the age of 12 years showed normal appearance of the thumbs, short fourth and fifth metacarpals, and fusion of proximal interphalangeal joint (PIPJ) and DIPJ of fingers 2, 4, and 5. The DIPJ of the third fingers appeared normal. All phalanges of the polydactylous fingers were fused with rudimentary metacarpals. Metacarpophalangeal joints (MPJ) of the second and third fingers were narrow bilaterally (Fig. 2B). Hand radiography at the age of 7 years showed brachymesophalangy of fingers 2–5. Cone-shaped epiphyses of the middle phalanges 2–5 were observed. The PIPJ of fingers 2–5 were narrow. The DIPJ of all fingers were wider than the proximal ones and narrower than those seen at the age of 12 years. Symphalangism was seen only at the PIPJ of the polydactylous fingers.

All toes were short. Postaxial polydactyly was found on the left foot; it could not be moved voluntarily. The first toes were broad with slightly dysplastic nails (Fig. 3A). Radiograph of the feet showed congenital absence of the distal phalanges of toes 2–5. The tufts of the distal phalanges of both great toes were not evident. The polydactylous toe appeared to have two phalanges but its metatarsal was absent (Fig. 2B). The metatarsal and tarsal bones appeared normal. A pelvic radiograph was unremarkable.

## MUTATIONAL ANALYSIS OF *NOG* AND *GDF5*

Genomic DNA was isolated from peripheral blood samples for mutation analysis of *NOG* and *GDF5*. *NOG* and *GDF5* contain only 1 and 9 coding exons, respectively. Amplification of the entire coding sequence of *NOG* was performed using primers NOG1/NOG5. PCR

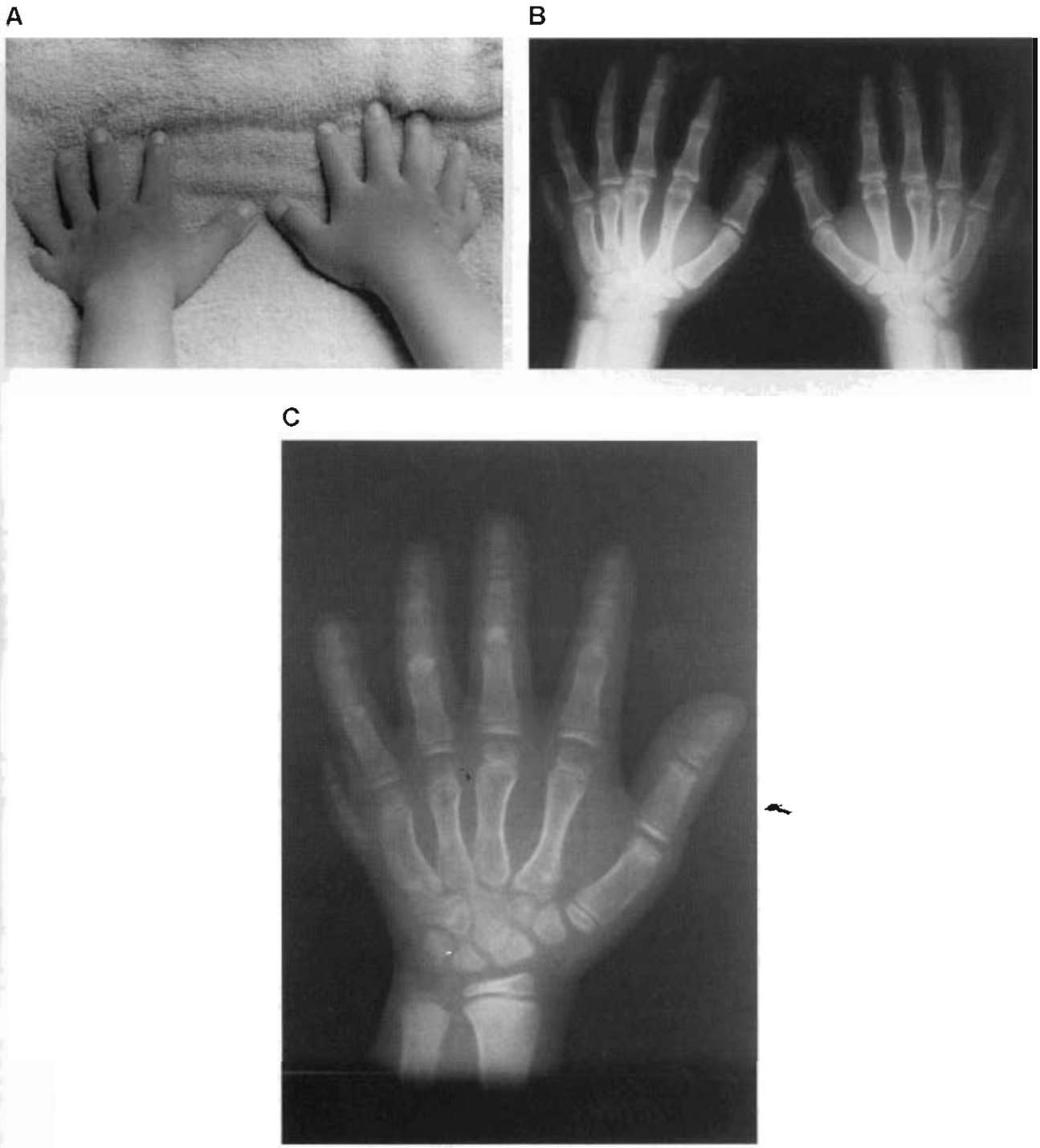


Fig. 2. A: Postaxial polydatyly of hands. B: PA radiograph of hands at the age of 12 years. Note proximal and distal symphalangism. C: PA radiograph at the age of 7 years. Short middle phalanges of all fingers. All IPJ spaces are visible except for that of the proximal IPJ of the polydactylous one.

conditions for NOG have been described elsewhere [Gong et al., 1999]. Direct sequencing did not identify a pathogenic mutation in *NOG* and *GDF5*.

## DISCUSSION

Symphalangism, a striking finding in this patient, is rare. Proximal symphalangism-associated syndromes are caused by heterozygous mutations in *NOG* [Gong et al., 1999]. Congenital stapes ankylosis syndrome with no symphalangism has recently been found to be caused by heterozygous nonsense and frameshift mutations in *NOG* [Brown et al., 2002]. Noggin, the protein product of *NOG*, is essential for human joint morphogenesis. It binds and inactivates members of the TGFβ superfamily, such as BMP4 [Brunet et al., 1998; Gong et al.,

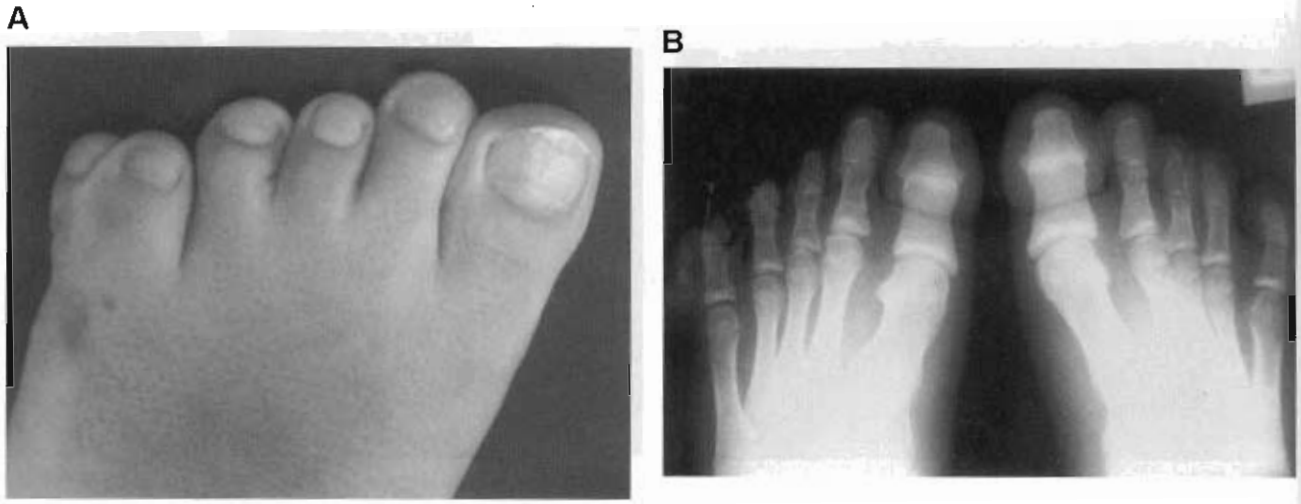


Fig. 3. A: Short toes. Postaxial polydactyly of the left foot. B: Short and broad distal phalanges of the great toes. Proximal IPJ spaces are narrow. Absence of the distal phalanges of toes 2–5. The polydactylous toe lacks metatarsal bone.

1999]. Patients with facio-audio-symphalangism syndrome and multiple synostosis syndrome, unlike those of proximal symphalangism syndrome, have facial manifestations, including a broad hemicylindrical nose, hypoplastic nasal alae, and a short upper lip [da-Silva et al., 1984; Hurvitz et al., 1985].

Symphalangism of both PIPJ and DIPJ in the same patient as reported here is very rare. Comparing the hand radiographs at the age of 7 and 12 years shows that symphalangism proceeds progressively in ulnar-radial [Strasburger et al., 1965; Polymeropoulos et al., 1995; Dixon et al., 2001] and proximo-distal directions [Krakow et al., 1998]. Proximal symphalangism, facio-audio-symphalangism syndrome, symphalangism with tarsal and carpal coalition, and multiple synostosis syndrome may have distal symphalangism as a rarely associated anomaly [Strasburger et al., 1965; Geelhoed et al., 1969; da-Silva et al., 1984; Drawbert et al., 1985; Hurvitz et al., 1985]. Similarly, distal symphalangism, as a distinct autosomal dominant disorder, may rarely have proximal symphalangism as an associated anomaly [Poush, 1991]. Although no mutations were found in *NOG* and *GDF5*, symphalangism in our patient could have resulted from overactivity of BMPs; increased BMP activity results in excess cartilage and failure in joint formation [Dixon et al., 2001; Tsumaki et al., 2002].

Morimoto et al. [2001] reported a patient sharing many similarities with our patient: proximal symphalangism of fingers 2–5, blepharoptosis, malformed ears, short stature, microdontia, high-arched eyebrows, and a prominent nose. However, distal symphalangism; multiple, hyperplastic frenula; hypodontia; malformed molars; polydactyly; absent distal phalanges of toes; absence of mental retardation, chronic renal failure, and hearing loss in our patient distinguish her from having the same syndrome reported by Morimoto et al. [2001]. Finger symphalangism of all IPJ, except for the third DIPJ found in our patient has been described in 9 members of a family [Moumoumi et al., 1991]. Distal symphalangism of the third finger appears to be the rarest form of the symphalangism [Letts et al., 1999]. Thus, we think the pathogenesis in our case may be different from others. Although some of the manifestations in our patient can be found in Ellis–van Creveld syndrome [da-Silva et al., 1980], Pallister–Hall syndrome [Biesecker et al., 1996], or oral-facial-digital syndromes [Toriello, 1993], the pattern of anomalies in our patient is at variance with these conditions.

## ACKNOWLEDGMENTS

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# Heterozygous Mutation in the SAM Domain of p63 Underlies Rapp-Hodgkin Ectodermal Dysplasia

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RESEARCH REPORTS
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# Heterozygous Mutation in the SAM Domain of p63 Underlies Rapp-Hodgkin Ectodermal Dysplasia

## ABSTRACT

Several ectodermal dysplasia syndromes, including Ectrodactyly-Ectodermal dysplasia-Clefting (EEC) and Ankyloblepharon-Ectodermal Dysplasia-Clefting (AEC) syndromes, are known to result from mutations in the *p63* gene. We investigated whether Rapp-Hodgkin syndrome (RHS) is also caused by mutations in the *p63* gene. We identified a heterozygous *de novo* germline missense mutation, S545P, in the sterile-alpha-motif (SAM) domain of p63, in a Thai patient affected with RHS. This is the first genetic abnormality to be described in RHS. The amino acid substitution is the most downstream missense mutation in *p63* reported thus far. Histological assessment of a skin biopsy from the patient's palm showed hyperkeratosis and keratinocyte cell-cell detachment in the upper layers of the epidermis, along with numerous apoptotic keratinocytes. Collectively, these investigations demonstrate that RHS is also caused by mutations in *p63* and that the clinical similarities to AEC syndrome are paralleled by the nature of the inherent mutation.

**KEY WORDS:** AEC syndrome, dental anomalies, palmoplantar keratoderma, *p63* gene, Rapp-Hodgkin syndrome.

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## INTRODUCTION

Rapp-Hodgkin syndrome (RHS), or Rapp-Hodgkin ectodermal dysplasia, was first described over 30 years ago in an affected mother, son, and daughter with a combination of anhidrotic ectodermal dysplasia, cleft lip, and cleft palate (Rapp and Hodgkin, 1968). The clinical syndrome is comprised of a characteristic facies (narrow nose and small mouth), wiry, slow-growing, and uncombable hair, sparse eyelashes and eyebrows, obstructed lacrimal puncta/epiphora, bilateral stenosis of external auditory canals, microsomia, hypodontia, cone-shaped incisors, enamel hypoplasia, dystrophic nails, and cleft lip/cleft palate. Approximately 45 cases of this developmental disorder, usually with autosomal-dominant inheritance, have been reported (Summitt and Hiatt, 1971; Wannarachue *et al.*, 1972; Stasiowska *et al.*, 1981; Silengo *et al.*, 1982; Salinas and Montes, 1988; Rodini *et al.*, 1990; Santos *et al.*, 1990; Breslau-Siderius *et al.*, 1991; Walpole and Goldblatt, 1991; Kantaputra *et al.*, 1998; Neilson *et al.*, 2002), thus defining RHS as a discrete clinical entity (Online Mendelian Inheritance in Man [OMIM] no. 129400).

Nevertheless, RHS does display some clinical overlap with other ectodermal dysplasia syndromes, notably Ectrodactyly-Ectodermal Dysplasia-Clefting (EEC) syndrome (OMIM 129900) and Ankyloblepharon-Ectodermal Dysplasia-Clefting (AEC), also known as Hay-Wells syndrome (Hay-Wells Syndrome, OMIM 106260) (van Bokhoven and McKeon, 2002). Differentiating features of AEC and RHS appear to be the presence of ankyloblepharon in AEC and microsomia in RHS, but making an accurate diagnosis on clinical grounds alone may be difficult. Indeed, this was highlighted in a case of a woman with RHS whose son had features of EEC syndrome (Moerman and Fryns, 1996). The affected child also had ankyloblepharon, a component of AEC syndrome. Other overlap syndromes have also been reported (Cambiaghi *et al.*, 1994; Rowan, 1996), suggesting that perhaps there might be a common genetic pathology to many of these and other related ectodermal dysplasia syndromes. It is noteworthy that some parents with AEC syndrome had affected children without ankyloblepharon. Furthermore, the ankyloblepharon of some affected individuals is so subtle or friable at birth that it often goes unrecognized, and these cases may subsequently be diagnosed as RHS.

The molecular basis of most cases of EEC syndrome has recently been shown to involve mutations in the *p63* gene (Celli *et al.*, 1999). The majority of mutations is comprised of heterozygous missense changes in the DNA-binding domain of p63 (van Bokhoven *et al.*, 2001). Moreover, AEC syndrome has subsequently been reported to result from missense mutations in *p63*, specifically within the sterile alpha motif (SAM) (McGrath *et al.*, 2001). Thus, both these disorders are caused by heterozygous germline mutations in p63, and a genotype-phenotype correlation for the site of the mutation (DNA-binding domain or SAM domain) has been defined. To date, no pathogenetic mutations in *p63* have been reported in patients with RHS. Therefore, in this study we investigated the molecular basis of RHS to further examine the possibility of allelic heterogeneity.

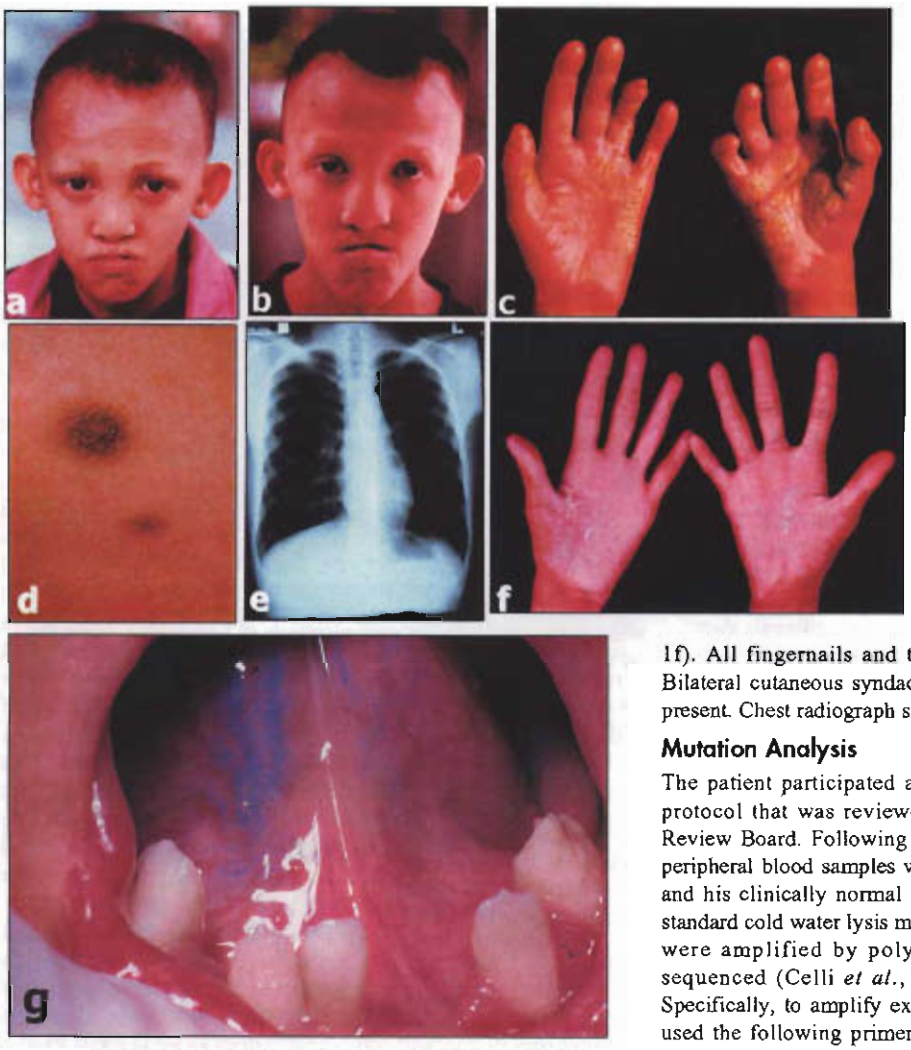


Figure 1. Clinical pictures of the patient. (a) Patient at 8 yrs old. Note repaired cleft lip and palate, microsomia, sparse hair, and diffuse dermatitis of the skin. (b) Patient at 14 yrs old. (c) Keratoderma of palms at 8 yrs. (d) Supernumerary nipple. (e) Hypoplastic scapulae. (f) Keratoderma of palms at 14 yrs. Note improvement of the condition as he aged. (g) Congenitally missing mandibular left central incisor. Enamel hypoplasia of the mandibular right lateral incisor and left canine.

## MATERIALS & METHODS

### Clinical Details

A 14-year-old Thai boy was seen in The Department of Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University. He had been diagnosed and reported previously as having Rapp-Hodgkin syndrome (Kantaputra *et al.*, 1998) (Figs. 1a, 1b). His clinical findings included characteristic facies, slow-growing and uncombable hair, sparse eyebrows and eyelashes, obstructed lacrimal puncta and epiphora, microsomia, repaired cleft lip and cleft palate (Figs. 1a, 1b), taurodontism, multiple caries, unerupted premolar, glossy tongue, hypodontia, enamel hypoplasia, and congenital absence of the lingual frenum and sublingual caruncles, including submandibular and sublingual salivary gland duct openings (Fig. 1g) (Kantaputra *et al.*, 1998). However, the abnormal scalp hair lacked pili canaliculi, a feature noted in some other cases of RHS (Salinas and Montes, 1988). The ears protruded, with evidence of bilateral bony external auditory canal stenoses, and he had a moderate mixed pattern of hearing loss. Hypoplastic and supernumerary nipples were observed (Fig. 1d). The skin was very dry, with some hypopigmented areas. Diffuse dermatitis of the scalp, face, and forearms was present (Figs. 1a, 1b). Palms and soles demonstrated keratoderma with multiple fissures, although the degree of keratoderma had improved when he became a teenager (Figs. 1c, 1f). All fingernails and toenails were dystrophic and ridged. Bilateral cutaneous syndactyly of the second and third toes was present. Chest radiograph showed hypoplastic scapulae (Fig. 1e).

### Mutation Analysis

The patient participated after providing informed consent to a protocol that was reviewed and approved by the Institutional Review Board. Following informed consent given by his father, peripheral blood samples were taken from the affected individual and his clinically normal parents, and DNA was extracted by a standard cold water lysis method. Individual exons of the *p63* gene were amplified by polymerase chain-reaction (PCR) and sequenced (Celli *et al.*, 1999; van Bokhoven *et al.*, 2001). Specifically, to amplify exon 13 and flanking introns of *p63*, we used the following primers: forward primer 5′-CTT ATC TCG CCA ATG CAG TTG G-3′, and reverse primer 5′-AAC TAC AAG GCG GTT GTC ATC AG-3′. The expected PCR product size was 241 base pairs (bp). For PCR amplification, 250 ng of genomic DNA was used as the template in an amplification buffer containing 6.25 pmol of the primers, 37.5 nmol $MgCl_2$, 5 mmol of each nucleotide triphosphate, and 1.25 U *Taq* polymerase (Applied Biosystems, Warrington, UK) in a total volume of 25 μL in an OmniGene thermal cycler (Hybaid, Basingstoke, UK). The amplification conditions were 94°C for 5 min, followed by 38 cycles of 94°C for 45 sec, 53°C for 45 sec, and 72°C for 45 sec. Aliquots (5 μL) of the PCR products were analyzed by 2% agarose gel electrophoresis. PCR products were then sequenced directly with the use of Big Dye labeling in an ABI 310 genetic analyzer (Applied Biosystems). Potential mutation was verified by restriction endonuclease digestion or by direct sequencing (forward and reverse) and assessed in 300 control chromosomes to exclude non-pathogenetic sequence variants.

### Skin Biopsy

Following informed consent, a 4-mm punch biopsy was taken from the patient's palm while he was under general anesthetic (given for dental surgery). Skin was fixed in 10% formalin and processed for routine light microscopy with paraffin-embedding. Five-micron sections were stained with hematoxylin and eosin and photographed.

## RESULTS

### Mutation Analysis

Sequencing of the PCR products spanning exon 13 with the use of DNA from the affected individual revealed a heterozygous T>C point mutation at nucleotide position 1633 within exon 13 (numbering based on the originally published TA-p63α sequence [Yang *et al.*, 1998], GenBank accession no. AF075430). The mutation converted a serine residue (TCC) to proline (CCC) and was designated S545P (Fig. 3). This mutation has not been reported previously in patients with EEC, AEC, or other *p63*-related disorders and was not detected in direct sequencing of DNA from this patient's father or from 150 control subjects. No other heterozygous or homozygous sequence variants for the remainder of the *p63* gene were found in the patient's DNA.

### Histological Findings

Light-microscopic examination of palmar skin showed acanthosis and hyperkeratosis with a mild upper dermal interstitial chronic inflammatory cell infiltration and some exocytosis of lymphocytes throughout the upper epidermis. Most notably, in the upper spinous layers of the epidermis there was evidence of abnormal keratinocyte differentiation with numerous apoptotic keratinocytes, acantholysis, and disruption of the granular layer (Fig. 2).

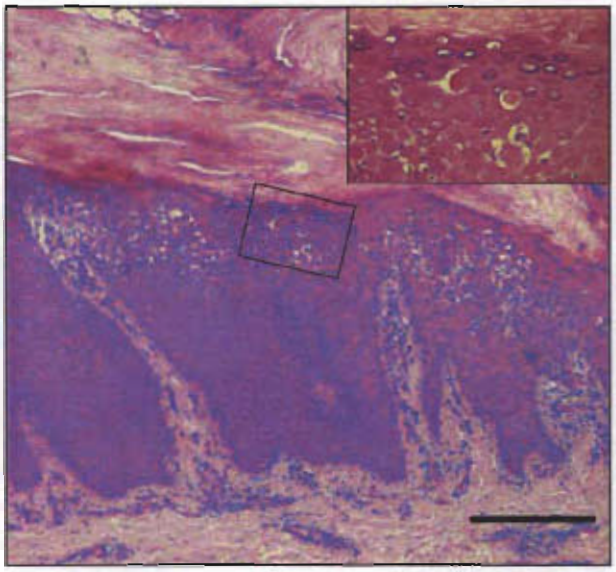

**Figure 2.** Histology of palmar skin shows acanthosis and hyperkeratosis. There is a mild upper dermal interstitial chronic inflammatory cell infiltrate and exocytosis of lymphocytes within the superficial dermis. Most notably, in the upper spinous cell layers there are numerous apoptotic keratinocytes (seen at higher magnification in the inset) with keratinocyte separation (acantholysis) and disruption of the granular cell layer. Bar = 100 microns.

## DISCUSSION

In this study, we have elucidated the molecular basis of RHS in one affected individual. Our findings provide evidence of further allelic heterogeneity for mutations in *p63* and the diverse clinical phenotypes therein. Indeed, we can now provide molecular data to support the earlier reports of clinical overlap between EEC syndrome, AEC syndrome, and RHS (Cambiaghi *et al.*, 1994; Moerman and Fryns, 1996; Rowan, 1996). RHS is clinically most similar to AEC syndrome, and this is borne out at a molecular level. In AEC syndrome, 8 heterozygous germline missense mutations in the SAM domain of p63 have been reported, all of which are clustered within the first 3 of 5 helical domains (McGrath *et al.*, 2001). In contrast, the missense mutation in our case of RHS occurs in the fourth helix of the SAM domain, albeit just 4 amino acids downstream from the most 3′ of the AEC mutations. This raises the fundamental question of whether this subtle difference is sufficient to justify labeling AEC syndrome and RHS as separate entities. To begin to answer this, it is clear that further patients with a clinical diagnosis of RHS will need to be screened for *p63* mutations. Interestingly, recent mutation analysis in one other RHS family (with mixed cleft type) did not reveal any *p63* mutations (Neilson *et al.*, 2002). Locus heterogeneity of RHS therefore cannot be ruled out, further adding to the complexity of attempts to unravel the molecular basis of this ectodermal dysplasia syndrome. From a clinical perspective, we believe that our patient has RHS rather than AEC syndrome because of the presence of the characteristic facies, microsomia, obstructed lacrimal puncta/epiphora, and palmoplantar keratoderma and the absence of ankyloblepharon. At a molecular level, it is necessary to be more circumspect, since little is currently known about the function of the SAM domain other than its potential as a site for protein-protein interactions (Thanos and Bowie, 1999). Characterization of the roles of individual amino acids in these interactions will be necessary to define more specific functions. However, attempts to define genotype-phenotype correlation for *p63*-mutations in ectodermal dysplasia syndromes are further complicated by reported diverse phenotypes arising from identical amino acid substitutions. For example, mutations in arginine 280 may give rise to EEC syndrome, split hand-split foot malformation, or, rarely, no detectable abnormalities (van Bokhoven *et al.*, 2001). Likewise, mutations in arginine 304 have been shown to underlie a spectrum of EEC syndrome phenotypes (Hamada *et al.*, 2002). Clearly, there are other influences on the phenotypic consequences of particular mutations in the TP63 transcription factor, and therefore a simple genotype-phenotype correlation based on a specific amino acid substitution is fundamentally flawed. Until the nature of these additional modifying factors or the full spectrum of inherent gene pathology becomes apparent, we believe that it is still more appropriate to retain the descriptive eponyms such as RHS, which is the most appropriate diagnosis that encompasses the clinical features present in our patient.

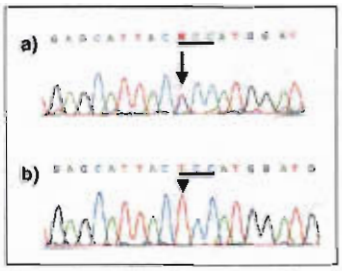


**Figure 3.** Nucleotide sequencing of *p63* exon 13 from normal (b) and the affected patient (a) shows a heterozygous T>C point mutation that converts a serine residue (TCC) to proline (CCC), designated as S545P.

In addition to disclosing the initial *p63* gene mutation in RHS, our study reports the first histological assessment of the erosive palm- and sole-skin thickening that is a characteristic feature of RHS. TP63 is known to have an important role in the regulation of epidermal stem cell proliferation (Schultz *et al.*, 1997; Mills *et al.*, 1999; Parsa *et al.*, 1999; Yang *et al.*, 1999; de Laurenzi *et al.*, 2000), and its expression in keratinocytes has been used to distinguish stem cells from transit amplifying cells and other keratinocytes (Pellegrini *et al.*, 2001). The missense mutation S545P in the p63 SAM domain clearly perturbs normal epidermal differentiation and maturation, leading to acanthosis (thickening) and hyperkeratosis (increased scale) as well as focal impairment of keratinocyte cell-cell attachment and, most notably, numerous apoptotic keratinocytes (colloid bodies). TP63 is known to influence keratinocyte apoptosis (Yang *et al.*, 1998) in response to certain stimuli such as ultraviolet irradiation (Liefer *et al.*, 2000) and in response to knockout mutations (Flores *et al.*, 2002), but inhibition of apoptosis appears to be abrogated in the presence of the heterozygous missense mutation S545P. Analysis of these clinico-pathological data may therefore provide insight into one of the functions of the p63 SAM domain in normal epithelial physiology. Specifically, our case demonstrates the first example of increased epidermal apoptosis in association with a human germline mutation in *p63*. This is clearly distinct from AEC syndrome, where no increase of apoptosis is observed (McGrath *et al.*, 2001). Palmoplantar keratoderma and mixed hearing loss, found in our patient, have not been reported in other patients affected with RHS (van Bokhoven and Brunner, 2002). The novel finding of hypoplastic scapula might have been related to *p63* mutation, or it might have been a coincidence.

The *p63* mutation detected may also be relevant to our understanding of the dental pathology present in some ectodermal dysplasia syndromes. The present patient has unerupted premolar and taurodontism, *i.e.*, large dental pulp and short roots, and although there are no previous reports of *p63* expression in dental pulps, the findings in our patient give credence to the significance of *p63* expression during dental development, since large pulp chambers are known to occur as a consequence of defective dentin formation in the dental pulp, a key role for normally functioning odontoblasts. This process is evidently disrupted in the RHS patient described here. The SAM domain mutation S545P, and its effects on oral mucosal development, may also be relevant to the pathogenesis of the patient's glossy tongue and absence of lingual frenum and of sublingual caruncles, since these are the rare clinical features in other *p63*-related syndromes.

In summary, this case discloses the molecular basis of RHS and provides new clinico-pathological insight into the consequences of a specific p63 SAM domain mutation on epithelial development.

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*Clinical Report*

# Thyroid Dysfunction in a Patient With Aglossia

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**We report a Thai girl who had aglossia, micrognathia, microsomia, collapse of mandibular arch, persistence of buccopharyngeal membrane, microcephaly, and mild developmental delay. Thyroid function tests indicated that she had subclinical hypothyroidism. Thyroid scan revealed normal uptake of the whole thyroid gland. Tongue morphogenesis is integrally linked to the normal development of thyroid gland, and abnormal tongue morphogenesis could potentially result in a functional thyroid disorder. We propose that micrognathia, microsomia, congenital absence of mandibular incisors, and collapse of the mandibular arch are the result of abnormal tongue development.** 

**KEY WORDS: high caries rate; hypodontia; hypothyroidism; micrognathia; persistence of buccopharyngeal membrane**

## INTRODUCTION

Thyroid gland develops from the foramen cecum on the back of the tongue. A relationship between thyroid dysfunction and abnormal tongue development would be reasonable but has never been reported. We report a Thai girl who had aglossia and subclinical hypothyroidism, which supports our hypothesis. In addition, we also propose that micrognathia, microsomia, congenital absence of mandibular incisors, and collapse of the mandibular arch are the result of abnormal tongue development.

## CLINICAL REPORT

A 13-year-old Thai girl came to the Department of Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University for dental care (Fig. 1b). She has had dental care regularly since she was 3 years old (Fig. 1a). Family history was unremarkable and the parents were not consanguineous. Birth weight, birth length, and head circumference (OFC) at birth were 3,430 g (>90th centile), 44 cm (3rd centile), and 37 cm (97th centile), respectively. Karyotype was 46,XX. At age 13 years, her weight, height, and OFC were 29 k. (10th–25th centile), 138 cm (<3rd centile), and 49 cm (<3rd centile), respectively. After birth, tracheostomy was initiated due to respiratory distress as the result of the presence of a buccopharyngeal membrane and oropharyngeal stenosis. At age 10 months, oropharyngeal reconstruction, using a median mandibulotomy approach, was initiated, and an anterior cricoid split with costal graft was performed in order to obtain a patent airway. It is noteworthy that during the surgical procedure, the tongue was not observed.

Oral examination at age 3 years revealed a persistent buccopharyngeal membrane, microsomia, aglossia, congenital absence of mandibular incisors, collapse of the mandibular arch, hypertrophy of the floor of mouth, and multiple carious teeth (Fig. 1c). Between the ages of 3 and 13 years, she kept losing her primary and permanent teeth as a result of rampant dental caries. Pit and fissure sealants were placed on the occlusal surfaces of all premolars and permanent molars in order to prevent occlusal caries. However, the uncontrollable dental caries severely and rapidly invaded all teeth from the mesial, distal, lingual, and buccal surfaces (Fig. 1d).

Her social development appeared delayed, possibly secondary to the physical handicap. She was able to speak, but not very well. She spoke only to her mother and close relatives and was too shy to speak with other people.

### Thyroid Function Tests

At age 8 years, thyroid function tests revealed slightly elevated thyrotropin-stimulating hormone (TSH) (6.61 μU/ml) (normal range = 0.5–4.0 μU/ml) and normal Free T4 (FT4) levels (1.06 ng/dl) (normal range = 0.6–1.5 ng/dl) without any clinical symptoms

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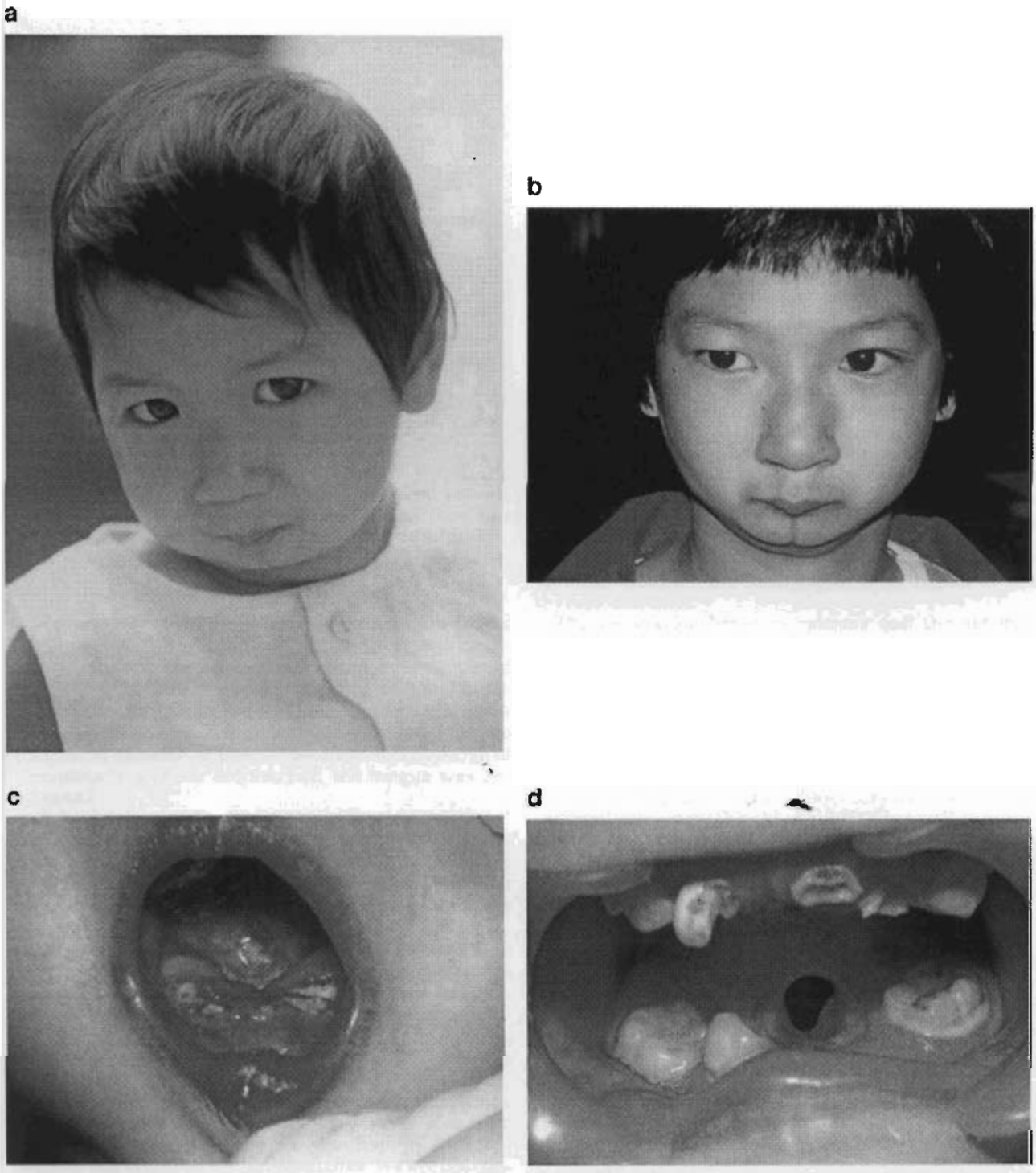


Fig. 1. a: Patient at age 3 years. b: Patient at age 13 years. c: Oral cavity at age 5 years. Note collapsed mandibular arch. Retained roots of primary teeth. d: Oral cavity at age 8 years. Note buccopharyngeal membrane with a very small orifice. Rampant dental caries.

of hypothyroidism. After treatment with levo-thyroxine (thyroid extract), the TSH level decreased to 4.7 μU/ml. Three months after levo-thyroxine was discontinued, TSH level elevated to 5.5 μU/ml. The TSH decreased to normal (3.4 μU/ml) once the thyroid supplement was reinstituted. During the treatment with levo-thyroxine the FT4 level stayed normal (1.5 ng/dl). All these lines of evidence indicated that she had subclinical hypothyroidism, which is characterized by elevated TSH level with normal serum FT4 and T3 [Cooper, 2001]. At age

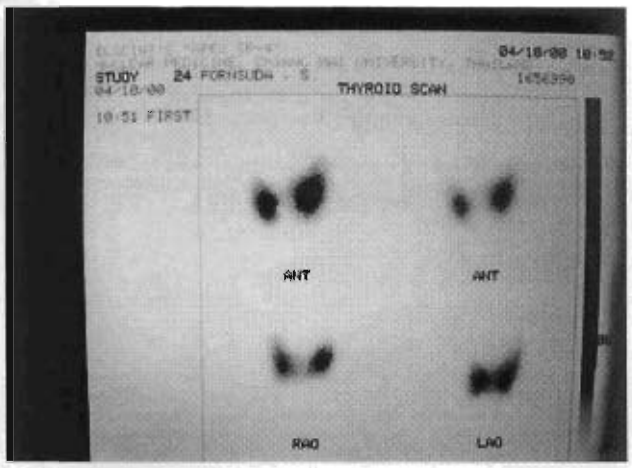


Fig. 2. Thyroid scan at age 10 years showing normal Technetium (Tc) uptake.

10 years, thyroglobulin level was normal (15 ng/ml) and the thyroid Technetium (Tc99) scan revealed normal uptake of the whole thyroid gland. The left lobe was slightly larger than the right (Fig. 2).

## DISCUSSION

We have reported a girl who had aglossia, subclinical hypothyroidism, persistence of buccopharyngeal membrane, subglottic stenosis, micrognathia, hypertrophy of the floor of mouth, congenital absence of mandibular permanent incisors, collapse of mandibular arch, and a very high caries rate. The patient was considered to have congenital absence of tongue because during surgical reconstruction of her oropharynx, the tongue was not observed.

Hypoglossia is rare. Most patients with "aglossia" actually have a small nubbin of tongue tissue on close examination [Hall, 1971; Gorlin et al., 2001]. We were not able to confirm the original diagnosis of aglossia because the tiny and opening did not permit the necessary visualization.

Many patients with aglossia or hypoglossia have been reported to have micrognathia, microsomia, congenital absence of mandibular incisors, and collapse of mandibular arch. Most of the patients have normal intelligence [Rosenthal, 1932; Kelln et al., 1968; Hall, 1971; Roth et al., 1972; Johnson and Robinow, 1978; Kuroda and Ohyama, 1981; Weckx, 1990].

The association between hypoglossia and persistent buccopharyngeal membrane is very rare. Flannery [1989] reported such a patient, who had, in addition, costovertebral and auricular anomalies. We are not convinced that a persistent buccopharyngeal membrane, even though found in our patient, is part of the abnormal tongue development since most reported patients with tongue anomalies have not had persistent buccopharyngeal membrane.

The thyroid gland develops from an unpaired primordium, which appears in the ventral midline of the pharynx between the first and second pouches. The thyroid origin later becomes the foramen cecum on the back of the tongue. During the fourth week, proliferation and later an invagination of the endothelium of the foramen cecum develops into the thyroid primordium. It commences at the foramen cecum as a blind-ended sac of thyroglossal duct, which becomes a hollow tube and subsequently enlarges caudally as a bilobed thyroid diverticulum. It then migrates caudally to its final destination by the seventh week at the level between the second and third tracheal cartilages. The thyroglossal duct has been reported to be filled with keratin, debris, and fluid emitted from accessory serous glands adjacent to the tract. The duct later constricts, becomes obliterated [Baughman, 1972], and breaks down by the end of the fifth week [Larsen, 1993], possibly by the programmed cell death. In almost 50% of the population, the distal portion of the thyroglossal duct persists and becomes the pyramidal lobe of the thyroid gland [Carlson, 1999]. Sauk [1971] found that 10% of 200 routine necropsies showed histologic remnants of thyroid tissue within the base of the tongue. In the cervical region, there are many "physiologic" migrations of the thyroid primordium in different directions. The pattern of thyroid migration is somewhat similar to that of the thymus and possibly the parathyroid glands [Carlson, 1999]. On occasion, a thyroid cyst, fistula, adenoma, or adenocarcinoma have been reported in region of the thyroglossal duct.

The association between aglossia and thyroid dysfunction has not been previously reported. Since the thyroid primordium is induced very early during embryonic development before any trace of the tongue appears, the two structures are probably under different modes of control. It is also possible that the tongue forms but disappears for some reason after the thyroid primordium develops.

A high caries rate with a rudimentary tongue has been reported previously [Khalil et al., 1995] as well as in our own patient. Causes of high caries could have been the lack of self-cleansing by the tongue or the composition of the saliva that might predispose to dental caries.

## ACKNOWLEDGMENTS

We thank the patient and her family for allowing us to see her and to use her medical and dental information for publication. Advice from Chanin Limwongse and Bruce M. Carlson is appreciated. This paper is dedicated to Prof. Robert J. Gorlin, my evergreen teacher.

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*Invited Comment*

# Thirteen-Year-Follow up Report on Mesomelic Dysplasia, Kantaputra Type (MDK), and Comments on the Paper of the Second Reported Family of MDK by Shears et al.

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## INTRODUCTION

In 1992, Professor Robert Gorlin, Professor Leonard Langer, and myself reported a new type of mesomelic dysplasia, which was later named as mesomelic dysplasia, Kantaputra type (MDK; OMIM *156232) [Kantaputra et al., 1992]. The condition was documented in a three-generation Thai family (Fig. 1). MDK is an autosomal dominant disorder, characterized by bilateral severe shortening of the ulnae and shortening and bowing of the radii (Figs. 2 and 3). The fibula is hypoplastic in most patients. Synostosis is observed between tibia and fibula, and the malformed calcaneus and talus. Fibulo–calcaneal complex, the prominent calcaneus fusing with the ventral surface of the distal fibula is a constant feature. It was found in all affected individuals (Figs. 4 and 7). Carpal and tarsal synostoses are present in some affected people, however, the pattern did not appear to be consistent. The affected patients stood on the tip of their toes [Kantaputra et al., 1992]. With great collaboration with Professor Niikawa's group at Nagasaki School of Medicine, the gene of MDK is successfully mapped to chromosome 2q24 and q32 [Fujimoto et al., 1998].

### Newly Born Member: What Does she Tell us About the Syndrome?

On examination at age 4 years, the newly born affected member (Patient V1) is an intelligent and cheerful girl. Her upper and lower limb abnormalities are similar to those of her mother and relatives (Figs. 5a–b and 7a–b). Flexion contracture involve more number of interphalangeal joint (IPJ) than that of any members of the family. It is found at the proximal interphalangeal joints (PIPJ) of the left fingers 2, 4, and 5 and the right finger 4. That of the distal interphalangeal joints (DIPJ) is found at the left thumb, left finger 3, and right fingers 2 and 3.

Radiographic findings consist of disproportionate shortening of the radii and ulnae. The ulnae were severely shortened. No distal epiphyses are evident. The distal humeri are large. Trapezium, trapezoid, scaphoid, lunate, and triquetrum are missing. Narrow joint spaces of the PIPJ of the left fingers 4 and 5 and the right finger 4 are observed (Fig. 5a,b).

Lower limb radiographs show shortening of tibiae and fibulae, large distal fibulae, and rectangular fibular prominence on the ventral surface of the left fibula (Fig. 7b). The right fibula does not appear to have fibular prominence (Fig. 7a). The calcaneus is malformed and in upright position, articulating with fibula. No synostosis is observed (Fig. 7a,b).

## THIRTEEN YEAR-FOLLOW UP OF PATIENT IV2 AND HIS RELATIVES

### Upper Limbs

The previous report was done when he was 8 years old. Thirteen-year-follow up examination of patient IV2 at age 21 years demonstrates progressively bowed radii and narrower spaces between carpal bones leading to their overlapping. Narrow PIPJs of fingers 4 were observed at age 8 years. At 21 years old, flexion contracture of the PIPJ are noted at fingers 4 and 5 bilaterally and narrowness of PIPJs and DIPJs 4 and 5 is observed radiographically on both sides. No symphalangism of fingers and thumbs is observed in him and other affected patients, except for the distal symphalangism of the left finger 5 of patient IV6. Carpal synostosis is not visible.

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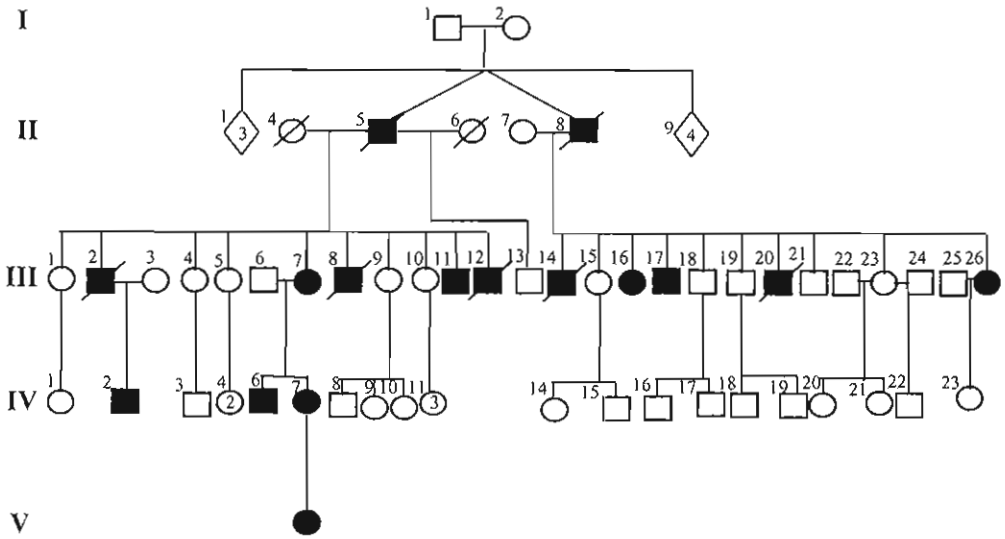



Fig. 1. Pedigree of the Thai family.


Fig. 2. **a**–**c**: Patient IV2 at ages 8, 15, and 21, respectively. **d**: Front row from **left** to **right**. Patients IV7, IV2, V1, III7, and Kantaputra.

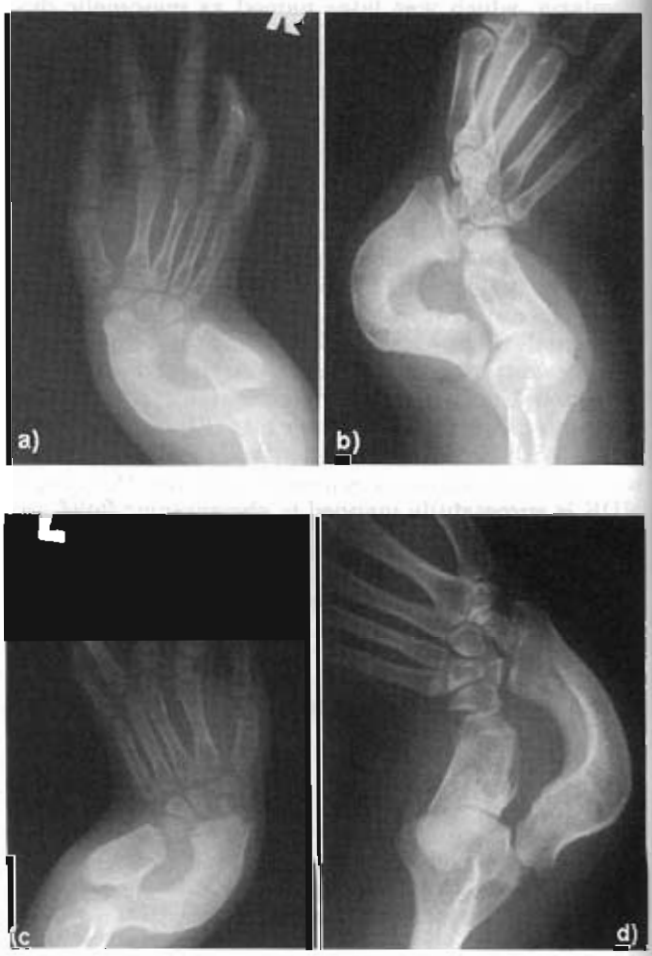
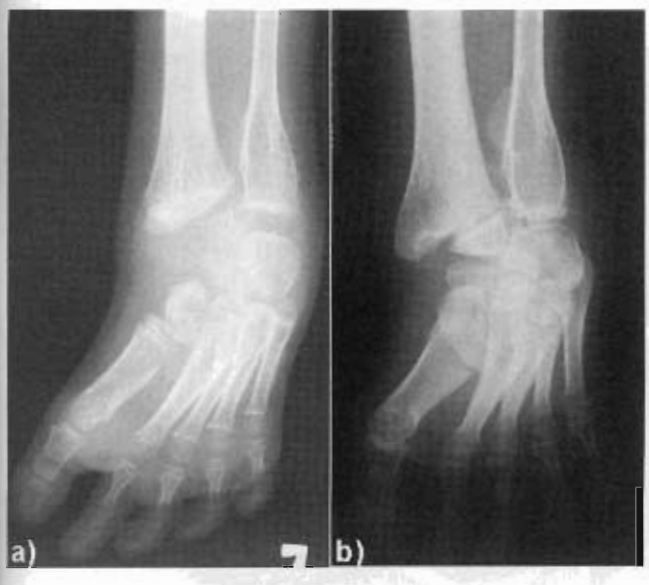



Fig. 3. Arm radiographs of patient IV2. Right arms at (**a**) age 8 and (**b**) 21 years. Left arm at ages (**c**) 8 and (**d**) 21 years. Note progressively bowed radii, large distal humeri, and overlapped carpal bones. Flexion contracture of the distal interphalangeal joints (DIPJ) of fingers 4 and 5 on both sides.



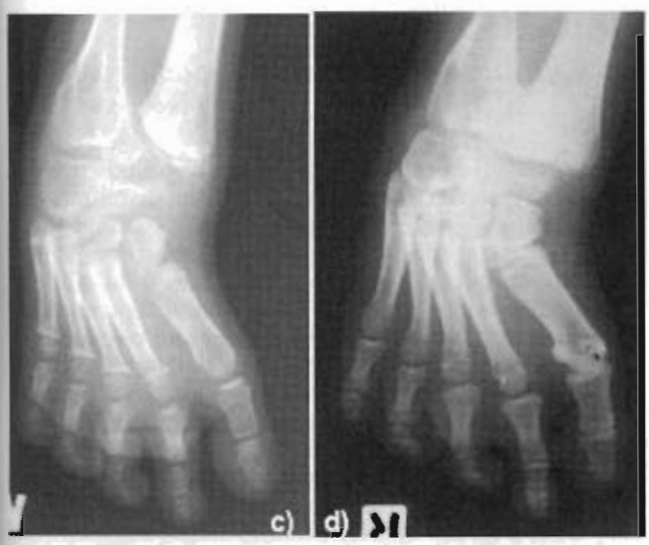




Fig. 4. Foot and ankle radiographs of patient IV2. **a**: Left foot at ages 8 and (**b**) 21 years. Note large distal end of fibula with "fibular prominence," malformed talus, and absence of calcaneus. Right foot at ages (**c**) 8 and (**d**) 21 years. Malformed talus is fused with fibula at age 8 years. Calcaneus is missing. Tibia-talus-fibula synostosis and overlapped tarsal bones with narrow spaces between them at age 21. Note distal symphalangism at toe 5 at age 21.

Distal humeri appear large with dumbbell-shape. This was seen in all affected adults.

### Lower Limbs

At age 21 years, right tibia, malformed talus, and distal fibula are fused. The distal ends of the fibulae appeared large especially that of the right one. Spaces between tarsal bones are narrow, resulting in overlapping of tarsal bones. Distal symphalangism of toes 5 is noted in him and patients III17, III26, and IV6. Distal symphalangism of toes 4 is found in Patient III26. In

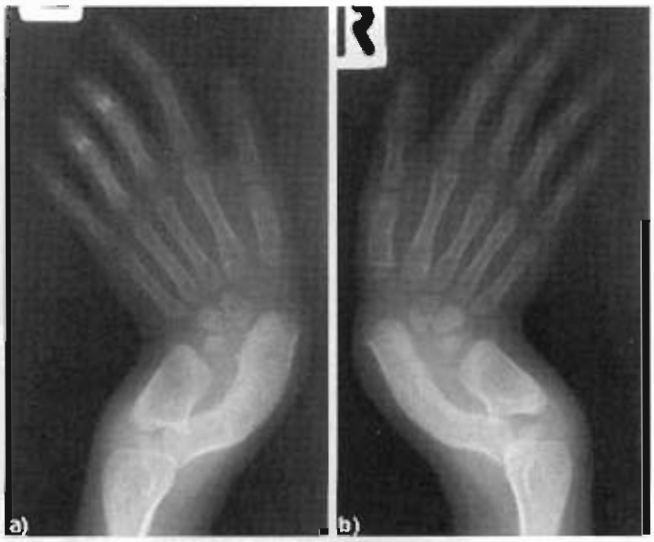



Fig. 5. Arm and hand radiographs of patient V1 at age 4 years. Absence of trapezium, trapezoid, scaphoid, lunate, and triquetrum. Distal humeri were large. No epiphyses of radii and ulnae were evident.

Patient III11, proximal and distal symphalangism are observed at the left toes 2 and 5.

## COMMENTS ON THE PAPER OF SHEARS ET AL.

I had the honor to review the very interesting paper of Shears et al. in this issue of *Am J Med Genet*, claiming to report the second reported family of MDK. The upper limb findings are similar to those of the original Thai family. However, the ulnae and radii of the Thai patients are more severely affected, showing more shortening and the radii are more severely bowed (Fig. 3a–d). The arrangement of the carpal bones in all Thai adult patients is more disorganized with narrow spaces among them or some fusion between them. This is not seen in the 4-year-old one. Interestingly as reported in the authors' patients, all Thai patients including the new member of the family have flexion contracture of the PIPJs of the 4th and 5th fingers (Figs. 3 and 5). Flexion contracture of other PIPJ and DIPJ is also observed in other patients. Report of flexion contracture was missed in my original paper.

The similarities between the Thai patients and those of the authors consist of shortening of radii and ulnae, flexion contracture of fingers 4 and 5, tarsal synostosis, talus–calcaneus fusion, and broad distal end of fibula. However, the authors' patients did not look disproportionately short as the Thai patients did. It appears that tibiae of the Thai patients are more severely affected. In addition, they also do not stand like "ballerinas" as the Thai patients do (Fig. 6a–g). I am convinced that "ballerina-like standing" is the result of misarticulation between tibia and talus leading to sharing of the same long axis of the tibia and tarsal bones. Most importantly, the authors' patients do not have fibular prominence,

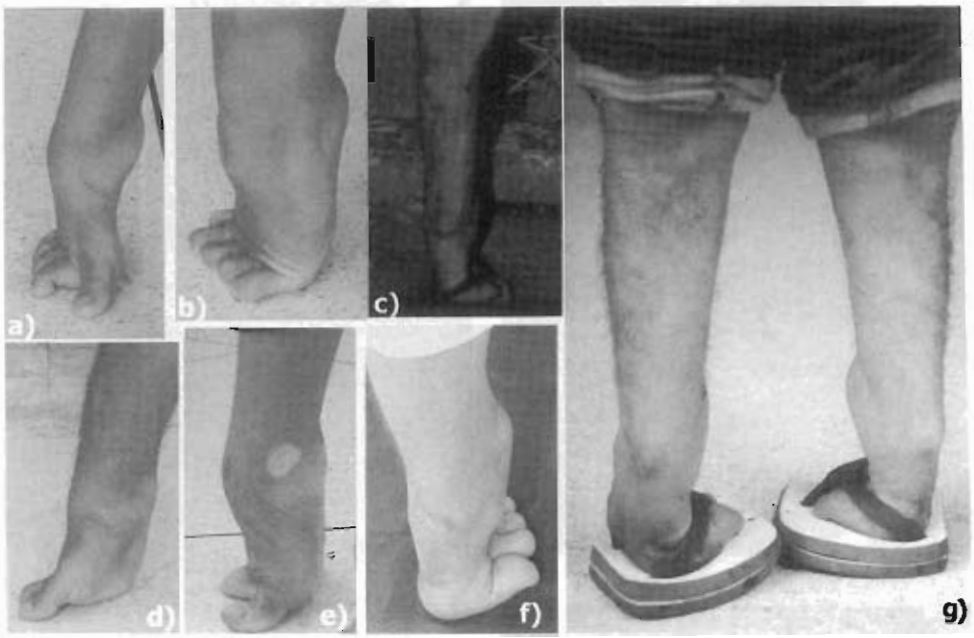



Fig. 6. Tip toe standing on the dorsal of Patients (**a**) and (**b**) V1. **d**: IV2. **e**: III7. Ballerina-like standing of Patients (**c**) III2, (**f**) III17, and (**g**) III11.

which is seen in all affected Thai patients even in the 4-year old one (Fig. 7). This finding appears to be the most consistent or "signature" finding of the syndrome.

The 4-year-old new member of the Thai family has provided us the better understanding of the syndrome. She is the youngest reported patient affected with MDK. Her findings have helped us to understand the development of this rare skeletal dysplasia; especially the prominent bone that we originally called "fibulo-calcaneal complex." The original understanding was based on the absence of a separate calcaneus and there was no fusion line in the large malformed bones. The radiograph of the 4-year-old showed left fibular prominence and a separate calcaneus, indicating that the prominent bone was solely fibula and calcaneus is missing in most Thai patients. Interestingly, the prominent fibula bone is not observed yet on the right fibula. Follow up report is needed in order to understand more on this endochondral bone formation. Regarding the fibula, which was hypoplastic in most Thai patients, in those with normal fibula, it articulated with tibial bony knot (Fig. 8a–d). This is found in four affected ones (II9, II10, III7, and III11). It is noteworthy that both original affected twins died of diabetic mellitus at the ages of 61 and 62.

In conclusion, with less severely affected radii and ulnae, the absence of ballerina-like standing, and the

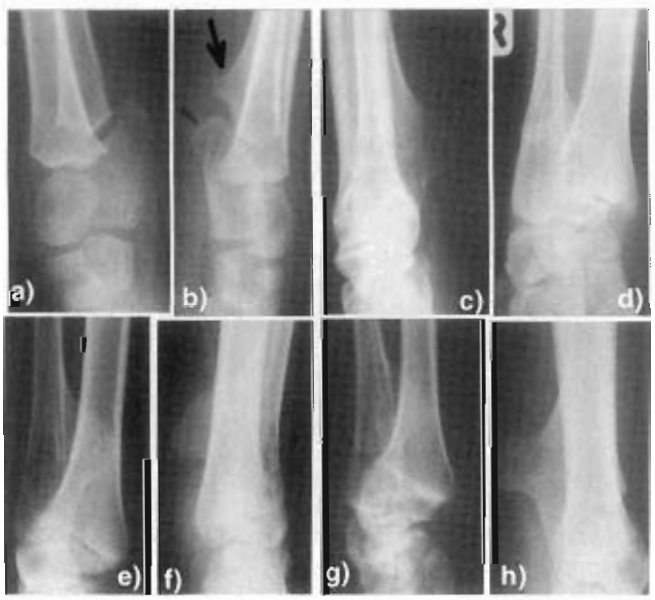



Fig. 7. Ankle radiographs of (**a**) Patient V1. Right ankle. Note vertical-positioned calcaneus. No fibular prominence. **b**: Patient V1. Left ankle. Note fibular prominence (arrow). Malformed and separate calcaneus (arrow head). Fibular prominences in Patients (**c**) IV2, (**d**) IV7, (**e**) IV6, (**f**) III11, (**g**) III7, (**h**) III17.

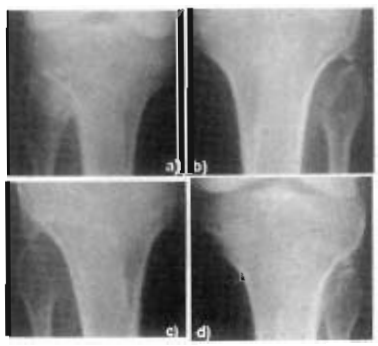



Fig. 8. **a–d**: Tibial bony knot articulates with fibula.

absence of characteristic fibular prominence, I believe the syndrome described by Shears et al. [2003] is unique and deserves its own distinct entity. However, I am convinced that the syndrome is allelic to MDK.

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I would like to thank all the patients for their friendship and help in making this project possible. This work is dedicated to Dr. Montri and Mrs. Penprapa Kantaputra, my father and mother.

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*Clinical Report*

# Microcephalic Osteodysplastic Primordial Dwarfism With Severe Microdontia and Skin Anomalies: Confirmation of a New Syndrome

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**We report two related Thai children having a new syndrome of microcephalic osteodysplastic primordial dwarfism (MOPD). The findings which classify them as having MOPD include IUGR, microcephaly, prominent nose and nasal bridge, small pinnae, short stature, cone-shaped and ivory-epiphyses, delayed bone age, slender long bones, and abnormal pelvis. The findings that distinguish them as having newly recognized syndrome consist of severe microdontia, malformed teeth, single-rooted or rootless teeth, severely hypoplastic alveolar bone, café au lait spots, acanthosis nigricans, and areas of hypo- and hyperpigmented skin. The reported patients appear to have the same condition as the family reported by Kantaputra [2002: Am J Med Genet 111:420–428]. This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html.** 

**KEY WORDS: hyperpigmented skin; hypopigmented skin; hypoplastic alveolar bone; malformed tooth; microdontia; rootless tooth**

## INTRODUCTION

In 2002, Kantaputra reported a newly recognized autosomal recessive syndrome of microcephalic osteodysplastic primordial dwarfism (MOPD). The uniqueness of that syndrome is the presence of dental, digital, and skin anomalies. The dental anomalies are striking, consisting of severe microdontia, opalescent and rootless molars, and an unerupted tooth. The mandibular premolars are unusually small and malformed, comprising many cusps. In addition, the alveolar process is severely hypoplastic. Digital anomalies include unusually long second toes, distal symphalangism of toes, and brachymesophalangy of fingers. Skin anomalies include café au lait spots, and areas of hypo- and hyperpigmented skin. The hair is dry and sparse [Kantaputra, 2002]. After reviewing all previously reported cases of MOPD, we found that 12 cases of MOPD with microdontia have been reported (Tables I and II). We hypothesize that MOPD with severe microdontia with or without skin anomalies should be classified as a distinct entity.

We report on two related Thai patients with MOPD syndrome, who share many characteristic findings of the novel syndrome reported by Kantaputra [2002].

## CLINICAL REPORT

### Patient 1

Patient 1 was a 7-year-old boy. He was the only child of healthy, nonconsanguineous parents (Figs. 1 and 2a). He was born at term with weight 1,270 g (<3 centile; <−7.4 SD), length 37 cm (<3 centile; <−8.6 SD), and OFC 28 cm (<3 centile; <−3 SD). At the age of 3, his growth was moderately delayed with a development quotient of 42.

On examination at age 7, he was a cheerful boy with microcephaly, prominent nose and nasal bridge, small pinnae, high-pitched voice, and proportionate short stature (Fig. 2a). His height, weight, and OFC were 83 cm (<3rd centile; <−8.5 SD), 9.5 kg (<3rd centile; <−6 SD), and 41.5 cm (<3rd centile; <−5.5 SD), respectively. His upper and lower ratio was appropriate for age (1.1:1), but his arm span (73 cm) was relatively shorter than his height due to limited elbow extension. Limited abduction of the hips was observed. Clinodactyly of the fifth fingers, deep palmar creases, and medially-deviated toes were noted. Penis and testis were unremarkable. Hearing via auditory brainstem response (ABR) was unremarkable, and development remained globally delayed. Growth hormone secretion following clonidine stimulation at age 3 was normal. Karyotype was 46,XY.

Skin complexion was darker than that of his parents. There were areas of hypo- and hyperpigmentation of the skin which did not follow lines of Blaschko (Fig. 2a,b). The palms appeared hyperpigmented (Fig. 2c). Café-au-lait spots were observed on his body, hands, and thigh (Fig. 2b). Hair was normal.

Oral examination revealed normal shape and color of deciduous crowns. All teeth were mobile. Most deciduous incisors appeared to have premature exfoliation. The remaining deciduous teeth were slightly small. The permanent teeth, especially the maxillary and mandibular permanent incisors were extremely small. The mesiodistal width of a mandibular incisor was 3 mm. The mamelons of all incisors were prominent (Fig. 3a). The carabelli cusp of the maxillary second deciduous molar was large. The mandibular and maxillary right deciduous second molars exfoliated at age 5. The pharynx was very narrow.

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TABLE I. Clinical Manifestations of MOPD Patients With Microdontia With/Without Skin Anomalies

| | Cervenka et al. [1979] | | Majewski et al. [1982] | | Shebib et al. [1991] | Lin et al. [1995] | | Majewski and Goecke [1998] | | Seymen et al. [2002] | Kantaputra [2002] | | Present cases | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pt1 | Pt2 | Pt1 | Pt2 | | Pt1 | Pt2 | Pt2 | Pt3 | | Pt1 | Pt2 | Pt1 | Pt2 |
| Mode of inheritance | AR | AR | Na | Na | AR | AR | AR | AR | Na | Na | AR | AR | AR | AR |
| Gender | M | M | M | F | F | M | M | M | M | M | M | F | M | F |
| Examination | | | | | | | | | | | | | | |
| General | | | | | | | | | | | | | | |
| Age when reported (year) | 8 | 11 | 2 | 3 | 7 | 10 | 15 | 2 | 2 | 7 | 18 | 16 | 8 | 9 |
| Weight (kg) | 8 | 15 | 6.3 | 4.2 | 5.4 | 18 | 31 | 5 | 6.1 | 7 | 14 | 12 | 9.5 | 9.5 |
| Height (cm) | 85 | 104 | 63.8 | 61 | 67 | 116 | 125 | 60.4 | 65.0 | 78 | 102 | 96 | 83 | 83 |
| OFC (cm) | 41 | 44 | 37 | 31.5 | 37.5 | 47.5 | 48 | 40.4 | 41 | 40.5 | 43 | 41 | 38.5 | 38.5 |
| Dis/proportionate short | P | P | P* | P* | D | P | P | D | D | P | P | P | P | P |
| Postnatal GR | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Dev delay/MR | Y | Y | Y | N | Y | N | Y | Y | Y | Y | N | N | Y | Y |
| Hypertonia | Y | Y | N | N | N | N | N | Y | N | N | N | N | N | N |
| High-piched voice | Y | Y | Y | Y | Na | Na | Na | Y | Y | N | N | N | Y | Y |
| Birth history | | | | | | | | | | | | | | |
| Birth weight (g) | 1,040 | 1,500 | 1,280 | 1,150 | 1,250 | 1,700 | 1,480 | 1,320 | 1,300 | 800 | 1,000 | 1,000 | 1,270 | 920 |
| Birth length (cm) | 34 | Na | 38 | Na | 30 | Na | Na | 37 | 39[a] | 35 | Na | Na | 37 | 31 |
| OFC (cm) | 25.5 | Na | 27.5 | Na | Na | Na | Na | 28 | 27.5 | 25 | Na | Na | 28 | 24.5 |
| IUGR | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Craniofacial | | | | | | | | | | | | | | |
| Microcephaly | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Craniosynostosis | Na | Na | Y | Y | Y | Na | Na | Na | N | Na | N | N | Y | Y |
| Strabismus | N | Na | Na | Na | Na | N | Na | Na | N | Na | N | N | N | Y |
| Small/abnormal pinnae | Y | Y | Y | Y | N | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Prominent nose | Y | Y | Y | Y | Y | N | N | Y | Y | Y | Y | Y | Y | Y |
| Small maxilla | Na | Na | Na | Na | Na | Na | Na | Na | Na | Y | Y | Y | Y | Y |
| Micrognathic mandible | Y | Y | Y | Y | Y | Na | Na | Y | Y | Y | Y | Y | Y | Y |
| Retrognathic mandible | Y | Na | Y | Na | N | Na | Na | Y | Y | Y | N | N | N | N |
| Teeth | | | | | | | | | | | | | | |
| Microdontia of prim teeth | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Microdontia of perm teeth | Y | Y | Na | Na | Y | Y | Y | Na | Na | Y | Y | Y | Y | Y |
| Hypodontia | N | Na | Na | Na | Y | N | N | Na | Na | Y | N | N | Y | Y |
| Taurodontism | N | Na | Na | Na | Na | N | N | Na | Na | Y | N | N | N | N |
| Malformed teeth | N | Na | N | N | Na | N | N | Na | Na | Y | Y | Y | Y | Y |
| Opalescent teeth | N | Na | Na | N | Na | N | N | Na | Na | N | Y | Y | N | N |
| Short root/rootless | Y | Y | Na | Na | Y | Y | Na | Na | Na | Y | Y | Y | Y | Y |
| Unerupted tooth | N | Na | Na | Na | N | N | N | Na | Na | N | N | Y | N | N |
| Hair and skin | | | | | | | | | | | | | | |
| Dry/sparse hair | Y | Y | Y | Y | N | Na | Na | Na | Na | Na | Y | Y | N | N |
| Hyperhirsute | Y | Y | Na | Na | N | Na | Na | Na | Na | Na | N | N | N | N |
| Skin hyperpigment | N | N | Na | Na | N | Na | Na | Na | Y | Na | Y | Y | Y | Y |
| Café au lait spots | N | N | Na | Na | Na | Na | Na | Y | Y | Na | Y | Y | Y | Y |
| Acanthosis nigricans | Na | Na | Na | Na | Na | Na | Na | Na | Na | Na | N | N | N | Y |
| Sclerotic skin | Y | N | Na | Na | Na | Na | Na | Na | Na | Na | N | N | N | N |
| Arms and hands | | | | | | | | | | | | | | |
| Bowed arms | Na | Na | N | Na | Y | N | N | Na | N | Y | N | N | Y | N |
| Radial head dislocation | Na | Na | Na | Na | N | N | N | Na | Na | Y | Y | Y | Y | N |
| Clinodactyly V | Y | Y | Y | Y | Y | Na | Na | Y | Y | Na | N | N | Y | Y |
| Legs | | | | | | | | | | | | | | |
| Bowed legs | N | N | N | N | N | Na | Na | N | N | Na | N | N | N | N |
| Coxa valga | Na | Na | N | N | N | Y | Y | N | N | N | Na | Na | N | N |
| Coxa vara | Na | Na | Y | Y | Y | N | N | Y | Y | Y | Na | Na | N | N |
| Genitalia (male) | | | | | | | | | | | | | | |
| Cryptorchidism | Y | N | N | — | — | Na | Na | Na | Na | Na | N | — | N | — |
| Small testis | Na | N | Y | — | — | Na | Na | Na | Na | Na | N | — | N | — |
| Small penis | N | N | Y | — | — | Na | Na | Na | Na | Na | N | — | N | — |

TABLE II. Radiographic Findings of MOPD Patients With Microdontia With/Without Skin Anomalies

| | Cervenka et al. [1979] | | Majewski et al. [1982] | | Shebib et al. [1991] | Lin et al. [1995] | | Majewski and Goecke [1998] | | Seymen et al. [2002] | Kantaputra [2002] | | Present cases | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pt1 | Pt2 | Pt1 | Pt2 | | Pt1 | Pt2 | Pt2 | Pt3 | | Pt1 | Pt2 | Pt1 | Pt2 |
| Craniofacial | | | | | | | | | | | | | | |
| Increased digital marking | Na | Na | Na | Na | Na | Na | Na | Na | Na | Na | Y | Y | Y | Y |
| Hypoplastic alv bone | Y | Y | Na | Na | Y | Y | Na | Na | Na | Y | Y | Y | Y | Y |
| Arms and Hands | | | | | | | | | | | | | | |
| Angular carpal bone | Na | Na | Na? | Na | Y | Na | PIC | Na | PIC | Na | Y | Y | Y | Y |
| Pseudoepiphysis of MC | Na | Na | Y | Y | Y | Y | Y | Y | Y | Na | N | N | N | Y |
| Ivory epiphyses | Y | N | N | Na | Y | N | Y | Na | N | Na | Y | Y | Y | Y |
| Cone-shaped epiphysis | N | N | N | Na | Y | Na | Y | Na | N | Na | Y | Y | Y | Y |
| Delayed bone age | N | N | Y | Y | Y | N | N | Y | Y | Y | Y | Y | Y | Y |
| Pelvis and legs | | | | | | | | | | | | | | |
| Hypoplastic/narrow pelvis | Na | Na | Y | Y | Y* | Y | Y | Y | N | Na | Y | Y | Y | Y |
| Epiphysiolysis of hips | Na | Na | Y | Y | N | N | N | N | Na | Na | N | N | Y | N |
| Triangular of distal femoral epiphyses | Na | Na | Y | Na | Y | N | N | Y | Y | Na | N | N | N | N |
| Flaring of distal femeral metaphysis | Na | Na | Y | Y | Y | Y | Y | Y | Y | Na | N | N | Y | Y |
| Feet | | | | | | | | | | | | | | |
| Ivory epiphysis | Na | Na | Na | Na | Y | Na | Na | Na | Na | Na | N | N | N | Y |
| Distal symphalangism of toes | Na | Na | Na | Na | Na | N | N | Na | Na | Na | Y | Y | Y | N |
| Chest | | | | | | | | | | | | | | |
| Long/slender clavicles | Na | Na | Na | Na | Y | Na | Na | Na | N | Na | Y | Y | Y | Y |
| Hypoplastic scapula | Na | Na | Na | Na | Na | Na | Na | Na | N | Na | Y | Y | Y | Y |
| 11 ribs | Na | Y | Na | Na | Y | Na | Na | Na | N | Na | Y | Y | N | N |

Y, present; N, absent; Na, not available.
Patients reported by Cervenka et al. [1979] were reported again in 1981 by Tsuchiya et al. [1981].
*Disproportionately short during the early years of life.

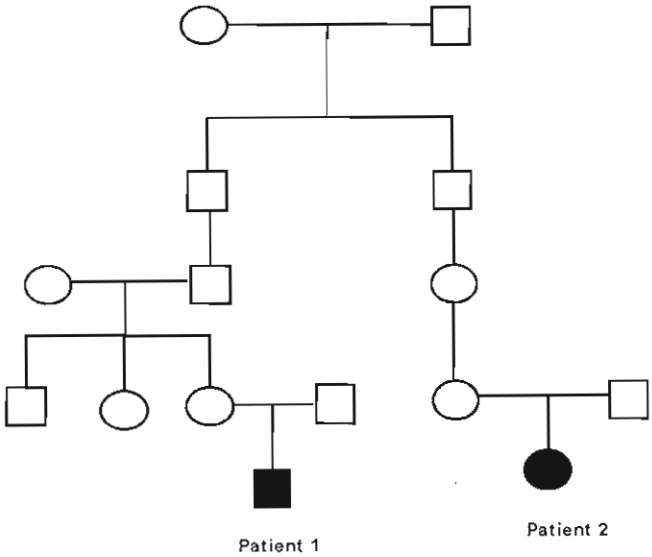
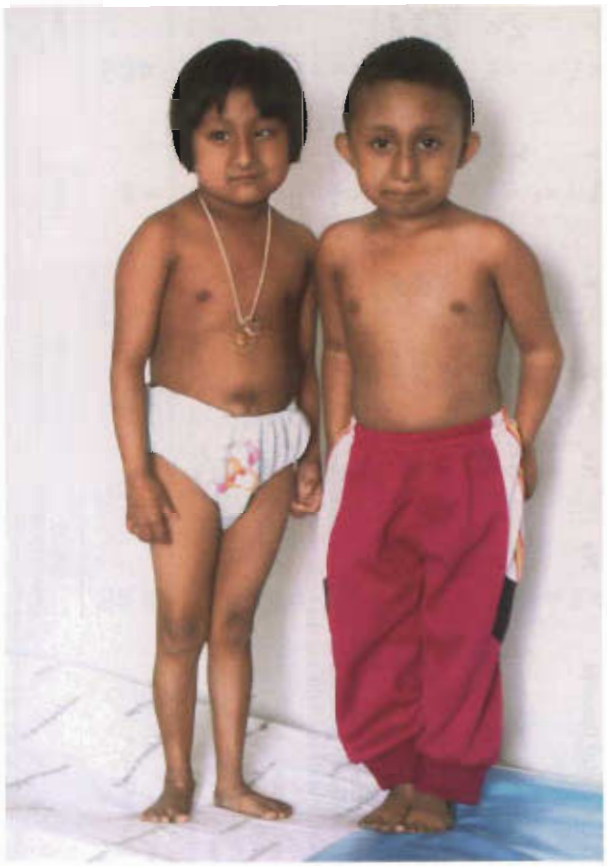



Fig. 1. Pedigree of the families.

Radiographic examination showed thin cranium. Molars were either single- and short-rooted, or rootless (Fig. 4a,b). Obliteration of the root canals was seen in all incisors (Fig. 5a,b). Alveolar process was severely hypoplastic and its cortical bone was not evident. Supernumerary maxillary right permanent molar was noted. All unerupted developing permanent teeth were malformed and very small mesiodistally. Digital marking of the skull was observed (Fig. 4a,b). Clavicles were long, straight, and slender. Scapulae were hypoplastic. There were 11 ribs on each side. The radius and ulna were slender. Radius was slightly bowed and the radial heads were dislocated. There was restricted supination and extension at the elbows.

According to Greulich and Pyle, the bone-age was delayed at age 3 years 3 months and estimated to around age 1 year (SD = 6.6 months). Middle phalanges of finger 5 appeared short and tapered proximo-distally. Epiphyses were observed only at the proximal phalanges of fingers 2–4 (see the online Figure 8a at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). At age 6, bone age was estimated to be of 5 years (SD = 9 months). Distal ulna was slightly short with ulnar deviation of the hand. Ivory epiphyses were observed at proximal phalanges of thumbs, all phalanges of fingers 3 and 4, and the middle and distal phalanges of fingers 2. Cone-shaped epiphyses of the proximal phalanges of fingers

a



b

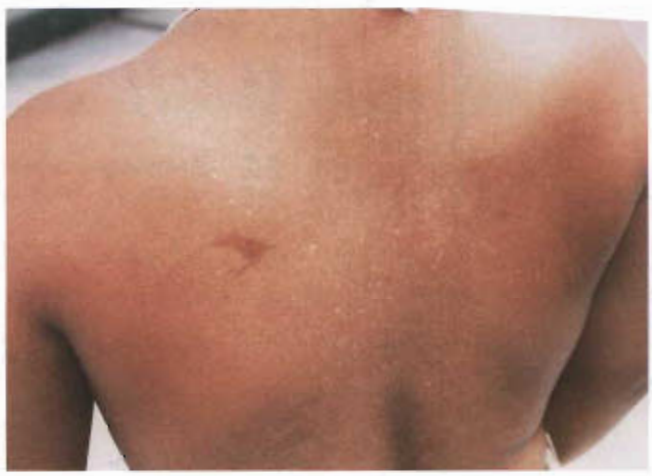


Fig. 2. a: Patient 2 (left) and 1 (right). Note prominent nose and nasal bridge, dark skin, exotropia of Patient 2's eye. b: Dark skin with areas of hypo- and hyperpigmented macules and café au lait spot. c: Hyperpigmented palms.

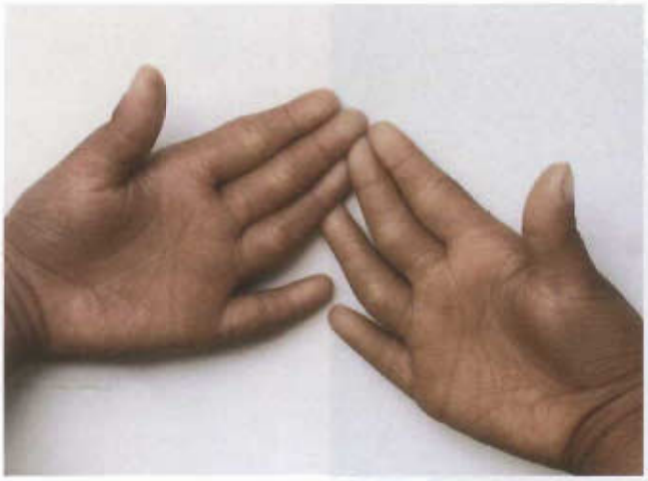


Fig. 2. (*Continued*)

2–4 were observed (see the online Figure 8b at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). Pelvic radiograph demonstrated narrow iliac wings, flat acetabular sockets, thin and elongated pubic and ischial bones, and bilateral epiphysiolysis of the femoral heads (see the online Figure 6b at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). Foot radiograph showed hypoplastic distal phalanges of toes 2–4, ivory epiphyses of proximal phalanges 5, and distal symphalangism of toe 5.

### Patient 2

Patient 2 was a 9-year-old girl. She was the only child of healthy and nonconsanguineous parents (Figs. 1 and 2a). Her maternal great grandfather was the younger brother of the great grandfather of Patient 1 (Fig. 1). The patient was born at 32 weeks of pregnancy. Her birth weight, birth length, and OFC were 920 g (<3rd centile; <−1.7 SD), 31 cm (<3 centile; <−2.5 SD), and 24.5 cm (<3rd centile; −1.8 SD), respectively. At birth she suffered from mild respiratory distress syndrome, hyperbilirubinemia, and retinopathy of prematurity, and was in an incubator for 2.5 months. Growth hormone stimulation test with glucagon revealed peak of growth hormone of 2.6 ng/ml (norm >10 ng/ml). IGF1 = 0 ng/ml (norm 7–21 ng/ml). IGBP3 = 2,096 ng/ml. The results indicated primary growth hormone deficiency.

On examination at age 8, her height, weight, and OFC were 83 cm (<3 centile; <−8.9 SD; 50 centile of age 20 months ), 8.3 k (<3 centile; <−5.6 SD; 50 centile of age 17 months), and 38.5 cm (<3rd centile; <−8.8 SD; 50 centile of age 2 months), respectively. Physical examination revealed microcephaly, left esotropia, prominent nose and nasal bridge, and small pinnae (Fig. 2a). Proportionate short stature was noted with 1:1 upper and lower segment ratio. Hands, arms, feet, and legs were proportionately short. Arm span was 81.5 cm. Movement of the shoulders, elbows, wrists, hips, knees, and ankles was unremarkable. Palms were dry, and deep palmar and finger flexion creases were noted. Clinodactyly of the fifth fingers was observed. The hair and hearing were normal.

Like that of Patient 1, her skin complexion was darker than that of her parents. Also there were areas of hypo- and hyperpigmented macules in the skin of her chest and back (Fig. 2a). Acanthosis nigricans was observed around her neck and armpits. She sat, stood, walked, and ran at

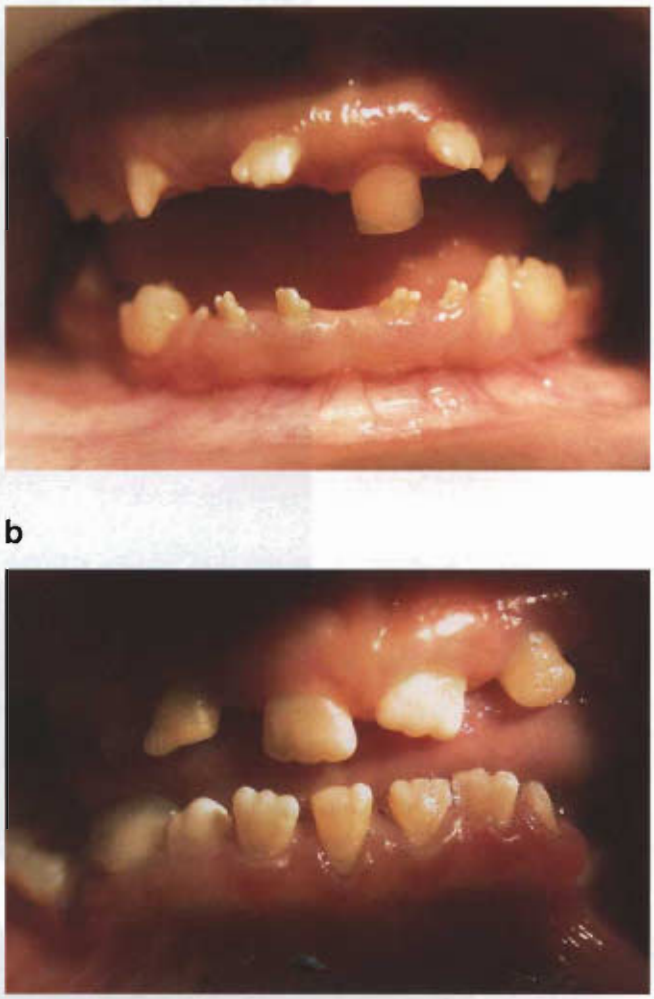


Fig. 3. **a**: Patient 1. Extremely small maxillary and mandibular permanent incisors with prominent mamelons. **b**: Patient 2. Very small and yellowish permenent incisors with prominent mamelons.

ages 9, 18, 22, and 28 months, respectively, and she used single words at 18 months. Her voice was high-pitched, and global developmental delay was observed, with cheerful disposition. Intelligence quotient was 56 according to Stanford Binet form. Karyotype was 46,XX.

Oral examination demonstrated very small and yellowish teeth. Their mamelons were prominent (Fig. 3b). All teeth were mobile. The maxillary right second deciduous molar exfoliated at age 5. The maxillary left first permanent molar had occlusal caries. The maxillary labial frenum was thick. Her gingiva did not appear hyperpigmented like her skin, and was otherwise normal. The pharynx was narrow.

Radiographic examination showed thin cranium, rootless molars, and severely hypoplastic alveolar bone. Cortical bone of the alveolar process was not observed. Rootless molars appeared to float in loose alveolar bone. The crowns of all permanent molars were tapered occluso-cervically. Increased digital marking of the skull was noted (Fig. 4c,d). The dental pulps of all incisors appeared obliterated (Fig. 5c,d). Clavicles were long, straight, and slender. They appeared to be located in horizontal position. Scapulae were hypoplastic. At age 6, hand radiograph showed ivory epiphyses at all

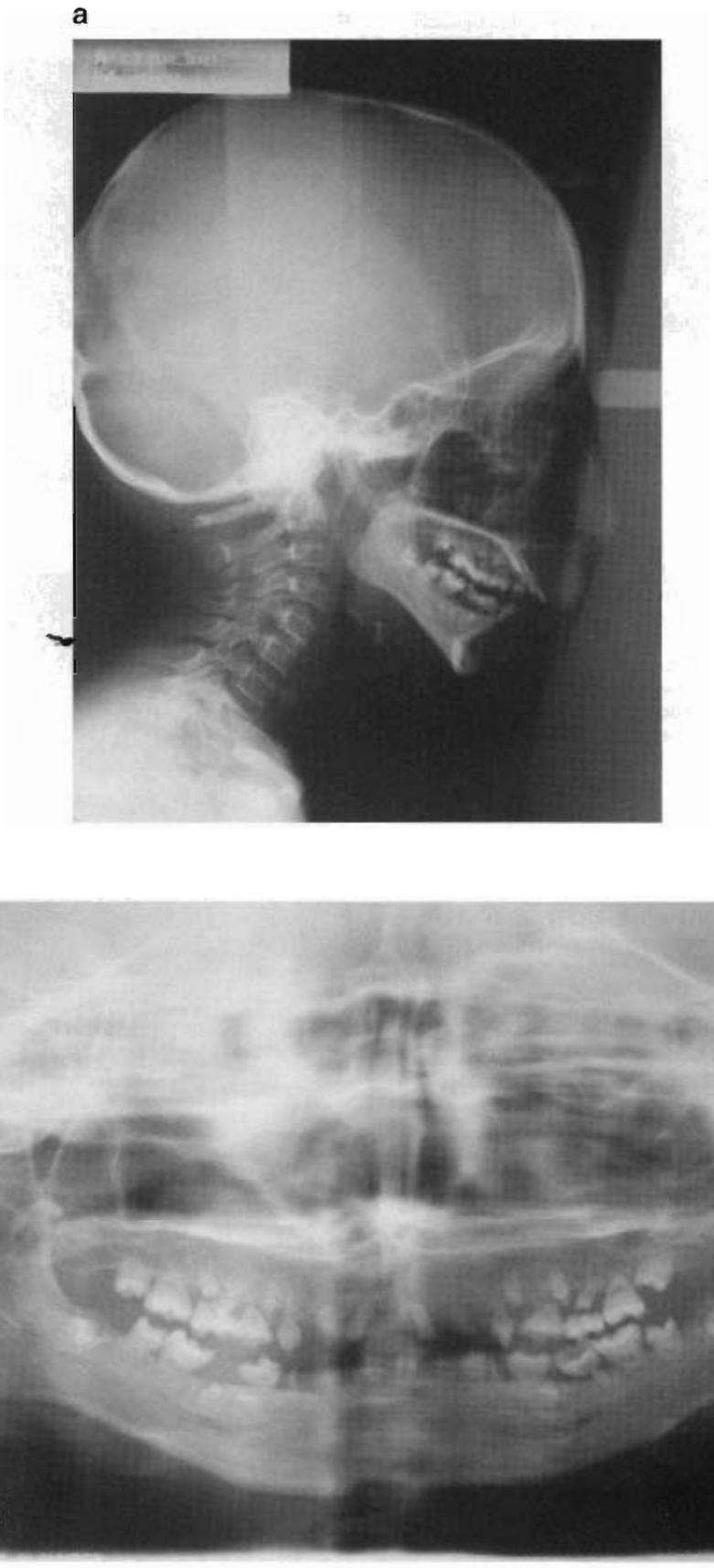



Fig. 4. Patient 1 (**a**) lateral cephalograph. Note increased digital marking of the skull. **b**: Panoramic radiograph. Supernumerary right maxillary permanent molar. Patient 2 (**c**) PA skull radiograph. **d**: Panoramic radiograph. Note single-rooted or rootless molars, severe microdontia of developing permanent premolars and molars, severely hypoplastic alveolar bone, and molar crowns converge occluso-cervically.

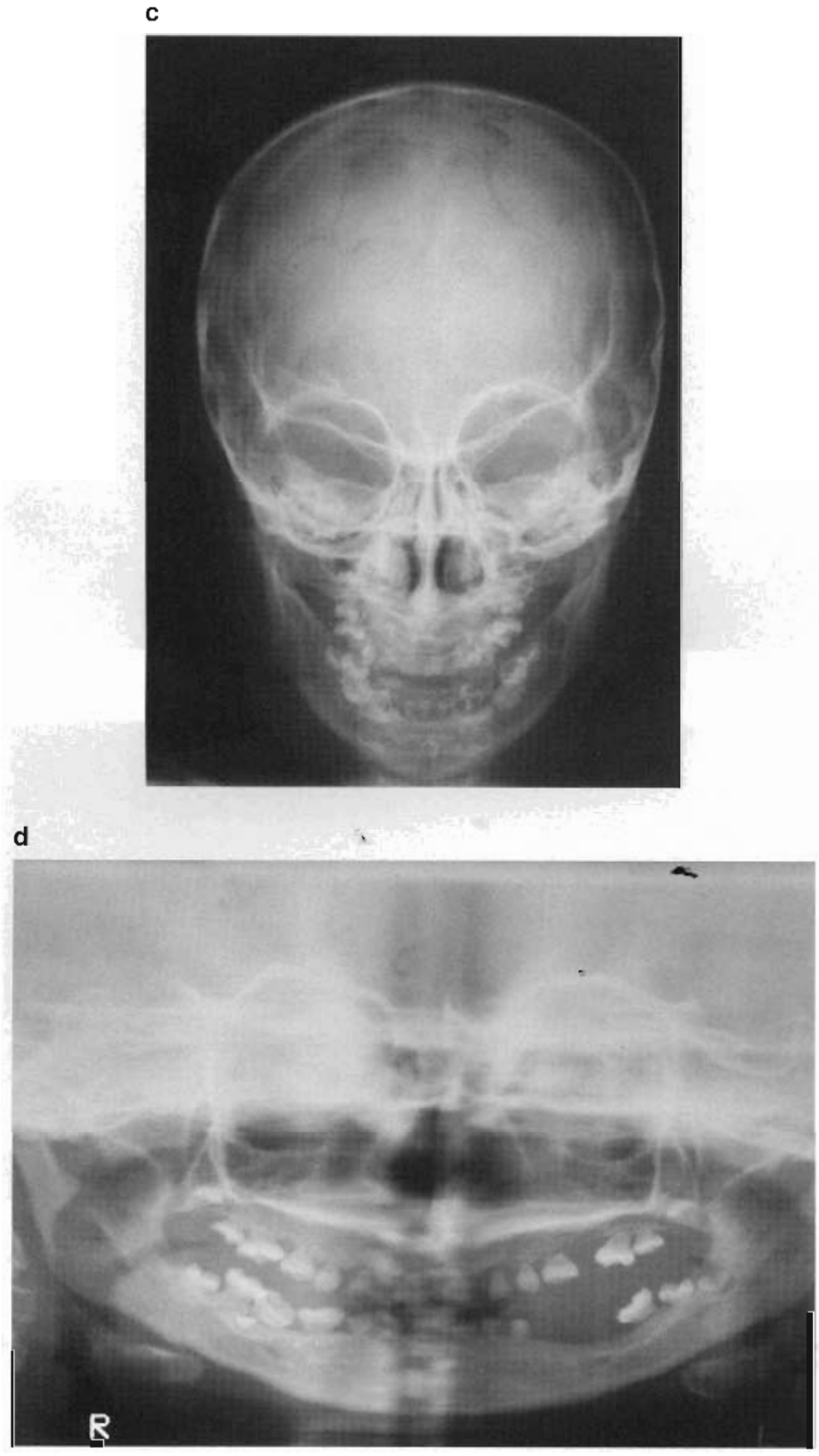



Fig. 4. (*Continued*)

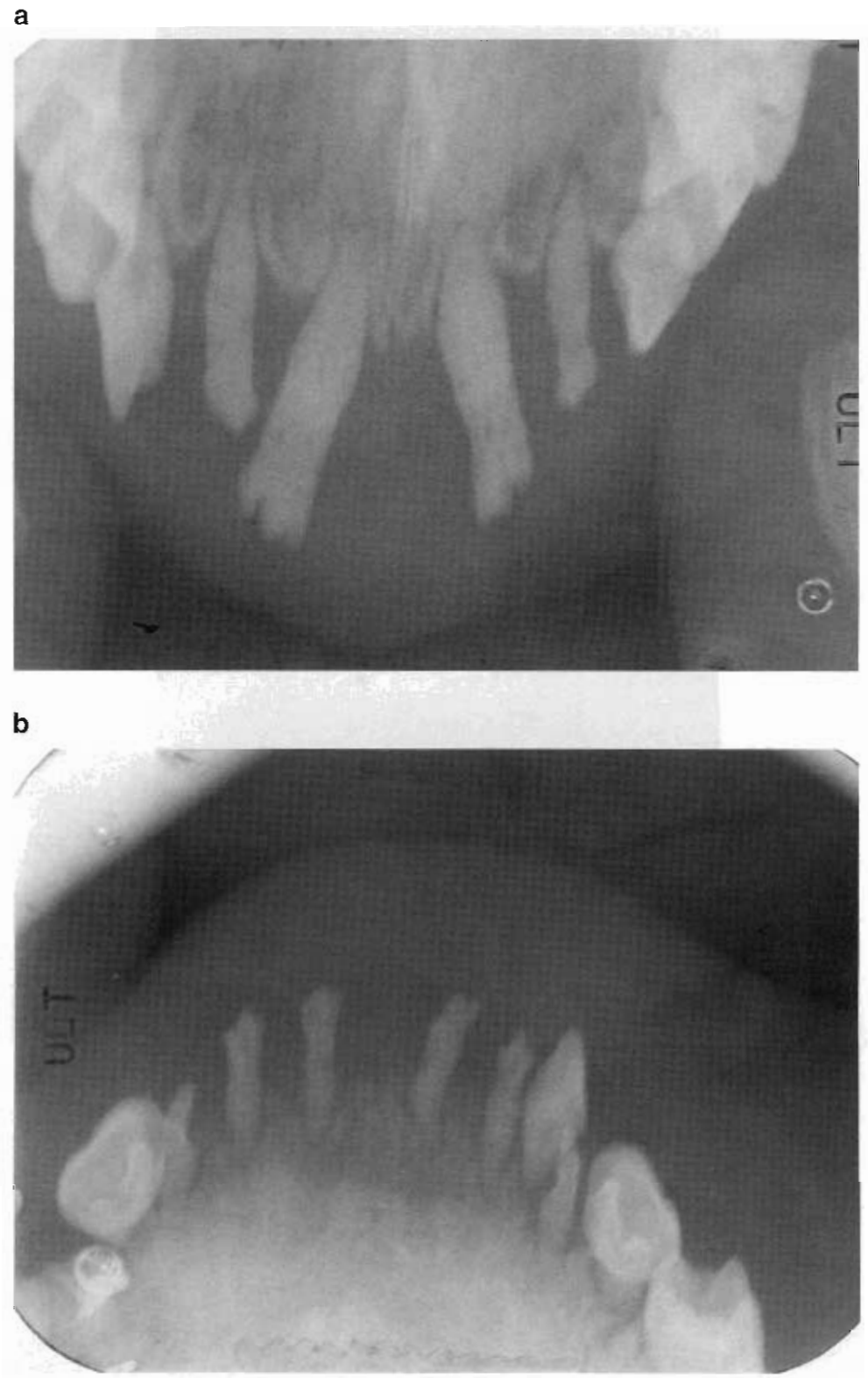



Fig. 5. Periapical radiographs of Maxillary and mandibular incisors of Patient 1 (a) and (b). Patient 2 (c) and (d). Note Severely hypoplastic alveolar bone. Obliteration of dental pulp chambers and root canals.

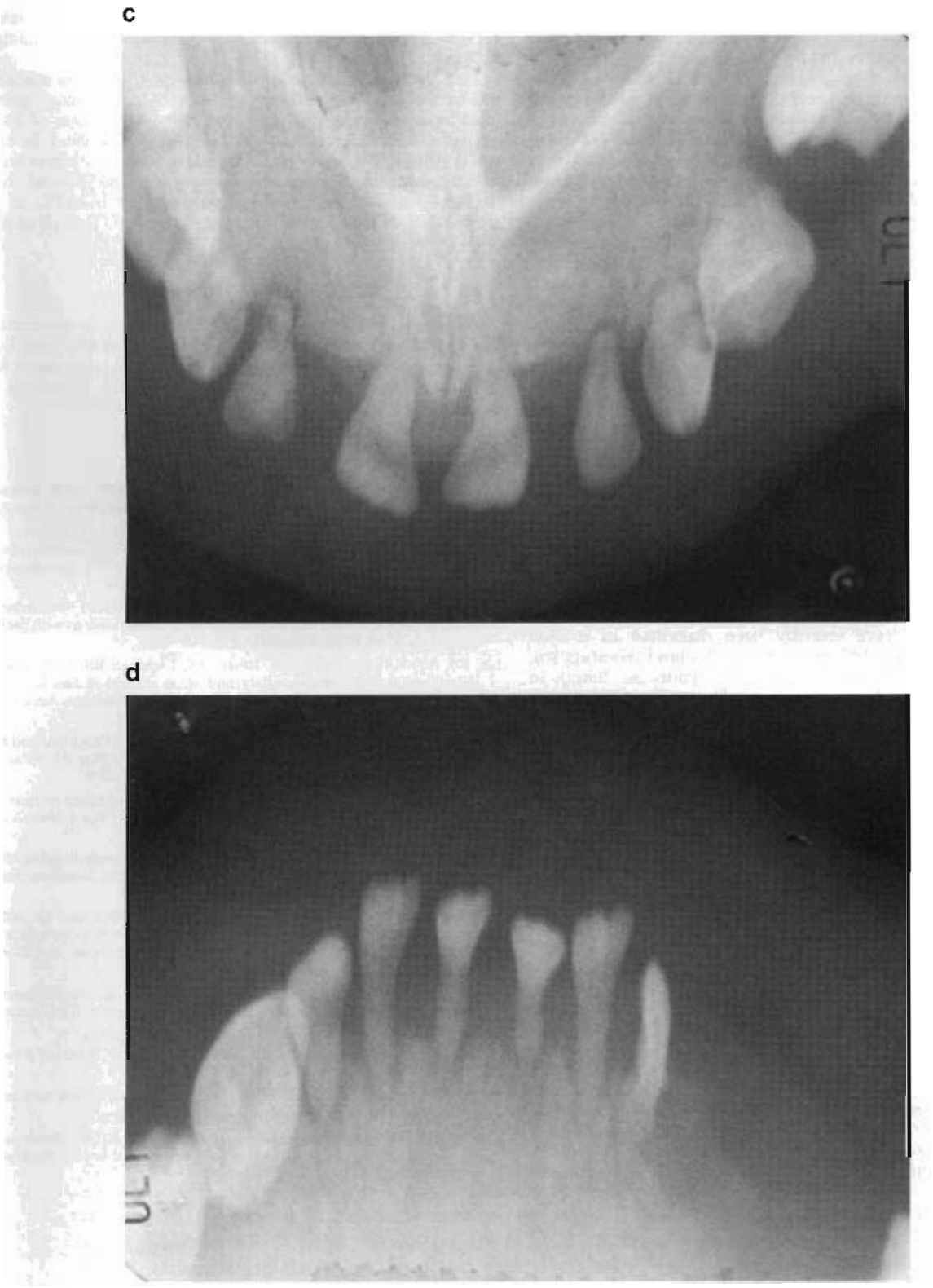



Fig. 5. *(Continued)*

phalanges except at the distal phalanges of her thumbs and the middle and distal phalanges of her 5th fingers, with bone age at 2.6–3 years (see the online Figure 8c at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). At age 8, ivory epiphyses were observed at all phalanges of the hands except for the distal phalanges of thumbs. Large pseudoepiphyses were noted at the proximal ends of metacarpals 2 and 5, and distal radial epiphyses appeared very dense (see the online Figure 8d at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). Eleven ribs were observed on each side. Like those of Patient 1, clavicles were straight and slender (see the online Figure 6a at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). Humerus, radius, ulna, tibia, and fibula were slender (see the online Figure 7b at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). Foot radiograph showed medially deviated toes and ivory epiphyses of all proximal phalanges and distal phalanges 2–5 (see the online Figure 9 at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html).

## DISCUSSION

We report on two additional Thai children affected with MOPD and severe microdontia, short and single-rooted molars, rootless molars, and severely hypoplastic alveolar bone, hyperpigmented skin, acanthosis nigricans, café au lait spots, and hypo- and hyperpigmented macules. The combination of dental and dermal features found in these two related children appears unique for MOPD. Interestingly, similar findings have recently been described in a Thai family living in the different part of Thailand [Kantaputra, 2002]. We are convinced that the syndrome found in our patients and that reported by Kantaputra [2002] represents a distinct entity.

Seckel syndrome and MOPD patients usually have normal teeth, and some affected patients even have large teeth [Seckel, 1960; Majewski, 1992; Kjær et al., 2001]. The permanent incisors found in the present patients especially those of Patient 1 were very small. Besides severe microdontia, these patients also had rootless or very short and single-rooted molars, with severely hypoplastic alveolar bone. We have reviewed the previously reported cases of MOPD with microdontia with or without skin anomalies and tabulated them in Tables I and II. Twelve MOPD cases were found to have microdontia. Seven of those had short roots or rootless teeth with severely hypoplastic alveolar processs (Table I). Based on both oral and skin manifestations, we believe the present cases and Thai sibs reported by Kantaputra [2002] have the same disorder.

It appears that the combination of severe microdontia, rootless molars, and severely hypoplastic alveolar bone, café au lait spots, hypo-hyperpigmented macules are the distinguishing features of this "newly" recognized syndrome. Seymen et al., 2002 reported a Turkish boy as having Seckel syndrome. However, with this phenotype and severe microdontia, hypodontia, abnormal molar roots, and hypoplastic alveolar bones, we suspect that he might have the same condition as our patients and those reported by Kantaputra [2002].

The mode of inheritance of this new disorder is suspected to be autosomal-recessive as their maternal great grandfathers were brothers. However, the fathers of both patients denied consanguinity, but they all lived in the same village. While our patients share several features with Seckel syndrome and since it has recently been found that Seckel syndrome is caused by mutations in ATR, it is worthwhile to look for mutation in the gene [O'Driscoll et al., 2003].

## ACKNOWLEDGMENTS

We thank Prof. John Graham for his very helpful comments. We appreciate the kindness of the patients and their families for allowing us to use their medical and dental information for publication and for the advancement of science. This study is solely dedicated to them.

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# A Novel Mutation in *IRF6* Underlies Hearing Loss, Pulp Stones, Large Craniofacial Sinuses, and Limb Anomalies in Van der Woude Syndrome Patients

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**Summary:** Van der Woude (VWS) and popliteal pterygium syndromes are caused by mutations in the interferon regulatory factor (*IRF6*) gene. Two Thai VWS families demonstrating newly recognized findings of VWS are reported. The phenotype in the first family includes sensorineural hearing loss, cleft lip and palate, lower lip anomalies, ankyloglossia, hypodontia, dental pulp stones, large craniofacial sinuses, and limb anomalies. Molecular analysis of *IRF6* revealed an 11 bp deletion in exon 4. This frameshift mutation truncates IRF6 just after the DNA binding domain. The mutation implies that IRF6 can affect dental pulp calcification, pneumatization of craniofacial sinuses, and ear and limb development. The second family consists of an affected brother and sister. Both have lower lip anomalies and the sister has cleft lip and palate. Interestingly, both have abnormal shape of the mandibular deciduous and permanent molars. Mutation analysis of *IRF6* was negative, suggesting that the mutations may be located outside of the coding exons or in other loci.

**Key words:** dental pulp stoneIRF6 genelimb anomalyhearing lossVan der Woude syndrome

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## INTRODUCTION

Van der Woude syndrome (VWS; OMIM 119300) is characterized by congenital lip pits, cleft lip with or without cleft palate, hypodontia, ankyloglossia, and cutaneous syndactyly of toes. The disorder is caused by mutations in interferon regulatory factor (*IRF6*) and mutations in the same gene also cause popliteal pterygium syndrome (PPS; OMIM 119500) (Kondo et al, 2002). In addition to the VWS phenotype, patients with PPS exhibit webbing of the limbs, toe nail dysplasia, and genital anomalies (Gorlin et al, 2001). From their analysis of 49 VWS mutations and 13 PPS mutations, Kondo et al (2002) suggested that VWS is caused by haploinsufficiency of *IRF6*, whereas PPS is caused by mutations that have a dominant negative effect on IRF6 function.

Recently, we reported a four-generation Thai family with a unique VWS-like phenotype. In addition to lip anomalies, hypodontia, and cleft lip and cleft palate, affected members of this family exhibited sensorineural hearing loss, large craniofacial sinuses, dental pulp stones, and minor limbs anomalies (Kantaputra et al, 2002). Given the VWS-like orofacial features in this

family, we hypothesized that affected members of the family would have a mutation in *IRF6*. With the unique anomalies in this family, we further hypothesized that the mutation would be novel and may provide clues to the structure and function of *IRF6*. Here we report a novel *IRF6* mutation found in this family and suggest that this mutation affects ear and limb development, calcification in the dental pulps, and pneumatization of craniofacial sinuses.

In addition, a new VWS family affected with abnormal shape of mandibular deciduous and permanent molars is reported. Abnormal shape of teeth has never

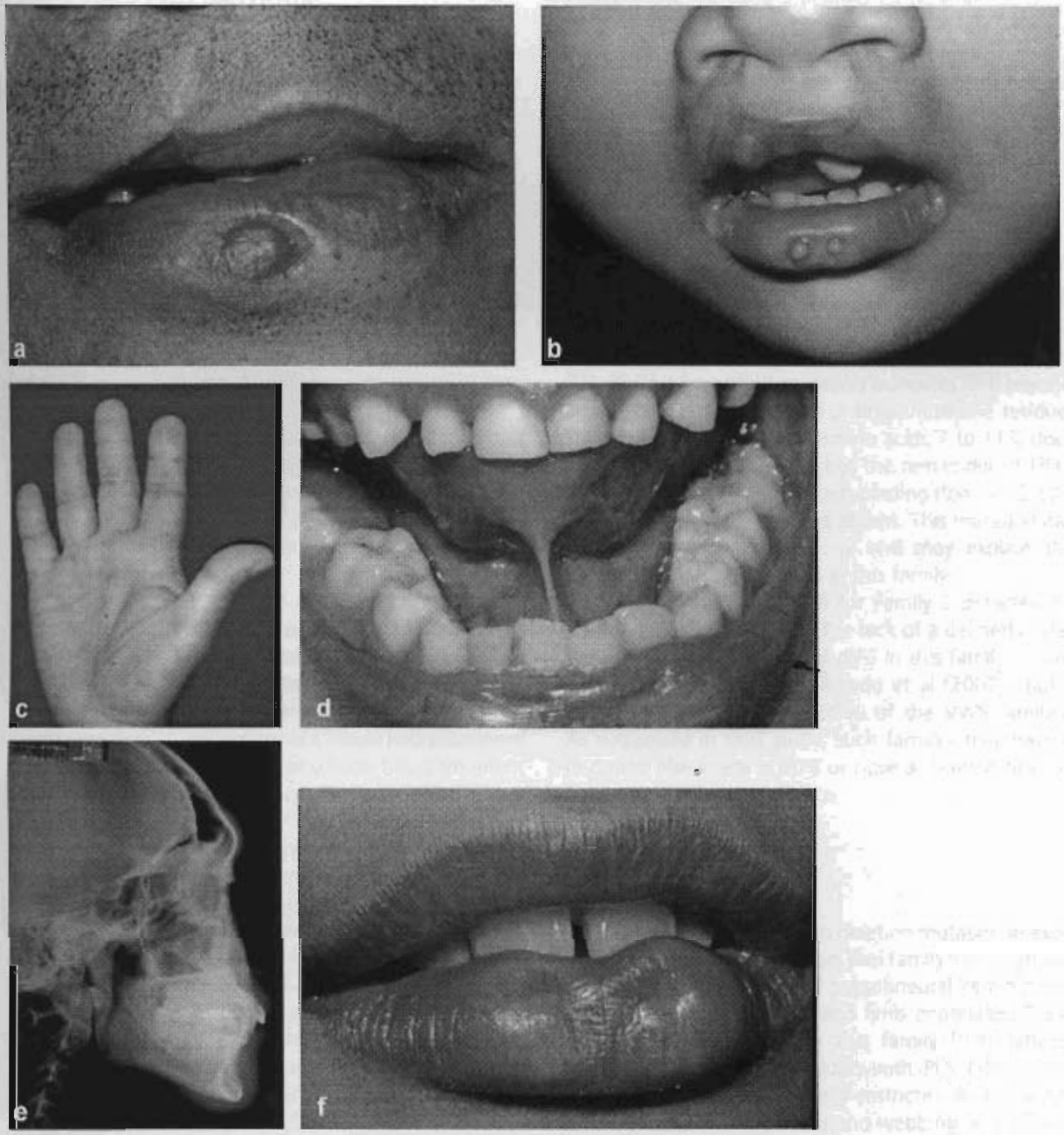


**Fig. 1** Family 1: a) Repaired unilateral cleft lip and palate. Lip pit and sulcus; b) Repaired bilateral cleft lip and palate. Lip nipples; c) Single flexion crease of the right fifth finger; d) Ankyloglossia; e) Large frontal sinus; f) Lip sulcuses.

been described to be associated with VWS. The molecular analysis of *IRF6* was negative, supporting the possibilty of VWS-causing mutations outside the coding region, or deletions or mutations in other loci (Kondo et al, 2002).

## MATERIALS AND METHODS

### *Clinical Details*

**Family 1:** This four-generation Thai family consists of 23 individuals, of which seven are affected with VWS. The clinical findings include cleft lip with cleft palate, lower lip anomalies, congenital absence of the mandibular second premolars, ankyloglossia, sensorineural hearing loss, large craniofacial sinuses, long tooth roots, and dental pulp stones. The limb anomalies include a single crease of the fifth finger (Figs. 1a-f), short middle phalanges of the fifth fingers, short middle phalanges of toe 4, short distal phalanges of toes 2 and 3, brachydactyly of fingers, cutaneous syndactyly of toes 2 and 3, and hyperphalangy of toes (Kantaputra et al, 2002).

**Family 2:** This family consists of an unaffected mother and father, and two affected children (Fig. 1a). The father died of an unrelated disease a few years ago. Clinical manifestations found in the affected daughter (Patient 2.1) were repaired unilateral cleft lip with cleft palate, supernumerary right maxillary lateral incisor, lower lip anomaly, and congenital absence of the mandibular second premolars (Figs. 1a,b). She had cosmetic surgery for her lower lip at age 12 years. The mandibular second deciduous molars had numerous secondary grooves, with prominent lingual cusps. The right mandibular first permanent molar had prominent distolingual cusps. The left mandibular first permanent molar had a very unusual shape. The occlusal table was round, with three prominent lingual cusps and many secondary grooves. The left mandibular second permanent molar was very small with an unusual occlusal configuration (Figs. 2d,e). She had crowding of the maxillary anterior teeth and anterior cross-bite as a result of the hypoplastic maxilla.

The affected son (Patient 2.2) had congenital lip pits and congenital absence of mandibular left second premolar. The shape of his right first permanent molar was unusual. The centrolingual cusp was very prominent, connecting with the mesiobuccal cusp with an oblique ridge (Fig. 2f). The left mandibular second deciduous molar was large mesiodistally, with very a prominent centrolingual cusp (Fig. 2g). The permanent teeth of both patients were affected with dental fluorosis, as a result of high fluoride consumption.

### *Mutation Analysis*

Blood was obtained from all affected individuals and the unaffected mother of Family 2 after the written informed consents were given. DNA was extracted using a conventional technique. Exons 1-8 and part of exons 9 and 10 were amplified by polymerase chain reaction (PCR), using primer sequences as reported elsewhere (Schutte et al, personal communication). The amplified products were purified (Qiagen) and directly sequenced using BigDye labelling in an ABI 377 automated DNA sequencer (Applied Biosystems).

## RESULTS

Mutation analysis of all coding exons of *IRF6* for Family 1 revealed an 11 bp deletion within exon 4, 358del(CAG GGC TCG AT), in all affected individuals (Fig. 3). This frameshift mutation truncates IRF6 beyond amino acid P119 and adds a single histidine residue. The DNA binding domain (amino acids 7 to 113) does not appear to be affected, but the remainder of IRF6, including the SMIR/IAD protein binding domain (Eroshkin and Mushegian, 1999) is absent. This mutation has not been reported previously and may explain the unique phenotypic features in this family.

Mutation analysis of *IRF6* for Family 2 detected no disease-causing mutations. The lack of a defined mutation in the coding region of *IRF6* in this family is consistent with the results of Kondo et al (2002); mutations were found in only 50% of the VWS families. As suggested in that study, such families may have a mutation elsewhere in *IRF6* or have a deletion or have a mutation in a second locus.

## DISCUSSION

We discovered a novel 11 bp deletion mutation in exon 4 of *IRF6* in a four-generation Thai family with a unique form of VWS that included sensorineural hearing loss, large craniofacial sinuses, and limb anomalies. These anomalies also distinguish this family from families with PPS. Although individuals with PPS exhibit limb anomalies, they are generally restricted to syndactyly of the second and third toes and webbing of the lower limbs which probably reflects a defect in skin development rather than skeletal development. Also, 40% of

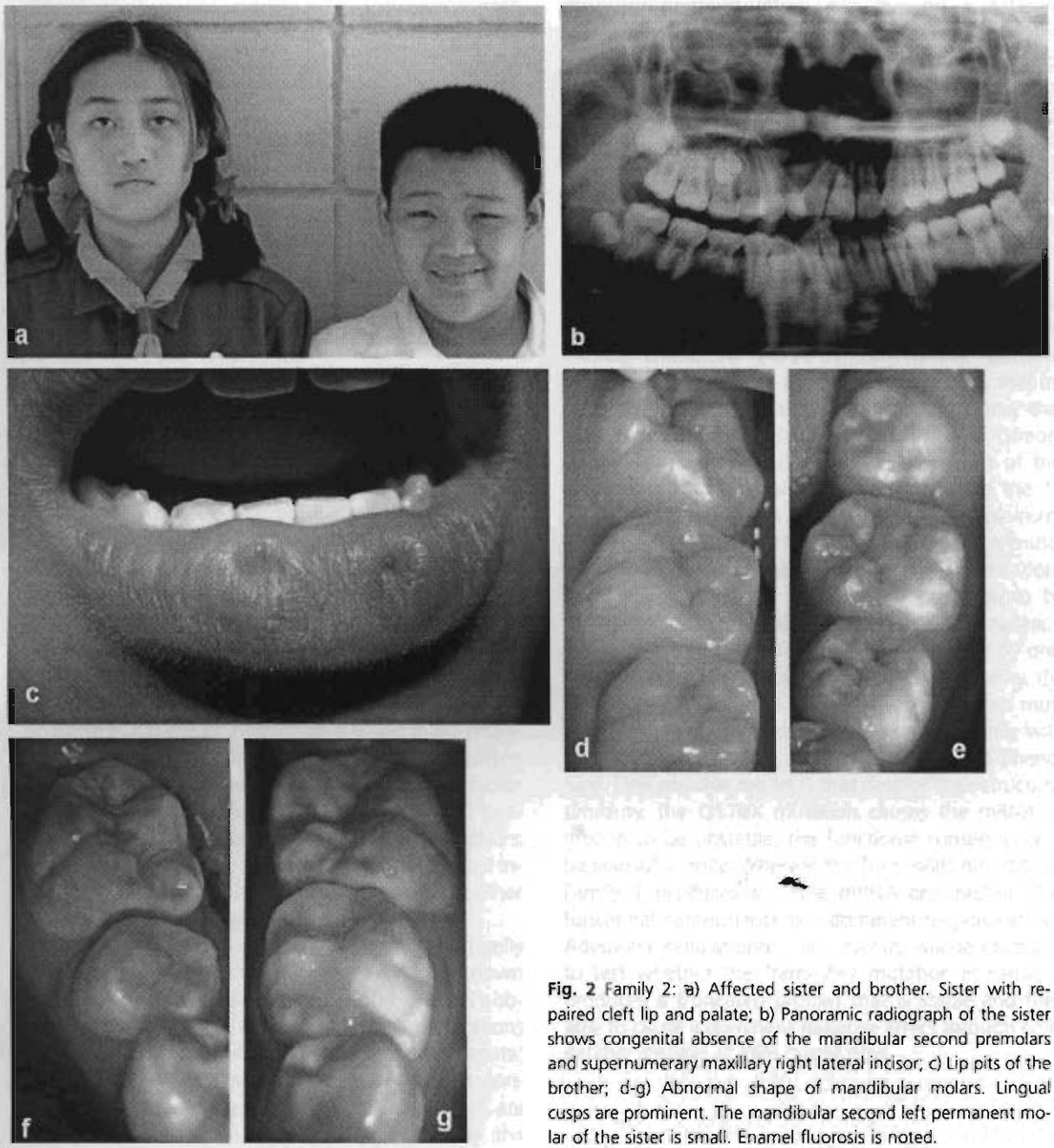


**Fig. 2** Family 2: a) Affected sister and brother. Sister with repaired cleft lip and palate; b) Panoramic radiograph of the sister shows congenital absence of the mandibular second premolars and supernumerary maxillary right lateral incisor; c) Lip pits of the brother; d-g) Abnormal shape of mandibular molars. Lingual cusps are prominent. The mandibular second left permanent molar of the sister is small. Enamel fluorosis is noted.

PPS cases exhibit genital anomalies which were not observed in this family. We conclude that this family displays a unique form of VWS. Is the novel mutation found in this family responsible for the unique phenotype?

First, we can try to exclude other genetic and environment factors that might contribute to the novel phenotype. As seen in other VWS families (Burdick et al, 1985), the phenotype in this family is extremely variable and is consistent with the existence of modifying

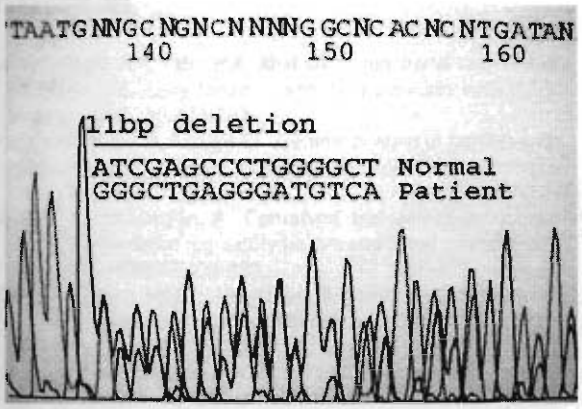


**Fig. 3** Sequence from VWS patient with deletion in exon 4 of IRF6. The reverse sequence is shown for the wild type (wt) deleted (del) alleles. The deleted allele is missing 11 nucleotides (lower case) and then picks up the wild type sequence (underlined).

factors. However, six of seven affected individuals in this family had a limb anomaly, four of six had dental anomalies and three of six exhibited deafness. The clustering of these additional anomalies in the affected members of this family suggest a single genetic factor with variable penetrance. The unique phenotype could be due to an intra-genic or linked modifier; however, no other mutations of interest were found in our mutation analysis of *IRF6* in this family. Also, the phenotype is probably not due to a common environment factor since the mothers in this family lived in different geographical regions, different socioeconomic conditions during their pregnancy, and the phenotype of most individuals with VWS in Thailand do not differ from other parts of the world (Shotelersuk et al, 2003).

Second, is this frameshift mutation in this family structurally and functionally distinct from the known PPS-causing mutation in *IRF6*? Kondo et al (2002) observed that nearly all (11/13) PPS-causing-mutations were located in exon 4. However, all of these mutations were missense mutations at residues that contacted the DNA. Consequently, the PPS mutations are predicted to abrogate DNA binding, whereas the frameshift mutation in Family 1 is predicted to leave the DNA-binding domain intact. We conclude that the frameshift mutation in Family 1 is structurally distinct from the known mutations that cause PPS. Functionally, the PPS mutations are predicted to have a dominant negative effect on IRF6 by forming a dimer with the wild type allele, but the dimer is unable to bind to its DNA sites (Kondo et al, 2002). We predict that the frameshift mutation in Family 1 could also cause a dominant negative effect, but through a different mechanism. Since the frameshift mutation is located immediately after the DNA binding domain, the truncated IRF6 could compete for binding of DNA sites, but would not be capable of transactivation. This mode of dominant negative effect was observed previously in cells that express truncated isoforms of IRF7 that only encode the DNA binding domain (Au et al, 2001). Future studies will need to address how dominant negative effects on the same gene can alter the development of distinct sets of tissues.

Third, is the frameshift mutation in this family functionally and structurally distinct from the known VWS-causing mutations in *IRF6*? Functionally, previous studies discovered that distinct microdeletions that include the entire *IRF6* cause VWS, demonstrating that VWS is caused by haploinsufficiency of *IRF6* (Sander et al, 1994; Schutte et al, 1999). In support of this conclusion, Kondo et al (2002) observed that the 49 VWS-causing mutations are consistent with haploinsufficiency, including 22 of 23 protein truncation mutations. In fact, one of the protein truncation mutations from the earlier study, Q118stop, is predicted to be nearly identical structurally to the frameshift mutation in family 1. The Q118stop mutation is shorter by only three amino acids. Despite this structural similarity, the functional consequence of these two mutations must differ, as the 6 affected individuals in the family with the Q118stop mutation exhibit the classic VWS phenotype. One possible model is that despite their structural similarity, the Q118X mutation causes the mRNA or protein to be unstable; the functional consequence is haploinsufficiency. Whereas the frameshift mutation in Family 1 produces a stable mRNA and protein; the functional consequence is a dominant negative effect. Additional cellular and *in vivo* studies will be necessary to test whether the frameshift mutation in Family 1 produces a truncated protein that is stable and then able to cause a dominant negative effect through competitive binding at IRF6 target sites.

## ACKNOWLEDGMENTS

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*Clinical Report*

# A Newly Recognized Syndrome Involving Limbs, Pelvis, and Genital Organs or a Variant of Al-Awadi/Raas-Rothschild Syndrome?

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**We report on a 3-year-old Thai boy with limb, pelvic, and genital malformations. The combination of findings found in this patient is similar to that of Al-Awadi/Raas-Rothchild syndrome (AARRS) or limb/pelvis hypoplasia/aplasia syndrome. The upper limbs are more severely affected than the lower ones. Unlike that of AARRS, the radial ray is more severely affected than the ulnar ray. The presence of humeroulnar synostosis and humero-ulnar-radial synostosis and the absence of a radius distinguishes it from AARRS. The similarities and dissimilarities between the features in the present patient and other limb-pelvic hypoplasia/aplasia syndromes are discussed. The findings in this group of patients appear to demonstrate limb-pelvis-genital organ developmental field defects.** © 2004 Wiley-Liss, Inc.

**KEY WORDS: genital anomaly; limb anomaly; pelvic hypoplasia; radius-ulna-humerua synostosis; ulna-humerus synostosis**

## INTRODUCTION

The syndrome of severe limb, pelvic, and genital malformation has been described in Al-Awadi/Raas-Rothschild syndrome (AARRS) or limb/pelvis-hypoplasia/aplasia syndrome [Al-Awadi et al., 1985; Richieri-Costa, 1987; Raas-Rothschild et al., 1988; Camera et al., 1993; Teebi, 1993], Roberts syndrome (RS) [Van Der Berg and Francke, 1993], Schinzel phocomelia syndrome [Olney et al., 2001], and femur-fibula-ulna (FFU) syndrome [Zlotogora et al., 1983] (Table I). We report on a 3-year-old boy affected with severe malformation of limbs, pelvis, and genital organs. The pattern of malformation appears unique but shares many findings with AARRS.

## CLINICAL REPORT

A 3-year-old Thai boy was seen at the Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand for physical evaluation and genetic counseling (Fig. 1a). He had multiple congenital anomalies and his parents were concerned if the inborn child of the pregnant mother would be affected with the same condition. He was the only child of healthy nonconsanguineous parents. Karyotype was 46,XY.

On examination at age 4 years, weight, height, and OFC were 8 kg (<3 centile), 64 cm (<3 centile), and 43.5 cm (<3 centile), respectively. His head and face were unremarkable. Severe malformation of bilateral upper and lower limbs was noted. The arms were severely short, making the hands appear connected with the shoulder (Fig. 1a,b). The left arm was more severely affected than the right one. Its forearm appeared absent. The left thumb was small, short, and free floating. Flexion contracture was observed at the proximal interphalangeal joints (IPJ) of fingers 2–4 (Fig. 1b). The right thumb was small and slender. Flexion contracture of the right fingers was observed at the proximal IPJ 3 and 4. Thenar muscles of both hands were hypoplastic (see the online Fig. 1c at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html).

His penis was unremarkable. There was absence of scrotum and testicles (Fig. 1a,d) (see the online Fig. 1d at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). The testes were neither palpable nor detected by ultrasonography. Rugae was evident. Both kidneys appeared normal. The anus was anteriorly displaced. A sacral dimple was observed. The legs were short. The right and left leg lengths were 24 cm (<3 centile) and 23 cm (<3 centile), respectively. The lower legs appeared connected with the pelvis without the presence of thighs and knees. Feet appeared normal (Fig. 1a).

At age 3 years, he walked independently for 10 steps and sat without support. He could use a spoon and get dressed, but could not undress completely by himself. He sang a song and asked for the meanings of words. He was very friendly and played cooperatively with other children. His gross and fine motor skill appeared delayed as the result of his limb anomalies.

Radiographic examination revealed severely short and malformed left humerus, fusing with the very short ulna (Fig. 2a). There was no evidence of a fusion line. The left radius was completely absent. Capitate-hamate synostosis and pisiform bone were visible. The left first proximal and distal phalanges were very short, and its metacarpal rudimentary (Fig. 2a,b) (see the online Fig. 2b at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). Right humerus was severely short, malformed, and bifurcated. It appeared fused with the severely shortened radius and short ulna with fusion lines. Capitate, hamate, and a very small ossification center were evident. The first metacarpal, proximal, and distal phalanges were short and slender (Fig. 2c,d) (see the online Fig. 2d at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). Eleven ribs were observed on each side. The scapulae appeared hypoplastic. The spine was unremarkable (Fig. 3a).

This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html.

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TABLE I. Important Features of Limb-Pelvis Hypoplasia/Aplasia Syndromes

| Syndromes anomalies | AARRS | Schinzel phocomelia | Roberts syndrome | FFU | Femoral hypoplasia-unusual facies | Present case |
|---|---|---|---|---|---|---|
| Mode of inheritance | AR | AR | AR | Sporadic | Sporadic | Sporadic |
| Brain malformation | − | − | + | − | − | − |
| Skull defect | − | ++ | + | − | − | − |
| Mental retardation | − | − | ++ | − | − | − |
| Face | | | | | | |
| Facial involvement | ++ | ++ | ++ | − | ++ | − |
| Facial hemangioma | + | − | ++ | − | − | − |
| Malformed ears | + | + | ++ | − | ++ | − |
| Abnormal eyes | + | − | ++ | − | ++ | − |
| Cleft lip | − | + | ++ | − | ++ | − |
| Cleft palate | − | + | + | − | + | − |
| Thorax | | | | | | |
| Abnormal clavicles | + | − | − | − | − | − |
| Abnormal ribs | + | − | + | − | − | − |
| Thoracic dystrophy | ++ | − | − | − | − | − |
| Upper limbs | | | | | | |
| Hypoplastic humerus | − | − | ++ | ++ | + | − |
| Bifurcated humerus | − | − | − | ++ | − | + |
| Phocomelia | ++ | ++ | ++ | + | − | + |
| A/hypoplastic thumb | + | − | + | − | − | + |
| Radial ray predominance | − | − | − | − | − | + |
| Ulnar ray predominance | ++ | ++ | ++ | + | − | − |
| Contracture of the elbow | ++ | − | ++ | − | ++ | − |
| Radius-humerus synostosis | ++ | − | − | ++ | + | + |
| Ulna-humerus synostosis | − | − | − | − | − | + |
| Radius-ulna-humerus synos | − | − | − | − | − | + |
| Hands | | | | | | |
| Very mobile wrist | + | − | − | − | − | − |
| Carpal a/hypoplasia | + | − | ++ | + | − | + |
| Carpal synostosis | + | − | − | − | − | + |
| Ectrodactyly | + | + | − | − | − | − |
| Malformed digits | ++ | + | − | ++ | − | + |
| Contracture of fingers | − | + | − | − | − | + |
| Oligodactyly | ++ | ++ | ++ | ++ | − | − |
| Genital organs | | | | | | |
| Cryptorchidism | ++ | + | + | − | + | + |
| Micropenis | ++ | + | − | − | + | − |
| Enlarged genitalia | − | − | ++ | − | − | na |
| A/hypoplastic scrotum | + | ++ | + | − | + | + |
| Hypoplastic labia | − | − | − | − | + | na |
| Upwardly displaced genitalia | + | − | − | − | − | − |
| Anal anomalies | + | − | − | − | − | + |
| Pelvis | | | | | | |
| Severely malformed pelvis | ++ | ++ | ++ | − | + | + |
| Lower limbs | | | | | | |
| A/hypoplastic femur | ++ | ++ | − | ++ | ++ | + |
| A/hypoplastic fibula | ++ | ++ | ++ | ++ | + | + |
| Unilateral involvement predominance | − | − | − | ++ | − | − |
| A/Hypoplastic tibia | ++ | ++ | ++ | + | − | + |
| Bifurcated femur | + | − | − | − | − | − |
| Stick-like appendage | ++ | ++ | − | − | − | − |
| Feet | | | | | | |
| Ectrodactyly | − | + | − | − | − | − |
| Polydactyly | − | + | − | − | + | − |
| Syndactyly | − | + | − | + | + | − |
| Oligodactyly | ++ | − | − | ++ | − | − |
| Hypoplastic feet | ++ | ++ | ++ | − | ++ | − |
| Miscelleneous | | | | | | |
| Hetrochromatin repulsion | − | − | ++ | − | − | − |
| Hypoplastic nails | ++ | ++ | + | − | − | − |
| Congenital heart defects | − | − | + | − | + | − |
| Kidney anomalies | + | + | + | − | + | − |
| Spine | − | + | − | ++ | ++ | |

Remarks: Common features, ++; occasional features, +; never been reported, −; not available, na.

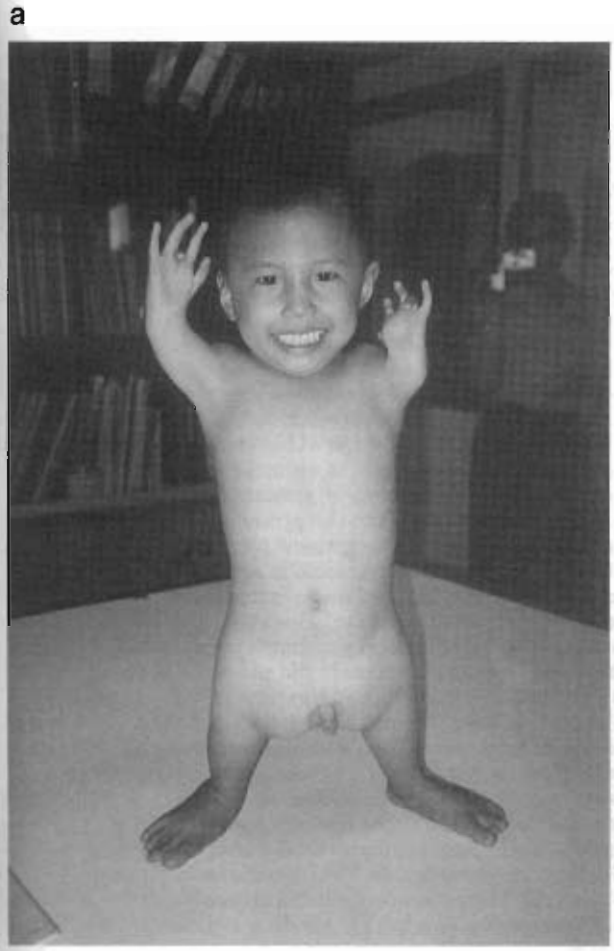


Fig. 1. **a**: Phocomelic appearance of the upper limbs, hypoplastic pelvis, and absent thighs and knees. **b**: Left hand. Hypoplastic thumb, flexion contracture of PIPJ 2–4. **c**: Right hand (see the online Fig. 1c at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). **d**: Cryptorchidism. Hypoplastic scrotum (see the online Fig. 1d at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html).

The pelvis was severely hypoplastic. The pubic bones were completely absent. The iliac bones appeared very small and narrow. The acetabula were triangle-shaped, comprising of iliac bones and ischia. The femora and fibulae were completely

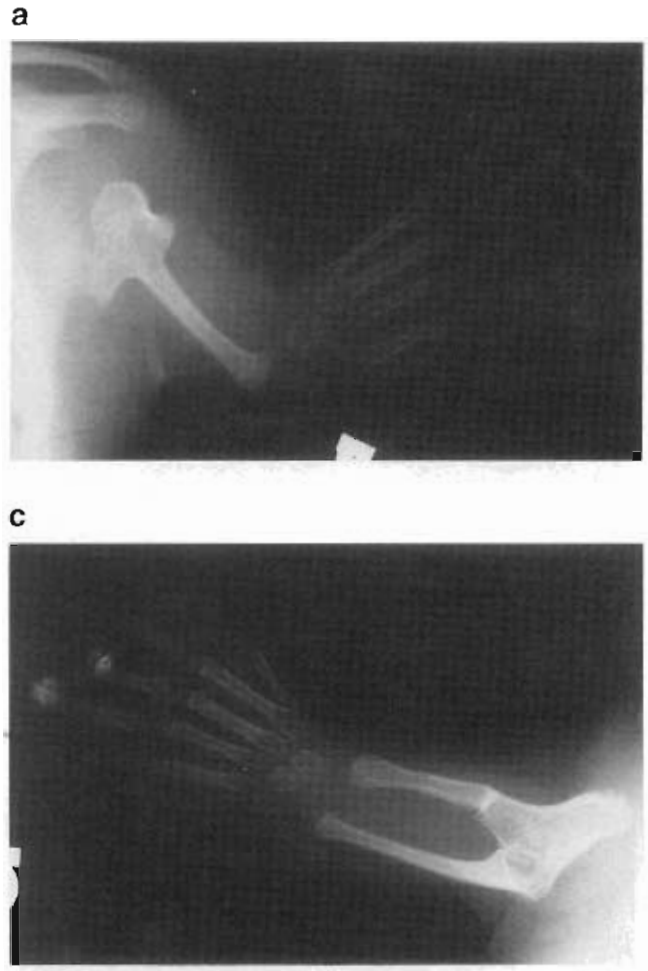


Fig. 2. Radiographs of upper limbs. **a**: Left arm. Malformed humerus fused with hypoplastic ulna. Humerus is dislocated from the shoulder. **b**: Left hand (see the online Fig. 2b at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html). Rudimentary first metacarpal. Hypoplastic first proximal phalanx. Capitate-hamate synostosis. Pisiform is observed. **c**: Right arm. Malformed humerus appears bifurcated. Humerus-radius-ulna synostosis. **d**: Right hand (see the online Fig. 2d at http://www.interscience.wiley.com/jpages/0148-7299/suppmat/index.html).

absent. Proximal tibiae were malformed and their shafts slender. They articulated distally with the tali. The proximal tibiae were dislocated from the poorly developed acetabula (Fig. 3b).

## DISCUSSION

The combination of findings found in our patient appears unique. However, some of the findings overlap with those of AARRS or limb/pelvis-hypoplasia/aplasia syndrome. AARRS is an autosomal recessive disorder, which is characterized by the presence of profound limb and pelvic deficiency, thoracic dystrophy, and genital anomalies. Limb anomalies consist of short and bowed radii, aplasia of ulna and fibula, hypoplastic or aplastic femur, hypoplasia/aplasia of tarsals, carpals, metatarsals, metacarpals, and phalanges [Al-Awadi et al., 1985; Richieri-Costa, 1987; Raas-Rothschild et al., 1988; Camera et al., 1993; Kumar et al., 1997]. The findings found in our patient appear similar to those of AARRS. However, the presence of humeroulnar synostosis, humero-ulnar-radial synostosis, and absence of radius distinguishes it from having AARRS. In addition, AARRS affects predominantly the ulnar

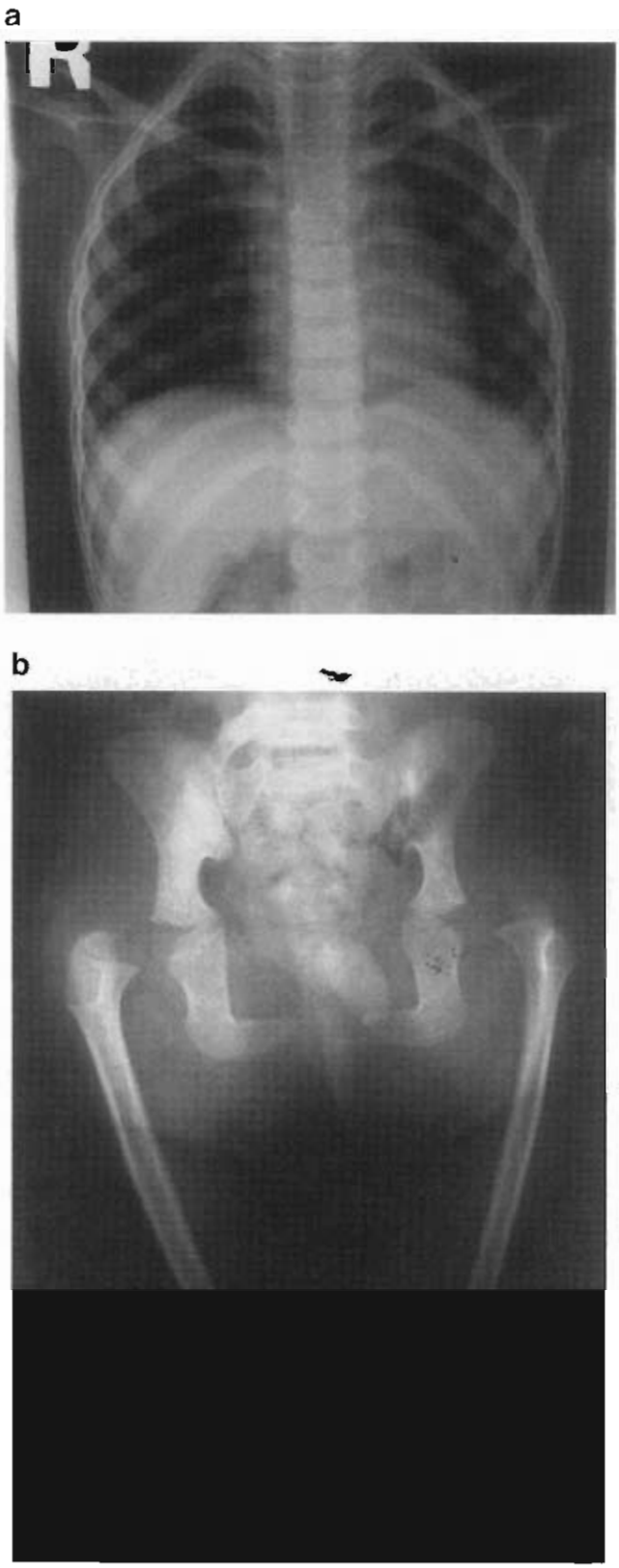


Fig. 3. **a**: Hypoplastic scapulae and dislocated left humerus. Normal spines and thoracic cage. **b**: Very small and narrow iliac bones. Severely hypoplastic pubic bones with absent superior pubic rami. Poorly formed acetabula comprise of iliac and ischium bones. Femora and fibulae are absent. Tibiae are slender with malformed proximal heads.

ray and some toes are almost always missing. In the present patient the radial ray was more severely affected and the feet were normal. Regarding hand anomalies, severity of the hand anomalies in the present patient appears to be very mild compared to those of most cases of AARRS. Hypoplastic nail which is common in AARRS was not present in our patient [Al-Awadi et al., 1985; Camera et al., 1993; Farag et al., 1993]. We are not convinced that unusual facies are component of the AARRS as they were not remarkable in the previously reported cases. We believe that the case reported by Mollica et al. [1995] represented a new autosomal recessive facio-skeleto-genital syndrome, not AARRS, as cleft lip and palate does not seem to be a component of AARRS.

The absence of craniofacial anomalies, especially craniosynostosis and cleft lip and palate, distinguishes it from Roberts syndrome (RS) and femoral hypoplasia-unusual facies syndrome. In addition, the characteristic cytogenetic phenomenon of RS consisting of replusion of heterochromatic regions near centromeres, particularly of chromosomes 1, 9, and 16 and splaying of the short arms of acrocentric chromosomes and of distal Yq was not found in the present patient [Van Der Berg and Francke, 1993]. The present patient resembled patients affected with Schinzel phocomelia syndrome, by virtue of his intercalary limb deficiencies, genitourinary anomalies, and severe pelvic malformation. However, the absence of skull defects rules out Schinzel phocomelia syndrome [Olney et al., 2001]. The pregnancy history did not include the ingestion of thalidomide [Smithhells and Newman, 1992] or the presence of diabetic mellitus [Grix, 1982]. Both conditions could lead to severe limb malformation and approximately 25% of patients with femoral hypoplasia-unusual facies syndrome have diabetic mothers [Grix, 1982; Urban et al., 1997]. The findings of femoral defects and upper limb anomalies found in the present patient can be found in femur-fibula-ulna (FFU) syndrome, but the limb involvement in FFU syndrome is more likely to be unilateral. The presence of genital anomalies and severe radial involvement rules out FFU syndrome [Zlotogora et al., 1983; Sorge et al., 1995].

The constellation of findings found in this patient is unique, even though the majority of them overlap with that of AARRS. However, the possibility of being its variant cannot be excluded. The anomalies found in AARRS, Schinzel phocomelia, Roberts syndrome, and femoral hypoplasia-unusual facies syndrome, and the present case appear to demonstrate limb-pelvis-genital organ developmental field defects.

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