was also responsible for the patient's gastrointestinal tract symptom: intussusception. Although some researchers have reported an association between intussusception and dietary factors,[7] no reports have suggested an association between intussusception and a type 1 food allergy. The pathogenesis of colonic invagination following ingestion is uncertain. One possible explanation of invagination is that ingestion of a food allergen provokes intestinal contraction,[5] which eventually results in invagination of the gastrointestinal tract.[8] Although there remains a possibility that a food allergy and intussusception occurred merely coincidently, the probability that these two rare events independently occurred is considerably low and we believe that a food hypersensitivity and intussusception in this patient could not be explained by chance alone.

Another atypical presentation in the present patient was the site of intussusception.[9,10] A previous study reported that, in children, colo-colic intussusceptions accounted for <3% of cases and could be related to PLP.[9] Enema was not suggestive of the presence of PLP in the present patient, but we might miss a PLP that could be identified on other imaging modalities such as ultrasonography. Although ultrasonography is widely used for the management of intussusception,[11] it is operator dependent and none of the present staff was sufficiently skilled as to detect intussusception due to an identifiable cause. This is one of the reasons why ultrasonography was not done, which may be a limitation of this report.

In conclusion, we report a case of intussusception secondary to anaphylactic reaction to salmon roe. This case report is the first to suggest a link between intussusception and a hypersensitive reaction to food, suggesting food allergy as a secondary cause of childhood intussusception. Additionally, we would like to emphasize that careful evaluation of an acute abdomen is critical, even when other potential causes such as a food allergy are present.

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Novel *CTSK* mutation resulting in an entire exon 2 skipping in a Thai girl with pycnodysostosis

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Abstract Pycnodysostosis is a rare autosomal recessive skeletal dysplasia characterized by osteosclerosis, short stature, acro-osteolysis of the distal phalanges, bone fragility and skull deformities. Mutations in the *cathepsin K (CTSK)* gene, which encodes a lysosomal cysteine protease highly expressed in osteoclasts, have been found to be responsible for the disease.

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We identified a Thai girl with pycnodysostosis. Her parents were first cousins. Polymerase chain reaction sequencing of the entire coding regions of *CTSK* of the proband's complementary DNA revealed that the whole exon 2 was skipped. We subsequently amplified exon 2 using genomic DNA, which showed that the patient was homozygous for a c.120G>A mutation. The mutation was located at the last nucleotide of exon 2. Its presence was confirmed by restriction enzyme analysis using *Ban*I. The skipping of exon 2 eliminates the normal start codon. The mutation has never been previously reported, thus the current report expands the *CTSK* mutational spectrum.

Key words cathepsin K, *CTSK*, exon skipping, mutation, pycnodysostosis.

Pycnodysostosis (MIM 265800) is a rare autosomal recessive skeletal dysplasia with less than 200 patients reported worldwide since the first description of the phenotype in 1962.[1] Mutations in the *CTSK* gene encoding cathepsin K, a member of the papain protease family, are responsible for the disease.[2,3] Since 1962, *CTSK* mutations have been identified in patients of various ethnic backgrounds.[4–8] Here, we report a Thai girl with pycnodysostosis and a novel causative *CTSK* mutation.

Case report

Clinical findings

A 7-year-old Thai girl, born in the northeast of Thailand, came to our clinic for the first time for evaluation of short stature. She had inappropriate fractures of left clavicle, left tibia and right tibia at the age of 2, 3, and 5 years, respectively. She complained of occasional bone pains at these previously fractured sites. She did well in the classroom, except in sports. Her parents were first cousins. Her height was 94.7 cm (<–5.1 SD), her weight was 12.7 kg (<–3.1 SD), and her head circumference was 49.5 cm (–1 SD). She had open anterior and posterior fontanels, widening of sagittal suture, frontal and parietal bossing, prominent eyes, beaked nose, obtuse mandibular angle, grooved palate, multiple dental caries, delayed eruption of permanent teeth, retention of primary teeth, and micrognathia. No blue sclera or scoliosis were seen. Examination of her hands showed brachydactyly and dysplastic nails (Table 1 and Fig. 1a,b). Skeletal survey showed generalized increased bone density, open fontanels, clavicular dysplasia, and bilateral tibial fractures (Fig. 1c,d).

CTSK *mutation analysis*

After written informed consent was obtained, DNA and RNA of the proband and her mother were extracted from peripheral blood using Qiagen Kit (Qiagen Inc, Valencia, CA, USA). Samples of the proband's father were unavailable. The proband's RNA was reverse transcribed using ImProm-II reverse transcriptase (Promega, Madison, WI, USA). The entire coding region of *CTSK* was amplified using a forward primer F1 (5′-GCCACCAAAGACAGTCTTGT-3′) and a reverse primer R1 (5′- ATGGGTGGAGAGAAGCAAAG-3′). Polymerase chain reaction (PCR) amplification was carried out using the following condition: 35 cycles of 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min. The PCR products were subsequently amplified by semi-nested-PCR using a forward primer F1 and a reverse primer R2 (5′-CTCAGTATCACCACATCTGC-3′). PCR amplification was carried out using the following condition: 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, OH, USA), according to the manufacturer's recommendations, and sent for direct sequencing at Macrogen (Seoul, Korea).

The identified mutation was further analyzed by sequencing of genomic DNA and restriction enzyme digestion. Exon 2 and its intronic flaking regions of *CTSK* from the proband, her mother and an unaffected control were amplified using a forward primer CTSK-ex2-F (5′-TTGCCTAAATCTCCCGAGAG-3′) and a reverse primer CTSK-ex2-R (5′-GAGGGACTGATTTGCTT GGA-3′). PCR amplification was carried out using the following amplification condition: 35 cycles of 94°C for 1 min, 60°C for

Table 1 Clinical features of previously reported patients with pycnodysostosis and our patient

Features	Previously reported cases[9]	Our patient
Short stature	100% (93/93)	Present
Increased bone density	100% (86/86)	Present
Open fontanels and sutures	100% (68/68)	Present
Frontal and parietal bossing	100% (67/67)	Present
Fractures	95% (65/68)	Present
Obtuse mandibular angle	100% (63/63)	Present
Hypoplasia of the jaws	100% (53/53)	Present
Stubby hands and feet	100% (43/43)	Present
Prominent eyes	100% (34/34)	Present
Grooved palate	100% (28/28)	Present
Dysplastic nails	100% (26/26)	Present
Clavicular dysplasia	100% (24/24)	Present
Beaked nose	100% (16/16)	Present

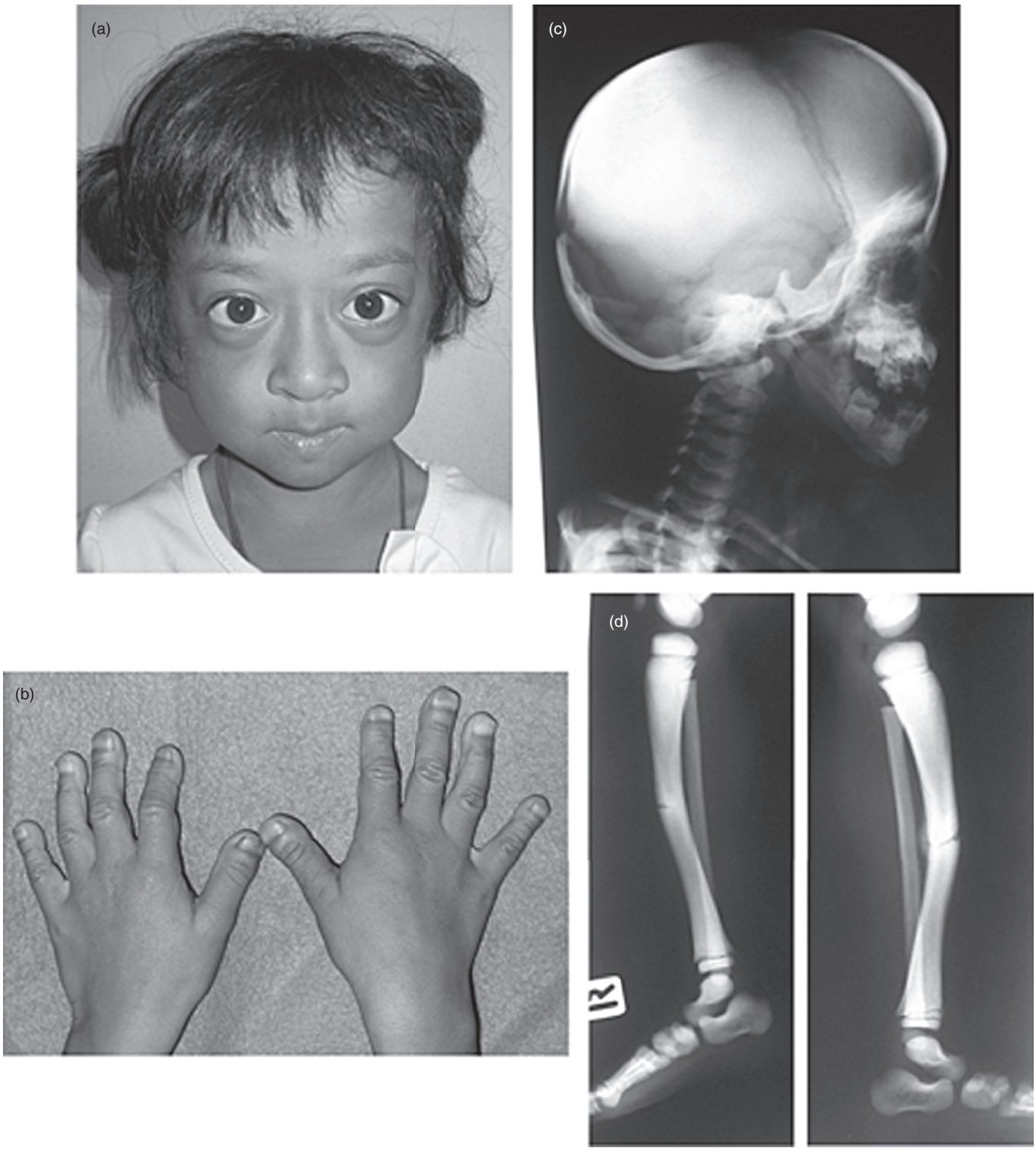

Fig. 1 Clinical and radiological features of a Thai girl with pycnodysostosis. (a) Prominent forehead and ocular proptosis. (b) Stubby hands with grooved and flattened nails. (c) Open anterior and posterior fontanels at 7 years of age. (d) Tibial fractures and osteosclerosis.

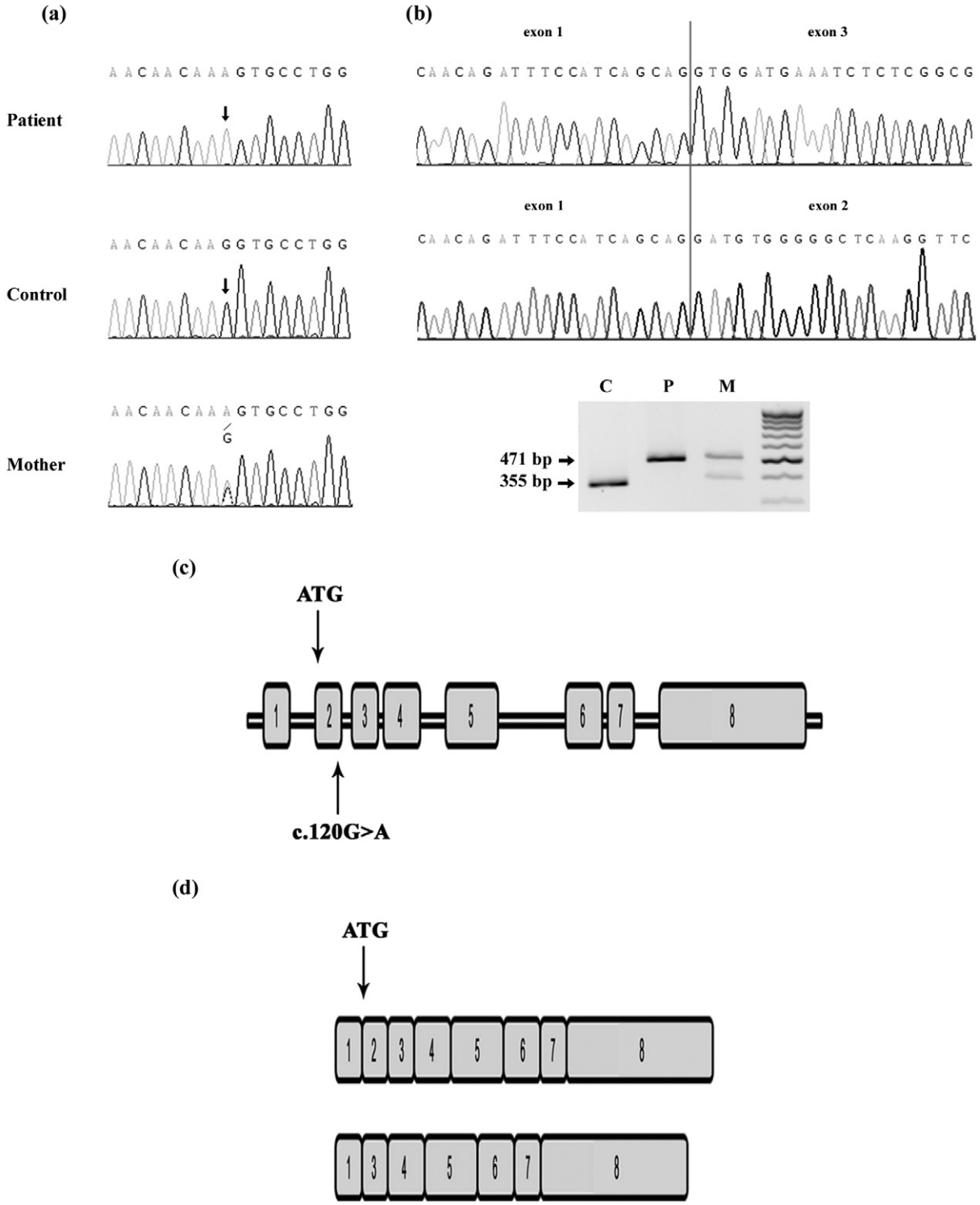

Fig. 2 Mutation analysis of the *CTSK* gene. (a) Electropherograms of genomic DNA of exon 2 of the *CTSK* gene show a G–A homozygous change at the nucleotide position 120 in the patient (upper panel), a normal sequence in an unaffected control (middle panel), and a heterozygous change in the proband's mother (lower panel). (b) Electropherograms of complementary DNA show a skip of exon 2 in the proband (upper panel) and a normal splicing in an unaffected control (middle panel). Restriction enzyme digestion analysis (lower panel) shows that the mutant allele which lacks the cleavage site for the restriction endonuclease *Ban*I has the undigested 471-bp band. The wild-type allele was digested and resulted in the 355-bp band. The other digested 116-bp band is not visualized. The mother carried a heterozygous c.120G>A mutation. (c) Schematic diagram of the *CTSK* gene showing genomic structure of *CTSK* with 8 exons. Grey boxes indicate non-coding sequences. The arrow shows the start codon in exon 2 and the c.120G>A mutation in our patient at the last nucleotide of exon 2. (d) Structure of *CTSK*'s mRNA: the upper represents the wild-type and the lower represents a skip of exon 2 eliminating the start codon found in our patient. C, control; P, patient; M, mother.

45 s, and 72°C for 1 min. PCR products were treated with ExoSAP-IT and sent for direct sequencing.

The identified mutation obliterated a *Ban*I site. The amplified product of *CTSK* exon 2 was digested by *Ban*I and separated by agarose gel electrophoresis with direct visualization using ethidium bromide.

Results

PCR-sequencing of the proband's complementary DNA (cDNA) of *CTSK* revealed that the whole exon 2 was skipped (Fig. 2a,b). Next, we amplified exon 2 using genomic DNA. The result showed that the patient was homozygous for a c.120G>A mutation, located at the last nucleotide of exon 2 (Fig. 2c). No other sequence alterations were found. PCR-sequencing of the mother's genomic DNA showed that she was heterozygous for the c.120G>A mutation. These were confirmed by restriction enzyme analysis using *Ban*I (Fig. 2).

Discussion

Pycnodysostosis is a potentially life-threatening genetic disease. Our patient had frequent fractures but the diagnosis of her underlying disease had not been given until she visited our genetics clinic at 7 years of age. Several findings, including a history of parental consanguinity, dysmorphic facial features and hand abnormalities, and plain X-rays, led to a clinical diagnosis of pycnodysostosis.[9] Raising awareness among physicians could lead to diagnosis, treatment, prevention of complications and proper genetic counseling at a younger age.

The *CTSK* gene, encoding a lysosomal cysteine protease and responsible for pycnodysostosis, was first identified by Gelb *et al*.[2] Until now, at least 34 different mutations in 60 unrelated families have been described.[9] These include 24 missense, four frameshift, three nonsense, two splicing and one termination codon mutations. Approximately 70% of reported mutations were located in the mature domain. Hotspot mutations of *CTSK* were Arg241 in exon 6 and Ala277 in exon 7.[9,10] In the present study, instead of sequencing genomic DNA of the *CTSK* gene, which contains eight exons, we chose to first sequence cDNA, using two primer pairs. It efficiently identified a homozygous skipping of exon 2. We then PCR-amplified genomic DNA of exon 2 and its intronic flanking regions. This identified a c.120G>A mutation, occurring at the last nucleotide of exon 2. PCR-sequencing of the mother's genomic DNA showed that she was heterozygous for the mutation. It was confirmed by restriction enzyme analysis.

If this mutation at the last nucleotide of exon 2 was only affected with the exon–intron splicing,[11] there might have been aberrant RNA species resulting from an alternative splice, which incorporated the whole or a part of intron 2. Nonetheless, we identified only the mRNA with skipping of exon 2. Using the ESE finder program, http://rulai.cshl.edu/tools/ESE2/, we found that this c.120G was also an exonic splicing enhancer. Interestingly, the c.120G>A mutation nullified it as an exonic splicing enhancer. Therefore, the exon 2 was presumably not recognized as an exon and was skipped. Skipping of the 121-bp exon 2 results in elimination of the normal start codon and an expected absence of the wild-type CTSK protein. The c.120G>A has never been previously reported.

In conclusion, we report a Thai patient with pycnodysostosis with a novel c.120G>A mutation in the *CTSK* gene, leading to skipping of the whole exon 2 and elimination of the normal start codon, expanding its mutational spectrum.

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