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Original Article

Novel and recurrent *COMP* gene variants in five Japanese patients with pseudoachondroplasia: skeletal changes from the neonatal to infantile periods

Kosei Hasegawa[1], Natsuko Futagawa[1, 2], Yuko Ago[1], Hiroyuki Miyahara[2], Daisuke Harada[3], Mari Miyazawa[4], Junko Yoshimoto[1], Kenji Baba[1], Tadashi Moriwake[5], Hiroyuki Tanaka[6], and Hirokazu Tsukahara[2]

[1]*Department of Pediatrics, Okayama University Hospital, Okayama, Japan*
[2]*Department of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*
[3]*Department of Pediatrics, JCHO Osaka Hospital, Osaka, Japan*
[4]*Department of Pediatrics, Kochi Health Sciences Center, Kochi, Japan*
[5]*Department of Pediatrics, Iwakuni Clinical Center, National Hospital Organization, Iwakuni, Japan*
[6]*Department of Pediatrics, Okayama Saiseikai General Hospital, Okayama, Japan*

Highlights

- Five *COMP* variants were identified in five Japanese pseudoachondroplasia patients.
- Four of five *COMP* variants were novel and involved a substitution of aspartic acid.
- Skeletal changes from the neonatal to infantile stage were retrospectively followed in one patient.

Abstract. Pseudoachondroplasia (PSACH) is an autosomal dominant skeletal dysplasia caused by pathogenic variants of cartilage oligomeric matrix protein (COMP). Clinical symptoms of PSACH are characterized by growth disturbances after the first year of life. These disturbances lead to severe short stature with short limbs, brachydactyly, scoliosis, joint laxity, joint pain since childhood, and a normal face. Epimetaphyseal dysplasia, shortened long bones, and short metacarpals and phalanges are common findings on radiological examination. Additionally, anterior tonguing of the vertebral bodies in the lateral view is an important finding in childhood because it is specific to PSACH and normalizes with age. Here, we report five Japanese patients with PSACH, with one recurrent (p.Cys351Tyr) and four novel heterozygous pathogenic COMP variants (p.Asp437Tyr, p.Asp446Gly, p.Asp507Tyr, and p.Asp518Val). These five pathogenic variants were located in the calcium-binding type 3 (T3) repeats. In four of the novel variants, the affected amino acid was aspartic acid, which is abundant in each of the eight T3 repeats. We describe the radiological findings of these five patients. We also retrospectively analyzed the sequential changes in the vertebral body and epimetaphysis of the long bones from the neonatal to infantile periods in a patient with PSACH and congenital heart disease.

Key words: infant, skeleton, spine, cartilage, growth

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Corresponding author: Kosei Hasegawa, M.D., Ph.D., Department of Pediatrics, Okayama University Hospital, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
E-mail: haseyan@md.okayama-u.ac.jp

Introduction

Pseudoachondrplasia (PSACH) is a rare autosomal dominant skeletal dysplasia caused by pathogenic variants of cartilage oligomeric matrix protein (COMP), which is one of the extracellular cartilage matrices (1–3). The COMP protein consists of an N-terminal domain, which is important for pentamerization of the protein, four type 2 epidermal growth factor-like domains, eight type 3 calcium binding repeat (T3 repeat) domains, and a C-terminal domain (4). COMP is expressed in various tissues, such as cartilage, ligaments, and tendons. The COMP protein interacts with cartilage extracellular matrix proteins, such as collagen I, II, IX, XII, and XIV; fibronectin; matrilins; and aggrecan. It also interacts with cell surface proteins, extracellular proteases, growth factors, and proteins of the complement system.

The clinical symptoms of PSACH include a short-limbed short stature, brachydactyly, joint laxity, joint pain since childhood, scoliosis, and a normal face. PSACH does not affect intelligence. Early osteoarthritis is also observed during childhood. Height is normal during the infantile period, but is severely impaired thereafter, and a severe short stature is present in adulthood (3, 5, 6).

The radiological skeletal characteristics of PSACH include metaphyseal and epiphyseal dysplasia of the shortened long and tubular bones (3). In the diagnosis of PSACH, the anterior tongue of the vertebral bone is also an important finding. This represents a delay in the ossification of the upper and lower segments in the anterior part of the vertebrae and is only observed during childhood. However, skeletal changes from the neonatal to infantile period have rarely been reported (7) because the diagnosis of PSACH is generally made from a waddling gait after the beginning of walking.

Here, we report the growth and radiological characteristics of five Japanese patients with PSACH. In these patients, we identified one recurrent and four novel COMP variants. We also report sequential radiological changes in the vertebral body and epimetaphysis of the long bones from the neonatal to infantile periods in one patient.

Table 1. Growth and genetic characteristics of the five patients with pseudoachondroplasia

	Patient number	1	2	3	4	5
Sex		Male	Female	Male	Male	Male
At birth	Gestational age	39 wk, 2 d	38 wk	40 wk	38 wk, 3 d	41 wk
	Height, cm (SDS)	49.9 (0.44)	50.0 (0.77)	49.0 (0.02)	47.5 (−0.68)	46.5 (−1.15)
	Weight, g (SDS)	3375 (0.83)	3084 (0.30)	3242 (0.45)	2750 (−0.56)	2954 (−0.10)
At the first visit	Age	4 y, 9 mo	3 y, 11 mo	4 y, 1 mo	2 y, 8 mo	2 y, 6 mo
	Height, cm (SDS)	89.5 (−3.70)	73.3 (−6.81)	85.0 (−4.02)	71.2 (−5.94)	80.5 (−2.81)
	Weight, kg (SDS)	14.0 (−1.64)	9.8 (−4.06)	14.3 (−0.76)	10.4 (−2.04)	10.1 (−2.11)
Recent growth date	Age	4 y, 9 mo	5 y, 9 mo	27 y, 10 mo	4 y, 10 mo	6 y, 3 mo
	Height, cm (SDS)	89.5 (−3.70)	78.5 (−7.18)	111.0 (−10.31)	76.2 (−6.92)	87.5 (−5.64)
	Weight, kg (SDS)	14.0 (−1.64)	11.0 (−5.28)	44.3 (−3.01)	11.8 (−3.48)	14.9 (−2.69)
Complication		None		Kyphosis	None	TA
Results of *COMP* gene analysis	Exon	10	13	13	14	14
	Base substitution	c.1052G>A	c.1309G>T	c.1337A>G	c.1519G>T	c.1553A>T
	Amino acid change	p.Cys351Tyr (12)	p.Asp437Tyr	p.Asp446Gly	p.Asp507Tyr	p.Asp518Val
	De novo or familial			*De novo*		
	Novel or recurrent	Recurrent		Novel		
	Pathogenic variant found in the same amino acids as in PSACH and MED	None	p.Asp437 to Gly (13) and Asn (14)	p.Asp446 to Asn (15) and His (10)	p.Asp507 to Asn* (16) and Gly (17)	p.Asp518 to Asn (17), Gly (18), His (13), and Tyr* (19)
	Affected amino acid			Highly conserved among species		
	Domain	T3-3		T3-6		T3-8
	PolyPhen-2			Probably damaging		
	Mutation Taster			Disease causing		
	FATHMM			Damaging		
	CADD score	26.5	27.6	34	27.1	32
	ACMG criteria	Pathogenic		Likely pathogenic		

PSACH, pseudoachondroplasia; MED, multiple epiphyseal dysplasia; SDS, standard deviation score; TA, tricuspid valve atresia; COMP, cartilage oligomeric matrix protein; T3, type 3 calcium-binding repeat (the number after the hyphen represents the number of eight T3 repeats); FATHMM, functional analysis through hidden Markov models; CADD, combined annotation-dependent depletion; ACMG, American College of Medical Genetics and Genomics. * Variants found in patients with MED.

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Materials and Methods

Five Japanese patients who were clinically and radiologically diagnosed with PSACH were included in this study. All patients were born to non-consanguineous healthy parents.

Genetic analysis was approved by the Ethics Committee of Okayama University Hospital (1701-038; January 6, 2017), and informed consent was obtained from the patients or their parents. All procedures in this study were performed in accordance with the 1964 Declaration of Helsinki and the 2003 Japanese Ethical Guidelines for Clinical Research and their later amendments.

All exon and exon-intron boundaries of the *COMP* gene were analyzed using Sanger sequencing. Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Hilden, Germany). Polymerase chain reaction (PCR) was conducted using AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) or PrimeSTAR GXL DNA Polymerase (Takara Bio Inc., Shiga, Japan) according to the manufacturer's standard protocol. PCR amplicons were purified using a QIAquick PCR Purification Kit (Qiagen Inc.) and sequenced using a BigDye Terminator Cycle Sequencing FS Ready Reaction Kit and a Genetic Analyzer (ABI Prism 310; Applied Biosystems). The sequences of the primer pairs used are listed in Supplementary Table 1. The sequence reads were aligned with reference sequences from GenBank (NM_000095.3). Data for the variants identified in this study are available at the Leiden Open Variation Database (https://databases.lovd.nl/shared/variants/COMP/unique).

Results

The growth characteristics of the five patients with PSACH are shown in **Table 1** and **Fig. 1**. For all patients, height was not impaired from the neonatal stage to 1 yr of age. However, growth was severely stunted, as previously reported (3, 5). The facial appearance of the five patients was not characteristic, with no minor anomalies.

Radiological analysis revealed anterior tonguing of the vertebral body; metaphyseal and epiphyseal dysplasia; and shortened long bones, phalanges, and metacarpals at the first visit in all patients (**Fig. 2**).

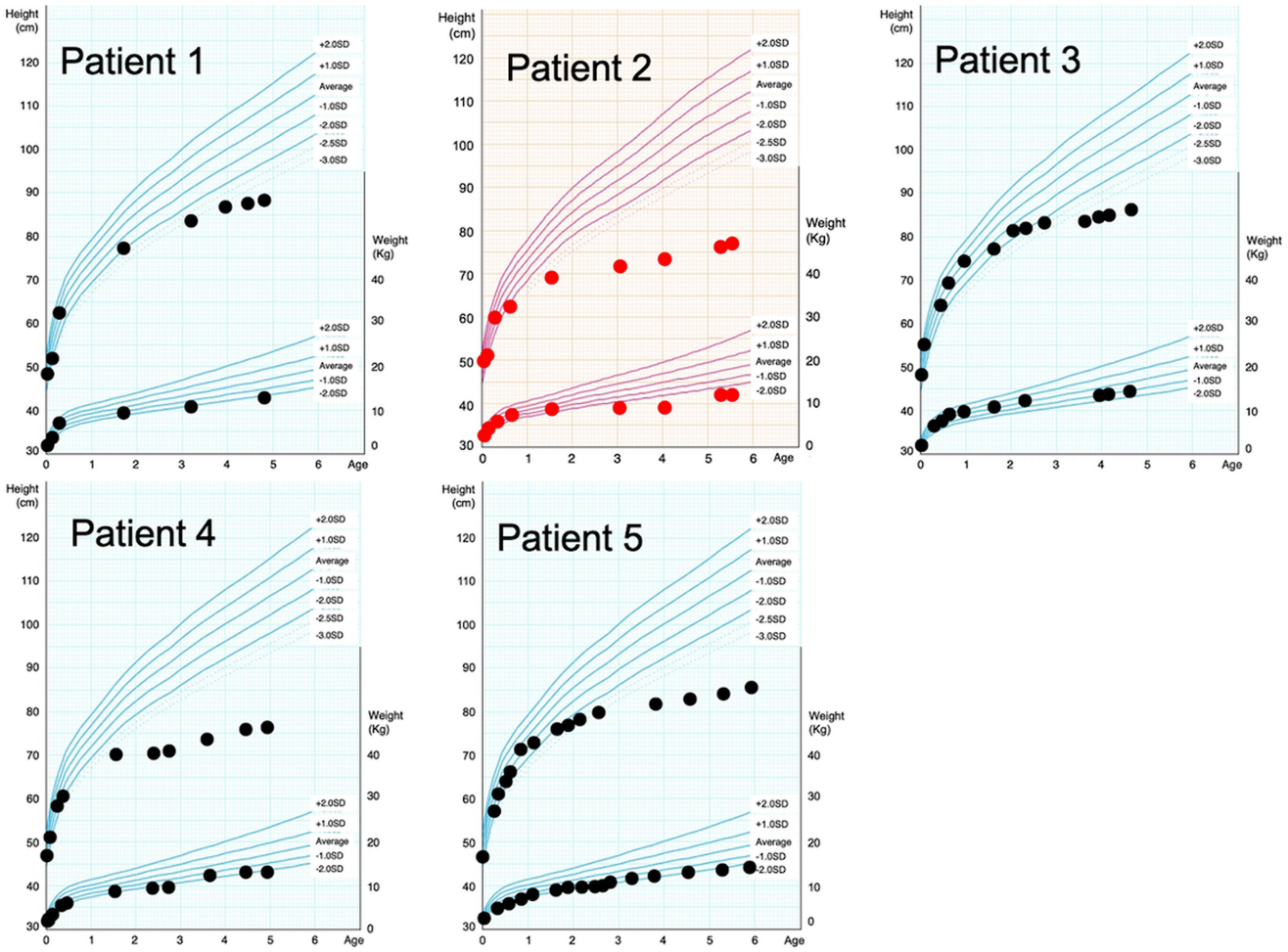

Fig. 1. Growth of the five patients plotted on a Japanese standard growth curve. Height was normal before 1 yr of age, but was stunted at approximately 1 to 2 yr of age in all patients.

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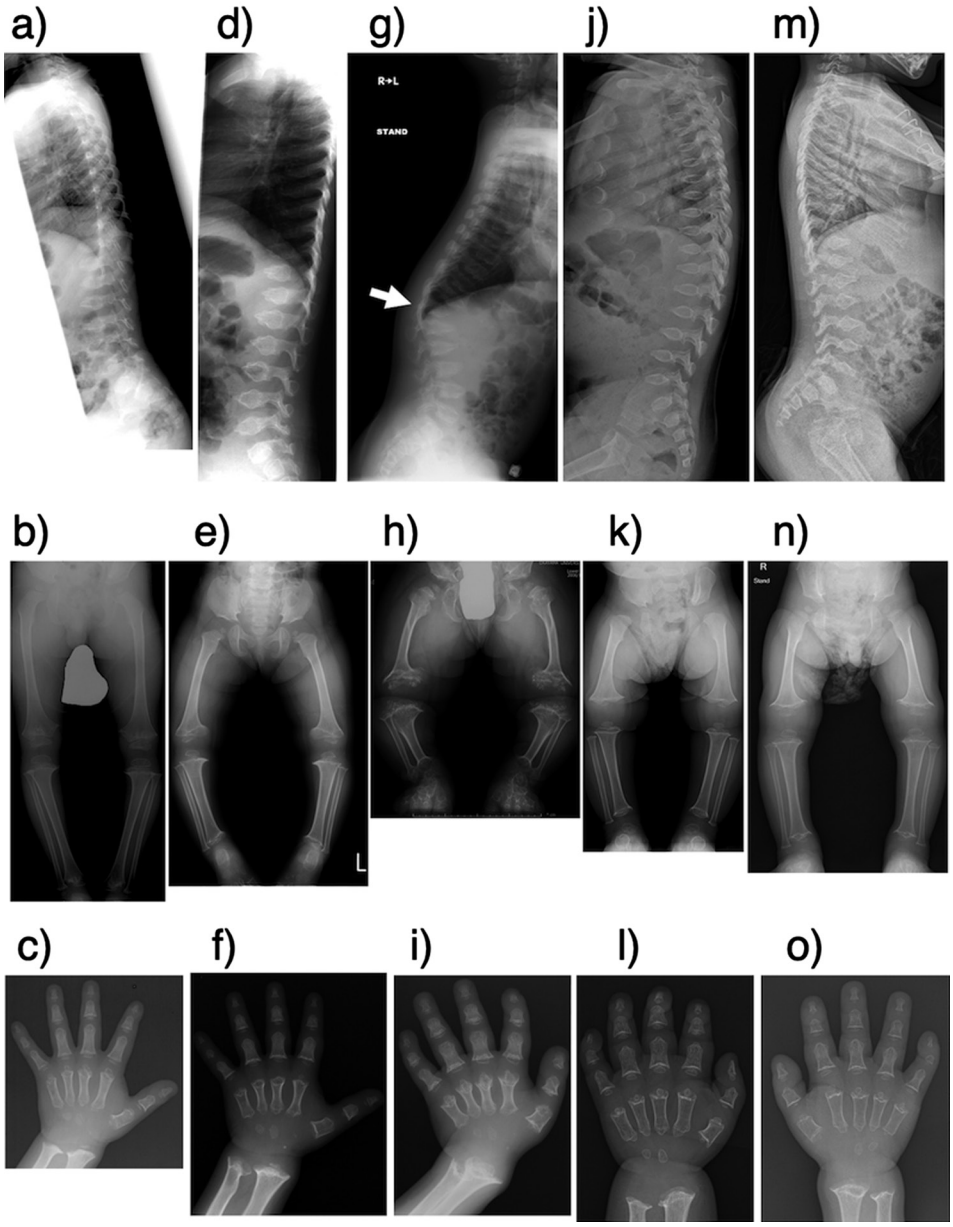

Fig. 2. Radiological skeletal characteristics of the five patients with pseudoachondroplasia (a–c: Patient 1 at 4 yr of age; d–f: Patient 2 at 3 (d and e) and 6 yr of age (f); g–i: Patient 3 at 13 (g), 11 (h), and 8 yr of age (i); j–l: Patient 4 at 1 yr and 7 mo of age; m–o: Patient 5 at 3 (m and n) and 2 yr and 6 mo of age (o). The vertebral body in all five patients (a, d, g, j, and m) shows characteristic anterior tonguing. Patient 3 shows kyphosis at the thoracolumbar junction (g, arrow). The lower extremities (b, e, h, k, and n) show metaphyseal and epiphyseal changes, and Patient 1 shows a milder phenotype than the other patients. All patients show genu varum. The carpal bone (c, f, i, l, and o) shows short metacarpals and phalanges. A delay in ossification in the carpal bone is obvious in Patients 1, 2, and 3 (c, f, and i).

Genu varum and shortening of the short tubular bones in the hand were less severe in Patient 1 (**Figs. 2b and c**) than in Patients 2 to 5 (**Figs. 2e, f, h, i, k, l, n, and o**).

In Patient 5, who had tricuspid atresia and required cardiac surgery, we retrospectively analyzed the sequential metaphyseal changes in the proximal humerus. Before 4 mo of age, metaphyseal changes were not obvious (**Figs. 3a–c**). However, after 7 mo of age (**Figs. 3d and e**), the irregularity of the metaphysis became obvious. No metaphyseal changes were observed in the femur during the neonatal period (**Fig. 3f**). Anterior tonguing of the vertebral body, which is an important finding in the diagnosis of PSACH, was not observed at 3 mo of age (**Fig. 3g**). However, distinct anterior tongues were observed at 23 mo (**Fig. 3h**).

We identified one recurrent variant (p.Cys351Tyr) and four novel variants (p.Asp437Tyr, p.Asp446Gly, p.Asp507Tyr, and p.Asp518Val; **Table 1** and **Fig. 4**). These variants were not identified in any single nucleotide polymorphism databases, including dbSNP

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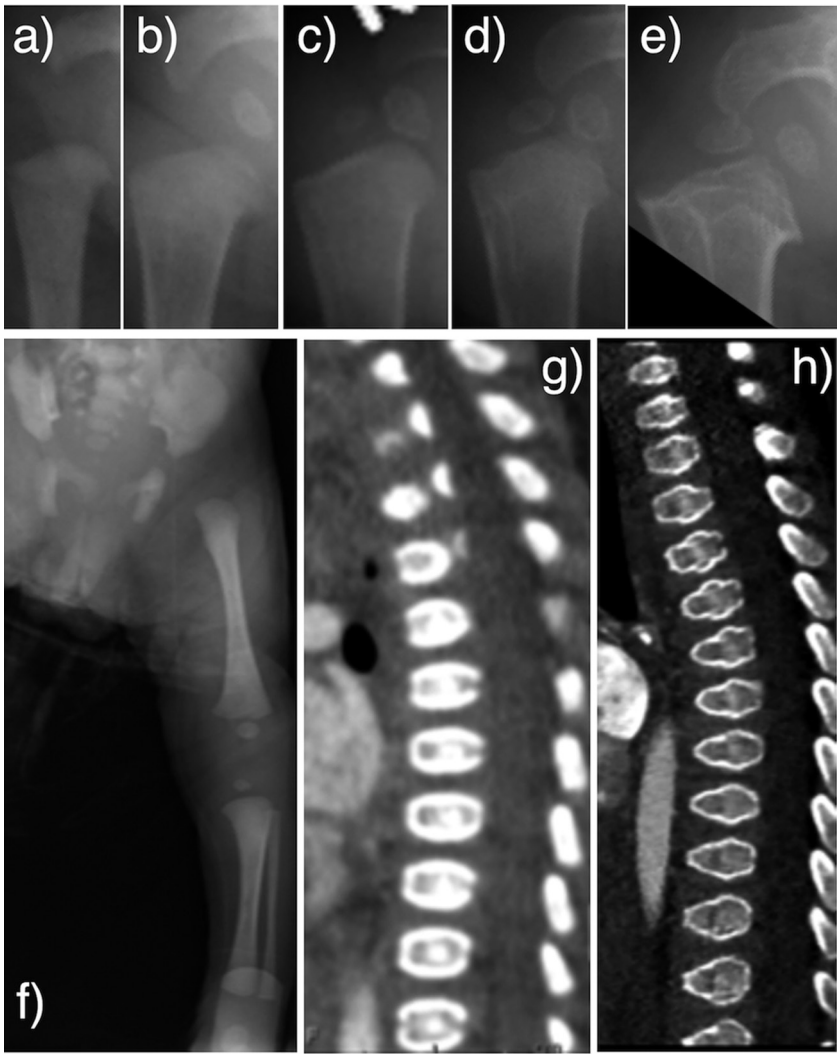

Fig. 3. Sequential skeletal changes in Patient 5. A proximal metaphyseal change in the right humerus (a: 1 d; b: 4 mo, c: 7 mo; d: 10 mo; e: 22 mo) was not obvious before 7 mo of age, but was gradually obvious after 10 mo of age. The appearance of the epiphyseal nucleus was delayed, and segmentation was observed after 7 mo of age. Metaphyseal changes in the left femur, tibia, and fibula were not obvious at 10 d of age (f). Chest computed tomography shows that the shape of the vertebral body was normal at 3 mo of age (g), but anterior tonguing became obvious at 23 mo of age (h).

(https://www.ncbi.nlm.nih.gov/snp/) and the Genome Aggregation Database (https://gnomad.broadinstitute.org). *In silico* analysis using Mutation Taster (http://www.mutationtaster.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and FATHMM (http://fathmm.biocompute.org.uk), determined these substitutions as "probably damaging", "disease causing", and "damaging", respectively. The CADD Scores (https://cadd.gs.washington.edu) of the five variants were distributed from 26.5 to 34. These variants are considered as "pathogenic" (Patient 1: PS1, PM2, PM6, PP3, and PP4) or "likely pathogenic" (Patients 2–5: PM1, PM2, PM5, PM6, PP2, PP3, and PP4) according to the American College of Medical Genetics and Genomics guidelines (8).

Discussion

We identified one recurrent and four novel COMP variants in Japanese patients with PSACH. All five patients were born with normal height and showed normal growth during the infantile period. However, growth disturbances were observed after 1 or 2 yr of age, and all patients were diagnosed with PSACH after the infantile period. These findings are consistent with those of previous studies on human and mouse models of PSACH (3, 5, 9).

All five variants identified in our patients were located in the T3 repeat of COMP. One recurrent variant affected cysteine, which is important for protein folding, but the other four variants affected aspartic acid residues. These four aspartic acid residues are evolutionarily conserved, and pathogenic variants other than those

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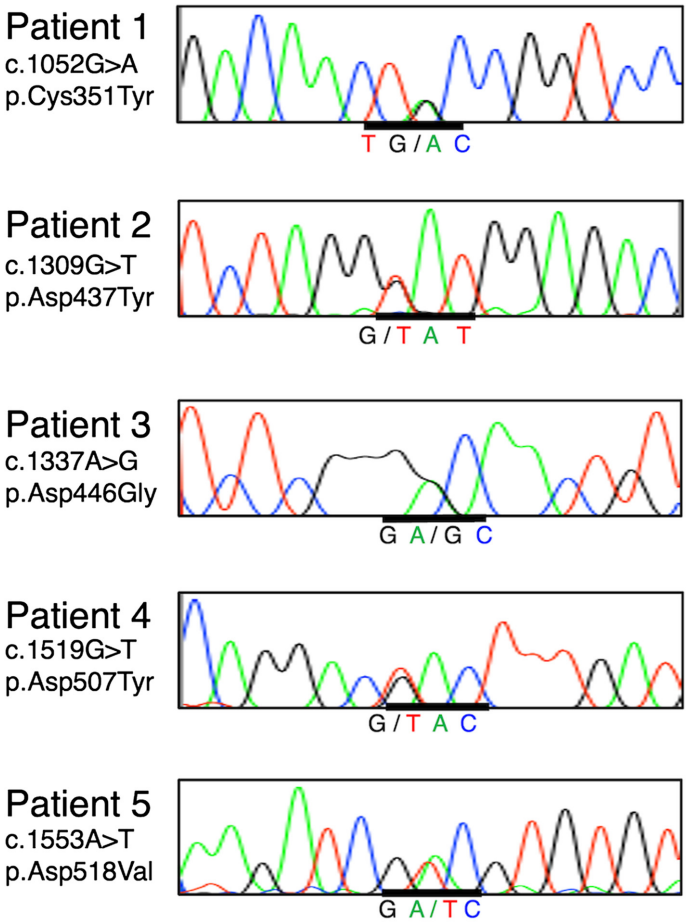

Fig. 4. Results of *COMP* gene analysis in the five patients. The bold bar indicates the affected codon. All five patients have heterozygous base changes.

found in this study have previously been found in these residues (**Table 1**). Aspartic acid residues in the T3 repeat are important for protein folding and binding to extracellular matrix proteins, such as type II and type IX collagen. Alterations in only one of the five aspartic acid residues in each T3 repeat resulted in PSACH or multiple metaphyseal dysplasia. *In silico* analysis suggested that these five variants were pathogenic.

A strict genotype–phenotype correlation is not observed in skeletal dysplasia caused by pathogenic COMP variants (10). However, there is a tendency for pathogenic variants located between the sixth and eighth T3 repeats to result in PSACH. In contrast, pathogenic variants located at the third and fourth T3 repeats result in multiple metaphyseal dysplasia, a genetically heterogeneous form of skeletal dysplasia caused by pathogenic genetic variants of *COMP*, *COL9A1*, *COL9A2*, *COL9A3*, *SLC26A2*, *MATN3*, and *CANT1* (10, 11), and characterized by a short stature and early-onset osteoarthropathy. This may be applicable to our patients, because Patient 1, who had a variant in the third T3 repeat, showed milder skeletal and growth phenotypes than the other four patients with variants in the sixth or eighth T3 repeats.

We retrospectively analyzed the sequential changes in the epimetaphysis and vertebral body from the neonatal to infantile periods in Patient 5. Skeletal radiography of the fetus and infant has rarely been reported in PSACH cases, with only one previous report (7). Patient 5 was not a familial case, but we were able to review the sequential changes from the neonatal period to childhood in this patient because of coexisting tricuspid valve atresia. A metaphyseal change in the long bones was not apparent in the neonatal to early infantile periods, but appeared during the infantile period before growth disturbance started. We could not track the skeletal changes in the vertebral body as closely as those in the humeral metaphysis. However, the vertebral bodies showed anterior tonguing at 23 mo, at which point humeral metaphyseal changes had already been observed. Therefore, we speculate that skeletal changes in the vertebral body may start at almost the same time as the metaphyseal changes in long bones. We also speculate that, in addition to joint laxity in the legs observed in patients with PSACH, skeletal changes in both the metaphysis and vertebral body may partly contribute to the decline in growth rate from 1 to 2 yr old.

In conclusion, we identified one recurrent and four novel COMP variants in Japanese patients with PSACH. To the best of our knowledge, this is the first report in which skeletal changes in a patient with PSACH were monitored from the neonatal period to childhood.

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