Pycnodysostosis: Novel Variants in *CTSK* and Occurrence of Giant Cell Tumor

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Abstract

Pycnodysostosis is an autosomal recessive skeletal dysplasia caused by pathogenic variants in the cathepsin K (*CTSK*) gene. We report seven patients from four unrelated families with this condition in whom we have identified three novel pathogenic variants, c.120 + 1G > T in intron 2, c.399 + 1G > A in intron 4, and c.148T > G (p.W50G) in exon 2, and a known variant, c.568C > T (p.Q190*) in exon 5 of *CTSK*. We present the clinical, radiographic, and molecular findings of all individuals with molecularly proven pycnodysostosis from the present cohort. We also report the occurrence of giant cell tumor in the skull of a patient with this condition.

Keywords

- pycnodysostosis
- cathepsin K
- giant cell tumor

Introduction

Pycnodysostosis (OMIM 265800) was first described by Maroteaux and Lamy in 1962 as an autosomal recessive condition with short stature, relatively moderate bone brittleness, and craniofacial deformity with a delayed fusion of fontanels.¹ Incidence of pycnodysostosis is 1 in 1.7 million individuals.² Other features include fractures, osteolysis of the distal phalanges, dysplastic nails, clavicular dysplasia, congenital pseudarthrosis of clavicle, spondylolysis, frontal and parietal bossing, beaked nose, prominent eyes, bluish sclera, hypoplasia of maxilla and mandible, craniosynostosis, nonpneumatized paranasal sinuses, Arnold–Chiari malformation, delayed eruption of permanent teeth, persistence of deciduous teeth, dental crowding, dental malocclusion, ob-

tuse mandibular angle, and grooved palate.³ Conductive hearing loss due to otosclerosis is also reported.⁴ Pycnodysostosis is caused by pathogenic variants in cathepsin K (*CTSK*), which is mapped to chromosome 1q21 and plays an important role in osteoclast function.⁵ The *CTSK* has 8 exons, and the translated protein has 329 amino acids with a molecular mass of 36 kDa. *CTSK* is a lysosomal cysteine proteinase and is involved in the remodeling and resorption of bone tissue. It is predominantly expressed in the osteoclasts. It is also shown to be significantly expressed in human breast cancer cells where it apparently is conducive to tumor invasion.⁶ To date, there have been approximately 55 different pathogenic variants reported in *CTSK* causing pycnodysostosis in the literature.^{7–19} It includes 35 missense variants, 5 nonsense variants, 10 frameshift variants, 4 splice site variants, and 1 stop variant.

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In this study, we performed mutation analysis in seven Indian patients from four unrelated families with clinical diagnoses of pycnodysostosis and found three novel *CTSK* variants and one known pathogenic variant in them. We also observed a giant cell tumor (GCT) in the occipital bone of one patient.

Materials and Methods

Appropriate informed consents were obtained from the patients and families for this study. Detailed clinical and radiological findings were recorded. Genomic DNA was isolated from peripheral blood using phenol–chloroform method. All the coding exons and flanking introns of *CTSK* (NM_000396.1) were amplified using polymerase chain reaction (PCR) with specifically designed primers. PCR amplicons were purified and sequenced by ABI PRISM 3130 genetic analyzer (Life Technologies, Foster City, California, United States). Multiple sequence alignment and *in silico* prediction of pathogenicity using MutationTaster, SIFT, and PolyPhen2 were used for bioinformatics analyses. This study was approved by the respective institutional ethics committees.

Results

We evaluated seven patients from four unrelated families. All of them were consanguineous (►Supplementary Material, available in the online version). The common clinical features include frontal prominence, micrognathia, short stature, and stubby digits (►Fig. 1). Skeletal survey revealed dense skull, persistently open anterior fontanel, delayed closure of the suture, obtuse angle of the mandible, brachydactyly, acro-osteolysis of hands, and osteosclerosis. Patient (P1) had a swelling over the occipital region and magnetic resonance imaging (MRI) of the brain revealed a focal, heterogeneously enhancing altered signal intensity extracalvarial lesion in the

subgaleal and subcutaneous planes in the left occipital region (►Supplementary Material, available in the online version). The swelling over the occipital region was surgically removed. Histopathological evaluation established the diagnosis of GCT (►Supplementary Material, available in the online version). Following surgery, the tumor recurred. Clinical and radiological features of patients described here are summarized in ►Table 1.

Mutation Analysis

Genomic deoxyribonucleic acid (DNA) from all seven patients was sequenced for the entire coding sequence and the splice sites of *CTSK*. Subsequent DNA analysis revealed three novel homozygous sequence variants: c.120 + 1G > T in intron 2 (P1), c.399 + 1G > A in intron 4 (P2), c.148T > G (p.W50G) in exon 2 (P3 and P4) (►Fig. 2A–I). A known variant, c.568C > T (p.Q190*) in exon 5, was detected in P5, P6, and P7 (►Fig. 2J–N).²⁰ The biallelic segregation was confirmed in parents in all three families. All four variants were not present in the Exome Aggregation Consortium (ExAC) database. Multiple sequence alignment performed by Clustal Omega for the homologs of human *CTSK* gene product showed that tryptophan amino acid is highly conserved among the species, whereas it has been replaced by glycine in P3 and P4 from family 3. This variant was also found to be damaging using pathogenicity prediction tools such as MutationTaster, SIFT, and PolyPhen-2.

Discussion

In this study, the most common clinical features observed in all seven patients are frontal prominence and micrognathia. Radiological features include persistent anterior and posterior fontanels, osteosclerosis, acro-osteolysis of distal phalanges, and brachydactyly. Other features include a prominent nose, wrinkled skin over dorsa of the fingers, hypoplasia of clavicle,

Fig. 1 Facial profiles of patients with pycnodysostosis. (Patient [P] 1, A, B; P2, C, D; P4, E; P5, F, G; P6 H, I; P7, J). All of them share prominent forehead, frontal and parietal bossing, and micrognathia. P1 also shows scar of operated giant cell tumor.

Table 1 Clinical features of patients (P1-P5) with pycnodysostosis

	Family 1	Family 2	Family 3		Family 4
	P1	P2	P3	P4	P5
Age at current evaluation (y)	26	5	13	7	3.5
Gender	Female	Male	Male	Female	Female
Height in cm (SD)	128 (−6)	NA	131 (−4)	NA	78 (−5)
Consanguinity	+	+	+	+	+
Clinical features					
Short stature	+	NA	+	NA	+
Frontal prominence	+	+	+	+	+
Occipital prominence	−	+	NA	NA	−
Micrognathia	+	+	+	+	+
Retrognathia	+	+	−	−	−
Prominent nose	+	+	+	−	−
Wrinkled skin over dorsa of fingers	+	+	NA	+	−
Radiological features					
Dense skull	+	+	NA	NA	+
Persistent open anterior fontanel	+	+	NA	NA	+
Wormian bones	+	+	NA	NA	−
Fracture	−	+	+	−	−
Delayed suture closure	+	+	NA	NA	+
Absent frontal sinus	+	+	NA	NA	−
Obtuse angle of mandible	+	+	NA	NA	+
Aplasia of clavicle	−	−	−	NA	−
Hypoplasia of clavicle	+	+	+	NA	−
Brachydactyly	+	+	+	+	−
Acro-osteolysis of hands	+	+	+	+	−
Scoliosis	+	+	+	NA	−
Osteosclerosis	+	+	+	+	+
Narrow ilia	NA	+	+	+	−
Genotype					
Homozygous variants in <i>CTSK</i> gene	c.120 + 1G > T	c.399 + 1G > A	c.148T > G	c.148T > G	c.568C > T

Abbreviations: +, present; −, absent; *CTSK*, cathepsin K; NA: not available; P, patient; SD, standard deviation.

the presence of Wormian bones, absent frontal sinus, scoliosis, and narrow ilia. Additionally, we observed GCT in the left occipital region in P1 which could be, however, coincidental.

GCT in patients with pycnodysostosis has not been reported previously, and the mechanism of tumorigenesis is yet to be identified. GCT of bone is generally benign and histologically characterized by the presence of osteoclast-like cells that are multinucleated. Although it is a benign lesion and locally aggressive, it can reappear after surgical resection.²¹ Lindeman et al established that *CTSK* is primarily responsible for osteolytic activity in GCT.²²

Sequence analysis of *CTSK* in seven patients with clinical diagnosis of pycnodysostosis identified three novel variants and one previously known variant. Two patients had homozygous

splice site pathogenic variants (c.120 + 1G > T in intron 2 and c.399 + 1G > A in intron 4), and another two patients belonging to the same family had a homozygous missense pathogenic variant c.148T > G in exon 2 of the *CTSK*. Splice site variants disrupt the canonical splice site in introns 2 and 4 in the *CTSK*. Canonical splice site variants are known to alter gene function by various mechanisms. The variant observed in the third family was a missense variant, c.148T > G, and that results in an amino acid substitution from tryptophan to glycine at the 50th codon in a highly conserved position. *In silico* analysis tools were consistent in predicting that the variant might damage *CTSK* protein function. In the fourth family, a known nonsense variant, c.568C > T, identified results in premature termination of translation at a residue 190.²⁰

Fig. 2 Electropherograms of families 1, 2, 3 and 4. Patient (P) 1 show homozygous splice site variant in intron 2 of cathepsin K (*CTSK*) gene (A), and the father is heterozygous for the same (B). P2 shows homozygous splice site variation in intron 4 (C), whereas parents are heterozygous for the same variant (D,E). P3 and P4 of family 3 show homozygous missense variation in exon 2 of *CTSK* gene (F,G), whereas parents are heterozygous for the same variant (H,I). P5 to P7 of family 4 show homozygous missense variation in exon 5 of *CTSK* gene (J–L), whereas parents are heterozygous for the same variant (M, N).

Very few mutation proven cases of pycnodysostosis have been reported from India.^{8,9} To date, only four splice site variants in the *CTSK* gene are reported in the literature.^{7,16,23,24} Therefore, our study widens the mutation spectrum of pycnodysostosis. Although *CTSK* has been associated with GCT in patients without pycnodysostosis, GCT in a patient with pycnodysostosis in our study has not been previously reported. This report expands the clinical spectrum of this condition as well.

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Conflict of Interest

None.

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References

- Maroteaux P, Lamy M. Pycnodysostosis [in French]. *Presse Med* 1962;70:999–1002
- Soliman AT, Ramadan MA, Sherif A, Aziz Bedair ES, Rizk MM. Pycnodysostosis: clinical, radiologic, and endocrine evaluation and linear growth after growth hormone therapy. *Metabolism* 2001;50(08):905–911
- Turan S. Current research on pycnodysostosis. *Intractable Rare Dis Res* 2014;3(03):91–93
- Mujawar Q, Naganoor R, Patil H, Thobbi AN, Ukkali S, Malagi N. Pycnodysostosis with unusual findings: a case report. *Cases J* 2009;2:6544. Doi: 10.4076/1757-1626-2-654
- Gelb BD, Edelson JG, Desnick RJ. Linkage of pycnodysostosis to chromosome 1q21 by homozygosity mapping. *Nat Genet* 1995 10(02):235–237
- Littlewood-Evans AJ, Bilbe G, Bowler WB, et al. The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. *Cancer Res* 1997;57(23):5386–5390
- Araujo TF, Ribeiro EM, Arruda AP, et al. Molecular analysis of the *CTSK* gene in a cohort of 33 Brazilian families with pycnodysostosis from a cluster in a Brazilian Northeast region. *Eur J Med Res* 2016;21(01):33
- Mandal K, Ray S, Saxena D, et al. Pycnodysostosis: mutation spectrum in five unrelated Indian children. *Clin Dysmorphol* 2016;25(03):113–120
- Singh A, Cuevas-Covarrubias S, Pradhan G, et al. Novel mutation and white matter involvement in an Indian child with pycnodysostosis. *Indian J Pediatr* 2015;82(05):471–473
- Xue Y, Wang L, Xia D, et al. Dental abnormalities caused by novel compound heterozygous *CTSK* mutations. *J Dent Res* 2015; 94(05):674–681
- Huang X, Qi X, Li M, et al. A mutation in *CTSK* gene in an autosomal recessive pycnodysostosis family of Chinese origin. *Calcif Tissue Int* 2015;96(05):373–378
- Pangrazio A, Puddu A, Oppo M, et al. Exome sequencing identifies *CTSK* mutations in patients originally diagnosed as intermediate osteopetrosis. *Bone* 2014;59:122–126
- Arman A, Bereket A, Coker A, et al. Cathepsin K analysis in a pycnodysostosis cohort: demographic, genotypic and phenotypic features. *Orphanet J Rare Dis* 2014;9:60
- Ozdemir TR, Atik T, Karaca E, Onay H, Ozkinay F, Cogulu O. A novel mutation in two families with pycnodysostosis. *Clin Dysmorphol* 2013;22(03):102–105

- 15 Zheng H, Zhang Z, He JW, Fu WZ, Zhang ZL. A novel mutation (R122Q) in the cathepsin K gene in a Chinese child with Pycnodysostosis. *Gene* 2013;521(01):176–179
- 16 Utokpat P, Panmontha W, Tongkobpetch S, Suphapeetiporn K, Shotelersuk V. Novel CTSK mutation resulting in an entire exon 2 skipping in a Thai girl with pycnodysostosis. *Pediatr Int* 2013; 55(05):651–655
- 17 Xue Y, Cai T, Shi S, et al. Clinical and animal research findings in pycnodysostosis and gene mutations of cathepsin K from 1996 to 2011. *Orphanet J Rare Dis* 2011;6:20
- 18 Fratzl-Zelman N, Valenta A, Roschger P, et al. Decreased bone turnover and deterioration of bone structure in two cases of pycnodysostosis. *J Clin Endocrinol Metab* 2004;89(04):1538–1547
- 19 Matsushita M, Kitoh H, Kaneko H, et al. Novel compound heterozygous mutations in the cathepsin K gene in Japanese female siblings with pycnodysostosis. *Mol Syndromol* 2012;2(06): 254–258
- 20 Hou WS, Brömme D, Zhao Y, et al. Characterization of novel cathepsin K mutations in the pro and mature polypeptide regions causing pycnodysostosis. *J Clin Invest* 1999;103(05):731–738
- 21 Chakarun CJ, Forrester DM, Gottsegen CJ, Patel DB, White EA, Matcuk GR Jr. Giant cell tumor of bone: review, mimics, and new developments in treatment. *Radiographics* 2013;33(01):197–211
- 22 Lindeman JH, Hanemaaijer R, Mulder A, et al. Cathepsin K is the principal protease in giant cell tumor of bone. *Am J Pathol* 2004; 165(02):593–600
- 23 Haagerup A, Hertz JM, Christensen MF, Binderup H, Kruse TA. Cathepsin K gene mutations and 1q21 haplotypes in at patients with pycnodysostosis in an outbred population. *Eur J Hum Genet* 2000;8(06):431–436
- 24 Donnarumma M, Regis S, Tappino B, et al. Molecular analysis and characterization of nine novel CTSK mutations in twelve patients affected by pycnodysostosis. *Mutation in brief #961*. Online. *Hum Mutat* 2007;28(05):524. Doi: 10.1002/humu.9490