A First-Case Report of Pycnodysostosis in an Omani Boy

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Abstract

Here we reported on the genetic findings of a 9-year-old Omani boy with a rare inherited bone disorder. The patient's clinical features include dysmorphic facial features, short stature, and skeletal abnormalities. Exome sequence of the patient's deoxyribonucleic acid revealed a variant in the cathepsin K gene, which was confirmed by Sanger sequencing. These findings established the diagnosis of pycnodysostosis (PKND). To the best of the authors' knowledge, this case is the first case to be reported in the Gulf Cooperative Region of the novel PKND with molecular confirmation.

Keywords

- pycnodysostosis (PKND)
- cathepsin K (*CTSK)*
- genetics

Introduction

Pycnodysostosis (PKND) is a rare autosomal recessive lysosomal disorder that is characterized by bone deformities, including short stature, osteopetrosis, and acro-osteolysis of the distal phalanges (OMIM: 265800). The disorder, originally described by Montarani in 1923 and then by Lamy in 1962, was first known as Maroteaux-Lamy syndrome.[1] The causative gene for PKND was not discovered until 1995 when linkage analysis studies mapped the gene to chromosome 1q21,[2] and then utilizing positional cloning identified it as cathepsin K (*CTSK*).[3] Since then, multiple variants in *CTSK* associated with PKND have been reported. These variants vary between missense, nonsense, small insertion-deletions, frameshifts, and splice sites (►**Table 1**).

Among the reported cases of PKND, one Pakistani family had three of five affected siblings with the condition caused by a pathogenic, exon 3 variant c.136C > T.[1] In another study from Istanbul,[4] 7 *CTSK* variants were identified in 16 patients from 14 families; 5 were missense, 1 a nonsense stop variant, and 1 a 301 bp insertion in intron 7. All of these families fit with the PKND autosomal recessive mode of inheritance. A further study, conducted by Pangrazio in 2014, reported on affected children in two Italian families from different ethnic backgrounds who presented with osteopetrosis and a PKND phenotype[5] (►**Table 2**). Recently, novel *CTSK* variants were reported in a 4-year-old Afghani boy (c.847T > C, p.Y283H)[6] and in an Iranian boy (c.905G > A, p.Trp320X).[7]

We presented here a 9-year-old Omani male child patient with a novel variant of the *CTSK* gene causing PKND. This is a rarely reported condition in the Middle East and North Africa region and is the first to be reported in the Gulf Cooperation Council (GCC) region with molecular diagnosis. Previous PKND reported cases from the region have reported about the phenotype of the patients but not the genetic defect.[8,9]

Clinical Presentation

The index patient was a 9-year-old male child patient with PKND who presented to the National Genetics Centre at the Royal Hospital in Muscat, Oman. The patient had dysmorphic facial features, short stature, scoliosis, and brachydactyly. He was born preterm, at 32 weeks of gestation, with a birth weight of 1.5 kg to consanguineous Omani parents and grandparents. A clinical examination at birth noted the dysmorphic facial features, and found a lateral inguinal hernia. Subsequently, in the Special Care Baby Unit, he was ventilated for 2 days and provided with preterm care. At age 6 months, he weighed 6.7 kg and his length was 68.3 cm. Findings then include plagiocephaly, oxycephaly, flat occiput, and temporal narrowing. These findings were consistent with Crouzon syndrome

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Table 1 Genetic variants of cathepsin K (*CTSK*) gene associated with pycnodysostosis (PKND) disorder

CTSK variants	ClinVar ID	Classification
NM_000396.4(CTSK):c.990A > G (p.Ter330Trp)	8420	Pathogenic
NM_000396.4(CTSK):c.436G > C (p.Gly146Arg)	8421	Pathogenic
NM_000396.4(CTSK):c.236G > A (p.Gly79Glu)	8424	Pathogenic
NM_000396.4(CTSK):c.154A > T (p.Lys52Ter)	8425	Pathogenic
NM_000396.4(CTSK):c.891–1G > T	623333	Pathogenic
NM_000396.4(CTSK):c.826C > T (p.His276Tyr)	801542	Likely pathogenic
NM_000396.4(CTSK):c.3G > A (p.Met1Ile)	551777	Likely pathogenic
NM_000396.4(CTSK):c.891–21_892dup	554307	Likely pathogenic
NM_000396.4(CTSK):c.669del (p.Tyr224fs)	550217	Likely pathogenic

Table 2 Reported cases of pycnodysostosis (PKND): summary of clinical features, characterizations, and genotypes of previously reported PKND patients and the presented 9-year-old Omani male child patient

Patient	Naeem et al (2009)[1]	Arman et al (2014)[4]	Pangrazio et al (2014)[5]	Reported case
Origin	Pakistan	Turkey	Pakistan Bangladesh Black Caribbean	Oman
Patients reported	3	14	3, 1, 1	1
Cathepsin K (*CTSK*) mutation	A277V	M1I, I249T, L7P, D80Y, D169N, R312X	R46W, K89del, R46W R47S, S246Cfs*4	Exon 5, donor splice site
Clinical features				
Short stature	+	+	+	+
Osteopetrosis	+	+	+	+
Osteosclerosis	+	+	+	+
Facial dysmorphism	ND	ND	ND	+
Brachydactyly	+	+	+	+
Skull deformities	+	ND	+	+
Acro-osteolysis of distal phalanges	+	+	+	+
Wrinkled skin	ND	Over the dorsa of distal fingers	ND	Over the dorsa of distal fingers
Intellectual disabilities	–	–	–	–
Visual impairment	ND	ND	+	–

Abbreviations: +, clinical signs present; –, clinical signs absent; ND, not determined.

(►**Fig. 1**). At 9 months, he was diagnosed with craniosynostosis. This defect was surgically corrected at the age of 5 years. At 6 years, a magnetic resonance imaging found cervical lordosis and a wrist radiograph showed increased bone density, consistent with osteopetrosis (►**Fig. 1A** and **1B**). A cardiac evaluation at this time was unremarkable.

Genetic Diagnosis

Cytogenetic analyses on the proband and his parents found normal karyotypes on all three individuals. Whole-exome sequencing (WES) and then Sanger sequencing were performed on all three. The results found that the patient had a homozygous, likely pathogenic intronic mutation variant c.618 + 2T > G (NM_000396.4, ClinVar_623333, dbSNP_rs75481239, GRCh37_1:150776495, GRCh38_1:150804019) in the *CTSK* gene. The parents were found to carry one copy each of this specific *CTSK* gene variant (►**Fig. 2**). These results confirm the genetic diagnosis of PKND (OMIM: 265800) in the proband.

Genotype–Phenotype Correlation

The *CTSK* gene is expressed exclusively in the osteoclasts, which are essential to bone remodeling, repair, and maintenance. The *CTSK* genomic deoxyribonucleic acid (DNA) spans 12 kb, covering 8 exons and 7 introns. The translation initiation codon methionine (Met1) is located in exon 2, whereas the termination codon is located in exon 8. The gene encodes a 329-amino

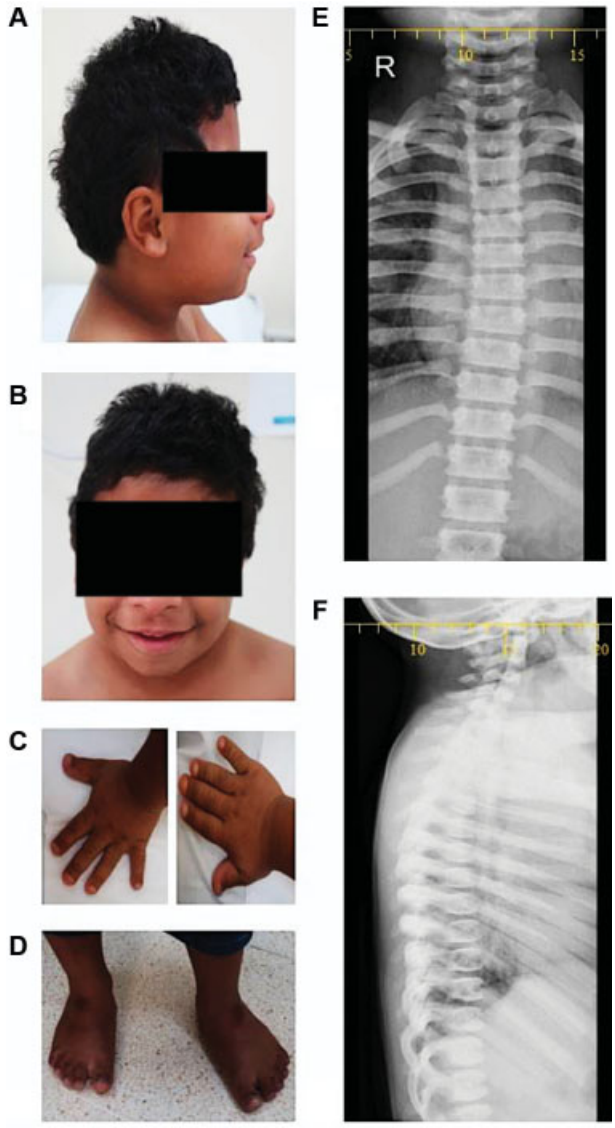

Fig. 1 Phenotypical features of the proband. A 9-year-old male child patient with pycnodysostosis. (**A**) A lateral and (**B**) a frontal image of the patient's head, neck, and face. Note the unicoronal synostosis, craniosynostosis, wide forehead, mid-face hypoplasia, low-set ears, and micrognathia. (**C**,**D**) Limb abnormalities, including brachydactyly, short distal phalanges, and wrinkly skin over the dorsa of the fingers. (**E**,**F**) Scoliosis and increased bone density, indicating osteopetrosis.

acid polypeptide, which is a member of the papain-cysteine protease family and is known to be involved in the degradation of bone matrix proteins at low pH levels, including collagen types I and II, osteopontin, and osteonectin.[10] The *CTSK* protein consists of a 15-amino acid signal peptides encoded by part of exon 2, a 99-amino acid pro-region encoded by the parts of exon 2, 3, and 4, and a main 215-amino acid mature active enzyme domain encoded by parts of exons 4, 5, 6, 7, and 8.[3] Functional studies performed in knockout mice (*Ctsk* $-/-$) have provided evidence that this gene is essential for normal bone development, as the defective mice were found to have osteopetrotic phenotype and structural abnormalities that closely match those described in PKND patients.[11] Furthermore, other studies indicate *Ctsk* $-/-$ mouse have abnormal bone matrix degradation and collagen defects.[12]

In our index patient, the disease-causing pathogenic mutation in the *CTSK* gene (c.618 + 2T > G) is theorized as having disturbed exon 5, a highly conserved donor splice site that is located at the start of the *CTSK* exons, and encodes for the primary mature active enzyme domain. Hence, this mutation is anticipated to result in the creation of a truncated *CTSK* protein with full inactivation of the protein's main function as a lysosomal cysteine protease in bone resorption. This mechanism is consistent with the previous studies on *CTSK* and the phenotype resulted from null protein function. The proband's genotype correlates with the observed PKND phenotype. While not published, the same variant has been mentioned to be associated with PKND phenotype in a family from Oman (ClinVar ID 623298).

Methods

Cytogenetics Analysis

Peripheral blood was cultured using Roswell Park Memorial Institute—RPMI—media, and cytogenetic analyses were performed on metaphase chromosomes obtained from phytohemagglutinin-stimulated lymphocyte cultures, according to standard procedure. Metaphases were stained with Giemsa, and chromosome analyses were performed and reported according to the standards of the International System for Human Cytogenetics.[13]

DNA Samples

DNA was extracted from whole blood using Hamilton's Genomic STARlet and following the manufacturer's instruction. DNA was then quantified using a NanoDrop (ThermoFisher, ND-2000) spectrophotometer.

Whole-Exome Sequence

WES was performed on the patient's DNA using HiSeq 2500 systems (Illumina, Centogene, Germany) and Agilent's SureSelect Human All Exon V6 kit.

Sanger Sequencing

Bi-directional Sanger sequencing was performed using an ABI310 sequencer. Amplified DNA was treated with ExoSap-IT (Thermo Fisher-78201) for enzymatic clean-up prior to sequencing preparations. This was followed by ethanol precipitation; then samples were treated with HiDi formamide (applied Biosystems-4311320), covered with septa, and loaded into the sequencer.

Conclusion

This report documented the clinical and molecular findings of a rare bone disorder in a patient from the Sultanate of Oman. These findings will contribute to our understanding with regard to PKND and the molecular basis of the disorder. Previous studies have reported patients with phenotype associated with PKND in the region.[9] However, this is the first case of PKND reported in Oman with molecular findings and has implications for genetic defects of PKND in the entire GCC region.

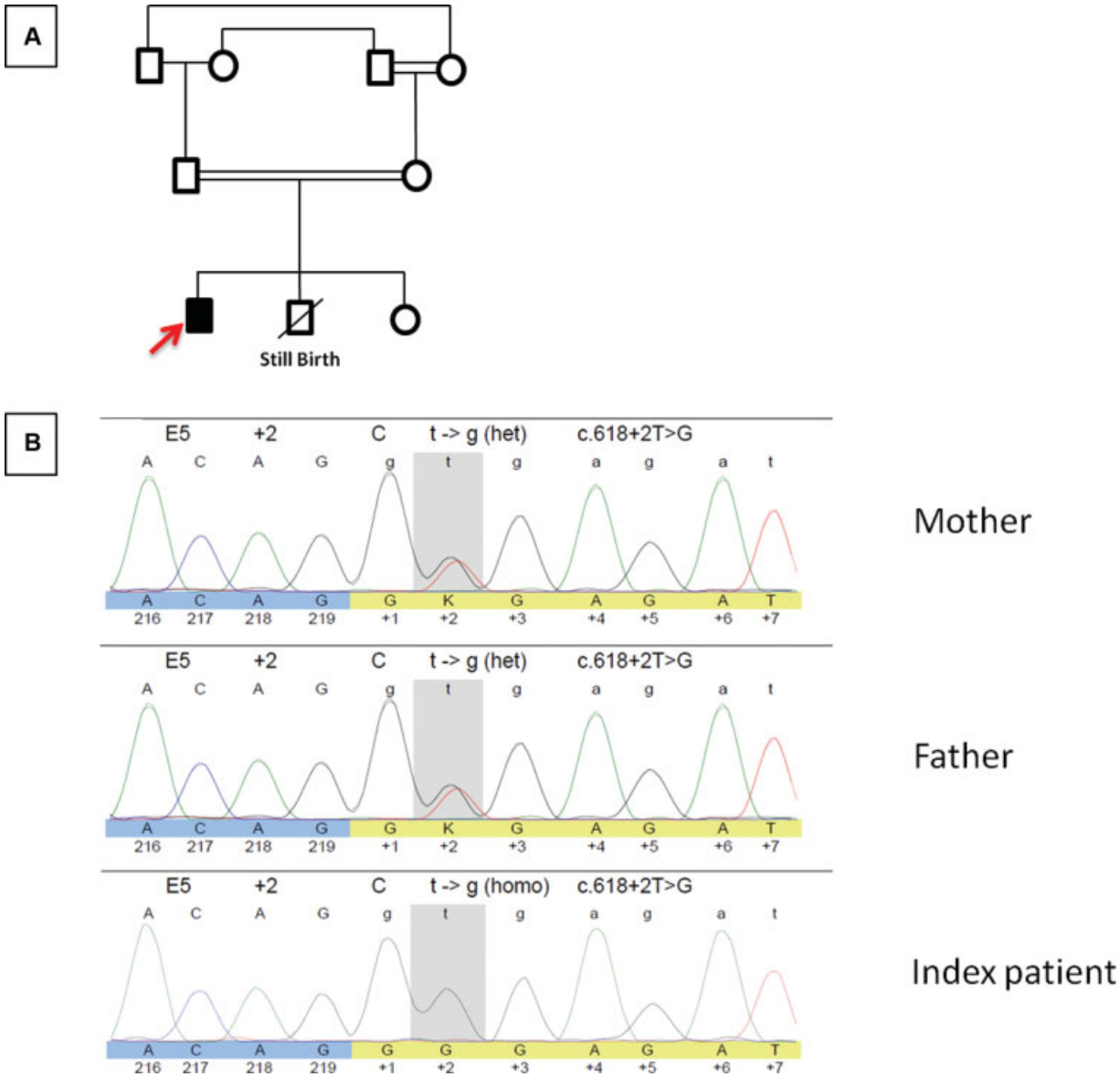

Fig. 2 Family pedigree and Sanger sequencing results of the proband. (**A**) Family pedigree of the index pycnodysostosis (PKND) patient (indicated by red arrow). The 9-year-old boy is the son of consanguineous parents (carriers) and grandparents. The parents also have a normal daughter and had a stillborn son. (**B**) Sanger sequencing of cathepsin K (*CTSK*) gene exon 5 mutation variant c.618 + 2T > G is shown for the parents and the index patient (mutation variant is highlighted in gray). The parents are carriers of this variant. Two peaks can be seen presenting DNA bases, (T) thymine (red peak) and (G) guanine (black peak), indicating heterozygous state in the parents. The index patient is homozygous for this mutation; only DNA base guanine (G) is detected.

Conflict of interest
None declared.

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References

1 Naeem M, Sheikh S, Ahmad W. A mutation in CTSK gene in an autosomal recessive pycnodysostosis family of Pakistani origin. BMC Med Genet 2009;10:76
2 Polymeropoulos MH, Ortiz De Luna RI, Ide SE, Torres R, Rubenstein J, Francomano CA. The gene for pycnodysostosis maps to human chromosome 1cen-q21. Nat Genet 1995;10 (02):238–239
3 Gelb BD, Shi GP, Chapman HA, Desnick RJ. Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. Science 1996;273(5279):1236–1238
4 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
5 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
6 Kochar IPS, Sethi A, Ahamad A. A novel variant c.847T>C in *CTSK* gene leading to pycnodysostosis: a case report. Clin Med Insights Case Rep 2019;12:1179547619837234
7 Razmara E, Azimi H, Bitaraf A, et al. Whole-exome sequencing identified a novel variant in an Iranian patient affected by pycnodysostosis. Mol Genet Genomic Med 2020;8(03):e1118

8 Mahmoudi A, Bouabdallah Y. Pycnodysostosis presented with tibial shaft fracture. Pan Afr Med J 2020;35:5
9 Soliman AT, Rajab A, AlSalmi I, Darwish A, Asfour M. Defective growth hormone secretion in children with pycnodysostosis and improved linear growth after growth hormone treatment. Arch Dis Child 1996;75(03):242–244
10 Tezuka K, Tezuka Y, Maejima A, et al. Molecular cloning of a possible cysteine proteinase predominantly expressed in osteoclasts. J Biol Chem 1994;269(02):1106–1109
11 Saftig P, Hunziker E, Wehmeyer O, et al. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proc Natl Acad Sci USA 1998;95(23):13453–13458
12 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
13 Simons A, Shaffer LG, Hastings RJ. Cytogenetic nomenclature: changes in the ISCN 2013 compared to the 2009 edition. Cytogenet Genome Res 2013;141(01):1–6