Whole-exome sequencing identified a novel variant in an Iranian patient affected by pycnodysostosis

Ehsan Razmara1 | Homeyra Azimi2 | Amirreza Bitaraf3 | Mohammad Ali Daneshmand4 | Mohammad Galehdari5 | Maryam Dokhanchi6 | Elika Esmaeilzadeh-Gharehdaghi7 | Masoud Garshasbi7

1Australian Regenerative Medicine Institute, Monash University, Clayton, VIC, Australia
2Dr. Azimi Genetic Counseling Center, Arak, Iran
3Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
4Daneshmand Pathology Laboratory, Arak, Iran
5Department of Biology, Faculty of Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran
6Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
7Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Correspondence
Masoud Garshasbi, Department of Medical Genetics; Faculty of Medical Sciences; Tarbiat Modares University, Tehran, Iran. Email: masoud.garshasbi@modares.ac.ir

Funding information
Tarbiat Modares University

Abstract

Background: Whole-exome sequencing (WES) has emerged as a successful diagnostic tool in molecular genetics laboratories worldwide. In this study, we aimed to find the potential genetic cause of skeletal disease, a heterogeneous disease, revealing the obvious short stature phenotype. In an Iranian family, we used solo-WES in a suspected patient to decipher the potential genetic cause(s).

Methods: A comprehensive clinical and genotyping examination was applied to suspect the disease of the patient. The solo clinical WES was exploited, and the derived data were filtered according to the standard pipelines. In order to validate the WES finding, the region harboring the candidate variant in the CTSK gene was amplified from genomic DNA and sequenced directly by Sanger sequencing.

Results: Sequence analysis revealed a rare novel nonsense variant, p.(Trp320*); c.905G>A, in the CTSK gene (NM_000396.3). In silico analysis shed light on the contribution of the variant to the pathogenicity of pycnodysostosis. This variant was confirmed by Sanger sequencing and further clinical examinations of the patient confirmed the disease.

Conclusion: The present study shows a rare variant of the CTSK gene, which inherited as autosomal recessive, in an Iranian male patient with pycnodysostosis. Taken together, the novel nonsense CTSK variant meets the criteria of being likely pathogenic according to the American College of Medical Genetics and Genomics-the Association for Molecular Pathology (ACMG-AMP) variant interpretation guidelines.

KEYWORDS
cathepsin K, nonsense variant, pycnodysostosis, rare disease, whole-exome sequencing
1 | INTRODUCTION

Rare syndromes are disorders affecting a scarce number of individuals in the world (Schieppati, Henter, Daina, & Aperia, 2008). The low number of patients or resources makes it difficult to detect the molecular cause of these conditions (Daneshjoo & Garshasbi, 2018). Instead, increasing knowledge in molecular biology in addition to using cutting-edge technologies such as next-generation sequencing (NGS) methods has enabled us to detect genetic defects causing some of the rare syndromes such as pycnodysostosis (PDO). The evident phenotype of PDO affecting the skeletal system is short stature (SS), and initially, based on this feature we selected the patient to detect the possible genetic cause(s).

We diagnosed a patient suspected of PDO as a rare autosomal recessive disease (OMIM 265800) (Figure 1a). Although the first case of this disease was described in 1923 by Sedano, Gorlin, and Anderson (1968), since 1962 to now, less than 200 cases have been detected or reported (Naeem, 2018).

**Figure 1** (a) This pedigree is comprised of four generations. The arrow appoints the proband of the family. The genetic status is shown as heterozygote: W/c.905G>A or homozygote: c.905G>A/c.905G>A; in this pedigree, white symbols: unaffected; red symbol: affected; squares: men; circles: females; parallel lines: consanguineous marriage, W: wild-type allele. (b) Schematic genetic and protein maps of the CTSK gene (NM_000396.3). c.905G>A variant is located in exon 8, which encodes the mature domain of CTSK protein. Similar to other most papain-like cysteine proteases, CTSK contains 329 amino acids that can be categorized in three distinct sections: a 15-amino acid preregion, a 99-amino acid proregion, and a 215-amino acid mature active enzyme (Toral-López et al., 2011). The low-PH environment changes inactive CTSK to the active form by the removal of the N-terminal proregion. (c) UCSC database used to show the conservation of specific nucleotide (G; highlighted as red) including the variant site in vertebrates, particularly in primates. The amino acid sequence of CTSK colored based on conservation scores by the ConSurf database. Scores ranged from 1 to 9, where a score of 9 represented a highly conserved residue. ConSurf demonstrates evolutionary conservation profiles for proteins of known/unknown structure according to the phylogenetic relations between homologous sequences as well as amino acid's structural and functional importance. (d) The 3D structure of CTSK is shown. The picture was rendered with PyMOL (v.0.99rc6). The original site of Trp302 is emphasized by a highlighted zone and locally zoomed.
Sheikh, & Ahmad, 2009). Thus, this disease is categorized as a rare disease showing an obvious SS phenotype. The universal prevalence of PDO is estimated to be 1 to 1.7 per million with equal sex distribution (Xue et al., 2011). Besides SS, the most common features of PDO are increased bone density of long bones, pathological fractures with poor healing, open fontanels, stubby hands and feet with dystrophic nails, and to some extent the obtuse mandibular angle (Xue et al., 2011).

Various mutations in CTSK gene have been reported in PDO patients which most of these mutations occur in the mature domain of CTSK protein (Xue et al., 2011) (Figure 1b); almost 70% of the mutations have been identified in the mature domain of CTSK, 24.24% in the proregion, and 6.06% in the preregion (Xue et al., 2011). The CTSK gene (NM_000396.3) spans around 12 kb and contains 8 exons (Figure 1b). The encoded protein, CTSK or cathepsin K, is a member of the papain-like cysteine protease family, and there is a high similarity between this protein and other cathepsins such as S and L (Gelb, Shi, Chapman, & Desnick, 1996). CTSK is highly expressed in osteoclasts (Xue et al., 2011), and its mRNA is detectable in macrophages and bone marrow-derived dendritic cells (Asagiri et al., 2008; Honey & Rudensky, 2003). Additionally, cathepsin K plays a vital role in osteoclast-mediated bone resorption by degrading the bone matrix proteins, such as type I collagen, osteopontin, and osteonectin (Hou et al., 1999; Mujawar et al., 2009). In a nutshell, impairment of CTSK-mediated osteoclast apoptosis/senescence may also be responsible for the higher bone density, which is a hallmark feature in patients affected by PDO (Chen et al., 2007).

In the present study, the main object was to resolve the genetic diagnosis of SS phenotype in an Iranian male patient with normal parents. By applying whole-exome sequencing (WES), a novel variant, p.(Trp320*), in the CTSK gene was identified. The physical assessments and medical evaluations confirmed the PDO due to the novel identified variant in this family. According to the American College of Medical Genetics and Genomics-the Association for Molecular Pathology (ACMG-AMP) variant interpretation guidelines (Biesecker & Harrison, 2018; Oza et al., 2018), this variant could be classified as a likely pathogenic variant, albeit functional studies to further confirm the pathogenicity of the variant in appropriate animal models will be recommended.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

The study protocol was approved by the local medical ethics committee of Tarbiat Modares University, Tehran, Iran. A written informed consent was obtained from all subjects before applying enrollment in this study. The family also provided written informed consent for publication of their pertinent images included in this paper. All of the patient’s clinical information and the medical histories were collected at the Dr. Azimy Genetic Center, Arak, Iran.

2.2 | Subject and clinical investigations

We enrolled 3 members of the family in our study (Figure 1a). The consanguineous family, originating from Arak province of Iran, was suspected to pycnodysostosis. The family was ascertained for the present study, and the affected subject underwent meticulous medical records including a comprehensive physical examination, bone density testing, and radiography tests. Some of the important clinical indices used in this study are summarized in Table 1.

2.3 | DNA extraction

To apply genetic tests, around 10 ml of blood samples was taken from the patient and his parents, and then, genomic DNA was isolated from the samples by Roche DNA Extraction Kit (Cat. No. 1181477001, Roche Life Science). Thence, DNA concentrations were measured by Thermo Scientific™ Nanodrop 2000 (Thermo Fisher Scientific).

2.4 | Whole-exome sequencing

About 1 μg of genomic DNA sample of the patient (IV.I) was subjected to high-throughput sequencing. RNA capture baits against approximately 60 Mb of the Human Exome (targeting > 99% of regions in CCDS, RefSeq, and Gencode databases) were used to enrich regions of interest from fragmented genomic DNA with Agilent's SureSelect Human All Exon V6 kit. The generated library was sequenced on an Illumina platform to obtain an average coverage depth of approximately 100x. Typically, around 97% of the targeted bases were covered >10x.

2.5 | Bioinformatics analysis

An end-to-end in-house bioinformatics pipeline including base calling, alignment of reads to GRCh37/hg19 genome assembly, primary filtering of low-quality reads and probable artifacts, and subsequent annotation of variants was applied. The reads were aligned to the reference genome (hg19/NCBI37.1) with SNP & Variation Suite version 8.0 (SVS v8.0) and DNASTAR Lasergene12 (DNASTAR Inc.). Small indel detection was used with the Unified Genotyper tool from GATK tools in Galaxy online database (http://www.usega
The missense, nonsense, silent, and indel variant rates were estimated by Galaxy online tool and finally were confirmed by Ivariantguide® (Chaudhry & Tainsky, 2019).

Regarding the pedigree and inheritance mode, we assumed that the variant(s) might be inherited as homozygous manner and we hereby excluded the heterozygous variants, and then, several filtering steps were applied to prioritize all variants: (a) Variants in dbSNP132 (https://www.ncbi.nlm.nih.gov/projects/SNP), 1000 Genomes Project (Siva, 2008), and GnomAD (Karczewski & Francioli, 2017) with allele frequencies more than 1% were excluded. (b) The rest of the variants underwent further exclusion in the Exome

laxy.org). The missense, nonsense, silent, and indel variant rates were estimated by Galaxy online tool and finally were confirmed by Ivariantguide® (Chaudhry & Tainsky, 2019).

Regarding the pedigree and inheritance mode, we assumed that the variant(s) might be inherited as homozygous manner and we hereby excluded the heterozygous variants, and then, several filtering steps were applied to prioritize all variants: (a) Variants in dbSNP132 (https://www.ncbi.nlm.nih.gov/projects/SNP), 1000 Genomes Project (Siva, 2008), and GnomAD (Karczewski & Francioli, 2017) with allele frequencies more than 1% were excluded. (b) The rest of the variants underwent further exclusion in the Exome

| TABLE 1 | Clinical features of the patient in the family |
| Features | IV.1 |
| Age at initial assessment/age at molecular assessment | 17/20 |
| Gender | Male |
| Father age at conception | 31 |
| Weight at birth | Below 5th percentile (3.1 ± 0.01 kg) |
| Height at birth | Below 5th percentile (45.2 ± 0.10 cm) |
| Head circumference at birth | 36.3 ± 0.10 cm |
| Height at assessment | 137 ± 0.10 cm |
| Weight at assessment | 34 ± 0.01 kg |
| Sexual maturity rating | Stage IV |
| Short stature (<150 cm) | Observed |
| Increase in bone density | Observed |
| Open fontanels and sutures | Open sutures of anterior fontanel and closed posterior fontanel |
| Frontal and parietal bossing | Observed |
| Fractures | Developed easy fractures for four times |
| Obtuse mandibular angle | Observed |
| Short fingers and hypodontia | Observed |
| Stubby hands and feet with osteolysis of the distal phalanges | Observed |
| Prominent eyes with bluish sclerae | Observed |
| Hypoplastic maxilla | Observed |
| Grooved palate | Observed |
| Scoliosis | Scoliosis was evident in the thoracic region |
| Dysplastic nails | Dysplastic nails were evident in this patient |
| Nonpneumatized paranasal sinuses | Not applicable |
| Prominent nose | Observed |
| Macrocephaly | Observed |
| Asymmetric skull | Observed |

| TABLE 2 | Several online databases that used to confirm the pathogenicity of the variant in CTSK |
| Variant | Zygosity | Gene | Index | Mother | Father | MutationTaster | SIFT | 1K Genome | Iranome | PROVEAN | ENTREPIE-X | Disease causing | N.R | Deleterious | N.R |
| Variant | Hom. Het. | Homzygote | Het. | Disease causing | N.R | Deleterious | N.R | Disease causing | N.R | Deleterious | N.R | Disease causing | N.R | Deleterious |
| p.(Trp320*) | CTSK | p.(Trp320*) | CTSK | CTSK | p.(Trp320*) | CTSK | p.(Trp320*) | CTSK | p.(Trp320*) | CTSK | p.(Trp320*) | CTSK | p.(Trp320*) | CTSK | p.(Trp320*) | CTSK |
| Hom. | Het. | Homzygote | Het. | Disease causing | N.R | Deleterious | N.R | Disease causing | N.R | Deleterious | N.R | Disease causing | N.R | Deleterious |
| Abbreviations: Het, Heterozygote; Hom, Homzygote; N.R, Not Reported.
Sequencing Project (ESP) (http://evs.gs.washington.edu/EVS) and Exome Aggregation Consortium (ExAC) database. (c) The intragenic, intronic, untranslated regions (UTRs), and synonymous variants were excluded from later analysis. (d) The SIFT (Ng & Henikoff, 2003), Provean (Choi & Chan, 2015), and MutationTaster (Schwarz, Rödelsperger, Schuelke, & Seelow, 2010) were used to predict the pathogenicity/prioritization of candidate variants (Table 2). To be on the safe side, the filtering was applied with assuming the heterozygous mode of inheritance, but this did not lead to candidate variant(s) in this family.

To narrow down the list of candidate variants as much as possible, we used Phenolyzer (Yang, Robinson, & Wang, 2015), Face2Gene (https://www.face2gene.com/), and Varcards (Li et al., 2017). All suspected pathogenic variants were double-checked in HGMD® (Stenson et al., 2003) and ClinVar (Landrum et al., 2015).

ConSurf server (Glaser et al., 2003) and UCSC database (Karolchik et al., 2003) were applied to provide an evolutionary conservation profile for CTSK protein and DNA sequence, respectively, to better predict the potential disrupting effects of the variant (Figure 1c and d). For further consideration, the frequency of the variant was checked out on Iranome as the local database (Fattahi et al., 2019) (Figure 2a). All information related to the in silico predictions such as allele frequency, cosegregation results, and pathological predictions is summarized in Table 2.

2.6 Segregation analysis

Sanger sequencing in forward and reverse directions was performed to validate the candidate variant, and then, segregation analysis was performed in the family. The
primers were designed by Primer3.0 (http://bioinfo.ut.ee/primer3-0.4.0) Web-based server (Table S1). The lack of SNPs in the genomic region corresponding to the 3’ ends of primers was inspected by looking through the dbSNP database. The specificity of primers was checked by the in silico-PCR tool in UCSC genome browser (https://genome.ucsc.edu/cgi-bin/hgPcr) and Primer blast on NCBI genome browser (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Finally, polymerase chain reaction (PCR) was performed in standard conditions, and then, PCR products were sequenced by ABI 730XL, using the conventional capillary system. Chromatograms were analyzed by GenomeCompiler online tool (http://www.genomecompiler.com) and Mutation Surveyor v.3.24 (Softgenetics) to identify the alternation. Variants were annotated based on the standards of the Human Genome Variation Society (HGVS) nomenclature (den Dunnen et al., 2016).

### 2.7 Three-dimensional structure modeling

The identified variant, p.(Trp320*), is located in the mature domain of cathepsin K (Figure 1e). The protein families and domains were analyzed using ScanProsite (Gattiker, Gasteiger, & Bairoch, 2002), and sequence alignments were recruited by using ClustalW (http://www.ebi.ac.uk/clustalw). The protein structures and possible effects of the novel variant on cathepsin K were analyzed by PyMOL (DeLano, 2002) after building the PDB structure file based on the Phyre2 (Kelley, Mezulis, Yates, Wass, & Sternberg, 2015) and SWISS-PROT (Bairoch & Boeckmann, 1992). The RAMPAGE online tool (https://www.mordred.bioc.cam.ac.uk/~rapper/rampage.php) was applied to check out the detailed residue-by-residue stereochemical quality on the basis of a Ramachandran plot to know whether the modeled structure was acceptable.

### 3 RESULTS

#### 3.1 Clinical findings

The patient, 18-year-old male, showed typical clinical features of the hereditary PDO, for example, abnormal skeletal system, scoliosis, SS with skeletal dysplasia, increased density of bones, open sutures of anterior fontanel and closed posterior fontanel, history of easy fractures for four times, frontal bossing, micrognathia, asymmetric skull and macrocephaly, short fingers with dysplastic flat nails, midface retrusion, and prominent nose. In addition, dental abnormalities including severe crowding, poor oral hygiene, periodontal problems, delayed exfoliation of primary teeth, an eruption of permanent teeth, enamel hypoplasia, obliteration of pulp chambers, and hypercementosis were evident. The patient’s radiographs exhibited an obtuse mandibular angle, the general increase in bone density, and open fontanels and sutures (Figure 2c).

In the assessment period, Tanner Stages, also known as Sexual Maturity Rating, was examined as stage IV. The history of short stature and growth retardation noted at 3 years of age, while at birth time weight (3.1 ± 0.01 kg; reference in an Iranian population: 3.26 ± 0.60 kg) and height (45.2 ± 0.10 cm; reference in an Iranian population: 51.9 ± 3.59 cm) were below 5th percentile in comparison with the normal values (Heydari, Emamghoreishi, & Amini, 2009) and head circumference (36.3 ± 0.1 cm; reference in an Iranian population: 35.13 ± 1.45 cm (Esmaeili & Esmaeili, 2015)), was above 95th percentile. Further bone evaluations showed increased bone density with the preservation of growth plate and sign of old fractures. Other important detected clinical features are summarized in Table 1. In addition to obvious dysplastic nails, radiographic examination of the index case verified the increased bone density and short fingers in comparison with the average values (Figure 2d).

Regarding the pedigree provided, the parents were consanguineous. Further laboratory analysis affirmed normal values for leukocyte and thrombocyte counts, plasma phosphate, and alkaline phosphatase. Additionally, no obvious phenotypic abnormality was observed in the parents.

#### 3.2 Genetic analysis

To elucidate the underlying genetic cause(s), genomic DNA was obtained from the patient and analyzed by solo clinical WES, and finally, p.(Trp320*) variant was confirmed by Sanger sequencing (Figure 2b).

In the patient, the detected SNVs and deletion/insertions were analyzed by several filtering steps. In total, 91,395 variants were found by WES in the proband after alignment and SNV calling. By applying several exclusion processes such as base quality filtering, and frequency in dbSNP132, 1000 Genomes Project, ESP, GnomAD, and ExAc databases, 51 variants were identified. These variants then prioritized based on the patient’s phenotypes.

Eventually, regarding the phenotypes by utilizing CentoMD (Trujillano), ClinVar, phenolyzer (Yang et al., 2015), Face2Gene, Human phenotype ontology (Robinson et al., 2008), and Varcards, only one relevant variant was identified. Consequently, the samples from the available members of the family were subjected to Sanger sequencing and segregation of the candidate nonsense variant of the CTSK gene was confirmed (Figure 2b).

Finally, we classified the variant based on ACMG-AMP guidelines (http://wintervar.wglab.org) into a likely pathogenic variant.

To caveat, besides considering autosomal recessive inheritance mode for this family, we considered autosomal dominant
inheritance, but this analysis did not result in any candidate variant. All details of filtering steps are accessible in Table 3, and all statistics regarding the variants are given in Table S2.

### 3.3 In silico predictions

We used RAMPAGE online tool to show the detailed residue-by-residue stereochemical quality of CTSK in accordance with the Ramachandran plot. The structural model of CTSK indicated almost 98.1% of residues in the most favored regions, around 0.7% of residues in allowed regions, and only 0.2% of residues in the outlier regions, which suggested that the modeled structure of this protein was acceptable.

Various in silico predictor databases such as SIFT, PolyPhen-2, MutationTaster, Provean, and ENTPRISE-X (Zhou, Gao, & Skolnick, 2018) were used to evaluate the possible pathogenicity of the variant. All detailed results are described in Table 2.

### 4 DISCUSSION

Bone development is a complex process in which a balance between bone formation and resorption is delicately maintained (Razmara et al., 2019). In fact, osteoblasts and also osteoclasts have an important hand in this process, and any perturbation influencing this procedure causes multidinous genetic bone diseases ranging from osteoporosis to osteopetrosis (Tanaka, Nakayamada, & Okada, 2005). To date, many genes have been identified related to skeletal abnormalities (Table S3), although the molecular and cellular basis of a great number of skeletal genetic disorders is still uncertain and this knowledge gap can be filled by using advanced technologies such as NGS, which has importantly expedited the detection of responsible genetic causes. Among NGS techniques, WES has been developed into a robust and cost-effective tool to identify the genetic cause in rare diseases (Razmara & Garshasbi, 2018). Using WES, we succeeded to identify a novel variant in the CTSK gene in an Iranian male patient presenting SS phenotype. We also provided evidence supporting the causative role of homozygous p.(Trp320*) in index case using in silico predictions. Nevertheless, the messy reality of unknown significant variants is not going away anytime soon, much as clinicians, geneticists, and patients might wish it would; in this study by applying different stages of filtering, 51 unknown significant variants were detected in the patient from which 6 genes were plausible showing an association with reported skeletal diseases. To narrow down as much as possible, we used the clinical data of the patient in various databases, for example, Face2gene (Table 3). This helped us to decrease the number of candidate variants to only one variant, thence confirmed by Sanger sequencing. The c.905G>A variant in the CTSK gene segregated with the condition in the family.

Cathepsin K deficiency does not influence on the function of osteoclast-mediated extracellular acidification (Chen et al., 2007), whereas CTSK mutations have been detected to halt the ability of osteoclasts to degrade collagen rather than demineralize the extracellular matrix. Furthermore, cathepsin K may also function as a potential regulator of apoptosis and senescence, controlling osteoclast numbers in vivo (Chen et al., 2007). Studies on the Ctsk−/− mice have revealed that the number of chondroclasts and osteoclasts is increased in joint tissues of the mice, whereas osteoclast numbers are increased in the mouse bones (Soki et al., 2018). Similarly, some scraps of evidence showed that the deletion of CTSK in osteoclasts enhances bone formation in vivo (Lotinun et al., 2013). These data can justify the increased bone density in the patients affected by pycnodysostosis.

The clinical presentation of the patient in the family was originally described as SS and solo-WES detected a novel variant in the CTSK gene. The hallmark features of this disease such as increased bone density, frontal bossing, micrognathia, prominent nose, short stature, delayed abnormal tooth eruption, and fragility fractures were observed in the index case. Indeed, one of the two original descriptions of PDO in 1962 named it an osteopetrosis variant (Pangrazio et al., 2014). However, PDO is usually a progressive but relatively benign condition. The applied clinical assessments confirmed that the patient is affected by pycnodysostosis, not osteopetrosis. Evaluation of the radiographs available for the patient showed the obtuse mandibular angle on a craniolateral view and absence of acrosteolysis of the hands (Figure 2c). Moreover, it seems that haploinsufficiency, a dominant phenotype in diploid organisms that are heterozygous for a loss-of-function allele, is not responsible for the impairment of CTSK, because none of the parents showed pertinent clinical symptoms. Thus, not only did this help us to exclude the osteopetrosis

| Patient                                      | IV.1 |
|---------------------------------------------|------|
| Total variants                             | 91,395 |
| Variants after base quality filtering       | 77,909 |
| Homozygous variants                        | 31,383 |
| Nonsynonymous/indel/splice site variants   | 8,928 |
| Novel variants (dbSNP132/1000GP queried)    | 51 |
| Genes with plausible disease association    | 6 |
| Phenotype analysis                          | 1 |
The p.(Trp320*) variant may lead to the production of a truncated protein with loss of downstream functional domain in the cell; as a result, cells should forestall this process by nonsense-mediated decay response (NMD response), which is increasingly appreciated as one of the central mechanisms of RNA surveillance, with a great role in the physiological control of gene expression. Chen et al. showed that a deficiency of cathepsin K prevents inflammation and bone erosion in rheumatoid arthritis and periodontitis and reveals its shared osteoimmune role (Hao et al., 2015). Based on the identified variant, we propose NMD or loss of downstream functional domain as judicial mechanisms of pathogenesis of p.(Trp320*) variant in the patient, but more studies need to unearth the exact molecular mechanisms that contribute to the pathogenicity.

Additionally, it has been shown that cathepsin K plays a role in hormone activation or degradation (Tepel, Bromme, Herzog, & Brix, 2000), glucose metabolism (Yang et al., 2006), and pathogenesis of obesity (Xiao et al., 2006). Thus, the identification of novel functional variants can broaden the horizons toward understanding the mechanisms in which cathepsin K plays roles.

To conclude, our results indicate that the novel nonsense variant, c.905G>A; p.(Trp320*), in the CTSK gene (NM_000396.3) might be the genetic cause of pycnodysostosis. The putative variant meets the criteria of being pathogenic, but we firmly advise applying functional analysis with animal models to inspect the distinctive pathological roles of this variant.

5 | CONCLUSION

In summary, we report the CTSK variant p.(Trp320*), which is associated with PDO in a male Iranian patient. We hope that this identification may also yield new insights into the mechanisms of human bone disease, especially in relation to metabolic alterations occurring during this process. We also suggest doing functional analysis by applying appropriate animal models to decipher the mechanism of pathogenesis of the variant prior to using in genetic counseling.

ACKNOWLEDGMENTS

We are especially grateful to staff of the DeNA laboratory for helping us in this research. Additionally, we appreciate supports from Dr. Morteza Dehghan, Shahrekord University of Medical Sciences, Shahrekord, Iran, for his comments on the clinical data. This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors stated no conflict of interest.

AUTHORS’ CONTRIBUTION

Conceived and designed the experiments: M.G, E.R, M.A.D. Conducted the experiments: M.G, M.A.D, and E.R. Analyzed and interpreted the data: E.R, M.A.D, H.A, and E.E.G. Contributed reagents/materials/analysis tools: E.R. Wrote the paper: E.R, M.G, A.R.B, M.GH, and M.D. Designed the figures: E.R. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher. Human variant and phenotypes have been reported to ClinVar (accession number: SCV000965583) and LOVD (individual ID: 00264073; https://databases.lovd.nl/shared/individuals/00264073), respectively. This study was also registered at Bioproject (PRJNA559970).

ORCID

Ehsan Razmara https://orcid.org/0000-0001-9926-3975
Amirreza Bitaraf https://orcid.org/0000-0002-1495-0870
Masoud Garshabi https://orcid.org/0000-0002-5508-7903

REFERENCES

Afzal, A. R., Rajab, A., Fenske, C. D., Oldridge, M., Elanko, N., Ternes-Pereira, E., … Jeffery, S. (2000). Recessive Robinow syndrome, allelic to dominant brachydactyly type B, is caused by mutation of ROR2. *Nature Genetics*, 25(4), 419. https://doi.org/10.1038/78107
Ahrens, W., Hiort, O., Staedt, P., Kirschner, T., Marschner, C., & Kruse, K. (2001). Analysis of the GNAS1 gene in Albright’s hereditary osteodystrophy. *The Journal of Clinical Endocrinology & Metabolism*, 86(10), 4630–4634.
Alazami, A. M., Al-Owain, M., Alzahrani, F., Shuaib, T., Al-Shamrani, H., Al-Falki, Y. H., … Alkuraya, F. S. (2012). Loss of function mutation in LARP7, chaperone of 7SK ncRNA, causes a syndrome of facial dysmorphism, intellectual disability, and primordial dwarfishism. *Human Mutation*, 33(10), 1429–1434. https://doi.org/10.1002/humu.22175
Ambrosetti, F., Palicelli, A., Bulfamante, G., & Rivasi, F. (2014). Langer mesomelic dysplasia in early fetuses: Two cases and a literature review. *Fetal and Pediatric Pathology*, 33(2), 71–83. https://doi.org/10.3109/15513815.2013.807322
Asagiri, M., Hirai, T., Kunigami, T., Kamano, S., Gober, H.-J., Okamoto, K., … Aoki, K. (2008). Cathepsin K-dependent toll-like receptor 9 signaling revealed in experimental arthritis. *Science*, 319(5863), 624–627.
Aytar, M. H., & Yilgör, Ç. (1992). *Kniest Displazi. Omurgayı Tutan Sendromlar*, 143.
Bairoch, A., & Boeckmann, B. (1992). The SWISS-PROT protein sequence data bank. *Nucleic Acids Research*, 20(Suppl), 1999–2022. https://doi.org/10.1093/nar/20.suppl.2019
Begemann, M., Zirn, B., Santen, G., Wirthgen, E., Soellner, L., Büttel, H.-M., ... Eggermann, T. (2015). Paternally inherited IGF2 mutation and growth restriction. *New England Journal of Medicine, 373*(4), 349–356.

Bernasconi, A., Marino, R., Ribas, A., Rossi, J., Ciaccio, M., Oleastro, M., ... Belgorosky, A. (2006). Characterization of immunodeficiency in a patient with growth hormone insensitivity secondary to a novel STAT5b gene mutation. *Pediatrics, 118*(5), e1584–e1592. https://doi.org/10.1542/peds.2005-2882

Biesecker, L. G., & Harrison, S. M. (2018). The ACMG/AMP reliable table source criteria for the interpretation of sequence variants. *Genetics in Medicine, 20*(12), 1687–1688. https://doi.org/10.1038/gim.2018.42

Bober, M. B., Niiler, T., Duker, A. L., Murray, J. E., Ketterer, T., Harley, M. E., ... Jackson, A. P. (2012). Growth in individuals with Majewski osteodysplastic primordial dwarfism type II caused by pericentromeric mutations. *American Journal of Medical Genetics Part A, 158*(11), 2719–2725. https://doi.org/10.1002/ajmg.a.35447

Bonaventure, J., Chaminade, F., & Maroteaux, P. (1995). Mutations in three subdomains of the carboxy-terminal region of collagen type X account for most of the Schmid metaphyseal dysplasias. *Human Genetics, 96*(1), 58–64. https://doi.org/10.1007/BF00214187

Borck, G., Hög, F., Denti, M. L., Tan, P. L., Sowada, N., Meideira, A., ... Lepri, F. (2015). BRF1 mutations alter RNA polymerase III-dependent transcription and cause neurodevelopmental anomalies. * Genome Research, 25*(2), 155–166.

Briggs, M. D., Hoffman, S., King, L. M., Olsen, A. S., Mohrenweiser, H., Leroy, J. G., ... Cohn, D. H. (1995). Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. *Nature Genetics, 10*(3), 330. https://doi.org/10.1038/ng0795-330

Chaudhry, S. R., & Tainsky, M. A. (2019). Utilizing iVariantGuide for variant assessment of next-generation sequencing. *Current Protocols in Bioinformatics, 65*(1), e73.

Chen, W., Yang, S., Abe, Y., Li, M., Wang, Y., Shao, J., ... Li, Y.-P. (2007). Novel pycnodysostosis mouse model uncovers cathepsin K function as a potential regulator of osteoclast apoptosis and senescence. *Human Molecular Genetics, 16*(4), 410–423. https://doi.org/10.1093/hmg/ddg474

Chistiakov, D. A., Voronova, N. V., & Chistiakov, A. P. (2009). Ligase IV syndrome. *European Journal of Medical Genetics, 52*(6), 373–378. https://doi.org/10.1016/j.ejmg.2009.05.009

Choi, Y., & Chan, A. P. (2015). PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics, 31*(16), 2745–2747. https://doi.org/10.1093/bioinformatics/btv195

Daneshjoo, O., & Garshahi, M. (2018). Novel compound heterozygote mutations in the ATP7B gene in an Iranian family with Wilson disease: A case report. *Journal of Medical Case Reports, 12*(1), 68.

DeLano, W. L. (2002). PyMol: An open-source molecular graphics tool. *CCP4 Newsletter on Protein Crystallography, 40*(1), 82–92.

den Dunnen, J. T., Dalgleish, R., Maglott, D. R., Hart, R. K., Greenblatt, M. S., McGowan-Jordan, J., ... Taschner, P. E. M. (2016). HGVS recommendations for the description of sequence variants: 2016 update. *Human Mutation, 37*(6), 564–569. https://doi.org/10.1002/humu.22981

Domené, H. M., Scaglia, P. A., Martínez, A. S., Keselman, A. C., Karabatas, L. M., Pipman, V. R., ... Ballestrini, M. G. (2013). Heterozygous IGFALS gene variants in idiopathic short stature and normal children: Impact on height and the IGF system. *Hormone Research in Paediatrics, 80*(6), 413–423.

Dunning-Davies, B., & Parker, A. (2016). Annual review of children with neurofibromatosis type 1. *Archives of Disease in Childhood-Education and Practice, 101*(2), 102–111. https://doi.org/10.1136/archdischild-2014-308084

Esmaeili, M., & Esmaeili, M. (2015). Head circumference in Iranian infants. *Iranian Journal of Neonatology, 6*(1), 28–32.

Fattahi, Z., Behshadian, M., Mohteni, M., Pourch, H., Sellars, E., Nezhadhi, S. H., ... Najmabadi, H. (2019). Irunome: A catalog of genomic variations in the Iranian population. *Human Mutation, 40*(11), 1968–1984. https://doi.org/10.1002/humu.23880

Foster, J. W., Domínguez-Steglich, M. A., Giolito, S., Kwok, C., Weller, P. A., Stevanović, M., ... Schafer, A. J. (1994).Camptomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature, 372*(6506), 525. https://doi.org/10.1038/372525a0

Fuqua, J. S., Derr, M., Rosenfeld, R. G., & Hwa, V. (2012). Identification of a novel heterozygous IGF1 splicing mutation in a large kindred with familial short stature. *Hormone Research in Paediatrics, 78*(1), 59–66.

Gannagé-Yared, M.-H., Klammt, J., Chouery, E., Corbani, S., Mégarbané, H., Abou Ghoch, J., ... Mégarbané, A. (2013). Homozygous mutation of the IGF1 receptor gene in a patient with severe pre-and postnatal growth failure and congenital malformations. *European Journal of Endocrinology, 168*(1), K1–K7. https://doi.org/10.1530/EJE-12-0701

Gattiker, A., Gasteiger, E., & Bairoch, A. M. (2002). ScanProsite: A reference implementation of a PROSITE scanning tool. *Applied Bioinformatics, 1*(2), 107–108.

Gelb, B. D., Shi, G.-P., Chapman, H. A., & Desnick, R. J. (1996). Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. *Science, 273*(5279), 1236–1238.

German Pasteris, N., Cadle, A., Logie, J. L., Porteous, M. E. M., Schwartz, C. E., Stevenson, R. E., ... Gorski, J. L. (1994). Isolation and characterization of the faciogenital dysplasia (Aarskog-Scott syndrome) gene: A putative RhofRac guanine nucleotide exchange factor. *Cell, 79*(4), 669–678. https://doi.org/10.1016/0092-8674(94)00552-5

Glaser, F., Pupko, T., Paz, I., Bell, R. E., Bechor-Shental, D., Martz, E., & Ben-Tal, N. (2003). ConSurf: Identification of functional regions in proteins by surface-mapping of phylogenetic information. *Bioinformatics, 19*(1), 163–164. https://doi.org/10.1093/bioinformatics/19.1.163

Gleghorn, L., Ramesar, R., Beighton, P., & Wallis, G. (2005). A mutation in the variable repeat region of the aggrecan gene (AGC1) causes a form of spondyloepiphysyeal dysplasia associated with severe, premature osteoarthritis. *The American Journal of Human Genetics, 77*(3), 484–490. https://doi.org/10.1086/444401

Gonzalo, S., Kreiekkamp, R., & Askaier, P. (2017). Hutchinson-Gilford Progeria Syndrome: A premature aging disease caused by LMNA gene mutations. *Ageing Research Reviews, 33*, 18–29. https://doi.org/10.1016/j.arr.2016.06.007

Hämäläinen, R. H., Avela, K., Lambert, J. A., Kallijärvi, J., Eyaid, W., Gronau, J., ... Lipsanen-Nyman, M. (2004). Novel mutations in the TRIM37 gene in Mubirey Nanism. *Human Mutation, 23*(5), 522–522.

Hao, L., Zhu, G., Lu, Y., Wang, M., Jules, J., Zhou, X., & Chen, W. (2015). Deficiency of cathepsin K prevents inflammation and bone erosion in rheumatoid arthritis and periodontitis and reveals its shared osteoimmune role. *FEBS Letters, 589*(12), 1331–1339. https://doi.org/10.1016/j.febslet.2015.04.008
He, H., Liyanarachchi, S., Akagi, K., Nagy, R., Li, J., Dietrich, R. C., … Xin, B. (2011). Mutations in U4atac snRNA, a component of the minor spliceosome, in the developmental disorder MOPD I. *Science*, 332(6026), 238–240.

Hellemans, J., Coucke, P. J., Giedion, A., De Paepe, A., Kramer, P., Beemer, F., & Mortier, G. R. (2003). Homozygous mutations in IHH cause acrocapitofemoral dysplasia, an autosomal recessive disorder with cone-shaped epiphyses in hands and hips. *The American Journal of Human Genetics*, 72(4), 1040–1046. https://doi.org/10.1086/374318

Heydari, S.-T., Emanghoreishi, F., & Amini, M. (2009). Infants' growth charts in Jahrom, Iran. *Iranian Journal of Pediatrics*, 19(1), 25–34.

Honey, K., & Rudensky, A. Y. (2003). Lysosomal cysteine proteases regulate antigen presentation. *Nature Reviews Immunology*, 3(6), 472. https://doi.org/10.1038/nri1110

Hood, R. L., Lines, M. A., Nikkel, S. M., Schwartzztruber, J., Beaulieu, C., Nowaczyk, M. J. M., … Boycott, K. M. (2012). Mutations in SRCAp, encoding SNF2-related CREBBP activator protein, cause Floating-Harbor syndrome. *American Journal of Human Genetics*, 90(2), 308–313. https://doi.org/10.1016/j.ajhg.2011.12.001

Hou, W.-S., Brömme, D., Zhao, Y., Mehler, E., Dushey, C., Weinstein, H., … Gelb, B. D. (1999). Characterization of novel cathepsin K mutations in the pro and mature polypeptide regions causing pycnodysostosis. *The Journal of Clinical Investigation*, 103(5), 731–738. https://doi.org/10.1172/JCI5163

Iida, K., Takahashi, Y., Kaji, H., Nose, O., Okimura, Y., Abe, H., & Chihara, K. (1998). Growth hormone (GH) insensitivity syndrome with high serum GH-binding protein levels caused by a heterozygous splice site mutation of the GH receptor gene producing a lack of intracellular domain. *The Journal of Clinical Endocrinology & Metabolism*, 83(2), 531–537.

Jee, Y. H., Andrade, A. C., Baron, J., & Nilsson, O. (2017). Genetics of short stature. *Endocrinology and Metabolism Clinics of North America*, 46(2), 259–281. https://doi.org/10.1016/j.ecl.2017.01.001

Kalev, I., Muru, K., Teek, R., Zordania, R., Reimand, T., Kõbas, K., & Õunap, K. (2010). LEOPARD syndrome with recurrent PTPN11 mutation Y279C and different cutaneous manifestations: Two case reports and a review of the literature. *European Journal of Pediatrics*, 169(4), 469–473. https://doi.org/10.1007/s00431-009-1058-1

Karczewski, K., & Francioli, L. (2017). The Genome Aggregation Database (gnomAD). MacArthur Lab.

Karolchik, D., Baertsch, R., Diekhans, M., Furey, T. S., Hinrichs, A., Lu, Y., … Thomas, D. J. (2003). The UCSC genome browser database. *Nucleic Acids Research*, 31(1), 51–54. https://doi.org/10.1093/nar/gkg129

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, 10(6), 845. https://doi.org/10.1038/nprot.2015.053

Khetarpal, P., Das, S., Panigrahi, I., & Munshi, A. (2016). Primordial dwarfism: Overview of clinical and genetic aspects. *Molecular Genetics and Genomics*, 299(1), 1–15. https://doi.org/10.1007/s00438-015-1110-y

Klopocki, E., Hennig, B. P., Dathe, K., Koll, R., de Ravel, T., Baten, E., … Mundlos, S. (2010). Deletion and point mutations of PTHHLH cause brachydactyly type E. *The American Journal of Human Genetics*, 86(3), 434–439. https://doi.org/10.1016/j.ajhg.2010.01.023

Landrum, M. J., Lee, J. M., Benson, M., Brown, G., Chao, C., Chitipiralla, S., … Hoover, J. (2015). ClinVar: Public archive of interpretations of clinically relevant variants. *Nucleic Acids Research*, 44(D1), D862–D868.

Le Goff, C., Mahaut, C., Wang, L. W., Allali, S., Abhyankar, A., Jensen, S., … Cormier-Daire, V. (2011). Mutations in the TGFRβ binding-protein-like domain 5 of PBN1 are responsible for achondroplastic and geleophysic dysplasias. *The American Journal of Human Genetics*, 89(1), 7–14. https://doi.org/10.1016/j.ajhg.2011.05.012

Lederer, D., Grisart, B., Digilio, M. C., Benoit, V., Crespin, M., Ghariani, S. C., … Verellen-Dumoulin, C. (2012). Deletion of KDM6A, a histone demethylase interacting with MLL2, in three patients with Kabuki syndrome. *The American Journal of Human Genetics*, 90(1), 119–124. https://doi.org/10.1016/j.ajhg.2011.11.021

Leduc, M. S., Niu, Z., Bi, W., Zhu, W., Miloslavskaya, I., Chiang, T., … Eng, C. (2016). CRIPT exonic deletion and a novel missense mutation in a female with short stature, dysmorphic features, microcephaly, and pigmentary abnormalities. *American Journal of Medical Genetics Part A*, 170(8), 2206–2211.

Lehmann, K., Seemann, P., Stricker, S., Sammar, M., Meyer, B., Suring, K., … Mundlos, S. (2003). Mutations in bone morphogenetic protein receptor 1B cause brachydactyly type A2. *Proceedings of the National Academy of Sciences*, 100(21), 12277–12282.

Li, J., Shi, L., Zhang, K., Zhang, Y., Hu, S., Zhao, T., … Ji, L. (2017). VarCards: An integrated genetic and clinical database for coding variants in the human genome. *Nucleic Acids Research*, 46(1), D1039–D1048.

Lindstrand, A., Grigelioniene, G., Nilsson, D., Pettersson, M., Hofmeister, W., Anderlid, B.-M., … Valta, H. (2014). Different mutations in PDE4D associated with developmental disorders with mirror phenotypes. *Journal of Medical Genetics*, 51(1), 45–54.

Linglart, A., Menguy, C., Couvineau, A., Auzan, C., Gunes, Y., Cancel, M., … Bougnères, P. (2011). Recurrent PRKAR1A mutation in acrodysostosis with hormone resistance. *New England Journal of Medicine*, 363(23), 2218–2226.

Lotunin, S., Kiviranta, R., Matsubara, T., Alzate, J. A., Neff, L., Lüth, A., … Baron, R. (2013). Osteoclast-specific cathepsin K deletion stimulates SIP-dependent bone formation. *Journal of Clinical Investigation*, 123(2), 668–680. https://doi.org/10.1172/JCI64840

Menke, L. A., van Belzen, M. J., Alders, M., Cristofoli, F., Study, D., Ehmke, N., … Hoffer, M. J. (2016). CREBBP mutations in individuals without Rubinstein-Taybi syndrome phenotype. *American Journal of Medical Genetics Part A*, 170(4), 2681–2693.

Mujavar, Q., Naganoor, R., Patil, H., Thorbi, A. N., Ukkali, S., & Malagi, N. (2009). Pycnodysostosis with unusual findings: A case report. *Cases Journal*, 2(1), 6544. https://doi.org/10.4076/1757-1626-2-6544

Naeem, S., Sheikh, S., & Ahmad, W. (2009). A mutation in CTSK gene in an autosomal recessive pycnodysostosis family of Pakistani origin. *BMC Medical Genetics*, 10(1), 76. https://doi.org/10.1186/1471-2350-10-76

Ng, P. C., & Henikoff, S. (2003). SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Research*, 31(13), 3812–3814. https://doi.org/10.1093/nar/gkg509

Nicole, S., Davoine, C.-S., Topaloglu, H., Cattolic, L., Barral, D., Beighton, P., … Fontaine, B. (2000). Perlecan, the major proteoglycan of basement membranes, is altered in patients with Schwartz-Jampel syndrome (chondrodystrophic myotonia). *Nature Genetics*, 26(4), 480. https://doi.org/10.1038/82638

Noonan, J. A. (2006). Noonan syndrome and related disorders: Alterations in growth and puberty. *Reviews in Endocrine and...*
Xiao, Y., Junfeng, H., Tianhong, L., Lu, W., Shulin, C., Yu, Z., … Min, L. (2006). Cathepsin K in adipocyte differentiation and its potential role in the pathogenesis of obesity. *The Journal of Clinical Endocrinology & Metabolism, 91*(11), 4520–4527. https://doi.org/10.1210/jc.2005-2486

Xue, Y., Cai, T., Shi, S., Wang, W., Zhang, Y., Mao, T., & Duan, X. (2011). Clinical and animal research findings in pycnodysostosis and gene mutations of cathepsin K from 1996 to 2011. *Orphanet Journal of Rare Diseases, 6*(1), 20. https://doi.org/10.1186/1750-1172-6-20

Yang, H., Robinson, P. N., & Wang, K. (2015). Phenolyzer: Phenotype-based prioritization of candidate genes for human diseases. *Nature Methods, 12*(9), 841. https://doi.org/10.1038/nmeth.3484

Yang, M., Sun, J., Zhang, T., Liu, J., Zhang, J., Shi, M. A., … Shi, G.-P. (2008). Deficiency and inhibition of cathepsin K reduce body weight gain and increase glucose metabolism in mice. *Arteriosclerosis, Thrombosis, and Vascular Biology, 28*(12), 2202–2208. https://doi.org/10.1161/ATVBAHA.108.172320

Zhou, H., Gao, M., & Skolnick, J. (2018). ENTPRISE-X: Predicting disease-associated frameshift and nonsense mutations. *PLoS ONE, 13*(5), e0196849. https://doi.org/10.1371/journal.pone.0196849

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

---

**How to cite this article:** 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:e1118. [https://doi.org/10.1002/mgg3.1118](https://doi.org/10.1002/mgg3.1118)