Research Article

Identification of a Novel Mutation in the Cathepsin K (*CTSK*) Gene in Iranian Patients with Pycnodysostosis

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Abstract:

Background: Pycnodysostosis is a rare autosomal recessive skeletal dysplasia, which is characterized by various skeletal malformations and distinctive facial characteristics. The disorder is caused by mutations in *CTSK* gene at chromosomal region 1q21, which encodes a lysosomal cysteine protease, cathepsin K.

Purpose: To describe the clinical, radiological, and molecular findings in a consanguineous Iranian family with 2 affected individuals demonstrating pycnodysostosis.

Methods: Whole exome sequencing (WES) was utilized to detect disease-causing variants. To prove the impact of the identified variants, *in silico* analysis using SIFT and PROVEAN web servers were carried out. The effects of the novel mutation on the protein structure was evaluated by Chimera software using normal and mutated structures derived from SWISS model web server.

Results: We identified the novel homozygous mutation, c.830C>A, in exon 7 of the *CTSK* gene. This missense mutation leads to the substitution of the amino acid alanine at position 277 by glutamic acid (p.A277E) in cathepsin K protease. Sanger sequencing analysis confirmed this mutation in the affected individuals and revealed that the parents were heterozygous for this gene mutation. Bioinformatics databases and the variant's protein structure proved the deleterious effect of this novel mutation on the enzymatic activity of cathepsin K protease.

Conclusion: In this study, we identified a novel mutation, c.830C>A, in *CTSK* gene in patients with pycnodysostosis. This finding adds more data to the genetic basis of pycnodysostosis and also reveals that WES is a powerful tool for diagnosis of this disease and provides the opportunity to for the patients to receive therapies as early as possible.

1. Background

Pycnodysostosis (OMIM #265800) is an uncommon, autosomal recessive skeletal dysplasia with a uniform clinical phenotype characterized by short stature, osteosclerosis, acroosteolysis of the distal phalanges, clavicular dysplasia, bone fragility, and skull deformities with delayed suture closure (1). This disorder was first described by Maroteaux and Lamy in 1962 as a skeletal malformation with normal life-span and intelligence and also normal sexual development (2). This disorder has been found to be caused by deficiency of the cathepsin K protease, which is involved in the degradation of collagen type I and other bone proteins (1, 3). The responsible gene was discovered by positional cloning strategy as *CTSK* gene on chromosome 1q21, which encodes the cysteine protease cathepsin K (4). Subsequent Studies revealed the existence of homozygous or compound heterozygous mutations in *CTSK* gene, which encodes a lysosomal cysteine protease highly expressed in osteoclasts (5). The *CTSK* gene spans over approximately 12 kb of the human genome and contains 8 exons (GenBank-EMBL No. NC_000001.8).

In recent years, the development of next-generation sequencing has led to a revolution in finding mutations underlying monogenic disorders, including skeletal malformations. In this regard, whole exome sequencing (WES) has successfully been exploited by several investigators to identify the causative gene(s) and/or mutation(s) underlying the occurrence of several skeletal malformations, namely pycnodysostosis. This approach, will help depict a more comprehensive figure of the molecular mechanisms, which could provide novel targets for the treatment of these diseases. In addition, WES is helpful in confirming the diagnosis in patients who originally diagnosed as intermediate osteopetrosis. Herein, we report the results of a WES analysis in two siblings with an initial clinical diagnosis of intermediate osteopetrosis, which identified a mutation in the *CTSK* gene, to cause pycnodysostosis.

2. Materials and methods

2.1. Patients and samples

This study involved a family with two pycnodysostosis. Written consent form was taken from all participants before being incuded to the study. Peripheral blood samples were then taken from each member of the family and collected in ethylenediamine tetraacetic acid (EDTA)-containing tubes.

2.2. DNA extraction, whole-exome sequencing and Sanger sequencing

DNA was extracted from the blood samples of two patients and their parents using GeneAll DNA extraction kit (GeneAll, Seoul, Korea), according to the manufacturer's instructions. The DNA samples were then quantified and their integrity was assessed using a NanoDrop®-1000-Detector device (NanoDrop-Technologies, Wilmington, DE, USA) and by running on 1% agarose gel electrophoresis, respectively.

The DNA samples were subjected to WES to determine inherited mutations. Exonic regions of the genome were enriched by the Agilent SureSelect Human All Exon V7 array. WES was conducted for patients by paired-end sequencing on a NovaSeq 6000 instrument (Macrogen Europe, Amsterdam, he Netherlands) with the coverage of more than 20X for 90% of the target regions, which resulted in ~6 Gb of raw data.

The BurrowsWheeler alignment (version 0.7.5a,18) was utilized to align cleaned reads to the human reference genome (hg19). Duplicate reads were detected by Picard (version 2.25) (http://broadinstitute.github.io/picard); Genome Analysis ToolKit (GATK), version 2.4–9 was used for realignment of insertions and deletions. Variants were called with both GATK and Samtools programs, which were then combined and annotated with various databases (dbSNP, 1000 Genomes Project, and ClinVar). The annotation analysis of detected variants was performed by ANNOVAR software. In order to recognize Indels and single nucleotide variants (SNVs), we took advantage of both probability and quality-based algorithms. For prioritization of gene/variant, a minimum depth of 30X and minor allele frequency of 1% were selected as the cut off. Nonsynonymous, frameshift, splice site, and missense variants were filtered in the WES annotation data of patients according to the predicted AR inheritance from the pedigree analysis.

Sanger sequencing was carried out on two affected patients and their healthy parents in order to validate WES findings and co-segregation of the pathogenic variants within the families. Sequencher (Gene Codes) program was chosen for the interpretation and analyzing of the sequencing results.

2.3. In silico analysis

The likely pathogenicity of the novel variants was investigated in silico to predict conformational changes in the protein product using online tools including PROVEAN (http://provean. jcvi.org) and Mutation Assessor (http://www.ngrl.org.uk/Manchester-/page/mutationassessor). I-Mutant software (http://folding.biofold.org/i-mutant/i-mutant2.0.html) was utilized to measure protein stability. Panther (http://pantherdb.org) and SNAP2 (https://rostlab.org/services/snap2web) tools were also used to predict the impact of nucleotide mutation. Finally, SWISS model and chimera software assessed three-dimensional structure of the mutated protein.

3. Results

In this study, one homozygous variant was found through filtering synonymous, non-coding, and common variants in WES annotated data. This variant was a novel missense mutation, c.830C>A, in the *CTSK* gene, which could explain the clinical manifestations related to pycnodysostosis. This novel variant results in replacing a highly conserved alanine amino acid at position 277 of cathepsin K protease to glutamic acid (p. Ala277Glu). According to the results from SIFT and PROVEAN bioinformatic tools, this variant changes the enzyme's structure and ability for binding to substrates. This variant was not recorded in any of the well-known human mutation databases, such as gnomAD and dbSNP (Figure 1).

Sanger sequencing confirmed the homozygote status of this variant in patients and its heterozygous state in the healthy parents. This finding is consistent with autosomal recessive (AR) inheritance pattern according to genetic pedigrees of families (Table 1 and Figure 1).
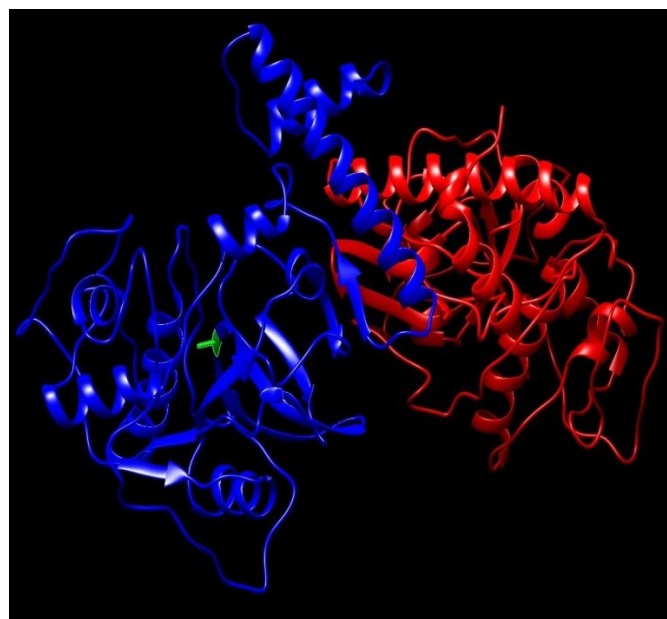

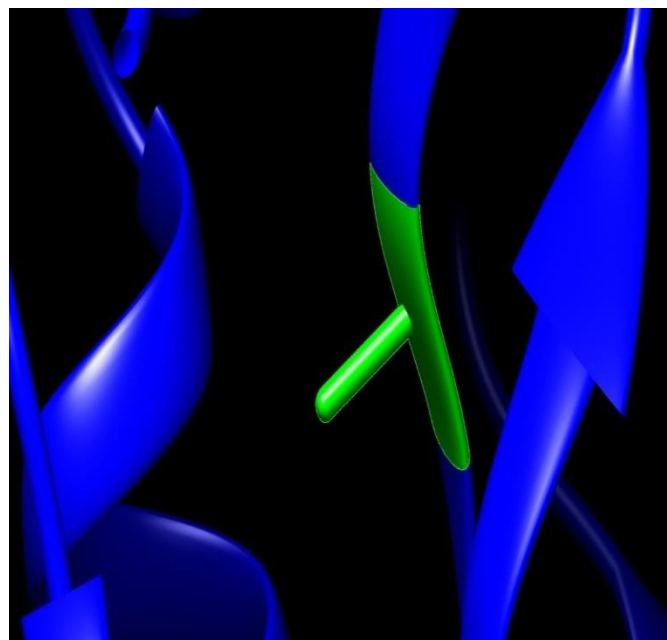**Figure 1. Wild type CTSK protein modelled by Chimera.**

Figure2. wild type CTSK protein modeled using by Chimera, closed up view.
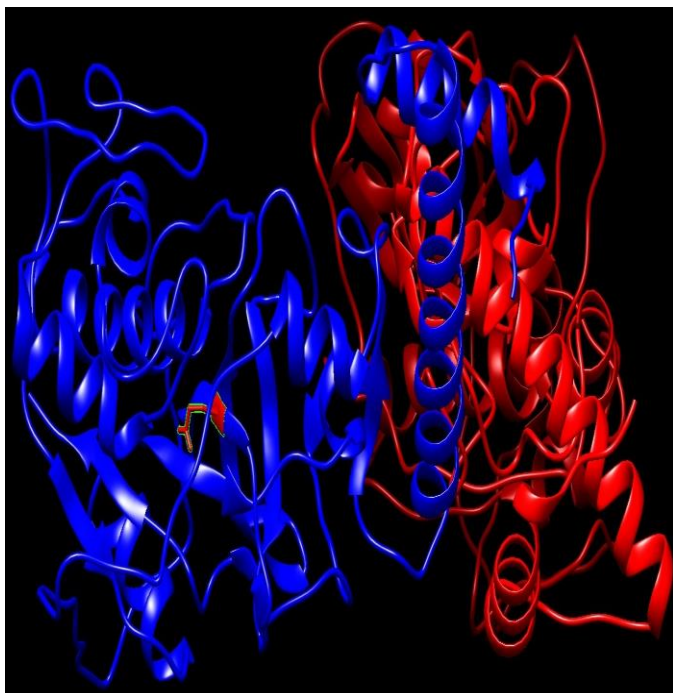
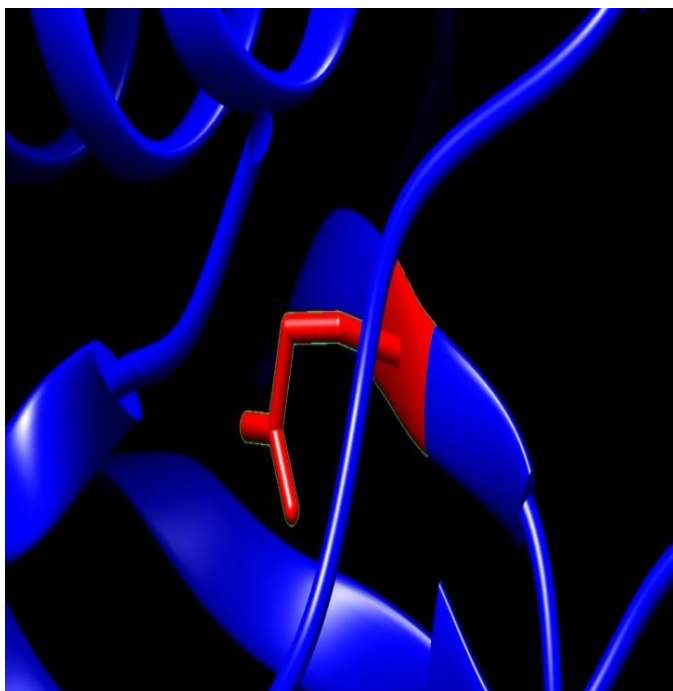**Figure 3. Mutated Mutated CSTK (p.A277E) protein modelled by Chimera.**

Figure 4. Mutated CSTK (p.A277E) protein modelled by Chimera, closed up view.

4. Discussion

In this study, we report the identification of a novel missense variant (c.830C>A) in CSTK gene in an Iranian family with two affected pycnodysostosis patients. This variant results in the substitution of glutamic alanine to glutamic acid at residue 277 of cathepsin K protein (p.Ala277Glu). Our result supports the role of exome sequencing in the differential diagnosis of genetically heterogeneous diseases.

Pycnodysostosis is a severe congenital skeletal disorder, which affects bone growth and results in various malformations. Symptoms of this disorder include a large head and high forehead, undeveloped form of facial bones, and

short fingers and toes (6). People with pycnodysostosis may have short stature, dental abnormalities, brittle bones, and delayed closure of the skull bones (7). In this disorder, bones may become more brittle with age. Other complications, such as trouble in breathing during sleep (sleep apnea) and bone infections may also be observed in the patients (8).

The diagnosis of pycnodysostosis is commonly based on clinical examination and X-ray findings and is confirmed by genetic testing. The genetic testing includes evaluation of *CTSK* gene and in patients with pycnodysostosis, *CSTK* gene mutations are usually observed in homozygous or compound heterozygous state. This gene encodes a cysteine protease from the papain family of proteases, called cathepsin K. This protease plays an important role in the degradation of protein components of the bone matrix. Consequently, it may present a potential target for therapy in osteoporosis. Interestingly, CTSK gene is a transcriptional target of Mitf and TFE3, which bind to this gene via 3 consensus elements in its promoter; Mitf and TFE3 have been reported to modulate age-dependent changes in osteoclast functions (9). The current study reports a novel missense variant (c.830C>A) in a family of Iranian origin with pycnodysostosis. As stated above, this variant results in the substitution of glutamic alanine to glutamic acid at residue 277 of cathepsin K protein (p.Ala277Glu). The markedly chemical difference between alanine and glutamic acid could significantly modify the protein folding and, consequently, affect the activity of cathepsin K. The atomic model assessment demonstrated that the A277E change highly affects stabilizing cathepsin K. This data justifies the occurrence of classic phenotype of pycnodysostosis in this family. In 2011, Xue et al performed a literature retrospective review on several papers which had been reported from 1996 and totally consisted of 159 pycnodysostosis patients. That study was mainly focused on the genetics of CTSK mutations and the clinical phenotypes of pycnodysostosis (10). According to their results, the hot spots for CTSK mutations were found in exons 6 and 7. mutations in these exons mostly resulted in total loss or inactivity of the CTSK protein, which causes abnormal degradation of type I collagen. In line with this study, our reported mutation is in exon 7 and results in a non-conservative amino acid change. This data further highlights the importance of exons 6 and 7 and the catalytic domain of cathepsin K on the pathogenicity of the disease and may help in the future to decide better about what to be targeted for the treatment of pycnodysostosis.

In conclusion, we report a novel variant (c.830C>A) in exon 7 of the cathepsin K protease in an Iranian family by WES. This results highlights the role of exome sequencing on finding novel mutations in skeletal malformations, which confirms the diagnosis of the disease and, hopefully, will help finding novel targets for future treatment of pycnodysostosis.

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