Report of second case and clinical and molecular characterization of Eiken syndrome

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Short running title: Second case of Eiken syndrome

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Conflict of interest

We declare that the authors do not have any conflict of interest and all have read and approved the final manuscript.

Abstract

We report a boy with Eiken syndrome caused by a homozygous missense variant in *PTH1R* c.103G>A [p.(Glu35Lys)]. Eiken syndrome is a very rare skeletal dysplasia due to bi-allelic variants in *PTH1R*. Only one affected family has been known to-date. The hallmarks include delayed ossification of bone including the epiphyses, pubic symphysis, and primary ossification centers of the short tubular bones, coarse bone trabeculae and modeling abnormalities. The phenotype being described here recapitulates the delayed ossification and modeling abnormalities of Eiken syndrome. In addition, supernumerary epiphyses of the tubular bones of the hands and primary failure of eruption of teeth were observed in our proband. This report characterizes Eiken syndrome and confirms that bi-allelic hypomorphic variants in *PTH1R* are likely to cause this condition.

Key words

Eiken syndrome, PTH1R, delayed ossification, bone remodeling, tooth eruption failure, skeletal dysplasia, pseudoepiphysis

Introduction

Pathogenic variants in *PTH1R* have been implicated in several skeletal dysplasias with diverse phenotypes. This include Jansen metaphyseal chondrodysplasia (MIM#156400), Blomstrand chondrodysplasia (MIM#215045) and Eiken syndrome (MIM#600002). Eiken syndrome is characterized by delayed ossification of bones, epiphyseal dysplasia and bone remodeling abnormalities. This disorder has been reported in only one Turkish family.[1] A homozygous truncating variant was identified in *PTH1R* as causative in this family.[2] Here, we report the second individual with Eiken syndrome and describe additional clinical and radiographic features.

Materials and methods

Subject

A seven-years-old boy was ascertained with failure of eruption of primary teeth. He was the first child of healthy first cousins. His birth history was unremarkable and he weighed 3 kg (normal). At six months of age, two pairs of lateral incisors erupted in both the upper and lower jaws. Thereafter, only the first premolar on the left and first molar on the right erupted in the upper jaw. The erupted primary teeth did not fall out. A prominent forehead was noted at around one year of age. He also had one episode of febrile seizures at age four years. He achieved age appropriate developmental milestones and his scholastic performance was average. His hearing and vision were normal. At seven years, he weighed 24 kg (normal), had a height of 119 cm (-1.2 SD) and occipito-frontal circumference of 54 cm (-1.5 SD). There was no disproportion of body segments. He had dolicocephaly, midface retrusion, short philtrum and everted thick lower lip (Figure 1). Oligodontia was observed. In the upper jaw, only the lateral incisor and first premolar on the left, lateral incisor and first molar on the right had erupted. In the lower jaw,

only the lateral incisors had erupted. He had long thumbs and great toes. Short middle phalanges and clinodactyly of fifth fingers were noted bilaterally. Limitation of extension at elbow and swan-neck deformity of fingers were also observed. Systemic examination was unremarkable.

Radiographs showed generalized severe delay in ossification and modeling defects of tubular bones (Figure 1). No carpal bones were visualized even at seven years and he had delayed tarsal ossification. Severe type A1 brachydactyly was observed in the hands and feet. Supernumerary epiphyses (pseudoepiphyses) were noted in the metacarpals and proximal phalanges of the hands. There was relative elongation of the proximal phalanges of the hands, and angel-shaped proximal phalanges of the 1st, 3rd, 4th, and 5th toes. The distal phalanges of the thumbs and great toes were also elongated. Coarse trabeculation was seen in the tubular bones. Radiographs of the pelvis showed delayed ossification of the pubic symphysis. Small, flattened epiphyses and metaphyseal undermodeling were noted in the femora, tibiae and forearm bones. The spine was normal (Supporting information figure S1). Delayed ossification of the patella and mild thickening of cranial vault were also noted. Orthopantomogram (OPG) showed missing, malpositioned and impacted multiple teeth in all the quadrants (Figure 1).

The proband's renal and liver function tests were normal. Serum calcium was 10.2 mg/dL (8.5-10.2), phosphorus was 5.3 mg/dL (2.5-4.5), alkaline phosphatase was 226 IU/L (up to 130). His thyroid function tests and blood glucose levels were normal. Parathyroid hormone (PTH) was elevated at 137.9 pg/mL (11.1-79.5). He had severe vitamin D deficiency and 25(OH)D was 4.5 ng/mL (20-100). Ophthalmological and hearing assessments did not reveal any abnormalities. Echocardiography and ultrasonography of abdomen were also normal.

His parents were of average stature. The father had widely spaced teeth and mother had a missing third molar tooth. They did not have delayed eruption of teeth. OPGs of the parents did

not show any tooth impaction (Supporting information figure S1). Written informed consent was obtained from the family. The study was approved by the institutional ethics committee.

Methods

Exome sequencing on genomic DNA of the proband was performed as described previously.[3] In brief, the libraries were prepared with the Nextera Rapid Capture Exome, Illumina and sequenced on HiSeq 4000 platform (Illumina, San Diego, CA, USA). Sequences were aligned to the GRCh37/hg19 human reference genome. Prioritization of variants was performed by an in-house bioinformatics pipeline based on the allele frequency in public and in-house databases, quality and functional annotation (Supporting information). Mean coverage of the exome was 103x and 96.9% of the targeted region had a depth of at least 10x. Validation and segregation analysis of the variant identified by exome sequencing was done by targeted Sanger sequencing. Structures of wild-type and mutant p.(Glu35Lys) human PTH1R protein (UniProt accession: Q03431) was modelled using the online web server SWISS-MODEL.[4] Structural impact of the mutant residue was predicted using HOPE.[5] ProSA (Protein Structure Analysis) web server was used for refinement and validation of protein structures.[6] The protein structure was visualized using Swiss- PDBViewer software, version: 4.1.0 (Supporting information).

Results

Exome sequencing in the proband identified a homozygous variant c.103G>A [p.(Glu35Lys)] in *PTH1R* (NM_000316.2). This variant is a missense substitution in the fourth exon of *PTH1R* and the same variant was present in both parents in heterozygous state. This novel variant has not been reported in population databases of normal variation (Exac, gnomAD, 1000 Genomes Project) and our in-house exome data of 417 families. This variant has a combined annotation dependent depletion (CADD) score of 34 and Glu35 is evolutionary conserved across multiple

species (Figure 2c). Several prediction tools (MutationTaster,[7] Polyphen 2,[8] SIFT[9]) predict this substitution to be damaging to PTH1R protein function (Supporting information).

In-silico protein modelling was used to assess the structural impact of the substitution of wild type Glu35 to mutant Lys35. HOPE prediction analysis showed that that the wild type Glu35 formed a salt bridge with lysine at position 34. It was predicted that the difference in charge due to the amino acid substitution will disturb the ionic interactions made by the original wild type residue (Figure 2b).

Discussion

When comparing the affected individuals reported by Eiken with our proband, we observe both similar and dissimilar features[1] (Supporting information Table T2). The common features include type A1 brachydactyly, delayed ossification of the pubic symphysis, carpal bones and the epiphyses of the hands and coarse bone trabeculae. Though pseudoepiphyses of metatarsals were observed in one of the patients reported by Eiken, our proband showed pseudoepiphyses in the hands and slightly different bone modeling abnormalities. Also, primary failure of tooth eruption was not observed in the Eiken family but is striking in our case. Elevated parathyroid hormone (PTH) with normal serum calcium levels are in concordance with the earlier report but our patient had nutritional deficiency of vitamin D. This may explain the dolichocephaly and coarse bone trabeculae observed in him.

Parathyroid hormone receptor 1 is encoded by *PTH1R* and binds to both PTH and parathyroid hormone related peptide (PTHrP). This leads to activation of downstream signaling pathways which have contrasting effects on chondrocyte proliferation and differentiation. The complexity is reflected by the spectrum of diverse phenotypes caused by pathogenic variants in this gene (Figure 2d).

Heterozygous gain-of-function variants result in the autosomal dominant Jansen metaphyseal chondrodysplasia (MIM#156400) which is characterized by severe short stature with short limbs, metaphyseal dysplasia and delayed ossification.[10,11] Bi-allelic loss-of-function variants lead to a different set of disorders. Blomstrand chondrodysplasia (MIM#215045) is characterized by accelerated bone maturation, osteosclerosis and is perinatally lethal. Interestingly, teeth impaction has also been reported in these fetuses.[12] Complete loss of PTH1R receptor function has been shown as causative of Blomstrand chondrodysplasia and the phenotype was reproduced in a knock-out mouse model.[2,13] Eiken syndrome (MIM#600002) results from bi-allelic hypomorphic variants in *PTH1R*.[2] The disease causing variant reported in the original Eiken family is a premature truncating variant c.1453C>T [p.(Arg485Ter)] in the C-terminal cytoplasmic tail (last exon) of *PTH1R* (NM_000316.2), most likely escaping nonsense-mediated RNA decay. The imbalance between the adenylcyclase/ protein kinase A (AC/PKA) and phospholipase C/ protein kinase C (PLC/PKC) pathways following PTH1R activation was shown in the DSEL mouse which resulted in a phenotype strikingly similar to Eiken syndrome.[14] Heterozygous inactivating variants in *PTH1R* also cause primary failure of tooth eruption (MIM#125350).[15] This was initially considered an autosomal dominant disorder, but recently, an Arab family has been reported where affected members had homozygous mutations.[16] The skeletal phenotype of the subjects in this study is not described. The heterozygous carriers did not have any dentition abnormalities.

The present patient has a missense substitution c.103G>A [p.(Glu35Lys)] in exon 4 of *PTH1R* (NM_000316.2) which is a part of the helix of PTH1R (UniProt accession: Q03431). This substitution of an acidic with a basic amino acid is expected to disrupt normal protein function as shown by *in-silico* analysis. No disease-causing variants have been reported in this domain

previously (Fig 2d). We speculate that this variant may result in hypomorphic alleles and partial loss-of-function of PTH1R as seen in the original family with Eiken syndrome.

We hereby report the second case of Eiken syndrome and delineate the phenotype further. The different phenotypic consequences of bi-allelic pathogenic variants in *PTH1R* may be domain-related or due to dosage-dependent activation of the complex downstream signaling pathways. Functional studies are needed for better insight into the underlying molecular mechanism.

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Figure legends

Figure 1. The proband has mild genu valgum (a), dolicocephaly, midface retrusion (b), and pseudoanodontia (partial eruption of only four teeth in upper jaw) (c). The middle phalanges of all digits are short, the thumbs and halluces are relatively long, and clinodactyly of the little finger is seen (d, e). Radiographs of the pelvis (j) and forearm (f) show delayed ossification of the pubic symphysis and metaphyseal undermodeling of the proximal femora (pelvis), metaphyseal undermodeling of the distal radius and ulna and distal radioulnar interosseous bridge (forearm); note the small and flattened epiphyses. Radiographs of the hands (h) and feet (i) show type A1 brachydactyly (shortening with absent epiphyses of the middle phalanges of the hands and aplasia of the middle phalanges of the feet), relative elongation with pseudoepiphyses of the proximal phalanges of the hands, and angel-shaped proximal phalanges of the 1st, 3rd, 4th, and 5th toes. The distal phalanges of the thumb and great toe are elongated. Supernumerary epiphyses are present in metacarpals of 1st, 2nd and 3rd fingers and proximal phalanges of all digits. Epiphyseal and carpal ossifications are markedly delayed. The trabecular pattern of the short tubular bones is very coarse. Orthopantomogram (g) shows malposition and impaction of multiple teeth (dental).

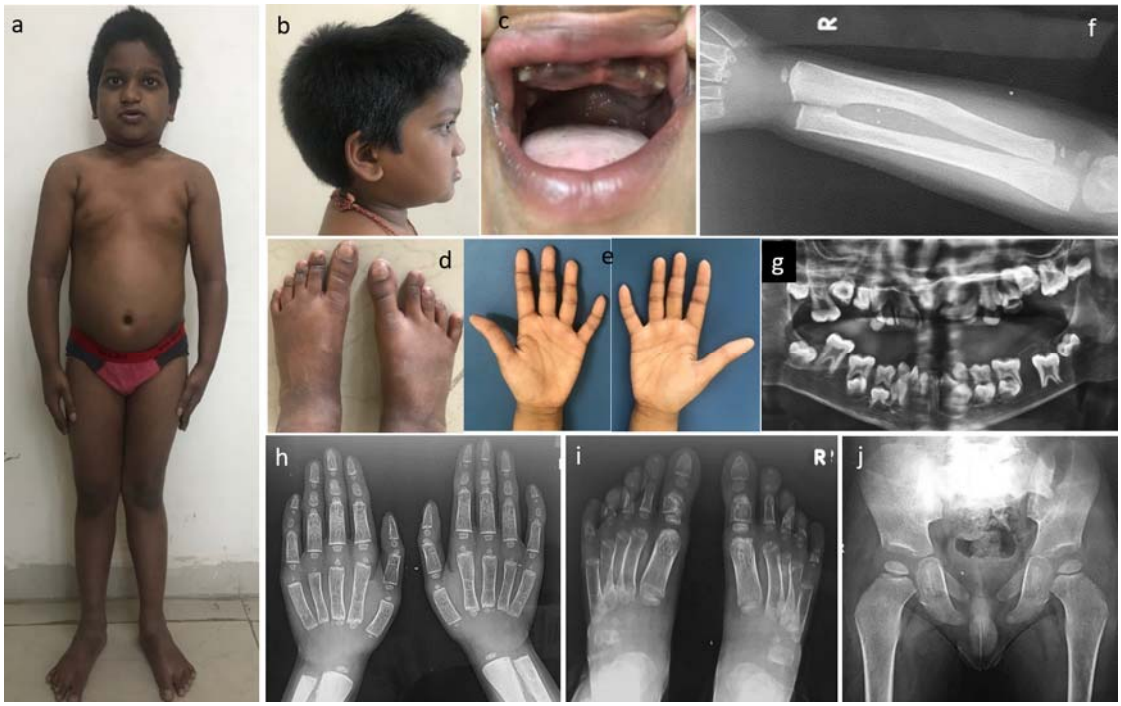

Figure 2. Pedigree of the family with segregation of the variant c.103G>A in *PTH1R* (NM_000316.2) (a). Predicted salt-bridge formation between Glu35 and Lys34 where the distance between the hydrogen donor atom of Lys34 and the hydrogen acceptor atom of Glu35 is 9.25 Å (left panel); replacement of the Glu35 with mutant Lys35 is predicted to lead to the loss of the salt-bridge between the wild type residue and Lys34 (right panel) (b). Multiple sequence alignment confirms evolutionary conservation of p.(Glu35Lys) in *PTH1R* across species (c). Spectrum of reported *PTH1R* variants corresponding to the associated disease/condition(s) in the schematic representation of coding exons from 3 to 16. PTH1R structural domains are color-coded (d).