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ORIGINAL PAPER

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Cleidocranial dysplasia with decreased bone density and biochemical findings of hypophosphatasia

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Abstract Cleidocranial dysplasia (CCD; MIM 119600) is an autosomal dominant skeletal dysplasia characterised by hypoplastic clavicles, patent fontanelles, short stature, tooth anomalies and other variable skeletal changes. Different mutations of the *RUNX2/CBFA1* gene (MIM 600211) have been detected in patients with CCD. We investigated a mother and daughter with features of CCD presenting with reduced plasma alkaline phosphatase activity, increased urinary phosphoethanolamine excretion and decreased bone density. The latter findings were suggestive of hypophophatasia but mutation analysis showed no mutation in the tissue-nonspecific alkaline phosphatase gene (*TNSALP*; MIM 171760). However, a heterozygous mutation (Arg169Pro caused by nucleotide change 506G > C) was detected in the *RUNX2* gene. Metabolic alterations gradually improved in both mother and daughter but bone-specific alkaline phosphatase remained low (less than 30% of normal) and mild phosphoethanolaminuria persisted. Recent studies in the *Cbfa1* knock-out mouse showed decreased expression of alkaline phosphatase in differentiating bone. **Conclusion:** we suggest that the observed metabolic alterations are secondary to the *RUNX2* gene mutation affecting early bone maturation and turnover. This is the first description of biochemical findings of hypophosphatasia in patients with cleidocranial dysplasia.

Keywords Alkaline phosphatase · Cleidocranial dysplasia · Hypophosphatasia · Pyridoxal-5-phosphate

Abbreviations *ALP* alkaline phosphatase · *CCD* cleidocranial dysplasia · *P5P* pyridoxal-5′ phosphate · *TNSALP* tissue non-specific alkaline phosphatase

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Introduction

Cleidocranial dysplasia (CCD; MIM 119600) is an autosomal dominant skeletal dysplasia characterised by patent fontanelles, hypoplastic clavicles, abnormal pelvic bones, supernumerary teeth and short stature. The diagnosis is based on the characteristic clinical and radiological features [1,10]. Although bone formation is clearly abnormal, metabolic studies are rarely performed. Studies in individuals and families affected with CCD suggest that the heterozygous loss of *RUNX2/CBFA1* gene (MIM 600211) function is sufficient for phenotypic development [11,14]. The gene is expressed in developing bone in the perichondrium, osteoblasts and chondrocytes. Heterozygous inactivation of *Runx2/Cbfa1* in mice results in a phenotype resembling that of CCD patients. Mice that lack both alleles of *Runx2/Cbfa1* show a complete lack of bone due to a block in the differentiation of precursors cells into mature osteoblasts. *Runx2–/–* mice do not express osteopontin or osteocalcin, and only weakly express alkaline phosphatase (ALP) [7,12]. Hypophosphatasia (MIM 241500) is a condition caused by mutations in the tissue non-specific ALP gene (*TNSALP;* MIM 171760) [3,5,9]. Overlapping features with CCD are short stature, late ossification of the cranial sutures and bone hypoplasia with metaphyseal abnormalities. Hypophosphatasia can be diagnosed by low activity of ALP, increased amount of inorganic pyrophosphate

and pyridoxal-5-phosphate (P5P) in serum and phosphoethanolaminuria [3,16].

We present a mother and daughter with CCD and metabolic alterations similar to those observed in hypophosphatasia.

Case reports

Case 1

The mother, born full-term in 1964 and a child of healthy, nonconsanguineous parents, had a birth weight of 3.28 kg (50th percentile) and a length of 51 cm (25th percentile). Macrocrania (37 cm; >97th percentile) and wide-open fontanelles were noted. Physical examination detected a narrow shoulder on the right suggestive of a clavicular fracture. The cranial X-ray film showed wide sutures and decreased calcification. She was started on a high dose of vitamin D_3. Dentition was retarded. At the age of 2 years, hypophosphatasia (serum ALP 27 U/l, reference value >60 U/l) was detected with normal serum calcium and phosphorus. Urinary thin-layer chromatographic screening was positive for phosphoethanolamine. Isoenzyme studies detected less than 5% of the bone fraction of total ALP [6] compared to control (20%–30% of total activity). Serum P5P levels were not measured. She was followed up with the diagnosis of late infantile-juvenile form of hypophosphatasia.

Her family was originally investigated in 1973 [8,15], but follow-up studies by thin-layer chromatography did not confirm phosphoethanolaminuria. Currently, at their age of 53 years neither clinical, nor metabolic alterations have been found in the parents (normal total ALP activity and fractions, no phosphoethanolaminuria by ion exchange chromatography, normal P5P, and normal vertebral DEXA in the patient's mother).

Currently, at the age of 33 years, the patient has mild phosphoethanolaminuria (20 µmol/mmol creatinine; controls <17.5 µmol/mmol creatinine), low serum ALP 68 U/l (controls <80 U/l) and normal serum P5P level [17] (80 nmol/l; controls <100 nmol/l). Her height is 159 cm (10th–25th percentile) and her head circumference is 57 cm (75th–90th percentile). She has a sloping shoulder on the right side. Eruption of secondary teeth was normal, and no supernumerary teeth are present. A DEXA study of the lumbar spine, left iliac bone and the left radius detected decreased bone mineral density (Z-score: –1.95; control >–1). Psychomotor development has been normal and no apparent health problems have so far been apparent.

A radiological survey at 2 years of age showed wide open fontanelles, Wormian bones near the lambdoid suture, a dysplastic right clavicle and hypoplastic pubic bones (Fig. 1a–c). At the current age, a spinal X-ray film showed hypoplastic posterior arches of the thoracic vertebrae, pseudo-joint of the hallux, pseudoepiphysis of the second metacarpals, persistent epiphyses of the cone-shaped distal carpal and pedal phalanges and mild generalised osteopenia (Fig. 1d). The features are consistent with the diagnosis of CCD.

Case 2

The daughter of Case 1 was born in 1993. The father was 28 years old and he had no apparent health problems. His metabolic evaluation revealed normal ALP levels and no phosphoethanolaminuria. The child was born at term by caesarean section with a birth

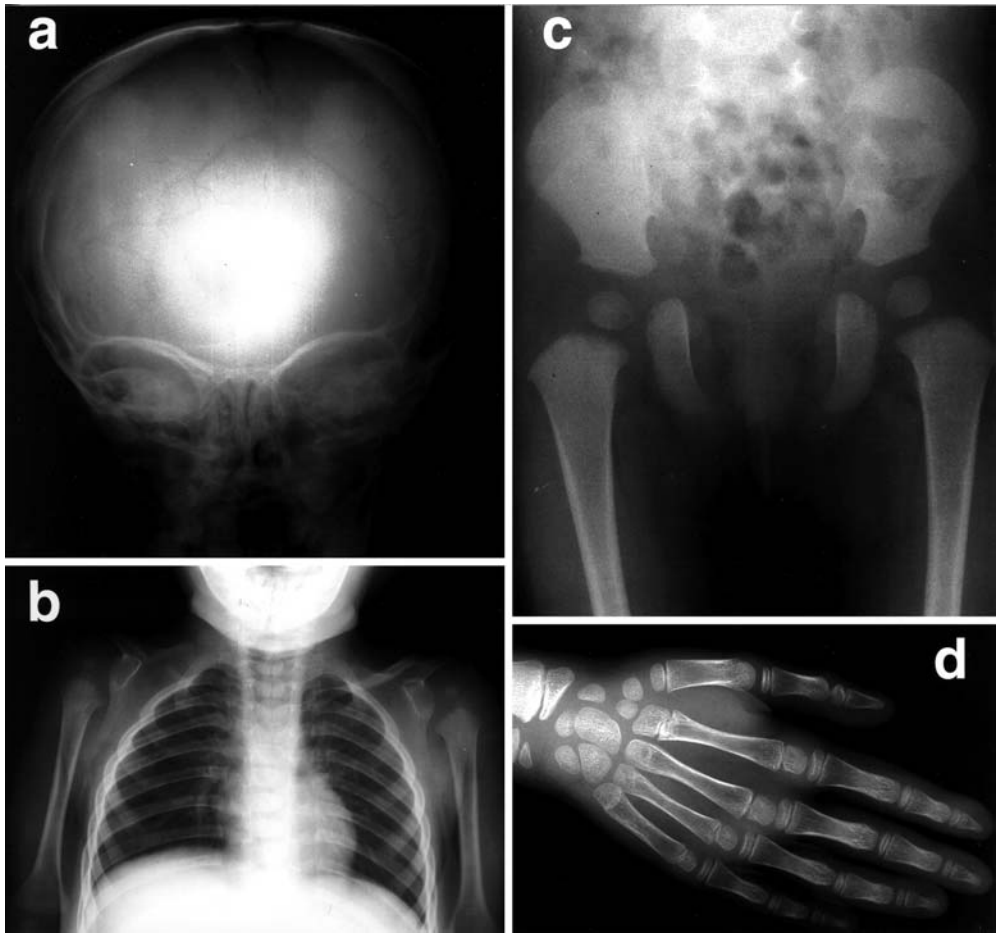

Fig. 1a–d. Radiological features of Case 1 at 2 years of age. **a** Plain X-ray film of the skull showing open fontanelles and Wormian bones near the lambdoid suture. **b** Chest film: the left clavicle is shorter, the right one is dysplastic and consists of two parts, and the proximal humeral epiphyses have two ossification centres. **c** Pelvic X-ray film: the inferior part of the pubic bone is missing and the superior part is dysplastic. **d** Plain X-ray film of the hand of Case 1at 31 years of age: the radial and ulnar epiphyses are not fused, pseudo-epiphyses at metacarpals, persistent phalangeal epiphyses, cone-shaped distal phalanges; note the remarkable delay in ossification!

weight of 3.1 kg (25th–50th percentile) and a length of 50 cm (25th percentile). Relative macrocrania (36 cm; 75th–90th percentile) with open sutures and fontanelles (anterior fontanelle: 3×4 cm) and narrow shoulders were noted. Serum ALP activity at birth was 67 U/l (controls >110 U/l). Serum calcium, phosphorus and amino acid values were normal; significant phosphoethanolaminuria (48 μmol/mmol creatinine; controls <25 μmol/mmol creatinine) was detected with normal urine electrolytes, pH, creatinine clearance and phosphorus reabsorption, and absent glucosuria and hyperaminoaciduria. She developed normally. Her cranial sutures closed at the age of 3–5 years. She had a fracture of her fifth finger with adequate healing. Her first teeth appeared at the age of 11 months and at the age of 9 years she has only three permanent teeth.

At the age of 6 years her weight was 17 kg (5th–10th percentile) and was 110 cm (25th percentile) tall. Serum analysis showed normal total ALP activity (146 U/l; controls >140 U/l) and increased P5P levels (320 nmol/l; controls <100 nmol/l). Isoenzyme studies found a 10% bone fraction of total ALP (control >28%). A DEXA examination of the lumbar spine, left iliac bone and the left radius showed a generalised decrease in bone mineral density (Z-score: –2,6; age matched control >–1).

A radiological survey at the age of 8 years showed that the sagittal suture of the skull was still visible, the right clavicle was dysplastic with a gothic shaped thorax. Narrow posterior arches of the thoracic vertebrae without complete fusion, hypoplastic pelvic bones with open symphysis, cone-shaped distal phalanges, partial closure of epiphyses of the first to fifth metacarpals were also observed. The findings were consistent with the diagnosis of CCD (Fig. 2a–c).

Due to the conflicting clinical and metabolic findings, DNA mutation analysis was performed. No mutation was found in the *TNSALP* gene in the mother and daughter, whereas analysis of the *RUNX2* gene revealed a heterozygous mutation (Arg169Pro due to nucleotide change 506G>C) supporting the diagnosis of CCD in both mother and daughter.

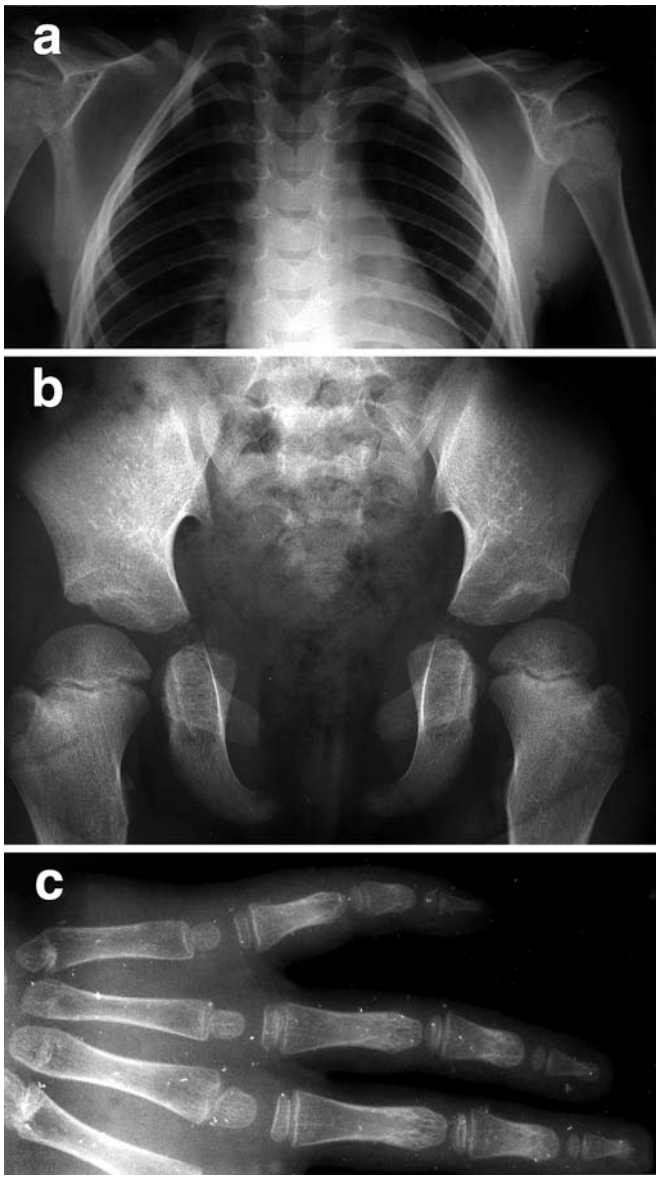

Fig. 2a–c. Radiological features of Case 2. **a** Chest film at 8 years of age: the posterior arches of the thoracic vertebrae are not completely fused, the right clavicle consists of two dysplastic parts. **b** Pelvic X-ray film at 8 years of age: narrow pelvis, dysplastic superior part and aplastic inferior part with open symphysis. **c** Plain film of the hand at 4 years of age: pseudo-epiphyses at metacarpals, clinodactyly of the fifth finger, cone-shaped distal phalanges

Discussion

Bone formation and homeostasis is a complex process involving a multitude of signalling pathways. Osteoblasts are constantly recruited from mesenchymal progenitor cells and contribute to the formation of new bone. The transcription factor Runx2 is a major regulator of this process as demonstrated by the complete lack of osteoblasts in *runx2*(–/–) mice. The Runx2 protein was shown to regulate a number of bone-related genes such as collagen type 1, osteocalcin, osteopontin and collagenase 1 [2]. On the cellular level, Runx2 interacts with the Smads, which serve as intracellular signalling molecules for the TGFβ pathway.

At least five different genes encode various forms of ALP. Depending on the expression pattern of those genes, we can distinguish embryonic, intestinal, or liver/bone/kidney (also known as TNSALP). Although the expression of the *TNSALP* gene in vivo is not restricted to bone, the upregulation of this gene in vitro has been generally associated with the onset of osteogenic differentiation. Inactivation of the gene results in hypophosphatasia, a metabolic bone disorder presenting with variable age of onset and severity. The late infantile-juvenile form has a mild clinical picture with premature loss of deciduous teeth, bone deformities, short stature and findings similar to rickets [16]. Characteristic metabolic findings are decreased activity of serum TNSALP, high levels of P5P and increased phosphoethanolaminuria. Low activity of ALP leads to abnormal bone metabolism, skeletal deformities and osteopenia. Increased amount of serum P5P and urine phosphoethanolamine levels indicate an increase in the non-metabolised substrate of the enzyme. Mutations in the *TSNALP* gene have been detected in all the different clinical forms of hypophosphatasia [3,5,9].

The family described here has clinical findings characteristic for CCD, in which we were able to identify a mutation in the *RUNX2* gene in both mother and

daughter. The mutation is located within the highly conserved DNA-binding domain and can be expected to interfere with normal binding/function [13,14]. In addition to the characteristic CCD phenotype, we observed metabolic signs of hypophosphatasia, i.e. low ALP and an increase in P5P and phosphoethanolaminuria. We suggest that the finding of low ALP with increased substrate accumulation in our patients is related to the mutation in the *RUNX2*/*CBFA1* gene. Expression of *Runx2* in a mouse fibroblastic cell line, C3H10T1/2, induces the expression of ALP activity [4] and expression of ALP is drastically reduced in *runx2*(–/–) mice, both findings suggesting a regulation of ALP by *Runx2* [7,12]. It remains to be determined if low ALP levels and the associated metabolic effects are frequent findings in CCD patients. The association of decreased bone mass and CCD has been described previously in a patient with severe osteopenia and multiple fractures [14]. It is possible that the reduced bone mass is caused by the reduced differentiation of precursor cells into osteoblasts. Alternatively, this may be a metabolic effect due to reduced ALP activity.

Metabolic studies are rarely performed in skeletal dysplasias. These routine biochemical studies are useful tools for screening purposes, however, they are nonspecific and should be interpreted cautiously. The present observation is the first description of biochemical features of hypophosphatasia in CCD.

Editorial note: the second observation of biochemical features of hypophosphatasia in CCD is in the following article [18].

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References

1. Cooper SC, Flaitz CM, Johnston DA, Lee B, Hecht JT (2001) A natural history of cleidocranial dysplasia. Am J Med Genet 104: 1–6
2. Ducy P, Zhang R, Geoffrey V, Ridall AL, Karsenty G (1997) Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 89: 747–754
3. Gehring B, Mornet E, Plath H, Hansmann M, Bartmann P, Brenner RE (1999) Perinatal hypophosphatasia: diagnosis and detection of heterozygote carriers within the family. Clin Genet 56: 313–317
4. Harada H, Tagashira S, Fujiwara M, Ogawa S, Katsumata T, Yamaguchi A, Komori T, Nakatsuka M (1999) Cbfa1 isoforms exert functional differences in osteoblast differentiation. J Biol Chem 274: 6972–6978
5. Henthorn PS, Raducha M, Fedde KN, Lafferty MA, Whyte MP (1992) Different missense mutations at the tissue nonspecific alkaline phosphatase gene locus in the autosomal recessively inherited forms of mild and severe hypophosphatasia. Proc Natl Acad Sci USA 89: 9924–9928
6. Hosenfeld D, Hosenfeld A (1973) Qualitative and quantitative Untersuchungen der Isoenzyme der alkalischen Serumphosphatase bei der Hypophosphatasie. Klin Padiätr 185: 437–443
7. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T (1997) Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89: 755–764
8. Méhes K, Klujber L, Lassu G, Kajtár P (1972) Hypophosphatasia: screening and family investigations in an endogamous Hungarian village. Clin Genet 3: 155–159
9. Mornet E (2000) Hypophosphatasia: the mutations in the tissue nonspecific alkaline phosphatase gene. Hum Mutat 15: 309–315
10. Mundlos S (1999) Cleidocranial dysplasia: clinical and molecular genetics. J Med Genet 36: 177–182
11. Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JH, Owen MJ, Mertelsmann R, Zabel BU, Olsen BR (1997) Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 89: 773–779
12. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB, Owen MJ (1997) Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 89: 765–771
13. Otto F, Kanegane H, Mundlos S (2002) Mutations in the RUNX2 gene in patients with cleidocranial dysplasia. Hum Mutat 19: 209–216
14. Quack I, Vonderstrass B, Stock M, Aylsworth AS, Becker A, Brueton L, Lee PJ, Majewski F, Mulliken JB, Suri M, Zenker M, Mundlos S, Otto F (1999) Mutation analysis of core binding factor A1 in patients with cleidocranial dysplasia. Am J Hum Genet 65: 1268–1278
15. Rubecz I, Méhes K, Klujber L, Bozzay L, Weisenbach J, Fenyvesi J (1974) Hypophosphatasia: screening and family investigation. Clin Genet 6: 155–159
16. Terheggen HG, Wischermann A (1984) Kongenitale Hypophosphatasie. Monatsschr Kinderheilkd 132: 512–522
17. Ubbink JB, Serfontein WJ, de Villiers LS (1985) Stability of pyridoxal-5-phosphate semicarbazone: applications in plasma vitamin B6 analysis and population surveys of vitamin B6 nutritional status. J Chromatogr 342: 277–284
18. Unger S, Mornet E, Mundlos S, Blaser S, Cole DEC (2002) Severe cleidocranial dysplasia can mimic hypophosphatasia. Eur J Pediatr DOI 10.1007/s00431-002-0978-9