CLINICAL REPORT

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Craniosynostosis in Pycnodysostosis: Broadening the Spectrum of the Cranial Flat Bone Abnormalities

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Pycnodysostosis is a rare autosomal recessive skeletal dysplasia caused by the absence of active cathepsin K, which is a lysosomal cysteine protease that plays a role in degrading the organic matrix of bones, acting in bone resorption and bone remodeling. The disease is primarily characterized by osteosclerosis, bone fragility, short stature, acro-osteolysis, and delayed closure of the cranial sutures. A differing feature, cranial synostosis, has occasionally been described in this disorder. We reviewed six unrelated patients with pycnodysostosis (mean age of 10 years and 4 months) in order to evaluate the presence of craniosynostosis. In addition to the typical findings of the condition, they all presented premature fusion of the coronal suture. Although none of them showed signs of cranial hypertension, one patient had had the craniosynostosis surgically corrected previously. These data suggest that the cranial sutures in pycnodysostosis can display contradictory features: wide cranial sutures, which are commonly described, and craniosynostosis. The clinical impact of this latter finding still remains to be elucidated. Further studies are necessary to address more precisely the role of cathepsin K in suture patency.

Key words: pycnodysostosis; cathepsin K; craniosynostosis

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INTRODUCTION

Pycnodysostosis, a rare autosomal recessive condition, is a member of the group of skeletal disorders characterized by increased bone density without modification of bone shape [Superti-Furga and Unger, 2007]. Besides osteosclerosis with bone fragility, other frequent clinical findings include short stature, persistent fontanels, wide cranial sutures, clavicular dysplasia, hypoplasia of the mandibular angle, spondylolysis, and acro-osteolysis. The craniofacial appearance includes a high cranial vault, proptosis, overhanging nasal tip, and micrognathia [Maroteaux and Lamy, 1962; Currarino, 1989]. Oral anomalies, with a high arched and grooved palate, and dental anomalies, have also been commonly found in this disorder [Hunt et al., 1998].

Loss-of-function mutations in the *CTSK* gene, which codes for cathepsin K, was identified in patients affected by this condition [Gelb et al., 1996]. The gene is located on chromosome 1q21 and codes for a lysosomal cysteine protease, with the highest levels of expression in osteoclasts. This enzyme has a role in degrading the organic matrix of bones, acting in bone resorption and bone remodeling. Its absence, seen in pycnodysostosis, explains the sclerosing aspect of the bones found in these patients.

In contrast, the pathomechanism of another hallmark of pycnodysostosis, failure of closure of cranial sutures, has not been addressed properly. Despite several studies of this disorder in animal models [Saftig et al., 1998; Gowen et al., 1999; Kiviranta et al., 2005; Li et al., 2006; Boskey et al., 2009], the complete scenario of pycnodysostosis was only recapitulated in the 129Sv inbred strain of *cathepsin K*$^{-}$/$^{-}$ mice [Chen et al., 2007]. These authors observed thinner calvarial bones in the mice and speculated that acro-osteolysis, spondylolysis, and calvarial thickness could be explained by increased bone resorption due to site-specific variations in cathepsin K action.

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These studies certainly enhance the understanding of the apparently paradoxical features, both osteolysis and osteosclerosis, seen in pycnodysostosis.

Another apparently conflicting characteristic, rarely described, is the presence of craniosynostosis in pycnodysostosis, a finding of uncertain clinical relevance [Bernard et al., 1980; Osimani et al., 2010]. Therefore, we herein review six patients with pycnodysostosis in order to evaluate the presence of craniosynostosis.

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Six unrelated patients (two males and four females), with ages ranging from 6 years and 8 months to 13 years and 10 months (mean age of 10 years and 4 months), were diagnosed with pycnodysostosis in our clinic. Consanguinity was present in two families, and in two other cases, the parents were from the same small village. All affected individuals had normal developmental milestones, short stature, bone sclerosis, obtuse angle of the mandible, and acro-osteolysis (Fig. 1). Except for the youngest patient, all of the others have shown multiple bone fractures throughout their lives (Table I). The anterior fontanel was still open at the age of 6 years in all affected individuals but one.

Three-dimensional cranial CT scans disclosed the following features in our cohort: all patients presented with wide lambdoidal sutures and premature synostosis of the coronal suture, either unilaterally or bilaterally, partially or totally (Fig. 2).

One of the cases (Patient 5) has a history of craniosynostosis, surgically corrected elsewhere when she was 15 months old. She was misdiagnosed as being affected by Crouzon syndrome. Thirteen years after surgery, she has midface hypoplasia and slight proptosis. She is scheduled for Lefort III advancement with distraction osteogenesis.

Another patient (#3) had facial asymmetry due to unilateral involvement of the coronal suture. He had no signs of high intracranial pressure and declined an offered procedure for correction of his facial asymmetry.

The other four affected individuals did not have any signs of intracranial hypertension assessed by cranial CT scans and/or ophthalmologic evaluation.

The coding region sequencing of the *CTSK* gene disclosed three different mutations in this cohort, in homozygosity in four patients and in heterozygosity in the two others: p.Arg241X, p.Gly146Arg, and p.Cys318Tyr (Table I). The latter (a novel mutation) is located in the region that codes for the mature domain of the protein, whereas the majority of the mutations involved in pycnodysostosis was described [Donnarumma et al., 2007]. PolyPhen program indicates that the p.Cys318Tyr mutation predicts damage to the protein (coot.embl.de/PolyPhen). The other two gene alterations (p.Arg241X and p.Gly146Arg) have already been found in patients with different ethnicity: Moroccan, Tunisian, Algerian, Spanish, Portuguese, and Italian [Gelb et al., 1996; Donnarumma et al., 2007; Osimani et al., 2010].

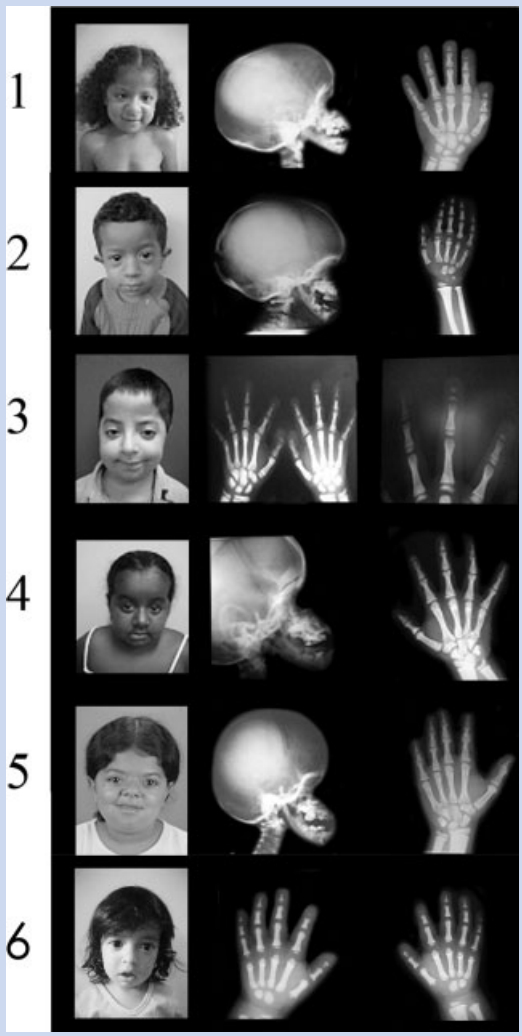

FIG. 1. Frontal view of the patients with pycnodysostosis showing high cranial vault, proptosis, and micrognathia with their corresponding cranial and/or hand X-rays (bone sclerosis, obtuse angle of the mandible and acro-osteolysis).

DISCUSSION

These six patients here presented have typical findings of pycnodysostosis and pathogenic mutations in the *CTSK* gene. Nevertheless, the intriguing issue is the presence of premature fusion of cranial sutures in all of them, a characteristic previously rarely described in the disorder [Bernard et al., 1980; Osimani et al., 2010].

The high prevalence of cranial suture synostosis in our cohort of pycnodysostotic patients suggests that this finding is part of the spectrum of the disorder. The possibility of co-occurrence of two separate disorders (pycnodysostosis and craniosynostosis) is highly unlikely, taking into consideration that craniosynostosis and, especially, pycnodysostosis are rare conditions.

Mouse models have repeatedly shown that cathepsin K, the enzyme affected in pycnodysostosis, plays a critical role in osteoclast -mediated bone resorption. The impairment of bone matrix degradation due to the osteoclastic dysfunction leads to an osteopetrotic phenotype and also a poorly organized bone microstructure, translating into poorer bone quality with brittleness of the long

TABLE I. Clinical and Radiographic Findings in Our Cohort of Six Patients With Pycnodysostosis

Clinical/radiological findings	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Sex	Female	Male	Male	Female	Female	Female
Age	12 y 8 m	7 y 8 m	10 y 8 m	11 y	13 y 10 m	6 y 8 m
Normal developmental milestones	+	+	+	+	+	+
Short stature	+	+	+	+	+	+
Bone sclerosis	+	+	+	+	+	+
Obtuse angle of the mandible	+	+	+	+	+	+
Acro-osteolysis/phalanges hypoplasia	+	+	+	+	+	+
Multiple bone fractures	+	+	+	+	+	−
Cranial bone abnormalities	+	+	+	+	+	+
Wide lambdoidal sutures	+	+	+	+	+	+
Coronal synostosis	+ (B, T)	+ (U, P)	+ (U, T)	+ (B, P)	+	+ (B, T)
Consanguinity	+	−	−[a]	−[a]	+	−
CTSK gene mutation	p.Cys318Tyr/ Cys318Tyr	p.Cys318Tyr/ Arg241X	p.Arg241X/ Gly146Arg	p.Cys318Tyr/ Cys318Tyr	p.Arg241X/ Arg241X	p.Arg241X/ Arg241X

B, bilateral; T, total; U, unilateral; P, partial; y, years; m, months.

[a]Parents are from the same small village.

bones [Saftig et al., 1998; Gowen et al., 1999; Kiviranta et al., 2005; Li et al., 2006].

Nevertheless, the skull bone abnormalities in pycnodysostosis have not been properly elucidated. The first mouse models for this condition did not show defects in these specific bones [Saftig et al., 1998; Gowen et al., 1999; Kiviranta et al., 2005], but other studies have demonstrated the role of cathepsin K in calvarial osteoclasts [Dodds et al., 1998; Everts et al., 2006; Chen et al., 2007]. In the latter, the authors generated *cathepsin K*$^{-/-}$ mouse strains in the 129/Sv background (different from the other studies) and recapitulated separated cranial sutures and thinner calvarial bones compared with those of wild-type mice. Although these studies discuss some aspects of skull abnormalities in pycnodysostosis, there are no comments regarding the defects in the cranial sutures.

The role of osteoclasts in cranial sutures patency is clearly elicited in a mouse model of another sclerosing bone disorder—osteopetrosis—in which there is a lack of osteoclastic cells due to a deficiency in macrophage colony-stimulating factor [Kaku et al., 1999; Byron, 2006].

We hypothesize that similar to what is observed in osteopetrosis, the impaired function of the osteoclasts produces unbalanced bone remodeling at the cranial sutural interface. Depending on the suture involved, this imbalance will provoke either craniosynostosis or wide cranial sutures.

This apparent incongruous finding, craniosynostosis in pycnodysostosis, rarely described previously, could be attributed mainly to two factors. The first is a lack of recognition due to the scarcity of detailed image studies of the cranial sutures in pycnodysostosis. Although anecdotal descriptions of epidural hematomas have been made, the focus has been the cerebrum itself and not the cranial sutures [Cirak et al., 1999; Olubaniyi et al., 2008]. The second factor is the absence of signs and symptoms of increased intracranial pressure. We can speculate that the presence of other cranial suture diastases, especially in the parieto-occipital interface, could compensate and hamper the development of increased intracranial pressure. Nevertheless, caution should be exercised regarding the apparent benign aspect of the craniosynostosis in these patients. In the two cases so far described [Bernard et al., 1980; Osimani et al., 2010], both presenting this skeletal dysplasia and synostotic involvement of the coronal sutures, they required a surgical decompression. One of our patients, with a previous diagnosis of Crouzon syndrome, also required surgical treatment. The description of other cases, as well as follow-up of our patients, will be important to clarify this matter.

The craniofacial features in pycnodysostosis are probably secondary to the cranial and facial bone abnormalities. In our cohort of pycnodysostosis patients there is a pattern of cranial abnormalities observed: wide lambdoidal sutures and synostosis of the coronal suture. These findings may explain, at least in part, the unusual cranial conformation observed in these patients, with a high cranial vault. Interestingly, none of the patients showed a marked brachycephaly usually seen in coronal synostosis, in which there is an arrest in fronto-occipital expansion and compensatory growth in the biparietal direction at the site of the sagittal suture [Oostra et al., 2005].

The genetic mechanisms involved in bone and calvaria suture formation are extremely complex, with an enormous network of transcription factors and signaling molecules, and the whole scenario is not yet completely understood [Passos-Bueno et al., 2008]. To date, there are several genes that lead unequivocally to craniosynostosis: those encoding fibroblast growth factor receptors (*FGFR1*, *FGFR2*, and *FGFR3*) are the most thoroughly studied, followed by *TWIST* and *MSX2*, both encoding transcription factors, and *EFBN1*, coding for a tyrosine kinase ligand of ephrin receptors. Other genes, although not directly involved in craniosynostosis, seem to play an important role in cranial suture formation, such as the genes coding for some of the bone morphogenetic proteins (BMP) [De Coster et al., 2007]. The description of craniosynostosis in pycnodysostosis implies that cathepsin K also plays a role in cranial suture patency, giving further support to the fact that

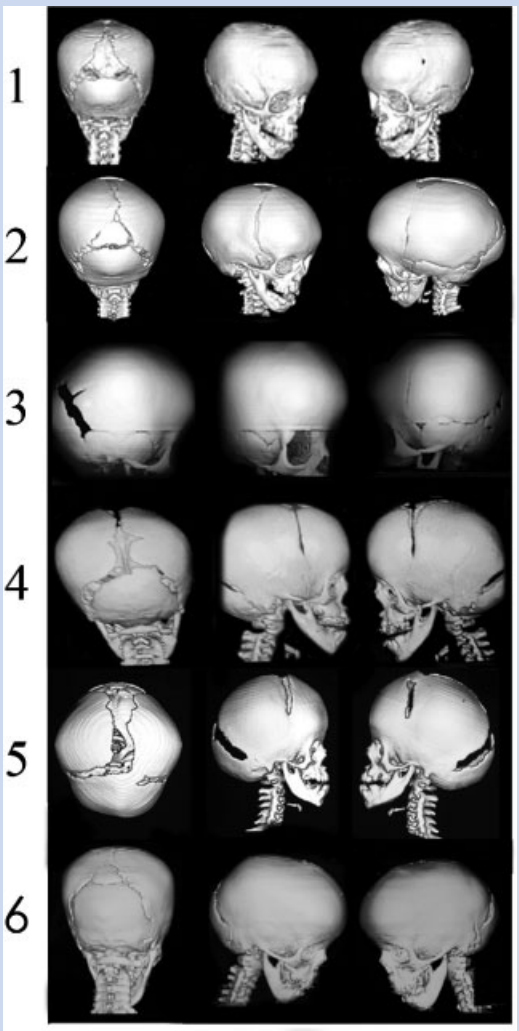

FIG. 2. Three-dimensional cranial computed tomography scans of the patients showing wide lambdoidal sutures and coronal synostosis.

osteoclasts are important cells in this process, as previously demonstrated [Kaku et al., 1999; Byron, 2006].

Although all the evidence of the effect of cathepsin K point toward osteoclast cells, we cannot rule out the possibility that a disturbance in osteoblasts also plays a role. Li et al. [2006] demonstrated that in the mouse model deficient in cathepsin K, bone formation was also altered. They suggested that this enzyme may be important for osteoblasts to synthesize and organize bone matrix properly.

To the recent description by Osimani et al. [2010] of a patient combining pycnodysostosis and craniosynostosis presenting with the p.Gly146Arg mutation in the *CTSK* gene, we add two other gene alterations (p.Arg241X and p.Cys318Tyr) within this particular phenotype. The two previously described mutations (p.Gly146Arg and p.Arg241X) have been found in patients with distinct ethnic backgrounds, interestingly all from the Mediterranean region [Gelb et al., 1996; Donnarumma et al., 2007; Osimani et al., 2010], which is compatible with the ancestry of the Brazilian population. These gene alterations are scattered through the coding region of the *CTSK* gene that codes for the mature domain of the protein. These findings show that craniosynostosis in the patients with pycnodysostosis is not associated with a specific genetic alteration, favoring the hypothesis that the lack of the gene product itself is responsible for the craniosynosotosis seen in these affected individuals. We cannot rule out for certainty that other genetic modifiers and/or environmental interactions are playing a role in this scenario.

The data shown here, documenting the apparently high frequency of craniosynostosis in the cohort of pycnodysostotic patients, are preliminary. Further studies comprising larger cohorts are important to corroborate this specific cranial finding, in order to verify if any adjustment in the current follow-up of these patients will be necessary, as suggested by Osimani et al. [2010]. Furthermore, appropriate animal models or, ideally, the study of the bone itself in patients who eventually require a surgical procedure will elucidate more precisely the role of cathepsin K in suture patency.

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