Title

Keratinocytic epidermal naevi associated with localised fibro-osseous lesions without hypophosphatemia

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Abstract

Keratinocytic epidermal nevi (KEN) are characterized clinically by permanent hyperkeratosis in the distribution of Blaschko's lines, and histologically by hyperplasia of epidermal keratinocytes. KEN with underlying RAS mutations have been associated with hypophosphataemic rickets and dysplastic bone lesions described as congenital cutaneous skeletal hypophosphatemia syndrome (CSHS). Here we describe two patients with keratinocytic epidermal naevi, in one associated with a papular naevus spilus, who presented with distinct localized congenital fibro-osseous lesions in the lower leg, diagnosed on both radiology and histology as osteofibrous dysplasia (OSFD), in the absence of hypophosphatemia or rickets, or significantly raised FGF23 levels but with distinct mosaic HRAS mutations. This expands the spectrum of cutaneous/skeletal mosaic RAS-opathies and alerts clinicians to the importance of evaluating for the evidence of bony disease even in the absence of bone profile abnormalities.

Background

The term epidermal naevus (EN) is used for mosaic congenital malformations of different components of the skin structures derived from the ectoderm, namely keratinocytic, or non-organoid EN, and sebaceous, follicular or apocrine/eccrine, collectively known as organoid EN. Keratinocytic epidermal nevi (KEN) are characterized clinically by permanent hyperkeratosis in the distribution of Blaschko's lines, and histologically by hyperplasia of epidermal keratinocytes. The co-presentation of a KEN with a papular naevus spilus is termed phakomatosis pigmentokeratotica (PPK), in which areas of linear sebaceous naevi (SN) can also sometimes be seen. Mosaic mutations in many genes have been demonstrated in KEN, including *KRT1* and *KRT10*, *PIK3CA, FGFR3, AKT1, HRAS* and *KRAS*[1]. PPK is caused by post-zygotic mutations in *HRAS*[2] or *BRAF*[3] in the few cases genotyped thus far. Importantly, some mosaic mutations in *KRT1*/10, and in *HRAS,* have been transmitted in heterozygous form to offspring, causing generalised epidermolytic ichthyosis or Costello syndrome[4] respectively. Typical features of Costello syndrome are coarse facial features, postnatal feeding problems and reduced growth, developmental and mental delay, hypotonia, cardiac and brain anomalies, curly and/or sparse hair, periorificial papillomata, cutis laxa and sometimes acanthosis nigricans. Although decreased bone mineral density has been described, cystic bone lesions are not.

Reports of bony disease in association with epidermal or congenital melanocytic naevi (CMN) have focused on the presentation of hypophosphataemic rickets, associated with elevated serum levels of FGF23, recently termed congenital cutaneous skeletal hypophosphatemia syndrome (CSHS)[5]. According to a review of the literature dysplastic

bone lesions were both in the appendicular and axial skeleton in more than 50% of the patients and caused fractures and deformities not seldom scoliosis. Almost all these cases had associated acquired hypophosphatasia/ rickets probably related to FGF23 related phosphate wasting[6]. This has been linked to the presence of different mosaic RAS gene mutations (*HRAS*, and rarely *NRAS,* although the latter has not yet been demonstrated within bony lesions themselves)[7,8]. Although radiologically the bony lesions in this metabolic presentation are sometimes interpreted as fibrous dysplasia, a lesion typically consisting of fibrous tissue with randomly oriented bony trabeculae, originating in the medulla, a recent review determined that in the four cases investigated the histological finding was severe osteomalacia without evidence of fibrous dysplasia (FD)[6]. This finding is supported by our review of radiological images in the literature. Here we describe two patients with widespread KEN, who presented with distinct localised congenital fibro-osseous lesions in the lower leg, diagnosed on both radiology and histology as osteofibrous dysplasia (OSFD), and characterised by severe bone pain (as is compatible with this diagnosis).
Fibro-osseous dysplasia is a deformity-inducing, fibro-osseous, circumscribed, lesion of the tibia and/or fibula, that originates in the cortex. It typically affects children under 10 years old. Histopathology shows, embedded in a loose fibrous tissue, a center of immature bony trabeculae, lined by a layer of osteoblasts surrounded by more mature lamellar bone. Notably, neither patient had hypophosphatemia or rickets, neither had significantly raised FGF23 levels, and the genetic mutations discovered were distinct from those in the CSHS literature.

Case reports

Full clinical features of the patients are detailed in **Figure 1** and **Table 1**. Briefly, in addition to the linear KEN, patient 2 also had segmental areas of papular naevus spilus and therefore fulfils the criteria for PPK. Neither had linear SN, or CMN. The biochemical investigations revealed normal serum calcium and phosphate in both cases, **slightly elevated** serum FGF23 (C-terminal assay immunotopics) in patient 1 but normal FGF23 in patient 2, and both with renal phosphate reabsorption within the normal range. Patient 1 underwent CT and MRI examinations at the age of 12 years, which demonstrated a multicentric lytic and slightly expansile lesion in the lateral aspect of the right lower tibia. Plain radiographs and MR imaging of patient 2 at the age of 10 months and 19 months respectively revealed anterior tibial bowing due to multicentric expansile lesions involving the left tibia and also fibula, with a mixed sclerotic and lytic picture, showing both cortical and medullary involvement, typical of OSFD (**Figure 2**). In both patients, no other lesions in the skeleton were detected. Histological examination demonstrated features of OSFD in both cases (**Figure 2**). Importantly from the clinical perspective, these lesions have had a severe impact on mobility due to pain despite surgical intervention, with patient 2 wheelchair-dependent and patient 1 on crutches with wheelchair use for longer distances.

High-sensitivity DNA sequencing techniques using DNA extracted directly from skin and bone biopsies from both patients demonstrated post-zygotic mosaicism in the gene *HRAS* (**Figure 3, Table S1**), *HRAS* c.34G>T, p.(Gly12Cys) in patient 1 and c.182A>T, p.(Gln61Leu) in patient 2. *HRAS* p.(Gln61Leu) has not previously been described in KEN or PPK, whereas p.(Gly12Cys) has been described in other cases of KEN with woolly hair[9]. Importantly, these specific mutations have not previously been described in patients with CSHS. We speculate the milder **general** bony phenotype (osteomalacy) in our patients, might be explained either because it

is a less-activating mutation of the downstream MAPK pathway, as has been shown for codon 12 serine substitutions[10] retrieved in patient 1 and/or the more restricted area and percentage of mosaicism and hence a lower systemic FGF23 production. The latter being the more likely explanation in patient 2 as codon 61 substitutions for Leucine, are commonly reported in tumours harbouring *HRAS* mutations and are highly activating [11]. Indeed laboratory values for FGF23 were either normal (patient 2) or slightly elevated (patient 1) and serum phosphate in the low normal range in patient 1 (normal in patient 2). Clinical and genetic aspects of previously published cases and those presented here, are included in **Table 2**.

Discussion

We describe focal tibial/tibial-fibular lesions with radiological and histopathological features of OSFD without hypophosphatemia, in two patients with *HRAS* mutations. This expands the spectrum of cutaneous/skeletal mosaic RASopathies.

Fibro-osseous lesions, which include OSFD and FD, as well as those described in CSHS, are to some degree non-specific and include much overlap, diagnosis depends on the clinical and genetic context, as was demonstrated in our cases. However, there are clear distinctions between our two cases presented here, with a diagnosis of OSFD, as this only affects the tibia/fibula and has distinct histology and radiology. Although previous cases of CSHS without low serum phosphate have been described [6,12,13], bone lesions, when histologically investigated, were non-specific and typically multiple. In those children presenting with extensive epidermal nevi, measurement of FGF23 and bone profile is advisable. If abnormal or in cases presenting with bone pain, limb length discrepancy, bone deformities, and impaired

mobility, imaging is necessary as well. Current management is largely supportive, with surgical intervention when needed. Supervised supplementation with cholecalciferol is advisable even in the absence of measurable bone profile disturbance, as this may have beneficial effects in the region of the mosaic abnormality. As cutaneous and skeletal involvement suggests an early embryonic mutation, the physician should be alert to the possibility of other organ involvement. The possibility of transmission of Costello syndrome to the next generation should prompt a referral for genetic counselling at an appropriate age[4] as there are at this moment no reliable criteria to predict this risk.

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Figure Legends

Figure 1 – Clinical Presentation of *HRAS* mosaicism

Patient 1 - (a) Woolly hair naevus at the age of 7 years and **(b)** epidermal naevus at the age of 12 years. Under the earlobe a tumoral proliferation is visible, histologically shown to be a papillomatous overgrowth.

Patient 2 - (c) Linear epidermal naevi, naevus spilus with papular lesions of left face and neck, consistent with phakomatosis pigmentokeratotica, at two years of age. **(d)** Aerial view at two years of age showing scalp epidermal naevus.

Figure 2 - Radiological and Histological Examination of Bone in *HRAS* mosaicism

Patient 1 - Axial **(a)** CT images of right lower leg and ankle at age of 12 years, demonstrates multicentric predominantly lytic and slightly expansile lesion in the lateral tibial cortex.

Patient 2 - Histology of excised tibial bone in patient 2 at 6 years of age, shown at 4x **(b)** and 10x **(c)** demonstrating normal bone architecture displaced by a bland spindle cell stroma containing irregular bony trabeculae showing osteoblast rimming consistent with the clinical and radiological diagnosis of osteofibrous dysplasia.

MRI examination of the right tibia at the age of 19 months in; Sagittal STIR **(d)** and post contrast water excited T1-weighted turbo spin echo images. There is a multicentric lesion (arrows) within the tibial diaphysis involving both cortex and medulla, and also involving the fibula (arrowhead), showing contrast enhancement, consistent with a diagnosis of osteofibrous dysplasia.

Figure 3 - Results of Genetic Investigations

Patient 1 - *HRAS* mosaicism confirmed by Sanger sequencing demonstrating *HRAS* c.34G>T, p.Gly12Cys **(a)** in skin (mutant allele load 34%) and **(b)** bone (mutant allele load 10%) not present in blood **(c)**

Patient 2 – (d) Next generation sequencing of affected skin showing somatic *HRAS* mutation, chr11:533874T>A, p.Glu61Leu, mutant allele load 36% (695/1905 reads) **(e)** Sanger sequencing trace of affected skin confirming somatic *HRAS* mutation c.182A>T, p.Glu61Leu which is not present in blood **(f)**.

Table 1 - Details of clinical features of patients 1 and 2

Table 2 - Summary of clinical and genetic profiling of previously-published patients with combined epidermal naevi and bone lesions

Table S1 - Details of molecular genetic findings in patients 1 and 2