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Melorheostosis: Exome Sequencing Of An Associated Dermatoses Implicates Postzygotic Mosaicism Of Mutated KRAS

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Abstract

Melorheostosis (MEL) is the rare sporadic dysostosis characterized by monostotic or polyostotic osteosclerosis and hyperostosis often distributed in a sclerotomal pattern. The prevailing hypothesis for MEL invokes postzygotic mosaicism. Sometimes scleroderma-like skin changes, considered a representation of the pathogenetic process of MEL, overlie the bony changes, and sometimes MEL becomes malignant. Osteopoikilosis (OPK) is the autosomal dominant skeletal dysplasia that features symmetrically distributed punctate osteosclerosis due to heterozygous loss-of-function mutation within *LEMD3*. Rarely, radiographic findings of MEL occur in OPK. However, germline mutation of *LEMD3* does not explain sporadic MEL.

To explore if mosaicism underlies MEL, we studied a boy with polyostotic MEL and characteristic overlying scleroderma-like skin, a few bony lesions consistent with OPK, and a large epidermal nevus known to usually harbor a *HRAS*, *FGFR3*, or *PIK3* gene mutation. Exome sequencing was performed to ~100× average read depth for his two dermatoses, two areas of normal skin, and peripheral blood leukocytes.

As expected for non-malignant tissues, the patient's mutation burden in his normal skin and leukocytes was low. He, his mother, and his maternal grandfather carried a heterozygous, germline, in-frame, 24-base-pair deletion in *LEMD3*. Radiographs of him and his mother revealed bony foci consistent with OPK, but she showed no MEL. For the patient, somatic variant analysis, using five algorithms to compare all 20 possible pairwise combinations of his five DNA samples, identified only one high-confidence mutation, heterozygous *KRAS* Q61H (NM_033360.3:c.183A>C, NP_203524.1:p.Gln61His), in both his dermatoses but absent in his normal skin and blood.

Thus, sparing our patient biopsy of his MEL bone, we identified a heterozygous somatic *KRAS* mutation in his scleroderma-like dermatosis considered a surrogate for MEL. This implicates postzygotic mosaicism of mutated *KRAS*, perhaps facilitated by germline *LEMD3* haploinsufficiency, causing his MEL.

Keywords

dysostosis; hyperostosis; *LEMD3*; linear epidermal nevus; malignancy; nevus sebaceous; osteopoikilosis; osteosclerosis; scleroderma

II) Introduction

Melorheostosis (MEL; OMIM #155950)⁽¹⁾ is the rare sporadic and frequently symptomatic dysostosis that features in mature lesions “flowing hyperostosis” likened to dripping candle-wax along one or more bones.^(2–4) Not infrequently, scleroderma-like skin change (SLS),^(5–11) nevi of various types,⁽¹²⁾ or vascular abnormalities⁽¹³⁾ overlie MEL bone. Sometimes MEL becomes malignant.^(14–16) The striking anatomical distribution of MEL, especially in the extremities, has suggested a pathogenesis involving sclerotomes,⁽¹⁷⁾ the body segments innervated by individual spinal sensory nerves.⁽¹⁸⁾ Postzygotic mosaicism, commonly involving a limb bud, is the prevailing hypothesis invoked for MEL.^(17,19)

Occasionally, radiographic findings of MEL appear sporadically in families with the benign autosomal dominant bone dysplasia called osteopoikilosis (OPK)^(1,20–22) or its variant with skin lesions designated Buschke-Ollendorff syndrome (BOS) (OMIM #166700).⁽¹⁾ OPK, literally “spotted bones”, features small sclerotic ossified foci distributed symmetrically at the ends of long bones, within the pelvis, and sometimes elsewhere in the skeleton.⁽¹⁾ BOS adds connective tissue nevi comprised of elastin or collagen^(23,24) that differ from the dermatoses associated with sporadic MEL. However, when MEL accompanies OPK/BOS, SLS may be present in this disorder as well.^(25–27) In 2004, Hellemans et al⁽²⁸⁾ discovered in OPK and BOS, including with associated MEL, germline haploinsufficiency of the LEM Domain Containing 3 gene (*LEMD3*) that encodes the nuclear membrane-associated protein MAN1. Nevertheless, we reported preliminarily in 2005^(29,30) and then fully in 2007⁽³¹⁾ that germline *LEMD3* mutation does not cause sporadic MEL. This was confirmed by others.⁽³²⁾ MEL in OPK or BOS had been postulated previously to reflect a somatic “second hit” mutation of some gene,^(28,31) subsequently including *LEMD3* itself,⁽³³⁾ but sporadic MEL remained unexplained.

In 2014, we were referred a boy with radiographic changes consistent with polyostotic MEL, overlying SLS, and punctate osteosclerotic lesions in keeping with OPK. Additionally, he had a large birthmark, a linear epidermal nevus (LEN) associated once previously with MEL.⁽³⁴⁾ In 2012, the etiology of LEN was discovered to most frequently involve somatic mutations in either *HRAS*, *FGFR3*, or *PIK3*,⁽³⁵⁾ and somatic mutations in *HRAS* and *KRAS* in nevus sebaceous⁽³⁶⁾ and the neurocutaneous disorder Schimmelpenning syndrome (OMIM #163200).⁽¹⁾ In 2014,⁽³⁷⁾ multilineage somatic activating mutations in *HRAS* and *NRAS* accounted for the entity that features mosaic cutaneous and skeletal lesions, elevated circulating FGF23, and hypophosphatemia. We then recalled that in 1972 Wagers et al⁽⁷⁾ stated for MEL: “It is likely that linear melorheostotic scleroderma represents skin involvement by the same proliferative disorder that produces the bony hyperostosis.” Hence, we reasoned our patient’s SLS overlying his MEL harbored the causal somatic mutation. Herein, we used exome sequencing of his two dermatoses to implicate postzygotic mosaicism of a *KRAS* missense mutation in his MEL.

III) Materials and Methods

Informed written consent for all studies, including language authorizing from the patient and his parents whole genome sequencing and data sharing, was obtained as sanctioned by the Human Research Protection Office, Washington University School of Medicine; St. Louis, MO, USA.

A) Patient

This 14-year-old American boy was admitted in 2014 to the Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for Children, St. Louis, MO, USA.

He was born at term after an uncomplicated pregnancy for a 33-year-old, gravida 2, para 1–2 mother. The father was 37 years old. At birth, a unilateral nevus covered much of his right posterior scalp, neck, thorax, flank, and lumbar region, and extended to his right thumb. At 6 months-of-age, the dermatosis was diagnosed as a LEN that respected the midline.

At 7 years-of-age, radiographs obtained after trauma reportedly showed a left tibial fracture and fibrous dysplasia. Soon after, the findings were reinterpreted as MEL that was also found in his right rib cage and right femur.

At 14 years-of-age, the patient told us that he had chronic intermittent pain in his left tibia. Physical examination revealed mild scoliosis, a two-centimeter leg-length discrepancy, and a shoe-size two greater on the right versus left in keeping with MEL (Figure 1).^(1–4) A large LEN extended from his right scalp and posterior neck to his right thorax, flank, and lumbar region following Blaschko lines (Figure 2A,B). Additionally, a large SLS lesion consisting of an atrophic hyperpigmented plaque (Figure 2C) extended from his right lumbar region to his right lateral thigh overlying radiographic changes of MEL (see Radiological Findings). Routine laboratory studies showed no disturbance of mineral homeostasis, including in fasting serum a normal level of fibroblast growth factor 23 (FGF23), i.e., intact molecule and c-terminal fragment, of 158 RU/ml (NI, 44–215: LabCorp; Burlington, NC, USA) and repeatedly normal phosphorus levels.

B) Radiological Studies

We completed a radiographic skeletal survey and reviewed all available previous radiological studies. Magnetic resonance imaging (MRI) has been performed at age 6 years for left anterior tibial pain and at age 13 years for right knee pain, and computed tomography (CT) had been undertaken at age 14 years to distinguish a persistent left tibial fracture versus a vascular channel suspected radiographically. Bone density was assessed by dual-energy x-ray absorptiometry (DXA) using a Discovery-A instrument (Hologic, Inc., Waltham, MA, USA). Selective skeletal radiographs of his mother were acquired to screen for MEL or OPK when we found she carried an *LEMD3* mutation (see Results).

C) Skin Biopsies

Five routine four-millimeter-diameter punch biopsies of his skin were performed to: i) obtain DNA from his LEN (Supplementary Appendix, Figure 1), ii) provide histopathological verification for the SLS, iii) obtain DNA from the SLS (Supplementary Appendix, Figure 2), and iv) obtain DNA from two separate regions of normal-appearing skin (one on each thigh). A fifth DNA sample was extracted from his peripheral blood leukocytes. We elected to spare him biopsy of his MEL bone and instead use the overlying SLS DNA as a surrogate specimen (see Discussion). Thus, five separate DNA specimens were studied (Figure 3).

D) Tissue Sample Preparation

The skin specimens for DNA extraction were separately frozen with liquid nitrogen and crushed using a mortar and pestle being careful against cross-contamination. Their DNA, and the DNA in blood lymphocytes, was extracted using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany). The five tissue DNA samples were coded as follows: “EN” the epidermal nevus, “SL” the SLSC area at his right thigh overlying MEL, “NL1” the apparently normal skin adjacent to the SLS lesion at the right thigh, “NL2” the healthy skin of the left thigh where there was no underlying MEL, and “PB” the peripheral blood as a germline comparator to detect any somatic mosaicism (Figure 3).

E) *LEMD3* Mutation Analysis

Because our patient had MEL and a few skeletal lesions consistent with OPK, we first used Sanger sequencing as previously described⁽³¹⁾ to study all 13 coding exons and adjacent mRNA splice sites of *LEMD3* in his LEN and SLS. When a deletion was found in exon 1 of *LEMD3* in both dermatoses (see *LEMD3* Mutation Analysis), we studied this exon selectively in his peripheral blood and in the two separate DNA samples, NL1 and NL2, from his healthy skin (Figure 3). We then tested, with informed consent, for this *LEMD3* mutation in both parents and maternal grandfather using their blood leukocyte DNA.

F) Exome Sequencing And Mutation Analysis

Exome capture was performed per the manufacturer's directions for a SeqCap EZ Human Exome Library v3.0 capture kit (Roche-Nimblegen, Inc., Basel, Switzerland). Exome sequencing of each of the five tissue DNA samples was conducted to ~100× average read depth (coverage at every exon base position of DNA) as previously described.^(38,39) Sequencing was performed on an Illumina HiSeq 2000 sequencing instrument in paired-end mode producing 2×100 base-pair (bp) reads.

Alignment of next-generation sequence reads to the human reference genome (build GRCh37), single nucleotide variant (SNV) detection, and small insertion and deletion (Indel) detection were performed using the Genome Modeling System (GMS) as previously described.^(38,39) Briefly, sequences were aligned to the human genome reference sequence (version GRCh37/hg19) using the aligner bwa-mem (v 0.7.10) (arXiv:13033997) and default parameters. Duplicate read alignments were collapsed using Picard MarkDuplicates (v 1.113) (<http://broadinstitute.github.io/picard/>). Somatic variant calling for pairs of tissue samples was carried out using the following four somatic variant callers: VarScan (v 2.3.6),⁽⁴⁰⁾ Somatic Sniper (v 1.0.4),⁽⁴¹⁾ Strelka (v 1.0.11),⁽⁴²⁾ and Mutect (v 1.1.4).⁽⁴³⁾ Each variant from the union of these callers was manually reviewed using the Integrative Genomics Viewer (IGV)^(44,45) to produce a final variant list for each pairwise comparison. Pairwise comparisons were made between all possible tissue sample combinations to identify candidate somatic variants (Supplementary Appendix, Figure 3A). A minimum coverage of 30× and minimum variant allele frequency of 2.5% were required for a somatic variant to be considered a candidate.

Somatic SNV and Indel calls from the pairwise comparisons were consolidated into a final unified list of SNV and Indel candidates. These candidates were subsequently reviewed manually by an experienced genome analyst using the IGV^(44,45) to filter out sites with low mapping quality, discrepant variant reads, highly discrepant regions, variants with only short insert read pairs, and/or sites having the majority of reads in only the forward or reverse orientation (Supplementary Appendix, Figure 3B).

Coverage of the targeted reference genome bases was assessed using the refcov tool⁽⁴⁶⁾ (Figure 4A). Allele frequencies for a set of 24 'identity' SNPs were analyzed to verify that no sample studied was significantly contaminated by DNA from an unrelated sample,⁽⁴⁷⁾ and were plotted using the GenVisR package (<http://biorxiv.org/content/early/2016/03/25/043604>) (Figure 4B).⁽⁴⁸⁾ Pairwise comparisons were each performed twice, by

swapping the control and variable sample, resulting in a total of 20 comparisons (Supplementary Appendix, Figure 3A).

To identify germline variants possibly contributing to the tissue phenotypes, variants called from each tissue sample were evaluated and prioritized using the GEMINI workflow pipeline.⁽⁴⁹⁾ Variants called on single bam files by SAMtools⁽⁵⁰⁾ and VarScan2⁽⁵¹⁾ were further annotated using Ensembl's Variant Effect Predictor (VEP)⁽⁵²⁾ (www.ensembl.org/info/docs/tools/vep/) and loaded into GEMINI (SQLite) databases used for complex queries from the imported variant annotations in combination with native GEMINI annotations (dbSNP, ClinVar) to identify and prioritize potential variants of interest. Common polymorphisms were removed from further analysis by excluding variants with an allele fraction >0.1% in 1000 Genomes⁽⁵³⁾ or the NHLBI Exome Sequencing Project.⁽⁵⁴⁾ Variants were prioritized in each tissue sample if they occurred within genes associated with osteogenesis or skeletal dysplasias, or were predicted to likely affect protein function ('medium' or 'high' impact). Any variant observed in four of the five samples was manually reviewed and evaluated for annotations in ClinVar.⁽⁵⁵⁾ These variants were then evaluated for their potential relevance to MEL.

Manual review using IGV was performed to visually inspect exome sequence data, across all five tissue samples on the *LEMD3* locus, for evidence of germline or somatic variants that may have potentially been missed by SNV/Indel callers.

The patient's germline variants in *AMPD1* and *MS4A12* identified by exome sequencing were then tested for in him and his parents using Sanger sequencing with primers we designed (available on request).

IV) Results

A) Radiological Findings

The patient's radiographic skeletal survey revealed bony changes most consistent with MEL in his right wrist, right ribs (#s 4, 6, 7, 9), right iliac bone, right proximal femur up to the femoral neck, both tibias (left worse than right), and left fibula (Figure 5). A few OPK densities were noted in his left wrist and right distal femoral epiphysis (Supplementary Appendix, Figure 4A). There was no evidence of rickets reported in some individuals with large LEN that in 2005 we postulated⁽⁵⁶⁾ emanated from the associated focal skeletal disease releasing FGF23 into the circulation. In 2006, we supported our hypothesis by reporting a girl with hypophosphatemic rickets accompanying the characteristic focal skeletal disease but without skin changes;⁽⁵⁶⁾ i.e., a *forme fruste*⁽⁵⁷⁾ (see Discussion).

CT of his left leg showed a thickened cortex in the mid shaft of the tibia, including external cortical thickening and obliteration of the medullary cavity, consistent with MEL. Additionally, dilated vascular channels were noted in the tibial cortex (Supplementary Appendix, Figures 5 and 6). MRI showed, as expected for MEL, low signal intensity in all sequences with no enhancement in the bone.

DXA BMD was normal for age and gender: lumbar spine (L₁–L₄) 0.793 gm/cm² (Z-score –0.2), left hip 0.840 gm/cm² (Z-score –0.7), and whole-body BMD 0.989 gm/cm² (Z-score 0.0).

The mother's skeletal radiographs showed a few OPK densities, but no evidence of MEL (Supplementary Appendix, Figure 4B).

B) Skin Histopathology

The patient's LEN, having classic appearance on inspection, was not studied histologically, and therefore we did not explore any potential impact on it from the subsequently discovered germline *LEMD3* mutation. His scleroderma-like dermatosis showed a normal epidermis with intact rete ridges. The reticular dermis was expanded by collagen that extended into the subcutaneous fat with no involvement of adnexal structures (Figure 6). Collagen fibers appeared normal with no significant thickening or homogenization. Elastic tissue staining highlighted a slightly decreased number of normal-appearing elastic fibers within the dermis in areas where the collagen was expanded. Dermal mucin was not significantly increased with colloidal iron staining. The findings were consistent with reports of the SLS overlying MEL.^(7,8)

C) *LEMD3* Mutation Analysis

Sanger sequencing of *LEMD3* revealed a unique germline heterozygous 24-bp deletion (NM_014319.4:c.240_263del, NP_055134.2:p.81_88delPAAAAAAG) in exon 1 in all five patient samples. This deletion was also carried by his mother and maternal grandfather, but not by his father or maternal grandmother. No additional *LEMD3* mutation, that could represent a “second hit”, was detected by this method or by exome sequencing (see below) in either dermatosis.

D) Exome Sequencing

Germline variant event evaluations revealed by exome sequencing confirmed the patient's heterozygous inframe 24-bp deletion in exon 1 of *LEMD3* (see *LEMD3* Mutation Analysis). It was verified during manual review of *LEMD3*, and was detected in all five tissue DNA samples (Figure 7A). This mutation is flanked by a short tandem repeat element, ‘GCCGCGGG’, which contains a six-base palindrome (CCGCGG). We hypothesize that replication slippage occurred in this region, deleting 16 bases flanked by the two tandem repeats plus one copy of the 8-bp tandem repeat, leaving a single copy of the tandem repeat. Because this *LEMD3* mutation involves compression at a tandem repeat, there are two equally valid representations for the deletion. However, one cannot know whether the left or right copy of the tandem repeat remains, but the predicted protein sequence and functional consequences are identical for both representations (we assume deletion of the right copy in our representation above).

Germline analysis by GEMINI of all five tissue DNA samples also identified: i) a stop gain in *AMPD1* (NP_000027.2:p.Gln45*), and ii) a frameshift in *MS4A12* (NP_060186.2:p.Ser63fs), as potentially relevant to this disease. However, using Sanger sequencing, both the stop gain in *AMPD1* and the frameshift in *MS4A12* were validated in

both the patient's and father's leukocyte DNA. The mother did not carry either mutation. The *AMPD1* "mutation" (p.Gln45Ter) is a common SNP with a minor allele frequency of 0.08714 (ExAC). The *MS4A12* "mutation" (Ser63fs) has a minor allele frequency of 0.002891 (ExAC) so it is a rare variant. An additional stop gain (c.219C>T, p.Gln71Ter) was identified in *MS4A12* in the mother's and patient's leukocyte DNA, but this is a common SNP (rs2298553, MAF 0.4760).

Somatic event evaluations of the patient's DNA identified *KRAS* mutation Q61H (NM_033360.3:c.183A>C, NP_203524.1:p.Gln61His) in his LEN and SLS, but not in his two samples of normal skin or blood leukocytes (Figure 4C,D and Figure 7B). This mutation had the highest variant allele frequency (VAF); i.e., the percentage of reads containing the variant nucleotide, among all predicted somatic events identified (Figure 4D) (see Supplementary Table 1 for all putative somatic variant candidates). The 30–60% VAFs observed indicated this variant was present within most cells comprising these tissue samples. *KRAS* Q61H was the only tissue-specific (potentially mosaic) event identified in the two dermatoses exclusively, and was located in a prominent cancer-associated hotspot (Figure 7C).

V) Discussion

Although MEL was described nearly a century ago in 1922⁽⁵⁸⁾ and more than 300 affected individuals are reported, its etiology and pathogenesis remain unknown.⁽¹⁾ Our patient harbored a heterozygous somatic missense mutation of *KRAS* within both his LEN and his SLS overlying MEL. As discussed below, this observation implicates postzygotic mosaicism of mutated *KRAS* in his MEL.

A) Postulated Etiology of MEL

Inflammation, infection, vasculopathy, neuropathy, and endocrinopathy have separately all been offered to explain MEL.⁽⁵⁹⁾ In 1972 Wagers et al⁽⁷⁾ suggested instead that MEL results from early postzygotic mutation. In 1979 Murray and McCredie⁽⁶⁰⁾ added that the skeletal distribution of MEL frequently mimics the innervation pattern of one or several sensory nerves, and therefore hypothesized sclerotomal involvement with MEL commonly initiating in mesenchyme before or when limb buds form. Postzygotic mosaicism in MEL was supported in 1995 by Fryns⁽¹⁷⁾ who noted in at least 5% of such patients vascular anomalies overlying the bony changes (e.g., capillary hemangiomas, lymphangiectasis, vascular nevi, glomus tumors, arteriovenous aneurysms). Soft tissue abnormalities overlying MEL⁽⁵⁹⁾ can also include skin changes;⁽¹⁷⁾ scleroderma-like patches over MEL have been documented since 1936.^(5–11) Furthermore, linear scleroderma with MEL in OPK/BOS and resembling OPK has been described.^(25–27) In 2003, Tinschert et al⁽³⁴⁾ reported an occurrence of extensive sebaceous nevus (LEN of the scalp) accompanying MEL and hypothesized non-allelic didymosis (i.e., twin spotting) as the genetic basis.

B) MEL In OPK and BOS

In occasional reports beginning in 1989, MEL has sporadically accompanied autosomal dominant OPK and BOS.^(20–22,33) Prior to the discovery in 2004 of *LEMD3* mutation

causing OPK and BOS,⁽²⁸⁾ Butkus et al⁽²⁰⁾ in 1997 hypothesized for such MEL a somatic “second hit” mutation within the putative gene causing OPK or BOS. In 1999 Nevin et al,⁽²¹⁾ and in 2003 Debeer et al,⁽²²⁾ agreed with this hypothesis. In 2004, Happle⁽³³⁾ described MEL as always occurring sporadically, but in OPK “may be best explained... as a type 2 segmental OPK, resulting from early loss of the wild-type allele at the gene locus...”, although a “two hit” etiology for classic sporadic MEL seemed unlikely.

C) LEMD3 in OPK and BOS

When Hellemans et al⁽²⁸⁾ in 2004 discovered heterozygous germline loss-of-function mutations in *LEMD3* in three unrelated individuals with OPK or BOS, they recognized that one family member could also manifest MEL. However, they found no somatic *LEMD3* mutation in the BOS skin lesions from two unrelated individuals (one with and one without MEL), and had not studied sporadic MEL. Beginning in 2005,^(29,30) we verified their finding that germline *LEMD3* mutation underlies OPK and BOS, including instances with MEL, but showed that germline *LEMD3* mutation does not explain sporadic MEL.⁽³¹⁾ Our conclusion was subsequently supported in 2006 by Hellemans et al.,⁽⁶¹⁾ and in 2009 by Zang et al.,⁽³²⁾ and then by others.⁽⁶²⁾ In 2010, we reported evidence that rarely BOS could be genetically heterogeneous.⁽⁶³⁾

LEMD3 protein (OMIM *607844),⁽¹⁾ also called MAN antigen 1 (MAN1),⁽⁶⁴⁾ occupies the inner nuclear membrane where it antagonizes TGF- β signaling by interacting with Smad 2 and Smad 3, and perhaps dampens bone morphogenetic protein (BMP) signaling by interacting with Smad family proteins.⁽⁶⁵⁾ Thereby, loss-of-function mutation within *LEMD3* could accelerate bone formation. In fact, all germline mutations that cause OPK and BOS are heterozygous termination or frame-shift defects scattered throughout the *LEMD3* coding region. No missense mutations of *LEMD3* have been reported, suggesting they cause no phenotype or one different from OPK or BOS. Haploinsufficiency of *LEMD3* is the etiology of OPK and BOS, whereas the skin lesions of BOS perhaps involve somatic mutation. In our patient and his mother, *LEMD3* haploinsufficiency from their unique 24-bp *LEMD3* inframe deletion would readily explain their subtle OPK, and perhaps facilitated the development of his MEL and SLS.

Our patient also carried germline heterozygous *AMPD1* and *MS4A12* variants. However, these variants likely did not contribute to his bone or skin changes because both were paternally derived and relatively common. Both the patient and his mother also harbored in their germline an additional common *MS4A12* SNP that creates a stop gain. Thus, he is compound heterozygous for truncating mutations in *MS4A12*. However, the ExAC database lists 13,909/121,290 individuals who are homozygous for the Gln71Ter variant. Therefore his MEL, that is extraordinarily rare, seems unrelated to such a common variant.

D) The Dermatoses of BOS and MEL

The skin lesions of BOS, sometimes called *dermatofibrosis lenticularis disseminata*, are skin-colored or yellow papules or plaques featuring increased density of elastic fibers within the mid- and lower-dermis (elastomas). These lesions may also show clumping of elastic fibers, thickened collagen bundles, or a wavy appearance to the epidermis. Less often, there

are abnormalities in collagen (collagenomas), rather than elastic tissue, but these lesions remain clinically indistinguishable from elastomas. Histopathology shows thickened collagen bundles arranged somewhat haphazardly throughout the dermis. In 1981, we showed increased desmosine from elastin within the BOS dermatosis.⁽²³⁾

The clinical and histological findings of the skin overlying our patient's MEL were not those of BOS, but instead the scleroderma-like changes of MEL. There was proliferation of normal-appearing collagen throughout the deep dermis and into the subcutis with a slight decrease in the density of elastic fibers. In 2000, Kim et al⁽⁶⁶⁾ used reverse dot-blot hybridization to study fibroblasts cultured from sclerodermatous skin overlying MEL and found diminished expression of several adhesion proteins, especially TGF β -induced gene product β ig-h3. In 2003, Endo et al⁽⁶⁷⁾ reported increased procollagen α 1(I) mRNA expression in aberrant skin overlying MEL.

E) Linear Epidermal Nevus and KRAS

Our patient's LEN that extended into his scalp was a conspicuous finding. LEN most frequently harbors a somatic mutation in *HRAS*, *FGF23*, or *PIK3*,⁽³⁵⁾ whereas nevus sebaceous often in the scalp typically harbors a mutation in *HRAS* or *KRAS*.^(36,68) The RAS genes encode GTP/GDP binding proteins that act as intracellular signal transducers.⁽⁶⁹⁾ Their somatic mutations are generally at or near amino acids 12, 13, and 61 and cause several malignancies.^(35,36,69) Along with other RAS mutations, KRAS Q61H, the defect in our patient's LEN and SLS, is prevalent in cancers.^(69–72)

Notably, the mosaic metabolic bone disorder “cutaneous skeletal hypophosphatemia syndrome”, that comprises epidermal/melanotic nevus associated with FGF23 overproduction and thus hypophosphatemic bone disease,⁽⁷³⁾ is caused by postzygotic activating *NRAS* or *HRAS* mutations in codons 13 and 61.⁽³⁷⁾ However, in 2016,⁽⁷³⁾ no direct evidence seemed to implicate the nevi of this disorder in its hypophosphatemia. Indeed, in 2005, we had postulated⁽⁵⁶⁾ instead that the hypophosphatemia emanated from the associated focal skeletal lesions in this disorder. Then, in 2006, we briefly reported a girl who supported our hypothesis having the characteristic focal bony changes and hypophosphatemic rickets but no skin changes and therefore seemed a *forme fruste* of this disorder. Mutation analysis of her skeletal lesion is underway. Our current patient with a large LEN instead harbors at codon 61 a postzygotic *KRAS* mutation, and showed no rickets radiographically and had normal blood levels of phosphorus and FGF23.⁽⁷³⁾ Thus, why he manifested MEL and SLS remains puzzling, but he carried a germline *LEMD3* defect as well.

Although RAS signaling is not well understood in bone development and remodeling, skeletal disease occurs in several “RASopathies” due to germline RAS mutations.⁽⁷⁴⁾ Two key aspects of RAS signaling potentially involved in MEL are: i) crosstalk with TGF β 1 signaling, and ii) *RUNX2* activation via the MAPK and PI3K/AKT pathways. Considerable crosstalk and synergism occur between the RAS and TGF β 1 signaling cascades in cancer/tumor progression.⁽⁷⁵⁾ Activated RAS signaling may switch on TGF β 1 signaling to promote tumor growth.⁽⁷⁵⁾ Furthermore, RAS signaling via MAPK and PI3K/AKT pathways activates *RUNX2* and can drive tumor growth.⁽⁷⁶⁾ *RUNX2* is a key transcription factor for

osteoblast and chondrocyte development, and deactivation of *RUNX2* causes autosomal dominant cleidocranial dysplasia.⁽⁷⁷⁾ *RUNX2* has also emerged as a factor in several cancers and in metastasis.⁽⁷⁶⁾ Therefore, perhaps our patient's *KRAS* mutation also activated *RUNX2* contributing to his MEL.

As discussed earlier, *LEMD3* inhibits TGF β 1 signaling. Therefore, deactivating *LEMD3* mutations (e.g. haploinsufficiency) could promote TGF β 1 signaling contributing to MEL. Our patient's somatic *KRAS* mutation could be the "second hit" upon the germline *LEMD3* defect that explains his MEL. In other MEL patients, perhaps this *KRAS* mutation or other activating mutations of *KRAS*, *HRAS*, *NRAS* (or in other genes for the RAS signaling pathway) explains this enigmatic dysostosis.

VI) Conclusion

For our patient with MEL, a somatic heterozygous *KRAS* missense mutation in his large linear epidermal nevus and scleroderma-like dermatosis overlying MEL suggests a role for postzygotic mosaicism of this mutation, perhaps facilitated by his germline *LEMD3* haploinsufficiency, in the etiology of his MEL. Our methodological approach will likely be helpful for the needed investigation of additional MEL patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University; Baltimore, MD: Dec. 2016 World Wide Web URL <http://omim.org>
2. Perlman MD. Melorheostosis: A case report and literature review. *J Foot Surg.* 1990; 29:353–6. [PubMed: 2229911]
3. Greenspan A, Azouz EM. Bone dysplasia series. Melorheostosis: Review and update. *Can Assoc Radiol J.* 1999; 50:324–30. [PubMed: 10555508]
4. Freyschmidt J. Melorheostosis: A review of 23 cases. *Eur Radiol.* 2001; 11:474–9. [PubMed: 11288855]
5. Thompson NM, Andrews GC, Gillwald FN. Scleroderma and melorheostosis: report of a case. *J Bone Jt Surg.* 1951; 33-B:430–3.
6. Muller SA, Henderson ED. Melorheostosis with linear scleroderma. *Arch Derm.* 1963; 88:142–5. [PubMed: 14043598]

7. Wagers LT, Young AW Jr, Ryan SF. Linear melorheostotic scleroderma. *Br J Derm.* 1972; 86:297–301.
8. Soffa DJ, Sire DJ, Dodson JH. Melorheostosis with linear sclerodermatous skin changes. *Radiol.* 1975; 114:577–8.
9. Siegel A, Williams H. Linear scleroderma and melorheostosis. *Br J Radiol.* 1992; 65:266–8. [PubMed: 1547459]
10. Birtane M, Eryavuz M, Unalan H, Tüzün F. Melorheostosis: report of a new case with linear scleroderma. *Clin Rheumatol.* 1998; 17:543–5. [PubMed: 9890690]
11. Moreno Alvarez MJ, Lazaro MA, Espada G, Barcelo HA, Maldonado Cocco A. Linear scleroderma and melorheostosis: case presentation and literature review. *Clinical Rheumatology.* 1996; 15:389–93. [PubMed: 8853175]
12. Miyachi Y, Horio T, Yamada A, Ueo T. Linear melorheostotic scleroderma with hypertrichosis. *Arch Dermatol.* 1979; 115:1233–4. [PubMed: 507873]
13. Kalbermatten NT, Vock P, Rufenacht, Anderson SE. Progressive melorheostosis in the peripheral and axial skeleton with associated vascular malformations: imaging findings over three decades. *Skeletal Radiol.* 2001; 30:48–52. [PubMed: 11289635]
14. Böstman OM, Holmström T, Riska EB. Osteosarcoma arising in a melorheostotic femur. *J Bone Jt Surg.* 1987; 69-A:1232–7.
15. Murphy M, Kerns S, Cavanagh M, O'Connell D, Hurson B. Occurrence of osteosarcoma in a melorheostotic femur. *Ir Med J.* 2003; 96(2):55–6. [PubMed: 12674159]
16. Brennan DD, Bruzzi JF, Thakore H, O'Keane JC, Eustace S. Osteosarcoma arising in a femur with melorheostosis and osteopathia striata. *Skeletal Radiol.* 2002; 31:471–4. [PubMed: 12172596]
17. Fryns JP. Melorheostosis and somatic mosaicism. *Am J Med Genet.* 1995; 58(2):199. [PubMed: 8533816]
18. Inman V, Saunders JBCM. Referred pain from skeletal structures. *J Nerv Ment Dis.* 1944; 99(5): 660–7.
19. Jha S, Laucis N, Bhattacharyya T. Pathophysiology of melorheostosis: a theoretical framework (abstract). *J Bone Miner Res.* 2015; 30(Suppl):S320.
20. Butkus CE, Michels VV, Lindor NM, Cooney WP 3rd. Melorheostosis in a patient with familial osteopoikilosis. *Am J Med Genet.* 1997; 72:43–6. [PubMed: 9295073]
21. Nevin NC, Thomas PS, Davis RI, Cowie GH. Melorheostosis in a family with autosomal dominant osteopoikilosis. *Am J Med Genet.* 1999; 82:409–14. [PubMed: 10069713]
22. Debeer P, Pykels E, Lammens J, Devriendt K, Fryns JP. Melorheostosis in a family with autosomal dominant osteopoikilosis: report of a third family. *Am J Med Genet A.* 2003; 119:188–93.
23. Uitto J, Starcher BC, Santa-Cruz DJ, Whyte MP, Murphy WA. Biochemical and ultrastructural demonstration of elastin accumulation in the skin of the Buschke Ollendorff syndrome. *J Invest Dermatol.* 1981; 76:284–7. [PubMed: 7205030]
24. Giro MG, Duvic M, Smith LT, et al. Buschke-Ollendorff syndrome associated with elevated elastin production by affected skin fibroblasts in culture. *J Invest Dermatol.* 1992; 99:129–37. [PubMed: 1629625]
25. Desproges-Totteron R, Labrousse C, Andrieu J, Lathelize H, Gallois M, Pitrou E, Treves R. Melorheostose avec aspect d'osteopécilie et sclera-atrophie extensive. *Revue du Rhumatisme.* 1976; 43:379–84.
26. Pascaud-Ged E, Rihouet J, Pascaud JL, Rousseau J. Melorheostosis, osteopoikilosis, and linear scleroderma. *Sem Hop Paris.* 1982; 58:1056–9. [PubMed: 6285483]
27. Massaolo F, Bertazzoni MG, Caroli A, Sardelli S, Cellini M, Mazzone E. Linear scleroderma melorheostotic with osteopoikilosis: a case report. *Ped Med Chir (Med Surg Ped).* 1989; 11:555–8.
28. Hellemans J, Preobrazhenska O, Willaert A, Debeer P, Verdonk PC, Costa T, Janssens K, Menten B, Van Roy N, Vermeulen SJ, Savarirayan R, Van Hul W, Vanhoenacker F, Huylebroeck D, De Paep A, Naeyaert JM, Vandesompele J, Speleman F, Verschueren K, Coucke PJ, Mortier GR. Loss-of-function mutations in LEMD3 result in osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis. *Nat Genet.* 2004; 36:1213–8. [PubMed: 15489854]

29. Mumm S, Zhang X, McAlister WH, Wenkert D, Whyte MP. Deactivating germline mutations in LEMD3 cause osteopoikilosis and Buschke-Ollendorff syndrome, but not melorheostosis. *J Bone Miner Res.* 2005; 20(S1):S418.
30. Mumm, S., Zhang, X., McAlister, WH., Wenkert, D., Whyte, MP. 2005 Deactivating germline mutations in LEMD3 cause osteopoikilosis and Buschke-Ollendorff syndrome, but not melorheostosis. The American Society of Human Genetics 55th Annual Meeting Proceedings; Salt Lake City, UT, USA. October 25–29, 2005;
31. Mumm S, Wenkert D, Zhang X, McAlister WH, Mier R, Whyte MP. Deactivating germline mutations In LEMD3 cause osteopoikilosis and Buschke-Ollendorff syndrome, but not sporadic melorheostosis. *J Bone Miner Res.* 2007; 22:243–50. [PubMed: 17087626]
32. Zang Y, Castori M, Ferranti G, Paradisi M, Wordsworth BP. Novel and recurrent germline LEMD3 mutations causing Buschke-Ollendorff syndrome and osteopoikilosis but not isolated melorheostosis. *Clin Genet.* 2009; 75:556–61. [PubMed: 19438932]
33. Happle R. Melorheostosis may originate as a type 2 segmental manifestation of osteopoikilosis. *Am J Med Genet.* 2004; 125A:221–3. [PubMed: 14994228]
34. Tinschert S, Stein A, Goldner B, Dietel M, Happle R. Melorheostosis with ipsilateral nevus sebaceous (didymosis melorheosebacea). *Eur J Dermatol.* 2003; 13:21–4. [PubMed: 12609776]
35. Hafner C, Toll A, Gantner S, Mauerer A, Lurkin I, Acquadro F, Fernandez-Casado A, Zwarthoff EC, Dietmaier W, Baselga E, Parera E, Vicente A, Casanova A, Cigudosa J, Mentzel T, Pujol RM, Landthaler M, Real FX. Keratinocytic epidermal nevi are associated with mosaic RAS mutations. *J Med Genet.* 2012; 49:249–53. [PubMed: 22499344]
36. Groesser L, Herschberger E, Ruetten A, Ruivenkamp C, Lopriore E, Zutt M, Langmann T, Singer S, Klingeisen L, Schneider-Brachert W, Toll A, Real FX, Landthaler M, Hafner C. Postzygotic HRAS and KRAS mutations cause nevus sebaceous and Schimmelpenning syndrome. *Nat Genet.* 2012; 44:783–7. [PubMed: 22683711]
37. Lim YH, Ovejero D, Sugarman JS, DeKlotz CMC, Maruri An, Eichenfield LF, Kelley PK, Juppner H, Gottschalk M, Tiffit CJ, Gafni RI, Boyce AM, Cowen EW, Bhattacharyya N, Guthrie LC, Gahl WA, Golas G, Loring EC, Overton JD, Mane SM, Lifton RP, Levy ML, Collins MT, Choate KA. Multilineage somatic activating mutations in HRAS and NRAS cause mosaic cutaneous and skeletal lesions, elevated FGF23 and hypophosphatemia. *Hum Mol Genet.* 2014; 23:397–407. [PubMed: 24006476]
38. Griffith M, Griffith OL, Smith SM, Ramu A, Callaway MB, Brummett AM, et al. Genome modeling system: a knowledge management platform for genomics. *PLoS Comput Biol.* 2015; 11(7):e1004274. [PubMed: 26158448]
39. Griffith M, Miller CA, Griffith OL, Krysiak K, Skidmore ZL, Ramu A, et al. Optimizing cancer genome sequencing and analysis. *Cell Syst.* 2015; 1(3):210–23. [PubMed: 26645048]
40. Koboldt DC, Chen K, Wylie T, Larson DE, McLellan MD, Mardis ER, Weinstock GM, Wilson RK, Ding L. VarScan: variant detection in massively parallel sequencing of individual and pooled samples. *Bioinformatics.* 2009; 25(17):2283–5. [PubMed: 19542151]
41. Larson DE, Harris CC, Chen K, Koboldt DC, Abbott TE, Dooling DJ, Ley TJ, Mardis ER, Wilson RK, Ding L. SomaticSniper: identification of somatic point mutations in whole genome sequencing data. *Bioinformatics.* 2012; 28(3):311–7. [PubMed: 22155872]
42. Saunders CT, Wong WS, Swamy S, Becq J, Murray LJ, Cheetham RK. Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics.* 2012; 28(14):1811–7. [PubMed: 22581179]
43. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, Gabriel S, Meyerson M, Lander ES, Getz G. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol.* 2013; 31(3):213–9. [PubMed: 23396013]
44. Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform.* 2013; 14(2):178–92. [PubMed: 22517427]
45. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. Integrative Genomics Viewer. *Nat Biotechnol.* 2011; 29(1):24–6. [PubMed: 21221095]

46. Griffith M, Griffith OL, Smith SM, Ramu A, Callaway MB, Brummett AM, Kiwala MJ, Coffman AC, Regier AA, Oberkfell BJ, Sanderson GE, Mooney TP, Nutter NG, Belter EA, Du F, Long RL, Abbott TE, Ferguson IT, Morton DL, Burnett MM, Weible JV, Peck JB, Dukes A, McMichael JF, Lolofie JT, Derickson BR, Hundal J, Skidmore ZL, Ainscough BJ, Dees ND, Schierding WS, Kandoth C, Kim KH, Lu C, Harris CC, Maher N, Maher CA, Magrini VJ, Abbott BS, Chen K, Clark E, Das I, Fan X, Hawkins AE, Hepler TG, Wylie TN, Leonard SM, Schroeder WE, Shi X, Carmichael LK, Weil MR, Wohlstadter RW, Stiehr G, McLellan MD, Pohl CS, Miller CA, Koboldt DC, Walker JR, Eldred JM, Larson DE, Dooling DJ, Ding L, Mardis ER, Wilson RK. Genome Modeling System: A Knowledge Management Platform for Genomics. *PLoS Comput Biol*. 2015; 11(7):e1004274. [PubMed: 26158448]
47. Pengelly RJ, Gibson J, Andreoletti G, Collins A, Mattocks CJ, Ennis S. A SNP profiling panel for sample tracking in whole-exome sequencing studies. *Genome Med*. 2013; 5(9):89. Erratum in: *Genome Med*. 7(1): 44, 2015. [PubMed: 24070238]
48. Skidmore ZL, Wagner AH, Lesurf R, Campbell KM, Kunisaki J, Griffith OL, Griffith M. GenVisR: Genomic Visualizations in R. *Bioinformatics*. 2016; 32(19):3012–4. [PubMed: 27288499]
49. Paila U, Chapman BA, Kirchner R, Quinlan AR. GEMINI: Integrative Exploration of Genetic Variation and Genome Annotations. *PLoS Comput Biol*. 2013; 9(7):e1003153. [PubMed: 23874191]
50. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homor N, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009; 25(16):2078–9. [PubMed: 19505943]
51. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, et al. VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Research*. 2012; 22(3):568–76. [PubMed: 22300766]
52. McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. *Bioinformatics*. 2010; 26(16): 2069–70. [PubMed: 20562413]
53. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012; 491(7422):56–65. [PubMed: 23128226]
54. Fu W, O'Connor TD, Jun G, Kang HM, Abecasis G, Leal SM, et al. Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. *Nature*. 2013; 493(7431):216–20. [PubMed: 23201682]
55. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Research*. 2016; 44(D1):D862–8. [PubMed: 26582918]
56. Heike CL, Steiner RD, Wenkert D, Hornung RL, Gruss JS, Cunningham ML, McAlister WH, Mumm S, Whyte MP. Skeletal changes in epidermal nevus syndrome: does focal bone disease harbor clues concerning pathogenesis? *Am J Med Genet*. 2005; 139A:67–77. [PubMed: 16222671]
57. Wenkert D, Novack D, McAlister WH, Whyte MP. Epidermal nevus syndrome with hypophosphatemic rickets and unilateral bone disease without dermatopathy : a forme fruste implicates the focal skeletal pathology in FGF-23-induced phosphaturia. (Abstract). *J Bone Miner Res*. 2006; 21(Suppl 1):S192.
58. Léri A, Joanny J. Une affection non décrite des os hyperostose en coulée sur toute la longueur d'un membre ou melorheostose. *Bull Mem Soc Hop Paris*. 1922; 46:1141–5.
59. Rozencwaig R, Wilson MR, McFarland GB. Melorheostosis. *Am J Orthop (Belle Mead NJ)*. 1997; 26(2):83–9. [PubMed: 9040882]
60. Murray RO, McCredie J. Melorheostosis and the sclerotomes: a radiological correlation. *Skeletal Radiol*. 1979; 4:57–71. [PubMed: 505048]
61. Hellemans J, Debeer P, Wright M, Janecke A, Kjaer KW, Verdonk PC, Savarirayan R, Basel L, Moss C, Roth J, David A, DePaepe A, Coucke P, Mortier GR. Germline LEMD3 mutations are rare in sporadic patients with isolated melorheostosis. *Hum Mutat*. 2006; 27:290.

62. Gutierrez D, Cooper KD, Mitchell AL, Cohn HI. Novel somatic mutation in LEMD3 splice site results in buschke-ollendorff syndrome with polyostotic melorheostosis and osteopoikilosis. *Pediatr Dermatol.* 2015; 32(5):e219–20. [PubMed: 26135202]
63. Yadegari M, Whyte MP, Mumm S, Phelps R, Shanske A, Totty WG, Cohen SR. Buschke-Ollendorff Syndrome: Absence of LEMD3 mutation in an affected family. *Arch Dermatol.* 2010; 146(1):63–8. [PubMed: 20083694]
64. Lin F, Morrison JM, Wu W, Worman HJ. MAN1, an integral protein of the inner nuclear membrane, binds Smad2 and Smad3 and antagonizes transforming growth factor-beta signaling. *Hum Mol Genet.* 2005; 14:437–45. [PubMed: 15601644]
65. Osada SI, Ohmori SY, Taira M. XMAN1, an inner nuclear membrane protein, antagonizes BMP signaling by interacting with Smad1 in *Xenopus* embryos. *Develop.* 2003; 130:1783–94.
66. Kim JE, Kim EH, Han EH, Park RW, Park IH, Jun SH, Kim JC, Young MF, Kim IS. A TGF- β -inducible cell adhesion molecule, β ig-h3, is downregulated in melorheostosis and involved in osteogenesis. *J Cell Biochem.* 2000; 77:169–78. [PubMed: 10723084]
67. Endo H, Katsumi A, Kuroda K, Utani A, Moriya H, Shinkai H. Increased procollagen alpha1(I) mRNA expression by dermal fibroblasts in melorheostosis. *Br J Dermatol.* 2003; 148(4):799–803. [PubMed: 12752142]
68. Igawa S, Honma M, Min Ami-Hori M, Tsuchida E, Iizuka H, Ishida-Yamamoto A. Novel postzygotic KRAS mutation in a Japanese case of epidermal nevus syndrome presenting with two distinct clinical features, keratinocytic epidermal nevi and sebaceous nevi. *The Journal of Dermatology.* 2016; 43:103–14. [PubMed: 26498739]
69. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. *Nat Rev Mol Cell Biol.* 2008; 9(7):517–31. [PubMed: 18568040]
70. Kharbanda A, Rajabi H, Jin C, Alam M, Wong KK, Kufe D. MUC1-C confers EMT and KRAS independence in mutant KRAS lung cancer cells. *Oncotarget.* 2014; 5(19):8893–905. [PubMed: 25245423]
71. Paliogiannis P, Cossu A, Tanda F, Palmieri G, Palomba G. KRAS mutational concordance between primary and metastatic colorectal adenocarcinoma. *Oncol Lett.* 2014; 8(4):1422–26. [PubMed: 25202344]
72. Aggarwal N, Swerdlow SH, Kelly LM, Ogilvie JB, Nikiforova MN, Sathanoori M, Nikiforov YE. Thyroid carcinoma-associated genetic mutations also occur in thyroid lymphomas. *Mod Pathol.* 2012; 25(9):1203–11. [PubMed: 22575864]
73. Ovejero D, Lim YH, Boyce AM, Gafni RI, McCarthy E, Nguyen TA, Eichenfield LF, DeKlotz CM, Guthrie LC, Tosi LL, Thornton PS, Choate KA, Collins MT. Cutaneous skeletal hypophosphatemia syndrome: clinical spectrum, natural history, and treatment. *Osteoporos Int.* 2016; 27:3615–26. [PubMed: 27497815]
74. Stevenson DA1, Yang FC. The musculoskeletal phenotype of the RASopathies. *Am J Med Genet C Semin Med Genet.* 2011; 157C(2):90–103. [PubMed: 21495174]
75. Grusch M, Petz M, Metzner T, Oztürk D, Schneller D, Mikulits W. The crosstalk of RAS with the TGF- β family during carcinoma progression and its implications for targeted cancer therapy. *Curr Cancer Drug Targets.* 2010; 10(8):849–57. [PubMed: 20718708]
76. Cohen-Solal KA, Boregowda RK, Lasfar A. RUNX2 and the PI3K/AKT axis reciprocal activation as a driving force for tumor progression. *Mol Cancer.* 2015; 14:137. [PubMed: 26204939]
77. Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JHM, Owen MJ, Mertelsmann R, Zabel BU, Olsen BR. Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. *Cell.* 1997; 89:773–9. [PubMed: 9182765]

Highlights

- Melorheostosis (MEL), a sporadic osteosclerosis, may represent postzygotic mosaicism
- Scleroderma-like skin (SLS) over MEL could reflect its etiology and pathogenesis
- MEL with SLS can occur in osteopoikilosis (OPK) due to germline *LEMD3* mutation
- In our OPK patient, *KRAS* somatic mutation in his SLS was implicated for MEL
- Our methodology using whole exome sequencing can investigate the etiology of MEL

Figure 1. Patient

(A,B) At 14 years-of-age our patient has slight knock-knee deformity from an enlarged and elongated right lower limb.

(C) The right foot is disproportionately big in keeping with melorheostosis.

Figure 2. Epidermal Nevus

A) A classic linear epidermal nevus involves his right neck and back.

B) This epidermal nevus extends to within his scalp.

Scleroderma-like Dermatositis:

C) This extensive dermatosis of his right thigh lies over the melorheostosis present in his right femur. It has clinical and histopathological changes (Figure 6) consistent with the scleroderma-like lesion associated with MEL, and is not an elastoma or collagenoma as found in Buschke-Ollendorff syndrome.

Figure 3.
Tissue designations, locations, and characteristics selected for DNA extraction and exome sequencing.

Figure 4. Exome Sequencing and Somatic Variant Detection

A) Sequencing depth and total bases sequenced in the target region by exome sequencing.

B) To demonstrate that all samples are derived from the same patient, the variant allele frequencies (VAFs) were plotted for 24 bi-allelic single nucleotide polymorphisms (SNPs) previously identified by Pengelly et al⁽⁴⁴⁾ to discriminate exome samples from multiple individuals. The highly correlated VAFs indicate all samples were derived from the same patient.

C) All genes with candidate somatic variants passing manual review (see Methods), along with mutation type and translational effect, are shown for each sample.

D) The scatter plots compare VAFs of somatic variants identified in each of the four tissue samples (y-axis) to their respective VAFs in the peripheral blood sample (x-axis). Variants with $\geq 8\%$ VAF are labeled with their respective gene name. *KRAS* mutations were only identified in the EN and SL samples, as labeled.

Figure 5. Patient's Skeletal Radiographs

A) Anteroposterior right chest: right ribs 4–9 (arrows) are sclerotic and slightly expanded. The fifth and eight ribs are less involved.

B) Postero-anterior left hand: an osteosclerotic lesion in the capitate (arrow) is in keeping with osteopoikilosis (OPK).

C) Anteroposterior pelvis: sclerosis affects the entire right iliac bone except near its crest (arrows) and has remarkable, almost “fluffy”, large, expanded, sclerotic areas. There is expansion of the body with an irregular lateral margin.

D) Anteroposterior femurs: linear sclerosis in the neck (**C**) with subtrochanteric cortical thickening, expansion, and medullary sclerosis (arrows) (**B and C**).

E) Anteroposterior right knee: a cluster of osteosclerotic lesions consistent with OPK is in the epiphysis of the distal femur (black ellipse).

F) Anteroposterior (left) and lateral (right) left tibia: the mid tibia has sharply demarcated oblique cortical thickening that extends into the medullary cavity (arrow, left panel), and is slightly expanded with anterior bowing (arrows, right panel). Cortical thickening extends both out and into the medullary cavity, which is obliterated in the distal fibula.

Figure 6. Scleroderma-like Dermatitis

A, B) The patient's scleroderma-like lesion shows a proliferation of normal-appearing collagen bundles within the reticular dermis extending into the subcutis that is **C, D)** associated with slightly decreased elastic fibers.

A) Hematoxylin and eosin, 40×. B) Hematoxylin and eosin, 100×. C) Elastin, 40×. D) Elastin, 100×.

Figure 7. Detailed View Of Relevant Somatic Variants

A) Integrative Genomics Viewer (IGV) screenshot of *LEMD3*, exon 1, 24 bp heterozygous deletion.

B) IGV screenshot of *KRAS* Q61H heterozygous mutation in EN and SL samples.

C) Schematic representations of the *KRAS* protein structure comparing the *KRAS* mutation observed in EN and SL samples to common *KRAS* mutations documented in cancer (COSMIC database). Each blue circle represents one of the top-ranked amino acid changes reported in *KRAS*.