Buschke-Ollendorff Syndrome

Absence of LEMD3 Mutation in an Affected Family

Michelle Yadegari, MD; Michael P. Whyte, MD; Steven Mumm, PhD; Robert G. Phelps, MD; Alan Shanske, MD; William G. Totty, MD; Steven R. Cohen, MD

Background: Buschke-Ollendorff syndrome (BOS), an autosomal dominant disorder, features small, acquired, asymptomatic, symmetrical foci of osteosclerosis detected radiographically in epimetafysisal bone (osteopoikilosis) (OPK) together with connective tissue nevi or juvenile elastomas. Heterozygous, loss-of-function, germline mutation in the LEMD3 gene (which encodes an inner nuclear membrane protein called LEMD3, or MAN1) has been repeatedly documented in patients with BOS or OPK.

Observations: We describe a father and son with multiple yellowish papules and nodules coalescing into cobblestone nevoid plaques consistent with nevus elasticus. Radiographs of the father show multiple, small, bone islands within the hands, wrists, distal femurs, proximal tibias, and left distal fibula consistent with OPK. Although the clinical findings are diagnostic of Buschke-Ollendorff syndrome, analysis of the LEMD3 gene showed no exonic mutations.

Conclusion: Absence of LEMD3 mutation in the exons and splice sites of a family with BOS suggests that there is genetic heterogeneity for this disorder.

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Methods

The propositus, a Bukari Jewish boy nearly 6 years old and living in New York City, was referred to dermatology for skin lesions noticed by his mother at about 3 to 6 months of age. At birth, he was the 3-kg product of an uneventful term pregnancy and delivery. The lesions were first observed on his abdomen and then slowly increased in number and size on his trunk over the next 2 years. The dermatosis was asymptomatic, and his general health and development were unremarkable.

Physical examination revealed a playful child. His height was 111 cm (25th percentile), and his weight was 23 kg (75th percentile). He had multiple, yellowish papules and nodules coalescing into cobblestone nevoid plaques on his left hypogastrium (Figure 1A). The plaques were irregularly shaped and sharply demarcated (Figure 1B). The remainder of the examination was unremarkable. Results from routine laboratory investigations (including complete blood

Osteopoikilosis (OPK) (OMIM 166700), which means “spotted bones,” is a rare, autosomal dominant condition that manifests radiographically during late childhood or early adult life with multiple, small, symmetric, epimetafysisal, osteosclerotic foci thought to represent old remodeled bone with a lamellar structure. These lesions are typically asymptomatic but persist, are sometimes associated with bone and joint pain, and can be mistaken for osteoblastic metastases to the skeleton.

Buschke-Ollendorff syndrome (BOS) (OMIM 166700) is a rare, autosomal dominant, typically benign disorder that combines OPK together with skin lesions consisting of connective tissue nevi (dermatofibrosis lenticularis disseminata) or juvenile elastomas.

In 2004, heterozygous, loss-of-function, germline mutations in the LEMD3 gene (also known as MAN1) were discovered to explain both OPK and BOS. LEMD3 is an inner nuclear membrane protein that antagonizes transforming growth factor β (TGF-β) and bone morphogenetic protein (BMP) signaling.

Additional studies confirmed this result and expanded the mutation spectrum regarding loss-of-function LEMD defects.

Recently, we evaluated a 5-year-old boy with nevus elasticus whose father was found to have similar skin lesions. Although radiographs of this child did not demonstrate OPK, likely because of his young age, radiographs of his father were consistent with BOS. As detailed herein, mutation analysis of the exons and adjacent messenger RNA (mRNA) splice sites of LEMD3 was negative in this family, suggesting genetic heterogeneity for BOS.

Author Affiliations: Division of Dermatology, Department of Medicine (Drs Yadegari and Cohen), and Center for Craniofacial Disorders, Department of Pediatrics, Children’s Hospital at Montefiore Medical Center (Dr Shanske), Albert Einstein College of Medicine, Bronx, New York; Division of Bone and Mineral Diseases (Drs Whyte and Mumm) and Musculoskeletal Section, Mallinckrodt Institute of Radiology (Dr Totty), Washington University School of Medicine, St Louis, Missouri; Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for Children, St Louis (Drs Whyte and Mumm); and Departments of Dermatology and Pathology, Mount Sinai School of Medicine, New York, New York (Dr Phelps).
cell count, biochemistry panel, urinalysis, thyroid function studies, and lipid profile) were within reference range.

A 3-mm punch biopsy specimen was taken from a representative skin lesion on the abdomen. Light microscopy revealed fibroplasia in the dermis and edema between collagen bundles. The reticular dermis is almost completely replaced by thickened, large, and haphazardly arrayed collagen fibers (Figure 2A). On higher power, a slight increase in interstitial cellularity (Figure 2B) can be seen. An elastica–van Gieson stain disclosed very thick elastic fibers that splay between and appear to envelop collagen bundles (Figure 2C). In the interfascicular spaces and in areas of enlarged elastic fibers, an Alcian blue stain revealed an increase in interstitial mucin (Figure 2D). The increase in other matrix components was unremarkable. The cumulative findings are most suggestive of nevus elasticus. Radiographs of the patient's hands and wrists, knees, and ankles do not show OPK.

Immediate family members were then examined. The father had more circumscribed, but comparable, skin lesions involving his lower extremities (Figure 3A). Microscopy of a punch biopsy specimen taken from an affected area on the dorsum of his right ankle shows similar changes to the propositus, although much more focally. In between the normal small thin collagen bundles, there are conspicuous large, thickened, and haphazardly arrayed collagen fibers (Figure 3B). In these areas, there is an increase in dermal cellularity with thin fibrocyte nuclei. The elastic fibers are massively thickened, irregular in size and shape, and clearly contrast with the small elastic fibers of the adjacent normal dermis (Figure 3C). No skin lesions suggesting BOS were observed. In the boy's mother or sister, the right ankle shows similar changes to the propositus, although much more focally. In between the normal small thin collagen bundles, there are conspicuous large, thickened, and haphazardly arrayed collagen fibers (Figure 3D). In the interfascicular spaces and in areas of enlarged elastic fibers, an Alcian blue stain revealed an increase in interstitial mucin (Figure 3E). The increase in other matrix components was unremarkable. The cumulative findings are most suggestive of nevus elasticus.

Radiographs of the patient's father show multiple, small, bone islands within the hands and wrists consistent with OPK (Figure 4). Similar findings are seen on the distal femurs, proximal tibias, and left distal fibula. The mother and sister were not evaluated radiographically.

**LEMD3 MUTATION ANALYSIS**

LEMD3 mutation analysis was performed after written informed consent approved by the committee on clinical investigations of the Albert Einstein College of Medicine, Bronx, New York. Whole blood from the propositus and his parents was collected separately using EDTA acid anticoagulant, and DNA was extracted from the leukocytes using the Puregene Kit (Gen- tra Systems Inc, Minneapolis, Minnesota).

The *LEMD3* gene spans approximately 78 kb. Using techniques that were reported in detail elsewhere, all 13 coding exons and adjacent mRNA splice sites were amplified by polymerase chain reaction (PCR) and sequenced in both directions. The DNA sequence was analyzed visually and with VectorNTI AlignX software (Invitrogen, Carlsbad, California). Owing to the large size of exon 1 of *LEMD3*, 2 primer sets were used. Owing to close proximity, 3 sets each of 2 exons (exons 5 and 6, exons 7 and 8, and exons 11 and 12) were PCR amplified and sequenced together. All remaining exons were amplified by PCR and sequenced individually. Primer sequences and conditions for PCR and DNA sequencing of *LEMD3* were kindly provided by Jan Hellemans, PhD, and Geert Mortier, PhD, MD (Ghent, Belgium). Some primers were modified to optimize PCR results (these primer sequences and PCR conditions are available from the authors on request).

All 13 coding exons and adjacent mRNA splice junctions of *LEMD3* were amplified by PCR and sequenced in both directions. A minimum of 30 base pairs (bp) of the adjacent intronic sequence was examined; for most splice junctions, about 40 to 85 bp were sequenced.

Mutation analysis showed no exonic mutations (exons 1-13) or splice site mutations in the *LEMD3* gene of the propositus. Three heterozygous intronic polymorphisms (IVS 4, rs11610822; IVS 7, rs10534559; IVS 11, rs3217456) were noted but were not thought to be functional because they are common in our patient cohort and are reported as polymorphisms in database single-nucleotide polymorphism. The presence of these 3 polymorphisms in the propositus, however, rules out the possibility of complete deletion of 1 *LEMD3* allele as the cause of this family's BOS. The polymorphisms in IVS 4 and 11 were found in the paternal DNA, and the polymorphism in IVS 7 was detected in the maternal DNA. Furthermore, all 13 exons and adjacent mRNA splice were also sequenced in both parents; no *LEMD3* mutation was found.

**COMMENT**

The association between OPK and connective tissue nevi was first reported by Buschke and Ollendorf in 1928. Buschke-Ollendorf syndrome is inherited as an autosomal...
dominant trait with variable expressivity. Affected individuals typically manifest both skin and bone findings, but some have involvement of only 1 of these tissues.

Although BOS is generally considered benign, this rare disorder has also been described in patients with diabetes mellitus; otosclerosis; ocular anomalies, including cataracts; peptic ulcer; cryptorchidism; congenital spinal stenosis; short stature with or without precocious puberty; and muscle contractures. However, many of these associations with BOS may be coincidental. In fact, BOS is usually asymptomatic and requires no specific therapy.

The differential diagnosis for BOS includes (1) pseudoxanthoma elasticum (PXE) (OMIM 264800), which has similar skin findings, but also serious retinal and vascular complications without bony involvement; (2) isolated elastoma, a sporadic condition with similar skin lesions in the absence of OPK; and (3) elastosis perforans serpiginosa (EPS) (OMIM 130100), which is differentiated clinically with ease from BOS by arcuate, hyperkeratotic papules and plaques.

The radiographic findings of OPK and BOS feature multiple, radiopaque, round or oval spots in the epiphyses and metaphyses of long bones, the pelvis, and bones of the hands and feet. Skull, ribs, and vertebrae are rarely involved, a finding that helps to distinguish OPK and BOS from other disorders. The bone lesions take several years to develop.

Figure 2. Biopsy specimen from the young patient. A, Biopsy findings of the skin lesion on left hypogastrium reveals fibroplasia in the dermis and edema between collagen bundles. The reticular dermis is almost completely replaced by thickened, large, and haphazardly arrayed collagen fibers (hematoxylin-eosin, original magnification × 4). B, On higher power view there is a slight increase in interstitial cellularity with enlarged fibrocyte nuclei between the collagen bundles (hematoxylin-eosin, original magnification × 40). C, Using an elastica–van Gieson stain there are very thick elastic fibers that splayed between and appeared to envelop collagen bundles (original magnification × 40). D, An Alcian blue stain reveals an increase of interstitial mucin in the interfascicular spaces and in the areas of the enlarged elastic fibers (original magnification × 40).
velop, reaching maturation at or near the time of puberty, although they are commonly detectable during late childhood. Typically, they change little after puberty and do not predispose to fractures. There is no evidence to suggest an increase in morbidity or mortality in patients with OPK and BOS. Nevertheless, documentation and appreciation of the bone lesions is important by early adult life to avoid confusion with osteoblastic skeletal metastases.

Two different cutaneous findings have been described in BOS. Some patients have symmetrical, yellow or skin-colored eruptions of small, uniform, lichenoid papules. More frequently, the lesions are larger, often grouped, yellowish nodules, which can be asymmetrically distributed. Both elastic-type nevi (juvenile elastoma) and collagen-type nevi (dermatofibrosis lenticularis disseminata) have been reported in BOS. However, the distinctive histologic feature of BOS skin lesions is an increase in morbidity or mortality in patients with OPK and BOS. Nevertheless, documentation and appreciation of the bone lesions is important by early adult life to avoid confusion with osteoblastic skeletal metastases.

Until recently, diagnosing BOS depended on a consistent medical history, careful clinical examination, radiographic studies of the patient, and sometimes assessment of the family. Now, OPK and BOS can be diagnosed by

Figure 3. The patient’s father. A, His right ankle shows a linear yellow plaque that is comparable to (but more circumscribed than) that found in the propositus. B, Skin biopsy specimen from the dorsum of the right ankle reveals similar changes to the propositus, although much more focally. In between the normal small thin collagen bundles, there are conspicuous large, thickened and haphazardly arrayed collagen fibers (arrows) (hematoxylin-eosin, original magnification × 2). C, An elastica–van Gieson stain reveals an increase in dermal cellularity with thin fibrocyte nuclei. The elastic fibers in these areas are massively thickened, irregular in size and shape, and clearly contrasted with the small elastic fibers of the adjacent normal dermis (arrows) (original magnification × 40).

Figure 4. This posteroanterior radiograph of the father’s right hand shows isolated sclerotic lesions (arrowheads), most prominently in the distal radius, the distal first metacarpal, the distal second middle phalanx, and overlying the head of the second and third metacarpals (note that the latter may also be within the bone, although these could be produced by sesamoid lesions in the flexor tendons). More subtle changes are also noted in the distal phalanges of the third, fourth, and fifth digits. Along the endosteal surface of the distal phalanges of the third, fourth, and fifth digits, there are irregular areas of sclerosis that resemble melorheostosis (asterisks).
LEMD3 mutation analysis. In 2004, Hellemans et al8 used whole genome linkage analysis of 3 affected families to map the chromosomal location of OPK to a large region on chromosome 12q13. Subsequently, a microdeletion was found and characterized in a patient with OPK together with proportionate short stature, microcephaly, learning disabilities, and ectopic kidneys.9 This narrowed the linkage interval to a 3.07-Mb critical region containing 23 genes. LEMD3 (MAN1), within this region, was considered a good candidate gene for OPK or BOS because it functioned in BMP signaling, which is important for skeletal development.10,11 Then, sequencing studies of LEMD3 identified heterozygous, loss-of-function mutations in all affected individuals within 3 OPK families, and in 3 additional unrelated individuals with OPK. To date, LEMD3 mutation has been reported in all 18 probands examined with OPK or BOS.9,12,13,25 In 2007, this discovery was confirmed in our study of 3 families affected by OPK and BOS and in 1 sporadic case.13 None of these reported cases of OPK or BOS failed to show a loss-of-function LEMD3 mutation.

LEMD3 functions in TGF-β and BMP signaling.10,11,26 Lin et al,10 in a series of LEMD3 overexpression experiments, showed that the carboxyterminus of the LEMD3 protein interacts with SMAD downstream elements of the BMP receptors to downregulate SMAD activation. When LEMD3 is deactivated, it cannot downregulate SMAD activation, and hence excess bone is produced. LEMD3 also antagonizes TGF-β signaling in human cells. Hence, increased signaling in the TGF-β pathway may also explain the skin lesions in patients with BOS. However, the focal nature of OPK and the connective tissue nevi in BOS remain an enigma.

The genodermatosis that we characterized in this father and son is clinically and histologically indistinguishable from BOS. Nevertheless, we found no LEMD3 mutation in the patient or in his parents. Therefore, our findings suggest genetic heterogeneity for BOS.12,25 There are, however, several alternative but rare genetic mechanisms that could involve LEMD3 in this family, including mutations within the remaining LEMD3 intronic sequences, defects within the LEMD3 promoter, or mutations upstream or downstream within regulatory sequences for LEMD3. Furthermore, we could not exclude complete deletion of 1 or more LEMD3 exons in our family, although the presence of the 3 polymorphisms in the proband shows that an entire LEMD3 allele is not deleted in the father or son. Instead, an entirely different gene, perhaps in BMP or TGF-β signaling, may be defective in this family. In fact, Giro et al,27 in 1992, suggested that the pathogenesis of BOS may be an altered response to cutaneous cytokine expression because cutaneous signals caused by cytokines can stimulate elastin production. Perhaps altered signaling, or a cytokine other than TGF-β, causes BOS in this unusual family.

In conclusion, the absence of an LEMD3 mutation in the exons and splice sites of a family with BOS suggests that there is genetic heterogeneity for this disorder.

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Correspondence: Steven R. Cohen, MD, Division of Dermatology, Montefiore Medical Center, 111 E 210th St, Bronx, NY 10467 (steven.cohen@aya.yale.edu).

Author Contributions: Dr Cohen had full access to all data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Yadegari, Whyte, Phelps, and Cohen. Acquisition of data: Yadegari, Whyte, Mummc, Phelps, and Cohen. Analysis and interpretation of data: Yadegari, Whyte, Mummc, Phelps, Sanscse, Tottv, and Cohen. Drafting of the manuscript: Yadegari, Whyte, Mummc, Phelps, Sanscse, and Cohen. Critical revision of the manuscript for important intellectual content: Whyte, Mummc, Tottv, and Cohen. Obtained funding: Whyte. Administrative, technical, and material support: Whyte, Mummc, and Cohen. Study supervision: Whyte and Cohen.

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Cock's Peculiar Tumor

A 79-year-old woman with a giant, cystic lesion of the scalp that had been at the site for 60 years had suddenly enlarged over the preceding 2 months (Figure). An excisional biopsy specimen showed the histologic features of a proliferating pilar cyst, also known as Cock's peculiar tumor. So who was Cock? And are we any wiser as to the nature of the entity that he first described in 1832?1

Edward Cock was born in Middlesex in 1805. He was apprenticed to his uncle, Astley Cooper, in 1821 and studied medicine at Edinburgh University in Scotland from 1829 to 1834. Then, he worked at Guy's and St Thomas's Hospital, London, England, where he was a demonstrator of anatomy, becoming Assistant Surgeon in 1838. During this time, he published Practical Anatomy of the Nerves and Vessels Supplying the Head, Neck and Chest, known to students as “Cock's Head and Neck.”2 He became a full surgeon in 1849 and was president of the Royal College of Surgeons, London, in 1869. He worked tirelessly, marrying late at the age of 62 years, and died in 1892. His name is connected to a range of important work in the field of surgery, including describing the pathologic changes that can cause congenital deafness, treating impermeable urethral stricture, and pioneering pharyngotomy, and he was one of the first surgeons to successfully trephine for middle meningeal hemorrhage. In dermatology, he described the clinical features in 4 cases of large proliferating scalp or face tumors that had been diagnosed as malignant but that he believed were benign.3

The microscopic reports on the tumors described by Cock were basic, but the drawings in his original article suggest the diagnosis of giant proliferative trichilemmal cyst. This tumor, which has been the subject of much discussion and debate in the dermatopathology literature since its initial description, has been also called proliferative pilar cyst, proliferating trichilemmal tumor, pilar tumor, and proliferating follicular cystic neoplasm. It classically occurs on the scalp of an elderly woman and varies in size up to 25 cm in diameter. Histologically, there is a circumscribed, pushing border, with 25% of cases exhibiting an epidermal connection. Confusion with squamous cell carcinoma is common, as recognized by Wilson Jones4 in 1966, when he first used the name proliferating epidermoid cyst. Mones and Ackerman5 stated that this entity is a variant of squamous cell carcinoma; however, the general consensus is that there is a possible spectrum of both clinical and histologic findings. Most cases appear to have a benign course, with no recurrence. The presence of a high mitotic rate, atypical mitoses, severe nuclear pleomorphism, and invasion of adjacent tissue suggest a malignant tumor when the name malignant proliferating trichilemmal cyst has been used. In these atypical cases, lymphatic spread and distant metastases have been reported. Complete surgical excision is recommended.

Sandeep Varma, MB, ChB, MRCP(UK)
Sandeep Varma, MB, ChB, MRCP(UK)

Contact Dr Affleck at andrew.affleck@nhs.net.