# Buschke-Ollendorf Syndrome: Report of a Case and a Brief Molecular Overview

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**Abstract:** Buschke-Ollendorf syndrome (BOS) is a rare autosomal dominant disorder characterized by localized increases in bone density manifesting as osteopoikilosis or melorheostosis and connective tissue nevi, collagenomas. Manifestations are highly variable. It is caused by loss-of-function mutations in the LEMD3 gene, which codes for an inner nuclear membrane protein that is also known as MAN1. Six different mutations have been described to date without a clear genotype-phenotype correlation. Buschke-Ollendorf syndrome exemplifies the importance of TGFß signaling for bone and connective tissue homeostasis. Here, we report on a father and his daughter with typical BOS syndrome caused by a known nonsense mutation and provide an overview of what is now known of this rare disorder.

**Keywords:** Buschke-Ollendorf, MAN1, LEMD3, nuclear envelope, TGF-beta.

## INTRODUCTION

Buschke-Ollendorf syndrome (BOS, MIM #166700) is characterized by the occurrence from early childhood of disseminated connective tissue nevi and circumscribed sclerotic areas near the ends of many bones known as osteopoikilosis [1]. The latter can also manifest as linear sclerotic bone lesions with fibrosis of the overlying soft tissues, a condition known as melorheostosis [2]. BOS was first described in 1915 by Albers-Schönberg. Buschke and Helen Ollendorff reported it in 1928 (R. Happle, personal communication, 2007).

BOS is caused by heterozygous loss of function mutations in the gene coding for the inner nuclear membrane protein MAN1, also known as LEMD3 because it contains a LEM-domain [3]. Mutations have also been described in some, but not all, cases of melorheostosis, suggesting that this condition may be genetically heterogeneous (G. Mortier, personal communication, 2007).

LEMD3 interacts with BMP and activin-TGFß receptor activated Smads [4] and antagonizes both signaling pathways. This observation is consistent with earlier results from our group that show deregulation of TGFß signaling in the context of nuclear envelope dysfunction [5]. Thus, BOS might be considered as a disruption of connective tissue homeostasis. All mutations observed so far have been truncating ones (nonsense, insertions and deletions) and a genotype-phenotype correlation has so far not been demonstrated. To illustrate the clinical phenotype, we describe a father and his daughter with classical manifestions of BOS caused by a nonsense mutation in exon 1 of the *LEMD3* gene.

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## CASE REPORT

The proposita, a 7-year old girl of Dutch descent, visited our outpatient clinic for evaluation of painless skin nodules on the lower back that had been present from the first year of life onwards. The lesions were slowly progressive. No other abnormalities had been noted. Her father noted that he had similar skin lesions on the medial sides of both knees, but had never paid much attention to them.

Upon examination, we saw a healthy-looking girl with no obvious dysmorphic traits. Hair, nails and teeth were normal. On the mid-thoracic and lumbar back we observed multiple partly confluent skin-colored nodules that were rather soft to palpation (Fig. **1**). We noted no other skin abnormalities. Examination of the father revealed similar skin lesions on the medial sides of both knees (Fig. **2**). Thinking of Buschke-Ollendorf syndrome, we acquired roentgenographs of the father's femur and pelvis. These showed clear metaphyseal osteopoikilosis.

Having confirmed the diagnosis, we performed mutation analysis of the *LEMD3* gene in both patients. Upon obtaining informed consent from the father, we isolated DNA from peripheral blood leucocytes using standard salt-precipitation methods as described previously [6]. Next, we amplified with PCR the coding regions and intron-exon boundaries of the *LEMD3* gene. PCR products were subjected to direct sequencing using the BigDyeDeoxyTerminator system on an ABI 3100 capillary sequencer (Applied Biosystems, Warrington, UK). Primer sequences are listed in Table **1**. Reaction conditions for PCR and sequencing are the same for all primer sets and are as follows: initial denaturation at 94ºC for 90 seconds followed by 30 cycles of 30 seconds at 94 ºC, 30 seconds at 62 ºC, 60 seconds at 72 ºC and termination at 72 ºC for 7 minutes. We append M13 sequencing tails to our primers and use those to sequence. In both patients, we found a transversion 1322C>A in the *LEMD3* gene leading to the truncating mutation Y441X that was previously described by Hellemans *et al.* [7].

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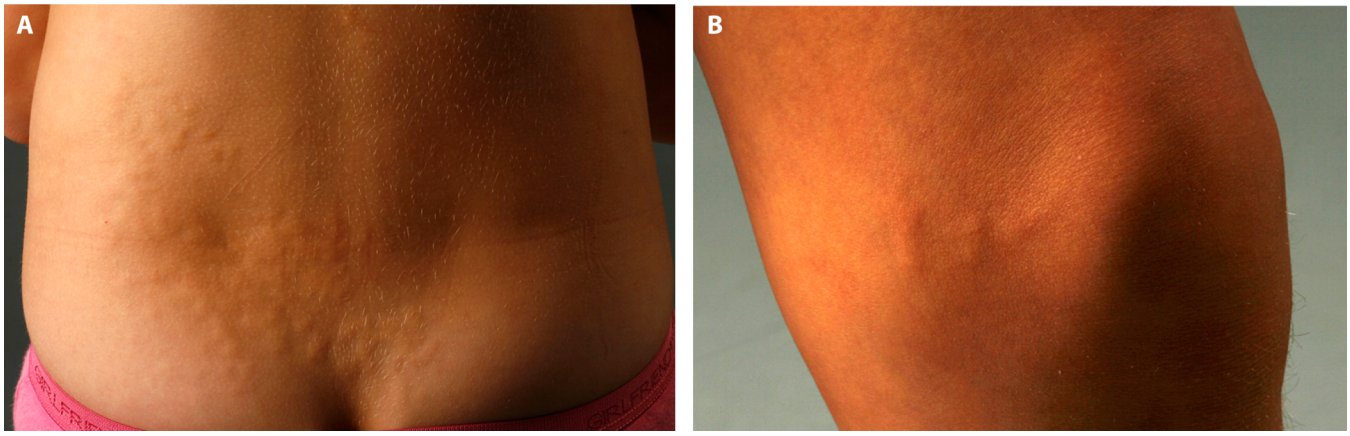


**Fig. (1).** The proposita. Subcutaneous aggregated nodules on the lower back. N. medial side of the father's left knee.

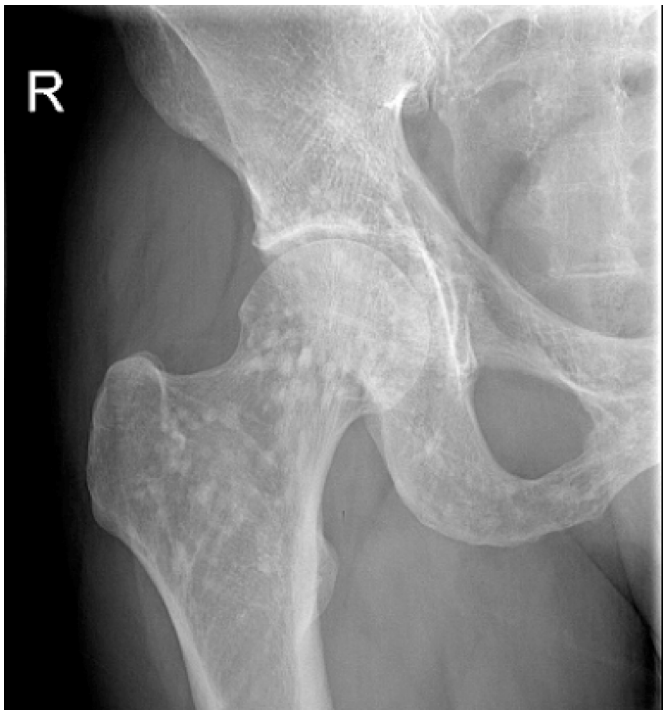


**Fig. (2).** Pelvic roentgenograph of the father showing osteopoikilosis.

## DISCUSSION

We found a known nonsense mutation in the *LEMD3* gene, causing classical Buschke-Ollendorf syndrome in a father and his daughter. All mutations described so far are likewise predicted to truncate the protein, causing a loss of function. The available functional data indicate that LEMD3 is involved in regulation of BMP- and TGFß-receptor mediated signaling. Specifically, LEMD3 can reduce the capacity of BMP4 to upregulate Smad6, Smad7, Id2 and Id3 [8]. Moreover, LEMD3 can also inhibit TGFß signaling [9]. The inhibition likely takes place through interactions between the LEMD3 C-terminus and the Smad MH2 domains. All known mutations, including the one we describe here, are expected to disrupt this interaction. Both the bone abnormalities and the connective tissue nevi are thought to result from dysregulation of TGFß signaling. In this respect, it is of interest to note that collagenomas can also occur in tuberous sclerosis (TSC). The connection between TSC and BOS may not be immediately obvious, until one realizes that the TSC1/2 complex is a negative regulator of mTOR, mammalian target

**Table 1. Primer Sequences (M13 Tails Not Shown for Clarity)**

| Primer Name | Sequence 5' -> 3' |
|---|---|
| **LEMD3F1** | CGGTAGCGCGGAGCTTGTAA |
| **LEMD3R1c** | GTTAGTCACCTCGCTGGCGG |
| **LEMD3F1a** | GGTCTCGGGCGACCTCTCCT |
| **LEMD3R1a** | CTATGGGTTCGTCTGGGCCG |
| **LEMD3F1b** | CTGCAGAGCGAAGGAAGCCC |
| **LEMD3R1b** | CGCACTGGGAGGGAGACTGT |
| **LEMD3F1c** | CCCCCGCCACTTACTGACAT |
| **LEMD3R1** | ATGCACGCACTGTTGCGTTT |
| **LEMD3F2** | CACCAGTTTGTTTACATTTGGTTCACA |
| **LEMD3R2** | CCAACAACTACAGGCAACAGGCA |
| **LEMD3F3** | GGGATTGGGAACATTGCTTTGG |
| **LEMD3R3** | CCTCTAATACAGATGGCAGGCAGGA |
| **LEMD3F4** | CTGGAGGGCGTCTTGTGTGC |
| **LEMD3R4** | ACCAAACAGCAGGCCCAAGC |
| **LEMD3F5** | AAAGGATACTTTACAGAGAGTCGAATG |
| **LEMD3R6** | TGTGACTTATGTGGCAACCATC |
| **LEMD3F7** | GCTAATTCAGCCATCTGTCTTGAAGG |
| **LEMD3R8** | GCAAGTCTAGTTGAGAAGGGTCACAGC |
| **LEMD3F9** | TCCTGAAGCAGCATCTTGACCC |
| **LEMD3R9** | TGATTCCTTCTACGAAACAGAACGAGA |
| **LEMD3F10** | CCTTTCAACAAACTAGAACAATGTCAA |
| **LEMD3R10** | TTGGCCAAATCTTTGCTTGGA |
| **LEMD3F11** | GGAAAGTGGGAGGAGGGCTG |
| **LEMD3R12** | TGGTAAAAGACATATGAGCACAAAACA |
| **LEMD3F12** | GCATTGCATGGCTCTTGGTTTG |
| **LEMD3R13** | TGCTGCCTCACTGCTAAATCCC |

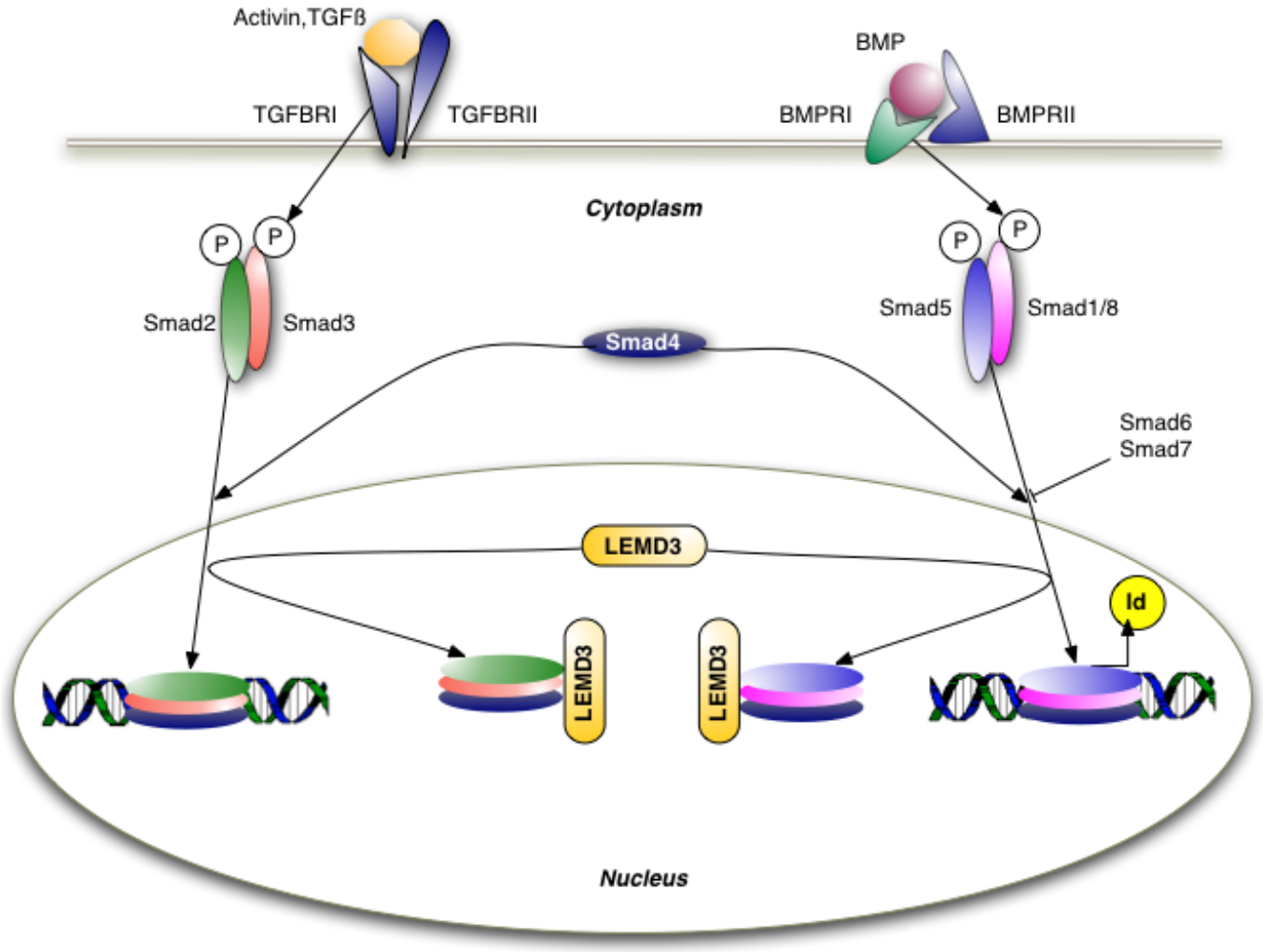


**Fig. (3).** Activin/TGFß/BMP signaling cascade. Activin/TGFß and BMPs signal through their respective receptor complexes to Smads that multimerize and act as transcription factors, regulating expression of among others, Smad family members (such as 6 and 7) and Id proteins. LEMD3 can sequester Smad complexes, preventing them from binding to their target sequences. P: phosphate group added by receptor complex. TGFß: transforming growth factor beta, BMP: bone morphogenetic proteins, Smad: suppressor of mothers against decapentaplegic, LEMD3: LEM domain containing 3 (aka MAN1), Id: inhibitor of differentiation.

of rapamycin [10]. A central regulator of cellular energy metabolism, mTOR seems to be directly involved in modulating Smad activity. For instance, rapamycin can induce Smad activity in prostate cancer cells [11]. Thus, dysregulation of mTOR can interfere with TGFß signaling, which possibly explains the collagenomas found in TSC.

Nosologically, BOS belongs to a family of connective tissue diseases characterized by abnornalities of TGFß signaling that affect bone density. For example, sclerosteosis (OMIM 269500) is caused by loss of fucntion of the *SOST* gene coding for the BMP antagonist sclerostin [12]. Camurati-Engelman disease, which is caused by activating mutations in the *TGFB1* gene [13-15], has some resemblance to BOS in that it is characterized by chronic thickening of the metaphyses. Considering that both disorders feature increased TGFß-signaling, this is not surprising. However, bone symptoms in Camurati-Engelmann syndrome are considerably more severe and the disorder will incapacitate patients unless treated with corticosteroids [16]. Skin abnormalities, on the other hand, are not found. This is rather unexpected, because sclerotic skin changes can be observed in individuals with melorheostosis [17]. Moreover, increased TGFß signaling has also been reported in scleroderma [18] and is partly responsible for the stiff skin syndrome phenotype (B. Loeys, personal communication, 2007). Scleroderma-miform skin disease is furthermore found in progeroid laminopathies caused by mutations in the *LMNA* gene or in *ZMPSTE24*, which codes for an enzyme that is required for one of the final steps in lamin A protein post-translational processing [19]. It was recently shown that A-type lamins are required for the regulation of TGF-ß1 mediated collagen production [5]. Lamin A can bind to LEMD3 through a distinct domain located towards the C-terminus [20]. The LEM domain binds to the DNA-binding protein BAF and LEMD3 can thus act as a bridge between the nuclear lamina and DNA. It is tempting to speculate that these interactions are required for proper suppression of Smad signaling and may be specific to the LEM domain, as closely related LEM domain proteins also appear to be involved in Smad interactions [21]. Disruption of binding to either lamin or BAF might explain part of the sclerosis observed in laminopathies

and BOS alike. However, no mutation described to date specifically affects either the lamin binding or LEM domains, rendering this explanation unlikely. Hellemans *et al.* described a patient with a microdeletion encompassing the LEMD3 gene [22]. While he had an extended phenotype with osteopoikilosis, his deletion affected several contigous genes. Thus it is not possible to attribute his more extensive phenotype to absence of the lamin binding and LEM domains. In conclusion, a genotype-phenotype correlation does not emerge from the known mutational spectrum. Hence, the clinical variability of BOS may be caused by individual variations in TGFß and/or BMP signaling.

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