LEMD3: The Gene Responsible for Bone Density Disorders (Osteopoikilosis)

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With completion of the human genome sequencing, many of the genes that cause genetic diseases and are involved in developmental at pathways are being tracked. A new developmental gene has recently been elucidated. Loss-of-function mutations of the *LEMD3* (*MAN1*) gene were shown to underlie disorders characterized by increased bone density, namely osteopoikilosis, Buschke-Ollendorff syndrome, and melorheostosis [1].

Osteopoikilosis is an autosomal dominant skeletal dysplasia characterized by a symmetric but unequal distribution of multiple hyperostotic areas in different parts of the skeleton [Figure 1] [2,3]. Osteopoikilosis can occur either as an isolated anomaly or in association with other abnormalities of skin and bone. BOS is an autosomal dominant disorder that results from the association of osteopoikilosis with disseminated connective-tissue nevi and manifests as skin lesions. Melorheostosis (often also associated with osteopoikilosis) is characterized by a 'flowing' (rheos) hyperostosis of the cortex of tubular bones and has a "dripping wax" appearance [Figure 1C].

The search for the disease-causing mutation started in a classic whole genome linkage analysis of three affected families,

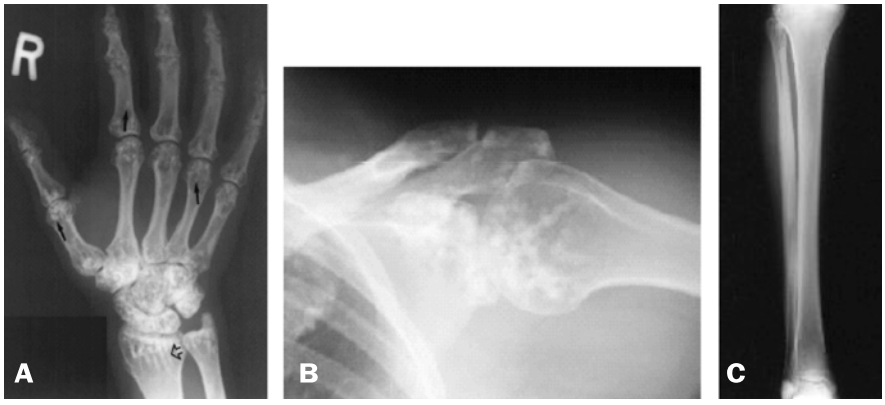

Figure 1. Examples of forms of increased bone density in osteopoikilosis and melorheostosis.
[A] Radiograph of hand shows small rounded radio-densities in phalanges, metacarpals,and carpal bones (closed arrows). Radio-densities are more linear in distal radius and ulna (open arrow). Both of these patterns are very characteristic of osteopoikilosis. This is the most common site of involvement in osteopoikilosis Taken from Kim et al., 2003 [3]; **[B]** Radiologic appearance of osteopoikilosis lesions in the left shoulder. Taken from Debeer et al., 2003 [2]; **[C]** Radiolograph of tibia. The lesions appear as clearly defined sclerotic densities, mainly cortical but also extending into the medullary portion of bone, having a linear pattern of distribution along the long axis of limb. The appearance of this hyperostosis has been linked to candle-wax flowing down the margin of affected bone. Taken from Debeer et al., 2003 [2].

and used polymorphic microsatellite markers. This analysis led to the identification of a large genomic region (~20 megabases) on chromosome 12q13 that harbored the mutation. However, only with the analysis of a particular individual affected with proportionate short stature, microcephaly, learning disabilities, ectopic kidneys and ostepoikilosis, was the genetic region considerably narrowed down. The researchers hypothesized that this individual might have a genomic microdeletion, resulting in the loss of several contiguous genes, including the gene mutated in osteopoikilosis. Indeed, the loss of heterozygosity of several markers confirmed such a microdeletion and defined a much shorter interval (~3 megabases) as the critical region of association with osteopoikilosis. Of the 23 genes within this region, 2 were good candidates for involvement in osteopoikilosis. The gene *WIF1* is involved in Wnt signaling and *LEMD3* functions in bone morphogenetic protein signaling. Both pathways are important in bone development [4,5]. *WIF1* was excluded since mutation detection analysis did not identify any abnormalities of the gene structure in the affected individuals. On the other hand, sequence analysis of *LEMD3* identified loss-of-function mutations in all affected individuals of the three families and in three unrelated individuals with osteopoikilosis. These findings strongly indicate that the mutated *LEMD3* gene is the disease-causing gene.

Bone morphogenetic protein and trans-

LEMD3 = LEM domain containing 3 (the LEM motif, a sequence of 40–50 amino acids, composed of two alpha helices, has been identified in a number of non-related proteins of the inner nuclear membrane. It is called the LEM domain after LAP2, Emerin and *Man1*.)
MAN1 = inner nuclear membrane protein *Man1* (LEM domain containing protein 3)
BOS = Buschke-Ollendorff syndrome

WIF1 = WNT inhibitory factor 1
Wnt = Wnt signaling pathway activation is important for induction of gene expression and cell morphogenesis throughout embryonic development

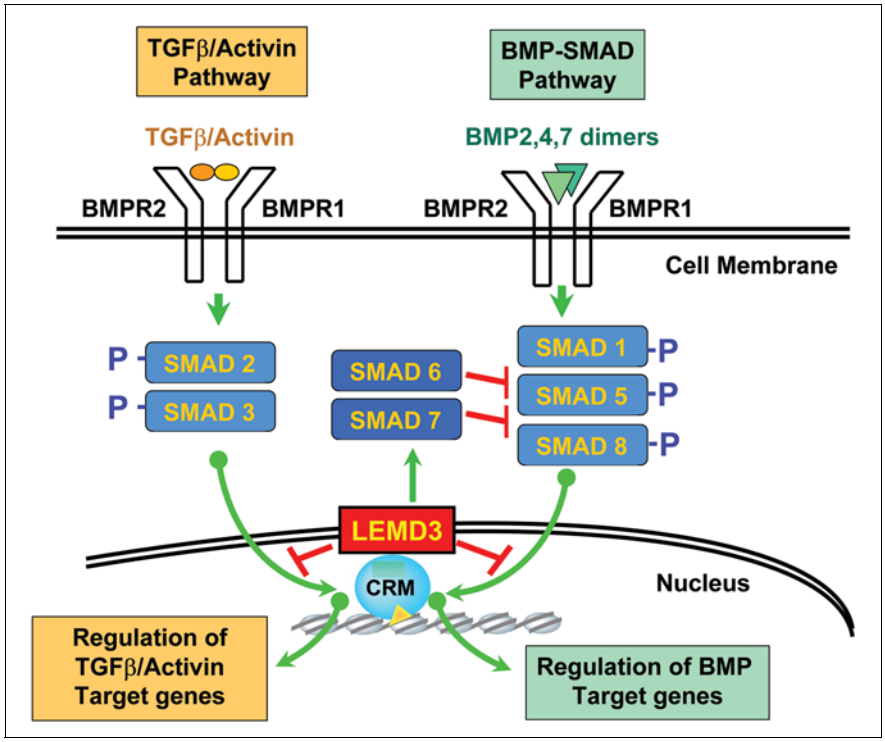

Figure 2. Simplified scheme presenting the involvement of the *LEMD3* gene in the BMP and TGFβ signaling pathways. In the BMP-SMADs pathway, BMP2, -4, or -7 dimers bind to the receptor complex (BMPR1-BMPR2), leading to phosphorylation/activation of the appropriate SMADs (1, 5, 8). This phosphorylation enables the SMADs complex to enter the nucleus and to activate or repress target genes, depending on which nuclear co-factors are present. In a similar way the dimer TGFβ/activin phosphorylates/activates other SMADs (2, 3) that enter the nucleus and affect the regulation of the corresponding target genes. This regulation may involve the chromatin-remodeling-machine (CRM) via DNA binding factors (yellow triangle). LEMD3 was shown to down-regulate the SMAD activation of both pathways and thus regulate bone synthesis. When *LEMD3* is mutated it cannot down-regulate the SMAD activation and hence excess of bone is produced. Based on von Bubnoff A, Cho KW, 2001 [6].

forming growth factor-beta signaling pathways play a key role in vertebrate embryonic development. Using yeast two-hybrid analysis, the authors showed that LEMD3, a nuclear membrane protein, interacts with the SMAD downstream elements of the BMP receptor TGFβ receptor pathways [Figure 2] [6]. Over-expression experiments in two cell lines have confirmed that LEMD3 can antagonize both BMP and TGFβ signaling, as measured by quantitative polymerase chain reaction and luciferase reporter assays. More specifically, the authors showed that it is the C-terminus of the LEMD3 protein that interacts with the SMADs, and the mutations either affect these interactions directly or result in a truncated protein that lacks the C-terminal domain. These findings, recently confirmed by Feng et al. [7], provide novel insights into the functional effects of BMP-signaling on bone development and may be relevant for therapeutic approaches for other, more frequent bone diseases like osteoporosis.

SMAD proteins are the homologs of the Drosophila MAD protein (SMAD is similar to MAD), which is activated by the decapentaplegic pathway and regulates the dorsal ventral axis in the embryonal development [8]. In vertebrates, an analogous pathway appears to have acquired a new role, i.e., regulation of bone formation. Interestingly, in vertebrates, SMADs interact also with Runx2, another key transcription factor, that regulates osteoblast and chondrocyte differentiation in both skeletal development and postnatal bone formation [9].

LEM-domain proteins, the family that includes LEMD3, are known to interact with barrier-to-autointegration factor. BAF is a member of the chromatin-remodeling complexes, ATP-utilizing enzymes that disrupt histone-DNA contacts [10]. In order to induce the transcription of genes that are involved in bone formation, chromatin has to be remodeled in order to ensure accessibility of transcription factors such as SMADs to their promoters. It is thus possible that the inhibitory action of LEMD3 on SMAD-related transcription is mediated by a chromatin-remodeling mechanism.

BMP = bone morphogenetic protein
TGFβ = transforming growth factor-beta

BAF = barrier-to-autointegration factor

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For more information on this gene, search for LEMD3 in http://GeneCards.Weizmann.ac.il