Ossification of the posterior longitudinal ligament: genetics and pathophysiology

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Ossification of the posterior longitudinal ligament (OPLL) is a disease of progressive ectopic calcification of
the PLL of the spine. It occurs most frequently in the cervical spine, followed by the thoracic spine. The disease
was first described in the Japanese population, and the prevalence of OPLL is highest in Japan at a rate of 1.9%–4.3%.
Note, however, that OPLL is also seen and is a known cause of cervical myelopathy in other Asian countries and
in the white population. Research into the underlying cause of OPLL over the past few decades has shown that it is
a multifactorial disease with significant genetic involvement. Genetic studies of OPLL have revealed several gene
loci that may be involved in the pathogenesis of this disease. Genes encoding for proteins that process extracellular
inorganic phosphate, collagen fibrils, and transcription factors involved in osteoblast and chondrocyte development
and differentiation have all been implicated in the pathophysiology of OPLL. In this paper, the authors review current
understanding of the genetics and pathophysiology of OPLL. (DOI: 10.3171/2010.12.FOCUS10271)

KEY WORDS • ossification of the posterior longitudinal ligament • enthesopathy • cervical myelopathy • diffuse idiopathic skeletal hyperostosis

Abbreviations used in this paper: BMP-2 = bone morphogenic protein–2; NPPS = nucleotide pyrophosphatase; OPLL = ossification of the posterior longitudinal ligament; PPi = inorganic pyrophosphate; SNP = single-nucleotide polymorphism; TGFβ = transforming growth factor–β; ttw = tip-toe walking.

OSSIFICATION of the posterior longitudinal ligament is a disorder of progressive ectopic calcification of
the cervical and thoracic segments of the PLL that results in a compressive myelopathy and/or radiculopathy.4 Belonging to the same pathological entity as diffuse idiopathic skeletal hyperostosis,18 OPLL was first described in detail by Tsukimoto and colleagues in 1960 and has a reported prevalence of 1.9%–4.3% in the Japanese population.13,12 Despite a long-standing predominance in Japan, this disease has also been recognized in other geographic regions and ethnicities, although its prevalence in the US and Europe is only 0.01%–1.7%.12 In both males and females, the average age at onset is approximately 50 years, and the reported male/female ratio is roughly 2:1. Ossification of the PLL is classically observed and categorized into 4 separate subtypes: segmental; continuous; mixed; and localized, circumscribed, or bridged, with segmental being the most common subtype.14 A diagnosis is typically made using
either plain film radiographs or CTs, on which calcification of the PLL is noted in the appropriate clinical setting.

Early clinical and epidemiological studies conducted in Japan have suggested that the underlying cause of
OPLL is multifactorial in nature, reflecting the interplay of numerous genetic and environmental factors.21,25 Over
the past several decades, however, a variety of genetic investigations, including pedigree studies, twin studies,
and detailed molecular analyses, have documented many genes or gene loci of interest involved in mediating
the molecular and genetic pathobiology of OPLL.2,5–7,9–11,13,15,17,22,23 Although a host of gene products has been
implicated as the root cause, several genes and proteins have emerged over the years as promising targets for fu-
ture investigation and intervention: NPPS, COL11A2 and COL6A1, and BMP-2 and TGFβ (Table 1). As OPLL is
believed to arise because of endochondral bone formation,7 each of the aforementioned genetic targets have
been shown to critically regulate a crucial step in chondrogenesis, osteogenesis, or bone mineralization.
Current treatment strategies for patients with myelopathic or radiculopathic symptoms due to OPLL are multifold and often dictated by the subtype of OPLL present, the extent of disease, and the duration of symptoms. Common surgical interventions include decompressive laminoplasty or laminectomy via a posterior approach or direct resection of the OPLL segment with spinal fusion through an anterior approach. However, as neurological surgeons obtain a deeper understanding of the genetic and molecular pathogenesis of OPLL, therapies targeted at preventing the initial formation and progression of OPLL may be pursued further. In the present report, we discuss the role of NPPS in the pathogenesis of OPLL and debilitating disease entity.

Genetics and Pathophysiology

NPPS

The ttkw, or tip-toe walking, mouse harbors a natural recessive mutation that results in progressive, ectopic spinal ligament calcification and ossification strikingly similar to that in human OPLL as well as in the ossification of other cartilaginous structures and peripheral ligaments. Naturally occurring mutants, the ttkw mice show spontaneous OPLL, which results in a myelopathic syndrome with an insidious motor disability as the most common manifestation of the disease. In 1998 Okawa et al. performed genetic analyses on ttkw mice and discovered a single-base mutation in the NPPS gene, which results in a shortened gene product with the loss of more than one-third of the native NPPS enzyme. Physiologically, NPPS is a Type II transmembrane metalloenzyme and regulates soft-tissue calcification and bone mineralization via the production of PPI, a known inhibitor of calcification. In humans, at least 3 isoforms of NPPS exist and are known to be involved in bone mineralization, ligament and joint capsule calcification, and cell motility. The NPPS subtype implicated in OPLL pathogenesis, NPP1, is the main enzyme that generates PPI in osteoblasts and chondrocytes and regulates bone mineralization by decreasing hydroxypatite crystal deposition. For reasons that have yet to be entirely elucidated, when extracellular PPI levels are low, pathological calcification of ligaments and joints occur.

To investigate the association between NPP1 and OPLL, Koshizuka et al. screened the human NPPS locus for SNPs in a case-control association study involving 180 patients with OPLL and 265 without. Their study demonstrated that a T→C substitution in intron 15 at position 14, upstream from the start of exon 16, was significantly associated with OPLL susceptibility and severity. However, a base-pair deletion in intron 20 at position 10, upstream from the start of exon 21, did not display a significant difference in allelic distribution between patients with OPLL and controls. In a prior case-control study in 323 patients with OPLL and 332 without, Nakamura et al. found a T deletion at 11 nucleotides upstream from the splice acceptor site of intron 20 that was significantly associated with OPLL susceptibility, suggesting again that NPP1 may be important in OPLL pathophysiology. Tábara et al. performed a similar case-control association study and likewise noted an association between certain SNPs in the NPPS gene and OPLL. Moreover, Tábara and colleagues discovered that other SNPs were strongly associated with disease severity when comparing patients with cervical OPLL versus those with both cervical and thoracic disease. Despite the important associations between these NPP1 sequence variants and OPLL disease susceptibility, these genetic studies did not examine NPP1 activity within the study participants. As a result, whether the expression and function of NPP1 is altered in these OPLL cohorts remains unknown. In 2001 Rutsch et al. did describe the case of a 2-year-old boy with a documented deficiency of NPP1 and a syndrome termed “idiopathic infantile arterial calcification,” a disease in which patients experience early, pathological arterial and articu- lar calcification. Ossification of the PLL was not noted in the boy, however. On the whole, while the phenotype of the ttkw mouse and the numerous case-control studies demonstrating an association between certain SNPs in NPP1 and OPLL susceptibility suggest a role for NPPS in the pathogenesis of OPLL, further molecular and functional studies are necessary to show a causative link.

### Table 1: Summary of Notable Genes, Transcription Factors, and Cytokines Implicated in OPLL Pathogenesis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Chromosome</th>
<th>Physiological Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPPS</td>
<td>nucleotide pyrophosphatase</td>
<td>6</td>
<td>regulates soft-tissue calcification and bone mineralization via the production of PPI, a known inhibitor of calcification</td>
</tr>
<tr>
<td>COL11A2</td>
<td>α2 chain, Type XI collagen</td>
<td>6</td>
<td>associates w/ Type II collagen &amp; is responsible for forming the size, diameter, &amp; growth rate of fibril networks; forms extracellular matrix scaffolds &amp; may contribute to the formation of ectopic bone by enhancing endochondral ossification</td>
</tr>
<tr>
<td>COL6A1</td>
<td>α1 chain, Type VI collagen</td>
<td>21</td>
<td>forms extracellular matrix scaffolds &amp; may contribute to the formation of ectopic bone by enhancing endochondral ossification</td>
</tr>
<tr>
<td>BMP-2</td>
<td>BMP-2</td>
<td>20</td>
<td>stimulates proteoglycan formation &amp; alkaline phosphatase activity in chondroblasts &amp; osteoblasts &amp; directs cellular differentiation</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>TGFβ1</td>
<td>19</td>
<td>mediates bone development &amp; metabolism</td>
</tr>
</tbody>
</table>

For reasons that have yet to be entirely elucidated, when extracellular PPI levels are low, pathological calcification of ligaments and joints occur.

**Table 1: Summary of Notable Genes, Transcription Factors, and Cytokines Implicated in OPLL Pathogenesis**
formed genetic linkage analyses on 90 sibling pairs from 53 families with a history of OPLL and deduced that genetic abnormalities in the COL11A2 gene were significantly associated with a predisposition to OPLL. Further gene mapping studies by this group demonstrated that the observed genetic mutations resulted in an aberrant N-propeptide of the α2 chain of Type XI collagen, and thus affecting the size and conformation of the resulting fibril structures. Physiologically, the N-propeptide of the α2 chain is purported to alter the conformation and shape of Type II collagen, which is responsible for bone and cartilage formation, and to mediate the interaction between extracellular matrix proteins and cell surface molecules. Moreover, Maeda et al.11 studied 195 patients with OPLL and 187 without and discovered an SNP on intron 6 of the COL11A2 gene that results in altered splicing in the region containing exons 6 through 8 with preservation of exon 7, which seems to confer a protective advantage in the development of OPLL. On the other hand, Koga et al.7 identified SNP variants of the COL11A2 promoter and intron 6 that were positively associated with OPLL susceptibility in their group of 124 sibling pairs from 53 families. Although the specific role of Type XI collagen in the pathogenesis of OPLL remains undefined, its role in the formation of extracellular matrix scaffolds may contribute to the formation of ectopic bone by enhancing endochondral ossification. Further, while the aforementioned studies do not provide a mechanistic link or explanation for Type XI collagen’s function in OPLL, the positive and negative association of various SNP variants with disease susceptibility supports the theory that COL11A2 is in fact important in the disease pathogenesis. In addition to COL11A2, COL6A1 is a gene that encodes the α1 chain of Type VI collagen and has also been implicated in OPLL susceptibility. Tanaka et al.21 performed a genome-wide study on 142 affected sibling pairs and discovered a high level of evidence of linkage on chromosome 21, localizing to the COL6A1 gene. Ultimately, future genetic, molecular, and functional studies focusing on the role of Types VI and XI collagen in OPLL may yield novel therapeutics for this progressive disease process.

**Bone Morphogenic Protein–2 and TGFβ**

Bone morphogenic proteins are multifunctional transcription factors that are members of the TGFβ superfamily of proteins and are involved in regulating cartilage and bone formation. During development, BMPs stimulate proteoglycan formation and alkaline phosphatase activity in chondroblasts and osteoblasts and direct cellular differentiation. In vitro studies indicate that BMP-2 is capable of augmenting osteoblast maturation and inducing mesenchymal cells to differentiate into osteoblasts. Moreover, BMP-2 has been shown to induce ectopic bone formation in rats. In 1997 Kon et al. examined the effect of recombinant BMP-2 in primary cultures of fibroblast cells derived from OPLL tissue and non-OPLL ligamentous cells. The authors demonstrated that the BMP-2 was capable of preferentially inducing alkaline phosphatase activity in OPLL cell lines as opposed to non-OPLL cell lines, indicating that BMP-2 is critically important in regulating osteogenic differentiation and function. Moreover, Yonemori et al.28 discovered that BMP receptors are expressed to a significantly greater degree in the ossified ligament of OPLL specimens than in non-OPLL tissue. In addition, the nonossified segment of the PLL in patients with OPLL also expressed the BMP receptors to an extent greater than non-OPLL tissue but less than ossified OPLL segments. This finding suggests either that observable molecular changes may precede the development of OPLL or that patients with OPLL have a genetic and molecular susceptibility to OPLL via the expression of the BMP receptors. Although BMP-2 and its respective receptors have not been the subject of such rigorous genetic studies as NPPS, COL11A2, and COL6A1, detailed molecular biological and functional studies have confirmed its role in the pathogenesis of OPLL and thus rendered it a promising therapeutic target.

Transforming growth factor–β, specifically TGFβ1, is an important mediator of bone development and metabolism and has been found in chondrocytes of the ossified cartilage in OPLL specimens. Transforming growth factor–β1 has been the subject of both genetic and molecular biological studies, and recent findings have highlighted its importance in the pathogenesis of OPLL. In a series of 46 patients with OPLL and 273 without, Kamiya et al.5 identified the association between a T869→C polymorphism in the TGFβ1 gene and susceptibility to OPLL in the Japanese population. Kawaguchi et al.6 further examined this SNP to determine if there was an association with the radiological appearance of OPLL. Ultimately, the group found that the T→C transition in the signal sequence did not predict a difference in the radiographic appearance of the ossified segment of PLL, but the difference in distribution of the SNPs was associated with the location of OPLL within the spinal column. While the physiological function of TGFβ1 hints at a potential role in the pathobiology of OPLL, additional genetic and functional studies are necessary to provide a true mechanistic link.

Over the past several decades, a host of genes, cytokines, and growth factors have emerged as potential factors underlying the molecular pathobiology of OPLL. While several studies have demonstrated an association between certain gene mutations and OPLL susceptibility and others have pointed to a functional role in OPLL pathogenesis, future detailed molecular and functional studies of these gene products are necessary to verify their appropriateness for therapeutic intervention.

**Conclusions**

Ossification of the PLL is a progressive and debilitating disease of spinal cord compression. While the underlying genetics and molecular pathobiology of the disease are currently under study, several genes, transcription factors, and cytokines have emerged as potential molecular causes and therapeutic targets. Although an array of factors have been studied and implicated over the years, NPP1, Types VI and XI collagen, BMP-2, and TGFβ have been identified by several groups and probably represent key proteins in the molecular pathogenesis of OPLL. As further discoveries are made in the field of molecular biology, targeted therapies can be implemented in the neu-
rosurgical arena to complement and augment the current anterior and posterior surgical approaches to the management of this insidious disease process.

**Disclosure**

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: all authors. Acquisition of data: Stapleton. Drafting the article: Hsieh, Stapleton. Pham. Critically revising the article: all authors. Reviewed final version of the manuscript and approved it for submission: Hsieh. Administrative/technical/material support: Attinello, Pham. Study supervision: Hsieh.

**References**


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