Review Article

Association between bone morphogenetic protein and ossification of posterior longitudinal ligament: a meta-analysis

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Received August 16, 2016; Accepted October 19, 2016; Epub January 15, 2017; Published January 30, 2017

Abstract: In the present study, we aimed to analyze the association between bone morphogenetic protein (BMP) polymorphisms and ossification of the posterior longitudinal ligament (OPLL). Relevant studies were identified by the search of PubMed, Embase, and Cochrane Library up to December 2015. The association between BMP and OPLL was assessed by pooled odds ratios (ORs) together with 95% confidence intervals (CIs). Heterogeneity was evaluated by the Chi-square test based on Q statistic and $I^2$ statistics. Results showed that 8 articles published between 2001 and 2014 were eventually identified. Meta-analysis showed that BMP2-rs2273073 (SNP location: Ser37Ala, SNP ID: rs2273073) was associated with OPLL. Specifically, G vs. T genotype (OR = 5.87, 95% CI: 4.15-8.30, $P<0.05$), GG vs. TT genotype (OR = 7.10, 95% CI: 4.95-10.19, $P<0.05$), and GG + GT vs. TT genotype (OR = 7.10, 95% CI: 4.95-10.19, $P<0.05$) were significantly associated with OPLL. In conclusion, BMP-2 is the predisposing gene of OPLL. The “TG” genotype in the BMP2-rs2273073 (SNP location: Ser37Ala, SNP ID: rs2273073) polymorphisms are associated with the occurrence of OPLL.

Keywords: Meta-analysis, bone morphogenetic protein, OPLL

Introduction

As a pathologic condition, Ossification of the posterior longitudinal ligament (OPLL) mainly affects cervical and thoracic spine [1]. Due to the chronic pressure on nerve roots and spinal cord, OPLL can lead to myelaradiculopathy [2]. OPLL is more common in East Asian. The incidence of OPLL in Japanese is 1.9%-4.3% for the general population over 30 years old [3]. In addition, the prevalence is 0.44%-8.92% with a mean prevalence of 3.08% in China [4], which is significantly higher than that in American and Europe (0.01%-1.7%) [5]. The specific feature of OPLL is heterotopic ossification of the spinal ligament, causing various degrees of myelopathy. Thus, it is important to find the accurate mechanisms of the OPLL development.

The development and progress of OPLL are associated with multiple genetic and environmental components [6], including transforming growth factor-β (TGF-β) [7], bone morphogenetic protein-2 (BMP2) [8], and bone morphogenetic protein-4 (BMP4) [9]. Among these factors, BMP has been reported to induce ectopic ossification after implantation subcutaneously [10]. A previous study on immunohistochemistry demonstrated that BMP was distributed in mesenchymal cells and periosteal cells of marrow stroma in healthy bone as well as mesenchymal cells and chondrocytes of the fracture site. Some studies [8, 11, 12] showed that TGF-β1, BMP-2 and BMP-4 were highly associated with OPLL. However, there are controversies about the association between BMP polymorphisms and OPLL. Kim et al indicated that BMP-2 might not directly influence the expression of OPLL [13]. Therefore, the controversial issue remains to be investigated.

Thus, in the current study, we performed a meta-analysis of eligible studies to better elucidate the association between BMP polymor-
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Materials and methods

Literature search

Several databases including PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Embase (http://www.embase.com), and Cochrane Library (http://www.update-software.com/Cochrane/default.htm) were retrieved up to December 2015. The search terms were as follows: bone morphogenetic protein or BMP-2 or BMP-4 or BMP-9 or TGF-β1 or TGF-β1; Ossification of Posterior Longitudinal Ligament or ossification of cervical posterior longitudinal ligament or cervical ossified posterior longitudinal ligament or OPLL.

Study selection

Studies were included if they met the following criteria: i) case-control study; ii) the case group was patient with OPLL and the control group was healthy people; iii) studies on correlations between OPLL and TGF-β, OPLL and BMP-2, as well as OPLL and BMP-4, which were written in English; (iv) the number of cases, controls and each gene could be obtained; (v) the cases group does not merge with other diseases including diabetes. Studies were excluded if they were review literatures, reports, comments or letters.

Data extraction

With the standard protocol, two investigators independently extracted the following data from the included studies: the first author, publication year, study time and region, diagnostic methods of OPLL, the number of cases and controls, and the number of distribution of each genotype. Literature assess was performed according to the Newcastle Ottawa scale (NOS) recommended by Agency for healthcare research and quality (AHRQ) [14]. Any disagreements were resolved by discussion between them or settled by a third reviewer.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) [15] in the controls was tested by the chi-square test. Gene model was analyzed using codominant model, dominant model, recessive model and allele model. Meta-analysis was carried out using R 3.12 software. The odds ratio (OR) and its 95% confidence interval (CI) were calculated for effect index. Heterogeneity test was evaluated by Chi-square based on Q statistic [16] and I² statistics [17]. The random effects model was used to combine the data for the heterogeneous outcomes (P<0.05 or I² ≥50%); otherwise, the fixed effects model was used [18]. Publication bias was evaluated through funnel plot visual analysis with the Egger’s tests [19, 20]. A P value <0.05 was considered statistically significant.

Results

Characteristics of included studies

The process of study selection was shown in Figure 1. Initially, totally 53 potentially relevant articles were retrieved from the databases (PubMed: 41; Embase: 11; Cochrane Library: 1). Then, 46 articles were left after eliminating the duplicate publication, and 28 of them were...
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### Table 1. Characteristics of included studies in the meta-analysis

<table>
<thead>
<tr>
<th>First Author</th>
<th>Public Year</th>
<th>Study Location</th>
<th>Study Year</th>
<th>The diagnosis of OPLL</th>
<th>Sample size</th>
<th>Study Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jia-Mou, Li</td>
<td>2014</td>
<td>China</td>
<td>2013.1-2013.2</td>
<td>Radiological findings including radiographs, CT, MRI of the cervical spine according to the criteria reported by Tsuyama.</td>
<td>36 18 18</td>
<td>Case-control study</td>
</tr>
<tr>
<td>Wang H</td>
<td>2008</td>
<td>China</td>
<td>2005.5-2007.1</td>
<td>Radiological findings including radiographs, CT, MRI of the cervical spine according to the criteria reported by Tsuyama.</td>
<td>192 57 135</td>
<td>Case-control study</td>
</tr>
<tr>
<td>Han IB</td>
<td>2013</td>
<td>South Korea</td>
<td>2008-2010</td>
<td>Radiographic criteria based on CT of the cervical spine.</td>
<td>298 98 200</td>
<td>Case-control study</td>
</tr>
<tr>
<td>Kamiya M</td>
<td>2001</td>
<td>Japan</td>
<td>NA</td>
<td>Radiograph films of the cervical spine by Tsuyama.</td>
<td>319 46 273</td>
<td>Case-control study</td>
</tr>
<tr>
<td>Liang</td>
<td>2013</td>
<td>China</td>
<td>NA</td>
<td>Radiologic findings including radiographs, CT, and MRI of the cervical spine according to the criteria reported by Tsuyama.</td>
<td>926 420 506</td>
<td>Case-control study</td>
</tr>
<tr>
<td>Kawaguchi Y</td>
<td>2003</td>
<td>Japan</td>
<td>NA</td>
<td>Radiologic findings including radiographs of the cervical, thoracic, and lumbar spine;tomogram; CT; MRI.</td>
<td>593 369 224</td>
<td>Case-control study</td>
</tr>
<tr>
<td>Ren Y</td>
<td>2012</td>
<td>China</td>
<td>NA</td>
<td>Based on imaging findings, which included radiographs, CT scans, MRI of the cervical spine, in accordance with the criteria reported by Tsuyama.</td>
<td>1000 450 550</td>
<td>Case-control study</td>
</tr>
<tr>
<td>Meng X-l</td>
<td>2010</td>
<td>China</td>
<td>2006.1-2008.3</td>
<td>Plain radiographs, CT and MRI of the cervical spine.</td>
<td>477 179 298</td>
<td>Case-control study</td>
</tr>
</tbody>
</table>

CT: computed tomogram; MRI: magnetic resonance imaging; NA: not available.
Table 3. The results of meta-analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Sample size</th>
<th>Test of association</th>
<th>Model</th>
<th>Test of heterogeneitya, b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Control</td>
<td>OR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>TGF-β1</td>
<td>A vs. C</td>
<td>1020</td>
<td>12963 [0.8716; 1.9281]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>AC vs. CC</td>
<td>355</td>
<td>1.7665 [0.7305; 4.2669]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>AA vs. CC</td>
<td>255</td>
<td>1.4531 [0.8644; 2.4426]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>AA vs. CC + AC</td>
<td>510</td>
<td>1.3631 [0.7302; 2.5447]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>AA + AC vs. CC</td>
<td>510</td>
<td>1.2112 [0.7027; 2.0879]</td>
<td>Random</td>
</tr>
<tr>
<td>BMP4</td>
<td>T vs. C</td>
<td>1258</td>
<td>0.6449 [0.1939; 2.1452]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>TT vs. CC</td>
<td>451</td>
<td>0.6555 [0.3036; 1.4154]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>TC vs. CC</td>
<td>305</td>
<td>0.9776 [0.5323; 1.7953]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>TT vs. CC + TC</td>
<td>629</td>
<td>0.8215 [0.3418; 1.9742]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>TT + TC vs. CC</td>
<td>629</td>
<td>0.7846 [0.4132; 1.4897]</td>
<td>Random</td>
</tr>
<tr>
<td>Arg190Ser</td>
<td>T vs. A</td>
<td>876</td>
<td>0.5956 [0.0500; 7.0901]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>TT vs. AA</td>
<td>322</td>
<td>1.0881 [0.8146; 1.4535]</td>
<td>Fix</td>
</tr>
<tr>
<td></td>
<td>TA vs. AA</td>
<td>201</td>
<td>1.5075 [0.6937; 3.2760]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>TT vs. AA + TA</td>
<td>438</td>
<td>0.9617 [0.1970; 4.6940]</td>
<td>Random</td>
</tr>
<tr>
<td></td>
<td>TT + TA vs. AA</td>
<td>438</td>
<td>0.7406 [0.2079; 2.6379]</td>
<td>Random</td>
</tr>
<tr>
<td>Ser37Ala</td>
<td>G vs. T</td>
<td>990</td>
<td>5.8452 [4.1357; 8.2614]</td>
<td>Fix</td>
</tr>
<tr>
<td></td>
<td>GG vs. TT</td>
<td>495</td>
<td>7.0758 [4.9324; 10.1505]</td>
<td>Fix</td>
</tr>
<tr>
<td></td>
<td>GT vs. TT</td>
<td>328</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>GG vs. TT + GT</td>
<td>495</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>GG + GT vs. TT</td>
<td>495</td>
<td>7.0758 [4.9324; 10.1505]</td>
<td>Fix</td>
</tr>
</tbody>
</table>

aRandom-effect model was used when the p-value for heterogeneity test <0.10, otherwise the fixed-effect model was used. bP-value <0.1 is considered statistically significant for Q statistics. OR: Odds ratio; CI: confidence interval.
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excluded after screening the title and abstracts. As a consequence, 18 articles were left and 10 (2 letter, 5 case series and overviews, 1 duplicated populations and 2 did not provide sufficient data) of them were excluded after screening the full text. Finally, 8 articles [9, 21-27] were included in this meta-analysis (Table 1). These included studies were published between 2001 and 2014. The study area was South Korea, Japan and Chinese. The diagnosis of OPLL mainly based on radiographs, computed tomogram (CT), magnetic resonance imaging (MRI) of the cervical spine according to the criteria reported by Tsuyama [28]. As shown in Table 2, a total of 3841 people including 1637 patients and 2204 healthy controls were collected. The BMP2 gene has two loci (rs2273073 and rs235768) and BMP4 genotype has one loci (rs17563). The results of HWE showed that p value was >0.05 for most of the studies except the study of Kamiya et al [23].

Merging quantitative data

Random-effect model was used when the P-value <0.10 for heterogeneity test, otherwise the fixed-effect model was used. The result of meta-analysis was shown in Table 3. There was no association between TGF-β1 and

Figure 2. Forest plot of associations between TGF-β1 and ossification of posterior longitudinal ligament (OPLL).

Figure 3. Forest plot of associations between MP4 and ossification of posterior longitudinal ligament (OPLL).
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**Study** | **Experimental Events Total** | **Control Events Total** | **Odds Ratio** | **OR** | **95%-CI** | **W(fixed)** | **W(random)**
--- | --- | --- | --- | --- | --- | ---
Ja-Mou, Li 2014 | 18 36 | 25 36 | 0.35 [0.13, 0.93] | 0.09 | 0.99 | 0.45 | 0.01
Wang H 2008 | 10 114 | 7 270 | 5.60 [2.34, 13.28] | 0.001 | 0.05 | 0.05 | 0.05
Liang 2013 | 140 440 | 135 490 | 3.75 [1.91, 7.47] | 0.001 | 0.05 | 0.05 | 0.05
Random effects model | | | | | | | |
Heterogeneity: I²=84.3%, Tau-squared=0.8437, p=0.004

**Study** | **Experimental Events Total** | **Control Events Total** | **Odds Ratio** | **OR** | **95%-CI** | **W(fixed)** | **W(random)**
--- | --- | --- | --- | --- | --- | --- | ---
Ja-Mou, Li 2014 | 10 75 | 7 73 | 0.57 [0.29, 1.14] | 0.24 | 0.24 | 0.24 | 0.24
Wang H 2008 | 19 157 | 9 135 | 1.77 [1.24, 2.56] | 0.01 | 0.01 | 0.01 | 0.01
Liang 2013 | 227 307 | 237 385 | 1.17 [1.21, 2.36] | 0.01 | 0.01 | 0.01 | 0.01
Random effects model | | | | | | | |
Heterogeneity: I²=74.2%, Tau-squared=0.735, p=0.003

**Figure 4.** Forest plot of associations between Ser37Ala and ossification of posterior longitudinal ligament (OPLL).

**Figure 5.** Forest plot of associations between Arg190Ser and ossification of posterior longitudinal ligament (OPLL).

**Discussion**

As a subset of “bone-forming” diseases, OPLL, first reported in 1839, was characterized by ectopic ossification in the spine ligaments. In the current study, a total of 8 articles published between 2001 and 2014 were included in this meta-analysis. The results showed that BMP2-rs2273073 (SNP location: Ser37Ala, SNP ID: rs22730-73) was associated with OPLL. Specifically, G vs. T genotype (OR = 5.87, 95% CI: 4.15-8.30, P<0.05), GG vs. TT genotype (OR = 7.10, 95% CI: 4.95-10.19, P<0.05), and GG + GT vs. TT genotype (OR = 7.10, 95% CI: 4.95-10.19, P<0.05) were significantly associated with OPLL.

BMP-2 is one of the members of TGF-β superfamily and acts as a potent stim-
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ulator of bone formation. It is reported that BMP-2 is an important regulator of bone metabolism [29]. A previous study using immunohistochemistry found that BMP-2 was expressed in the ossifying matrix and chondrocytes in cartilaginous areas next to the OPLL tissues [29]. However, BMP-2 was not found in the unossified posterior longitudinal ligament, which hinted that BMP might play essential roles in initiating the differentiation of mesenchymal progenitor cells in different stages of ectopic ossification development [29]. Moreover, expression of BMP2 and its receptor have been identified using cyclic stretch induce in OPLL cells in vitro [30, 31]. However, a previous study reported that there was no significant association between BMP-2 polymorphism and OPLL in the cervical spine [32]. But only 18 patients with OPLL were included in that study, and no single nucleotide polymorphisms of BMP-2 were analyzed. In the present meta-analysis including 3841 people (1637 patients and 2204 healthy controls), we found that BMP2-rs2273073 (SNP location: Ser37Ala, SNP ID: rs2273073) was associated with OPLL.

Many studies have shown polymorphisms of the BMP-2 gene [33, 34]. One of the polymorphism is a T vs. G transition at nucleotide 116 in exon 2 of BMP-2, causing substitution of Ser vs. Ala at 37 amino acid position. Another polymorphism is a same sense mutation with Ser87Ser vs. G. These two mutations are closely associated with Osteoporosis and Osteoarthritis [33, 34]. As a genetic disease related to abnormal calcium phosphate metabolism, OPLL occurs genetically associated with metabolic diseases. Previous studies have found that Ser87Ser (A vs. G) SNP is involved in bone mineral density and increased risk of osteoarthritis disease in women [34, 35]. Moreover, Ser37Ala (T vs. G) polymorphism is significantly related to osteoporosis [33]. Consistent with these reports, in our present study, we found that the “TG” genotype in the BMP2-rs2273073 (SNP location: Ser37Ala, SNP ID: rs2273073) polymorphisms were associated with the occurrence of OPLL.

Some limitations of this study should be addressed. First, only published studies were included and no correction for covariates was detected. Second, no further sub group analysis was performed. Third, although BMP4 and BMP2 genotypes have different SNP location, few relevant literatures were included in this study. Therefore, larger and well-designed studies about different SNP locations are warranted to validate our results.

In conclusion, the present results demonstrate that BMP-2 is the predisposing gene of OPLL. The “TG” genotype in the BMP2-rs2273073 (SNP location: Ser37Ala, SNP ID: rs2273073) polymorphisms are associated with the occurrence of OPLL.

Disclosure of conflict of interest

None.

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