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Review Article

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

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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 OPLLwas 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 myeloradiculopathy [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-

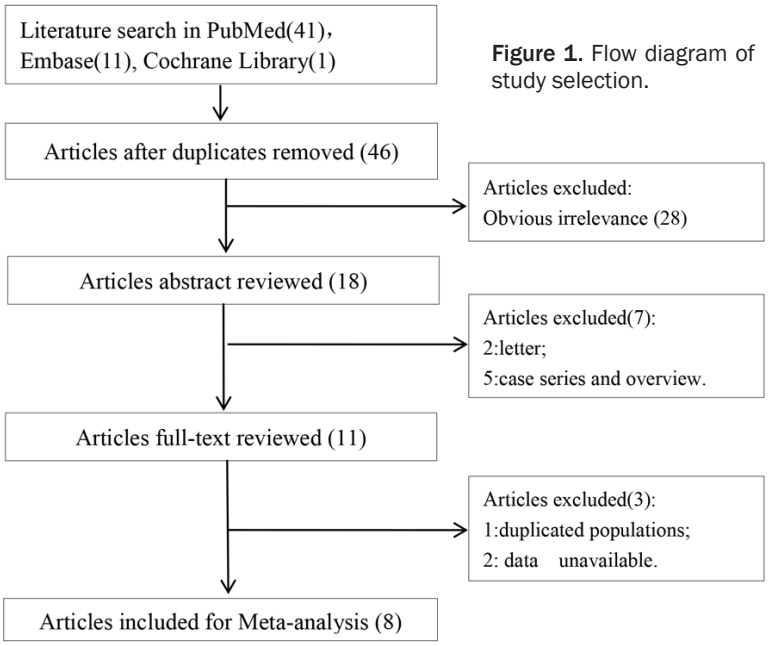

Figure 1. Flow diagram of study selection.

phisms and OPLL. Findings of this study may provide a theoretical basis for clinical treatment of OPLL in future.

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 I^2 statistics [17]. The random effects model was used to combine the data for the heterogeneous outcomes (P<0.05 or I^2 ≥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

Table 1. Characteristics of included studies in the meta-analysis

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

CT: computed tomogram; MRI: magnetic resonance imaging; NA: not available.

Table 2. Genotype frequencies in studies included in the meta-analysis

Study	Year of Publication	Gene	SNP location	SNP ID	Case genotype			Control genotype			HWE[a]	
					TT	TG	GG	TT	TG	GG	chi-square test	*P* value
Jia-Mou, Li	2014	BMP2	Ser37Ala	rs2273073	10	8	0	17	1	0	0.015	0.9035
		BMP2	Arg190Ser	rs235768	AA	AT	TT	AA	AT	TT	0.126	0.7227
					5	10	3	2	7	9		
Wang H	2008	BMP2	Ser37Ala	rs2273073	TT	TG	GG	TT	TG	GG	0.161	0.6887
					38	19	0	126	9	0		
Han IB	2013	TGF-β1	-	-	TT	TC	CC	TT	TC	CC	0.077	0.7816
					25	45	25	53	98	49		
Meng X-l	2010	BMP4	6007 C>T	rs17563	CC	CT	TT	CC	CT	TT	3.430	0.0640
					40	89	50	81	133	84		
Kamiya M	2001	TGF-β1	-	-	TT	TC	CC	TT	TC	CC	4.162	0.0413
					5	26	15	85	149	39		
Liang	2013	BMP2	Ser37Ala	rs2273073	TT	TG	GG	TT	TG	GG	0.612	0.4342
					280	140	0	472	34	0		
		BMP2	Arg190Ser	rs235768	AA	AT	TT	AA	AT	TT	1.856	0.1731
					80	227	113	148	237	121		
Kawaguchi Y	2003	TGF-β1	-	-	TT	TC	CC	TT	TC	CC	0.862	0.3531
					70	184	115	43	118	63		
Ren Y	2012	BMP4	6007 C>T	rs17563	CC	CT	TT	CC	CT	TT	0.969	0.3250
					87	235	128	62	230	258		

[a]HWE: Hardy-Weinberg equilibrium, it was evaluated using the goodness-of-fit chi-square test. P<0.05 was considered representative of a departure from HWE; SNP: Single Nucleotide Polymorphism.

Table 3. The results of meta-analysis

Gene		Gene Sample size		Test of association	Model	Test of heterogeneity[a,b]		
		Cases	Control	OR (95% CI)		*Q*	*P* value	I^2 (%)
TGF-β1	A vs. C	1020	1394	1.2963 [0.8716; 1.9281]	Random	8.33	0.0155	76
	AC vs. CC	355	546	1.7655 [0.7305; 4.2669]	Random	9.17	0.1108	54.5
	AA vs. CC	255	332	1.4531 [0.8644; 2.4426]	Random	5.75	0.0102	78.2
	AA vs. CC + AC	510	697	1.3631 [0.7302; 2.5447]	Random	6.32	0.0565	65.2
	AA + AC vs. CC	510	697	1.2112 [0.7027; 2.0879]	Random	4.40	0.0424	68.4
BMP4	T vs. C	1258	1696	0.6449 [0.1939; 2.1452]	Random	13.86	<0.0001	93.8
	TT vs.CC	451	506	0.6555 [0.3036; 1.4154]	Random	9.88	0.0409	76.1
	TC vs. CC	305	485	0.9776 [0.5323; 1.7953]	Random	0.1467	0.0002	92.8
	TT vs. CC + TC	629	848	0.8215 [0.3418; 1.9742]	Random	9.82	0.0017	89.9
	TT + TC vs. CC	629	848	0.7846 [0.4132; 1.4897]	Random	0.2009	0.0017	89.9
Arg190Ser	T vs. A	876	1048	0.5956 [0.0500; 7.0901]	Random	5.57	0.0092	85.3
	TT vs.AA	322	394	1.0881 [0.8146; 1.4535]	Fix	4.85	0.2507	24.2
	TA vs. AA	201	280	1.5075 [0.6937; 3.2760]	Random	0.1551	0.0183	82.0
	TT vs. AA + TA	438	524	0.9617 [0.1970; 4.6940]	Random	3.3	0.0277	79.4
	TT + TA vs. AA	438	524	0.7406 [0.2079; 2.6379]	Random	0.73	0.0006	69.2
Ser37Ala	G vs. T	990	1318	5.8452 [4.1357; 8.2614]	Fix	0.25	0.8826	0
	GG vs. TT	495	659	7.0758 [4.9324; 10.1505]	Fix	0.34	0.8427	0
	GT vs. TT	328	615	-	-	-	-	-
	GG vs. TT + GT	495	659	-	-	-	-	-
	GG + GT vs. TT	495	659	7.0758 [4.9324; 10.1505]	Fix	0.34	0.8427	0

[a]Random-effect model was used when the *p*-value for heterogeneity test <0.10, otherwise the fixed-effect model was used. [b]*P*-value <0.1 is considered statistically significant for Q statistics. OR: Odds ratio; CI: confidence interval.

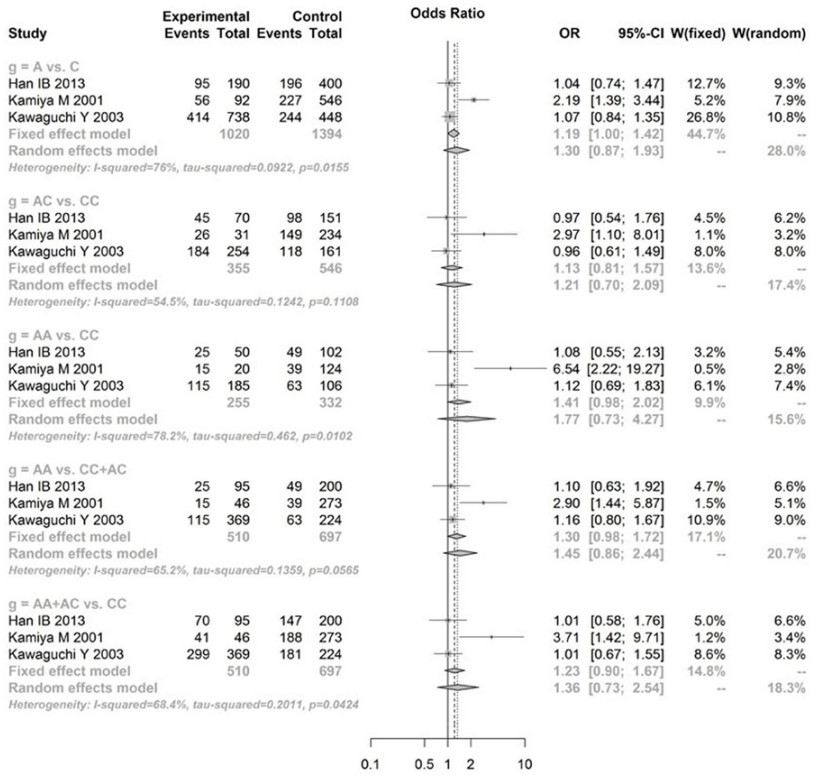

Figure 2. Forest plot of associations between TGF-β1 and ossification of posterior longitudinal ligament (OPLL).

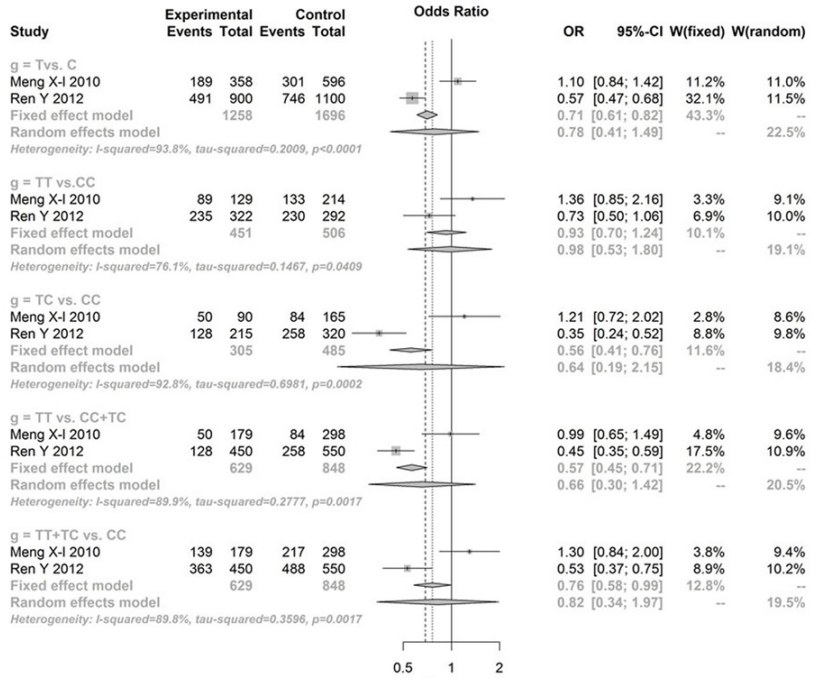

Figure 3. Forest plot of associations between MP4 and ossification of posterior longitudinal ligament (OPLL).

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 (rs227-3073 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

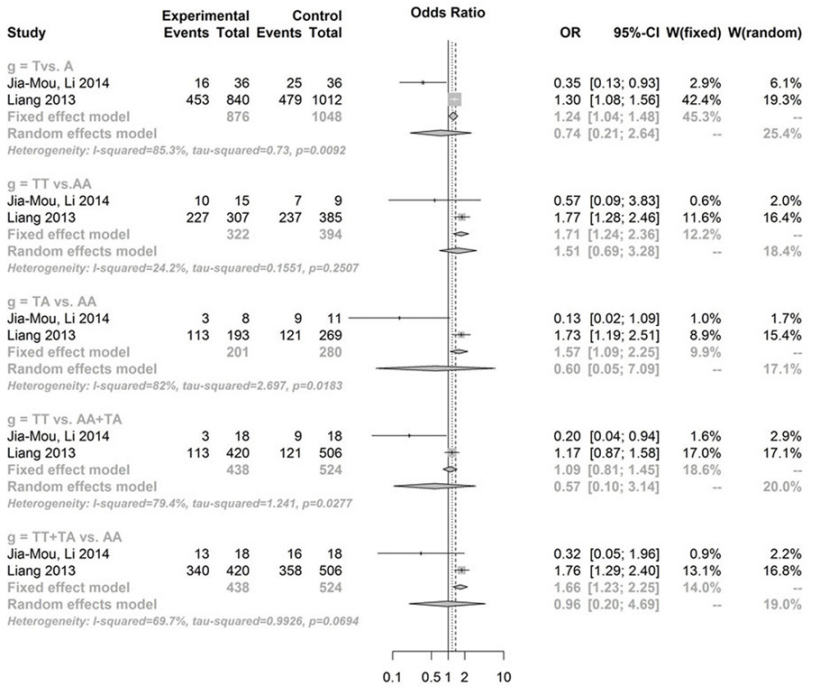

Figure 4. Forest plot of associations between Ser37Ala and ossification of posterior longitudinal ligament (OPLL).

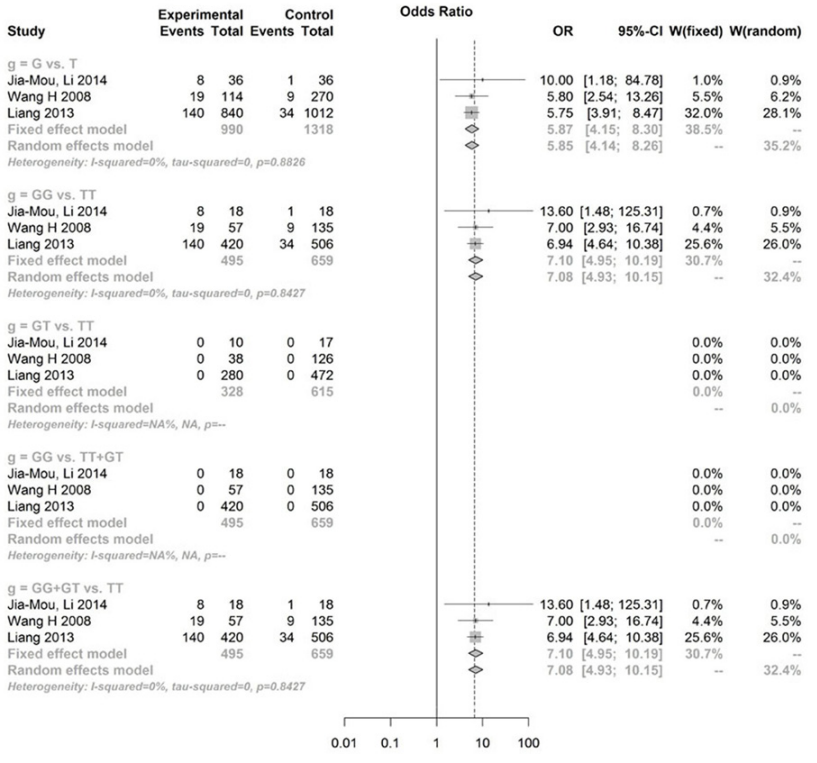

Figure 5. Forest plot of associations between Arg190Ser and ossification of posterior longitudinal ligament (OPLL).

OPLL (**Figure 2**), *BMP4* and OPLL (**Figure 3**), as well as BMP2-Arg190Ser and OPLL (**Figure 4**) at any mutational site. However, meta-analysis showed that BMP2-rs2273073 (SNP location: Ser37Ala, SNP ID: rs2273073) was associated with OPLL (**Figure 5**). 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.

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: 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.

BMP-2 is one of the members of TGF-β superfamily and acts as a potent stim-

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 Ser87 Ser A 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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