

Association of Bone Morphogenetic Protein Receptor IA (BMPIA) Gene Polymorphism with Ossification of Posterior Longitudinal Ligament (OPLL) of the Cervical Spine in a Chinese Han Population

Weitao Jin¹, Hui Yang¹, Chunli Zhao², Xin Lin^{2*} and Hao Wang²

¹Beijing Institute for Neuroscience, Beijing Center of Neural Regeneration and Repair, Key Laboratory for Neurodegenerative Disease of the Ministry of Education, Capital Medical University (CMU), Beijing, China

²Beijing Tiantan Hospital, Capital Medical University (CMU), Beijing, 100050 China

Abstract

Study design: A case-control study using radiograph findings and the PCR assay with regard to the susceptibility and the severity of ossification of posterior longitudinal ligament of the spine (OPLL).

Objective: To analyze whether the single nucleotide polymorphisms in the Bone Morphogenetic Protein Receptor IA (BMPIA) gene predisposed to increased the frequency and severity of OPLL.

Methods: Analysis of 292 OPLL patients and 586 non-OPLL controls was performed. Radiographs of the cervical spine were analyzed to determine whether OPLL was present and to what degree. Genomic DNA was extracted from all participants. Polymorphisms of the BMPRIA gene were analyzed using the PCR assay. The association of the polymorphisms with the development and extent of OPLL were statistically evaluated.

Results: Significant associations were found between two single nucleotide polymorphisms (rs34755052, rs11528010) and the existence of OPLL in BMPRIA genes. Both of rs34755052 (C/T) and rs11528010 (C/A) were more frequent in patients with OPLL. Regarding the rs11528010 (C/A), patient with A Allele had a significantly greater number of ossified cervical vertebrae compared with patients without the A allele, but rs34755052 didn't show the similar tendency.

Conclusion: The present results suggested that rs34755052(C/T) was associated with the occurrence of OPLL, but not associated with the severity of OPLL. rs11528010(C/A) was associated with more extensive OPLL and the frequency with which it occurred.

Keywords: Bone Morphogenetic Protein Receptor IA gene (BMPIA); Ossification of the posterior longitudinal (OPLL); Single Nucleotide Polymorphism (SNP); Cervical spine; Case-control study

Introduction

Ossification of the posterior longitudinal ligament (OPLL) is characterized by ectopic calcification of ligamentous tissues of the spine. The new calcificated clot often compressed the spinal cord and nerve roots which resulted in various degrees of myeloradiculopathy [1,2]. OPLL of the spine had been reported in Asian populations with a prevalence of 1.9%–4.3% in Japanese (aged >30 years) and 0.44%–8.92% in Chinese [3–7]. Although a lot of factors, such as high body mass index (BMI), high peripheral bone mineral density (BMD), dietary habits, aging and glucose intolerance, had been reported to have various extent relationships with OPLL, the etiology of OPLL was still not clear until now [8–12]. Genetic background was considered to be an important factor in the development of OPLL on the basis of pedigree surveys, family study of twins and analysis of human leukocyte antigen haplotypes [13–16]. And previous studies had shown that single nucleotide polymorphisms (SNPs) in the transforming growth factor- β , nucleotide pyrophosphatase (NPPS), leptin receptor, collagen 6A1 (COL6A1), bone morphogenetic protein 2 (BMP2) and bone morphogenetic protein 4 (BMP4) as well as estrogen receptor (ER) and interleukin-1 (IL-1) genes were associated with the development of OPLL [17–21]. However, only BMPs were able to induce ectopic ossification when these factors were injected into animal models [22]. Our group had previously demonstrated that some SNPs in BMP2 and BMP4 gene were associated with the occurrence and severity of OPLL in a Chinese population, although little evaluation of the clinical and demographic backgrounds of the participants was carried out [18,23]. In this study we focused on the role of Bone morphogenetic protein receptor IA (BMPIA) in the development of OPLL.

BMPIA was one subtype of the bone morphogenetic protein receptor family [24]. Previous studies had demonstrated that BMPIA was an important regulator in the signal transduction of BMP pathway [25,26]. Analysis of the osteogenic activities of the bone marrow stromal cells demonstrated that over expression of BMPIA was one of the most potent inducers of osteogenic differentiation *in vitro* [27] and in animal model [28]. Additional case-control studies of fibrodysplasia ossificans progressiva and adolescent idiopathic scoliosis also showed BMPIA to be a potent modulator of cartilage development *in vivo* and inhibition of BMP type I receptor activity might be useful in treating heterotopic ossification syndromes [29,30]. Interestingly, pathological studies found that bone morphogenetic protein receptors were highly expressed in ossified ligament tissues of patients with ossification of the posterior longitudinal ligament [31,32], which implied BMPIA might play an important role in the development of OPLL. Most recently, Liu et al. observed the variation in BMPRIA gene would result in over expression of BMPIA, which had been considered to be an indicator to cells' osteogenic potential [33]. Up to know no previous studies had assessed BMPIA variations. In the current study we hypothesized

*Corresponding author: Xin Lin, Beijing Tiantan Hospital, Capital Medical University (CMU), Beijing, 100050 China, E-mail: drlinxin@gmail.com

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that BMPRIA might be involved in OPLL, and we evaluated the overall polymorphisms of the BMPRIA gene in a large group of Chinese patients with OPLL.

Materials and Methods

Participants

A total of 292 cervical OPLL patients and 586 age- and sex-matched non-OPLL controls were investigated in this study. All the characteristics of the OPLL patients and controls were shown in table 1. This study was approved by the Ethical Committee of Beijing Tiantan Hospital, Capital Medical University. Informed consent was obtained from all the participants including in this study. The diagnosis of OPLL was established based on the sagittal section of computed tomography (CT) of the cervical spine according to the criteria reported by Tsuyama. The severity of OPLL was determined based on the number of ossified vertebrae on these CT films, and patients were also stratified according to the extent of ossification [34]. Based on the radiological and laboratory examinations, patients with ankylosing spondylitis and metabolic diseases, such as hypophosphate rickets, osteomalacia, osteoporosis, diffuse idiopathic skeletal hyperostosis (DISH) and hyperparathyroidism were excluded. Patients who have taken medicines such as estrogen, progesterone, glucocorticoids, bisphosphonates, alfacalcidol and calcitriol were also excluded from this study.

Blood samples were collected from all participants. The study protocol was approved by the ethical committee of Beijing Tiantan Hospital, Capital Medical University and written informed consent was obtained from all participants before the study.

Genomic DNA analysis

Peripheral blood samples were collected in tubes containing ethylene diamine tetraacetic acid (EDTA, 50 mmol/L of disodium salt), and stored at -20°C until use for genomic DNA extraction and genotyping. Genomic DNA was extracted from peripheral blood leukocytes using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The BMPRIA gene was amplified by the polymerase chain reaction (PCR) with a standard protocol [35] in a total of 5 overlapping fragments and sequenced. The reactions were performed. Different thermocycling parameters were used for different primer pairs. Sequencing reactions were performed using the

Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and the extension products were analyzed on an ABI 3730XL POP7 DNA sequencing analysis 5.2 system (Applied Biosystems). All described data about polymorphisms were discovered in our study, but before our experiments, all these rs-ID (rs 34755052, rs 113347633, rs 11528010, rs 35619497, rs 17231982, rs 6586039, rs 11202203, rs 10887666, rs 7922846, rs 2354354, rs 10788528, rs 19947684) have also been reported in GeneBank. SNPs with unknown heterozygosity and minor allele frequencies below 5% were excluded.

Statistical analysis

Hardy-Weinberg equilibrium and the genotypic and allelic distribution were evaluated using χ^2 tests. The characteristics of two groups were compared using Student unpaired *t* test and the genotype distribution in BMPRIA between two groups were compared by chi-squared tests. The nonparametric Mann-Whitney *U* test was used to compare the number of ossified cervical vertebrae between the two groups. Data were expressed as means \pm SD, and analyzed with SPSS 16.0 software (SPSS, Chicago, IL) A *p* value less than 0.05 was considered statistically significant.

Results

The clinical and demographic characteristics of the patients with OPLL and control were shown in table 1. There were no significant differences in age, gender, height, body weight, or personal history between the case and control subjects. The BMPRIA gene of all participants were sequenced and 12 SNPs (minor allele frequency in cases >0.05) were identified and genotyped (Table 2). Alleles and genotype frequencies for all SNPs were in Hardy-Weinberg equilibrium in both groups. Single marker test, which were based on an allergic model, revealed association between an increased risk of OPLL and the minor alleles of rs34755052 (C/T) (*p*<0.05) and rs11528010 (C/A) (*p*<0.05).

The Genotype distribution in BMPRIA between OPLL and control groups was compared by Pearson chi-squared test. The result showed significant associations between the occurrence of OPLL and rs34755052 (CC vs. CT vs. TT) (*p*<0.05) and rs11528010 (CC vs. CA vs. AA) (*p*<0.05) (Table 3).

To study the contribution of these polymorphisms to the severity or promotion of OPLL, a nonparametric test (Mann-Whitney exact

Indices	All		Male		Female	
	OPLL	Controls	OPLL	Controls	OPLL	Controls
Number	292	586	162	295	130	291
Age (years)	31-77 (55.2 \pm 9.9)	28-79 (54.8 \pm 7.6)	31-77 (56.9 \pm 10.2)	31-79 (55.7 \pm 5.7)	34-75 (52.7 \pm 9.0)	28-78 (51.6 \pm 7.2)
Weight (kg)	51-84 (59.7 \pm 6.4)	50-85 (59.1 \pm 8.0)	63-84 (60.1 \pm 9.4)	61-85 (61.3 \pm 6.3)	51-71 (57.7 \pm 9.6)	50-72 (56.9 \pm 7.8)
Height (cm)	150-178 (163.1 \pm 6.6)	152-180 (164.1 \pm 9.8)	165-178 (168.6 \pm 6.3)	167-180 (169.3 \pm 3.6)	150-170 (158.6 \pm 3.5)	152-169 (159.0 \pm 4.4)
Social History						
Smoking (yes)	118	256	102	234	16	22
Alcohol use (\geq once a week)	86	125	72	121	16	4
Sub-type of OPLL						
Continuous	104		56		48	
Mixed	52		28		24	
Segmental	116		62		54	
Localized	20		11		09	

Age, body weight and height are expressed as means \pm SD

Table 1: Clinical and demographic characteristics of the participants in the entire study.

No.	rs-ID	Location and nucleotide position	Allele	Amino acid substitution	MAF (OPLL)	MAF (Control)	$p(\chi^2)$
1	rs 34755052	5'UTR 88516595	C/T		0.45	0.32	0.012'
2	rs 113347633	5'UTR 88635969	C/T		0.22	0.23	0.826
3	rs 11528010	Exon 3 88635779	C/A	Pro2Thr	0.39	0.31	0.032'
4	rs 17231982	Intron 1 88557849	C/G		0.43	0.44	0.378
5	rs 6586039	Intron 1 88568565	A/G		0.49	0.48	0.419
6	rs 10788528	Intron 2 88616771	A/G		0.43	0.45	0.382
7	rs 11202221	Intron 2 88562314	A/G		0.36	0.34	0.647
8	rs 7922846	Intron 4 88641501	A/T		0.40	0.38	0.419
9	rs 10887666	Intron 6 88659047	C/T		0.28	0.26	0.704
10	rs 35619497	Exon 11 88681437	C/T	Arg443Cys	0.30	0.28	0.482
11	rs 55845713	Exon 12 88683185	G/C	Pro465Pro	0.46	0.44	0.419
12	rs 182970149	3'UTR 88683010	C/T		0.39	0.37	0.638

$p(\chi^2)$ calculated by chi-squared test (Continuity correction)
Rs-IDs and Location of the 12 SNPs in BMPRIA gene, as well as nucleotide position, amino acid substitution and p -values of allele in our cohort are indicated

Table 2: The 12 Identified single nucleotide polymorphism locus in BMPRIA gene.

SNP No.	rs-ID	Number (AA/AB/BB)		$p(\chi^2)$
		OPLL	Control	
1	rs 34755052(C/T)	58/146/88	70/234/282	0.008'
2	rs 113347633(C/T)	23/82/187	41/188/357	0.336
3	rs 11528010(C/A)	56/117/119	47/270/269	0.015'
4	rs 17231982(C/G)	53/146/93	100/316/170	0.478
5	rs 6586039(A/G)	58/169/65	105/352/170	0.519
6	rs 10788528(A/G)	50/152/90	105/293/188	0.282
7	rs 11202221(A/G)	35/140/117	64/270/252	0.337
8	rs 7922846(A/T)	35/140/117	94/258/234	0.289
9	rs 10887666 (C/T)	35/93/164	53/199/334	0.684
10	rs 35619497(C/T)	53/70/169	94/141/351	0.572
11	rs 55845713(G/C)	70/128/94	152/211/223	0.369
12	rs 182970149(C/T)	53/123/116	94/246/246	0.458
	total	292	586	

$p(\chi^2)$ calculated by Pearson chi-squared test
rs 34755052(C/T)(CC vs. CT vs. TT) and rs 11528010(C/A) (CC vs. CA vs. AA) show statistical significance ($p<0.05$)

Table 3: Analysis of genotype distribution in BMPRIA gene between OPLL and control groups.

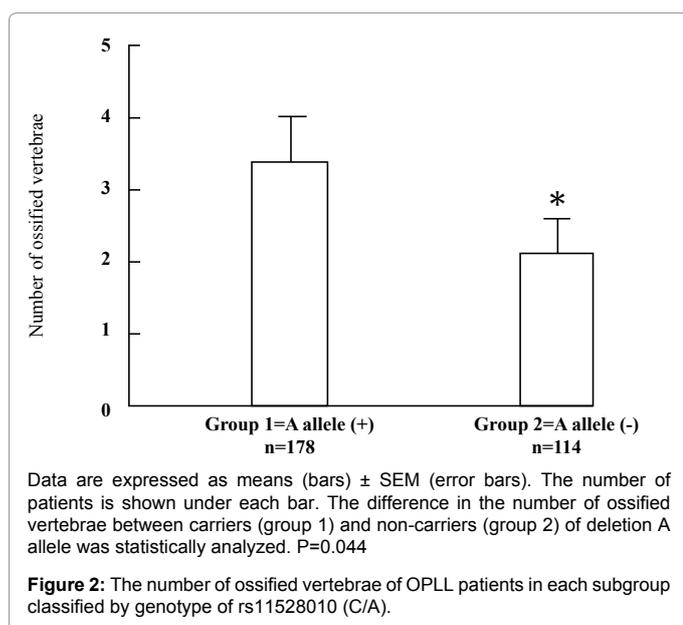
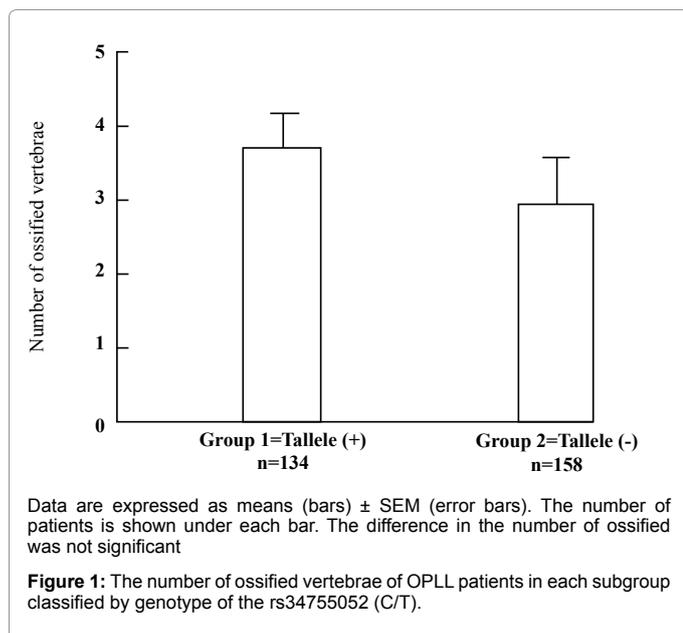
test) was employed to examine the association between the allele distribution of 12 SNPs and the number of ossified vertebrae in OPLL patient (Figures 1 and 2). There was no significant association between the extent of OPLL and rs34755052 (C/T) ($p=0.131$). But regarding the rs11528010 (C/A) polymorphism, patient with A Allele (genotype, AC or AA) had a significantly greater number of ossified cervical vertebrae compared with patients without the A allele (genotype, CC $p<0.05$). The present results indicate that the A allele promotes ectopic ossification in the cervical spine among patients with OPLL.

Discussion

OPLL was age related, characterized by ectopic ossification in the spine ligaments, and resulted in spinal myelopathy in Asian populations [1]. However, the etiology of this disease had not been clarified. Multiple genetic and environmental components contributed

to the development of OPLL. Previous studies indicated that genetic background may play an important role in OPLL pathogenesis.

BMPRIA was one subtype of the bone morphogenetic protein receptor family [24]. Previous studies had demonstrated that BMPRIA was one of the most potent inducers of osteogenic differentiation *in vitro* [27] and in animal model [28]. Previous case-control studies of fibrodysplasia ossificans progressiva and adolescent idiopathic scoliosis had proved that BMPRIA was a potent modulator of cartilage development in human being, inhibition of BMP type I receptor activity might be useful in treating heterotopic ossification syndromes [29,30] and SNPs changes of BMPRIA might be responsible to the onsets of these diseases. Interestingly, pathological studies found that, BMPRIA was also highly expressed in the ossified ligament tissues of patients with ossification of the posterior longitudinal ligament [31,32].



In this study we focused on the role of Bone morphogenetic protein receptor IA (BMPRIA) in the development of OPLL. To confirm that the BMPRIA locus is associated with the occurrence of OPLL, we sequenced the complete 12 kb BMPRIA genomic region and identified 12 SNPs (Table 2). We analyzed the association between these gene variations and the occurrence of OPLL and found that the alleles of rs34755052 (C/T) and rs11528010 (C/A) increased risk of OPLL. However, the mechanisms that underlined these associations remained unclear. Given that rs34755052 (C/T) was located in upstream of BMPRIA gene, the functional impact of this SNPs on genetic susceptibility to OPLL was still uncertain. Liu et al. [33] found that there were some transcription factor binding sites in the upstream of BMPRIA gene, which impacted on the expression of BMPRIA and resulted in calcification in the cells used to have a poor osteogenetic activity [33,36]. The gene locus of rs34755052 (C/T) was exactly located in one of the transcription factor binding site. We concluded

that the change of nucleotide in rs34755052 (C/T) may have effect on the binding of transcription factor with BMPRIA, through which the expression level of the BMPRIA gene changed, and thus resulted in the calcification of the posterior longitudinal ligament. On the other hand, rs11528010 (C/A), which located in the exon 3 of BMPRIA gene, would result in an amino acid substitution (Pro to Thr). This variation occur in the area where BMPs binding with BMPRIA and may change the structure of BMPRIA, which could change the binding force of BMPRIA with BMP [37]. However, previous study of rs11528010 (C/A) reported that this SNPs had an association with the susceptibility of glycometabolism. And hyperglycemia was one of the most important risk factor with the occurrence of OPLL. So alleles of rs11528010 (C/A) may not be causative itself but a marker for high risk factor related to OPLL.

Moreover, we analyzed the distribution of the number of ossified vertebrae in relation to alleles of rs34755052 (C/T) and rs11528010 (C/A) in OPLL patients. The alleles of rs34755052 (C/T) showed non-relationship with the severity of OPLL, but rs11528010 (C/A) increased the severity of OPLL significantly. As mentioned before, it is unknown as yet how this SNPs impact on the osteogenetic activity of the ligament cells. Additional studies were needed to reveal the exactly function of this SNPs in BMPRIA gene.

In conclusion, our study provided evidence to support an association between SNPs in BMPRIA and the occurrence of OPLL. The allele T of rs34755052(C/T) is associated with the severity of OPLL, but not associated with the severity of OPLL. However, the allele A of rs11528010(C/A) is not only associated with the occurrence of OPLL, but also associated with the severity of OPLL. The result of the current study may contribute to understand the underlying mechanism and genetic etiology of OPLL and lead to further biochemical or gene functional studies.

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