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# A single nucleotide polymorphism in the human bone morphogenetic protein-2 gene (109T>G) affects the Smad signaling pathway and the predisposition to ossification of the posterior longitudinal ligament of the spine

**Dingjun Hao, Baorong He, Liang Yan**

**Hong Hui Hospital, Xi'an Jiaotong University College of Medicine, Xi'an, Shaanxi 710054, China**



# ABSTRACT


## PURPOSE

Although various systemic and local factors such as abnormal carbohydrate or calcium metabolism, aging, and hormonal disturbances have been suggested as causes of ossification of the posterior longitudinal ligament (OPLL), the etiology of OPLL is not fully understood. The purpose of this study was to investigate whether bone morphogenetic protein (BMP)-2 is a candidate gene to modify the susceptibility of OPLL and the mechanism of signal transduction in ossification.



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## METHODS

A total of 420 OPLL patients and 506 age- and sex-matched controls were studied. The complete coding sequence of the human BMP-2 gene was analyzed using polymerase chain reaction (PCR) and direct sequencing. All single nucleotide polymorphisms (SNPs) were detected and genotyped. BMP-2 expression vectors containing positive polymorphisms were constructed and transfected into the C3H10T1/2 cells. The expression of BMP-2 and the Smad signal pathway in positive cell clones were detected by We stern blotting. The alkaline phosphatase (ALP) activity was determined using quantitative detection kits.

## RESULTS

The frequencies for the 109T>G and 570A>T polymorphisms were different between the case and control groups. The “TG” genotype in 109T>G polymorphism is associated with the occurrence of OPLL, the frequency of the “G” allele is significantly higher in patient s with OPLL than in control subjects (P <0.001). The “AT” genotype in 570A>T polymorphism is associated with the occurrence of OPLL, the frequency of the “T” allele is significantly higher in patients with OPLL than in control subjects (P=0.005). Western blotting analysis revealed that the expression of P-Smad1/5/8 protein transfected by wild-type or mutant expression vectors were significantly higher than control groups (P <0.05),

## RESULTS

but there was no statistical difference in each experimental group (P >0.05). The expression of Smad4 protein transfected by wild-type or mutant expression vectors was significantly higher than control groups (P <0.05). The expression of Smad4 protein transfected by pcDNA3.1-BMP2 (109G) and pcDNA3.1-BMP2 (109G, 570T) was significantly higher than the other experimental groups (P <0.05). The increase in ALP activity has been detected in pcDNA3.1-BMP2 (109G) and pcDNA3.1-BMP2 (109G, 570T) transfected cells up to 4 weeks after stable transfection. Activity of ALP was (30.56±0.46) nmol/min/mg protein and (29.62 ±0.68) nmol/min/mg protein, respectively. This was statistically different compared with the other experimental groups (P <0.05).

## CONCLUSIONS

BMP-2 is the predisposing gene of OPLL. The “TG” genotype in the 109T>G and the “AT” genotype in the 570A>T polymorphisms are associated with the occurrence of OPLL. The 109T>G polymorphism in exon-2 of the BMP-2 gene is positively associated with the level of Smad4 protein expression and the activity of ALP. The Smad mediated signaling pathway plays an important role during the pathological process of OPLL induced by SNPs of BMP-2 gene.

**Keywords** ossification of the posterior longitudinal ligament; single nucleotide polymorphisms; bone morphogenetic protein-2; signal transduction

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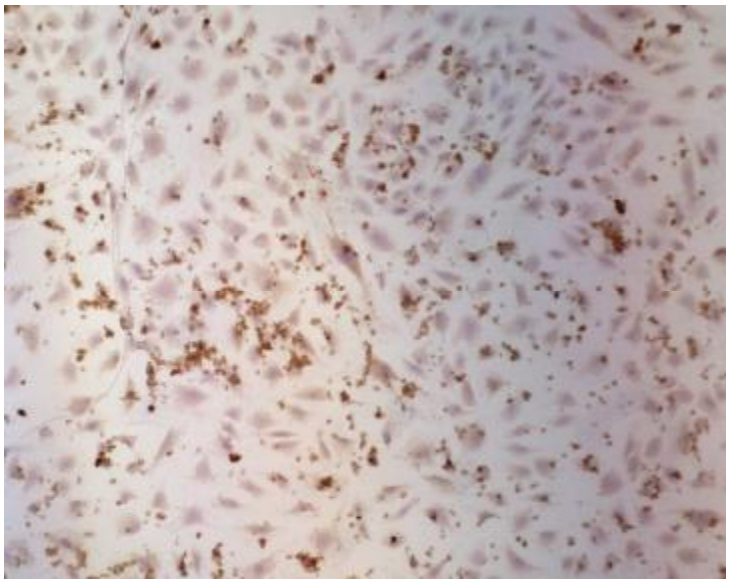

Figure1. In situ hybridization of BMP-2 transfected C3H10T1/2 cells.

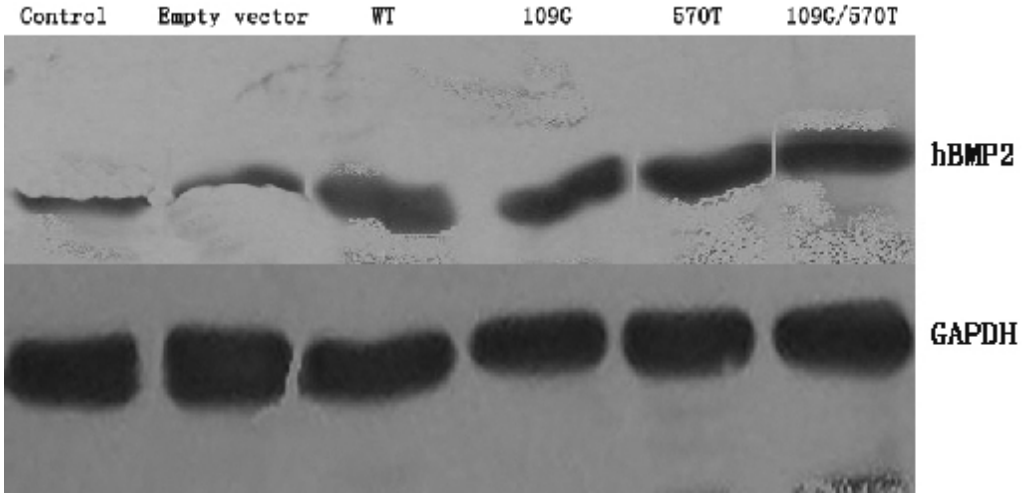


Figure 2. Western blotting analysis of hBMP2 protein levels in different groups following transfection.

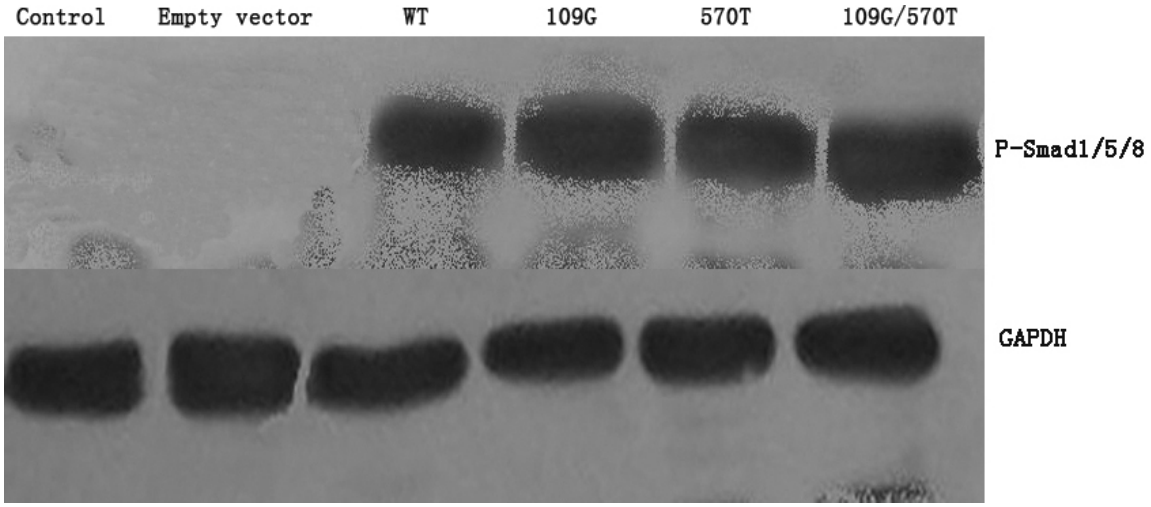


Figure 3. Western blotting analysis of P-Smad1/5/8 protein levels in different groups following transfection.

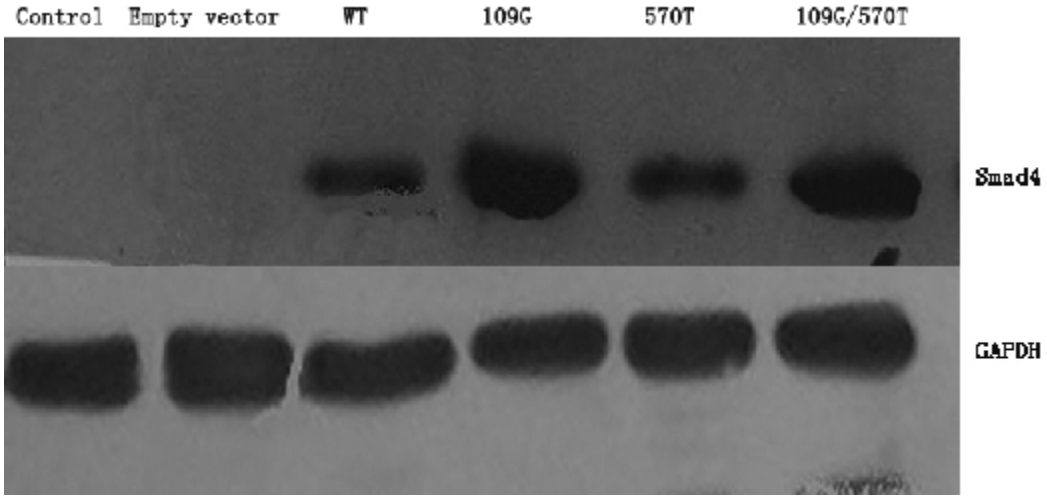


Figure 4. Western blotting analysis of Smad4 protein levels in different groups following transfection

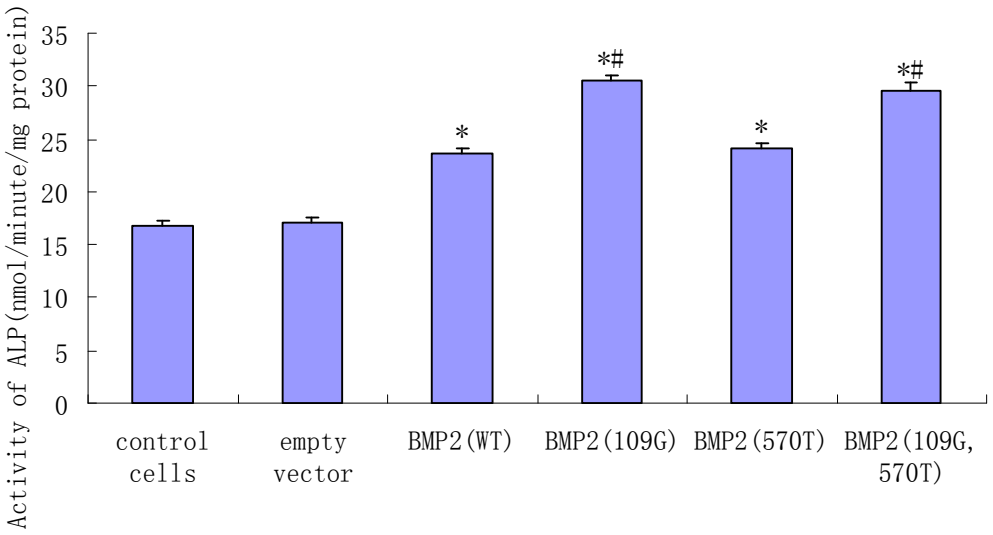


Figure 5. Quantified ALP activity assay 4 weeks after transfection. *P< 0.05 compared with empty vector transfected cells and control cells; # P< 0.05 compared with other experimental groups.

Table 1. Characteristics of OPLL cases and healthy control subjects

| | All | | Male | | Female | |
|---|---|---|---|---|---|---|
| | OPLL | Controls | OPLL | Controls | OPLL | Controls |
| Number | 420 | 506 | 232 | 288 | 188 | 218 |
| Age(years) | 32-77 | 31-79 | 32-77 | 31-79 | 34-75 | 32-78 |
| | (55.2±9.9) | (54.8±7.6) | (56.9±10.2) | (55.7±5.7) | (52.7±9.0) | (51.6±7.2) |
| Body weight(kg) | 51-84 | 50-85 | 63-84 | 61-85 | 51-71 | 50-72 |
| | (59.7±6.4) | (59.1±8.0) | (60.1±9.4) | (61.3±6.3) | (57.7±9.6) | (56.9±7.8) |
| Height(cm) | 150-178 | 152-180 | 165-178 | 167-180 | 150-170 | 152-169 |
| | (163.1±6.6) | (164.1±9.8) | (168.6±6.3) | (169.3±3.6) | (158.6±3.5) | (159.0±4.4) |
| Personal history | | | | | | |
| Smoking | 167 | 221 | 151 | 204 | 16 | 17 |
| Alcohol use | 68 | 94 | 52 | 81 | 16 | 13 |
| Food preference | 28 | 47 | 16 | 22 | 12 | 25 |
| Type of OPLL | | | | | | |
| Continuous | 155 | | 78 | | 77 | |
| Mixed | 88 | | 52 | | 36 | |
| Segmented | 139 | | 81 | | 58 | |
| Localized | 38 | | 21 | | 17 | |

Age, body weight and height are expressed as means ± SD

Table 2. Gentypic and allelic distributions of two SNPs between OPLL cases and controls

| SNPs | Ser37Ala (T/G) | | | Arg190Ser (A/T) | | |
|---|---|---|---|---|---|---|
| Genotype, *n* (%) | TT | TG | GG | AA | AT | TT |
| OPLL (*n*=420) | 280 (67) | 140 (33) | 0 (0) | 80 (19) | 227 (54) | 113 (27) |
| Control (*n*=506) | 472 (93) | 34 (7) | 0 (0) | 148 (29) | 237 (47) | 121 (24) |
| *P* | | ＜0.001 | | | 0.002 | |
| Allele n (%) | T | | G | A | | T |
| OPLL (*n*=420) | 700 (83) | | 140 (17) | 387 (46) | | 453 (54) |
| Control (*n*=506) | 978 (97) | | 34 (3) | 533 (53) | | 479 (47) |
| *P* | | ＜0.001 | | | 0.005 | |

## Disclosure of Conflicts of Interest

We certify that all our affiliations with or financial involvement in, within the past 5 years and foreseeable future, any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript are completely disclosed.