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Original Article

Association between bone morphogenetic protein 2 polymorphisms and osteoporotic fracture

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Abstract: Objective: To explore the association between bone morphogenetic protein 2 (*BMP2*) polymorphisms and osteoporotic fracture. Methods: A total of 115 osteoporotic fracture patients treated at our hospital from Jan 2014 to Sep 2014 were enrolled as case group and 131 healthy individuals as control group. Direct sequencing was performed to analyze *BMP2* rs967417 and rs79417223 polymorphisms. And ELISA was used to measure serum BMP2 level. Results: In case group, the proportion of BMI ≤ 24.0 kg/m^2, smoking and alcohol intake were higher than that in control group. Both mutant genotype (TT/GT) and G allele frequencies of rs967417 were higher in case group than in control group, similar to mutant genotype (GG) and G allele of rs79417223. For the onset risk of osteoporotic fracture, rs967417 TG+GG carriers who smoke and drink was 6.923 and 6.154 times as high as wild genotype (TT) carriers; rs79417223 AG+GG carriers who smoke was 6.75 times as high as AA carrier. BMP2 level of rs967417 TT, TC and GG carriers was lower in case group than in control group, similar to rs79417223 AA, AG, GG carriers. In case group, BMP2 level in GG and TC carriers were lower than TT genotype carriers. Logistic regression analysis showed that rs79417223 GG genotype and low BMP2 level were positively associated with the risk of osteoporotic fracture, while rs967417 TG/TT genotypes and BMI < 24.0 kg/m^2 were negatively associated with the risk of osteoporotic fracture. Conclusion: The polymorphisms of *BMP2* rs967417 and rs79417223 were associated with osteoporotic fracture. Additionally, rs967417 TG/TT genotype might be protective factor for osteoporotic fracture, while rs79417223 GG genotype might increase the risk of osteoporotic fracture.

Keywords: Bone morphogenetic protein 2, osteoporotic fracture, genetic polymorphism, occurrence risk, smoking

Introduction

Osteoporosis (OP), characterized by decreased bone mass, micro-architectural deterioration and fragility fractures, is a widespread chronic metabolic disease of the bone and affects people of every ethnic backgrounds especially elder women and men [1]. As a common complication of OP, osteoporotic fracture seriously threatens human health since social aging is coming. Approximately, 21 million men and 137 million women aged over 50 years or more worldwide were under high risk of osteoporosis fracture by 2010 and is set to double in 2040 [2]. By 2050, World Health Organization (WHO) predicted that half of the world's osteoporotic hip fractures will occur in Asia [3, 4]. Hence, it is the primary focus to determine reasonable and effective diagnosis and treatment for osteoporotic fracture.

Bone morphogenetic proteins (BMPs) belongs to the transforming growth factor β (TGF-β) superfamily, which plays a critical role in bone development, postnatal bone growth and fracture repair [5, 6]. In addition, over forty proteins have been obtained from BMPs family [7]. As an important member of BMPs, BMP2 whose sequence contains 12166 bp is located on the genome of 20p12 and participates in the early development of skeletal system and tissue construction [7]. BMP-2 is highly involved in inducing osteoblast differentiation and enhancing bone matrix production by osteoblastic cells, even in the signal transduction of apoptosis [8, 9]. Mutations in *BMP2* gene and the disorder of *BMP2* expression are easy to cause bone diseases and fracture. Additionally, some unhealthy lifestyles are closely related to OP, such as smoking and alcohol intake [10]. Currently, several studies have found that smoking had an important influence on occurrence and development of OP [11, 12]. And alcohol abuse reduces osteoblasts viability, osteogenic capacity and bone formation (poor bone quality and quantity) [13].

Table 1. PCR primer design for rs967417 and rs79417223

Locus	Primer sequence	Length of PCR products (bp)	Annealing temperature (°C)
rs967417	F: 5'-CAGATTACTCATGGATCTGC-3'	566	58
	R: 5'-AGGACTGATGGATGCGGTT-3'		
rs79417223	F: 5'-TGATTCCCTGGGGACTTTC-3'	393	61
	R: 5'-GCTAAATGATTAGATAAAT-3'		

Notes: F, forward; and R, reverse.

Table 2. General information comparison between case group and control group

General information	Case group (n = 115)	Control group (n = 131)	t/χ^2	*P*
Gender (male/female)	58/57	81/50	3.237	0.072
Age (year)	56.2 ± 6.8	55.2 ± 6.4	1.071	0.286
BMI ≤ 24.0 kg/m^2	26 (22.6%)	89 (77.4%)	50.55	< 0.001
Smoking	56 (48.7%)	35 (30.4%)	12.69	< 0.001
Alcohol intake	58 (50.4%)	42 (36.5%)	8.569	0.003

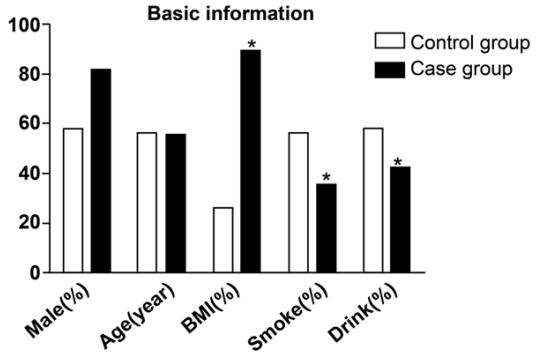

Figure 1. General information comparison between case group and control group. Notes: Compare between case group and control group, $P < 0.05$.

This study was aimed to verify the association of *BMP2* polymorphisms rs967417 and rs79417223 and osteoporotic fractures. Meanwhile, this study analyzed the association of smoking and alcohol intake with osteoporotic fractures.

Materials and methods

Study subjects

A total of 115 patients with osteoporotic fracture (58 males and 57 females; mean age, 56.2 ± 6.8 years; median age, 62 years) admitted in our hospital from January 2011 to September 2014 were recruited in this study, including 89 cases with vertebral fractures and 116 cases with hip fractures. A total of 131 healthy individuals were selected as control group, including 81 males and 50 females (age from 51 to 83 years; mean age 55.2 ± 6.4 years; median age 63 years). Based on the diagnostic criteria for OP formulated by WHO, bone mineral density (BMD) is 2.5 standard deviations (SD) lower than the peak bone mass of the same gender [14]. Inclusion criterion: BMD was 1 SD higher than the peak bone mass of the same gender. Exclusion criteria: patients with diseases affecting bone metabolism (metabolic bone disease, disuse bone disease and other bone diseases caused by some drugs and malnutrition; bone tumor; idiopathic osteoporosis; etc.) or patients who took drugs affecting bone metabolism; patients with diabetes, thyroid disease, parathyroid glands disease, pituitary disease, Cushing syndrome, severe anemia, cancer or patients living in absence of sunshine for a long time. This study was approved by our Ethics Committee of our hospital, and all patients signed the written informed consent.

Specimen collection

All subjects took venous blood (10 ml) in early morning after being fasting for 10 to 12 hours. Taking 3 ml blood, we added ethylene diaminetetraacetic acid (EDTA) for anticoagulation. Genomic DNA was extracted from whole blood sample using Whole Blood Genomic DNA Extraction Kit (Tiangen Biotech Co. Ltd.) according to the manufacturer's instructions. Without adding anticoagulant, the rest of blood samples went centrifugation at 3000 r/min for 10 min at room temperature after 1-hour standing to extract serum. And the serum sample was kept at -20°C.

Detection by SNP

Direct sequencing was performed to detect *BMP2* gene polymorphisms (rs967417 and rs79417223). Polymerase chain reaction (PCR)

Table 3. The genotype distribution and allele frequency distribution of rs967417 and rs79417223 SNPs in case group and control group

Genotyp/allele	Case group (n = 115)	Control group (n = 131)	χ^2	*P*	OR (95% CI)
rs967417					
TT	79 (68.7%)	108 (82.5%)	6.606	0.037	Ref.
TG	26 (22.6%)	18 (13.7%)			1.975 (1.013-3.849)
GG	10 (8.7%)	5 (3.8%)			2.734 (1.296-8.316)
TG+GG	36 (31.3%)	23 (16.0%)	6.348	0.012	2.140 (1.176-3.893)
T	184 (80.0%)	234 (89.3%)			Ref.
G	46 (20.0%)	28 (10.7%)	8.313	0.004	2.089 (1.257-3.473)
rs79417223					
AA	4 (3.5%)	10 (7.6%)	8.432	0.015	Ref.
AG	20 (17.4%)	61 (46.6%)			0.638 (0.189-2.142)
GG	91 (79.1%)	60 (45.8%)			4.608 (1.479-14.36)
AG+GG	111 (96.5%)	121 (92.4%)	1.97	0.180	2.293 (0.699-7.524)
A	28 (12.2%)	81 (30.9%)			Ref.
G	202 (87.8%)	181 (69.1%)	3.543	0.025	1.761 (1.070-2.899)

Notes: OR: odd ratio.

primer was designed by Primer Premier 5.0 software and synthesized by TsingKe (Beijing) Biological Technology Co. Ltd. Moreover, all primers were synthesized based on loci distribution of SNPs. And DNA fragments with polymorphic locus were extended by PCR into 400-800 bp. For cDNA sequence of *BMP2* gene, it included *BMP2* signal peptide fragment and corresponding sequence of active protein synthesized in the end whose primer was from http: www.ncbi.nlm.nih.gov (NT011387). Direct sequencing was applied to analyze gene sequence of target gene fragments extended by PCR. Primer sequences and length were shown in **Table 1**.

Protein detection

Enzyme-linked Immuno Sorbent Assay (ELISA) was used to detect serum BMP2 level according to instructions of ELISA kit (Jingmei Biological Products Co. Ltd.). Absorbance (OD value) was measured in each barrel at 450 nm. With linear regression equation calculated by the standard concentration and OD value, t the corresponding concentration of samples was obtained when OD value came in equation. All specimens were measured twice and took the average value. The detection was performed in accordance with laboratory quality control criteria.

Statistical analysis

Statistical analysis was performed using the SPSS 20.0 (SPSS Inc, Chicago, IL, USA) software. Genetic equilibrium was checked by Hardy-Weinberg equilibrium. Expressing as mean ± SD, measurement data were analyzed by t-test; enumeration data were expressed as percentage and ratio using χ^2 test to analyze; both homogeneity test for variance and one-way analysis of variance were applied to the comparison among multiple sets of mean. Logistic regression was used to analyze risk factors of osteoporotic fracture. $P < 0.05$ was considered statistically significant.

Results

General information comparison

In case group, the proportion of BMI ≤ 24.0 kg/m^2, smoking and alcohol intake were higher than control group (all $P < 0.05$), while differences in gender, age and other factors were out of statistical significance (all $P > 0.05$). Hence, the significant differences in BMI, smoking and alcohol intake between case group and control group indicated that non genetic factors like BMI, smoking and alcohol intake might be associated with the occurrence of osteoporotic fracture (**Table 2**; **Figure 1**).

Distribution of genotype and allele frequency of rs967417 and rs79417223SNPs in case group and control group

Frequency distribution of rs967417 and rs79417223 genotypes in case group and control group was performed using χ^2-type goodness of fittest. Frequency distribution of rs967417 and rs79417223 in both groups conformed to Hardy-Weinberg equilibrium (all $P > 0.05$). Comparing with control group, both mutant genotype (TT/GT) and G allele frequen-

Table 4. Analysis of different genotypes and clinical data

Clinical data	rs967417		OR (95% CI)	rs79417223		OR (95% CI)
	TT (Ref.)	TG+GG		AA (Ref.)	AG+GG	
BMI ≤ 24.0 kg/m^2				Ref.		
Case group	23	3	1.321 (0.324-5.386)	3	23	0.361 (0.07-1.728)
Control group	81	8		4	85	
Smoking						
Case group	26	30	6.923 (2.344-20.45)	2	54	6.75 (1.314-34.69)
Control group	30	5		7	28	
Alcohol intake						
Case group	26	32	6.154 (2.350-16.12)	1	57	7.703 (0.865-68.62)
Control group	35	7		5	37	

Notes: BMI: body mass index; CI: confidence interval; OR: odd ratio.

Table 5. Expression of serum BMP2 at different genotypes

Genotype	Case group	Control group	t	*P*
rs967417				
TT	67.85 ± 14.46	87.25 ± 18.42	7.460	< 0.001
TG	55.24 ± 13.19*	85.25 ± 16.13	4.487	0.002
GG	45.21 ± 11.54*,#	85.23 ± 14.20	4.419	0.047
rs79417223				
AA	69.89 ± 7.46	87.86 ± 17.87	8.677	< 0.001
AG	64.72 ± 4.31	87.63 ± 17.15	3.501	0.013
GG	62.27 ± 4.30	86.82 ± 9.62	4.374	0.049

Notes: *Compare with wild genotype, $P < 0.05$; #compare with heterozygote, $P < 0.05$.

cies of rs967417 were higher in case group (both $P < 0.001$), and G allele may be the risk factor of atrial fibrillation (TG vs. TT: OR = 1.975, 95% CI = 1.013~3.849; GG vs. TT: OR = 2.734, 95% CI = 1.296~8.316; TG+GG vs. TT: OR = 2.140, 95% CI = 1.176~3.893; G vs. T: OR = 2.089, 95% CI = 1.257~3.473). In case group, mutant genotype (GG) and G allele of rs79417223 were higher than that in control group, and the difference was statistically significant (both $P < 0.05$). Moreover, G allele might be the risk factor of atrial fibrillation as well (GG vs. AA: OR = 4.608, 95% CI = 1.479-14.36; G vs. A: OR = 1.761, 95% CI = 1.070~2.899) (**Table 3**).

Analysis of different genotypes and clinical data

Significant differences in smoking and alcohol intake were found between polymorphic wild genotype (TT) and mutant genotype (TG+GG) of rs967417 in case group and control group. Moreover, mutant genotype carriers who smoke and drink was 6.923 and 6.154 times as high as wild genotype carriers for the onset risk of osteoporotic fracture (95% CI = 2.344~20.45; 95% CI = 2.350~16.12). Besides, between polymorphic wild genotype (AA) and mutant genotype (AG+GG) of rs79417223 in case group and control group, smoking was also significantly different. Furthermore, for the onset risk of osteoporotic fracture, mutant genotype carriers who smoke was 6.75 times as high as wild genotype carrier (95% CI = 1.314~34.69) (**Table 4**).

Serum BMP2 level indifferent genotypes

BMP2 level of patients carrying TT, TC and GG genotypes of rs967417 in case group was lower than that in control group, and differences were statistically significant (all $P < 0.05$). In addition, when compared with control group, BMP2 level of patients carrying AA, AG, GG of rs79417223 in case group was higher (all $P < 0.05$). In case group, GG and TC genotypes carriers were lower than TT genotype carriers in terms of BMP2 level, and differences between groups were statistically significant (all $P < 0.05$). While either in case group or control group, there was no significant differences in BMP2 level in subjects carrying rs79417223 AA, AG and GG genotypes (all $P > 0.05$) (**Table 5**; **Figure 2**).

Logistic regression analysis

Logistic regression was performed to get updated odds ratio-Exp (B) with OF as dependent variable and BMI ≤ 24.0 kg/m^2, smoking, alco-

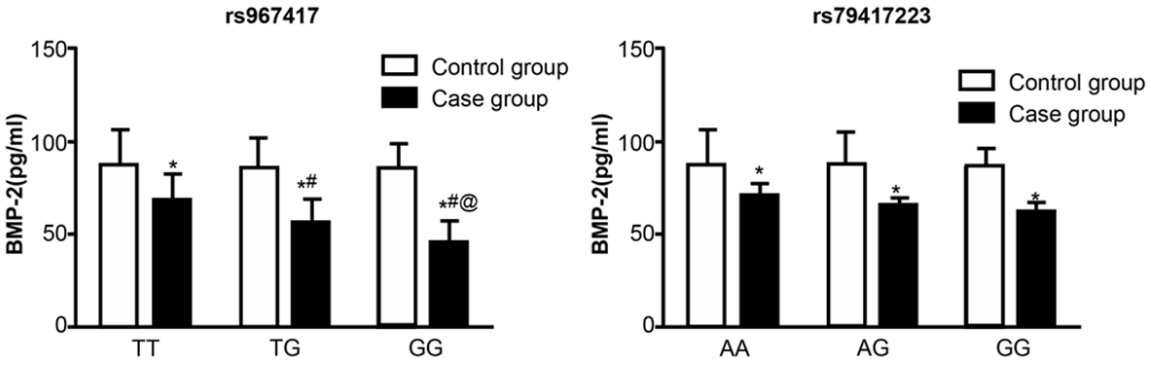

Figure 2. Expression of serum BMP2 at different genotypes. Notes: Compare with control group, $P < 0.05$; #: compare with wild genotype, $P < 0.05$; @: Compare with heterozygote, $P < 0.05$.

Table 6. Analysis of risk factors of osteoporotic fracture

Variables	B	S.E	Wald	Sig	Exp (B)	95% CI
rs967417TG/TT genotype	-1.578	0.376	17.649	< 0.001	0.206	0.099-0.431
rs79417223GG genotype	0.392	0.146	6.897	0.009	1.480	1.104-1.984
Low BMP2 level	0.05	0.01	26.315	< 0.001	1.051	1.031-1.072
Alcohol intake	1.253	0.802	2.441	0.118	3.500	0.727-16.848
BMI < 24 kg/m^2	-0.751	0.187	16.092	< 0.001	0.472	0.327-0.681
Smoking	-0.818	0.820	0.997	0.318	0.441	0.088-2.199

B, partial regression coefficient; S.E, standard error; Wald, Wald χ^2; df, degree of freedom; Sig, *P* value; Exp (B), odds ratio.

hol intake, genotypes and alleles of rs967417 and rs79417223 and serum BMP2 level as independent variables. The analysis results showed that rs79417223 GG genotype and low BMP2 level were positively associated with the occurrence risk of osteoporotic fracture, while rs967417 TG/TT genotype and BMI < 24.0 kg/m^2 were negatively associated with the occurrence risk of osteoporotic fracture (**Table 6**).

Discussion

Osteoporosis risk increases with age, and the elder is likely to suffer osteoporotic fracture which seriously affects their health [14]. Previous studies showed that osteoporotic fracture was associated with polymorphism in genes [15-17] which mainly functioned in collagen synthesis and regulation in bone and bone tissue formation including apolipoprotein E (*ApoE*) [18], methyl enetetra hydrofolate reductase (*MTHFR*) [19], vitamin D receptor (*VDR*) [15], collagen type I alpha 1 (*COL1A1*) [20]. But *BMP2*, another important factor, did not catch enough attentions yet. In this study, it was confirmed that mutation of related polymorphic loci was correlated with osteoporotic fracture, which will provide new basis for the diagnosis and treatment of osteoporotic fracture.

In this study, polymorphic loci of *BMP2* mainly focused on rs967417 and rs79417223. According to NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp), rs967417 is mostly located in the upstream of *BMP2*; rs79417223 is located in the intron of *BMP2*, whose mutation is indel and frame-shift one. On the basis of polymorphism frequency results, comparing with control group, mutant genotype frequencies of rs967417 (TG+GG) and rs79417223 (AG+GG) were significantly higher in case group. What's more, genotype mutation has significant effects on genetic expression. Once mutation occurred in rs967417 (TG+GG) and rs79417223 (AG+GG) in OF patients, the expression quality of *BMP2* will decrease greatly. (Evidentially, these two genetic polymorphic loci exerted effects on the expression of *BMP2*. In 5' untranslated region (UTR), rs967417 (TG+GG) could not only save mRNA from degradation by excision enzyme of 5' end, but also provide export signal for mRNA to improve stability and efficiency of translation template; and mutation of rs967417 (TG+GG) in 5' UTR is likely to affect the recognition of transcription factor, which exerts great impact on the expression quantity of mRNA to lower the mRNA translation level [21, 22]. While rs79617223 mainly has mutation in intron that

helps sequence to develop specific secondary structure to protect and stabilize primary transcript [23]. Besides, intron functions as an enhancer by promoting these sequences binding with some protein to enhance initiation and extension of transcript [24]. Consequently, changes of intron in rs79617223 (AG+GG) will decrease the translation efficiency indirectly [23, 24]. Hence mutation in rs967417 and rs79417223 might exert effects on the expression quantity of mRNA template, which decreased the expression quantity of *BMP2* indirectly and directly. During osteogenic differentiation, *BMP2* functions by regulating Wnt signaling to promote bone development and differentiation [25, 26]. After *BMP2* activates the channel, there is a great increase in both specific activity of alkaline phosphatase (ALP) and phosphorylation level of Smad1/5/8 protein, which strengthens bone differentiation [27]. Therefore, the decreasing expression quantity of BMP2 will weaken the channel for bone differentiation so as to cause bone mass loss, which will lead to a big increase in the occurrence of osteoporotic fracture.

Additionally, this study carried out a comprehensive analysis at smoking and alcohol intake. The analysis results showed that with mutation in *BMP2* polymorphic loci, smoking and alcohol intake will increase the occurrence possibility of osteoporotic fracture. According to current studies, reasons why smoking leads to OP were toxic effects of tobacco on bone, interference in calcium homeostasis, imbalance in sex hormones level, changes in expression quantity of cytokines, muscle injury and effects of complication [12, 28-32]. Furthermore, alcohol abuse interfered in bone metabolism and reduced bone mass which would increase the fracture risk; alcohol abuse will weaken coordination ability of muscles, and it is likely to have accidents like fall down which increased the risk of fracture [33]. So to that extent, with the decreasing expression quantity of *BMP2* which will lead to a decrease in bone mass and the loose structure of bone, unhealthy lifestyle such as smoking and alcohol intake will trigger osteoporotic fracture.

In conclusion, mutation in polymorphic loci of *BMP2* (rs967417; rs79417223) increased the risk of developing osteoporotic fracture by decreasing the expression quantity of *BMP2*, which has a certain correlation with smoking and alcohol intake as well. Therefore, establishing healthy lifestyle as well as making the best of *BMP2* polymorphic loci as clinically diagnostic target will effectively help to prevent osteoporotic fracture.

Disclosure of conflict of interest

None.

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