

PFNI Gene Polymorphisms and the Bone Mineral Density Response to Alendronate Therapy in Postmenopausal Chinese Women with Low Bone Mass

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Purpose: Alendronate is a widely used anti-osteoporotic drug. *PFNI* gene is a newly identified early-onset Paget's disease pathogenic gene. The purpose of this study is to study whether the genetic variations in this gene affect the clinical efficacy of alendronate in postmenopausal Chinese women with low bone mass.

Patients and Methods: Seven single nucleotide polymorphisms in *PFNI* gene were genotyped. A total of 500 postmenopausal women with osteoporosis or osteopenia were included. All participants were treated with weekly alendronate 70 mg for 12 months. A total of 466 subjects completed the follow-up. Bone mineral density (BMD) of lumbar spine, femoral neck and total hip were measured at baseline and after treatment.

Results: After 12 months of treatment, the BMD of lumbar spine, femoral neck and total hip all increased significantly (all $P < 0.001$), with an average increase of $4.72 \pm 5.31\%$, $2.08 \pm 4.45\%$, and $2.42 \pm 3.46\%$, respectively. At baseline, there were no significant differences in BMD at lumbar spine, femoral neck and total hip between different genotype groups ($P > 0.05$). We failed to identify any significant association between the genotypes or haplotypes of *PFNI* and the BMD response to alendronate therapy.

Conclusion: Genetic polymorphisms of *PFNI* may not be a major contributor to the therapeutic response to alendronate treatment in Chinese women with low bone mass.

Keywords: alendronate, bone mineral density, osteoporosis, *PFNI* gene, single-nucleotide polymorphism

Introduction

Osteoporosis is a common skeletal disease characterized by low bone mass, reduced bone strength, deteriorated bone microarchitecture. Genetic factors contribute 60–80% of the variance in bone mineral density (BMD).^{1–3} With the development of the aging population, the incidence of osteoporosis and osteoporotic fracture has been increasing. An analysis of National Health and Nutrition Examination Survey 2005–2008 data revealed that 19% of older men and 30% of older women in the United States require antiosteoporosis therapy.⁴ With regard to osteoporosis in China, the rate of hip fractures in China is progressively increased approximately 10% per year from 2002 to 2006.⁵ Our study in 2019 indicated that the prevalence of vertebral fractures in the elderly in Shanghai was 17.2%, that of men was 17.0%, and that of women was 17.3%.⁶

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Alendronate is a potent and specific inhibitor of osteoclast-mediated bone resorption. Because of its beneficial effects in increasing bone mass and reducing the risk of fracture, it is widely used as a first-line drug in postmenopausal women with osteoporosis in Europe, the Americas and Asia.^{7–12} Studies have demonstrated that treatment with 70 mg of alendronate once weekly decreases the risk of vertebral fractures by 50% and the risk of peripheral fractures by 20–30%.^{13,14} Nevertheless, the efficacy and safety of alendronate vary among different patients, with approximately 5–10% of cases having poor or no clinical response.¹⁵ The identification of the factors that influence the response to alendronate therapy can help optimize drug therapy. Variations in the human genome are considered the key cause of variable drug responses and adverse reaction development.^{16,17} The *COL1A1* gene¹⁸ and *VDR* gene^{19–21} have been shown to be involved in the response to bisphosphonate therapy. Our previous research proved that *MVK* and *FDFT1* polymorphisms are genetic determinants for response to alendronate therapy in postmenopausal Chinese women.²² We also clarified that there is no association between polymorphisms in *OPG*, *RANKL* and *RANK* gene and BMD response to alendronate.²³ Recently, we demonstrated that the common variation of *SOST* gene is associated with the response to alendronate treatment in postmenopausal women with low BMD.²⁴ However, to date, pharmacogenomics reports on alendronate are still scattered.

The *PFN1* gene, located at 17p13.3, encodes the highly evolutionarily conserved protein Profilin 1. Profilin 1 is a small actin monomer-binding protein which is considered to be an essential control element for actin polymerization and cell migration.^{25,26} Profilin 1 is ubiquitously expressed in all cell types and in organs and tissues throughout the body, and is involved in the control of a variety of cell functions.^{25,27–31} We and two other research teams have reported that mutations in the *PFN1* gene could lead to early-onset Paget's disease with or without giant cell tumors.^{32–34} This finding indicates that *PFN1* mutations can cause the imbalance of skeletal homeostasis and changes in bone mass and bone turnover status. In addition, Shirakawa et al have demonstrated in animal experiments that *Pfn1* plays critical role in inhibiting osteoclast motility and bone resorption, thereby affecting the skeletal homeostasis of mice.³⁵ So, we believe that *PFN1* gene may be a key gene regulating the proliferation, differentiation and activity of osteoclasts.

We hypothesized that genetic polymorphisms in *PFN1* gene is good candidate to explain the variable responses to alendronate treatment. Therefore, we conducted this study to explore the association between *PFN1* gene variations and the BMD response to alendronate therapy in postmenopausal Chinese women with low mass.

Materials and Methods

Subjects

All participants were recruited by the department of Osteoporosis and Bone Disease in Shanghai Jiaotong University Affiliated Sixth People's Hospital. Five hundred women were enrolled in this study. All study subjects were postmenopausal Han Chinese women with primary osteoporosis or osteopenia, without spontaneous menses for at least 1 year. All participants were treated with 70 mg of alendronate (Merck, Fosamax, Hangzhou, China) once weekly, 600 mg of calcium and 125 IU of vitamin D daily for 12 months. The study protocol was approved by the Committee of the Ethics of Human Research in the Shanghai Jiaotong University Affiliated Sixth People's Hospital and was conducted in accordance with the Declaration of Helsinki. All study participants provided written informed consent.

The inclusion criteria were as follows: natural menopause after 40 years of age and a BMD of at least 1.0 SD below the peak mean bone density of healthy young women (−1.0 T-score) at the posterior–anterior L1–4, femoral neck, or total hip. The diagnosis of osteoporosis was based on L1–4, femoral neck or total hip with a T-score less than −2.5, or a history of fragility fractures of the spine or hip. Among women with osteopenia, the decisions of treatment were made depended on the high risk of osteoporotic fractures. We evaluated the high risk of fracture according to the history of low trauma fracture at humerus or radius, the 10-year probability of hip fracture $\geq 3\%$ or of a major osteoporotic fracture (clinical spine, hip, forearm and humerus fracture) $\geq 20\%$ by FRAX[®].³⁶

The exclusion criteria²⁴ were as follows: a history of (1) chronic renal disease manifested by an endogenous creatinine clearance of < 35 mL/min; (2) acute inflammation of the gastrointestinal tract (eg, gastritis and ulcerations); (3) esophagitis or certain malformations and malfunctions of the esophagus (eg, strictures and achalasia); (4) proton-pump inhibitor usage along with alendronate treatment; (5) inability to stand, walk or sit for 30 min after oral administration

of alendronate; (6) hypersensitivity to alendronate or another ingredient in the therapeutic compound; (7) hypocalcemia (serum calcium (Ca) < 2.08 mmol/l) or hypophosphatemia (serum phosphorus (P) < 0.80 mmol/l); (8) increased serum parathyroid hormone (PTH) levels (normal values: 15–65 pg/mL); (9) serious residual effects of cerebral vascular disease; (10) diabetes mellitus, except for adult asymptomatic hyperglycemia controlled by diet; (11) chronic liver disease or alcoholism; (12) 12 weeks of corticosteroid therapy at pharmacologic levels; (13) 6 months of treatment with anticonvulsant therapy; (14) evidence of other metabolic or inherited bone diseases (eg, hyperparathyroidism or hypoparathyroidism, Paget's disease, osteomalacia or osteogenesis imperfecta); (15) rheumatoid arthritis or collagen disease; (16) significant disease of any endocrine organ that would affect bone mass (eg, Cushing's syndrome or hyperthyroidism); (17) any neurological or musculoskeletal condition that would be a nongenetic cause of low bone mass; (18) a body mass index (BMI) of <18 kg/m² or >30 kg/m²; and (19) any previous treatment with bisphosphonate, sodium fluoride, calcitonin, a selective estrogen receptor modulator, strontium ranelate, or the recombinant form of PTH or current use of hormone replacement therapy.

BMD Measurements

Lunar prodigy dual-energy X-ray absorptiometry densitometer (DXA; GE Healthcare, Madison, WI, USA) was used to measure the BMD of lumbar spine (L1–4), left femoral neck and total hip at baseline and after 12 months of treatment, respectively. The BMD of the right hip was measured only in patients with a history of left hip fracture or surgery. The Prodigy encore software (ver. 6.70, standard-

array mode; GE Healthcare) were used for data analysis. The machines were calibrated daily and coefficient of variance (CV) values for BMD of the L1–4, femoral neck and total hip were 1.39%, 2.22% and 0.70%, respectively.³⁷ The long-term reproducibility of our DXA instrument during the study based on weekly repeated phantom measurements was 0.45%. Besides, we chose the least significant change (LSC) to estimate the effectiveness of treatment. And the LSC in BMD at L1–4, femoral neck and the total hip were 3.85%, 6.15% and 1.94%.³⁷ Weight and height were measured using a calibrated balance beam scale and a calibrated stadiometer. The BMI was defined as weight/height² in kg/m².

PFNI Genotyping

Seven tagSNPs in *PFNI* gene (rs13204, rs78224458, rs238243, rs238242, rs113460962, rs4790714 and rs117337116) were selected from dbSNP (<http://www.ncbi.nlm.nih.gov/snp/>) and HapMap (<http://hapmap.ncbi.nlm.nih.gov/>) based on the following criteria: (1) minor allele frequency (MAF) higher than 5%; (2) pairwise linkage disequilibrium (LD) exceeding the threshold of 0.8 ($r^2 > 0.8$) (Table 1). Genomic DNA was isolated from peripheral blood leukocytes via isopropanol-precipitating method. Genotyping was performed using the ABI PRISM SNaPshot multiplex kit (Applied Biosystems), an Mx3000p real-time PCR system (Stratagene), and GeneMapper 4.1 (Applied Biosystems).

Statistical Analyses

Hardy–Weinberg equilibrium (HWE) was tested for each SNP using the χ^2 test. The linkage disequilibrium block structure was assessed using the Haploview 4.2. PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>) was utilized in quality control filtering and haplotype association test. SNPs

Table 1 Information on the 7 SNPs in This Study

SNP	Physical Position	Gene Location	Major Allele	Minor Allele	MAF	P-value of HWE
rs13204	4849284	Exon3	G	A	0.180	0.410
rs78224458	4849546	Intron2	T	C	0.126	0.924
rs238243	4849635	Intron2	A	G	0.469	0.004
rs238242	4849774	Intron2	G	A	0.313	0.107
rs113460962	4850748	Intron1	T	C	0.093	1.000
rs4790714	4852463	5'-flanking	C	T	0.175	0.583
rs117337116	4853945	5'-flanking	T	C	0.052	0.723

Abbreviations: SNP, single nucleotide polymorphism; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium.

Table 2 Characteristic Changes of 466 Postmenopausal Women

	Height (cm)	Weight (kg)	BMI (kg/cm ²)	L1-4 BMD (g/cm ²)	Femoral Neck BMD (g/cm ²)	Total Hip BMD (g/cm ²)
Baseline	153.76±6.38	54.29±8.26	22.96±3.08	0.814±0.149	0.673±0.104	0.717±0.107
12 months	153.52±6.51	54.32±8.39	23.04±3.13	0.850±0.147	0.686±0.105	0.733±0.109
Percent change (%)	—	—	—	4.72±5.31	2.08±4.45	2.42±3.46
P-value	0.587	0.844	0.119	< 0.001	< 0.001	< 0.001

Notes: Data presented as mean ± SD. Significant values ($P < 0.05$) are presented in bold.

Abbreviations: BMI, body mass index; BMD, bone mineral density.

with call rates <75% and HWE tests with P -values <0.01 were excluded from further analysis. Descriptive statistics were given as the mean ± SD for normally distributed data and as the median and interquartile range for the non-normally distributed data. The BMD value at L1–4, femoral neck and total hip before and after alendronate treatment were compared by using the paired t -test. SPSS 22.0 (SPSS, Chicago, IL, USA) was used to analyze these data. The response to alendronate treatment is estimated by the % change in BMD, defined as (12-months BMD – baseline BMD)/baseline BMD × 100%. The baseline and % change after 12 months treatment in BMD among different genotype groups were tested using PLINK in the ADD linear model. The differences in BMD at baseline and % change after 12 months treatment among haplotype groups were analyzed using PLINK in the GLMs linear model. According to the LSC in BMD, the participants were divided into two groups: responder(s) and non-responder(s). The association between genotype groups and the opportunity of being a responder after treatment was analyzed using PLINK in the ADD logistic regression model. The association between the haplotype groups and the opportunity of being a responder was analyzed using PLINK in the GLMs logistic regression model. All data were adjusted by the age and BMI covariates. P -values <0.05 were defined as statistically significant. The Bonferroni correction was used to adjust for multiple testing. For SNP analysis, P -value thresholds for statistical significance were 0.008; for haplotype analysis, P -value thresholds for statistical significance were 0.025.

Results

Basic Characteristics of Study Subjects

Among the 500 subjects participating in the study, 466 subjects completed 12 months of alendronate treatment and had BMD measurement at baseline and 12-months treatment, respectively. The average baseline age, height, weight and BMI were 64.88 ± 8.11 years, 153.76 ± 6.38 cm, $54.29 \pm$

8.26 kg and 22.96 ± 3.08 kg/m², respectively. The mean BMD of the L1–4, femoral neck and total hip was 0.814 ± 0.149 g/cm², 0.673 ± 0.104 g/cm², 0.717 ± 0.107 g/cm², respectively. After 12 months of treatment, there were no significant difference between baseline and follow-up height, weight or BMI. As expected with alendronate treatment, the BMD of L1–4, femoral neck and total hip all increased significantly (all $P < 0.001$), with average increases of $4.72 \pm 5.31\%$, $2.08 \pm 4.45\%$, and $2.42 \pm 3.46\%$, respectively (Table 2).

Allele Frequencies and Haplotype Structures

All 466 participants were genotyped. All tagSNPs were successfully genotyped and none of them failed the frequency test ($MAF < 0.01$). In the study, the population distribution of rs238243 deviated from HWE ($P < 0.01$) and were excluded from further analysis. The other 6 SNPs (rs13204, rs78224458, rs238242, rs113460962, rs4790714 and rs117337116) were compatible with HWE. Two haplotype blocks have been constructed from 6 SNPs (Figure 1).

Association Between Genetic Polymorphisms and the Therapeutic Response to Alendronate Treatment

At baseline, there were no significant differences in BMD at L1–4, femoral neck and total hip between different genotype groups ($P > 0.05$). The associations between the 6 SNPs and the % change in BMD at L1–4, femoral neck or total hip were analyzed as the first step. Two haplotype blocks have been constructed from 6 SNPs (Figure 1). The rs13204, rs78224458 and rs238242 constituted block 1, and rs4790714, rs117337116 constituted block 2. After Bonferroni correction, no association was observed between any SNP or haplotype and BMD % change after 12 months of alendronate therapy in postmenopausal Chinese women with low BMD (Tables 3 and 4). In order to estimate the effectiveness of treatment, subjects

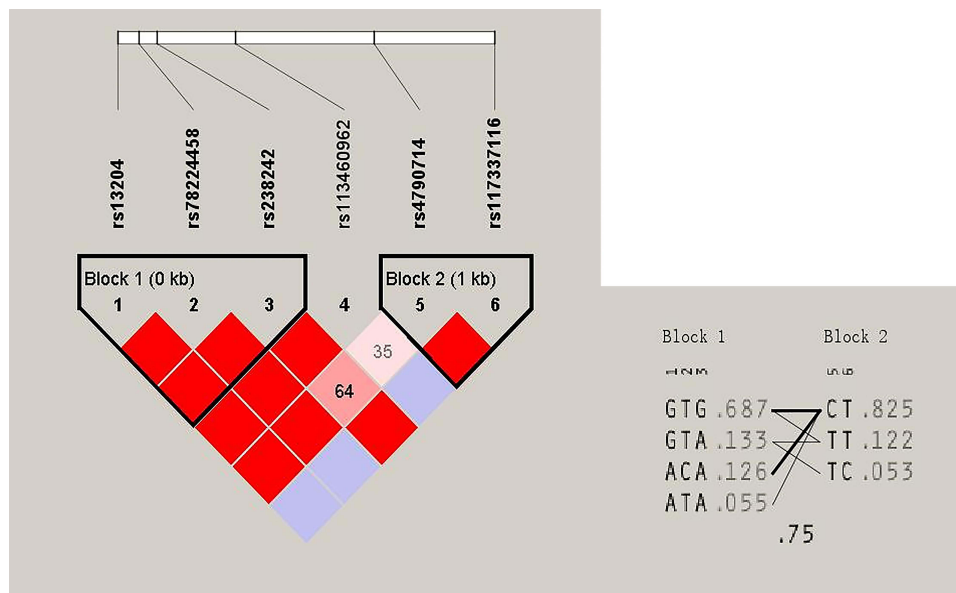


Figure 1 Haplotype linkage disequilibrium (LD) blocks in 466 postmenopausal women. Two LD blocks connecting SNP pairs are shaded based on the LD strength between SNPs by using the disequilibrium coefficient r^2 . The increasing degree of red of the cells represents the increasing strength of LD. LD plots with r^2 values were generated using Haploview. Figure 1 shows the LD pattern in the *PFNI* gene based on the 466 postmenopausal women with low bone mineral density.

were divided into responder(s) group and non-responder(s) group according to the LSC in BMD. The associations between 6 SNPs and being a responder to alendronate treatment at the L1–4, femoral neck or total hip were analyzed in the second step. In response to 70 mg weekly of alendronate, 55.0% subjects in spine BMD, 12.3% subjects in femoral neck, 57.8% subjects in total hip arrived an effective therapeutic range. Unfortunately, no association was found between any SNP or haplotype and treatment response in postmenopausal Chinese women with low BMD (Tables 5 and 6).

Discussion

Osteoporosis is an important health problem worldwide. The effect of anti-osteoporosis treatment is determined by the bone-remodeling process, including the resorption of osteoclasts and the formation of osteoblasts. Alendronate is an important inhibitor of bone resorption and is the most commonly used bisphosphonate worldwide. It can increase BMD and decrease the risk of fragility fracture.³⁸ It is well known that the treatment response to this drug is highly variable.²² Pharmacogenetics studies the genetic bases of individual drug response, determines the most effective drugs and the most beneficial doses for each patient. It has an important pharmaco-economic impact on chronic diseases that require long-term treatment.³⁹ As we all know, the response to alendronate treatment has a strong

genetic component. Therefore, it is very important to clarify the genetic factors involved in anti-osteoporosis treatment, which will help to significantly improve the treatment effect. In previous studies, studies involving bone loss control, mevalonate pathway, farnesyl diphosphate synthase gene, geranylgeranyl diphosphate and other genes were analyzed.^{18,22,24,40–45} Despite all the advances in the field of pharmacogenetics in recent years, so far, these studies have found only a few SNPs that may be related to the response to anti-osteoporosis treatments. The pharmacogenetics of osteoporosis is still in its infancy, and pharmacogenomics research on anti-osteoporosis treatment is still insufficient.¹⁶

The *PFNI* gene is a recently discovered pathogenic gene for early-onset Paget's disease.^{32–34} It is widely expressed and highly expressed in blood and bone marrow cells.⁴⁶ The *PFNI* gene plays a central role in regulating de novo actin polymerization by enhancing ADP-ATP exchange on G-actin.⁴⁷ Cell studies have confirmed that the *PFNI* gene at least partially regulates the function of stem cells in bone marrow through the *PFNI*/*Ga13*/*EGRL* axis.⁴⁸ Many studies have shown that *PFNI* can stimulate the migration of endothelial cells, chondrocytes, human mesenchymal stem cells and neuronal cells.^{49,50} Zoidakis et al found that *PFNI* plays an important role in skeletal development, and is a key molecule in sternal development and trabecular bone formation.⁵¹ Cell research found

Table 3 The Association Between 6 Single Nucleotide Polymorphisms and the % Change in BMD

SNP	Genotype	Counts	L1-4 (%)			Femoral Neck (%)			Total Hip (%)		
			Mean	SD	P-value	Mean	SD	P-value	Mean	SD	P-value
rs13204	G/G	310	4.52	5.45	0.215	2.14	4.77	0.696	2.49	3.65	0.501
	G/A	144	5.16	4.84		1.96	3.70		2.18	3.04	
	A/A	12	4.73	6.93		2.08	4.32		3.45	2.98	
rs78224458	T/T	357	4.71	5.53	0.911	2.05	4.67	0.814	2.49	3.62	0.309
	C/T	101	4.67	4.43		2.02	3.69		2.01	2.79	
	C/C	8	5.92	5.62		4.16	2.96		4.29	3.01	
rs238242	G/G	212	4.38	5.79	0.372	1.82	4.88	0.814	2.28	3.68	0.918
	G/A	216	5.05	4.38		2.32	4.08		2.60	3.31	
	A/A	38	4.79	6.95		2.18	3.99		2.15	2.96	
rs113460962	T/T	383	4.77	5.45	0.328	1.94	4.49	0.269	2.45	3.57	0.177
	C/T	79	4.56	4.65		2.53	3.98		2.25	2.91	
	C/C	4	3.30	3.73		7.06	6.87		2.69	1.75	
rs4790714	C/C	315	4.74	5.37	0.529	1.82	4.51	0.866	2.35	3.55	0.901
	C/T	139	4.81	5.15		2.54	4.35		2.54	3.27	
	T/T	12	3.23	5.55		3.75	3.52		2.64	3.35	
rs117337116	T/T	419	4.73	5.39	0.432	2.02	4.39	0.703	2.41	3.54	0.677
	C/T	45	4.60	4.57		2.38	4.79		2.34	2.59	
	C/C	2	9.29	—		8.20	7.56		6.19	0.58	

Abbreviations: BMD, bone mineral density; SNP, single nucleotide polymorphism.

that in osteoblasts MC3T3-E1 (MC), the expression of *PFNI* mRNA was down-regulated with osteogenic differentiation.⁵² And in MC cells, siRNA knocking out *PFNI* can enhance the alkaline phosphatase activity

induced by bone morphogenetic protein (BMP). Therefore, it can be considered that *PFNI* is a new target of BMP, which can inhibit the differentiation of osteoblasts induced by BMP through transcription.⁵² Wanting

Table 4 The Association Between 2 Haplotypes and the % Change in BMD

SNPs			Haplotype	L1-4		Femoral Neck		Total Hip	
				Beta	P-value	Beta	P-value	Beta	P-value
rs13204	rs78224458	rs238242	ACA	0.055	0.911	-0.092	0.814	-0.299	0.309
			ATA	1.280	0.067	-0.213	0.748	0.187	0.681
			GTA	-0.100	0.835	0.044	0.907	0.182	0.537
			GTG	-0.332	0.372	0.071	0.814	0.023	0.918
rs4790714	rs117337116		TC	-0.533	0.432	0.212	0.703	-0.179	0.677
			TT	-0.076	0.879	-0.031	0.938	0.049	0.875
			CT	0.271	0.529	-0.058	0.866	0.033	0.901

Abbreviations: BMD, bone mineral density; SNP, single nucleotide polymorphism; Beta, regression coefficient.

Table 5 The Association Between 6 Single Nucleotide Polymorphisms and BMD Response

SNP	L1-4			Femoral Neck			Total Hip		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
rs13204	1.066	0.749–1.518	0.722	0.823	0.477–1.421	0.485	0.844	0.594–1.199	0.344
rs78224458	1.019	0.684–1.519	0.926	0.882	0.479–1.624	0.687	0.813	0.548–1.207	0.305
rs238242	0.960	0.713–1.292	0.786	0.915	0.586–1.427	0.695	0.966	0.718–1.301	0.820
rs113460962	0.905	0.575–1.425	0.666	1.191	0.625–2.269	0.596	0.868	0.507–1.367	0.540
rs4790714	0.815	0.569–1.167	0.264	1.013	0.598–1.713	0.963	1.191	0.832–1.707	0.340
rs117337116	0.645	0.355–1.172	0.150	1.013	0.427–2.407	0.976	1.238	0.679–2.258	0.486

Abbreviations: BMD, bone mineral density; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table 6 The Association Between 2 Blocks and BMD Response

SNPs			Haplotype	L1-4		Femoral Neck		Total Hip	
				OR	P-value	OR	P-value	OR	P-value
rs13204	rs78224458	rs238242	ACA	1.020	0.926	0.882	0.687	0.813	0.305
			ATA	1.140	0.652	0.759	0.571	0.982	0.951
			GTA	0.855	0.443	1.080	0.795	1.160	0.454
			GTG	1.040	0.786	1.090	0.695	1.040	0.820
rs4790714	rs117337116		TC	0.645	0.150	1.010	0.976	1.240	0.486
			TT	0.942	0.778	1.010	0.974	1.140	0.535
			CT	1.230	0.264	0.988	0.963	0.839	0.340

Abbreviations: BMD, bone mineral density; SNP, single nucleotide polymorphism; OR, odds ratio.

et al conditionally knocked out profilin1 by using *DMPI-CRE* and *PFNI*-floxed mice. This conditional deletion of profilin1 specifically in osteocytes led to reduction in the levels of bone volume and BMD.⁵³ This study indicated that *PFNI* was expressed in osteocytes and regulates cell shape, migration and bone mass. In addition, Shirakawa et al investigated the osteoclast-specific functions of *Pfn1* in mice and in cultured osteoclasts, and revealed its negative function in osteoclast movement and bone-resorbing activity.³⁵ These studies have shown that *PFNI* is closely related to the function of osteoblasts and osteoclasts as well as the regulation of bone mass. Therefore, we hypothesized *PFNI* gene polymorphism as a candidate factor for the efficacy of alendronate.

To our knowledge, this is the first study reported a population-based association analysis of *PFNI* gene polymorphism and response to alendronate treatment in postmenopausal Chinese women. In the present study,

500 postmenopausal Chinese women with osteoporosis or osteopenia were given alendronate at a dose of 70 mg weekly for 12 months, and 466 completed the therapy. In total, 7 SNPs in the *PFNI* gene were genotyped in all patients. Initially, the association between candidate polymorphisms and baseline BMD, % change in BMD at L1–4, femoral neck and total hip were analyzed. No association was observed between any SNP or haplotype and baseline BMD or % change in BMD to 12 months of alendronate therapy in postmenopausal Chinese women with low BMD. Next, we considered that the % change of BMD may not represent the pharmacologic treatment response. According to the standards and guidelines established by ISCD 2007 Adult and Pediatric Official Positions, LSC should be considered to eliminate the error effect.⁵⁴ Therefore, we divided the patients into responder(s) group and non-responder(s) group depending on our LSC at L1–4, femoral neck and total hip.

Unfortunately, no SNP or haplotype was associated with the opportunity of being a responder at L-4, femoral neck or total hip. Although *PFNI* has been proved to be the pathogenic gene of early-onset Paget's disease, and it has been confirmed in cell and animal experiments that it is closely related to the skeletal homeostasis, we were unable to find a clear association between the *PFNI* gene polymorphism and the response of Chinese postmenopausal women to alendronate treatment.

The results of this study may be due to the fact that although the *PFNI* gene is considered to be closely related to the function of osteoblasts and osteoclasts, these studies have mainly focused on the cellular and animal levels. At present, the relationship between *PFNI* and human bone mass is not clear. Another possible reason for failure to observe association of *PFNI* polymorphism with the efficacy of alendronate is that unknown environmental factors (including other phenotypes or lifestyle factors such as vitamin D status, dietary characteristics, or medication compliance) may be confounding correlation between *PFNI* polymorphism and the efficacy of alendronate, thereby masking the presence of a genetic association. And the number of subjects in our study was moderate. The rs238243 was detected deviated from HWE and excluded from further analysis. It was likely due to the relatively insufficient sample size. Therefore, it is necessary to testify the relationship between the excluded SNP and efficacy of alendronate treatment by enlarging samples size.

This is the first study to explore the genotype-treatment interaction between genetic variations in the *PFNI* gene and BMD response to alendronate therapy in postmenopausal Chinese women with low BMD. However, we also acknowledge that our study has limitations. First, bone turnover markers were not evaluated. Bone turnover markers are also an important parameter to evaluate the effects of alendronate treatment. Second, 12 months follow-up time might not enough to detect the significant change of BMD. We believe that further studies with longer follow-up may be necessary.

Conclusion

In conclusion, this study demonstrated that polymorphisms of *PFNI* gene may not be a major contributor to therapeutic response to alendronate in Chinese postmenopausal women with low BMD. Further studies that consider additional confounding factors or other types of polymorphisms such as insertion-deletions, copy number variants, and less common SNPs are needed for testing in a larger sample.

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Disclosure

The authors report no conflicts of interest in this work.

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