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Genetic polymorphisms and their influence on therapeutic response to alendronate-a pilot study

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Abstract

Introduction: Osteoporosis has a strong genetic contribution, and several genes have been shown to influence bone mineral density. Variants in the human genome are considered important causes of differences in drug responses observed in clinical practice. In terms of bone mineral density, about 26–53% of patients do not respond to amino-bisphosphonate therapies, of which alendronate is the most widely used.

Material and method: The current study is prospective, observational, analytical, longitudinal and cohort type. It included 25 postmenopausal women treated with alendronate for 1 year. Bone mineral density at lumbar spine and proximal femur was measured and bone turnover markers (C-terminal telopeptide of type I collagen and procollagen 1N-terminal propeptide) were evaluated at 0 and 12 months of treatment. Six single nucleotide polymorphisms in osteoporosis-candidate genes were genotyped (FDPS *rs2297480*, LRP5 *rs3736228*, SOST *rs1234612*, VKORC1 *rs9934438,* GGPS1 *rs10925503* and RANKL *rs2277439)*. Treatment response was evaluated by percentage changes in bone mineral density and bone turnover markers.

Results: The heterozygous CT of FDPS *rs2297480* showed lower increases in BMD values in the lumbar spine region and the homozygous CC of the GGPS1 *rs10925503* showed lower increases in terms of BMD at the total hip region. No association was found for LRP5 *rs3736228*, SOST *rs1234612*, VKORC1 *rs9934438* and RANKL *rs2277439.*

Conclusions: Romanian postmenopausal women with osteoporosis carrying the CT genotype of FDPS *rs2297480* or the CC genotype of GGPS1 *rs10925503* could have an unsatisfactory response to alendronate treatment.

Key words: *osteoporosis; genetic polymorphism; alendronate; bone mineral density; bone turnover markers,*

Introduction

Osteoporosis (OP) is a systemic skeletal disorder characterized by progressive bone loss, leading to deteriorated bone microstructure, compromised bone strength and increased risk of fracture (1). A genetic contribution to OP is well established by linkage and genome-wide association studies that have identified several genes that influence bone mineral density (BMD) variation or fracture risk (2-5).

As it is well established, amino-bisphosphonates (N-BPs) are the first-line therapeutic choice for OP, which is characterized by differing clinical responses (good, moderate or no response). In terms of BMD, about 26–53% of patients do not respond to BPs therapy (6). Among the N-BPs, alendronate (ALN) is the most widely used, as it acts by inhibition of the farnesyl pyrophosphate synthase in osteoclasts, of which the nitrogen moiety makes it more affinitive with bone mass (7).

Variants in the human genome are considered as important causes of differences in drug responses (8-11). To date, there are few and small sampled studies on pharmacogenetics of OP therapies, in which

polymorphisms in genes like *VDR* (12-14), *ER* (15), *LRP5* (16), SOST (17), *DKK1* (18), OPG/RANK/RANKL (19,20), FDPS (21-24) or GGPS1 (25) have been investigated with regard to response to ALN. However, the genes that may determine bone response to ALN have not yet been precisely defined, as studies are under-powered and lack information from different ethnic populations (26).

The effects of antiosteoporotic treatment are determined by the remodeling process of the bone, which involves both resorption by osteoclasts and formation by osteoblasts.

Stimulation of bone formation is necessary to achieve improvements in bone mass, architecture and strength (27).

Recently, the mevalonate signaling pathway had been found to be an important regulator of osteoclast. FDPS and GGPS1 belong to the mevalonate pathyway and are the target of N-BPs in osteoclasts, making them viable candidates for pharmacogenetic studies in OP (23,24).

The wingless integration (Wnt) signaling pathway has been well known as a key regulatory component of bone formation (27). LRP5 and SOST are essential elements of the Wnt pathway, which plays a pivotal role in regulating osteoblast differentiation and bone formation (16,17).

Cell–cell contact between osteoclasts and osteoblasts through the receptor activator of the NF-κβ ligand (RANKL)/receptor activator of the NF-κβ (RANK)/osteoprotegerin (OPG) pathway plays an important role in regulating bone remodeling (28,29). Three major proteins, RANKL, RANK and OPG are key players in this pathway. Several studies have suggested that polymorphic variants in the RANKL, RANK, and OPG genes may influence bone density and turnover (30-33).

There could be other pathways involved in bone homeostasis. Recent evidence suggest that vitamin K also plays an important role in maintaining bone strength and that mutations in the vitamin K epoxi reductase (VKORC1) gene may modify the gamma-carboxylation of osteocalcin and may influence BMD (34,35).

Therefore, the aim of this study is to explore the possible influence of six gene variations on the response to ALN therapy in postmenopausal Romanian women with OP.

Material and method

The current study is prospective, observational, analytical, longitudinal, cohort type. It included 25 postmenopausal women recruited from the Clinical Rehabilitation Hospital in Cluj Napoca, Romania, during June 2016-June 2018.

The inclusion criteria were as follows: (1) women aged 41-75 years old; (2) years since menopause (YSM) more than one year; (3) age at menopause more than 40 years old; (4) BMD T-score at lumbar spine (2-4) (LS) or femoral neck (FN) less than -2.5; (5) could stand up or ambulate at least 30 minutes every day.

Exclusion criteria were as follows: (1) severe liver or renal disease; (3) significant gastrointestinal diseases; (4) treatment with N-BPs or parathyroid hormone within 12 months; (5) treatment with hormone replacement therapy, raloxifene, active vitamin D or calcitonin within 6 months; (6) corticosteroid or anticonvulsant therapy for more than 2 weeks within 3 months; (7) other metabolic or inherited bone diseases; (8) rheumatoid arthritis or other connective tissue disease.

A total of 25 postmenopausal women diagnosed with OP met the inclusion criteria, were enrolled in the study and were prescribed by their physician 70 mg of oral ALN weekly for 1 year. The need for treatment was established by the patient's physician and was not considered an intervention derived from the study protocol. The women were instructed to take ALN with plain water at least half an hour before the first intake of food or oral medication while sitting or standing and not to lie down for at least 30 minutes after dosing. They also took 5600 IU of vitamin D3 daily.

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca (approval no. 249/09.06.2016). All participants were informed of the characteristics of the study and all gave signed informed consent regarding the genetic testing and clinical data collection prior to inclusion.

BMD measurement

BMD at lumbar spine and proximal femur was measured at 0 and 12 months of treatment by Dual-energy x-ray absorptiometry (DXA) (General Electric Lunar Prodigy Advance). According to the definition of World Health Organization, OP was defined as BMD T-score ≤ -2.5 SD at lumbar spine, femoral neck or total hip.

Serum biomarkers of bone turnover

Fasting blood sample was collected from each participant. Bone turnover markers (C-terminal telopeptide of type I collagen - β-CTX, as a bone resorption marker and procollagen 1N-terminal propeptide – PINP, as a bone formation marker) were evaluated at baseline and after 12 months of ALN treatment.

Serum CTX and PINP were measured in the laboratory of the County Hospital Cluj Napoca by ELISA method. The lowest detection limit of β-CTX and PINP was 125 pg/ml and 15 ng/ml, respectively. The intraassay and interassay coefficients of variance were ≤8% for β-CTX and ≤10% for PINP, respectively.

Genotyping

Fasting blood sample was collected on EDTA from each participant and genomic DNA was obtained, using commercially available kits (PureLink Genomic DNA Mini Kit, Invitrogen, Thermo Fisher, USA). We genotyped one SNP in six osteoporosis-candidate genes in all patients (FDPS *rs2297480*, LRP5 *rs3736228*, SOST *rs1234612*, VKORC1 *rs9934438,* GGPS1 *rs10925503* and RANKL *rs2277439)*, using TaqMan SNP Genotyping assays purchased from Applied Biosystems, according to manufacturer's instructions, and run on a QuantStudio 3 real-time PCR machine (Applied Biosystems, Thermo Fisher, USA).

Statistical analysis

Data of normal distribution were presented as mean ± standard deviation. All follow-up measurements were related to the baseline values, and the percentage changes were calculated for each participant. The percentage changes of serum biomarkers and BMD at each site after treatment were calculated by the following formula: change percentage = (parameters after treatment - parameters at baseline)/parameters at baseline × 100%. The percentage changes in BMD and BTM were analyzed using an ANOVA model. Comparisons within-groups were performed on the final visit relative to baseline using paired student's *t* test. Association between the genotype and change percentages of BMD, ALP and β-CTX was calculated by ANCOVA. The statistical analyses were performed using MedCalc Statistical Software version 19.0.6 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2019). A p-value <0.05 was considered statistically significant.

Results

A total of 25 postmenopausal women met the inclusion criteria and were enrolled in the study. The baseline and after treatment characteristics of participants are shown in *Table 1*. The average age, BMI and years since menopause were 67.7 ± 7.05 years, 26.7 ± 4.84 kg/m2 and 22.1 ± 7.2, respectively. The average BMD of LS L1-L4, FN, and TH values was at baseline 0.842 ± 0.104, 0.755 ± 0.112 and 0.785 ± 0.092, respectively. There was significant changes in terms of BMD after 1 year of ALN treatment (all $p<0.05$), except for the lumbar spine region ($p=0.381$). Fracture risk score and values of BTM were significantly lower after 1 year of ALN treatment compared to baseline (all $p<0.05$).

The heterozygous CT of FDPS *rs2297480* SNP showed lower increases in BMD values in the lumbar spine region than the homozygous CC or TT ($p=0.05$), but not in the other measured sites (all $p>0.05$) after 1 year of ALN treatment. Also, the homozygous CC of the GGPS1 *rs10925503* showed lower increases in terms of BMD at the total hip region, compared to the other genotypes ($p=0.03$). Percentage changes in BMD or BTM among genotypes are shown in *Figure 1*. The BMD values increased and the BTM decreased in all patients treated, but no significant association was found when comparing the data among genotypes of LRP5 *rs3736228*, SOST *rs1234612*, VKORC1 *rs9934438* or RANKL *rs2277439*. Correlations between genotypes and BMD or BTM are shown in *Table 2*.

Discussion

The pathophysiology of OP is complex, involving a broad spectrum of endogenous and environmental factors (36,37). When talking about chronic diseases like OP, that require long-term drug therapy, it is desired to choose the most efficient and risk-free pharmacological agent. The large variability among individuals in terms of drug efficiency and treatment response to a pharmaceutical agent is a major challenge in current clinical practice, drug development, and drug regulation (38).

It was previously reported that genetic inheritance accounts for 20% to 95% of the difference in the drug response. If common polymorphisms in the candidate genes for OP modify the effects of ALN on BMD or BTM, identifying and characterizing them might help physicians assess the pharmacogenetic profile of ALN therapy (39). For postmenopausal women with

OP, pharmacological therapy aims to prevent fragility fractures and optimize function, in order to increase health-related quality of life, which is low in this category of patients (40).

The primary pathological mechanism of OP is a bone remodeling imbalance. Therapies mainly focus on the agents that inhibit bone resorption or that promote bone formation. Identification of the potential nonresponders to ALN may be helpful in preventing an exorbitant medical cost and providing the best pharmacological therapy for these patients. To date, it is not been precisely established which genes may influence bone response to ALN (26).

In the present study, we identified SNPs in six osteoporosis candidate genes (FDPS, GGPS1, SOST, LRP5, RANKL, VKORC1) in Romanian postmenopausal women, and evaluated the relationship between *genotypes* and bone response in terms of BMD and BTM to ALN treatment. We found that BMD values increased and the BTM decreased after 1 year of treatment in all patients, but the heterozygous CT of FDPS *rs2297480* SNP showed lower increases in BMD values in the lumbar spine region than the homozygous CC or TT. A study conducted on Danish postmenopausal women with OP showed that the CC genotype for rs2297480 *FDPS* polymorphisms tended to have a lower response in terms of BMD to N-BPs therapy (21). These results are also consistent with the findings of Olmos et al whose research showed that women with CC genotype for rs2297480 *FDPS* lost 1.6% of BMD after 1 year of treatment (23). On the contrast, two studies conducted on Chinese and Korean women showed no association between rs2297480 *FDPS* genotypes and treatment response to ALN in terms of either BMD nor BTM (22,41). The contrasting results could be explained by the differences regarding allelic or genotypic frequencies due to ethnicity, as studies conducted on Caucasians indicate that the presence of the C allele could predict an undesired response to OP therapies, but more research is needed in this area.

It is known that ALN inhibits GGPS1 in the mevalonate pathway, which is why genetic variance of GGPS1 gene is speculated to affect the skeletal response to ALN (42). In a recent study on 540 Chinese women, BMD at the lumbar spine and femoral neck had no obvious difference among all genotypes of GGPS1 gene after ALN treatment (43). Also, a study on Korean postmenopausal women evaluated 2 SNPs in the GGPS1 gene and found that *rs3840452* SNP is correlated to the femoral neck BMD response to N-BPs (41). Results from the present study show that the homozygous CC of the GGPS1 *rs10925503* had lower increases in terms of BMD at the total hip region, compared to the other genotypes, although the strength of association was weak due to small sample size.

Sclerostin, encoded by SOST gene, antagonizes Wnt signaling in osteocytes and osteoblasts by binding to the lipoprotein receptor-related protein (LRP5/6) coreceptor (44). LRP5 is the most important membrane receptor of the Wnt signaling pathway and it was reported as a candidate gene for treatment response to risedronate in men (45). Zhou et al (2015) showed that changes in lumbar spine BMD were correlated to rs1234612 and changes of femoral neck BMD were correlated to rs865429 polymorphism in 545 Chinese postmenopausal women (17). The same group of authors conducted a study on the rs3736228 polymorphism of the LRP5 gene and showed that the presence of the C allele led to a larger increase in lumbar spine BMD after 6 and 12 months of treatment, and that women that are T homozygotes may have a poor response to ALN treatment (16). In the current research, we found no association between genotypes of LRP5 *rs3736228* or SOST *rs1234612* and percentage changes in BMD or BTM.

Some studies have suggested that polymorphic variants in the RANKL, RANK, and OPG genes may influence bone density and bone turnover in men (30). Recently, Zheng et al (2016) evaluated 40 SNP's in the OPG, RANKL, and RANK genes regarding their influence on treatment response to ALN, but no association was observed between any SNP and markers of treatment response in 501 postmenopausal Chinese women with osteoporosis or osteopenia (19). We did not find an association for RANKL *rs2277439* and treatment response to ALN in Romanian postmenopausal women with OP.

It is presumed that there are also other pathways that could influence OP therapeutic response. Even though its haemostatic effect and implication in warfarin sensitivity are well known, there has been evidence that vitamin K also plays an important role in maintaining bone strength and that mutations in the VKORC1 gene may modify the gamma-carboxylation of osteocalcin and may influence BMD (34,35). No association was found between VKORC1 *rs9934438* SNP and BMD or BTM changes after 1 year of ALN treatment.

Our study has several limitations. First, the size of the study was not large enough and limited the statistical power. Second, the treatment period was relatively short. Third, we only evaluated *one* SNP from each gene regarding their involvement in ALN response. Other SNPs should be tested to add more information in the field. Another limitation is that we used a candidate gene approach and included small numbers of participants and very few of the genes have been replicated in two or more independent cohorts. Also, genetic differences between ethnic groups and the complexity of human genome are the main reasons as why no definite gene variations have been conclusively shown to be responsible for the regulation of any anti-osteoporotic drug response. Perhaps by widening the cohort or evaluating more SNPs from the candidate-genes of interest could shed some light in the future regarding pharmacogenetics of OP in Romanian postmenopausal women. These results could be used to build a basis of genotype-tailored guidelines for osteoporosis treatment, such as genotype screening before initiation of bisphosphonate therapy to prevent potential "non-responders" from taking unnecessary medications.

Conclusions

Our study showed that BMD values increased and BTM decreased in all patients treated with ALN for 1 year. The CT genotype of FDPS *rs2297480* SNP had an unsatisfactory response to ALN treatment, as the BMD values in the lumbar spine region did not increase as much as in the other genotypes. Also, the homozygous CC of the GGPS1 *rs10925503* showed lower increases in terms of BMD at the total hip region. No association was found for the other SNPs studied regarding their influence on BMD increases after 1 year of ALN treatment in postmenopausal Romanian women with OP.

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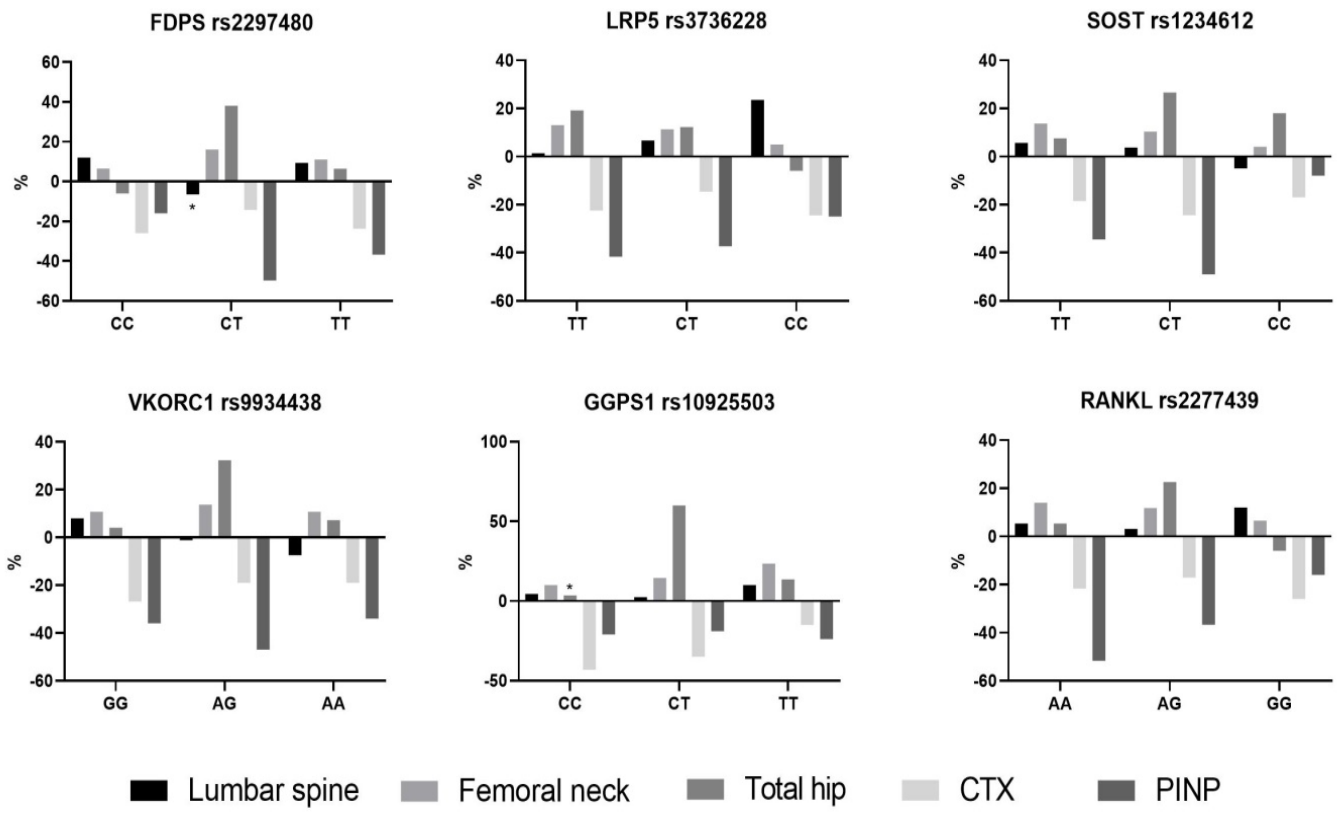

Fig. 1. Percentage changes of BMD and BTM according to genotypes after 1 year of ALN treatment

Table 1. Clinical characteristics of women included in the study at baseline and after 1 year of ALN treatment

Variables	Baseline	After 1 year of ALN	*p*-value
Age, mean ± SD [years]	67.7 ± 7.05	-	-
BMI, mean ± SD [kg/m^2]	26.7 ± 4.84	-	-
Age at menopause, mean ± SD [years]	45.5 ± 5.3	-	-
Years since menopause, mean ± SD [years]	22.1 ± 7.2	-	-
Lumbar spine (L1-L4) BMD, mean ± SD [g/cm^2]	0.842 ± 0.104	0.869 ± 0.103	0.381
Femoral neck BMD, mean ± SD [g/cm^2]	0.755 ± 0.112	0.839 ± 0.110	**<0.001**
Total hip BMD, mean ± SD [g/cm^2]	0.785 ±0.092	0.880 ± 0.186	**0.05**
FRAX - 10 year risk of major osteoporotic fracture, mean ± SD [%]	8.1 ± 3.8	6.7 ± 3.0	**0.008**
FRAX - 10 year risk of hip fracture, mean ± SD [%]	2.6 ± 2.5	1.6 ± 1.9	**0.004**
CTX, mean ± SD [%]	1734 ± 487	1337 ± 293	**<0.0001**
PINP, mean ± SD [%]	305 ± 195	148 ± 46	**0.0002**

Table 2. Correlation between genotypes and percentage changes in bone mineral density and bone turnover markers after 12 months of ALN treatment

Gene/ SNP	Genotypes	Bone mineral density (% change)			Bone turnover markers (% change)	
		LS	FN	TH	CTX	PINP
FDPS rs 2297480	CC (n=2)	12 ± 15.5	6.5 ± 6.3	-6 ± 8.4	-26 ± 9.8	-16 ± 1.4
	CT (n=8)	-6.5 ± 15.9	16 ± 9.9	38.3 ± 74.7	-14 ± 7.7	-49.8 ± 25.4
	TT (n=15)	9.3 ± 14.2	10.6 ± 14.3	6.4 ± 11.9	-23.6 ± 15.3	-36.7 ± 22.9
	p-value	**0.05**	0.524	0.212	0.246	0.169
LRP5 rs 3736228	TT (n=17)	1.4 ± 17.8	13	19.2	-22.5	-41.6
	CT (n=6)	6.6 ± 10.7	11.3	12.3	-14.6	-37.3
	CC (n=2)	23.5 ± 12	5	-6	-24.5	-25
	p-value	0.18	0.704	0.749	0.447	0.655
SOST rs 1234612	TT (n=14)	5.7 ± 16.2	13.7 ± 14.6	7.5 ±15.9	-18.5 ± 10.7	-34.5 ± 24.4
	CT (n=10)	3.7 ± 17.4	10.4 ± 9.9	26.6 ± 68.3	-24.4 ± 16.9	-49 ± 20.9
	CC (n=1)	-5 ± 0	4 ± 0	18 ± 0	-17 ± 0	-8 ± 0
	p-value	0.813	0.389	0.605	0.565	0.146
VKORC1 rs 9934438	GG (n=6)	8 ± 9.5	10.8 ± 15.5	4.1±10.8	-26.8 ± 17.1	-36 ± 27.3
	AG (n=9)	-1.1 ± 19.2	13.8 ± 11.6	32.4 ± 71	-19 ± 12.6	-47.3 ± 25.1
	AA (n=10)	7.4 ± 16.4	11 ± 12.7	7.3 ± 16.7	-18.8 ± 11.8	-34 ± 21.6
	p-value	0.469	0.468	0.377	0.864	0.449
GGPS1 rs 10925503	CC (n=18)	4.4 ± 15.8	10 ± 10.5	3.5 ± 11.9	-43.1 ± 25.4	-20.9 ± 13.8
	CT (n=5)	2.4 ± 22	14.6 ± 15.8	60 ± 89.7	-35 ± 18.8	-19 ± 9.6
	TT (n=2)	10 ± 4.2	23.5 ± 23.3	13.5 ± 19	-15 ± 2.8	-24 ± 25.4
	p-value	0.864	0.326	**0.03**	0.275	0.909
RANKL rs 2277439	AA (n=7)	5.4 ± 16.6	14 ± 15.2	5.4 ± 9.1	-27.7 ± 17.7	-51.7 ± 23.9
	AG (n=16)	3.1 ± 16.8	11.8 ± 12.4	22.7 ± 54.4	-17.1 ± 10.6	-36.7 ± 23.4
	GG (n=2)	12 ± 15.5	6.5 ± 6.3	-6 ± 8.4	-26 ± 9.8	-16 ± 1.4
	p-value	0.768	0.771	0.554	0.189	0.142

Data expressed as mean ± standard deviation
CTX = C-terminal telopeptide of type I collagen ; PINP= procollagen 1N-terminal propeptide; LS= lumbar spine; FN=femoral neck; TH=total hip; ALN= alendronate