Genetic Polymorphism of Geranylgeranyl Diphosphate Synthase (GGSP1) Predicts Bone Density Response to Bisphosphonate Therapy in Korean Women

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Purpose: Genetic factor is an important predisposing element influencing the susceptibility to osteoporosis and related complications. The purpose of the present study is to investigate whether genetic polymorphisms of farnesyl diphosphate synthase (FDPS) or geranylgeranyl diphosphate synthase (GGPS) genes were associated with the response to bisphosphonate therapy. Materials and Methods: In the present study, 144 Korean women with osteoporosis were included. Among 13 genetic polymorphisms found within the FDPS and GGPS1 gene, 4 genetic polymorphisms with frequencies > 5% were selected for further study. Bone mineral density (BMD) response after 1 year treatment of bisphosphonate therapy was analyzed according to the genotypes. Results: Women with 2 deletion allele of GGPS1 -8188A ins/del (rs3840452) had significantly higher femoral neck BMD at baseline compared with those with one or no deletion allele (0.768 ± 0.127 vs. 0.695 ± 0.090 respectively; \( p = 0.041 \)). The response rate of women with 2 deletion allele of GGPS1 -8188A ins/del (28.6%) was significantly lower than the rate of women with one (81.4%) or no deletion allele (75.0%) (\( p = 0.011 \)). Women with 2 deletion allele of GGPS1 -8188A ins/del had 7-fold higher risk of non-response to bisphosphonate therapy compared with women with other genotypes in GGPS1 -8188 after adjusting for baseline BMD (OR = 7.48; 95% CI = 1.32-42.30; \( p = 0.023 \)). Other polymorphisms in FDPS or GGPS1 were not associated with lumbar spine BMD or femoral neck BMD. Conclusion: Our study suggested that GGPS1 -8188A ins/del polymorphism may confer susceptibility to femoral neck BMD response to bisphosphonate therapy in Korean women. However, further study should be done to confirm the results in a larger population.

Key Words: Polymorphism, osteoporosis, bisphosphonate

INTRODUCTION

Osteoporosis is a common disease that affects the majority of older women and men. The pathophysiology of osteoporosis involves a broad spectrum of endogenous...
and environmental factors. At any particular age, about 70% of the bone phenotype variance is explained by genetic factors. Although no single major determinant has been established, polymorphisms in vitamin D receptor gene, collagen type I alpha 1 gene, estrogen receptor gene, interleukin-1 receptor antagonist gene, interleukin-6 gene, transforming growth factor receptor gene, transforming growth factor beta 1 gene, insulin-like growth factor-I pathway, calcitonin receptor gene, apolipoprotein E gene and chromosomal loci 11q 12-13 have been shown to be associated with bone mineral density (BMD).

Recent clinical trials have shown that response rates to bisphosphonate therapy, range from approximately 70% to 75% based on changes in BMD. Although bisphosphonate is known as a potent inhibitor of bone resorption and used for the treatment and prevention of osteoporosis, non-responders to bisphosphonate therapy is of potential concern to clinicians and patients. Several studies have been conducted to identify high risk groups of non-responder(s), based on baseline characteristics, early changes in biochemical markers of bone turnover and early changes in BMD. However, little is known about the common characteristics of non-responder(s) to help clinicians decide whether to initiate bisphosphonates or not. Variability in therapeutic response may reflect complex genetic factors. Indeed, several lines of evidence demonstrated that genetic factors were associated with response of treatment with bisphosphonate. For instance, Qureshi, et al. have identified significant associations between genetic polymorphisms of collagen type I alpha 1 gene and BMD response to alendro-nate treatment. In addition, femoral neck BMD response to cyclic etidronate therapy has been shown to be associated with bone mineral density (BMD).

Genotyping
From all subjects, 10 mL of peripheral blood was obtained. The genomic DNA was extracted from peripheral blood samples with using a PUREGENE blood DNA kit (Gentra, Sanofi-aventis) at a dose of 5 mg/day. Patients were instructed to take the medication orally in the morning at least 30 min before breakfast with abundant water and on an empty stomach after an overnight fast, and to remain upright for at least 30 min after dosing. All patients were supplemented with calcium (200-600 mg elementary calcium per day) and vitamin D (200-800 IU per day).

BMD (g/cm²) of lumbar spine and femoral neck was measured at baseline by dual energy X-ray absorptiometry (DXA) using Lunar Prodigy Densitometer or Lunar Expert-XL densitometer (GE Medical. Systems, Madison, WI, USA). After 12 months of treatment, participants were followed up for BMD measurements.

Materials and Methods

Subjects
The study group comprised pre- and post-menopausal women with newly diagnosed osteoporosis, who were given treatment with bisphosphonate therapy for the first time, and were admitted to two teaching hospitals located in Korea (Seoul National University Hospital and Ajou University Hospital) between September 1998 and January 2005. Clinical, laboratory and radiological studies were performed to exclude secondary causes of osteoporosis, such as hypogonadism, hyperthyroidism, multiple myeloma, and endogenous or exogenous hypercortisolism. Diagnosis of osteoporosis was made using manufacturer-provided reference values for Koreans and World Health Organization T-score criteria (T-score ≤ -2.5 at any site). Among total 182 patients, 38 patients dropped out during study period and 144 patients were finally analyzed, based on per protocol. The study was approved by the committee on human research of Seoul National University Hospital and written informed consent from all subjects was obtained at the time of blood sampling.

Treatment and diagnosis
All women received alendronate (Fosamax, Merck Sharp) at a dose of 10 mg/day or risedronate (Actonel, Sanofi-aventis) at a dose of 5 mg/day. Patients were instructed to take the medication orally in the morning at least 30 min before breakfast with abundant water and on an empty stomach after an overnight fast, and to remain upright for at least 30 min after dosing. All patients were supplemented with calcium (200-600 mg elementary calcium per day) and vitamin D (200-800 IU per day).

Genotyping
From all subjects, 10 mL of peripheral blood was obtained. The genomic DNA was extracted from peripheral blood samples with using a PUREGENE blood DNA kit (Gentra Inc. Minneapolis, MN, USA) following the manufacturer’s protocol.

For the SNP discovery, we screened for genetic variations in the FDPS and GGPSI gene, including 2kb promoter region, using direct sequencing of 24 control samples and sequences were analyzed using an Applied Biosystems (Foster City, CA, USA) 3730xl DNA Analyzer. All SNPs and sequence alignments were analyzed by the Polyphred 5.04 (University of Washington, Seattle, WA, USA; http://droog.mbt.washington.edu/PolyPhred.html). Then, to investigate common polymorphisms, 4 single nucleotide polymorphisms with frequencies > 5% were selected among
the 13 polymorphisms (Table 1).

The FDPS gene polymorphisms were determined by SNP-ITTM assays with the SNPstream 25KTM System (Orchid Biosciences, Princeton, NJ, USA). Briefly, the genomic DNA region spanning the polymorphic site was amplified using one each of phosphotiolated primer and regular PCR primer (Table 2). The genotypes of GGPS1 gene were assayed by single base primer extension assay by using an ABI PRISM SNaPShot Multiplex kit and ABI Prism 3730xl DNA analyzer (ABI, Foster City, CA, USA) according to the manufacturer’s recommendation.

Statistical analysis
Genotype distributions were tested for Hardy-Weinberg equilibrium using $\chi^2$ analysis. Difference in variables between responders and non-responders were tested using t-test for independent samples. Differences in continuous variables were assessed by linear regression analyses with or without adjusting for baseline BMD. Age and body mass index (BMI) were tested as covariates in the model, however, the association between genetic polymorphisms and BMD were not significantly changed. Therefore, they were omitted in the final model for statistical stability. Results are presented as mean ± standard deviation (SD). A $p$ value of $< 0.05$ was considered statistically significant.

Non-responder(s) to treatment were defined as subjects who experienced any decrease in first one year following BMD measurements. Non-responders in respect of lumbar spine BMD or femoral neck were analyzed separately. Association between response rate and genotype was calculated using Fisher’s exact test. The risk of non-response to treatment was estimated with odds ratios (ORs) and 95% confidence intervals (CIs).

**Table 1. Results of Discovery of Genetic Polymorphisms among 48 Control Samples**

| Gene | rs number | Location | Genotype | Frequency (n = 48) |
|------|-----------|----------|----------|------------------|
| FDPS | rs2297480*| Promoter | -99 C / A | C : A = 0.766 : 0.234 |
| FDPS | rs16836819| Exon2    | Asn68Asn | C : T = 0.989 : 0.011 |
| FDPS | DL1000995| Intron5  | IVS5 + 86G / C | G : C = 0.99 : 0.01 |
| FDPS | DL1000990| Intron6  | IVS6 + 42C / T | C : T = 0.99 : 0.01 |
| FDPS | rs11264361*| Intron8 | IVS8 + 66G / T | G : T = 0.793 : 0.207 |
| GGPS1 | rs3840452*| Promoter | -8188T ins / del | Ins : del = 0.67 : 0.33 |
| GGPS1 | DL1001049| Promoter | -7908C ins / del | Ins : del = 0.99 : 0.01 |
| GGPS1 | DL1001050| Promoter | -6757G / ins[G] | G : Ins[G] = 0.969 : 0.031 |
| GGPS1 | DL1001051| Exon1 (5’UTR) | -6677C / T | C : T = 0.969 : 0.031 |
| GGPS1 | DL1000992| Intron2  | IVS2 + 23T / C | T : C = 0.979 : 0.021 |
| GGPS1 | DL1000993| Intron2  | IVS2 + 114T / C | T : G = 0.989 : 0.011 |
| GGPS1 | DL1000994| Intron2  | IVS2 + 404A / G | A : G = 0.969 : 0.031 |
| GGPS1 | rs3841735*| Intron3  | IVS3 - 13A del / ins | Del : Ins = 0.75 : 0.25 |

DL number represents Korean specific SNP.
*Selected SNPs for further analysis.

**Table 2. Primer Sequence for PCR and SNP-ITTM assay and/or SNaPShot**

| Gene | rs number | Primer | Sequence(5’ - 3’) |
|------|-----------|--------|------------------|
| FDPS | rs2297480| PCR forward | TAATTGTCCCCAAGCACATT |
| FDPS | rs2297480| PCR reverse | AAAGGCAGAGGCATGGTG |
| FDPS | rs2297480| Genotyping | AGGGAGGGGCACTCTGGGCTAAGGC |
| FDPS | rs11264361| PCR forward | TAGCAGAGACAAGGCACCA |
| FDPS | rs11264361| PCR reverse | AAAGAGTGCAAGGTAATCATCCT |
| FDPS | rs11264361| Genotyping | TTAACCTCTCCTCTGGCCAGGAAACC |
| GGPS1 | rs3840452| PCR forward | AAGTCACAAACCACACACTTCT |
| GGPS1 | rs3840452| PCR reverse | CTCGGCCCTCAGGTGT |
| GGPS1 | rs3840452| Genotyping | TTTGCGGCTGCGCCTGGT |
| GGPS1 | rs3841735| PCR forward | AGCTCTGCAAGTAGGTAATGG |
| GGPS1 | rs3841735| PCR reverse | TCTCTTCAGGTGGGCAAGT |
| GGPS1 | rs3841735| Genotyping | AATAAGTGAATTTTCATATTTT |

The FDPS gene polymorphisms were determined by SNP-ITTM assays with the SNPstream 25KTM System (Orchid Biosciences, Princeton, NJ, USA).
confidence intervals (95% CIs), using logistic regression models adjusting for baseline BMD. Statistical analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

General characteristics and results of measurements of the 144 participants are shown in Table 3. The average age, height, weight and BMI were 61.5 ± 9.9 yrs, 153.6 ± 5.6 cm, 55.5 ± 8.6 kg, and 23.5 ± 3.3 kg/m², respectively. The average baseline and 1 year follow-up BMD of lumbar spine were 0.789 ± 0.122 g/cm² and 0.839 ± 0.126 g/cm², respectively. The average percent change of lumbar spine was 6.7 ± 7.0%. The average baseline and 1 year follow-

### Table 3. Characteristics of Study Subjects before and after 12 Months of Treatment with Bisphosphonate (n = 144)

| Characteristics | Mean ± SD |
|-----------------|-----------|
| Age (yrs)       | 61.5 ± 9.9|
| Height (cm)     | 153.6 ± 5.6|
| Weight (kg)     | 55.5 ± 8.6|
| BMI (kg/m²)     | 23.5 ± 3.3|

Lumbar spine BMD
- Baseline BMD (g/cm²) 0.789 ± 0.122
- Follow-up BMD (g/cm²) 0.839 ± 0.126
- Percent change (%) 6.7 ± 7.0

Femoral neck BMD
- Baseline BMD (g/cm²) 0.698 ± 0.093
- Follow-up BMD (g/cm²) 0.724 ± 0.097
- Percent change (%) 3.7 ± 7.2

BMD, bone mineral density.

### Table 4. Association between FDPS and GGPS1 Genetic Polymorphisms and Bone Mineral Density (BMD) before and after 12 Months of Treatment with Bisphosphonate (10 mg/day) (mean ± SD)

| Gene | rs number and position | Number | Major homozygous | Heterozygous | Minor homozygous | p values* |
|------|-------------------------|--------|------------------|--------------|-----------------|-----------|
| FDPS | rs2297480 -99C / A | Lumbar Baseline | 788 ± 0.120 | 784 ± 0.132 | 821 ± 0.129 | 0.473 |
|      |                         | Follow-up | 840 ± 0.117 | 833 ± 0.145 | 887 ± 0.113 | 0.368 |
|      |                         | % change | 7.0 ± 7.6 | 6.0 ± 5.5 | 8.9 ± 9.7 | 0.264 |
|      |                         | Femoral Baseline | 696 ± 0.089 | 693 ± 0.095 | 730 ± 0.135 | 0.346 |
|      |                         | Follow-up | 720 ± 0.094 | 718 ± 0.098 | 810 ± 0.133 | 0.060 |
|      |                         | % change | 3.6 ± 6.4 | 3.4 ± 8.6 | 8.5 ± 10.0 | 0.059 |
| FDPS | rs11264361 IVS8 + 66G / T | Lumbar Baseline | 787 ± 0.120 | 787 ± 0.132 | 812 ± 0.115 | 0.540 |
|      |                         | Follow-up | 837 ± 0.119 | 839 ± 0.145 | 864 ± 0.116 | 0.825 |
|      |                         | % change | 6.7 ± 7.5 | 6.5 ± 5.2 | 8.0 ± 8.8 | 0.724 |
|      |                         | Femoral Baseline | 697 ± 0.089 | 694 ± 0.097 | 730 ± 0.112 | 0.268 |
|      |                         | Follow-up | 718 ± 0.094 | 722 ± 0.099 | 790 ± 0.106 | 0.310 |
|      |                         | % change | 3.4 ± 6.3 | 3.7 ± 8.8 | 6.7 ± 8.0 | 0.108 |
| GGPS1 | rs3840452 -8188T ins / del | Lumbar Baseline | 793 ± 0.114 | 782 ± 0.141 | 791 ± 0.036 | 0.961 |
|      |                         | Follow-up | 838 ± 0.115 | 844 ± 0.146 | 821 ± 0.029 | 0.999 |
|      |                         | % change | 6.0 ± 6.7 | 8.1 ± 7.4 | 2.2 ± 2.2 | 0.101 |
|      |                         | Femoral Baseline | 705 ± 0.091 | 681 ± 0.086 | 768 ± 0.127 | 0.041 |
|      |                         | Follow-up | 736 ± 0.097 | 700 ± 0.092 | 771 ± 0.112 | 0.374 |
|      |                         | % change | 3.2 ± 6.0 | 5.0 ± 8.9 | 0.8 ± 4.6 | 0.533 |
| GGPS1 | rs3841735 IVS3-13A del / ins | Lumbar Baseline | 796 ± 0.128 | 777 ± 0.125 | 777 ± 0.051 | 0.805 |
|      |                         | Follow-up | 842 ± 0.130 | 837 ± 0.131 | 814 ± 0.056 | 0.430 |
|      |                         | % change | 6.1 ± 6.4 | 8.0 ± 7.7 | 3.4 ± 4.2 | 0.158 |
|      |                         | Femoral Baseline | 706 ± 0.091 | 681 ± 0.094 | 708 ± 0.098 | 0.732 |
|      |                         | Follow-up | 738 ± 0.100 | 701 ± 0.091 | 717 ± 0.099 | 0.329 |
|      |                         | % change | 3.4 ± 6.0 | 5.1 ± 9.4 | 1.3 ± 4.4 | 0.372 |

BMD, bone mineral density.

*p values were calculated by linear regression analysis (recessive model, major homozygous + heterozygous vs. minor homozygous); p values of follow-up and % changes were adjusted for baseline BMD.
up BMD of femoral neck were 0.698 ± 0.093 g/cm² and 0.724 ± 0.097 g/cm², respectively. The average percent change of lumbar spine was 3.7 ± 7.2%. Responders at lumbar spine BMD were older than non-responder(s) (62.5 ± 9.8 vs. 55.6 ± 9.1; \( p < 0.05 \)). At femoral neck, the age between responders and non-responder(s) was not significantly different. There were no significant differences in the height, weight and BMI between responders and non-responder(s), at lumbar spine and femoral neck.

All genotype frequencies of \( FDPS \) and \( GGPS1 \) were in Hardy-Weinberg equilibrium. For \( FDPS \) rs2297480, \( FDPS \) rs11264361, \( GGPS1 \) rs3840452 and \( GGPS1 \) rs3841735, Hardy-Weinberg equilibrium \( \chi^2 \) (p-value) were 0.08 (0.96), 3.12 (0.21), 0.62 (0.73) and 0.01 (0.99), respectively. Height and weight were significantly associated with the genetic polymorphisms of \( FDPS \) and \( GGPS1 \). Women with \( FDPS \) AA genotype (rs2297480) or TT genotypes (rs11264361) had higher weight than women with other genotypes (\( p < 0.01 \)). Women with \( GGPS1 \) -8188A two del alleles (rs3840452) had higher height than women with other genotypes (\( p = 0.017 \)) (data not shown). Associations between BMD and genetic polymorphisms of \( GGPS1 \) and \( FDPS \) are shown in Table 4. Participants with two deletion allele of \( GGPS1 \) -8188A ins/del had significantly higher baseline femoral neck BMD than those with one or no deletion allele (0.768 ± 0.127 vs 0.695 ± 0.090; \( p = 0.041 \) recessive model). This association remained significant after adjusting for age and BMI (\( p = 0.018 \)), but not significant after adjustment for either height or weight (data not shown).

Based on the response criteria at femoral neck BMD, the

| Variant | Gene / Locus | Genotype | Non-responder n (%) | Responder n (%) | Response rate (%) | Recessive Model OR* (95% CI) |
|---------|-------------|----------|---------------------|----------------|------------------|----------------------------|
| **Lumbar spine BMD** | | | | | | |
| rs2297480 | \( FDPS \) | CC | 14 (70) | 71 (60) | 83.5 | 0.90 |
| -99 | CA | 5 (25) | 41 (35) | 89.1 | (0.10 - 8.01) |
| AA | | 1 (5.0) | 6 (5.1) | 85.7 | p = 0.926 |
| rs11264361 | \( FDPS \) | GG | 15 (71) | 77 (64) | 83.7 | 1.42 |
| IVS8 + 66 | GT | 4 (19) | 36 (30) | 90.0 | (0.28 - 7.27) |
| TT | | 2 (9.5) | 8 (6.6) | 80.0 | p = 0.671 |
| rs3840452 | \( GGPS1 \) | Ins / Ins | 16 (76) | 64 (53) | 80.0 | 1.18 |
| -8188A | Ins / Del | 4 (19) | 52 (43) | 92.9 | (0.13 - 10.66) |
| Del / Del | | 1 (4.8) | 5 (4.1) | 83.3 | p = 0.883 |
| rs3841735 | \( GGPS1 \) | Del / Del | 14 (70) | 66 (56) | 82.5 | 1.02 |
| IVS3 - 13T | Del / Ins | 5 (25) | 46 (39) | 90.2 | (0.12 - 9.02) |
| Ins / Ins | | 1 (5.0) | 6 (5.1) | 85.7 | p = 0.983 |
| **Femoral neck BMD** | | | | | | |
| rs2297480 | \( FDPS \) | CC | 16 (57) | 50 (63) | 75.8 | N / A |
| -99 | CA | 12 (43) | 24 (30) | 66.7 | |
| AA | | 0 (0) | 5 (6) | 100 | |
| rs11264361 | \( FDPS \) | GG | 17 (61) | 52 (63) | 75.4 | N / A |
| IVS8 + 66 | GT | 11 (39) | 22 (27) | 66.7 | |
| TT | | 0 (0) | 8 (9.8) | 100 | |
| rs3840452 | \( GGPS1 \) | Ins / Ins | 15 (54) | 45 (55) | 75.0 | 7.48 |
| -8188A | Ins / Del | 8 (29) | 35 (43) | 81.4 | (1.32 - 42.30) |
| Del / Del | | 5 (18) | 2 (2) | 28.6 | p = 0.023 |
| rs3841735 | \( GGPS1 \) | Del / Del | 15 (56) | 46 (58) | 75.4 | 4.41 |
| IVS3 - 13T | Del / Ins | 8 (30) | 30 (38) | 78.9 | (0.89 - 21.79) |
| Ins / Ins | | 4 (15) | 3 (3.8) | 42.9 | p = 0.068 |

BMD, bone mineral density.

*ORs were calculated by logistic regression model (recessive model, major homozygous + heterozygous vs. minor homozygous) adjusting for baseline BMD. Nonresponders to treatment was defined as subjects who have any decrease in the 1 year following BMD measurements. N / A, not applicable.
We found five and eight genetic polymorphisms of response to treatment.

Analyses including other phenotypes or lifestyle factors other influence or confounding factors. Due to the study covariates (baseline BMD, age and BMI), there could be ins/del and response rate was significant after adjusting for target molecules for bisphosphonate treatment.23

GGPS1 plausible to expect that the polymorphism could alter and GGPS1 were not associated with either baseline BMD or age and BMI) (< 0.05). The sensitivity of GGPS1 -8188A ins/del polymorphism genotyping for predicting responder at the femoral neck for the “ins/ins” or “ins/del” genotype was 97.6% (80/82) with a specificity of 17.9% (5/28), and a positive predictive value of 77.7% (80/103).

Analyses with additive or dominant models did not show significant results. Other polymorphisms in FDPS or GGPS were not associated with either baseline BMD or response to treatment.

**DISCUSSION**

We found five and eight genetic polymorphisms of FDPS and GGPS, respectively, in Korean women and investigated whether the genetic factors were associated with BMD as well as the response to bisphosphonate treatment. Among Korean women, GGPS1 -8188A ins/del genetic polymorphism was associated with BMD at baseline and the response rate to bisphosphonate therapy at femoral neck.

FDPS and GGPS are important enzymes in the isoprenoid biosynthesis pathway, which is important target of bisphosphonate treatment. In this study, we were not able to provide mechanistic insight into the functional consequences of the GGPS1 -8188A ins/del polymorphism. However, since GGPS1 -8188A ins/del polymorphism is located at the promoter region of GGPS1 gene, it may be plausible to expect that the polymorphism could alter GGPS1 gene transcription level and alter the GGPS concentration in turn. The efficacy of bisphosphonate to induce apoptosis of osteoclasts could be changed when GGPS concentration changes, since GGPS is one of the target molecules for bisphosphonate treatment.21

Even though the association between GGPS1 -8188A ins/del and response rate was significant after adjusting for covariates (baseline BMD, age and BMI), there could be other influence or confounding factors. Due to the study design and limitation to obtain patient information, further analyses including other phenotypes or lifestyle factors such as serum bone turnover markers, vitamin D status, PTH level, fracture history, smoking or alcohol history, dietary characteristics, or compliance to medication were not feasible. Future study including these factors could enhance the knowledge regarding the pharmacogenetic effect of FDPS and GGPS polymorphisms on bisphosphonate therapy. In addition, given that multiple comparison testing was performed in small number of participants, this study may have high risk of type I error (false positive).

GGPS1 -8188A ins/del was significantly associated with response rate at femoral neck, but not at lumbar spine. Similar to the result at femoral neck, the response rate of subjects with rare homozygote (del/del) at lumbar spine (83.3%) was also lower than that of subject with common homozygote (ins/ins) or heterozygote (ins/del) (85.3%), although not statistically significant. Small sample size might be the cause of insignificant statistical result regarding lumbar spine. The effect of this polymorphism on cortical bone and trabecular bone might be different, as many previous studies have suggested that cortical bone and trabecular bone have different drug effect and different regulating genes.24,25 Further study with larger sample size and mechanism analyses could clarify this issue.

No gene-dose effect was found regarding the association between GGPS1 -8188A ins/del and response rate at femoral neck. This lack of gene-dose effect could result from mechanism by which this polymorphism influences the bisphosphonate response. Since the GGPS1 gene encodes an enzyme, haploinsufficiency might not have enough effect to alter the response rate. Further study regarding the GGPS enzyme activity and the substrate levels would reveal the mechanism.

GGPS1 -8188A ins/del was significantly associated with response rate at femoral neck and baseline femoral neck BMD, but no significant difference was found in the % change in BMD at femoral neck. In accordance with the response rate, the % change in BMD at femoral neck of subject with two del allele was lower than that of subject with one or no del allele, although not statistically significant. This might also be due to small sample size, and further studies with larger sample size are needed for the verification.

The baseline BMD of subject with two del allele of GGPS1 -8188A ins/del polymorphism was significantly higher than those with one or no deletion allele. However, this association was not significant when height was included as a covariate. And GGPS1 -8188A ins/del polymorphism was significantly associated with height. Also, FDPS polymorphisms were significantly associated with weight. Since isoprenoid biosynthesis pathway including FDPS and GGPS enzyme is essential in the steroid synthesis, genetic difference in FDPS or GGPS gene could
alter the height and weight. However this association regarding height and weight is beyond the scope of present study and further study is needed to investigate this issue.

Since the effect of bisphosphonate appears slowly in the course of treatment, more than two years follow-up BMD data could be adequate enough to determine a true response rate to bisphosphonate. However, a well designed study to predict subsequent BMD response to bisphosphonate from early BMD response suggested that any improvement in BMD at 6 or 12 months is strongly indicative of subsequent BMD response. Therefore, we assumed that one year follow-up BMD would be sufficient to classify “non-responder(s)” and “responders”.

In the present study, we used “0%” (any decrease in BMD) as a cut-off value for identifying “non-responder(s)”, consistent with previous studies. Other investigators also used “3% increase” as a cut-off value for significant BMD increase (least significant change) due to innate precision error of the test, and suggested that a “0-3% increase” should be considered as an “indeterminable response” rather than a significant response. We chose 0% as our cut-off value for two reasons. First, as the main objective of the present study was to identify definite “non-responder(s)” (any decrease in BMD) in the early course of therapy, the use of 0% as a cut-off value would be more reasonable to separate “indeterminable response” patients (0-3% increase in BMD) from the definite “non-responder(s)” (any decrease in BMD). Second, in light of the fact that more than 1 year is often required for bisphosphonate to sufficiently increase BMD by more than 3%, the use of cut-off value of 3% would underestimate the response rates in our short-term follow-up study design (1 year).

There are several limitations to the present study. Considerably small sample size does limit the statistical power of the present study. In particular, the number of subjects with two del/del allele of GGPS1 -8188A ins/del polymorphism was too small to make a firm conclusion. Lack of information regarding the risk factors for osteoporosis (level of physical activity, serum bone turnover markers, PTH level, fracture history, smoking or alcohol history, dietary characteristics, or compliance to medication) is an another important limitation to the present study. Other limitations include multiple testing issue and lack of biochemical results to support the underlying mechanisms. Future studies with larger population and functional mechanisms are needed and we are in a process to pursue this project.

In conclusion, our present study indicates that GGPS1 -8188A ins/del polymorphism is associated with the femoral neck BMD response rate to bisphosphonate therapy, although the strength of association was weak due to small sample size. 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-responder(s)” from taking unnecessary medications.

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