J Bone Miner Metab (2010) 28:578–584
DOI 10.1007/s00774-010-0167-2

ORIGINAL ARTICLE

High level of serum undercarboxylated osteocalcin in patients with incident fractures during bisphosphonate treatment

Masataka Shiraki · Yasushi Yamazaki · Yumiko Shiraki · Takayuki Hosoi · Naoko Tsugawa · Toshio Okano

Received: 8 December 2009 / Accepted: 3 February 2010 / Published online: 11 March 2010
© The Japanese Society for Bone and Mineral Research and Springer 2010

Abstract To evaluate the possible interaction of metabolic effects in the mevalonate pathway between amino-bisphosphonates (amino-BP) and vitamin K, the serum level of undercarboxylated osteocalcin (ucOC) was measured in amino-BP users in relationship to incident fracture occurrence. Osteoporotic patients (mean age, 70.7 ± 9.1 years; $n = 231$) treated with alendronate or risedronate were followed for 3.4 ± 2.1 years, and observations regarding the presence or absence of incident fractures in their vertebrae were made based on vertebral X-ray films every year. During the observation period, new fractures were found in a total of 71 patients (incident vertebral fracture, $n = 61$; the remaining 10 patients had long bone fractures). The baseline data of the patients with incident fractures indicated that incident fractures are more likely to occur in older patients who have a higher number of prevalent vertebral fractures and lower baseline lumbar bone mineral density (LBMD) as compared to patients without incident fractures. There was no significant difference in the changes of LBMD and urinary excretion of NTX after treatment. On the other hand, the serum level of ucOC in patients with incident fractures and with amino-BP treatment was significantly higher (2.75 ± 0.19 ng/ml) than that in patients without incident fractures and with amino-BP treatment (2.28 ± 0.13 ng/ml) ($P = 0.038$). These results indicate that older age, a greater number of prevalent fractures and higher ucOC levels, and lower LBMD are risks for incident fractures despite use of amino-BP. The time-dependent incident fracture rate was higher in accordance with an increase in the number of risk items ($P < 0.001$ in log-rank and Wilcoxon tests). In conclusion, measurement of undercarboxylated osteocalcin may be useful for assessing fracture risk in patients receiving amino-BP treatment.

Keywords Undercarboxylated osteocalcin (ucOC) · Bisphosphonates treatment · Osteoporosis · Incident fractures · Bone mineral density

M. Shiraki (✉) · Y. Yamazaki · Y. Shiraki
Research Institute and Practice for Involutional Diseases, 1610-1 Meisei, Misato, Azumino, Nagano 299-8101, Japan
e-mail: ripid@fc4.so-net.ne.jp

T. Hosoi
Department of Clinical Research and Development, National Center for Geriatrics and Gerontology, Aichi, Japan

N. Tsugawa · T. Okano
Department of Hygienic Sciences, Kobe Pharmaceutical University, Hyogo, Japan

Introduction

Recent progress in key pathogenesis of osteoporosis has focused on bone resorption through increased osteoclastic activity. Bisphosphonates specifically inhibit osteoclastic activity through inhibition of the mevalonate pathway [1], achieving a decrease in bone turnover followed by an increase in secondary mineralization of bone. Such actions of bisphosphonates are connected to prevention of bone fractures in osteoporosis [2] because bisphosphonates turn the negative bone balance positive. Although bisphosphonate has been established as a first-line drug for preventing fractures in osteoporosis, complete inhibition of new fractures in osteoporosis has not been achieved [3–5]. This failure may be partly explained by the concept that complex pathogenesis of osteoporosis and the reduction in bone turnover or increase in bone density induced by bisphosphonates may not be sufficient to achieve thorough inhibition of incident fractures. In fact, deficiencies of many

nutrients such as vitamin D, calcium, and vitamin K have accounted for possible risk factors of incident fractures in osteoporosis [6, 7]. Among these nutrients, vitamin K deficiency or insufficiency has been consistently reported as a risk factor for osteoporotic fractures [7–9]. Vitamin K is thought to maintain bone strength through gamma-carboxylation of matrix glutamic acid residues of protein. In vitamin K insufficiency or deficiency, a small amount of undercarboxylated osteocalcin (ucOC) is released from the osteoblasts into circulation. Thus, the serum concentration of ucOC has been recognized as being a sensitive marker of vitamin K deficiency in bone. Serum ucOC decreases significantly after menatetrenone (vitamin K_2) [10, 11] or vitamin K_1 [12] treatment, suggesting that vitamin K homologues may improve bone osteocalcin content and may be linked to reduction of the incident fracture rate. Furthermore, Okano et al. [13] reported that phylloquinone (vitamin K_1) can be converted to menaquinone (vitamin K_2) in various cells, including osteoblasts, through geranylgeranylation in the side chain, and that menaquinone 4 was considered to be an active form of vitamin K because menaquinone 4 was reported to bind to nuclear receptor SXR [14]. It is possible that this metabolic process of vitamin K activation may be inhibited by bisphosphonates as a result of inhibition of geranylgeranylation of protein through reduction of farnesyl diphosphate (FPP) synthase activity [1, 2]. Therefore, there may be a close relationship between the effect of bisphosphonates on cell function and vitamin K activation in the same cell. However, until now, there have been no data regarding the relationship between the state of vitamin K and the effect of bisphosphonates on fracture prevention. In this study, the authors attempted to investigate preliminarily whether the state of vitamin K in bone modulates the effect of bisphosphonates on fracture prevention.

Materials and methods

Subjects

Ambulatory postmenopausal women more than 45 years old with primary osteoporosis and undergoing amino-bisphosphonates (amino-BP) treatment during the period from January 2000 to June 2008 were eligible for participation in the study. Exclusion criteria consisted of endocrine disorders such as hyperthyroidism or hyperparathyroidism, a history of extensive gastrointestinal surgery or chronic renal failure, and current use of medications known to result in secondary osteoporosis. The patients were participants in a Nagano cohort study, and therefore baseline examinations such as bone density measurement and measurements of serum levels of calcium, phosphate, and urinary excretion of N-telopeptide of type I collagen (NTX) had been performed for baseline data with informed consent. Baseline X-ray examinations to confirm the presence or absence of preexisting fractures were also performed at the time that the patients registered for the Nagano cohort study. The period for conducting follow-up observations of each participant was calculated as the time from their inclusion into the study up to their death, minus 1 year after the occurrence of incident fractures or to the end of June 2009, whichever occurred first. Follow-up was conducted on all the subjects in this study for more than 1 year.

Intervention

Study subjects were started on amino-BP treatment, either alendronate 5 mg/day orally or risedronate 2.5 mg/day orally by Japanese dosage regulation. Alternatively, some patients received equivalent weekly doses of alendronate (35 mg/week) or risedronate (17.5 mg/week). Patients were continued on amino-BP treatment for the duration of their participation in the study. Vitamin K_2 administration was prohibited in the participants. All the patients were treated with amino-BP alone during the entire observation period.

Bone mineral density (BMD) measurements

Lumbar spine bone mineral density (LBMD) was measured by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX-L or DPX-IQ (Lunar Corporation, Madison, WI, USA). The interassay variance of LBMD in the laboratory was 0.5 ± 0.5% [coefficient of variation (CV) ± SD] [15]. To guard against machine drift, a quality assurance test was carried out for every measurement. The baseline value of LBMD was used to diagnose osteoporosis, and measurements of LBMD were repeated every 6 months. The value of the last observation was used as the value of LBMD after the treatment.

Detection of prevalent and incident vertebral fractures

Prevalent and incident vertebral fractures were diagnosed by a semiquantitative visual method using lateral thoracolumbar spine radiographs in accordance with the method described by Genant et al. [16]. To detect incident vertebral fractures, spine radiographs were routinely taken at 1-year intervals, and additional X-rays were taken whenever the subjects complained of symptoms suggestive of new clinical vertebral fractures. Both new clinical and morphometric fractures were counted as incident vertebral fractures. Incident long bone fractures were identified from medical records or confirmed using X-ray films. Although incident clinical (symptomatic) fractures in vertebrae or

other parts of the bone structure were easily recognized when they occurred, morphometric vertebral fractures were sometimes difficult to detect clinically. Because the exact timing when a morphometric incident vertebral fracture occurred could not be determined for some of the patients with such fractures, the time of the spinal radiograph showing the fractures was considered as being the time of the fracture. Fractures induced by major trauma were excluded from the analysis; namely, fractures induced by a fall from standing height were categorized as incident fractures, but fractures induced by a fall from a point higher than body height were excluded.

Diagnosis of osteoporosis

Diagnosis of osteoporosis was made in accordance with the osteoporosis diagnostic criteria (2000 version) proposed by the Japanese Society for Bone and Mineral Research [17]. Osteoporosis is diagnosed as the presence of fragility fractures in any bone lesion in a person with a BMD less than 80% (–1.63 SD) of the BMD of the young adult mean (YAM). Osteoporosis is also diagnosed when the LBMD is less than 70% (–2.45 SD) of the BMD of a YAM, even if the person has no prevalent fragility fractures.

Biochemical indices

Nonfasting serum and urine samples were collected as baseline data at the time of enrollment. Routine biochemical data including serum levels of calcium and phosphate were analyzed immediately using an autoanalyzer. Urinary N-terminal telopeptides of type I collagen (NTX) were measured with an enzyme-linked immunosorbent assay (ELISA) kit (Osteomark Ostex, Princeton, NJ, USA), and the value of NTX was standardized by the concentration of creatinine in the same urine sample. Urine samples were collected during the second voiding of the day. Urinary NTX was measured before and at the end of the observation.

Measurement of undercarboxylated osteocalcin

Serum level of undercarboxylated osteocalcin (ucOC) was measured using a new electrochemiluminescence immunoassay (Sanko Junyaku, Ibaraki, Japan) [8]. Because ucOC measurements were not available when the study began, serum level of ucOC could not be measured at baseline for any of the participants, but during treatment values could be obtained for all participants. Measurement of ucOC in patients without incident fracture was conducted at the end of the observations. On the other hand, for patients with incident fractures, serum samples were taken 1 or more years after the occurrence of incident fractures to determine ucOC.

Ethical considerations

The study protocol was reviewed by the ethical committee of the Research Institute and Practice for Involutional Diseases (RIPID), and comprehensive written informed consent was obtained from all study subjects.

Statistical analysis

In the descriptive analysis of the baseline characteristics, numerical data are expressed as mean ± SD. Comparisons of baseline characteristics between subjects with and without incident fractures were performed using two levels of one-way analysis of variance (ANOVA). Comparisons between the values before and after treatment were based on a paired t test. To assess confounding effects of the risks, stepwise multiple regression analysis was used. After confirmation of independent risks for incident fractures in amino-BP users, secondary analyses were carried out: the sum of the existing risk factors in individual subjects was calculated, and the patients were categorized by the calculated number of risks. Subsequently, time-dependent incident fracture rates were analyzed using a Kaplan–Meier plot. Here, the number of patients with high ucOC in each category of risk was tested by Pearson's Chi-square test. The level of significance was set at less than 0.05 (Table 1).

Results

Demography of the subjects

From among the patients visiting the outpatient care unit of Research Institute and Practice for Involutional Diseases, a total of 269 patients with osteoporosis were recruited for this study. Of these patients, 38 were excluded from the study because of lack of baseline data or missing follow-up data. The remaining 231 patients were followed for 1 or more years and were adapted to the following analyses. The mean ± SD age of participants was 70.0 ± 9.1 years old, and 140 subjects (60.6%) had prevalent fractures. The average observation period was 3.4 ± 2.1 years, with the longest observation period being 9 years. After bisphosphonate treatment, urinary excretion of NTX decreased significantly, from 55.2 to 30.0 nM/mM Cr (45.7% of the baseline; $P < 0.0001$ in paired t test), and LBMD increased significantly, from 0.774 to 0.844 g/cm^2 (+9.0% increase from baseline; $P < 0.0001$ in paired t test).

Table 1 Comparison of baseline data and data at end of observation of subjects

Item	Baseline	End of observation
Age (years)	70.7 ± 9.1	74.1 ± 8.9*
Body weight (kg)	49.1 ± 7.4	47.5 ± 7.6
Body height (cm)	149.2 ± 6.3	148.2 ± 5.8
Serum Ca (mg/dl)	9.19 ± 0.41	9.24 ± 0.43
Serum Pi (mg/dl)	3.49 ± 0.46	3.50 ± 0.50
NTX (nM/mM Cr)	55.2 ± 30.7	30.0 ± 19.3*
Initial bone mineral density (BMD) (g/cm^2)	0.774 ± 0.129	0.844 ± 0.144*

Values are expressed as mean ± SD. *$P < 0.0001$ versus baseline in paired t test

Table 2 Fracture outcomes during the observation

Site of fractures	Baseline	Incident fractures
None	208	160
Vertebrae	117	61
Colles	9	4
Hip	5	2
Other sites	9	4

Multiple prevalent fractures in multiple bone sites were observed in 14 cases; incident fractures were counted as the first incident fracture

Prevalent and incident fractures in the participants

A total of 154 sites of prevalent fractures were counted in 140 patients, indicating that 14 cases had multiple prevalent fractures. Incident fractures were observed in 71 cases during the observation period, and the most prominent fracture site was the vertebral body, with both morphometric and clinical symptomatic fractures (Table 2).

Baseline data of patients with and without incident fractures

To screen for risks for incident fractures in amino-BP users, comparisons were made on baseline data between patients with incident fractures and those without incident fractures during the observation period. Table 3 shows the comparison of baseline data between patients with and without incident fractures. Patients with incident fractures during amino-BP treatment were characterized by older age and a lower initial lumbar BMD as compared to the patients without incident fractures. The number of prevalent vertebral fractures in the patients with incident fractures was higher than that of the patients without incident fractures, suggesting that incident fractures may occur in more severe cases of osteoporosis even during bisphosphonate treatment.

Bone outcomes after treatment in patients with and without incident fractures

Follow-ups were conducted on all the patients treated with amino-BP in the form of LBMD, urinary NTX, serum levels of calcium and phosphate, and measurement of ucOC at the end of the observation period. Comparisons were made in the values obtained between patients with and without incident fractures to determine what kinds of changes occurred in bone parameters after the treatment in association with incident fractures (Table 4).

Among the various outcomes related to bone metabolism, only serum level of ucOC was significantly higher in the patients with incident fractures than in those without, suggesting that vitamin K deficiency in bone may exist in the patients with incident fractures.

Stepwise regression analysis for the risk of future fractures in amino-BP users

From the primary analyses, the baseline age, LBMD, number of prevalent vertebral fractures, and ucOC after treatment were considered to be risks for fracture susceptibility in bisphosphonate users. To exclude confounding factors, multiple stepwise regression analysis among these risks was performed. The four risks just mentioned were recognized as independent risks for incident fractures in bisphosphonates users (Table 5).

Logistic regression analysis and receiver operating characteristic (ROC) analysis for each risk to evaluate the risk assessment of each patient (Fig. 1)

To evaluate the time-dependent fracture rate, secondary analyses using a Kaplan–Meier plot analysis were performed. After deciding the cutoff value for each risk using ROC analysis, the sum of the risks (0–4) was calculated for each participant. The cutoff values for each risk were as follows: 75 years or older for age, 0.763 g/cm^2 or less for LBMD, two or more for number of prevalent vertebral fractures, and 2.6 ng/ml or more for ucOC. The patients were divided into five categories in accordance with the presence of risks. Group 0 consisted of patients without any risk ($n = 31$), group 1 consisted of patients with one risk ($n = 67$), group 2 consisted of patients with two risks ($n = 75$), group 3 consisted of patients with three risks ($n = 47$), and group 4 consisted of patients with four risks ($n = 11$). Groups 0, 1, 2, 3, and 4 consisted of 2, 11, 20, 24,

Table 3 Baseline data in the patients with or without incident fractures during amino-bisphosphonates (amino-BP) treatment

Items	Incident fracture (–)	Incident fracture (+)	*P*
Number of cases	160	71	–
Age (years)	69.0 ± 0.7	74.3 ± 1.1	<0.0001
BMI (kg/m^2)	21.8 ± 0.2	22.3 ± 0.4	0.272
Serum Ca (mg/dl)	9.23 ± 0.03	9.14 ± 0.05	0.060
Serum P (mg/dl)	3.53 ± 0.04	3.41 ± 0.05	0.083
NTX (nM/mM Cr)	54.1 ± 2.5	57.2 ± 3.7	0.448
Initial BMD (g/cm^2)	0.788 ± 0.010	0.743 ± 0.015	0.0157
Number of prevalent vertebral fractures	1.09 ± 0.16	2.58 ± 0.25	<0.0001

The baseline data were compared between the patients with or without incident fractures, retrospectively. A total of 71 patients had new fractures during amino-BP treatment. The patients with incident fractures were of higher age, with a greater number of prevalent vertebral fractures and lower lumbar bone mineral density (LBMD) at baseline. Data are expressed as mean ± SE. Statistical analysis was made by analysis of variance (ANOVA)

Table 4 Bone-related outcomes at the end of observation in the patients with or without incident fractures

Outcomes	Incident fracture (–)	Incident fracture (+)	*P*
LBMD (g/cm^2)	0.854 ± 0.011	0.823 ± 0.017	0.131
Ca (mg/dl)	9.26 ± 0.03	9.16 ± 0.05	0.091
P (mg/dl)	3.54 ± 0.04	3.43 ± 0.06	0.140
NTX (nM/mM Cr)	28.6 ± 1.5	33.2 ± 2.3	0.104
ucOC (ng/ml)	2.28 ± 0.13	2.75 ± 0.19	0.038

Data listed in Table 4 were obtained at the end of the observations. In a case having incident fracture, serum and urinary samples were taken 1 or more years after the occurrence of incident fracture or 1 or more years after the recognition of morphometric fracture (2.6 ± 0.6 years after the recognition of incident fracture). Data are expressed as mean ± SE. ucOC, undercarboxylated osteocalcin

Table 5 Stepwise regression analyses of the risks for fracture susceptibility in amino-BP users

Risk	χ^2	*P*	R^2
Number of prevalent vertebral fracture	21.999	0.0000	0.0772
Age (years)	9.1164	0.0025	0.1092
ucOC (ng/ml)	4.4385	0.0351	0.1247
Baseline LBMD (g/cm^2)	3.8923	0.0485	0.1384

Stepwise regression analysis was performed among the four risks, which may relate to having incident fractures during amino-BP treatment. All four risks were significantly associated with incident fractures

and 9 patients with incident fractures, respectively. The Kaplan–Meier plot indicated a significantly higher susceptibility of incident fractures in accordance with an increasing number of risks (see Fig. 1). Furthermore, the number of patients with high ucOC (>2.6 ng/ml) in each category is shown in Table 6. The percentages for the patients with high ucOC were significantly increased in

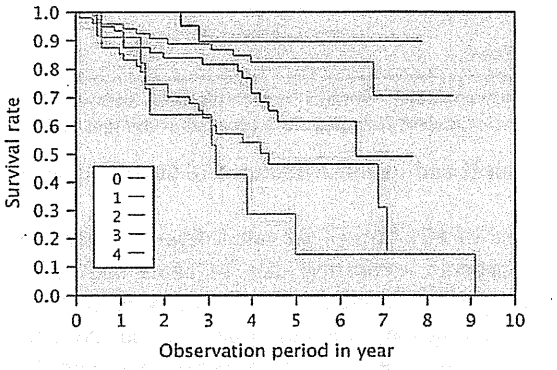

Fig. 1 Kaplan–Meier plot of the categorized patients under amino-bisphosphonate (amino-BP) treatment in accordance with the number of fracture risks (0–4). The lower survival rate from incident fractures was observed in accordance with the number of fracture risk in patients with amino-BP-treated osteoporosis ($P < 0.001$ in log-rank and Wilcoxon test)

accordance with increase in number of risks ($P < 0.0001$), providing further proof that high ucOC was an independent risk factor for incident fractures in osteoporosis during bisphosphonates treatment.

Discussion

In this study, traditional risk factors, such as older age, low BMD, and the presence of preexisting fractures [18], for incident fractures in osteoporosis were also recognized as risks for incident fractures in bisphosphonate users. However, no significant contribution of incident fractures to changes in LBMD and bone resorption markers was seen, although such changes considerably favored fracture prevention; that is, LBMD increased by about 11% and 8% for patients with and without incident fractures, respectively, and urinary excretion of NTX decreased by about 60% and

Table 6 Rate of patients with high ucOC in five risk categories

Risk category	ucOC < 2.6 ng/ml	ucOC ≥ 2.6 ng/ml	Percent (%)
0	31	0	0
1	48	19	28.4
2	46	29	38.7
3	19	28	59.6
4	0	11	100

The trend for the rate of patients with high ucOC was apparently increased in accordance with increase in number of risks. $\chi^2 = 49.1$ and $P < 0.0001$ by Peason's Chi-square test

50% for patients with and without incident fractures, respectively. These changes in LBMD and urinary NTX were not significantly different between patients with and without incident fractures. These changes in parameters were in good agreement with previous reports obtained from Japanese postmenopausal osteoporosis [19]. Therefore, the biological effects of bisphosphonates on bone density and resorption were identical regardless of the occurrence of new fractures during treatment. Previous reports have indicated that increase in BMD and decrease in bone markers were not strong predictors for future fractures in bisphosphonates users [20–24].

On the other hand, the serum level of ucOC of fractured patients was significantly higher than that of patients without any new fractures. Secondary analysis using stepwise regression analysis revealed that the level of ucOC in amino-BP users was a significant independent risk for incident fractures (Table 5). Because ucOC was measured after an incident fracture occurred, it was thought that the difference in ucOC levels between patients with and without incident fracture may have reflected the occurrence of a fracture but not a vitamin K deficiency in bone. However, this possibility is unlikely because urinary excretion of NTX did not differ between these two groups, indicating that there was no significant difference in bone resorption. It has been recently reported that the biological action of amino-BP and activation of vitamin K have a point of contact, that is, the geranylgeranylation of molecules. Amino-BP inhibits this process [1, 2] and, in contrast, vitamin K activation requires geranylgeranylation of the side chain [13]. Therefore, it is possible that bisphosphonates may interfere with vitamin K activation in bone cells, especially in patients with preexisting vitamin K deficiency. This hypothesis prompted the authors to measure ucOC in patients treated with amino-BP. Results indicate that patients with incident fractures during amino-BP treatment may be lacking vitamin K in bone cell levels consequent to a higher level of ucOC. However, Hirano et al. [24] reported that amino-BP treatment caused a decrease in ucOC level but resulted in no change in carboxylated osteocalcin (cOC), suggesting that amino-BP did not decrease the carboxylation of osteocalcin. So, further clarification is required whether amino-BP treatment affects carboxylation on osteocalcin in both patients with or without vitamin K deficiency in a prospective study design. This is the first report that suggests the usefulness of measuring ucOC in amino-BP users in terms of fracture prediction. Because ucOC decreased after vitamin K_2 treatment [11], concurrent use of vitamin K_2 with amino-BP may be effective in preventing the occurrence of new fractures in patients with higher ucOC levels during treatment with only amino-BP.

The limitations of this study were as follows. First, the present results must be evaluated as a prospective study design because the present study was a retrospective study. Second, a larger number of participants is necessary to increase statistical power. Third, direct evidence that shows that bisphosphonates inhibit vitamin K activation in bone cells is required in vitro, and we have to measure serum carboxylated osteocalcin level together with ucOC to assess gamma-carboxylation during amino-BP treatment in vivo. Last, because other risk factors for fracture such as the physical activity of the participants was not evaluated in the present study, those factors related to increased susceptibility to fractures should be incorporated into the model in future.

In conclusion, it is useful to evaluate the serum level of ucOC in patients with amino-BP to predict future fracture occurrence.

Acknowledgments The authors thank Sanko Junyaku Co., Ltd., for measuring serum undercarboxylated osteocalcin concentrations; we also thank Choju Aoki for his assistance. M Shiraki, T Hosoi, and T Okano have received honoraria for lectures from Eizai Pharmaceutical Co.

References

1. Luckman SP, Hughes DE, Coxon FP, Russell RGG, Rogers MJ (1998) Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP binding proteins, including ras. J Bone Miner Res 13:581–589
2. Russell RGG, Watts NB, Ebetino FH, Rogers MJ (2008) Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. Osteoporos Int 19:733–759
3. Liberman UA, Weiss SR, Broll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW, Dequeker J, Favus M, Seeman E, Recker R, Capizzi T, Santora AC, Lombardi A, Shah RV, Hirsch LJ, Karpf DB, For the Alendronate Phase III Osteoporosis Treatment Study Group (1995) Effects of oral alendronate on bone mineral density and the incidence of fracture in postmenopausal osteoporosis. N Engl J Med 333:1437–1443
4. Black DM, Cummings SR, Karpf DB, Cauley JA, Thompson DE, Nevitt MC, Bauer DC, Genant HK, Haskell WL, Marcus R, Ott SM, Torner JC, Quandt SA, Reiss TF, Ensrud KE, For the Fracture Intervention Trial Research Group (1996) Randomized

trial of effect of alendronate on risk of fracture in women with existing vertebral fracture. Lancet 348:1535–1541
5. Harris ST, Watts NB, Genant HK, McKeever CD, Hangartner T, Keller M, Chesnut CH 3rd, Brown J, Eriksen EF, Hoseyni MS, Axelrod DW, Miller PD (1999) Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial. Vertebral Efficacy with Risedronate Therapy (VERT) Study Group. JAMA 282:1344–1352
6. Tang BM, Eslick GD, Nowson C, Smith C, Bensoussan A (2007) Use of calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. Lancet 370:657–666
7. Cockayne S, Adamson J, Lanham-New S, Shearer MJ, Gilbody S, Torgerson DJ (2006) Vitamin K and the prevention of fractures: systemic review and meta-analysis of randomized controlled trials. Arch Intern Med 166:1256–1261
8. Tsugawa N, Shiraki M, Suhara Y, Kamao M, Ozaki R, Tanaka K, Okano T (2008) Low plasma phylloquinone concentration is associated with high incidence of vertebral fracture in Japanese women. J Bone Miner Metab 26:79–85
9. Kaneki M, Hedges SJ, Hosoi T, Fujiwara S, Lyons A, Crean SJ, Ishida N, Nakagawa M, Takechi M, Sano Y, Mizuno Y, Hoshino S, Miyao M, Inoue S, Horiki K, Shiraki M, Ouchi Y, Orimo H (2001) Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K_2: possible implications for hip-fracture risk. Nutrition 17:315–321
10. Shiraki M, Shiraki Y, Aoki C, Miura M (2000) Vitamin K_2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. J Bone Miner Res 15:515–521
11. Shiraki M, Itabashi A (2009) Short-term menatetrenone therapy increases gamma-carboxylation of osteocalcin with a moderate increase of bone turnover in postmenopausal osteoporosis: a randomized prospective study. J Bone Miner Metab 27:333–340
12. Binkley N, Harke J, Krueger D, Engelke J, Vallarta-Ast N, Gemar D, Checovich M, Chappell R, Suttie J (2009) Vitamin K treatment reduces undercarboxylated osteocalcin but does not alter bone turnover, density or geometry in healthy postmenopausal North American women. J Bone Miner Res 24:983–991
13. Okano T, Shimomura Y, Yamane M, Suhara Y, Kamao M, Sugiura M, Nakagawa K (2008) Conversion of phylloquinone (vitamin K_1) into menaquinone 4 (vitamin K_2) in mice. Two possible routes for menaquinone-4 accumulation in cerebra in mice. J Biol Chem 283:11270–11279
14. Tabb MM, Sun A, Zhou C, Grun F, Errandi J, Romero K, Pham H, Inoue S, Mallick S, Lin M, Forman BM, Blumberg B (2003) Vitamin K_2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. J Biol Chem 278:43919–43927
15. Shiraki M, Shiraki Y, Aoki C, Hosoi T, Inoue S, Kaneki M, Ouchi Y (1997) Association of bone mineral density with apolipoprotein E phenotype. J Bone Miner Res 12:1438–1445
16. Genant HK, Jergas M, Palermo L, Nevitt M, Valentin RS, Black D, Cummings SR (1996) Comparison of semiquantitative visual and quantitative morphometric assessment of prevalent and incident vertebral fractures in osteoporosis. The Study of Osteoporotic Fractures Research Group. J Bone Miner Res 11:984–996
17. Orimo H, Hayashi Y, Fukunaga M, Sone T, Fujiwara S, Shiraki M, Kushida K, Miyamoto S, Soen S, Nishimura J, Oh-hashi Y, Hosoi T, Gorai I, Tanaka H, Igai T, Kishimoto H (2001) Osteoporosis diagnostic criteria for primary osteoporosis: year 2000 revision. J Bone Miner Metab 19:331–337
18. Shiraki M, Kuroda T, Nakamura T, Fukunaga M, Hosoi T, Orimo H, Makino K (2006) The sample size required for intervention studies on fracture prevention can be decreased by using a bone resorption marker in the inclusion criteria: prospective study of a subset of the Nagano Cohort, on behalf of the Adequate Treatment of Osteoporosis (A-TOP) Research Group. J Bone Miner Metab 24:219–225
19. Shiraki M, Kushida K, Fukunaga M, Kishimoto H, Taga M, Nakamura T, Kaneda K, Minaguchi H, Inoue T, Morii H, Tomita A, Yamamoto K, Nagata Y, Nakashima M, Orimo H (1999) A double-masked multicenter comparative study between alendronate and alfacalcidol in Japanese patients with osteoporosis. Osteoporos Int 10:183–192
20. Eastell R, Barton I, Hannon RA, Chines A, Garnero P, Delmas PD (2003) Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate. J Bone Miner Res 18:1051–1056
21. Seibel MJ, Naganathan V, Barton I, Grauer A (2004) Relationship between pretreatment bone resorption and vertebral fracture incidence in postmenopausal osteoporotic women treated with risedronate. J Bone Miner Res 19:323–329
22. Bauer DC, Garnero P, Hochberg MC, Santora A, Delmas P, Ewing SK, Black DM, For the Fracture Intervention Research Group (2006) Pretreatment levels of bone turnover and the antifracture efficacy of alendronate: the fracture intervention trial. J Bone Miner Res 21:292–299
23. Seibel MJ (2005) Biochemical markers of bone turnover. Part I: Biochemistry and variability. Clin Biochem Rev 26:97–122
24. Hirano M, Hashimoto J, Ando W, Ono T, Yoshikawa H (2008) Response of serum carboxylated and undercarboxylated osteocalcin to alendronate monotherapy and combined therapy with vitamin K_2 in postmenopausal women. J Bone Miner Metab 26:260–264

J Bone Miner Metab (2010) 28:601–607
DOI 10.1007/s00774-010-0217-9

REVIEW ARTICLE

Genetic aspects of osteoporosis

Takayuki Hosoi

Received: 21 May 2010 / Accepted: 7 July 2010 / Published online: 10 August 2010
© The Japanese Society for Bone and Mineral Research and Springer 2010

Abstract The multiple factors contributing to the pathogenesis of osteoporosis include genetic and environmental factors. Because decrease in bone mineral density (BMD) is the major clinical indicator and a useful quantitative trait, many association and linkage studies of BMD have been conducted. Although the series of studies showed apparently significant associations, the genes have not been found that can be utilized in clinical practice. Several genes identified in robust genome-wide association studies will be the new cutting edge in genetic studies of osteoporosis. Our recent reports of functional single nucleotide polymorphism in the tissue-nonspecific alkaline phosphatase gene and gamma-carboxylase gene are presented in this review to discuss the future prospects in the genetic research of osteoporosis from the point of view of genome–nutrition interaction.

Keywords Osteoporosis · Single nucleotide polymorphism · Bone mineral density · Genetics

Introduction

Osteoporosis brings about deterioration in activities of daily living (ADL) and quality of life (QOL) of the affected patients. Although fragility fractures resulting from osteoporosis continue to increase in the current aging society, it is assumed that this disease is still undertreated [1]. In the 1990s, osteoporosis was defined as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk [2]. This definition reflects the importance of bone mass and microarchitecture in determining bone strength. Because there have not been practical measures of microarchitecture, bone mass or bone mineral density (BMD) has been used as a quantitative trait in searching the genes for osteoporosis. It seems reasonable that a vast series of association and linkage studies have been conducted with BMD, but one should keep in mind that BMD is one of the complex traits of osteoporosis and one of the surrogate markers for bone fragility.

Recently, osteoporosis was redefined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture [3]. In the previous definition of osteoporosis, low BMD was not considered as a sole factor of osteoporosis, but the new definition declares more clearly that bone strength is determined not only by BMD but also by factors other than BMD [3]. According to the new definition, the genes of osteoporosis should be a group of genes contributing to the multiple aspects of pathogenesis. Although case–control studies by defining the case with the diagnostic criteria of this disease are suggested, the diagnosis of osteoporosis might not be suitable as a "phenotype" in genetic studies because the diagnosis contains biologically heterogeneous components. In this review, genetic aspects of osteoporosis are discussed mainly using BMD as one of the measurable phenotypes of osteoporosis.

T. Hosoi is a recipient of the 2002 JSBMR Distinguished Scientist Award.

T. Hosoi (✉)
Department of Clinical Research and Development,
National Center for Geriatrics and Gerontology, Aichi, Japan
e-mail: t-hosoi@ncgg.go.jp

Genetic aspects of bone phenotypes

Predisposing factors of osteoporosis include both lifestyle factors and genetic factors. The first step in preventing osteoporosis should be the reduction of lifestyle-related risk factors. On the other hand, the genetic factors cannot be removed even when these are identified. However, it will be useful if one can learn that she or he has the genetically predisposing factor(s) and is thus motivated to avoid certain lifestyle risk factors.

Family history of fractures is included among the established risk factors for osteoporotic fractures [4], indicating the importance of the genetic background of osteoporosis. Twin studies also supported the heritability of BMD [5], which is the most valuable indicator of bone strength. On the other hand, it was reported that the possibility that the genetic determinants of BMD and those of fractures might be different [6]. Factors contributing to the variation in bone quality will be studied further from genetic aspects. For example, femoral neck cross-sectional geometry was successfully used as a clinical measure in quantitative trait locus analyses [7].

Approaches for the pathogenesis of diseases can be classified as deductive or inductive. Analyses about the roles of known substances or genes would be classified into deductive approaches and belong to the genetic approach. On the other hand, the recent availability of whole genome information has made the inductive approach possible, which is named a genome-wide association study (GWAS). In this mini-review, the recent genetic and genomic approaches for osteoporosis are reviewed, and our studies on the functional single-nucleotide polymorphisms (SNPs) related to osteoporosis are introduced.

Candidate gene approaches to the determinants of bone mineral density

Until now, BMD has been utilized most widely as a quantitative measure in genetic and genomic studies for osteoporosis. Quite a few association studies with BMD have been done with so-called candidate gene approaches [8]. Candidate genes have been chosen based on basic bone cell biology and clinical observations. In addition, the genome-wide linkage and association studies will show novel series of candidate genes that should be investigated further.

Association studies with the polymorphisms of these genes were done using genetic polymorphisms. Among the polymorphisms, SNPs were most commonly utilized. SNPs in the regulatory region (rSNPs) and those in coding regions (cSNPs) could be related to quantitative or qualitative variations of the gene expressions. In addition, other SNPs, for example, those in introns, could affect the gene expression or could be markers for genomic study. Microsatellite polymorphisms (e.g., dinucleotide repeat or triple repeat) are other kinds of polymorphisms that have also been utilized in osteoporosis research.

Association studies with candidate gene polymorphisms have been published by many groups including ours [9–40]. If you search the database using the key words "gene polymorphisms and bone mineral density," 1,000 and more articles will be hit. The genes analyzed are classified into nuclear receptors and related molecules, collagen and other matrix proteins, receptor activator of nuclear factor-kappa B ligand (RANKL)/RANK system, cytokines and related molecules, hormones and related molecules, enzymes, cell cycle-related molecules, lipoprotein receptor-related peptides (LRPs) and Wnt signals, cell-surface molecules, transcription factors, and others (Table 1). However, the contribution of most genes to determining BMD is small and the result is not always reproducible [41, 42]. Lifestyle-related factors as confounding factors against genetic factors should be managed in the association studies. In addition, ethnic factors have to be considered appropriately [43].

The vitamin D receptor gene has been studied most extensively, but the implications of vitamin D receptor gene polymorphisms have not been established [44]. Recent extensive meta-analyses [45] showed that the effects of the vitamin D receptor gene polymorphisms seem modest, although the significant effects of the polymorphisms on BMD and osteoporotic fractures were proved.

Searches for functional SNPs affecting variation in bone metabolism

When polymorphisms of genes were significantly and reproducibly associated with bone phenotypes, biological relevancy should be confirmed, and the methods of clinical application should be considered following that process. In other words, it would be a rational method in the genetic approach for osteoporosis to examine the association of functional polymorphisms with bone phenotypes. Although the contribution of each polymorphism to BMD would be small, the significant effects of each polymorphism supported by functional studies will be a clue suggesting that the gene should play important roles in the pathogenesis of osteoporosis.

We reported two functional SNPs in two genes that are related to the variation in BMD of the elderly. The first one was an SNP in the tissue-nonspecific alkaline phosphatase (TNSALP) gene [46]. TNSALP resides in the plasma membrane of osteoblasts and supplies phosphate to the

Table 1 Genes studied in candidate gene approaches for bone mineral density (BMD)

Nuclear receptors and related molecules
Vitamin D receptor
Estrogen receptor-α
Estrogen receptor-β
Androgen receptor
Glucocorticoid receptor
Peroxisome activator receptor-γ
Nuclear receptor co-activator-3
Erα co-factor retinoblastoma-interacting zinc finger protein
Collagen and other matrix protein
Type I collagen-α1
Type I collagen-α2
Osteocalcin
Matrix gla protein
Alpha 2-HS glycoprotein
RANKL/RANK system
RANKL
RANK
Osteonectin/SPARC
Cytokines and related molecules
Transforming growth factor-β1
Insulin-like growth factor-1
Tissue necrosis factor-α
TNFRSF1B
TNFRSF11B
TNF receptor-associated factor-6
Bone morphogenetic protein-2
Bone morphogenetic protein-4
LTBP3
Interleukin-6
Interleukin-1
Interleukin-1 receptor antagonist
Interleukin-1β
Interleukin-10
Tissue necrosis factor
Tissue necrosis factor receptor
Smad 6
TGF-β receptor-3
Adiponectin
Myostatin
Hormones and related molecules
Calcitonin
Calcitonin receptor
Thyroid hormone receptor
TSH receptor
Calcium-sensing receptor
PTH
PTH/PTHRP receptor
Dopamine receptor D4
Prepro-NPY
Growth hormone
Growth hormone receptor
POMC
Cannabinoid receptor type 2
Leptin receptor
β3-adrenergic receptor
Vitamin D-binding protein
SHBG
Enzymes
Adenyl cyclase
Methylene tetrahydrofolate reductase
Methionine synthase
Catalase
Farnesyl diphosphate synthase
Farnesyl pyrophosphate synthase
FMS-related tyrosine kinase
Aromatase
p450
Carbon anhydrase
Angiotensin-converting enzyme
CYP1A1
CYP1B1
CYP3A7
CYP3A4*18
CYP17
CYT19
COMT
eNOS
GGCX
Urokinase
PAI-1
ALDH2
Pituitary glutamyl cyclase
Phosphodiesterase 40
Tissue-nonspecific alkaline phosphatase
CYP1A1
ALOX15
ALOX12
Lactase
Paraoxonase
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase
Rho GTPase-Rho REF
WRN
Matrix metalloproteinase-1
Cathepsin K
Mature metalloproteinase-9
Delta-aminolevulinic dehydrogenase
Uridine diphosphate glucuronyl transferase 2B7

Table 1 continued

Cell cycle-related molecules
p57
Cdx-2
Cyclin D1
CD38
LRPs and Wnt signals
LRP5
LRP6
WISP 1
FZD 1
"multiple Wnt pathway genes"
SOST
Cell-surface molecules
Duffy antigen receptor
Toll-like receptor 4
GALR3 receptor
CC domain receptor 2
CD38
CD40
CLCN7 (chloride channel)
Osteoclast-associated receptor
Vascular proton pomp
Purinergic P2RX7 receptor
Semaphorin 7
GALR3 receptor
Transcription factors
RUNX2/CBRA1
Microphthalmia-associated transcription factor
Forkhead box C2
Others
Perilipin

calcification site. We searched for nonsynonymous and functional SNPs in the exons of this gene. As a result, an SNP in exon 7 (787C > T), which replaces tyrosine at codon 246 to histidine, gives the biochemical differences between the products of each genotype. The K_m value of 787 His was smaller than that of 778 Tyr, which means that persons with 787 His may supply phosphate to the calcification site more efficiently. Elderly Japanese women with 787 His had higher radial BMD than those with other genotypes. This study demonstrated the importance of phosphate metabolism in bone metabolism in the elderly. Additional in vitro experiments supported the biochemical variations resulting from this polymorphism [47, 48]. Further studies are underway to examine the clinical meaning of this variation, for example, the effects of this genotype on the relationship between phosphate intake and hormones in calcium metabolism and aging.

Another gene is vitamin K-dependent gamma-glutamyl carboxylase (GGCX) [49]. GGCX carboxylates vitamin K-dependent proteins including bone Gla protein (osteocalcin) and matrix Gla protein. Functional polymorphisms in the GGCX gene, if any, might explain the variation in bone metabolism and BMD. Also in this case, polymorphisms in the exons were screened in Japanese elderly women and a nonsynonymous SNPs was found: about 8762 G > A (Arg325Gln). When the kinetic parameters of GGCX325-Gln and GGCX325-Arg were compared in vitro, V_{max}/K_m was significantly higher for GGCX325-Gln than for GGCX325-Arg. Association study of this polymorphism with radial BMD of Japanese postmenopausal women showed that the body mass index (BMI)-adjusted Z score in the subpopulation older than 75 years was higher in those with 325 Gln than those with 325 Arg/Gln or 325 Arg. In this study, we first reported the different activities of GGCX between the common genotypes and their association with BMD. Vitamin K deficiency is known as a nutritional risk factor for osteoporotic fractures, and a regimen of vitamin K_2 is utilized for osteoporosis treatment. The common allelic variation in the GGCX gene may explain the individual variation in the response to nutritional and/or pharmacological intervention with vitamin K. It would be rational to utilize the allele information in finding the level of vitamin K intake at which the effects of the genotype with lower enzymatic activity can be avoided. We have already reported that this GGCX gene polymorphism affects the correlation between the vitamin K status and gamma-carboxylation of osteocalcin in young males [50], and this kind of study is awaited in the group of elderly.

Implication from monogenic bone diseases

There are rare diseases involving bone that are caused by mutations of single genes and considered to be monogenic diseases. The causative genes of these diseases were identified by linkage analyses of the affected families. These genes would have important implications for the variations of bone phenotype also in the general population. A distinguished example is the gene for osteoporosis-pseudoglioma syndrome (OPPG) [51]. Positional cloning with the affected pedigrees showed that rare mutations in lipoprotein receptor-related peptide 5 (LRP5) gene cause the disease. In addition, another mutation in the same gene was demonstrated to cause a syndrome with high BMD [52]. It is also interesting that the LRP5 gene resides in the locus that has been among the loci related to BMD in the linkage studies [53]. Several groups including ours examined the relationship between the polymorphisms of LRP5 gene and BMD, and the results were reproducible [54–56].

Table 2 Candidate genes suggested by genome-wide association studies

Genes	Chromosome location
ADAMTS18 (ADAM metallopeptidase with thrombospondin type 1 motif, 18)	16q23
TGFBR3 (transforming growth factor-beta receptor III)	1p33–32
TNFRSF11B (tumor necrosis factor receptor superfamily, member 11b, osteoprotegerin)	8q24
LRP5 (lipoprotein receptor-related protein 5)	11q13.4
Receptor activator of nuclear factor-kappa B ligand (RANKL)	13q14
Osteoprotegerin (OPG)	8q24
Estrogen receptor-1 gene (ESR1)	6q25.1
Zinc finger and BTB domain containing 40 genes (ZBTB40)	1q36
Major histocompatibility complex region	6p21

In addition, the LRP5 gene was screened out by the recent GWAS, as mentioned below [57]. These results strongly suggest that variations in this gene would contribute to the variation of BMD in the general population.

Genes suggested by genome-wide association studies

Systematic search for the genes for osteoporosis has been done by genome-wide linkage studies with pedigrees, which have shown some hotspots linked to BMD, for example, those on chromosome 11 [53]. Further fine mappings were required to specify the genes contributing to the pathophysiology of osteoporosis and consequent analyses of their functions in bone biology. Recent advances in analyzing SNPs distributing to the whole genome area made it possible to conduct a GWAS (Table 2). One of the GWAS studies identified two SNPs, rs4355801 on chromosome 8 and rs3736228 on chromosome 11 [57]. The former is close to the osteoprotegerin gene and the latter nonsynonymous SNP is in the LRP5 gene, both of which are major components in bone biology. In the series of candidate gene approach, the significant correlation between the polymorphisms in LRP5 gene has been reproducible. The identification of the LRP5 gene in the GWAS study further strengthens the importance of this gene in the pathogenesis of osteoporosis. Osteoprotegerin was also identified to be correlated with BMD in another GWAS study [58].

Other examples of genes identified in GWAS studies are RANKL [58], estrogen receptor 1 (ESR1) [58], ADAM metallopeptidase with thrombospondin type 1 motif, 18 (ADAMTS18) [59], and transforming growth factor-beta receptor III (TGFBR3) [59].

Prevention of osteoporotic fractures is the major clinical goal of osteoporosis therapy, and the incidence of osteoporotic fractures should be an ideal phenotype used in the genetic studies searching the genes for osteoporosis. Recently, Kung et al. [60] reported the association of the JAG1 gene with osteoporotic fractures as well as BMD with GWAS study. They also demonstrated the possible molecular mechanism with which the genetic variation of this gene affects bone metabolism [60].

Discussion

Selection of candidate genes for polymorphism studies of osteoporosis is rather arbitrary. This situation cannot be avoided because we do not know how many genes are involved in the pathogenesis of osteoporosis or in the determination of BMD. Recent genome-wide studies with a large population size are successfully overcoming this issue, and several genes were identified for osteoporosis. These genes include the novel series of candidate genes whose implications should be studies. So far the new list of genes contains "previous" candidate genes that are well known in the field of bone biology.

BMD is a surrogate marker for bone fragility, and one should not consider the genes for low BMD as immediately being those for osteoporosis. Although BMD is still a useful quantitative measure in genetic studies for osteoporosis, other phenotypes, particularly the incidence of fractures, should be kept in mind. Further studies are required to utilize the products of genetic studies for the advancement of osteoporosis practice.

References

1. Kroth PJ, Murray MD, McDonald CJ (2004) Undertreatment of osteoporosis in women, based on detection of vertebral compression fractures on chest radiography. Am J Geriatr Pharmacother 2:112–118
2. Consensus Development Center (1993) Consensus Development Conference V, 1993. Diagnosis, prophylaxis and treatment of osteoporosis. Am J Med 94:646–650

3. NIH Consensus Development Panel (2001) NIH consensus development panel on osteoporosis prevention, diagnosis, and therapy. JAMA 285:785–793
4. Kanis JA, Johnell O, De Laet C, Jonsson B, Oden A, Ogelsby AK (2002) International variation in hip fracture probabilities: implication for risk assessment. J Bone Miner Res 17:1237–1244
5. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S (1987) Genetic determinants of bone mass in adults. A twin study. J Clin Invest 80:706–710
6. Andrew T, Antioniades L, Scurrah KJ, Macgregor AJ, Spector TD (2005) Risk of wrist fracture in women is heritable and is influenced by genes that are largely independent of those influencing BMD. J Bone Miner Res 20:67–74
7. Xiong DH, Shen H, Xiao P, Gua YF, Long JR, Zhao LJ, Liu YZ, Deng HY, Li JL, Recker RR, Deng HW (2006) Genome-wide scan identified QTLs underlying femoral neck cross-sectional geometry that are novel studied risk factors of osteoporosis. J Bone Miner Res 21:424–437
8. Liu YJ, Shen H, Xiao P, Xiong DH, Li LH, Recker RR, Deng HW (2006) Molecular genetics of gene identification for osteoporosis: a 2004 update. J Bone Miner Res 21:1511–1535
9. Sano M, Inoue S, Hosoi T, Ouchi Y, Emi M, Shiraki M, Orimo H (1995) Association of estrogen receptor dinucleotide repeat polymorphism with osteoporosis. Biochem Biophys Res Commun 217:378–383
10. Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H (1996) Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res 11:306–311
11. Shiraki M, Shiraki Y, Aoki C, Hosoi T, Inoue S, Kaneki M, Ouchi Y (1997) Association of bone mineral density with apolipoprotein E phenotype. J Bone Miner Res 1:1438–1445
12. Mizunuma H, Hosoi T, Okano H, Soda M, Tokizawa T, Kagami I, Miyamoto S, Ibuki I, Inoue S, Shiraki M, Ouchi Y (1997) Estrogen receptor gene polymorphism and bone mineral density at the lumbar spine of pre- and postmenopausal women. Bone (NY) 21:379–383
13. Hosoi T, Miyao M, Inoue S, Hoshino S, Shiraki M, Orimo H, Ouchi Y (1999) Association study of parathyroid hormone gene polymorphism and bone mineral density in Japanese postmenopausal women. Calcif Tissue Int 64:205–208
14. Miyao M, Hosoi T, Inoue S, Hoshino S, Shiraki M, Orimo H, Ouchi Y (1998) Polymorphism of insulin-like growth factor I gene and bone mineral density. Calcif Tissue Int 63:306–311
15. Ota N, Hunt SC, Nakajima T, Suzuki T, Hosoi T, Orimo H, Shirai Y, Emi M (1999) Linkage of interleukin 6 locus to human osteopenia by sibling pair analysis. Hum Genet 105:253–257
16. Ogawa S, Urano T, Hosoi T, Miyao M, Hoshino S, Fujita M, Shiraki M, Orimo H, Ouschi Y, Inoue S (1999) Association of bone mineral density with a polymorphism of the peroxisome proliferator-activated receptor gamma gene: PPAR gamma expression in osteoblasts. Biochem Biophys Res Commun 260:122–126
17. Nakajima T, Ota N, Shirai Y, Hata A, Yoshida H, Suzuki T, Hosoi T, Orimo H, Emi M (1999) Ethnic difference in contribution of Sp1 site variation of COL1A1 gene in genetic predisposition to osteoporosis. Calcif Tissue Int 65:352–353
18. Tsukamoto K, Orimo H, Hosoi T, Miyao M, Yoshida G, Watanabe S, Suzuki T, Emi M (2000) Association of bone mineral density with polymorphism of the human matrix Gla protein locus in elderly women. J Bone Miner Metab 18:27–30
19. Tsukamoto K, Orimo H, Hosoi T, Miyao M, Ota N, Nakajima T, Yoshida H, Watanabe S, Suzuki T, Emi M (2000) Association of bone mineral density with polymorphism of the human calcium-sensing receptor locus. Calcif Tissue Int 66:181–183
20. Urano T, Hosoi T, Shiraki M, Toyoshima H, Ouchi Y, Inoue S (2000) Possible involvement of the p57 (Kip2) gene in bone metabolism. Biochem Biophys Res Commun 269:422–426
21. Yamada Y, Harada A, Hosoi T, Miyauchi A, Ikeda K, Ohta H, Shiraki M (2000) Association of transforming growth factor beta1 genotype with therapeutic response to active vitamin D for postmenopausal osteoporosis. J Bone Miner Res 15:415–420
22. Ogawa S, Hosoi T, Shiraki M, Orimo H, Emi M, Muramatsu M, Ouchi Y, Inoue S (2000) Association of estrogen receptor beta gene polymorphism with bone mineral density. Biochem Biophys Res Commun 269:537–541
23. Miyao M, Morita H, Hosoi T, Kurihara H, Inoue S, Hoshino S, Shiraki M, Yazaki Y, Ouchi Y (2000) Association of methylenetetrahydrofolate reductase (MTHFR) polymorphism with bone mineral density in postmenopausal Japanese women. Calcif Tissue Int 66:190–194
24. Miyao M, Hosoi T, Emi M, Nakajima T, Inoue S, Hoshino S, Shiraki M, Orimo H, Ouchi Y (2000) Association of bone mineral density with a dinucleotide repeat polymorphism at the calcitonin (CT) locus. J Hum Genet 45:346–350
25. Ota N, Hunt SC, Nakajima T, Suzuki T, Hosoi T, Orimo H, Shirai Y, Emi M (2000) Linkage of human tumor necrosis factor-alpha to human osteoporosis by sib pair analysis. Genes Immun 1:260–264
26. Ota N, Nakajima T, Nakazawa I, Suzuki T, Hosoi T, Orimo H, Inoue S, Shirai Y, Emi M (2001) A nucleotide variant in the promoter region of the interleukin-6 gene associated with decreased bone mineral density. J Hum Genet 46:267–272
27. Ogata N, Shiraki M, Hosoi T, Koshizuka Y, Nakamura K, Kawaguchi H (2001) A polymorphic variant at the Werner helicase (WRN) gene is associated with bone density, but not spondylosis, in postmenopausal women. J Bone Miner Metab 19:296–301
28. Ogata N, Matsumura Y, Shiraki M, Kawano K, Koshizuka Y, Hosoi T, Nakamura K, Kuro-O M, Kawaguchi H (2002) Association of klotho gene polymorphism with bone density and spondylosis of the lumbar spine in postmenopausal women. Bone (NY) 31:37–42
29. Ohmori H, Makita Y, Funamizu M, Hiooka K, Hosoi T, Orimo H, Suzuki T, Ikari K, Nakajima T, Inoue I, Hata A (2002) Linkage and association analyses of the osteoprotegerin gene locus with human osteoporosis. J Hum Genet 47:400–406
30. Kawano K, Ogata N, Chiano M, Molloy H, Kleyn P, Spector TD, Uchida M, Hosoi T, Suzuki T, Orimo H, Inoue S, Nabeshima Y, Nakamura K, Kuro-o M, Kawaguchi H (2002) Klotho gene polymorphisms associated with bone density of aged postmenopausal women. J Bone Miner Res 17:1744–1751
31. Ota N, Nakajima T, Ezura Y, Iwasaki H, Suzuki T, Hosoi T, Orimo H, Inoue S, Ito H, Emi M (2002) Association of a single nucleotide variant in the human tumor necrosis factor-alpha promoter region with decreased bone mineral density. Ann Hum Biol 29:550–558
32. Ezura Y, Nakajima T, Kajita M, Ishida R, Inoue S, Yoshida H, Suzuki T, Shiraki M, Hosoi T, Orimo H, Emi M (2003) Association of molecular variants, haplotypes, and linkage disequilibrium within the human vitamin D-binding protein (DBP) gene with postmenopausal bone mineral density. J Bone Miner Res 18:1642–1649
33. Feng D, Ishibashi H, Yamamoto S, Hosoi T, Orimo H, Machida T, Koshihara Y (2003) Association between bone loss and promoter polymorphism in the Japanese women with hip fracture. J Bone Miner Metab 21:225–228
34. Omasu F, Ezura Y, Kajita M, Ishida R, Kodaira M, Yoshida H, Suzuki T, Hosoi T, Inoue S, Shiraki M, Orimo H, Emi M (2003) Association of genetic variation of the RIL gene, encoding a PDZ-LIM domain protein and localized in 5q31.1, with low bone

mineral density in adult Japanese women. J Hum Genet 48:342–345
35. Ezura Y, Kajita M, Ishida R, Yoshida S, Yoshida H, Suzuki T, Hosoi T, Inoue S, Shiraki M, Orimo H, Emi M (2004) Association of multiple nucleotide variations in the pituitary glutaminyl cyclase gene (QPCT) with low radial BMD in adult women. J Bone Miner Res 19:1296–1301
36. Ishida R, Ezura Y, Emi M, Kajita M, Yoshida H, Suzuki T, Hosoi T, Inoue S, Shiraki M, Ito H, Orimo H (2003) Association of a promoter haplotype (-1542G/-525C) in the tumor necrosis factor receptor associated factor-interacting protein gene with low bone mineral density in Japanese women. Bone (NY) 33:237–241
37. Urano T, Shiraki M, Fujita M, Hosoi T, Orimo H, Ouchi Y, Inoue S (2005) Association of a single nucleotide polymorphism in the lipoxygenase ALOX15 5'-flanking region (-5229G/A) with bone mineral density. J Bone Miner Metab 23:226–230
38. Sudo Y, Ezura Y, Kajita M, Yoshida H, Suzuki T, Hosoi T, Inoue S, Shirak M, Ito H, Emi M (2005) Association of single nucleotide polymorphisms in the promoter region of the pro-opiomelanocortin gene (POMC) with low bone mineral density in adult women. J Hum Genet 50:235–240
39. Mori S, Kou I, Sato H, Emi M, Ito H, Hosoi T, Ikegawa S (2008) Association of genetic variations of genes encoding thrombospondin, type I, domain-containing 4 and 7A with low bone mineral density in Japanese women with osteoporosis. J Hum Genet 53:694–697
40. Mori S, Kou I, Sato H, Emi M, Ito H, Hosoi T, Ikegawa S (2009) Nucleotide variations in genes encoding carbonic anhydrase 8 and 10 associated with femoral bone mineral density in Japanese females with osteoporosis. J Bone Miner Metab 27:213–216
41. Shen H, Liu YJ, Liu PY, Recker RR, Deng HW (2005) Non-replication in genetic studies of complex diseases: lessons learned from studies of osteoporosis and tentative remedies. J Bone Miner Res 20:365–376
42. Ionnidis JP, Trikalinos TA, Ntzani EE, Contopoulos-Ionnidis DG (2003) Genetic association in large versus small studies: an empirical assessment. Lancet 361:567–571
43. Deng HW (2001) Population admixture may appear to mask, change or reverse genetic effects of genes underlying complex traits. Genetics 159:1319–1323
44. Thakkinstian S, D'Este C, Eisman J, Nguen T, Attia J (2004) Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study. J Bone Miner Res 19:419–426
45. Uitterlinden AG, Ralston SH, Brandi ML, Carey AH, Grinberg D, EPOS Investigators; EPOLOS Investigators; FAMOS Investigators; LASA Investigators; Rotterdam Study Investigators; GENOMOS Study et al (2006) The association between common vitamin D receptor gene variations and osteoporosis: a participant-level meta-analysis. Ann Intern Med 145:255–264
46. Goseki-Sone M, Sogabe N, Fukushi-Irie M, Mizoi L, Orimo H, Suzuki T, Nakamura H, Orimo H, Hosoi T (2005) Functional analysis of the single-nucleotide polymorphism (787T>C) in tissue-nonspecific alkaline phosphatase gene associated with bone mineral density. J Bone Miner Res 20:773–778
47. Sogabe N, Oda K, Nakamura H, Orimo H, Watanabe H, Hosoi T, Goseki-Sone M (2008) Molecular effects of the tissue-nonspecific alkaline phosphatase gene polymorphism (787T>C) associated with bone mineral density. Biomed Res 29:213–219
48. Orimo H, Goseki-Sone M, Hosoi T, Shimada T (2008) Functional analysis of the mutant tissue-nonspecific alkaline phosphatase gene using U2OS osteoblast-like cells. Mol Genet Metab 94:375–381
49. Kinoshita H, Nakagawa K, Narusawa K, Goseki-Sone M, Fukushi-Irie M, Mizoi L, Yoshida H, Okano T, Nakamura T, Suzuki T, Inoue S, Orimo H, Ouchi Y, Hosoi T (2007) A functional single nucleotide polymorphism in the vitamin K-dependent gamma-glutamyl carboxylase gene (Arg325Glu) is associated with bone mineral density in elderly Japanese women. Bone (NY) 40:451–456
50. Sogabe N, Tsugawa N, Maruyama R, Kamao M, Kinoshita H, Okano T, Hosoi T, Goseki-Sone M (2007) Nutritional effects of gamma-glutamyl carboxylase gene polymorphism on the correlation between the vitamin K status and gamma-carboxylation of osteocalcin in young males. J Nutr Sci Vitaminol (Tokyo) 53:419–425
51. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Osteoporosis-Pseudoglioma Syndrome Collaborative Group et al (2001) LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107:513–523
52. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP (2002) High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346:1513–1521
53. Koller DL, Econs MJ, Morin PA, Christian JC, Hui SL, Parry P, Curran ME, Rodriguez LA, Conneally PM, Joslyn G, Peacock M, Johnston CC, Foroud T (2000) Genome screen for QTLs contributing to normal variation in bone mineral density. J Clin Endocrinol Metab 85:3116–3120
54. Urano T, Shiraki M, Ezura Y, Fujita M, Sekine E, Hoshino S, Hosoi T, Orimo H, Emi M, Ouchi Y, Inoue S (2004) Association of a single-nucleotide polymorphism in low-density lipoprotein receptor-related protein 5 gene with bone mineral density. J Bone Miner Metab 22:341–345
55. Audrey Koay M, Brown MA (2005) Genetic disorders of the LRP5–Wnt signalling pathway affecting the skeleton. Trends Mol Med 11:129–137
56. Ezura Y, Nakajima T, Urano T, Sudo Y, Kajita M, Yoshida H, Suzuki T, Hosoi T, Inoue S, Shiraki M, Emi M (2007) Association of a single-nucleotide variation (A1330V) in the low-density lipoprotein receptor protein 5 gene (LRP5) with bone mineral density in adult Japanese women. Bone (NY) 40:997–1005
57. Xiong DH, Liu XG, Guo YF, Tan LJ, Wang L et al (2009) Genome-wide association study and follow-up replication studies identified ADAMTS18 an TGFBR3 as bone mass candidate genes in different ethnic groups. Am J Hum Genet 84:388–398
58. Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N et al (2008) Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet 371:1505–1512
59. Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, Jonsdottir T, Saemundsdottir J, Center JR, Nguyen TV, Bagger Y, Gulcher JR, Eisman JA, Christiansen C, Sigurdsson G, Kong A, Thorsteinsdottir U, Stefansson K (2008) Multiple genetic loci for bone mineral density and fractures. N Engl J Med 358:2355–2365
60. Kung AW, Xiao SM, Chemy S, Li GH, Gao Y et al (2010) Association of JAG1 with bone mineral density and osteoporotic fractures: a genome-wide association study and follow-up replication studies. Am J Hum Genet 86:229–239

Bone 47 (2010) 1064–1070

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

The Fracture and Immobilization Score (FRISC) for risk assessment of osteoporotic fracture and immobilization in postmenopausal women—A joint analysis of the Nagano, Miyama, and Taiji Cohorts

Shiro Tanaka [a,*], Noriko Yoshimura [b], Tatsuhiko Kuroda [c], Takayuki Hosoi [d], Mitsuru Saito [e], Masataka Shiraki [f]

[a] Division of Clinical Trial Design and Management, Translational Research Center, Kyoto University, Kyoto, Japan
[b] Department of Joint Disease Research, 22nd Century Medical and Research Center, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[c] Public Health Research Foundation, Tokyo, Japan
[d] Department of Clinical Research and Development, National Center for Geriatrics and Gerontology, Obu, Japan
[e] Department of Orthopaedic Surgery, Jikei University School of Medicine, Tokyo, Japan
[f] Research Institute and Practice for Involutional Diseases, Nagano, Japan

ARTICLE INFO

Article history:
Received 12 May 2010
Revised 20 August 2010
Accepted 27 August 2010
Available online 8 September 2010

Edited by: David Fyhrie

Keywords:
Bone mineral density
Bed-bound
Fracture probability
FRAX
Japan

ABSTRACT

Introduction: We aimed to (i) explore risk factors for major osteoporotic fracture or immobilization; (ii) develop a prediction model that can be used to assess the risk of fracture and immobilization; and (iii) assess external validity of the final model.

Methods: A total of 1787 postmenopausal Japanese women were followed in a hospital-based cohort study. Endpoints included the annual incidence of major osteoporotic fracture and immobilization. For each endpoint, multivariate Poisson regression models were fitted separately and risk factors were screened through backward variable selection. The predictive accuracy of the final model (FRISC) was evaluated in two independent community-based cohorts.

Results: Over a median follow-up of 5.3 years, a total of 383 major osteoporotic fractures (279 clinical vertebral, 44 hip, 60 distal forearm) and 83 immobilizations occurred in the developmental dataset. Backward variable selection confirmed that the following are risk factors for major osteoporotic fracture: age, weight, prior fracture, back pain, and lumbar bone mineral density (BMD). Age, prior fracture and dementia were significant risk factors for immobilization. Hosmer–Lemeshow tests did not indicate any significant deviation between the observed fracture frequency and prediction from the FRISC in the independent validation dataset. The C statistic for the FRISC was 0.727 (95% confidence interval: 0.660 to 0.794) and was higher than that for BMD alone significantly ($p=0.03$).

Conclusions: We developed a novel prediction model for fracture and immobilization, FRISC, and the clinical risk factors in the FRISC allows better identification of populations at high risk of fracture than BMD alone. A web application is available at http://www.biostatistics.jp/prediction/frisc.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Fracture due to osteoporosis results in increased mortality, morbidity and medical expense in the US and Japan [1–3]. As an adjunct to the development of effective treatments, early identification of populations at high risk of fracture is regarded as an effective strategy for decreasing both the burden of illness and the associated cost in countries with aging populations [4]. The US and Japanese guidelines both recommend that bone mineral density (BMD) should be used to determine when to intervene in patients with osteoporosis [5,6]. However, epidemiological studies have demonstrated that many major osteoporotic fractures occur among individuals with a BMD T score value above the intervention threshold value, although the incidence of fracture certainly increases with decreasing BMD [7,8]. As a solution to this problem, a WHO scientific group proposed the use of 10-year probabilities of osteoporotic or hip fracture, calculated using multiple risk factors. The result was the WHO fracture risk assessment tool (FRAX) [9]. Several other prediction models/risk assessment tools were also developed based on data from cohort studies and clinical trials [10–13].

Abbreviations: BMD, bone mineral density; FRAX, fracture risk assessment tool; FRISC, Fracture and Immobilization Score; CHD, coronary heart disease; CVD, cerebrovascular disease; ROC, receiver operating characteristic; CI, confidence interval.

* Corresponding author. Department of Clinical Trial Design & Management, Translational Research Center, Kyoto University Hospital, 54 Shogoin Kawahara-Cho, Sakyo-ku, Kyoto 606-8507.
E-mail address: shiro@kuhp.kyoto-u.ac.jp (S. Tanaka).

8756-3282/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2010.08.019

Although case-finding strategies optimized by risk assessment tool for fracture appear to be promising, there are several problems which must be addressed before clinical application. First, whether combination of BMD and clinical risk factors allows better discrimination of fracture than BMD alone is still controversial; the discriminatory power of the FRAX was excellent in eleven population-based cohorts [14] but was similar to BMD alone in a cohort study [15]. Second, since the Japanese version of FRAX was validated only in the community dwelling cohorts in Japan [9], there has been no available data whether the FRAX probability performs well in hospital-based population. As Justice et al. pointed out, the effectiveness of prediction models in clinical practice depends on the extent to which they can be generalized to the population in question [16]. In general practice, the FRAX was often adapted to assess fracture risk in a patient who has a more complicated risk for fracture, such as atherosclerosis, diabetes or other potential risks to deteriorate bone strength. In this regard, it is necessary to evaluate prediction models for fracture not only in community but also hospital-based populations. Third, outcomes other than fracture, such as immobilization, appear to be also important for optimizing osteoporotic treatment from the health economic perspective. In fact, we have found that osteoporotic fracture was an independent risk for immobilization and this morbid state would require a large amount of resources of care [17].

The present study was therefore performed with three main aims. The first was to explore risk factors for the incidence of major osteoporotic fracture or immobilization. This was done using data from the Nagano Cohort, a hospital-based cohort study of postmenopausal Japanese women. The second aim of this study was to develop a prediction model named as the Fracture and Immobilization Score (FRISC), to assess the risk of major osteoporotic fracture and immobilization, based on the risk factors confirmed in the first part of the study. Finally, we assess the external validity of the FRISC and investigate whether the predictive accuracy was improved from BMD alone using pooled data from the Miyama and Taiji Cohorts, which followed 400 Japanese women from communities over a 10-year period.

Methods

Development and validation datasets

We used two independent datasets in the current analysis; a developmental dataset from the Nagano Cohort and a validation dataset from the Miyama and Taiji Cohorts. Profiles of these three cohorts have been detailed previously [17–28]. The Nagano Cohort recruited and followed up postmenopausal women who were receiving medical care as outpatients or visitors at a medical institute in Nagano Prefecture, Japan since April 1993 [16–20]. A total of 1787 participants were included in the developmental dataset; exclusion criteria were (i) metabolic bone disease and (ii) secondary osteoporosis (e.g. hyperparathyroidism, hyperthyroidism other than patients on T4 replacement and with euthyroid for more than one year, chronic renal failure or osteomalacia). We excluded those who met the exclusion criteria regardless of BMD.

However, steroid users were enrolled to the present study because the history of steroid use was required in the FRAX. The protocol was approved by the ethics committee at the Research Institute and Practice for Involutional Diseases and we obtained written informed consent from all participants. The Miyama Cohort was set up in 1988 as subsets of nationwide community-based cohort studies sponsored by the Ministry of Education or Ministry of Health and Welfare [21–23]. A total of 1453 inhabitants aged 40–79 years in Miyama Village were listed from the resident registration in December 1988. Then, 200 men and 200 women were recruited and followed up between 1990 and 2000. The Taiji Cohort is a community-based cohort study in Taiji Town, Wakayama Prefecture, Japan [25–27]. From a list of 2261 inhabitants aged 40–79 years obtained from the resident registration in June 1992, 50 men and 50 women in each decade age group between 40 and 79 years (a total of 400 participants) were recruited randomly and followed up between 1993 and 2003. All the sampled participants were contracted and agreed to participate. The validation dataset included all the women in the Miyama and Taiji Cohorts.

Data collection in the Nagano Cohort

At baseline, anthropometric indices, including body weight and body height, were obtained for all patients. Subjects were also interviewed to obtain data about age at menopause, smoking habit, alcohol consumption, past and present occupation, presence of pain, medical history (including rheumatoid arthritis, diabetes mellitus, hypertension, dyslipidemia, cancer, dementia, coronary heart disease [CHD] and cerebrovascular disease [CVD]). Pain was defined as any symptom of pain in the back, hip muscles, ribs, legs, knees, neck, shoulders, wrists, or other joints. Back pain was defined as any symptom of pain in the back trunk area, regardless of the degree or consistency of the pain [18]. Rheumatoid arthritis was diagnosed according to the diagnostic criteria proposed by the American Rheumatism Association [28]. The Japanese version of the Mini-mental State Examination (MMSE) was performed in the subjects who were suspicious dementia and a subject with the total score of MMSE less than 20 points was considered to have obvious cognitive dysfunction [29]. A history of CHD was defined as any previous acute coronary symptoms or events requiring coronary intervention, and was confirmed by coronary angiography, echocardiogram, or MD-CT imaging study. A history of CVD was diagnosed based on evidence of apparent brain attack or an existing brain lesion as observed by magnetic resonance imaging or computer-assisted X-ray tomography. Self-reports of a history of malignancy were confirmed by referring to the patient's medical records [19]. The BMD of the lumbar spine was measured at baseline using dual-energy X-ray absorptiometry (Lunar DPX-L or DPX-IQ; Lunar Corporation, Madison, WI) and a quality assurance test was carried out for every measurement to detect machine drift. The inter-assay variance of the lumbar BMD measurements in our laboratory was $0.5 \pm 0.5\%$ (coefficient of variation $\pm$ standard deviation) [20]. T score was calculated by using Japanese standard values [30].

Data collection in the Miyama and Taiji Cohorts

A self-administered questionnaire was used for baseline data collection in the Miyama Cohort, while both self-administered and interviewer-administered questionnaires were used in the Taiji Cohort [22–27]. The items in these questionnaires included birth date, body weight, body height, current smoking status, current alcohol intake, presence of back pain, use of steroids, and medical history such as rheumatoid arthritis. Parental history of fracture was asked only in the Taiji Cohort. The BMD of L2–4 and BMD at femoral neck, Ward's triangle and the trochanteric region were measured by dual-energy X-ray absorptiometry (Lunar DPX; Lunar Corporation, Madison, WI in the Miyama Cohort, Hologic QDR-1000; Hologic Inc., Crosby Drive Bedford, MA in the Taiji Cohort) and treated as T scores. The incidence of clinical fracture was evaluated in both the cohorts. However, radiographs for morphometrical vertebral fracture were available only in the Miyama Cohort. Consequently, parental history or morphometrical vertebral fracture was missing data in either cohort systematically and thus we assumed that participants with these missing data did not have parental history or prior fracture. We calculated the 10-year probability of major osteoporotic fracture by entering the following data into online version of the FRAX; age, sex, weight, height, previous fracture, parental history of hip fracture, current smoking status, glucocorticoid use, rheumatoid arthritis, alcohol intake and femoral neck BMD.

Endpoints

Endpoints included the annual incidence of major osteoporotic fracture and immobilization. Major osteoporotic fracture was defined

as first occurrence of any clinical fracture (hip fracture, surgical neck fracture of the humerus, distal forearm fracture, or clinical vertebral fracture). We also evaluated a radiographical vertebral fracture by the semi-quantitative visual method [31], but major osteoporotic fracture does not include morphometrical fracture by definition. A validation analysis of our semi-quantitative method for analyzing incident vertebral fracture has been reported elsewhere [32]. Prior vertebral fractures were defined as those fractures for which the ratio of the height of the central or anterior vertebral body to that of the posterior vertebral body was less than 0.8, or when any of these three vertebral body heights was less than 80% of the height of the adjacent vertebral body [6]. Immobility was defined in accordance with the subject's locomotive ability. Subjects bed-bound at home (lying in bed almost all day) for more than 6 months or institutionalized in nursing homes (lying in bed or using a wheelchair for locomotion), were defined as immobile [17]. Some immobile subjects could be sitting on a bed and could be going to a portable toilet which located besides the bed. Participants who died from any cause, moved to the home of a relative because they were not able to perform the activities of daily living independently, were lost to follow-up, or were followed up until June 1, 2009 were treated as censored. For each endpoint, the accumulation of person-years at risk started from registration of each patient.

Statistical considerations

For each endpoint, we fitted multivariate Poisson regression models separately and rate ratios for risk factors estimated by the Poisson regression models were reported with 95% confidence intervals (CI) and p values. The following variables were initially identified from the literature as the traditional risk factors for osteoporotic fracture: covariates included in the FRAX other than femoral neck BMD (age, height, weight, prior fracture, parental history of fracture, current smoking status, use of steroids, rheumatoid arthritis, alcohol intake), lumbar BMD, presence of back pain, presence of any pain, and drug treatment for osteoporosis [9,18,22]. These covariates were screened via backward variable selection with a significance level of $p=0.2$. We constructed a prediction model for immobilization using the same procedure, except that three covariates (dementia, history of CVD, and history of malignancy) were used in addition to those used in the osteoporotic fracture analysis. Finally, the FRISC was developed using the following formula:

$$\mathrm{Prob}(t) = \int_0^t \lambda \exp(\mathbf{X}\beta) \exp\left[-\int_0^u \{\lambda \exp(\mathbf{X}\beta) + m(v)\} dv\right] du$$

Here, t is a time point for prediction (i.e. the formula calculates 10-year probability if $t=10$), β is a vector of log-rate ratios for covariates $\mathbf{X}$, λ denotes baseline incidence rate, and $m(v)$ is mortality at time v obtained from sex- and age-specific mortality in Vital Statistics of Japan in 2008 [33].

We assessed the predictive accuracy of the FRISC in terms of calibration and discrimination [34] using occurrence of major osteoporotic fracture within a 10-year period, which was treated as a binary event, in the validation dataset. Calibration, namely how closely the prediction reflects actual events, was assessed using ratio of observed and predicted events and the Hosmer–Lemehshow test. Discrimination, the ability to distinguish between those who experience the event and those who do not, was evaluated using receiver operating characteristic (ROC) curves and Harrell's C statistic. Improvement in the C statistics of the two models from BMD alone was assessed by using contrast tests.

All reported p values for statistical tests are two-tailed, and $p<0.05$ was taken to indicate statistical significance. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Results

Characteristics of participants and follow-up

Baseline characteristics of participants in the Nagano, Miyama and Taiji Cohorts are summarized in Table 1. The Nagano Cohort included older participants and mean lumbar BMD in this cohort was lower than the other cohorts. Prior fracture, back pain and parental history were observed more frequently in the Miyama and Taiji Cohorts. In the Nagano Cohort, 37.4% of participants were being treated with bone resorption inhibitors (bisphosphonates or a selective estrogen receptor modulator), and 16.7% were receiving 1-alpha-OH vitamin D_3 or vitamin K_2 at baseline. Table 2 describes the incidence of fractures and immobilization. In the Nagano Cohort, over a median follow-up time of 5.3 years (range, 0.03–16.5 years), a total of 383 major osteoporotic fractures occurred (279 clinical vertebral fractures, 44 hip fractures, 60 distal forearm fracture, Table 2). In the Miyama and Taiji Cohorts, 337 of 400 participants completed the planned follow-up of the 10-year period (84%) and a total of 60 major osteoporotic fractures occurred (44 clinical vertebral fractures, 8 hip fractures, 8 distal forearm fracture, Table 2). Incidence rates of fractures in the two cohorts were much lower than the Nagano Cohort possibly due to the difference in average age and lumbar BMD at baseline (Tables 1 and 2). Immobilization occurred in 83 participants in the Nagano Cohort.

Risk factors for fracture and immobilization

We fitted multivariate Poisson regression models to the validation dataset of 1787 participants. Backward variable selection identified the following six risk factors for major osteoporotic fracture: age, weight, lumbar BMD, prior fracture and presence of back pain (Table 3). That is, parental history of fracture, smoking status, alcohol consumption, rheumatoid arthritis, and use of steroids, which are all

Table 1
Characteristics of participants in the three cohorts.

		The Nagano Cohort (N=1787)			The Miyama and Taiji Cohorts (N=400)		
		Mean	SD	5–95 percentile	Mean	SD	5–95 percentile
Age (years)		63.4	11.1	45–81	59.5	11.3	41–77
Height (cm)		150.9	6.6	140–161	150.2	6.2	140–159
Weight (kg)		51.1	8.5	38–65	51.2	9.3	37–66.5
Lumbar BMD (T score)		−1.55	1.22	−3.5–0.5	−1.36	1.19	−3.85–1.57
Femoral neck BMD (T score)		*			−1.61	1.84	−3.29–0.53
				Frequency	%	Frequency	%
Prior fracture				403	22.6	49†	25.0
Presence of pain	Back			572	32.0	251	63.0
	Other sites			449	25.1	†‡	
Parental history				22	1.2	20‡	10.0
Current smoker				38	2.1	16	4.0
Current alcohol drinker				137	7.7	46	11.5
Medication	Bone resorption inhibitors			369	37.4	†‡	
	Active vitamin D_3 or vitamin K_2			299	16.7	†‡	
	Steroids			27	1.5	0‡	0.0
Rheumatoid arthritis				224	12.5	0	0.0
Dementia				97	5.4	†‡	

SD: standard deviation; BMD: bone mineral density.
*Not measured in the Nagano Cohort. †Not measured in the Miyama Cohort (N=200).
‡Not measured in the Taiji Cohort (N=200).

Table 2
Frequencies and incidence rates of fracture and immobilization in participants in the three cohorts.

	The Nagano Cohort (N = 1787)				The Miyama and Taiji Cohorts (N = 400)			
	Frequency	IR	95% CI		Frequency	IR	95% CI	
Major osteoporotic fracture	383	34.1	30.9	37.7	60	16.1	12.5	20.7
Clinical vertebral facture	279	24.9	22.1	28.0	44	11.8	08.8	15.9
Hip fracture	44	3.9	2.9	5.3	8	2.2	1.1	4.3
Immobilization	83	7.4	6.0	9.2	–			

IR, incidence rate per 1,000 person-years; CI, confidence interval.

included in the FRAX, were excluded based on having p values less than 0.2. Importantly, incidence rate of major osteoporotic fracture increased as weight elevated and this direction is opposite to the FRAX and this trend remains significant even when all the other risk factors listed initially in the variable selection procedure are adjusted for (rate ratio for 10 kg increase in weight: 1.22, 95% CI: 1.07 to 1.40, $p<0.01$). Multivariate analysis for immobilization, using the same variable selection procedure, showed that age, prior fracture and dementia were associated with the incidence of immobilization (Table 3).

Input and output of the FRISC

All the risk factors that were retained through the variable selection procedure were incorporated into the final prediction model named as the FRISC. Interface of web application of the FRISC is displayed in Fig. 1. The input comprises the sex risk factors and, menopausal status and secondary osteoporosis which were used only for assessment of the applicability. The output comprises the 1, 3, 5 and 10-year probabilities of major osteoporotic fracture and those of immobilization and is calculated by using the algorithm described in Supplementary Data.

External validation of the FRISC

Fig. 2 displays histograms of the calculated 10-year probabilities of major osteoporotic fracture for the 400 participants in the validation dataset (upper: the FRISC, lower: the FRAX). An apparent difference was observed in the left tail of the two histograms; in the upper figure participants with fracture probability less than 0.05 were very few, while the FRAX gave the fracture probability less than 0.05 to a substantial portion of the participants. As a result, the fracture probabilities from the FRISC were much higher on average. Table 4 compares the predictive accuracy of the two prediction models and prediction from BMD alone. Over the 10-year follow-up, major osteoporotic fracture developed in 60 of 400 participants in the validation dataset. The predicted event frequency calculated from the FRISC was slightly higher than the observation (observed/predicted ratio: 0.74), while the FRAX tended to underestimate (observed/predicted ratio: 1.59). The Hosmer–Lemeshow test did not indicate any significant deviation between the observed event frequency and prediction from the FRISC. The C statistics for the FRISC was 0.727, indicating that the discriminatory power of the FRISC is moderate, while that for prediction from BMD alone was 0.651. That is, the discriminatory power of the FRISC, which combines BMD and additional clinical risk factors, was better than BMD alone significantly even in independent community-based cohort studies ($p=0.03$, Table 4). Fig. 3 shows ROC curves for major osteoporotic fracture probability from the FRISC (solid curve), the FRAX (dashed curve) and BMD alone (dotted curve). Both the ROC curves of the prediction models increased almost identically at first, but the curve for the FRISC was slightly above the curve for the FRAX where sensitivity is higher than 0.7 and where lower probability is used as a cutoff point (i.e. 16% or lower in the FRISC, 14% or lower in the FRAX), indicating that the FRISC is advantageous over the FRAX for screening of low-risk osteoporotic patients.

Table 3
Multivariate Poisson regression analysis of risk factors for major osteoporotic fracture and immobilization in the development dataset of 1,787 participants.

	Major osteoporotic fracture				Immobilization			
	Rate ratio	95% CI		p	Rate ratio	95% CI		p
Age, + 10 years	1.62	1.43	1.83	<0.01	2.80	2.09	3.73	<0.01
Weight, + 10 kg	1.25	1.10	1.42	<0.01	–	–	–	–
Lumbar BMD, + 1 T score point	0.85	0.76	0.94	<0.01	–	–	–	–
Prior fracture, yes/no	2.00	1.57	2.54	<0.01	2.04	1.21	3.44	0.01
Back pain, yes/no	1.58	1.27	1.96	<0.01	–	–	–	–
Dementia, yes/no	–	–	–	–	2.09	1.32	3.29	<0.01

BMD: bone mineral density; CI: confidence interval.

Discussion

In the current study, we explored clinical risk factors for major osteoporotic fracture and immobilization and developed a novel prediction model, the FRISC. Importantly, the assessment of external validity showed that the FRISC allows accurate prediction of major osteoporotic fracture even in the community-based setting and after a long-term follow-up of ten years, although it was developed in a hospital-based cohort study (i.e. for outpatients and visitors to a clinic). Therefore, the FRISC is useful both not only for patients who have a more complicated risk for fracture, such as atherosclerosis, diabetes or other potential risks to deteriorate bone strength, but also general postmenopausal women. Further the discriminatory power of the FRISC was shown to be better than BMD alone. We have previously noted that there is a close relationship between bone fractures and subsequent immobilization in postmenopausal Japanese women, and that these two conditions are morbid states that require a large amount of health resources [17]. Therefore, an accurate measure to predict these two conditions is particularly valuable in the context of an aging society. A web application of the FRISC is available at http://www.biostatistics.jp/prediction/frisc (Fig. 1).

The major finding of the current study is that inclusion of the four clinical risk factors, namely age, weight, prior fracture and back pain, in addition to BMD significantly improved the accuracy of the prediction model for major osteoporotic fracture. In contrast, parental history of fracture, smoking status, alcohol consumption, rheumatoid arthritis and use of steroids, which are all included in the FRAX, were not associated with incidence of fracture in the present analysis. The reason for this observation does not appear to be a lack of power given the number of observed events in the Nagano Cohort. Diet and other lifestyle factors, which were Westernized among smokers in this cohort, may have contributed to this unexpected result. One implication of these findings is that the association between lifestyle factors and fracture risk is possibly biased due to confounding factors, and it is necessary for prediction models to reflect the multidimensional nature of lifestyles. Although there were smokers and drinkers in the present population, the extent of their smoking and drinking was very mild, and smaller percentages of patients had these habits than in comparable Caucasian populations. In the practical point of view, a more parsimonious model is desirable and the FRISC would therefore provide a simple but sufficiently accurate measure for prediction of major osteoporotic fracture.

The present results indicated that incidence of fracture increases with heavier body weight, although low BMI has been considered as a significant risk factor of fracture as proposed in the FRAX. This trend remained even after the adjustment for the other risk factors

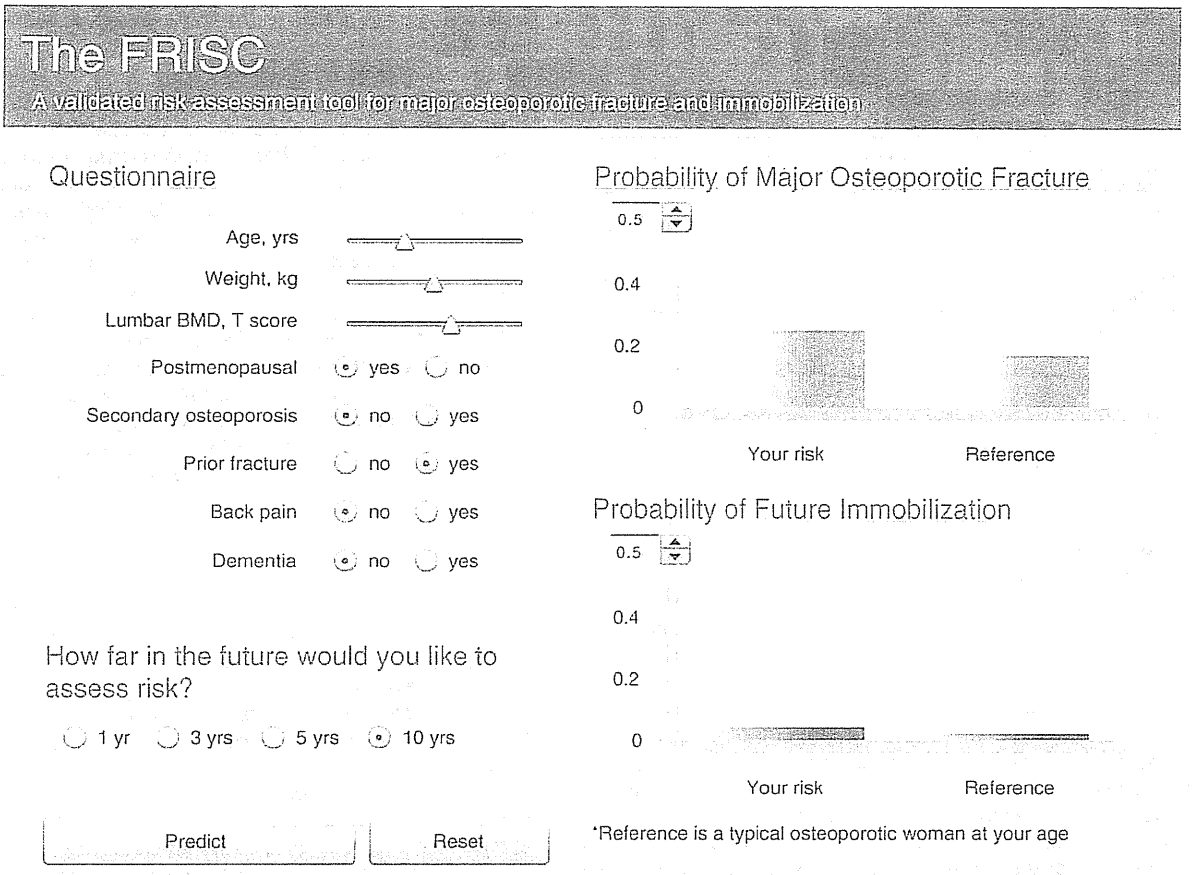

Fig. 1. Input and output of the web application of the FRISC.

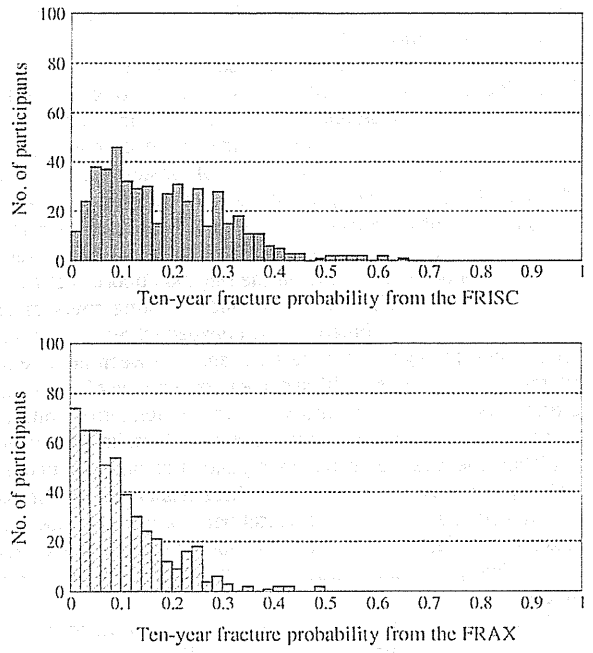

Fig. 2. Histogram of 10-year probabilities of major osteoporotic fracture from the FRISC (upper) and the FRAX (lower) in the Miyama and Taiji Cohorts.

($p<0.01$) and therefore seemed to be attributable to confounders. This may be one of the causes of discrepancy in 10-year probability between the FRAX and the actual fracture rate in the three cohorts. Recent report indicated that morbid obesity had a higher susceptibility of fractures comparing to the postmenopausal women with normal weight although the BMD of the obesity was higher than the controls [35]. As it is well known that obesity will connect to have diabetes mellitus or at least to have glucose intolerance and diabetes may deteriorate bone quality due to an increase in non-enzymatic glycation induced cross-links of collagen, which increased collagen

Table 4
Predictive accuracy of major osteoporotic fracture probability from the FRISC compared the FRAX evaluated in the validation dataset from general population.

	Calibration			Discrimination			
	Predicted no. of cases	Observed/ predicted ratio	p^*	C statistics†	95% CI		$p^‡$
BMD alone	–	–	–	0.651	0.575	0.728	–
The FRAX	37.8	1.59	<0.01	0.699	0.629	0.768	0.23
The FRISC	81.2	0.74	0.17	0.727	0.660	0.794	0.03

CI: confidence interval.

* Hosmer–Lemeshow test, p value less than 0.05 indicates a significant deviation between the observed and predicted event frequencies. Number of strata and degree of freedom are 10 and 8, respectively.

† The proportion of all patient pairs in which prediction and observed occurrence of event are concordant.

‡ Contrast test comparing C statistics of the FRAX and FRISC from that of BMD alone, p value less than 0.05 indicates a significant improvement from BMD alone.

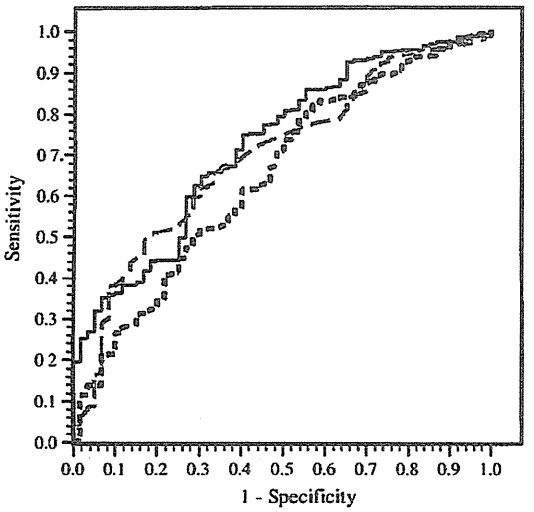

Fig. 3. Receiver operating characteristic curve for major osteoporotic fracture probability from the FRISC (solid curve), the FRAX (dashed curve) and BMD alone (dotted curve) in the Miyama and Taiji Cohorts.

brittleness [36,37]. Lifestyle factors such as diet and exercise may also be other explanations for this observation.

Although the intrinsic properties of the bone are important components of fracture risk, assessment of these factors alone does not adequately reflect the full range of factors associated with the occurrence of fracture [38]. Loss of bone mass and impaired bone quality are commonly held to be the two major causes of increased bone fragility in osteoporosis [2]; however, existing prediction models do not directly take bone quality into consideration. Despite recent progress in understanding the composition and structure of the bone, there are currently no standard assessments of bone quality. Novel bone quality-related markers such as homocysteine [39,40] and pentosidine [41] appear to improve predictive accuracy, but further research is required to determine whether they will be useful in the context of predicting osteoporotic fracture.

The incidence of clinical vertebral fracture in the Japanese population is substantially high (Table 2). As a result, the 10-year fracture probabilities generated using the FRISC is much higher than the FRAX (Fig. 2). The major underlying cause of the discrepancy in the 10-year probabilities is likely to be the difference in population. The FRISC was developed in a cohort study conducted at one medical institute and included subjects who were receiving treatment for osteoporosis, whereas the FRAX was developed using data from a community-based population. Although the effectiveness of bisphosphonate and selective estrogen receptor modulators in reducing fracture risk has been demonstrated, in the current analysis, drug treatment for osteoporosis was not a significant factor at the site of major osteoporotic fracture after adjustment for other risk factors, suggesting that its influence on risk is smaller than that of the risk factors. People who visit a hospital or clinic possibly have a higher prevalence of co-morbid conditions than people in the general population, yielding an increased incidence of fracture because of deterioration in both bone quality and quantity.

Given the large difference in incidence rates of fracture between the Nagano Cohort and the Miyama and Taiji Cohorts (Table 2), it may not seem to be sensible to choose the Miyama and Taiji Cohorts as validation cohorts since a good fit is unexpected. However, as shown in Table 1, the Nagano cohort included older participants and the mean lumbar BMD in this cohort was lower than the other cohorts. Therefore the difference in participants' characteristics may be attributable to the higher incidence rate in the Nagano cohort relative to the other cohorts. Further, the Miyama and Taiji Cohorts followed participants over a 10-year period and are more suitable for the validation analysis. A limitation of our validation analysis was that parental history or morphometrical vertebral fracture was missing data in either of the validation cohorts systematically. We assumed that participants with these missing data did not have parental history or prior fracture, yielding a somewhat lower 10-year probability of major osteoporotic fracture. Given that we did not find any evidence of deviation between the observed fracture frequency and prediction from the FRISC even in independent community-based cohort studies, the FRISC appears to allow accurate prediction of major osteoporotic fracture both in community-based and hospital-based settings.

Supplementary materials related to this article can be found online at doi:10.1016/j.bone.2010.08.019.

Conclusion

We developed a novel prediction model for fracture and immobilization, FRISC, and the clinical risk factors in the FRISC allows better identification of populations at high risk of fracture than BMD alone.

References

[1] Braithwaite RS, Col NF, Wong JB. Estimating hip fracture morbidity, mortality and costs. J Am Geriatr Soc 2003;51:364–70.
[2] Hayashi Y. Health economics of treatment of osteoporosis (in Japanese). Geriatr Med 2004;42:613–8.
[3] NIH Consensus Development Panel on Osteoporosis Prevention. Diagnosis and therapy. JAMA 2001;285:785–95.
[4] Kanis JA, Borgstrom F, De Laet C, et al. Assessment of fracture risk. Osteoporos Int 2005;16:581–9.
[5] Clinician's Guide to Prevention and Treatment of Osteoporosis. National Osteoporosis Foundation, Washington, D.C., http://www.nof.org/professionals/Clinicians_Guide.htm; 2010 [accessed April 1, 2010].
[6] Orimo H. New diagnostic criteria of primary osteoporosis. Clin Calcium 2001;11:1133–9.
[7] Kanis JA, Johnell O, Oden A, et al. Risk of hip fracture according to the World Health Organization criteria for osteopenia and osteoporosis. Bone 2000;27:585–90.
[8] Siris ES, Miller PD, Barrett-Connor E, et al. Identification and fracture outcomes of undiagnosed low bone mineral density in postmenopausal women: results from the National Osteoporosis Risk Assessment. JAMA 2001;285:2815–22.
[9] Fujiwara S, Nakamura T, Orimo H, et al. Development and application of a Japanese model of the WHO fracture risk assessment tool (FRAX). Osteoporos Int 2008;19:429–35.
[10] Robbins J, Aragaki AK, Kooperberg C, et al. Factors associated with 5-year risk of hip fracture in postmenopausal women. JAMA 2007;28:2389–98.
[11] Pongchaiyakul C, Panichkul S, Songpatanasilp T, et al. A nomogram for predicting osteoporosis risk based on age, weight and quantitative ultrasound measurement. Osteoporos Int 2007;18:525–31.
[12] Díez-Pérez A, González-Macías J, Marín F, et al. Prediction of absolute risk of non-spinal fractures using clinical risk factors and heel quantitative ultrasound. Osteoporos Int 2007;18:629–39.
[13] Nguyen ND, Frost SA, Center JR, et al. Development of prognostic nomograms for individualizing 5-year and 10-year fracture risks. Osteoporos Int 2008;19:1431–44.
[14] Kanis JA, Oden A, Johnell O, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int 2007;18:1033–46.
[15] Trémollieres FA, Pouillès JM, Drewniak N, Laparra J, Ribot CA, Dargent-Molina P. Fracture risk prediction using BMD and clinical risk factors in early postmenopausal women: sensitivity of the WHO FRAX tool. J Bone Miner Res 2010;25:1002–9.
[16] Justice AC, Covinsky KE, Berlin JA. Assessing the generalizability of prognostic information. Ann Intern Med 1999;16:515–24.
[17] Shiraki M, Kuroda T, Shiraki Y, Aoki C, Sasaki K, Tanaka S. Effects of bone mineral density of the lumbar spine and prevalent vertebral fractures on the risk of immobility. Osteoporos Int 2010;21:1545–51.
[18] Kuroda T, Shiraki M, Tanaka S, et al. The relationship between back pain and future vertebral fracture in postmenopausal women. Spine 2009;34:1984–9.
[19] Kuroda T, Shiraki M, Tanaka S, et al. Contributions of 25-hydroxy vitamin D, co-morbidities and bone mass to mortality in Japanese post-menopausal women. Bone 2009;44:168–72.
[20] Shiraki M, Shiraki Y, Aoki C, et al. Association of bone mineral density with apolipoprotein E phenotype. J Bone Miner Res 1997;12:1438–45.
[21] Tanaka S, Matsuyama Y, Shiraki M, et al. Effects of time-varying osteoporosis treatments on incidence of fractures among Japanese postmenopausal women. Epidemiology 2007;18:529–36.
[22] Kasamatsu T, Morioka S, Hashimoto T, et al. Epidemiological study on bone mineral density of inhabitants in Miyama Village, Wakayama Prefecture (Part I). Background of study population and sampling method. J Bone Miner Metab 1991;9:50–5.