んでいるが，証明はされていない。一つの仮説としてカルシウム摂取が増えることにより，副甲状腺ホルモンの分泌が抑制され，また1,25$(OH)_2$ビタミンDの濃度も低下する。これらのホルモンの影響により脂肪細胞での脂肪合成が抑えられるとともに，脂肪が分解される方向にシフトするという説がある[20]。また，カルシウムが脱共役タンパク質UCPの発現を促進し，そのため体温が上昇，代謝が亢進し，エネルギーが消費される方向にシフトするという説もある[21]。しかしこれらはまだ仮説の段階である。カルシウムや乳製品が直接抗肥満効果を有する可能性とともに，食生活，ライフスタイル全体が，抗肥満効果に有効である可能性があり，牛乳・乳製品の摂取と他の食品群の摂取との関係，運動や喫煙などのライフスタイルとの関係など，牛乳・乳製品以外の影響についても十分に考慮する必要がある。

牛乳・乳製品にはさまざまな機能性ペプチドが含まれていることが報告されている。たとえば先に示した血圧低下作用を有するペプチドのほかにカルシウム吸収促進ペプチド（CPP）などが存在する[22]。CPPの存在は牛乳・乳製品に含まれるカルシウムをより効率よく体内に吸収させることになり，カルシウムの効果をより高めている可能性が考えられる。牛乳・乳製品はカルシウムの供給源として非常に有用な食品である。今回の対象者でも牛乳・乳製品の摂取量が多い者は，カルシウム摂取量も多くなっており，カルシウム摂取量全体に占める牛乳・乳製品の寄与率も高くなっている。したがって，カルシウムによる効果の一部は牛乳・乳製品によって説明できると考えられる。

近年，海外では牛乳・乳製品がメタボリックシンドロームを抑制するという研究が発表されてきている。しかし，日本では牛乳・乳製品の摂取量，カルシウムの摂取量が少なく，海外の結果をそのままあてはめることには問題も多い。今回日本人成人を対象として同様の検討を行ったところ，非喫煙女性では牛乳・乳製品の摂取量が増えるにしたがい，メタボリックシンドロームのリスクは有意に減少していた。非喫煙男性でも同様の傾向がみられた。

なお，健診で肥満やメタボリックシンドロームを指摘された者が牛乳・乳製品の摂取を控えた可能性も考えられるが，次の点からその可能性は低いと考えられる。すなわち，今回の調査は特定保健指導が実施される前に行われ，健診結果を基にした特別な食事指導などは行われてこなかったこと，過去の牛乳・乳製品摂取状況と現在の摂取状況をみる限り，摂取状況は大きく変わっておらず，指摘によっての変化は少ないと考えられることである。

以上，これまでの海外の報告と，今回のわれわれの研究結果を合わせて考えると，メタボリックシンドロームの予防に，牛乳・乳製品が有用である可能性があり，このことは前向きの追跡研究さらには介入研究で検討する価値のある課題と考えられる。

今後の課題

今回，乳業4社の健診受診済み従業員のみを対象とした場合のアンケート回収率は35.8%であった。日本での地域住民を対象とした同様の調査の回収率では46.5%との報告がある[23]。これは千葉県の鴨川市の住民を対象とした調査である。このときの回収率と比べ，今回の回収率は若干低いが，ポピュレーションベースの調査としては解析可能な水準と考えられる。

今回の調査では健診結果を自己申告させた。アンケートは無記名で回収したので，健診結果の作為的な誤記入は避けられたものと考えられる。

今回の対象者は，乳業企業に勤務する者とその家族である。その点から，一般の人を対象とした場合よりも，牛乳・乳製品摂取状況は良好である可能性がある。しかし，牛乳・乳製品摂取が身体に与える影響については，職業による違いはない。今回の対象者の性別，年齢階級別の体重やBMI，さらにメタボリックシンドロームの該当者（判定基準は若干異なる）の割合は，平成18年国民健康・栄養調査の結果と同水準であり，対象者の大きなバイアスはないと考えられる[24]。また，今回の結果から，日本人の平均的な牛乳・乳製品摂取量に相当する第二四分位でもメタボリックシンドロームのリスクは下がっており，全体の結果と同様の傾向を示していた。

今回の結果は，横断研究によるものであり，実際に牛乳・乳製品がメタボリックシンドローム関連の各指標項目を改善するかどうかは今後の課題である。

本研究は平成20年度牛乳・乳製品の機能性等に関する調査・研究事業の一部として実施されたものである。

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Original Paper

Milk, Dairy Products and Metabolic Syndrome: A Cross-sectional Study of Japanese

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Summary: We conducted a cross-sectional study to examine the relationship between milk and dairy product consumption by Japanese adults and the development of metabolic syndrome. The subjects were employees working for four groups of milk and dairy product manufacturing companies and their families (aged 20–69 yr). We sent self-administered questionnaire forms to them by post, requesting them to answer the questions on the forms. Responses were collected from 3,252 non-smoking men, 3,296 non-smoking women and 2,111 smoking men, and subjected to analysis. Eighteen percent of non-smoking men were identified as having some form of metabolic syndrome symptoms and having access to "positive support" (through consultation provided by doctors, national registered dietitians, public health nurses, etc. on a continuous basis) to alleviate their conditions, while non-smoking women who measured 80 cm or more around the waist and who were identified as having access to "positive support" to alleviate their metabolic syndrome symptoms accounted for 10%. The total respondents were classified into quartiles, according to their volumes of milk and dairy product consumption. When the ratio of metabolic syndrome sufferers in the group consuming the lowest amount of milk and dairy products was regarded as 1, the ratios in the other three groups were significantly lower among non-smoking women as they consumed more milk and dairy products, while non-smoking men showed a similar trend. Our findings indicate that milk and dairy product consumption may be useful for prevention of metabolic syndrome in non-smokers.

Key words: milk, dairy products, metabolic syndrome, Japanese, cross-sectional study

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ORIGINAL ARTICLE

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

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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

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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

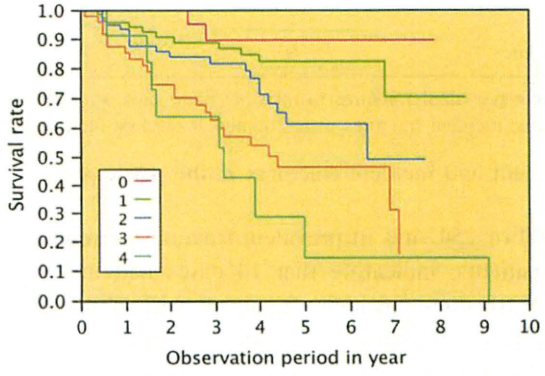

Fig. 1 Kaplan–Meier plot of the categorized patients under amino-bisphosphonate (amino-BP) treatment in accordance with the number of fracture risks (0–4). The lower survival rate from incident fractures was observed in accordance with the number of fracture risk in patients with amino-BP-treated osteoporosis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

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REVIEW ARTICLE

Genetic aspects of osteoporosis

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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.

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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.

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ORIGINAL ARTICLE

Design of a pragmatic approach to evaluate the effectiveness of concurrent treatment for the prevention of osteoporotic fractures

Rationale, aims and organization of a Japanese Osteoporosis Intervention Trial (JOINT) initiated by the Research Group of Adequate Treatment of Osteoporosis (A-TOP)

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Abstract The aim of osteoporosis treatment is to prevent future fractures. Although concurrent treatment has been used very frequently for osteoporosis in clinical practice, there are no data on accurate and verified effectiveness of concurrent treatment for fracture prevention in patients with osteoporosis. To clarify the clinical usefulness of concurrent treatment, the Japan Osteoporosis Society has authorized the establishment of the A-TOP (Adequate Treatment of Osteoporosis) research group. The objective of this research is to establish a design for a clinical trial to prove whether concurrent treatment using both alfacalcidol (1-alpha-hydroxycholecalciferol) and alendronate is more effective as compared to treatment using alendronate alone

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in terms of fracture prevention. The present study was named JOINT (Japanese Osteoporosis Intervention Trial) and is based on a method using national, prospective, randomized, open-labeled, blinded endpoints focusing on postmenopausal osteoporosis with a high risk for fracture. The patients were mainly selected by practitioners and allocated randomly by a central registration system into two groups, of which one received 5 mg/day of alendronate alone, and the other received 1 μg/day of 1-alpha-hydroxycholecalciferol (alfacalcidol) in addition to the alendronate. The endpoints focused primarily on fracture prevention, and the patients' quality of life (QOL) and change in body height, as well as adherence and the adverse events of the treatments were evaluated secondarily. To obtain sufficient statistical power in the events during a 2-year observation period, the patients who are expected to have higher risk were selected to participate in this study, and it was decided that the final plan would involve 890 patients per group (two-sided alpha = 0.05, power = 0.8). Data collection began in November 2003. Correspondence regarding the registration of the investigator and the progress of the study was conducted through a web system from the Public Health Research Foundation to practitioners.

Keywords Alendronate · Alfacalcidol · Concurrent treatment · Fracture prevention · Osteoporosis

Introduction

Osteoporosis, which is characterized by compromised bone strength and increased susceptibility to fractures, which lead to deterioration in the QOL and increased mortality, is a national burden on an aging society [1, 2]. However, recent studies indicate that treatment with a parathyroid hormone, bisphosphonates or a selective estrogen receptor modulator (SERM) [3–9] may decrease the risk of fractures in patients with osteoporosis.

Although bisphosphonate treatment currently represents the most powerful form of treatment available for fracture prevention in osteoporotic patients, it has not succeeded in completely preventing osteoporotic fractures [3–5, 7–9]. Therefore, concurrent treatment of osteoporosis has been frequently used by Japanese practitioners without any concrete evidence regarding fracture reduction. Since the concept of evidence-based medicine (EBM) has been introduced to clinical practice since the 1990s [10], the Japan Osteoporosis Society and the Japanese Society of Bone Mineral Research have edited the clinical guideline for treatment of osteoporosis (Chief editor: Hajime Orimo [11]). However, the writers recognized that there was a lack of evidence in the effectiveness of concurrent treatment of osteoporosis. Furthermore, it was expected that the patients who visit clinics have varying degrees of risk of fracture, which may differ from the degree of those who participated in development trials for bisphosphonates. This possibility would make it easier to obtain pragmatic evidence in general clinical practice.

Starting in 2000, the Japan Osteoporosis Society had planned to investigate the effectiveness of treatment of osteoporosis in order to provide evidence to general practitioners. Before constructing evidence, some feasibility studies were required to confirm the consensus in the diagnosis of incident fractures among the researchers and to elucidate the risk of future fractures in the patient population. In addition to these efforts in the field of osteoporosis, the Japanese government also established an

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ethical guideline for clinical trials [12], and the International Committee of Medical Journal Editors launched a clinical trial registry [13]. Such kinds of progress in the circumstances of clinical trials have enabled for investigator-initiated clinical trials in general practice.

The Adequate Treatment of Osteoporosis (A-TOP) study group was established in 2000 [11] in affiliation with the Japan Osteoporosis Society and organized a team for clinical trial management. The team consisted of clinical investigators (planning and analysis), foundation (funding) managers, officers from non-profit organization (data management) and several companies (data collection). This was the first joint team to create the post-making evidence for osteoporosis. In November 2003, A-TOP initiated a randomized clinical trial referred to as the Japanese Osteoporosis Intervention Trial (JOINT). The purpose of JOINT was to confirm the clinical significance of concurrent use of osteoporotic drugs. The first protocol, named JOINT-01, was initiated in 2002, but was suspended the following year due to a change in drug labeling. A second protocol, named JOINT-02, was established to clarify the effect of adding 1-alpha-hydroxycholecalciferol (alfacalcidol) to alendronate (ALN), using the incident fracture rate as the primary endpoint. In this paper, the rationale, organization and study design of JOINT-02 are introduced.

Rationale and aims

In 2002, the Japan Society of Osteoporosis sent a letter to randomly selected practitioners and enclosed a questionnaire regarding whether concurrent treatment using bisphosphonate and another drug was being utilized to treat osteoporosis. Surprisingly, 87.8% (79/90 practitioner) of the doctors who responded did have experience using concurrent treatment [14]. The most frequent drugs used in concurrent treatment with amino-bisphosphonate were alfacalcidol (93.7%), followed by calcitonin (50.6%), as there were expectations for these drugs to exhibit more potent inhibition of fracture occurrence or more significant increase in BMD, even though there was no apparent evidence. In addition to the lack of evidence related to fracture prevention, the safety profile of concurrent treatment had not been evaluated. Thus, evaluations of the effectiveness and safety of concurrent treatment were urgently required. Etidronate [15] and ALN [8, 9] were used as the drugs to confirm anti-fracture effectiveness in comparison to alfacalcidol in Japanese osteoporotic patients. However, these clinical trials were carried out at specific institutions and were initiated by experts in accordance with tight regulations. As a result, there may have been differences in the selected treatment and in the backgrounds of the patients between treatments conducted at these institutions and those conducted in general practice. In addition, the adherence of the treatment is expected to be lower in general practice than in institutions with experts who are committed to developmental trials. Thus, pragmatic study is urgently needed to evaluate whether bisphosphonates are effective to the same extent at the level of general practitioners as compared to the prior study (Phase III study).

Feasibility studies

The Japan Osteoporosis Society started discussions to execute a national clinical trial for obtaining evidence regarding the effectiveness of concurrent treatment in 2000. An executive committee of A-TOP was organized in 2002 and planned on forming a consensus regarding judgment standards for pre-existing fractures and incident vertebral fractures [16]. Morphometric criteria for incident fractures combined with a semi-quantitative assessment were thought to provide useful information on the study of clinical osteoporosis, especially for international comparisons. Next, to assume the number of participants in the clinical trial, the incident fracture rate and the risk of incident fracture were analyzed in the patient population, and the number of participants with sufficient statistical power [17] was calculated. Bone resorption marker was an independent risk factor for incident vertebral fractures in Japanese women. When the newly discovered risk factor was incorporated into the inclusion criteria in addition to conventional selection criteria such as age, prevalent fractures and bone mineral density, a reduction of about 40% in the estimated sample size was achieved. Thus, measurement of bone resorption markers is useful in reducing the sample size and the observation period in fracture-prevention studies carried out for developing drugs used to treat osteoporosis.

Materials and methods

Study design

Objective

JOINT was the first national, prospective, randomized, multicenter, open-labeled, blinded endpoints, controlled trial for osteoporosis made up mainly of practitioners of investigators in Japan. The objective of JOINT-02 was to clarify additive efficacy in terms of fracture prevention and safety, QOL and adherence in simultaneous use of alfacalcidol and ALN.

Subjects, intervention and endpoints

Confirmations regarding the patients were made by practitioners based on the inclusion and exclusion criteria (Table 1) after obtaining written informed consent. The