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

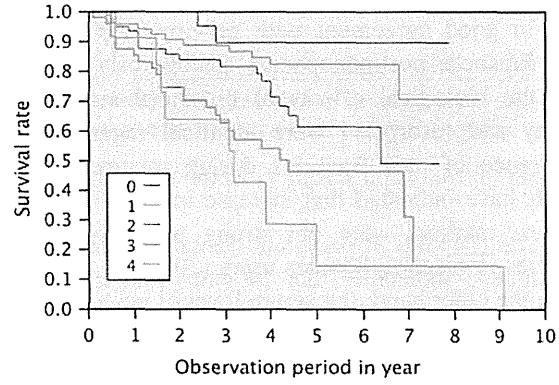

Fig. 1 Kaplan–Meier plot of the categorized patients under amino-bisphosphonate (amino-BP) treatment in accordance with the number of fracture risks (0–4). The lower survival rate from incident fractures was observed in accordance with the number of fracture risk in patients with amino-BP-treated osteoporosis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

Table 1 Inclusion and exclusion criteria

Inclusion criteria
Postmenopausal osteoporosis[a]
Over 70 years old
Ambulatory patients who do not require any help
Able to answer QOL questionnaire
Corresponds to more than one of A-TOP's risk factors for fracture[b]
Exclusion criteria
Metabolic bone diseases other than osteoporosis[c]
Contraindication to the drugs (ALN or alfacalcidol)
Dysfunction in communication of intentions
Severe degenerative deformation of vertebra
Abnormal heart function
Abnormal hepatic function
Abnormal kidney function
Treatment of osteoporosis by bisphosphonate within 6 months prior to the present study

These thresholds were decided by risk analysis by the A-TOP research group

[a] Over 1 year after menopause

[b] Pre-existing vertebral fracture number ≥ 1; BMD $\leq$ Young Adult Mean -3 SD; Urinary DPD ≥ 7.6 nmol/mmol Cr; or NTX $\geq$ 54.3 nmol BCE/mmol Cr

[c] Hyperparathyroidism and hyperthyroidism were excluded

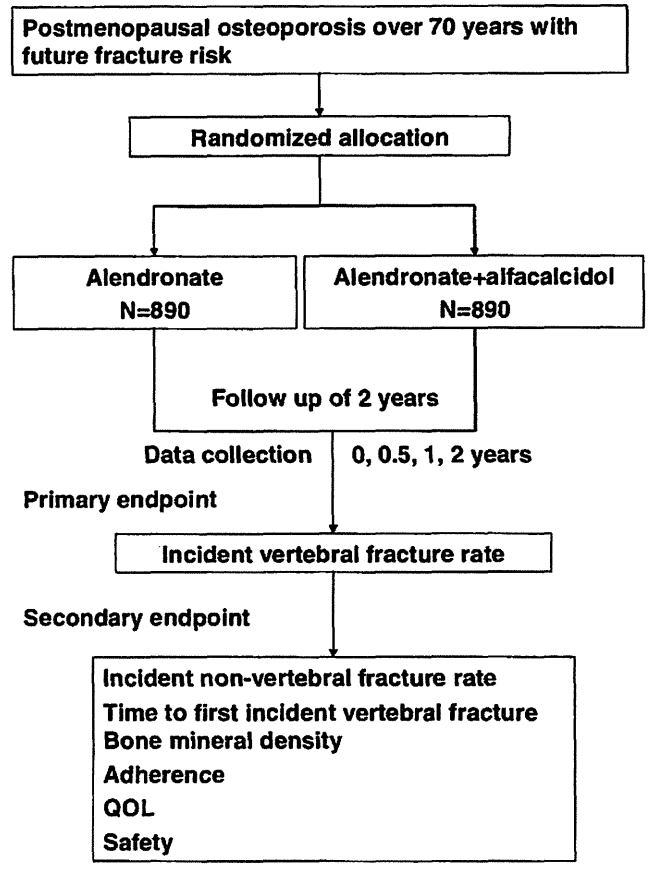

Fig. 1 Study design and outcomes

participants were selected by the practitioners and registered by Japan Clinical Research Support Unit (JCRSU), and then randomly allocated with a modified minimization method using age, number of pre-existing vertebral fracture number, bone mineral density (BMD) and value of bone metabolic marker into the group to be administered only ALN or the group that was to be administered both ALN and alfacalcidol. Registration and allocation of the participants were carried out on the Internet. After initiation of the assigned treatment, clinical data were collected at intervals of half a year for 2 years by I'cros Co., Ltd., through their visiting data collection service. Data were input to the database using a web system developed by ING Corporation. The primary endpoint was to compare the incident vertebral fracture rate between the intervention arms. The secondary endpoints were to compare the differences in the time to first incident vertebral fracture, non-vertebral fracture rate, bone mineral density, adherence, QOL and safety (Fig. 1). In addition, sub-group analyses categorized by baseline characteristics such as age, body mass index (BMI), serum 25-hydroxyl vitamin D levels, the number of pre-existing vertebral fractures and fracture grade were candidate factors. If participants wanted to change the designated treatment because of side effects or occurrence of fractures, they were permitted to do so, and follow-up observations were continued. Please see Table 1 and Fig. 1.

Sample size

Assumptions regarding the fracture rate in the ALN group were made based on a paper by Kushida et al. (Phase III trial for alendronate), in which it is reported that there was a 12.2% fracture rate during observations conducted over 2 years [8]. Since there are not much data on concurrent use of ALN and alfacalcidol [18], the authors' expectations were such that the effects of ALN would be added to those of alfacalcidol and that the hazard ratio of the alfacalcidol combined arm to ALN alone would be 0.64 [19]. The sample size was then estimated to be 890 cases per arm (two-sided alpha = 0.05, power = 0.8), taking account of a dropout rate of 10% referring to the value of the prior clinical trial of fracture intervention [4].

Fracture evaluation

X-ray films of conventional lateral radiographs of lumbar and thoracic vertebrae were taken and collected by I'cros Co., Ltd. After masking the patient's information, two

independent readers (orthopedist, TN and radiologist, MF) simultaneously reviewed films from T4 to L4 in chronological sequence and graded vertebral fracture based on a semi-quantitative method [20]. Before the start of the study, these two observers held meetings to make adjustments between their own criteria for grading vertebral fractures. When the diagnosis of pre-existing fractures made by the reviewers differed from those made by the practitioners, the reviewers' diagnosis was adopted preferentially. If inconsistencies arose between the readers in diagnosing pre-existing and incident vertebral fractures, the two readers negotiated between themselves to reach a consensus. Incident bone fractures other than those of the vertebrae were comprehended from the chart, and the occurrence of fractures was confirmed based on X-ray films or a record of the operation.

Clinical data

BMD at the lumbar vertebrae, hip (proximal femur), distal radius (dual energy X-ray absorptiometry) or left-sided second metacalpal bone (microdensitometry) was measured at baseline and at 6-month intervals for 2 years at each institute. The data of BMD obtained from the different machines and from different bone sites were calculated as the percentage change in each time point from the baseline value. The statistical difference in change of BMD between the group that received combined treatment and the group that was administered only ALN was compared based on each set of data for BMD for the different bone sites. Body height was measured at baseline, 12 and 24 months. Bone turnover markers (urinary type I collagen cross-linked N-telopeptide or urinary excretion of deoxypyridinoline) were measured at baseline and again 6 months after initiating treatment. QOL was assessed by using self-administered questionnaires (JOQOL and EQ-5D) at baseline and at 6, 12 and 24 months after initiating treatment [21]. Serum samples were sent to the central laboratory (SRL Co., Japan), and 25-hydroxyl vitamin D concentrations were measured. Other routine biochemical examinations were carried out at baseline and at 2 years after initiating treatment in order to estimate biochemical adverse events. All adverse events were reported to JCRSU, coded by MedDRA, and categorized as either "known" or "unknown." If an unknown adverse effect occurred, it was reported to the investigator and ethical committee.

Ethics and registration

Ethical issues regarding protocol were reviewed by the ethical committee for JOINT under the Declaration of Helsinki (Dr. Rikushi Morita, Chairman). If it turned out that a patient was at a disadvantage under observation, the ethical committee was given permission to stop the protocol. This study was registered at UMIN-CTR (University Hospital Medical Information Network—Clinical Trial Registry) with the number C000000001.

Statistical analysis

Analysis of the intent to treat principle was applied to the statistical analysis. Efficacy analysis uses a full analysis set (FAS), and all of the enrolled patients are applied to the analysis except for patients without efficacy data, patients who do not correspond to inclusion criteria and patients who do not receive treatment. The PPS (protocol per set) group is defined as consisting of patients without any serious protocol violation.

Recruitment of the practitioner

The explanatory meeting of the protocol and registration was held in all of Japan. The executive members of the A-TOP research group were responsible for the presentation of the protocol and for the recruitment of study institutions and practitioners. The A-TOP committee created the WEB site on the Internet so that the registration of the study could be executed directly. I'cros Co., Ltd., was also involved as a collaborator in calling practitioners.

Results and discussion

Several principles had to be considered for acceptance of concurrent treatment: firstly, the concurrent treatment should be clinically and statistically significantly more effective than the basic treatment; secondly, the concurrent treatment should be of the same level of safety as single treatment; thirdly, the concurrent treatment should be cost-effective. Although these principles should be evaluated before adopting concurrent treatment, the authors have applied concurrent treatment to osteoporosis widely, without any background evidence. Among these principles, the authors have decided to evaluate the first two issues, effectiveness and safety, in the present study. Since this type of evaluation is absolutely required by a clinician, a researcher-initiative study was considered as being the most suitable type of evaluation. This was the reason why the authors decided to use a researcher-initiative clinical trial in determining the effectiveness of concurrent treatment for osteoporosis. The JOINT-02 protocol was the first randomized, controlled trial conducted nationwide for osteoporosis in Japan initiated by researchers, and its scale was also the largest ever. It was therefore necessary for various organizations to collaborate together, and there

have been no previous reports on how to manage the PROBE trial in Japan. This is why we wanted to report the design of JOINT-02. In this paper, we have presented the organization of the A-TOP research group and execution of the JOINT 02 protocol. This is because we believe that this report should help a researcher who is willing to build a new nationwide investigation that is constructed by an organization of clinical research work.

Since the primary aim of JOINT-02 was to determine whether concurrent treatment using ALN and alfacalcidol is superior to treatment using ALN alone in terms of fracture prevention, true ("hard") endpoints such as vertebral fractures or long bone fractures were selected as the primary endpoint. The diagnosis of whether vertebral fractures were present or not on the X-ray films was made by two independent reviewers who did not have any information about the patient; when the judgment of fractures was split between the two reviewers, the reviewers negotiated with each other. Identifying vertebral fractures is more difficult than identifying long bone fractures due to some cases of new vertebral fractures not showing clinical symptoms and the shape of the vertebral body making it difficult at times to recognize whether there is a fracture. Therefore, to avoid misdiagnosis, diagnosis of pre-existing and incident vertebral fractures was made by two different observers. In cases where there was a discrepancy in the diagnosis of vertebral fractures between the reviewers and the practitioner, which occurred with regard to pre-existing fractures, the reviewers' judgment was given priority over that of the practitioner.

Surrogate ("soft") endpoints such as change in BMD or bone turnover markers were considered to be inadequate as primary endpoints in making conclusive statements regarding the efficacy of concurrent treatment. In this type of study, the soft endpoint (BMD or biomarkers) will connect dropout bias less effectively than the case-selected hard endpoint, because such markers are not able to be made blind to the clinicians. Furthermore, previous studies indicate that changes in BMD or bone markers do not predict future fractures [22–24].

In recent literature, it has been reported that poor adherence of bisphosphonates leads to a decline in the beneficial effects of this drug on bone [25–28]. It is expected that in contrast to prior developmental trials, this current study may have a higher dropout rate, since in a researcher-initiative study, the registered practitioner is not forced to maintain adherence very strictly. As a result, adherence in the present study may resemble the actual circumstances of adherence to bisphosphonate treatment by a general practitioner. It will be interesting to determine whether adherence in this study modifies fracture prevention by alendronate.

Although a careful plan for this study has been set up, the results will be applied to osteoporosis patients with the same background as the present study population, but not adapted to the entire osteoporosis population. Despite this limitation, we believe that the results will give us very important information regarding the concurrent treatment of osteoporosis.

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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

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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.

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.

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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:

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

Here, t is a time point for prediction (i.e. the formula calculates 10-year probability if $t = 10$), $\boldsymbol{\beta}$ 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).