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ORIGINAL ARTICLE

Japanese orthopedists' interests in prevention of fractures in the elderly from falls

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Abstract The aim of the present study was to survey the interest of Japanese orthopedists in preventing fractures in the elderly, and investigate their awareness with regard to main prevention strategies such as medications and hip protectors. From the list of 20,899 members of the Japanese Orthopedic Association, we randomly selected a sample of 2035 people. Each orthopedist was sent an anonymous survey consisting of 12 questions during July to August 2001. At that time, risedronate, raloxifene, and parathyroid hormone had not been approved for clinical use in Japan, and even alendronate had just been approved. Of the survey forms sent, 1011 responses were received, for a response rate of 50%. Analysis of these responses showed a very high interest in osteoporosis, fractures in the elderly from falls, and the prevention of such fractures. This interest was associated with physician age, with those above the age of 50 years being 2.3 times more likely to have an interest in each of these than physicians below that age. The respondents considered the most promising measure for the prevention of fractures in the elderly from falls to be fall prevention, followed by exercise and osteoporosis medications. The medication considered to be effective as a monotherapy by the overwhelming number of respondents was bisphosphonates, followed by vitamin D_3 and calcitonin. Combination agents cited were vitamin D_3, bisphosphonates, and calcitonin, in that order. Forty-two percent of respondents had some knowledge of hip protectors, but confidence in them as a means to prevent fractures was still low. The practical information from our survey should serve as a starting point for comparison to periods when new bisphosphonates or hip protectors become commonly available to Japanese orthopedists. The overall results indicate that Japanese orthopedists are very positive toward fracture prevention.

Keywords Fall · Fracture · Hip protector · Medication · Osteoporosis · Survey

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Introduction

As the proportion of elderly continues to increase, the aging of Japan's population outpaces that of most countries in the world. People over the age of 65 years accounted for 18% of the total population in 2001, an increase of 1.5-fold over 10 years. This remarkable increase in the proportion of the elderly population has resulted in an increase in diseases characteristic of the elderly, with striking escalations in osteoporosis and fragility fractures. For example, new hip fractures increased a dramatic 1.7-fold in the 10 years from 1987 to 1997 in Japan [1]; worldwide, such fractures are expected to increase from an estimated 1.26 million people in 1990 to 2.60 million in 2025 [2].

Unless efficient and effective measures to prevent such increases in fragility fractures due to osteoporosis in the elderly are carried out comprehensively, the medical economic burden is foreseen to be great, and post-fracture mortality and morbidity will become a troublesome burden on society [3,4,5]. In fact, according to a 1998 national survey by the Japanese government, fall fractures accounted for 10% of the underlying causes requiring people over the age of 65 to receive care. This was the second leading underlying cause. This percentage also increased with age, reaching 17% in those aged

over 85, ranking fall fractures together with cerebrovascular diseases in the top position [6].

To alleviate these problems it is essential to curb the occurrence and accumulation of fractures among the elderly. For orthopedists, who are in the forefront in the management of elderly patients with fragility fractures or those at high risk of such fractures, simply treating the fracture without addressing the underlying weakened skeleton is not enough [7,8]. Orthopedists should not leave the patient at risk for the accumulation of fractures. However, there have been few surveys of the actual state of the care orthopedists provide for prevention of fractures [9,10,11]. In the present study, therefore, we surveyed the interest of Japanese orthopedists in preventing fractures in the elderly, and investigated their attitudes toward main prevention strategies such as medications and their level of knowledge of hip protectors.

Materials and methods

Selection of subjects

The subjects of the survey were physicians comprising 10% of the membership of the Japanese Orthopedic Association (JOA) as of June 2001. Before selecting the subjects, we sent a letter to the president of the JOA requesting permission to use the membership directory and digital data from the list of printed address labels, with a copy of the questionnaire also enclosed, and obtained his consent.

From the list of 20,899 members, we automatically selected every ninth person on the list starting with the first person, for a randomly selected sample of 2035 people, or about 10% of the membership. The sex and year of graduation from medical school of each person were ascertained from the JOA directory, and an individual identification number was allocated to each.

In Japan, physicians are allowed to freely establish a practice in any field of specialty, and following university research or the accumulation of clinical experience in a hospital, many orthopedists go into private practice to treat motor diseases in community residents. According to the JOA, 25% of its members are in private practice.

Questionnaire survey

Each orthopedist was sent a one-page anonymous survey consisting of 12 questions, along with a covering letter providing details of the proposed study and a prepaid return envelope, in July to August 2001.

The respondents were assured that the information would be used in aggregate form only and that no individual or unit would be identified. All questionnaires received by the end of September 2001 were included for analysis. A reminder was not sent to non-responders.

Three main areas were addressed in the study questionnaire: interest in the prevention of elderly fractures by falls, strategies for fracture prevention including hip protectors, and demographic items. At the time of the survey, the bisphosphonates for the treatment of osteoporosis that had been approved for use in Japan were etidronate and alendronate (sales approval overlapped with the survey period).

Statistical analysis

Data handling and statistical analysis were performed using Statview (SAS Institute, Cary, N.C., USA). Associations between categorical variables were tested with chi-squared distribution, and differences between means for continuous variables were analyzed using the *t*-test. A *P*-value of 0.05 (two-tailed) was used to define statistical significance. Logistic regression was used to adjust significant findings for multiple variables.

In an analysis using a logistic regression model, the physicians' level of interest in osteoporosis, fall fractures, and fall fracture prevention was classified as "very much" or "less than very much." For the investigation of demographic data, the proportion of elderly patients was divided into "50% or more" and "less than 50%," physician age as "50 years or more" and "less than 50 years," and workplace as "private practice" and "non-private practice" (physicians employed at university institutions or non-university hospitals).

Results

By the end of September 2001, 1011 responses had been received for a response rate of 50%. Of the responses, 976 were complete (complete response rate 48%), and these were used in the analysis.

The mean number of years since graduation from medical school of the respondents was 22.9, greater than the 17.0 years for non-respondents ($P < 0.0001$). In addition, 50% of males and 36% of females responded ($P = 0.0278$).

The main demographic data for the physicians are shown in Table 1. Those in their 30s and 40s accounted for more than half, at 57%, and private practitioners for less than half, at 39%. Among all members of the JOA, the percentages working in university hospitals, other hospitals, private practice and others was 21%, 52%, 25% and 2%, respectively. In terms of this distribution, the reply rate in the present study was lower in the physicians working in the university hospitals and higher in those in private practice. Seventy-two percent of physicians responded that more than half of their patients were elderly.

The intensity of orthopedists' interest in osteoporosis, fractures in the elderly from falls, and prevention of fractures in the elderly from falls was very high overall (Table 2). More than half had "very much" interest in

Table 1 Characteristics of respondents

	Number	Percentage of complete respondents
Sample population	2035	–
Complete respondents	976	–
Age		
20-	54	6%
30-	239	24%
40-	312	32%
50-	179	18%
60-	137	14%
70-	55	6%
Gender		
Male	953	98%
Female	23	2%
Current workplace		
University hospital	98	10%
Public hospital	157	16%
Private hospital	307	31%
Private practitioners	382	39%
Other	32	3%
Ratio of elderly patients[a]		
90%-	24	2%
70%-	219	22%
50%-	458	47%
30%-	206	21%
10%-	48	5%
9% or less	21	2%

[a]Ratio of patients aged 65 years or more to all patients

all three items; those with interest "to some extent" or greater exceeded 90% for all of three items. Orthopedists reporting no interest at all were equal to or less than 1% for each.

A significant association excluding gender was found between these interests and the demographic data of the doctors. After adjustment with a logistic correction model, there was a consistent correlation between age and these three interests; the interest in each was about 2.3 times greater in orthopedists over the age of 50 than in those below that age. There was also a greater interest in osteoporosis and the prevention of fractures in the elderly from falls among orthopedists in private practice than among those not in private practice. Physicians whose patients were more than 50% elderly had a greater interest in prevention of fractures from falls in the elderly than did physicians with fewer than 50% elderly patients.

Next, when asked to name promising strategies to prevent fractures in the elderly from falls, the most common responses was fall prevention measures, followed by exercise and osteoporosis medications (Table 2). The most common combination strategy, determined from multiple responses, was exercise and fall prevention (179 respondents), followed by osteoporosis medications, exercise, and fall prevention (149 respondents), a combination of all strategies (142 respondents), and osteoporosis drugs and fall prevention (135 respondents). A great many doctors thus regarded fall prevention measures as necessary.

However, when those who responded that fall prevention measures were promising were asked if they were actually implementing such measures with their patients, 303 (39%) reported that they were and 472 (61%) that they were not. Fall prevention measures were carried out by significantly more physicians who had very much interest in osteoporosis, fractures in the elderly from falls, and their prevention.

In response to questions on promising medications for the prevention of fractures in the elderly from falls, 685 responded with the name of some drug (Table 3). The agents overwhelmingly mentioned as being promising as a monotherapy were bisphosphonates, followed by vitamin D_3 and calcitonin. These three agents accounted for 86% of responses.

The number of physicians responding with drug combinations was 255, and the above three agents again had the top three selection rates. The order, however, was reversed with vitamin D_3 first and bisphosphonates second. Combinations were selected by 32% of orthopedists in private practice and 23% of those not in private practice, so there was a higher rate of selection of multidrug treatment among those in private practice ($P=0.0042$). In addition, older physicians selected multidrug treatment at a higher rate. The percentage of elderly patients was not related with the choice of multidrug treatment.

The contribution of physicians' demographic data to level of interest was investigated using a logistic regression model (Table 4). Age showed a significant associ-

Table 2 Frequencies of responses regarding interests and strategies in prevention of elderly fractures

Question	Number of replies				
	Very much	To some extent	Little	*None*	
Do you have an interest in osteoporosis?	494 (51%)	417 (43%)	57 (6%)	8 (1%)	
Do you have an interest in fractures in the elderly from falls?	553 (57%)	379 (39%)	39 (4%)	5 (1%)	
Do you have an interest in prevention of fractures in the elderly from falls?	510 (52%)	405 (41%)	57 (6%)	4 (0%)	
–	*Osteoporosis drugs*	*Nutrition guidance*	*Exercise*	*Fall prevention*	*Other*
Please select strategies considered to be promising for the prevention of fractures in the elderly from falls[a]	624 (64%)	237 (24%)	690 (71%)	767 (79%)	64 (7%)

[a]Multiple answers are possible

Table 3 Drugs or supplements the respondents found the most promising for prevention of elderly fractures

Number of replies	Monotherapy 430	Multiple drug or supplement 255	No response 291
	Number of responses for drug or supplement	Number of responses for drug or supplement	
Vitamin D_3	43 (10%)	174 (68%)	
Vitamin K_2	9.(2%)	81 (32%)	
Calcitonin	43 (10%)	135 (53%)	
Bisphosphonate	284 (66%)	159 (62%)	
Iprifravon	1 (0%)	10 (4%)	
Estrogen	20 (5%)	64 (25%)	
Ca supplements	5 (1%)	60 (24%)	
Other	25 (6%)	10 (4%)	

ation with level of interest in each of the three items mentioned above. The interest of Japanese orthopedists above the age of 50 years in each of these items was more than 2.3 times greater than that in orthopedists below that age. Physician workplace was also associated with interest in osteoporosis and prevention of fractures in the elderly from falls. Private practitioners were more likely to have greater interest in these items. A significant association was also seen between percentage of elderly patients and level of interest in prevention of fall fractures.

When promising strategies to prevent elderly fractures from falls were analyzed similarly, significant associations were found between age and drugs, age and nutrition guidance, and workplace and exercise. With regard to promising drugs to prevent fractures in the elderly from falls, physician age showed significant associations with vitamin D, calcitonin, bisphosphonates, and calcium. Similarly, workplace was associated with multidrug treatment and calcitonin (Table 4).

Finally, in response to questions on hip protectors, 20% reported being very familiar with hip protectors. With the addition of those who had seen hip protectors, altogether 42% of respondents had a certain level of knowledge of hip protectors. However, the most common response was having heard of hip protectors only. This together with the 18% who knew nothing at all of hip protectors indicated that the majority of respondents lacked knowledge of hip protectors (Table 5).

To the question of whether hip protectors can prevent hip fractures, fewer than 10% of the orthopedists who reported that they were very familiar with hip protectors, had seen hip protectors, or had heard of hip protectors, responded that hip protectors were sufficiently able to prevent such fractures. The great majority had a lower assessment, while 20% responded that they did not know (Table 5).

The contributions of level of doctor interest and demographic data to a response of being very familiar with hip protectors were examined with a logistic regression model. The results showed that only level of interest in preventing fall fractures was significantly associated with this response (OR: 2.18, 95%CI: 1.32, 3.61).

Discussion

In this survey, we were able to gather practical information on the interests of Japanese orthopedists in preventing fractures in the elderly, as well as their awareness with regard to main prevention strategies

Table 4 Significant OR (95%CI) defined by logistic regression in demographic data of physicians. The interest of physicians in each item was treated as a dependent variable, and demographic data as an independent variable. Similarly, each strategy or each drug was treated as a dependent variable, and demographic data as an independent variable, in analyzing the associations between promising strategies or drugs and demographic data

		Age	Workplace	Percentage of elderly patients
Interest in osteoporosis		2.32 (1.75, 3.08)	1.94 (1.47, 2.57)	–
Interest in fractures in the elderly from falls		2.34 (1.75, 3.12)	–	–
Interest in prevention of fractures in the elderly from falls		2.37 (1.79, 3.14)	1.41 (1.07, 1.86)	1.36 (1.02, 1.82)
Promising strategies to prevent fractures in the elderly from falls	Drugs	1.39 (1.02–1.88)	–	–
	Nutrition guidance	0.68 (0.49–0.93)	–	–
	Exercise	–	0.71 (0.52–0.97)	–
	Fall prevention measures	–	–	–
Promising drugs or supplements to prevent fractures in the elderly from falls	Multidrug treatment	–	1.37 (1.01–1.87)	–
	D	1.84 (1.32–2.56)	–	–
	CT	1.76 (1.23–2.6)	2.00 (1.41–2.85)	–
	Bis	0.45 (0.34–0.61)	–	–
	Ca	1.87 (1.08–3.23)	–	–

Table 5 Knowledge and confidence about hip protectors among respondents

Question	Number of replies				
	Yes, very familiar	I have seen it	I have heard of it	Never heard of it	–
Are you familiar with this device?	193 (20%)	217 (22%)	388 (40%)	178 (18%)	–
Do you think that a hip protector can prevent hip fractures?[a]	Quite possible 57 (8%)	To some extent possible 374 (51%)	Not very possible 130 (18%)	Impossible 25 (3%)	Don't know 150 (20%)

[a]Question to doctors who are very familiar with hip protectors, have seen or heard of them

such as medications and hip protectors. This should serve as a starting point for comparison to periods when new bisphosphonates or hip protectors become commonly available to Japanese orthopedists.

Patients with fragility fractures represent a unique opportunity for treatment intervention. Failure to treat them for osteoporosis at the time of the fracture is a missed opportunity for prevention of additional fragility fracture [12]. According to several surveys, however, the rate at which diagnostic evaluation or treatment aimed at secondary prevention of fragility fractures is implemented is not high. One study reported that only 13% of patients with hip fracture were treated with osteoporosis medication at discharge [13], and others reported rates of osteoporosis follow-up for patients with wrist fracture of 24% [14] and 50% [15]. In addition, 24% of women with fractures of various sites received an osteoporosis drug [16] and 49% were evaluated or treated for osteoporosis [17] during the 1 or 2 years following fracture.

Writing about the attitudes of orthopedists to the prevention of fragility fractures, the editor of one orthopedics journal stated that, "historically, orthopedists have readily treated fragility fractures, but they have rarely followed through and initiated care and treatment of the porous skeleton. Fixation of fractures is not enough. Orthopedists must strive to prevent fractures rather than treating them once they occur" [7].

To the best of our knowledge, there are not a great many surveys on the interests or attitudes of orthopedists toward the prevention of osteoporotic fractures. However, from a 1998 British survey of 70 orthopedic surgeons it was reported that "only a small percentage of orthopedic surgeons advised their patients routinely on various preventive measures for osteoporotic fractures" [9]. A 2000 survey of 89 orthopedic surgeons in Ireland reported that these orthopedists had a passive stance with regard to secondary prevention following hip fractures [10]. In the clinical scenario of the questionnaire, 83% of the orthopedic surgeons responded that they would not initiate or recommend investigation of the extent of the underlying osteoporosis in the hypothetical case of a 72-year-old female with a hip fracture after a minor fall. Looking only at these surveys, the pessimism of the editor cited above is quite understandable.

From a comparison of our results with these other surveys, it would seem that Japanese orthopedists are much more positive toward fracture prevention. No similar surveys were conducted in the past, so the generational changes in prevention awareness cannot be known; however, it is possible that orthopedists are instinctively coming to recognize the importance of prevention as the number of fractures in the elderly in Japan rapidly increases.

However, the real attitude or practice seems to be different from the interest or awareness. Even among the orthopedists in the present survey who responded that fall prevention is promising, only 39% actually implemented fall prevention measures, revealing a chasm between thinking and implementation. This gap between interest and implementation in Japanese orthopedists may also be seen in other strategies such as medication, nutrition guidance or exercise, although the precise rates are unknown due to a limitation of the present study design. However, the high interest in preventing fractures among the respondents will surely provide a strong basis for the early improvement of the low implementation rate.

One reason for the forward-looking interest of Japanese orthopedists in fracture prevention may be the influence of orthopedists in private practice. Many of them treat outpatients with non-surgical methods, and so may have greater occasion to consider and implement preventive measures than do hospital doctors who are pushed toward surgery. Of the present respondents, 39% were private practitioners, and their interest in osteoporosis and fracture prevention was higher than that of physicians in other employment systems.

Measures thought by Japanese orthopedists to be particularly important for the prevention of fractures in the elderly from falls were fall prevention, exercise, and drugs, in that order. Among these measures, fall prevention is most commonly taken up in combination with several other fall fracture prevention methods, indicating that fall prevention occupies a central position in approaches to fracture prevention. The British survey mentioned above [9] revealed a similar tendency in that a majority (69%) of orthopedists agreed that physiotherapy and occupational therapy were very important to minimize. They advised physiotherapy and occupational therapy at a higher rate than other measures such as diet (19%), exercise (17%), calcium supplement (3%), vitamin D alone (0%), vitamin D with calcium (7%), bisphosphonates alone (0%), bisphosphonates with calcium (4%) or calcitonin (1%). Although the data from the present survey do not permit us to clarify why

the majority of Japanese orthopedists believe that fall prevention is more important than medical management, some reasons may be suggested. First, the circumstances of orthopedists may make them consider fractures of the elderly to be injuries due mainly to the accident force rather than the underlying osteoporosis. Most patients with fractures other than asymptomatic spinal fractures visit or are transported to orthopedists as accident patients. Consequently, orthopedists may be prone to regard fall prevention as the strategy to be adopted first. Secondly, the delay of approval in Japan for new osteoporosis medicines such as risedronate, raloxifene, and parathyroid hormone, for which there is strong evidence of fragility fracture prevention, may be related to such results. Because none of these medicines was approved for clinical use and even alendronate had just recently been approved in Japan at the time of our survey, Japanese orthopedists did not at the time have sufficient knowledge or confidence in the power of these new osteoporosis medicines to prevent fractures. Therefore, the difference in attitudes toward fall prevention and medication would likely be reduced if a similar survey were to be conducted today.

The relationship between physician demographic data and responses about level of interest in fractures among the elderly and promising measures and medications to prevent such fractures was investigated in a multivariate analysis. The most consistent influence on these items was the age of the physician him- or herself. This differs from a survey of English orthopedic surgeons in which no difference was seen according to age [9]. Japanese doctors over the age of 50 have a significantly greater interest in fractures and their prevention than do doctors below that age, and believe that medications are a promising measure for such prevention. The agents most commonly selected by them were vitamin D, calcitonin, and calcium, with few doctors selecting bisphosphonates. This age-dependent influence reflects the experienced judgment based on long years of medical practice of these physicians, and possibly a tendency as well for older doctors to regard osteoporosis and fractures from falls as being problems closer to them personally. The hesitation seen in older physicians to select bisphosphonates, which are relatively new drugs in Japan, may indicate their conservative tendencies toward new drugs.

The effectiveness of hip protectors is still not highly evaluated by Japanese orthopedists, even though their preventive efficacy against hip fractures has also been reported in Japan [18]. Forty-two percent of physicians in the present study knew something about hip protectors, and 60% of these physicians were aware that they had some real effect in fracture prevention. Even though the level of awareness is still low, knowledge over a certain level was found to exist. Be that as it may, at the time of the survey there was a large gap between knowing about and actually recommending that high-risk patients wear hip protectors. The confidence of Japanese orthopedists in hip protectors still seems to be low, and information should continue to be provided regarding the reliability of hip protectors.

A limitation of the present study is thought to be the moderately low response rate, so that the results possibly do not reflect overall trends. For example, the results may be biased toward the stratum of older males. They may also have been biased by the lower percentage of responses from orthopedists in university hospitals and the higher percentage from those in private practice. However, considering that female orthopedists account for a very low proportion of only 3.2% of all Japanese orthopedists, and that the 2–4 years after graduation from medical school is a period of training, the study subjects would seem to approximate the stratum of orthopedists that is actually involved in daily orthopedic treatment in Japan. The present analysis results may therefore be a fairly accurate reflection of the current approaches to the prevention of fractures in the elderly from falls among Japanese orthopedists.

Another possible limitation is that the special circumstances of Japanese orthopedics may have made the results of the survey pertain primarily to the Japanese. Orthopedics in Japan is different from most other countries in that there are many non-surgical orthopedic practitioner. This fact should be taken into consideration when comparing the results of our survey with those of similar surveys from other countries. However, considering the results of the British survey cited above and ours, the tendency to regard fall prevention as the first strategy for preventing fractures in the elderly may be common in orthopedists of many countries.

In conclusion, our survey showed that Japanese orthopedists had a very high interest in osteoporosis, fractures in the elderly from falls, and the prevention of such fractures. They considered the most promising measure for the prevention of fractures in the elderly from falls to be fall prevention, and the most effective agents to be bisphosphonates, vitamin D_3 and calcitonin. Their confidence in hip protectors as a means to prevent fractures was still low.

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特集
高齢者の歩行障害
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転倒リスクの多因子評価

新野　直明*

KEY WORD
高齢者
転倒
危険要因
多因子評価
縦断的検討

POINT
- 高齢者の転倒は寝たきりの主因の一つである．
- 転倒発生に関連する要因あるいは転倒の危険性を増す要因(転倒要因)として多数の要因が報告されている．
- 転倒の予防には，転倒要因を取り除くことが必要であり，多数の要因の転倒に対するリスクを正確に評価する系統的な調査研究が重要である．

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はじめに

高齢者の転倒は，打撲傷，骨折など様々な外傷を引き起こし，寝たきりの大きな原因となる[1]．高齢化が急速に進行しているわが国において，高齢者の日常生活動作(ADL)の低下，寝たきりを引き起こす転倒について検討することは大きな意義がある．

ところで，高齢者の転倒発生に関連する要因あるいは転倒の危険性を増す要因，すなわち転倒要因としては，これまでにも多種類の要因が挙げられている．しかし，様々な領域にわたる多くの要因と転倒の関係を同時に検討した研究は少ない．本論文では転倒要因について簡単にふれるとともに，複数の要因と転倒の関連を調べた著者の研究の一部を報告する．

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高齢者の転倒要因

高齢者の転倒は，加齢による心身機能の変化・低下と周囲の環境的要素が相互に関係しあって発生するものであり，多数の要因が関与すると考えられる．表1は，転倒の危険要因の可能性が指摘されている要因を簡単にまとめたものである[2]．

この表は要因を単純に列記したものだが，江藤はより系統的に転倒要因を分類している[3](図1)．その分類では，転倒要因を大きく内的要因と外的要因に分け，内的要因を心理要因と身体要因とし，さらに感情，高次，感覚，運動に分類する．外的要因は生活環境・習慣と薬物を考える．

以上の図表を見ると，転倒要因としては，身体的な問題や段差などだけではなく，幅広い分野にわたる多種類の要因があることが改めてわかる．しかし，転倒要因については，多数の要因の相互影響を考慮した評価，あるいは因果関係を推定するために重要な縦断的検討は少ない

表1　転倒の危険要因となる可能性のある要因(新野，1998：文献2より引用)

1．年齢(加齢)	ただし超高齢では転倒が減少する場合もある
2．女性	ただし男性がリスクが高い，性差なしとする研究もある
3．社会的要因	無配偶者(独身，離婚，死別)，閉じ込もり，など
4．身体的，精神的疾患	起立性低血圧，高血圧，不整脈，脳卒中(後遺症)，パーキンソン症候群，視力障害，聴力障害，関節疾患，排尿障害，排便障害，痴呆，うつ病，など
5．薬剤	睡眠剤，鎮静剤，降圧剤，利尿剤，など
6．(特別な)行動	単独歩行，ベッド昇降，車椅子乗り降り，入浴，排泄，など
7．環境的要因	段差，凹凸のある床，滑る床，不十分な照明，履物，介護・看護者数の減少，不適切な補助具，慣れない環境，など
8．転倒の既往	

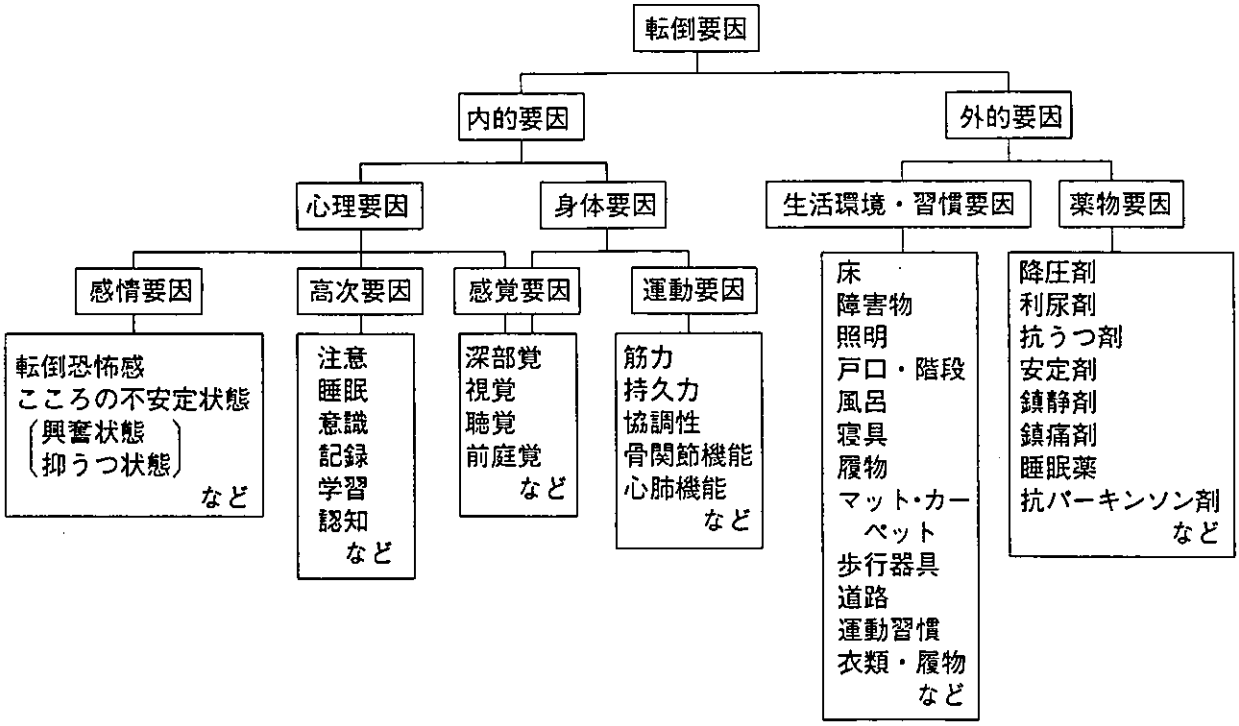

図1　転倒要因の分類(江藤真紀，2003：文献3より引用)

ため，これらの要因のすべてが実証的な研究で確認されているとは言い難い．そこで筆者は，複数の転倒要因と転倒の関係に関する縦断的研究を進めている．次章ではその研究の一部を紹介する．

高齢者の転倒要因に関する多因子評価

筆者は，厚生労働省長寿科学総合研究の一環として，静岡県浜松市保健所の協力を得て，浜松市内のM町とH町において縦断的な転倒調査を実施した[4]．その調査における転倒要因評価に関する結果を紹介する．

1．浜松市M町における調査

a．調査の概要：浜松市M町の65歳以上住民719名を対象として，転倒の関連要因について面接と質問紙による調査を行った．約1年後に，初年度調査参加者を対象に1年間の転倒既往について調べた．初年度の調査項目は以下の通りである．①過去1年間の転倒の有無，②日常生活動作能力(ADL)，③主観的健康度，④受療状況，既往歴，⑤うつ状態(GDS：Geriatric Depression scale)，⑥社会的活動，⑦身体測定

表2　転倒の関連要因：M町の結果（χ^2検定）

		転倒者%(n)
性	男性	18.2(28)
	女性	21.3(52)
年齢	65-74歳	15.0(36)**
	75歳以上	27.4(43)
過去1年の転倒既往	なし	16.3(56)**
	あり	45.1(23)
ADL(食事，歩行，トイレ，着替え，入浴の5行動)	全部自立	18.0(67)**
	要介助あり	63.2(12)
	全部できる	12.6(19)**
老研式活動能力指標	できないことあり	24.8(60)
主観的健康度	良い	16.8(52)
	悪い	31.5(28)
治療中疾患	なし	9.0(10)**
	あり	24.4(70)
脳卒中既往	なし	19.8(77)
	あり	30.0(3)
心臓病既往	なし	19.2(68)
	あり	27.9(12)
高血圧既往	なし	16.3(43)**
	あり	27.6(37)
糖尿病既往	なし	20.4(76)
	あり	16.0(4)
骨粗鬆症既往	なし	19.5(74)
	あり	31.6(6)
膝関節症既往	なし	19.4(68)
	あり	25.5(12)
緑内障既往	なし	19.5(74)
	あり	33.3(5)
白内障既往	なし	17.4(49)
	あり	24.5(27)

		男性%(n)
眼手術既往	なし	20.5(76)
	あり	12.5(3)
内服薬	なし	27.8(5)
	あり	24.2(65)
降圧剤服用	なし	20.3(31)
	あり	29.1(39)
安定剤・睡眠薬服用	なし	23.7(64)
	あり	35.3(6)
Ca剤服用	なし	23.1(62)
	あり	42.1(8)
うつ状態	なし(GDS≦5)	16.3(44)**
	あり(GDS≧6)	30.2(29)
現在の仕事	している	19.0(58)
	していない	23.7(22)
自治会活動	している	33.3(4)
	していない	19.7(76)
老人クラブ活動	している	22.8(28)
	していない	18.9(52)
肥満	なし(BMI<25)	19.6(64)
	あり(BMI≧25)	22.9(16)
遠見常用視力	良好(≧0.3)	15.6(23)*
	不良(<0.3)	22.7(57)
立体視	良好	18.2(43)
	不良	24.3(36)
動体視力	良好(>0.1)	14.3(23)*
	不良(≦0.1)	24.4(53)
握力	高(平均以上)	15.5(30)*
	低(平均未満)	24.5(50)

**：$p<0.01$，*：$p<0.05$，＋：$p<0.1$.

(身長，体重，握力，血圧など)，⑧視力(常用・矯正遠見視力，常用・矯正近見視力，動体視力，立体視)．

これら初年度に調べた項目がその後1年間の転倒の有無に関連するかをχ^2検定により調べた．さらに，これらの検定で転倒に有意に関連した項目を説明変数，転倒の有無を目的変数としてロジスティック回帰分析を行った．

b．結果：初年度調査回答者は481名(男性196名，女性285名，平均年齢73.5歳，回答率66.9%)，2年度回答者は421名(男性164名，女性257名，平均年齢75.2歳，初年度回答者の87.5%)であった．

2回の調査に参加した421名中，初回調査から2回目調査までの1年間に転倒した人は80名(20.1%)であった．単変量の分析では，転倒既往あり，高齢，ADL不良，主観的健康度不良，治療中疾患あり，高血圧既往あり，うつ状態あり，常用遠見視力不良，動体視力不良，握力平均以下の場合に有意に転倒者が多く，前述したように多数の要因が転倒発生の危険要因となる可能性が見られた(表2)．しかし，ロジスティック回帰分析により要因相互の影響を考慮したところ，転倒の既往あり，ADL不良，高

表3　転倒の関連要因：M町の結果

（多重ロジスティック回帰分析，転倒なし＝0，あり＝1）

要因	オッズ比	95%CI	P
性（男性＝0，女性＝1）	0.71	(0.38, 1.35)	ns
年齢（65-74＝0，75以上＝1）	1.36	(0.71, 2.63)	ns
転倒既往（なし＝0，あり＝1）	3.43	(1.57,7.47)	<0.01
ADL（良好＝0，不良＝1）	6.00	(1.67, 21.63)	<0.01
老研式活動能力（良好＝0，不良＝1）	0.92	(0.39,2.16)	ns
主観的健康（良好＝0，不良＝1）	1.28	(0.63, 2.58)	ns
治療中疾患（なし＝0，あり＝1）	2.03	(0.85, 3.16)	ns
高血圧既往（なし＝0，あり＝1）	1.98	(1.06, 3.69)	<0.05
うつ状態（なし＝0，あり＝1）	1.32	(0.66, 2.63)	ns
遠見常用視力（良好＝0，不良＝1）	1.25	(0.61, 2.55)	ns
動体視力（良好＝0，不良＝1）	1.16	(0.54, 2.50)	ns
握力（平均以上＝0，以下＝1）	1.16	(0.61, 2.20)	ns

95%CI：95%信頼区間　ns：not significant

表4　転倒の関連要因：H町の結果

（多重ロジスティック回帰分析，転倒なし＝0，あり＝1）

説明変数	オッズ比	95%CI
遠見常用視力（良好＝0，不良＝1）	2.54*	1.02-6.35
性（男性＝0，女性＝1）	1.61	0.90-2.89
年齢（65-74＝0，75以上＝1）	0.55	0.30-1.04
ADL（良好＝0，不良＝1）#1	2.33	0.98-5.58
握力（平均以上＝0，以下＝1）	1.97*	1.08-3.60
うつ状態（なし＝0，あり＝1）#2	1.83	0.97-3.44
転倒既往（なし＝0，あり＝1）	3.33**	1.84-6.03

95%CI：95% 信頼区間　*p<0.05，**p<0.01.

#1 不良：歩行，食事，入浴，排泄，着替えのいずれかに介護が必要

#2 うつ状態あり：GDS≧6

血圧既往ありの場合に有意に転倒ありが多く，最終的には転倒の既往，ADL，高血圧既往が，その後1年間の転倒と独立して関連することが示された（表3）．

2．浜松市H町における調査

a．調査の概要：浜松市H町の65歳以上住民885名を対象とし，前述のM町とほぼ同様の調査内容，分析方法による検討を行った．

b．結果：2回の調査の両方で情報の得られた人は417名（男性160名，平均年齢73.4歳，女性257名，平均年齢73.8歳）であった．初回調査から2回目調査までの1年間に転倒した人は87名（20.9%）であった．単変量の分析では，転倒既往あり，高齢，ADL不良，うつ状態あり，常用遠見視力不良，握力平均以下の場合に転倒者の割合が有意に高かった．しかし，多変量の分析結果では，転倒既往あり，遠見常用視力不良，握力平均以下の場合に転倒ありが多く，最終的には，転倒既往，遠見常用視力，握力の3要因が調査後1年間の転倒発生を予測する要因となる可能性が示された（表4）．

以上の結果を概観すると，最終的な結果として得られた転倒要因はきわめて妥当なものだったという印象である．さらに，転倒の既往，握力，常用視力などは，測定が比較的容易であることから，転倒のハイリスク高齢者を見つける場合に有用性は高いと考えられる．また，多因

子評価の結果残った有意な転倒要因の数はそれほど多くはないことがわかる．限られた研究から結論を出すことはできないが，転倒リスクの多因子評価を厳密に実施するならば，独立した危険要因として抽出されるものはそれほど多くはないのかもしれない．

おわりに

高齢者における寝たきりの主因である転倒を予防するには，その危険要因を取り除くことが必要である．そのためには，転倒発生に関わる要因を特定することが欠かせない．多数の要因の転倒に対するリスクを正確に評価する系統的な調査研究をさらに積み重ねていくことが重要であろう．

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ORIGINAL ARTICLE

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Association of polymorphisms of the androgen receptor and klotho genes with bone mineral density in Japanese women

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Abstract Genetic variants of the androgen receptor and klotho protein may contribute to variation in bone mass as well as to predisposition to osteoporosis. The relationship of a CAG repeat polymorphism of the androgen receptor gene (*AR*) and of a –395G→A polymorphism of the klotho gene (*KL*) to bone mineral density (BMD) in Japanese women was examined in a population-based study. The subjects (1,101 and 1,110 women for *AR* and *KL* polymorphisms, respectively) were aged 40–79 years and were randomly recruited to a population-based prospective cohort study of aging and age-related diseases. BMD for the total body, lumbar spine, right femoral neck, right trochanter, and right Ward's triangle was measured by dual-energy X-ray absorptiometry. Genotypes for the *AR* and *KL* polymorphisms were determined by polymerase chain reaction based assays. The number of CAG repeats of *AR* was inversely correlated with BMD for the lumbar spine in premenopausal women but not in postmenopausal women. The $(CAG)_{n\leq 22}$ and $(CAG)_{n\geq 23}$ alleles were designated *S* and *L*, respectively. Among premenopausal women, BMD for the total body was significantly lower in subjects with the *LL* genotype than in those with the *SS* genotype or those in the combined group of *SS* and *SL* genotypes. In contrast, BMD was not associated with *AR* genotype in postmenopausal women. Among all women, BMD for the lumbar spine was significantly lower in subjects with the *GG* genotype of the –395G→A polymorphism of *KL* than in those with the *AA* genotype. BMD was not associated with –395G→A genotype among premenopausal women. In postmenopausal women, BMD for the total body or lumbar spine tended to be lower in subjects with the *GG* genotype than in those with the *AA* genotype or those in the combined group of *GA* and *AA* genotypes. These results suggest that *AR* is a susceptibility gene for reduced BMD in premenopausal Japanese women, and that *KL* is a susceptibility gene for reduced BMD in all women.

Keywords Bone density · Androgen receptor · Klotho protein · Genetics · Osteoporosis

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Abbreviations *AR:* Androgen receptor · *BMD:* Bone mineral density · *PCR:* Polymerase chain reaction

Introduction

Osteoporosis, a major health problem of the elderly, is characterized by a reduction in bone mineral density (BMD) and a deterioration in the microarchitecture of bone, both of which result in predisposition to fractures [1]. Although reproductive, nutritional, and life-style factors influence BMD, family and twin studies have suggested that this parameter is largely heritable and under the control of multiple genes [2, 3, 4]. Genetic linkage analyses [5, 6, 7] and candidate gene association studies [8, 9, 10] have thus implicated several loci and candidate genes in the regulation of bone mass and the prevalence of osteoporosis or osteoporotic fractures. Such candidate genes include those for the androgen receptor (*AR*) and klotho [11, 12].

Androgens play important roles in the development and metabolism of bone [13]. The *AR* is expressed in human osteoblastic cells as well as in human osteoclasts, suggesting that androgens exert direct effects on bone cells [14]. The gene encoding the AR (AR), which is located on human chromosome Xq11-q12, is thus an important candidate susceptibility gene for osteoporosis. Variation in the size of the microsatellite region in the first exon of *AR* is attributable to a CAG repeat polymorphism that encodes a polyglutamine tract comprising 9–35 residues in the amino-terminal domain of the receptor protein [15, 16]. In vitro transfection assays have demonstrated that *AR* proteins with shorter polyglutamine tracts possess greater transactivation activity [17, 18, 19] whereas tract size does not affect the binding of androgens to the receptor [20]. Although the CAG repeat polymorphism of *AR* was shown to be associated with BMD in women or in men in some studies [11, 21, 22, 23, 24], other studies have failed to detect an effect of this polymorphism on BMD or fracture risk [25, 26]. Furthermore, racial differences in the number of CAG repeats have been demonstrated, with African-Americans exhibiting a higher prevalence of short CAG repeat sequences than other ethnic groups [15, 27]. Given the ethnic differences in CAG repeat length as well as in other genetic or environmental influences on BMD, it is important to examine the relationship of the CAG repeat polymorphism of *AR* to BMD in each ethnic group.

Klotho is a type I membrane protein that shares sequence similarity with members of the glycosidase family [28]. Mice deficient in this protein exhibit multiple aging phenotypes and age-related disorders, including a shortened life span, reduced spontaneous activity, arteriosclerosis, infertility, skin atrophy, premature thymic involution, pulmonary emphysema, and osteopenia, although the function of klotho remains to be determined [28]. The osteopenia observed in klotho-deficient mice is accompanied by a reduced turnover of bone; a decrease in bone formation exceeds a decrease in bone resorption, resulting in substantial bone loss that resembles that in aging humans [29]. A human homolog of the mouse klotho gene has been isolated and its structure determined [30]. The human gene (*KL*) comprises five exons and spans approx. 50 kb on chromosome 13q12. Ogata et al. [31] examined the relationship of a CA repeat polymorphism downstream of *KL* to BMD and showed that the alleles corresponding to 22 and 24 repeats are associated with low and high BMD, respectively. Kawano et al. [12] identified eight and six polymorphisms of *KL* in white and Japanese women, respectively, and showed that the –395G→A polymorphism in the promoter of *KL* is associated with BMD in postmenopausal (≥65 years) women of each ethnicity. The sizes of the populations in which this association was detected were only small (55 white, 215 Japanese), however. Large-scale population-based studies are thus required to assess the effect of this polymorphism on BMD.

We attempted to identify genes significantly associated with BMD in Japanese women in a population-based study. *AR* and *KL* are both candidates for genes that confer susceptibility to osteoporosis. We thus examined the relationship of polymorphisms of these genes to BMD in the present study, although there is no apparent biological link between the two genes. Our aim was to identify a single polymorphism significantly associated with BMD for each gene. Among several polymorphisms previously identified in *KL*, only the –395G→A polymorphism has been shown to potentially affect gene function. We therefore selected this polymorphism for our analysis. We have now examined whether the CAG repeat polymorphism of *AR* or the –395G→A polymorphism of *KL* is associated with BMD in Japanese women in a population-based study.

Methods

Study population

The National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA) is a population-based prospective cohort study of aging and age-related diseases [32]. The present study represents a cross-sectional analysis within the NILS-LSA. The subjects of the NILS-LSA are stratified by both age and gender and were randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan [32, 33]. The life-style of residents of this area is typical of that of individuals in most regions of Japan. The NILS-LSA aimed to recruit equal numbers of men and women. Age at the baseline was 40–79 years, and the numbers of participants in each age decade (40s, 50s, 60s, and 70s) were similar. The planned number of participants was 2,400, that is, approx. 300 men and 300 women in each age decade. A total of 7,855 men and women was randomly selected from the community-dwelling population; of these selected individuals 16 were already deceased and 49 had moved away. The remaining 7,790 individuals were invited to attend an explanatory meeting by mail; a total of 3,434 replied, 881 of whom declined to attend the meeting, 2,553 agreed to attend, and 2,513 actually did attend. After the explanatory meeting, 2,267 individuals participated in the initial examination. Thus of the 7,790 individuals contacted by mail and the 34,34 individuals who replied, 29.1% and 66.0%, respectively, enrolled in the study. The subjects will be followed up every

2 years. All participants are subjected at a special center to a detailed examination, which includes not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. Among the 2,267 participants 1,128 are women. Eighteen women who had disorders known to cause abnormalities of bone metabolism, including diabetes mellitus, renal diseases, rheumatoid arthritis, and thyroid, parathyroid, and other endocrine diseases, or who had taken drugs such as estrogen, progesterone, glucocorticoids, and bisphosphonates were excluded from the present study. Nine women whose *AR* genotype was not successfully determined were also excluded from the analysis of the relationship of the *AR* polymorphism to BMD.

We examined the relationship of BMD at various sites to the CAG repeat polymorphism of *AR* and to the −395G→A polymorphism of *KL* in 1,101 and 1,110 women, respectively. The study protocol complies with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of National Chubu Hospital and the NILS. Written informed consent was obtained from each subject.

Measurement of BMD

BMD for the total body, lumbar spine (L2–L4), right femoral neck, right trochanter, and right Ward's triangle was measured by dual-energy X-ray absorptiometry (QDR 4500; Hologic, Bedford, Mass., USA). The coefficients of variance of the machine were 0.9% (total body), 0.9% (L2–L4), 1.3% (femoral neck), 1.0% (trochanter), and 2.5% (Ward's triangle).

Determination of genotypes

The polymorphic region in exon 1 of *AR* was amplified by the polymerase chain reaction (PCR) with a sense primer labeled at the 5′ end with 6-carboxyfluorescein (5′-ACCTCCCGGCGCC-AGTTTG-3′) and with an antisense primer (5′-CTGCTGCTGC-CTGGGGCTAG-3′). The reaction mixture (25 μl) contained 20 ng DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 mmol/l $MgSO_4$, and 0.4 U KODplus DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s and annealing-extension at 68°C for 30 s; and a final extension at 68°C for 2 min. The size of microsatellite-containing DNA fragments amplified by PCR was determined with a Prism 3100 DNA sequencer with GeneScan and Genotyper software (Applied Biosystems, Foster City, Calif., USA).

Genotypes for *KL* were determined with a fluorescence-based allele-specific DNA primer assay system [34]. The polymorphic region of *KL* was amplified by PCR with allele-specific sense primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-GGCGCCGACCAACTTXCC-3′) or Texas red (5′-GGCGCC-GACCAACTTXTC-3′) and with an antisense primer labeled at the 5′ end with biotin (5′-CTAGGGCCCGGCAGGATC-3′). The reaction mixture (25 μl) contained 20 ng DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 mmol/l $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo) in polymerase buffer. The amplification protocol comprised initial denaturation at 95°C for 5 min; 40 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 30 s, and extension at 68°C for 30 s; and a final extension at 68°C for 2 min. The amplified DNA was incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature. The plate was then placed on a magnetic stand, and the supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/l NaOH and were measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

Statistical analysis

Since quantitative data were not necessarily all distributed normally, they were compared by both parametric and nonparametric tests. Comparisons between two groups were performed with the unpaired Student's *t* test or the Mann-Whitney *U* test, and those among three or more groups were compared by one-way analysis of variance and the Tukey-Kramer post hoc test or by the Kruskal-Wallis test (SAS, SAS Institute, Cary, N.C., USA). Since the results obtained with parametric and nonparametric tests were similar, statistical analyses with the former are shown in Tables 1, 2, 3, and 4. BMD values were analyzed with adjustment for age, height, and body weight by the least squares method in a general linear model. Allele frequencies were estimated by the gene-counting method, and the χ^2 test was used to identify significant departure from Hardy-Weinberg equilibrium. The effects of the CAG repeat genotype of *AR*, the −395 G→A genotype of *KL*, or both genotypes on BMD at various sites for all women were evaluated by regression analysis; R^2 and *P* values were calculated from analysis of *AR* genotype and/or *KL* genotype. We considered a *P* value of 0.005 or less to be statistically significant for the multiple comparisons of genotypes with BMD. For other background data, a *P* value of 0.05 or less was considered statistically significant. We also calculated the statistical power to detect differences in BMD among women with different genotypes, where α=0.0167 among three groups, α=0.0083 among four groups, and β=0.1.

Results

The distribution of the number of CAG repeats in *AR* for all women ranged from 12 to 37 (22.8±2.9; Fig. 1). The number of CAG repeats was significantly related to L2–L4 BMD for premenopausal women, but not for postmenopausal or total women (Fig. 2). Among premenopausal women BMD for the lumbar spine decreased as the number of CAG repeats increased. Since the mean number of CAG repeats was 22.8, we designated $(CAG)_{n\leq 22}$ and $(CAG)_{n\geq 23}$ alleles as short (*S*) and long (*L*) alleles, respectively.

The distributions of *SS*, *SL*, and *LL* genotypes of *AR* were in Hardy-Weinberg equilibrium, and age, height,

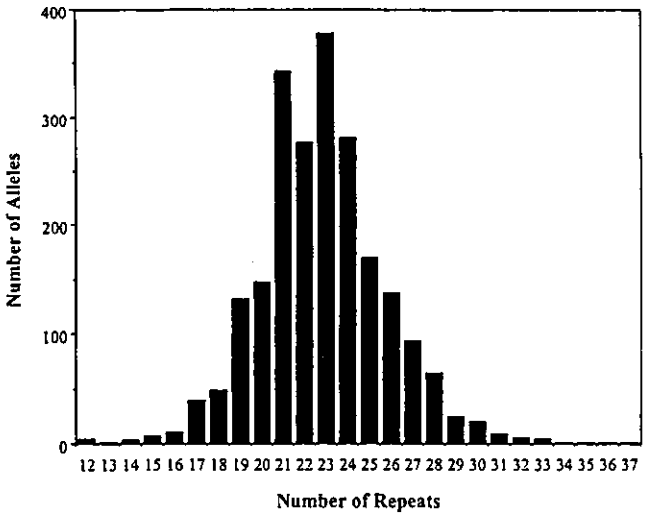

Fig. 1 Distribution of the number of CAG repeats in *AR* in 1,101 women (2,202 alleles)

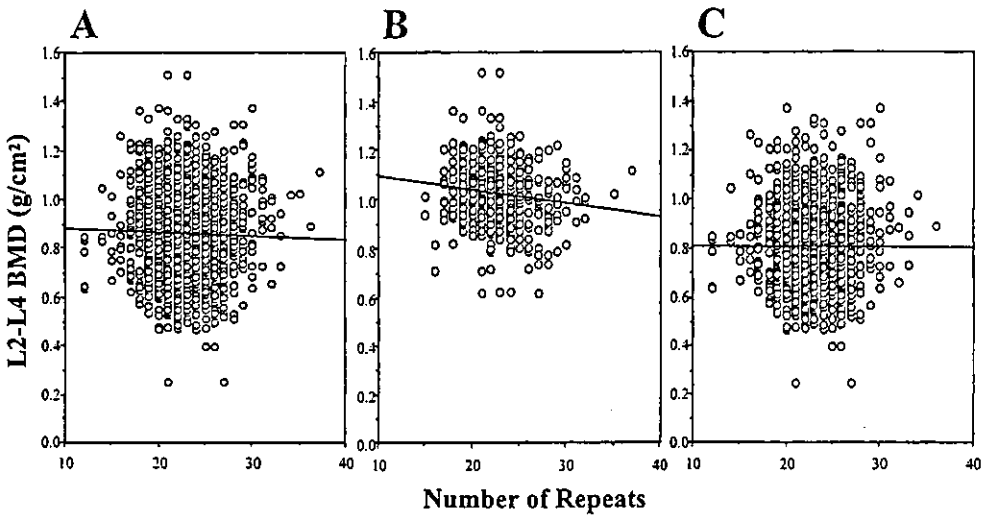

Fig. 2 Relationship between the number of CAG repeats in *AR* and L2-L4 BMD. A All women (n=1,101, 2,202 alleles); r=–0.01967, P=0.3584. B Premenopausal women (n=275, 550 alleles); r=–0.14455, P=0.0007. C Postmenopausal women (n=809, 1,618 alleles); r=0.00751, P=0.7644

Table 1 BMD and other characteristics of all women (n=1,101) according to the CAG repeat genotype of *AR*. BMD values are adjusted for age, height, and body weight

	SS (n=238, 21.6%)	*SL* (n=535, 48.6%)	*LL* (n=328, 29.8%)	*SS* + *SL* (n=773, 70.2%)	*SL* + *LL* (n=863, 78.4%)
Age (years)	58.9±0.7	59.1±0.5	59.9±0.6	59.1±0.4	59.4±0.4
Height (cm)	151.8±0.4	151.2±0.3	151.0±0.3	151.4±0.2	151.1±0.2
Body weight (kg)	52.3±0.5	52.4±0.4	52.6±0.5	52.4±0.3	52.5±0.3
BMD (g/cm^2)					
Total body	0.972±0.006	0.965±0.004	0.961±0.005	0.967±0.003	0.963±0.003
L2–L4	0.884±0.008	0.861±0.005*	0.860±0.007	0.868±0.005	0.860±0.004**
Femoral neck	0.686±0.006	0.677±0.004	0.675±0.005	0.680±0.003	0.676±0.003
Trochanter	0.576±0.005	0.570±0.004	0.568±0.005	0.572±0.003	0.569±0.003
Ward's triangle	0.514±0.008	0.506±0.005	0.505±0.006	0.508±0.004	0.506±0.004

* $P \leq 0.05$, ** $P \leq 0.01$ vs. *SS* (statistical power to detect differences in BMD among women with *SS*, *SL*, or *LL* genotypes is 0.1% of the largest value)

and body weight did not differ among genotypes, for all women (Table 1). BMD for the lumbar spine with adjustment for age, height, and body weight tended to be lower in the combined group of women with the *SL* or *LL* genotypes or in women with the *SL* genotype than in those with the *SS* genotype; the *P* values for these differences, however, did not achieve statistical significance.

To examine the possible influence of menopause on the relationship between genotype and BMD, we analyzed BMD and other characteristics for premenopausal and postmenopausal women independently. Because of their small number (n=17) perimenopausal women were excluded from the analysis. The distributions of *SS*, *SL*, and *LL* genotypes of *AR* were in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for premenopausal or postmenopausal women (Table 2). For premenopausal women, BMD for the total body was significantly ($P \leq 0.005$) lower in those with the *LL* genotype than in those with the *SS* genotype or those in the combined group of *SS* and *SL* genotypes. The difference in BMD for the total body between the *SS* genotype and the *LL* genotype was 3.9% (expressed as a proportion of the larger value). In contrast, BMD was not associated with *AR* genotype in postmenopausal women.

The distribution of –395G→A genotypes of *KL* was in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes for all women (Table 3). BMD for the lumbar spine was significantly ($P \leq 0.005$) lower in women with the *GG* genotype than in those with the *AA* genotype; the difference in L2–L4 BMD between these two groups (expressed as a percentage of the larger value) was 7.9%.

We also analyzed the relationship of BMD and other characteristics to *KL* genotype for premenopausal and postmenopausal women independently (Table 4). The distributions of –395G→A genotypes of *KL* were in Hardy-Weinberg equilibrium, and age and body weight did not differ among genotypes in premenopausal or postmenopausal women. Height did not differ among *KL* genotypes in premenopausal women, but postmenopausal women with the *GG* genotype were taller than were those with the *GA* genotype or those in the combined group of *GA* and *AA* genotypes. In premenopausal women, BMD was not associated with –395G→A genotype. In postmenopausal women, although there was a trend ($P \leq 0.05$) for BMD for the total body or lumbar spine to be lower in subjects with the *GG* genotype than in those with the *AA* genotype or those in the combined group of *GA* and *AA*

Table 2 BMD and other characteristics of women (n=1,084) according to menopausal status and the CAG repeat genotype of *AR*. BMD values are adjusted for age, height, and body weight

	Premenopausal women (n=275)				Postmenopausal women (n=809)			
	SS (n=62, 22.6%)	*SL* (n=134, 48.7%)	*LL* (n=79, 28.7%)	*SS* + *SL* (n=196, 71.3%)	*SS* (n=173, 21.4%)	*SL* (n=393, 48.6%)	*LL* (n=243, 30.0%)	*SS* + *SL* (n=566, 70.0%)
Age (years)	46.2±0.6	46.0±0.4	46.6±0.5	46.0±0.3	63.6±0.7	63.8±0.4	64.4±0.6	63.7±0.4
Height (cm)	154.4±0.6	154.4±0.4	154.5±0.5	154.4±0.3	150.8±0.5	150.0±0.3	149.8±0.4	150.3±0.3
Body weight (kg)	53.9±1.0	54.4±0.7	54.6±0.9	54.2±0.6	51.7±0.6	51.7±0.4	51.8±0.5	51.7±0.3
BMD (g/cm^2)								
Total body	1.111±0.010	1.102±0.007*	1.068±0.009**, *****	1.105±0.006	0.922±0.007	0.916±0.004	0.921±0.006	0.918±0.004
L2–L4	1.050±0.014	1.031±0.010	0.997±0.013****	1.037±0.008	0.826±0.010	0.801±0.006	0.809±0.008	0.809±0.005
Femoral neck	0.780±0.011	0.777±0.008	0.762±0.010	0.778±0.006	0.654±0.006	0.640±0.004	0.643±0.005	0.645±0.004
Trochanter	0.668±0.010	0.664±0.007	0.642±0.009***	0.665±0.006	0.544±0.006	0.537±0.004	0.541±0.005	0.539±0.003
Ward's triangle	0.674±0.015	0.666±0.010	0.641±0.013	0.668±0.008	0.457±0.009	0.449±0.006	0.456±0.007	0.452±0.005

*$P\leq0.01$, **$P\leq0.005$ vs. *SS*, ***$P\leq0.05$, [4]*§$P\leq0.01$, [5]*$P\leq0.001$ vs. *SS* + *SL* (statistical power to detect differences in BMD among premenopausal or postmenopausal women with *SS*, *SL*, or *LL* genotypes is 0.2% or 0.1% of the largest value, respectively)

Table 3 BMD and other characteristics in all women (n=1,110) according to the −395G→A genotype of *KL*. BMD values are adjusted for age, height, and body weight

	GG (n=812, 73.2%)	*GA* (n=268, 24.1%)	*AA* (n=30, 2.7%)	*GA* + *AA* (n=298, 26.8%)
Age (years)	59.4±0.4	58.9±0.7	58.8±2.0	58.9±0.6
Height (cm)	151.5±0.2	150.7±0.4	151.0±1.1	150.7±0.4
Body weight (kg)	52.5±0.3	52.1±0.5	53.2±1.5	52.2±0.5
BMD (g/cm^2)				
Total body	0.962±0.003	0.970±0.005	0.994±0.016	0.973±0.005
L2–L4	0.860±0.004	0.872±0.008	0.934±0.023**,***	0.878±0.007*
Femoral neck	0.678±0.003	0.675±0.005	0.692±0.016	0.677±0.005
Trochanter	0.569±0.003	0.572±0.005	0.601±0.015	0.575±0.005
Ward's triangle	0.504±0.004	0.511±0.007	0.537±0.021	0.513±0.007

* $P\leq0.05$, **$P\leq0.005$ vs. *GG*, ***$P\leq0.05$ vs. *GA* (statistical power to detect differences in BMD among women with *GG*, *GA*, or *AA* genotypes is 0.1% of the largest value)

Table 4 BMD and other characteristics in women (n=1093) according to menopausal status and the −395G→A genotype of *KL*. BMD values are adjusted for age, height, and body weight.

	Premenopausal women (n=278)				Postmenopausal women (n=815)			
	GG (n=199, 71.6%)	*GA* (n=71, 25.5%)	*AA* (n=8, 2.9%)	*GA* + *AA* (n=79, 28.4%)	*GG* (n=602, 73.9%)	*GA* (n=191, 23.4%)	*AA* (n=22, 2.7%)	*GA* + *AA* (n=213, 26.1%)
Age (years)	46.3±0.3	46.0±0.5	45.5±1.6	45.9±0.5	63.9±0.3	64.0±0.6	63.6±1.8	63.9±0.6
Height (cm)	154.4±0.3	154.7±0.6	152.9±1.7	154.5±0.5	150.5±0.2	149.1±0.4*	150.4±1.3	149.2±0.4**
Body weight (kg)	54.4±0.6	53.8±1.0	55.0±2.9	53.9±0.9	51.9±0.3	51.4±0.6	52.5±1.7	51.5±0.6
BMD (g/cm^2)								
Total body	1.094±0.006	1.087±0.010	1.133±0.029	1.092±0.009	0.914±0.004	0.928±0.006	0.946±0.018	0.930±0.006*
L2–L4	1.023±0.008	1.023±0.013	1.110±0.040	1.032±0.013	0.803±0.005	0.818±0.009	0.874±0.027*	0.824±0.009*
Femoral neck	0.774±0.006	0.765±0.011	0.781±0.032	0.767±0.010	0.643±0.003	0.643±0.006	0.662±0.018	0.645±0.006
Trochanter	0.661±0.006	0.646±0.010	0.684±0.029	0.650±0.009	0.536±0.003	0.547±0.006	0.572±0.017	0.549±0.006
Ward's triangle	0.656±0.008	0.658±0.014	0.714±0.042	0.664±0.013	0.450±0.005	0.458±0.008	0.475±0.025	0.459±0.008

* $P\leq0.05$, **$P\leq0.01$ vs. *GG* (statistical power to detect differences in BMD among premenopausal or postmenopausal women with *GG*, *GA*, or *AA* genotypes is 0.2% or 0.1% of the largest value, respectively)

Table 5 Effects of the CAG repeat genotype of *AR*, the −395G→A genotype of *KL*, or both genotypes on BMD in all women (n=1,110). The R^2 and P values were derived from regression analysis of *AR* genotype (0=*SS*, 1=*SL*=*LL*) and/or KL genotype (0=*GG*=*GA*, 1=*AA*)

	AR genotype		*KL* genotype		*AR* and *KL* genotypes	
	R^2	P	R^2	P	R^2	P
Total body						
AR	0.0023	0.1255	0.0015	0.2151	0.0026	0.1016
KL					0.0015	0.2157
L2–L4						
AR	0.0045	0.0307	0.0045	0.0287	0.0048	0.0256
KL					0.0046	0.0281
Femoral neck						
AR	0.0031	0.0735	0.0008	0.3457	0.0034	0.0621
KL					0.0008	0.3464
Trochanter						
AR	0.0013	0.2399	0.0027	0.0921	0.0016	0.1991
KL					0.0027	0.0958
Ward's triangle						
AR	0.0015	0.2124	0.0013	0.2382	0.0017	0.1856
KL					0.0013	0.2432

genotypes, the P values for these relationships did not achieve statistical significance.

Finally, the effects of the CAG repeat genotype of *AR*, the −395G→A genotype of *KL*, or both genotypes on BMD at various sites in all women were evaluated by regression analysis (Table 5). Although there was a trend ($P \leq 0.05$) that *AR* genotype and *KL* genotype affected BMD for the lumbar spine, this difference was not statistically significant. The effects of the two polymorphisms on BMD were statistically independent.

Discussion

The CAG repeat polymorphism of *AR* has previously been shown to be associated with osteoporosis in men. In a study of white men, repeat length was inversely correlated with BMD, with long repeats [$(CAG)_{n>21}$] being associated with lower phalangeal BMD, higher bone turnover, and increased bone loss [21]. A study of Finnish men, however, did not detect an association between this polymorphism of *AR* and BMD [26]. In women overrepresentation of certain *AR* genotypes (combinations of alleles with 22, 23, 24, or 25 repeats) was found among pre- or perimenopausal individuals with low BMD [11]. A Danish study demonstrated a higher frequency of long alleles in women with osteoporotic fractures and a negative correlation between allele size and BMD [22]. In contrast, no association was observed between the *AR* polymorphism and BMD in a study of Finnish women [25]. The effects of the CAG repeat polymorphism of *AR* on BMD have not previously been determined for premenopausal and postmenopausal women independently in the same ethnic group.

We have now shown that the number of CAG repeats in *AR* is inversely correlated with BMD for the lumbar spine in premenopausal Japanese women, and that BMD for the total body is significantly lower in premenopausal women with two $(CAG)_{n\geq 23}$ alleles than in those with one or two $(CAG)_{n\leq 22}$ alleles. Our observation that long repeat alleles are associated with reduced BMD is consistent with the similar previous observation in Danish women [22].

This association between BMD and the CAG repeat polymorphism is possibly attributable to the fact that the transactivation activity of the *AR* is inversely correlated with the number of CAG repeats [17, 18, 19]. In vitro observations thus suggested that a decrease of six CAG repeats results in a 12% increase in ligand-dependent transactivation activity of the *AR* [18]. This relationship between repeat length and transactivation activity is due in part to variation in the basal activity of the *AR* and to functional interaction of the polyglutamine tract with coactivators [35, 36]. In addition, the serum concentration of androgens is related to the CAG repeat polymorphism of *AR*, with short alleles being associated with higher levels of androgens in premenopausal women [37]. This finding supports our observation that the *AR* polymorphism is associated with BMD in premenopausal, but not postmenopausal, women, although the definition of short alleles differed between this previous study [$(CAG)_{n\leq 19}$] [37] and our study [$(CAG)_{n\leq 22}$] and postmenopausal women were not examined in the previous study [37].

The mean number of CAG repeats for the *AR* in our population (22.8) was greater than that previously reported in Danish women (21.9) [24] or in Danish normal (20.5) or osteoporotic (21.0) women [22]. Furthermore, the mean number of CAG repeats in African-American men (20.1) was smaller than that in white men (22.1) or Asian men (22.1) [15]. These differences in repeat number may account at least in part for the differences in BMD or in the prevalence of osteoporosis among ethnic groups. Since the mean number of CAG repeats was 22.8 in our study population, we designated $(CAG)_{n\leq 22}$ and $(CAG)_{n\geq 23}$ alleles as short (*S*) and long (*L*) alleles, respectively. The cutoff value for the CAG repeat number in our study was thus greater than that in previous studies: $(CAG)_{n\leq 21}$ [24], $(CAG)_{n\leq 20}$ [22], $(CAG)_{n\leq 19}$ [37], and $(CAG)_{n\leq 18}$ [25] for the *S* allele.

The somatic cells of most females contain two X chromosomes, only one of which is active. The process of X chromosome inactivation, which occurs early in de-