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
高齢者の転倒事故
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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．

*にいの　なおあきら：桜美林大学大学院国際学研究科老年学

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	

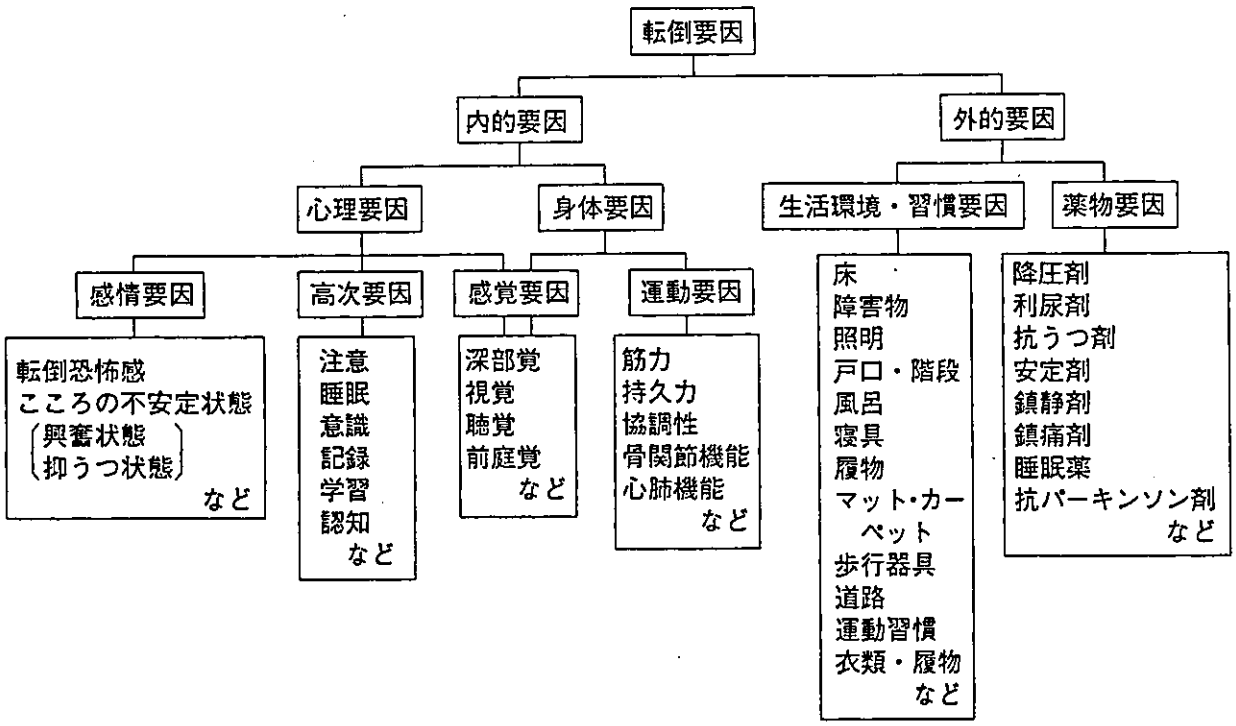

図1　転倒要因の分類(江藤真紀，2003：文献3より引用)

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	なし	16.3(56)**
転倒既往	あり	45.1(23)
ADL(食事，歩行，	全部自立	18.0(67)**
トイレ，着替え，	要介助あり	63.2(12)
入浴の5行動)	全部できる	12.6(19)**
老研式活動能力	できないことあり	24.8(60)
指標		
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	なし	23.7(64)
服用	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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(執筆者連絡先)　新野直明　〒194-0294　東京都町田市常盤町 3758　桜美林大学大学院国際学研究科老年学

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ORIGINAL ARTICLE

Yoshiji Yamada · Fujiko Ando · Naoakira Niino · Hiroshi Shimokata

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

Yoshiji Yamada received his M.D. degree in 1982 and his Ph.D. degree in 1990 in internal medicine from Nagoya University Graduate School of Medicine in Nagoya, Japan. He carried out postdoctoral research at the Eccles Institute of Human Genetics, University of Utah, Salt Lake City, Utah, USA. He is presently Professor and Director of the Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu, Mie, Japan. His research interests include genomic epidemiology and functional genomics of cardiovascular disease, stroke, osteoporosis, diabetes mellitus, and cancer.

Hiroshi Shimokata received his M.D. degree in 1977 and his Ph.D. degree in 1983 in internal medicine from Nagoya University Graduate School of Medicine in Nagoya, Japan. He carried out postdoctoral research at the National Institute of Aging, NIH, Baltimore, Maryland, USA. He is presently Director of the Department of Epidemiology, National Institute for Longevity Sciences, Obu, Aichi, Japan. His research interests include molecular epidemiology and geriatrics.

Y. Yamada (✉)
Department of Human Functional Genomics,
Life Science Research Center,
Mie University, 1515 Kamihama, Tsu, 514-8507 Mie, Japan
e-mail: yamada@gene.mie-u.ac.jp
Tel.: +81-59-2315387, Fax: +81-59-2315388

F. Ando · N. Niino · H. Shimokata
Department of Epidemiology,
National Institute for Longevity Sciences,
Obu, Aichi, Japan

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,

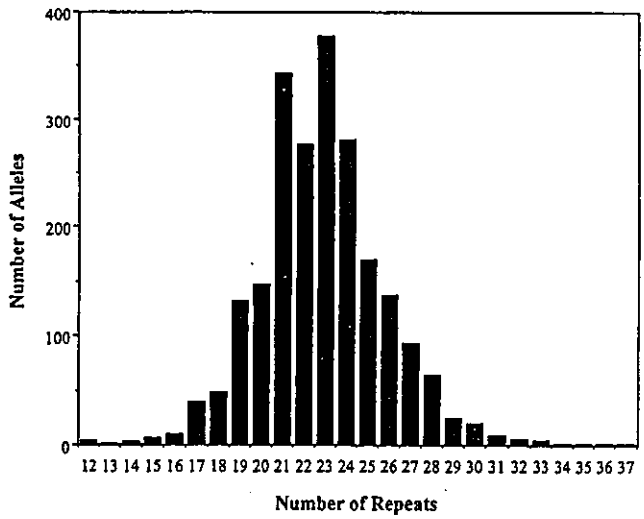

Fig. 1 Distribution of the number of CAG repeats in *AR* in 1,101 women (2,202 alleles)

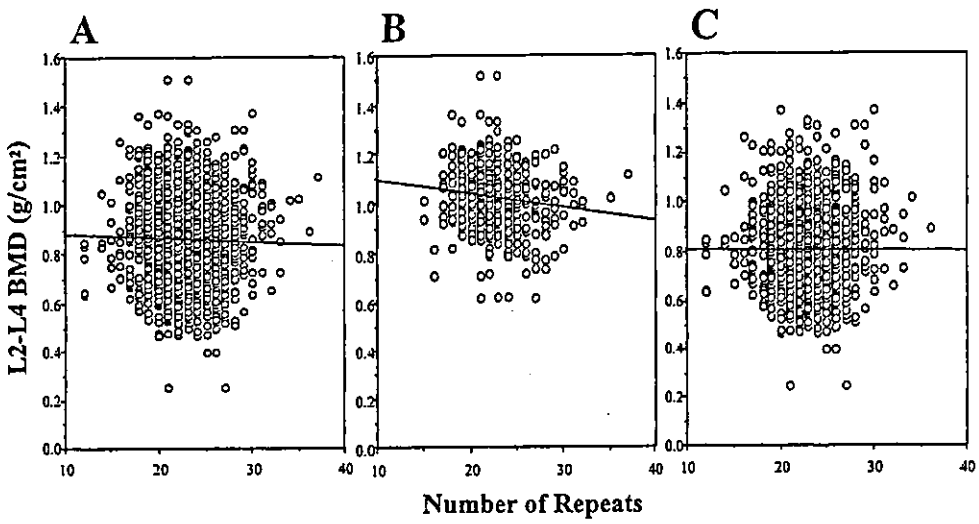

Fig. 2 Relationship between the number of CAG repeats in *AR* and L2-L4 BMD. A All women (n=1,101, 2,202 alleles); r=–0.01967, P=0.3584. B Premenopausal women (n=275, 550 alleles); r=–0.14455, P=0.0007. C Postmenopausal women (n=809, 1,618 alleles); r=0.00751, P=0.7644

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/cm²)					
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-

velopment, is usually random, resulting in the generation of tissues with approximately equal numbers of cells in which the active X chromosome is of maternal or paternal origin [38]. Deviation from such an equal distribution of the two cell types can occur, however. A skewed pattern of X chromosome inactivation affecting the CAG repeat polymorphism of *AR* has been associated with other hormone-related diseases in women [38, 39, 40]. Given that no information is available on the relative extents of inactivation of the *S* and *L* alleles of *AR* in the present study, the evaluation of BMD in individuals with the *SL* genotype requires caution.

The −395G→A and 1818C→T polymorphisms of *KL* have previously been associated with BMD for the total body in white women aged 65 years or older and with that for the distal radius in Japanese women of the same age group, with BMD decreasing according to the rank orders of genotypes *GG* >*GA* >*AA* for the −395G→A polymorphism and *CC* >*CT* >*TT* for the 1818C→T polymorphism [12]. In the present study we examined the relationship of BMD at various sites to the −395G→A polymorphism but not to the 1818C→T polymorphism, since the latter is a synonymous polymorphism (His→His) and appears not to have a functional effect. We found that the −395G→A polymorphism of *KL* is significantly associated with BMD for the lumbar spine in all women, with the *GG* genotype representing a risk factor for reduced BMD. However, when premenopausal and postmenopausal women were analyzed separately, this polymorphism was not significantly related to BMD in either group, although there was a trend for the *GG* genotype to be associated with low BMD in postmenopausal women. The alleles of the −395G→A polymorphism associated with reduced BMD thus differ between the present study (*G* allele) and the previous study (*A* allele) [12]. Although the reason for this discrepancy is unclear, there are two major differences between the two studies: (a) The number of subjects in which the association was detected was greater in our study (n=1,110) than in the previous study (n=55 for white women, n=215 for Japanese women). (b) BMD was compared among *KL* genotypes with adjustment for age, height, and body weight in our study, but BMD was not adjusted in the previous study. However, it is possible that the −395G→A polymorphism of *KL* is in linkage disequilibrium with other polymorphisms of *KL* or of nearby genes that are actually the determinants of BMD. Although we adopted a strict criterion of statistical significance ($P \leq 0.005$) for the association of genotypes with BMD, we cannot completely exclude the possibility of statistical errors such as false positives.

Evidence suggests that the −395G→A polymorphism of *KL* affects promoter function [12]. Electrophoretic mobility-shift analysis revealed that the amount of DNA-protein complex formed by the *G* allele of the promoter was greater than that formed by the *A* allele, suggesting that the binding of one or more proteins to the promoter is impaired by the G→A substitution, which may affect the expression of *KL*. The effect of this polymorphism on the transcriptional activity of *KL*, however, remains to be determined.

There were no subjects with clinical vitamin D deficiency such as osteomalacia in the present population. However, National Nutrition Survey in 2001 suggested that in approximately 25% of Japanese individuals, the amount of vitamin D taken was smaller than that of daily requirement (100 IU). Serum concentrations of free thyroxine in three subjects (0.3%) slightly exceeded the normal range (0.77–1.93 ng/dl). It is thus possible that subclinical vitamin D deficiency or thyrotoxicosis affected the results obtained in the present study.

In conclusion, our present results suggest that *AR* is a determinant of BMD in premenopausal Japanese women, with the $(CAG)_{n \geq 23}$ allele representing a risk factor for reduced BMD. *KL* is also a determinant for bone mass in Japanese women, with the *G* allele being a risk factor for reduced BMD. The effects of both polymorphisms on BMD were statistically independent.

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ELSEVIER

Cholecystokinin A Receptor Gene Promoter Polymorphism and Intelligence

HIROSHI SHIMOKATA, MD, PHD, FUJIKO ANDO, MD, PHD, NAOAKIRA NIINO, MD, PHD, KYOKO MIYASAKA, MD, PHD, AND AKIHIRO FUNAKOSHI, MD, PHD

PURPOSE: To study the association between Cholecystokinin A receptor (CCKAR) genotypes and intelligence in community-living men and women.
METHOD: Subjects were 2251 community-dwelling Japanese men and women aged 40 to 79 years. The CCKAR gene promoter polymorphisms A-81G and G-128T were determined. Intelligence was assessed by Japanese Wechsler Adult Intelligence Scales – Revised Short Forms (JWAIS-R SF). The difference in intelligence between wild type and mutation was tested.
RESULTS: There were no subjects with *AA/GT*, *AA/TT*, or *AG/TT* genotypic combinations. Both A-81G and G-128T genotypes were related to intelligence quotient (IQ) estimated by JWAIS-R SF. The mean and SE of IQ levels of subjects with the wild-type allele and the mutation allele at nucleotide -128 were 103.4 ± 0.3 and 101.6 ± 0.6, respectively. There was a significant difference in IQ for G-128T ($p = 0.008$). The difference in IQ for A-81G was also significant ($p = 0.011$). The IQ level was 103.6 ± 0.4 in the subjects with the wild-type allele and 102.0 ± 0.5 in the subjects with the mutation. Differences in IQ levels by haplotypes for combinations of A-81G/G-128T were examined. IQ significantly decreased with an increasing number of mutation alleles ($p = 0.018$).
CONCLUSION: There were statistically significant differences in IQ for CCKAR gene promoter polymorphisms A-81G and G-128T in community-living Japanese.
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KEY WORDS: Cholecystokinin, Intelligence, Genotype, Epidemiology.

INTRODUCTION

It is suspected that various genes influence intelligence, but the association between gene polymorphism and intelligence is still unclear. Cholecystokinin (CCK) is one of the major physiologic substances of gallbladder contraction and pancreatic enzyme secretion. CCK also plays an important role in the central nervous system (CNS) by interacting with dopamine and other neurotransmitters (1). CCK receptors have been classified into two subtypes, CCK type-A receptor (CCKAR) and type-B receptor (CCKBR). CCKAR has been found in the CNS (2). Associations with feeding disorders (3), anxiety (4), and schizophrenia (5) have been reported. It was also reported that learning and memory functions were impaired in CCKAR gene-knockout (OLETF) rats (6, 7). The CCKAR gene may be related to intelligence in humans. We examined the association between CCKAR gene promoter polymorphisms and intelligence in a group of 2251 community-dwelling Japanese men and women.

METHODS

Subject Selection

The subjects in this study were participants in the National Institute for Longevity Sciences – Longitudinal Study of Aging (NILS-LSA) (8). The NILS-LSA started in November 1997. The first phase of examinations was finished by the end of March 2000, and followed-up every 2 years. Participants in the NILS-LSA were independent residents in Obu city and Higashiura town in Aichi prefecture, central Japan. Data on all residents in the area are maintained in a Resident Registration System by local governments. Residents aged 40 to 79 years old were selected using Resident Registration. Samples of 7790 males and females were selected by age and gender stratified random sampling and invited to an explanatory meeting by mail. The number of replies was 3434. Of these, 881 refused to attend the meeting, 2553 agreed to attend, and 2513 actually attended. After the meeting, 2267 participated in the first phase examination. At the meeting, the procedures

From the Department of Epidemiology, National Institute for Longevity Sciences, Obu Aichi, Japan (H.S., F.A., N.N.); Department of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan (K.M.); and Department of Gastroenterology, National Kyushu Cancer Center, Fukuoka, Japan (A.F.).
Address correspondence to: Hiroshi Shimokata, M.D., Ph.D., Department of Epidemiology, National Institute for Longevity Sciences, 36-3 Gengo, Morioka-cho, Obu Aichi 474-8522, Japan. Tel.: +81-562-46-2311, ext. 815; Fax: +81-562-44-6593. E-mail: hiroshi@nils.go.jp
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360 Park Avenue South, New York, NY 10010

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Selected Abbreviations and Acronyms

BMI = body mass index
CCK = cholecystokinin
CCKAR = cholecystokinin A receptor
CNS = central nervous system
DNA = deoxyribonucleic acid
GLM = general linear model
IQ = intelligence quotient
JWAIS-R-SF = Japanese Wechsler Adult Intelligence Scales – Revised Short Forms
NILS-LSA = National Institute for Longevity Sciences – Longitudinal Study of Aging
PCR-RFLP = polymerase chain reaction – restriction fragment length polymorphism
OLETF = Otsuka Long-Evans Tokushima Fatty
SE = standard error
WAIS-R = Wechsler Adult Intelligence Scales – Revised

for each examination and follow-up schedule were fully explained. Written informed consent to participate in all procedures was obtained from each subject. All persons in the Resident Registration list had Japanese nationality, and there were no persons who had a foreign name among the subjects. The subjects in this study were supposed to be ethnically homogenous Japanese.

Among the 2267 participants in the first phase examination, 2251 men and women were evaluated for CCKAR genotypes and intelligence. These subjects were analyzed for cross-sectional associations between genotype and intelligence. The number of the subjects by gender and age was almost equal (Table 1). The mean and standard deviation for age was 59.2 ± 10.9 years. Among the subjects, 26.7% had an educational background of college or greater. The Ethical Committee of Chubu National Hospital approved all procedures of the NILS-LSA.

Evaluation of Intelligence and Other Variables

The Wechsler Adult Intelligence Scales – Revised (WAIS-R) is one of the most popular tools used to assess intelligence (9). A Japanese version of the WAIS-R (JWAIS-R) has been developed and is widely used in Japan (10). In this study, intelligence was assessed by the Japanese Wechsler Adult Intelligence Scales - Revised - Short Forms (JWAIS-R-SF) (11). The JWAIS-R-SF consists of the following four subtests: Information, Similarities, Picture Completion, and Digit Symbol. Scaled scores of subtests were used in the analysis. The intelligence quotient (IQ) was estimated from the combination of these four subtests. Psychologists conducted the interviews and JWAIS-R-SF tests. Height and weight were measured while wearing lightweight clothes, and body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Life-style and medical history including annual income, education, and smoking status were checked by questionnaires. The questionnaires were checked by a physician at the medical examination. All drugs used during the previous 2 years were to be documented by participants; the physician confirmed them at an interview and coded the drugs used during the last 2 weeks. Among the 2251 subjects in the study, 213 had used drugs acting on the CNS, that is, hypnotic sedative agents, antianxiety agents, antiepileptic agents, stimulant drugs, antihypnotic drugs, anti-Parkinson drugs, and anti-psychotic drugs during the previous 2 weeks. The IQ was less than 70 in 11 subjects, and only one of them used drugs acting on the CNS.

TABLE 1. Distribution of the subjects by gender and age

	Age (years)				
Gender	40–49	50–59	60–69	70–79	Total
Males	291	282	281	280	1134
Females	278	278	283	278	1117
Total	569	560	564	558	2251

CCKAR Genotype Analysis

Genomic DNA was extracted from peripheral blood lymphocytes by a standard procedure. A mismatch PCR-RFLP method was used to analyze polymorphisms in the upstream region of the CCKAR gene [GenBank Accession No. U23427 (5)]. One pair of primers, sense primer = 5′-GCATATGTACACATGTGTGTAAAAAGCAGCCAGAC-3′, anti-sense primer = 5′-GCCCTTTCCTGGGCCAGACT-3) was designed to amplify a 103-base pair product, digested with restriction enzyme Hinf I, and analyzed by 3% agarose gel electrophoresis. Two sequence changes were detected: a G to T change at nucleotide -128, and an A to G change at nucleotide -81 (12).

Statistical Analysis

All values were expressed as the mean ± SE, if not specified. Both polymorphisms at nucleotides -128 and -81 were divided into two groups; as wild-type and mutation. Hetero groups were classified as mutation. The difference between wild-type and mutation groups was tested by the *t*-test for continuous variables and the 2 × 2 chi-square test for categorical variables. The difference in IQ and JWAIS-R subtests score by genotype was also tested by the *t*-test excluding subjects who had used drugs acting on the CNS or subjects with IQ less than 70. The trend among the three groups was tested by the general linear model (GLM) and the probability for trend (p for trend) was shown. Statistical analyses were performed using the SAS system (SAS Institute Inc., Cary, NC). All p-values were two-tailed.

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RESULTS

Distribution of CCKAR Promoter Genotypes

The distributions of CCKAR promoter single nucleotide polymorphisms A-81G and G-128T were both in Hardy-Weinberg equilibrium. The distribution of genotype combination was examined (Table 2). These polymorphisms were in linkage disequilibrium. There were no subjects with *AA/GT*, *AA/TT*, or *AG/TT* genotypic combinations. Thus, subjects with a mutation at -128 always had a mutation at -81.

Background Characteristics and CCKAR Genotype

Figure 1 shows the IQ distribution. The distribution was slightly skewed to the left (lower IQ) and close to a normal distribution. The mean value of the IQ of the all subjects was 103.0, and the median was also 103. The difference between the mean and median was very small. The lowest IQ was 43 and the highest IQ was 142 among the subjects. The number of subjects with IQ less than 70 was 11, and those with IQ 135 or over was 13. Background characteristics were compared by CCKAR G-128T and A-81G genotypes (Table 3). Age, body weight, body mass index, annual income, education, and smoking status did not differ between wild-type (GG) and mutation (*GT* or *TT*) for the CCKAR G-128T genotype. These variables also did not differ for the CCKAR A-81G genotype except for education status. Education status in the wild-type (AA) group was significantly higher than that in the mutation-type (AG or GG) group ($p = 0.009$). The IQ was significantly different by education status ($p < 0.001$). The IQ for the low education group was 100.3 ± 0.3 and that for the high education group was 110.6 ± 0.5.

Intelligence and CCKAR Genotype

The IQ levels in subjects with wild-type and mutation alleles at nucleotide -128 were 103.4 ± 0.3 and 101.6 ± 0.6, respectively. There was a significant difference in IQ for the G-128T genotype ($p = 0.008$). The score of Digit Symbol was lower in subjects with a mutation ($p = 0.003$). There was no difference in the scores of Information, Picture Completion, and Similarities subtests for polymorphism G-128T. The IQ level was 103.6 ± 0.4 in subjects with wild-type (AA) and 102.0 ± 0.5 in subjects with mutation (AG or GG) at nucleotide -81. The difference in IQ for the A-81G polymorphism was significant ($p = 0.011$). The Picture Completion and Digit Symbol subtest scores were significantly lower in subjects with the mutation ($p = 0.043$ and $p = 0.008$, respectively). The Similarities subtest score was marginally lower for a mutation at nucleotide -81 ($p = 0.051$).

In the low education group, IQ was 100.5 ± 0.4 in the -128 wild-type group and 99.5 ± 0.6 in the -128 mutation-type group. There was no significant difference in IQ between the wild- and mutation-type of G-128T genotype. However, the IQ for the -81 wild-type group was 100.8 ± 0.4, which was significantly higher than that for the mutation group (99.4 ± 0.4) ($p = 0.038$). In the high education group, the IQ was 111.5 ± 0.6 in the -128 wild-type group and 107.9 ± 1.1 in the -128 mutation-type group. There was a significant difference between the wild and mutation groups ($p = 0.004$). The IQ in the -81 wild-type group (111.1 ± 0.7) did not differ from that in the mutation group (109.8 ± 0.9).

Intelligence was compared excluding subjects who had used drugs acting on the CNS and subjects with IQ less than 70 (Table 4). The number of excluded subjects was 223. Differences in IQ between in the wild-type and mutation groups were still significant both for A-81G and G-128T

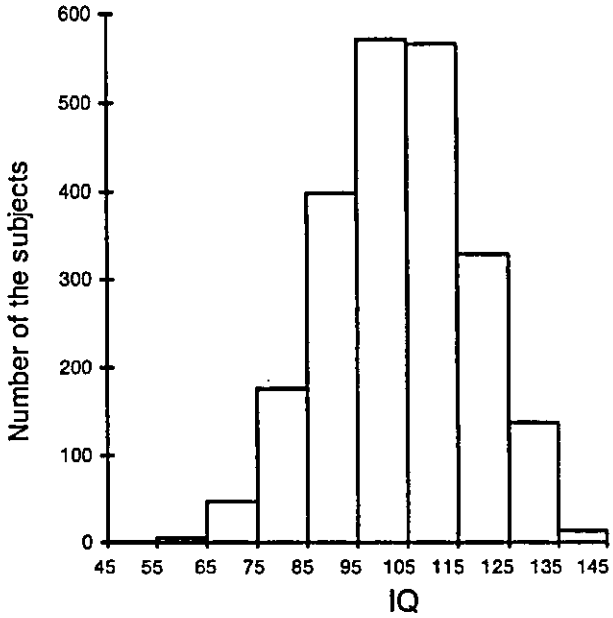

FIGURE 1. Distribution of IQ levels in the subjects.

TABLE 2. Distribution of CCKAR G-81T and A-128G genotypes

CCKAR G-128T	CCKAR A-81G AA	AG	GG	Total
GG	1317 (58.5%)	307 (13.6%)	26 (1.2%)	1650 (73.3%)
GT	0 (0.0%)	491 (21.8%)	61 (2.7%)	552 (24.5%)
TT	0 (0.0%)	0 (0.0%)	49 (2.2%)	49 (2.2%)
Total	1317 (58.5%)	798 (35.5%)	136 (6.0%)	2251 (100.0%)