Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Archives of Gerontology and Geriatrics 42 (2006) 47–58
ARCHIVES OF GERONTOLOGY AND GERIATRICS
www.elsevier.com/locate/archger

Effects of cognitive function on functional decline among community-dwelling non-disabled older Japanese

Tatsuro Ishizaki [a,*], Hideyo Yoshida [b], Takao Suzuki [b], Shuichiro Watanabe [c], Naoakira Niino [c], Kazushige Ihara [d], Hunkyung Kim [b], Yoshinori Fujiwara [b], Shoji Shinkai [b], Yuichi Imanaka [a]

[a] *Department of Healthcare Economics and Quality Management, School of Public Health, Kyoto University Graduate School of Medicine, Yoshida Konoe-cho, Sakyo-ku, Kyoto 606 8501, Japan*
[b] *Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*
[c] *Institute of Aging and Human Development, Obirin University, Tokyo, Japan*
[d] *Toho University School of Medicine, Tokyo, Japan*

Received 6 February 2005; accepted 1 June 2005
Available online 2 August 2005

Abstract

This study examined whether cognitive impairment, falls, and urinary incontinence (UI) were independent predictors of functional decline using a 2-year observation of a non-disabled older Japanese cohort living in a community from 1999 to 2001. A total of 139 men and 214 women aged 70–94 years at the baseline who were independent in both activities of daily living (ADL) and instrumental activities of daily living (IADL) were analyzed in this study. Independent variables, such as cognitive impairment, falls, UI, and other possible factors associated with functional decline were obtained from an interview survey at the baseline. A dependent variable was functional status in ADL and IADL obtained at the time of the 2-year follow-up. During the 2-year follow-up, cognitive function was a significant predictor for both IADL dependence and ADL and/or IADL dependence. Using a group of subjects with Mini Mental State Examination (MMSE) scores of 30–27 points as a reference group, a significant correlation was identified between lower MMSE scores and an increased odds ratio for functional decline. Lower cognitive function was a significant predictor of functional decline, even among those older Japanese whose cognitive function was deemed to be within the normal range.

Keywords: Instrumental activities of daily living; Aged; Cognitive impairment; Follow-up studies; Japan

* Corresponding author. Tel.: +81 75 753 4454; fax: +81 75 753 4455.
E-mail address: tatsuro@pbh.med.kyoto-u.ac.jp (T. Ishizaki).

0167-4943/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.archger.2005.06.001

1. Introduction

The rapid rise in the number of older Japanese in recent years means that public health policies should pay particular attention to conditions and disorders unique to the elderly. Some of the most common disorders among the elderly persons defined as "old–old (aged 75 years and older)" are jointly referred to as geriatric syndrome, which generally includes senile dementia, urinary incontinence (UI), immobility, malnutrition, pressure sores, and iatrogenic disorders (Tinetti et al., 1995; Kane et al., 1999). Geriatric syndrome is known to diminish not only the long-term quality of life, but also the physical functioning in older people (Tinetti et al., 1995). Although several studies have examined whether cognitive impairment, falls, and/or UI are independent predictors of functional decline (Stuck et al., 1999; Aguero-Torres et al., 2002), few such studies have simultaneously assessed the prevalence of these conditions among community-living older adults to address the question of whether the latter are possible predictors of functional decline (Tinetti et al., 1995). Most studies dealing with the association between geriatric syndrome and functional decline use the activities of daily living (ADL) scale instead of the instrumental activities of daily living (IADL) scale as an outcome measure (Stuck et al., 1999); the latter provides an essential basis for determining whether an elderly individual is capable of living independently in the community (Aguero-Torres et al., 2002; Sauvaget et al., 2002). Measures for preventing dependence on IADL are expected to contribute to preventing ADL dependence because IADL dependence is a predictor of ADL dependence (Spector et al., 1987; Kai et al., 1991; Strawbridge et al., 1996; Nourhashemi et al., 2001). This is because individuals with IADL limitations are more likely to regain independence than those with ADL limitations (Crimmins and Saito, 1993; Ishizaki et al., 2000a, 2004). We therefore examined whether cognitive function, UI, experience of falls affected functional decline in either IADL only or ADL and/or IADL using a 2-year observation of a non-disabled older Japanese cohort living in a community.

2. Subjects and methods

2.1. Data source and study subjects

Following approval by the Institutional Review Board of the Tokyo Metropolitan Institute of Gerontology (TMIG), the study was conducted in a village in Akita Prefecture in the northern area of Honshu, one of the four main islands in Japan. In 2000, the total population of the village was 3538. A survey was taken first in 1999 and then again 2 years later. In the autumn of 1999, a face-to-face interview survey was carried out in a community center, for subjects with difficulty reaching the center, at their homes, to obtain baseline data. Because the questions in this study contained sensitive items, including UI and cognitive function, we were careful to protect participants' privacy by using screens between interviewer–participant pairs. Of the 786 people aged 70 years and older (320 men and 466 women) living in the village in 1999, 605 (77%) participated in the survey (256 men and 349 women). The vital status of the cohort was identified in 2001, using information for the residence registration records provided by the village government. Two

years after the baseline survey, and using the same method that had been used at the baseline, the subjects were surveyed again in relation to their survival status, ADL, and IADL. We limited the subjects of this particular study to those who were independent in both ADL and IADL at the baseline survey.

2.2. Assessment of functional status

ADL questions included walking, feeding, bathing, using the toilet, and dressing. IADL questions were derived from the instrumental self-maintenance scale of the TMIG index for competence (Koyano et al., 1991), and included going out using public transportation, shopping for daily necessities, preparing meals, paying bills, and depositing or withdrawing money from a bank account. The response to each item of these indices was simply "yes" (able to do without the help of another person or special equipment) or "no" (unable to do without the help of another person or special equipment). In this study, only those subjects who were assessed as being independent in all ADL (or IADL) items listed above were regarded as being ADL (or IADL)-independent. All other subjects were defined as ADL (or IADL)-dependent. Because the objective of this study was to examine the effects of geriatric syndrome on functional decline among older people during the 2-year follow-up period, we used functional status obtained from the follow-up survey conducted in 2001 as the outcome. Each subject's degree of functional independence at the time of the 2-year follow-up was categorized into the following three levels: independent in both ADL and IADL, dependent in only IADL, and dependent in ADL. In this study, functional decline was defined a change from independent in both ADL and IADL to either dependent in only IADL or dependent in ADL.

2.3. Assessment of geriatric syndrome

We collected information about the presence of UI, cognitive impairment, or experience of falls as geriatric syndrome. The questions regarding UI were related to the presence of UI and the frequency of incontinent episodes. The first UI question asked whether the subject had ever experienced urinary leakage before reaching a toilet. Answer choices for this question were: never, occasionally, and wearing diapers at all times. All subjects who chose alternatives indicating that they experienced urinary leakage occasionally or that they wore diapers at all times were questioned about the frequency of incontinence episodes. The answer choices were: almost daily, once every 2 days, once or twice a week, 1–3 times a month, and several times a year. Those subjects indicating that they experienced urinary leakage more than once a week were defined as having UI.

The Japanese version of the Mini Mental State Examination (MMSE) (Otsuka and Homma, 1991) was modified to evaluate the cognitive function of subjects living in a community (Folstein et al., 1985). The contents of the modified Japanese MMSE differ from the original in several ways. First, while the orientation question in the original version asks about the name and floor of the hospital where a respondent receives treatment, the same question in the modified version asks about the name of the community center where the interview was carried out. Second, the serial-sevens test, wherein the respondent starts with the number 100 and proceeds downward by subtracting seven each

time, was replaced by a backward spelling of the Japanese word "*FU-JI-NO-YA-MA*" (a five-syllable Japanese word "Mt. Fuji"). This substitution was made because of difficulty encountered explaining the rules of a serial-sevens test to both interviewers and respondents. Third, a copying task (copying of a complex figure) was given before a writing task because it was expected that many of the respondents in the present survey may not have immediately understood what exactly they were expected to write when asked to write a sentence. An MMSE score of 23 points or less and a score of 19 points or less were considered to be indicative of low cognitive skills and very low cognitive skills, respectively (McDowell and Newell, 1996).

The question regarding falls asked the subjects if they had experienced any falls during the past year. All subjects who had experienced falls were then asked about the frequency of falls during that period of time.

2.4. Potential predictors of functional decline

The interview also included questions regarding potential predictors of functional decline: age, gender, educational status, presence of visual impairment, presence of hearing impairment, intellectual activity, social role, and self-rated health. In terms of educational status, the subjects in the present study were divided into those with 6 years of education or less (elementary school level), and those with 7 years of education or more. A question about visual impairment was asked "Do you have any difficulties with visual activities?" The answer choices were: No, Yes, Yes with glasses. A question about hearing impairment was asked "Do you have any difficulties with hearing capability?" The answer choices were: No, Yes, Yes with a hearing device. Subscales derived from the TMIG index for competence (Koyano et al., 1991) were used to assess the subjects' intellectual activity (four items: filling out pension forms, reading a newspaper, reading books or magazines, and being interested in news stories or programs dealing with health) and social role (four items: visiting the homes of friends, being called on for advice, being able to visit sick friends, and initiating conversations with young people). A subject's intellectual activity and social role were defined as "good" only if a subject was assessed as being independent in all subscale items, and were defined as "poor" if a subject was assessed as being dependent in any of the subscale items. A question about self-rated health was asked "How would you rate your present health?" The answer choices were: good, fair, poor, and very poor. In this particular study, the self-rated health was categorized as either 'good' (good or fair) or 'poor' (poor or very poor).

2.5. Statistical methods

Functional and cognitive status of the subjects at the baseline survey were categorized according to the level of functional independence. Possible predictors of functional decline were examined by conducting the χ^2-test and a backward-stepwise multiple logistic regression analysis using either functional decline in only IADL or functional decline in ADL and/or IADL after 2 years (reference category: remaining independent in both ADL and IADL after 2 years) as a dependent variable, and presence of UI, cognitive function and experience of falls as explanatory variables. Other explanatory variables used included age,

gender, educational status, presence of hearing impairment, presence of visual impairment, self-rated health, intellectual activity, and social role. Whereas a P-value of 0.15 was used for variable retention for the backward-stepwise procedure, gender, age, cognitive function, presence of UI, and fall experience were always used as independent variables regardless of P values. The association between functional decline and possible predictors was assessed by odds ratio (OR) and 95% confidence interval (CI). We performed the goodness-of-fit tests developed by Hosmer and Lemeshow on the final model to measure how well the model fit the data (Hosmer and Lemeshow, 1989). A sensitivity analysis was conducted to determine the effects of drop-outs on the analysis results (Heitjan, 1997). Other sensitivity analysis was performed to examine the effect of the absence of the variable regarding educational attainment on the results. All analytical procedures were performed using SPSS Version 10.0 (SPSS Inc., 1999). All reported P values were two-tailed, and the level of significance was $P < 0.05$.

3. Results

3.1. Functional and cognitive status of the followed-up subjects at the baseline

Of all the subjects who participated in the 1999 baseline survey, a total of 526 (204 men and 322 women) provided answers for all question items related to functional abilities and the presence of geriatric syndrome. Of those, 81% (425 respondents) were assessed as both ADL- and IADL-independent, 14% were assessed as IADL-dependent only, and 5% were assessed as ADL-dependent.

The mean MMSE scores of 407 subjects who were both ADL- and IADL-independent at the baseline was 26.2 points (standard deviation = 3.6, median = 27, and range = 11–30). Table 1 illustrates the distribution of the subjects' MMSE scores, the proportion of subjects who experienced UI more than once a week, and the proportion of subjects who experienced falls during the past year.

3.2. Changes in functional independence during the 2-year interval

Those subjects who were both ADL- and IADL-independent at the baseline were examined for changes in functional independence during the 2-year period (Table 2). Because 18 of 425 subjects did not have a clear educational status, the following analyses were conducted among 407 subjects. Of the 407 subjects who were initially independent in both ADL and IADL (163 men and 244 women), two had died during the interval between the two surveys and 55 (29 men and 31 women) did not participate in the follow-up survey. Although we did not have the detailed information on the reason for the lost to follow-up among the cohort, we confirmed that the reason was neither migration nor death. Although the majority of the surveyed subjects maintained functional independence over the 2-year period, a certain degree of functional decline was observed among 11% of the male and female subjects. Less than half of the female subjects who were aged 80 years and older remained functionally independent over the 2-year period.

Table 1
Basic characteristics of subjects who were independent in both ADL and IADL in the baseline survey of 1999 ($n = 407$)

Basic characteristics		
Mean age (years)		74.9 (standard deviation 4.4)
Age range (years)		70–94
Age class (% ≥80)		15.5
Gender (% women)		60.0
Educational status (% 6 years or less)		88.9
Self-rated health (% poor)		20.1
Intellectual activity (% poor)		45.7
Social role (% poor)		35.1
Hearing impairment (% present)		7.9
Visual impairment (% present)		1.5
Geriatric syndrome		
Cognitive function: MMSE score (%)	30–27	51.6
	26–24	26.3
	23–20	17.4
	19–0	4.7
Presence of UI (% present)		9.3
Falls experienced during the past year (% present)		9.1

3.3. Relationship between functional decline and geriatric syndrome

Univariate analyses and a stepwise multiple logistic regression analysis were performed to compare subjects who were ADL- and IADL-independent in both 1999 and 2001 ($n = 304$) and those who were ADL- and IADL-independent in 1999 and either became IADL-dependent only in 2001 ($n = 37$) or became ADL-dependent in 2001 ($n = 9$). These analyses revealed that only MMSE score was significantly associated with functional decline after 2 years (Tables 3 and 4). As shown in Model 2 (Table 4), using a group of subjects with MMSE scores of 30–27 points as a reference group, the ORs for any functional decline after 2 years were estimated as 3.20 ($P = 0.015$), 5.66 ($P < 0.001$) and

Table 2
Functional transition over a 2-year period among Japanese elderly who were initially ADL- and IADL-independent at the baseline survey in 1999 ($n = 407$)

	Age group	Functional status in 2001				
		Independent in ADL and IADL (%)	Dependent in IADL only (%)	Dependent in ADL (%)	Dead (%)	Loss to follow-up (%)
Men	70–79 ($n = 135$)	75.6	9.6	0.7	0.0	14.1
	≥80 ($n = 28$)	60.7	10.7	3.6	0.0	25.0
	Total ($n = 163$)	73.0	9.8	1.2	0.0	16.0
Women	70–79 ($n = 209$)	82.3	3.3	2.4	1.0	11.0
	≥80 ($n = 35$)	37.1	40.0	5.7	0.0	17.1
	Total ($n = 244$)	75.8	8.6	2.9	0.8	11.9

Table 3
Results of univariate analyses that examined effects of geriatric syndrome on functional decline in IADL among Japanese elderly who were initially ADL- and IADL-independent over a 2-year period ($n = 350$)

Predictors	Category	n	Functional status in 2001			
			Independent in ADL and IADL (%)	Dependent in only IADL (%)	Dependent in ADL (%)	P-value[a]
MMSE score	30–27	185	95.1	4.9	0.0	<0.001
	26–24	91	85.7	12.1	2.2	
	23–20	61	73.8	18.0	8.2	
	19–0	13	38.5	46.2	15.4	
UI	Absent	317	87.1	10.1	2.8	0.432
	Weekly or daily	33	84.8	15.2	0.0	
Falls experienced during the past year	Absent	317	87.4	9.8	2.8	0.219
	Present	33	81.8	18.2	0.0	

[a] χ^2-test.

16.50 ($P < 0.001$) for groups of subjects with MMSE scores of 26–24 points, 23–20 points and 19–0 points, respectively (test for trend, $P < 0.001$). MMSE scores were also significantly associated with only IADL decline.

Table 4
Effect of cognitive function assessed by the MMSE on functional decline among Japanese elderly who were initially independent in both ADL and IADL during the 2-year interval, 1999–2001

Predictors	Category	Model 1[a] ($n = 341$)			Model 2[b] ($n = 350$)		
		OR	95% CI	P-value	OR	95% CI	P-value
MMSE score	30–27	1.00			1.00		
	26–24	2.65	0.99, 7.10	0.052	3.20	1.25, 8.20	0.015
	23–20	3.51	1.25, 9.86	0.017	5.66	2.19, 14.64	<0.001
	19–0	13.29	2.69, 65.79	0.002	16.50	3.68, 73.96	<0.001
			Test for trend	<0.001		Test for trend	<0.001
UI	Absent	1.00			1.00		
	Weekly or daily	1.30	0.38, 4.40	0.673	1.84	0.56, 6.07	0.317
Falls experienced during the past year	Absent	1.00			1.00		
	Present	0.48	0.16, 1.42	0.184	0.59	0.20, 1.73	0.331
Hosmer–Lemeshow test		$\chi^2 = 2.59$ ($P = 0.957$)			$\chi^2 = 3.30$ ($P = 0.914$)		

[a] Model 1: Stepwise multiple logistic regression analysis was performed to compare subjects who were ADL- and IADL-independent in both 1999 and 2001 ($n = 304$) and those who were ADL- and IADL-independent in 1999 and became dependent in only IADL in 2001 ($n = 37$). The OR was adjusted for gender, age, and educational status.

[b] Model 2: Stepwise multiple logistic regression analysis was performed to compare subjects who were ADL- and IADL-independent in both 1999 and 2001 ($n = 304$) and those who were ADL- and IADL-independent in 1999 and became dependent in IADL and/or ADL in 2001 ($n = 46$). The OR was adjusted for gender, age, educational status, and social roles.

Table 5
Results of sensitivity analysis to examine effects of loss-to-follow-up and lack of educational information on functional decline over the 2-year period

Predictors	Category	Model 1[a] ($n = 405$)			Model 2[b] ($n = 363$)		
		OR	95% CI	P-value	OR	95% CI	P-value
MMSE score	30–27	1.00			1.00		
	26–24	1.86	1.03, 3.38	0.041	3.25	1.31, 8.08	<0.001
	23–20	2.98	1.54, 5.78	0.001	5.72	2.25, 14.51	<0.001
	19–0	8.84	2.69, 29.09	<0.001	21.44	5.13, 89.53	<0.001
			Test for trend	<0.001		Test for trend	<0.001
UI	Absent	1.00			1.00		
	Weekly or daily	1.44	0.60, 3.45	0.412	1.79	0.54, 5.87	0.339
Falls experienced during the past year	Absent	1.00			1.00		
	Present	0.86	0.37, 1.96	0.711	0.63	0.22, 1.84	0.400
Hosmer–Lemeshow test		$\chi^2 = 5.11$ ($P = 0.746$)			$\chi^2 = 7.33$ ($P = 0.501$)		

[a] Model 1: As a sensitivity analysis, subjects who were alive but did not participate in the follow-up survey were regarded as those who became dependent in IADL and/or ADL in 2001. Stepwise multiple logistic regression analysis was performed to compare subjects who were ADL- and IADL-independent in both 1999 and 2001 ($n = 304$) and those who were ADL- and IADL-independent in 1999 and either became dependent in IADL and/or ADL in 2001 ($n = 46$) or lost-to-follow-up in 2002 ($n = 55$). The OR was adjusted for gender, age, educational status, and social roles.

[b] Model 2: Other sensitivity analysis included subjects who did not have information about educational status ($n = 18$). Stepwise multiple logistic regression analysis was performed to compare subjects who were ADL- and IADL-independent in both 1999 and 2001 ($n = 315$) and those who were ADL- and IADL-independent in 1999 and either became dependent in IADL and/or ADL in 2001 ($n = 48$). The OR was adjusted for gender, age, and social roles.

3.4. Sensitivity analysis

Two kinds of sensitivity analyses (Table 5) were conducted to determine the effects of either drop-out or lack of information about educational status on the analysis results (Heitjan, 1997). First, we assumed that 55 subjects who did not participate in the follow-up survey were dependent in ADL and/or IADL and they were included in the multiple logistic regression analysis. Other sensitivity analysis was performed to examine the effect of not having information on educational attainment in the results. In both models, lower MMSE scores were significant predictors of functional decline and a statistically significant association was observed between lower MMSE scores and increased OR for functional dependence (test for trend, $P < 0.001$).

4. Discussion

This study examined whether cognitive impairment, falls, and UI were independent predictors for functional decline by means of a 2-year observation of a non-disabled older Japanese cohort living in a community from 1999 to 2001. Multivariate logistic regression

analysis revealed that cognitive function was a significant predictor for functional decline during the 2-year follow-up. Analysis also revealed that, using a group of subjects with MMSE scores of 30–27 points as a reference group, a significant association was found between lower MMSE scores and increased OR for functional decline. These results indicate that lower cognitive function was a significant predictor for functional decline even among those older non-disabled Japanese whose cognitive function was deemed to be within the normal range.

All subjects included in the analysis were both ADL- and IADL-independent at the baseline; nevertheless, lower MMSE scores were significantly associated with any decreased functional independence 2 years later. This result indicates that normal cognitive function as assessed by MMSE does not necessarily guarantee long-term functional independence, especially if the elderly concerned have lower than average MMSE scores. Several longitudinal studies point to dementia (Aguero-Torres et al., 1998; Sauvaget et al., 2002) and cognitive impairment (Moritz et al., 1995; Gill et al., 1996, 1997; Aguero-Torres et al., 1998) as predictors of decreased ADL-independence; others revealed dementia to be a predictor of decreased IADL-independence (Sauvaget et al., 2002). One study also indicated that elderly people with normal cognitive function, but lower MMSE scores, were likely to have decreased ADL independence in the future (Greiner et al., 1996). The present study is unique in that it examined whether lower cognitive function is a possible predictor for ADL and/or IADL decline and in that it indicates that elderly with normal cognitive function, but lower MMSE scores, are likely to have decreased functional independence in the future.

Several issues may need to be considered when addressing cognitive function as a possible predictor of decreased functional independence. According to Lawton's model (Lawton, 1972), IADL performance requires higher cognitive functioning than ADL performance, since IADL deals with execution of more complicated tasks. A person with low cognitive performance is considered to have limitations in performing IADL tasks, and is therefore at increased risk of impaired IADL. In our previous study, a survey carried out in another village targeted older adults who were both ADL- and IADL-independent at the baseline (Ishizaki et al., 2000b). In that study, poor intellectual activity was identified as a significant predictor for IADL decline among older ADL- and IADL-independent subjects; however, the subjects' cognitive functions were not measured. To compare the results from our previous study with the present study, cognitive function assessed by MMSE was excluded from a multiple logistic regression analysis in this study. The results indicated that poor intellectual activity was a significant predictor of IADL dependence in the follow-up 2 years, supporting the understanding that IADL requires a certain level of intellectual capacity.

In this study, the present of UI was not associated with functional decline. Most female subjects with UI who participated in this survey replied that they used a special pad for urinary leakage (data not shown). Thus, such women were able to maintain IADL despite having UI. The prevalence rate of UI in this study (10%) was lower that that of previous studies because subjects in this study were limited to those with any disability in both ADL and IADL, while previous epidemiological studies were included both non-disabled and disabled populations. When we added disabled subjects at the time of the baseline survey to the original analyzable subjects, the prevalence of UI was about 40%. In addition, when we

asked respondents about UI, we were careful to protect their privacy. Therefore, we can say that the effect of underreporting UI in this study was not significant.

In interpreting the study results, the following limitations must be considered. First, 10% of the participants at the baseline did not participate in the follow-up, and 10 of the followed-up subjects provided no answers to some of the key questions related to functional status and were therefore excluded from the analysis. The 55 subjects who were lost to follow-up were significantly more likely to have poor social role, to have hearing impairment, and to have visual impairment than the 350 analyzable subjects ($P < 0.05$, data not shown). Sensitivity analysis was conducted to determine the effects of drop-outs on the analysis results based on two assumptions. In all cases, lower MMSE scores were significant predictors of IADL dependence and a statistically significant association was observed between lower MMSE scores and increased OR for functional dependence ($P < 0.001$). It can therefore be concluded that the effects of drop-outs on the results of the analysis were relatively small. Second, although the MMSE score of an individual is known to vary greatly depending on his/her educational attainment, educational attainment was not included in the logistic model regardless of the sensitivity analysis. The result indicated that lower MMSE scores were still a significant predictor of functional dependence. Finally, the subjects in the present study were selected from an agricultural village in Japan, and they therefore do not represent the entire elderly population of the country. However, the elderly adults who participated in the baseline survey constituted 77% of all elderly aged 70 years and older living in the surveyed district; thus, our study represents at least the elderly population living within the village surveyed. Despite the limitations listed above, the present study reliably demonstrates that lower cognitive function is a significant predictor of increased functional dependence over a 2-year period among elderly Japanese whose cognitive function is deemed to be within the normal range and who are both ADL- and IADL-independent.

5. Conclusion

To prevent lifestyle-related diseases, such as cerebral stroke, heart attack, and cancer, among middle aged and older adults, every Japanese citizen aged 40 years and older is entitled to receive an annual health examination under the Health Care Law for the Aged (Nakahara, 1997). As the Japanese population is graying rapidly, we believe the annual health examination should also be used as a means either to detect or prevent functional decline among the elderly. In order to ensure functional independence among the elderly, health examinations for them should include regular assessment of functional and cognitive status (Rubenstein and Rubenstein, 1992; Ebrahim, 1999). If a person is both ADL- and IADL-independent, but is assessed as exhibiting relatively poor cognitive performance, such an individual should be examined more closely and followed up to ensure that both his/her functional and cognitive status are maintained or improved. We believe the findings in this study can be instrumental in promoting healthy changes and preventing functional decline among non-disabled older adults living in communities in Japan.

Acknowledgement

This study was supported in part by a Grant-in-Aid for Comprehensive Research on Aging and Health from the Ministry of Health, Labour and Welfare, Japan.

References

Aguero-Torres, H., Fratiglioni, L., Guo, Z., Viitanen, M., von Strauss, E., Winblad, B., 1998. Dementia is the major cause of functional dependence in the elderly: 3-year follow-up data from a population-based study. Am. J. Public Health 88, 1452–1456.

Aguero-Torres, H., Thomas, V.S., Winblad, B., Fratiglioni, L., 2002. The impact of somatic and cognitive disorders on the functional status of the elderly. J. Clin. Epidemiol. 55, 1007–1012.

Crimmins, E.M., Saito, Y., 1993. Getting better and getting worse. J. Aging Health 5, 3–36.

Ebrahim, S., 1999. Disability in older people: a mass problem requiring mass solutions. Lancet 353, 1990–1992.

Folstein, M., Anthony, J.C., Parhad, I., Duffy, B., Gruenberg, E.M., 1985. The meaning of cognitive impairment in the elderly. J. Am. Geriatr. Soc. 33, 228–235.

Gill, T.M., Williams, C.S., Richardson, E.D., Tinetti, M.E., 1996. Impairments in physical performance and cognitive status as predisposing factors for functional depoendence among nondisabled older persons. J. Gerontol. Med. Sci. 51A, M283–M288.

Gill, T.M., Williams, C.S., Richardson, E.D., Berkman, L.F., Tinetti, M.E., 1997. A predictive model for ADL dependence in community-living older adults based on a reduced set of cognitive items. J. Am. Geriatr. Soc. 45, 441–445.

Greiner, P.A., Snowdon, D.A., Schmitt, F.A., 1996. The loss of independence in activities of daily living: the role of low normal cognitive function in elderly Nuns. Am. J. Public Health 86, 62–66.

Heitjan, D.F., 1997. Annotation: what can be done about missing data? Approaches to imputation. Am. J. Public Health 87, 548–550.

Hosmer, D., Lemeshow, S., 1989. Applied Logistic Regression. John Wiley and Sons Inc., New York, pp. 140–145.

Ishizaki, T., Kobayashi, Y., Kai, I., 2000a. Functional transitions in instrumental activities of daily living among older Japanese. J. Epidemiol. 10, 249–254.

Ishizaki, T., Watanabe, S., Suzuki, T., Shibata, H., Haga, H., 2000b. Predictors for functional decline among non-disabled older Japanese living in a community during a 3-year follow-up. J. Am. Geriatr. Soc. 48, 1424–1429.

Ishizaki, T., Kai, I., Kobayashi, Y., Matsuyama, Y., Imanaka, Y., 2004. The effect of aging on functional decline among older Japanese living in a community: a 5-year longitudinal data analysis. Aging Clin. Exp. Res. 16, 233–239.

Kai, I., Ohi, G., Kobayashi, Y., Ishizaki, T., Hisata, M., Kiuchi, M., 1991. Quality of life: a possible health index for the elderly. Asia Pac. J. Public Health 5, 221–227.

Kane, R.L., Ouslander, J.G., Abrass, I.B., 1999. Essentials of Clinical Geriatrics, fourth ed. McGraw-Hill, New York, pp. 14–16.

Koyano, W., Shibata, H., Nakazato, K., Haga, H., Suyama, Y., 1991. Measurement of competence: reliability and validity of the TMIG index of Competence. Arch. Gerontol. Geriatr. 13, 103–116.

Lawton, M.P., 1972. Assessing the competence of older people. In: Kent, D., Kastenbaum, R., Sherwood, S. (Eds.), Research, Planning, and Action for the Elderly. Behavioral Publications, New York, pp. 122–143.

McDowell, I., Newell, C., 1996. Measuring Health, second ed. Oxford University Press, New York, pp. 314–323.

Moritz, D.J., Kasl, S.V., Berkman, L.F., 1995. Cognitive functioning and the incidence of limitations in activities of daily living in an elderly community sample. Am. J. Epidemiol. 141, 41–49.

Nakahara, T., 1997. Public health policies and strategies in Japan. In: Detels, R., Holland, W.W., McEwen, J., Omenn, G.S. (Eds.), Oxford Textbook of Public Health. third ed. Oxford University Press, New York, pp. 323–329.

Nourhashemi, F., Andrieu, S., Gillette-Guyonnet, S., Vellas, B., Albarede, J.L., Grandjean, H., 2001. Instrumental activities of daily living as a potential marker of frailty: a study of 7364 community-dwelling elderly women (the EPIDOS study). J. Gerontol. A: Biol. Sci. Med. Sci. 56, M448–M453.

Otsuka, T., Homma, A., 1991. Assessment Manual of Intellectual Function for the Demented Elderly. World Planning, Tokyo (in Japanese).

Rubenstein, L.Z., Rubenstein, L.V., 1992. Multidimensional geriatric assessment. In: Brocklehurst, J.C., Tallis, R.C., Fillet, H.M. (Eds.), Textbook of geriatric Medicine and Gerontology, fourth ed. Churchill Livingstone, Edinburgh, pp. 151–159.

Sauvaget, C., Yamada, M., Fujiwara, S., Sasaki, H., Mimori, Y., 2002. Dementia as a predictor of functional disability: a four-year follow-up study. Gerontology 48, 226–233.

Spector, W.D., Katz, S., Murphy, J.B., Fulton, J.P., 1987. The hierarchical relationship between activities of daily living and instrumental activities of daily living. J. Chron. Dis. 40, 481–489.

SPSS Inc., 1999. SPSS Regression Models 10.0. SPSS Inc., Chicago.

Strawbridge, W.J., Cohen, R.D., Shema, S.J., Kaplan, G.A., 1996. Successful aging: predictors and associated activities. Am. J. Epidemiol. 144, 135–141.

Stuck, A.E., Walthert, J.M., Nikolaus, T., Bula, C.J., Hohmann, C., Beck, J.C., 1999. Risk factors for functional status decline in community-living elderly people: a systematic literature review. Soc. Sci. Med. 445–469.

Tinetti, M.E., Inouye, S.K., Gill, T.M., Doucette, J.T., 1995. Shared risk factors for falls, incontinence, and functional dependence. J. Am. Med. Assoc. 273, 1348–1353.

Association of polymorphisms in forkhead box C2 and perilipin genes with bone mineral density in community-dwelling Japanese individuals

YOSHIJI YAMADA[1], FUJIKO ANDO[2] and HIROSHI SHIMOKATA[2]

[1]Department of Human Functional Genomics, Life Science Research Center, Mie University, Tsu, Mie;
[2]Department of Epidemiology, National Institute for Longevity Sciences, Obu, Aichi, Japan

Received January 16, 2006; Accepted March 22, 2006

Abstract. Evidence suggests the existence of a close relation between lipid metabolism and bone remodeling. We hypothesized that polymorphisms of genes that play a role in lipid metabolism, such as those for forkhead box C2 (*FOXC2*) and perilipin (*PLIN*), might affect bone mineral density (BMD). We thus examined the possible relationships between a -512C→T polymorphism of *FOXC2* and a 1243C→T polymorphism of *PLIN* to BMD in community-dwelling Japanese women and men. The subjects (1129 men, 1114 women for *FOXC2*; 1122 men, 1112 women for *PLIN*) were aged 40 to 79 years and were randomly recruited to a population-based prospective cohort study of aging and age-related diseases in Japan. Genotypes for *FOXC2* and *PLIN* were determined with a fluorescence-based allele-specific DNA primer assay system. The -512C→T polymorphism of *FOXC2* was associated with BMD for the distal and proximal radius in men and in premenopausal women as well as with BMD for the distal radius and total body in postmenopausal women, with the T allele being related to reduced BMD. The 1243C→T polymorphism of *PLIN* was associated with BMD for the total body, lumbar spine, femoral neck, and trochanter in men, with the C allele being related to reduced BMD. This polymorphism of *PLIN* was not associated with BMD in all women. These results suggest that *FOXC2* is a susceptibility locus for reduced BMD in Japanese men and women, and that *PLIN* constitutes such a locus in Japanese men.

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 lifestyle factors influence BMD, family and twin studies have suggested that BMD is largely heritable and under the control of multiple genes (2-4). Personalized prevention of osteoporosis and osteoporotic fractures is an important public health goal, one approach to which is to identify disease susceptibility genes. Although genetic linkage analyses (5-7) and candidate gene association studies (7-10) have implicated various loci and genes in predisposition to osteoporosis or fractures, the genes that confer susceptibility to this disease remain to be identified definitively. In addition, because of ethnic differences in gene polymorphisms as well as in lifestyle and environmental factors, it is important to examine polymorphisms related to BMD in each ethnic group.

Bone loss is associated with an expansion of adipose tissue in bone marrow (11), and osteoblasts and adipocytes share a common progenitor derived from stromal cells in bone marrow (12). Products of lipoprotein oxidation and an atherogenic diet also inhibit preosteoblast differentiation and result in reduced bone mineralization (12,13). In addition, lipid-lowering agents (statins) stimulate bone formation and inhibit bone resorption, resulting in the prevention of both bone loss and osteoporotic fractures (14). Early postmenopausal women with an atherogenic serum lipid profile were found to have a lower BMD for the lumbar spine or femoral neck and an increased risk of osteopenia compared with those with a normal lipid profile (15). Postmenopausal women with increased plasma concentrations of low density lipoprotein (LDL) cholesterol have also been shown to be at greater risk of developing osteopenia than are those with normal concentrations, suggesting that an increased plasma level of LDL-cholesterol is a risk factor for reduced BMD (16). These observations suggest the existence of a close relationship between lipid metabolism and bone remodeling as well as demonstrating adverse effects of an atherogenic lipid profile on the latter. We thus hypothesized that polymorphisms of genes that play a role in lipid metabolism, such as those for forkhead box C2 (*FOXC2*) and perilipin (*PLIN*), might affect BMD.

FOXC2, a forkhead-winged helix transcription factor, is a key regulator of adipocyte metabolism (17). Transgenic mice that overexpress *FOXC2* specifically in white and brown adipocytes manifest a lean and insulin-sensitive phenotype.

Correspondence to: Dr Yoshiji Yamada, Department of Human Functional Genomics, Life Science Research Center, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan
E-mail: yamada@gene.mie-u.ac.jp

Key words: polymorphism, genetics, osteoporosis, bone density, forkhead box C2, perilipin

In these mice, deposition of white adipose tissue in the abdomen is reduced, with such tissue also exhibiting a brown fat-like histology, whereas interscapular brown adipose tissue is hypertrophic. Increased expression of *FOXC2* in adipocytes had pleiotropic effects on the expression of genes important in cellular differentiation and metabolism, insulin action, adrenergic sensitivity, and intracellular signaling. These various *FOXC2*-dependent changes appeared to confer protection against obesity. Furthermore, increased *FOXC2* expression induced by a high-fat diet in wild-type mice seems to counteract most of the signs associated with obesity, including hypertriglyceridemia and diet-induced insulin resistance, suggesting that this gene might also protect against type 2 diabetes (17). These findings suggest that *FOXC2* is a candidate gene for predisposition to obesity and type 2 diabetes mellitus, and that it may also play a role in bone remodeling.

Perilipins are a family of proteins that coat intracellular lipid droplets. Expression of *PLIN* is largely restricted to adipocytes and steroidogenic cells (18,19), and it appears to function primarily in the regulation of intracellular lipolysis in adipocytes (20,21). Perilipin promotes cellular storage of triglycerides by reducing the rate of triglyceride hydrolysis, and it also controls the release of triglycerides when requried. The absence of *PLIN* confers a lean phenotype and counterbalances both genetic and diet-induced obesity in mice (22,23). In contrast, increased expression of *PLIN* correlates with increased adiposity in humans (24). *PLIN* is thus implicated as a candidate gene for human obesity, and it may therefore also affect bone remodeling.

We have been attempting to identify gene polymorphisms that are significantly associated with BMD in Japanese women or men in a population-based study. In the present study, we examined the relationship with polymorphisms of *FOXC2* and *PLIN* to BMD, even though there is no apparent biological link between these genes. Our aim was to identify a single polymorphism significantly associated with BMD for each gene. Among several polymorphisms previously identified, the -512C→T polymorphism of *FOXC2* and the 1243C→T polymorphism of *PLIN* have been shown to potentially affect gene function (25,26). We thus examined the relations of these polymorphisms to BMD in Japanese women and men in our population-based cohort.

Materials and methods

Study population. The National Institute for Longevity Sciences-Longitudinal Study of Aging is a population-based prospective cohort study of aging and age-related diseases (27). The subjects are stratified by both age and sex, and are randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan (28, 29). The lifestyle of residents in this area is typical of that of Japanese individuals. The numbers of men and women recruited were similar and ages at the baseline were 40 to 79 years, with similar numbers of participants in each decade (40s, 50s, 60s, 70s). The subjects were followed up every 2 years. All participants were subjected at a special center to a detailed examination, which included not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. Individuals with disorders known to cause abnormalities of bone metabolism, including diabetes mellitus, chronic renal failure, rheumatoid arthritis, as well as thyroid, parathyroid, adrenal, and other endocrine diseases, or those who had taken drugs that affect bone metabolism, such as estrogen, glucocorticoids, bisphosphonates, vitamin D, and statins, were excluded from the present study. Individuals whose genotypes were not successfully determined (nine individuals for *PLIN*) were also excluded from the analysis.

We examined the relationship of BMD at various sites to the -512C→T polymorphism of *FOXC2* (NCBI, Y08223, nt685) in 2243 individuals (1129 men, 1114 women) and to the 1243C→T polymorphism of *PLIN* (NCBI, dbSNP, rs2304796) in 2234 individuals (1122 men and 1112 women). In addition, to uncover potential differences between women according to menopausal status, we conducted all analyses separately for premenopausal and postmenopausal women. Menopausal status was evaluated with a detailed questionnaire, and menopause was defined as complete cessation of menstruation. The study protocol complies with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Institute for Longevity Sciences. Written informed consent was obtained from each subject.

Measurement of BMD. BMD at the radius was measured by peripheral quantitative computed tomography (pQCT) (Desiscan 1000; Scanco Medical, Bassersdorf, Switzerland) and was expressed as D50 (BMD for the inner 50% of the cross-sectional area of the distal radius, comprising mostly cancellous bone), D100 (BMD for the entire cross-sectional area of the distal radius, including both cancellous and cortical bone), and P100 (BMD for the entire cross-sectional area of the proximal radius, consisting mostly of cortical bone). BMD for the total body, lumbar spine (L2-L4), right femoral neck, and right trochanter was measured by dual-energy X-ray absorptiometry (DXA) (QDR 4500; Hologic, Bedford, MA). The coefficients of variation of the pQCT instrument for BMD values were 0.7% (D50), 1.0% (D100), and 0.6% (P100), and those of the DXA instrument were 0.9% (total body), 0.9% (L2-L4), 1.3% (femoral neck), and 1.0% (trochanter).

Determination of genotype. Genotypes for *FOXC2* and *PLIN* were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) (30). The polymorphic region of *FOXC2* was amplified by the polymerase chain reaction with sense primers labeled at the 5' end with either fluorescein isothiocyanate (5'-AACT CGCTTTCAGCAAGAAGXCT-3') or Texas red (5'-ACTC GCTTTCAGCAAGAAGXTT-3') and with an antisense primer labeled at the 5' end with biotin (5'-AGGCCAAGTCC CTTTTAGGGA-3'). The polymorphic region of *PLIN* was amplified with allele-specific sense primers labeled at the 5' end with either fluorescein isothiocyanate (5'-CCTCCCCTT GGTTGAGGXGA-3') or Texas red (5'-GCCCTCCCC TTGGTTGAGGXAA-3') and with an antisense primer labeled at the 5' end with biotin (5'-AGGGAGGGTGC TGCACCTCAC-3'). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 4 (for *FOXC2*) or 3.5 (for *PLIN*) mmol/l $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The ampli-

Table I. BMD and other characteristics for all men (n=1129) according to *FOXC2* genotype.[a]

Characteristic	CC	CT	TT	CT+TT
Number (%)	543 (48.1)	468 (41.5)	118 (10.5)	586 (51.90
Age (years)	59.4±0.5	58.8±0.5	60.0±1.0	59.0±0.5
Height (cm)	164.6±0.3	164.6±0.3	164.6±0.6	164.6±0.3
Body weight (kg)	62.8±0.4	61.9±0.4	63.1±0.8	62.1±0.4
BMD measured with pQCT (mg/cm^3)				
D50	273.0±2.8	262.5±3.1[b]	254.4±6.1[c]	260.8±2.7[d]
D100	548.3±3.9	536.3±4.2	524.7±8.5[e]	533.9±3.8[f]
P100	1194.7±6.0	1176.5±6.5	1175.7±12.9	1176.3±5.8[g]
BMD measured with DXA (g/cm^2)				
Total body	1.090±0.004	1.085±0.004	1.086±0.008	1.085±0.004
L2-L4	0.989±0.006	0.974±0.007	0.989±0.014	0.977±0.006
Femoral neck	0.753±0.004	0.756±0.005	0.743±0.009	0.753±0.004
Trochanter	0.669±0.004	0.668±0.004	0.667±0.009	0.667±0.004

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE. [b]P=0.0307, [c]P=0.0158, [d]P=0.0020, [e]P=0.0306, [f]P=0.0084, [g]P=0.0270 versus CC.

fication protocol comprised initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 sec, annealing at 60°C (for *FOXC2*) or 67.5°C (for *PLIN*) for 30 sec, and extension at 72°C for 30 sec; and a final extension at 72°C for 2 min. The amplified DNA was then incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature, and the plate was placed on a magnetic stand. 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. Statistical analysis was performed with SAS software (SAS Institute, Cary, NC). Data were compared among three genotype groups by one-way analysis of variance and the Tukey-Kramer post hoc test, and between two groups (dominant or recessive model) by the unpaired Student's t- test. BMD values were compared among genotypes for each polymorphism 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 chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. The effect of genotype for each polymorphism on BMD was evaluated by single regression analysis; P values and R^2 were calculated from analysis of genotype for *FOXC2* (CC=0, CT=TT=1 for men; CC=CT=0, TT=1 for women) or *PLIN* (CC=CT=0, TT=1). A P value of <0.05 was considered statistically significant.

Results

Relationship between the -512C→T polymorphism of FOXC2 and BMD. The distribution of -512C→T genotypes of *FOXC2* was in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all men (Table I). Among all men, BMD for D50, with adjustment for age, height, and body weight, was greater in individuals with the CC genotype than in those with the CT genotype, the TT genotype, or in the combined group of CT and TT genotypes (Table I). BMD for D100 was greater in individuals with the CC genotype than in those with the TT genotype or in the combined group of CT and TT genotypes. BMD for P100 was also greater in individuals with the CC genotype than in the combined group of CT and TT genotypes. The differences in BMD for D50 and D100 between individuals with the CC genotype and those with the TT genotype (expressed as a percentage of the larger value) were 6.8 and 4.3%, respectively. The difference in BMD for P100 between individuals with the CC genotype and the combined group of CT and TT genotypes was 1.5%.

The distribution of -512C→T genotypes of *FOXC2* was in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all women (Table II). Among all women, BMD for D50, with adjustment for age, height, and body weight, was greater in the combined group of CC and CT genotypes than in individuals with the TT genotype (Table II). BMD for D100 was greater in individuals with the CC genotype, in individuals with the CT genotype, or in the combined group of CC and CT genotypes than in those with the TT genotype. BMD for the total body was also greater in the combined group of CC and CT genotypes than in individuals with the TT genotype. The differences in BMD for

Table II. BMD and other characteristics for all women (n=1114) according to *FOXC2* genotype.[a]

Characteristic	CC	CT	TT	CC+CT
Number (%)	525 (47.1)	479 (43.0)	110 (9.9)	1004 (90.1)
Age (years)	59.2±0.5	59.4±0.5	59.1±1.0	59.3±0.3
Height (cm)	151.5±0.3	151.1±0.3	151.8±0.6	151.3±0.2
Body weight (kg)	52.5±0.4	52.6±0.4	52.7±0.8	52.6±0.3
BMD measured with pQCT (mg/cm^3)				
D50	187.2±2.7	185.9±2.8	172.8±6.0	186.6±1.9[b]
D100	487.5±3.9[c]	489.2±4.1[d]	463.2±8.6	488.3±2.8[e]
P100	1156.6±6.3	1156.4±6.6	1130.3±13.9	1156.5±4.5
BMD measured with DXA (g/cm^2)				
Total body	0.967±0.004	0.968±0.004	0.948±0.008	0.968±0.003[f]
L2-L4	0.869±0.006	0.866±0.006	0.854±0.012	0.867±0.004
Femoral neck	0.680±0.004	0.679±0.004	0.666±0.008	0.679±0.003
Trochanter	0.574±0.004	0.570±0.004	0.560±0.008	0.572±0.003

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE [b]P=0.0284, [c]P=0.0275, [d]P=0.0174, [e]P=0.0056,[f]P=0.0200 versus TT.

Table III. BMD and other characteristics for premenopausal women (n=281) according to *FOXC2* genotype.[a]

Characteristic	CC	CT	TT	CT+TT
Number (%)	132 (47.0)	122 (43.4)	27 (9.6)	254 (90.4)
Age (years)	45.7±0.4	46.9±0.4	45.1±0.9	46.3±0.3
Height (cm)	155.7±0.4[b]	153.4±0.4	153.7±0.9	154.6±0.3
Body weight (kg)	54.4±0.7	54.4±0.7	53.9±1.6	54.4±0.5
BMD measured with pQCT (mg/cm^3)				
D50	248.3±4.9	244.1±5.1	236.6±10.8	246.3±3.5
D100	614.0±6.8[c]	603.0±7.1	571.6±15.2	608.8±4.8[d]
P100	1374.7±10.1[e]	1354.7±10.6	1315.3±22.6	1365.1±7.2[f]
BMD measured with DXA (g/cm^2)				
Total body	1.098±0.007	1.093±0.007	1.073±0.016	1.095±0.005
L2-L4	1.032±0.010	1.018±0.010	1.020±0.022	1.025±0.007
Femoral neck	0.772±0.008	0.770±0.008	0.773±0.017	0.771±0.006
Trochanter	0.662±0.007	0.655±0.008	0.643±0.016	0.659±0.005

[a]BMD is adjusted for age, height and body weight. Data are means ± SE. [b]P=0.0004 versus CT; [c]P=0.0308, [d]P=0.0209, [e]P=0.0448, [f]P=0.0368 versus TT.

D50, D100, and total body between the combined group of CC and CT genotypes and individuals with the TT genotype were 7.4, 5.1, and 2.1%, respectively.

To examine the possible influence of menopause on the relationship between *FOXC2* genotype and BMD, we analyzed premenopausal and postmenopausal women independently.

Table IV. BMD and other characteristics for postmenopausal women (n=816) according to *FOXC2* genotype.[a]

Characteristic	CC	CT	TT	CT+TT
Number (%)	384 (47.1)	352 (43.1)	80 (9.8)	736 (90.2)
Age (years)	64.0±0.4	63.8±0.5	64.1±1.0	63.9±0.3
Height (cm)	150.0±0.3	150.2±0.3	151.0±0.7	150.1±0.2
Body weight (kg)	51.9±0.4	51.9±0.4	52.4±0.9	51.9±0.3
BMD measured with pQCT (mg/cm^3)				
D50	165.4±3.3	164.9±3.4	149.1±7.2	165.2±2.3[b]
D100	442.7±4.7	447.0±4.8	426.7±10.4	444.8±3.4
P100	1079.4±7.8	1083.7±8.0	1063.0±17.2	1081.4±5.6
BMD measured with DXA (g/cm^2)				
Total body	0.921±0.004	0.922±0.005	0.901±0.010	0.92±0.003[c]
L2-L4	0.810±0.007	0.811±0.007	0.795±0.014	0.810±0.005
Femoral neck	0.646±0.004	0.645±0.004	0.629±0.009	0.646±0.003
Trochanter	0.543±0.004	0.539±0.004	0.531±0.009	0.541±0.003

[a]BMD is adjusted for age, height and body weight. Data are means ± SE. [b]P = 0.0342, [c]P = 0.0492 versus TT.

Table V. BMD and other characteristics for all men (n=1122) according to *PLIN* genotype.[a]

Characteristic	CC	CT	TT	CC+CT
Number (%)	630 (56.1)	418 (37.3)	74 (6.6)	1048 (93.4)
Age (years)	58.9±0.4	59.7±0.5	59.0±1.3	59.2±0.3
Height (cm)	164.7±0.3	164.4±0.3	164.8±0.7	164.6±0.2
Body weight (kg)	62.6±0.4	62.5±0.4	61.1±1.1	62.6±0.3
BMD measured with pQCT (mg/cm^3)				
D50	265.2±2.7	267.2±3.2	276.1±7.7	266.0±2.1
D100	537.8±3.7	542.6±4.5	554.0±10.6	539.7±2.8
P100	1178.3±5.6	1193.7±6.8	1187.7±16.1	1184.5±4.3
BMD measured with DXA (g/cm^2)				
Total body	1.083±0.004	1.090±0.004	1.107±0.010	1.086±0.003[b]
L2-L4	0.975±0.006	0.988±0.007	1.017±0.017	0.980±0.005[c]
Femoral neck	0.749±0.004[d,h]	0.752±0.005	0.790±0.011	0.750±0.003[e]
Trochanter	0.663±0.004[f]	0.671±0.005	0.696±0.011	0.666±0.003[g]

[a]BMD is adjusted for age, height, and body weight. Data are mean ± SE. [b]P=0.0461, [c]P=0.0396, [d]P=0.0021, [e]P=0.0007, [f]P=0.0140, [g]P=0.0106 versus TT; [h]P=0.0052 versus CT.

Because of their small number (n=17), perimenopausal women were excluded from this analysis. The distribution of -512C→T genotypes of *FOXC2* was in Hardy-Weinberg equilibrium in premenopausal (Table III) and postmenopausal (Table IV) women. Age and body weight did not differ among genotypes for premenopausal or postmenopausal women. Height was greater in premenopausal women with the CC genotype that in those with the CT genotype (Table III), but it

Table VI. BMD and other characteristics for all women (n=1112) according to *PLIN* genotype.[a]

Characteristic	CC	CT	TT	CC+CT
Number (%)	609 (54.8)	415 (37.3)	88 (7.9)	1024 (92.1)
Age (years)	59.7±0.4	58.8±0.5	57.9±1.2	59.4±0.3
Height (cm)	151.3±0.2	151.4±0.3	151.8±0.6	151.3±0.2
Body weight (kg)	52.3±0.3	53.1± 0.4	52.7±0.9	52.6±0.3
BMD measured with pQCT (mg/cm^3)				
D50	185.2±2.5	186.3±3.0	180.6±6.5	185.7±1.9
D100	483.4±3.6	486.5±4.4	497.9±9.5	484.7±2.8
P100	1151.2±5.9	1154.4±7.1	1168.2±15.3	1152.5±4.5
BMD measured with DXA (g/cm^2)				
Total body	0.966±0.003	0.963±0.004	0.968±0.009	0.965±0.003
L2-L4	0.864±0.005	0.868±0.006	0.863±0.014	0.866±0.004
Femoral neck	0.680±0.003	0.677±0.004	0.672±0.009	0.678±0.003
Trochanter	0.572±0.003	0.572±0.004	0.561±0.009	0.572±0.003

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE.

Table VII. Effects of genotypes for FOXC2 and PLIN on BMD.[a]

Genotype	D50	D100	P100	Total body	L2-L4	Femoral neck	Trochanter
Men							
FOXC2	**0.0027** (0.0083)	**0.0170** (0.0053)	0.0661	0.3055	0.0787	0.7315	0.4283
PLIN	0.2709	0.2305	0.7867	0.1800	0.2188	**0.0207** (0.0048)	0.1239
All women							
FOXC2	0.0780	0.0526	0.2333	0.1683	0.5003	0.2771	0.3362
PLIN	0.8510	0.0566	0.0919	0.2873	0.6016	0.8517	0.8361
Premenopausal women							
FOXC2	0.7208	0.1008	0.2751	0.2353	0.8690	0.8614	0.4129
PLIN	0.1660	0.8853	0.8262	0.9462	0.6911	0.1224	0.1015
Postmenopausal women							
FOXC2	**0.0372** (0.0055)	0.0947	0.2346	0.1393	0.4126	0.1810	0.4181
PLIN	0.9305	0.2306	0.4186	0.7807	0.8573	0.7448	0.8836

[a]Data were analyzed by single regression analysis of genotype for *FOXC2* (CC=0, CT=TT=1 for men; CC=CT=0, TT=1 for women) or *PLIN* (CC=CT=0, TT=1). Data are P values (R^2). P values of <0.05 are shown in bold.

did not differ among genotypes for postmenopausal women (Table IV). For premenopausal women, BMD for D100 or P100 was greater in individuals with the CC genotype or in the combined group of CC and CT genotypes than in subjects with the TT genotype (Table III). The differences in BMD for D100 and P100 between individuals with the CC genotype and those with the TT genotype were 6.9 and 4.3%, respectively. For postmenopausal women, BMD for D50 or the total body was greater in the combined group of CC and CT genotypes than in individuals with the TT genotype (Table IV).

The differences in BMD for D50 and the total body between the combined group of CC and CT genotypes and individuals with the TT genotype were 9.7 and 2.2%, respectively.

Relationsip between the 1243C→T polymorphism of PLIN and BMD. The distribution of 1243C→T genotypes of *PLIN* was in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all men (Table V). Among all men, BMD for the total body or lumbar spine, with adjustment for age, height, and body weight, was significantly greater in individuals with the TT genotype than in the combined group of CC and CT genotypes (Table V). BMD for the femoral neck or trochanter was greater in individuals with the TT genotype than in those with the CC genotype or in the combined group of CC and CT genotypes. BMD for the femoral neck was also greater in individuals with the CT genotype than in those with the CC genotype. The differences in BMD for the total body and lumbar spine between individuals with the TT genotype and the combined group of CC and CT genotypes were 1.9 and 3.6%, respectively. The differences in BMD for the femoral neck and trochanter between individuals with the TT genotype and those with the CC genotype were 5.2 and 4.7%, respectively.

There was no significant relationship between *PLIN* genotype and BMD for all women (Table VI). For premenopausal women, BMD for D100 was greater in the combined group of TT and CT genotypes than in individuals with the CC genotype (data not shown). For postmenopausal women, no relationship was detected between *PLIN* genotype and BMD (data not shown).

Effects of genotypes for FOXC2 and PLIN on BMD. The effects of -512C→T genotype for *FOXC2* and 1243C→T genotype for *PLIN* on BMD at various sites were evaluated by single regression analysis (Table VII). This analysis revealed that -512C→T genotype for *FOXC2* affected BMD for D50 and D100 in men and BMD for D50 in postmenopausal women, and that 1243C→T genotype for *PLIN* affected BMD for the femoral neck in men.

Discussion

We have examined the relationship of the -512C→T polymorphism of *FOXC2* and the 1243C→T polymorphism of *PLIN* with BMD at various sites in community-dwelling Japanese women and men. Our results show that the T allele of *FOXC2* is associated with reduced BMD in both men and women, and that the C allele of *PLIN* is associated with this condition in men.

Association of the -512C→T polymorphism of FOXC2 with BMD. *FOXC2*-deficient mice show multiple defects of skeletal tissue. In the craniofacial skeleton of these animals, for example, the supraoccipital bone is missing and other bones are reduced in size (31,32). *FOXC2* is expressed in the early stage of chondrogenic differentiation both *in vivo* and *in vitro*, and bone morphogenetic proteins regulate *FOXC2* expression in skeletal precursor cells (33). Expression of *FOXC2* in mesenchymal condensation and subsequently in cartilaginous tissue, as well as the phenotype of *FOXC2*-deficient mice, indicate that *FOXC2* contributes to the proliferation and differentiation of skeletal cells.

The T allele of the -512C→T polymorphism in the 5' untranslated region of *FOXC2* was shown to be associated with enhanced insulin sensitivity and lower plasma triglyceride concentration in women (25). A higher level of expression of *FOXC2* in visceral fat than in subcutaneous fat was also apparent only in individuals homozygous for the T allele. These observations suggest that increased expression of *FOXC2* may protect against insulin resistance, and that the -512C→T polymorphism of this gene may influence insulin sensitivity (25). We have now shown that this polymorphism of *FOXC2* is associated with BMD in men and women, with the T allele being related to reduced bone mass. The mechanism responsible for the association of the T allele both with enhanced insulin sensitivity and lower plasma triglyceride concentrations in women (25) and with reduced bone mass in men and women (the present study) remains to be elucidated. The molecular mechanism of the effect of this polymorphism on bone remodeling also remains unclear.

Association of the 1243C→T polymorphism of PLIN with BMD. The AA genotype of the 11,482G→A polymorphism of *PLIN* (rs894160) was shown to be associated with a decreased *PLIN* content and increased lipolytic activity in adipocytes of women (34). Individuals with the 11,482A variant of *PLIN* were also found to manifest both a lower baseline body weight and resistance to weight loss in response to a low-energy diet (35). Haplotypes of several polymorphisms of *PLIN* have been related to the risk of obesity, but the extent of this relationship differs between men and women (36) and among ethnic groups (37). The 1243C→T polymorphism of *PLIN* was associated with total cholesterol levels in Chinese (26). Men with the 1243T variant (CT or TT genotype) had higher plasma concentrations of total cholesterol, high density lipoprotein (HDL)-cholesterol, and LDL-cholesterol than did male CC homozygotes, suggesting that the 1243C→T polymorphism of *PLIN* may affect lipid metabolism. Our present results show that the 1243C→T polymorphism of *PLIN* was associated with BMD in men, with the C allele being related to reduced bone mass. This polymorphism was not associated with body weight or body mass index (data not shown) in the present study, making it unlikely that its association with BMD in men was attributable to an effect on these parameters. The mechanism responsible for the association of the C allele with both lower plasma cholesterol concentrations in men (26) and reduced bone mass in men (the present study) remains to be elucidated. The effects of the 1243C→T polymorphism of *PLIN* on gene expression, the function of the encoded protein, or bone remodeling have not been determined.

Given the multiple comparisons of genotypes with BMD at various sites in the present study, it is not possible to exclude potential statistical errors such as false positives. It is also possible that the polymorphisms associated with reduced BMD in our study are in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for the development of this condition. Furthermore, the relevance of the polymorphisms to gene transcription or to protein structure or function and their effects on bone remodeling were not

determined in the present study. Despite these limitations, our present results suggest that *FOXC2* is a susceptibility locus for reduced BMD in Japanese men and women and that *PLIN* constitutes such a locus in Japanese men. Determination of genotypes for these polymorphisms may prove informative for assessment of the genetic risk for reduced BMD.

Acknowledgments

Our research was supported in part by a research grant for Comprehensive Research on Aging and Health (H17-Chojyu-039) from the Ministry of Health, Labor, and Welfare of Japan.

References

1. Kanis JA, Melton LJ III, Christiansen C, Johnston CC and Khaltaev N: The diagnosis of osteoporosis. J Bone Miner Res 9: 1137-1141, 1994.
2. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN and Eberl S: Genetic determinations of bone mass in adults: a twin study. J Clin Invest 80: 706-710, 1987.
3. Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J and Siest G: Segregation analysis and variance components analysis of bone mineral density in healthy families. J Bone Miner Res 10: 2017-2022, 1995.
4. Howard GM, Nguyen TV, Harris M, Kelly PJ and Eisman JA: Genetic and environmental contributions to the association between quantitative ultrasound and bone mineral density measurements: a twin study. J Bone Miner Res 13: 1318-1327, 1998.
5. Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB and Recker RB: Linkage of a gene causing high bone mass to human chromosome 11 (11q12-13). Am J Hum Genet 60: 1326-1332, 1997.
6. Econs MJ, Koller DL, Hui SL, Fishburn T, Conneally PM, Johnston CC Jr, Peacock M and Foroud TM: Confirmation of linkage to chromosome 1q for peak vertebral bone mineral density in premenopausal white women. Am J Hum Genet 74: 223-228, 2004.
7. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN and Eisman JA: Prediction of bone density from vitamin D receptor alleles. Nature 367: 284-287, 1994.
8. Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FEA, Grant SFA, Hofman A, van Leeuwen JPTM, Pols HAP and Ralston SH: Relation of alleles of the collagen type Iα1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. N Engl J Med 338: 1016-1021, 1998.
9. Yamada Y, Ando F, Niino N and Shimokata H: Transforming growth factor-ß1 gene polymorphism and bone mineral density. JAMA 285: 167-168, 2001.
10. Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB, Langdahl B, van Meurs JB, Mosekilde L, Scollen S, Albagha OM, Bustamante M, Carey AH, Dunning AM, Enjuanes A, van Leeuwen JP, Mavilia C, Masi L, McGuigan FE, Nogues X, Pols HA, Reid DM, Schuit SC, Sherlock RE and Uitterlinden AG; GENOMOS Study: Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. JAMA 292: 2105-2114, 2004.
11. Bergman RJ, Gazit D, Kahn AJ, Gruber H, McDougall S and Hahn TJ: Age-related changes in osteogenic stem cells in mice. J Bone Miner Res 11: 568-577, 1996.
12. Parhami F, Jackson SM, Tintut Y, Le V, Balucan JP, Territo M and Demer LL: Atherogenic diet and minimally oxidized low density lipoprotein inhibit osteogenic and promote adipogenic differentiation of marrow stromal cells. J Bone Miner Res 14: 2067-2078, 1999.
13. Diascro DD Jr, Vogel RL, Johnson TE, Witherup KM, Pitzenberger SM, Rutledge SJ, Prescott DJ, Rodan GA and Schmidt A: High fatty acid content in rabbit serum is responsible for the differentiation of osteoblasts into adipocyte-like cells. J Bone Miner Res 13: 96-106, 1998.
14. McFarlane SI, Muniyappa R, Francisco R and Sowers JR: Clinical review 145: Pleiotropic effects of statins: lipid reduction and beyond. J Clin Endocrinol Metab 87: 1451-1458, 2002.
15. Orozco P: Atherogenic lipid profile and elevated lipoprotein (a) are associated with lower bone mineral density in early postmenopausal overweight women. Eur J Epidemiol 19: 1105-1112, 2004.
16. Poli A, Bruschi F, Cesana B, Rossi M, Paoletti R and Crosignani PG: Plasma low-density lipoprotein cholesterol and bone mass densitometry in postmenopausal women. Obstetr Gynecol 102: 922-926, 2003.
17. Cederberg A, Gronning LM, Ahren B, Tasken K, Carlsson P and Enerback S: *FOXC2* is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. Cell 106: 563-573, 2001.
18. Greenberg AS, Egan JJ, Wek SA, Moos MC Jr, Londos C and Kimmel AR: Isolation of cDNAs for perilipins A and B: sequence and expression of lipid droplet-associated proteins of adipocytes. Proc Natl Acad Sci USA 90: 12035-12039, 1993.
19. Servetnick DA, Brasaemle DL, Gruia-Gray J, Servetnick DA, Schultz CJ, Levin DM and Kimmel AR: Perilipins are associated with cholesteryl ester droplets in steroidogenic adrenal cortical and Leydig cells. J Biol Chem 270: 16970-16973, 1995.
20. Souza SC, de Vargas LM, Yamamoto MT, Line P, Franciosa M, Moss L and Greenberg A: Overexpression of perilipin A and B blocks the ability of tumor necrosis factor alpha to increase lipolysis in 3T3-L1 adipocytes. J Biol Chem 273: 24665-24669, 1998.
21. Brasaemle DL, Rubin B, Harten IA, Gruia-Gray J, Kimmel A and Londos C: Perilipin A increases triacylglycerol storage by decreasing the rate of triacylglycerol hydrolysis. J Biol Chem 275: 38486-38493, 2000.
22. Martinez-Botas J, Anderson JB, Tessier D, Lapillojnne A, Hung-Junn Chang B, Quast M, Gorenstein D, Chen KH and Chan L: Absence of perilipin results in leanness and reverses obesity in Lepr (db/db) mice. Nat Genet 26: 474-479, 2000.
23. Tansey JT, Sztalryd C, Gruia-Gray J, Roush D, Zeo J, Gavrilova O, Reitman M, Deng CX, Ki C, Kimmel A and Londos C: Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. Proc Natl Acad Sci USA 98: 6494-6499, 2001.
24. Kern PA, Di Gregorio G, Lu T, Rassouli N and Ranganathan G: Perilipin expression in human adipose tissue is elevated with obesity. J Clin Endocrinol Metab 89: 1352-1358, 2004.
25. Ridderstrale M, Carlsson E, Klannemark M, Cederberg A, Kosters C, Tornqvist H, Storgaard H, Vaag A, Enerback S and Groop L: *FOXC2* mRNA expression and a 5' untranslated region polymorphism of the gene are associated with insulin resistance. Diabetes 51: 3554-3560, 2002.
26. Yan W, Chen S, Huang J, Shen Y, Qiang B and Gu D: Polymorphisms in PLIN and hypertension combined with obesity and lipid profiles in Han Chinese. Obes Res 12: 1733-1737, 2004.
27. Shimokata H, Ando F and Niino N: A new comprehensive study on aging-the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidemiol 10: S1-S9, 2000.
28. Yamada Y, Ando F, Niino N and Shimokata H: Association of polymorphisms of interleukin-6, osteocalcin, and vitamin D receptor genes, alone or in combination, with bone mineral density in community-dwelling Japanese women and men. J Clin Endocrinol Metab 88: 3372-3378, 2003.
29. Yamada Y, Ando F, Niino N and Shimokata H: Association of polymorphisms of androgen receptor and klotho genes with bone mineral density in Japanese women. J Mol Med 83: 50-57, 2005.
30. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, Sone T, Tanaka M and Yokota M: Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med 347: 1916-1923, 2002.
31. Winnier GE, Hargett L and Hogan BL: The winged helix transcription factor MFH1 is required for proliferation and patterning of paraxial mesoderm in the mouse embryo. Genes Dev 11: 926-940, 1997.
32. Iida K, Koseki H, Kakinuma H, Kato N, Mizutani-Koseki Y, Ohuchi H, Yoshioka H, Noji S, Kawamura K, Kataoka Y, Ueno F, Taniguchi M, Yoshida N, Sugiyama T and Miura N: Essential roles of the winged helix transcription factor MFH-1 in aortic arch patterning and skeletogenesis. Development 124: 4627-4638, 1997.
33. Nifuji A, Miura N, Kato N, Kellermann O and Noda M: Bone morphogenetic protein regulation of forkhead/winged helix ZC1 and in skeletal precursor cells. J Bone Miner Res 16: 1765-1771, 2001.