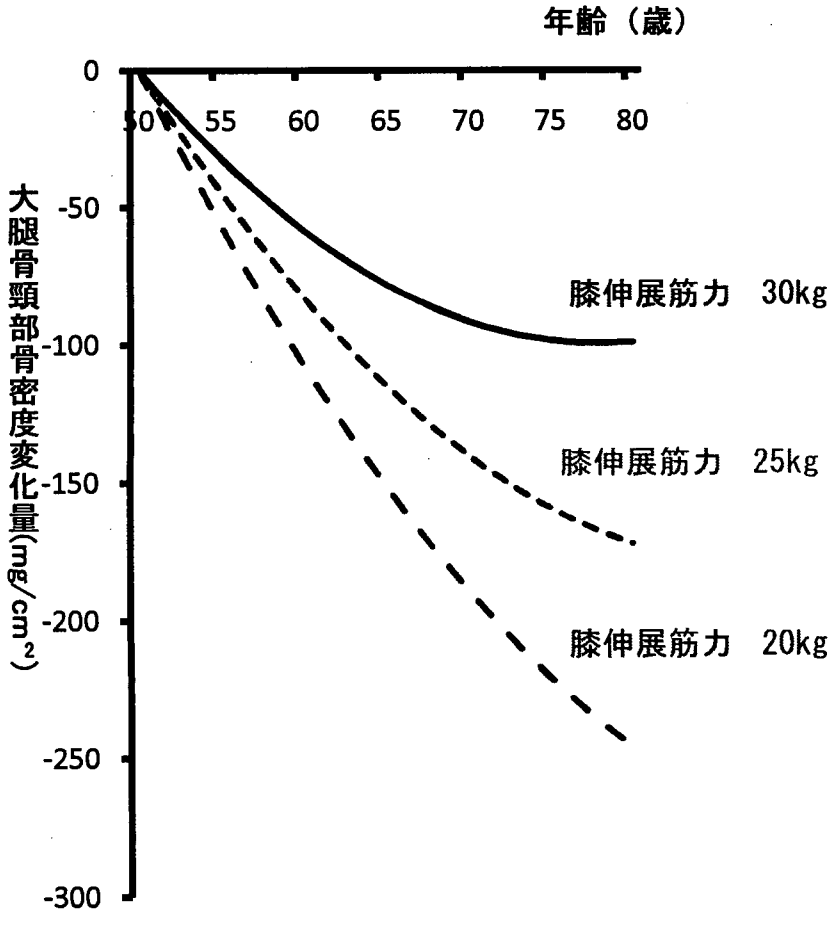
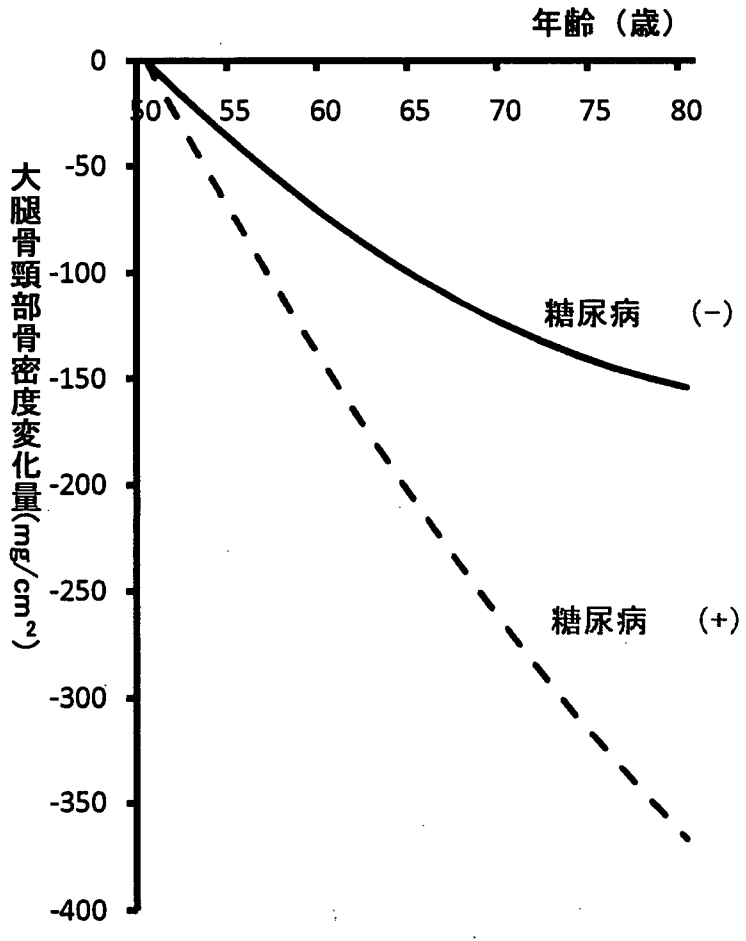
表3. 大腿骨頸部骨密度低下率の将来予測と生活習慣改善による効果の推定アルゴリズム

（閉経女性、Mixed Effect Model）

	遺伝子多型	糖尿病	推定値	F検定(p値)
Intercept			−19.2123	
SNP1	AA		2.2187	0.0026
SNP1	AG/GG		0	
SNP2	AA		−2.1532	0.0030
SNP2	AG/GG		0	
SNP3	II/ID		2.4655	0.0029
SNP3	DD		0	
SNP4	CC		−9.0695	0.0041
SNP4	CA/AA		0	
SNP5	CC		−8.4325	0.0028
SNP5	CG/GG		0	
SNP6	CC		0.5891	0.0006
SNP6	CT/TT		0	
糖尿病		あり	3.7982	0.2747
糖尿病		なし	0	
膝伸展筋力			−0.1596	0.0079
膝伸展筋力＊SNP4	CC		0.3554	0.0030
膝伸展筋力＊SNP4	CA/AA		0	
膝伸展筋力＊SNP5	CC		0.284	0.0072
膝伸展筋力＊SNP5	CG/GG		0	
糖尿病＊SNP6	CC	あり	−10.7946	0.0022
糖尿病＊SNP6	CC	なし	0	
糖尿病＊SNP6	CT/TT	あり	0	
糖尿病＊SNP6	CT/TT	なし	0	
年齢			0.2467	＜ 0.0001

図4. 大腿骨頸部骨密度低下率の将来予測と生活習慣改善による効果の推定アルゴリズム
（閉経女性、Mixed Effect Model）





膝伸展筋力の影響を受けやすい遺伝子多型群を有している場合は膝伸展筋力によって将来の骨密度変化量は大きく異なる（左）。
同様に糖尿病既往の影響を受けやすい遺伝子多型を有している場合は糖尿病既往の有無によって将来の骨密度変化量が大きく異なる（右）。

Ⅱ．研究成果の刊行に関する一覧表

雑　誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yamada Y, Ando F, Niino N, Shimokata H	Association of polymorphisms of the androgen receptor and klotho genes with bone mineral density in Japanese women	J Mol Med	83	50-57	2005
Kozakai R, Doyo W, Tsuzuku S, Yabe K, Miyamura M, Ikegami Y, Ando F, Niino N, Shimokata H	Relationships of muscle strength and power with leisure-time physical activity and adolescent exercise in middle-aged and elderly Japanese women	Geriatrics and Gerontology International	5	182-188	2005
小笠原仁美, 新野直明, 安藤富士子, 下方浩史	中年期地域住民における転倒の発生状況	保健の科学	47	301-305	2005
小坂井留美、道用亘、安藤富士子、下方浩史、池上康男	中高年者における余暇身体活動および青年期の運動経験と骨密度との関連	総合保健体育科学	28	1-7	2005
道用亘、小坂井留美、安藤富士子、下方浩史、布目寛幸、池上康男	中高年者における歩行動作の特徴	総合保健体育科学	28	37-45	2005
Yamada Y, Ando F, Niino N, Shimokata H	Association of a -1997G→T polymorphism of the collagen I α 1 gene with bone mineral density in postmenopausal Japanese women	Hum Biol	77	27-36	2005
Yamada Y, Ando F, Shimokata H	Association of polymorphisms in CYP17, MTP, and VLDLR with bone mineral density in community-dwelling Japanese women and men	Genomics	86	76-85	2005
西田裕紀子, 新野直明, 小笠原仁美, 安藤富士子, 下方浩史	地域在住中高年者における転倒恐怖感の要因に関する縦断的検討	日本未病システム学会雑誌	11	101-103	2005
Okura T, Nakata Y, Lee DJ, Ohkawara K, Tanaka K	Effects of aerobic exercise and obesity phenotype on abdominal fat reduction in response to weight loss	Int J Obes	29	1259-1266	2005
中田由夫, 田中喜代次, 大藏倫博, 大河原一憲, 李東俊	ADRB3遺伝子多型が減量抵抗性に及ぼす影響: The SMART Study	肥満研究	11	301-305	2005
今井具子, 安藤富士子, 新野直明, 下方浩史	四訂および五訂日本食品標準成分表を用いて算出した栄養素等摂取量推定値の比較	日本栄養・食糧学会誌	59	21-29	2006
下方浩史	超高齢者医療の重要性. 公衆衛生・社会医学的視点から	J Integrated Med	16	102-105	2006
Shigematsu R, Okura T, Kumagai S, Hiyama T, Amagai H, Tanaka K	Cutoff and target values for intra-abdominal fat area for prevention of metabolic disorders in pre- and post-menopausal obese women before and after weight reduction	Ciculation Journal	70	110-114	2006
Ishizaki T,Yoshida H, Suzuki T, Watanabe S, Niino N, Ihara K, Kim H, Fujiwara Y, Shinkai S, Imanaka Y	Effects of cognitive function on functional decline among community-dwelling nondisabled older Japanese	Archives of Gerontology and Geriatrics	42	47-58	2006

Yamada Y, Ando F, Shimokata H	Association of polymorphisms in forkhead box C2 and perilipin genes with bone mineral density in community-dwelling Japanese individuals.	Int J Mol Med	18(1)	119-127	2006
下方浩史、安藤富士子、今井具子、中村美詠子	栄養摂取と骨密度減少との関連への遺伝子の影響に関する研究.	日本未病システム学会雑誌	12(1)	180-184	2006
安藤富士子、小坂井留美、道用亘、下方浩史	閉経女性の体力と骨密度の関連にMMP-12(A-82G)が及ぼす影響.	日本未病システム学会雑誌	12(1)	188-191	2006
Imai T, Nakamura M, Ando F, Shimokata H	Dietary supplement use by community-living population in Japan: Data from the National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA)	J. Epidemiol	16	249-260	2006
Kozakai R, Doyo W, Ando F, Shimokata H	Age-related changes of postural stability and physical function in middle-aged and elderly Japanese.	Japanese Journal of Physical Fitness and Sports Medicine	55(S)	S227-230	2006
下方浩史	高齢者の生活習慣はどこまで是正すべきか	日本老年医学会雑誌	43(4)	462-464	2006
Itho Z, Harada A, Matsui Y, Takemura M, Wakao N, Suzuki T, Nihashi S, Kawatsu S, Shimokata H, Ishiguro N	Can you diagnose for vertebral fracture correctly in plain X-ray?	Osteoporos Int	17(1)	1584-1591	2006
新野直明、福川康之	転倒予防	総合リハ	34	1035-1039	2006
Okura T, Rankinen T, Gagnon J, Lussier-Cacan S, Davignon J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C.	Effect of regular exercise on homocysteine concentrations: the HERITAGE Family Study.	European Journal of Applied Physiology	98	394-401	2006
Shigematsu R, Okura T.	A novel exercise for improving lower-extremity functional fitness in the elderly.	Aging Clinical and Experimental Research	18	242-248	2006
田中喜代次, 林容市, 中田由夫, 大藏倫博	メタボリックシンドローム診断における運動療法の基本コンセプト	日本臨床	64	574-579	2006
魏丞完, 大藏倫博, 中田由夫, 大河原一憲, 沼尾成晴, 片山靖富, 田中喜代次	肥満度と介入方法の違いが内臓脂肪型肥満者の減量効果に及ぼす影響	肥満研究	12	47-53	2006
鈴木恵美, 大藏倫博, 中田由夫, 大河原一憲, 片山靖富, 沼尾成晴, 田中喜代次	食行動意識が肥満男性の活力年齢および減量効果に及ぼす影響	肥満研究	12	112-118	2006
片山靖富, 中田由夫, 大河原一憲, 沼尾成晴, 松尾知明, 大藏倫博, 田中喜代次	食事制限と運動の実践による血清脂質の変化が血液流動性に及ぼす影響—減量前後の変化に着目して	肥満研究	12	225-233	2006

Kuzuya M, Ando F, Iguchi A, Shimokata H	Preproghrelin Leu72Met variant contributes to overweight in middle-aged men of a Japanese large cohort.	Intern J Obes	30	1609-1614	2006
竹村真里枝、松井康素、原田敦　安藤富士子、下方浩史	地域在住中高者年の骨代謝マーカーによる骨量減少/骨粗鬆症予測	Osteoporosis Japan	15(1)	28-32	2007
下方浩史	老化および老年病の疫学的研究	Geriatric Medicine	45(1)	13-17	2007
安藤富士子、北村伊都子、甲田道子、大藏倫博 、下方浩史	一般地域住民における腹部肥満感受性因子の網羅的検討	日本未病システム学会誌	13(1)	144-147	2007
Kuzuya M, Ando F, Iguchi A, Shimokata H	Age-specific change of prevalence of metabolic syndrome: Longitudinal observation of large Japanese cohort	Atherosclerosis	191	305-312	2007
Kitamura I, Ando F, Koda M, Okura T, Shimokata H	Effects of the interaction between lean tissue mass and estrogen receptor α gene polymorphism on bone mineral density in middle-aged and elderly Japanese	Bone	40	1623-1629	2007
下方浩史	食生活と長寿	日本老年医学会雑誌	44(2)	209-211	2007
Yoshiji Yamada, Fujiko Ando, Hiroshi Shimokata	Association of candidate gene polymorphisms with bone mineral density in community-dwelling Japanese women and men	Int J Mol Med	19	791-801	2007
亀井智子、梶井文子、山田艶子、川上千春、久代和加子、杉本知子、大藏倫博、小坂井留美、新野直明	都市部に居住する高齢者のための転倒骨折予防アウトリーチプログラムの実践ー市民主導型介護予防をめざしたプログラム開発と評価ー	聖路加看護大学紀要	33	74-84	2007
Numaoa S, Hayashi Y, Katayama Y, Matsuo T, Tomita T, Ohkawara K, Nakata Y, Okura T, Tanaka K	Plasma fat concentration increases in visceral fat obese men during high-intensity endurance exercise	Obesity Research & Clinical Practice	1	273-279	2007
Okura T, Nakata Y, Ohkawara K, Numao S, Katayama Y, Matsuo T, Tanaka K	Effect of aerobic exercise on metabolic syndrome improvement in response to weight reduction	Obesity	15	2478-2484	2007
松尾知明，室武由香子，齋藤義浩，大藏倫博，中田由夫，田中喜代次	減量介入前の体格，食事摂取量，身体活動量が体重減少量に及ぼす影響	肥満研究	13	154-163	2007
Okura T, Nakata Y, Ohkawara K, Numao S, Katayama Y, Ono Y, Matsuo T. Tanaka K	Effects of weight reduction on concentration of plasma total homocysteine in obese Japanese men	Obesity Research & Clinical Practice	1	213-221	2007
Matsuo T, Okura T, Nakata Y, Yabushita N, Numao S, Sasai H, Tanaka K	The influence of physical activity-induced energy expenditure on the variance in body weight change among individuals during a diet intervention	Obesity Research & Clinical Practice	1	109-117	2007

柳 久子、奥野純子、戸村成男、大藏倫博、田中喜代次	軽度要介護者の血中ビタミンDレベルの分布状況とビタミンD・カルシウム製剤補充による介護予防効果－生活機能・身体機能と血中ビタミンDレベルとの関連より－	Osteoporosis Japan	15	677-681	2007
Sugiura M, Nakamura M, Ogawa K, Ikoma Y, Ando F, Yano M	Bone mineral density in post-menopausal female subjects is associated with serum antioxidant cartenoids	Osteoporosis International	19(2)	211-219	2008
Yamada Y, Ando F, Shimokata H	Association of genetic variants of *MAOA* and *SH2B1* with bone mineral density in community-dwelling Japanese women	Mol Med Rep	1	269-274	2008
松井康素、下方浩史	ビタミンAと骨	THE BONE			印刷中

書　籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
下方浩史、安藤富士子	老いるということ／個人差	井藤英喜	看護のための最新医学講座(第2版)第17巻	中山書店	東京	2005	56-61
下方浩史	高齢者の栄養と食生活	ウエルネス公衆栄養学　第6版	老年医学 update 2002	医歯薬出版	東京	2005	199-210
石田裕美、今枝奈保美、高橋東生、伊達ちぐさ、徳留裕子、中村美詠子、福井充、横山徹爾、吉池信男、由田克士	(共同執筆)	伊達ちぐさ，徳留裕子，吉池信男	食事調査マニュアル	南山堂	東京	2005	(共同執筆)
安藤富士子	高齢者の看護・介護	飯島節、鳥羽研二	老年学テキスト	南江堂	東京	2006	225-234
安藤富士子、中村美詠子	骨と栄養	津志田藤二郎、高城孝助、小久保貞之、横山理雄	アクティブシニア社会の食品開発指針	Science Forum	東京	2006	128-137
小坂井留美、下方浩史	スポーツと長寿	長寿科学健康財団	Advances in Aging and Health Research 2006 健康長寿と運動	長寿科学健康財団	愛知	2006	7-13
新野直明	老化と老年病	飯島節、鳥羽研二	老年学テキスト	南江堂	東京	2006	10—18
新野直明	老化の生物学説	柴田博、長田久雄、杉澤秀博	老年学要論ー老いを理解するー	建帛社	東京	2007	33-37
大藏倫博	全身持久性体力の測定と評価　C間接法　自転車運動	田中喜代次、木塚朝博、大藏倫博	健康づくりのための体力測定評価法	金芳堂	京都	2007	15-21
大藏倫博	メタボリックシンドロームの概念と診断基準	田中喜代次、木塚朝博、大藏倫博	健康づくりのための体力測定評価法	金芳堂	京都	2007	150-153
Yamada Y	Genomics of osteoporosis and related phenotypes	Dhavendra Kumar	Genomics and Clinical Medicine	Oxford University Press	New York		in press
Yamada Y, Ichihara S, Takemura M	Human functional genomics and proteomics	Dhavendra Kumar	Genomics and Clinical Medicine	Oxford University Press	New York		in press
中村美詠子	疫学指標・疫学の方法	佐々木敏、伊達ちぐさ	公衆栄養学	南江堂	東京		in press

Ⅲ. 研究成果の刊行物・別刷

J Mol Med (2005) 83:50–57
DOI 10.1007/s00109-004-0578-4

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

Received: 12 March 2004 / Accepted: 18 June 2004 / Published online: 4 November 2004

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,

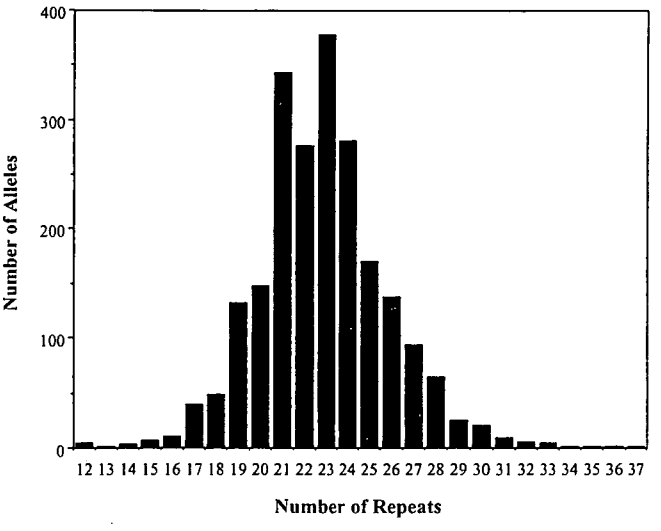


Fig. 1 Distribution of the number of CAG repeats in *AR* in 1,101 women (2,202 alleles)

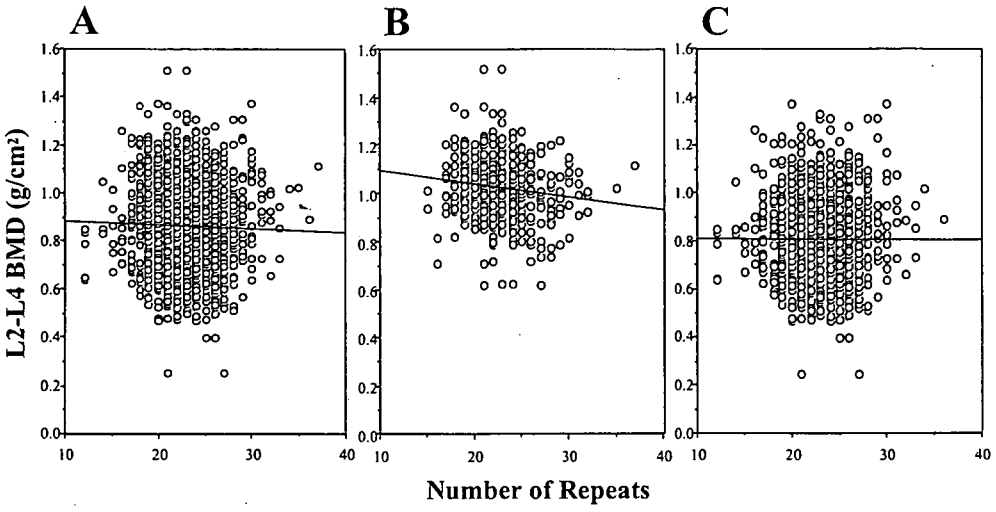


Fig. 2 Relationship between the number of CAG repeats in *AR* and L2-L4 BMD. **A** All women (n=1,101, 2,202 alleles); r=–0.01967, P=0.3584. **B** Premenopausal women (n=275, 550 alleles); r=–0.14455, P=0.0007. **C** Postmenopausal women (n=809, 1,618 alleles); r=0.00751, P=0.7644

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/cm^2)					
Total body	0.972±0.006	0.965±0.004	0.961±0.005	0.967±0.003	0.963±0.003
L2–L4	0.884±0.008	0.861±0.005*	0.860±0.007	0.868±0.005	0.860±0.004**
Femoral neck	0.686±0.006	0.677±0.004	0.675±0.005	0.680±0.003	0.676±0.003
Trochanter	0.576±0.005	0.570±0.004	0.568±0.005	0.572±0.003	0.569±0.003
Ward's triangle	0.514±0.008	0.506±0.005	0.505±0.006	0.508±0.004	0.506±0.004

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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.

Acknowledgements This work was supported in part by Research Grants for Health and Labor Sciences Research Grants for Comprehensive Research on Aging and Health (H15-Chojyu-014) from the Ministry of Health, Labor, and Welfare of Japan.

References

1. Kanis JA, Melton LJ III, Christiansen C, Johnston CC, Khaltaev N (1994) The diagnosis of osteoporosis. J Bone Miner Res 9:1137–1141
2. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S (1987) Genetic determinations of bone mass in adults: a twin study. J Clin Invest 80:706–710
3. Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J, Siest G (1995) Segregation analysis and variance components analysis of bone mineral density in healthy families. J Bone Miner Res 10:2017–2022
4. Howard GM, Nguyen TV, Harris M, Kelly PJ, Eisman JA (1998) 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
5. Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB, Recker RB (1997) Linkage of a gene causing high bone mass to human chromosome 11 (11q12–13). Am J Hum Genet 60:1326–1332
6. Devoto M, Shimoya K, Caminis J, Ott J, Tenenhouse A, Whyte MP, Sereda L, Hall S, Considine E, Williams CJ, Tromp G, Kuivaniemi H, Ala-Kokko L, Prockop DJ, Spotila LD (1998) First-stage autosomal genome screen in extended pedigrees suggests genes predisposing to low bone mineral density on chromosomes 1p, 2p and 4p. Eur J Hum Genet 6:151–157
7. Econs MJ, Koller DL, Hui SL, Fishburn T, Conneally PM, Johnston CC Jr, Peacock M, Foroud TM (2004) Confirmation of linkage to chromosome 1q for peak vertebral bone mineral density in premenopausal white women. Am J Hum Genet 74:223–228
8. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA (1994) Prediction of bone density from vitamin D receptor alleles. Nature 367:284–287
9. Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FEA, Grant SFA, Hofman A, van Leeuwen JPTM, Pols HAP, Ralston SH (1998) 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

10. Yamada Y, Ando F, Niino N, Shimokata H (2001) Transforming growth factor-β1 gene polymorphism and bone mineral density. JAMA 285:167–168
11. Sowers M, Willing M, Burns T, Deschenes S, Hollis B, Crutchfield M, Jannausch M (1999) Genetic markers, bone mineral density, and serum osteocalcin levels. J Bone Miner Res 14:1411–1419
12. Kawano K, Ogata N, Chisano M, Molloy H, Kleyn P, Spector TD, Uchida M, Hosoi T, Suzuki T, Orimo H, Inoue S, Nabeshima Y, Nakamura K, Kuro-o M, Kawaguchi H (2002) Klotho gene polymorphisms associated with bone density of aged postmenopausal women. J Bone Miner Res 17:1744–1751
13. Vanderschueren D, Bouillon R (1995) Androgens and bone. Calcif Tissue Int 56:341–346
14. Pederson L, Kremer M, Judd J, Pascoe D, Spelsberg TC, Riggs BL, Oursler MJ (1999) Androgens regulate bone resorption activity of isolated osteoclasts in vitro. Proc Natl Acad Sci USA 96:505–510
15. Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E (2000) Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. J Natl Cancer Inst 92:2009–2017
16. Hsing AW, Gao Y-T, Wu G, Wang X, Deng J, Chen Y-L, Sesterhenn IA, Mostofi FK, Benichou J, Chang C (2000) Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case-control study in China. Cancer Res 60:5111–5116
17. Chamberlain NL, Driver ED, Miesfeld RL (1994) The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 22:3181–3186
18. Kazemi-Esfarjani P, Trifiro MA, Pinsky L (1995) Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenetic relevance for the (CAG) n-expanded neuronopathies. Hum Mol Genet 4:523–527
19. Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL (1997) Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab 82:3777–3782
20. Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, Rouleau G, Pinsky L (1993) Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. Nat Genet 5:184–188
21. Zitzmann M, Brune M, Kornmann B, Gromoll J, Junker R, Nieschlag E (2001) The CAG repeat polymorphism in the androgen receptor gene affects bone density and bone metabolism in healthy males. Clin Endocrinol (Oxf) 55:649–657
22. Langdahl BL, Stenkjaer L, Carstens M, Tofteng CL, Eriksen EF (2003) A CAG repeat polymorphism in the androgen receptor gene is associated with reduced bone mass and increased risk of osteoporotic fractures. Calcif Tissue Int 73:237–243
23. Chen H-Y, Chen W-C, Wu M-C, Tsai F-J, Tsai C-H (2003) Androgen receptor (AR) gene microsatellite polymorphism in postmenopausal women: correlation to bone mineral density and susceptibility to osteoporosis. Eur J Obstet Gynaecol Reprod Biol 107:52–56
24. Tofteng CL, Kindmark A, Brändström H, Abrahamsen B, Petersen S, Stiger F, Stilgren LS, Jensen JEB, Vestergaard P, Langdahl BL, Mosekilde L (2004) Polymorphisms in the CYP19 and AR genes—relation to bone mass and longitudinal bone changes in postmenopausal women with or without hormone replacement therapy: the Danish Osteoporosis Prevention Study. Calcif Tissue Int 74:25–34
25. Salmén T, Heikkinen A-M, Mahonen A, Kröger H, Komulainen M, Pallonen H, Saarikoski S, Honkanen R, Mäenp PH (2003) Relation of androgen receptor gene polymorphism to bone mineral density and fracture risk in early postmenopausal women during a 5-year randomized hormone replacement therapy trial. J Bone Miner Res 18:319–324
26. Remes T, Väisänen SB, Mahonen A, Huuskonen J, Kröger H, Jurvelin JS, Penttilä IM, Rauramaa R (2003) Aerobic exercise and bone mineral density in middle-aged Finnish men: a controlled randomized trial with reference to androgen receptor, aromatase, and estrogen receptor α gene polymorphisms. Bone 32:412–420
27. Irvine RA, Yu MC, Ross RK, Coetzee GA (1995) The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. Cancer Res 55:1937–1940
28. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima Y (1997) Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390:45–51
29. Kawaguchi H, Manabe N, Miyaura C, Chikuda H, Nakamura K, Kuro-o M (1999) Independent impairment of osteoblast and osteoclast differentiation in klotho mouse exhibiting low-turnover osteopenia. J Clin Invest 104:229–237
30. Matsumura Y, Aizawa H, Shiraki-Iida T, Nagai R, Kuro-o M, Nabeshima Y (1998) Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein. Biochem Biophys Res Commun 242:626–630
31. Ogata N, Matsumura Y, Shiraki M, Kawano K, Koshizuka Y, Hosoi T, Nakamura K, Kuto-o M, Kawaguchi H (2002) Association of klotho gene polymorphism with bone density and spondylosis of the lumbar spine in postmenopausal women. Bone 31:37–42
32. Shimokata H, Ando F, Niino N (2000) A new comprehensive study on aging—the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). J Epidemiol 10:S1–S9
33. Yamada Y, Ando F, Niino N, Shimokata H (2003) 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
34. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, Sone T, Tanaka M, Yokota M (2002) Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med 347:1916–1923
35. Irvine RA, Ma H, Yu MC, Ross RK, Stallcup MR, Coetzee GA (2000) Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. Hum Mol Genet 9:267–274
36. Hsiao P-W, Lin D-L, Nakao R, Chang C (1999) The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutamine region-associated coactivator. J Biol Chem 274:20229–20234
37. Westberg L, Baghaei F, Rosmond R, Hellstrand M, Landén M, Jansson M, Holm G, Björntorp P, Eriksson E (2001) Polymorphisms of the androgen receptor gene and the estrogen receptor β gene are associated with androgen levels in women. J Clin Endocrinol Metab 86:2562–2568
38. Buller RE, Sood AK, Lallas T, Buekers T, Skilling JS (1999) Association between nonrandom X-chromosome inactivation and BRCA1 mutation in germline DNA of patients with ovarian cancer. J Natl Cancer Inst 91:339–346
39. Vottero A, Stratakis CA, Ghizzoni L, Longui CA, Karl M, Chrousos GP (1999) Androgen receptor-mediated hypersensitivity to androgens in women with nonhyperandrogenic hirsutism: skewing of X-chromosome inactivation. J Clin Endocrinol Metab 84:1091–1095
40. Kristiansen M, Langerød A, Knudsen GP, Weber BL, Børresen-Dale A-L, Ørstavik KH (2002) High frequency of skewed X inactivation in young breast cancer patients. J Med Genet 39:30–33

Geriatrics and Gerontology International 2005; 5: 182–188

ORIGINAL ARTICLE

Relationships of muscle strength and power with leisure-time physical activity and adolescent exercise in middle-aged and elderly Japanese women

Rumi Kozakai,[1] Wataru Doyo,[1] Shigeki Tsuzuku,[2] Kyonosuke Yabe,[3] Miharu Miyamura,[4] Yasuo Ikegami,[5] Naoakira Niino,[6] Fujiko Ando[1] and Hiroshi Shimokata[1]

[1]Department of Epidemiology, National Institute for Longevity Sciences: NILS, [2]Body Design Medical Institute Japan, [3]Graduate School of Health Sport Sciences, Osaka University of Health and Sport Sciences, [4]Department of Human Wellness, Tokai-Gakuen University, [5]Research Center of Health, Physical Fitness & Sports, Nagoya University, [6]Graduate School of Gerontology, Obirin University, Japan

Aim: The purpose of the present study is to assess the relationships of muscle strength and power with recent leisure-time physical activity and exercise during adolescence in middle-aged and elderly Japanese women.

Methods: The subjects consisted of 1128 community-dwelling women aged 40–79 years. They were interviewed about their physical activity habits during leisure time in the past 12 months and exercise they engaged in during adolescence. Muscle function was measured as grip strength, knee extension strength and leg extension power. Subjects were grouped into three intensity levels for leisure-time physical activity and as to whether or not they engaged in adolescent exercise. The relationships of muscle strength and power with leisure-time physical activity and adolescent exercise were assessed using analysis of covariance controlled for age, smoking status, annual income and education level.

Results: The proportion of subjects that participated in leisure-time physical activity was 67.1% (light, 33.7%; moderate or heavy, 33.4%). The subjects that engaged in adolescent exercise represented 41.9% of the total. There was a significant relationship between leisure-time physical activity and adolescent exercise. In the analysis of covariance controlled for age, smoking status, annual income and education level, leisure-time physical activity and adolescent exercise had significant main effects on all muscle strength and power measurements. However, there was no interaction effect between leisure-time physical activity and adolescent exercise.

Conclusion: The results suggest that current leisure-time physical activity and adolescent exercise benefit muscle function in middle-aged and elderly women.

Keywords: adolescent exercise, leisure-time physical activity, middle-aged and elderly, muscle power, muscle strength.

Introduction

Regular physical activity and exercise are closely associated with muscle function. Previous cross-sectional studies suggest that regular physical activity, such as leisure-time physical activity or playing sports, is positively

Accepted for publication 14 January 2005.

Correspondence: Dr Rumi Kozakai, Department of Epidemiology, National Institute for Longevity Sciences, 36-3 Gengo, Morioka-cho, Obu city, Aichi 474-8522, Japan. Email: kozakai@nils.go.jp

associated with muscle strength and power,[1,2] and some longitudinal studies showed that elderly women who were very physically active maintained their knee extensor strength at a higher level.[3,4] Intervention studies also documented the effects of strength training on the improvement of muscle strength.[5–11] The stimuli of physical activity or exercise on skeletal muscle may help maintain or improve muscle function.

On the other hand, since muscle function develops rapidly during childhood and adolescence, reaching a peak during adulthood, the beneficial effects of exercise on muscle development seem to be greater during this period. Moreover, Malina noted that tracking of physical activity in youth was associated with physical performance in later life.[12] Therefore, it is important to pay attention not only to current physical activity but also to adolescent physical activity to prevent a decline of muscle strength and power in the elderly. However, little is known about the contribution of both current and adolescent physical activity on muscle function in middle-aged and elderly people.

The purpose of the present study was to assess the relationships of muscle strength and power with current leisure-time physical activity (LTPA) and past adolescent exercise (AEX) in middle-aged and elderly Japanese women. Although the age-associated changes in muscle strength and power were similar by gender, women are generally weaker than men across the adult life span.[13–18] Since women have a longer period of dependency than men, in spite of women's longer life expectancy,[19,20] poor muscle strength and power may be a more serious physical problem for elderly women, resulting in disability or difficulty in performing basic daily tasks. For these reasons, we focused on women in this study.

Methods

Subjects

The data for the present study were derived from baseline data collected as part of the initial survey of the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA). In this project, the normal aging process has been assessed using detailed questionnaires and examinations including clinical evaluations, blood chemistries, anthropometrical measurements, physical fitness tests, nutritional analysis, and psychological tests. Details of the study are reported elsewhere.[21] The initial survey of NILS-LSA involved 2267 men and women aged 40–79 years. They were gender- and decade age-stratified random samples living in Obu city and Higashiura-cho Aichi Prefecture, Japan. Written informed consent was obtained from all the participants. Out of these 2267 participants, 1128 women were used as subjects in this study.

Muscle function

Grip strength (GS): a handgrip dynamometer (Takei Co., Japan) was used to assess grip strength in kilograms. The subjects stood holding a handgrip dynamometer with their hands by their sides while squeezing with maximum force alternating the left and right hands. The average of two readings from each hand was used as the measurement result.

Knee extension strength (KES): the subjects were seated in an adjustable straight-back chair (Takei Co., Japan) with the pelvis, knee and ankle fixed at 90°. A strain gauge was attached to the distal leg by a strap just above the ankle. The subjects tried to extend their legs using maximum isometric force with the knee flexed at 90° while the amplified output from the strain gauge was recorded. The average of the maximum force that each leg attained after three attempts was used as the measurement result in kilograms.

Leg extension power (LEP): leg extension power was measured with the help of a sledge ergometer in a sitting position (Takei Co., Japan). The acceleration of the sledge was 0.73 m/s and the sledge stroke was 0.79 m. The subjects were fastened by a seat belt to the chair. In the starting position, the feet were placed on a footplate attached perpendicularly to a rail, and the knee angle was adjusted to 90°. The subjects were asked to extend their legs as quickly and powerfully as possible, so that the footplate started sliding horizontally on the rail. The highest result of eight attempts was taken as the measurement result in watts.

A medical doctor asked the subjects about their health condition before the muscle function tests. Subjects with serious pains, physical injuries or illness of the orthopedic or cardiovascular systems were excluded. All muscle function tests were performed on the same day.

Physical activity

Leisure-time physical activity (LTPA): trained interviewers using a questionnaire developed by the Japanese Lifestyle Monitoring Study Group asked subjects about the frequency and duration of their physical activity habits during leisure time for the past 12 months.[22] This questionnaire was modified from the Minnesota Leisure-time Physical Activity Questionnaire, one of the most widely used physical activity questionnaires.[23] Activities that were performed at least once a week and for 10 min were defined as LTPA, and classified into three levels: light (approximate physical intensity; 2.5 METs [metabolic equivalents]); moderate (4.5 METs); heavy (> 6.5 METs) (Table 1). Sedentary activities in LTPA, for example, bonsai, were excluded.

Adolescent exercise (AEX): subjects were also interviewed in the same questionnaire about the frequency and duration of their participation in physical exercise

Table 1 The classification of leisure-time physical activity

Level	Approximate intensity (METs)	Description	Examples
Light	2.5	Activity such as walking	Walking, gymnastic exercise, gardening, etc.
Moderate	4.5	Sweating activity that one can do comfortably	Brisk walking, dancing, swimming for pleasure, etc.
Heavy	≥ 6.5	Vigorous exercise with heavy breathing	Several sports activities (swimming, tennis, badminton etc.)

METs, metabolic equivalents.

Table 2 Characteristics of the subjects

	n	Mean ± SD
Age (years)	1128	59.3 ± 10.9
Height (cm)	1128	151.3 ± 6.1
Weight (kg)	1128	52.4 ± 8.2
Body mass index (kg/m^2)	1128	22.9 ± 3.3
Body fat (%)	1120	31.5 ± 5.2
Grip strength (kg)	1106	23.8 ± 5.1
Knee extension strength (kg)	780	25.2 ± 6.8
Leg extension power (w)	1048	301.4 ± 107.1
Smoking status (%; currently)	1126	7.3
Annual income (%; ≥ ¥6 500 000)	1055	54.7
Education level (%; > high school)	1123	23.1

or sports, such as club activities, in addition to compulsory physical exercise at school from 12 to 20 years of age. Activities that were engaged in at least once a week over 1 year were defined as AEX.

Other parameters

Height and weight were measured using a digital scale. Body mass index was calculated by weight divided by height squared (BMI; kg/m^2). Body fat mass was assessed by dual X-ray absorptiometry (DXA; QDR-4500A, Hologic, USA). Lifestyle factors including smoking status, annual income and education level were also determined by questionnaire.

Statistical analysis

The participation rate in physical activity was calculated as the percentage of subjects who reported such activities in a multiple response format. The subjects were divided into three groups according to the intensity of LTPA: no LTPA, LTPA (N); participation in only light activities, LTPA (L); participation in moderate or heavy activities, LTPA (H). Because there were only a few subjects who participated in heavy LTPA, we combined the subjects who engaged in moderate LTPA and heavy LTPA together as LTPA (H). They were also divided into those who engaged in adolescent exercise, AEX (+), and those that did not, AEX (–). The Cochran–Mantel–Haenszel method was used to examine the relationship between LTPA and AEX. The relationship of muscle function with LTPA and AEX was analyzed using the analysis of covariance controlled for age, smoking status, annual income and education level. Statistical testing was performed using the Statistical Analysis System release.8.2 (SAS Institute Inc. NC, USA).[24] Significant probability levels were considered to be less than 0.05.

Table 3 The participation rates in leisure-time physical activity (LTPA) and adolescent exercise (AEX)

	Levels	*n* (%)
LTPA	None	371 (32.9)
	Light	380 (33.7)
	Moderate or heavy	377 (33.4)
AEX		473 (41.9)†

†Total number of the subjects who participated in AEX.

Results

The characteristics of the subjects are summarized in Table 2. The mean and standard deviation (SD) of age was 59.3 ± 10.9 years. The averages of the anthropometric parameters, height, weight, BMI and percent body fat, were 151.3 ± 6.1 cm, 52.4 ± 8.2 kg, 22.9 ± 3.3 kg/m^2 and 31.5 ± 5.2%, respectively. The averages for muscle strength and power, GS, KES and LEP, were 23.8 ± 5.1 kg, 25.2 ± 6.8 kg and 301.4 ± 107.1 w, respectively. The proportions of people who currently smoked, had an annual income of over 6 500 000 yen, and had an education beyond high school were 7.3, 54.7 and 23.1%, respectively.

Table 3 shows the participation rates in LTPA and AEX. Subjects who did not participated in leisure-time