通常歩行時の膝・足関節角度範囲、速歩行時の足関節角度範囲においても、有意な性差が認められた。結果を表３、４に示す。また通常歩行時における股関節角度範囲と年齢との間の相関係数は男性－0.10、女性－0.13、速歩行時における股関節角度範囲と年齢との間の相関係数は男性－0.12、女性－0.15であり、通常歩行時および速歩行時ともに、男女とも有意な負の相関が認められた（$p<0.05$, $p<0.01$, $p<0.001$）。通常歩行時および速歩行時の足関節角度範囲においても、男女とも年齢との間に有意な負の相関が認められた。結果を表５、６に示す。

下肢関節ピークトルク

通常歩行時の足関節底屈ピークトルクは男性 86.6 ± 18.0Nm、女性 66.8 ± 13.1Nm、速歩行時の足関節底屈ピークトルクは男性 87.3 ± 20.3Nm、女性 64.4 ± 13.4 Nm であり、通常歩行時および速歩行時ともに有意な性差が認められた（$p<0.001$）。通常歩行時の股関節伸展／屈曲ピークトルク、膝関節屈曲／伸展（前半）ピークトルク、膝関節屈曲（後半）ピークトルク、速歩行時の股関節伸展／屈曲ピークトルク、膝関節屈曲／伸展（前半）ピークトルク、膝関節屈曲／伸展（後半）ピークトルクにおいても、有意な性差が認められた。

表３　男女別にみた通常歩行時の歩行変量（Student t 検定）

			男性		女性		p<	
歩行速度		(m/秒)	1.43 ± 0.18		1.42 ± 0.18		n.s.	
歩幅		(m)	0.72 ± 0.07		0.67 ± 0.06		0.001	
歩調		(Hz)	1.99 ± 0.16		2.14 ± 0.16		0.001	*
時間成分		(秒)						
支持時間			0.654 ± 0.050		0.621 ± 0.049		0.001	
遊脚時間			0.377 ± 0.034		0.357 ± 0.045		0.001	
1サイクル時間			1.034 ± 0.068		0.979 ± 0.077		0.001	
単脚支持時間			0.365 ± 0.032		0.345 ± 0.031		0.001	
両脚支持間(支持期後半)			0.144 ± 0.020		0.138 ± 0.020		0.001	
下肢関節角度範囲		(度)						
股関節			45.6 ± 4.6		44.5 ± 4.7		0.001	
膝関節			65.2 ± 4.8		64.3 ± 5.2		0.01	
足関節			32.4 ± 7.4		34.7 ± 7.7		0.001	*
下肢関節ピークトルク		(Nm)						
股関節	伸展		88.1 ± 23.1		74.1 ± 22.5		0.001	
	屈曲		－ 28.5 ± 8.6		－ 24.9 ± 7.5		0.001	
膝関節	屈曲(前半)		29.7 ± 9.8		23.1 ± 8.3		0.001	
	伸展(前半)		－ 25.6 ± 22.3		－ 19.6 ± 17.3		0.001	
	屈曲(後半)		26.4 ± 14.1		22.0 ± 11.8		0.001	
	伸展(後半)		－ 4.6 ± 5.2		－ 4.1 ± 5.8		n.s.	
足関節	低屈		86.6 ± 18.0		66.8 ± 13.1		0.001	

1)平均値±標準偏差
2)下肢関節ピークトルクの股関節屈曲、膝関節伸展は負の値ほど大きいことを示す
3)n.s.は有意でないことを示す
4)＊は男性より女性が有意に大きいことを示す

結果を表3、4に示す。また通常歩行時における足関節底屈ピークトルクと年齢との間の相関係数は、男性−0.36、女性−0.32、速歩行時における足関節底屈ピークトルクと年齢との間の相関係数は、男性−0.36、女性−0.32であり、通常歩行時および速歩行時ともに、男女とも有意な負の相関が認められた（$p<0.001$）。通常歩行時の股関節伸展／屈曲ピークトルク、膝関節屈曲／伸展（前半）ピークトルク、膝関節屈曲（後半）ピークトルク、速歩行時の股関節伸展／屈曲ピークトルク、膝関節伸展（前半）ピークトルク、膝関節屈曲／伸展（後半）ピークトルクにおいても、男女とも年齢との間に有意な正の相関が認められた。結果を表5、6に示す。

考　察

速度、歩幅、歩調

本研究において、男性より女性の方が速歩行時の速度、通常歩行時および速歩行時の歩幅は小さかった。小坂井ら[7]は、速度、歩幅ともに女性の方が早期に加

表4　男女別にみた速歩行時の歩行変量（Student t 検定）

			男性			女性			p<	
歩行速度		(m/秒)	1.86	±	0.25	1.75	±	0.24	0.001	
歩幅		(m)	0.80	±	0.07	0.71	±	0.07	0.001	
歩調		(Hz)	2.33	±	0.24	2.48	±	0.24	0.001	*
時間成分		(秒)								
支持時間			0.553	±	0.056	0.537	±	0.050	0.001	
遊脚時間			0.345	±	0.039	0.323	±	0.043	0.001	
1サイクル時間			0.901	±	0.082	0.862	±	0.082	0.001	
単脚支持時間			0.323	±	0.039	0.309	±	0.033	0.001	
両脚支持時間(支持期後半)			0.116	±	0.018	0.114	±	0.018	n.s.	
下肢関節角度範囲		(度)								
股関節			50.3	±	5.6	47.3	±	5.1	0.001	
膝関節			63.6	±	5.4	63.0	±	5.5	n.s.	
足関節			33.7	±	6.7	36.1	±	7.1	0.001	*
下肢関節ピークトルク		(Nm)								
股関節	伸展		109.7	±	25.0	93.8	±	25.6	0.001	
	屈曲		−39.3	±	13.4	−32.5	±	10.6	0.001	
膝関節	屈曲(前半)		36.3	±	12.7	28.3	±	9.7	0.001	
	伸展(前半)		−29.0	±	29.8	−24.7	±	19.6	0.001	
	屈曲(後半)		30.0	±	16.1	22.5	±	12.6	0.001	
	伸展(後半)		−6.4	±	7.9	−5.2	±	7.1	0.01	
足関節	低屈		87.3	±	20.3	64.4	±	13.4	0.001	

1)平均値±標準偏差

2)下肢関節ピークトルクの股関節屈曲、膝関節伸展は負の値ほど大きいことを示す

3)n.s.は有意でないことを示す

齢低下が出現することを報告している。本研究の女性における小さい速度、歩幅は加齢低下の影響があったのかもしれない。男性より女性の歩調が大きいことは先行研究の結果と一致した[11]。また、速度、歩幅と年齢との間に負の関連が認められたことは先行研究の結果[1) 2) 3) 4) 7) 8) 10) 15)]と一致しており、顕著な中高年者の歩行動作の特徴であると考えられる。さらに通常歩行時および速歩行時とも歩調と年齢との間に負の関連が認められた。Himann JE, et al.[4]は、19—102歳の438名の対象において加齢に伴う歩調の低下を示しており、本研究の結果を支持すると考えられる。歩調と年齢の関連は認められないという本研究の結果と異なる先行研究も報告されているが、対象が少数であり、体力レベルが優位であるため[2)]、本研究の結果と異なったと考えられる。

時間成分

本研究において、通常歩行時および速歩行時ともに支持時間、遊脚時間、1サイクル時間、単脚支持時間は男性より女性の方が低値を示した。通常歩行時の両

表5　男女別にみた通常歩行時の歩行変量と年齢との関係（ピアソンの相関係数）

年齢		男性	p<	女性	p<
歩行速度		− 0.27	0.001	− 0.44	0.001
歩幅		− 0.31	0.001	− 0.43	0.001
歩調		− 0.08	0.05	− 0.17	0.001
時間成分					
支持時間		0.12	0.001	0.25	0.001
遊脚時間		− 0.04	n.s.	0.03	n.s.
1サイクル時間		0.04	n.s.	0.18	0.001
単脚支持時間		− 0.01	n.s.	0.05	n.s.
両脚支持間(支持期後半)		0.15	0.001	0.26	0.001
下肢関節角度範囲					
股関節		− 0.10	0.05	− 0.13	0.01
膝関節		− 0.12	0.01	− 0.07	n.s.
足関節		− 0.12	0.01	− 0.26	0.001
下肢関節ピークトルク					
股関節	伸展	− 0.12	0.01	− 0.17	0.001
	屈曲	0.24	0.001	0.27	0.001
膝関節	屈曲(前半)	− 0.29	0.001	− 0.26	0.001
	伸展(前半)	0.08	0.01	0.08	0.01
	屈曲(後半)	− 0.23	0.001	− 0.27	0.001
	伸展(後半)	0.06	n.s.	0.10	0.01
足関節	低屈	− 0.36	0.001	− 0.32	0.001

n.s. は年齢と有意でないことを示す

脚支持時間は男性より女性の方が低値を示したが、速歩行時に性差が認められなかった。このことは1）絶対値のみ評価していること、2）女性の歩調が大きいことに起因すると考えられ、今後1サイクルあたりの相対値（%）も検討することが必要であろう。また、通常歩行時および速歩行時ともに、支持時間、両脚支持時間と年齢との間に正の関連が認められたことは先行研究の結果と一致したと考えられる[10) 15)]。Winter DA[15)]は高年者における両脚支持時間の増加は、安全で安定した歩行パターンを行なうための適合であることを示唆している。古市ら[10)]は、両脚支持期に後方の足から前方の足に体重を移動させるため、両脚支持時間の増加は体幹の不安定な期間を減少させると考察している。両脚支持時間と年齢の間に正の関連が認められたことは、本研究の高年者が歩行中の姿勢保持の不安定さを補償するため、両脚支持時間を増加させた可能性がある。今後、加齢変化に関する詳細な分析が必要であると考えられる。

表6　男女別にみた速歩行時の歩行変量と年齢との関係（ピアソンの相関係数）

年齢		男性	p<	女性	p<
歩行速度		− 0.41	0.001	− 0.56	0.001
歩幅		− 0.36	0.001	− 0.44	0.001
歩調		− 0.20	0.001	− 0.35	0.001
時間成分					
支持時間		0.29	0.001	0.40	0.001
遊脚時間		0.04	n.s.	0.09	0.05
1サイクル時間		0.22	0.001	0.31	0.001
単脚支持時間		0.13	0.001	0.17	0.001
両脚支持間（支持期後半）		0.30	0.001	0.38	0.001
下肢関節角度範囲					
股関節		− 0.12	0.001	− 0.15	0.001
膝関節		0.01	n.s.	0.00	n.s.
足関節		− 0.21	0.001	− 0.23	0.001
下肢関節ピークトルク					
股関節	伸展	− 0.20	0.001	− 0.23	0.001
	屈曲	0.30	0.001	0.30	0.001
膝関節	屈曲（前半）	− 0.27	0.001	− 0.33	0.001
	伸展（前半）	− 0.00	n.s.	0.16	0.001
	屈曲（後半）	− 0.28	0.001	− 0.25	0.001
	伸展（後半）	0.09	0.05	0.10	0.05
足関節	低屈	− 0.36	0.001	− 0.32	0.001

n.s. は年齢と有意でないことを示す

下肢関節角度範囲

本研究において、男性より女性の股関節角度範囲は小さいことを示したが、足関節角度範囲は女性が大きい値を示した。また、通常歩行時および速歩行時ともに、股・足関節角度範囲と年齢との間に負の関連が認められた。DeVita P, and Hortobagyi T[1] は若年群と高年群を同速度で歩行させた際、若年群と比較し、高年群の足関節角度範囲は小さいが、股関節角度範囲は大きくすることが可能であると示唆している。本研究の股関節角度範囲と年齢との間に負の関連が認められたことは、高年者に股関節機能の低下が認められることを示唆するものであり、先行研究の結果と異なると考えられる。この知見の相違は、先行研究における高年群の健康度、体力レベルが優位なことに起因する[1] と推察される。膝関節角度範囲は通常歩行時のみ有意な性差が認められたものの、顕著な年齢との関連は認められなかった。

下肢関節ピークトルク

本研究において、通常歩行時の膝関節伸展ピークトルク（後半）を除いたすべてのピークトルクは男性より女性の方が小さかった。また通常歩行時の膝関節伸展ピークトルク（後半）および速歩行時の膝関節伸展ピークトルク（前半）を除いたすべてのピークトルクは、男女とも年齢が高くなるほど、運動が小さくなることを示したことは先行研究の結果と一致した[11]。植松[14] や Judge OJ, et al.[6] は若年者と比較して歩行中の高齢者の足関節底屈トルクが低下することを示している。Shultz AB[13] は筋力に関する先行研究を検討し、25—30歳の若年群と60—85歳の高年群の足関節底屈筋力を比較したところ、若年群より高年群が低値を示し、男性より女性の方が低値を示したことを報告している。本研究の対象において、歩行中の足関節底屈ピークトルクと年齢の間に負の関連が認められたことは、高年者の足関節底屈筋力低下に関連する可能性が推察され、今後、対象の筋機能との関連を評価する必要があると考えられる。

まとめ

本研究において３次元映像解析法を用いて歩行動作記録した結果、中高年者の運動学的・運動力学的歩行パターンが示された。また中高年者の歩行パターンは性差および年齢に関連することが認められた。今後、歩行変量間の関連、対象の背景因子の影響、加齢変化を評価することにより、さらに中高年者の歩行動作の特徴や機序が明らかにできると考えられる。

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(2004年11月19日受付)

Association of a −1997G → T Polymorphism of the Collagen Iα1 Gene with Bone Mineral Density in Postmenopausal Japanese Women

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Abstract Genetic variants that affect collagen Iα1 metabolism may be important in the development of osteoporosis or osteoporotic fractures. A −1997G → T polymorphism in the promoter of the collagen Iα1 gene (COL1A1) was shown to be associated with bone mineral density (BMD) for the lumbar spine in postmenopausal Spanish women. The relation of this polymorphism to BMD in Japanese women or men has now been examined in a population-based study. The subjects (1,110 women, 1,126 men) were 40 to 79 years of age and were randomly recruited for a population-based prospective cohort study of aging and age-related diseases. BMD for the lumbar spine, right femoral neck, right trochanter, and right Ward's triangle was measured using dual-energy x-ray absorptiometry. Genotypes for the −1997G → T polymorphism of COL1A1 were determined with a fluorescence-based allele-specific DNA primer assay system. When all women were analyzed together, BMD for the lumbar spine and trochanter was significantly lower in subjects with the *COL1A1*G/*G* genotype than in those in the combined group of *COL1A1*G/*T* and *COL1A1*T/*T* genotypes. When postmenopausal women were analyzed separately, BMD for the femoral neck and trochanter was also significantly lower in those with the *COL1A1*G/*G* genotype than in those with the *COL1A1*G/*T* genotype or those in the combined group of *COL1A1*G/*T* and *COL1A1*T/*T* genotypes. BMD was not associated with −1997G → T genotype in premenopausal women or in men. Multivariate regression analysis revealed that −1997G → T genotype affected BMD at various sites with a variance of 0.46–0.62% for all women and 0.61–1.01% for postmenopausal women. The −1997G → T genotype was not related to the serum concentration of osteocalcin, the serum activity of bone-specific alkaline phosphatase, or the urinary excretion of deoxypyridinoline or cross-linked N-telopeptides of type I collagen in men or in premenopausal or postmenopausal women. These results suggest that COL1A1 is a susceptibility locus for reduced BMD in postmenopausal Japanese women.

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Human Biology, February 2005, v. 77, no. 1, pp. 27–36.

KEY WORDS: BONE MINERAL DENSITY, COLLAGEN Iα1, COLA1A, OSTEOPOROSIS, JAPAN.

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 (Kanis et al. 1994). Although several environmental factors, including diet, smoking, and physical exercise, influence BMD, a genetic contribution to this parameter has been recognized (Peacock et al. 2002). Genetic linkage analyses (Morrison et al. 1994; Johnson et al. 1997; Devoto et al. 1998; Koller et al. 1998, 2000; Niu et al. 1999) and candidate gene association studies (Morrison et al. 1994; Kobayashi et al. 1996; Uitterlinden et al. 1998; Yamada et al. 2001; Ishida et al. 2003) have implicated several loci and candidate genes in the regulation of bone mass and the prevalence of osteoporosis or osteoporotic fractures. However, the genes that contribute to genetic susceptibility to osteoporosis remain to be identified definitively.

Type I collagen is the most abundant protein of bone matrix. Mutations in the coding regions of the genes for the two type I collagen chains (COL1A1 and COL1A2) result in a severe autosomal dominant pediatric condition known as osteogenesis imperfecta (Sykes 1990). A G → T single nucleotide polymorphism (SNP) at the first base of a consensus binding site for the transcription factor Sp1 in the first intron of COL1A1 was associated not only with BMD in white women (Grant et al. 1996) but also with osteoporotic fractures in postmenopausal women (Langdahl et al. 1998; Uitterlinden et al. 1998). The *COL1A1*T* allele of this polymorphism affects collagen gene regulation in such a manner that it increases the production of the α1(I) collagen chain relative to that of the α2(I) chain and leads to reduced bone strength by a mechanism that is partly independent of bone mass (Mann et al. 2001). These observations thus implicate genetic variants that affect collagen Iα1 metabolism as important determinants of the development of osteoporosis and osteoporotic fractures. Other studies, however, have shown only a weak association of the Sp1 binding site polymorphism with BMD or osteoporotic fractures in premenopausal French women (Garnero et al. 1998) or a lack of association in postmenopausal women in Sweden (Liden et al. 1998), in American women (Hustmyer et al. 1999), or in postmenopausal Danish women (Heegaard et al. 2000).

A −1997G → T SNP in the promoter of COL1A1 was also associated with BMD for the lumbar spine in postmenopausal Spanish women, and this SNP and the G → T SNP of the Sp1 binding site of COL1A1 were shown to be in linkage disequilibrium (Garcia-Giralt et al. 2002). Given the ethnic divergence of gene polymorphisms, it is important to examine polymorphisms potentially related to BMD in each ethnic group. We have now examined whether the −1997G → T SNP of COL1A1 is associated with BMD in Japanese women or men in a population-based study.

Materials and 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 (Shimokata et al. 2000). The subjects of the NILS-LSA are stratified by both age and sex and are randomly selected from resident registrations in the city of Obu and the town of Higashiura in central Japan (Yamada et al. 2003a, 2003b). Individuals with disorders known to cause abnormalities of bone metabolism, including diabetes mellitus, renal diseases, rheumatoid arthritis, and thyroid, parathyroid, and other endocrinologic diseases, were excluded from the study. Women who had taken drugs such as estrogen, progesterone, glucocorticoids, and bisphosphonates were also excluded.

We examined the relation of BMD at various sites to the $-1997G \rightarrow T$ SNP of COL1A1 in 2,236 participants (1,110 women, 1,126 men). All analyses were performed separately for men and for 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 by a detailed questionnaire, and menopause was defined as complete cessation of menstruation. Furthermore, the relation of biochemical markers of bone turnover to $-1997G \rightarrow T$ genotype of COL1A1 was examined for men or premenopausal or postmenopausal women separately. The study protocol was approved by the Committee on Ethics of Human Research of National Chubu Hospital and the NILS, and written informed consent was obtained from each subject.

Measurement of BMD. BMD for the lumbar spine (L2–L4), right femoral neck, right trochanter, and right Ward's triangle was measured using dual-energy x-ray absorptiometry (DXA) (QDR 4500; Hologic, Bedford, Mass.). The coefficients of variation (CVs) of the DXA instrument were 0.9% (L2–L4), 1.3% (femoral neck), 1.0% (trochanter), and 2.5% (Ward's triangle); these values were determined by measurement of BMD three times at each site in 10 healthy subjects (mean age ± SE, 38.7 ± 2.4 years).

Determination of Genotypes. Genotypes were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) (Yamada et al. 2002). The polymorphic region of COL1A1 was amplified using the polymerase chain reaction with allele-specific sense primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-TGGGTCAGTTCCAAGAGX*C*C-3′) or Texas red (5′-TGGGTCAGTTCCAAGAGX*A*C-3′) and with an antisense primer labeled at the 5′ end with biotin (5′-TCTAAATGTCTGTTCCCTCCAA-3′). The reaction mixture (25 μL) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate, 3.5 mmol/L $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol was initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 60°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 measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 nm and 538 nm, respectively, for fluorescein isothiocyanate and of 584 nm and 612 nm, respectively, for Texas red.

Measurement of Biochemical Markers of Bone Turnover. Venous blood and urine samples were collected in the early morning after the subjects had fasted overnight. Blood samples were centrifuged at 1,600 × *g* for 15 min at 4°C, and the serum fraction was separated and stored at −80°C until analysis. The serum concentration of intact osteocalcin was measured with an immunoradiometric assay kit (Mitsubishi Chemical, Tokyo, Japan). The activity of bone-specific alkaline phosphatase in serum was measured with an enzyme immunoassay kit (Metra Biosystems, Mountain View, Calif.). Urine samples were collected in plain tubes and stored at −80°C. Urinary deoxypyridinoline was measured with an enzyme immunoassay kit (Metra Biosystems); the values were corrected for urinary creatinine and expressed as picomoles per micromole of creatinine. The urinary concentration of cross-linked N-telopeptides of type I collagen (NTx) was measured with an enzyme-linked immunosorbent assay kit (Mochida Pharmaceutical, Tokyo, Japan); the values were expressed as picomoles of bone collagen equivalents per micromole of creatinine. Urinary creatinine was enzymatically measured with a creatinine test kit (Wako Chemical, Osaka, Japan).

Statistical Analysis. Quantitative data were compared among the three groups using one-way analysis of variance and the Tukey-Kramer post hoc test and between two groups using the unpaired Student's *t* test. BMD values were analyzed with adjustment for age and body mass index (BMI) using the least-squares method in a general linear model. The effect of −1997G → T genotype on BMD at various sites was evaluated using multivariate regression analysis; R^2 and *P* values were calculated from the analysis including age, BMI, and COL1A1 genotype (0 = *COL1A1*G/*G*, 1 = *COL1A1*G/*T* = *COL1A1*T/*T*) . Allele frequencies were estimated using the gene-counting method, and the chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. A *P* value less than 0.05 was considered statistically significant.

Results

The distribution of −1997G → T genotypes was in Hardy-Weinberg equilibrium, and age and BMI did not differ among genotypes for all women (Table 1). BMD for the lumbar spine and trochanter was significantly lower in women with the **G/*G* genotype than in those in the combined group of **G/*T* and

Table 1. BMD and Other Characteristics of All Women (n = 1,110) According to the −1997G → T Genotype of COL1A1

Characteristic	**G/*G*	**G/*T*	**T/*T*	**G/* T + *T/*T*
Number (%)	407 (36.7)	526 (47.4)	177 (15.9)	703 (63.3)
Age (years)	60.0 ± 0.5	58.9 ± 0.5	58.4 ± 0.8	58.8 ± 0.4
BMI (kg/m^2)	22.9 ± 0.2	22.8 ± 0.1	23.0 ± 0.2	22.9 ± 0.1
BMD values (g/cm^2)				
L2–L4	0.855 ± 0.006	0.870 ± 0.006	0.878 ± 0.010	0.872 ± 0.005[a]
Femoral neck	0.672 ± 0.004	0.681 ± 0.004	0.680 ± 0.007	0.681 ± 0.003
Trochanter	0.564 ± 0.004	0.575 ± 0.004	0.574 ± 0.006	0.575 ± 0.003[b]
Ward's triangle	0.500 ± 0.006	0.512 ± 0.005	0.508 ± 0.009	0.511 ± 0.004

Data are means ± SE. BMD values are adjusted for age and BMI.
a. P = 0.039 vs. **G/*G*.
b. P = 0.033 versus **G/*G*.

**T/*T* genotypes; the difference in BMD between the **G/*G* genotype and the combined group of **G/*T* and **T/*T* genotypes (expressed as a percentage of the corresponding larger value) was 1.9% for both the lumbar spine and the trochanter.

We also analyzed BMD and other characteristics for premenopausal and postmenopausal women independently. The distributions of −1997G → T genotypes were in Hardy-Weinberg equilibrium, and age and BMI did not differ among genotypes for premenopausal or postmenopausal women (Table 2). For postmenopausal women there was no difference in years after menopause among genotypes. For premenopausal women BMD was not associated with −1997G → T genotype. In contrast, BMD for the femoral neck or trochanter was significantly lower in postmenopausal women with the **G/*G* genotype than in those with the **G/*T* genotype or those in the combined group of **G/*T* and **T/*T* genotypes; the differences in BMD between the **G/*G* genotype and the combined group of **G/*T* and **T/*T* genotypes were 2.5% for the femoral neck and 2.2% for the trochanter.

The distribution of −1997G → T genotypes was in Hardy-Weinberg equilibrium, but BMD did not differ among these genotypes in men (Table 3).

The effect of −1997G→T genotype on BMD was evaluated using multivariate regression analysis (Table 4). The analysis revealed that the −1997G → T genotype affected BMD at various sites with a variance of 0.46–0.62% for all women and of 0.61–1.01% for postmenopausal women.

The relation of biochemical markers of bone turnover to −1997G → T genotype of COL1A1 was also examined. No association of −1997G → T genotype with the serum concentration of intact osteocalcin, serum activity of bone-specific alkaline phosphatase, or urinary excretion of deoxypyridinoline or NTx was apparent for men or premenopausal or postmenopausal women (Table 5).

Table 2. BMD and Other Characteristics of Women (n = 1,093) According to Menopausal Status and the −1997G → T Genotype of COL1A1

	Premenopausal Women (n = 278)				*Postmenopausal Women* (n = 815)			
Characteristic	**G/*G*	**G/*T*	**T/*T*	**G/*T + *T/*T*	**G/*G*	**G/*T*	**T/*T*	**G/*T + *T/*T*
Number (%)	94 (33.8)	140 (50.4)	44 (15.8)	184 (66.2)	306 (37.5)	377 (46.3)	132 (16.2)	509 (62.5)
Age (years)	45.9 ± 0.4	46.3 ± 0.4	45.8 ± 0.6	46.2 ± 0.3	64.6 ± 0.5	63.8 ± 0.4	62.8 ± 0.7	63.5 ± 0.4
Years after menopause					15.4 ± 0.5	15.1 ± 0.5	13.7 ± 0.8	14.7 ± 0.4
BMI (kg/m^2)	22.8 ± 0.3	22.9 ± 0.3	22.4 ± 0.5	22.8 ± 0.2	23.0 ± 0.2	22.8 ± 0.2	23.2 ± 0.3	22.9 ± 0.1
BMD values (g/cm^2)								
L2–L4	1.018 ± 0.012	1.026 ± 0.010	1.044 ± 0.018	1.030 ± 0.009	0.798 ± 0.007	0.813 ± 0.007	0.821 ± 0.011	0.815 ± 0.006
Femoral neck	0.782 ± 0.009	0.767 ± 0.008	0.771 ± 0.014	0.768 ± 0.007	0.634 ± 0.005	0.650 ± 0.004[a]	0.647 ± 0.007	0.650 ± 0.004[b]
Trochanter	0.656 ± 0.009	0.661 ± 0.007	0.656 ± 0.013	0.660 ± 0.006	0.532 ± 0.005	0.544 ± 0.004	0.545 ± 0.007	0.544 ± 0.004[c]
Ward's triangle	0.663 ± 0.012	0.657 ± 0.010	0.658 ± 0.018	0.657 ± 0.009	0.444 ± 0.007	0.459 ± 0.006	0.455 ± 0.010	0.458 ± 0.005

Data are means ± SE. BMD values are adjusted for age and BMI.

a. P = 0.034 vs. **G/*G*.

b. P = 0.011 vs. **G/*G*.

c. P = 0.033 vs. **G/*G*.

Table 3. BMD and Other Characteristics of Men (n = 1,126) According to the −1997G → T Genotype of COL1A1

Characteristic	**G/*G*	**G/*T*	**T/*T**	*G/*T + *T/*T*
Number (%)	457 (40.6)	511 (45.4)	158 (14.0)	669 (59.4)
Age (years)	58.5 ± 0.5	59.7 ± 0.5	59.2 ± 0.9	59.6 ± 0.4
BMI (kg/m^2)	22.9 ± 0.1	23.0 ± 0.1	22.9 ± 0.2	22.9 ± 0.1
BMD values (g/cm^2)				
L2–L4	0.990 ± 0.007	0.975 ± 0.007	0.983 ± 0.012	0.977 ± 0.006
Femoral neck	0.754 ± 0.005	0.754 ± 0.004	0.744 ± 0.008	0.751 ± 0.004
Trochanter	0.672 ± 0.005	0.665 ± 0.004	0.667 ± 0.008	0.665 ± 0.004
Ward's triangle	0.557 ± 0.006	0.552 ± 0.005	0.540 ± 0.010	0.549 ± 0.005

Data are means ± SE. BMD values are adjusted for age and BMI.

Discussion

The −1997G → T SNP of the COL1A1 promoter has previously been associated with BMD for the lumbar spine and, to a lesser extent, with BMD for the femoral neck in postmenopausal Spanish women, and with the **T/*T* genotype, which represents a risk factor for reduced BMD (Garcia-Giralt et al. 2002). We have now shown that the −1997G → T SNP is associated with BMD for the femoral neck and trochanter in postmenopausal Japanese women and with the **G/*G* genotype, which represents a risk factor for reduced BMD. The −1997G → T genotype affected BMD at various sites with a variance of 0.61–1.01% for postmenopausal women, although this SNP was not associated with biochemical markers of bone turnover.

The alleles of the −1997G → T polymorphism associated with reduced

Table 4. Effects of the −1997G → T Genotype of COL1A1 on BMD for All Women (n = 1,110) or Postmenopausal Women (n = 815)

Site	R^2	P
All women		
L2–L4	0.0061	0.0102
Femoral neck	0.0047	0.0243
Trochanter	0.0062	0.0093
Ward's triangle	0.0046	0.0262
Postmenopausal women		
L2–L4	0.0061	0.0263
Femoral neck	0.0101	0.0044
Trochanter	0.0076	0.0137
Ward's triangle	0.0071	0.0172

The R^2 and P values were derived from multivariate regression analysis including age, BMI, and COL1A1 genotype (0 = **G/*G*, 1 = **G/*T* = **T/*T*).

Table 5. Biochemical Markers of Bone Turnover for Women or Men According to the −1997G → T Genotype of COL1A1

Marker	**G/*G*	**G/*T*	**T/*T*
Premenopausal women			
Osteocalcin (ng/mL)	6.35 ± 0.29	6.46 ± 0.24	6.93 ± 0.42
Bone-specific alkaline phosphatase (U/L)	19.6 ± 0.5	20.3 ± 0.5	19.0 ± 0.8
dPyr (pmol/μmol Cr)	5.54 ± 0.15	5.35 ± 0.12	5.50 ± 0.22
NTx (pmol BCE/μmol Cr)	33.5 ± 1.5	33.6 ± 1.3	38.3 ± 2.3
Postmenopausal women			
Osteocalcin (ng/mL)	10.53 ± 0.21	10.30 ± 0.19	10.25 ± 0.32
Bone-specific alkaline phosphatase (U/L)	31.6 ± 0.6	31.5 ± 0.6	30.5 ± 0.9
dPyr (pmol/μmol Cr)	4.08 ± 0.06	4.01 ± 0.05	3.99 ± 0.10
NTx (pmol BCE/μmol Cr)	60.4 ± 1.6	60.2 ± 1.5	59.4 ± 2.5
Men			
Osteocalcin (ng/mL)	7.67 ± 0.11	7.64 ± 0.11	7.64 ± 0.20
Bone-specific alkaline phosphatase (U/L)	26.3 ± 0.4	25.6 ± 0.4	26.2 ± 0.7
dPyr (pmol/μmol Cr)	4.08 ± 0.06	4.01 ± 0.05	3.99 ± 0.10
NTx (pmol BCE/μmol Cr)	36.6 ± 0.7	36.2 ± 0.7	36.4 ± 1.2

Data are means ± SE. dPyr, deoxypyridinoline; Cr, creatinine; NTx, cross-linked N-telopeptides of type I collagen; BCE, bone collagen equivalents.

BMD thus differ between the present study (**G* allele) and the previous study (**T* allele) (Garcia-Giralt et al. 2002). Although the reason for this apparent discrepancy is unclear, there are three major differences between the two studies. First, the subjects were older in our study (mean age of 64 years for postmenopausal women) than in the previous study (mean age, 51 years), and years since menopause were significantly greater in our study (mean, 15.0 years) than in the previous study (mean, 3.6 years). Given that bone resorption markedly increases during 10 years after menopause, genetic effects on BMD might differ between women for short and long time after menopause. Second, the number of subjects in which the association was detected was greater in our study ($n = 815$ for postmenopausal women) than in the previous study ($n = 256$). The results of association studies with small sample sizes are prone to bias compared with those with large sample sizes. Finally, the distribution of −1997G → T genotypes differed significantly ($P < 0.0001$; chi-square test) between our study (postmenopausal women: **G/*G*, 38%; **G/*T*, 46%; **T/*T*, 16%) and the previous study (**G/*G*, 76%; **G/*T*, 22%; **T/*T*, 2%), possibly reflecting the difference in ethnicity. The difference in genetic influences on BMD between different ethnic groups might be attributable, at least in part, to the difference in the distribution of genotypes. It is also possible that the −1997G → T SNP of COL1A1 is in linkage disequilibrium with other polymorphisms of COL1A1 or with polymorphisms of other nearby genes that are actually responsible for the observed association with BMD. Given the multiple comparisons of genotype performed, we cannot completely exclude the possible occurrence of statistical errors such as

false positives, although we observed a significant association of this SNP with BMD at different sites.

Evidence suggests that the $-1997G \rightarrow T$ SNP of COL1A1 may affect promoter function (Garcia-Giralt et al. 2002). A double-stranded oligonucleotide containing the $-1997G \rightarrow T$ site bound osteoblast nuclear factors; however, the extent of factor binding was even more pronounced with a single-stranded antisense DNA probe, suggesting the involvement of a protein selective for single-stranded DNA. The extent of factor binding observed with a probe corresponding to the **G* allele was greater than that apparent with a probe based on the **T* allele. The effect of this SNP on COL1A1 transcription, however, remains to be determined.

In conclusion, our present results suggest that the $-1997G \rightarrow T$ SNP of COL1A1 is associated with BMD for the femoral neck and trochanter in postmenopausal Japanese women and that the alleles associated with reduced BMD differ between postmenopausal Japanese (**G* allele) and Spanish (**T* allele) women, although the contribution of this SNP to bone mass appears relatively small.

Acknowledgments This study was supported in part by a Research Grant for Longevity Sciences (grant 12C-01 awarded to Y. Yamada and H. Shimokata) and by a Health and Labor Sciences Research Grant for Comprehensive Research on Aging and Health (grant H15-Chojyu-014 awarded to Y. Yamada, F. Ando, N. Niino, and H. Shimokata) from the Ministry of Health, Labor, and Welfare of Japan.

Received 22 March 2004; revision received 16 July 2004.

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Genomics 86 (2005) 76–85

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Association of polymorphisms in *CYP17A1, MTP,* and *VLDLR* with bone mineral density in community-dwelling Japanese women and men

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Received 20 January 2005; accepted 14 March 2005
Available online 15 April 2005

Abstract

We examined whether a −34T → C polymorphism of the gene for cytochrome P450, family 17, subfamily A, polypeptide 1 (*CYP17A1*), a −493G → T polymorphism of the microsomal triglyceride transfer protein gene (*MTP*), and a CGG repeat polymorphism of the very low density lipoprotein receptor gene (*VLDLR*) were associated with bone mineral density (BMD) in community-dwelling Japanese women and men. The −34T → C polymorphism of *CYP17A1* was associated with BMD in postmenopausal women, with the *CC* genotype being related to increased BMD. The −493G → T polymorphism of *MTP* was associated with BMD in premenopausal women, with the *TT* genotype being related to increased BMD. The CGG repeat polymorphism of *VLDLR* was associated with BMD in men, with two $(CGG)_{n \geq 8}$ alleles being related to increased BMD. These results suggest that *CYP17A1* and *MTP* are susceptibility loci for increased BMD in postmenopausal and premenopausal Japanese women, respectively, and that *VLDLR* constitutes such a locus in Japanese men.

Keywords: Polymorphism, genetic; Genetics; Osteoporosis; Bone density; Steroid 17α-hydroxylase; Microsomal triglyceride transfer protein; VLDL receptor

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 and treatment of osteoporosis and osteoporotic fractures are important public health goals, 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 genetic susceptibility to this condition remain to be identified definitively. In addition, because of both ethnic divergence of gene polymorphisms and gene–environment interactions, it is important to examine polymorphisms related to BMD in each ethnic group.

Cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1) is an enzyme with both 17α-hydroxylase and 17,20-lyase activities that is essential for the production of gonadal and adrenal androgens [11]. Loss-of-function mutations in *CYP17A1,* which is located at chromosome 10q24.3, result in reduced skeletal growth and diffuse osteoporosis [12]. The 5′ untranslated region of *CYP17A1* contains a common polymorphism (−34T → C) located 34 bp upstream of the translation initiation site [13]. The presence of C at this site creates a potential Sp1-like binding motif (CCACC box) that has been proposed to increase both *CYP17A1* expression and consequent androgen biosynthesis [13]. In vitro experiments, however, failed to detect a difference in transcriptional activity between the two allelic variants [14,15]. Men homozygous for the *C* allele of this polymorphism were found to have higher bioavailable testosterone concentrations as well as an increased adult stature and femoral size compared with those homozygous for the *T* allele [16]. In contrast, postmenopausal women with the *CC* genotype had a

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doi:10.1016/j.ygeno.2005.03.005

lower BMD at the femoral neck compared with those harboring a *T* allele [17]. In a study of Danish women, although lean individuals homozygous for the *C* allele had a lower BMD for the lumbar spine or femoral neck than did those with the *T* allele, this association was not detected in overweight women [18]. Given the apparently inconsistent results of previous studies, the effect of the −34T → C polymorphism of *CYP17A1* on BMD remains unclear.

Recent observations suggest that lipid and bone metabolism are closely related and that an atherogenic lipid profile has adverse effects on bone remodeling [19–24]. We thus hypothesized that polymorphisms in genes that play a role in lipid metabolism, such as those for microsomal triglyceride transfer protein (*MTP*) and the very low density lipoprotein receptor (*VLDLR*), might affect BMD. MTP is a heterodimeric lipid transfer protein that is essential for both the assembly of apolipoprotein B-containing lipoproteins and their secretion from the liver and intestine [25]. Mutations in the coding region of *MTP* prevent the production of apolipoprotein B-containing lipoproteins, resulting in the rare genetic disorder abetalipoproteinemia [26]. *MTP* is located at chromosome 4q22–q24 and is polymorphic, with several genetic variants existing in linkage disequilibrium [27]. A common polymorphism (−493G → T) has been identified in the promoter region of *MTP* (located 493 bp upstream of the transcription start site), with the less prevalent *T* variant having been associated with a reduced plasma concentration of low density lipoprotein (LDL)-cholesterol [28]. Functional analysis of the −493G → T polymorphism with the use of promoter constructs revealed that the promoter activity of the *T* variant was greater than that of the *G* variant [28]. The −493G → T polymorphism was also recently shown to be associated with the prevalence of coronary heart disease [29]. Furthermore, a haplotype marker of *MTP* was associated with longevity, suggesting that *MTP* might modify human life span [30]. The effect of the −493G → T polymorphism of *MTP* on BMD, however, has not been examined.

The very low density lipoprotein receptor (VLDL-R) binds apolipoprotein E-containing lipoproteins such as VLDL, intermediate density lipoprotein, and β-VLDL and is expressed in heart, muscle, and adipose tissue, but not in the liver, suggesting that it might contribute to the metabolism of triglyceride-rich lipoproteins [31]. The structure of *VLDLR*, which is located at chromosome 9p24, is similar to that of the LDL-R gene [32]. The 5′ untranslated region of *VLDLR* contains a polymorphic triplet (CGG) repeat sequence, with 4 to 11 repeat units having been identified in population samples [32–34]. This polymorphism has been related to plasma concentrations of lipoprotein E:B and lipoprotein A-I [33] as well as to the prevalence of sporadic Alzheimer disease in Japanese [34]. The possible effect of the CGG repeat polymorphism of *VLDLR* on BMD has not been examined, however.

We have been attempting to identify genes significantly associated with BMD in Japanese women or men in a population-based study. *CYP17A1*, *MTP*, and *VLDLR* are candidates for genes that might affect BMD. In the present study, we examined the relations of polymorphisms of these three genes to BMD, even though there is no apparent biological link among them. Our aim was to identify a single polymorphism significantly associated with BMD for each gene. Among several polymorphisms previously identified, the −34T → C polymorphism of *CYP17A1*, the −493G → T polymorphism of *MTP*, and the CGG repeat polymorphism of *VLDLR* have been shown to have the potential to affect gene function. We thus examined the relations of these polymorphisms with BMD in Japanese women and men in a population-based study.

Results

Association of the −34T → C polymorphism of CYP17A1 with BMD

The distribution of −34T → C genotypes of *CYP17A1* was in Hardy–Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all women (Table 1), premenopausal women (data not shown), or postmenopausal women (Table 2). Among all women, BMD for the femoral neck, with adjustment for age, height, and body weight, was significantly ($p < 0.01$) greater in individuals with the *CC* genotype than in those in the combined group of *TT* and *TC* genotypes (Table 1). To examine the possible influence of menopause on the relation between *CYP17A1* genotype and BMD, we analyzed premenopausal and postmenopausal women independently. Because of their small number ($n = 16$), perimenopausal women were excluded from this analysis. *CYP17A1* genotype was not associated with BMD in premenopausal women (data not shown). For postmenopausal women, however, BMD for the femoral neck was significantly greater in individuals with the *CC* genotype than in those in the combined group of *TT* and *TC* genotypes (Table 2). The difference in BMD for the femoral neck between individuals with the *CC* genotype and those in the combined group of *TT* and *TC* genotypes (expressed as a percentage of the larger value) was 2.9%.

The distribution of −34T → C genotypes of *CYP17A1* was in Hardy–Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for men (data not shown). No significant association was detected between *CYP17A1* genotype and BMD in men (data not shown).

Association of the −493G → T polymorphism of MTP with BMD

The distribution of −493G → T genotypes of *MTP* was in Hardy–Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all women

Table 1
BMD and other characteristics for all women (n = 1108) according to *CYP17A1* genotype

Characteristic	*TT*	*TC*	*CC*	*TT* + *TC*
Number (%)	307 (27.7)	544 (49.1)	257 (23.2)	851 (76.8)
Age (years)	58.3 ± 0.6	59.6 ± 0.5	59.7 ± 0.7	59.1 ± 0.4
Height (cm)	151.6 ± 0.3	151.4 ± 0.3	150.8 ± 0.4	151.5 ± 0.2
Body weight (kg)	52.1 ± 0.5	52.9 ± 0.4	52.7 ± 0.5	52.6 ± 0.3
BMD measured with pQCT (mg/cm^3)				
D50	183.7 ± 3.5	185.7 ± 2.7	186.3 ± 3.9	185.0 ± 2.1
D100	488.5 ± 5.1	485.3 ± 3.8	485.1 ± 5.6	486.5 ± 3.1
P100	1161.3 ± 8.2	1145.1 ± 6.2	1161.7 ± 8.9	1151.0 ± 4.9
BMD measured with DXA (g/cm^2)				
Total body	0.965 ± 0.005	0.962 ± 0.004	0.972 ± 0.005	0.963 ± 0.003
L2–L4	0.861 ± 0.007	0.862 ± 0.005	0.879 ± 0.008	0.861 ± 0.004[a]
Femoral neck	0.676 ± 0.005	0.673 ± 0.004[b]	0.691 ± 0.005	0.674 ± 0.003[c]
Trochanter	0.569 ± 0.005	0.569 ± 0.004	0.578 ± 0.005	0.569 ± 0.003

BMD is adjusted for age, height, and body weight. Data are means ± SE.
[a] $p = 0.047$ versus *CC*.
[b] $p = 0.019$ versus *CC*.
[c] $p = 0.006$ versus *CC*.

(Table 3), premenopausal women (Table 4), or postmenopausal women (data not shown). There were no differences in BMD among *MTP* genotypes for all women (Table 3). For premenopausal women, however, BMD for the distal radius (D100) was significantly greater in individuals with the *TT* genotype than in those with the *GG* genotype or those in the combined group of *GG* and *GT* genotypes (Table 4). The difference in BMD for D100 between individuals with the *TT* genotype and those in the combined group of *GG* and *GT* genotypes (expressed as a percentage of the larger value) was 12.5%. *MTP* genotype was not associated with BMD in postmenopausal women (data not shown).

The distribution of −493G → T genotypes of *MTP* was in Hardy–Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for men (data not shown). No relation was detected between *MTP* genotype and BMD for men (data not shown).

Association of the CGG repeat polymorphism of VLDLR with BMD

The number of CGG repeats in *VLDLR* ranged from 5 to 10 (6.9 ± 1.5, mean ± SD; n = 4410 alleles) for all women and men (Table 5). The number of CGG repeats tended to be related ($p < 0.05$) to BMD for the distal radius (D100) or trochanter for women and to BMD for the distal radius (D100), lumbar spine, femoral neck, or trochanter for men, with the polymorphism explaining 0.2 to 0.3% of the variance in BMD (Table 6). At each of these sites, BMD increased as the number of CGG repeats increased. Given that the mean and median numbers of CGG repeats were 6.9 and 8,

Table 2
BMD and other characteristics for postmenopausal women (n = 813) according to *CYP17A1* genotype

Characteristic	*TT*	*TC*	*CC*	*TT* + *TC*
Number (%)	218 (26.8)	401 (49.3)	194 (23.9)	619 (76.1)
Age (years)	63.2 ± 0.6	64.3 ± 0.4	63.9 ± 0.6	63.9 ± 0.4
Height (cm)	150.3 ± 0.4	150.3 ± 0.3	149.8 ± 0.4	150.3 ± 0.3
Body weight (kg)	51.2 ± 0.6	52.2 ± 0.4	52.6 ± 0.6	51.8 ± 0.3
BMD measured with pQCT (mg/cm^3)				
D50	164.7 ± 4.3	162.5 ± 3.2	165.6 ± 4.6	163.3 ± 2.6
D100	448.2 ± 6.2	441.1 ± 4.6	443.8 ± 6.6	443.6 ± 3.7
P100	1089.4 ± 10.3	1067.9 ± 7.5	1094.4 ± 10.9	1075.4 ± 6.1
BMD measured with DXA (g/cm^2)				
Total body	0.919 ± 0.006	0.915 ± 0.004	0.927 ± 0.006	0.916 ± 0.003
L2–L4	0.810 ± 0.009	0.803 ± 0.006	0.818 ± 0.009	0.806 ± 0.005
Femoral neck	0.639 ± 0.006[a]	0.640 ± 0.004[b]	0.659 ± 0.006	0.640 ± 0.003[c]
Trochanter	0.538 ± 0.006	0.537 ± 0.004	0.548 ± 0.006	0.537 ± 0.003

BMD is adjusted for age, height, and body weight. Data are means ± SE.
[a] $p = 0.045$ versus *CC*.
[b] $p = 0.030$ versus *CC*.
[c] $p = 0.006$ versus *CC*.

Table 3
BMD and other characteristics for all women (n = 1108) according to *MTP* genotype

Characteristic	*GG*	*GT*	*TT*	*GG* + *GT*
Number (%)	763 (68.9)	313 (28.2)	32 (2.9)	1076 (97.1)
Age (years)	59.0 ± 0.4	60.1 ± 0.6	57.3 ± 1.9	59.3 ± 0.3
Height (cm)	151.3 ± 0.2	151.4 ± 0.3	152.4 ± 1.1	151.3 ± 0.2
Body weight (kg)	52.5 ± 0.3	52.6 ± 0.5	54.1 ± 1.4	52.5 ± 0.2
BMD measured with pQCT (mg/cm^3)				
D50	185.4 ± 2.2	185.4 ± 3.5	184.8 ± 11.0	185.4 ± 1.9
D100	483.8 ± 3.2	489.5 ± 5.0	507.5 ± 15.9	485.5 ± 2.7
P100	1151.2 ± 5.2	1161.1 ± 8.1	1154.5 ± 25.7	1154.1 ± 4.4
BMD measured with DXA (g/cm^2)				
Total body	0.968 ± 0.003	0.961 ± 0.005	0.964 ± 0.015	0.966 ± 0.003
L2–L4	0.866 ± 0.005	0.863 ± 0.007	0.885 ± 0.022	0.866 ± 0.004
Femoral neck	0.679 ± 0.003	0.674 ± 0.005	0.703 ± 0.015	0.677 ± 0.003
Trochanter	0.573 ± 0.003	0.566 ± 0.005	0.581 ± 0.014	0.571 ± 0.003

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

respectively, we designated $(CGG)_n \leq 7$ and $(CGG)_n \geq 8$ alleles as short (*S*) and long (*L*) alleles, respectively.

The distribution of *SS*, *SL*, and *LL* genotypes of *VLDLR* was in Hardy–Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all women, premenopausal women, or postmenopausal women (data not shown). There was no significant relation between *VLDLR* genotype and BMD for all women, premenopausal women, or postmenopausal women (data not shown).

For men, the distribution of *SS*, *SL*, and *LL* genotypes of *VLDLR* was in Hardy–Weinberg equilibrium, and age, height, and body weight did not differ among genotypes (Table 7). Among men, BMD for the lumbar spine was significantly greater in individuals with the *LL* genotype than in those with the *SL* genotype or those in the combined group of *SS* and *SL* genotypes (Table 7). The difference in BMD for the lumbar spine between individuals with the *LL* genotype and those in the combined group of *SS* and *SL* genotypes (expressed as a percentage of the larger value) was 2.8%.

Effects of genotypes for CYP17A1, MTP, and VLDLR on BMD

The effects of the −34T → C genotype of *CYP17A1*, the −493G → T genotype of *MTP*, and the CGG repeat genotype of *VLDLR* on BMD at various sites were evaluated by multiple regression analysis (Table 8). The analysis revealed that the −34T → C genotype of *CYP17A1* significantly affected BMD for the femoral neck with an R^2 variance of 0.6% in postmenopausal women, the −493G → T genotype of *MTP* affected BMD for the distal radius (D100) with an R^2 variance of 2.9% in premenopausal women, and the CGG repeat genotype of *VLDLR* affected BMD for the lumbar spine with an R^2 variance of 0.7% in men.

Table 4
BMD and other characteristics for premenopausal women (n = 279) according to *MTP* genotype

Characteristic	*GG*	*GT*	*TT*	*GG* + *GT*
Number (%)	200 (71.7)	72 (25.8)	7 (2.5)	272 (97.5)
Age (years)	46.1 ± 0.3	46.6 ± 0.5	46.3 ± 1.7	46.2 ± 0.3
Height (cm)	154.4 ± 0.3	154.8 ± 0.6	155.1 ± 1.8	154.5 ± 0.3
Body weight (kg)	54.3 ± 0.6	54.3 ± 1.0	53.8 ± 3.1	54.3 ± 0.5
BMD measured with pQCT (mg/cm^3)				
D50	245.7 ± 3.9[a]	240.6 ± 6.5[b]	294.8 ± 20.1	244.3 ± 3.3[c]
D100	601.1 ± 5.4[d]	610.1 ± 9.1[e]	689.9 ± 28.3	603.5 ± 4.6[f]
P100	1353.5 ± 8.1	1367.5 ± 13.7	1445.5 ± 42.5	1357.2 ± 7.0[g]
BMD measured with DXA (g/cm^2)				
Total body	1.097 ± 0.006	1.080 ± 0.010	1.135 ± 0.030	1.093 ± 0.005
L2–L4	1.028 ± 0.008	1.016 ± 0.013	1.033 ± 0.043	1.025 ± 0.007
Femoral neck	0.772 ± 0.006	0.766 ± 0.010	0.838 ± 0.034	0.770 ± 0.005[h]
Trochanter	0.660 ± 0.006	0.647 ± 0.010	0.701 ± 0.031	0.657 ± 0.005

BMD is adjusted for age, height, and body weight. Data are means ± SE.
[a] $p = 0.044$ versus *TT*.
[b] $p = 0.028$ versus *TT*.
[c] $p = 0.013$ versus *TT*.
[d] $p = 0.006$ versus *TT*.
[e] $p = 0.020$ versus *TT*.
[f] $p = 0.002$ versus *TT*.
[g] $p = 0.041$ versus *TT*.
[h] $p = 0.047$ versus *TT*.

Table 5
Distribution of the number of CGG repeats in *VLDLR*

	Repeat number					
	5	6	7	8	9	10
Women (n = 1097; 2194 alleles)	851 (38.79%)	1 (0.05%)	0 (0%)	1226 (55.88%)	98 (4.47%)	18 (0.82%)
Men (n = 1108; 2216 alleles)	823 (37.14%)	1 (0.05%)	1 (0.05%)	1260 (56.86%)	108 (4.87%)	23 (1.04%)

Relation of serum lipid profile to genotypes for CYP17A1, MTP, or VLDLR or to BMD

Finally, the relation of serum lipid profile to *CYP17A1*, *MTP*, or *VLDLR* genotypes or to BMD was examined. There were no significant differences in the serum concentrations of total cholesterol, high density lipoprotein (HDL)-cholesterol, LDL-cholesterol, or triglycerides among *CYP17A1*, *MTP*, or *VLDLR* genotypes for women or men (data not shown). For women, BMD for the distal (D100) or proximal (P100) radius or total body was significantly related to the serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides, and BMD for the distal radius (D50), lumbar spine, femoral neck, or trochanter was significantly related to the serum concentrations of total cholesterol, LDL-cholesterol, and triglycerides (Table 9). For men, BMD for D50 or D100 was significantly related to the serum concentrations of LDL-cholesterol or total cholesterol, respectively, and BMD for the femoral neck or trochanter was significantly related to the serum concentration of HDL-cholesterol (Table 9).

Discussion

We have examined the relations of the −34T → C polymorphism of *CYP17A1*, the −493G → T polymorphism of *MTP*, and the CGG repeat polymorphism of *VLDLR* to BMD at various sites in community-dwelling Japanese women and men. Our results now show that the *CC* genotype of *CYP17A1* and the *TT* genotype of *MTP* are associated with increased BMD in postmenopausal and premenopausal women, respectively, and that two $(CGG)_{n \geq 8}$ alleles are associated with increased BMD in men.

Table 6
Relation between the CGG repeat number of *VLDLR* and BMD

	Women (n = 1097; 2194 alleles)		Men (n = 1108; 2216 alleles)	
	p	R^2	p	R^2
BMD measured with pQCT (mg/cm^3)				
D50	0.279		0.075	
D100	0.038	0.002	0.045	0.002
P100	0.064		0.267	
BMD measured with DXA (g/cm^2)				
Total body	0.085		0.168	
L2–L4	0.161		0.035	0.002
Femoral neck	0.085		0.013	0.003
Trochanter	0.048	0.002	0.028	0.002

Data were analyzed by simple regression analysis.

Association of the −34T → C polymorphism of CYP17A1 with BMD

The −34T → C polymorphism of *CYP17A1* has been associated with polycystic ovary syndrome [13] and breast cancer [35,36], with the *C* allele representing a risk factor for these diseases. The *C* variant was suggested to create a novel Sp1 recognition site that might increase *CYP17A1* gene expression [13]. In vitro experiments, however, did not detect a difference in binding of recombinant Sp1 to, or in transcriptional activity of, DNA sequences corresponding to the two allelic variants of *CYP17A1* [14,15]. We have now shown that the *CC* genotype of the −34T → C polymorphism of *CYP17A1* was associated with increased BMD for the femoral neck in postmenopausal women.

Several lines of evidence suggest that this polymorphism might influence endogenous sex hormone levels [37,38]. Premenopausal nulliparous women with the *CC* genotype were thus found to have higher concentrations of serum estradiol than those with the *TT* genotype [37], and postmenopausal women with the *CC* genotype had higher levels of serum estrone and estradiol than did those with the *TT* genotype [38]. In addition, postmenopausal women with the *CC* genotype were shown to be less likely to be current users of hormone replacement therapy [39]. These previous observations showing a relation between the *CC* genotype and higher serum concentrations of estrogen support our present data, given that estrogen exhibits beneficial effects on bone remodeling by inhibiting bone resorption and stimulating bone formation [40].

Men with the *CC* genotype were previously found to exhibit a 20% increase in bioavailable testosterone concentrations, a 3-cm increase in height, and a 5% increase in cross-sectional area of the femoral neck compared with men with the *TT* genotype, although *CYP17A1* genotype was not related to BMD at the femoral neck [16]. In our study, BMD was not related to *CYP17A1* genotype in men. We also did not detect a relation between *CYP17A1* genotype and the serum concentrations of total testosterone or free testosterone in men (data not shown). The lack of an association between *CYP17A1* genotype and BMD for men in the present study may thus be attributable, at least in part, to the absence of a relation between *CYP17A1* genotype and serum testosterone concentrations.