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1. 食生活と長寿

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第48回日本老年医学会学術集会記録
〈市民公開シンポジウム：高齢者の健康と食〉

1. 食生活と長寿

下方　浩史

要　約　肥満はさまざまな生活習慣病の原因であり，最近はメタボリックシンドロームとしてその病態が注目されている．健康長寿には健全な食生活によって肥満を防止することが重要である．しかしやせているほど健康というわけではなく，人間には理想的な肥満度がある．この理想的な肥満度は年齢によって異なり，高齢者では生命予後を考えた場合，肥満の予防よりもむしろやせの予防の方が重要である．栄養摂取の不足は高齢者では寿命を短くすることが多い．高齢者の栄養のかかえるさまざまな栄養問題，栄養評価に関する考え方を述べるとともに，健康な長寿を目指すための理想的な肥満度，内臓脂肪や体脂肪分布と健康，そして急激な体重変動が健康障害をもたらす等の知見を示し，長寿と食生活，栄養との関連について幅広く紹介する．

Key words：長寿，肥満，食事，老化，栄養

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はじめに

厚生労働省による平成16年度の簡易生命表では日本人の平均寿命は，女性が85.59歳，男性が78.64歳であった[1]．男女とも5年連続で過去最高を更新したことになる．女性は20年連続の世界一であり，男性は前年の3位から香港を抜いて2位となり，世界最速のペースで長寿化が進んでいる．

日本人の長寿には食生活が重要な要因となっていると考えられる．日本には独特の食習慣がある．先進諸国中で脂肪摂取量が飛び抜けて少なく，米飯を中心として炭水化物の摂取が多い．また魚の摂取が多いことも特徴である．豆腐や納豆，味噌などの大豆製品の摂取が多く，これらは動脈硬化の進行を防ぐには理想に近い食習慣である．またカテキンやビタミンCなどの抗酸化物質が多く含まれる緑茶の摂取は，動脈硬化や癌を防いでいる可能性がある．ここでは長寿や高齢者の健康と栄養との関わりについて述べてみる．

理想的肥満度

自由無制限の食餌を与えたラットより食餌を制限したラットの方が長生きするという結果は基礎老化の研究者の間ではよく知られている[2]．しかし他の動物においても食餌制限が有効かどうかについては議論のあるところで，サルを使ったプロジェクトがアメリカ国立老化研究所で行われつつあるが，サルの寿命は長く最終的な結論がでるのはまだまだ先である[3]．

人間ではやせていればいるほど健康にいいのか，もしそうでないなら，どの程度の体重であるのが医学的には理想なのか．Andresは米国の生命保険会社のデータから，体重（kg）を身長（m）の二乗で割って求めたBody Mass Index（BMI）を身長とは無相関の肥満の指標として用い，各年代で最も死亡率の低いBMIをもとめた[4]．この結果死亡率を縦軸，BMIを横軸にとった時，きれいなU字を描くことに示した．BMIの小さいやせた人では，肺炎や結核などの感染症の発病率が高く，BMIの大きな太った人では糖尿病や心臓病などの発病率が高くなる．男女別に，年齢ごとにこのようなグラフを作成し，死亡率の最も低い肥満度を求めてみると，この理想的な肥満度の値は加齢とともに大きくなっている[5]．男女で大きな差はなく年齢とともにほぼ直線的に理想的なBMIの値が大きくなっていく．例えば，身長170cmの45歳の男性で67kg位の体重であると死亡率，疾患の罹患率が最も低くなる．

日本での検討では，生活習慣病の発生率の最も少ないのは，BMIが22.2であることが示されており[6]，この値は米国の40歳代における最も死亡率の低いBMIの値とほぼ同じ値である．日本人でも，理想的BMIは，米国

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表1　年齢別にみたBMIによる痩せの基準値

年齢	BMI
20～29歳	18
30～39歳	19
40～49歳	20
50～59歳	21
60～69歳	22
70歳以上	23

での場合と同様に，加齢とともに高くなっていると思われるが，残念ながら日本ではこうした加齢による，理想的肥満度の変化についての十分な検討は行われていない．

高齢者の栄養問題

1）老化に伴う生理学的変化

消化吸収という生体機能は原始的機能であり，基本的には予備力が大きい．しかし加齢によって消化吸収に関連する機能は少しずつ低下し，いろいろな疾患や病態を引き起こす．老化により唾液分泌が低下することが多い．唾液が出にくくなれば食物の咀嚼も悪くなる．また食物を飲み込みにくくなり，嚥下障害となる．さらに口腔内の衛生状態も悪くなり，慢性の口内炎や慢性の舌炎，歯槽膿漏の原因となる．口内炎や歯肉炎は入れ歯があわない場合にも起きやすい．口腔内の炎症があれば，不快感や疼痛のため食事が十分とれなくなる．

胃の支持組織の緊張低下により胃液が食道に逆流し，食道にびらんや潰瘍を形成する逆流性食道炎は老人に多い．胃の粘膜が萎縮し胃酸の分泌が悪くなる．鉄やビタミンの吸収が低下し，また胃酸は細菌の増殖を抑える作用があるが，酸が低下すれば消化管への細菌感染の危険が増加する．

消化液の分泌能の低下はとくに油脂類の消化に負担を与える．また歯の脱落や咀しゃく筋の筋力低下による咀しゃく能の低下により，堅い食品を避け，柔らかいものを好むようになる．柔らかい食品には糖質を主体とするものが多く，たんぱく質やカルシウムなどが不足してしまう．消化管の筋組織の筋力低下や支持組織の緊張低下に起因する消化管運動機能の低下によって便秘となりやすい．さらに消化管の栄養素の吸収能，肝臓における処理能力の低下もみられる．このような老化による変化は個人により進行の程度に差はあるとはいえ避けがたいものである．

2）高齢者の食欲不振

高齢者では若年者に比べて食欲が低下することが多い，これにはいくつかの要因がある．高齢者では心肺機能が低下し運動を十分にすることができなくなり，身体活動によるエネルギー消費が少なくなる．運動を行わないため骨格筋が萎縮し体脂肪が増加する．骨格筋は多くのエネルギーを消費するが脂肪組織ではエネルギーはほとんど消費されず，体脂肪率の上昇とともに全身の基礎代謝率は低下する．エネルギー要求量が低くなり，その結果，食欲が低下することが多い．感覚機能，特に食欲に密接に関わる味覚，臭覚，視覚などの機能の低下がいっそう食欲不振を増強させる．高齢者に多い心疾患に対して使われるジギタリス剤などには食欲を減退させる副作用が往々にしてみられる．また亜鉛欠乏は味覚障害を起こし食欲低下の原因となる．

高齢者の栄養状態の評価

1）血液検査による評価

血清アルブミンは高齢者の栄養状態を示す指標として最も有用なものである．健康な高齢者では加齢に伴う血清アルブミンの低下はみられない．血清アルブミンは生命予後の有用な指標でもある[7]．アルブミン値が3.5g/dl以下の状態では骨格筋の消耗が始まっている可能性が強い．高脂血症，特に高コレステロール血症は虚血性心疾患のリスクとなるが，血清コレステロール値が300mg/dlを越えるような場合は家族性の高脂血症であることがほとんどで，治療しない限り老年に達する前に心疾患などで死亡してしまうことが多い．しかし高齢者では低コレステロール血症がむしろ死亡や日常生活の活動能力が低下することにつながることが知られている[8]．

2）体格による評価

高齢者では生命予後を考えた場合，肥満よりもやせの方が重要である．肥満は糖尿病や高血圧の原因のひとつであり，肥満者では心臓病や脳卒中の発生率が高くなる．しかし肥満者の死亡が多いのは主に中年期である．高齢者では中年に比べて肥満は健康を害したりする危険や死亡に結びついたりすることが少ない．表1にBMIでの年齢別にみたやせの基準値を示した[9][10]．年齢が高くなるにつれて基準となるBMIの値が高くなっている．高齢者では椎間の狭小化，椎骨の圧迫骨折による脊椎前弯の増強などにより，身長が年齢とともに低くなっていく．このためBMIは本来あるべき値よりも大きくなっていることにも注意しなければならない．高度の肥満に伴う高血圧症や糖尿病などがないかぎり高齢者に食事制限を勧めるべきではない．高齢者では肥満よりもやせの重要性を認識すべきである．

おわりに

高齢者では一般成人と異なった視点からの栄養管理が必要である．加齢とともに肥満よりもやせのリスクが高くなる．やせた高齢者が寝たきりになると褥創ができやすく，また感染症も治りにくい．低栄養に十分に留意する必要がある．耐糖能は年齢とともに低下する．高齢者で食後血糖やHbA1cが高くなることは高頻度にみられる．40代，50代では糖尿病合併症の進行を抑えるためにも厳格な血糖のコントロールが必要だが，高齢者では過度な制限はむしろ栄養のバランスを崩し，低栄養をきたすこともある．高齢者では血圧も高くなることが多い．しかし食事療法で，無理な減塩を行えば食事が取れなくなってしまい，かえって健康を害することもある．高コレステロールは高齢者ではむしろ生命予後を良くしている．こうした高齢者の特性を考えて，栄養管理を行うことが重要であろう．

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Dietary habit and longevity

Hiroshi Shimokata

Abstract

Obesity is one of the most important causes of life-style related diseases, and recently its pathophysiology is emphasized as metabolic syndrome. Preventing obesity by good dietary habit is a key to achieve healthy longevity. However, a lean body is not always good for health. There is an ideal body size for each person. This ideal body size differs according to age. Especially in the elderly, to prevent weight loss is more important for maintaining health and longevity than to be obese. Malnutrition is a critical factor of diseases and death in the elderly. Problems in nutritional status, and dietary intake, and methods of nutritional assessment in the elderly are discussed. Ideal body size for health and longevity, the relationship of body fat distribution and intra-abdominal fat accumulation health, and the effects of rapid weight change are also discussed to clarify the association of dietary habit and nutrition with longevity.

Key words: *Longevity, Obesity, Diet, Aging, Nutrition*
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Association of gene polymorphisms with blood pressure and the prevalence of hypertension in community-dwelling Japanese individuals

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Abstract. Hypertension is a complex multifactorial disorder that is thought to result from an interaction between genetic background and environmental factors. Although various loci and genes have been implicated in predisposition to hypertension by genetic linkage analyses and candidate gene association studies, the genes that confer susceptibility to this condition remain to be identified definitively. We examined the relations of nine candidate gene polymorphisms to blood pressure (BP) and the prevalence of hypertension in a population-based study. The 2238 subjects (1110 women, 1128 men) were aged 40 to 79 years and were randomly recruited for a population-based prospective cohort study of aging and age-related diseases in Japan. BP was measured with subjects having rested in a sitting position for at least 15 min. Genotypes for the 160C→T (Arg54Trp) polymorphism of *QPCT*, the C→T (Pro198Leu) polymorphism of *GPX1*, the 137,346T→C polymorphism of *FYN*, the -344C→T polymorphism of *CYP11B2*, and the A→G (Ser49Gly) polymorphism of *ADRB1* were determined with a fluorescence-based allele-specific DNA primer assay system; those for the A→G polymorphism of *CNR2*, the I/D (22,375delAC) polymorphism of *CAV1*, and the -1213T→C polymorphism of *ESR2* by melting curve analysis, and that for the $(GT)_n$ polymorphism of *COL1A2* were determined by DNA fragment analysis. The polymorphism of *FYN* was associated with systolic and diastolic BP in women. In men, polymorphisms of *CNR2*, *QPCT*, *GPX1*, *COL1A2*, *CYP11B2*, and *ESR2* were associated with systolic and diastolic BP, those of *CAV1* and *FYN* with systolic BP, and that of *ADRB1* with diastolic BP. The polymorphisms of *QPCT* and *CYP11B2* were also associated with the prevalence of hypertension in men. These results suggest that polymorphisms of *QPCT* and *CYP11B2* are determinants of BP and the development of hypertension in Japanese men.

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Key words: blood pressure, hypertension, genetics, polymorphism

Introduction

Hypertension is a complex multifactorial disorder that is thought to result from an interaction between an individual's genetic background and various environmental factors (1). Given that hypertension is a major risk factor for coronary heart disease, stroke, and chronic renal failure, personalized prevention of hypertension is an important public health goal. One approach to personalized prevention of, and selection of the most appropriate treatment for, hypertension is to identify genes that confer susceptibility to this condition. Although genetic linkage analyses (2-5) and candidate gene association studies (6-9) have implicated various loci and genes in the predisposition to hypertension, the genes that confer genetic susceptibility to this condition remain to be identified definitively. In addition, because of ethnic divergence of gene polymorphisms as well as of environmental factors and lifestyle, it is important to examine polymorphisms related to hypertension in each ethnic group.

We have been attempting to identify genes significantly associated with blood pressure (BP) in Japanese women or men with a population-based approach. In the present study, we selected nine candidate genes that might be expected to contribute to the regulation of BP (Table I) and examined the relations of polymorphisms of these genes to BP, even though there was no apparent biological link among these genes. Our aim was to identify a single polymorphism significantly associated with BP for each gene. Among several polymorphisms previously identified, we selected those that might be expected to affect gene function. We thus examined the relations of these polymorphisms to BP and the prevalence of hypertension in community-dwelling Japanese women and men.

Materials and methods

Study population. The National Institute for Longevity Sciences, the Longitudinal Study of Aging, is a population-

Table I. The nine gene polymorphisms examined in the present study.

Locus	Gene	Symbol	Polymorphism	dbSNP
1p36.11	Cannabinoid receptor 2	*CNR2*	A→G	rs2501431
2p22.2	Glutaminyl-peptide cyclotransferase	*QPCT*	160C→T (Arg54Trp)	rs2255991
3p21.3	Glutathione peroxidase	*GPX1*	C→T (Pro198Leu)	rs1050450
6q21	FYN oncogene related to SRC, FGR, YES	*FYN*	137,346T→C	rs706895
7q22.1	Collagen, type I, α-2	*COL1A2*	$(GT)_n$	ND
7q31.1	Caveolin 1	*CAV1*	I/D (22,375delAC)	rs3840634
8q21-q22	Cytochrome P450, subfamily Y XIB, polypeptide 2	*CYP11B2*	-344C→T	rs1799998
10q24-q26	ß-1-adrenergic receptor	*ADRB1*	A→G (Ser49Gly)	rs1801252
14q23.2	Estrogen receptor 2	*ESR2*	-1213T→C	ND

ND, not detected in dbSNP.

based prospective cohort study of aging and age-related diseases (10). The subjects were unrelated individuals stratified by both age and sex, and were randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan (11-13). The lifestyle of residents of this area is typical of that of individuals in most regions of Japan. The numbers of men and women recruited were similar and the age at baseline was 40-79 years, with similar numbers of participants in each decade (40, 50, 60 and 70s). The subjects are being followed up every 2 years. All participants were subjected at a special center to a detailed examination, which included not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. Individuals with coronary heart disease, valvular heart disease, cardiomyopathies, or renal or endocrinologic diseases that cause secondary hypertension were excluded from the present study. We thus examined the relations of gene polymorphisms to BP or the prevalence of hypertension in 2238 individuals (1110 women, 1128 men). Individuals whose genotypes were not successfully determined were excluded from the analysis. The study protocol complies with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Institute for Longevity Sciences. Written informed consent was obtained from each subject.

Measurement of BP. BP was measured with an automatic sphygmomanometer (BP-203RV-II; Colin, Tokyo, Japan) in subjects having rested in a sitting position for at least 15 min. BP in each subject was confirmed with the measurement made by a physician with a mercury manometer according to the guidelines of the American Heart Association (14). Normal BP was defined as both a systolic BP of <140 mmHg and a diastolic BP of <90 mmHg. Hypertension was defined as a systolic BP of ≥140 mmHg or a diastolic BP of ≥90 mmHg (or both), or the use of antihypertensive medication.

Determination of genotype. Genotypes for polymorphisms of *QPCT*, *GPX1*, *FYN*, *CYP11B2*, and *ADRB1* were determined with a fluorescence-based allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan) (15). Primers and other conditions for genotyping are shown in Table II. The polymorphic region of each gene was amplified by the polymerase chain reaction (PCR) with allele-specific sense primers labeled at the 5' end with either fluorescein isothiocyanate or Texas red and with an antisense primer labeled at the 5' end with biotin. The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5-4 mmol/l $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 95˚C for 5 min; 35 cycles of denaturation at 95˚C for 30 sec, annealing at 60-70˚C for 30 sec, and extension at 72˚C for 30 sec; and a final extension at 72˚C for 2 min. The amplified DNA was incubated with streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature, and the plate was then placed on a magnetic stand. The supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/l NaOH and were measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

Genotypes for polymorphisms of *CNR2*, *CAV1*, and *ESR2* were determined by melting curve analysis (intercalater-mediated fluorescence resonance energy transfer probe method). The polymorphic region of each gene was amplified by PCR (Table II) in a reaction mixture (25 μl) containing 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2 mmol/l $MgCl_2$, and 1.25 U of rTaq DNA polymerase in polymerase buffer. The amplification protocol comprised initial denaturation at 95˚C for 5 min; 45 cycles of denaturation at 95˚C for 30 sec, annealing at 65˚C for 30 sec, and extension at 72˚C for 30 sec; and a final extension at 72˚C for 2 min. A mixture (2 μl) of 10 pmol of probe and SYBR-Green was added to the PCR products, which were then transferred to a PRISM 7700 instrument (Applied Biosystems, Foster City, CA) for measurement of melting temperature. The program for analytic melting comprised incubation at 95˚C for 30 sec, 40˚C for 1 min, and temperatures increasing to 80˚C over 10 min. The fluorescence signals were detected at excitation and emission wavelengths of 485 and 612 nm, respectively.

Table II. Primers, probes, and other PCR conditions for genotyping.

Gene	Polymorphism	Sense primer with FITC	Sense primer with Texas red
QPCT	160C→T (Arg54Trp)	TTTGAATTCATCGGCTCTxCG	TTTGAATTCATCGGCTCTxTG
GPX1	C→T (Pro198Leu)	GCGCCCTAGGCACAGCTxAG	GCGCCCTAGGCACAGCTxGG
FYN	137,346T→C	GGAGTAATTGACAAGGCTCAxCG	AGGAGTAATTGACAAGGCTCAxTG
CYP11B2	-344C→T	TATTAAAAGAATCCAAGGxCC	GTCTATTAAAAGAATCCAAGGxTC
ADRB1	A→G (Ser49Gly)	GAGACAGCGGCTCGGGGxCT	GACAGCGGCTCGGGGxTT

	Antisense primer with biotin	Annealing (°C)	Cycles	Mg^{2+} (mM)	Taq/KOD
QPCT	GGTATCGCTCTATCAGCAATGG	62.5	35	2.5	Taq
GPX1	GTGTGCCCCTACGCAGGTACA	65.0	35	2.5	Taq
FYN	CCTTTCCTCATGCCCCCTAAT	67.5	35	4.0	Taq
CYP11B2	GGACTTTATCTTATCGTGAGATGA	60.0	35	3.0	Taq
ADRB1	GCCGCCCGCCTCGTTG	70.0	35	3.5	Taq

Gene	Polymorphism	Sense primer	Antisense primer
CNR2	A→G	GGGCAGGTAGGAGACTAGTGCTGAGAG	CTCACCCGTGGAAGGGCACTG
CAV1	I/D (22.375delAC)	AAAGGTGATGGATCATTTCCCATTATACAC	TGGGCAATGGTCATCCATGACTG
ESR2	-1213T→C	GAACAGGAGCCAGGGGCACAG	CCTGAAGACAAGTACCTTGCAGCTGAG

	Probe	Annealing (°C)	Cycles	Mg^{2+} (mM)	Taq/KOD
CNR2	CACATGATGCCCAGGGTC	65.0	45	2.0	Taq
CAV1	CAAAATGTGTGTCCATTTCAGG	65.0	45	2.0	Taq
ESR2	AACAGTAAAATTCTGCCTGGG	65.0	45	2.0	Taq

Gene	Polymorphism	Sense primer with FAM	Antisense primer
COL1A2	$(GT)_n$	CAGCACGGTGTCTACCACTGC	ATTACTCCTTAGTATCCACAGTATGTATAC

	Annealing (°C)	Cycles	Mg^{2+} (mM)	Taq/KOD
COL1A2	60.0	35	1.2	KOD

FITC, fluorescein isothiocyanate; FAM, 6-carboxyfluorescein. Oligonucleotide sequences are 5'→3'.

The GT repeats [$(GT)_n$] in the first intron of *COL1A2* were amplified by PCR with a sense primer labeled at the 5' end with 6-carboxyfluorescein and with an antisense primer (Table II). The reaction mixture (25 μl) contained 20 ng of DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 1.2 mmol/l $MgSO_4$, and 0.4 U of KOD plus DNA polymerase (Toyobo) in polymerase buffer. The amplification protocol comprised initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 15 sec, annealing at 60°C for 30 sec, and extension at 68°C for 30 sec; and a final extension at 68°C for 2 min. The size of the $(GT)_n$-containing PCR products was determined with a PRISM 3100 DNA sequencer and with GeneScan and Genotyper software (Applied Biosystems).

Statistical analysis. Age and BP were compared among three groups by one-way analysis of variance and the Tukey-Kramer *post hoc* test, and between two groups by the unpaired Student's t-test. BP values were analyzed in individuals who were not taking antihypertensive drugs. The prevalence of hypertension was compared between two groups (2x2) or among three groups (3x2) by the Chi-square test in all individuals. Allele frequencies were estimated by the gene-counting method, and the Chi-square test was used to identify significant departure from Hardy-Weinberg equilibrium. A P value of <0.05 was considered statistically significant.

Results

Relation of the A→G polymorphism of CNR2 to BP. For men, the distribution of genotypes for the A→G polymorphism of *CNR2* was in Hardy-Weinberg equilibrium and individuals in the combined group of *AG* and *GG* genotypes were younger than those with the *AA* genotype (Table III). Systolic and diastolic BP were significantly higher in men with the *GG* genotype than in those with the *AA* genotype or with the *AG* genotype or in those in the combined group of *AA* and *AG*

Table III. Blood pressure (BP) for male subjects according to *CNR2* genotype.

Characteristics	*AA*	*AG*	*GG*	*AA + AG*	*AG + GG*
Number of subjects (n=874)[a]	295	425	154	720	579
Age (years)	60.2±0.6	58.5±0.5	59.2±0.8	59.2±0.4	58.7±0.4[b]
Systolic BP (mmHg)	120.5±1.0[c]	120.3±0.8[d]	125.0±1.4	120.4 ±0.6[e]	121.6±0.7
Diastolic BP (mmHg)	74.8±0.6[f]	74.9±0.5[g]	78.0±0.9	74.9±0.4[h]	75.8±0.4
Number of all subjects (n=1122)	387	549	186	936	735
Hypertension (%)	34.4	33.5	37.6	33.9	34.6

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *AA* and *AG* genotypes (*AA + AG*) were compared with those for individuals with the *GG* genotype (recessive genetic model). Data for the combined group of *AG* and *GG* genotypes (*AG + GG*) were compared with those for individuals with the *AA* genotype (dominant genetic model). [b]P=0.0313 versus AA; [c]P=0.0230, [d]P=0.0101, [e]P=0.0025, [f]P=0.0071, [g]P=0.0065, [h]P=0.0010 versus *GG*.

Table IV. Blood pressure (BP) for male subjects according to *QPCT* genotype.

Characteristics	*CC*	*CT*	*TT*	*CC + CT*	*CT + TT*
Number of subjects (n=878)[a]	387	379	112	766	491
Age (years)	58.8±0.5	59.8±0.5	58.4±0.9	59.3±0.4	59.5±0.4
Systolic BP (mmHg)	119.5±0.9	122.4±0.9	121.7±1.7	121.1±0.6	122.3±0.8[b]
Diastolic BP (mmHg)	74.6±0.5	76.3±0.5	75.1±1.0	75.5±0.4	76.1±0.5[c]
Number of all subjects (n=1128)	487	501	140	988	641
Hypertension (%)	29.2[e]	39.5[e]	35.0[e]	34.4	38.5[d]

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *CC* and *CT* genotypes (*CC + CT*) were compared with those for individuals with the *TT* genotype (recessive genetic model). Data for the combined group of *CT* and *TT* genotypes (*CT + TT*) were compared with those for individuals with the *CC* genotype (dominant genetic model). [b]P=0.0233, [c]P=0.0434, [d]P=0.0010 versus CC. [e]P=0.0027 (*CC* versus *CT* versus *TT*, 3x2 Chi-square test).

genotypes. The differences in systolic and diastolic BP between individuals with the *GG* genotype and those in the combined group of *AA* and *AG* genotypes (expressed as a percentage of the larger value) were 3.7 and 4.0%, respectively. The prevalence of hypertension did not differ among *CNR2* genotypes for men. For women, neither systolic or diastolic BP nor the prevalence of hypertension differed among *CNR2* genotypes (data not shown).

Relation of the 160C→T (Arg54Trp) polymorphism of QPCT to BP. The distribution of genotypes for the160C→T polymorphism of *QPCT* was in Hardy-Weinberg equilibrium and age did not differ among genotypes for men (Table IV). Systolic and diastolic BP were significantly greater for men in the combined group of *CT* and *TT* genotypes than for those with the *CC* genotype; the differences in systolic and diastolic BP between these groups were 2.3 and 2.0%, respectively. The prevalence of hypertension also differed significantly among genotypes (*CC* versus *CT* versus *TT*), being greater for men in the combined group of *CT* and *TT* genotypes than for those with the *CC* genotype. The odds ratio of the *T* allele compared with the *C* allele for predisposition to hypertension was 1.3. There were no differences in systolic or diastolic BP or in the prevalence of hypertension among *QPCT* genotypes in women (data not shown).

Relation of the C→T (Pro198Leu) polymorphism of GPX1 to BP. Among men, the distribution of genotypes for the C→T (Pro198Leu) polymorphism of *GPX1* was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table V). Systolic and diastolic BP were significantly higher for men with the *CT* genotype or for those in the combined group of *CT* and *TT* genotypes than for those with the *CC* genotype. The differences in systolic and diastolic BP between individuals in the combined group of *CT* and *TT* genotypes and those with the *CC* genotype were 3.6 and 3.7%, respectively. The prevalence of hypertension did not differ among *GPX1* genotypes for men. Among women, no difference in systolic or diastolic BP or in the prevalence of hypertension was detected among *GPX1* genotypes (data not shown).

Relation of the 137,346T→C polymorphism of FYN to BP. For women, the distribution of genotypes for the 137,346T→C polymorphism of *FYN* was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table VI). Systolic and diastolic BP were significantly higher in women with the *TC* genotype or in those in the combined group of *TC* and *CC* genotypes than in those with the *TT* genotype. The differences in systolic and diastolic BP between individuals in the combined group of *TC* and *CC* genotypes and those

Table V. Blood pressure (BP) for male subjects according to *GPX1* genotype.

Characteristics	*CC*	*CT*	*TT*	*CC + CT*	*CT + TT*
Number of subjects (n=879)[a]	750	126	3	876	129
Age (years)	59.3±0.4	58.7±0.9	59.0±6.3	59.2±0.4	58.7±0.9
Systolic BP (mmHg)	120.5±0.6	125.0±1.5[b]	122.0±9.9	121.2±0.6	125.0±1.5[c]
Diastolic BP (mmHg)	75.0±0.4	78.0±0.9[d]	71.7±6.1	75.4±0.4	77.9±0.9[e]
Number of all subjects (n=1128)	971	154	3	1125	157
Hypertension (%)	34.0	37.7	0	34.5	36.9

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *CC* and *CT* genotypes (*CC + CT*) were compared with those for individuals with the *TT* genotype (recessive genetic model). Data for the combined group of *CT* and *TT* genotypes (*CT + TT*) were compared with those for individuals with the *CC* genotype (dominant genetic model). [b]P=0.0188, [c]P=0.0072, [d]P=0.0078, [e]P=0.0040 versus *CC*.

Table VI. Blood pressure (BP) for female subjects according to *FYN* genotype.

Characteristics	*TT*	*TC*	*CC*	*TT + TC*	*TC + CC*
Number of subjects (n=883)[a]	338	423	122	761	545
Age (years)	59.0±0.5	59.6±0.5	58.1±0.9	59.3±0.4	59.3±0.3
Systolic BP (mmHg)	117.9±1.0	121.6±0.9[b]	119.6±1.7	119.9±0.7	121.1±0.8[c]
Diastolic BP (mmHg)	71.5±0.6	73.8±0.5[d]	72.4±1.0	72.8±0.4	73.5±0.5[e]
Number of all subjects (n=1109)	426	532	151	958	683
Hypertension (%)	32.4	37.3	31.8	35.1	36.1

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *TT* and *TC* genotypes (*TT + TC*) were compared with those for individuals with the *CC* genotype (recessive genetic model). Data for the combined group of *TC* and *CC* genotypes (*TC + CC*) were compared with those for individuals with the *TT* genotype (dominant genetic model). [b]P=0.0217, [c]P=0.0138, [d]P=0.0132, [e]P=0.0104 versus *TT*.

Table VII. Blood pressure (BP) for male subjects according to *FYN* genotype.

Characteristics	*TT*	*TC*	*CC*	*TT + TC*	*TC + CC*
Number of subjects (n=875)[a]	339	409	127	748	536
Age (years)	59.0±0.5	59.4±0.5	59.1±0.9	59.2±0.4	59.4±0.4
Systolic BP (mmHg)	119.5±1.0	122.3±0.9	121.3±1.6	121.2±0.6	122.1±0.7[b]
Diastolic BP (mmHg)	74.7±0.6	75.8±0.5	76.1±0.9	75.3±0.4	75.9±0.5
Number of all subjects (n=1122)	441	527	154	968	681
Hypertension (%)	32.4	36.6	31.8	34.7	35.5

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *TT* and *TC* genotypes (*TT + TC*) were compared with those for individuals with the *CC* genotype (recessive genetic model). Data for the combined group of *TC* and *CC* genotypes (*TC + CC*) were compared with those for individuals with the *TT* genotype (dominant genetic model). [b]P=0.0344 versus *TT*.

with the *TT* genotype were 2.6 and 2.7%, respectively. The prevalence of hypertension did not differ among *FYN* genotypes for women.

For men, the distribution of *FYN* genotypes was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table VII). Systolic BP was significantly higher for men in the combined group of *TC* and *CC* genotypes than for those with the *TT* genotype; the difference in systolic BP between these groups was 2.1%. The prevalence of hypertension did not differ among *FYN* genotypes for men.

Relation of the $(GT)_n$ polymorphism of COL1A2 to BP. Given that the mean and median numbers of GT repeats for *COL1A2* in the study subjects were 14.2 and 12, respectively, we designated $(GT)_{n\leq12}$ and $(GT)_{n\geq13}$ as short (*S*) and long (*L*) alleles, respectively. The distribution of the *SS*, *SL*, and *LL*

Table VIII. Blood pressure (BP) for male subjects according to *COL1A2* genotype.

Characteristics	*SS*	*SL*	*LL*	*SS + SL*	*SL + LL*
Number of subjects (n=854)[a]	252	415	187	667	602
Age (years)	59.7±0.6	59.4±0.5	58.7±0.7	59.5±0.4	59.2±0.4
Systolic BP (mmHg)	123.1±1.1	120.4±0.9	120.6±1.3	121.4±0.7	120.5±0.7[b]
Diastolic BP (mmHg)	76.8±0.7	74.7±0.5[c]	75.4±0.8	75.5±0.4	74.9±0.4[d]
Number of all subjects (n=1095)	308	543	244	851	787
Hypertension (%)	33.3	34.6	36.1	34.2	35.1

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. *S*, short repeat allele [$(GT)_n \leq 12$]; *L*, long repeat allele [$(GT)_n \geq 13$]. Data for the combined group of *SS* and *SL* genotypes (*SS + SL*) were compared with those for individuals with the *LL* genotype (recessive genetic model). Data for the combined group of *SL* and *LL* genotypes (*SL + LL*) were compared with those for individuals with the *SS* genotype (dominant genetic model). [b]P=0.0460, [c]P=0.0341, [d]P=0.0178 versus *SS*.

Table IX. Blood pressure (BP) for male subjects according to *CAV1* genotype.

Characteristics	*II*	*ID*	*DD*	*II + ID*	*ID + DD*
Number of subjects (n=879)[a]	796	82	1	878	83
Age (years)	59.1±0.3	60.6±1.1	48.0	59.2±0.4	60.4±1.1
Systolic BP (mmHg)	120.8±0.6	125.0±1.9	126.0	121.2±0.6	125.1±1.9[b]
Diastolic BP (mmHg)	75.2±0.4	77.4±1.2	75.0	75.4±0.4	77.3±1.2
Number of all subjects (n=1128)	1028	99	1	1127	100
Hypertension (%)	34.2	36.4	0	34.4	36.0

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *II* and *ID* genotypes (*II + ID*) were compared with those for individuals with the *DD* genotype (recessive genetic model). Data for the combined group of *ID* and *DD* genotypes (*ID + DD*) were compared with those for individuals with the *II* genotype (dominant genetic model). [b]P=0.0325 versus *II*.

genotypes of *COL1A2* was in Hardy-Weinberg equilibrium and age did not differ among genotypes for men (Table VIII). Systolic BP was significantly higher in men with the *SS* genotype than in those in the combined group of *SL* and *LL* genotypes, whereas diastolic BP was significantly higher in men with the *SS* genotype than in those with the *SL* genotype or in those in the combined group of *SL* and *LL* genotypes. The differences in systolic and diastolic BP between individuals with the *SS* genotype and those in the combined group of *SL* and *LL* genotypes were 2.1 and 2.5%, respectively. The prevalence of hypertension did not differ among *COL1A2* genotypes for men. There were no differences in systolic or diastolic BP or in the prevalence of hypertension among *COL1A2* genotypes in women (data not shown).

Relation of the I/D (22,375delAC) polymorphism of CAV1 to BP. For men, the distribution of genotypes for the 22.375 I/D polymorphism of *CAV1* was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table IX). Systolic BP was significantly higher for men in the combined group of *ID* and *DD* genotypes than for those with the *II* genotype; the difference in systolic BP between these groups was 3.4%. The prevalence of hypertension did not differ among *CAV1* genotypes for men. For women, neither systolic or diastolic BP nor the prevalence of hypertension differed among *CAV1* genotypes (data not shown).

Relation of the -344C→T polymorphism of CYP11B2 to BP. The distribution of genotypes for the -344C→T polymorphism of *CYP11B2* was in Hardy-Weinberg equilibrium and age did not differ among genotypes for men (Table X). Systolic and diastolic BP were significantly higher in men with the *CT* genotype or with the *TT* genotype or in those in the combined group of *CT* and *TT* genotypes than in those with the CC genotype. The difference in systolic or diastolic BP between individuals in the combined group of *CT* and *TT* genotypes and those with the *CC* genotype was 4.9%. The prevalence of hypertension also differed significantly among genotypes (*CC* versus *CT* versus *TT*), being greater for men in the combined group of *CT* and *TT* genotypes than for those with the *CC* genotype. The odds ratio of the *T* allele compared with the *C* allele for predisposition to hypertension was 1.2. Although there were no differences in systolic or diastolic BP among *CYP11B2* genotypes in women (data not shown), the prevalence of hypertension differed among genotypes [*CC* (26.6%) versus *CT* (33.9%) versus *TT* (38.3%), P=0.0333], being greater for women in the combined group of *CT* and *TT* genotypes (36.0%) than for those with the *CC* genotype (26.6%, P=0.0272) as well as greater for women with the *TT* genotype (38.3%) than for those in the combined group of *CC* and *CT* genotypes (32.3%, P=0.0387). The odds ratio of the *T* allele compared with the *C* allele for predisposition to hypertension was 1.3.

Table X. Blood pressure (BP) for male subjects according to *CYP11B2* genotype.

Characteristics	*CC*	*CT*	*TT*	*CC + CT*	*CT + TT*
Number of subjects (n=876)[a]	109	418	349	527	767
Age (years)	59.5±1.0	59.1±0.5	59.2±0.5	59.2±0.4	59.1±0.4
Systolic BP (mmHg)	115.9±1.7	122.6±0.8[b]	121.1±0.9[c]	121.2±0.8	121.9±0.6[d]
Diastolic BP (mmHg)	72.2±1.0	76.2±0.5[e]	75.5±0.6[f]	75.3±0.5	75.9±0.4[g]
Number of all subjects (n=1125)	130	541	454	671	995
Hypertension (%)	23.9[i]	35.3[i]	36.6[i]	33.1	35.9[h]

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *CC* and *CT* genotypes (*CC + CT*) were compared with those for individuals with the *TT* genotype (recessive genetic model). Data for the combined group of *CT* and *TT* genotypes (*CT + TT*) were compared with those for individuals with the *CC* genotype (dominant genetic model). [b]P=0.0010, [c]P=0.0171, [d]P=0.0007, [e]P=0.0014, [f]P=0.0115, [g]P=0.0007, [h]P=0.0053 versus *CC*. [i]P=0.0188 (*CC* versus *CT* versus *TT*, 3 x 2 Chi-square test).

Table XI. Blood pressure (BP) for male subjects according to *ADRB1* genotype.

Characteristics	*AA*	*AG*	*GG*	*AA + AG*	*AG + GG*
Number of subjects (n=876)[a]	627	233	16	860	249
Age (years)	59.1±0.4	59.7±0.6	59.2±2.4	59.3±0.4	59.7±0.6
Systolic BP (mmHg)	120.6±0.7	122.6±1.1	125.9±4.3	121.1±0.6	122.8±1.1
Diastolic BP (mmHg)	75.0±0.4	76.4±0.7	79.5±2.7	75.4±0.4	76.6±0.7[b]
Number of all subjects (n=1125)	804	301	20	1105	321
Hypertension (%)	33.7	35.9	45.0	34.3	36.5

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *AA* and *AG* genotypes (*AA + AG*) were compared with those for individuals with the *GG* genotype (recessive genetic model). Data for the combined group of *AG* and *GG* genotypes (*AG + GG*) were compared with those for individuals with the *AA* genotype (dominant genetic model). [b]P=0.0430 versus *AA*.

Relation of the A→G (Ser49Gly) polymorphism of ADRB1 to BP. For men, the distribution of genotypes for the A→G polymorphism of *ADRB1* was in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table XI). Diastolic BP was significantly higher for men in the combined group of *AG* and *GG* genotypes than for those with the *AA* genotype, the difference in diastolic BP between these groups being 2.1%. The prevalence of hypertension did not differ among *ADRB1* genotypes for men. Although systolic and diastolic BP did not differ among *ADRB1* genotypes for women (data not shown), the prevalence of hypertension differed significantly among genotypes [*AA* (32.6%) versus *AG* (41.4%) versus *GG* (29.6%), P=0.0270], being greater for women in the combined group of *AG* and *GG* genotypes (40.3%) than for those with the *AA* genotype (32.6%, P=0.0156). The odds ratio of the *G* allele compared with the T allele for predisposition to hypertension was 1.3.

Relation of the -1213T→C polymorphism of ESR2 to BP. In men, the distribution of genotypes for the -1213T→C polymorphism of *ESR2* was not in Hardy-Weinberg equilibrium and age did not differ among genotypes (Table XII). Systolic and diastolic BP were significantly higher in men with the *TC* genotype or in those in the combined group of *TC* and *CC* genotypes than in those with the *TT* genotype. The differences in systolic and diastolic BP between individuals in the combined group of *TC* and *CC* genotypes and those with the *TT* genotype were 3.3 and 4.2%, respectively. The prevalence of hypertension did not differ among *ESR2* genotypes for men. For women, there was no difference in systolic or diastolic BP or in the prevalence of hypertension among *ESR2* genotypes (data not shown).

Discussion

The regulation of blood pressure involves the integration of a variety of biological systems that control the structure and tone of the vasculature as well as the volume and composition of body fluid. It also involves the adaptation of these systems to constantly changing physiological needs (16). We have now examined the relations of nine candidate gene polymorphisms to BP and the prevalence of hypertension in community-dwelling Japanese women and men. Our results show that the polymorphism of *FYN* was associated with systolic and diastolic BP in women as well as with systolic BP in men; the polymorphisms of *CNR2*, *QPCT*, *GPX1*, *COL1A2*, *CYP11B2*, and *ESR2* with systolic and diastolic BP in men; and those of *CAV1* and *ADRB1* with systolic and diastolic BP, respectively, in men. The polymorphisms of *QPCT* and *CYP11B2* were also associated with the prevalence

Table XII. Blood pressure (BP) for male subjects according to *ESR2* genotype.

Characteristics	*TT*	*TC*	*CC*	*TT* + *TC*	*TC* + *CC*
Number of subjects (n=879)[a]	773	97	9	870	106
Age (years)	59.4±0.3	57.4±1.0	61.7±3.3	59.2±0.4	57.7±0.9
Systolic BP (mmHg)	120.7±0.6	125.5±1.7[b]	117.2±5.7	121.2±0.6	124.8±1.7[c]
Diastolic BP (mmHg)	75.0±0.4	79.0±1.1[d]	70.9±3.5	75.5±0.4	78.3±1.0[e]
Number of all subjects (n=1128)	991	126	11	1117	137
Hypertension (%)	33.5	42.1	27.3	34.5	40.9

[a]Subjects not taking antihypertensive medication. Data for age and BP are means ± SE. Data for the combined group of *TT* and *TC* genotypes (*TT* + *TC*) were compared with those for individuals with the *CC* genotype (recessive genetic model). Data for the combined group of *TC* and *CC* genotypes (*TC* + *CC*) were compared with those for individuals with the *TT* genotype (dominant genetic model). [b]P=0.0257, [c]P=0.0212, [d]P=0.0014, [e]P=0.0027 versus *TT*.

of hypertension in men. These observations thus suggest that polymorphisms of *QPCT* and *CYP11B2* are determinants of BP and the development of hypertension in Japanese men.

Given that selection bias can influence the results of genetic association studies, it is important that study populations be genetically and ethnically homogeneous. Our study subjects were recruited randomly from individuals residing in the city of Obu and town of Higashiura in central Japan, where the population is thought to share the same ethnic ancestry and to possess a homogeneous genetic background. We also showed that, with the exception of *ESR2*, the genotype distributions of the examined polymorphisms were in Hardy-Weinberg equilibrium in the study population. We thus appeared to avoid admixture and selection bias.

We detected associations of all nine polymorphisms with BP in men, whereas only the *FYN* polymorphism was associated with BP in women. The reason for this sex difference remains unclear. It might, however, be attributable, at least in part, to the difference in the plasma concentration of estrogen between men and women, given that estrogen exerts various favorable effects on vasomotor function, including stimulation of the production of nitric oxide and prostaglandin I_2 as well as inhibition of the release of endothelin-1 by vascular endothelial cells (17).

The formation of amino-terminal pyroglutamate from its glutaminyl precursor is an important posttranslational or cotranslational event in the processing of numerous bioactive neuropeptides, hormones, and cytokines during their maturation in the secretory pathway. These regulatory peptides require the amino-terminal pyroglutamate to develop the correct conformation for binding to their receptors or to protect their amino termini from exopeptidase-mediated degradation (18,19). The glutaminyl cyclases are acyltransferases that catalyze this posttranslational modification (20,21). They are abundant in mammalian neuroendocrine tissues, such as the hypothalamus and pituitary gland (21,22), and are highly conserved from yeast to humans. In humans, the glutaminyl-peptide cyclotransferase (glutaminyl cyclase) gene (*QPCT*) is located at chromosomal position 2p22.2. Ezura *et al* (23) examined the relations of 13 polymorphisms in this region to bone mineral density (BMD) in adult women and detected associations between the genotypes of six polymorphisms and BMD for the radius. The 160C→T (Arg54Trp) polymorphism of *QPCT* showed the most pronounced association, with the *T* allele being associated with low BMD. These results indicate that genetic variation in *QPCT* is an important determinant of BMD in adult women and may therefore contribute to susceptibility to osteoporosis. We have now shown that the 160C→T (Arg54Trp) polymorphism of *QPCT* was associated both with systolic and diastolic BP and with the prevalence of hypertension in Japanese men, with the *T* allele being associated with high BP. The effect of this polymorphism on gene expression or protein function has not been determined. This is the first demonstration of an association of this polymorphism of *QPCT* with BP or the prevalence of hypertension, but the underlying molecular mechanism of the association remains to be elucidated.

The cytochrome P450, subfamily Y XIB, polypeptide 2 (aldosterone synthase) gene (*CYP11B2*) encodes an enzyme that participates in the terminal steps of aldosterone synthesis in the zona glomerulosa cells of human adrenal glands, and its expression is regulated by angiotensin II and potassium (24). The candidacy of this gene in the present study is based on its pathogenic role in the syndrome of glucocorticoid-remediable aldosteronism (25). Several common polymorphisms of *CYP11B2* have been described (26-28). The -344C→T polymorphism, which is located in a putative binding site for a steroidogenic transcription factor, has been associated with hypertension (29-31) and with other hypertensive intermediate phenotypes such as plasma aldosterone level (32), urinary aldosterone excretion rate (30), and the aldosterone/renin ratio (27,28). Although some studies have not confirmed these relations (33,34), this locus may be important in the regulation of BP and the development of hypertension (35). We have now shown that the -344C→T polymorphism of *CYP11B2* was associated with both systolic and diastolic BP and the prevalence of hypertension in Japanese men, with the *T* allele being associated with high BP. Our results are thus consistent with previous observations (29-31).

Given the multiple comparisons of genotypes with BP or the prevalence of hypertension in the present study, it is not possible to completely exclude potential statistical errors such as false positives. It is also possible that one or more of the polymorphisms associated with BP or the prevalence of hypertension in our study are in linkage disequilibrium with

other polymorphisms of the same genes or of nearby genes that are actually responsible for the development of high BP. Furthermore, the relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study.

In conclusion, our results implicate *QPCT* and *CYP11B2* in the regulation of BP and the development of hypertension in Japanese men. Determination of genotypes for these polymorphisms may prove informative for assessment of the genetic risk for hypertension and may contribute to the personalized prevention of this condition. Given that multiple variants, each having a small effect, will likely ultimately be found to be responsible for a large fraction of the genetic component of essential hypertension, identification of additional hypertension susceptibility genes will allow more accurate assessment of the genetic component of this condition.

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Effects of the interaction between lean tissue mass and estrogen receptor α gene polymorphism on bone mineral density in middle-aged and elderly Japanese

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Abstract

Because both genetic and environmental factors influence bone mass, it is important to examine the effect of gene-environment interactions on bone mineral density (BMD) for the prevention of osteoporosis at an individual level. Estrogen receptor α (ERα) plays an important role in increasing BMD via mechanical strain and muscle mass is a reflection of the forces the muscle applies to the bone. The aim of this study is to investigate the effect of the interaction between lean tissue mass (LTM) and the ERα polymorphisms T→C (*Pvu*II) [dbSNP: rs2234693] and A→G (*Xba*I) [dbSNP: rs9340799] on BMD in middle-aged and elderly individuals. Subjects were 2209 community-dwelling Japanese men and women, ages 40 to 79 years. ERα polymorphisms in the first intron, T→C and A→G were identified and lumbar spine and femoral neck BMD and LTM were measured by dual-energy X-ray absorptiometry. Both T→C and A→G polymorphisms were divided into two genotype groups (TT vs. TC/CC; AA vs. AG/GG). In postmenopausal women, the effect of LTM on femoral neck BMD was significantly larger for those with the TC/CC genotype than for those with the TT genotype for the T→C polymorphism, and larger for those with the AG/GG genotype than for those with the AA genotype for the A→G polymorphism. This gene–LTM interaction was observed at the femoral neck, but not at the lumbar spine. For men and premenopausal women, no gene–LTM interaction was found. In conclusion, there was an interaction between LTM and the ERα T→C and A→G polymorphisms with respect to their effect on femoral neck BMD in postmenopausal women and those with the TC/CC and AG/GG genotypes had larger effects of LTM than those with TT and AA genotypes.

Keywords: Single nucleotide polymorphism; Estrogen receptor alpha; BMD; Lean tissue mass; Postmenopausal women

Introduction

It is generally accepted that dynamic loading acts as an osteogenic stimulus [1] and that the forces applied to bone are primarily the result of muscular contraction [2]. Therefore, muscular weakness is an important factor contributing to osteoporosis [3]. The importance of skeletal muscle in preserving bone [4] and the relation between low skeletal mass and poor structural parameters of bone in elderly men [5] have been reported. A previous study suggested that physical exercise maintains bone mineral density (BMD) in postmenopausal women [6]. Vainionpaa et al. showed that the intensity of exercise was significantly correlated with BMD changes [7] and Kerr et al. reported that postmenopausal bone mass can be significantly increased by strength training, but not by endurance training [8].

Animal studies have suggested that mechanical strain stimulates osteoblast proliferation through estrogen receptor α (ERα) [9], and osteoblast-like cells from ERα knockout mice have deficient responses to mechanical strain [10]. Thus, it is thought that ERα plays an important role in increasing BMD via mechanical strain [11,12]. Although the association between ERα genotype and the risk of osteoporosis in humans remains controversial [13], many studies have suggested a

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relation between ERα polymorphism and BMD [14–16]. A study previously carried out in our laboratory also showed that the ERα gene was a susceptibility locus for reduced bone mass, especially at the femoral neck, in elderly Japanese women [17].

Because the effects of environment on individuals might differ in accordance with individuals' different genetic make-ups, it is important to examine the effects of the gene-environment interaction on BMD, particularly for the prevention of osteoporosis at an individual level. Some studies have investigated the effect of ERα polymorphism on the relationship between exercise and BMD. These studies have shown an effect of the ERα gene (*Pvu*II)–exercise interaction on BMD in middle-aged men [18] and prepubertal and early pubertal girls [19].

Because magnetic resonance imaging (MRI)-measured muscle area correlates with muscle strength [20], and the differences between MRI-measured and dual-energy X-ray absorptiometry (DXA)-predicted skeletal muscle mass are small [21], DXA-predicted total body lean mass can be legitimately used as an index of skeletal load. As mentioned above, a few studies have investigated the effects of the ERα gene–exercise interaction on BMD. However, the effects of the ERα gene–lean tissue mass (LTM) interaction were unknown. Furthermore, these previous studies involved single-sex populations within a limited age range. This study investigated for the first time the effects of the interaction between LTM and the typical ERα polymorphisms T→C (*Pvu*II) and A→G (*Xba*I) on BMD in both men and women in a large population.

Materials and methods

Subjects

Study subjects were 1119 men and 1090 women, ages 40–79 years, who participated in the first wave (from April 1998 to March 2000) of the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA), which is a population-based prospective cohort study of aging and age-related diseases. Participants in the NILS-LSA were randomly selected age and sex stratified individuals selected from the pool of independent residents in the NILS neighborhood, Obu city and Higashiura town, Aichi Prefecture, central Japan. Details of the NILS-LSA have been given elsewhere [22]. The study protocol was approved by the Committee of Ethics of Human Research of the National Center for Geriatrics and Gerontology. Written informed consent was obtained from all subjects.

Anthropometric variables

Body weight was measured to the nearest 0.01 kg using digital scales, height was measured to the nearest 0.1 cm using a stadiometer, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Menstrual status

Menopause was confirmed as the absence of menses by a questionnaire.

Dual-energy X-ray absorptiometry

Whole-body fat mass, LTM, bone mineral content (BMC), and BMD of the femoral neck and lumbar spine (L2–4) were assessed by DXA (QDR-4500; Hologic, Madison, OH, USA). Lean tissue mass is equal to the fat-free mass minus BMC, and is assumed to be an index of the amount of muscle mass.

ERα genotype analysis

DNA was extracted from peripheral blood lymphocytes by using a standard procedure. ERα genotypes were determined in accordance with a study by Yamada et al. [17] The ERα genotypes were analyzed by using an automated fluorescent allele-specific DNA primer assay system (Toyobo Gene Analysis, Tsuruga, Japan). To determine the T→C (*Pvu*II) genotype, the polymorphic region of the gene was amplified by polymerase chain reaction (PCR) using allele-specific sense primers labeled at the 5′ end either with fluorescein isothiocyanate (5′-AGTTCCAAATGTCCCAGXTG-3′) or with Texas red (5′-AGTTCCAAATGTCCCAGXCG-3′) and an antisense primer labeled at the 5′ end with biotin (5′-TCTGGGAAACAGAGACAAAGC-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 1U DNA polymerase (rTaq; Toyobo, Osaka, Japan) in rTaq buffer. The amplification protocol consisted of initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 62.5 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 2 min. For determination of the A→G (*Xba*I) genotype, the polymorphic region of the gene was amplified by PCR using a sense primer labeled at the 5′ end with biotin (5′-CTGTTTCCCA-GAGACCCTGAG-3′) and allele-specific antisense primers labeled at the 5′ ends either with fluorescein isothiocyanate (5′-CCAATGCTCAT-CCCAACTXTA-3′) or with Texas red (5′-CCAATGCTCATCCCAACTXCA-3′). The reaction mixture (with the exception of the primers) and the amplification protocol (with the exception that the annealing temperature was 65 °C) were identical to those used for genotyping of the T→C polymorphism.

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 placed on a magnetic stand and the supernatant was discarded. After two washings, 0.01 M NaOH was added to the wells and mixed well. The plate was placed on a magnetic stand again and the supernatants were transferred to the wells of a new 96-well plate. The fluorescence was measured by using 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 584 nm and 612 nm, respectively, for Texas red.

Haplotype analysis

The haplotype distribution was calculated by using Haplotyper, a software program for haplotype inference, with the Bayesian algorithm [23,24].

Statistical analysis

Values are expressed as the mean±standard error (SE). The chi-squared test was used to identify significant departures from Hardy-Weinberg equilibrium. Both T→C and A→G polymorphisms were divided into two genotype groups (TT vs. TC/CC; AA vs. AG/GG). The differences between genotype groups were analyzed using one-way analysis of variance and the Tukey–Kramer post hoc test. A general linear model was employed to evaluate the effect of the LTM–genotype interaction on BMD (adjusted for age and BMI). When the effect of the interaction on BMD was significant for both T→C and A→G polymorphisms, further analysis (in accordance with haplotype groups) was

Table 1
Distribution of T→C and A→G genotypes of the ERα gene

	AA		AG		GG		Total	
	n	%	*n*	%	*n*	%	*n*	%
TT	787	35.6	1	0.1	0	0.0	788	35.7
TC	584	26.4	465	21.1	5	0.2	1054	47.7
CC	120	5.4	174	7.9	73	3.3	367	16.6
Total	1491	67.5	640	29.0	78	3.5	2209	100.0

Table 2
Physical characteristics of subjects with reference to the T→C and A→G genotypes of the ERα gene

	Men (n=1119)		Premenopausal women (n=278)		Postmenopausal women (n=812)	
	TT (n=398)	TC/CC (n=721)	TT (n=98)	TC/CC (n=180)	TT (n=292)	TC/CC (n=520)
Age (years)	58.9±0.6	59.3±0.4	46.2±0.5	46.2±0.3	62.8±0.5	64.6±0.4*
Weight (kg)	62.9±0.5	62.2±0.3	53.9±0.8	54.7±0.6	52.5±0.5	51.7±0.4
BMI (kg/m2)	23.2±0.1	22.9±0.1	22.5±0.3	22.9±0.2	23.1±0.2	23.0±0.2
LTM (kg)	47.2±0.3	46.6±0.2	36.3±0.4	36.5±0.3	33.9±0.2	33.7±0.2
L2–4 BMD (kg/cm^2)	0.99±0.01	0.98±0.01	1.03±0.01	1.02±0.01	0.82±0.01	0.80±0.01
Femoral neck BMD (g/cm^2)	0.76±0.01	0.75±0.004	0.78±0.01	0.77±0.01	0.66±0.01	0.64±0.004*
	AA (n=769)	AG/GG (n=350)	AA (n=192)	AG/GG (n=86)	AA (n=530)	AG/GG (n=282)
Age (years)	59.2±0.4	59.1±0.5	46.3±0.3	46.0±0.5	63.7±0.4	64.2±0.5
Weight (kg)	62.7±0.3	61.9±0.5	53.5±0.5	56.4±1.0	51.9±0.3	52.2±0.5
BMI (kg/m^2)	23.1±0.1	22.8±0.1	22.3±0.2	23.7±0.4**	22.9±0.1	23.3±0.2
LTM (kg)	47.0±0.2	46.5±0.3	36.1±0.3	37.0±0.5	33.8±0.2	33.7±0.2
L2–4 BMD (kg/cm^2)	0.99±0.01	0.97±0.01	1.03±0.01	1.02±0.01	0.81±0.01	0.81±0.01
Femoral neck BMD (g/cm^2)	0.75±0.004	0.75±0.01	0.77±0.01	0.78±0.01	0.65±0.004	0.64±0.01

Data are mean±SE. *p<0.05 vs. TT genotype, **p<0.01 vs. AA genotype.

carried out. Values of p<0.05 were considered to indicate statistical significance. Data were analyzed with the Statistical Analysis System (SAS) release 8.2 (SAS Institute Inc., Cary, NC, USA).

Results

Distribution of ERα genotypes

The distribution of genotype combinations was examined (Table 1). The distributions of ERα T→C and A→G genotypes were both in Hardy–Weinberg equilibrium. There were no subjects with the TT and GG genotypic combination and few with the TT/AG or TC/GG genotypic combination.

Physical characteristics

Physical characteristics of the subjects were compared with reference to the ERα T→C and A→G genotype groups (Table 2). For men and premenopausal women, age, weight, BMI, LTM, L2–4 BMD, and femoral neck BMD did not differ between subjects with the TT and TC/CC genotypes. In contrast, in postmenopausal women, age was significantly higher and femoral neck BMD was significantly lower in individuals with the TC/CC genotype than in those with the TT genotype. After adjusting for age, statistical significance was not achieved for the difference in femoral neck BMD in postmenopausal women (data not shown). In men and postmenopausal women, there were no differences in age and physical characteristics between subjects with the AA and AG/GG genotypes. In premenopausal women, age, weight, LTM, and BMD did not differ between subjects with the AA and AG/GG genotypes, whereas BMI was significantly greater in those with the AG/GG genotype than in those with the AA genotype. After adjusting for BMI, the relationship of L2–4 and femoral neck BMD between AA and AG/GG genotypes still did not show a significant difference in premenopausal women (data not shown).

ERα genotype and association between LTM and BMD

To investigate whether an interaction between ERα genotype and LTM had an effect on L2–4 and femoral neck BMDs, general linear models for BMD were analyzed using LTM, ERα

Table 3
General linear model for bone mineral density (BMD) with interaction between the ERα genotype and LTM

Dependent variables	Independent variables	Men		Premenopausal women		Postmenopausal women	
		F	p value	F	p value	F	p value
L2–4 BMD	LTM	45.65	<0.0001	24.73	<0.0001	25.53	<0.0001
	T→C genotype	0.91	ns	1.36	ns	2.41	ns
	LTM×(T→C genotype)	0.83	ns	1.29	ns	2.55	ns
Femoral neck BMD	LTM	63.90	<0.0001	15.07	<0.0001	25.35	<0.0001
	T→C genotype	0.03	ns	0.13	ns	8.15	0.004
	LTM×(T→C genotype)	0.03	ns	0.06	ns	7.48	0.007
L2–4 BMD	LTM	45.27	<0.0001	24.36	<0.0001	25.41	<0.0001
	A→G genotype	0.10	ns	0.16	ns	2.20	ns
	LTM×(A→G genotype)	0.05	ns	0.26	ns	2.14	ns
Femoral neck BMD	LTM	64.07	<0.0001	14.95	<0.0001	24.95	<0.0001
	A→G genotype	0.38	ns	0.07	ns	8.15	0.004
	LTM×(A→G genotype)	0.45	ns	0.05	ns	8.03	0.005

ns=not significant. Adjusted for age and BMI.

genotype, and the interaction between ERα genotype and LTM as independent variables, adjusting for age and BMI (Table 3). Lean tissue mass was significantly associated with L2–4 and femoral neck BMDs in both sexes and irrespective of menstrual status. In postmenopausal women, genotype and the interaction between genotype and LTM were significantly associated with femoral neck BMD for both the T→C and A→G genotypes, but not with L2–4 BMD. In men and premenopausal women, the effects of genotype and the interaction between genotype and LTM on BMD were not significant.

To clarify the influence of LTM on femoral neck BMD for T→C and A→G ERα genotypes in postmenopausal women, a general linear model for BMD was analyzed by each genotype, using LTM as an independent variable, adjusting for age and BMI. Fig. 1 shows the regression lines between femoral neck BMD and LTM for the ERα T→C and A→G genotype groups in postmenopausal women. For the T→C genotype (Fig. 1A), the slope was significantly larger ($p<0.01$) for TC/CC (slope=0.0071, $p<0.0001$) than for TT individuals (slope=0.0015, not significant). For the A→G genotype (Fig. 1B), the slope was significantly larger ($p<0.01$) for AG/GG (slope=0.0081, $p<0.0001$) than for AA individuals (slope=0.0033, $p=0.012$).

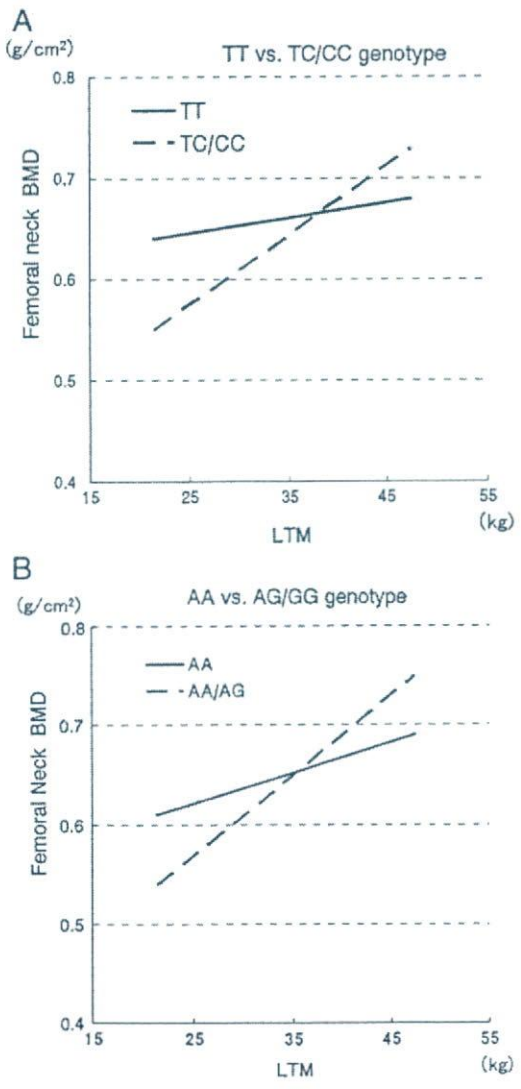

Fig. 1. Relationship between femoral neck BMD and LTM with reference to ERα T→C and A→G genotypes in postmenopausal women, adjusted for age and BMI. (A) T→C genotype. Solid line, TT genotype; Dotted line, TC/CC genotype. (B) A→G genotype. Solid line, AA genotype; Dotted line, AG/GG genotype.

Table 4
Physical characteristics of postmenopausal women with reference to ERα T→C and A→G haplotype

	Haplotype		
	TA	CA	CG
N	965	349	307
Age (years)	63.4±0.3	64.8±0.5*	64.3±0.5
Weight (kg)	52.2±0.3	51.6±0.4	51.8±0.5
BMI (kg/m²)	23.0±0.1	22.9±0.2	23.2±0.2
LTM (kg)	33.8±0.1	33.7±0.2	33.6±0.2
BMD L2–4 (g/cm²)	0.81±0.004	0.80±0.01	0.80±0.01
BMD femoral neck (g/cm²)	0.65±0.003	0.64±0.01	0.64±0.01

Data are mean±SE. *$p<0.05$ vs. TA.

ERα haplotype and association between LTM and BMD in postmenopausal women

Because there were significant genotype-LTM interactions on femoral neck BMD for both T→C and A→G polymorphisms in postmenopausal women, further analysis was carried out to evaluate the effect of the haplotype–LTM interaction. The distribution of haplotypes is shown in Table 4. The possible haplotype combinations for the ERα T→C and A→G polymorphisms were TA, CA, TG, and CG, but very few subjects had the TG haplotype. For postmenopausal women, the number of TA haplotype was 965; CA was 349; and CG was 307. Physical characteristics and BMD were compared with reference to these three haplotypes (Table 4). Age was significantly higher for those with the CA haplotype than for those with the TA haplotype. Weight, BMI, LTM, and L2–4 and femoral neck BMDs did not differ among haplotypes.

To clarify the influence of the interaction between ERα haplotype and LTM on femoral neck BMD in postmenopausal women, general linear models for BMD were analyzed using LTM, ERα haplotype, and the interaction between ERα haplotype and LTM as independent variables, adjusting for age and BMI (Table 5). Lean tissue mass, haplotype, and the interaction between haplotype and LTM were significantly

Table 5
General linear model for femoral neck BMD with interactions between ERα haplotype and LTM in postmenopausal women

Dependent variables	Independent variables	Postmenopausal women	
		F	*p*
Femoral neck BMD	LTM	49.80	<0.0001
	haplotype	6.63	0.001
	LTM × haplotype	6.23	0.002

Adjusted for age and BMI.

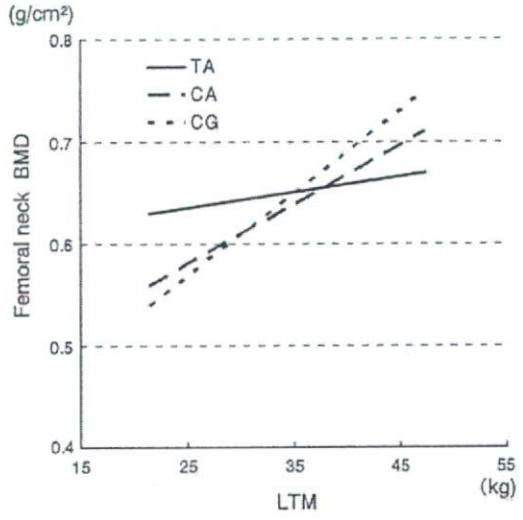

Fig. 2. Relationship between femoral neck BMD and LTM with reference to ERα haplotype in postmenopausal women, adjusted for age and BMI. Solid line, TA haplotype; Dotted line, CA haplotype; Fine dotted line, CG haplotype.

associated with femoral neck BMD. To evaluate the extent of the influence of LTM on BMD with respect to different haplotypes, general linear models were analyzed by each haplotype, using LTM as an independent variable, adjusting for age and BMI. Fig. 2 presents the relationship between femoral neck BMD and LTM with respect to the ERα haplotypes in postmenopausal women. The slope was significantly larger ($p<0.01$) for subjects with the CG (slope=0.0081, $p<0.0001$) and CA (slope=0.0063, $p<0.0001$) haplotypes than for those with the TA haplotype (slope=0.0035, $p=0.0003$), but there was no difference between the CG and CA haplotypes.

Discussion

We found that ERα polymorphisms influence the relationship between LTM and femoral neck BMD in postmenopausal women and that the effect of LTM on BMD was significantly larger in individuals with the TC/CC genotype of the T→C polymorphism than in those with the TT genotype, and larger in those with the AG/GG genotype of the A→G polymorphism than in those with the AA genotype. Haplotype analysis revealed that the effect was significantly larger for those with the CG and CA haplotypes than for those with the TA haplotype. This is the first study to investigate the effect of ERα gene–LTM interaction on BMD and to reveal the significant interaction in postmenopausal women.

In this study, a significant gene–LTM interaction in postmenopausal women was found at the femoral neck, but not at the lumber spine. It has been reported that hip joint compression forces reach 2.5 to 3 times body weight during walking [25,26]. The significant results found only at the femoral neck could be explained by the fact that high loading occurs at this site even in ordinary daily life.

We also analyzed data for different combinations of genotypes (TC/TT vs. CC; AG/AA vs. GG) (data not shown). In premenopausal women, no significant gene–LTM interaction was found. In postmenopausal women, significant interaction was found at femoral neck between TT/TC and CC genotype groups; however, no significant genotype–LTM interaction was found between GG and AA/AG genotype groups. This might be due to the small number in the GG genotype group ($n=28$). In men, when divided into TT/TC and CC genotypes, significant interaction was found at L2–4 and the femoral neck (L2–4, $p=0.04$; femoral neck, $p=0.02$) and the effect of LTM on BMD was larger for those with CC genotype than for those with TC/TT genotype. However, these significant interactions in men were weak in spite of the large number and the coefficients of determination (R^2) in the analysis model were low in men compared with postmenopausal women (men at the femoral neck, 0.28; men at L2–4, 0.18; postmenopausal women at the femoral neck, 0.38). Therefore, there might be other related factors in men and we considered that these results in men are insufficient to draw a clear conclusion about the effect of ERα gene–LTM interaction between TT/TC and CC genotype groups. We will examine this problem by adding other factors and analyzing the data longitudinally.

There have been some human studies investigating the effects of ERα gene polymorphism on exercise-induced effects on BMD. In a 4-year exercise intervention study, Remes et al. [18] reported that middle-aged Finnish men with the Pp (TC) or PP (CC) ERα genotype had increased lumbar spine BMD values. In the study of Remes et al., the subjects were middle-aged men, and the exercise intervention group spent 45–60 min on prescribed aerobic exercise five times a week for 4 years. Because we did not intervene as in the research of Remes et al., there might not have been a significant interaction between TT and TC/CC genotype groups in men.

Suuriniemi et al. [19] found that prepubertal and early pubertal Finnish girls with the Pp (TC) ERα genotype and high levels of physical activity had significantly higher bone mass and BMD at loaded bone sites. The subjects were 10- to 13-year-old prepubertal and early pubertal girls, whose estrogen concentrations were low, like those of postmenopausal women. A previous report indicated that estrogen can affect bone strength and mass by lowering the remodeling threshold, and that loss of estrogen would raise the threshold and help cause postmenopausal bone loss [27]. ERα expression in osteoblasts and osteocytes depends on estrogen concentration [28]. The increase in the potential of mechanical loading to stimulate bone gain in the peripubertal period is associated with marked increases in serum estrogen [11]. Thus, in the peripubertal period, when estrogen concentrations are high, the response to mechanical loading might be greater than in the prepubertal and postmenopausal periods, when estrogen concentrations are low. However, in the present study, significant interactions were found for postmenopausal women and in the study of Suuriniemi et al. [19] significant interactions were found for prepubertal and early pubertal girls. Accordingly, the effect of the gene–LTM interaction on BMD, that is, differences between individuals with different single nucleotide polymorphisms, might more readily appear in groups with low estrogen concentrations than in those with high concentrations.

In the study of Suuriniemi et al. [19], the interaction was found only for individuals with the Pp (TC) genotype (heterozygotes). This was not in agreement with our results. In this previous study, girls with low levels of physical activity bearing the Pp genotype had lower values for bone parameters compared with other groups. Because there are differences in the subject characteristics, age, sex, lifestyle, and study design, it is difficult to simply compare the results of the present study with those of the study by Suuriniemi et al. Further investigations are necessary to clarify these differences.

The mechanisms by which the ERα T→C and A→G polymorphisms might affect the femoral neck BMD are not clear, because the affected regions lie in an intronic and non-functional area of the gene. However, single nucleotide polymorphisms are usually linked to each other, so the two polymorphisms in intron 1 may be in linkage disequilibrium with causal polymorphisms elsewhere in the ERα gene or in genes nearby. In this regard, it is known that the T→C and A→G polymorphisms are in linkage disequilibrium with an upstream TA repeat polymorphism in the promoter region of the ERα gene [29]. An association between the TA repeat polymorphism and BMD has been shown in postmenopausal Japanese [30] and Italian women [29]. The number of TA repeats could be important in ERα gene transcription [31].

There are conflicting results regarding the association between ERα genotype and BMD in previous studies. Gene–LTM interactions might be one of the reasons for these differing results. That is, differences in the amount of muscle mass might change the association between ER genotype and BMD. For example, as shown in Fig. 1A, it is considered that in the group with low LTM, postmenopausal women with the TT genotype for T→C polymorphism have higher femoral neck BMD than those with the CC genotype. Conversely, in the group with high LTM, postmenopausal women with the CC genotype have higher BMD than those with the TT genotype.

In the present study, we evaluated the relationship between LTM and BMD with reference to ERα genotype, but we did not evaluate cause and effect directly. A previous exercise intervention study [18] showed an interaction between ERα genotype and exercise (i.e. the effect of mechanical loading) on BMD in men. Because LTM correlates with muscle strength [20] and it can be used as an index of skeletal load, the result of this previous exercise intervention study supports our present results regarding the gene–LTM interaction.

The strengths of the present study are the large sample size, the inclusion of both sexes, and the wide range of ages. Previous research has evaluated only one sex and a limited age range [18,19]. So far, the ERα gene–environment interaction concerning mechanical loading has not been investigated in postmenopausal women, who are particularly susceptible to osteoporosis. In the present study, both T→C and A→G ERα polymorphisms were examined and haplotype analysis was carried out.

As already described, previous studies have investigated the effects of ERα gene polymorphism on exercise-induced effects on BMD, but there has been no study that has evaluated the effect of the ERα gene–LTM interaction on BMD. The well-known phenomenon of reduction in muscle mass with aging is known as sarcopenia. Recent studies have reported a high prevalence of sarcopenia in postmenopausal woman with osteoporosis [32]. It would be very useful to identify individuals in this group who would experience a marked effect from increasing muscle mass, and the results of this study might assist in developing this process.

ERα plays an important role in the increase of BMD via mechanical strain [9–11]. A recent study has suggested that the effect of chronic immobility might be more marked on bone formation than bone resorption [33]. On the basis of our results, we can speculate that the influence of the mechanical loading increase on BMD via ER is different according to the ERα T→C and A→G polymorphisms in postmenopausal women.

In conclusion, there was an interaction between LTM and the T→C and A→G ERα polymorphisms with respect to their effect on femoral neck BMD in postmenopausal women and those with the TC/CC and AG/GG genotypes had larger effects of LTM than those with TT and AA genotypes.

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