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特集 老年医学の展望

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老化および老年病の疫学的研究

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KEY WORD

老化
老年病
疫学
ゲノム研究
長期縦断疫学研究

POINT

- 老化を観察し老年病の成因を明らかにするために長期縦断疫学研究が必要である.
- 老化を目標にした長期縦断疫学研究は膨大な費用と時間を要するため，世界的にみても今までほとんど行われていなかった.
- これからの長期縦断疫学研究には，分子疫学の手法も取り入れた新しい方法論が必要となる.

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はじめに

老化および老年病の疫学的研究には，老化に関連する健康問題の検討と，正常な老化による変化を観察するという２つの大きな目的がある[1-3]．老年病や運動機能障害などの発症のリスクファクターについての検討を目的とした調査，老年病の予防とその判定，健康を守り，長寿を全うするための生活指針を探る健康医学的研究，寿命を規定する要因の検討などが，老化に関連した健康問題の研究として特に重要である.

加齢とともに様々な生体機能は低下していく．正常な老化の過程を明らかにし，また老化の研究での共通する基礎資料として加齢による身体機能や精神活動の変化についての詳細なデータを集積していくことも極めて重要である．例えば加齢による検査値の変化についての基準値作成は，高齢者の診療に当たって欠くことができないものである．こうした疫学研究の方法論は老年学，老年医学の最も基本をなすものであるといってよい.

研究の実際の方法としては，大きく分けて横断的方法と縦断的方法の２つがある[4]．前述のように若年者から高齢者まで，なるべく多数の集団で種々の検査を一度に実施し，検討を行う方法が横断的研究である．一方，縦断的研究は同一の個人を継続して観察し，加齢による実際の変化，加齢に関連する要因，寿命などをとらえようとするものである．縦断的研究は長期にわたっての継続が必要で，一度の調査で終了してしまう横断的研究に比べて実施が困難であることが多い.

老化の縦断的研究

経時的な追跡を行う縦断的研究は横断的方法に比べて，結論が出るまでに一般に10年以上もの期間を要し，調査を継続するための費用や人材の確保も必要である．しかし，老化の観察を行うためには，後述するように横断的観察の

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表1　コホート研究と老化の縦断研究の比較

	コホート研究	老化の縦断研究
目的	曝露要因とエンドポイントの因果関係を証明	検査値の縦断的変動を観察
対象者数	曝露要因に関する有意差を得るのに十分な数のエンドポイント発症者が生ずる数．比較的稀な疾患をエンドポイントにすれば，膨大な対象者数が必要	検査値の縦断的変動が有意となる数で，通常数千人の範囲
開始時検査項目	曝露要因に限って実施	加齢に関連する詳細な項目
追跡検査項目	エンドポイントを追跡	詳細な検査項目を繰り返し実施
追跡期間	曝露要因に関する有意差を得るのに十分な数のエンドポイント発症者が生ずる期間	世代が交代する 30 年間をめどに
多施設協同研究	限られた共通の検査を実施しエンドポイントに関する追跡を多数の対象者に行うことは多施設協同研究に適している	多くの詳細な検査項目を多数の施設で，全く同じ方法，精度で行うのは事実上不可能
実施方法	調査項目を絞り，できるだけ多数の対象を調査	対象者数を絞り，できるだけ詳細な検査項目を実施

みでは，多くのバイアスを生じることがあり，加齢による変化を正確にとらえることができない．このため，加齢研究には縦断的方法が欠かせない．同一対象者に同じ検査項目を一定期間ごとに繰り返し行い，加齢による検査値の縦断的変動を観察する老化の縦断的研究は，正常な老化過程の評価の基礎データとして極めて重要である[5]．

縦断的方法を用いて，疾患や死亡などのリスクファクターを検討する研究方法にコホート研究がある．正常な老化の過程を観察するための縦断的研究と疾病のリスクファクターを探ることを目的としたコホート研究は，その方法や対象が大きく異なることに注意せねばならない．老化の縦断的研究は繰り返し検査を行い，検査値の縦断的変動を観察することが重要であり，コホート研究は曝露要因と疾病の罹患や死亡などのエンドポイントとの因果関係を求めるものである．このため老化の縦断的研究では，対象者数は検査値の縦断的変動が有意となる数で，通常数千人の範囲となるが，コホート研究では曝露要因に関する有意差を得るのに十分な数のエンドポイントの発症者が生ずる数の対象者が必要であり，比較的稀な疾患をエンポイントにすれば，数十万人の対象者数が必要となることもある．コホート研究では調査項目を絞り，できるだけ多数の対象を調査することが望ましく，一方，老化の縦断研究では対象者数を絞り，できるだけ詳細な老化に関連する検査を実施することが望ましい．多施設共同研究は限られた共通の検査を実施し，エンドポイントに関する追跡を多数の対象者に行うコホート研究には適しているが，老化の縦断的研究の場合，多くの詳細な検査項目を多数の施設で，全く同じ方法，同じ精度で行うのは事実上不可能であり，多施設共同研究として実施するのは極めて困難である(表1)．

縦断的方法がなぜ必要か

高齢者は長期間，数々の致命的な疾患に罹らずにきたエリートである．死亡に結びつく様々

表2　国内外の代表的な老化の縦断的研究[6]

名称	開始年	調査機関	対象	人数	追跡サイクル	対象年齢	特徴
Duke Study	1955	Duke大学	地域在住男女	267	2～4年	60～90歳	歴史的縦断研究
BLSA	1958	NIA(米国国立老化研究所)	米国内ボランティア	1,200	2年	20歳～	包括的老化縦断研究の象徴的存在
Normal Aging Study	1963	Boston退役軍人病院	ボストン近郊の退役軍人	2,032	5年	25～75歳	対象者は健常人
Rotterdam Study	1990	Erasmus大学	ロッテルダムの地域住民	11,854	2年	55～98歳	神経老年病,心疾患,運動器疾患,眼科疾患を対象
小金井Study	1976	東京都老人総合研究所	東京都小金井市住民	477	5年	69～71歳	日本の縦断研究の草分け的存在,社会・心理面も考慮
NILS-LSA	1997	国立長寿医療センター	愛知県大府市・東浦町住民	2,267	2年	40～79歳	日本で最初の施設型の包括的な老化の縦断研究

な危険因子をもつ人たちは早期に死亡し，健康で疾病罹患の危険因子をもたない人たちが選択的に生き残り高齢者となる．この選択効果のため，横断的研究では加齢による変化を実際よりも過小評価してしまう危険性がある．

出生年代による測定値への影響をコホート効果という．例えば，身長は60歳を超える頃から年齢とともに少しずつ低くなっていく．これは，脊椎の彎曲の増強や骨量の減少などによるものである．現在の若者は高齢者に比べて身長が高いが，横断的にみた身長の年齢による差は，身長の加齢変化よりもむしろ，成長期の栄養改善の影響によるものだと推測される．

このように，老化の観察を行うためには，そのときの集団の平均のみを観察する横断的研究のみでは，観察結果に偏りを生じることがあり，老化による変化を正確にとらえることができない．

縦断的疫学調査の中でも保健所をベースとして，あるいは地域の公民館などに住民を集めて，数日間，医師や研究者が泊まり込んで，聞き取り調査や，栄養調査，血液検査，心電図などの簡単な臨床検査を行い，これを何年間かにわたって毎年繰り返すという形での地域においての調査は，日本でもいくつか行われ，優れた成果も出ている．特に離島や山村など限られた地域の特色を描き出すためには，こうした地域での調査は極めて重要である．しかし，老化に伴う数多くの変化をできるだけ広範囲にとらえ観察するには，最新の機器を利用した医学検査と詳細な生活調査に加え，食事調査，運動機能調査，心理検査など，学際的な精度の高い調査・検査を繰り返し同一の参加者に行うことが必要である．加齢・老化による変化を多くの設備の整った施設での検査，調査によって詳細に観察し，疾患や障害の発症をとらえて，その病因を探す長期縦断疫学研究を実施することが必要である．

フラミンガム・スタディのような世界各地で行われている大規模疫学研究の多くは，癌や循環器疾患などの特定の疾患をエンドポイントとしたコホート研究であり，老化の研究を目指したものではない．国内外での代表的な老化の縦断的研究を表2に示した[6]．施設での設備を利用した総合的な老化に関する縦断的研究は，国際的にみても米国国立老化研究所(NIA)における Baltimore Longitudinal Study of Aging(BLSA)など少数に限られている．

縦断疫学研究の新たな課題

老化の疫学研究の目的は，積極的介入による寿命の延長を目指した老化制御だけでなく，む

しろ高齢者の日常生活に関与する機能(ADL)および生活の質(QOL)の維持を目指している．老年症候群，特に高齢者の自立に影響を与えるような軽度の認知機能障害(Mild Cognitive Impairment：MCI)や，軽度の身体機能障害(frailty)は最近の老年医学の重要な課題にもなっている．

高齢化社会への対応には医学ばかりでなく，高齢者の経済，人権，介護，ソーシャルサポート，家族関係，死別体験，ストレス，自尊心，自立などの研究も重要である．高齢者と若年者，健常者と障害者，すべてが共存できる共生社会を目指す社会学的研究が重要な意味をもってくるだろう．これからの長期縦断疫学研究も，こうした社会学的側面を包括した学際的研究でなければならない．

環境要因や文化，生活習慣などの老化・老年病への影響を観察するためには，世界で行われている老化の疫学的調査研究と国際比較研究を行っていく必要もある．

分子生物学から社会学まで学際的展開，さらには研究の国際的展開が，老化の疫学的研究の中心となる縦断研究にも，今求められている．

老化・老年病の分子疫学と縦断研究

最近の急速なゲノム科学の進歩は，老化や老年病罹患の素因としての遺伝子多型の探索を可能にした．小児期に起こってくる稀な遺伝性疾患は単一の原因遺伝子がはっきりしており，その遺伝子の変異があれば必ず疾患が発症する．しかし老化や老年病に関連する遺伝子の多型は，単一ではなく数多くの遺伝子が関わっており，それぞれの遺伝子多型間の相互作用や，さらには加齢や環境要因の影響もあり解析が難しい．老年病に関連する遺伝子多型は疾患の発症への寄与率が一般に低く，多くの生活環境因子との交絡があるため，解析を行うのに十分な対象者数が必要である．例えば高脂血症でも食事や体格，年齢，運動量などを一定に調整した上での遺伝子多型の寄与の推定が求められる．こうした検討を行うためには，多変量解析や多くの検査結果の時間的変化を重視した縦断的解析が必要である[7]．

このようなことを考慮すると，老化や老年病の分子疫学的研究には少なくとも数千人規模の基礎集団を設定することが望ましい．できれば無作為抽出された中高年の一般住民を対象とし，老化や老年病に関連する多数の遺伝子多型の検査を行うと同時に，様々な環境因子，医学的所見，疾患マーカーの検査や臨床検査を実施する．さらに環境因子の経時的な影響をみるために，継続的に繰り返して調査を行う包括的な縦断研究を実施していく．一般の調査では，多くの遺伝子多型について検査を行おうとすると，検体が枯渇してしまう危険性があるが，縦断研究では同一の人が繰り返し参加するため，遺伝子検体の繰り返しの採取が可能であり，検体量を心配することなく研究を行うことができるという利点もある[8,9]．

国立長寿医療センター長期縦断疫学研究

平成8年度に，国立長寿医療センター研究所(NILS)に長期縦断疫学研究室が設置され，平成9年度の11月より「老化に関する長期縦断疫学研究(NILS-LSA)」を開始した[10-12]．対象者は，観察開始時年齢が40～79歳までの男女である．1日の検査人数は7名で，毎日年間を通して詳細な老化に関連する検査を行っている．平成12年4月に2,267名の基礎集団が完成し，以後は2年ごとに検査を繰り返し実施している．対象者は国立長寿医療研究センター周辺の地域住民とし，地方自治体(大府市および東浦町)の協力を得て，地域住民から年齢・性別に層化した無作為抽出を行っている．抽出によって選定された者を説明会に招いて，検査の目的や方法などを十分に説明し，インフォームドコンセントを得た上で検査を実施している．

検査および調査は，ほとんどすべて施設内に設けた専用の検査センターで行っている．朝9時から夕方4時までの間に分刻みでスケジュールを組み，頭部MRI検査や心臓および頸動脈超音波断層検査，骨密度測定，腹部CT検査などの最新の機器を利用した医学検査のみならず，

詳細な生活調査，栄養調査，運動機能調査，心理検査など広汎で学際的な，しかも精度の高い調査・検査を実施している．

調査開始当初より調査参加者のほぼ全員からの血液サンプルを用いて，DNA を自動抽出装置で抽出し蓄積している．これほど背景因子が詳細に検討されている一般住民の DNA 検体の蓄積は，国内外でも他にはないと思われる．現在，老化・老年病に関連する 172 の遺伝子多型について検討を行っており，様々な老化関連疾患への罹患，疾患や老化のマーカーなどとの関連について数多くの背景因子を考慮した検討を行っており[13, 14]，その成果が期待される．

おわりに

高齢化が急速に進む日本の社会において，高齢になってもできる限り元気に過ごしたいという国民の共通の願いを実現することは急務である．高齢者の健康を増進させ，疾病を予防し，医療費を低減させることが求められている．さらに今後は医学だけでなく，心理学や社会システムまでをも含む学際的な研究の展開も必要であろう．特に最近のゲノム科学の進歩を取り入れた分子疫学の分野は，老化の進行や疾患罹患のリスク予測と効果的な予防法の開発には欠かせない．分子生物学の手法を老化の疫学的研究の中に取り入れていくことで，今後の老化および老年病に関わる遺伝子多型の探索，環境因子，生活習慣との相互作用など，今後の老年医学における新たな展開が期待できよう．

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研究論文●31

一般地域住民における腹部肥満感受性因子の網羅的検討

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1．目的

肥満，特に腹部肥満はメタボリックシンドロームの源流にある病態として近年注目されている[1,2)]。メタボリックシンドロームに関連する病態(肥満，高脂血症，高血圧，耐糖能異常)はいずれも多因子疾患と考えられており，多くの遺伝子多型との関係が報告されている[3,4)]。従来このような高脂血症，高血圧，耐糖能異常との関連が報告されている遺伝子多型が，腹部肥満にも影響を及ぼしている可能性がある。

本研究では，腹部肥満指標(内臓脂肪面積およびウエスト周囲径)と126種の老化・老年病関連候補遺伝子多型との関係を網羅的に検討し，腹部肥満感受性遺伝子多型を抽出することを目的とした。

2．対象および方法

対象は，「国立長寿医療センター研究所・老化に関する長期縦断疫学研究(NILS-LSA：National Institute for Longevity Sciences-Longitudinal Study of Aging)[5)]」の第一次調査(1997～2000年)，第二次調査(2000～2002年)にともに参加した地域在住中高年男女1,813人(第二次調査時42～82歳，平均年齢60.5±10.6歳，男性944人，女性869人)の中で，本研究に必要な調査項目を完遂した約1,750人である(遺伝子多型の判定が可能であった人数が遺伝子多型によって異なるため，解析人数は一定ではない)。

腹部肥満指標として臍位腹部CTスキャンにおける内臓脂肪面積(WCT)，および前日午後9時より欠食で午前中に測定したウエスト周囲径(WC)を用いた。腹部肥満の有無の判定には，わが国のメタボリックシンドローム診断基準(2005年)[6)]に基づき，内臓脂肪面積100 cm^2 (WCT-J)，ウエスト周囲径男性85 cm，女性90 cm (WC-J)をカットオフポイントとして用いた。

遺伝子多型は，NILS-LSAで2005年度までに測定された老化・老年病関連候補遺伝子多型145種の中で，解析に必要な多型の分布が得られた126種である(詳細割愛)。各遺伝子多型について頻度の高いalleleを野生型，頻度の低いalleleを変異型とし，ホモ野生型/ヘテロ・ホモ変異型間で腹部肥満指標を比較した。解析は性別，女性ではさらに閉経の有無別で行い，年齢で調整した一般線形モデルで内臓脂肪面積，ウエスト周囲径と遺伝子多型との関係を検討した後，関係が有意であった遺伝子多型について，腹部肥満の有無との関係を年齢を調整した多重ロジスティック回帰分析を用いて検討した。統計解析にはSAS 8.2を用い，$p<0.05$を統計的有意とした。

3．結果

126種の候補遺伝子多型中，24種の遺伝子多型で，多型により内臓脂肪面積(WCT)，ウエスト周囲径(WC)が有意に異なっていた(表1)。これらの遺伝子多型の中で8種の遺伝子多型では，年齢を調整した多重ロジスティック回帰分析で検討した結果，腹部肥満のリスクが遺伝子

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表1　内臓肥満と遺伝子多型

遺伝子多型		性	閉経の有無	従属変数	遺伝子多型間の有意差 (p value)
略　号	名称(多型部位) (rs No.)			腹腔内脂肪面積(WCT)/ウエスト周囲径(WC)	
ADR	Androgen receptor (CAG repeat) (rs4045402)	女性	未閉経	WCT	0.022
CAL	Calcitonin receptor (C1377T) (rs1801197)	女性	未閉経	WCT	0.047
CP10	Calpain10 (G-43A) (rs3792267)	女性	閉経	WCT	0.021
CYP17	Cytochrome P450, family 17, subfamily A, polypeptide 1 (T-34C) (rs743572)	女性	閉経	WC	0.029
DRD2	Dopamine receptor D_2 CG (Ser311Cys) (rs1801028)	女性	未閉経	WC	0.046
EDN1	Endothelin-1 (Lys198Asn)	女性	未閉経	WC	0.041
		女性	未閉経	WCT	0.048
GNB	Guanine nucleotide-binding protein β3 (C825T) (rs5443)	女性	閉経	WC	0.045
GP1BA	Glycoprotein Ⅰbα (C1018(Thr145Met)) (rs6065)	男性		WC	0.018
		男性		WCT	0.008
IRAK1	Interleukin-1 receptor-associated kinase 1	女性		WCT	0.043
	(T587C(F196S)) (rs1059702)	女性	閉経	WC	0.031
		女性	閉経	WCT	0.029
KLOT	Klotho (G-395A) (rs1207568)	男性		WC	0.047
		男性		WCT	0.045
MMP12	Matrix metalloproteinase-12 (A-82G) (rs2276109)	男性		WC	0.019
		男性		WCT	0.010
MAOB	Monoamine oxidase B [GA(intron13/exon14)] (rs1799836)	女性	未閉経	WC	0.021
		女性	未閉経	WCT	0.027
Mt15497	MT15497 (G/A)	女性		WC	0.013
		女性		WCT	0.019
		女性	閉経	WC	0.009
		女性	閉経	WCT	0.016
Mt15524	MT15524 (A/G)	女性	閉経	WC	0.049
		女性	閉経	WCT	0.034
NOSID	Nitric oxide synthase 3 (ID)	男性		WCT	0.010
PAI	Plasminogen activator inhibitor 1 (4G/5G) (rs1799889)	男性		WC	0.025
PRC	24 kDa protein of complex I (Ala29Val) (rs906807)	女性	閉経	WC	0.035
RAGE1	Receptor of advanced glycation end products (AGER) (1704G/T) (rs184003)	男性		WCT	0.029
RIL	Reversion-induced LIM (T-333C) (rs453602)	男性		WC	0.041
		男性		WCT	0.007
TNF	Tumor necrosis factor α (C-863A) (rs1800630)	男性		WCT	0.030
TOM40	TOM40 polymorphism SNP988 (T5328C) (rs157581)	女性		WC	0.009
		女性		WCT	0.020
		女性	閉経	WC	0.001
		女性	閉経	WCT	0.003
UCP1	Uncoupling protein 1 (A-3826G) (rs1800592)	男性		WC	0.013
VDR1	Vitamin D receptor (T2C)	女性	閉経	WC	0.049
VEGF4	Vascular endothelial growth factor (G-1154A) (rs1570360)	男性		WC	0.008
		男性		WCT	0.048

腹腔内脂肪面積(WCT)もしくはウエスト周囲径(WC)を従属変数，遺伝子多型を説明変数，年齢を調整変数とした，性別・閉経の有無別の一般線形モデルによる分析。

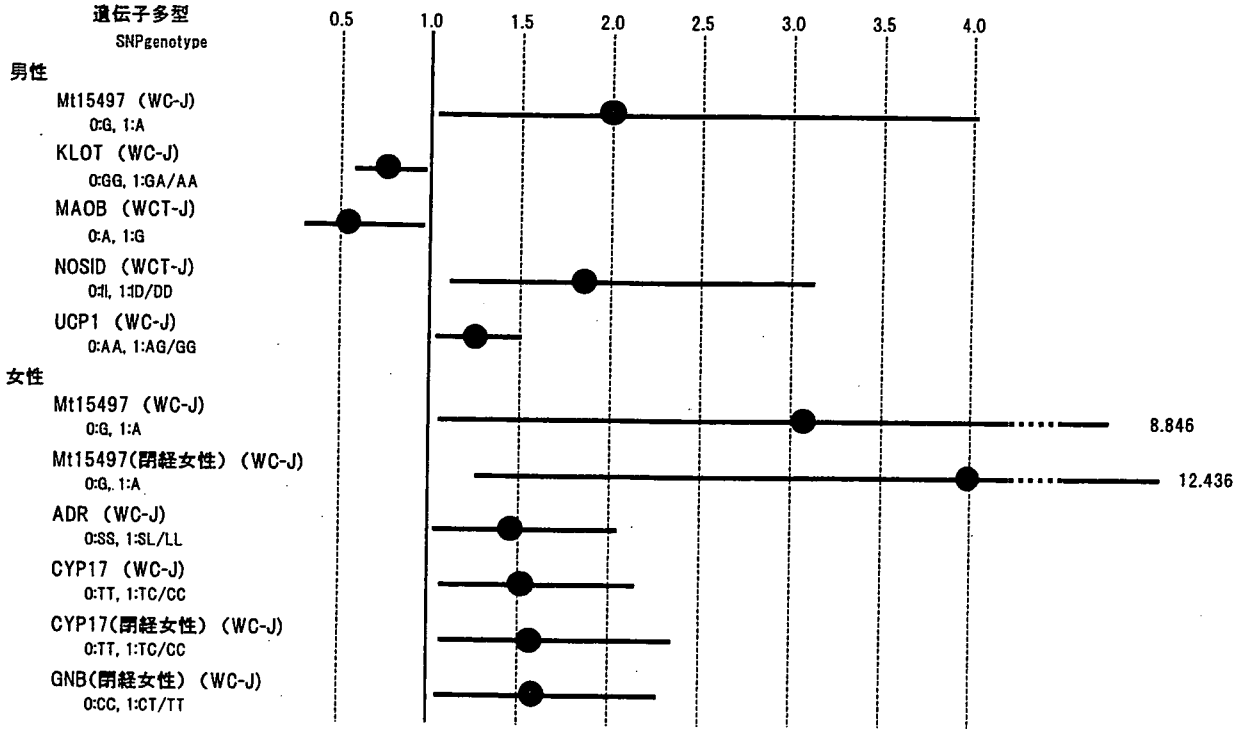

図1 ロジスティック解析(性別，閉経の有無別，年齢調整済み)
結果変数：腹部肥満(WC-JもしくはWCT-J) 無：0，有：1。

多型によって有意に増大することが示された(図1)。

呼吸・代謝に関連するミトコンドリアの遺伝子多型の1つであるMt15497のG alleleを有する者では，A alleleを有する者より，ウエスト周囲径で判定される腹部肥満を示す危険性が男性では約2倍，女性では約3倍，特に閉経女性では約4倍高かった。

そのほかに男性ではKLOT，MAOB，NOSID，UCP1，女性ではADR，CYP17，GNBの遺伝子多型で腹部肥満を示す危険性が異なる可能性が示された。

4. 考察，結論

老化・老年病に関わる複数の遺伝子多型が中高年者の腹部肥満に関連していると考えられた。肥満のみならず，腹部肥満も多因子疾患であり，腹部肥満に影響を与える遺伝子多型は男女共通のものと，男性，女性にそれぞれ特異なものがあると考えられた。Mt15497と肥満との関係についてわれわれは既に一部報告しているが[7]，今回，わが国でのメタボリックシンドロームのカットオフポイントとの関連も明らかになった。しかし，腹部CTあるいはウエスト周囲径と遺伝子多型との関係と，わが国のメタボリックシンドローム腹部肥満診断基準と遺伝子多型との関係は，必ずしも一致していなかった。腹部肥満診断基準の妥当性については現在，論議されているところであり，個別の遺伝子多型が腹部肥満に与える影響のカットオフポイントがあるかどうかも検討すべきであろう。今後，これらの遺伝子多型の機能や腹部肥満への影響の性・年齢特異性，さらにはこれらの遺伝子多型の集簇により腹部肥満の危険性が増大するかどうかについて検討が必要である。

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Age-specific change of prevalence of metabolic syndrome: Longitudinal observation of large Japanese cohort

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Abstract

To examine real age-related changes in the prevalence of metabolic syndrome, we studied longitudinal changes in the prevalence of metabolic syndrome in a single cohort of individuals. The participants included 112,960 Japanese (70,996 men, 14–94 years and 41,946 women, 17–85 years), who had received annual examinations between 1989 and 2004. Metabolic syndrome was defined according to the Japan Metabolic Syndrome Criteria Study Group and the US National Cholesterol Education Program (NCEP) guidelines. Overweight was defined as BMI $\geqq 25$ kg/m^2. Longitudinal changes indicated a birth cohort effect in the prevalence rate of metabolic syndrome with a lower or higher prevalence in the younger birth cohort than in the older for females or males, respectively. The estimation of the age-specific prevalence of metabolic syndrome demonstrated that in males, the prevalence of metabolic syndrome increased up to 50 decades of life for the Japanese and 60 decades of life for the NCEP criteria. In females, the prevalence increased with age up to 80 years old for both criteria. The estimated secular trends suggested that the prevalence rate of metabolic syndrome decreased in females and increased in males during study periods.

Keywords: Metabolic syndrome; Aging; Secular trends; Longitudinal study

Metabolic syndrome has become one of the major public-health challenges worldwide [1,2]. The most important dimension of metabolic syndrome is its association with the risk of developing type 2 diabetes mellitus and atherosclerotic cardiovascular disease [3–9]. A number of metabolic syndrome definitions have been proposed, including the World Health Organization (WHO) Consultation for diabetes and its complications [10], the European Group for the Study of Insulin Resistance [11], the National Cholesterol Education Program (NCEP) Expert Panel [12], and, more recently, the International Diabetes Federation (IDF) [13] have formulated definitions for metabolic syndrome. In addition, the American Heart Association in conjunction with the National Heart, Lung, and Blood Institute have proposed a revised version of the NCEP-ATPIII definition [14]. In Japan, the National Metabolic Syndrome Criteria Study Group has proposed new criteria for metabolic syndrome in the Japanese [15].

Since several definitions of the syndrome are in use, it is difficult to compare the prevalence and impact between countries. However, a very consistent finding is that the prevalence of metabolic syndrome is highly age-dependent [16–18]. These previous findings were based on the cross-sectional observations, which may represent cohort, period, and/or survivorship effects rather than a true aging effect. Although longitudinal studies are required to examine real age-related changes in the prevalence of metabolic syndrome, to our knowledge, no studies have examined the longitudinal changes in the prevalence of metabolic syndrome in individuals over time.

We therefore studied longitudinal changes in the prevalence of metabolic syndrome in a single cohort of individuals to observe the effect of the natural aging process on the prevalence of metabolic syndrome as well as on obesity,

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hypertension, impaired glucose tolerance, and dyslipidemia as components of metabolic syndrome.

1. Methods

1.1. Study population

The study population consisted of office workers and their families residing in Aichi Prefecture in central Japan. The subjects included 112,960 Japanese (70,996 men and 41,946 women) with an average age of 44.6 years in men and 43.4 years in women, who had received annual examinations at a health examination center in Japan between 1989 and 2004 (Table 1). About 57% of the cohort (41,709 men and 23,001 women) had attended at least one follow-up examination. The average visits for the follow-up examinations were 3.4 times for men and 3.0 times for women.

1.2. Procedures and laboratory methods

The examinations included a questionnaire, physical examination, blood pressure measurement, an anthropometric measurement, and laboratory analysis of blood samples, all taken on the same day as described previously [19–21]. The anthropometric measurements included height and body weight. The body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Blood pressure was measured after the participants had been comfortably seated for at least 5 min.

All serum samples were obtained following a 12–14 h fast. The serum was separated promptly, and all lipid analyses were conducted at the clinical laboratory in the health examination center. Serum glucose and triglycerides were measured using enzymatic methods. HDL-cholesterol was measured after dextran sulfate-magnesium precipitation. No differences were seen in the sample collection, laboratory apparatus, or techniques used between 1989 and 2004.

1.3. Definition of metabolic syndrome

We applied both the Japanese criteria of metabolic syndrome [15] and the NCEP Expert Panel on the Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III; ATPIII) criteria [12]. According to the Japanese definition, someone has metabolic

Table 1
Characteristics of participants

	Males	Females
Number of subjects	70996	41946
Total number of measurements for 16 years	239879	122624
Number of subjects for whom measurements were taken at least twice	41709	23001
Number of measurements per subject for 16 years, mean (S.D.)	3.4 (3.4)	3.0 (2.9)
Average follow-up periods (years), mean (S.D.)	3.4 (4.3)	3.1 (4.1)
Initial measurements		
Age (years), mean (S.D.), age range (years)	44.6 (9.3), 14–94	43.4 (9.5), 17–85
Number of subjects in each age group, *n* (%)		
10–29 years	1914 (2.7)	2183 (5.2)
30–39 years	21173 (29.8)	1398 (31.5)
40–49 years	26583 (37.4)	15287 (36.4)
50–59 years	16783 (23.6)	9199 (21.9)
60–69 years	4152 (5.8)	1830 (4.4)
70–79 years	361 (0.5)	230 (0.6)
≥80 years	30 (0.04)	19 (0.05)
Hight (cm), mean (S.D.)	168.6 (6.0)	156.0 (5.5)
Body weight (kg), mean (S.D.)	65.7 (9.4)	52.4 (7.4)
BMI (kg/m^2), mean (S.D.)	23.1 (2.9)	21.6 (2.9)
Total cholesterol (mg/dl), mean (S.D.)	199.4 (35.0)	199.0 (36.5)
Triglyceride (mg/dl), mean (S.D.)	142.0 (103.5)	87.0 (49.7)
HDL-cholesterol (mg/dl), mean (S.D.)	55.2 (13.3)	67.8 (14.6)
Fasting plasma glucose levels (mg/dl), mean (S.D.)	98.5 (18.6)	91.4 (11.1)
Systolic blood pressure (mmHg), mean (S.D.)	121.2 (16.2)	113.7 (7.4)
Dyastolic blood pressure (mmHg), mean (S.D.)	73.0 (11.7)	66.5 (5.5)
Prevalence of obesity (%, BMI ≧ 25 kg/m^2)	24.1	11.5
Prevalence of hypertension (%, BP ≧ 130/85 mmHg or treated)	30.6	17.0
Prevalence of glucose intolerance (%, FSG ≧ 110 mg/dl or treated)	11.4	4.0
Prevalence of high triglyceride (%, ≧150 mg/dl)	32.2	8.0
Prevalence of low HDL (%, HDL male < 40, female < 50 mg/dl)	9.1	9.1
Prevalence of dyslipidaemia (%, TG ≧ 150 or HDL < 40 mg/dl)	35.1	8.5
Prevalence of metabolic syndrome		
Modified Japanese criteria, % (95% CI)	7.8 (7.6–8.0)	2.2 (2.0–2.3)
ATPIII-BMI25, % (95% CI)	11.6 (11.4–11.9)	4.0 (3.8–4.1)

BMI: body mass index, BP: blood pressure, FSG: fasting serum glucose, TG: triglyceride.

syndrome if he or she has central adiposity plus two or more of the following three factors [15]: (1) raised concentration of triglycerides, ≥150 mg/dl or reduced concentration of HDL-cholesterol, <40 mg/dl; (2) raised blood pressure: systolic blood pressure, ≥130 mmHg or diastolic blood pressure, ≥85 mmHg or treatment of previously diagnosed hypertension; and (3) raised fasting plasma glucose concentration, ≥110 mg/dl. The thresholds for waist circumference to define central adiposity: ≥85 cm for men and ≥90 cm for women. The ATPIII proposed the following five abnormalities to define metabolic syndrome [12]: (1) abdominal obesity (abdominal circumference >102 cm for men and >88 cm for women); (2) elevated serum triglyceride level (≥150 mg/dl); (3) decreased HDL-cholesterol level (<40 mg/dl for men and <50 mg/dl for women); (4) elevated blood pressure (systolic and diastolic blood pressure 130/85 mmHg); and (5) an elevated fasting glucose level (≥110 mg/dl). Individuals with three or more of the five abnormalities were considered to have metabolic syndrome. Because waist measurements were not available for the entire study sample, we substituted a BMI of 25 kg/m^2 or greater for all participants as an index of obesity for both criteria (the modified Japanese criteria and ATPIII-BMI25). A BMI of 25 kg/m^2 or greater has been proposed as a cutoff for the diagnosis of obesity in Asian people [22]. Individuals who were using antihypertensive or antidiabetic medications met the criteria for high blood pressure or high fasting glucose.

1.4. Data analysis

The data were analyzed with the Statistical Analysis System (SAS), release 8.2. We demonstrated that there is a birth cohort effect on the prevalence rate of metabolic syndrome based on a 16-year longitudinal analysis of the same cohort. Therefore, the pooled cross-sectional data at the initial examination of each subject from 1989 through 2004 were adjusted for the year of the examination using the logistic regression model, and estimated for the examination in 1997. The prevalence rate of metabolic syndrome for the modified Japanese and ATPIII-BMI25 definitions was estimated from an age younger than 40 years through age 70 years and older at 10-year intervals, and compared between these two definitions in each age group by paired *t*-test.

Longitudinal changes in the prevalence of metabolic syndrome were analyzed by a generalized-estimating-equation (GEE), which adjusts for repeated measurements in the same persons. For the longitudinal analyses, the subjects who did not receive follow-up examination were excluded. Age-related changes in the prevalence rate of metabolic syndrome, obesity, hypertension, impaired glucose tolerance and dyslipidemia were estimated by quadratic curve of age controlling for the observation year during which the subjects attended at least one follow-up examination. The GEE was also used to test for trends in the prevalence of metabolic syndrome during 1989–2004. The year of examination was used to test for temporal trends in prevalence. Age adjustment was performed by a least-squares regression approach. The model included age, square of age and year of examination as independent variables. A result was considered statistically significant if the P value was less than 0.05.

2. Results

Based on the pooled cross-sectional data of each subject at initial examination from 1989 through 2004, the mean prevalence rate of metabolic syndrome defined by the modified Japanese or ATPIII-BMI25 criteria was 7.8% in males and 2.2% in females, or 11.6% in males and 4.0% in females, respectively (Table 1). The prevalence of metabolic syndrome defined by two criteria was shown by age group and gender after adjusting for the examination year (Table 2). The prevalence rate of metabolic syndrome increased with age, with the highest rate in the 60–69 years group followed by a decline in the 70 years and older group in females with both criteria. In males, the highest prevalence rate was observed in the 50–59 years group or the 60–69 years group in the modified Japanese or ATPIII-BMI25 criteria, respectively. There was a significant difference between the two definitions in both genders and any age group with a higher prevalence rate in the ATPIII-BMI25 definition.

Longitudinal changes for 16 years in the prevalence rate of metabolic syndrome by birth cohort using the both criteria indicate the birth cohort effect in the prevalence rate of metabolic syndrome for females from the fifth decade of life, since at those ages, the prevalence rate of the younger

Table 2
The cross-sectional data of prevalence of metabolic syndrome at initial examination of each subject from 1989 through 2004

Age (years)	Females					Males				
	N	Modified Japanese		ATPIII-BMI25		*N*	Modified Japanese		ATPIII-BMI25	
		Mean (%)	95% CI	Mean (%)	95% CI		Mean (%)	95% CI	Mean (%)	95% CI
≤39	15381	0.5	(0.4, 0.6%)	1.1	(0.9, 1.3%)	23087	5.7	(5.4, 6.0%)	8.3	(7.9, 8.6%)
40–49	15287	1.9	(1.7, 2.1%)	3.5	(3.2, 3.7%)	26583	8.1	(7.7, 8.4%)	11.9	(11.5, 12.3%)
50–59	9199	4.0	(3.6, 4.5%)	7.4	(6.9, 8.0%)	16783	9.9	(9.4, ,10.3%)	15.0	(14.4, 15.5%)
60–69	1830	7.8	(6.6, 9.1%)	13.7	(12.1, 15.2%)	4152	9.6	(8.7, 10.5%)	15.2	(14.1, 16.3%)
≥70	249	7.2	(4.0, 10.5%)	12.1	(8.0, 16.1%)	391	7.4	(4.8, 10.0%)	13.6	(10.2, 17.0%)

Data were adjusted for year of initial examination, and estimated for the examination in 1997. Significant difference between two definitions in both genders and any age group with higher prevalence rate in ATPIII-BMI25 definition $P < 0.0001$.

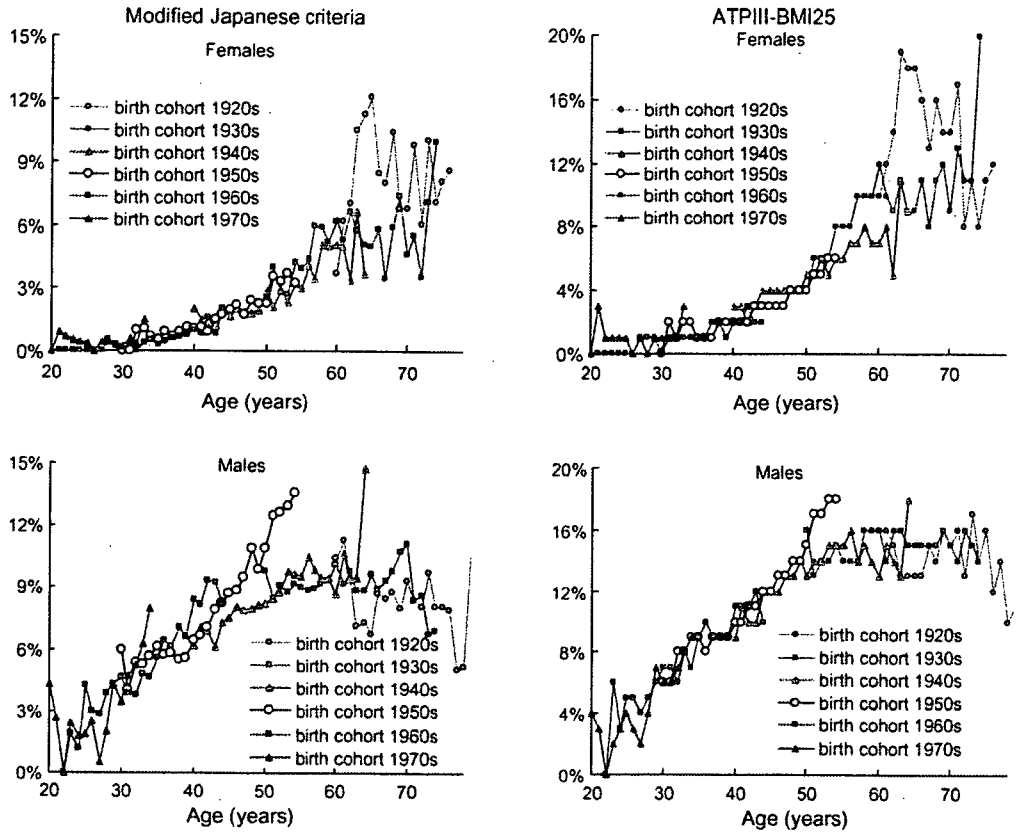

Fig. 1. Longitudinal changes for 16 years in the prevalence rate of metabolic syndrome by birth cohort in females and males using the modified Japanese and ATPIII-BMI25 criteria.

birth cohorts were lower than those of the older birth cohorts in both definitions (Fig. 1). There was a birth cohort effect in males at least between 40 and 55 years, indicating that the younger birth cohorts scored higher than the older birth cohorts in both criteria (Fig. 1).

Fig. 2 shows the prevalence rates of the individual components of metabolic syndrome in the modified Japanese criteria. There was no birth cohort effect on the prevalence rate of obesity in females, except for the cohort of the 1930s, which demonstrated a higher prevalence rate than that of the 1940s between age 50 and 64 years. However, the apparent birth cohort effect on the prevalence rate of obesity was detected in males with a higher prevalence in the younger cohort than the older one. Regarding the prevalence rate of hypertension, no apparent birth cohort effect was detected in either gender. There was no apparent birth cohort effect of the prevalence rate of impaired glucose tolerance in females, but there was an apparent effect in males with a higher prevalence rate in the younger cohort than that the older one. There seemed to be a birth cohort effect of the prevalence rate of dyslipidemia in both genders, with a lower prevalence rate in the younger cohort than the older one, at least for the birth cohort of the 1950s and older cohorts.

Fig. 3A shows the estimated prevalence rate of metabolic syndrome at individual ages according to the two different criteria after adjusting for the examination year (estimation at 1997). In male, the highest prevalence rate of metabolic syndrome was observed around 60 years for the ATPIII-BMI25 criteria and around 55 years for the modified Japanese criteria. In females, the highest rate was detected at the 70 years and older age group for both criteria.

Fig. 3B showed the estimated prevalence rate of each component of metabolic syndrome defined in the modified Japanese criteria at individual ages after adjusting for the examination year (estimation at 1997). The prevalence rates of obesity and dyslipidemia increased between 20 and 50 years, or 70 years in males, or females, respectively. The prevalence rate of hypertension increased in both genders from 20 years through the 80 years. Regarding impaired glucose tolerance, the prevalence rate increased up to the 60th or 70th decade and then declined in males or females, respectively.

Fig. 4A shows the secular change in the prevalence rate of metabolic syndrome defined by two different criteria from 1989 to 2004 from age younger than 40 years through age 70 years and older at 10-year intervals. In ATPIII-BMI25 criteria in females except for younger than 40 and 70 years and older age groups the prevalence rate of metabolic syndrome decreased (trends: 40–49 years, $P<0.01$; 50–59 and 60–69 years, $P<0.0001$). In males aged 40–49 and 50–59 years the

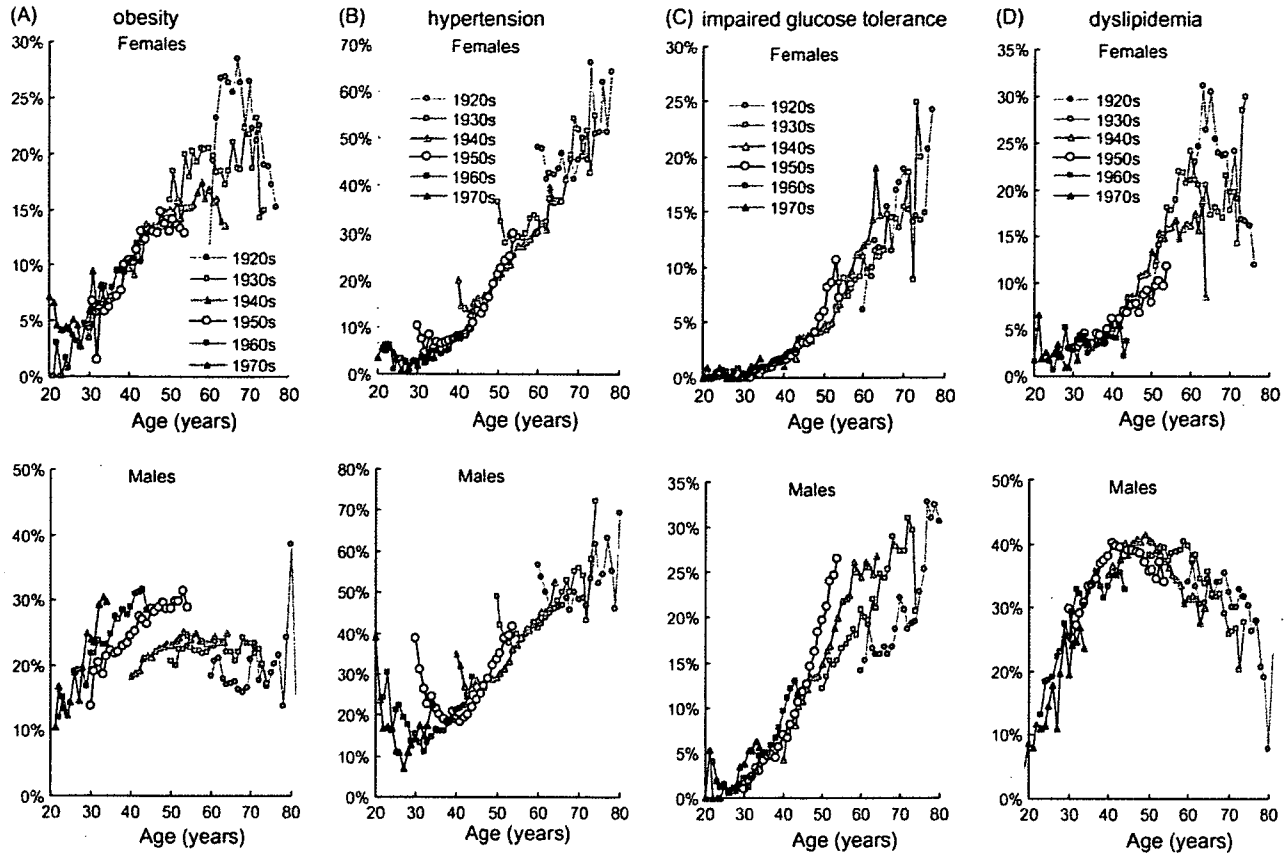

Fig. 2. Prevalence rates of individual component of metabolic syndrome in the modified Japanese criteria.

prevalence increased during study periods (trends, $P < 0.001$). According to the modified Japanese definition, the prevalence of metabolic syndrome decreased in females aged 50–59 and 60–69 years during study periods (trends, $P < 0.01$ and 0.001, respectively), and increased in males of youngest group, aged 40–49 and 50–59 years. Fig. 4B shows the trends in age-adjusted prevalence rate of metabolic syndrome defined by two criteria. The data were estimated age at 50 years. In both criteria the prevalence rate of metabolic syndrome decreased in females and increased in males, respectively.

3. Discussion

The cross-sectional observations suggested similar age-specific changes of the prevalence of metabolic syndrome with two different metabolic syndrome definitions. In addition, our results agree with the previous cross-sectional observations of age-specific prevalence of metabolic syndrome from other countries and ethnicities. The Third National Health and Nutrition Survey indicated that the prevalence of metabolic syndrome increased from 20–29 to 60–69 years [16]. A survey in Iran suggested that the prevalence increased with age, with the lowest prevalence at 20–29 years and the highest at 60–69 years in both genders [18]. A cross-sectional survey in China demonstrated that the prevalence of metabolic syndrome increased among men and women until age 65 years, when the prevalence decreased slightly among men and remained constant among women [17]. Although there are some differences in the peak age of the prevalence in males, it was surprising to find that there is a similarity of age-specific changes in the prevalence rate of metabolic syndrome among different ethnic groups and in different countries, which have different cultures, lifestyles, food habits, and longevity. This consistency may suggest that the aging effect highly regulates the prevalence of metabolic syndrome even if genetic and environmental influences may exist.

We clearly showed that the prevalence rate of metabolic syndrome was much higher in both genders as well as in all age groups with the ATPIII-BMI25 definition than with the modified Japanese definition. This is not surprising, since there are several noteworthy differences. The Japanese definition requires the presence of obesity [15]. In contrast, the NCEP definition makes obesity one of the five equally weighted criteria [12]. Furthermore, the thresholds for HDL-cholesterol levels under the modified Japanese definition are <40 mg/dl in both genders, but those under the NCEP definition are different between genders: men, <40 mg/dl; women, <50 mg/dl.

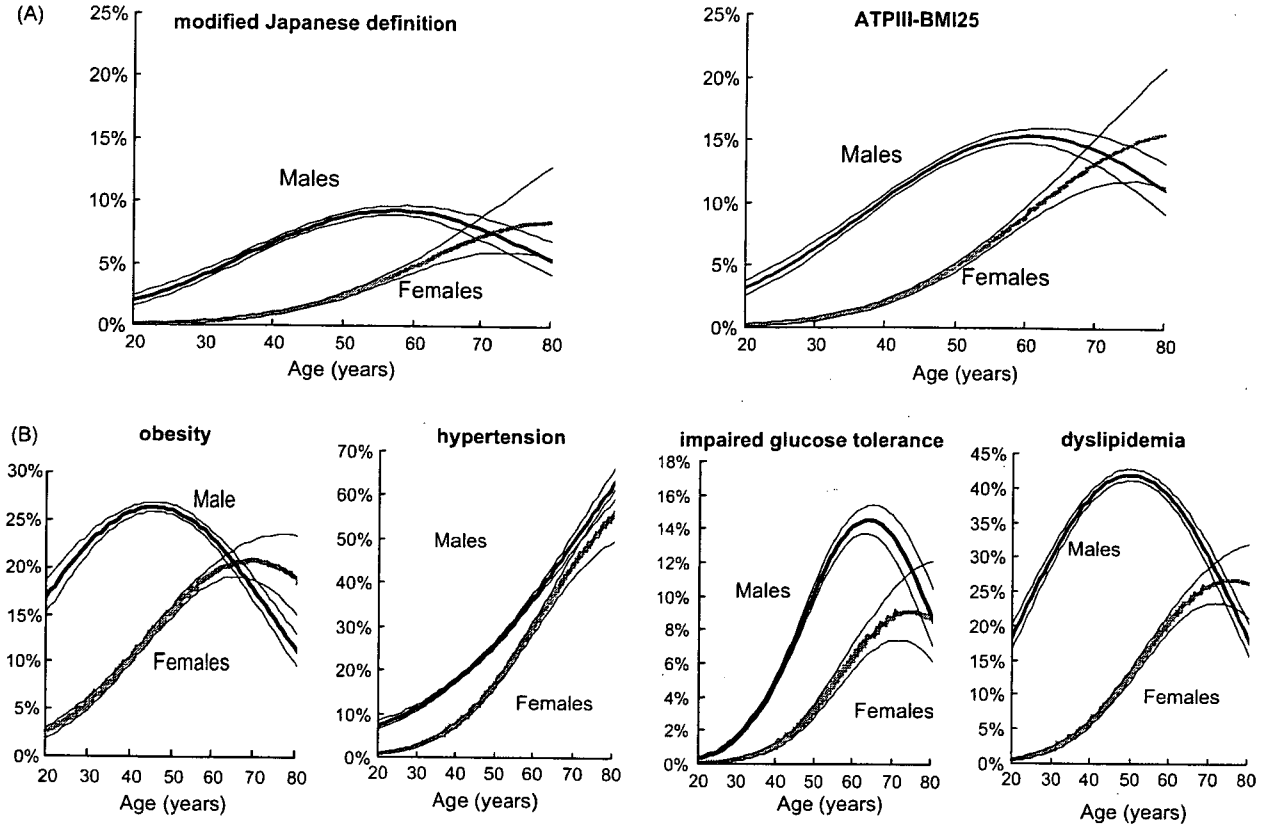

Fig. 3. (A) Estimated prevalence rate of metabolic syndrome at individual age according to the two different criteria after adjusting for examination year (estimation at 1997). (B) Estimated prevalence rate of each component of metabolic syndrome defined by the modified Japanese criteria at individual age after adjusting for examination year (estimation at 1997). Dotted curves indicate 95% CI.

Longitudinal observations demonstrated that there is a birth cohort effect on the prevalence rate of metabolic syndrome as well as the each of the components of metabolic syndrome in a large Japanese cohort. The estimated prevalence of metabolic syndrome increased from 20 up to 80 years in females both with the modified Japanese and ATPIII-BMI25 definitions. In males, the prevalence of metabolic syndrome gradually increased from 20 up to 50–59 years or up to 60–69 years followed by a decline at 70 years and older with the modified Japanese or ATPIII-BMI25 definition, respectively. It is obvious that the increasing trend of metabolic syndrome with age can be attributed to a similar age-related trend in each of the components of metabolic syndrome. The similar age-specific change of the prevalence rate was observed in obesity, impaired glucose tolerance, and dyslipidemia in males. The difference of the peak ages of the prevalence rate of metabolic syndrome in the ATPIII-BMI25 and modified Japanese criteria in males may be due to the requirement of obesity in the modified Japanese criteria, since the prevalence of obesity decreased after 50 years of age in males. In contrast to males, consistent age-specific changes of each component of the metabolic syndrome in females showed a consistent increase in the prevalence rate with age.

We showed the age-specific secular trends of the prevalence of metabolic syndrome in both genders from 1989 through 2004. In both definitions a similar trends of the prevalence of metabolic syndrome were demonstrated in both genders during study periods. The prevalence of metabolic syndrome decreased in females aged 50–69 years, and increased in males aged 40–59 years. Consistently the age-adjusted trends estimated at 50 years based on the longitudinal analysis decreased in females and increased in males in both criteria during study periods. Only a few reports regarding secular trends in the prevalence of metabolic syndrome are available. From two national surveys: the National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999–2000, it has been demonstrated that the prevalence of metabolic syndrome increased significantly in females from 1988–1994 through 1999–2000 in US but increased much smaller without statistical significance in males [23]. The recent the Mexico City Diabetes Study, a population-based study, revealed that the prevalence of the metabolic syndrome has not increased in both genders between 1990–1992 and 1997–1999 [24]. These taken together with our findings suggest the secular trends of metabolic syndrome are dependent on each country or ethnicity which has differences in genetic background, social status, and diet. Two important determinants of the metabolic syndrome are obesity and physical activity. In our cohort the secular trends of the prevalence of obe-

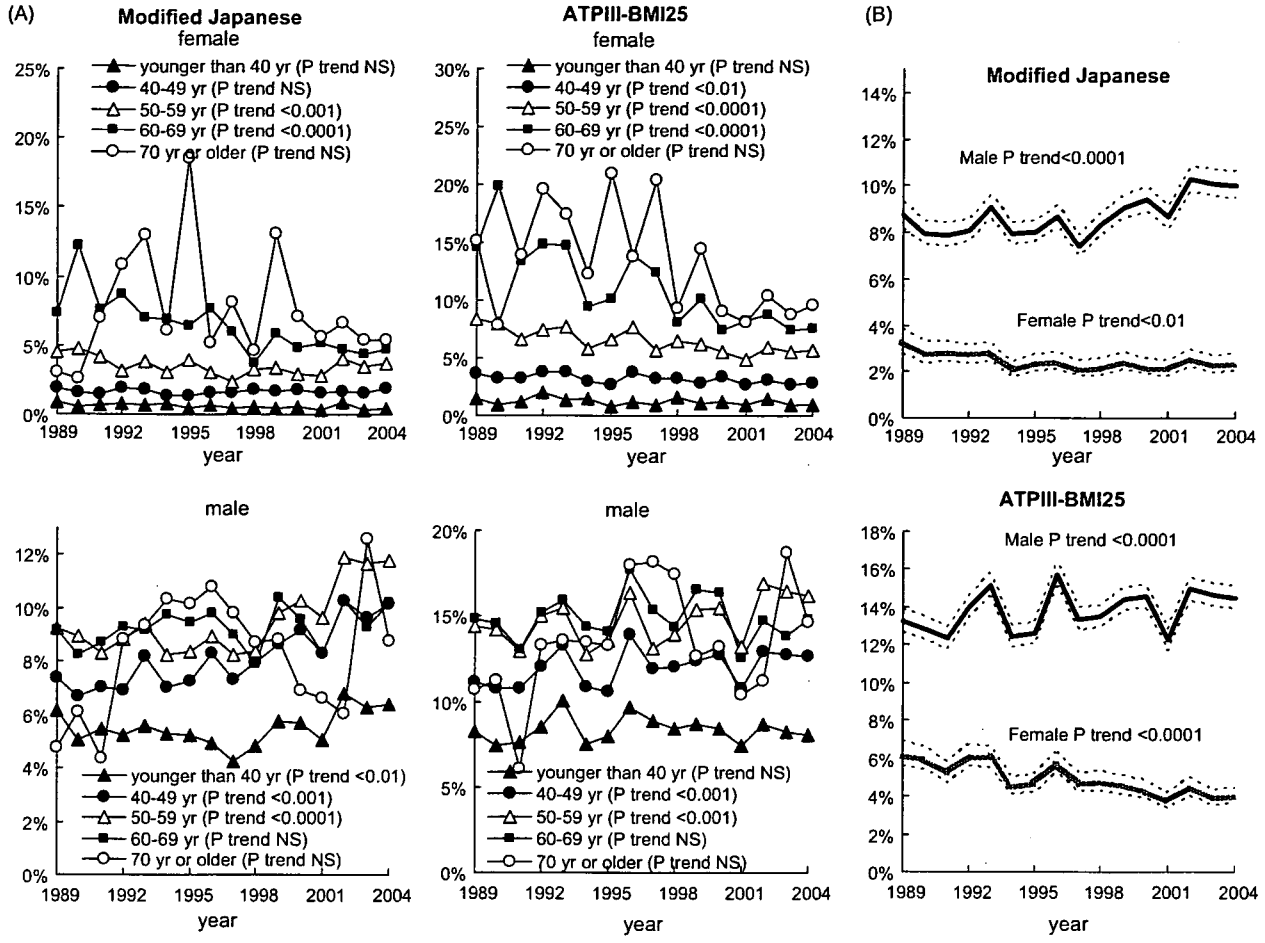

Fig. 4. (A) Secular change in the prevalence rate of metabolic syndrome defined by two different criteria from 1989 to 2004 from age younger than 40 years through age 70 years and older at 10-year intervals. (B) Trends in age-adjusted prevalence rate of metabolic syndrome defined by two criteria. The data were estimated age at 50 years.

sity decreased in females and increased in males in most of age groups during study periods (data not shown). This seems to affect the trends of metabolic syndrome in our cohorts. The data of physical activity in the participants are unavailable.

There are several limitations in the present study. The BMI was used as an index of obesity instead of waist circumference. This substitution appears to affect the prevalence rate of metabolic syndrome, although it has been reported that BMI may be useful in making comparisons pertaining to metabolic syndrome in the Japanese as the index of visceral obesity, rather than waist circumference, and that the ATPIII-BMI25 definition is suitable for the determination of metabolic syndrome for the Japanese [25]. Some selection bias such as a healthy worker bias may exist in our study, since most of the subjects were healthy office workers. In addition, the subjects may have been aware of the impact of body weight, blood pressure, glucose and lipid levels on their health, since they had been receiving annual examinations at a health examination center.

Acknowledgment

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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 (*PvuII*) [dbSNP: rs2234693] and A→G (*XbaI*) [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.