総　説

長期縦断疫学で分かったこと

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要　約　長期縦断疫学研究は一定の集団を長期にわたって縦断的に追跡し，老化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の集積することを目的にしている．縦断疫学研究は老化に関連する健康問題や正常な老化による変化を明らかにするだけでなく，認知症や骨粗鬆症などの老年病の実態，発症のリスクファクター，予防と早期診断の方法を見出すために重要である．「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）」は1997年に開始された．第1次調査への参加者はNILS-LSA周辺の地域から無作為抽出された40歳から79歳までの地域在住男女2,267人であり，2年ごとに追跡されている．毎日7名の参加者がNILS-LSA調査センターで検査を受け，老化に関しての詳細な質問票，診察，生理機能検査，身体計測，運動機能，栄養調査，心理調査が実施されている．これらのデータを縦断的に解析し，遺伝子多型，身体的および心理的要因，生活習慣および環境要因などの老化，老年病について影響を解明している．本総説では縦断研究の方法論を概説するとともに，NILS-LSAの概要と研究の成果について紹介する．

Key words：縦断研究，老化，老年病，予防，健康長寿

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はじめに

老化とともにさまざまな生体機能は変化していく．基礎資料として老化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の集積をしていくことはきわめて重要である．これにより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ことが可能となる．老化に関する観察研究は，さらに認知症や骨粗鬆症などの老年病の発症のリスクファクターの検討，予防と早期診断，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数多くの研究につながっていく．

筆者らは10年以上にわたって国立長寿医療センターにおいて長期縦断疫学研究を実施してきた．加齢研究の方法論としての縦断的研究を紹介し，その意義と必要性について述べるとともに，研究の成果について紹介する．

加齢変化に関する縦断的研究

加齢による変化を疫学的に検討する方法には大きく分けて横断的方法と縦断的方法のふたつがある．縦断的研究は同一の個人を継続して観察し，加齢による実際の心身の変化，加齢に関連する要因，老化，寿命などをとらえようとするものである[1)-4)]．一方，さまざまな年齢を含む集団を設定し，検査を一度に実施して1歳ごとのあるいは5歳，10歳ごとの年齢群で検査値がどのように異なるのかを検討し，その差を加齢変化とする方法が横断的研究である．

一度の調査で終了してしまう横断的研究に比べて経時的な追跡を行う縦断的研究は結論が出るまでに一般に数年から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，加齢変化の観察を行うためには，後述するように横断的観察のみでは加齢による変化を正確にとらえることが出来ない．

縦断的方法がなぜ必要か

横断的調査での検査値を縦軸，年齢を横軸にしてプロットしてみると，本来，加齢とともに検査値が悪化していく場合でも，高齢者では検査値には加齢変化がみられなかったり，むしろ高齢になるほど検査値が良くなったりしている．これを「選択効果」という[5)]．身体機能が悪い人が早く死亡する一方で，高齢まで生き残っている人の検査値は良いために，見かけ上のこのような変化が起きてしまう．

生まれ育った時代の生活環境の影響についても考える

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必要がある．出生年代によって生活環境などが異なれば検査値に変化が生じることがある．身長は成長が止まれば，通常は高齢期になるまで変化しない．しかし出生世代によって平均的な身長には明らかな差がある．高齢者では加齢によって椎間の狭小化や，胸腰椎の変形，円背などが生じ，身長は低くなっていくが，横断的調査での加齢による身長の差のほとんどは，発育期の栄養状態による世代間の差である．こうした世代間の差を「コホート効果」という．

また戦争などの異常体験，飢餓の経験，戦後の急激な栄養や生活環境の変化の影響など出生世代に関わりなく，時代の影響を受けている検査値もある．例えば血清コレステロールは戦後の生活の欧米化，特に食事の変化により，すべての世代で1970年代から1980年代にかけて血清コレステロールの値は大きく増加している．このようにライフスタイルの変化で身体機能は変わっていく．これを「時代効果」という．これらの「選択効果」，「コホート効果」，「時代効果」は横断的研究だけでは正確に評価できない．老化による本来の変化を正確に評価するためには，横断的研究に加えてには縦断的研究が必要である．

縦断的観察では，実際の変化をみることができることによる利点がある．例えば，身体活動量が多ければ体力は向上するはずであるが，体力には個人差が大きく，横断的研究では，身体活動量が多い人で必ずしも体力が優れている結果が得られるわけではない．しかし運動を続けている人は，運動をしない人に比べて体力が向上する．身体活動が体力向上につながることが，縦断的観察ではよりはっきりわかる．亜鉛欠乏症は味覚障害の原因のひとつと言われている．亜鉛摂取量と味覚障害の間に関連性が認められた場合，亜鉛欠乏が味覚障害を引き起こしたとも考えられるが，味覚障害があって食事がおいしく食べられず亜鉛欠乏となった可能性もある．亜鉛欠乏の人と亜鉛が十分取れている人を縦断的に観察して，亜鉛欠乏の人に味覚障害が生じれば亜鉛が味覚障害の原因であると推定できる．横断的調査だけでは時間的変化が不明で，このような因果関係を推定することはできない．

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

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「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）」が開始された[6)~9)]．対象者は観察開始時年齢が40歳から79歳までの男女である．一日の検査人数は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（図1）．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，現在は第6次調査を実施している．対象者は長寿医療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．追跡中の80歳未満のドロップアウトは新たに無作為抽出を行い，同じ年齢，性別で新たな補充を行っている．また，どの時点でも若い世代との比較ができるように無作為抽出で40歳の男女を毎回新たに加えて，定常状態として約2,400人のダイナミックコホートを目指している（図2）．

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．

終了した第1次から5次調査までの調査結果をモノグラフとしてインターネット上で公開している（http://www.nils.go.jp/department/ep/index-j.html）．NILS-LSAのデータを用いた解析によって，医学，心理，運動，栄養，身体組成などの分野で成果をあげており，医学調査開始以来，現在までに専門学術雑誌への発表や学会発表など約600件の成果発表を行っている．その一部をここで紹介する．

分子疫学研究

老化に関連する疾患は慢性的に経過し，日常生活活動に障害を与え，治療が難しいものが多い．老化や老年病には多くの遺伝子が関与し，また多くの環境要因によって影響を受ける．老化や老年病の素因を明らかにするために，分子疫学の手法を用いて多数の集団での遺伝子多型や環境要因の影響の検討を行っていく必要がある[10)]．

NILS-LSAでは，ほとんどすべての調査参加者からDNA試料を得ており，これほど詳細な縦断的背景要因を調査された一般住民のDNAの蓄積は他には例がないと思われる[11)]．これらの試料を用いて現在までに224種類の老化，老年病関連遺伝子多型についてタイピングを終え，骨粗鬆症，認知機能障害，脳梗塞，高血圧症，肥

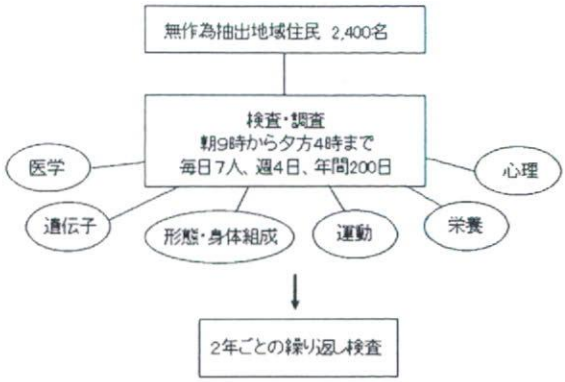

図1　国立長寿医療センター研究所・老化に関する長期縦断疫学研究（NILS-LSA）の概要

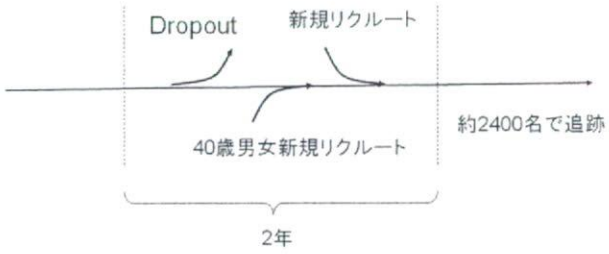

図2　NILS-LSA では追跡中の 80 歳未満のドロップアウトは新たに無作為抽出を行い，同じ年齢，性別で新たな補充を行っている．また，どの時点でも若い世代との比較ができるように無作為抽出で 40 歳の男女を毎回新たに加えて，定常状態として約 2,400 人のダイナミックコホートを目指している．

満，高脂血症，肝障害などに関連する遺伝子多型を明らかにした．

骨粗鬆症に関連しては，これまでに骨密度と有意な関連にあった 31 種類の遺伝子多型について新たに発見，あるいは確認の報告を行っている（表 1）[12)~27)]．これらは各遺伝子多型と骨密度との関連を検討した結果である．これらのうちいくつかの遺伝子多型は男性の骨形成や骨塩減少に関連することが初めて明らかになった．

高血圧については，トランスフォーミング増殖因子（TGF）-β1 の遺伝子多型が日本人の高血圧に関連することを明らかにしたのをはじめ[28)]，アポ蛋白 A5，ミクロゾームトリグリセリド転移蛋白（MTP）など 12 の遺伝子の多型が高血圧症と関連していることを報告している[29)~31)]．また，メチレンテトラヒドロ葉酸還元酵素（MTHFR）アルコール脱水素酵素（ADH）の各遺伝子多型が，無症候性の脳梗塞に関連することを明らかにした[32)33)]．ADH 遺伝子多型は脂質異常症にも関連していることも報告している[34)]．

中高年者の肥満に関しては，コレシストキニン A（CCK-A）受容体遺伝子，グレリン遺伝子多型が肥満に関連していることを見出した[35)36)]．エストロゲン受容体 α の変異が閉経後の女性の肥満に関連すること[37)]，β3 アドレナリン受容体と CCK-A 受容体の遺伝子多型の組合せが男性の「中年太り」に関与することを明らかにした[38)]．また，ミトコンドリア遺伝子多型の肥満への影響を明らかにした[39)]．グルタチオンペルオキシダーゼの遺伝子多型がメタボリックシンドロームの発症要因であることも見出している[40)]．一方，米国フラミンガムスタディでの 10 万 SNP の解析の結果，肥満との関連が明らかにされた rs7566605 遺伝子多型について，日本人では肥満と関連性が認められなかったことを報告している[41)]．さらに，認知機能の関連する遺伝子多型としてクロトー遺伝子，CCK-A 受容体遺伝子の多型を新たに見出した[42)43)]．クロトー遺伝子はマウスでの老化に関連することが明らかになっているが，ウェクスラー成人知能検査（WAIS-R）で推定した知能を用いて，認知機能と遺伝子多型との関連を解析した結果，クロトー遺伝子の多型は 60 歳未満では認知機能には影響を与えなかったが，60 歳以上では認知機能と強い関連がみられた．一方 CCK-A 受容体遺伝子多型は若い年代から認知機能と関連していた．その他，アルコール依存症，パニック症候群，膵癌，肝障害に関連する多型を見出した[44)~47)]．

老化や老年病への遺伝子多型の影響は，直接的な影響よりもむしろ生活習慣や環境因子による影響を遺伝子多型が修飾する部分が大きい可能性がある．閉経女性の DXA による骨密度と除脂肪体重との関係へのエストロゲン受容体（ERα）遺伝子 *XbaI* 多型の影響について検討した（図 2）[48)]．除脂肪体重として求めた筋量が多ければ骨密度は高いが，その影響は AA 型よりも AG/GG 型の方が強い．AG/GG 型の多型を持つ者は筋量を増やすことが AA 型の者よりも骨粗鬆症の予防には効果的であることがわかる．筋量が少ない集団では AA 型の方が骨密度は高いが，筋量が多い集団では AG/GG 型の方が骨密度は高いという逆転が生じており，このため対象集団の筋量が異なれば，遺伝子多型の骨密度との関係は全く逆になってしまう．遺伝子以外の個体差が十分に検討されていないことが，ゲノム研究での再現性が乏しいことの要因のひとつになっている可能性がある．

特定の疾患への感受性遺伝子多型を持つ人でも，その疾患を発症しない人もいる．発症しない要因を探るというアプローチもある．感受性遺伝子多型を持つ人の中で発症した人，発症していない人について生活習慣などの要因を詳細に比較検討することで，感受性遺伝子を持っていても疾病をどうすれば予防できるかを明らかにする

表1　NILS-LSA において骨密度との関連を新たに発見または確認した遺伝子多型

略号	遺伝子多型	骨密度への影響	文献
カルシウム向性ホルモンおよび受容体			
VDR	Vitamin D receptor（A-3731G）	男性の CC 型で大腿骨頸部の骨密度が高い	12
ESR1	Estrogen Receptor α（*PP/pp*）	高齢女性の CC 型で骨密度が低い	13
ESR1	Estrogen Receptor α（*XX/xx*）	高齢女性の GG 型で骨密度が低い	14
OST	Osteocalcin（C298T）	閉経女性の TT 型で骨密度が低い	12
ADR	Androgen receptor（CAG repeat）	未閉経女性の CAG リピートが多いと骨密度が低い	14
CYP17A1	Cytochrome P450, family 17, subfamily A, polypeptide 1（T-34C）	閉経女性の CC 型で骨密度が低い	15
サイトカイン，成長ホルモンおよび受容体			
IL6	Interleukin-6（C-634G）	閉経女性の GG 型で橈骨遠位の骨密度低い	12
TGFB	Transforming growth factor-β1（T29C）	高齢女性の TT/TC 型で橈骨の骨密度が低い	16
OPG	Osteoprotegerin（T950C）	未閉経女性の CC 型で橈骨近位の骨密度が低い	17
OPG	Osteoprotegerin（T245G）	閉経女性の GG 型で大腿骨頸部骨密度が低い	17
CCR	Chemokine receptor 2（G190A）	若年男性と閉経女性の GG/GA で骨密度が低い	18
骨基質関連蛋白			
MMP1	Matrix metalloproteinase-1（1G/2G at-1607）	閉経女性の GG/GG 型で橈骨遠位骨密度が低い	19
MMP9	Matrix metalloproteinase-9（C-1562T）	男性の CT/TT 型で骨密度が低い	20
COL	Collagen type1（G-1997T）	閉経女性の GG 型で骨密度が低い	21
ICAM1	Intercellular adhesion molecule-1（Lys469Glu）	閉経女性の AA 型で骨密度低が低い	22
PLOD1	Procollagen-lysine 2-oxyglutarate 5-dioxygenase（Ala99Thr）	未閉経・閉経女性の GA/AA 型で骨密度が低い	22
CX37	Connexin 37（Pro319Ser）	男性の TT 型で骨密度が低い	22
その他			
KLOT	Klotho（G-395A）	閉経・未閉経女性の GG 型で骨密度が低い	14
MTP	Microsomal triglyceride transfer protein（G-493T）	未閉経女性の TT 型で骨密度が高い	15
VLDLR	VLDL receptor（triplet repeat）	男性の CGG リピート 8 以上で骨密度が高い	15
ALAP	Adipocyte-derived leucine aminopeptidase（Lys528Arg）	未閉経女性の GA/AA 型で骨密度が低い	22
LIPC	Hepatic lipase（C-514T）	閉経女性の TT 型で骨密度が低い	22
CNR2	Cannabinoid receptor 2 gene（A/G, rs2501431）	未閉経・閉経女性の AA/AG 型で骨密度が低い	22
PON1	Paraoxonase-1（Gln192Arg）	閉経女性の GG 型で骨密度が低い	23
PON1	Paraoxonase-1（Met55Leu）	閉経女性の TT 型で骨密度が低い	23
PON2	Paraoxonase-2（Cys311Ser）	閉経女性の CC 型で骨密度が低い	23
DRD4	Dopamine D4 Receptor（C-521T）	男性の CC 型で骨密度が低い	24
FOXC2	Forkhead box C2（C-512T）	男女ともに T アリルで骨密度が低い	25
PLN	Perilipin（C1243T）	男性の C アリルで骨密度が低い	26
MAOA	Monoamine oxidase A（uVNTR）	未閉経・閉経女性のリピート 4 未満で骨密度低い	26
SH2B1	Src-homology-2-B（Ala484Thr）	未閉経・閉経女性の A アリルで骨密度が低い	26

（文献 27 より改変）

ことができる．さらに生活習慣などの修飾可能な危険要因については，その縦断的変化についての検討も必要である．特定の遺伝子多型を持つ人が，例えば身体活動量を2倍にしたとき骨密度はどう変化するのか，遺伝子多型によってその効果にどのような差があるのかを明らかにすることが，遺伝子多型を利用した実際の予防指導の際には重要である．こうしたデータを蓄積するためには NILS-LSA のような多数の集団で長期にわたった詳細な生活習慣や環境要因の調査が必要である[10]．

老年医学分野

NILS-LSA は学際的な研究であり，医学関係の研究も，内科・老年科だけでなく眼科，耳鼻咽喉科，整形外科，泌尿器科，歯科の各分野で，それぞれの専門家が解析を行っている．頸動脈の内中膜肥厚の加齢変化について検討し，総頸動脈内中膜肥厚は加齢によって増大するが，頸動脈分岐部のプラークは加齢の影響はそれほど強くないことを示した[49]．皮膚知覚の加齢変化[50]，皮膚知覚が耐糖能異常者で過敏になっていること[51]，空気置換法と二重エネルギー X 線吸収法（DXA 法）による体脂肪量

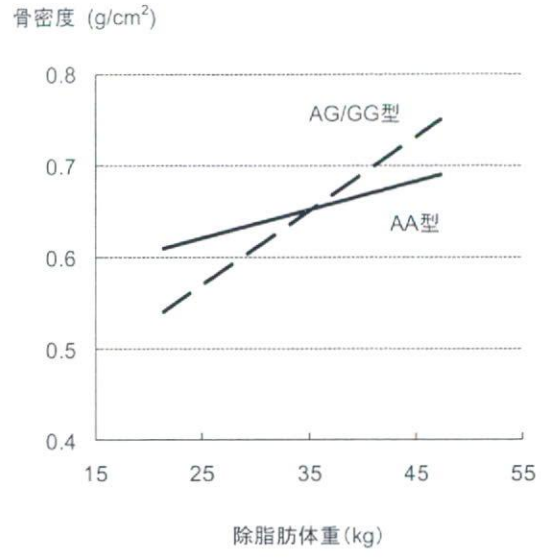

図3　閉経女性のDXAによる骨密度と除脂肪体重との関係へのエストロゲン受容体（ERα）遺伝子 *XbaI* 多型の影響．除脂肪体重すなわち筋量が多ければ骨密度は高いが，その影響はAA型よりもAG/GG型の方が強い（文献48より引用）．

測定の比較と加齢の影響[52][53]，高年男性の安静時代謝量は腹部の脂肪蓄積と，高年女性では全身の脂肪蓄積と関連しており，安静時代謝量は高年者の身体組成，体脂肪分布に影響を与える可能性があること[54]，米国国立老化研究所（NIA）との共同研究として行った冠動脈心疾患危険因子の人種差[55]，テストステロンレベルの加齢変化，男性更年期障害の分布についても明らかにした[56]．転倒の頻度と要因[57]，末梢骨定量CT（pQCT）で計測した橈骨骨密度の加齢変化[58]，骨代謝マーカーによる骨量減少の予測[59]，口腔所見の加齢変化[60]などさまざまな検討も行っている．

眼科関連の研究として，視力の加齢変化を検討するとともに[61]，約1割の対象者が日常生活視力0.5未満であり，そのほとんどは適切な屈折矯正により，0.5以上へ視力の改善がみられた．中高年者では不適切な眼鏡により日常生活視力に障害が生じている可能性が高いことを明らかにした[62]．近視などの屈折障害の要因の検討も行っている[63]．また，縦断的な解析で加齢により眼圧が下がっていることを報告した[64]．さらに加齢に伴う眼圧変化の要因について検討し，肥満及び高血圧，角膜中心厚の増大が眼圧を上げ，一方対象者の年齢が眼圧を下げていることがわかった[65]．さらに，加齢と乱視との関係[66]，コントラストを見分ける能力の加齢変化を明らかにした[67]．

聴力に関連する研究では聴力や中耳機能の加齢変化の検討を行っている[68]．また，高齢者では聴力障害があっても自覚していないことが多いことを明らかにした[69]．騒音は聴覚障害を引き起こす危険因子の中で最も良く研究され報告されている環境要因である．NILS-LSAにおいても騒音曝露歴の聴力に対する単独効果を確認し[70]，続いて喫煙，動脈硬化の聴力への効果を騒音曝露との関連に注目して解析し報告してきた[71]．全身性基礎疾患について，いくつかの疾患の聴力障害へ影響与えるかどうかの基礎的，臨床的アプローチによる研究が以前より進められてきた．NILS-LSAではさまざまな全身性基礎疾患と純音聴力レベルで表される聴力障害との関連を検討した[72]．全身性疾患のうち統計学的に有意に難聴の有病率を高めたのは，糖尿病，虚血性心疾患，腎疾患で，年齢と独立した聴力障害の危険因子となることが示唆された．NILS-LSAでは内耳機能として耳音響反射の検査も行っている．内耳機能の加齢変化[73]，中耳機能と内耳機能の関連を明らかにした[74]．しかし耳音響反射の検査で，純音聴力検査で捉えきれない変化を検出することは困難であった[75]．この他，全身疾患と内耳機能との関連などについての検討も行っている[76]．耳鳴についてはMRIで判定した脳梗塞のある群で有意な耳鳴のオッズ比の低下を認めた．部位別では被殻，視床，橋で有意なオッズ比の低下を認めた．中枢聴覚経路を含む部位の脳梗塞が耳鳴の発生を抑制している可能性が示唆された[77]．

運動生理学分野

歩行は高齢者の日常生活活動（ADL）の重要な因子である．歩行が困難となれば車イスでの生活を余儀なくされ，日常生活活動に大きな障害となる．NILS-LSAでは調査開始当初から歩行動作を3次元動作解析により定量化し，中年期から高齢期の連続的な歩行動作の加齢変化の検討を行っている[78]．2,000人を超える一般住民でのこれほど多数の動作解析データの蓄積は他にはないと思われる．また加齢による歩行速度の変化が脚筋力とどのように関わっているかを明らかにした[79]．若い頃の運動習慣は，中高年になってからの女性の筋力維持に重要であることを明らかにした[80]．中高年女性の現在の握力，脚伸展パワー，膝進展筋力は，若い頃の運動歴がある群で有意に高く，身体活動量と有意に相関していた．また，平衡機能は4年間で低下し，平衡機能の維持には体幹の筋力の強いこと，下肢の筋力の高いこと，普段素早く歩くこと等が関連することを示した[81]．

栄養分野

食事調査は，日々の食物摂取を正確に記録し，そのデー

タをすべて食品コードに割り振り，摂取量を決定するという膨大な作業が必要となる．このため大規模な集団で正確な食事調査を行い，栄養摂取量を推定することは極めて難しい．NILS-LSAでは秤量法による3日間の食事調査を継続的に行っている．3日間に摂取したすべての食品を秤量し，調査票に記入するとともに3日間のすべての食事の前後に写真撮影を行っている[82]．これにより摂取した食品や量について正確な情報を得ることが可能となる．これだけの精度の高い食事調査を，2,000名以上の対象者に継続して10年以上行っている例は世界的にも他にはほとんどないと思われる．この3日間の秤量法食事調査による栄養素摂取量の評価結果[82]，食物摂取頻度調査との比較[83]の結果について報告を行っている．また食事調査の結果を用いて日本食品標準成分表の改訂で特定の栄養素摂取量に有意な系統誤差が生じることを明らかにした[84]．

サプリメントについても，過去1年間に使用したサプリメントを参加者に持ってきていただき，栄養士の面接で聞き取りを行い，サプリメント摂取量について調査を行っている．年間のサプリメントの摂取割合は男性の55%，女性61%と高いことが明らかになった[85]．特にビタミン類やミネラル類の摂取量については，食事調査だけでは正確な評価はできない．サプリメント調査の重要性を報告するとともに，サプリメント摂取の要因を明らかにした[85]．調査で作成したサプリメントのデータベースはホームページ上に公開している（http://www.nils.go.jp/department/ep/index-j.html）．

心理学分野

「老い」は近くのものが見えにくくなる，音が聞き取りにくくなるなど身体的な衰えによって自覚されることが多いと思われていたが，実際には家族特に子供とのトラブルの結果など精神的な問題を通して老いを感じることが多いという結果が得られた[86]．高齢期には抑うつが生活の質（QOL）に大きな影響を与えることが多い．抑うつの要因の性差や年齢差を検討するとともに[87]，健康問題が活動性や抑うつに及ぼす影響に年代差があることを明らかにした[88]．また，さまざまなサポートが中高年者の抑うつを予防すること[89]，友人との死別が引き起こす抑うつを周りのサポートで軽減できること[90]，歩行が特に高年期の抑うつ低減に効果があることを報告した[91]．

転倒の心理学的側面からの研究にも力を入れてきた．転倒に対して恐怖感を持つ者の頻度と恐怖感を引き起こす要因[92][93]，転倒予防おける社会的サポートの役割[94]についての検討結果を報告している．この他，認知症で早期から障害がみられるエピソード記憶についての障害の実態と程度[95]，WHOの評価システムを用いたQOLの評価[96]，主観的幸福感に傷病経験が及ぼす影響の検討[97]などを行っている．

対人関係と健康，配偶者や身近の人の死などのライフイベント体験の年代差，抑うつとの関連等を中心に老化とストレスに関わる数多くの検討の結果を1冊の本にまとめて出版をしている[98]．

おわりに

大規模で詳細な老化の縦断的調査を行う疫学研究は，高齢化への対策が急がれる日本の社会での果たす役割がきわめて大きい．短期間の現地調査が中心の「フィールド型」の調査とは異なり，施設で年間を通して検査を行う「施設型」の縦断研究は，調査を行うための専用の施設が必要であり，また学際的な調査を行うため臨床検査技師，放射線技師，看護師，管理栄養士，心理学，運動生理学の関連などさまざまな職種のアシスタントスタッフが必要である．常勤の研究者，長寿医療センター病院から研究参加を行っている医師，外来研究員や研究生など研究者約30名がNILS-LSAに参加し，データ収集，管理，解析を行っており，さらに50名をこえるアシスタントスタッフの協力で調査が実施できている．このような研究は大学や民間の研究機関では実施が難しく，国立の老化・老年病の研究機関である長寿医療センターでしか行えない，また行っていかねばならない研究であろう．NILS-LSAは調査開始後10年をこえて，これから本格的な縦断的なデータ解析が可能となってくる．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への展開を目指して，今後も研究を続けていきたい．

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Findings from the long-term longitudinal epidemiological study

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Abstract

The purpose of long-term longitudinal epidemiological studies is to follow a certain cohort longitudinally, and collect detailed data on age-related changes in physical functions and mental activities. Longitudinal epidemiological studies are important not only to clarify the health problems associated with aging and the changes accompanying normal aging, but also to investigate the prevalence, risk factors, prevention, and early diagnosis of geriatric diseases such as dementia and osteoporosis. The National Institute for Longevity Science-Longitudinal Study of Aging (NILS-LSA) started in 1997. The participants in the NILS-LSA of the first wave were 2,267 men and women aged 40 to 79 years, randomly selected from the NILS area. Seven participants were examined every day at the NILS-LSA examination center, and followed up every two years. The aging process is assessed by detailed questionnaires and examinations including clinical evaluation, physiological functions body composition and anthropometry, physical functions, nutritional survey, and psychological assessments. The effects of genotypes, physical and psychological factors, and life-style and environment factors on aging and geriatric diseases were investigated by longitudinal analysis of these detailed and extensive data. In this review, methodologies of longitudinal study on aging and an outline of the system and examinations of the NILS-LSA are shown. The various results from the NILS-LSA research are also presented.

Key words: *Longitudinal study, Aging, Geriatric disease, Prevention, Healthy longevity*
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Association of the genetic variants of *APOA5* and *PRKCH* with hypertension in community-dwelling Japanese individuals

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Abstract. Hypertension is a complex multifactorial disorder that is thought to result from the interaction between genetic background and environmental factors. Although various loci and genes have been implicated in the 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 have now examined the relation of five candidate gene polymorphisms to blood pressure (BP) and the prevalence of hypertension in an 8-year population-based longitudinal cohort study. The 2267 subjects (1128 women, 1139 men) were aged 40-79 years and were randomly recruited to a population-based prospective cohort study of aging and age-related diseases in Japan. BP was measured after subjects had rested in a sitting position for at least 15 min. Genotypes for the -765G→C polymorphism of *PTGS2* and the 67G→A (Ala23Thr) polymorphism of *CCL11* were determined using a fluorescence-based allele-specific DNA primer assay system, and those of the 1444T→C (3'-UTR) polymorphism of *CRP*, the -1131T→C polymorphism of *APOA5* and the 1425G→A (Val374Ile) polymorphism of *PRKCH* using melting curve analysis. Longitudinal analysis of the relation between systolic or diastolic BP and the five polymorphisms with a mixed-effect model revealed that the polymorphism of *CRP* was significantly related to systolic BP in all subjects, that of *APOA5* to systolic BP in men, and that of *PRKCH* to diastolic BP in women. Longitudinal analysis of the relation between the prevalence of hypertension and the five polymorphisms with a generalized estimating equation revealed that the *CRP*, *APOA5* and *CCL11* polymorphisms were significantly related to the prevalence of hypertension in men, the *PTGS2* polymorphism to its prevalence in all subjects, and the *PRKCH* polymorphism to its prevalence in all subjects and in women. The *APOA5* and *PRKCH* polymorphisms were thus associated with both BP and the prevalence of hypertension in men and women, respectively. These results suggest that the *APOA5* and *PRKCH* polymorphisms are determinants of BP and the development of hypertension in Japanese men and women, respectively.

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Key words: hypertension, genetics, polymorphism, *APOA5*, *PRKCH*

Introduction

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

We have been attempting to identify the genes associated with blood pressure (BP) and the prevalence of hypertension in Japanese women and men recruited to a population-based prospective cohort study with a candidate gene approach. In the present study, we selected five candidate genes that might be expected to contribute to the regulation of BP (Table I). Although there is no apparent biological link between these genes, we examined the relation of their polymorphisms to systolic and diastolic BP and to the prevalence of hypertension. Among the various polymorphisms previously identified, we selected those that might be expected to affect gene function. We thus examined the relation of these polymorphisms to systolic and diastolic BP and to the prevalence of hypertension in community-dwelling Japanese women and men.

Materials and methods

Study population. The National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA) is a population-based prospective cohort study of aging and age-related diseases, the details of which have been described previously (10-13). We examined the relation of gene polymorphisms to

Table I. The five gene polymorphisms examined in the study.

Locus	Gene	Symbol	Polymorphism	dbSNP
1q21-q23	C-reactive protein, pentraxin-related	*CRP*	1444T→C (3'-UTR)	rs1130864
1q25.2-q25.3	Prostaglandin-endoperoxide synthase 2	*PTGS2*	-765G→C	rs20417
11q23	Apolipoprotein A-V	*APOA5*	-1131T→C	rs662799
14q22-q23	Protein kinase C, η	*PRKCH*	1425G→A (Val374Ile)	rs2230500
17q21.1-q21.2	Chemokine (C-C motif) ligand 11	*CCL11*	67G→A (Ala23Thr)	rs3744508

BP and the prevalence of hypertension in 2267 individuals (1128 women, 1139 men) recruited to the NILS-LSA. Individuals whose genotypes were not successfully determined were excluded from the analysis. The study protocol complied with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of the National Center for Geriatrics and Gerontology. 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 who had been resting in a sitting position for at least 15 min. The BP of each subject was confirmed by measurement performed with a mercury manometer by a physician 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 as the taking of antihypertensive medication.

Genotyping of polymorphisms. Genotypes for the *PTGS2* and *CCL11* polymorphisms 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 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 (for *PTGS2*) or 6 (for *CCL11*) 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 65˚C (for *PTGS2*) or 67.5˚C (for *CCL11*) for 30 sec, and extension at 72˚C for 30 sec, followed by 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 respective excitation and emission wavelengths of 485 and 538 nm for fluorescein isothiocyanate, and 584 and 612 nm for Texas red.

Genotypes for the *CRP*, *APOA5* and *PRKCH* polymorphisms were determined by melting curve analysis (intercalater-mediated fluorescence resonance energy transfer probe method) (Table II). The polymorphic region of each gene was amplified by PCR in a reaction mixture (25 μl) containing 20 ng DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 3 (for *CRP*) or 2 (for *APOA5* and *PRKCH*) 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 (for *CRP* and *PRKCH*) or 40 (for *APOA5*) cycles of denaturation at 95˚C for 30 sec, annealing at 65˚C for 30 sec, and extension at 72˚C for 30 sec, followed by a final extension at 72˚C for 2 min. A mixture (2 μl) of 10 pmol of probe labeled at the 5' end with Texas red and 1/400 diluted SYBR-Green I 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, then temperatures increasing to 80˚C over 10 min. The fluorescence signals were detected at excitation and emission wavelengths of 485 and 612 nm, respectively.

Statistical analysis. Age, body mass index and BP were compared in subjects with hypertension to the controls using the unpaired Student's t-test, and the prevalence of smoking was compared in the two groups by the χ^2 test. BP values were analyzed in individuals who were not taking antihypertensive drugs. We examined the effects of the genetic variants of *CRP*, *PTGS2*, *APOA5*, *PRKCH* and *CCL11* on systolic and diastolic BP and on the prevalence of hypertension based on an 8-year longitudinal cohort study. The data for the examination of each subject in the first wave (November 1997 to April 2000), in the second wave (April 2000 to May 2002), in the third wave (May 2002 to May 2004) and in the fourth wave (June 2004 to July 2006) were pooled and analyzed. Systolic and diastolic BP and the prevalence of hypertension were evaluated in terms of all subjects, women, or men. Longitudinal changes in BP were analyzed with a mixed-effect model (16), a type of statistical analysis commonly used for repeated measurements. Responses for points close in time are usually more highly correlated with each other than responses for points far apart in time. Special methods of analysis are therefore usually needed to accommodate the correlation structure of the repeated measurements. This autoregressive structure was controlled in the mixed-effect model. Systolic and diastolic BP were thus compared in the two groups (dominant or recessive model) by a mixed-effect model with adjustment for age and sex in all

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

Gene	Polymorphism	Sense primer with FITC	Sense primer with Texas red	Antisense primer with biotin	Annealing (°C)	Cycles	Mg^{2+} (mmol/l)
PTGS2	-765G→C	GAGGAGAATTTACCTTTCCXGC	GTATTATGAGGAGAATTTACCTTTCCXCC	GTTCTCCGTACCTTCACCCCCT	65.0	35	2.5
CCL11	67G→A	GGGGCTTACCTGGCCCAXTG	GGGGCTTACCTGGCCCAXCG	CCTCCAACATGAAGGTCTCCGCAG	67.5	35	6.0
Gene	Polymorphism	Sense primer	Antisense primer	Probe with Texas red	Annealing (°C)	Cycles	Mg^{2+} (mmol/l)
CRP	1444T→C	GAGCTCGTTAACTATGCTGGGA	TTATCTCCAAGATCTGTCCAACTTG	GCTGGGAAACGGTCCAAA	65.0	45	3.0
APOA5	-1131T→C	GGGACTCTGAGCCCCAGGAACTG	CGAGTGGAGTTCAGCTTTTCCTCATG	GAGCGAAAGTGAGATTTGCC	65.0	40	2.0
PRKCH	1425G→A	CCTCCTTTTGCTTTGCCATAGGTG	TCAGCACCTTCACAGCATAGAGGTCTC	TGCTTGCAAGAGTAAAGAAACA	65.0	45	2.0

FITC, fluorescein isothiocyanate. Oligonucleotide sequences are 5'→3'.

subjects or for age in women or in men. Longitudinal changes in the prevalence of hypertension were compared in the two groups by a generalized estimating equation (17) with adjustment for age and sex in all subjects, or for age in women or in men. Age-related changes in systolic or diastolic BP or in the prevalence of hypertension were estimated by quadratic curve controlling for the observation year during which the subjects attended at least one follow-up examination. Allele frequencies were estimated by the gene-counting method, and the χ^2 test was used to identify significant departure from Hardy-Weinberg equilibrium. A P-value of <0.05 was considered statistically significant. Statistical analysis was performed with SAS software release 9.13 (SAS Institute, Cary, NC).

Results

The baseline characteristics (first wave) of the 2267 study subjects are shown in Table III. Age, body mass index and systolic and diastolic BP were greater in subjects with hypertension than in the controls in the case of both the men and women. The prevalence of smoking was greater in the controls than in the hypertensive subjects in the case of the men.

The relation of the five polymorphisms to systolic and diastolic BP was analyzed with a mixed-effect model in all subjects, in women, and in men (Table IV). The 1444T→C (3'-UTR) polymorphism of *CRP* was significantly related to systolic BP in all subjects, with the *CC* genotype reflecting a higher BP. The -1131T→C polymorphism of *APOA5* was significantly related to systolic BP in men, with the *CC* genotype being associated with a lower BP. The 1425G→A (Val374Ile) polymorphism of *PRKCH* was significantly related to diastolic BP in women, with the *A* allele being associated with a higher BP.

The relation of the five polymorphisms to the prevalence of hypertension was analyzed with a generalized estimating equation for all subjects, for women, and for men (Table V). The 1444T→C (3'-UTR) polymorphism of *CRP*, the -1131T→C polymorphism of *APOA5*, and the 67G→A (Ala23Thr) polymorphism of *CCL11* were associated with the prevalence of hypertension among men in a recessive model. The *CC* genotype of *CRP* and the *AA* genotype of *CCL11* were risk factors for hypertension, whereas the *CC* genotype of *APOA5* was protective against this condition. The -765G→C polymorphism of *PTGS2* was associated with the prevalence of hypertension in all subjects, with the variant *C* allele protecting against this condition. The 1425G→A (Val374Ile) polymorphism of *PRKCH* was associated with the prevalence of hypertension among all subjects and women, with the variant *A* allele representing a risk factor for this condition. The genotype distributions of the five polymorphisms in male and female controls were all in Hardy-Weinberg equilibrium.

Given that the -1131T→C polymorphism of *APOA5* and the 1425G→A (Val374Ile) polymorphism of *PRKCH* were associated with both BP and the prevalence of hypertension in men and women, respectively, the relation between systolic or diastolic BP and age was analyzed longitudinally, according to genotypes for *APOA5* in men and those for *PRKCH* in women, with a mixed-effect model (Fig. 1) and the relation between the prevalence of hypertension and age was analyzed longitudinally with a generalized estimating equation (Fig. 2).

Table III. Baseline characteristics (first wave, n=2267) of male and female subjects with hypertension and the controls.

Characteristic	Men			Women		
	Hypertension	Control	P-value	Hypertension	Control	P-value
Number of subjects (n=2267)	358	781		377	751	
Age (years)	63.2±0.6	57.4±0.4	<0.0001	64.5±0.5	56.6±0.4	<0.0001
Body mass index (kg/m^2)	23.6±0.1	22.6±0.1	<0.0001	23.9±0.2	22.4±0.1	<0.0001
Smoking [n (%)]	100 (27.9)	333 (42.6)	<0.0001	19 (5.0)	63 (8.4)	0.0673
Number of subjects (n=1847)[a]	156	781		159	751	
Systolic BP (mmHg)	145.2±1.0	115.9±0.5	<0.0001	148.4±1.0	113.6±0.5	<0.0001
Diastolic BP (mmHg)	89.3±0.7	72.2±0.3	<0.0001	86.6±0.7	69.5±0.3	<0.0001

Data for age, body mass index, and BP are represented as the means ± SE. [a]Subjects not taking antihypertensive medication.

Systolic and diastolic BP and the prevalence of hypertension was lower in men with the *CC* genotype of *APOA5* than in the combined group, 40-80 years of age, of those with the *TT* or *TC* genotypes. Systolic and diastolic BP and the prevalence of hypertension was higher in the combined group of women of 40-80 years of age with the *GA* or *AA* genotypes of *PRKCH* than in those with the *GG* genotype

Discussion

The regulation of BP 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 (18). We have now examined the relation of five candidate gene polymorphisms to systolic and diastolic BP, and the prevalence of hypertension in community-dwelling Japanese women and men. Our results show that the -1131T→C polymorphism of *APOA5* and the 1425G→A (Val374Ile) polymorphism of *PRKCH* are associated with both BP and the prevalence of hypertension in men and women, respectively. These observations suggest that *APOA5* and *PRKCH* are, respectively, susceptibility loci for the development of hypertension in Japanese men and women.

APOA5 is located approximately 27 kb upstream of the well-characterized *APOA1-APOC3-APOA4* gene cluster at chromosome 11q23 (19). The -1131T→C polymorphism in the promoter region of human *APOA5* was found to be associated with plasma triglyceride levels in populations of various ethnicities, with the *C* allele being a risk factor for increased triglyceride concentrations (20-23). This polymorphism was also associated with high-density lipoprotein (HDL)-cholesterol levels, in addition to triglyceride levels in both Asian and Caucasian populations, with individuals with the *C* allele exhibiting reduced HDL-cholesterol concentrations (21-23). A peroxisome proliferator response element (PPRE) has been identified at a position 328 bp downstream of the -1131T→C polymorphism in the promoter region of *APOA5* (24,25). The expression of *APOA5* was also found to be increased by fibrates acting through peroxisome proliferator-activated receptor α and the PPRE. These observations suggest that the -1131T→C polymorphism of *APOA5* might influence gene expression and thereby affect the circulating concentrations of triglycerides and HDL-cholesterol. We have now shown that the -1131T→C polymorphism of *APOA5* was significantly related to systolic BP and the prevalence of hypertension in men, with the variant *CC* genotype being associated with a lower BP and protecting against hypertension. The molecular mechanism responsible for this association remains to be elucidated.

Protein kinase C (PKC) is a serine-threonine kinase that regulates a wide variety of important cellular functions, including proliferation, differentiation, and apoptosis. The η isoform of PKC (PRKCH) is regulated by diacylglycerol and phospholipids, but is insensitive to Ca^{2+} (26,27). Although its specific substrates remain to be identified, PRKCH has been implicated in the cellular response to oxidative stress. The overexpression of PRKCH in human monocytic cells resulted in the induction of inducible nitric oxide synthase and nitric oxide production in response to the exposure of the cells to endotoxin (28). Evidence also suggests that PRKCH promotes cell growth through the suppression of cyclin E expression (29) and caspase-3 activity (30), as well as through the activation of the Akt signaling pathway (31). The 1425G→A (Val374Ile) polymorphism of *PRKCH* was shown to be associated with the incidence of cerebral infarction in a Japanese population (32), with the *A* allele being a risk factor for this condition. This polymorphism is located within the ATP binding site of PRKCH (27). The Val374Ile substitution enhances the autophosphorylation and kinase activity of PRKCH induced by cell stimuli, thereby promoting signaling by this enzyme (32). We have now shown that the 1425G→A (Val374Ile) polymorphism of *PRKCH* was significantly related to diastolic BP and the prevalence of hypertension in women, with the variant *A* allele being associated with a higher BP and a risk factor for hypertension. This association might be attributable to an effect of this polymorphism on vascular inflammation, although the underlying molecular mechanism remains to be elucidated.

Given the multiple comparisons of genotypes with BP or the prevalence of hypertension in the present study, it is not possible to exclude completely potential statistical errors,

Table IV. Relation of five polymorphisms to systolic and diastolic BP (mmHg) analyzed with a mixed-effect model (first wave to fourth wave).[a]

Gene	Polymorphism			Dominant model		P-value	Recessive model		P-value
CRP	1444T→C (3'-UTR)			*TT*	*TC + CC*		*TT + TC*	*CC*	
		All subjects	No. of samples	4820	687		5484	23	
			Systolic BP	119.9±0.4	120.3±1.0	0.7343	119.9±0.4	132.5±5.3	**0.0183**
			Diastolic BP	73.8±0.2	73.9±0.6	0.9460	73.8±0.2	79.7±3.2	0.0663
		Women	No. of samples	2337	348		2676	9	
			Systolic BP	118.5±0.6	119.4±1.5	0.5466	118.5±0.5	131.9±7.7	0.0822
			Diastolic BP	72.1±0.3	72.9±0.9	0.3478	72.1±0.3	77.1±4.5	0.2761
		Men	No. of samples	2483	339		2808	14	
			Systolic BP	121.3±0.5	121.1±1.5	0.9055	121.2±0.5	131.8±7.4	0.1530
			Diastolic BP	75.5±0.3	74.7±0.9	0.3872	75.4±0.3	81.4±4.5	0.1791
PTGS2	-765G→C			*GG*	*GC + CC*		*GG + GC*	*CC*	
		All subjects	No. of samples	5129	374		5501	2	
			Systolic BP	120.1±0.4	118.1±1.4	0.1705	120.0±0.4	112.9±16.6	0.6710
			Diastolic BP	73.9±0.2	73.1±0.8	0.3556	73.9±0.2	71.9±10.0	0.8415
		Women	No. of samples	2473	207		2680	0	
			Systolic BP	118.9±0.6	116.2±1.9	0.1842	118.7±0.5		ND
			Diastolic BP	72.3±0.3	71.4±1.1	0.4337	72.2±0.3		ND
		Men	No. of samples	2656	167		2821	2	
			Systolic BP	121.3±0.5	120.0±2.1	0.5366	121.3±0.5	116.7±16.2	0.7785
			Diastolic BP	75.5±0.3	74.7±1.3	0.5655	75.4±0.3	75.0±9.8	0.9668
APOA5	-1131T→C			*TT*	*TC + CC*		*TT + TC*	*CC*	
		All subjects	No. of samples	2384	3118		4809	693	
			Systolic BP	119.3±0.6	120.5±0.5	0.1156	120.0±0.4	119.6±1.0	0.6845
			Diastolic BP	73.4±0.3	74.2±0.3	0.0639	73.9±0.2	73.6±0.6	0.6452
		Women	No. of samples	1135	1549		2321	363	
			Systolic BP	117.6±0.8	119.4±0.7	0.0935	118.3±0.6	120.9±1.5	0.1062
			Diastolic BP	71.6±0.5	72.6±0.4	0.1075	72.1±0.3	72.9±0.9	0.3822
		Men	No. of samples	1249	1569		2488	330	
			Systolic BP	120.9±0.7	121.5±0.7	0.5211	121.6±0.5	118.4±1.4	**0.0332**
			Diastolic BP	75.1±0.5	75.7±0.4	0.2601	75.6±0.3	74.3±0.9	0.1684
PRKCH	1425G→A (Val374Ile)			*GG*	*GA + AA*		*GG + GA*	*AA*	
		All subjects	No. of samples	3528	1983		5260	251	
			Systolic BP	119.8±0.5	120.3±0.6	0.5487	119.9±0.4	121.7±1.7	0.2971
			Diastolic BP	73.6±0.3	74.2±0.4	0.2100	73.8±0.2	75.6±1.0	0.0664
		Women	No. of samples	1729	959		2567	121	
			Systolic BP	118.0±0.7	119.8±0.9	0.0941	118.6±0.5	119.4±2.4	0.7455
			Diastolic BP	71.7±0.4	73.1±.5	**0.0311**	72.1±0.3	73.4±1.4	0.3804
		Men	No. of samples	1799	1024		2693	130	
			Systolic BP	121.6±0.6	120.6±0.8	0.3124	121.1±0.5	124.0±2.3	0.2167
			Diastolic BP	75.6±0.4	75.2±0.5	0.5941	75.3±0.3	77.9±1.4	0.0715
CCL11	67G→A (Ala23Thr)			*GG*	*GA + AA*		*GG + GA*	*AA*	
		All subjects	No. of samples	4196	1286		5400	82	
			Systolic BP	120.1±0.4	119.4±0.8	0.4401	119.9±0.4	121.6±2.9	0.5699
			Diastolic BP	73.8±0.3	73.8±0.5	0.8746	73.8±0.2	74.1±1.8	0.8873
		Women	No. of samples	2055	613		2630	38	
			Systolic BP	118.5±0.6	118.6±1.1	0.9520	118.6±0.5	115.6±4.4	0.4959
			Diastolic BP	72.1±0.4	72.3±0.7	0.7400	72.2±0.3	69.8±2.6	0.3583
		Men	No. of samples	2141	673		2770	44	
			Systolic BP	121.5±0.6	120.3±1.0	0.2676	121.1±0.5	126.4±3.8	0.1715
			Diastolic BP	75.5±0.3	75.2±0.6	0.6233	75.4±0.3	77.7±2.3	0.3310

[a]Systolic or diastolic BP was compared between two groups (dominant or recessive model) for each polymorphism, with adjustment for age and sex in all subjects or for age in women and in men. P-values <0.05 are shown in bold. ND, not determined.

Table V. Relation of five polymorphisms to the prevalence of hypertension analyzed with a generalized estimating equation (first wave to fourth wave).[a]

Gene	Polymorphism			Hypertension (%)	Control (%)	P-value (dominant)	P-value (recessive)
CRP	1444T→C (3'-UTR)						
		All subjects	*TT*	2051 (87.4)	4182 (87.5)	0.6198	0.0690
			TC	274 (11.7)	580 (12.1)		
			CC	21 (0.9)	15 (0.3)		
		Women	*TT*	927 (84.4)	2057 (87.3)	0.1111	0.6673
			TC	164 (14.9)	293 (12.4)		
			CC	8 (0.7)	6 (0.3)		
		Men	*TT*	1124 (90.1)	2125 (87.8)	0.3698	**0.0360**
			TC	110 (8.8)	287 (11.9)		
			CC	13 (1.0)	9 (0.4)		
PTGS2	-765G→C						
		All subjects	*GG*	2235 (95.23)	4442 (93.10)	**0.0363**	ND
			GC	111 (4.73)	327 (6.85)		
			CC	1 (0.04)	2 (0.04)		
		Women	*GG*	1045 (95.0)	2161 (92.0)	0.0640	ND
			GC	55 (5.0)	188 (8.0)		
			CC	0 (0)	0 (0)		
		Men	*GG*	1190 (95.4)	2281 (94.2)	0.2882	ND
			GC	56 (4.5)	139 (5.7)		
			CC	1 (0.1)	2 (0.1)		
APOA5	-1131T→C						
		All subjects	*TT*	1002 (42.6)	2094 (43.9)	0.4507	0.5061
			TC	1051 (44.7)	2078 (43.6)		
			CC	298 (12.7)	598 (12.5)		
		Women	*TT*	434 (39.3)	1016 (43.2)	0.0712	0.1970
			TC	494 (44.8)	1030 (43.8)		
			CC	176 (15.9)	307 (13.1)		
		Men	*TT*	568 (45.6)	1078 (44.6)	0.5598	**0.0287**
			TC	557 (44.7)	1048 (43.4)		
			CC	122 (9.8)	291 (12.0)		
PRKCH	1425G→A (Val374Ile)						
		All subjects	*GG*	1440 (61.3)	3072 (64.3)	**0.0324**	0.8568
			GA	813 (34.6)	1492 (31.2)		
			AA	98 (4.2)	215 (4.5)		
		Women	*GG*	667 (60.4)	1546 (65.6)	**0.0178**	0.5752
			GA	390 (35.3)	708 (30.0)		
			AA	47 (4.3)	103 (4.4)		
		Men	*GG*	773 (62.0)	1526 (63.0)	0.4975	0.8233
			GA	423 (33.9)	784 (32.4)		
			AA	51 (4.1)	112 (4.6)		
CCL11	67G→A (Ala23Thr)						
		All subjects	*GG*	1839 (79.0)	3640 (76.5)	0.2896	0.4509
			GA	446 (19.1)	1047 (22.0)		
			AA	44 (1.9)	71 (1.5)		
		Women	*GG*	846 (77.5)	1809 (77.3)	0.7159	0.1258
			GA	237 (21.7)	495 (21.2)		
			AA	8 (0.7)	36 (1.5)		
		Men	*GG*	993 (80.2)	1831 (75.7)	0.0734	**0.0256**
			GA	209 (16.9)	552 (22.8)		
			AA	36 (2.9)	35 (1.5)		

[a]The prevalence of hypertension was compared between two groups (dominant or recessive model) for each polymorphism, with adjustment for age and sex in all subjects or for age in women and in men. P-values <0.05 are shown in bold. ND, not determined.

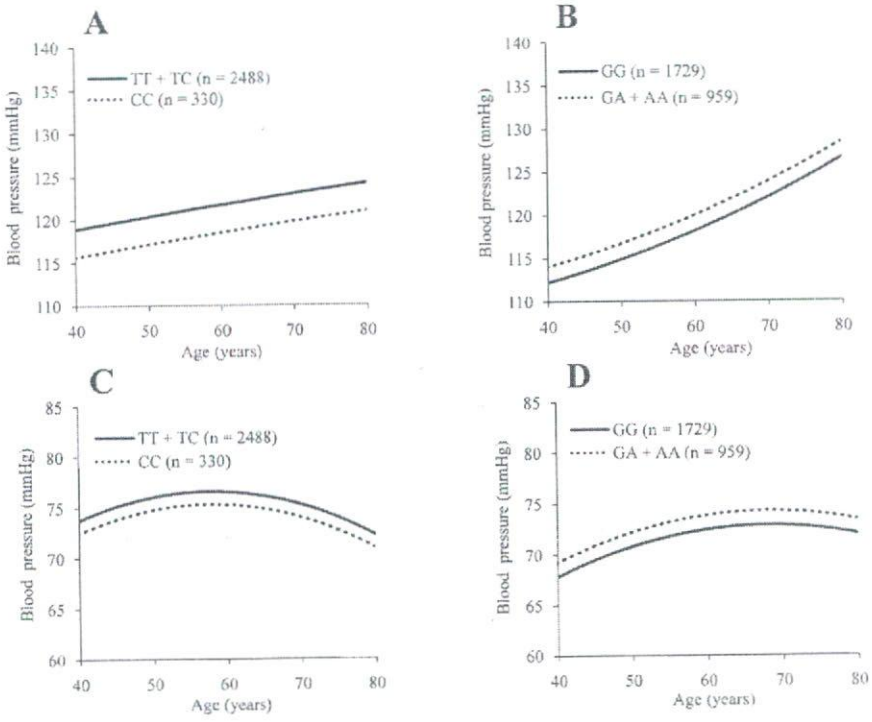

Figure 1. Longitudinal analysis of relations between systolic (A and B) or diastolic (C and D) BP and age according to the genotype for *APOA5* (*TT + TC* versus *CC*) in men (A and C) or to the genotype for *PRKCH* (*GG* versus *GA + AA*) in women (B and D) with a mixed-effect model.

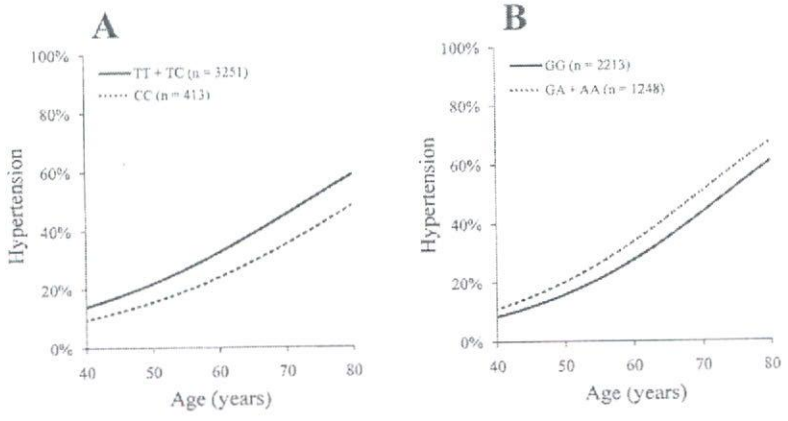

Figure 2. Longitudinal analysis of relations between the prevalence of hypertension and age according to the genotype for *APOA5* (*TT + TC* versus *CC*) in men (A) or to the genotype for *PRKCH* (*GG* versus *GA + AA*) in women (B) with a generalized estimating equation.

such as false positives. It is also possible that one or more of the polymorphisms associated with the BP or the prevalence of hypertension in our study is in linkage disequilibrium with other polymorphisms of the same genes or of nearby genes that are actually responsible for the development of hypertension. 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 the -1131T→C polymorphism of *APOA5* and the 1425G→A (Val374Ile) polymorphism of *PRKCH* in the regulation of BP and the development of hypertension in Japanese men and women, respectively. The determination of genotypes for these polymorphisms may prove to be informative to the assessment of the genetic risk for hypertension. 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 for a more accurate assessment of the genetic risk for this condition.

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Glutathione peroxidase 1 Pro198Leu variant contributes to the metabolic syndrome in men in a large Japanese cohort[1–3]

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ABSTRACT

Background: There has been much interest in the role of free radicals and oxidative stress in the pathogenesis of metabolic syndrome (MetS). Cellular antioxidant enzymes such as glutathione peroxidase 1 (GPX1) play a central role in the control of reactive oxygen species.

Objective: We examined whether GPX1 polymorphism (Pro198Leu) is associated with MetS as well as with each component of MetS.

Design: The study was a cross-sectional analysis of randomly selected, community-dwelling Japanese persons aged 40–70 y (1128 M, 1105 F).

Results: The genotype frequencies for the GPX1 Pro198Leu polymorphism in this cohort were 0.846, 0.151, and 0.003 for *CC*, *CT*, and *TT*, respectively. The *CT/TT* genotypes had significantly higher waist-hip ratios, triacylglycerol concentrations, homeostasis model assessment for β-cell function, and systolic and diastolic blood pressures in men ($P = 0.045$, 0.012, 0.011, 0.004, and 0.003, respectively) than did the *CC* genotype; the *CC/TT* genotypes also had higher insulin in both sexes ($P = 0.019$ for men, $P = 0.010$ for women) and higher body fat mass ($P = 0.027$) and homeostasis model assessment for insulin resistance ($P = 0.008$) in women. The *CT/TT* genotypes showed significant association with higher prevalence of MetS as defined by 2 commonly used criteria in men [odds ratio (OR): 2.02; 95% CI: 1.30, 3.15 by the International Diabetes Federation criteria; OR: 1.49; 95% CI: 1.02, 2.18 by the modified National Cholesterol Education Program criteria) but not in women. The *CT/TT* genotypes showed a higher prevalence of central obesity (OR: 1.93; 95% CI: 1.31, 2.85) and hypertriglyceridemia (OR: 1.52; 95% CI: 1.08, 2.15) in men but not in women; there were no differences in other components of MetS between the *CC* and *CT/TT* genotypes in either sex.

Conclusion: GPX1 Pro198Leu variants are associated with the prevalence of MetS in Japanese men but not in women. *Am J Clin Nutr* 2008;87:1939–44.

INTRODUCTION

Accumulating evidences suggest that oxidative stress is involved in various hyperglycemia-induced diabetic complications as well as insulin resistance (1–3). Furthermore, in recent years, there has been much interest in the role of free radicals and oxidative stress in the pathogenesis of metabolic syndrome (MetS) (4, 5). It has been shown that obesity per se may induce systemic oxidative stress and that increased oxidative stress in accumulated fat is, at least in part, the underlying cause of the dysregulation of adipocytokines and the development of MetS (6, 7). In addition, the imbalance between reactive oxygen species and antioxidants improves insulin resistance in mice and humans (8, 9). It has been proposed that increased oxidative stress also underlies the pathophysiology of hypertension (10).

Oxidative stress may be defined as an imbalance between the production and degradation of reactive oxygen species. Enzymatic inactivation of reactive oxygen species is achieved mainly by antioxidative enzymes including glutathione peroxidase (GPX), superoxide dismutase, and catalase. In mammalian cells, glutathione and the GPX constitute the principal antioxidant defense system. There are at least 6 different GPX isoenzymes, all of which contain selenocysteine at their active sites (11).

The most abundant of these isoenzymes is GPX1, a ubiquitous intracellular form and key antioxidant enzyme within most cells, which uses glutathione to reduce hydrogen peroxide to water and lipid peroxides to their respective alcohols, and also acts as a peroxynitrite reductase (12, 13). GPX1 is polymorphic at codon 198 (at nucleotide 594, a cytosine-to-thymine (C→T) substitution (rs1050450), which results in either a proline or a leucine at that position, and the frequency of the *leu* allele is strongly associated with an increase in the risk of various kinds of cancer (14–18). The identity of the amino acid at codon 198 (proline or leucine) has functional consequences with regard to the level of enzyme activity in response to the provision of increasing amounts of selenium to cells in culture (15). In fact, it has been reported that human erythrocyte GPX activity was lowered according to the *T* allele dose (17). In addition, the 198Leu polymorphism in the coding region of the *GPX1* gene had a 40% decrease in enzyme activity in vitro functional analyses (18). The overexpression of GPX1 in cultured endothelial cells rescued the endothelial dysfunction induced by homocysteine-mediated oxidative

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stress, which suggests that GPX1 constitutes the principal antioxidant defense system (19)

However, the contribution of the GPX1 Pro198Leu variant to the prevalence of MetS or the components of MetS remains unknown. In the present study, we used 2233 randomly selected, community-dwelling, middle-aged and older Japanese people to examine whether GPX1 Pro198Leu is associated with MetS as well as with each component of MetS among Asian populations.

SUBJECTS AND METHODS

Study population

The present study consisted of a cross-sectional analysis of 1105 women and 1128 men who participated in the first wave of examinations at the National Institute for Longevity Sciences–Longitudinal Study of Aging (NILS-LSA) from April 1998 to March 2000. The subjects of the NILS-LSA were local men and women ranging in age from 40 to 79 y. The populations of the city of Obu and the town of Higashiura in the Aichi prefecture in central Japan were stratified by both age and sex, and the subjects were randomly selected from resident registrations in cooperation with the local governments. The number of men and women was to be the same so that sex difference could be tested. Age at the baseline was to be 40–79 y, and the number of participants in each decade (40–49, 50–59, 60–69, and 70-79 y old) was to be the same. The examinations included various areas of gerontology and geriatrics, such as medical examination; anthropometric measurements; analysis of body composition, physical function, and physical activity; psychological assessment; nutritional analysis; and molecular epidemiology. The subjects will be followed up every 2 y. The details of the NILS-LSA were reported elsewhere (20–22). Randomly selected men and women were invited by mail to attend an explanatory meeting. At that meeting, the procedures for each examination and the follow-up schedule were fully explained.

Written informed consent to the entire procedure was obtained from each participant. The study was approved by the Ethics Committee of NILS.

Anthropometric variables

Body weight was measured to the nearest 0.01 kg by using a digital scale, height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer, and body mass index (BMI; in kg/m^2) was calculated. Waist circumference (WC) and waist-to-hip ratio (WHR) were used as the indexes of body fat distribution in this study. WHR was calculated as the ratio of waist circumference measured at the midpoint between the anterior superior iliac crest to the lowest rib-to-hip circumference. Whole-body fat mass, assessed by using dual-energy X-ray absorptiometry (DXA, QDR-4500; Hologic, Waltham, MA) was used as an index for determining body composition.

Biochemical assays of blood

An antecubital blood sample was drawn from each subject after an overnight fast. Serum total cholesterol, triacylglycerols, and LDL cholesterol were determined enzymatically, whereas serum HDL cholesterol was measured by using the heparin-manganese precipitation method. Fasting plasma glucose was assayed by using the glucose oxidase method (23). Plasma insulin, an immunoreactive insulin (IRI), was measured by radioimmunoassay (24). Homeostasis model assessments of insulin resistance (HOMA-IR) and β-cell function (HOMA-β) were calculated as described by Matthews et al (25).

Measurement of blood pressure

Blood pressure was measured with an automatic sphygmomanometer (BP-203RV-II; Colin, Tokyo, Japan) in subjects who had rested in a sitting position for ≥15 min. The blood pressure in each subject was confirmed by a mercury manometer recording made by a physician according to the guidelines of the American Heart Association (26).

Definition of the metabolic syndrome

We applied both the International Diabetes Federation (IDF) and the modified National Cholesterol Education Program (NCEP) definitions of MetS. MetS, according to the modified NCEP criteria (27), included any 3 of the following: *1*) WC > 90 cm in men and > 80 cm in women; *2*) triacylglycerol concentrations > 1.7 mmol/L or drug treatment for elevated triacylglycerol; *3*) HDL-cholesterol concentrations < 1.0 mmol/L in men and < 1.3 mmol/L in women or drug treatment for reduced HDL cholesterol; *4*) systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg or antihypertensive drug treatment; and *5*) fasting plasma glucose concentrations ≥ 5.6mmol/L or drug treatment for elevated glucose. The IDF definition of MetS includes central obesity (WC > 90 cm in men and > 80 cm in women) plus any other 2 criteria in the modified NCEP criteria as proposed (28).

Determination of *GPX1* genotypes

Genotypes were determined by using a fluorescence-based, allele-specific DNA primer assay system (Toyobo Gene Analysis Co, Ltd, Osaka, Japan). The polymorphic regions of rs1050450 were amplified by polymerase chain reaction with allele-specific sense primers labeled at the 5′-end with either fluorescein isothiocyanate (5′-GCGCCCTAGGCACAGCTxAG-3′) or Texas red (5′- GCGCCCTAGGCACAGCTxGG-3′) as allele-specific hybridization probe and with an antisense primer labeled at the 5′-end with biotin (5′-GTGTGCCCCTACGCAGGTACA-3′). The reaction mixtures (25 μL) contained 20 ng DNA, 10 pmol fluorescein isothiocyanate– and biotin-labeled primer, 5 pmol Texas red–labeled primer, 2.5 mmol/L of each deoxynucleoside triphosphate, 2.5 mmol $MgCl_2$/L, and 0.625 U rTaq DNA polymerase (Toyobo Gene Analysis Co, Ltd) in polymerase buffer. The amplification protocol consisted of initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 65 °C for 30 s, and extension at 72 °C for 30 s; a final extension was conducted at 72 °C for 2 min. Our genotyping error rate was ≈0.1%.

Data analysis

Quantitative data were compared between the 2 groups by the unpaired Student's *t* test. In the analyses of the association between genotypes and glucose, lipid metabolisms, or blood pressure, participants who were being treated with oral hypoglycemic agents or insulin, hypolipidemic agents, or antihypertensive agents were excluded, respectively. Allele frequencies were estimated by the gene-counting method, and the chi-square test was used to identify any significant departure from Hardy-Weinberg